

Characterisation of Cardiovascular Risk in Adults with Turner Syndrome

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Abstract

Turner Syndrome (TS) results from the complete or partial absence of one X chromosome in females. Short stature and gonadal dysgenesis are characteristic, with increased risks of cardiovascular disease, diabetes and obesity. This thesis investigates the prevalence and pathogenesis of factors contributing to cardiovascular risk. Because women with TS differ from normals in terms of their X-chromosome defect and oestrogen deficiency, they were compared to similarly-aged normal women, and a second control group of similarly-aged women with 46,XX oestrogen deficiency was also recruited.

A cross-sectional study investigated the spectrum of structural disease in the heart, aorta and conduit vessels using various imaging techniques. Intima media thickness (IMT), arterial stiffness and endothelial function were also assessed. Progression of aortic dilatation was investigated in a longitudinal echocardiography study.

Metabolic abnormalities in TS were investigated by measuring anthropometric parameters and serum markers of adiposity and comparing them in all three groups. Adipose tissue distribution was further investigated in a subgroup of women with TS and normal controls.

A longitudinal oestrogen dose-ranging study was performed in women with TS and 46,XX primary amenorrhoea to assess oestrogen effects on various parameters pertaining to cardiovascular risk.

Women with TS had greater height-adjusted arterial diameters than controls, and greater IMT, the latter amenable to reduction by increasing doses of oestrogen. The rate of aortic dilatation was greater than in the normal population. Endothelial function did not differ significantly.

Women with TS have some features of the metabolic syndrome, but fasting insulin, glucose and leptin concentrations are surprisingly low, despite increased C-reactive

protein and Interleukin-6 concentrations, greater central obesity and increased visceral fat than controls.

Age, bicuspid aortic valve, blood pressure and oestrogen status were the most important predictors of cardiovascular disease in TS. This knowledge should aid identification of therapeutic targets to improve care in this population.

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Chapter 1

Introduction

General Background

Turner syndrome (TS), the most commonly occurring chromosomal abnormality in females, results from complete or partial absence of one X chromosome in a phenotypic female. It is usually accompanied by characteristic clinical features, of which the most consistent are short stature and primary amenorrhoea.

The Italian anatomist Morgagni first described TS in 1768. There were further descriptions by Funke in 1902 and Ullrich in 1930, but the syndrome is named after the American endocrinologist Henry Turner, who described seven women with typical features of the syndrome in 1938 (1). He drew attention to the presence of primary amenorrhoea in the syndrome, and also pioneered treatment with oestrogen (2).

Approximately 1:2500 live female births have TS with an estimated worldwide prevalence of 1.5 million women (3). Mortality rates in TS are three times higher than in the general population, with a reduction in life expectancy of up to 13 years (4). This is mainly accounted for by higher rates of cardiovascular disease, both structural and atherosclerotic (4-6).

Although the majority of women with TS will have been diagnosed in childhood or adolescence, about 10% are not diagnosed until adulthood (see Chapter 2, Figure 2.1). This variability reflects the wide clinical spectrum of the syndrome, which ranges from the severe phenotype with short stature, primary amenorrhoea, lymphoedema and characteristic dysmorphic appearance, to the more mildly affected with minimal impact on stature or secondary amenorrhoea.

1.1. Clinical Manifestations of Turner Syndrome in Adults

1.1.1. Genetics

The Turner karyotype is characterised by absence of one X chromosome, monosomy X or

45,X, the most common karyotype found in approximately 50% (7)) or presence of an abnormal X chromosome such as isochromosome X, a partial deletion or ring X. In addition, various mosaic forms exist, which include 45,X/ 46,XX and 45,X/46,XY mosaicism. Many adult units reassess karyotype in adults as low-grade mosaicism may have been missed in the past if too few cells were counted. To some extent, an accurate karyotype has a bearing on future morbidity. Women with monosomy X generally have the most severe phenotype, while isochromosome X is more frequently associated with autoimmunity (8,9) and inflammatory bowel disease (10). Ring X karyotype is also associated with a particularly severe phenotype, as well as psychological and learning difficulties. This appears to be related to failure of X inactivation which is more likely with a smaller X ring (11,12). Mosaicism generally results in a milder phenotype, with up to 40% of such women spontaneously entering puberty, although premature ovarian failure may ensue (13). Women with Y-chromosome mosaicism have an increased risk of gonadoblastoma, and a minority may be virilised.

In monosomy X (45,X), the X-chromosome is of maternal origin in 68-80% of women, and of paternal origin in 20-32%, indicating that paternal sex chromosome loss is the most common cause of this condition (14-16). Parental origin of the X-chromosome has been associated with cardiovascular disease, height, webbing of the neck and psychological profiles (16,17), suggesting that genetic imprinting of certain genes may affect function.

In a normal woman with 46,XX karyotype, one X chromosome in each cell is inactivated by the process of lyonisation, which occurs at an early stage of development in the foetus. It has become clear, however, that this random process is not absolute, in that some genes, which also have homologues on the Y chromosome, are required in duplicate for normal development. Absence of a second copy, or 'haploinsufficiency' of certain genes, of which the majority have been found to map to the short arm of the X chromosome, is considered to be largely responsible for the Turner phenotype (18).

The search for specific genes which account for the Turner phenotype has focused particularly on the two predominant features of the syndrome, short stature and ovarian dysgenesis. The SHOX gene (short stature homoeobox-containing gene), situated in pseudoautosomal regions on the X chromosome, encodes for a protein found mainly in bone fibroblasts, and is favoured as a gene responsible for short stature (19-22). There

have been extensive searches in the 'critical regions' of both the long and short arms of the X chromosome for genes governing ovarian dysgenesis, but none have been conclusive (22,23). Conversely, it may be that any abnormality of the X-chromosome results in impaired cell division and hence oocyte atresia (24).

1.1.2. Ovarian Dysgenesis, Fertility and Oestrogen Replacement

In normal development, germ cell numbers fall progressively from their peak of approximately 7,000,000 at 5 months' gestation so that only half remain at full gestation, and these continue to decline until menopause (25). In TS the process of accelerated oocyte apoptosis and increased ovarian stromal fibrosis begins after the third month of gestation. Ovarian failure ensues within the first few months or years of life with a consequent rise in serum gonadotrophin concentrations (26,27). The Middlesex Hospital series found the overall incidence of spontaneous puberty to be 12% in the Adult Turner Clinic population, ranging from 8% in those with monosomy X, to 10% in those with isochromosome X and 47% in those with 45,X/ 46,XX mosaicism (26). A recent study has demonstrated follicles in eight of nine adolescent girls investigated with ovarian biopsy (28). Fertility is poor, however, and unassisted pregnancy occurs in only 2-8% of women (13,29,30). Of these, approximately one third end in spontaneous abortion or perinatal death, and a further third have chromosomal abnormalities, particularly Down's or Turner's syndrome, and congenital malformations (29,31). Earlier studies are probably subject to publication bias, but a recent Danish study suggests that, with prospective monitoring, the outlook may be better than previously thought (30).

Long-term requirement for oestrogen replacement therapy is the norm in adults with TS. After induction of puberty, which some will attain spontaneously, this will take the form of cyclical oestrogen with a progestogen. Oestrogen replacement has been shown to reduce the risks of osteoporosis (32), aspects of vascular risk (33-35), and may improve liver function (36,37). The relative benefits of different oestrogen preparations including transdermal oestrogen, continuous oral natural oestrogens such as conjugated oestrogen or oestradiol valerate, and oral contraceptive preparations (OCP) with regard to liver function in TS have yet to be clarified. The ethinyloestradiol-containing OCP has been associated with an increased risk of liver disease (38), but in TS, ethinyloestradiol has been shown to be more efficient than oestradiol valerate in suppressing raised hepatic

enzymes (37). Improvement of cognitive function (39,40), and reduction in risk of colonic carcinoma (41,42) with oestrogen replacement therapy in TS remain to be confirmed. The use of oestrogen replacement in oestrogen deficient women is not thought to cause an increased risk of breast cancer above that of the general population (5).

1.1.3. Osteoporosis

It has been postulated that there is a primary defect in bone formation, such as an abnormality of chondroplasia, which may explain the short stature and apparently high incidence of osteoporosis in TS. This may be related to oestrogen deficiency or other endocrine/ paracrine derangement (43). Measurement of BMD is affected by stature, and it therefore remains to be clarified whether the low BMD found in women with TS reflects true osteoporosis or is, in fact, an artefact of short stature. Volumetric correction of BMD suggests that this may be normal or only slightly reduced, particularly in cortical bone (44-46). Fracture risk at characteristic fracture sites is certainly increased in TS by a factor of two to three (5,47,48), and especially the forearm has been implicated (49). It has been suggested that the increased fracture risk is due to inadequate bone strength relative to body weight (45) or to an increased risk of falls, and recently a selective deficiency of forearm cortical bone has been demonstrated which is apparently independent of oestrogen exposure (50).

In adulthood, oestrogen deficiency is proposed as the most important factor affecting BMD, with oestrogen replacement resulting in improvement of BMD (32,51). Treatment with growth hormone and oestrogen during childhood and adolescence has been found to contribute to higher bone mass, combination of the two having a greater effect (52). Earlier start age of oestrogen (before age 12) and growth hormone treatment for over one year are beneficial (53). Those with spontaneous puberty have relatively preserved bone mass (26), and it may be that after correction for bone volume and oestrogen deficiency, there is, in fact, no evidence for osteoporosis in TS (51).

1.1.4. Endocrine Dysfunction

The most common endocrine abnormalities in TS are thyroid disease, abnormalities of the growth hormone axis, diabetes, dyslipidaemia and oestrogen deficiency. The latter three will be examined more closely under the heading of cardiovascular risk (see below).

The incidence of autoimmune thyroid disease increases with age in TS. Approximately half of an adult TS population have positive thyroid autoantibodies, and 25-30% are hypothyroid, compared with 1.5% of the general population (9,54,55). Graves' disease does not show increased incidence despite its similar pathogenetic mechanism. Isochromosome X (46,Xi(Xq)) karyotype is particularly associated with autoimmune thyroid disease.

There is continuing debate about the abnormalities of the growth hormone (GH) axis in TS. It is thought that girls with TS are relatively resistant to GH, but may also be relatively deficient. One study showed that GH levels before the age of nine years in girls with TS are the same as in controls, but the levels are lower in those with TS aged 9-20 years. IGF-1 levels, however, were found to be lower through the entire age range (56). A relationship between reduced GH levels and higher body weight has been reported, although this appeared to apply to both TS patients and controls, whilst there was no difference in IGF-1 levels in TS and controls (57,58). Gravholt *et al.* showed that although total IGF-1 levels in TS may be normal, free IGF-1 levels are reduced compared to controls (59). It has also been demonstrated that TS fibroblasts secrete less IGF-1 and IGF-2 in response to GH stimulation (60), and indeed that TS monocytes and T-lymphocytes are relatively resistant to the action of GH and IGF-1 (61). This relative resistance to GH is reflected by the reports from the Dutch experience with GH treatment in TS, which have suggested that their recent improvement in long-term and final height results may be due to higher GH doses and/or younger start age (62). There is no evidence to suggest that adults with TS are deficient or resistant to GH.

1.1.5. Renal Abnormalities

Renal disorders in TS are generally categorised as congenital and renovascular. With a nine-fold increased incidence of congenital anomalies (5), the most common

malformations are horseshoe kidney and collecting system abnormalities, both of which occur in approximately 7-8% of girls with TS. Of those patients with congenital renal pathology, 8% require surgical intervention (63). There is a weak association between renal malformations and monosomy X, but any karyotype may be affected (64). With regard to the sequelae of renal abnormalities, there is an increased risk of pyelonephritis and pelvi-ureteric obstruction, which may result in chronic renal failure. Although horseshoe kidney is considered to confer greater susceptibility to renal malignancy in the normal population, no excess risk of neoplasia has been found in TS (65). Renovascular disease may contribute to the development of hypertension in TS as discussed below (Section 1.2.2.1.).

1.1.6. Gastrointestinal Disorders

A variety of gastrointestinal problems affect women with TS with greater frequency. These include inflammatory bowel disease, liver dysfunction, intestinal telangiectasia, colonic carcinoma (see Section 1.1.10) and possibly coeliac disease.

Inflammatory bowel disease (IBD) is estimated to be two to three times more common in TS than in the general population (5,66). In our series the prevalence was found to be 2.6%, with the prevalence of Crohn's disease being twice that of ulcerative colitis, a reversal of the general population trend (7). Three fatal cases of IBD in TS have been reported, reflecting the frequent severity of the condition in TS. Complications are common and include colectomy in 40% of reported cases, fistula formation and sepsis. Threshold for suspecting IBD should be low in TS patients with unexplained diarrhoea or gastrointestinal bleeding, and conversely a diagnosis of Turner Syndrome should be considered in adolescents with IBD and short stature.

The association with karyotype isochromosome Xq (found in 52% of reported cases of IBD in TS) (10) mirrors that of autoimmune thyroid disease in TS (see above). This lends weight to the theory that the cause of IBD may be immune dysfunction. There is still debate surrounding reports of an increased prevalence of coeliac disease in TS, reports ranging from 4 to 10% (67,68), and 6.4% in the largest, most recent study (69).

Intestinal telangiectasia, thought to be a developmental defect, may increase the risk of gastrointestinal bleeding in TS (70).

Hepatic dysfunction, initially manifest as derangement of liver enzymes, has been reported in up to 80% of women with TS (54), although in the Middlesex Hospital series the figure was 44% (36). The findings included elevated transaminases, alkaline phosphatase and particularly gamma glutamyl transferase, but no demonstrable association with karyotype, body mass index, immune markers or history of HRT use (type and duration). Alcohol excess and infectious hepatitis are not thought to be aetiological factors (71). The risk of progression to cirrhosis, the prevalence of which is five times that in the general population (5), is unknown. Histology from liver biopsies varies from fatty infiltration to hepatic fibrosis and vascular abnormalities. Similarities to neonatal hepatic morphology have been proposed, suggesting that lifelong oestrogen deficiency may be a causative factor (72).

The relationship of hepatic dysfunction to oestrogen administration remains controversial. The suggestion that the abnormalities found in TS may be due to oestrogen deficiency is based on the finding that oestrogen improves the disturbance (36,37); but others have documented a deterioration in liver function with oestrogen therapy (73). It is notable that in a recent series of hepatic abnormalities in TS, almost one third of the patients had never been treated with oestrogen (74). The optimal type of oestrogen replacement is still in question. The combined oral contraceptive pill is thought to cause liver dysfunction in women with a normal karyotype whilst natural oestrogens such as oestradiol valerate are not. It has been suggested that transdermal oestrogens which avoid the hepatic first pass effect may be preferable (75). Others have found that the overall oestrogen dose may be the crucial factor, a higher dose being more effective in normalising liver enzymes (37).

1.1.7. Neurological Complications

Hearing loss, comprising conductive (CHL), sensorineural deafness (SNHL) or a mixed picture, is very common in TS. Over 90% of middle aged women are affected and 60% have clinically significant deficit (76).

CHL is attributed to congenital craniofacial abnormalities which cause distortion of the eustachian tubes and defective ventilation, as well as impaired mucociliary transport (77). The resulting otitis media affects up to 68-78% of patients (7), and of these, 43% have been reported to have conductive hearing loss (78). SNHL is less well understood and its aetiology is not fully explained. It has been reported in 58% of girls, down to the age of 6 years (78), and in 61% of women over 35 years of age (54) and is known to progress with age.

Oestrogen receptors have been demonstrated in the inner ear which may be of significance (79), and an association between hearing loss and the growth hormone axis has also been suggested. In a study of girls and women with TS, Barrenas *et al.* showed a weak inverse correlation between otitis media and serum insulin-like growth factor (IGF-1) concentration, the latter being positively correlated with stature (77), although another group has shown a greater incidence and severity of otitis media in TS patients receiving GH treatment than in a placebo group (80). It has recently been suggested that there may be an association between the thrifty phenotype hypothesis and SNHL, with low foetal concentrations of IGF-1 as the possible mechanism, programming future hearing problems in early life (81). An *in vitro* study in chick embryos has demonstrated that IGF-1 stimulates growth of the otic vesicle and cochleo-vestibular ganglion (82). Our own study found no association between either CHL or SNHL and history of previous oestrogen deficiency or growth hormone therapy (83).

An association between CHL and karyotypes monosomy X and isochromosome Xq has been reported, and a 'dose-response' relationship has been proposed between the degree of Xp (short arm of the X-chromosome) mosaicism and hearing function (84). Our own study confirmed only the association with monosomy X (83). The influence of karyotype on SNHL in the literature is difficult to interpret. Some papers offer no statistical testing of an apparent adverse effect of isochromosome X (85,86). While SNHL is more prevalent in monosomy X compared to mosaics, this might reflect the generally more severe phenotype in the former, rather than an effect specific to the short arm of X (Xp) (84). We found no association between SNHL and karyotype in our study (83).

Ophthalmological disorders are present in up to 63% of women with TS(87) and are thought to result from foetal lymphoedema. Strabismus is found in up to 37% of women,

with amblyopia (41%), hypermetropia (41%), bilateral epicanthus (10-45%), ptosis (16-29%) and impairment of colour vision (10%) also occurring with greater frequency than in the general population (88,89).

1.1.8. Respiratory Disease

Until fairly recently, the respiratory system was believed to be spared from any effects in TS. A recent study has shown, however, that the relative risk of mortality due to respiratory disease, especially pneumonia, is 7.9 in TS patients of all ages, although the effect is predominantly in adults (6). Another study demonstrated increased bronchial reactivity in TS which is ameliorated by oestrogen therapy (90), and there have been case reports of ciliary aplasia causing bronchiectasis, ciliary dyskinesia and sleep apnoea syndrome (91-93).

1.1.9. Psychosocial Issues

Cognitive impairment in the form of generalised intellectual impairment is not a marked feature of TS except in those with karyotype ring X (11). Specific areas of intellectual functioning may be affected, however. Verbal skills are generally well preserved, but deficits may be apparent in non-verbal skills such as visuo-spatial processing, motor coordination, perceptual abilities, affect recognition, and attentional abilities (94,95). As a result, women with TS may have difficulty with arithmetic, constructional tasks, sense of direction and learning to drive (96).

The relative contributions of an abnormal X-chromosome and oestrogen deficiency to differences in brain structure have not been clarified. An 'X-chromosome dosage' effect has been proposed, whereby the amount of X chromosome missing determines the severity of cognitive impairment (97,98). This is supported by the finding of cognitive deficits in oestrogen-replete women with TS which were not apparent in women with premature ovarian failure or normal control women (99). It is interesting to note that functional and structural neuroimaging studies have detected abnormalities of neural development in TS females, including smaller size of the cerebellum and pons, thalamus and hippocampal, caudate and lenticular nuclei (100). Reductions in parieto-occipital areas, corpus callosum white matter tracts and the right temporal lobe have also been

noted (101). These structural differences appear to be at least partly related to the degree of monosomy X (97).

It has also been suggested that cognitive abilities and social functioning are influenced by an imprinted gene on the X-chromosome which escapes X-inactivation. Those with a paternally-derived X-chromosome were reported to have superior verbal and executive skills than those with a maternally-derived X-chromosome (16). Despite the genetic influences, oestrogen replacement has been demonstrated to improve nonverbal and visuo-motor skills (102), but growth hormone treatment has not (103).

The difficulties with non-verbal communication may partly account for the fact that women with TS characteristically find it more difficult to make friends and embark on sexual relationships. Poor self-image which derives from short stature and delayed sexual maturation is a contributory factor (104) and this may be improved by commencing oestrogen replacement therapy before the age of 12-14 years (105). Women with TS tend to leave the parental home and become sexually mature at a later age than their peers (106). Another trait seen in women with TS is that although over one third may have a university education, they are often employed in jobs for which they are overqualified. Childcare or healthcare professions appear to be a frequent choice (7,54).

1.1.10. Risk of Malignancy

The risk of gonadoblastoma increases ten-fold between the ages of 10 and 30 years (2% to 27.5%) in those women with 45,X/ 46,XY mosaicism (107). 50% of these tumours have been reported to develop into dysgerminomas, and a further 10% into other malignant germ cell tumours in women with levels of Y-chromosome mosaicism detected by conventional cytogenetics (108), prompting recommendations of early prophylactic gonadectomy in these patients. It has recently been found that a further 5% of TS individuals have low levels of Y-chromosome material when tested by the more sensitive polymerase chain reaction technique, but the clinical significance of this is not clear (109).

Breast, ovarian and endometrial cancers are not thought to occur with greater frequency in TS than in the general population (5), although the use of unopposed oestrogens has been

associated with reports of endometrial carcinoma (110). It is recommended that women with TS should be prescribed a combination of oestrogen and progesterone in physiological doses. The practice by some of using continuous combined preparations as a means of avoiding endometrial shedding has been shown to be safe with regard to endometrial carcinoma only in an older age group with a greater degree of uterine atrophy, although the risk of ovarian carcinoma is increased in postmenopausal women (111). Safety evidence in younger women is currently not available.

The relative risk of colon cancer in women with TS has been reported to be between five and seven (5,112), although there is no excess of reports in the literature. Both inflammatory bowel disease and oestrogen deficiency are known to be possible risk factors but, in fact, IBD did not precede the development of colon cancer in the reported cases. Oestrogen receptors are found in colonic mucosa and epidemiological studies in postmenopausal women have found a reduced risk of colonic malignancy in those treated with HRT (113). The effect of growth hormone on colonic cancer in TS is unknown. A recent report showed an increased incidence of colorectal cancer in children treated with human pituitary growth hormone treatment before 1985, but it is not clear whether any of these patients had TS (114). It should be noted that, except for a small number of patients (115,116), use of growth hormone in TS became commonplace only in the late 1980's when synthetic growth hormone had replaced the human form. It may be some years before a definite answer to this question is available.

Women with TS are at increased risk of multiple melanocytic naevi, although these do not appear to be related to sun exposure and do not have an increased risk of malignant transformation (117). Excision is therefore recommended only for those naevi which cause irritation with clothing or if there is concern about malignant transformation, and women should be counselled about the increased risk, albeit anecdotal, of keloid scar formation in TS.

1.2. Cardiovascular Risk in Turner Syndrome

Cardiovascular complications are the main cause of increased mortality in Turner Syndrome (TS), in which life expectancy may be reduced by up to 13 years (4), with a ten-fold increased risk of mortality from circulatory disease (6). Cardiovascular disease (CVD) in TS may be both congenital and acquired (4-6).

1.2.1. Structural Heart Disease

1.2.1.1. Congenital Heart Disease

Congenital heart disease is estimated to occur in 22-40% of patients with TS (118-121). It is most common in those with karyotype monosomy X and least common with isochromosome Xq, suggesting that the long arm of the X chromosome may harbour genes which influence cardiac development (118-120,122). A maternally derived monosomic X chromosome in TS may be associated with more frequent congenital heart defects compared to a paternally derived X, suggesting an effect from an imprinted gene on cardiac development (17). Both coarctation and bicuspid aortic valve have been shown to be associated with developmental central lymphoedema (characterised by webbing of the neck), independent of karyotype (123).

Bicuspid Aortic Valve

The congenital cardiac anomalies are predominantly left-sided, and bicuspid aortic valve (BAV) is the most common, occurring in 12-38% of TS patients (118,119,124-128). An association has been reported in TS between BAV and the ring X chromosome karyotype in one series (121), although this has not been confirmed by others. BAV may occur in isolation (which is more usual) or in association with other cardiac abnormalities (approximately 30% (119)). This differs from BAV in the general population, where the overall prevalence is just 0.24% in females and 0.75% in males, association with other cardiac abnormalities occurring in 70% of cases (129,130).

The prevalence of BAV in TS patients with aortic root dilatation on echocardiography has been quoted at 25-50% (126,128) (the prevalence of BAV in patients with aortic root dilatation in the general population is approximately 20% (131)). An association with

coarctation of the aorta has also been demonstrated in TS, with BAV occurring in 19/49 patients with coarctation in one study (124), whilst patients with a BAV were shown to have a relative risk for coarctation of 2.6 in another (119). Calcification of the BAV, which occurs with age, may cause functional impairment with either aortic stenosis or regurgitation. There is also a putative risk of infective endocarditis, although there are no reports in the literature of this having occurred in patients with TS.

Coarctation of the aorta

Coarctation of the aorta has been reported to occur in 6-12% of women with TS (125,132), compared to a prevalence of 0.03% in the general population (133,134). Coarctation in TS is frequently associated with webbing of the neck and with severe lymphoedema in monosomy X aborted fetuses (123,135,136). This has prompted the suggestion that in TS it is caused by abnormal lymphatic flow which compresses the ascending aorta and alters intracardiac blood flow. Coarctation is an important cause of hypertension in TS. When identified clinically, coarctation is usually surgically corrected in childhood, but screening takes place in only a minority of girls.

1.2.1.2. Aortic dilatation and dissection

Aortic dilatation and dissection are more prevalent in TS than had previously been recognised (128). The prevalence of aortic dilatation has been reported as between 8 and 42% (126,128,137). Risk factors for aortic root dilatation include hypertension, aortic valve pathology and other cardiac malformations which have been reported in 90% of patients (128,137).

Aortic root dilatation itself is an important risk factor for aortic dissection, and the heightened awareness of this catastrophic complication in recent years has prompted greater attention to predisposing factors. In a survey of death certification, 3 out of 156 patients died from aortic dissection, and a fourth from aortic rupture with coarctation (4), whilst the deaths of 8% of women in another mortality study were attributed to aortic aneurysm (6). 50% of those with aortic dilatation and/or dissection were under the age of 21 years in a recent literature review (128), and dissection has been catastrophic in four pregnant women with TS (138-140). This is a particularly worrying finding in an era of

increasing demand for assisted reproduction. Indeed, a recent study has estimated that the mortality rate for TS women achieving pregnancy through ovum donation may be as high as 2% from aortic dissection or rupture (141).

Atherosclerosis is a well documented risk factor for aortic dissection in the general population (142,143), although a mesenchymal defect may be more important in TS (128). There have been at least 44 case reports of aortic dissection, with histology available in 26 cases: 65% of these had evidence of cystic medial necrosis (118,124,144). This prompts an analogy to Marfan syndrome, which therefore provides a valuable paradigm by which to study the risk of aortic dissection in TS. In Marfan's, abnormal microfibrils are thought to alter load-bearing by the aorta and predispose to aortic dilatation by degeneration in the elastic laminae. Previous studies have shown that the aorta in Marfan syndrome has abnormal elastic properties causing it to be stiff with reduced compliance (145-147). Other risk factors for aortic dissection in TS include coarctation (as in Noonan syndrome, another condition in which webbing of the neck may result from foetal lymphatic obstruction (135,148)), BAV and hypertension (126,128,137). BAV and hypertension are known associations of aortic dissection in the general population. The prevalence of BAV in one series of patients with aortic dissection under the age of 40 years was 9% (149).

1.2.2. Atherosclerosis

Ischaemic heart disease is thought to be twice as common in adults with TS as in the general population (5). Women with TS have a high prevalence of risk factors for atherosclerosis in addition to hypertension, including both insulin dependent (IDDM) and non-insulin dependent diabetes mellitus (NIDDM), insulin resistance, obesity and dyslipidaemia which will be considered below. Oestrogen deficiency and supplementation are also thought to contribute and these will be considered in Section 1.2.3.

1.2.2.1 Hypertension

Hypertension is three times more common in TS (5), occurring in up to 21% of girls and 50% of women (3,87,128,150). A blunted circadian rhythm has been demonstrated in the

blood pressure of even normotensive girls who may lose their nocturnal 'dip' (150). Hypertension may be secondary to renal disease or coarctation in only 20% of patients (7). Small vessel renovascular disease has been proposed as a possible explanation (3), given the elevated renin activity which may be found before oestrogen therapy is instituted (150). Reduced arterial compliance and obesity are other potential contributors (34,35,151). Karyotype does not appear to influence the prevalence of hypertension, which may be underestimated without the use of age-related normal ranges (3,151,152). Hypertension is exacerbated by ethinyl oestradiol but not by natural oestrogens or growth hormone (35,153).

1.2.2.2 Glucose Homoeostasis

Diabetes mellitus has been reported as the underlying cause of death in 25% of TS patients (154). IDDM is nearly 12 times more common than in the general population (5), although an excess of islet cell antibodies has not been documented (155,156). The prevalence of NIDDM in TS is increased more than four-fold (5), and the prevalence of impaired glucose tolerance has been quoted at 10-78% (33,157-161). It has also been reported to occur at a younger age than in the general population, and a karyotype effect has been noted, with normal glucose tolerance predominating in patients with mosaicism (160).

An analysis of the literature with regard to glucose homoeostasis in TS (and indeed in the general population) is not straightforward because of the varying methodologies used in different studies. Some use oral or intravenous glucose tolerance tests (OGTT/ IVGTT) or a glucose infusion test to derive absolute values, or calculate area under the curve for insulin and glucose (33,157-162); others use insulin tolerance tests (162,163) or euglycaemic clamps (164-166). Furthermore, calculations to derive values for glucose oxidation (eg by indirect calorimetry) or non-oxidative glucose disposal (storage) are then of varying complexity, and the results and conclusions drawn are therefore not necessarily comparable, and indeed sometimes contradictory. Lastly, the degree of oestrogen deficiency in different study groups has been variable.

An additional problem which applies to many studies in TS patients is the difficulty of finding a suitable comparison group. With regard to glucose homoeostasis, body mass

index (BMI) matching is frequently attempted, but most studies do not achieve this (33,164). It should also be noted that women with TS have a higher waist-hip ratio for a given BMI (167), and this has been taken to mean greater central obesity. Some studies compare only TS patients not treated with oestrogen replacement to normal controls (33), others have sought to match for oestrogen deficiency by comparing to women with 46XX premature ovarian failure (157,161).

Thus there are studies which demonstrate increased stimulated insulin concentrations during OGTT, IVGTT or glucose infusion in TS (33,157,159,163), decreased insulin concentrations (160,162,165), increased first phase insulin response (157) and decreased first phase insulin response (33,159,161-163). Reduced response to insulin secretagogues has also suggested insulin deficiency (168). Several studies have found increased glucose concentrations during OGTT or IVGTT analysis (33,160,169), but normal results have also been reported (162). There are studies which show no difference in fasting insulin and glucose concentrations in TS compared to normal controls (33,162,164) or raised fasting insulin concentrations (166), whilst a study comparing TS to women with premature ovarian failure found both decreased fasting insulin and glucose concentrations in TS (161). Euglycaemic clamp studies have suggested that the defect may be one of glucose storage (164), or perhaps even a muscle receptor defect (166). There have also been conflicting reports regarding the relationship between greater BMI and impaired glucose metabolism in TS, with some groups finding an association (159), while others do not (164).

There are few reports in the literature of pancreatic histology from TS patients, but one study found definite hyperplasia of the islets of Langerhans with signs of decreased activity of the β -cells in an elderly woman with TS, whilst there was also a tendency to hyperplasia in a neonate (162).

Some previous work has suggested an increased incidence of diabetes amongst the relatives of patients with TS (168,170,171) but this has not been corroborated by other studies (157,162).

It is interesting that, as early as 35 years ago, it was suggested that the pathogenesis of diabetes in TS may be distinct from type 2 diabetes mellitus (157). Various mechanisms

for the defects in glucose homeostasis have been invoked, ranging from an insulin secretory defect to true insulin resistance and a more specific glucose storage defect (as distinct from a problem of glucose oxidation). The latter has also been demonstrated in other insulin-resistant states: not only in type 2 diabetics (172,173) and in the early pre-diabetic state (174), but also in first degree relatives of type 2 diabetics (175), and in insulin resistant subjects with normal glucose tolerance (176). This glucose storage defect may be partly due to decreased glycogen synthesis (177-179).

To conclude from this literature review, if two of the most recent, and perhaps most carefully matched studies are to be believed (33,161), untreated women with TS do appear to have impaired first phase insulin secretion and insulin resistance. Fasting glucose and insulin concentrations are neither consistently higher or lower than in controls. Neither of these studies examined the relationship between oestrogen-treated women with TS and either normal or 46,XX premature ovarian failure controls.

The effects of oestrogen treatment on glucose homeostasis in TS have already been alluded to, and will be considered further below. It should be noted, however, that growth hormone and oxandrolone, the other hormones widely used in paediatric practice in the treatment of TS to maximise growth, also influence glucose metabolism. Growth hormone therapy has been shown to cause hyperinsulinaemia although not glucose intolerance (180,181), and it is reported that the effects are reversed after 6-12 months (182). Oxandrolone may further exacerbate hyperinsulinaemia (183), and in fact, 44% of girls taking either oxandrolone alone or in combination with growth hormone have been found to have abnormal glucose tolerance tests (184).

1.2.2.3. Obesity

Obesity is widespread in TS. In young adult populations, the BMI has been reported to average at 25-27kg/m², significantly higher than in age-matched control groups (33,151), and fat free mass is also reduced (33). Although the relationship between BMI and glucose homeostasis in TS has yet to be clarified, obesity undoubtedly contributes to the cardiovascular risk in these women. Obesity has been found to be associated with serum triglyceride and cholesterol concentrations (151,185), and also systolic and diastolic blood

pressure (151). The weight problem in TS may be partly attributable to reduced physical fitness (33).

The obesity in TS appears to be predominantly central, with an increased waist-hip ratio, and a reduced fat free mass has been demonstrated on bioelectrical impedance measures (33). Given that the relationship between obesity and glucose homeostasis does not directly mirror that in the general population, further investigation of the nature of the obesity in TS would appear warranted. The present study therefore set out to clarify body fat distribution in TS using bioelectrical impedance and whole body MRI.

1.2.2.4 Dyslipidaemia

Dyslipidaemia in TS has been documented in girls from the age of 11 years and has been reported to be independent of age, karyotype or BMI (185). The prevalence of hypercholesterolaemia has been reported to be 50% in a young adult TS population (186), although in this study a significant proportion of women were hypothyroid, and the effect of BMI on the results was not investigated. There have been reports of higher concentrations of total cholesterol, LDL and HDL subfractions in paediatric and adult series (185,186), but these are not universal findings (33,37,151,187). Hypertriglyceridaemia, related to obesity, has also been noted (151).

Growth hormone therapy has been shown to lower total and LDL cholesterol, and increase HDL cholesterol (180,188), but this does not appear to be a sustained effect on cessation of treatment (182). The effect of oestrogen will be discussed further below.

1.2.3 Cardiovascular Risk and Oestrogen

Women with TS may have a potentially increased cardiovascular risk as a result of both oestrogen deficiency in the untreated state, and oestrogen therapy in the oestrogen-replete state. This section will consider the possible risks from both. Data regarding oestrogen treatment in young women are scant and the validity of extrapolation of data from the studies in postmenopausal women, where the bulk of the evidence lies, is unknown.

Premenopausal women in the general population are relatively protected from cardiovascular disease, and even after menopause, women have a lower cardiovascular risk than men with similar other cardiovascular risk factors (189). This gave rise to the theory that female sex steroids were protective from a cardiovascular risk point of view, a hypothesis which was supported by observational epidemiological studies (190-193) and even angiographic studies (194,195). Until relatively recently, therefore, the effect of exogenous oestrogen in postmenopausal women was thought to be neutral at worst, but probably beneficial, despite evidence to the contrary from the Framingham study nearly 20 years ago (196,197).

Over the last few years, more studies have been published suggesting that oestrogen replacement in postmenopausal women confers no cardiovascular benefit and may even increase risk with regard to both primary and secondary prevention of cardiovascular disease (198-204). The deleterious effect of HRT on cardiovascular disease appears to be more marked in the 60-69 years age-group than in 50-59 year-olds (205). One explanation for this paradox is that oestrogen may slow atherogenesis in young women while a prothrombotic effect predominates in the older postmenopausal age-group.

1.2.3.1 Oestrogen Deficiency

There are very few data on cardiovascular disease in women with premature ovarian failure, but one study has shown that there is an increased cardiovascular mortality which is thought to be due to oestrogen deficiency (206). This is supported by data from studies in hypopituitarism, which have shown increased cardiovascular mortality in women (207,208), and particularly in those with gonadotrophin deficiency (209). The mortality data reflecting increased incidence of cardiovascular disease in TS stem from a time when oestrogen replacement in adults was less widespread, and it may be that current cardiac risks are substantially reduced. Nevertheless, it should be noted that up to 24% of adults with TS default from oestrogen replacement therapy (210).

1.2.3.2. Oestrogen replacement therapy – general considerations

As indicated above, the overwhelming majority of studies on the effects of exogenous oestrogen in oestrogen-deficient women have been carried out in middle-aged

postmenopausal women, who are thought to be a very different category from young women with premature or primary ovarian failure. The use of exogenous oestrogens must be considered in terms of their various effects, which include symptom control for oestrogen deficiency, and effects on the cardiovascular system, breast and other cancers, primary or secondary prevention of osteoporosis, haemostasis and metabolism, liver and cognition. Since the subject of this study is cardiovascular disease, this will be the main focus of discussion, but brief consideration of the impact of HRT on other systems is warranted to illustrate the overall balance of risk and benefit.

There have been several studies in post-menopausal women showing that the use of HRT increases the risk of breast cancer and is unsafe, even for short periods, in women with a previous history of breast cancer (204,211,212). The WHI but not HERS studies demonstrated an absolute risk reduction of colorectal cancer with use of HRT (204,213). The impact of HRT on osteoporosis in postmenopausal women is still disputed, however, with evidence that its effects are either beneficial (204) or neutral (213). It is no longer recommended as first line treatment for osteoporosis as any fracture protection appears to be short-lived on cessation of treatment (214). Further important points from the large prospective studies are that HRT increased the rates of venous thromboembolism (215) and biliary tract surgery (204,213). Thus the overall consensus must be that, whilst HRT is certainly beneficial in terms of reducing symptoms of oestrogen deficiency in postmenopausal women (216), its use is not indicated for primary or secondary prevention of other conditions, and this also includes cardiovascular disease (see above).

It is interesting, however, that although the large prospective studies with hard clinical endpoints have failed to support the use of oestrogen replacement for cardiovascular disease prevention, there have been many studies suggesting benefit on surrogate markers of cardiovascular disease. These will be considered in further detail below.

1.2.3.3 Oestrogen, Lipids and Metabolism

The literature on the effects of oestrogen replacement on lipids and metabolism in postmenopausal women is vast. It has been documented that oestrogen replacement induces a more favourable plasma lipid profile, with reduced total cholesterol and low density lipoprotein (LDL) concentrations and increased high density lipoprotein (HDL)

concentrations (198,202,217-219). The effect of oestrogen replacement is complex, however, and has been shown to vary according to the type of regimen used – whether conjugated equine oestrogen, 17- β oestradiol or oestradiol valerate, with or without progesterone, and whether oral or non-oral (eg transdermal, implants).

There is no absolute consensus in the literature as to which is better, although oral regimens, which exert greater effects on the liver through the first pass mechanism, are generally considered to result in greater changes in plasma lipoproteins, and may therefore result in increased HDL (both HDL-2 and HDL-3 subfractions (220)) and apolipoprotein A concentrations, and decreased lipoprotein (a) and apolipoprotein B concentrations, but also greater plasma triglyceride concentrations (219,221-223). Whilst LDL concentrations may be lower with oral oestrogen replacement, the particle size may also be smaller than with transdermal oestrogen, hence making the particles more susceptible to oxidation (224). Interpretation of these studies must nevertheless be cautious in view of the fact that oral and transdermal oestrogen groups were frequently studied on different progestogen regimens and may therefore not be directly comparable (218,222,225).

Whilst HRT has thus been shown to improve lipid profiles in postmenopausal women, this effect has not been demonstrated in short-term studies of oestrogen replacement in TS (33,35).

With regard to insulin sensitivity and glucose homeostasis, there have been a number of studies assessing the effects of oestrogen in healthy women, and also a few in TS. Oral contraceptive therapy in young normal women can result in relative insulin resistance, which may be due to a combination of oestrogen-induced insulin resistance and progestogen-associated changes in insulin half-life (226).

In postmenopausal women both endogenous and exogenous oestrogen may be associated with insulin resistance (227,228). The effect of the latter again appears to be associated with route of delivery, with oral oestrogen more likely to induce a deterioration in glucose tolerance than transdermal, possibly resulting from increased hepatic insulin uptake without a compensatory increase in first-phase pancreatic insulin secretion. There have been some studies suggesting that HRT reduces insulin resistance (229-231), even in

women with type 2 diabetes mellitus (232,233), but overall the picture remains unclear (234-236). A paradox has been noted between a reduction in fasting plasma glucose but impaired glucose tolerance in women taking oestrogen (237). This may be due to an inhibitory effect of oestrogen on the action and secretion of glucagon and a stimulatory effect on glucocorticoid activity, with the reduced fasting insulin concentrations then being attributable to reduced gluconeogenesis resulting from glucagon antagonism (238). Insulin resistance has been reported to be greater during the combined oestrogen-progesterone phase of a cyclical preparation than the oestrogen-only phase (228). Higher doses of oestrogen and progestogens may attenuate any beneficial effect on insulin sensitivity (230,239,240).

Studies in TS women thus far have also not shown entirely consistent results. In the study by Elsheikh *et al.*, fasting glucose and insulin were reduced after three months of treatment with oestradiol valerate and levonorgestrel (35). In the study by Gravholt *et al.*, women were treated with 17 β -oestradiol via both oral and transdermal routes, using norethisterone as a progestogen. This regimen resulted in an increased area under the curve for glucose on oral glucose tolerance testing, but lower fasting serum insulin and acute plasma glucose response on intravenous glucose tolerance testing (33). The authors raised the question as to whether it was the norethisterone which had deleterious effects on glucose metabolism in the latter study.

It has been noted that oestrogen replacement therapy may affect body fat distribution, and this may contribute to the effect on insulin sensitivity. Visceral adipose tissue was found to be reduced in studies of postmenopausal women on HRT (241-244). This has also been demonstrated in TS. Elsheikh and Conway reported that oestrogen reduced waist-hip ratio without a change in body mass index (35), whilst Gravholt *et al.* found an increased proportion of total fat free mass after oestrogen therapy (33).

A further aspect of oestrogen therapy which must be considered is its effect on circulating concentrations of sex hormone binding globulin (SHBG). An association has been reported between insulin resistance and low concentrations of SHBG in both men and women (245-247), and in women (but not men) low levels of SHBG have been shown to predict the development of type 2 diabetes (248). Oral oestrogen therapy in particular is known to increase SHBG concentrations (225).

Leptin, which reflects adiposity, should also be considered at this point. Gender differences, with concentrations of leptin being higher in women, have been documented, (249), and also minor variations through the menstrual cycle in rats (250). Some *in vitro* studies suggested that leptin concentrations are influenced by short-term oestrogen administration (251,252), but this has not been substantiated by others (250). *In vivo* studies in postmenopausal women have shown that, although leptin concentrations do increase after the menopause (253), this effect is not independent of fat mass and insulin concentrations (254-256). Oestrogen administration may, however, prevent the increase in fat mass and thereby maintain premenopausal leptin concentrations (257).

1.2.3.4 Oestrogen, Inflammation and Haemostasis

Atherosclerosis, thrombosis and inflammation are linked through a number of complex molecular pathways (258,259). Numerous inflammation-sensitive factors have been found to predict increased cardiovascular risk, and of these, high sensitivity C-reactive protein (hs-CRP) has been found to be the strongest predictor (260), although recent work suggests it may not be quite as useful as previously thought (261). Other markers of the inflammatory cascade which predict future risk of plaque rupture include cellular adhesion molecules such as E-selectin and P-selectin, interleukin-6 (IL-6), tumour necrosis factor- α (TNF- α) and soluble intercellular adhesion molecule-1 (ICAM-1) (262). Factors more specific to the coagulation cascade include prothrombin, (a risk factor for venous thrombosis), von Willibrand factor (vWF) and coagulation factor VIIIc, and plasminogen and plasminogen activator inhibitor-1 (PAI-1) which participate in fibrinolysis. Fibrinogen and P-selectin are involved in both thrombosis and inflammation (263,264).

The effects of oestrogen replacement therapy on these factors have been assessed as a means of testing surrogate markers for cardiovascular risk. Once again, the evidence has been somewhat divergent. CRP has been shown to increase rapidly with oral oestrogen therapy (263,265-267). It has been suggested that this may explain the adverse early effects of oestrogen therapy (266). By contrast, oestrogen induced a more favourable cardiovascular risk profile with increases in plasminogen (263) and increases in E-selectin (266,268). With regard to different routes of oestrogen, the transdermal route does not cause the same increase in CRP (269). Overall, the effects of oral oestrogen on inflammation and haemostasis appear to be more marked than with transdermal oestrogen

– several studies have demonstrated an increase in fibrinolytic activity coupled with potentially antiatherogenic changes in lipids, but this is counterbalanced by a trend towards coagulation activation and hypercoagulability (268,270).

1.2.3.5 Oestrogen and Vascular Physiology

In addition to the effects on lipids, metabolism and markers of inflammation and haemostasis mentioned above, oestrogen has also been shown to exert effects directly on vascular physiology and the endothelium. There are oestrogen receptors on the walls of both endothelial and smooth muscle cells (271-274).

Arterial endothelial dysfunction, particularly the reduction in bioavailability of endothelium-derived nitric oxide, is a key early event in atherogenesis, and may occur long before the appearance of structural atherosclerotic changes (275,276). Nitric oxide is thought to function as an endogenous antiatherogenic molecule by maintaining low arterial tone at rest, inhibiting leucocyte-endothelial interactions, attenuating platelet aggregation and inhibiting smooth muscle proliferation (277). The testing of endothelial nitric oxide release therefore provides important information about normal arterial physiology, and has been shown to be an independent predictor of coronary artery disease (278-281).

Endothelium-dependent flow-mediated dilatation (FMD) can be non-invasively measured at the brachial artery by ultrasound techniques. It has been shown to vary during the menstrual cycles of normal women. One study found FMD to be higher during the follicular and luteal phases when serum oestrogen concentrations are higher, and lower during the menstrual phase (282), whilst another group has shown the nadir to be in the early luteal phase (283). The difference in FMD between men and women at the age of 35 years but not at the age of 55 years (when women were assumed to be postmenopausal) suggests that differences between the sexes are at least partly attributable to sex steroid differences (284). Both oral and transdermal oestrogen therapy improve endothelium-dependent FMD in postmenopausal women (285-289), although the effect is more convincing with oral oestrogen (290). The improvement may be evident within one month of commencement in postmenopausal women (288), and has also been shown in young women with premature ovarian failure after six months of oestrogen

therapy (291). Vaginal micronised progesterone does not appear to attenuate the beneficial effect of oestrogen (287), but oral progesterone therapy may do so (292). With regard to assessing FMD in women with TS, it is of interest to note that FMD has been found to be negatively correlated with vessel size in both men and women, but the effect may be more marked in women (284,293).

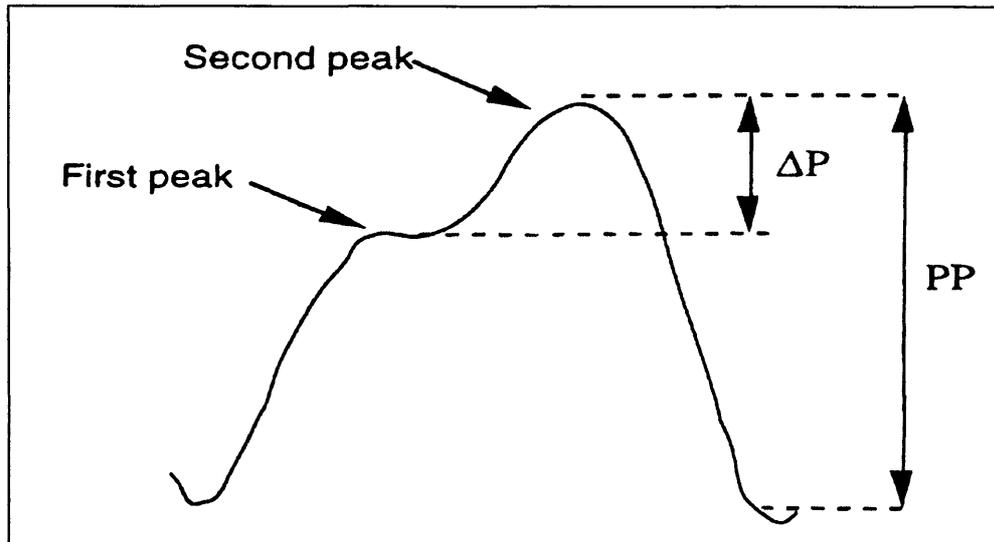
Another method of assessing progression to atherosclerosis is through ultrasound measurement of the thickness of the arterial intima-media complex, particularly in the carotid artery. The normal intimal thickness consists of a single layer of endothelial cells over a thin layer of subendothelial connective tissue, but for the purposes of ultrasound measurement, the tunica media is also included (294). The spectrum of atherosclerotic lesions from the fatty streak to the fibrous plaque and finally the complicated lesion can be seen to increase intima media thickness (IMT) and changes in IMT may thus precede the development of localised atherosclerotic plaques. This measure can provide predictive information on future vascular risk. A study in middle-aged men has shown that for each 0.1mm increase in common carotid IMT, the risk of anterior myocardial infarction was increased by 11% (294).

Oestrogen has been shown to exert effects on IMT. An interventional study in healthy postmenopausal women found a reduction in IMT of 0.0017mm (295). A study in postmenopausal women with elevated LDL cholesterol has shown a reduction in IMT in those taking oestrogen replacement compared to those who were not after three years, although this effect was not independent of the effect of a statin (296). The benefit of oestrogen has also been demonstrated in a cross-sectional study of postmenopausal women with and without diabetes (297).

Another independent predictor of cardiovascular events is increased elastic artery stiffness. This can also be assessed non-invasively by ultrasound. Pulse wave velocity (PWV) refers to the speed of a pulse wave travelling along a conduit vessel such as the carotid or radial arteries and is related mainly to structural factors such as increased collagen and medial smooth muscle (298). Augmentation index (Aix), which is derived from pulse wave analysis, is calculated as the difference between the second reflected peak and the first systolic peak as a percentage of pulse pressure (299)(see Figure 1.1.). Aix reflects structural factors, but also dynamic factors such as endogenous

vasoconstrictor tone and endothelium-derived nitric oxide (298). Greater values of both PWV and AIx indicate stiffer arteries or reduced compliance.

Figure 1.1. Augmentation Index is calculated as the difference between the second reflected peak and the first systolic peak as a percentage of pulse pressure ($\Delta P/PP$) (299)



PWV is an independent marker of cardiovascular risk (300,301) and is associated with other cardiovascular risk factors such as blood pressure, insulin resistance, central obesity, greater carotid IMT (302) and coronary plaques (303). Data on the prognostic value of AIx are still emerging (304,305) and it has been suggested to have greater predictive power than PWV (306).

With regard to the effect of oestrogen on markers of arterial stiffness, a recent study has shown elastic artery stiffness to be less in younger premenopausal women compared to men, but greater in older postmenopausal women than men of a similar age (307). Augmentation index was found to be reduced in a cross-sectional study comparing postmenopausal women on and off HRT, despite no difference in their blood pressures (308). A study of PWV at different time points through the menstrual cycle of healthy premenopausal women found no variation, however (283), and another cross-sectional study of postmenopausal users and non-users of hormone replacement therapy found no difference in either PWV or AIx (309). It should be noted that augmentation index may also be influenced by both heart rate and body height (299,310).

Oestrogen administration also results in an acute increase in peripheral blood flow in postmenopausal women, with reduced peripheral vascular resistance, possibly due to a direct relaxing effect on vascular myocytes or endothelium-dependent relaxation (311).

Thus far there have been very few studies of vascular physiology/ endothelial function in TS. Elsheikh *et al.* showed that augmentation index was significantly lower in women with TS taking oestrogen therapy than those not taking oestrogen; this effect was seen within three months and was independent of changes in blood pressure or serum lipids (35). Chan *et al.* showed in a venous plethysmography study that the vasodilator response to bradykinin on forearm blood flow was significantly reduced within six weeks of stopping oestrogen replacement in women with TS, and improved within six weeks of recommencement. The effect was endothelial, since GTN responses were unchanged (34). There have been no longer-term studies or studies with hard clinical endpoints in TS.

In this study we aimed to assess various markers of vascular physiology/ endothelial function both in our cross-sectional study of women with TS compared to normal control women, and also in our oestrogen dose-ranging study comparing women with TS and women with primary amenorrhoea (PA), karyotype 46,XX.

1.2.4 Cardiovascular Imaging in Turner Syndrome

Optimal screening tools for the assessment of cardiovascular anomalies in women with TS have yet to be determined. Transthoracic echocardiography is commonly used, but images are frequently poor due to chest wall anomalies. Furthermore, aortic root diameter (AR_{dm}) on echocardiography is recognised to correlate with body size which has a major impact in women with TS who have a mean height deficit of about 20 cm (87). Adjustment for body surface area (BSA) appears most relevant to normal children and young adults in whom growth contributes to variability (312-314), whereas in older age-groups, adjustment for height, weight and age may be more relevant (314-316). There have been various recommendations on how best to correct for stature in subjects with TS. Traditionally, criteria used to define aortic root dilatation (ARD) on echocardiography have been derived by correcting individual measurements of aortic diameter for body surface area (BSA) using the Geigy correction tables (317). Because of

their short stature, however, this correction method may result in women with TS actually being compared to children in normal population tables, potentially leading to an overestimate of the prevalence of ARD in TS. This study aims to explore the effect of different strategies to account for the effect of short stature in TS on echocardiographic measurements.

Magnetic resonance imaging (MRI) can detect degrees of dilatation and coarctation which are not apparent on echocardiography (125). Previous work has suggested use of the ascending to descending aortic ratio (Asc:Desc ratio) as a measure of aortic root dilatation, a ratio of greater than 1.5 being considered abnormal in children on autopsy (318), normal adults on CT (319) and TS children on MRI (137). This means of 'intra-subject' correction for size, which circumvents one problem of using echocardiography in which correction for body size is more crude, is itself prone to error if anomalies occur in the descending aorta.

Another aspect of echocardiography surveillance which requires clarification is the frequency of interval scanning. Current guidelines for the management of adults with TS recommend 3-5 yearly echocardiography for monitoring of the aortic root, but this advice is not based on any clear evidence (7,320,321).

1.3. Aims of this Research Project

This research project aimed to characterise the cardiovascular risk in women with TS with regard to congenital and acquired disease. The setting for the studies undertaken was the Middlesex Hospital Adult Turner Syndrome Clinic which is one of the largest such clinics in the world, serving a population drawn from all over England. At commencement of the research project, the clinic population totalled 254 women, and this has since risen to 384 women. Approximately 60% of these patients are referred from the local paediatric clinic, and a further 40% are from primary care. This large clinic population has made it possible to assess various aspects of cardiovascular risk both cross-sectionally and longitudinally within the constraints posed by the distances at which some patients lived from the clinic.

1.3.1 Echocardiography and Magnetic Resonance Imaging of the Aorta

In the Middlesex Hospital Adult TS clinic it became evident that protocols for cardiac screening in paediatric practice vary considerably and moreover, that some anomalies such as aortic root dilatation are likely to progress through adulthood. This research project aimed to compare measurements of aortic dimensions assessed by transthoracic echocardiography and by MRI in an adult TS population with controls, and to explore their relationships with clinical parameters. A further aim was to define the distribution of aortic dimensions found in an asymptomatic adult TS population as a reference source for future risk intervention studies.

A longitudinal study aimed to gain information about the rate of progression of aortic root dilatation on echocardiography in women with TS in order to verify whether the current interval guidelines of 3-5 years are appropriate.

1.3.2. Cross-sectional Vascular Physiology Study

The precise mechanisms of the increased cardiovascular risk in TS are unclear. We hypothesised that women with TS have a fundamental arterial wall defect which may extend beyond the arch of the aorta and which may be related to genetic factors or oestrogen deficiency. In order to characterise the vasculopathy of TS this study assessed arterial structure (carotid and brachial artery dimensions and carotid artery intima media thickness), arterial stiffness (pulse wave velocity and augmentation index) and endothelial function (flow-mediated dilatation) in a cross-sectional cohort of women with TS. The TS women were compared to normal controls and to karyotypically normal women of normal stature with 46,XX primary amenorrhoea. This was to permit comparisons to women with a similar history of oestrogen deficiency but without the genetic abnormalities inherent in TS.

1.3.3. Cross-sectional Metabolic Study

Most of the studies assessing risk of diabetes and cardiovascular risk in TS have been small. This research project approached the issue from a different angle, by screening a large TS population for anthropometric and metabolic markers of cardiovascular disease.

The aim was again to compare not only to normal controls, but also to women with 46XX premature ovarian failure (322), a subgroup of whom also had primary amenorrhoea.

Subgroups of this larger cross-sectional cohort were recruited to participate in more detailed analyses of body composition using bioelectrical impedance and MRI scanning for fat content.

1.3.4. Longitudinal oestrogen dose-ranging study

It has been suggested that women with TS are relatively resistant to the actions of oestrogen and benefit from higher doses with regard to bone markers and liver function (37) and uterine development (323). The hypothesis of this part of the project was that higher doses of oestrogen may be beneficial to markers of cardiovascular risk and metabolism. In order to assess the relative oestrogen resistance of women with TS, they were once again compared to women with 46,XX primary amenorrhoea (PA), who are similarly oestrogen deficient but have a normal karyotype.

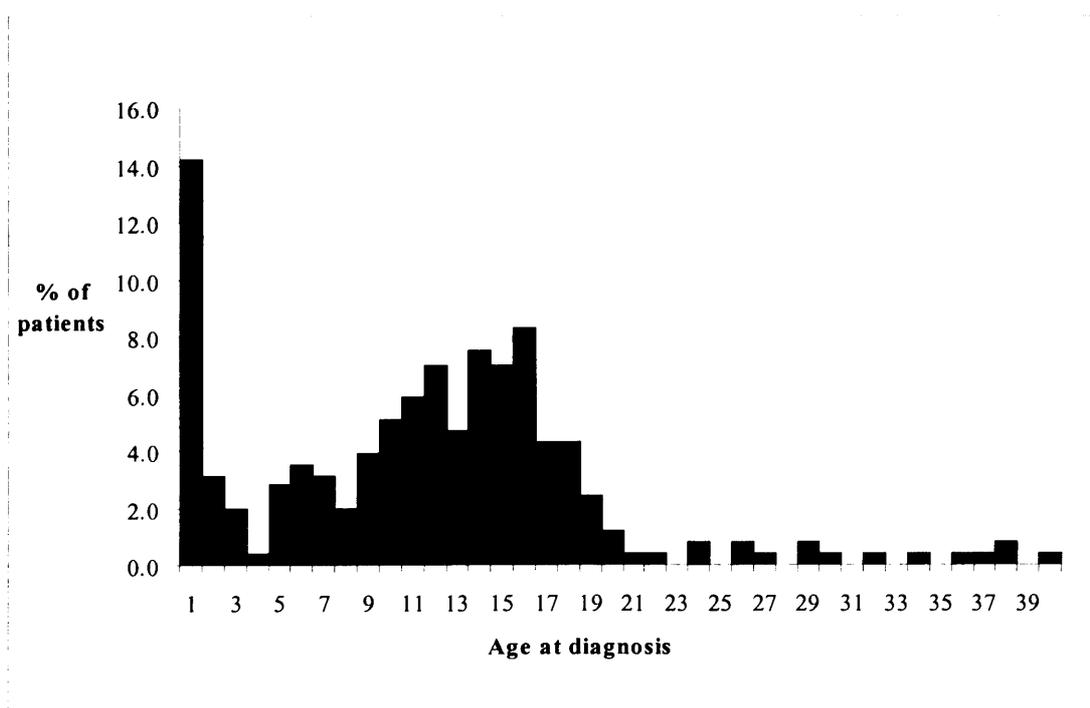
Chapter 2

General Methods

2.1. Patient Characteristics

All subjects with TS were recruited from the Middlesex Hospital Adult TS clinic. This clinic comprised a total of 254 women with TS, including women who had previously attended the Middlesex Hospital paediatric endocrine clinic, as well as new referrals. Figure 2.1 demonstrates the age of diagnosis in this clinic population. The women, from all over the country, annually attend a monthly morning clinic, with travelling distances of up to 300 miles. For this reason, every effort was made to combine as many aspects of the study as possible with a clinic visit, although some patients were willing to attend for further visits to complete other parts of the study. All aspects of the study were coordinated and organised by a single investigator (JEO). Women were sent a patient information sheet approximately two weeks before the date of their clinic appointment. This was followed up with a telephone call, and a further discussion on the day of their clinic visit before obtaining written informed consent from participants. The study was approved by the UCL Hospitals Ethics Committee.

Figure 2.1. Age at diagnosis of 254 patients attending the Adult Turner Clinic at the Middlesex Hospital



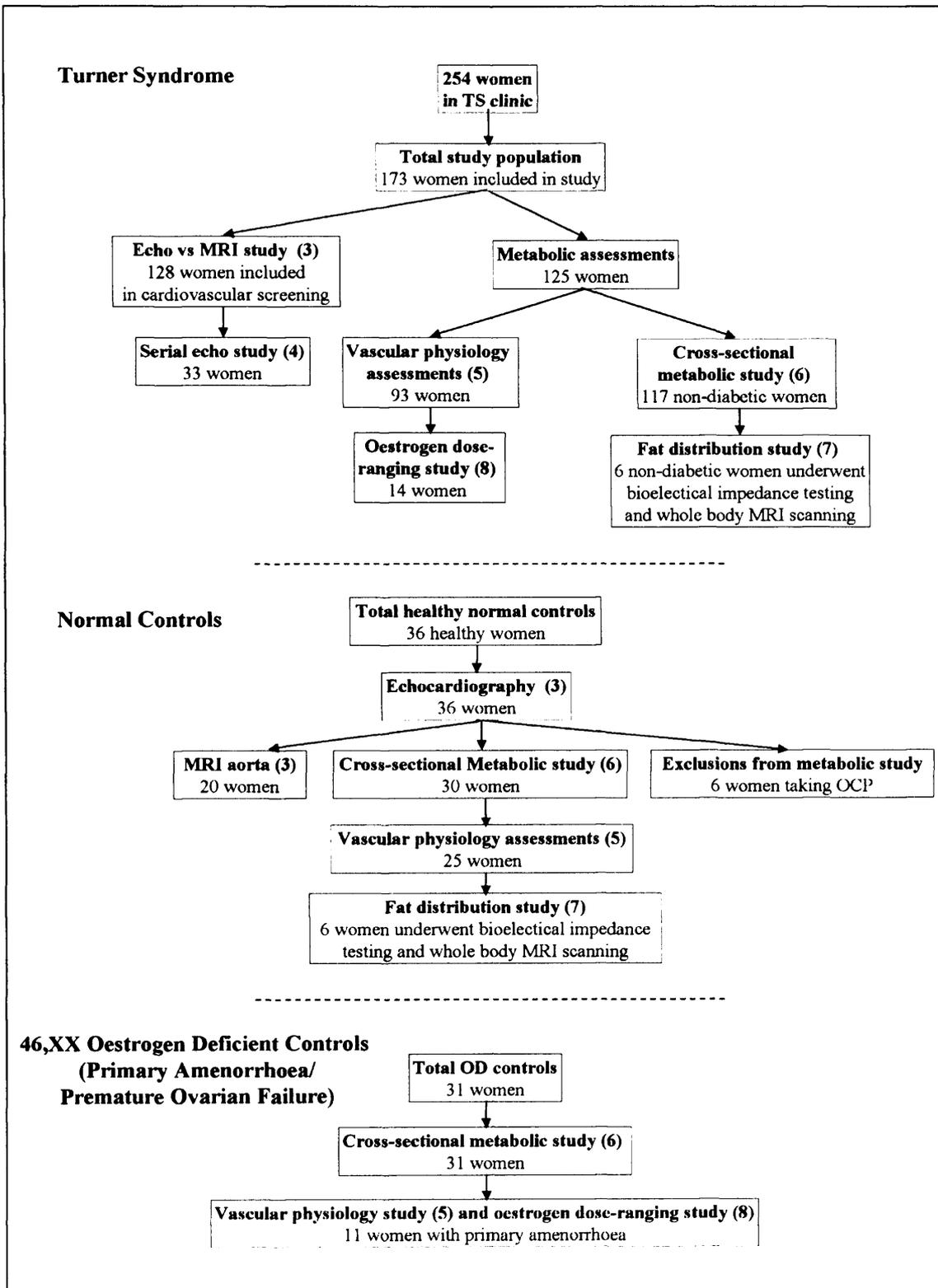
173 unselected women in the Middlesex Adult TS clinic were included in the overall cross-sectional study (karyotype distribution illustrated in Table 2.1. and Appendix 1), and subpopulations of this cohort took part in various aspects of it (see Figure 2.2.). Every attempt was made to study women during the oestrogen-only phase of their hormone replacement in order to standardise the analysis. This was relevant for the cross-sectional vascular physiology study (Chapter 5), the cross-sectional metabolic study (Chapter 6) and the longitudinal oestrogen study (Chapter 8), since phase in the cycle would not be expected to have an impact on the other imaging studies. This will be discussed in each of the relevant methods sections.

Clinical history and case notes were reviewed for previous medical history and surgery, particularly cardiac. History of oestrogen replacement, previous growth hormone and oxandrolone administration was recorded, and current medications, especially the use of antihypertensives, were noted. Oestrogen-deficient years were defined as the cumulative years after the age of 11 during which oestrogen-deficient subjects were not treated with oestrogen. Subjects were asked about their history of smoking and exercise. (These data are quoted in each individual chapter; Table 1, Appendix 1 shows a summary of these patient characteristics in each subpopulation.)

Table 2.1. Karyotype distribution of total study population

| Karyotype | % of TS women, n=173 |
|--------------------|---------------------------------|
| Monosomy X (45,X) | 50 |
| Isochromosome X | 22 |
| Partial X deletion | 3 |
| Ring X | 8 |
| Any Y fragment | 6 |
| Mosaic 45,X/46,XX | 8 |
| Complex | 3 |

Figure 2.2. Distribution of the sample population. The relevant chapter discussing each section of the study is shown in parentheses.



Clinical parameters recorded included anthropometric assessments such as height, weight, waist and hip measurements. *Height* was measured to the nearest 1 cm (Harpenden stadiometer, Holtain, Crosswell, Crymmych, Pembs., UK). *Weight* was measured in normal clothing, after removal of shoes and jacket or coat, using a digital weighing machine, to the nearest 0.1 kg (Tanita BWB-620, Marsden, London, UK). Height and weight measurements were used to calculate *body mass index* (BMI) ($\text{weight (kg) / height (m)}^2$) and *body surface area* (BSA) (square root of [(height in centimetres x weight in kilograms) / 3600]) using the Mosteller formula (324).

Waist circumference (cm) was measured at the narrowest part of the torso and *hip* circumference (cm) was measured in a horizontal plane at the level of the maximal circumference of the buttocks. Waist and hip were measured with a plastic tape in triplicate and the mean recorded. The *waist:hip ratio* (WHR) was calculated from these measurements.

Recumbent blood pressure was recorded when subjects had rested for at least 15 minutes at the right and left brachial arteries, and the left dorsalis pedis artery using an automated sphygmomanometer (Dinamap, Critikon, Tampa, Florida, USA). Five readings were taken at each site and mean systolic and diastolic blood pressure calculated. A large cuff was used for obese individuals.

Normal Controls

TS women were compared to 36 normal control women of similar age who were recruited from amongst the departmental and hospital staff. The stature of this control group at mean 1.60m was notably shorter than the population mean of 1.66m in order to lessen the impact of height adjustment. Again subpopulations of this cohort took part in different aspects of the study (Figure 2.2.). All normal control women were studied in the follicular phase of their menstrual cycle.

Oestrogen-deficient Controls

TS women were also compared to a second control group which comprised 31 karyotypically normal (46,XX) amenorrhoeic women of normal stature. 11 of these women had 46,XX primary amenorrhoea (PA), nine with gonadal dysgenesis and two with hypogonadotropic hypogonadism, and these took part in the cross-sectional

vascular physiology study and the longitudinal oestrogen dose-ranging study. The other women in this group of 31 women all had premature ovarian failure (POF) and where the whole group is referred to, they will be described, for convenience, as oestrogen deficient (OD). All 46,XX PA women were studied in the oestrogen-only phase of their hormone replacement therapy; every attempt was made to study the other women with OD, many of whom also had long travelling distances, in the same phase (see further discussion in Section 2.7.).

2.2. Blood sampling

Subjects were asked to fast over night (12 hours) prior to blood sampling. Serum (taken in plain tubes containing granules to assist clot retraction) and plasma (tubes containing lithium-heparin anticoagulant) were collected for measurement of renal, liver, bone and lipid profiles, insulin concentrations and thyroid function. In the case of the women participating in the oestrogen dose-ranging study, serum gonadotrophins (follicle stimulating hormone, FSH, and luteinising hormone, LH) were also measured. Samples for glucose measurement were collected in fluoride tubes. EDTA samples were taken for full blood count and renin measurement and lithium heparin samples were collected for karyotype analysis. All samples were immediately stored on ice before being processed in the laboratory. The homoeostasis model insulin resistance index (HOMA-R) was calculated as the product of fasting insulin and glucose concentrations divided by 22.5.

Further samples were taken for storage of serum and plasma, the latter collected in both citrated and pre-chilled EDTA tubes. These were immediately stored on ice and centrifuged at 2,000 x g at 2°C for 7.5 minutes within one hour of collection. They were then stored at -80°C.

2.3 Assays

Renal function (urea, electrolytes and creatinine), liver function (alkaline phosphatase, alanine transferase, gamma glutamyl transferase, albumin, bilirubin), calcium and phosphate concentrations were measured on a Roche Modular Autoanalyser, as was plasma glucose (using glucose oxidase reagent). Total cholesterol, high density cholesterol and triglyceride concentrations were determined enzymatically (LDL was

estimated as described by Friedewald *et al.*(325)) and serum insulin, gonadotrophins, oestradiol and testosterone were measured by electrochemiluminescence immunoassay (Roche Modular Autoanalyser, Modular Analytics E170). Plasma renin activity was measured by radioimmunoassay (RENCTK, DiaSorin, Italy). Full blood count and fibrinogen were measured on an automated analyser.

C-reactive protein was determined with an in-house enzyme immunoassay using rabbit antihuman antibodies (X0293) from Dako Diagnostics (Ely, Cambs., UK), validated against the UK Reference Preparation, with an assay range of 0.15-0.48 mg/l and intra- and inter-assay correlations of coefficient of <10% as previously described (326).

Interleukin-6 (Il-6) was measured by high sensitivity two-site ELISA (R&D Systems). This is a quantitative sandwich enzyme immunoassay technique utilising a monoclonal antibody specific for Il-6. The inter- and intra-assay coefficients of variation (CV) were <10%. The minimum detectable dose of IL-6 was 0.09 pg/ml. This method has previously been described (327).

Leptin was measured in serum using a well-validated in-house radioimmunoassay, with an antihuman rabbit monoclonal antibody and I¹²⁵-labelled leptin. The assay was sensitive to 0.1µg/l with inter- and intra-assay CVs <10% as previously described (328).

2.4. Method for Echocardiography and Magnetic Resonance Imaging (MRI) of the Aorta – Cross-sectional Study

128 women in the TS clinic were offered transthoracic echocardiography and MRI of the aorta, and successful images were obtained in 107 women using both modalities. These women were compared to 36 normal control women of similar age, all of whom completed echocardiography whilst 20 had MRI of the aorta (see Figure 2.2).

M-mode and two-dimensional echocardiography was completed in 120/128 (94%) women with TS (Acuson 'Aspen' echocardiography machine, Acuson, Mountain View, CA, USA). Echocardiography data were technically inadequate in 8/128 (6%) women. Measurements of aortic root diameter (ARdm) at the level of the annulus, estimates of left

ventricular mass and the presence of BAV were recorded. Other abnormalities visualised on echocardiography were also noted.

Axial and sagittal oblique MR images of the aorta, as well as cine and phase contrast sequences were obtained (Siemens Magnetom plus 1.5T scanner, Siemens, New York, NY, USA) in 115/128 (89.8%) women with TS. 13/128 women (10.2%) were unable to tolerate MRI. Ascending (AAdm) and descending (DAdm) aortic diameters were measured at the level of the bifurcation of the pulmonary artery with calipers on hard copy images and corrected for the appropriate scale. The presence of ARD on MRI was defined as a ratio (Asc:Desc ratio) of >1.5 (137,318,319). The presence and degree of coarctation and flow disturbance at the classical aortic coarctation site immediately distal to the origin of the left subclavian artery (ligamentum arteriosum site) was noted. Other morphological abnormalities of the aorta were also recorded.

2.5. Method for Interval Assessment of Aortic Dilatation using Echocardiography

An initial pilot study for this project was commenced in 1997, and these women were rescanned as part of the current project to determine the rate of change of aortic dimensions, having also been included in the cross-sectional echocardiography study (see Figure 2.2). Interval echocardiography was performed in 43 TS women, but owing to technical difficulties, only 33 women (mean \pm SD age 29.1 ± 6.6 years, height 1.47 ± 0.08 m.) had two successful scans permitting measurement of the aortic root with a time interval of 3.8 ± 1.4 years. The presence or absence of a bicuspid aortic valve was recorded. In order to reflect routine clinical practice, echocardiograms were performed as a standard request from the services at the Middlesex Hospital by duty echocardiographers.

29 women in this cohort also underwent Magnetic Resonance Imaging of the aorta around the time of their second echocardiogram and the presence of coarctation site abnormalities in particular was noted.

2.6. Method for Vascular Physiology Assessments – Cross-sectional study

Vascular physiology assessments for the cross-sectional analysis were performed in 93 women with TS, 25 normal controls and 11 women with 46,XX PA. As previously mentioned, every attempt was made to assess women in the oestrogen-only phase of their hormone replacement. This was not always possible because of the fact that women often had to travel long distances to attend. 55 women were studied in the oestrogen-only phase of their hormone replacement therapy, 19 in the oestrogen and progesterone phase, 10 in the pill-free week, 7 on a continuous combined preparation and cycle phase was unknown in 2 women. All normal and 46,XXPA controls were studied in the follicular and oestrogen-only phases respectively.

All measurements were performed in a warm, temperature-controlled room at the Vascular Physiology Laboratory at Great Ormond Street. Antihypertensive medication was withheld for at least 24 hours. Subjects attended after a 12-hour overnight fast. On arrival they were allowed to rest for at least 15 minutes. The blood sample was then drawn and clinical parameters recorded as described above, including a baseline blood pressure and pulse. All vascular physiology measurements were performed by two experienced vascular technologists (AED and CS) and recorded on software. Tape-recorded images were analysed and computing of results performed by a single analyst (JEO).

Pulse Wave Velocity (PWV) and Augmentation Index (AIx)

Pulse wave velocity was determined with the subject supine, using a transcutaneous pressure tonometer (Sphygmocor system, ScanMed, Draycott, Gloucestershire, UK) to record the pressure pulse waveform consecutively at the carotid and femoral artery. The distance travelled by the pulse wave was measured over the body surface, and the pulse wave velocity (in metres/ second) was obtained from the mean time difference between the R-wave measured in a simultaneously recorded ECG and pressure wave in relation to the arterial path length.

Augmentation Index (AIx) was calculated using the radial pulse pressure waveform (see Figure 1.1.) to derive the central pressure waveform using a validated generalised transfer function. This was then corrected for heart rate.

Intima Media Thickness (IMT)

Carotid IMT was measured in the far wall, 1cm below the common carotid bifurcation in plaque free segments using high resolution B-mode real-time ultrasound (Acuson XP10 ultrasound system, Acuson, Mountain View, CA, USA) with a 5-10 MHz linear array transducer. The intima media thickness was defined by the lumen-intima and media-adventitia interfaces. Ultrasound images were recorded on videotape and later masked interpretation of scans was performed. Carotid artery diameter was calculated from the end-diastolic distance between the lumen-intima interfaces measured in three sequential R-wave-triggered frames, whilst IMT was defined by the lumen-intima and media-adventitia interfaces. Three measurements were taken for both parameters on each side, and the mean of right and left taken as the overall average carotid artery diameter and IMT respectively.

Brachial artery diameter and Flow-mediated dilatation (FMD)

Brachial artery diameter and FMD were assessed after completion of PWV/ PWA and IMT measurements. Subjects had been supine for at least 20 minutes. Three monitoring ECG electrodes were attached to the chest. The brachial artery was imaged in longitudinal section 5-10 cms proximal to placement of a blood pressure cuff, just below the antecubital fossa. An Acuson XP10 ultrasound system was used with a 10-MHz linear-array transducer supported by stereotactic clamp. The image was magnified using a resolution box function and gated with the R-wave of the ECG. When the clearest B-mode image through the centre of the vessel was obtained with optimal contrast between the anterior and posterior vessel walls and the lumen of the vessel, the stereotactic clamp was fixed in place. A Doppler signal was recorded from the centre of the vessel with the range gate set at 1.5mm. Fine adjustments in the position of the transducer were made by means of micrometer screws attached to the base of the clamp to maintain image quality throughout the study. Sequential end-diastolic images of the artery were acquired every 3 seconds throughout each study using data acquisition software (Brachial Tools, Medical Imaging Applications, Iowa, USA), and the diameter of a 1- to 2-cm segment was determined for each image using semiautomatic edge-detection algorithms. Blood flow at the vessel diameter measurement site was recorded continuously throughout the study using pulsed wave Doppler. Systemic blood pressure was measured in the contralateral arm at regular intervals throughout each study using an automated sphygmomanometer (Dinamap).

Endothelium-Dependent (Flow-Mediated) Vasodilation

The baseline image and Doppler signal were recorded for 60 seconds, following which the blood pressure cuff was inflated to suprasystolic pressure (300mmHg) for 5 minutes. The cuff was then rapidly deflated and the artery imaged and Doppler signal recorded for 5 minutes after cuff deflation. Brachial artery FMD was calculated as the maximum change in diameter from baseline, expressed as a percentage. It is accepted that errors are inherent in the flow velocity measures at a Doppler angle of 70 degrees in the centre of the vessel but relative changes are accurate.

Blood flow was expressed as reactive hyperaemia – the ratio of change from baseline. The velocity time integral (VTI) in metres was determined at baseline, during cuff inflation, and at prespecified time points (at 5 and 15 seconds, and then every 15 seconds for the first 2 minutes of reactive hyperaemia).

Flow was calculated as:

$$\text{VTI} * \text{HR} * \pi r^2 * \cos\theta \text{ ml/minute}$$

where r = vessel radius and $\theta = 70^\circ$ (the angle of insonation of the blood vessel).

On cuff deflation the resultant reactive hyperaemia was calculated as the flow change from baseline, expressed as a percentage change in blood flow. This measurement was used to assess whether the studies between the different groups (in this case TS and control groups) were comparable.

Baseline vessel diameter (mm) was calculated as the mean of 20 measurements during the first minute of each study. Dilatation (maximal after reactive hyperaemia and mean of 1 minute during steady-state conditions) was measured using the baseline average and the maximum of three measures averaged at peak, expressed as a percentage change from the baseline diameter.

Endothelium-independent (GTN mediated) dilatation

The effect of endothelium-independent stimulation was assessed by administration of a sublingual dose of 25µg glyceryl trinitrate (GTN). Although many studies have used 200-400µg doses of GTN, a 25µg dose gives a dilatation equivalent to the degree of FMD dilatation seen in healthy controls and was therefore used in this study. This small dose

reduces the safety and ethical concerns regarding potential side effects from large doses of GTN.

As with assessment of FMD, the baseline image and Doppler signal were recorded for 60 seconds. GTN was then administered sublingually, and the image and Doppler signal were recorded for a further 5 minutes. GTN-mediated brachial artery dilatation is calculated as the maximum change from baseline diameter, expressed as a percentage.

Blinded replicate measures for the FMD and GTN measurements were performed to assess reliability and found inter- and intra-observer correlation coefficients to be 0.96 and 0.99 respectively.

2.7. Methods for Metabolic Assessments – Cross-sectional study

117 women with TS and normal fasting serum glucose were compared to 30 normal control women of similar age who were taking no medication and had regular spontaneous menstrual cycles, and also to the 31 karyotypically normal, oestrogen-treated amenorrhoeic women, also of similar age. The latter group, which will be referred to as the 46,XX oestrogen deficient (OD) group, comprised 12 women with primary amenorrhoea (two of these having hypogonadotrophic hypogonadism), whilst the remainder had premature ovarian failure (POF). Eight women in the TS clinic with known diabetes mellitus were excluded from the study.

As previously mentioned, every attempt was made to study all women in the oestrogen-only hormone replacement phase or follicular phase as appropriate. Amongst the women with TS, 57 were studied in the oestrogen-only phase of their hormone replacement therapy, 23 in the oestrogen and progesterone phase, 10 in the pill-free week, 8 on a continuous combined preparation, and cycle phase was unknown in 19 women. Amongst the women with 46,XXOD, 26 were studied in the oestrogen-only phase of their hormone replacement therapy, 4 in the pill-free week and 1 on a continuous combined preparation. All normal controls were studied in the follicular phase of their menstrual cycles. Subjects attended after a 12-hour overnight fast. Clinical parameters and blood sampling were performed as previously described.

2.8. Methods for Fat Distribution Study

Six non-diabetic oestrogen-treated women with TS were compared to six age-matched normal control women of similar BMI who were taking no medication and had regular spontaneous menstrual cycles (see Figure 2.2.). As previously, clinical history and case notes were reviewed, and clinical parameters recorded.

Subjects attended after a 12-hour overnight fast, although they were allowed to drink water until 3 hours before the test and a blood sample was taken as previously described.

Total body adipose tissue content

Rapid T₁ weighted MR images (repetition time 36ms, echo time 14ms) were acquired as previously described (329). Subjects lay in the magnet in a prone position with arms straight above the head, and were moved through the magnet on a purpose-built platter. They were scanned from their fingertips to their toes by acquiring 10mm-thick transverse images with 30mm gaps between slices in the arms and legs, and 10mm gaps between slices in the trunk. Images were analysed for fat and non-fat components using SliceOmatic (Tomovision, Montreal, Quebec, Canada) (330). Total body adipose tissue, subcutaneous, total internal, subcutaneous abdominal and intra-abdominal adipose tissue volumes were measured (329). The coefficient of variation varies between different depots, but the data analysis method is generally highly reproducible: 3% for internal fat, 5% for visceral fat, and less than 1% for total, subcutaneous and subcutaneous abdominal fat (331).

MRS of the liver

¹H MR spectra were acquired on a 1.5T Eclipse multinuclear system (Phillips Medical Systems, Cleveland, Ohio) using a flexible body coil. Spectra were obtained from the right lobe of the liver using a PRESS sequence (repetition time 1500ms, echo time 135ms) without water saturation and with 128 signal averages. Transverse images of the liver were used to ensure accurate positioning of the 20x20x20mm voxel in the liver, avoiding blood vessels, the gall bladder and fatty tissue. Spectra were analysed as previously described, with intrahepatocellular lipids (IHCL) measured relative to liver water content after correcting for relative variations in T₁ and T₂ components (332). The coefficient of variation for repeated determinations of this measurement was 7% (332).

MRS of muscle

Subjects were supine with the left leg immobilised in a 30cm diameter quadrature bird cage coil. ¹H MR spectra were obtained from the soleus and tibialis muscles with repetition time 1500ms, echo time 135 ms, 256 averages (333). Intramyocellular lipids (IMCL) were measured relative to the total muscle creatine signal after correcting for relative variations in T1 and T2 components. The coefficient of variation for repeated determinations of this measurement was $13.6 \pm 3.5\%$ (333).

Bioelectrical impedance for body fat composition

Bioelectrical impedance was assessed in all women. This was measured from foot to foot using a Tanita BC-418MA body-fat analyser (Tanita Corp., Tokyo, Japan). Subjects stood on the metal sole plates of the machine without shoes, socks and heavy items of clothing. An allowance of 1kg was made for the weight of clothing. All measurements were made after asking subjects to urinate, and allowing a period of at least 10 minutes standing to minimise potential errors from acute shifts in fluid distribution. The machine provided a print-out of measured impedance and calculated body fat after entering the subjects' sex, height and age. Standard prediction equations were used (334).

2.9. Methods for Longitudinal Oestrogen Dose-ranging Study

Fourteen women with TS from the original cross-sectional cohort were recruited (see Figure 2.2.), 13 of whom experienced primary amenorrhoea and one secondary amenorrhoea. They were compared to 11 women with 46,XX primary amenorrhoea (PA) of similar age; nine of the PA group had 46,XX gonadal dysgenesis, two had hypogonadotrophic hypogonadism. The latter two women were excluded from the analysis of gonadotrophin measurements, but their exclusion for all other analyses did not alter the results and data presented for all other parameters are therefore inclusive of these two women. All women taking part in the study were generally fit and well.

Clinical history and case notes were reviewed as previously discussed. All subjects entered the study recording good compliance with their routine oestrogen replacement. At each visit they were questioned about compliance with oestrogen therapy and intercurrent illness.

All women sequentially received for three periods of twelve weeks (three 28-day cycles) each: oral 17- β oestradiol 1, 2 and 4mg daily in the form of Femoston 1/10, Femoston 2/10 and two daily tablets of Femoston 2/10 respectively (Solvay Pharmaceuticals, Southampton, UK). The progestogen in these preparations is dydrogesterone 10mg, which was contained in the tablets taken from days 15-28 in each cycle.

All assessments were carried out in the oestrogen-only phase of oestrogen replacement during week 10 of each oestrogen dose (between days 8 and 14 of the third pack). As in the cross-sectional study, women attended after a 12-hour overnight fast; clinical parameters were recorded and blood samples were taken. Vascular physiology measurements were recorded according to the methods described above. These included pulse wave velocity and augmentation index to assess arterial stiffness, intima media thickness measurement to assess arterial structure, and flow-mediated dilatation to assess endothelial function. Notes on the position of the arm for FMD studies and ultrasound images of the brachial and carotid artery were recorded and matched to ensure consistency between sites of analysis for FMD and IMT on each of the three visits.

2.10. Power Calculations

The initial design of this study used aortic root dilatation as the primary outcome measure. Power calculations were performed to estimate the number of subjects required to provide meaningful outcomes in subgroup analysis. Further power calculations were then performed for other aspects of the study.

Power Calculations for Echocardiography and Magnetic Resonance Imaging (MRI) of the Aorta – Cross-sectional study

Pilot data suggested that in a total of 100 subjects, the estimated sizes of subgroups were: 50 with karyotype monosomy X, 25 with isochromosome X, 25 with oestrogen deficiency for more than five years, and 25 who had received growth hormone treatment. There were no data available which allowed us to predict a clinically significant difference in aortic root diameter in TS comparable to Marfan syndrome. 2mm differences in aortic root diameter on echocardiography between groups were therefore taken to be significant as this represents the change seen over two years in Marfan syndrome (335,336). A

standard deviation of 4mm was used as determined by our pilot data in adult patients with TS. In order to detect a 2mm difference in aortic root diameter with 80% power, we calculated that 63 subjects would be required. To find differences in subgroups defined by 1:1 or 1:4 ratios, such as monosomy X or growth hormone users in childhood, a requirement of 126 subjects was calculated.

Power Calculations for Interval Assessment of Aortic Dilatation using Echocardiography

Using power calculations based on pilot data of interval echocardiography in 10 TS subjects which were available from clinic prior to this study, the mean \pm SD change in aortic root diameter over three years was 0.27 ± 0.29 cm. With a significance of 0.05 and a power of 90%, it was estimated that 14 subjects would be required to detect a significant difference.

Power Calculation for Vascular Physiology Assessments (Intima-Media Thickness) – Cross-sectional study

Published data in the general population have shown that an increase in IMT in the common carotid artery correlates with an 11% increase in risk of myocardial infarction (294). Pilot data in 18 subjects with TS compared to controls showed a mean \pm SD difference in IMT of 0.4 ± 0.4 mm. We calculated that for a power of 90% and a significance level of 0.05, 22 subjects would be needed in subgroup analysis to detect a 0.4mm difference in IMT. With a total population cohort of 93 patients, this would allow us to demonstrate differences in IMT in the larger subgroups such as karyotype (monosomy X in 50%) and childhood treatment with growth hormone (used in approximately 25% of women with TS), defined by 1:1 and 1:4 ratios respectively. 11 women with 46,XX PA were recruited for illustration of the effect of oestrogen deficiency.

Power Calculations for Longitudinal Oestrogen Dose-ranging study

The power calculation for this study was based on published sources documenting IMT changes in response to oestrogen. A study in postmenopausal women showed that IMT is reduced by 0.012 ± 0.012 mm/year with oestrogen replacement therapy (296). A power calculation based on a difference of 0.012 mm, a standard deviation of 0.012 mm, a

significance level of 0.05 and a power of 90% estimated a requirement of 13 subjects to determine a significant within-subject difference. We recruited 14 TS subjects and 11 46,XX PA subjects.

2.11. Statistical Analysis

Statistical analysis was performed using SPSS Version 11.0 for Windows. Associations between variables were assessed using Pearson's correlation coefficient and ANOVA or Student's t-test for continuous variables with log transformation where appropriate and controlling for other variables as cofactors where indicated. Spearman's correlation coefficient was used for non-parametric variables and categorical variables, with the χ^2 test also being used for the latter. Linear regression analysis (entry method) was performed to assess independent associations of continuous variables, and binary logistic regression analysis was used for categorical variables.

In Chapter 3, linear regressions and 95% prediction intervals in Figure 3.2 were plotted using SigmaPlot 8.0; the differences between these regression lines were assessed by comparing 95% confidence intervals.

Normally distributed data such as the differences in mean aortic root diameter with time in Chapter 4, were assessed using a paired Student's t-test, whilst duration of oestrogen exposure in TS and 46,XX PA groups was compared by Mann Whitney U test (Chapters 5, 6 and 8).

It has been noted that in the vascular physiology and metabolic studies in chapters 5 and 6, the majority but not all TS and 46,XX OD women were studied in the oestrogen-only phase of cyclical hormone replacement therapy. All statistical analysis in both chapters was performed initially using the 'oestrogen-only phase' women and then on the entire cohort. Since the inclusion of the additional women did not significantly alter the results, but increased the power of the study, the results presented are all inclusive.

Additional specific points regarding statistical analysis in Chapter 6 were that Scheffe post-hoc analysis was used as appropriate and that all cross-sectional analyses were performed first using data from the entire OD cohort and again including only the twelve

46,XX primary amenorrhoea women. Since there was no significant difference in the results, the findings for the total OD cohort are presented, since the greater number of subjects increased the statistical power of the comparisons. As the OD group differed from normal controls only with respect to triglycerides, these two groups were combined in Figure 6.1. for clarity of illustration. Interaction analysis was performed on univariate analysis of variance to assess the effect of TS status in regression equations.

In Chapter 8, to assess the effects of varying doses of oestrogen on the parameters tested, a repeated-measures ANOVA was used with log transformation as appropriate. The comparisons quoted throughout are of the 1mg and 4mg doses of oestradiol.

Chapter 3

Echocardiography and Magnetic Resonance Imaging (MRI) of the Aorta – Cross-sectional Study

3.1 Aims

The aim of this study was to compare measurements of aortic dimensions obtained by echocardiography and MRI in women from our clinic population compared to controls, and to explore their relationships with clinical parameters and their sources of error. A further aim was to define the distribution of aortic dimensions found in an asymptomatic TS population as a reference source for future risk intervention studies.

128 women with TS were included in this study. Echocardiographic images were obtained in 120 women, MRI images in 115 women and successful images using both techniques in 107 women. The women with TS were compared to 36 normal control women, all of whom underwent echocardiography, and 20 had MRI of the aorta.

3.2. Results

Comparison of TS and control women – general, metabolic and cardiac findings

Women with TS were shorter, had greater BMI, systolic and diastolic blood pressures (BP) at the right and left brachial and left dorsalis pedis arteries, and were less likely to smoke or take exercise compared to controls (Tables 3.1. and 3.2.). There was no difference between TS and control women with regard to BP differences between the left and right brachial arteries, or the left brachial and left dorsalis pedis arteries (Table 3.2.). Diabetes mellitus was present in 7 women with TS and no controls. Plasma renin activity and triglyceride concentrations were greater in women with TS compared with controls.

Table 3.1. Comparison of clinical characteristics in women with Turner Syndrome and controls

| Clinical Characteristics | Turner Syndrome (n=128) | Controls (n=36) | p-value |
|--|----------------------------|--------------------|---------------------|
| Age, years ^a | 31.1 ± 8.5 | 33.5 ± 7.3 | 0.124 |
| Height, m ^a | 1.47 ± 0.07 | 1.60 ± 0.06 | <0.001 |
| BMI, kg/m ^{2a} | 27.1 ± 5.6 | 23.6 ± 3.1 | 0.001 |
| BSA, m ^{2a} | 1.50 ± 0.18 | 1.62 ± 0.14 | <0.001 |
| Right brachial Diastolic Blood Pressure, mmHg ^a | 76 ± 10 | 69 ± 9 | <0.001 |
| Right brachial Systolic Blood Pressure, mmHg ^a | 122 ± 14 | 114 ± 10 | 0.002 |
| Total cholesterol, mmol/l ^a | 5.4 ± 1.0 | 5.2 ± 0.9 | 0.326 |
| HDL cholesterol, mmol/l ^a | 1.9 ± 0.5 | 1.9 ± 0.3 | 0.550 |
| LDL cholesterol, mmol/l ^a | 3.0 ± 0.8 | 2.9 ± 0.9 | 0.643 |
| Triglycerides, mmol/l ^a | 1.1 ± 0.6 | 0.8 ± 0.3 | 0.006 |
| Plasma renin activity, nmol/h/l ^b | 1.79 ± 2.76 | 0.89 ± 2.38 | 0.001 |
| Prevalence of Diabetes Mellitus, % | Type 1 1.6 | 0 | 0.450 |
| | Type 2 3.9 | 0 | 0.228 |
| Smoking History, % | Never 84.6 | 52.8 | <0.001 ^d |
| | Ex 6.7 | 36.1 | |
| | Current 8.7 | 11.1 | |
| Proportion taking Exercise, % (Mild= 1 hour per week, Moderate= 2 hours per week, Vigorous= 3 or more hours per week) | None 59.6 | 38.9 | <0.001 ^d |
| | Mild 26.0 | 16.7 | |
| | Moderate 10.6 | 11.1 | |
| | Vigorous 3.8 | 33.3 | |
| Age at starting oestrogen, years ^c | 14 (5-47) | | |
| Years of oestrogen deficiency ^c | 3 (0-41) | | |
| Previous GH treatment, % | 31 | | |
| Age of starting GH, years ^c | 11 (6-17) | | |
| Duration of GH treatment, years ^c | 4.0 (0.3-9.0) | | |
| Karyotype distribution, % | Monosomy X | 49.2 | |
| | Isochromosome X | 22.7 | |
| | Partial X deletion | 2.3 | |
| | Ring X | 10.2 | |
| | Any Y fragment | 6.3 | |
| | Mosaic 45,X/ 46,XX | 5.5 | |
| | Complex | 3.9 | |

Key: ^amean ± SD; ^bgeometric mean ± SD; ^cmedian (range); ^dp-value refers to overall χ^2 test

Table 3.2. Mean \pm SD blood pressures and blood pressure differences at brachial and dorsalis pedis arteries in TS and normal control women

| | TS | Normal controls | p-value |
|--|------------------|------------------------|----------------|
| Mean left brachial BP | 105 \pm 12 | 98 \pm 9 | 0.003 |
| Mean right brachial BP | 106 \pm 12 | 99 \pm 9 | 0.001 |
| Mean left dorsalis pedis BP | 127 \pm 15 | 116 \pm 14 | 0.001 |
| Mean left brachial – mean right brachial systolic BP | -1.5 \pm 5.9 | -0.8 \pm 3.7 | 0.460 |
| Mean left brachial – mean left dorsalis pedis BP | -21.8 \pm 13.3 | -18.3 \pm 10.4 | 0.163 |

A normal echocardiogram was found in 63/120 (53%) women with TS and all controls. Abnormalities detected by echocardiography are shown in Table 3.3.

Table 3.3. Abnormalities detected by Echocardiography in the TS women

| Cardiovascular abnormalities on Echocardiography | Frequency, % (n=120) |
|---|-----------------------------|
| Congenital | |
| Aortic Root Dilatation | 16 |
| Bicuspid Aortic Valve | 18 |
| Aortic Stenosis | 10 |
| BAV with AS | 5 |
| BAV without AS | 13 |
| Coarctation | 2 |
| Ventriculo-septal defect | 1 |
| Functional | |
| Valve regurgitation | |
| Aortic Regurgitation | 18 |
| Mitral Regurgitation | 16 |
| Pulmonary Regurgitation | 2 |
| Tricuspid Regurgitation | 12 |
| Ventricular/ atrial abnormalities | |
| Left ventricular/ septal hypertrophy ^a | 11 |
| Left ventricular dysfunction ^b | 1 |
| Dilated left atrium | 2 |
| Right ventricular hypertrophy | 1 |
| Dilated right heart | 1 |
| Other abnormalities | |
| Aortic aneurysm and dissection | 1 |

Key: AS=Aortic Stenosis; BAV=Bicuspid Aortic Valve; ^aSecondary to hypertension, AS or coarctation; ^bHistory of cardiac surgery

A normal MRI (ie no coarctation, aortic root dilatation (ARD) or other dysmorphology) was found in 39/115 (34%) women with TS compared with 19/20 (95%) controls. Entirely normal appearances on both echo and MRI were found in 26/107 (24%) women with TS who underwent both studies. Representative MRI scans in Figure 3.1 show the two major patterns of aortic pathology – a dilated aortic root and coarctation site narrowing.

Figure 3.1 MR images of (a) a normal aorta, (b) aortic root dilatation (Ascending:Descending Aortic ratio marked by white arrows), (c) coarctation (white arrow indicates coarctation site), (d) severe aortic dilatation with dissection.

Figure 3.1a.



Figure 3.1b.

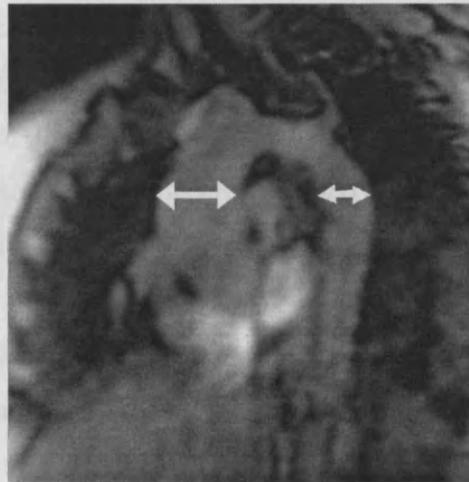


Figure 3.1c.

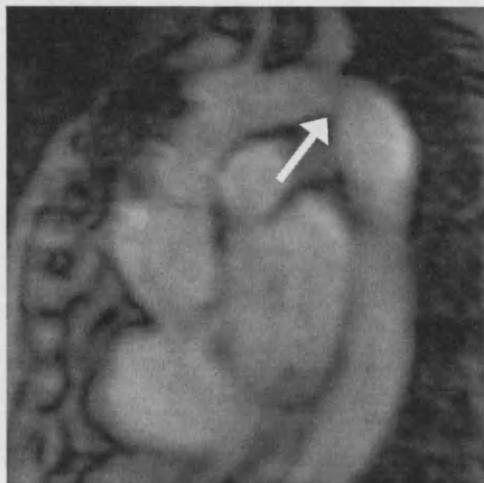
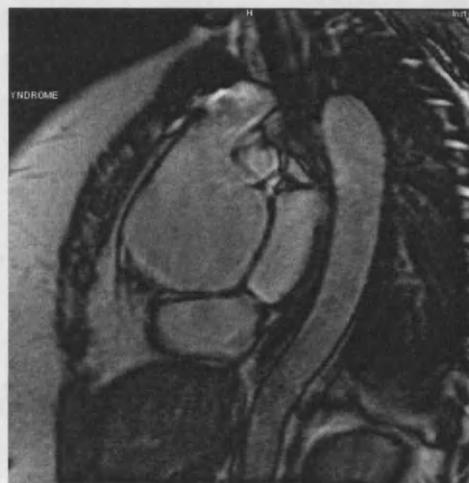


Figure 3.1d.



The karyotype distribution of the women with TS is shown in Table 3.1. Women with monosomy X tended towards greater likelihood of a cardiovascular imaging abnormality on echo or MRI (i.e. bicuspid AV, other valve abnormalities, ARD, coarctation site abnormalities and other aortic dysmorphism) compared with other karyotypes (prevalence of monosomy X 52 vs 31%, $p=0.054$ for abnormal vs normal).

The individual with an incidental finding of aortic dissection on routine screening (Figure 3.1d) was immediately referred for surgery. The inclusion of data from this individual was found to have no major influence on any of the findings when calculations were repeated after exclusion (data not shown). Inclusive data are therefore used throughout, except in Figure 3.2 where they were considered inappropriate.

Comparison of TS and control women – Aortic Dimensions

With regard to echocardiography, absolute measurements of ARdm were similar in TS compared to controls (Table 3.4), despite their slightly higher blood pressure. When controlling for differences in BSA and height between TS and controls, however, ARdm was significantly greater in TS, with height adjustment having the greatest effect (Table 3.4).

Table 3.4. Comparison of aortic measurements in women with Turner Syndrome and controls

| All measurements in cm | Turner Syndrome | Controls | p-value |
|---|--------------------------|-------------|---------|
| Echocardiography^a | | | |
| Aortic root diameter (ARdm) | 2.86 ± 0.46 | 2.76 ± 0.22 | 0.242 |
| ARdm adjusted for BSA | 2.88 ± 0.04 | 2.70 ± 0.07 | <0.001 |
| ARdm adjusted for height | 2.90 ± 0.04 | 2.62 ± 0.09 | 0.010 |
| MRI^b | | | |
| Ascending Aortic diameter (AAdm) | 2.83 ± 0.61 | 2.52 ± 0.23 | 0.029 |
| Descending Aortic diameter (DAdm) | 1.97 ± 0.27 | 1.86 ± 0.27 | 0.092 |
| Ascending:Descending (Asc:Desc) ratio | 1.44 ± 0.23 | 1.40 ± 0.11 | 0.483 |
| MRI Excluding coarctation site abnormalities^c | | | |
| AAdm | 2.63 ± 0.36 | | |
| DAdm | 1.87 ± 0.18 | | |
| Asc:Desc ratio | 1.41 ± 0.18 | | |
| MRI Coarctation abnormalities only^d | | | |
| AAdm | 3.12 ± 0.81 ^e | | |
| DAdm | 2.06 ± 0.30 ^e | | |
| Asc:Desc ratio | 1.51 ± 0.22 ^f | | |

Key: Values quoted are mean ± SD; ^an=120 for TS, n=36 for controls; ^bn=115 for TS, n=20 for controls; ^cn=66; ^dn= 40; ^ep<0.001, and ^fp=0.019 for comparison to those without coarctation site abnormalities.

To apply height correction for ARdm we used the equation: Adjusted ARdm=ARdm+(1.6–height)*1.74 with 1.6 being the mean height of controls and 1.74 being the slope of the regression line of ARdm and height in women with TS. Using this adjustment and applying the common criterion for ARD on echocardiography of 3.4cm (incidentally the upper limit of ARdm in our control group), 19/120 (16%) women qualified for ARD. Absolute measurements of ARdm were used to derive TS-specific reference ranges shown in Figure 3.2a.

Figure 3.2. The distribution of (a) Absolute Aortic Root diameter (ARdm) on echocardiography and (b) Ascending Aortic diameter (AAdm) on MRI with age (subject with aortic dissection excluded).

Turner syndrome: filled circles and solid lines, Controls: open circles and dashed lines. Lines represent the linear regression plot with 95% prediction lines for the distribution.

Figure 3.2a.

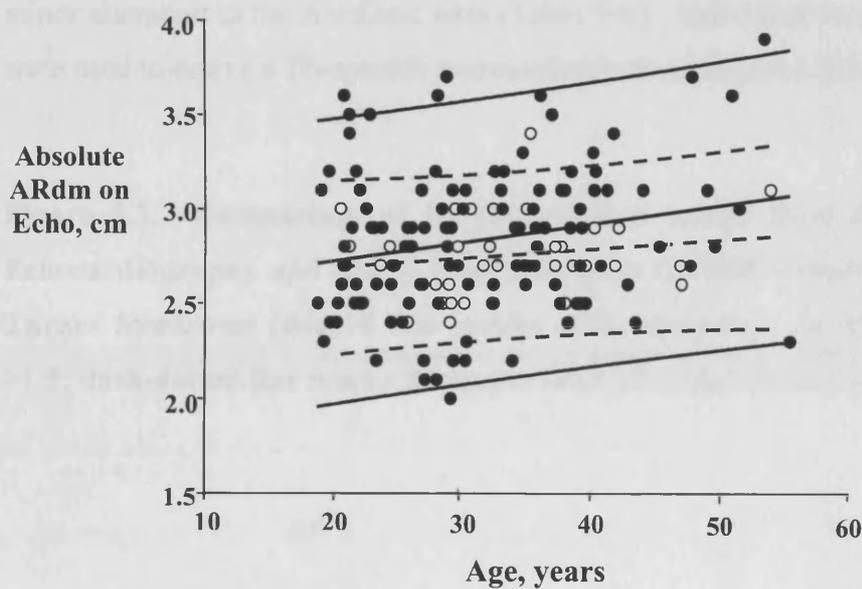
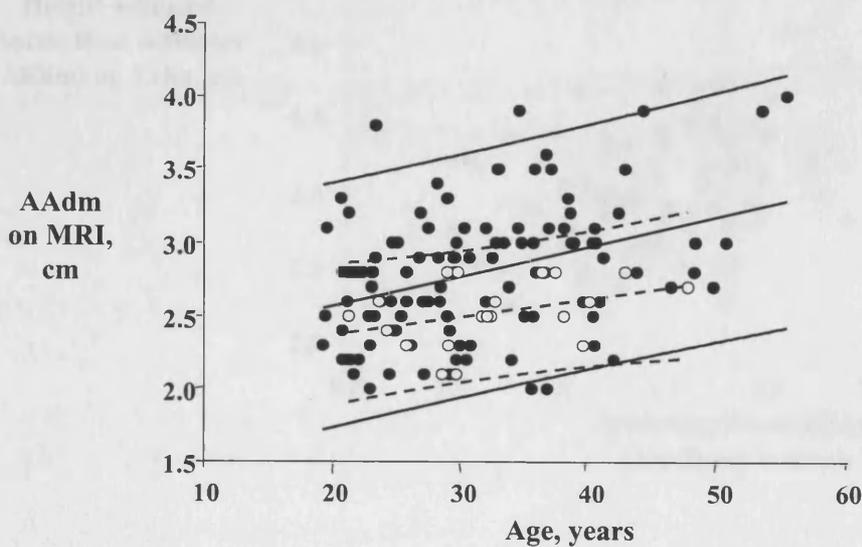
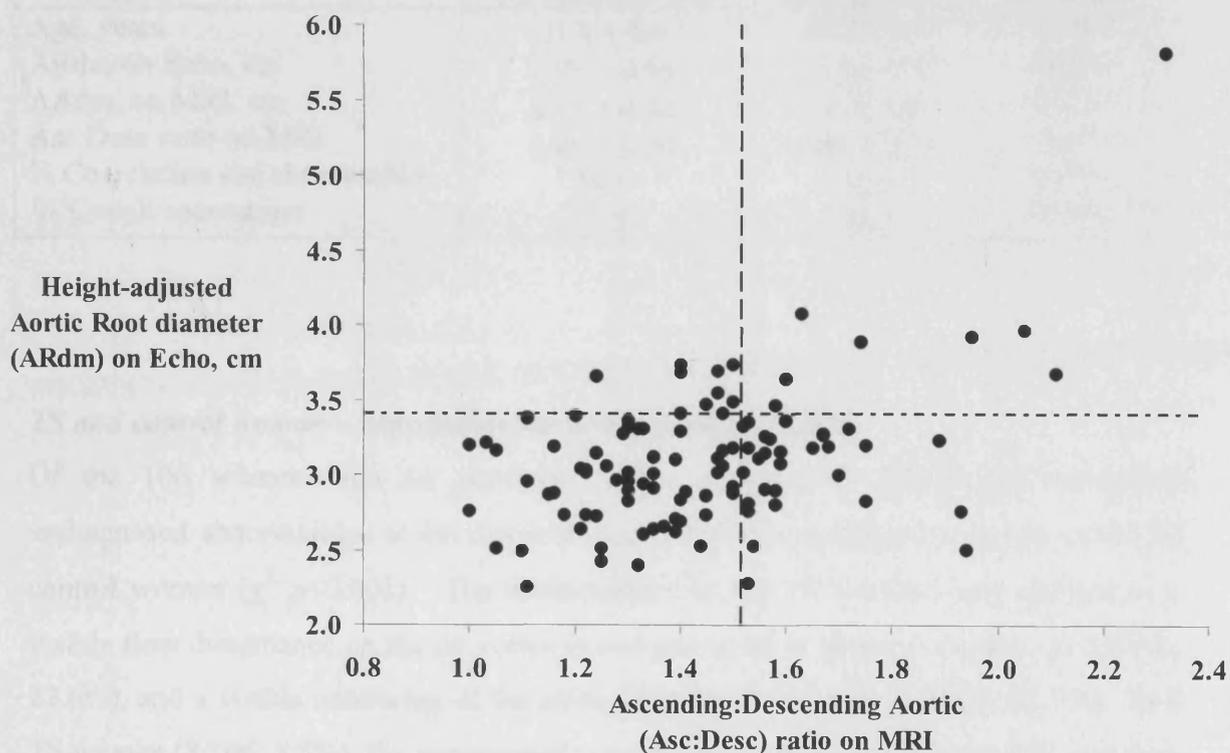


Figure 3.2b.



With regard to MRI, AAdm, but not DAdm or the Asc:Desc ratio, was significantly greater in women with TS than controls. The Asc:Desc ratio was not associated with either BSA or height. ARD (defined as Asc:Desc ratio >1.5) was found in 38/115 (33%) women with TS – significantly more than the 16% for echocardiography ($p<0.01$) (Figure 3.3). Coarctation site abnormalities may be associated with pre- and post-stenotic dilatation, potentially confounding the Asc:Desc ratio (Figure 3.1c). This aspect was investigated in 106 women with TS, excluding the nine who had had previous coarctation surgery. Both AAdm and DAdm were significantly greater in 40 women with coarctation site abnormalities compared to 66 women with TS who had no coarctation but with only minor alteration to the Asc:Desc ratio (Table 3.4.). Individual ascending aortic diameters were used to derive a TS-specific normal distribution (Figure 3.2b).

Figure 3.3. Comparison of Height-Adjusted Aortic Root Diameter (ARdm) on Echocardiography and Aortic Root Dilatation by MRI criteria in 107 women with Turner Syndrome (dashed line marks aortic dilatation on MRI at Asc:Desc ratio >1.5 ; dash-dotted line marks the upper limit of ARdm in the control group)



There was a positive correlation between ARdm on echocardiography and aortic dimensions on MRI (AAdm: $r=0.502$, $p<0.001$; DAdm: $r=0.318$, $p=0.001$; Asc:Desc ratio: $r=0.323$, $p=0.001$) although each modality identifies different subjects with ARD (Figure 3.3).

Determinants of aortic dilatation

Age and bicuspid aortic valve were the only two variables which had significant associations with aortic measurements. The strength of association between age and aortic dimensions was more pronounced in women with TS than in controls (Figure 3.2). Aortic dimensions were greater in those women with TS who had a BAV than those without (Table 3.5). There was also a positive association between ARdm and LV mass ($r=0.317$, $p=0.004$), which persisted when women with a BAV were excluded ($r=0.286$, $p=0.016$).

Table 3.5. Association of aortic abnormalities with bicuspid AV in women with TS (mean \pm SD)

| Variable | No Bicuspid AV | Bicuspid AV | p-value |
|--------------------------------|-----------------------|--------------------|----------------|
| Age, years | 31.0 \pm 8.4 | 32.3 \pm 9.1 | 0.488 |
| ARdm on Echo, cm | 2.80 \pm 0.36 | 3.12 \pm 0.71 | 0.003 |
| AAdm, on MRI, cm | 2.71 \pm 0.40 | 3.33 \pm 1.02 | <0.001 |
| Asc:Desc ratio on MRI | 1.40 \pm 0.20 | 1.61 \pm 0.29 | <0.001 |
| % Coarctation site abnormality | 34.1 | 58.8 | 0.055 |
| % 'Occult coarctation' | 3.3 | 46.2 | <0.001 |

TS and control women – coarctation site abnormalities on MRI

Of the 106 women with no previous cardiac surgery, 40 (38%) had previously undiagnosed abnormalities at the classical coarctation site, compared with one of the 20 control women (χ^2 $p<0.001$). The abnormalities in the TS women were defined as a visible flow disturbance on the jet vortex at this site in 24 of these 40 women (or 24/106; 22.6%), and a visible narrowing of the aortic lumen in 23 women (23/106; 21.7%). In 8 TS women (8/106; 7.5%), the impingement on the aortic lumen was at least 30% and flow disturbance was sufficiently great to warrant classification as 'occult coarctation' (Figure

3.2c). One control (1/20; 5%) had a small notch at the coarctation site with no associated flow disturbance which was deemed to be haemodynamically insignificant.

Presence of a bicuspid AV was more likely both amongst women with any coarctation site abnormality (25 vs 11%, although not quite achieving statistical significance) and amongst those with 'occult coarctation' (75 vs 11%, $p < 0.001$) compared to women without coarctation site abnormality respectively (Table 3.5.). There were no consistent differences between TS women with and without coarctation site abnormalities with regard to right and left or upper and lower body blood pressures.

3.4 Discussion

This study has demonstrated a high prevalence of occult aortic abnormalities in a cohort of adults with Turner Syndrome using both echocardiography and MRI, with only 25% of women having normal results on both imaging modalities. The two techniques are complementary in that they identify different aspects of aortic pathology, and technical problems may arise in either. It has been demonstrated that aortic dimensions assessed by echocardiography in women with TS can be simply adjusted for height deficit and while the Asc:Desc ratio on MRI avoids this problem, it in turn may be affected by anomalies in the descending aorta. Reference ranges have been produced for aortic root diameter on echocardiography and ascending aortic diameter on MRI for women with TS.

This study evaluated the cardiovascular system by both imaging modalities in adults with TS, allowing the relative merits of each to be characterised. The two modalities differ in the site of measurement of the aorta: Echocardiography usually measures the ARdm at the annulus, whilst AAdm on MRI is measured slightly distal, at the level of bifurcation of the pulmonary arteries. It is therefore, not surprising that the two imaging modalities identify different individuals qualifying for ARD (Figure 3.3). With long term follow up of this cohort it may be possible to determine which group has the greatest risk of dissection. In this regard it is notable that the effect of age on the aorta was a particular feature of the Turner women (Figure 3.2).

Amongst the few studies which have evaluated the use of cardiovascular MRI in TS, the prevalence of ARD on MRI has varied between 12.5% and 33.3% depending on the exact

criteria used (125,132) compared with 33% in this study. Data from the control group used in the present study confirmed the CT-derived definition of Asc:Desc ratio >1.5 as a reliable criterion for ARD on MRI. The utility of this ratio is hampered by the fact that the DAdm is slightly increased in women with TS, eradicating a significant difference in the mean ratio when compared with controls. This would imply that the wall of the entire aorta might be defective in TS and the use of the ratio might be falsely reassuring. Also, coarctation site defects are associated with increases in both AAdm and DAdm – increases which may cancel each other out on calculation of the ratio. In women with any degree of coarctation, therefore, absolute values of ascending aortic diameter are likely to be better predictors of the risk of dissection.

MRI has not been advocated as the investigation of first choice for evaluation of the aorta in TS in view of its limited availability, greater cost, and the fact that a significant number of women with TS (10.2% in this study) are unable to tolerate the scan because of claustrophobia. MRI is of greatest advantage in the assessment of the coarctation site which is not always visible on echocardiography because of the shape of the chest wall in women with TS. There is clearly a spectrum of pathology at the coarctation site and there is no consensus as to what constitutes a ‘significant’ defect worthy of intervention. The finding in the present study of 8% of women with an ‘important’ occult coarctation agrees with similar studies quoting 6% (132) and 12% (125). The absence of consistent blood pressure differences accompanying the imaging abnormalities may be explained by the small number of subjects. The goal now will be to develop a strategy to define risk in terms of flow disturbance.

In conclusion, this study has shown that aortic imaging by echocardiography and MRI provides complementary information, but it is only with longitudinal study of this population that it will be possible to determine how each relates to future morbidity. Ideally all women with TS will have cardiovascular imaging by both techniques as a routine, and certainly echocardiography should be universal. Accepting that MRI may not be available for all women with TS, a practical way forward would be to target high risk subgroups such as those with a BAV, those pursuing ovum donation or those in whom clear echocardiographic imaging is unobtainable. Given the difficulties of correcting for short stature, this study has defined TS-specific reference ranges for absolute measurements of both aortic root diameter on echocardiography and ascending aortic

diameter on MRI in the hope that they will aid future interpretation of these measurements and help define thresholds for risk management strategies.

Chapter 4

Interval Assessment of Aortic Root Dilatation using Echocardiography

4.1. Aims

Increasing age has been demonstrated as one of the primary determinants of aortic root diameter, both in TS (337) and in the general population (313,314,316). Current guidelines recommend that adults with TS should have 3- to 5-yearly echocardiography for monitoring of the aortic root, although there is currently no evidence on which to base this timing interval (7,26,320,338).

This study assessed echocardiographic aortic root dimensions longitudinally in order to determine the rate of change of aortic diameter in TS, and investigated whether any clinical associations might predict the rate of aortic dilatation. Successful sequential imaging was obtained in 33 women at a mean time interval of 3.8 ± 1.4 years.

4.2. Results

Patient Characteristics

Two women had undergone previous coarctation surgery. All women were taking oestrogen replacement throughout the study, but they varied in the number of cumulative years of previous oestrogen deficiency (median [range] 3 [0-22] years). Five women had previously received growth hormone treatment. Twenty-five women had never smoked and 18 did not take any exercise. Nine women were taking antihypertensive medication, and of these, three were commenced in the interval between scans. Twenty (61%) women had karyotype monosomy X.

Changes in ARdm and clinical parameters with time

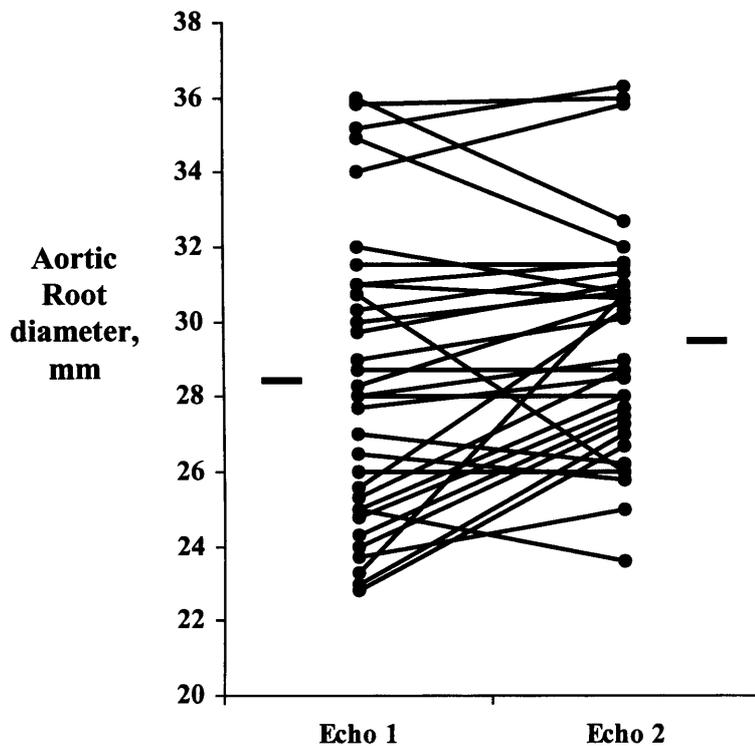
Mean \pm SD aortic root diameter in the cohort increased by 0.91 ± 3.0 mm between the first (ARdm1) and second (ARdm2) scans (Table 4.1), which represented a change of 0.33 ± 0.84 mm ($1.5 \pm 3.3\%$) per year. Adjustment of ARdm2 values to represent ARdm1 + difference after one year showed that there was a significant increase in diameter after

one year (ARdm1 vs adjusted ARdm2: 28.5 ± 4.0 vs 28.8 ± 3.5 mm, $p=0.030$). To illustrate this, Figure 4.1 shows ARdm2 adjusted to a 3-year interval which approximates more closely to the actual mean time interval studied.

Table 4.1. Changes in clinical parameters at time of first and second echocardiograms (mean \pm SD)

| Patient Characteristics | Echo1 | Echo2 |
|---------------------------------|----------------|----------------|
| Aortic Root diameter (ARdm), mm | 28.5 ± 4.0 | 29.4 ± 3.1 |
| Systolic Blood Pressure, mmHg | 128 ± 18 | 123 ± 15 |
| Diastolic Blood Pressure, mmHg | 78 ± 12 | 80 ± 11 |
| Mean Blood Pressure, mmHg | 95 ± 10 | 94 ± 9 |

Figure 4.1. Changes of aortic root diameter at the time of the first echo with time, with the second value adjusted to a standard period of 3 years. Means are shown as bold dashes.



In the interval between scans, blood pressure did not change significantly (Table 4.1), weight increased (55.5 ± 8.8 to 58.1 ± 12.1 kg, $p=0.034$), but there was no significant change in body surface area (1.50 ± 0.14 to 1.53 ± 0.18 m², $p=0.055$).

Associations between the ARdm difference and clinical parameters

There was no association between absolute or percentage increase in ARdm with age, bicuspid AV (present in 12 women), coarctation site abnormalities, blood pressure, anthropometric parameters, previous growth hormone and oestrogen therapy, karyotype, smoking, exercise history or metabolic markers. ARdm increased in all three women who started antihypertensive therapy after their first echo.

4.4. Discussion

This longitudinal study has demonstrated an overall increase in aortic root diameter of 0.33 ± 0.84 mm per year in women with TS. This rate of change is three-fold greater than that reported in a cross-sectional study of normal subjects of 0.1mm per year (314), but similar to that of 0.3-1.0 mm/year reported in a longitudinal study of subjects with bicuspid AV in the general population (339). These findings are consistent with the data from the previous cross-sectional part of this study (Chapter 3) which revealed age as one of the primary determinants of aortic root diameter (337).

This study was too small to identify a particular risk group for close monitoring. The absence of an association of bicuspid aortic valve with aortic diameter increment is interesting, since this was the other main predictor of aortic dilatation in the previous study (Chapter 3) (337).

Analysis of the individual data reveals that the pattern of change was variable with some women showing increases of over 11% per year, whilst in others there was an apparent decrease of 5.4% per year. This heterogeneity suggests that there may have been considerable inter- and perhaps also intra-observer error. Given the length of time between scans, and the fact that they were performed in a hospital setting, it was not possible to ensure a single operator, and this is likely to be representative of most clinical practice. It may be that the difficulties of echocardiography in women with TS increase the variability of measurements. In particular, chest wall anomalies limit the window of access to the aortic root.

An analogy has been made between TS and Marfan Syndrome (MS), prompted by the reports of cystic medial necrosis in 65% of TS cases of aortic dissection in which histology was available (118,124,144). The natural history of aortic dilatation in MS is reported to be more accelerated than in this TS study (2mm/year on average (336)), but there are several other similarities between TS and MS. In MS, too, aortic dilatation is related to age (340), and a longitudinal echocardiography study likewise found no relationship between the initial aortic diameter and the rate of dilatation (336).

In MS, it is recommended that patients with an aortic root diameter of 40-45 mm should have annual echocardiography, whilst those in whom it is >45mm should have

individualised follow-up with a view to aortic surgery at an early stage (336). None of the TS women in this study had an aortic root >40 mm, but it is known that aortic root diameter is height-dependent, both in the general population (314) and in women with TS (337). Extrapolation of recommendations from MS may therefore require height-adjustment of ARdm values before application to TS. Furthermore, as in MS, the possibility of more sudden 'non-linear' aortic dilatation cannot be excluded (336), and this is supported by the multiple case reports of aortic dilatation and dissection in young women with TS (128). A comparison of echocardiography and MR imaging in MS has suggested asymmetry of the aortic root, which may be more obvious on MRI than echocardiography, as a possible cause of unexpected aortic dissection (341).

In conclusion, this study has shown that the rate of progression of aortic dilatation in TS is approximately three-fold greater than that reported in normal subjects, but less than in Marfan Syndrome. No predictive clinical features of aortic dilatation in TS have been identified in this study, suggesting that all women should continue to be screened. An interval of 3-5 years would seem to be appropriate in women whose ARdm remains below 36 mm, the maximum ARdm recorded in this study. More frequent scanning seems advisable over this threshold. At the present time echocardiography remains the mainstay of serial screening in view of its greater availability, but serial MRI screening may become more widespread in the future as this may circumvent the problems of operator error. MRI is certainly required in women whose echocardiographic window is poor. Further studies are required to assess the effects of antihypertensive medication, and particularly beta blockade (by analogy with Marfan Syndrome (342)) on deceleration of aortic dilatation in TS.

Chapter 5

Vascular Physiology Assessments – Cross-sectional study

5.1. Aims

Although the aortic abnormalities of TS are well recognised as previously described (337,343), we hypothesized that women with TS have a fundamental arterial wall defect extending beyond the arch of the aorta. This may be related to genetic factors or oestrogen deficiency. The present study set out to characterize the vasculopathy of TS by assessing arterial structure (carotid and brachial artery diameter and carotid artery intima media thickness), arterial stiffness (pulse wave velocity and augmentation index) and endothelial function (flow-mediated dilatation). The arterial diameters in this study were compared to echocardiography and MRI assessments undertaken in the women with TS and normal controls as previously described. 93 women with TS were compared to 25 normal controls and 11 women with 46,XX primary amenorrhoea (PA).

5.2. Results

General comparisons and observations of clinical and metabolic factors

Women with TS were shorter and had greater body mass index, waist circumference, systolic and diastolic blood pressures, heart rate, serum CRP and IL-6 concentrations than women in the normal control and 46,XX PA groups (Table 5.1.). They smoked less (85% vs 60% vs 36% had never smoked in TS, normals and 46,XX PA groups respectively, $p < 0.001$) and took less exercise (15% vs 48% vs 36% took regular moderate or vigorous exercise in the three groups respectively, $p < 0.001$). Four women with TS had type 2 diabetes mellitus.

All women with TS and 46,XX PA were on routine physiological oestrogen replacement and reported good compliance. TS women started oestrogen treatment at an earlier age (median [range] 14 [5-25] vs 17 [12-22] years, $p = 0.001$) and had fewer years of oestrogen deficiency (3 [0-27] vs 6 [1-11] years, $p = 0.001$) than the 46,XX PA women. In the TS group, proportions taking oral synthetic, oral natural and patch estrogens were 19.1%, 71.9% and 9.0% respectively, with 100% of women in the 46,XX PA group taking oral natural oestrogens (χ^2 test $p = 0.127$). 30.1% of TS women had previously been treated

with growth hormone (GH), with a median [range] start age of 10 [5-17] years and 4.5 [0.5-10] years' duration of treatment. 48.4% of the women with TS had karyotype monosomy X.

Table 5.1. Comparison of clinical characteristics in women with Turner Syndrome (TS), normal controls and women with 46,XX primary amenorrhoea (PA).

| Clinical Characteristics | TS (n=93) | Normal (n=25) | 46,XX PA (n=11) | p-value |
|---|--------------|------------------|--------------------|---------|
| Age, years ^a | 32.6 ± 8.2 | 33.3 ± 6.9 | 30.2 ± 8.4 | 0.556 |
| Height, m ^a | 1.47 ± 0.06 | 1.61 ± 0.05 | 1.67 ± 0.09 | <0.001 |
| BMI, kg/m ² ^a | 27.0 ± 5.8 | 24.3 ± 3.1 | 21.8 ± 3.3 | 0.002 |
| Waist, cm ^a | 80.8 ± 12.8 | 74.5 ± 6.9 | 74.6 ± 6.7 | 0.026 |
| Diastolic Blood Pressure, mmHg ^a | 76 ± 10 | 67 ± 6 | 63 ± 6 | <0.001 |
| Systolic Blood Pressure, mmHg ^a | 121 ± 14 | 112 ± 8 | 108 ± 8 | <0.001 |
| Heart rate, beats/minute ^a | 75 ± 13 | 65 ± 9 | 65 ± 13 | 0.001 |
| Total cholesterol, mmol/l ^a | 5.4 ± 1.0 | 5.0 ± 1.0 | 5.0 ± 0.9 | 0.088 |
| LDL cholesterol, mmol/l ^a | 3.1 ± 0.9 | 2.8 ± 1.0 | 2.5 ± 0.8 | 0.104 |
| HDL cholesterol, mmol/l ^a | 1.9 ± 0.5 | 1.9 ± 0.3 | 2.0 ± 0.5 | 0.843 |
| Triglycerides, mmol/l ^a | 1.0 ± 0.4 | 0.8 ± 0.2 | 0.9 ± 0.3 | 0.034 |
| Glucose, mmol/l ^a | 4.8 ± 1.2 | 5.0 ± 1.1 | 4.7 ± 1.1 | 0.351 |
| Insulin, mIU/l ^b | 4.9 ± 2.4 | 6.6 ± 3.2 | 6.4 ± 1.9 | 0.087 |
| HOMA-R ^b | 1.0 ± 0.5 | 1.5 ± 0.7 | 1.3 ± 0.4 | 0.070 |
| C-reactive protein, mg/l ^a | 3.0 ± 1.5 | 0.8 ± 1.1 | 0.9 ± 1.0 | <0.001 |
| Interleukin-6, pg/ml ^b | 1.5 ± 0.8 | 0.9 ± 0.3 | 0.7 ± 0.3 | <0.001 |

Key: ^amean ± SD; ^bgeometric mean ± SD; p-values refer to group differences on ANOVA

A bicuspid aortic valve (BAV) was identified in 17 (18%) women with TS, and six women were known to have significant aortic coarctation from the MRI study (Chapter 3). Two women with TS had aortic stenosis (peak aortic valve gradients 31 and 42 mmHg respectively), of whom one also had bicuspid aortic valve, and these subjects were excluded from the analyses of augmentation index. 20 women were taking

antihypertensive medication (9 on atenolol, 5 on an angiotensin converting enzyme inhibitor, 4 on bendrofluazide, 1 on an angiotensin II receptor antagonist and 1 on a calcium channel antagonist). Both exclusion of the women with each category of complications individually, and of all TS women with any complication (n=32), did not alter the relationship between TS status and the vascular parameters studied except augmentation index as mentioned above. The data presented for TS throughout the study are therefore inclusive of all TS women unless otherwise stated.

Comparison of the vascular phenotype in TS, 46,XX PA and normal controls

Comparison of arterial structure

Women with TS had greater absolute and height-adjusted carotid diameters than women in the normal control and 46,XX PA groups (Table 5.2). This relationship remained after adjusting for factors which were associated with carotid diameter in women with TS (Table 5.3.): height, waist and IMT ($p < 0.001$). Group differences were also significant for absolute and height-adjusted brachial artery diameters. Relative differences in arterial diameters between women with TS compared to controls are shown in Figure 5.1.

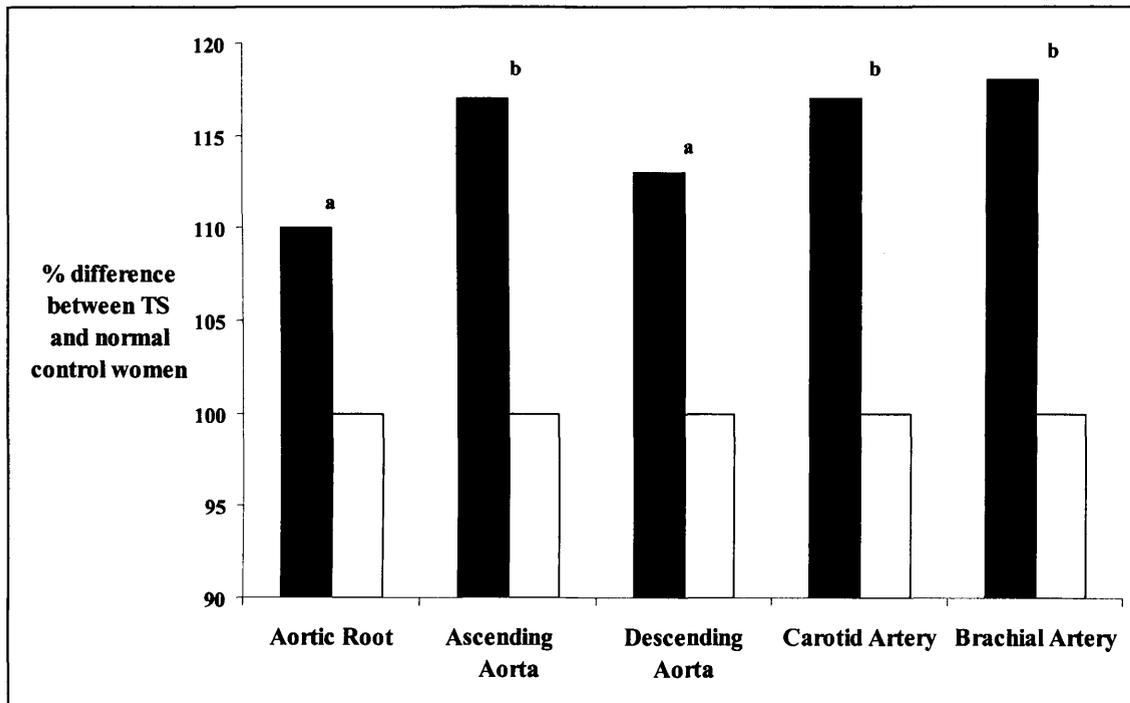
IMT was greater in TS than in normal control women ($p < 0.001$) but similar in the TS and 46,XX PA groups ($p = 0.837$) (Table 5.2). These group differences remained significant after adjustment for factors which were associated with IMT in women with TS (Table 5.3.): age, diastolic blood pressure and carotid artery diameter ($p = 0.002$).

Table 5.2. Comparison of absolute and height-adjusted measures of arterial structure and stiffness in women with Turner Syndrome (TS), normal controls and women with 46,XX primary amenorrhoea (PA). (Note IMT and PWV are not height-related variables.)

| | Absolute Values | | | | Height-adjusted Values | | | |
|---|-----------------------------|------------------|-----------------------|--------------------------|------------------------|----------------|-----------------------|--------------------------|
| | TS (n=93) | Normal (n=25) | 46,XX GD (n=11) | p- value ^a | TS (n=93) | (n=25) | 46,XX GD (n=11) | p- value ^a |
| Structure | | | | | | | | |
| Carotid Artery diameter, mm | 5.71 ^b ± 0.64 | 5.27 ± 0.34 | 5.22 ± 0.38 | <0.001 | 5.85 ± 0.63 | 5.00 ± 0.65 | 4.74 ± 0.67 | <0.001 |
| Brachial Artery diameter, mm | 3.29 ± 0.44 | 3.06 ± 0.36 | 2.97 ± 0.30 | 0.006 | 3.39 ± 0.46 | 2.87 ± 0.48 | 2.65 ± 0.49 | <0.001 |
| Carotid Intima Media Thickness (IMT), mm | 0.61 ± 0.07 | 0.55 ± 0.06 | 0.60 ± 0.05 | <0.001 | - | - | - | - |
| Stiffness | | | | | | | | |
| Pulse Wave Velocity (PWV), m/sec | 6.57 ± 1.25 | 6.44 ± 0.90 | 5.82 ± 0.75 | 0.129 | - | - | - | - |
| Augmentation Index (AIx), % | 21.7 ^b ± 12.2 | 10.1 ± 12.7 | 10.1 ± 10.8 | <0.001 | 19.5 ± 13.9 | 14.8 ± 13.3 | 18.0 ± 14.9 | 0.379 |

Key: Values shown are mean ± SD; ^ap-values refer to group differences on ANOVA; ^bindicates the value which differs from the others on ANOVA with Scheffe post-hoc analysis, p<0.05

Figure 5.1. Comparison of percentage differences in height-adjusted arterial diameters in women with TS compared to control values set at 100%. Solid black bars represent women with Turner Syndrome (TS), unshaded bars represent normal control women. (Vascular physiology and Echocardiography performed in all TS and control women, MRI performed in 76 women with TS and 20 normal controls.)



Key: ^a $p < 0.05$, ^b $p < 0.01$ for the comparison between TS and controls.

Comparison of arterial stiffness

Pulse wave velocity (PWV) was similar in all three groups of women. Augmentation index (AIx) was greater in women with TS than in the other two groups, but the difference between these groups was no longer significant after adjustment for height (Table 5.2).

Table 5.3. Associations between clinical and metabolic variables and structural arterial factors (carotid artery diameter and IMT) in women with TS. Selected variables showing significant simple correlations were included in multiple regression analysis and the resulting partial correlation coefficients are shown.

| Clinical Characteristics | Carotid Artery diameter, correlation coefficients, r | | Carotid Artery IMT, correlation coefficients, r | |
|---------------------------|--|--------------------|---|--------------------|
| | Simple | Partial | Simple | Partial |
| Age, years | 0.102 | | 0.488 ^c | 0.427 ^c |
| Height, m | 0.340 ^b | 0.315 ^b | -0.137 | |
| Weight, kg | 0.281 ^b | | -0.061 | |
| BMI, kg/m ² | 0.314 ^c | | -0.001 | |
| Waist, cm | 0.372 ^c | 0.236 ^a | 0.047 | |
| Diastolic Blood Pressure | 0.004 | | 0.370 ^c | 0.239 ^a |
| Systolic Blood Pressure | 0.118 | | 0.325 ^b | |
| Heart rate | 0.003 | | 0.018 | |
| Total cholesterol | 0.228 ^a | 0.108 | 0.118 | |
| HOMA | 0.067 | | 0.027 | |
| C-reactive protein, mg/l | 0.125 | | 0.038 | |
| Interleukin-6, pg/ml | 0.088 | | 0.057 | |
| Oestrogen deficient years | 0.058 | | 0.132 | |
| Aortic Root diameter | 0.189 ^a | 0.064 | 0.122 | |
| Carotid Artery IMT | 0.254 ^a | 0.271 ^b | 1.000 | |
| Carotid Artery diameter | 1.000 | | 0.254 ^a | 0.221 ^a |
| Brachial Artery diameter | 0.269 ^b | -0.066 | 0.079 | |
| Flow-mediated dilatation | -0.211 ^a | -0.127 | -0.128 | |
| Pulse Wave Velocity | 0.170 | | 0.127 | |
| Augmentation Index | 0.114 | | 0.413 ^c | 0.134 |

Key: ^a p<0.05; ^b ≤0.01; ^c p≤0.001

Comparison of brachial artery vasomotor function

FMD was similar in the three groups, with and without adjustment for the magnitude of the flow stimulus (Table 5.4.) and brachial artery diameter. Vasodilator responses to GTN were also similar.

Table 5.4. Comparison of endothelial function measured at the brachial artery in women with Turner Syndrome (TS), normal controls and women with 46,XX primary amenorrhoea (PA)

| | TS (n=93) | Normal (n=25) | 46,XX PA (n=11) | p-value |
|--|----------------------|--------------------------|----------------------------|----------------|
| Absolute flow-mediated dilatation, mm | 0.27 ± 0.11 | 0.27 ± 0.10 | 0.22 ± 0.07 | 0.344 |
| % flow-mediated dilatation (FMD) | 8.3 ± 3.8 | 9.1 ± 3.6 | 7.5 ± 2.7 | 0.469 |
| Absolute GTN-mediated dilatation, mm | 0.33 ± 0.14 | 0.32 ± 0.12 | 0.33 ± 0.11 | 0.979 |
| % GTN-mediated dilatation | 10.3 ± 4.6 | 10.7 ± 4.4 | 10.8 ± 3.8 | 0.881 |
| Baseline flow, ml/min | 10.8 ± 8.6 | 8.4 ± 5.4 | 6.7 ± 3.5 | 0.139 |
| Max flow increase, ml/min | 56.4 ± 26.5 | 66.6 ± 19.3 | 42.2 ± 11.0 | 0.033 |
| Max flow increase, % | 1174 ± 3150 | 1171 ± 565 | 889 ± 400 | 0.946 |

Key: Values shown are mean ± SD; p-values refer to group differences on ANOVA.

Factors associated with the vascular phenotype in TS

Factors associated with arterial structure

Height, waist circumference and IMT were independently associated with carotid artery diameter (Table 5.3.). Similar clinical associations were observed with the brachial artery diameter. Carotid and brachial artery diameters were associated with aortic root diameter (mean ± SD 2.8 ± 0.4 cm) ($r=0.208$ for carotid; $r=0.228$ for brachial; both $p \leq 0.05$), and there was a weak correlation between carotid and brachial artery diameters which did not achieve significance ($r=0.199$, $p=0.059$).

Aortic root diameter was greater in the 17 women with BAV compared to those without (3.0 ± 0.4 vs 2.8 ± 0.4 cm, $p=0.040$), but carotid and brachial artery diameters were not influenced by BAV (carotid 5.6 ± 0.6 vs 5.7 ± 0.6 cm, $p=0.416$ and brachial 3.3 ± 0.3 vs 3.3 ± 0.5 cm, $p=0.882$ in women with and without BAV respectively).

Increasing age, diastolic blood pressure and carotid artery diameter were independently associated with increased IMT (Table 5.3.). History of smoking, exercise, diabetes mellitus, previous growth hormone treatment, cumulative oestrogen-deficient years, karyotype and the presence of a bicuspid aortic valve, aortic stenosis and coarctation site abnormalities were not associated with carotid artery diameter or IMT. AIx was associated with IMT, but not after adjustment for age. Serum CRP and IL-6 concentrations were not associated with IMT or arterial diameters.

Factors associated with arterial stiffness

PWV and AIx increased significantly with age (PWV $r=0.326$, $p=0.002$; AIx $r=0.397$; $p<0.001$) and both systolic and diastolic blood pressure ($p\leq 0.001$). Neither PWV nor AIx were associated with waist circumference, lipids, smoking history, exercise, CRP, IL-6 or karyotype. Variation in the use of growth hormone in childhood and cumulative years of oestrogen deficiency were not associated with PWV or AIx after adjustment for age. PWV and AIx were similar in those with and those without bicuspid aortic valve.

Determinants of Endothelial Function

FMD was independently associated with waist circumference (partial correlation coefficient $r=-0.206$, $p=0.039$), total cholesterol concentration ($r=-0.215$, $p=0.032$) and heart rate ($r=0.303$, $p=0.002$). There was no association with CRP or IL-6 concentrations.

5.4. Discussion

This study demonstrates the widespread structural vascular differences in women with TS characterized by enlargement of conduit arteries and increased carotid intimal thickening compared to normal controls. Of note, arterial enlargement appears to involve multiple vessels and is associated with increased intimal thickening. Although this suggests that common underlying mechanisms may be, at least in part, responsible, the similar increase in IMT without arterial dilatation that was observed in 46XXPA subjects supports a more selective contribution from oestrogen deficiency to the intimal hyperplasia seen in TS. It was also found that FMD was similar in TS, 46XXPA and normal control women, suggesting that conduit artery endothelial dysfunction does not contribute significantly to the large vessel abnormalities seen in young women with TS and early oestrogen deficiency.

The occurrence of aortic dilatation in TS is well established as has been previously mentioned (126,128,137). It has been associated with the presence of bicuspid aortic valve, aortic coarctation and hypertension but its aetiology is poorly understood. In this study it was observed that although aortic root diameter was greater in women with TS who have bicuspid aortic valves, arterial dilatation is not restricted to these individuals and also occurs in other large conduit vessels, such as the carotid and brachial arteries, to a similar extent in those with and without bicuspid valves.

This study has demonstrated that carotid artery diameter is independently associated with TS status, height, waist circumference and carotid IMT. However, in contrast to women with TS and despite a greater degree of oestrogen deficiency, karyotypically normal women with primary amenorrhoea had normal carotid and brachial artery diameters, thus, suggesting that genetic factors rather than oestrogen deficiency have a greater influence on arterial dilatation in TS. The absence of an independent association between peripheral arterial diameter and bicuspid aortic valve, aortic stenosis or blood pressure, suggests an intrinsic abnormality of the arterial wall in TS. An analogy to the arterial dilatation seen in Marfan Syndrome has been postulated as previously mentioned (137,344). Indeed, cystic medial necrosis similar to that in Marfan Syndrome has been reported in 65% of TS case reports of aortic dissection, where histology was available (118,124,144).

The apparent relationship between generalized arterial dilatation and increased IMT, may, in part, be consistent with the 'Glagov phenomenon' (345) This refers to the outward remodelling and enlargement of atherosclerotic arteries as a consequence of complex inflammatory changes in the vascular wall that compensates for luminal occlusion in the earlier stages of disease development. For example, increased carotid artery diameter was independently associated with IMT as well as the presence of atherosclerotic plaques and increased blood pressure in a population based study of older subjects (346). Increased IMT is a marker of early carotid atherosclerosis and an independent predictor of an adverse cardiovascular prognosis in the general population (294) In the present study, IMT was greater in both TS and 46,XX PA groups compared to controls. However, the fact that carotid artery diameter was increased only in the TS group suggests that factors other than the 'Glagov phenomenon' were involved.

In the general population, IMT has been shown to be associated with traditional risk factors for ischaemic heart disease, such as age, blood pressure, serum lipids, smoking, diabetes mellitus and BMI (294,347-349) and also CRP (350-353). In this study of younger women, in addition to age and blood pressure, the major influences on variation of IMT measurements were TS status and oestrogen deficiency. Although women with TS had raised blood pressure, CRP and IL-6 concentrations compared to the other two groups, these did not account for their greater IMT. Indeed, the similar increase in IMT seen in TS and 46,XX PA women implicates oestrogen deficiency as the key determinant of neointimal hyperplasia in these subjects. Furthermore, as CRP and IL-6 do not appear to be associated with IMT, it may be that factors in the arterial wall such as local inflammation, oedema, extracellular matrix and foam cells are contributing to intimal thickening (354), or that CRP and IL-6 are less direct markers of ischaemic risk than previously thought (261,355). The local factors alluded to may in turn be influenced by oestrogen therapy (356-359). CRP and IL-6 will be considered further in Chapter 6.

Oestrogen deficiency may, therefore, be an appropriate target for early intervention in both TS and 46,XX PA to reduce progression of intimal thickening, and ultimately clinical atherosclerotic disease. Although there is a precedence in the literature, with exogenous oestrogen reducing IMT in older postmenopausal women (295-297) this is not a universal finding (344,360,361). It has been suggested that the reason for this discrepancy is a lack of benefit in women with established coronary artery disease, or insufficient duration of oestrogen therapy (356).

The comparisons of measures of aortic stiffness in this study in TS, normal controls and women with 46,XX PA should be interpreted with caution. Although there was a trend towards greater arterial stiffness, as measured by PWV, this did not achieve statistical significance. It has previously been suggested that PWV may be underestimated in the general population when the abdominal aorta becomes more tortuous with age (298), which may be an important factor in women with TS who frequently have greater tortuosity of the descending aorta (362) and elongation of the transverse arch (343). AIx was greater in women with TS than the other two groups, suggestive of an increased contribution of wave reflection to central aortic systolic blood pressure and left ventricular afterload. However, AIx is influenced by both heart rate and body height and should therefore be corrected for both (299,310). Although the difference in AIx between TS and

controls was lost by height-adjustment, there may, however, still be relevance for central aortic pathophysiology and increased cardiac loading, since neither the transfer function used, nor height-adjustment with regard to this measure, have yet been validated specifically in women with TS.

PWV is an independent marker of cardiovascular risk (300,301) and is associated with other cardiovascular risk factors such as blood pressure, insulin resistance, central obesity and greater carotid IMT in the general population (302). Data on the prognostic value of AIx are still emerging and some studies suggest that it may have even greater predictive power than PWV (305,306). Invasive studies would be required for accurate clinical validation of these measures in TS.

It has been shown that endothelium-dependent vasodilator function reflects underlying cardiovascular risk factor burden (363) and independently predicts cardiovascular prognosis (278,364). The present study found, however, that FMD was similar in women with TS compared to normal or 46,XX PA controls suggesting that endothelial dysfunction is not an important mediator of arterial disease in TS.

As previously mentioned, there have thus far been very few studies of arterial dynamics in TS. Two studies have explored the effects of exogenous oestrogen in women with TS. Both augmentation index (35) and the vasodilator response to bradykinin in a plethysmography study (34) have been shown to improve when oestrogen deficient women with TS are treated with oestrogen. The results of the present study are similar to those in a recent smaller study which focused on both children and women with TS (365). In general, our findings extend those of Baguet *et al.* by virtue of the increased power and homogeneous adult oestrogen-replete population in the present study. For instance, IMT and carotid diameter in TS compared to controls only achieved significance when corrected for height in the previous study – an adjustment absolutely required in a paediatric population. The current study has demonstrated differences in IMT and carotid diameter without height correction. In addition the observation of arterial dilatation has been extended to include the brachial, and women with 46,XXPA have also been studied to control for the oestrogen deficiency of TS.

In conclusion, women with TS have arterial dilatation not only involving the aorta, but also other major conduit arteries, which does not appear to be a consequence of oestrogen deficiency. IMT is also greater in TS than normal controls, and appears to be related to the increased arterial dilatation. This raises the possibility of a common pathway for these two processes, although oestrogen deficiency does appear to contribute to intimal thickening in TS. FMD was similar in all groups, excluding an important contribution from conduit arterial endothelial dysfunction. The findings in this study suggest that both genetic factors and oestrogen deficiency influence the vasculopathy of TS. These data suggest that blood pressure and oestrogen deficiency are likely to be the most appropriate modifiable therapeutic targets for cardiovascular risk reduction in TS, but interventional studies will now be required.

Chapter 6

Metabolic Assessments - Cross-sectional study

6.1. Aims

The increased prevalence of ischaemic heart disease and diabetes mellitus have already been discussed (Section 1.2.3.). Obesity is also common in TS (33,151) and is associated with elevated serum triglyceride and cholesterol concentrations (151,185) which may contribute to the increased cardiovascular risk. The obesity in TS appears to be predominantly central, with an increased waist-hip ratio and a reduced fat free mass (33).

The associations between obesity and type 2 diabetes mellitus (366,367), as well as other features of the insulin resistance/ metabolic syndrome such as hypertension, hypertriglyceridaemia and low HDL concentrations are well established in the general population (368,369). Since central obesity, hypertension, dyslipidaemia and impaired glucose homeostasis are common in TS (33,151), the aim of this study was to determine whether these are indeed features of 'metabolic/ insulin resistance syndrome' in TS. Relationships between markers of the metabolic syndrome and obesity were investigated, with particular reference to the adipokines IL-6 and leptin, and also C-reactive protein. 117 TS women with normal fasting serum glucose were compared to 30 normal control women and 31 women with 46,XX oestrogen deficiency (OD) of similar age.

6.2. Results

Subject characteristics

Table 6.1. compares clinical characteristics between the three groups of subjects. In addition, women with TS smoked less (84.5 vs 56.7 vs 51.6% non-smokers amongst TS, normals and 46,XX OD respectively, $p < 0.001$) and were more sedentary (60.0 vs 43.3 vs 48.4% took no exercise amongst TS, normals and 46,XX OD respectively, $p = 0.002$). The prevalence of karyotype monosomy X was 49.6% in women with TS. 35.0% of TS women had received previous growth hormone therapy and 26.5% had previously been treated with oxandrolone. TS women started oestrogen therapy at a younger age than women with OD (median [range] 14 [5-34] vs 20 [12-38] years, $p < 0.001$), but the overall years of oestrogen deficiency were similar (3 [0-22] vs 5 [0-16], $p = 0.164$). In the TS

group, proportions taking oral synthetic, oral natural and patch oestrogens were 20%, 69% and 11% respectively, with 13%, 80% and 7% respectively in the 46,XX POF group (χ^2 test $p=0.517$). Twenty-two (18.8%) women with TS but none of the normal or 46,XX OD controls were taking antihypertensive medication.

Comparisons between TS, normal controls and women with 46,XX OD

Women with TS had greater BMI, waist-hip ratio, absolute waist circumference and blood pressure than women in the other two groups (Table 6.1.). Triglyceride concentrations in the TS group were similar to those in the 46,XX OD group and greater than in normal controls. The group differences in triglycerides were no longer significant when adjusted for waist circumference ($p=0.071$), but remained so when adjusted for BMI ($p=0.030$).

Fasting glucose and insulin concentrations and calculated HOMA-R score were lower in women with TS. CRP and IL-6 concentrations were greater and leptin concentrations lower in TS women than in both other groups (see Figure 6.1.). On interaction analysis, there was no difference in the relationship between waist and insulin, leptin, IL-6 or CRP in TS and control women (Figure 6.1.). TS status was associated with insulin, HOMA-R, CRP and leptin concentrations, independent of either waist circumference or BMI ($p<0.001$ for all). The group differences in IL-6 concentrations were not significant, however, after adjustment for these physical markers of obesity.

Table 6.1. Characteristics and Results for women with Turner Syndrome (TS), Normal controls and 46,XX oestrogen deficient women (OD).

| | TS (n=117) | Normals (n=30) | 46,XX OD (n=31) | p-value ^c |
|---|--------------------------|------------------------|--------------------|----------------------|
| Clinical characteristics | | | | |
| Age, years ^a | 31.4 ± 8.3 | 33.5 ± 6.5 | 33.0 ± 7.0 | 0.306 |
| Height, m ^a | 1.48 ± 0.06 ^f | 1.60 ± 0.06 | 1.64 ± 0.08 | <0.001 |
| Weight, kg ^a | 58.7 ± 14.4 | 60.9 ± 9.3 | 61.6 ± 10.5 | 0.450 |
| BMI, kg/m ^{2a} | 26.8 ± 5.8 ^f | 23.7 ± 3.2 | 22.9 ± 3.4 | <0.001 |
| Waist, cm ^a | 79.9 ± 12.4 ^e | 73.5 ± 6.9 | 74.7 ± 8.6 | 0.005 |
| Waist-hip ratio ^a | 0.84 ± 0.05 ^f | 0.76 ± 0.05 | 0.78 ± 0.04 | <0.001 |
| Systolic Blood Pressure, mmHg ^a | 121 ± 14 ^f | 111 ± 9 | 113 ± 12 | <0.001 |
| Diastolic Blood Pressure, mmHg ^a | 76 ± 10 ^f | 67 ± 6 | 61 ± 9 | <0.001 |
| Biochemical markers | | | | |
| Total cholesterol, mmol/l ^a | 5.4 ± 0.9 | 5.1 ± 1.0 | 5.0 ± 1.0 | 0.075 |
| LDL cholesterol, mmol/l ^a | 3.0 ± 0.8 | 2.8 ± 0.9 | 2.7 ± 0.9 | 0.098 |
| HDL cholesterol, mmol/l ^a | 1.8 ± 0.5 | 1.9 ± 0.3 | 1.8 ± 0.5 | 0.679 |
| Triglycerides, mmol/l ^a | 1.1 ± 0.6 | 0.8 ± 0.3 ^d | 1.1 ± 0.6 | 0.024 |
| Insulin, mIU/l ^b | 4.7 ± 2.3 ^e | 6.3 ± 3.0 | 6.9 ± 2.9 | 0.004 |
| Glucose, mmol/l ^a | 4.6 ± 0.6 ^f | 5.0 ± 0.4 | 5.0 ± 0.4 | <0.001 |
| HOMA-R ^b | 1.0 ± 0.5 ^e | 1.4 ± 0.7 | 1.5 ± 0.7 | 0.001 |
| Leptin, ng/ml ^b | 10.2 ± 6.3 | 14.4 ± 7.6 | 14.8 ± 8.1 | 0.048 |
| C-Reactive Protein, mg/l ^a | 2.9 ± 1.5 ^f | 0.8 ± 1.0 | 1.2 ± 0.9 | <0.001 |
| Interleukin-6, pg/ml ^b | 1.5 ± 0.7 ^d | 1.0 ± 0.5 | 1.2 ± 0.5 | 0.014 |

Key: ^aMean or ^bgeometric mean ± SD. ^cp-values refer to group differences on ANOVA; ^{d, e, f} indicate the value which differs from the others on ANOVA with Scheffe post-hoc analysis: ^dp<0.05; ^ep<0.01; ^fp<0.001

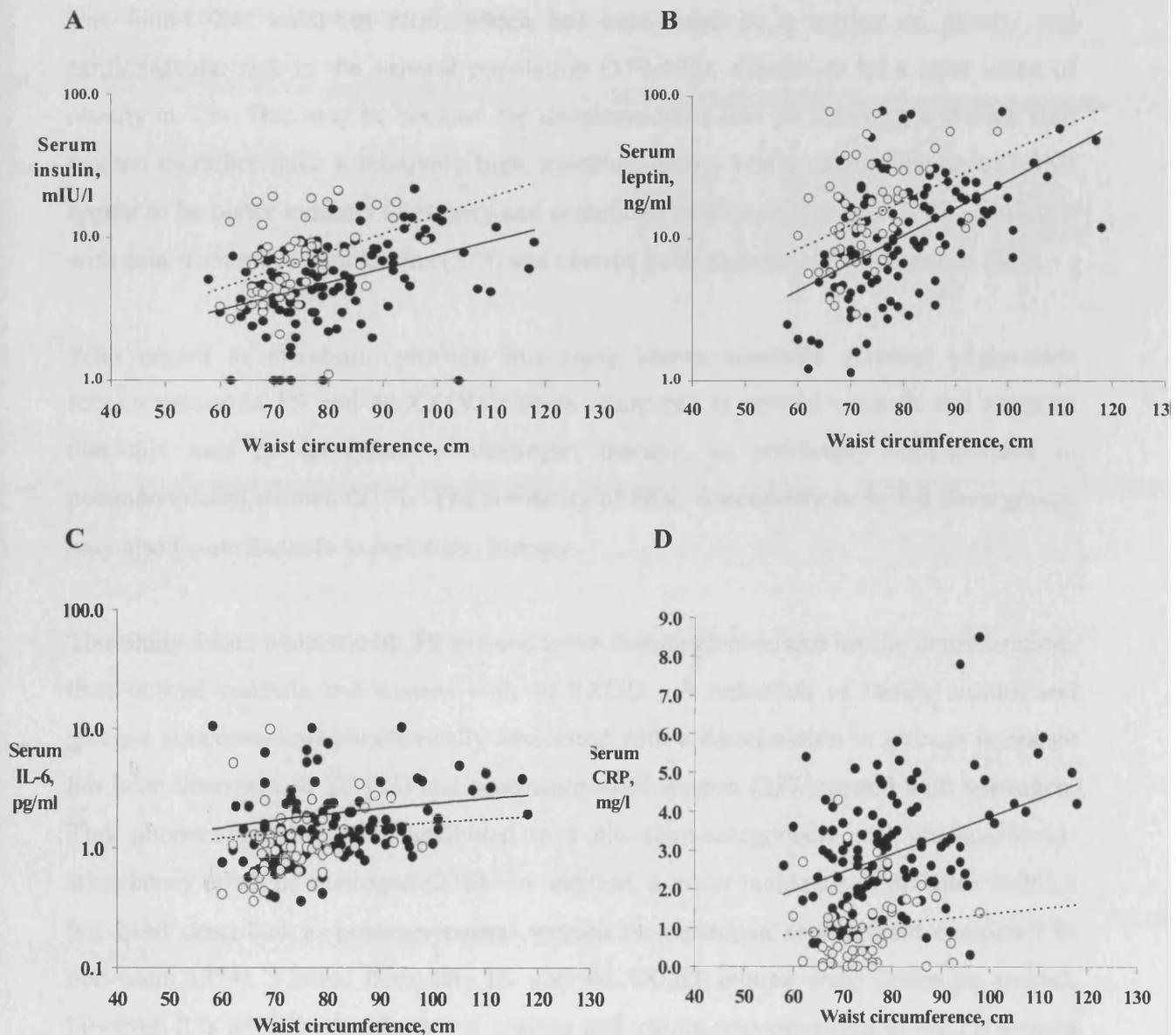
Analyses within the TS cohort

Anthropometric measures of obesity (weight, waist circumference and BMI) were strongly intercorrelated ($r \geq 0.800$, $p < 0.001$ for all) and were associated with biochemical markers of obesity. In comparison, waist-hip ratio was weakly associated with other markers of obesity (correlation with BMI $r=0.243$, $p=0.010$; weight $r=0.228$, $p=0.016$). Waist circumference was associated with height ($r=0.263$, $p=0.005$), BMI ($r=0.855$,

$p < 0.001$), total, LDL and HDL cholesterol ($r = 0.215$, $p = 0.024$; $r = 0.229$, $p = 0.016$; and $r = -0.262$, $p = 0.006$ respectively). There were also associations between waist circumference and triglycerides ($r = 0.410$, $p < 0.001$), fasting insulin ($r = 0.494$, $p < 0.001$) and HOMA-R ($r = 0.491$, $p < 0.001$) but not fasting glucose ($r = 0.154$, $p = 0.110$). Serum leptin, CRP and IL-6 were all strongly correlated with waist circumference ($r = 0.556$, 0.402 and 0.370 respectively, $p < 0.001$ for all) (see Figure 1). In addition, leptin was strongly and CRP and IL-6 were weakly correlated with insulin concentrations ($r = 0.267$, $p = 0.004$; $r = 0.182$, $p = 0.055$; and $r = 0.185$, $p = 0.051$ respectively). The associations between waist and all three adipokines/ pro-inflammatory markers were independent of the association with insulin.

There was no significant association between measures of obesity and karyotype or previous history of growth hormone and oxandrolone therapy. There was no significant difference in any metabolic parameter between groups of women with either TS or POF taking different types of oestrogen replacement.

Figure 6.1. Relationships between serum insulin (A), leptin (B), IL-6 (C) and CRP (D) and waist circumference in women with TS compared to normal and 46,XXOD controls. Trend lines have been added and logarithmic axes used where appropriate. Filled circles and solid lines represent TS, open circles and dashed lines represent normal and 46,XXOD controls combined.



6.4. Discussion

This study compared metabolic markers of obesity and cardiovascular risk in oestrogen-replete adults with TS, 46,XXOD and normal controls, demonstrating that, whilst women with TS are more centrally obese than normal and 46,XXOD control women of similar

age, the evidence for an insulin resistance/ metabolic syndrome based is unconvincing when based on fasting insulin measurements. In addition, there appears to be a discrepancy between the adipokines leptin and IL-6 in women with TS, with leptin concentrations being lower than expected for the degree of adiposity.

The present findings confirm increased central adiposity in women with TS (33,151). It was found that waist-hip ratio, which has been used as a marker of obesity and cardiovascular risk in the general population (370-372), appears to be a poor index of obesity in TS. This may be because hip development is less pronounced, and even slim women therefore have a relatively high waist-hip ratio. Waist circumference and BMI appear to be better markers of obesity and associated cardiovascular risk in TS, consistent with data from other populations (373) and current guidelines for risk assessment (369).

With regard to metabolic profiles, this study shows similarly elevated triglyceride concentrations in TS and 46,XXOD women compared to normal controls and suggests that this may be an effect of oestrogen therapy, as previously demonstrated in postmenopausal women (217). The similarity of HDL concentrations in the three groups may also be attributable to oestrogen therapy.

This study found women with TS to have lower fasting glucose and insulin concentrations than normal controls and women with 46,XXOD. A reduction of fasting insulin and glucose concentrations paradoxically associated with a deterioration in glucose tolerance has been described in TS (33) and postmenopausal women (237) treated with oestrogen. This phenomenon has been attributed to a glucagon-antagonistic and glucocorticoid-stimulatory effect of oestrogen (238). In addition, a lower incidence of diabetes mellitus has been described in postmenopausal women on oestrogen replacement compared to non-users (374). Since both the TS and 46,XXOD groups were oestrogen-treated, however, it is unlikely that the lower glucose and insulin concentrations in the TS women are attributable to oestrogen therapy.

The high prevalence of impaired glucose tolerance in women with TS (5,33) appears to be secondary to an insulin secretory defect (161) or a glucose storage defect (164). It should be noted that the use of diabetes terminology, in particular IDDM and NIDDM in earlier studies (33), may not necessarily be exactly equivalent to the currently used type 1 and

type 2, hence complicating interpretation of earlier studies. As early as 35 years ago, however, it was suggested that the pathogenesis of diabetes in TS may be distinct from 'maturity-onset type' diabetes mellitus (157), as mentioned previously. The absence of fasting hyperinsulinaemia, despite excess central obesity, is consistent with this (375). The role of the 'metabolic syndrome' therefore becomes questionable in TS.

This study reports raised CRP and IL-6 and reduced leptin concentrations in TS. These parameters were measured as indicators of body fat (376-379) and inflammatory markers of cardiovascular risk (260,380). CRP and IL-6 are predominantly associated with visceral fat (381-383), although the relationship is complex. Oral oestrogen therapy itself is known to increase CRP concentrations via an effect on the liver (384), but CRP concentrations have been shown to predict cardiovascular risk independent of oestrogen therapy (385). The effect of oral oestrogen on IL-6 concentrations is inconsistent (384,386-388).

Given the significantly higher CRP concentrations in TS women compared to both other groups, it is unlikely that they can be attributed to the effect of oestrogen, since the 46,XXOD women were also oestrogen-treated. IL-6 concentrations were elevated in TS women compared to normal controls, but not compared to 46,XXOD women, so an oestrogen effect is difficult to determine and may contribute. Notably both CRP and IL-6 were strongly correlated with waist circumference. Thus their elevated concentrations in the TS women are likely to be attributable to the increased central adiposity, although other factors may also contribute since they are non-specific inflammatory markers.

The concentration of leptin, which originates predominantly from subcutaneous fat (376,389) but is generally elevated in people of increased adiposity, was lower in women with TS than in the other two groups. A previous study did not demonstrate but inferred hypoleptinaemia in TS women compared to normal controls because of the greater percentage of body fat (167). It could be postulated that low leptin in TS results from reduced subcutaneous adiposity, and this prompted further investigation of fat distribution as discussed in Chapter 7. Another possible explanation is that the reduced leptin concentrations are related to low fasting insulin concentrations, since leptin production is regulated by chronic rather than acute insulin concentrations (390). The correlation between leptin and insulin in TS women in this study was not independent of the effect of

waist size, however. Metabolic syndrome is typically, but not unequivocally, associated with increased leptin concentrations (391,392). A unifying hypothesis to explain the low insulin and leptin concentrations in the context of greater obesity would be that the insulin secretory defect is the primary abnormality, causing low leptin concentrations, which then results in inhibited satiety mechanisms (393,394).

In conclusion, women with TS have various physical and biochemical features suggestive of the metabolic/ insulin resistance syndrome, but the evidence that they are all interrelated in a true 'metabolic syndrome' is questionable. An excess prevalence of central obesity in TS is evident. Hypertriglyceridaemia in TS may be partly related to oestrogen therapy, although obesity probably contributes. The abnormalities of glucose homeostasis do not follow the classical pattern associated with the metabolic syndrome and may result from a unique metabolic defect. The elevated CRP and IL-6 concentrations are consistent with visceral adiposity, but the hyperleptinaemia often associated with the metabolic syndrome was not evident. Although these markers are undoubtedly related to obesity in TS, other factors such as oestrogen therapy, low fasting insulin, and perhaps other sources of pro-inflammatory cytokines appear to contribute. The features compatible with the metabolic syndrome in TS are not fully explained by the obesity, the history of oestrogen deficiency nor indeed current oestrogen therapy, and it may be that one or more TS-specific metabolic defects explain this apparent paradox.

Chapter 7

Fat Distribution Study

7.1. Aims

The high prevalence of obesity in TS (33,151) with a predominantly central distribution (33) has already been alluded to (Chapter 1, Section 1.2.2. and Chapter 6). This is in the context, however, of unexpectedly low circulating insulin concentrations (Chapter 6) (33,161).

Various techniques are available to assess obesity and fat distribution. These include anthropometric measures, magnetic resonance imaging (MRI) and bioelectrical impedance. MRI is being increasingly used to localise fat depots in different populations (329), whilst bioelectrical impedance has been shown to correlate well with other markers of obesity in the general population (334). On MRI the most sensitive markers of insulin resistance are abdominal adipose tissue, intrahepatocellular lipids and intramyocellular lipids in the soleus muscle (395,396).

The aim of this study was to characterise body fat content and distribution in six TS subjects and six normal control women by different physical and imaging techniques, and to compare these to biochemical markers of obesity in order to gain a greater understanding of the unusual metabolic profile of TS.

7.2. Results

Subject characteristics

Table 7.1. compares clinical characteristics between the two groups of subjects. Women with TS were shorter and had greater systolic blood pressure and waist circumference. All but one woman in each group were non-smokers, and there was also no difference between the groups with regard to exercise taken (3 vs 4 women in TS and control groups respectively took no regular exercise). TS women had a median (range) oestrogen start age of 14 (11-19) years, with 2 (0-8) cumulative years of oestrogen deficiency. 2 women had karyotype monosomy X, 2 had isochromosome X, one had ring X and one had a

complex karyotype. Total cholesterol, CRP and Il-6 concentrations were greater in the TS women compared to the controls.

Table 7.1. Baseline characteristics in women with TS and normal controls.

| Measurement | TS (n=6) | Control (n=6) | p |
|--|--------------|------------------|-------|
| Age, years ^a | 34.9 ± 8.2 | 34.7 ± 6.5 | 0.966 |
| Height, m ^a | 1.47 ± 0.08 | 1.60 ± 0.04 | 0.008 |
| Systolic blood pressure, mmHg ^a | 123 ± 10 | 111 ± 6 | 0.038 |
| Diastolic blood pressure, mmHg ^a | 76 ± 8 | 67 ± 6 | 0.056 |
| Waist circumference, cm ^a | 91.5 ± 10.7 | 75.8 ± 9.0 | 0.020 |
| BMI, kg/m ^{2a} | 30.0 ± 5.3 | 25.7 ± 4.5 | 0.168 |
| Total cholesterol, mmol/l ^a | 6.0 ± 0.9 | 4.6 ± 1.1 | 0.035 |
| HDL cholesterol, mmol/l ^a | 1.9 ± 0.6 | 1.8 ± 0.4 | 0.867 |
| LDL cholesterol, mmol/l ^a | 3.4 ± 1.0 | 2.4 ± 0.9 | 0.094 |
| Triglycerides, mmol/l ^a | 1.2 ± 0.4 | 0.7 ± 0.3 | 0.060 |
| Fasting glucose, mmol/l ^a | 4.4 ± 0.2 | 5.0 ± 0.4 | 0.015 |
| Fasting insulin, mIU/l ^b | 5.4 ± 2.8 | 6.2 ± 3.3 | 0.772 |
| Fasting HOMA-R ^b | 1.1 ± 0.6 | 1.4 ± 0.8 | 0.137 |
| Leptin, ng/ml ^b | 20.2 ± 9.8 | 21.7 ± 11.4 | 0.868 |
| CRP, mg/l ^a | 4.0 ± 2.3 | 0.9 ± 1.2 | 0.015 |
| Il-6, pg/ml ^b | 2.3 ± 1.3 | 1.0 ± 0.3 | 0.049 |
| Bilirubin, µmol/l ^b | 8.1 ± 2.4 | 10.4 ± 3.3 | 0.268 |
| Alanine transferase, U/l ^b | 24.1 ± 14.7 | 18.4 ± 6.1 | 0.533 |
| Alkaline phosphatase, U/l ^b | 100.2 ± 39.2 | 59.6 ± 5.5 | 0.031 |
| Gamma glutamyl transferase, U/l ^b | 70.6 ± 32.2 | 14.4 ± 3.2 | 0.001 |

Key: Values shown are ^amean or ^bgeometric mean ± SD.

Comparisons between TS and control groups

With regard to absolute values for MRI and bioelectrical impedance, the only difference between the two groups was the excess of IHCL in the TS women (Table 7.2.). There was, however, a strong correlation between visceral adipose tissue and height ($r=0.886$,

$p=0.019$), and height adjustment was therefore performed for all physical parameters of fat distribution. After correction for height differences, women with TS still had an excess of IHCL, but in addition they were shown to have greater ratios of total, visceral and internal (all non-subcutaneous, non-hepatic) fat to subcutaneous and subcutaneous abdominal fat, and a lower ratio of total to internal fat (the relationship between total and visceral fat did not quite achieve significance) (Figure 7.1., Table 7.2.). Height correction had no effect on the comparisons of bioelectrical impedance measures between the two groups.

Figure 7.1. Representative T¹-weighted MR images from the abdomen (just below the umbilicus) of (a) a woman with TS (BMI 29.0 kg/m²) and (b) a normal control woman (BMI 30.6 kg/m²), demonstrating increased visceral adipose tissue in the woman with TS despite slightly lower BMI. Adipose tissue is shown in white.

Figure 7.1a.

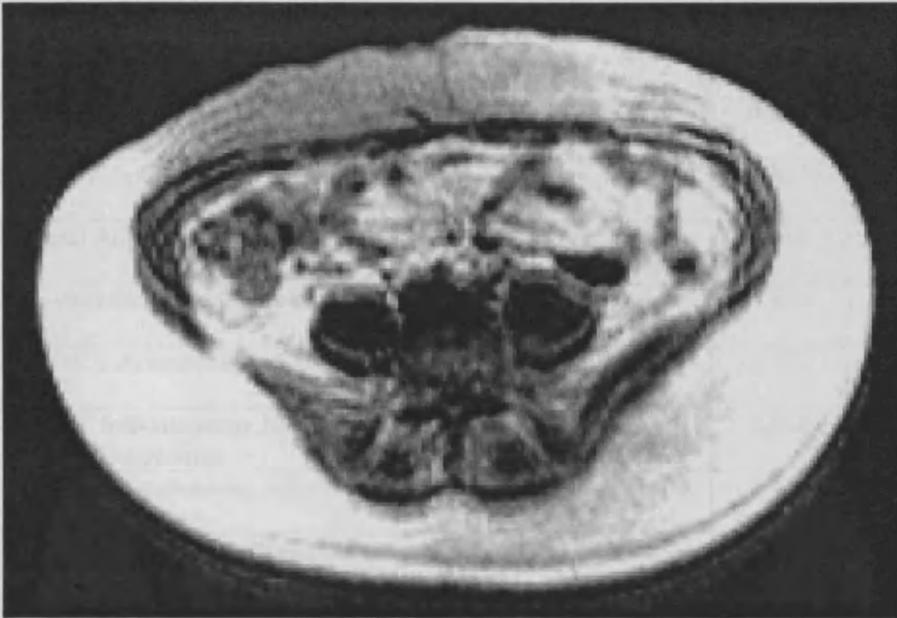


Figure 7.1b.

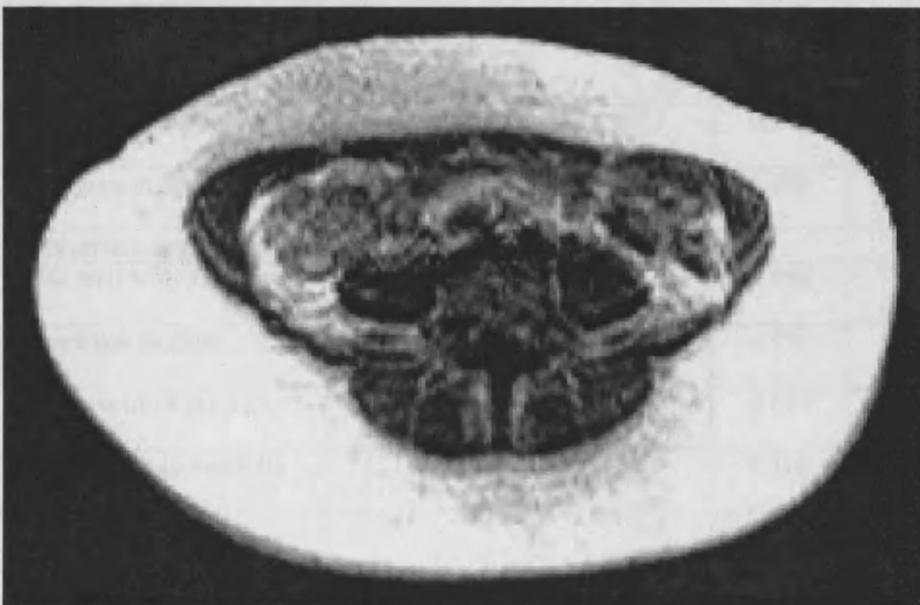


Table 7.2. Absolute and height-adjusted values of MRI and bioelectrical impedance results in women with TS and normal controls

| Measurement | Absolute values | | | Height-adjusted values | | |
|---|------------------|------------------|---------|------------------------|------------------|---------|
| | TS (n=6) | Control (n=6) | p-value | TS (n=6) | Control (n=6) | p-value |
| MRI data | | | | | | |
| Total Adipose Tissue (litres) | 31.60 ± 8.93 | 28.35 ± 9.96 | 0.565 | 31.3 ± 13.5 | 28.7 ± 13.5 | 0.791 |
| Subcutaneous Adipose Tissue (litres) | 25.90 ± 8.11 | 23.60 ± 7.91 | 0.629 | 24.7 ± 10.8 | 24.8 ± 10.8 | 0.989 |
| Subcutaneous Abdominal Adipose Tissue (litres) | 8.39 ± 2.59 | 6.85 ± 3.28 | 0.388 | 7.9 ± 4.2 | 7.3 ± 4.2 | 0.837 |
| Subcutaneous Peripheral Adipose Tissue (litres) | 17.51 ± 5.69 | 16.75 ± 4.66 | 0.805 | 16.8 ± 7.4 | 17.5 ± 7.4 | 0.891 |
| Internal Adipose Tissue ^a (litres) | 5.69 ± 1.56 | 4.75 ± 2.32 | 0.427 | 6.6 ± 2.6 | 3.9 ± 2.6 | 0.176 |
| Visceral Adipose Tissue (litres) | 2.77 ± 0.90 | 2.30 ± 1.44 | 0.515 | 3.3 ± 1.6 | 1.8 ± 1.6 | 0.191 |
| Non-visceral Internal Adipose Tissue (litres) | 2.92 ± 0.77 | 2.44 ± 0.91 | 0.353 | 3.2 ± 1.1 | 2.1 ± 1.1 | 0.194 |
| Internal: Subcutaneous Adipose Tissue ratio | 0.2 ± 0.06 | 0.2 ± 0.05 | 0.352 | 0.27 ± 0.06 | 0.15 ± 0.06 | 0.014 |
| Visceral: Subcutaneous Abdominal Adipose Tissue ratio | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.914 | 0.4 ± 0.1 | 0.2 ± 0.1 | 0.030 |
| Visceral: Subcutaneous Adipose Tissue ratio | 0.11 ± 0.04 | 0.09 ± 0.04 | 0.404 | 0.14 ± 0.04 | 0.07 ± 0.04 | 0.045 |
| Total Adipose: Subcutaneous Tissue ratio | 1.2 ± 0.06 | 1.2 ± 0.05 | 0.352 | 1.3 ± 0.06 | 1.2 ± 0.06 | 0.014 |
| Total: Subcutaneous Abdominal Adipose Tissue ratio | 3.8 ± 0.4 | 4.5 ± 0.9 | 0.129 | 4.0 ± 1.0 | 4.3 ± 1.0 | 0.742 |
| Total: Visceral Adipose Tissue ratio | 12.0 ± 3.8 | 14.6 ± 5.8 | 0.375 | 8.9 ± 6.0 | 17.8 ± 6.0 | 0.062 |
| Total: Internal Adipose Tissue ratio | 5.7 ± 1.3 | 6.4 ± 1.4 | 0.372 | 4.6 ± 1.5 | 7.4 ± 1.5 | 0.024 |
| Intramyocellular Lipids Soleus | 11.04 ± 4.78 | 10.02 ± 6.20 | 0.755 | 14.9 ± 6.4 | 6.1 ± 6.4 | 0.081 |
| Intramyocellular Lipids Tibialis | 15.48 ± 14.67 | 9.31 ± 2.08 | 0.332 | 8.4 ± 12.3 | 16.4 ± 12.3 | 0.373 |
| Intrahepatocellular Lipids | 2.93 ± 1.50 | 0.42 ± 0.25 | 0.002 | 3.0 ± 1.5 | 0.4 ± 1.5 | 0.038 |
| Bioelectrical Impedance data | | | | | | |
| Tanita total body fat (kg) | 23.1 ± 7.6 | 22.7 ± 11.6 | 0.938 | 22.8 ± 14.0 | 23.1 ± 14.0 | 0.975 |
| Tanita trunk fat (kg) | 10.0 ± 3.8 | 10.1 ± 4.8 | 0.974 | 10.5 ± 6.2 | 9.7 ± 6.2 | 0.858 |
| Tanita non-trunk fat (kg) | 13.1 ± 4.0 | 12.6 ± 7.0 | 0.874 | 12.3 ± 8.1 | 13.4 ± 8.1 | 0.849 |
| Tanita total body water (l) | 29.9 ± 3.4 | 32.9 ± 2.2 | 0.100 | 31.2 ± 3.8 | 31.7 ± 3.8 | 0.849 |
| Tanita % body fat | 35.4 ± 5.9 | 31.0 ± 8.3 | 0.315 | - | - | - |
| Tanita trunk % fat | 29.4 ± 7.1 | 27.3 ± 9.3 | 0.668 | - | - | - |

Key: ^aRefers to all non-subcutaneous, non-hepatic fat. Values shown are mean ± SD

Associations within TS and control groups

MRI and bioelectrical impedance

In TS women, there were strong correlations between bioelectrical impedance measures of total and trunk fat mass and MRI measures of total and subcutaneous AT on MRI ($r>0.800$, $p<0.05$ for all) (see Figure 7.2.). There was no association, however, between bioelectrical impedance trunk fat mass and MRI internal or visceral AT (Figure 7.3.).

In the control group there were strong associations both between bioelectrical impedance total and trunk fat mass and MRI total, subcutaneous, visceral and internal AT and also waist circumference ($r>0.800$, $p<0.05$ for all) (Figure 7.2.). In particular, and in contrast to the TS group, the relationship between bioelectrical impedance trunk fat mass and MRI visceral and internal AT was very strong in the control group ($r>0.900$, $p<0.01$) (Figure 7.3.), although there was no association with IHCL.

Figure 7.2. Comparison of total body fat measurements in TS and normal control women. Filled circles and solid line represent TS, open circles and dashed line represent controls.

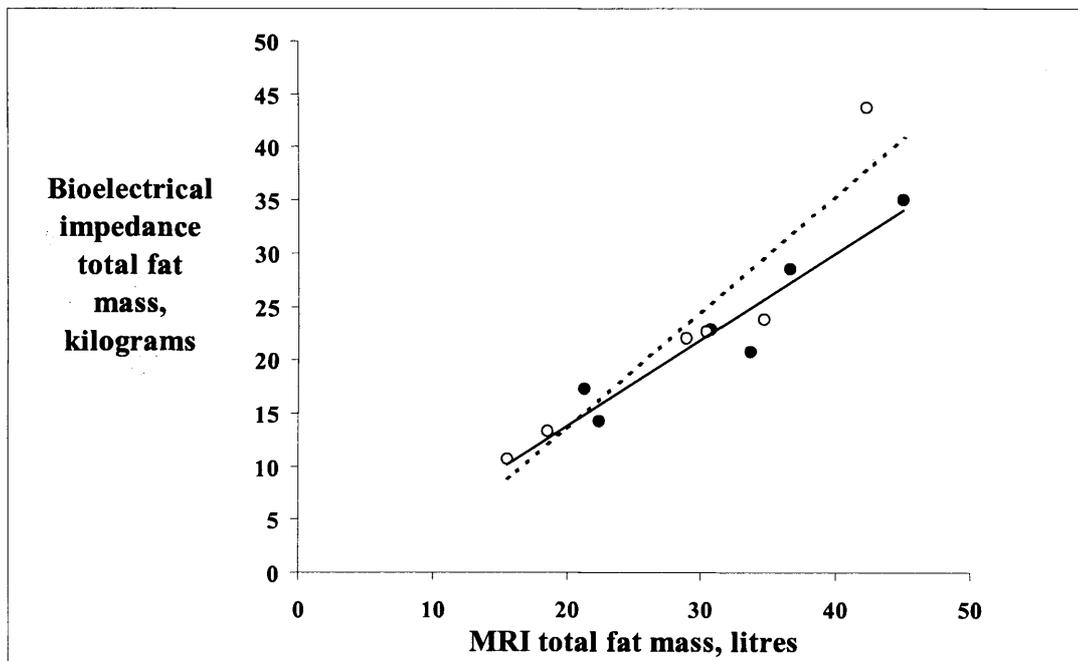
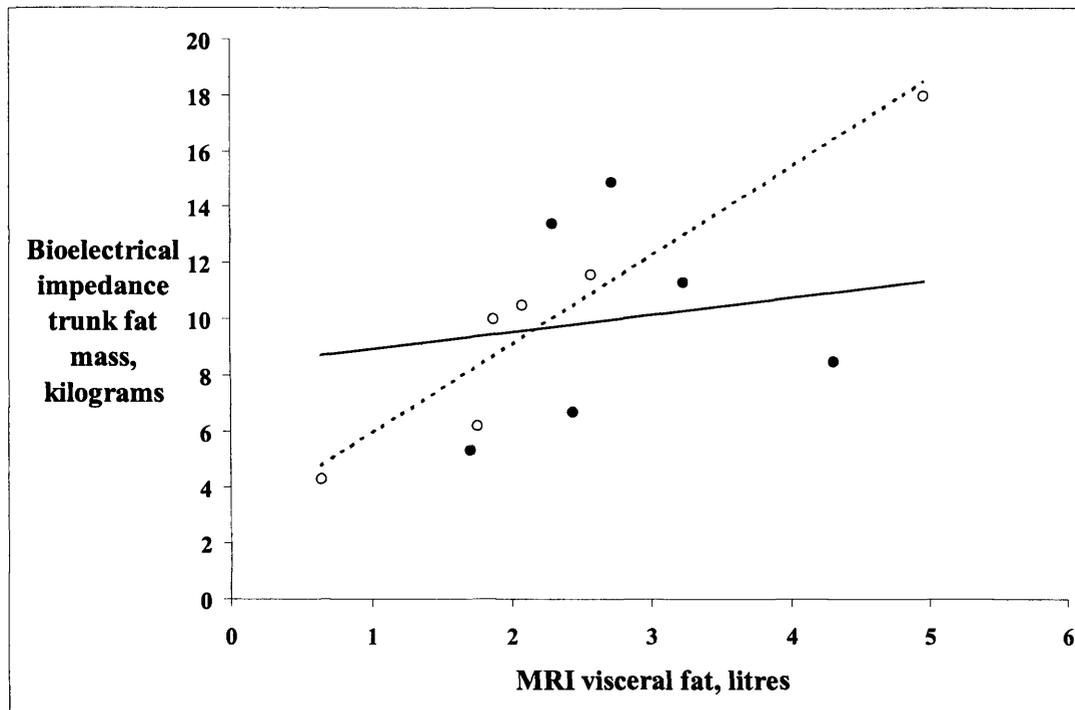


Figure 7.3. Comparison of trunk and visceral fat measurements in TS and normal control women. Filled circles and solid line represent TS, open circles and dashed line represent controls.



Physical and biochemical markers of adiposity

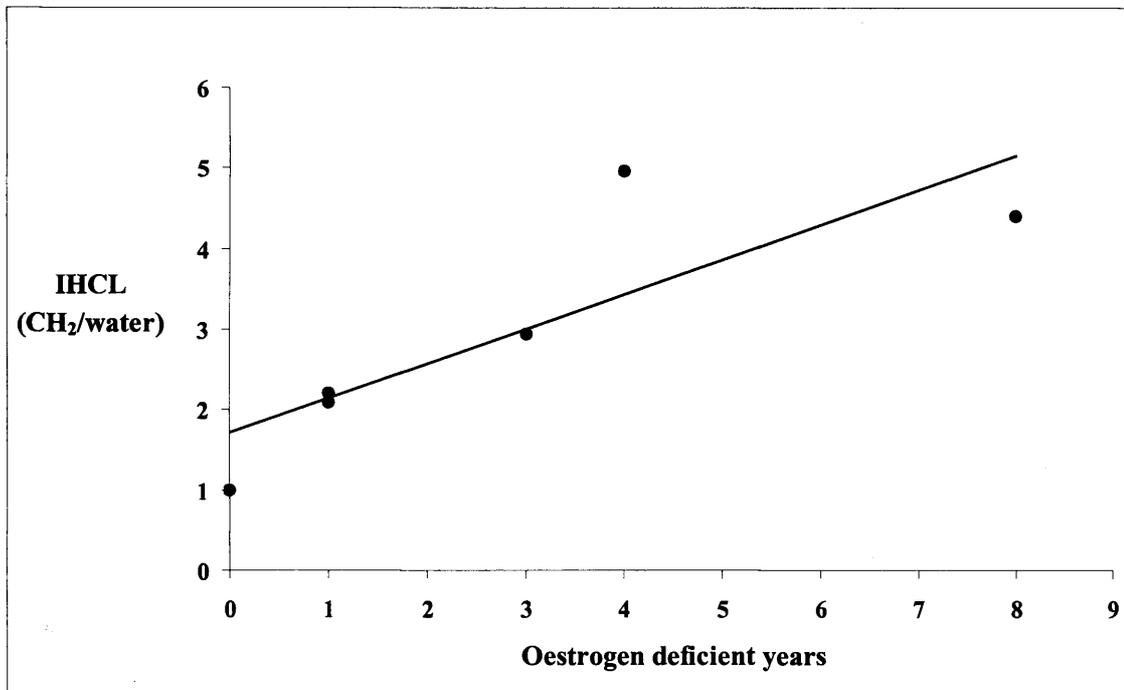
With regard to associations between MRI measures of adiposity and biochemical markers of obesity within the TS group, the only correlations were with triglyceride concentrations. These were positively correlated with visceral:subcutaneous abdominal AT ratio on MRI ($r=0.928$, $p=0.008$) and visceral: total AT ratio ($r=0.841$, $p=0.036$), but were negatively correlated with peripheral subcutaneous AT: total AT ratio ($r=-0.928$, $p=0.008$). No other parameter, physical or metabolic, had a significant association with MRI measurements, nor bioelectrical impedance measurements.

Within the control group, MRI visceral AT was strongly correlated with insulin, HOMA-R and CRP concentrations; bioelectrical impedance trunk mass was associated with insulin and HOMA-R ($r>0.800$, $p<0.05$ for all). There were no associations between soleus IMCL or IHCL and biochemical markers of obesity.

Previous oestrogen, growth hormone and oxandrolone therapy in TS women

Within the TS cohort there was an association between both oestrogen start age and cumulative years of oestrogen deficiency and IHCL ($r=0.900$, $p=0.037$ and $r=0.928$, $p=0.008$ respectively) (Figure 7.4.). Previous history of growth hormone and oxandrolone therapy or karyotype had no association with physical or biochemical markers of adiposity.

Figure 7.4. The relationship between intrahepatocellular lipids (IHCL) on MR spectroscopy and oestrogen deficient years in women with TS.



7.3. Discussion

This study characterises the obesity of Turner Syndrome using both MRI and bioelectrical impedance techniques. Women with TS and controls had similar BMI, although women with TS had greater absolute waist circumference. This greater central obesity was confirmed on MRI by an excess of visceral and internal AT and intrahepatocellular lipids after adjustment for height.

Bioelectrical impedance measures of adiposity did not reflect the excess trunk mass in TS detected by MRI, even after height-adjustment, although there were good correlations with total and subcutaneous AT on MRI. This contrasted with the findings in the control group, in whom visceral and trunk measures of adiposity were well correlated between the two techniques. Our findings suggest that whilst bioelectrical impedance may be useful to gain an overall impression of total adiposity, fat localisation is poor in women with TS. MRI, however, which has been well validated in patient and control groups (329), is a useful method of assessing different fat depots in TS.

Women with TS not only had physical but also biochemical markers of excess adiposity with greater total cholesterol, CRP and Il-6 concentrations than controls. In the larger cross-sectional metabolic study (Chapter 6) a greater triglyceride concentration had also been demonstrated. The excess central adiposity in TS was associated with triglyceride concentrations. There is thus a discrepancy between the finding of increased visceral AT and intrahepatocellular lipids on MRI, two of the three measures most associated with insulin resistance (395) in the context of some elevated markers of obesity but an absence of hyperinsulinaemia. A possible explanation for this would be that the centrally distributed adipose tissue in TS is predominantly subcutaneous, but our findings have ruled this out. The excess visceral adipose tissue shown here infers an abnormality of adipose function/deposition, although the present study does not explain the mechanism. A further possibility is the existence of an insulin secretory defect in TS which has been previously suggested (33,161) and the findings in the present study are consistent with this.

Perhaps the most remarkable finding in this study was the association between oestrogen deficiency and intrahepatocellular lipids. Women with TS are known to have an increased prevalence of abnormal liver function (36,37), and it has been suggested that this may be related to oestrogen deficiency (37,74). It is possible that oestrogen deficiency in childhood years may affect adipose tissue lipolysis and/or alter adipocyte differentiation and function, which in turn would lead to increased liver fat content later in life (397). Accumulation of ectopic fat, especially in the muscle and liver has been implicated in the development of type II diabetes (398). Liver biopsies have revealed a variety of abnormalities including fatty infiltration, fibrosis and cirrhosis (7). It is therefore interesting to note the excess of intrahepatocellular lipids in women with TS

compared to controls in this study, and the association with oestrogen deficiency, in the context of elevated liver enzymes in TS women. The sample size may have been too small to demonstrate a relationship between serum liver function biochemistry and hepatic fat (332).

In conclusion, this study has confirmed that the central obesity seen in women with TS on anthropometric measurements is reflected by excess total, internal and visceral adipose tissue on MRI, best demonstrated by adjustment for height. This fat distribution is associated with established biochemical markers of central adiposity but not hyperinsulinaemia. Intrahepatocellular lipids are elevated in TS and this is associated with history of oestrogen deficiency, which may explain the beneficial effect of oestrogen therapy on liver function. Bioelectrical impedance may be useful to estimate total body fat, but does not reliably localise fat depots in women with TS. Larger studies are now required to explore these relationships further.

Chapter 8

Longitudinal Oestrogen Dose-ranging Study

8.1 Aims

The increased cardiovascular risk in TS may be related either to genetic factors (resulting from the abnormal karyotype) or to oestrogen deficiency. The impact of oestrogen deficiency can be extrapolated from comparable other groups of women with early oestrogen deficiency, for instance, women with premature ovarian failure and hypopituitarism. The improvement of surrogate markers of cardiovascular risk with oestrogen therapy in postmenopausal women as previously discussed is in contrast to the greater cardiovascular morbidity (204), a paradox which remains unexplained at present.

It has been suggested that women with TS are relatively resistant to the actions of oestrogen and benefit from higher doses with regard to bone markers, liver function (37) and uterine development (323). The hypothesis of this study was that higher doses of oestrogen may be beneficial to markers of cardiovascular risk and metabolism in women with TS. In order to assess the oestrogen sensitivity of women with TS, 14 women with TS were compared to 11 women with 46,XX primary amenorrhoea (PA), who were similarly oestrogen deficient but with a normal karyotype. All women were sequentially treated with three months each of oral oestrogen replacement preparations containing 1mg, 2mg and 4mg of 17 β -oestradiol respectively.

8.2. Results

Comparisons of women with TS and 46,XX PA (see Table 8.1.)

The two groups were of similar age but not height-matched (mean age \pm SD 33.3 \pm 6.1 vs 30.2 \pm 8.6 years, $p=0.306$; height 1.48 \pm 0.06 vs 1.67 \pm 0.09 metres, $p<0.001$, in TS and 46,XXPA women respectively). Women with TS smoked less (79% vs 36% had never smoked in TS and 46,XXPA groups respectively, $p=0.032$) but there was no difference with regard to exercise taken (64% vs 46% took no exercise, $p=0.346$). Women with TS started oestrogen replacement therapy at a younger age (median [range] years 15 [10-19] vs 17 [12-22], $p=0.033$) and had fewer cumulative years of oestrogen deficiency (3.5 [0-8] vs 6 [1-11] years, $p=0.005$) than women with 46,XXPA.

Table 8.1. Comparison of characteristics on 1mg oestradiol in women with TS and 46,XXPA.

| | TS (n=14) | 46,XXPA (n=11) | p-value |
|--|----------------------|---------------------------|----------------|
| Anthropometric parameters | | | |
| Systolic BP, mmHg ^a | 116 ± 9 | 106 ± 7 | 0.002 |
| Diastolic BP, mmHg ^a | 73 ± 9 | 63 ± 4 | 0.008 |
| Weight, kg ^a | 56.1 ± 10.9 | 60.8 ± 11.8 | 0.290 |
| BMI, kg/m ^{2a} | 25.7 ± 4.4 | 21.8 ± 3.4 | 0.023 |
| Waist, cm ^a | 79.4 ± 11.4 | 73.6 ± 7.4 | 0.157 |
| Waist-hip ratio ^a | 0.85 ± 0.05 | 0.78 ± 0.03 | 0.001 |
| Lipids and Metabolism | | | |
| Total cholesterol, mmol/l ^a | 5.6 ± 0.9 | 4.9 ± 0.9 | 0.057 |
| LDL cholesterol, mmol/l ^a | 3.2 ± 0.8 | 2.6 ± 0.9 | 0.091 |
| HDL cholesterol, mmol/l ^a | 1.9 ± 0.4 | 1.8 ± 0.5 | 0.690 |
| Triglycerides, mmol/l ^a | 1.2 ± 0.5 | 1.1 ± 0.7 | 0.694 |
| Glucose, mmol/l ^a | 4.7 ± 0.4 | 5.0 ± 0.3 | 0.067 |
| Insulin, mIU/l ^b | 4.7 ± 2.4 | 7.6 ± 2.6 | 0.058 |
| HOMA-R ^b | 1.0 ± 0.5 | 1.7 ± 0.6 | 0.041 |
| CRP, mg/l ^a | 1.9 ± 0.8 | 1.1 ± 0.8 | 0.012 |
| Leptin, ng/ml ^b | 15.8 ± 9.9 | 11.6 ± 5.1 | 0.365 |
| IL-6, pg/ml ^b | 1.4 ± 0.9 | 1.0 ± 0.5 | 0.268 |
| Sex Hormones | | | |
| LH, IU/l ^b | 17.8 ± 12.9 | 31.6 ± 24.7 | 0.281 |
| FSH, IU/l ^b | 36.0 ± 20.0 | 39.3 ± 26.3 | 0.610 |
| Oestradiol, pmol/l ^b | 204 ± 52 | 297 ± 65 | 0.028 |
| Testosterone, nmol/l ^b | 1.8 ± 0.5 | 2.8 ± 1.0 | 0.005 |
| Liver Function | | | |
| Gamma glutamyl transferase, U/l ^b | 54.1 ± 33.4 | 18.8 ± 8.0 | 0.003 |
| Alkaline phosphatase, U/l ^b | 93.1 ± 67.1 | 68.2 ± 18.1 | 0.024 |
| Alanine transferase, U/l ^b | 40.2 ± 15.5 | 20.7 ± 7.1 | 0.002 |
| Bilirubin, µmol/l ^b | 8.7 ± 1.4 | 9.8 ± 4.6 | 0.501 |
| Albumin, g/l ^a | 46.1 ± 2.5 | 47.2 ± 2.6 | 0.296 |
| Urea, mmol/l ^a | 4.6 ± 1.2 | 4.4 ± 0.7 | 0.609 |
| Haematocrit, l/l ^a | 0.397 ± 0.018 | 0.405 ± 0.036 | 0.342 |
| Fibrinogen, g/l ^a | 2.58 ± 0.52 | 2.35 ± 0.45 | 0.260 |

Key: ^amean ± SD; ^bgeometric mean ± SD

2/14 (14.3%) women with TS had previously received growth hormone treatment and 28.6% had karyotype monosomy X. The remainder had a variety of TS karyotypes including 42.9% isochromosome X, 7.1% ring X, 14.3% any Y fragment and 7.1% mosaic 45,X/46,XX. None of the women in either group took antihypertensive medication.

Other group differences were assessed at the 1mg oestradiol dose. Women with TS had greater BMI and waist-hip ratio than women in the 46,XXPA group. Lipid concentrations between the two groups were similar, as were insulin, leptin and Il-6 concentrations, whilst calculated HOMA-R was slightly lower and CRP concentrations somewhat higher in TS women than 46,XXPA women.

There were no significant differences in gonadotrophin concentrations between the two groups. The TS group had lower oestradiol and testosterone concentrations on 1mg oestradiol.

Serum gamma glutamyl transferase (γ GT), alanine transferase (ALT) and alkaline phosphatase (ALP) concentrations were all higher in TS than 46,XXPA women on 1mg oestradiol. Fibrinogen concentration did not differ between the two groups.

Parameters of vascular physiology were similar between the two groups of women except for AIx which was higher in TS.

Oestrogen dose-ranging within TS and 46,XXPA groups (Tables 8.2. and 8.3.)

Anthropometric parameters

Increasing dose of oestrogen had no effect on blood pressure or weight over the period studied in either TS or 46,XXPA women.

Lipids and Metabolism

There was an increase in HDL cholesterol in both groups with increasing oestrogen dose, whilst insulin concentrations and calculated HOMA-R score were reduced in the women with 46,XXPA. Other metabolic markers were unaffected in both groups.

Table 8.2. Variations in clinical, metabolic and hormonal parameters with increasing doses of oestrogen in women with TS

| Turner Syndrome (n=14) | Dose of oestradiol | | | p-value |
|--|--------------------|---------------|---------------|---------|
| | 1mg | 2mg | 4mg | |
| Anthropometric parameters | | | | |
| Systolic BP, mmHg ^a | 116 ± 9 | 113 ± 7 | 114 ± 7 | 0.352 |
| Diastolic BP, mmHg ^a | 73 ± 9 | 71 ± 6 | 72 ± 8 | 0.598 |
| Weight, kg ^a | 56.1 ± 10.9 | 56.1 ± 10.4 | 56.9 ± 11.6 | 0.218 |
| Waist, cm ^a | 79.4 ± 11.4 | 79.3 ± 11.6 | 78.4 ± 12.3 | 0.297 |
| Waist-hip ratio ^a | 0.85 ± 0.05 | 0.85 ± 0.06 | 0.84 ± 0.06 | 0.174 |
| Lipids and Metabolism | | | | |
| Total cholesterol, mmol/l ^a | 5.6 ± 0.9 | 5.8 ± 0.7 | 5.8 ± 1.0 | 0.720 |
| LDL cholesterol, mmol/l ^a | 3.2 ± 0.8 | 3.1 ± 0.9 | 2.8 ± 0.9 | 0.106 |
| HDL cholesterol, mmol/l ^a | 1.9 ± 0.4 | 2.1 ± 0.5 | 2.2 ± 0.2 | 0.003 |
| Triglycerides, mmol/l ^a | 1.2 ± 0.5 | 1.3 ± 0.4 | 1.6 ± 0.6 | 0.070 |
| Glucose, mmol/l ^a | 4.7 ± 0.4 | 4.6 ± 0.3 | 4.5 ± 0.4 | 0.633 |
| Insulin, mIU/l ^b | 4.7 ± 2.4 | 6.2 ± 2.9 | 5.2 ± 2.8 | 0.187 |
| HOMA-R ^b | 1.0 ± 0.5 | 1.3 ± 0.6 | 1.0 ± 0.6 | 0.814 |
| CRP, mg/l ^a | 1.9 ± 0.8 | 2.2 ± 0.6 | 3.7 ± 5.6 | 0.279 |
| Leptin, ng/ml ^b | 15.8 ± 9.9 | 15.6 ± 9.6 | 13.4 ± 9.4 | 0.413 |
| IL-6, pg/ml ^b | 1.4 ± 0.9 | 1.2 ± 0.7 | 1.5 ± 0.9 | 0.933 |
| Sex Hormones | | | | |
| LH, IU/l ^b | 17.8 ± 12.9 | 14.1 ± 8.7 | 4.7 ± 3.6 | 0.001 |
| FSH, IU/l ^b | 36.0 ± 20.0 | 23.6 ± 12.8 | 7.6 ± 4.6 | <0.001 |
| Oestradiol, pmol/l ^b | 204 ± 52 | 240 ± 96 | 398 ± 166 | <0.001 |
| Testosterone, nmol/l ^b | 1.8 ± 0.5 | 1.4 ± 0.7 | 0.5 ± 0.3 | 0.005 |
| Liver Function | | | | |
| Gamma glutamyl transferase, U/l ^b | 54.1 ± 33.4 | 40.9 ± 26.5 | 33.8 ± 20.9 | 0.002 |
| Alkaline phosphatase, U/l ^b | 93.1 ± 67.1 | 84.0 ± 26.6 | 86.9 ± 22.3 | 0.104 |
| Alanine transferase, U/l ^b | 40.2 ± 15.5 | 30.1 ± 11.6 | 27.1 ± 11.7 | 0.008 |
| Bilirubin, µmol/l ^b | 8.7 ± 1.4 | 7.5 ± 1.7 | 7.0 ± 1.8 | 0.016 |
| Albumin, g/l ^a | 46.1 ± 2.5 | 45.3 ± 2.7 | 43.3 ± 2.7 | 0.002 |
| Urea, mmol/l ^a | 4.6 ± 1.2 | 4.3 ± 0.9 | 4.1 ± 1.2 | 0.104 |
| Haematocrit, l/l ^a | 0.397 ± 0.018 | 0.394 ± 0.020 | 0.382 ± 0.022 | 0.109 |
| Fibrinogen, g/l ^a | 2.58 ± 0.52 | 2.59 ± 0.49 | 2.93 ± 0.70 | 0.152 |

Key: ^amean ± SD; ^bgeometric mean ± SD

Table 8.3. Variations in clinical, metabolic and hormonal parameters with increasing doses of oestrogen in women with 46,XXPA

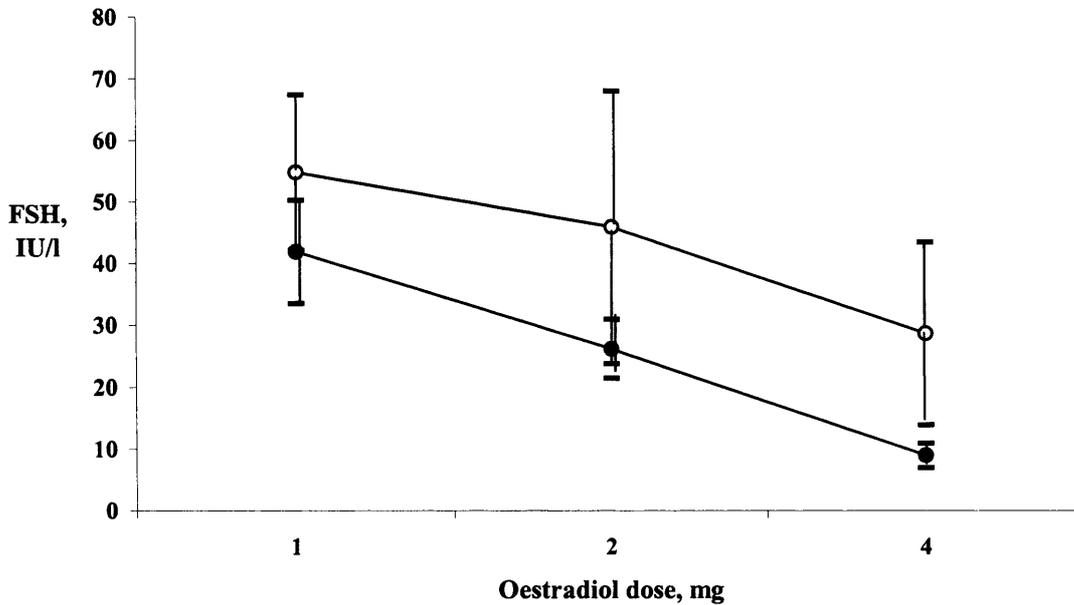
| 46,XXPA (n=11) | Dose of oestradiol | | | p-value |
|--|--------------------|---------------|---------------|---------|
| | 1mg | 2mg | 4mg | |
| Anthropometric parameters | | | | |
| Systolic BP, mmHg ^a | 106 ± 7 | 108 ± 8 | 106 ± 8 | 0.948 |
| Diastolic BP, mmHg ^a | 63 ± 4 | 63 ± 6 | 61 ± 7 | 0.373 |
| Weight, kg ^a | 60.8 ± 11.8 | 60.9 ± 10.7 | 61.1 ± 11.7 | 0.421 |
| Waist, cm ^a | 73.6 ± 7.4 | 74.6 ± 6.7 | 73.1 ± 7.3 | 0.422 |
| Waist-hip ratio ^a | 0.78 ± 0.03 | 0.79 ± 0.04 | 0.77 ± 0.04 | 0.441 |
| Lipids and Metabolism | | | | |
| Total cholesterol, mmol/l ^a | 4.9 ± 0.9 | 5.0 ± 0.9 | 5.2 ± 1.0 | 0.263 |
| LDL cholesterol, mmol/l ^a | 2.6 ± 0.9 | 2.5 ± 0.8 | 2.6 ± 1.0 | 0.919 |
| HDL cholesterol, mmol/l ^a | 1.8 ± 0.5 | 2.0 ± 0.5 | 2.2 ± 0.5 | 0.001 |
| Triglycerides, mmol/l ^a | 1.1 ± 0.7 | 1.0 ± 0.4 | 1.2 ± 0.7 | 0.824 |
| Glucose, mmol/l ^a | 5.0 ± 0.3 | 4.7 ± 0.3 | 4.6 ± 0.8 | 0.130 |
| Insulin, mIU/l ^b | 7.6 ± 2.6 | 6.4 ± 1.8 | 5.1 ± 2.3 | 0.031 |
| HOMA-R ^b | 1.7 ± 0.6 | 1.3 ± 0.4 | 1.0 ± 0.5 | 0.030 |
| CRP, mg/l ^a | 1.1 ± 0.8 | 0.9 ± 1.0 | 1.5 ± 1.5 | 0.279 |
| Leptin, ng/ml ^b | 11.6 ± 5.1 | 13.7 ± 6.6 | 13.7 ± 7.8 | 0.401 |
| IL-6, pg/ml ^b | 1.0 ± 0.5 | 0.7 ± 0.3 | 1.0 ± 0.5 | 0.957 |
| Sex Hormones | | | | |
| LH, IU/l ^b | 31.6 ± 24.7 | 19.2 ± 15.2 | 9.0 ± 7.1 | <0.001 |
| FSH, IU/l ^b | 39.3 ± 26.3 | 22.8 ± 16.8 | 12.2 ± 9.2 | 0.001 |
| Oestradiol, pmol/l ^b | 297 ± 65 | 355 ± 154 | 611 ± 311 | 0.007 |
| Testosterone, nmol/l ^b | 2.8 ± 1.0 | 2.8 ± 0.8 | 1.7 ± 0.6 | 0.012 |
| Liver Function | | | | |
| Gamma glutamyl transferase, U/l ^b | 18.8 ± 8.0 | 17.1 ± 6.5 | 14.6 ± 6.5 | 0.035 |
| Alkaline phosphatase, U/l ^b | 68.2 ± 18.1 | 66.1 ± 18.8 | 66.5 ± 18.1 | 0.501 |
| Alanine transferase, U/l ^b | 20.7 ± 7.1 | 20.8 ± 9.4 | 19.1 ± 6.5 | 0.386 |
| Bilirubin, µmol/l ^b | 9.8 ± 4.6 | 9.3 ± 4.4 | 8.9 ± 3.6 | 0.403 |
| Albumin, g/l ^a | 47.2 ± 2.6 | 46.8 ± 2.6 | 45.0 ± 2.6 | 0.030 |
| Urea, mmol/l ^a | 4.4 ± 0.7 | 4.2 ± 0.9 | 4.4 ± 0.6 | 0.934 |
| Haematocrit, l/l ^a | 0.405 ± 0.036 | 0.390 ± 0.024 | 0.399 ± 0.029 | 0.136 |
| Fibrinogen, g/l ^a | 2.35 ± 0.45 | 2.09 ± 0.49 | 2.40 ± 0.68 | 0.828 |

Key: ^amean ± SD; ^bgeometric mean ± SD

Sex hormone concentrations

In both groups, sex hormone concentrations reflected greater oestradiol concentrations and gonadotrophin suppression with increasing oestrogen dose (see Figure 8.1), and this was accompanied by a reduction in testosterone concentration.

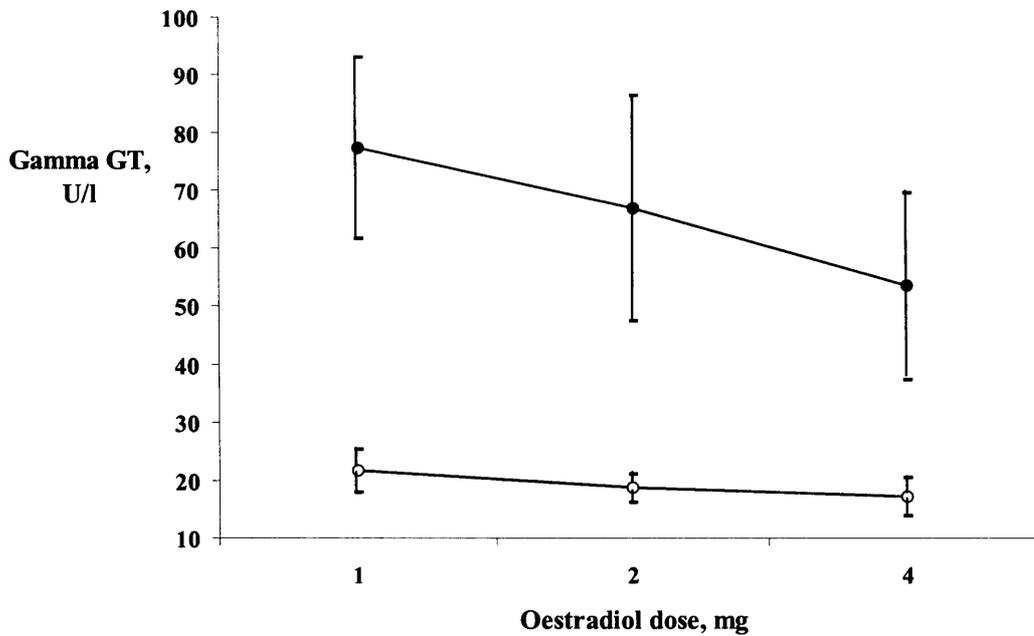
Figure 8.1. Serum Follicle Stimulating Hormone (FSH) in women with TS and 46,XXPA at varying doses of oestradiol. Filled circles represent TS, open circles represent 46,XXPA (mean \pm SE).



Liver Function

Changes in liver function were more marked in the TS group (see Figure 8.2.). They showed a reduction in γ GT and ALT concentrations, with concurrent decreases in bilirubin and albumin concentrations. In 46,XXPA women, there was a small significant reduction in γ GT and albumin. Urea and haematocrit concentrations, recorded as additional markers of fluid status, did not alter in either group, and a similar pattern was seen for fibrinogen concentration.

Figure 8.2. Serum Gamma Glutamyl Transferase (GT) in women with TS and 46,XXPA at varying doses of oestradiol. Filled circles represent TS, open circles represent 46,XXPA (mean \pm SE).



Vascular physiology markers

IMT decreased with increasing oestrogen dose in both groups of women (Table 8.4., Figure 8.3.). FMD, PWV and AIx did not alter significantly, although in the 46,XXPA group there was a slight reduction in GTN-mediated vasodilatation with greater oestrogen dose. There was no difference in baseline brachial diameter, baseline flow or maximum flow increase in either TS or 46,XXPA group at the 1mg and 4mg doses of oestradiol.

Figure 8.3. Intima Media Thickness measurements in TS (black bars) and 46,XXPA women (white bars) with oestrogen deficiency at varying doses of oestradiol (mean \pm SE). Dotted lines show reference range (mean \pm SE) derived from 25 normal control women of similar age (Chapter 5).

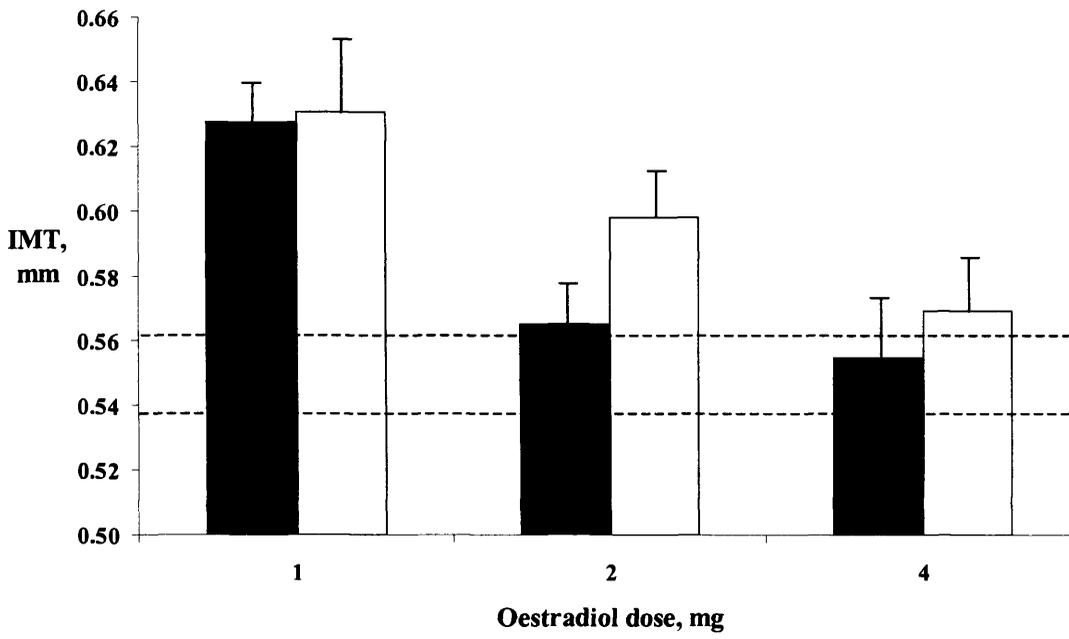


Table 8.4. Variations in vascular physiology markers during oestrogen dose-ranging study in women with TS and 46,XX PA (mean \pm SD)

| Turner Syndrome (n=14) | Dose of oestradiol | | | p-value |
|---------------------------|--------------------|------------------|------------------|---------|
| | 1mg | 2mg | 4mg | |
| IMT, mm | 0.63 \pm 0.05 | 0.57 \pm 0.06 | 0.56 \pm 0.07 | 0.003 |
| FMD % | 7.50 \pm 3.55 | 8.69 \pm 2.42 | 9.14 \pm 3.97 | 0.092 |
| GTN % | 11.31 \pm 5.10 | 9.84 \pm 3.53 | 10.80 \pm 4.54 | 0.645 |
| PWV | 6.3 \pm 1.0 | 6.0 \pm 1.2 | 5.9 \pm 0.9 | 0.065 |
| AIx | 24.5 \pm 8.8 | 23.5 \pm 11.3 | 23.1 \pm 9.9 | 0.401 |
| 46,XX PA | | | | |
| (n=11) | Dose of oestradiol | | | p-value |
| | 1mg | 2mg | 4mg | |
| IMT, mm | 0.63 \pm 0.08 | 0.60 \pm 0.05 | 0.57 \pm 0.05 | 0.038 |
| FMD % | 8.44 \pm 2.84 | 7.48 \pm 2.73 | 8.95 \pm 4.05 | 0.485 |
| GTN % | 11.83 \pm 3.30 | 10.84 \pm 3.81 | 8.84 \pm 2.94 | 0.030 |
| PWV | 6.0 \pm 0.7 | 5.8 \pm 0.7 | 6.1 \pm 0.8 | 0.703 |
| AIx | 10.1 \pm 10.4 | 10.1 \pm 10.8 | 10.6 \pm 11.8 | 0.792 |

8.4. Discussion

This study assessed the effects of varying doses of the same oral oestrogen preparation in young oestrogen-deficient women with TS and 46,XXPA. The most important findings are the reduction of intima media thickness in both groups, accompanied by an increase in HDL concentrations but with no increase in blood pressure or weight. There was also an improvement in liver function which was more marked in women with TS. There was thus no evidence for a greater degree of oestrogen resistance in women with TS compared to women with normal karyotype.

Vascular Physiology parameters

In the general population, a 0.1mm increase in IMT in the common carotid artery has been shown to be associated with an 11% increased risk of anterior myocardial infarction (294). The reductions of 0.07mm and 0.06mm in TS and 46,XXPA women respectively

may therefore be important, particularly considering the absence of a change in blood pressure and the short duration of the study. A dose-dependent improvement has not previously been demonstrated in either postmenopausal or young oestrogen-deficient women. Previous work has shown that IMT improves in postmenopausal women receiving oestrogen therapy (295-297), but the reductions of 0.0017mm per year (295) and 0.012 mm per year (296) described are considerably less than the improvements seen over 6 months in this study. In the HERS sub-study, no difference in IMT was seen between oestrogen-treated and untreated women, although these subjects had established coronary artery disease (360). One explanation for this difference may be that the average age in the postmenopausal studies was around 60 years or more, compared with 32 years in the present study. Our results may therefore indicate that young women with oestrogen deficiency are more sensitive to oestrogen-mediated improvements of IMT than older women.

This finding supports the notion that the antiatherogenic effects of oestrogen in young women confer a net cardioprotective effect compared to the predominant prothrombotic effect of oestrogen in older women. With this in mind it is reassuring that no parallel increase in fibrinogen or blood pressure was found in this study. Furthermore, the reduction in IMT with increasing oestrogen dosing does not appear to be cytokine or inflammation-mediated as no change in IL-6 or CRP was found. Although the mechanism for such a dramatic reduction in IMT is unclear, our observations may, in part, be explained by increased responsiveness of vascular cells to the antiatherogenic effects of oestrogen in earlier developing atherosclerotic lesions compared to more established disease (356).

With regard to endothelial function, no major oestrogen-sensitive effects were noted. FMD, PWV and AIx showed no change with increasing oestrogen dose in the 46,XXPA group, in agreement with an FMD dose-ranging study in postmenopausal women (285). The trends towards an increase in FMD and a decrease in PWV in the TS women, although not significant in this small group, may be physiologically significant. One explanation may be that, whilst FMD is significantly greater in oestrogen-treated women compared to those who are oestrogen deficient as demonstrated in postmenopausal women (285,288), women with premature ovarian failure (291), and women with TS (34), the differences between oestrogen replete groups are less dramatic. It is possible that

larger population samples and perhaps longer time intervals might strengthen these relationships.

Lipids, Metabolism and Blood Pressure

The increase in HDL cholesterol observed in this study is consistent with trends in previous studies in postmenopausal women (399). Markers of glucose homeostasis did not change in the women with TS, but fasting insulin and calculated HOMA-R were reduced with increasing dose in 46,XXPA women. A reduction of fasting insulin and glucose concentrations paradoxically associated with a deterioration in glucose tolerance has been described in TS (33) and postmenopausal women (237) treated with oestrogen as previously discussed in Chapter 6. This phenomenon has been attributed to a glucagon-antagonistic and glucocorticoid-stimulatory effect of oestrogen (238). Although dynamic testing was not performed in our study, the absence of a deleterious effect of increasing oestrogen dose on fasting glucose and insulin concentrations in TS is reassuring.

The absence of an effect of increasing oestrogen dose on blood pressure or measures of obesity is consistent with previous studies in postmenopausal women which showed no significant difference either in oestrogen-treated women compared to controls (204), or in cross-over oestrogen studies (285), where the oestrogen is a form of hormone replacement therapy rather than the oral contraceptive pill. Although the duration of treatment in the present study was relatively short, the absence of a detrimental effect over a 6-month period is nevertheless encouraging.

Sex hormones

Women with TS and 46,XXPA showed similar responses to increasing dose of exogenous oestradiol with regard to increasing serum oestradiol and decreasing gonadotrophin concentrations, although TS women had significantly lower serum oestradiol concentrations than 46,XXPA women on 1mg oestradiol. The changes in testosterone in both groups are interesting. A previous study has shown that untreated women with TS have reduced androstenedione and testosterone concentrations but normal DHEAS concentrations when compared to normal women, and it was suggested that these findings might indicate insufficient extraadrenal and extragonadal conversion of DHEAS to other androgens (400). This group also demonstrated that both free and total testosterone concentrations were reduced by the administration of oestrogen therapy. Our study has

shown that total testosterone concentrations were lower in women with TS than those with 46,XXPA, but both were reduced with increasing doses of oestradiol. This may be related to an increase in SHBG or to an inhibitory effect of 17β -oestradiol on conversion of DHEAS by 3β -hydroxysteroid dehydrogenase (400).

Liver Function

The raised liver enzymes commonly found in women with TS are known to be suppressed by exogenous oestrogen (36,37). A lesser suppression of liver enzymes has been shown in normal postmenopausal women given a variety of oestrogen preparations (401). The use of oestrogen therapy in women with liver disease in the general population has traditionally been discouraged because of concerns about cholestasis, but this view was mainly derived from early data of the oral contraceptive pill using high doses of ethinyloestradiol (254). More recently, hormone replacement therapy using other forms of oestrogen such as oestradiol, especially by the transdermal route, have been found to be relatively safe, even in women with liver disease (402).

The significance of elevated liver enzymes in TS has been unclear and significant liver disease was thought to be rare (72). A recently published series has highlighted the possibility of severe liver disease, even requiring transplantation, developing in women with TS. In this study, it is interesting to note that the most severe architectural changes were noted particularly in women who had never received oestrogen therapy (74). Taken together, the present study and those of Guttman *et al.* (37) and Roulot *et al.* (74) are consistent in suggesting that exogenous oestrogen may be protective against progressive liver disease in women with TS.

Fibrinogen, which is also produced by the liver, is a useful marker of the haemostatic process which contributes to inflammation, being part of the final common pathway of the coagulation cascade, although the anticoagulant and fibrinolytic systems also come into play. Neither TS nor 46,XXPA women in this study demonstrated significant changes in the concentration of fibrinogen. Data from previous studies have shown inconsistent effects of exogenous oestrogen replacement on plasma fibrinogen (403-406). Whilst there are obviously other contributors to haemostasis and coagulation, it may be somewhat reassuring that, in the context of the other benefits observed with increasing dose of

oestrogen, there was no evidence of an increased procoagulant state, at least with regard to fibrinogen.

In conclusion, this study has shown that women with TS and 46,XXPA are similarly sensitive to increasing dose of oral oestradiol therapy with regard to IMT, a marker of cardiovascular risk. This was in the absence of changes in other markers of cardiovascular risk, such as blood pressure, waist-hip ratio, glucose homeostasis, adipokines, pro-inflammatory cytokines, and fibrinogen, albeit with a small increase in HDL cholesterol concentrations. Elevated liver enzymes are also sensitive to increasing dose of oestradiol, and demonstrate a dose-dependent improvement.

In the light of recent evidence in postmenopausal women (201,204,213), there has been a tendency to err on the side of caution and treat young and older oestrogen-deficient women with the minimum oestrogen dose tolerated. Some young women with TS or 46,XXPA undoubtedly feel symptomatically better on higher doses of oestradiol, and it must be remembered that oestrogen therapy in these cases is replacing the natural premenopausal oestrogen-replete state rather than extending years of oestrogen exposure beyond their 'normal menopause' in older women. This study suggests that greater dosage of oestradiol in young women, particularly those with TS, may be beneficial with regard to several organ systems.

Chapter 9

General Discussion, Conclusions and Future Directions

This is the first large study to characterise various aspects of the cardiovascular risk in adults with Turner Syndrome. These have included structural cardiovascular disease which may be both congenital and acquired, atherosclerosis, metabolic factors and obesity. The effects of the widely used treatments oestrogen and growth hormone have been assessed, both as they relate to historic use and more specifically in an oestrogen dose-ranging study. Furthermore, insights have been gained into liver disease in TS, both through its association with obesity and the effects of oestrogen.

Structural Cardiovascular Disease

It has been shown that both echocardiography and magnetic resonance imaging are required in order to effectively screen for aortic root dilatation, bicuspid aortic valve and coarctation, and that the two techniques provide complementary information. It has been demonstrated that height correction is a useful means of correcting for the short stature of TS, and reference ranges have been produced for both echocardiographic and MRI measurements of aortic root diameter in order to aid future interpretation. The longitudinal echocardiography study has confirmed that a screening interval of three to five years is probably appropriate, at least in TS women whose aortic root does not exceed 3.6cm at the first scan. It is to be hoped that the cohort in this longitudinal study can continue to be followed for many years to come, so that these preliminary findings can be extended. Furthermore, a refined echocardiography protocol involving more detailed measurements of aortic dimensions at different levels of the ascending aorta, may be useful to gain greater understanding of the pattern of aortic dilatation in a follow-up series.

Although women with TS had raised blood pressures, the present study found no direct association between blood pressure and aortic diameters. Nevertheless, the obvious analogies with aortic dilatation in Marfan Syndrome (342,407) suggest that a prophylactic beta-adrenergic blockade blood pressure intervention trial would be useful in TS.

The vascular physiology study has demonstrated that the dilatation previously noted in the aorta on echocardiography and MRI is also found in other conduit vessels, notably the common carotid and brachial arteries, suggesting that there may be a fundamental arterial wall defect throughout the vascular tree in TS. The absence of this dilatation in other young oestrogen deficient women with normal karyotype suggests that oestrogen deficiency is not the cause of this abnormality. Furthermore, the similarity of IMT but disparity of arterial diameters between the TS and 46,XXPA women suggests that a simple relationship resulting from arterial remodelling does not provide the explanation. The implications of this apparently generalised conduit artery dilatation now require clarification. The diameter of more distal arteries such as the retinal arteries could also be analysed to determine the degree to which arterial dilatation is a generalised phenomenon in TS. A study of arterial distensibility and resistance artery flow may help to explain the hypertension of TS in the context of such conduit artery dilatation.

The findings of this study show that important 'occult' coarctation is present in a significant number of TS women. It is not known, however, how these imaging findings relate to clinical risk. Since no significant associations were found between resting blood pressure and coarctation site abnormalities, an exercise blood pressure study would be useful to investigate this further. In addition, the use of imaging techniques can be refined to quantify the flow disturbance on both echocardiography (using Doppler techniques) and MRI (using flow quantification techniques).

Bicuspid aortic valve has been shown to be an important risk factor for both aortic root dilatation and coarctation site abnormalities. Clinicians attending to TS women should be made aware of this, and clinic protocols should highlight the need for particular attention in the surveillance of women with a bicuspid AV.

Atherosclerosis

Women with TS have been shown to have greater IMT than normal control women, but not compared to a similarly oestrogen-deficient young cohort of karyotypically normal women, indicating that this aspect of the vasculopathy of TS may be related to oestrogen deficiency. Greater strength is given to this hypothesis in that the intima-media thickening in both TS and 46,XX oestrogen-deficient women responds to increasing doses

of oestrogen in a dose-dependent manner. It is, however, interesting to note that systemic markers of inflammation, which are elevated in women with TS, do not appear to have a significant relationship with IMT. In fact, CRP tended to rise with oestrogen use. This suggests that local oestrogen-sensitive factors in the arterial wall may contribute to intimal thickening, independent of systemic markers of inflammation. Investigation of other markers relevant to vascular risk which reflect activity and integrity of the extracellular matrix, such as matrix metalloproteinases and cellular adhesion molecules, in future studies may shed some light on this apparent paradox. The role of oestrogen will be considered further below.

Since blood pressure, another possible therapeutic target, has also been shown to be an independent positive association of IMT in women with TS, the need for a blood pressure intervention study in this population is further strengthened. Although intuition might dictate that aggressive antihypertensive treatment would be beneficial, it is difficult to justify what is likely to be lifelong therapy in such a young population without strong evidence.

It is interesting that flow-mediated dilatation, a marker of endothelial function, did not differ significantly between the TS, 46,XXPA and normal control women, nor indeed did it improve significantly in either TS or 46,XXPA women during the oestrogen dose-ranging study. Possible explanations may be that the differences between an oestrogen-deficient state and oestrogen repletion are greater than those between different oestrogen-treated women, as also noted in postmenopausal women (285). A study of larger power may help to clarify these issues further, but the data presented here do not confirm FMD to be an important differentiating marker of cardiovascular risk in oestrogen-treated women.

Metabolic factors and Obesity

With regard to metabolic aspects of the cardiovascular risk in TS, this study has compared markers of obesity and cardiovascular risk in oestrogen-treated women with TS and 46,XX and untreated normal controls. Furthermore, the obesity of TS has been characterised using MRI and bioelectrical impedance techniques. Women with TS have various features consistent with the insulin resistance/ metabolic syndrome. These

include their central obesity, confirmed in this study to be an excess of visceral and intrahepatocellular adipose tissue, along with hypertriglyceridaemia, hypertension and elevated CRP and IL-6 concentrations. However, these occur in the context of low fasting serum insulin, glucose and leptin. This study has shown that the hypertriglyceridaemia but not the hypertension may be oestrogen-related, in view of the comparisons to 46,XX women similarly treated with oestrogen. The apparent features of the metabolic syndrome are, however, not fully explained by the obesity or oestrogen deficiency, nor indeed oestrogen treatment, and one or more TS-specific metabolic defects may explain this paradox.

The fat distribution study has shown that women with TS do have relatively, but not absolutely less subcutaneous adipose tissue than normal controls, thus not adequately explaining the lower leptin concentrations. An insulin secretory defect has been previously proposed, and this may explain the low leptin concentrations in TS, whilst the obesity in turn could be related to inhibited satiety mechanisms. The elevated CRP and IL-6 concentrations may be accounted for by the increased visceral obesity in TS which has been demonstrated in the MRI fat distribution study, and an abnormality of visceral adipose function or deposition could also account for the absence of hyperinsulinaemia, although the mechanism has yet to be elucidated. Assessment of other markers of adipose function may be useful, for instance, adiponectin which may help to clarify these relationships further (408,409). Adipose tissue biopsies would allow further characterisation of the distribution and possible dysregulation of adipokine production from visceral and subcutaneous fat depots through in vitro studies.

Given that this study has shown average fasting serum glucose to be low in TS women, but previous work has suggested an insulin secretory defect with glucose intolerance, this suggests that fasting glucose measurements are a poor screening tool for assessing glucose homeostasis in TS. It should therefore be recommended that clinic protocols be amended so that all women undergo oral glucose tolerance testing as part of their cardiovascular risk surveillance.

Effects of Oestrogen Treatment

This study has assessed the effects of oestrogen therapy on aspects of cardiovascular risk

in various ways. First, the history of cumulative years of oestrogen deficiency and oestrogen start age were recorded and correlated with parameters of cardiovascular risk being studied. Second, women with TS were compared to similarly oestrogen-treated women with 46,XXOD in cross-sectional studies to assess the influence of the oestrogen therapy in relation to normal controls, and to control for the oestrogen deficiency and replacement in order to elucidate the genetic influence. Finally, the effect of oestrogen was studied in the dose-ranging study of TS and 46,XXPA women.

History of oestrogen therapy appeared to have no demonstrable impact on present aortic, carotid or brachial artery diameter or on the rate of aortic dilatation. Similarly, no association was demonstrated between history of oestrogen deficiency and carotid artery IMT, or other indices of vascular physiology such as FMD and aortic stiffness. There was also no association between serum markers of obesity and oestrogen deficiency, but it is interesting to note that hepatocellular adipose tissue was greater with increased oestrogen deficient years. This is consistent with previous work which has suggested that abnormal liver function in TS may be related to oestrogen deficiency (37,74).

The comparisons of TS women with 46,XXOD women in chapters 5 and 6 show that the greater conduit vessel diameter noted in TS does not appear to be related to oestrogen deficiency or treatment, since the latter group were similarly oestrogen treated but more oestrogen deficient than TS women. IMT, however, was similar in the two groups of oestrogen deficient women and greater than in normal controls, thus suggesting that oestrogen is an important factor determining IMT. It is interesting that this relationship was not confirmed by an association between oestrogen-deficient years and IMT in the TS group, but this discrepancy may be explained by the fact that 'cumulative oestrogen deficient years' is only a calculated estimate of true oestrogen deficiency. No evidence of an oestrogen association with parameters of aortic stiffness was detected.

Neither obesity nor hypertension in TS can be accounted for by oestrogen therapy. Triglyceride concentrations were greater in both oestrogen treated groups than in normal controls, supporting a role for oestrogen therapy as suggested by previous work in postmenopausal women (217). Oestrogen did not appear to explain the low fasting glucose, insulin and leptin concentrations in TS, however, nor the increased CRP and IL-6 concentrations.

The longitudinal oestrogen dose-ranging study demonstrated that HDL cholesterol concentrations increased in a dose-dependent manner in both TS and 46,XXPA women, whilst blood pressure and weight remained unchanged. Sex hormones were increased with a concomitant reduction in gonadotrophins in both groups, and liver enzyme concentrations were reduced in both groups. The magnitude was, however, greater in TS women. The baseline comparison of liver function in the two groups suggests that, although oestrogen deficiency may contribute to abnormalities of liver function, other factors must also be involved to explain the discrepancy between similarly treated TS and 46,XXPA women.

As already alluded to, perhaps the most important finding of the longitudinal oestrogen study was the dose-dependent improvement in IMT, with a suggestion that this can be almost normalised within six months of therapy. The fact that the magnitude of the reduction appeared to be greater than in previous work on postmenopausal women indicates that the scope for improvement, perhaps into the normal range, in young women with oestrogen deficiency may be greater than in postmenopausal women, in whom the atherosclerotic process may already have caused greater arterial wall damage. This study has not fully investigated possible prothrombotic effects of increasing oestrogen dose, but the absence of an increase in fibrinogen is reassuring. Future work should now focus on including a group of postmenopausal 46,XX women for direct comparison to the other groups, investigating the timescale of the oestrogen-replacement effect on IMT, and gaining further insight into the prothrombotic effects of oestrogen dose-ranging in young women. A greater understanding of the functional effects of reduced IMT may be gained by a study of arterial distensibility at increasing oestrogen doses. In the longer term, other aspects of risk and benefit at differing doses of oestrogen in young women should be addressed, including the risk of breast cancer.

Effects of Growth Hormone Treatment

This study has assessed the impact of previous growth hormone therapy on each parameter of cardiovascular risk studied. It is of interest that no aspect of arterial structure or function studied, nor indeed any metabolic parameter was found to be associated with previous growth hormone use. Clearly this was not an interventional

study, but the findings are nevertheless important in that they do not indicate a role for future cardiovascular benefit in TS girls receiving the treatment, and also do not raise concerns about a negative longterm impact of growth hormone, particularly with regard to metabolic parameters.

Difficulties arising in this study

A theme throughout this study has been the difficulty of finding appropriate control groups. In order to control both for their genetic differences and the oestrogen deficiency, women with TS were compared to both normal women and to 46,XX oestrogen deficient women. The situation is more complex than this, however. Women with TS are of shorter stature, and also generally more obese than women of similar age in the normal population. Women with 46,XX oestrogen deficiency, by contrast, tend to be of greater stature than normal controls.

Finding a normal control group at the lower end of the normal range for stature, with women who were also of similar BMI and age, not on the oral contraceptive pill, and of similar smoking and exercise status, proved to be extremely difficult. It was accepted that it would not be possible to truly match for height. Adjustment for height appeared to be the most useful means of interpreting the data, particularly with respect to anthropomorphically dependent variables such as arterial diameters and analysis of body fat distribution.

Another methodological problem in this study was the fact that many of the subjects lived at considerable distance from the study centre. Thus, in the larger cross-sectional studies, despite considerable efforts of coordination, investigations could not always be uniformly timed at the optimal phase of the cycle, accounting for a certain degree of heterogeneity in cycle stage. Data were analysed both by applying strict cycle stage criteria, and also using the whole cohort in order to increase statistical power as described. Since there was no significant difference between the analyses in the cross-sectional studies, the inclusive data were used as indicated.

Conclusion

In conclusion, the findings of this study should contribute to a greater understanding of cardiovascular risk factors in women with Turner Syndrome. The advantages and disadvantages of echocardiography and magnetic resonance imaging in the screening of structural heart disease have been discussed and TS-specific reference ranges for aortic diameters have been produced. The concept of a 'vasculopathy' in TS has been introduced with the finding that arterial dilatation is not confined to the heart, but also occurs in other vessels such as the brachial and carotid arteries. Metabolic insights have been gained, demonstrating that women with TS do have increased visceral adipose tissue, in spite of the fact that the full spectrum of features associated with the metabolic syndrome is not present. This lends further weight to the hypothesis that women with TS have specific metabolic defects which may account for this discrepancy. This study has gone some way towards elucidating the contributions of the genetic effect and the influence of oestrogen deficiency on the anomalies observed by the comparisons not only to normal control women, but also to oestrogen-deficient women of normal karyotype. Finally, a beneficial effect of increasing oestrogen dose has been demonstrated in women with TS and 46,XX oestrogen-deficient controls, both with respect to arterial IMT, and also liver dysfunction.

It is to be hoped that these advances will now be developed further and will translate into improved recognition and surveillance of problems specific to women with Turner Syndrome in the many newly emerging adult TS clinics.

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Appendix 1

Table 1. Distribution of patient characteristics in subpopulations of the total cohort of women with Turner Syndrome

| | Total cohort (n=173) | Chapter 3 (n=128) | Chapter 4 (n=33) | Chapter 5 (n=93) | Chapter 6 (n=117) | Chapter 7 (n=6) | Chapter 8 (n=14) |
|--|-------------------------|----------------------|---------------------|---------------------|----------------------|--------------------|---------------------|
| Karyotype (%) | | | | | | | |
| Monosomy X (45,X) | 50 | 49 | 61 | 49 | 50 | 33 | 29 |
| Isochromosome X | 22 | 23 | 27 | 29 | 25 | 33 | 43 |
| Partial X deletion | 3 | 2 | 0 | 2 | 2 | 0 | 7 |
| Ring X | 8 | 10 | 6 | 9 | 8 | 2 | 0 |
| Any Y fragment | 6 | 6 | 3 | 4 | 6 | 0 | 14 |
| Mosaic 45,X/46,XX | 8 | 6 | 0 | 4 | 7 | 0 | 7 |
| Complex | 3 | 4 | 3 | 3 | 3 | 2 | 0 |
| Oestrogen start age, median (range) years | | 14 (5-47) | 14 (8-34) | 14 (5-25) | 14 (5-34) | 14 (11-19) | 15 (10-19) |
| Oestrogen-deficient median (range) years | | 3 (0-41) | 3 (0-22) | 3 (0-27) | 3 (0-22) | 2 (0-8) | 3.5 (0-8) |
| % treated with Growth Hormone | | 31 | 15 | 30 | 35 | 0 | 14 |
| Smoking history, % | | | | | | | |
| Never smoked | | 85 | 76 | 85 | 85 | 83 | 79 |
| Ex-smokers | | 7 | 10 | 5 | 5 | 0 | 7 |
| Current smokers | | 8 | 14 | 10 | 10 | 17 | 14 |
| Proportion taking Exercise, % | | | | | | | |
| No exercise | | 60 | 55 | 58 | 60 | 50 | 64 |
| Mild exercise | | 26 | 21 | 27 | 25 | 33 | 7 |
| Moderate exercise | | 10 | 24 | 10 | 10 | 17 | 21 |
| Vigorous exercise | | 4 | 0 | 5 | 5 | 0 | 7 |

Appendix 2

Attribution of Work

The research reported in this study was based in the Department of Endocrinology, Middlesex Hospital (UCLH), London.

The author was involved with the application for funding and coordinated the entire study, arranged patient visits, took histories and consent, performed clinical examinations, took blood samples, and prepared these samples in the laboratory for freezing. The author accompanied patients and controls to all aspects of the study, including echocardiography at the Middlesex Hospital, MRI of the aorta at the Middlesex Hospital and Medical Alliance Imaging Centre, London (Chapters 3 and 4), Vascular Physiology at the Vascular Physiology Laboratory, Great Ormond Street (Chapter 5) and adipose tissue MRI at the Hammersmith Hospital, London (Chapter 7). The author was responsible for administering the oestrogen treatment to patients in Chapter 8. All data collation and statistical analysis were performed by the author.

Dr Gerard Conway, Consultant Endocrinologist and Honorary Senior Lecturer at UCLH, supervised this thesis and was closely involved throughout. He conceived the original idea for the thesis and was in charge of the funding applications to the British Heart Foundation and the Turner Syndrome Support Society.

Carolyn McCarthy, Echocardiographer, performed echocardiograms and Dr Jocelyn Brookes, Consultant Radiologist, UCLH, analysed the aortic MRIs. Ann Donald and Clare Storry, Vascular Technologists, Institute of Child Health, UCL, performed the vascular physiology studies and provided the data for analysis. Dr Julian Halcox, Senior Lecturer and Honorary Consultant in Cardiology, UCLH, and Ann Donald were involved with interpretation of the vascular physiology data.

Dr M Javad Hosseinzadeh Attar performed the serum analyses for C-reactive protein, Interleukin-6 and leptin, and Dr Vidya Mohammed-Ali was involved with the interpretation of these results. Nadia Payne and Emma West helped to centrifuge and

aliquot the samples, and Christine West and Cherie Nelson helped to collect blood samples. All other blood analysis was performed by the Biochemistry and Haematology laboratories of UCLH.

Dr Louise Thomas, Dr Jimmy Bell and Dr Gavin Hamilton performed and interpreted the adipose tissue MRIs at the Hammersmith Hospital.

Appendix 3

Papers published from findings reported in this thesis

(copies appended)

1. JE Ostberg, JAS Brookes, C McCarthy, J Halcox, GS Conway
A Comparison of Echocardiography and Magnetic Resonance Imaging in Cardiovascular Screening of Adults with Turner Syndrome
Journal of Clinical Endocrinology and Metabolism 2004; 89(12):5966-71
2. JE Ostberg, EL Thomas, G Hamilton, MJ Hosseinzadeh Attar, JD Bell, GS Conway
Excess Visceral and Hepatic Adipose Tissue in Turner Syndrome determined by Magnetic Resonance Imaging: Oestrogen Deficiency associated with Hepatic Adipose Content
Journal of Clinical Endocrinology and Metabolism 2005; 90(5):2631-2635
3. JE Ostberg, MJ Hosseinzadeh Attar, V Mohammed, GS Conway
Adipokine dysregulation in Turner Syndrome: comparison of circulating interleukin-6 and leptin concentrations with measures of adiposity and C-reactive protein
Journal of Clinical Endocrinology and Metabolism 2005; 90(5):2948-2953
4. JE Ostberg, JPJ Halcox, AE Donald, C Storry, C McCarthy, GS Conway
Vasculopathy in Turner Syndrome: Arterial Dilatation and Intimal Thickening without Endothelial Dysfunction
Journal of Clinical Endocrinology and Metabolism 2005; 90(9): 5161-5166

Scientific Abstracts presented at International Meetings

1. J Ostberg, A Sahdev, J Brookes, GS Conway
Surveillance of the aorta in women with Turner's Syndrome – a comparison of Echocardiography and Magnetic Resonance Imaging
Poster presented at the British Endocrine Society Meeting, Birmingham, March 2000;
Abstract in *Journal of Endocrinology Abstract Supplement* Vol. 164, March 2000: P21
2. J Ostberg and GS Conway
A Comparison of Echocardiography and Magnetic Resonance Imaging of the Aorta in Women with Turner's Syndrome
Oral Presentation and poster at the 5th International Turner Symposium, Naples, Italy, March 2000;
Abstract in *Abstracts* for the 5th International Turner Symposium, Naples, Italy, March 2000: B1
3. JE Ostberg and GS Conway
Accelerated Aortic Root Dilatation in Turner Syndrome
Poster presented at the British Endocrine Society Meeting, Harrogate, April 2002;
Abstract in *Endocrine Abstracts* Vol. 3, 21st Joint Meeting of the BES, April 2002: P171
4. JE Ostberg, J Brookes, and GS Conway
Optimisation of Aortic Imaging in Adults with Turner Syndrome
Poster presented at The Endocrine Society's 84th Annual Meeting, San Francisco, USA, June 2002;
Abstract in *Program and Abstracts, ENDO 2002*: P3-47
5. JE Ostberg, JAS Brookes, C McCarthy, GS Conway
Classification of the Spectrum of Aortic Dymorphology in Adults with Turner Syndrome
Oral Presentation at the British Endocrine Society Meeting, Glasgow, March 2003;
Abstract in *Endocrine Abstracts* Vol. 5, 22nd Joint Meeting of the BES, March 2003: OC8
6. JE Ostberg, JAS Brookes, GS Conway
High Prevalence of Aortic Dymorphology on Magnetic Resonance Imaging in Adults with Turner Syndrome – a Cross-Sectional Analysis of 104 Women
Poster and conference television broadcast presented at The Endocrine Society's 85th Annual Meeting, Philadelphia, USA, June 2003;
Abstract in *Program and Abstracts, ENDO 2003*: P2-221
7. Julia E Ostberg, Ann E Donald, Clare Storry, Natalie Lloyd, Carolyn McCarthy, Julian Halcox, Gerard S Conway
Bicuspid Aortic Valve is associated with increased arterial stiffness in adult women with Turner Syndrome
Oral Presentation at the Society for Endocrinology 194th Meeting, London, November 2003 (short-listed for Clinical Oral Communication Prize);

- Poster Presentation at the Society for Endocrinology 194th Meeting joint Endocrinology and Diabetes Day, London, November 2003;
Abstract in *Endocrine Abstracts*, Vol. 6, 194th Meeting of the Society for Endocrinology joint Endocrinology and Diabetes Day in association with Diabetes UK, November 2003: OC1 and DP31
8. JE Ostberg, J Attar, V Mohamed-Ali, GS Conway
Diabetes Risk in Turner Syndrome – a unique metabolic defect?
Poster Presentation at the British Endocrine Society Meeting, Brighton, March 2004
Abstract in *Endocrine Abstracts* Vol. 7, 23rd Joint Meeting of the BES, March 2004: P69
9. JE Ostberg, J Attar, V Mohamed-Ali, GS Conway
Glucose Homeostasis in Turner Syndrome – a unique metabolic defect?
Poster presented at The Endocrine Society's 86th Annual Meeting, New Orleans, USA, June 2004;
Abstract in *Program and Abstracts, ENDO 2004*: P2-368
10. JE Ostberg, AE Donald, C Storry, C McCarthy, JP Halcox, GS Conway
Vasculopathy in Turner Syndrome: Arterial Dilatation and Intimal Thickening without Endothelial Dysfunction
Poster Presentation at the Society for Endocrinology 195th Meeting and Joint Endocrinology and Diabetes Day, London, November 2004;
Abstract in *Endocrine Abstracts*, Vol. 8, 195th Meeting of the Society for Endocrinology Meeting and Joint Endocrinology and Diabetes Day in association with Diabetes UK, November 2004: P36 and DP16.
11. JE Ostberg, C Storry, AE Donald, JP Halcox, GS Conway
Increasing oestrogen dose reduces intima media thickness in women with Turner Syndrome
Poster Presentation at the Society for Endocrinology 195th Meeting and Joint Endocrinology and Diabetes Day, London, November 2004;
Abstract in *Endocrine Abstracts*, Vol. 8, 195th Meeting of the Society for Endocrinology Meeting and Joint Endocrinology and Diabetes Day in association with Diabetes UK, November 2004: P37 and DP17.
12. JE Ostberg, AE Donald, JP Halcox, C Storry, C McCarthy, GS Conway
Characterisation of Vasculopathy in Turner Syndrome: Arterial Dilatation and Intimal Thickening without Endothelial Dysfunction
Poster presented at The Endocrine Society's 87th Annual Meeting, San Diego, USA, June 2005;
Abstract in *Program and Abstracts, ENDO 2005*: P129-11
13. JE Ostberg, C Storry, AE Donald, JP Halcox, GS Conway
Increased Intima Medial Thickness in women with Turner Syndrome is normalised by oestrogen – a dose-response study
Poster presented at The Endocrine Society's 87th Annual Meeting, San Diego, USA, June 2005;
Abstract in *Program and Abstracts, ENDO 2005*: P129-9

Collaborative work during the time of study of the thesis but not included in the thesis

Scientific Papers

1. JE Ostberg, A Beckman, B Cadge, GS Conway
Oestrogen Deficiency and Growth Hormone Treatment in Childhood are not associated with Hearing in Adults with Turner Syndrome
Hormone Research 2004; 62(4): 182-186
2. JE Ostberg, T Damjanovic, N Dimkovic, D Byrne, DP Mikhailidis, GM Prelevic
Effect of Tibolone on Markers of Cardiovascular Risk in Post-Menopausal Women on Haemodialysis – A Pilot Study
Fertility and Sterility 2004; 81(6): 1624-1631

Abstracts presented at International Meetings

1. JE Ostberg, A Beckman, B Cadge, GS Conway
Does Growth Hormone Reduce Sensorineural Deafness in Adults with Turner Syndrome?
Oral Presentation at the Society for Endocrinology 193rd Meeting, London, November 2002 (short-listed for Clinical Oral Communication Prize);
Poster Presentation at the Society for Endocrinology 193rd Meeting joint Endocrinology and Diabetes Day, London, November 2002;
Abstract in *Endocrine Abstracts*, Vol. 4, 193rd Meeting of the Society for Endocrinology joint Endocrinology and Diabetes Day in association with Diabetes UK, November 2002: OC7 and DP10
2. JE Ostberg, GS Conway
Screening for Iron Deficiency and Anaemia in Adults with Turner Syndrome
Poster presented at the British Endocrine Society Meeting, Glasgow, March 2003;
Abstract in *Endocrine Abstracts* Vol. 5, 22nd Joint Meeting of the BES, March 2003: P201
3. JE Ostberg, T Damjanovic, N Dimkovic, D Byrne, DP Mikhailidis, GM Prelevic
Effect of Tibolone on Markers of Cardiovascular Risk in Post-Menopausal Women on Haemodialysis – A Pilot Study
Poster presented at The Endocrine Society's 85th Annual Meeting, Philadelphia, USA, June 2003;
Abstract in *Program and Abstracts, ENDO 2003*: P2-212