UNIVERSITY OF LIVERPOOL

Understanding the cardiovascular risk profile in paediatric patients with Turner Syndrome

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Master in Philosophy by Lily Rose Jones

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Abstract

Introduction

Turner Syndrome (TS) is a chromosomal disorder associated with an increased risk of cardiovascular disease and metabolic ill health. Patients exposed to glucocorticoid excess have a number of cardiovascular risk factors common in TS, however there are very few data describing cortisol concentrations in patients with TS.

Aims

To investigate the acceptability of a study protocol that describes the circadian profile of salivary cortisol and cortisone in girls with TS, compare these measurements to profiles obtained from healthy children and adolescents, and describe associations between salivary cortisol and cortisone and markers of evolving cardiovascular risk.

Methods

Patients were introduced to the study during routine appointments at Alder Hey Children's Hospital. The study consisted of two visits involving the following activities: venepuncture, body composition measurement, vascular scanning, continuous glucose monitoring (CGM), ambulatory blood pressure monitoring (ABPM), saliva sample collection and Likert-style acceptability questionnaires. From study questionnaires, a mean score above five defined acceptability. Experimental data was compared to matched healthy control subjects or reference intervals.

Results

20 patients were identified and provided with information about the study, and 11 (18.2% 45,X0) consented to participate (mean age 13.8 ± 2.4 years). All study activities were rated as acceptable. Not all participants completed all aspects of the protocol. Participants' blood tests results revealed: five (45.5%) proatherogenic lipid profiles, three (27.3%) Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) >3, seven (63.6%) raised clotting and/or inflammatory markers, six (54.5%) increased leptin concentrations. The correlation between serum leptin and percentage body fat mass (%BFM) was statistically significant (+0.905). Body composition data showed six participants (54.5%) had a body mass index (BMI) > +1 SDS and seven (63.6%) a muscle-to-fat ratio (MFR) <0.80.

Ambulatory hypertension was observed in three participants (37.5%). No participant had a physiological BP circadian rhythm. One participant (10.0%) had an abnormal glucose profile. Late-night salivary cortisol was detectable in three out of five participants. The mean total area under the curve for salivary cortisol was statistically significantly greater in STARRY study participants (mean 49.7 nmol/L; 95%CI [18.3-81.1 nmol/L]) compared to the matched control group (42.3 nmol/L; 95%CI [16.6-67.9 nmol/L]; *p*=0.005). The cortisol circadian rhythm of STARRY study participants appeared non-physiological.

Conclusion

This thesis supports the existing literature, demonstrating an adverse cardiovascular risk profile in paediatric patients with TS. Compared to healthy controls, participants also demonstrated a disrupted salivary cortisol circadian rhythm and increased cortisol exposure. Further adequately powered studies are required to validate these novel cortisol findings and investigate the impact on the cardiovascular profile of paediatric patients with TS. Our acceptability and feasibility data give some confidence that a larger, multicentre study is likely to have an acceptable recruitment rate of 50% or more.

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Outputs arising from this thesis

Publications

Lily Jones, Joanne Blair, Daniel B. Hawcutt, *et al*. Hypertension in Turner Syndrome: A review of proposed mechanisms, management and new directions. Rebuttal submitted to Journal of Hypertension on 17th May 2022.

Lily Jones, Julie Park, Joanne Blair, *et al*. 20 Years on – The measurement of blood pressure and detection of hypertension in children and adolescents: A national descriptive survey. Submitted to Archives of Disease in Childhood on 2nd May 2022.

Presentations

Lily Jones, Julie Park, Joanne Blair, *et al*. Poster presentation at the Royal College of Paediatrics and Child Health (RCPCH) Conference (June 2022), British Society of Paediatric Endocrinology and Diabetes Specialty. Challenges in identification of hypertension in children and young people with Turner Syndrome

Lily Jones, Julie Park, Joanne Blair, *et al*. Poster presentation at the Royal College of Paediatrics and Child Health (RCPCH) Conference (June 2022), British Association of General Paediatrics Specialty. Measuring blood pressure in children and adolescents: 20 years of change

Lily Jones, Julie Park, Joanne Blair, *et al.* Poster presentation at the British and Irish Hypertension Society Conference (September 2022). Identifying adolescents with Turner Syndrome at risk of poor cardiovascular outcomes: a pilot study

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List of Abbreviations

11βHSD	11β-hydroxysteroid dehydrogenase
AASI	Ambulatory arterial stiffness index
ACT	Activity
ACTH	Adrenocorticotropic hormone
ADHD	Attention deficit hyperactivity disorder
Ag	Antigen
ALP	Alkaline phosphatase
ANXA1	Annexin A1
AS	Dr Shantsila
ASD	Autism spectrum disorder
AUC	Area under the curve
BAV	Bicuspid aortic valve
BFM	Body fat mass
BK	Voltage-activated potassium channels
BMI	Body mass index
BP	Blood pressure
bpm	Beats per minute
CBG	Corticosteroid-binding globulin
CGM	Continuous glucose monitor
cIMT	Carotid intima-media thickness
cm	Centimetres
CMR	Cardiac magnetic resonance
CoA	Coarctation of the aorta
COCP	Combined oral contraceptive pill
CRF	Clinical Research Facility
CRH	Corticotrophin-releasing hormone
CT	Computerised tomography
CV	Coefficient of variation
CVD	Cardiovascular disease
del	Deletion of chromosome
DH	Dr Hawcutt
ECG	Electrocardiogram

ESR	Erythrocyte sedimentation rate
FMD	Flow-mediated dilatation
FPG	Fasting plasma glucose
FVIII	Factor VIII
G:I	Glucose to insulin ratio
g/L	Grams per litre
GAPR-UKI	General and Adolescent Paediatric Research in the United Kingdom & Ireland
GL	Professor Lip
GR	Glucocorticoid receptor
HDL-C	High-density lipoprotein cholesterol
hGH	Human growth hormone
HOMA-IR	Homeostatic model assessment for insulin resistance
HPA	Hypothalamic-pituitary-adrenal
Hrs	Hours
HRT	Hormone replacement therapy
i	Isochromosome
IBD	Inflammatory bowel disease
ID	Identifier
IDF	International Diabetes Federation
idic	Isodiscentric chromosome
IQR	Interquartile range
IR	Insulin resistance
JB	Professor Blair
JP	Dr Park
LDL-C	Low-density lipoprotein cholesterol
LJ	Miss Jones
MFR	Muscle-to-fat ratio
mmHg	Millimetre of mercury
mmol/L	Millimoles per litre
MR	Mineralocorticoid receptor
n	Number
NIHR	National Institute for Health and Care Research
nmol/L	Nanomoles per litre
NVLD	Non-verbal learning disability
p	Short arm of chromosome

POMC	Proopiomelanocortin
PP	Pulse pressure
PRA	Plasma renin activity
q	Long arm of chromosome
QoL	Quality of life
QTc	Corrected QT interval
r	Ring chromosome
REC	Research Ethics Committee
SD	Mr Dliso
SDS	Standard deviation score
SGA	Small-for-gestational age
SHOX	Short stature homeobox
SMMa	Appendicular skeletal muscle mass
SPSS	Statistical Package for the Social Science
StAR	Steroidogenic acute regulatory protein
STARRY	a pilot study of Salivary corTisol and cortisone profiles and associations with cArdiovascular Risk profile in paediatric patients with tuRner sYndrome
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TG	Triglyceride
TS	Turner syndrome
US	United States of America
vWF	Von Willebrand Factor
WC	Waist circumference
WHO	World Health Organisation
WTH	Waist-to-hip
X	X Chromosome

1. Introduction

1.1. Background

Turner Syndrome (TS) is a chromosomal disorder associated with an increased risk of cardiovascular disease and metabolic ill health. The introductory chapter will discuss TS in detail, cardiovascular health in children and young people in general, and in TS specifically. In addition, other factors related to cardiovascular health such as the hypothalamic-pituitary-axis and cortisol will be discussed.

This project explored the acceptability of a study protocol designed to investigate cortisol levels and cardiovascular risk in paediatric patients with TS.

1.2. Turner Syndrome - pathogenesis and aetiology

1.2.1. Genetics

TS, a chromosomal disorder which affects approximately 1 in 2,000 live born female infants, (1-3) results from partial or complete loss of genetic material from the second X chromosome. (4) It is estimated that 40-50% of patients diagnosed with TS display monosomy X on karyotyping. (4) Phenotypic males and women over 50 years of age with less than 5% 45,X0 mosaicism are excluded from the diagnosis. (5, 6) TS is a sporadic condition, unrelated to advanced maternal age, with no known ethnic predisposition. (7, 8) The 45, X0 karyotype accounts for 10% of all first-trimester miscarriages, (8, 9) and it has been hypothesised that some degree of mosaicism is required for early pregnancy survival. (10, 11)

1.2.2. Clinical Features

The phenotype of TS demonstrates marked heterogenicity (Figure 1.1), with many features showing only a weak association with genotype (Figure 1.2). (4, 12) Clinical features such as webbed neck, peripheral oedema, and short fourth and fifth metacarpals, may raise the possibility of diagnosis of TS, (13) however short stature and premature ovarian failure are the most consistent features. (4, 14) In any female presenting with an unexplained short stature or low growth velocity and primary ovarian failure, TS is an important differential diagnosis that should always be excluded.

Figure 1.1 | A group of girls with Turner Syndrome. Permission for use from the Turner Syndrome Support Society UK Executive Officer.

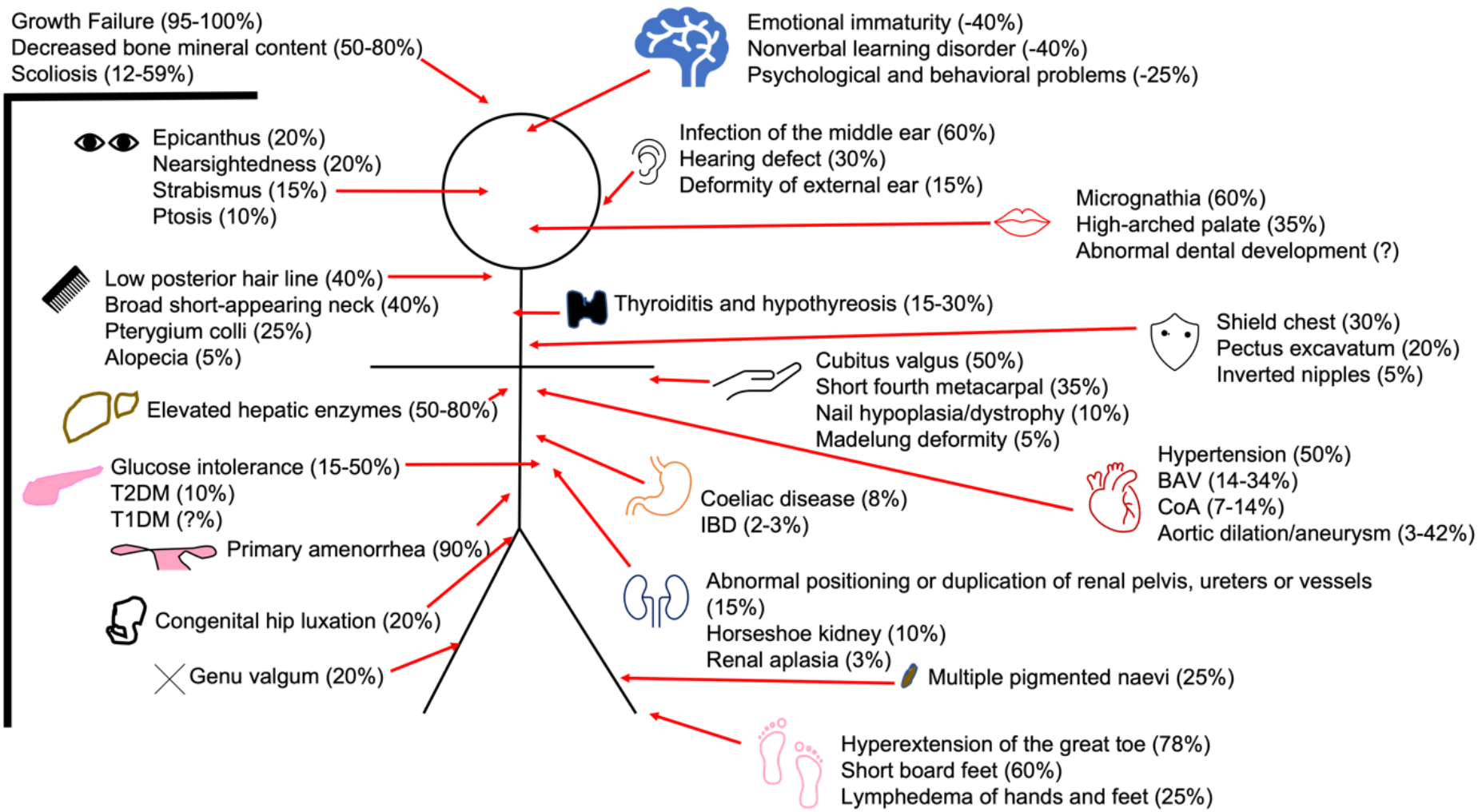


Figure 1.2 | Phenotypic features associated with Turner Syndrome and their approximate prevalence. Image adapted from Gravholt, C. H., et. Al (2017) (4). T2DM: type 2 diabetes mellitus; T1DM: type 1 diabetes mellitus; BAV: bicuspid aortic valve; CoA: coarctation of the aorta; IBD: inflammatory bowel disease.

At an individual level, a specific TS karyotype is not predictive of phenotype, however genotype-phenotype correlations for specific karyotype subgroups have been demonstrated. (4) For example, 45,X0/47,XXX mosaicism is associated with milder phenotypic features and the presence of a ring X chromosome can be associated with intellectual disability. (15, 16) Phenotypic features in 45,X0 individuals have been compared more generally to those in individuals with mosaic karyotypes: in a study of 522 patients with TS over 12 years old, only 14% of girls with the 45, X0 karyotype experienced spontaneous thelarche, whereas this figure was higher (32%) for girls with mosaic karyotypes. (17)

Diagnostic delay for individuals with TS can be substantial. The average age of diagnosis is 15 years old. (2, 18) International guidelines encourage oestrogen replacement from 11 to 12 years of age and recommend that recombinant human growth hormone (hGH) therapy is commenced around four to six years of age. (4) The assessment and timely initiation of these medical interventions before adolescence can go some way to minimising the physical and psychosocial difficulties associated with short stature and delayed puberty. TS is associated with problems affecting multiple organ systems (mentioned below), and early diagnosis can also allow for timely screening and detection of subclinical disease.

1.2.2.1. Premature Ovarian Failure

The majority of girls with TS demonstrate hypergonadotropic hypogonadism as a result of gonadal dysgenesis (Figure 1.3). Compared to 46XX females, a reduced number of oogonia and increased frequency of apoptotic cells have been observed in the early second trimester of fetal development, (19) a time-period before the maximum number of follicles are typically formed. (20) Specimens from TS mosaic ovaries demonstrate less severe impairment, (19) and higher spontaneous menarche rates in this sub-group compared to 45,X0 females have also been demonstrated. (21) The cause of ovarian dysgenesis in TS remains unknown. It has been hypothesised that improper homologous X-chromosome pairing at a meiotic checkpoint could cause oocyte apoptosis. An alternative theory to pairing defects, describes haploinsufficiency of genes found on the pseudo-autosomal region of the X chromosome (genes that would ordinarily escape X inactivation) which are important in ovarian maintenance. (22)

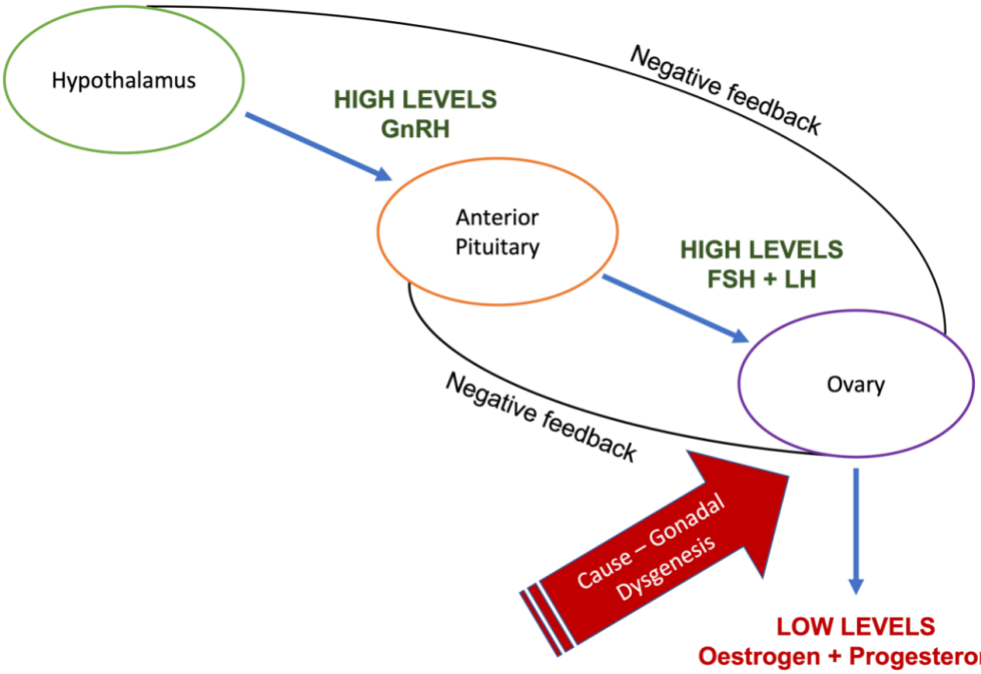


Figure 1.3 | Hypergonadotropic hypogonadism in Turner Syndrome. Gonadal dysgenesis causes low oestrogen and progesterone levels. Lack of negative feedback to the hypothalamus and pituitary resulting in high GnRH and FSH and LH levels, respectively. GnRH: Gonadotrophin-releasing hormone; FSH: Follicle Stimulating Hormone; LH: Luteinizing hormone.

Most girls with TS require oestrogen for pubertal induction, (4) however, the optimal treatment protocol has been considerably debated, reflecting of the lack of robust evidence in this area. International guidelines recommend transdermal natural oestrogen regimens since these have been shown to produce the most physiological hormone profile. (4, 23)

Following menarche, or two years after commencing oestrogen replacement, progesterone is added to the hormone replacement therapy (HRT) regimen. Oestrogen plus progesterone is required for secondary sexual characteristic maintenance, normal uterine growth, and optimal bone density and cardiovascular health. (4) International guidelines recommend continuing HRT throughout life until the risks outweigh the benefits of treatment, which is usually around the average age of menopause. (4)

The impact of delayed diagnosis and subsequent oestrogen deficiency during adolescence on long term cardiovascular and bone health is unknown. Oestrogen deficiency, through regulation of serotonin and norepinephrine, has also been associated with low mood. (24) A large study of young women with TS (n=566) showed that delayed puberty and lack of spontaneous puberty adversely

impacted romantic relationships. (25) Interestingly, however, the age at onset of puberty in a study of 49 young women (19.6 ± 3.0 years) did not influence health-related quality of life (QoL) outcomes. (26) It may be that the duration of HRT is more important in mood regulation: women with TS who did not receive required HRT continuously throughout their adult life (n=8) scored significantly lower on the Psychological General Well-Being Index than women who had sufficient HRT (n=55). (27)

As a consequence of premature ovarian failure, most women with TS are unable to have biological children. Infertility may be one of the greatest factors impacting QoL, being expressed as the most prevalent and painful challenge associated with TS by adult women interviewed in a qualitative study. This study also, somewhat unexpectantly, provided evidence that knowledge of infertility may be a stressor present in childhood too. (28)

1.2.2.2. Short Stature

Disorders of the hypothalamic-pituitary-somatotrophic axis have not been linked to short stature in TS, nor have low sex steroid levels, instead, haploinsufficiency of the SHOX (short stature homeobox) gene is thought to be significant. The SHOX gene is located on the pseudo-autosomal region 1 of the X and Y chromosomes and, through chondrocyte proliferation and maturation, plays an important dose-dependent role on linear long-bone growth. (29)

Heterozygous loss of the SHOX gene is also associated with other features common to TS including shortening of the fourth and fifth metacarpals, cubitus valgus and craniofacial abnormalities. (29) It has been suggested that oestrogen deficiency in TS is protective against some of the bony malformations, such as Madelung deformity, typically seen in isolated SHOX gene deficiency. (30)

Compared to corresponding female populations the final adult height deficit averages 20cm in TS. (31) The use of hGH therapy to increase final adult height is standard treatment for girls diagnosed prior to epiphyseal fusion. Compared to their mean projected adult height or untreated controls, the average height gain from hGH therapy in TS, over study periods ranging from 4.9 to 7.6 years, is between 5.0 and 8.4cm. (32-34) Minimising physical limitations for daily living is the main rationale behind treatment. However, although short stature can cause functional difficulties, it may be that its greatest impact is on confidence, self-esteem and psychosocial functioning, particularly during childhood and adolescence.

1.2.2.3. Ear, Nose and Throat Disorders

Individuals with TS may face hearing challenges continuously throughout life. Low self-esteem and decreased QoL has been associated with hearing difficulties in young women with TS. (25, 27)

Prolonged periods of conductive hearing loss, with varying degrees of permanent damage, are common during childhood. (35) This is a consequence of recurrent episodes of acute and serous otitis media, which may result from abnormal auricular anatomy, eustachian tube dysfunction and subnormal immune response observed in TS. (36, 37) Compared to unaffected children, there is not only an increased prevalence of middle ear pathologies in TS, but the incidence continues for a longer-time period often declining only during and after puberty. (38)

A pattern of early-onset sensorineural hearing loss has been demonstrated during early adulthood, but children as young as six years of age may also be affected. (39, 40) This hearing loss is life-long and progressive. (35) The pathophysiology of sensorineural hearing loss in TS is largely unknown. Oestrogen deficiency and SHOX gene haploinsufficiency are possible contributing factors. (41)

Decreased expression of genes on the X chromosome are thought to be linked to several abnormalities in tooth development: smaller tooth size, thinner enamel and reduced dentine. (42, 43) Micrognathia, common to TS, can also cause crowding of the lower teeth. (4) Interestingly, increased eruption of permeant teeth and advanced dental age has been demonstrated in girls with TS. (44, 45) This advanced dental age is paradoxical to the delayed skeletal age typically observed. (46)

1.2.2.4. Kidney Disease

The prevalence of congenital malformations of the urinary tract in patients with TS is approximately 30-40%, with horseshoe kidney and duplex collecting systems being the most common abnormalities. (47, 48) Mechanical fusion during the metanephric phase, abnormal migration of the kidney to the pelvis, a teratogenic event, or the compressive effects of lymphatic stasis are possible mechanisms of abnormal kidney development during embryogenesis. (49) These congenital anomalies do not usually result in a significant secondary morbidity, however they can predispose patients to hypertension, pyelonephritis, and hydronephrosis, increasing the risk of chronic renal disease. (50)

1.2.2.5. Autoimmune Diseases

Individuals with TS are at an increased risk of several autoimmune diseases: thyroid disease, coeliac disease, T1DM and IBD. (37, 51) Hashimoto's thyroiditis, which has an annual incidence of approximately 3% in TS, is the most prevalent autoimmune disease observed. (4, 52) Abnormal proportions of T cell subsets, with a low CD4 to CD8 lymphocyte ratio, and low circulating Immunoglobulin G levels has been demonstrated in TS, which may be associated with the autoimmune predisposition. (53)

1.2.2.6. Mental Health Difficulties

TS is not a defined DSM-V psychiatric condition. (54) Patients and their families have favoured using the term nonverbal learning disability (NVLD) to informally describe the typical neuropsychological profile of individuals with TS. (55, 56) However NVLD has a definition and existence debated in the literature, (57, 58) and when treatment is indicated, the complex neuropsychological profile of many individuals with TS is better characterised by well-established psychiatric disorders. (56) Indeed, a large retrospective cohort study concluded that individuals with TS are at an increased risk of receiving a psychiatric disorder diagnosis. (59)

A recent systematic review of 35 articles found that individuals with TS may be at least at an equal risk of depression or depressive symptoms compared to the general population. (60) These symptoms were more prevalent in recent studies and were reported to have an onset in adolescence, becoming more established by adulthood. (60) Higher levels of anxiety and decreased self-esteem have also been observed in TS populations. (61-63) Individuals with TS have self-reported high levels of social difficulties, (61, 62, 64) which has been replicated, if not further emphasised, in studies using parent-reported scales. (55, 61, 65-67)

The literature presents a number of case reports of anorexia nervosa in TS. (68-72) An association between the two disorders has been considered, (71) however, it is the overall consensus that the two disorders do not occur more often than by chance in the same patient. (59, 68, 73) The dual diagnosis of anorexia nervosa and TS is an interesting area of study as many of the risk factor for the development of anorexia nervosa are common to TS: social difficulties, anxiety, and cognitive rigidity. (62, 74-76) It is also temping to speculate that the body habitus typical to TS, with a higher body mass index (BMI) and increased fat mass, (77, 78) may predispose individuals to disordered eating in order to conform to "modern beauty standards". (79)

There are many factors that might contribute to poorer mental health in girls and women with TS including the physical phenotype of TS, the impact of living with chronic illness, the administration of daily medication(s), and specific neurocognitive difficulties related directly to the TS genotype.

1.2.2.7. Neurodevelopmental Disorders

Infants with TS generally show a neurodevelopmental profile indistinguishable to their unaffected peers. (80) As girls develop, however, the neurocognitive profile characteristic of TS demonstrates relative strengths in verbal skills, and relative weakness in areas of executive function and visual-spatial skills. (81-87)

Certain neurodevelopmental conditions are not uncommon in TS: compared to their female peers the prevalence of attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder is increased 18-fold and four-fold increased respectively. (59, 88) These conditions are primary male-biased in the wider population, and interestingly, an association was found between poor performance on measures of social cognition in young people with TS with a maternally derived X chromosome. (89) Through exploring possibilities related to sex hormone deficiency and haploinsufficiency of genes on the X chromosome, there is potential to understand more about the mechanisms of neurodevelopmental disorders in both genders. (90-93)

1.2.2.8. Cardiovascular Disease

There are several cardiovascular disease issues relevant to TS, which are discussed fully in section 1.3.2.

1.3. Cardiovascular Disease

1.3.1. The Current Crisis

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels. These diseases can further be divided into congenital and acquired disease. Acquired CVDs are largely preventable and the majority are caused by atherosclerosis. (94)

CVDs are the leading cause of disease burden in the world. The absolute CVD prevalence increased between 1990 and 2019 and further rises are predicted. Population growth and aging are thought to be the major drivers for the increase in total CVD, since total global age-standardised deaths decreased during this time-period. It is concerning that in some locations of the United Kingdom age-standardised ischaemic heart disease death rates increased between 2014 and 2019, suggesting that preventative measures and improved treatment were no longer effective in these regions. (95)

In both developed and developing countries, there is an increasing prevalence of children with cardiovascular risk factors for atherosclerotic disease. (96) Patients with diagnoses such as familial hypercholesterolaemia, TS, and T1DM are known to be at risk of premature acquired CVD, subsequently, primary and secondary CVD prevention measures during childhood are especially important in these population groups. (97-99)

1.3.1.1. The pathogenesis of atherosclerosis

Atherosclerosis is a chronic pathological process of lesion development in medium and large-sized arterial walls. The process begins during childhood and has a long asymptomatic period, typically only manifesting clinically in middle and late adulthood (Figure 1.4) (100)

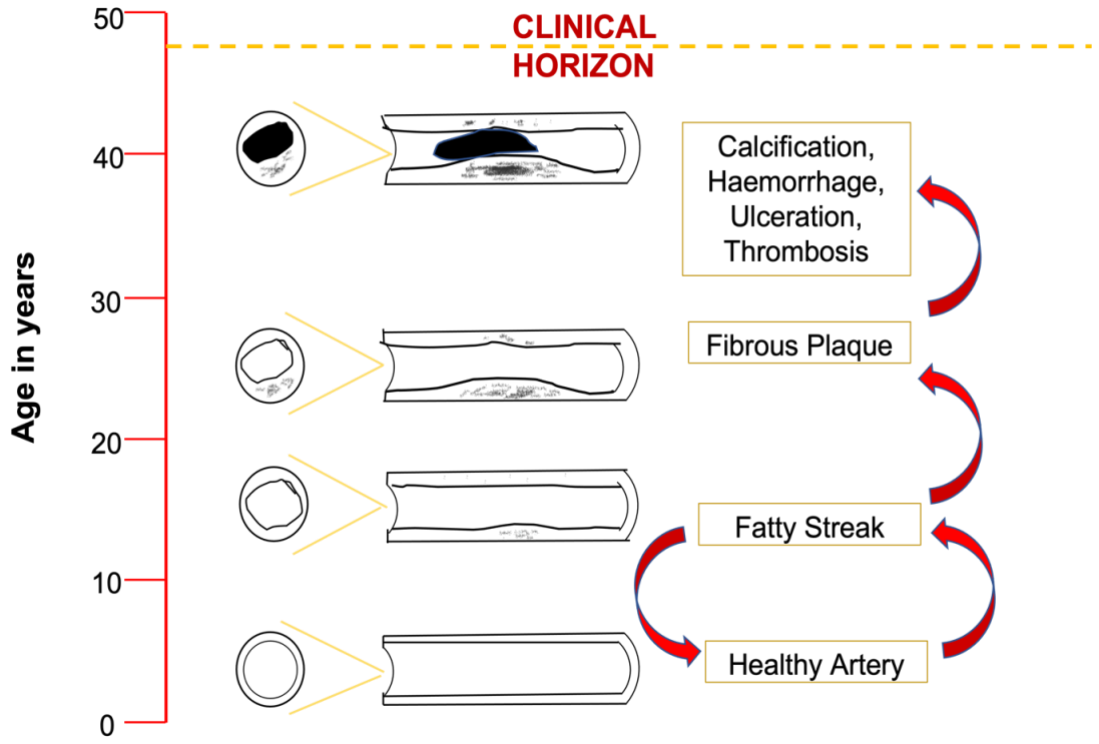


Figure 1.4 | The natural progression of atherosclerosis with age. Image adapted from Hong, Y. M., et. Al (2010) (100).

The pathogenesis and progression of atherosclerosis is described differently by different scholars. Multiple biological pathways are implicated, complicated by several environmental factors that modify the expression of disease. (101)

The 'response to injury' hypothesis is a widely accepted as the mechanism initiating the development of atherosclerosis. (102) Exposure to toxins, such as those found in cigarette smoke, or a mechanical injury to the endothelium leads to endothelial cell dysfunction. Endothelial cells lose the ability to produce vasoactive molecules, such as nitric oxide, required for response to physiological stimuli. Additionally, there is loss of non-thrombogenic surface-properties, promoting adherence of inflammatory cells, especially macrophages and T cells, subsequently allowing their passage into the intima. The permeability of the endothelial wall increases, and low-density lipoproteins (LDL's) enter the intima where they are oxidised and digested by macrophages. Lipid-laden macrophages deposit under the endothelium as foam cells, which accumulate forming a fatty streak. Macrophages also produce numerous growth factors, such as platelet derived growth factor, which promote proliferation of vascular smooth muscle cells and their secretion from the media into the intima. With

time, fatty streaks may enlarge, undergo calcification, and develop a fibrous cap. Thin-cap fibroatheromas are at risk of rupture and subsequent thrombotic luminal occlusion. The clinical features that result are dependent on the arterial lumen affected: acute coronary syndrome, cerebrovascular event, or peripheral arterial disease. (103)

1.3.1.2. Non-invasive assessment of preclinical atherosclerosis

1.3.1.2.1. Flow-mediated dilation

The technique of flow-mediated dilation (FMD) can be used to evaluate endothelial function indirectly. FMD of the brachial artery utilises high-resolution ultrasonography to measure nitric oxide-mediated vasodilation produced via increased blood following an blood restriction period. (100) A diminished arterial vasodilator response is observed in the early stages of atherogenesis. (104) Decreased FMD has been demonstrated in young people with T1DM and a family history of premature coronary artery disease. (105, 106)

1.3.1.2.2. Carotid intima-media thickness

Common carotid intima-media thickness (cIMT), defined as the thickness of the carotid wall in millimetres, between the lumen-intima and media-adventitia interfaces measured by high-resolution ultrasonography (Figure 1.5), is a structural marker of arterial atherosclerotic changes. In the early phases of atherosclerosis intimal thickness increases due to foam cell accumulation. (101) cIMT increases as atherosclerosis becomes more advanced, and there is extensive data to support the association between increased cIMT with cardiovascular and cerebrovascular events. (107, 108)

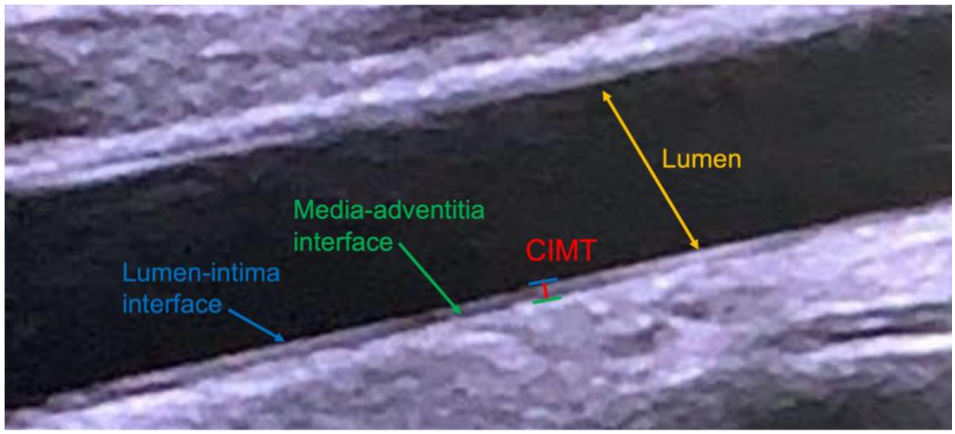


Figure 1.5 | Image demonstrating common carotid intima-media thickness (usually measured in distal 1cm of artery, just before the bifurcation into the internal and external carotid arteries). CIMT: Carotid intima-media thickness. (Please see Chapter 4).

1.3.1.2.3. Ambulatory arterial stiffness index

Ambulatory arterial stiffness index (AASI) is a functional marker of systemic arterial elasticity measured via oscillometric technology. A study including 156 asymptomatic healthy volunteers demonstrated that increased AASI correlated with subclinical atherosclerosis. (109) Both functional and structural endothelial factors are thought to contribute to the reduced elasticity in atherosclerotic arteries. (101)

1.3.1.3. Modifiable behavioural risk factors for CVD in childhood

Atherosclerotic CVD risk factors can develop during childhood and adolescence. Environmental and genetic factors are important, and when behavioural risk factors develop at a young age, they are likely to persist throughout life. Prevention of adverse health behaviours (described below) is important. It is unusual for these risk factors to exist in isolation and they often interact in the same individual. (100)

1.3.1.3.1. Obesity

Obesity acts an independent risk factor for CVD development. Atherosclerosis is accelerated in obese individuals, thought to be primarily due to the pro-inflammatory state. High levels of leptin in obese individuals are another possible contributor: recent evidence suggested that cholesterol uptake by macrophages, resulting in increased foam cell formation, is stimulated by leptin. (110) Obesity-related

hypoadiponectinemia is also likely to be significant, as adiponectin has anti-atherogenic, insulin-sensitising and vasodilatory activities. (111)

The prevalence of childhood and adolescent obesity is increasing globally. (112) The burden of excess weight on the coronary system is evident at a young age: an autopsy study of 204 young people demonstrated a statistically significant positive correlation between extent of atherosclerotic lesions in the aorta and carotid arteries with BMI. (113) Preventative measures are important from a young age, as the risk of overweight children developing into overweight adults is at least double that of children that are a normal weight. (114)

1.3.1.3.2. Diet

It is well-known that diet can influence the development of atherosclerosis. This may be via direct mechanisms, such as excessive proinflammatory cytokine production, or indirectly through effects on risk factors such as excessive weight, hypertension, hyperglycaemia, and hyperlipidaemia.

A diet high in calories, red meat, saturated fats and salt is associated with the highest risk of CVD. Systemic inflammation and increased oxidative stress are mechanisms in which these foods accelerate atherosclerosis. (115) Diet also affects the composition of gut microbiota and gastrointestinal dysbiosis may have a role in the development of CVD. (116)

Dietary fibre has beneficial effects on the risk of developing CVD: it is proposed that exposure of the gut microbiome to fibre decreases glucose absorption and the inflammatory response. (116) Phytosterols, naturally present in nuts, vegetable oil and whole grains, reduce serum LDL-C levels primarily by reducing absorption in the small intestine. Omega-3 polyunsaturated fatty acids, found in fish, nuts and seeds, also regulate the lipoprotein profile, having a protective effect against atherosclerosis by reducing inflammation, oxidative stress and platelet formation and aggregation. (115)

Polyphenols, abundant in fruit and vegetables, are powerful antioxidants. Oxidative stress influences the progression of atherosclerosis, both through oxidation of LDL and endothelial dysfunction, however, given polyphenol's low bioavailability it is unlikely that the cardiovascular benefits are due to this mechanism alone. (117) Polyphenols have also demonstrated anti-atherosclerotic properties through oestrogen receptor binding, anti-inflammatory and anti-platelet effects. (115)

Childhood dietary habits are likely to persist into adulthood. (118) Establishing a healthy relationship with nutrient-dense food in early life has many benefits, including for cardiovascular health.

1.3.1.3.3. Physical inactivity

Physical activity is a preventative measure in the development of atherosclerosis. Individuals who regularly exercise display a low-risk cardiovascular lipoprotein profile and increased nitric oxide-mediated vessel vasodilation, however the mechanisms responsible for these protective changes are unknown. (119)

Even after control of other cardiovascular risk factors, an inverse relationship between physical activity and CVD was demonstrated in a large adult cohort. (120) It is concerning that the prevalence of sedentary lifestyle in European adults is rapidly increasing. (121) Continuous physical activity, for several years, in youth is predictive of physical activity in adulthood. (122)

1.3.1.3.4. Smoking

Smoking tobacco is estimated to cause almost 10% of all CVD. (123) Cigarette smoke exposure accelerates atherosclerosis and clinical disease through different mechanisms, including pro-inflammatory and pro-thrombotic effects and decreasing nitric-oxide dependent vasodilatory function. Cigarette smoke also has an unfavourable impact on lipid profile and increases oxidation of LDL, which is likely a contributing factor to the increased atherosclerosis. (124)

Second-hand smoke causes CVD through multiple interacting mechanisms: the effects of even brief passive smoking are almost as large as those from chronic active smoking. (125) Additionally, parental smoking, particularly maternal smoking, is likely to adversely influence children's health-related behaviours and increase risk of CVD. It has been reported that, if either parent smoked, children's fat intake was significantly higher and they were less likely to be physically active and spend more time watching television. (126)

1.3.2. Turner Syndrome – Clinical Consequences

Compared with the general population, the life expectancy for individuals with TS is reduced by at least ten years. (2, 127, 128) Those diagnosed at a young age and women with the classical 45,X0 karyotype generally have the greatest mortality risk. (127) Approximately 50% of the increased

mortality is due to CVD, of which congenital disease accounts for only eight percent. (127, 129, 130) The remainder of the increased risk of premature death from CVD is accounted for by acquired diseases. (127)

Many of the risk factors for acquired CVDs are evident in TS from childhood. An observational study of 98 girls with TS who were followed for 12-years, reported a progressive increase in systolic and diastolic blood pressure (BP), BMI and impaired glucose metabolism and abnormal lipid profiles with age. (99)

1.3.2.1. Congenital abnormalities of the cardiovascular system

Congenital cardiac abnormalities occur in 23-39% of individuals, (131, 132) most frequently in patients with the 45,X0 karyotype and most commonly involve the left side of the heart. (4, 129, 133) It has been hypothesised that fetal lymphoedema, as well as haploinsufficiency of the TIMP1 gene located on the X chromosome, contributes to the development of these cardiac defects. (1, 134)

1.3.2.1.1. Bicuspid Aortic Valve

The prevalence of bicuspid aortic valve (BAV) in TS is 15-30%, which is approximately 15 times greater than that observed in the general population. (135) Higher diagnostic sensitivity, achieved with the use of cardiac magnetic resonance imgaging (CMR), means that the diagnosis of BAV in TS is increasing. (136)

Clinical manifestations relating to BAV disease typically develop in adulthood. Abnormal sheer stress in the aorta accelerates valve calcification, often resulting in premature valvular stenosis. Additional abnormalities of BAV leaflets can result in aortic valve regurgitation. Dilation of the aorta is commonly associated with BAV disease, and aortic valve regurgitation may also develop secondary to this. (135) In the general population, the annual incidence of endocarditis in individuals with a BAV is increased compared to individuals without a BAV. (137) Due to the frequent occurrence of BAV and other congenital heart disease in TS, it is assumed that the risk of endocarditis is increased in these females too. (135)

1.3.2.1.2. Coarctation of the aorta

Coarctation of the aorta (CoA) is described in approximately one in six women with TS. (135) It is more commonly found in individuals with the 45,X0 karyotype and often co-exists with BAV. It has been suggested that CoA in newborn females should viewed as an independent predictor of TS. (138)

Surgical or transcatheter procedures are used to eliminate the narrowed segment. Largely for unknown reasons, a longer operative and post-operative period for CoA repair has been demonstrated in TS patients compared to age matched non-TS patients. (139)

1.3.2.2. Hypertension

Hypertension is one of the most important risk factors for acquired CVDs. (140-143) Observational studies have described hypertension in 21-40% of girls and adolescents with TS, (99, 144, 145) and a pragmatic, observational 12-year study, of 102 women age 18-62 years, demonstrated that age was positively associated with systolic BP. (146)

The aetiology of hypertension in TS is largely unknown. It is possible that dysfunction of the autonomic nervous system has a role in the pathogenesis. Compared to the general population, women with TS are reported to have an increased resting baseline sympathetic tone, (147) and an impaired ability to modulate their sympathovagal tone in response to change in body position. (148, 149) Furthermore, the embryonic origin of the autonomic nervous system, the neural crest, (150) gives rise to a number of the congenital abnormalities seen in TS, including CoA, BAV and nuchal oedema. (151-153) It may be that congenital differences in the development of this neural pathway are important in the development of hypertension in TS. (135)

Secondary hypertension can occur as a consequence of congenital renal or cardiac abnormalities. (4) Aortic coarctation occurs in approximately 7-14% of patients, (4) and hypertension can persist or reoccur after coarctation repair in TS even without residual obstruction. (154, 155)

1.3.2.3. Abnormal Blood Pressure Circadian Rhythm

A 10-20% nocturnal fall in systolic and diastolic BP from daytime values is expected in the general population. (156) Individuals with this reproducible BP circadian profile (Figure 1.6) are termed ‘dippers’, and those who exhibit less than a 10% fall in BP are termed ‘non-dippers’. (156-159) In a study of 75 girls with TS, 57% of girls were ’non-dippers’, (144) which is important as loss of the normal

circadian profile, and therefore the protective mechanism of reduced pressure load on the arterial walls at night, is an independent predictor of cardiovascular risk. (160, 161)

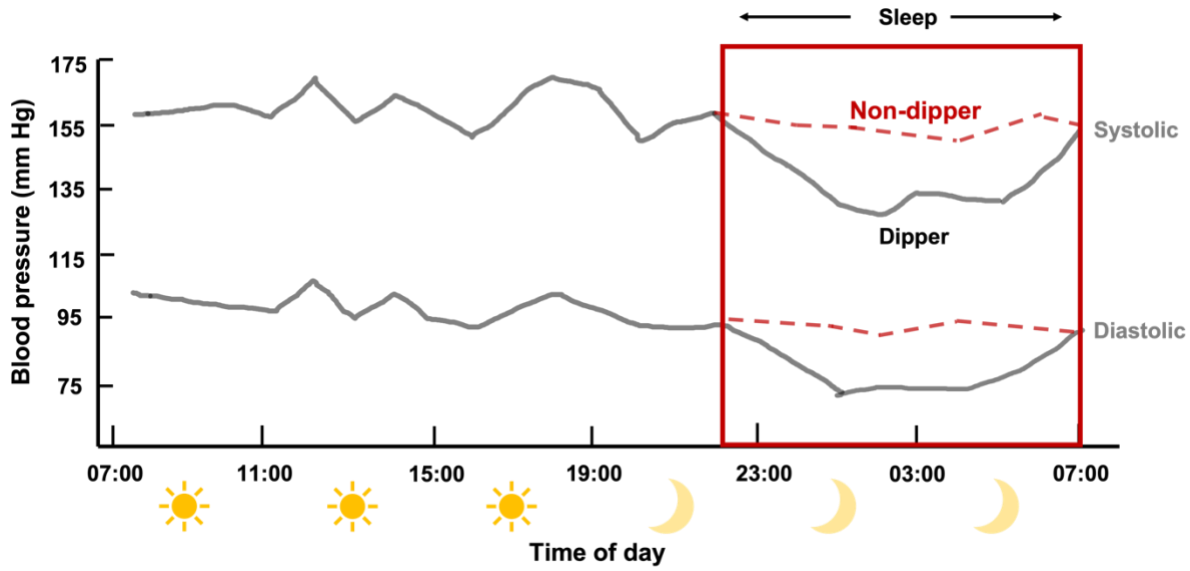


Figure 1.6 | Blood pressure circadian rhythm. Image adapted from Larochelle, P., et. Al (2002) (159). Healthy individuals have a reproducible circadian blood pressure profile (solid line), consisting of a drop at night, a steep surge during the transition from sleep to wakefulness, and a peak in the transition from sleep to wakefulness, and a peak in the late afternoon.

Increased sympathetic activity, or failure of night-time sympathetic tone reduction, may contribute to the pathogenesis of abnormal BP circadian rhythm in TS. (162)

1.3.2.4. Obesity and Physical Activity

Body habitus in TS is unfavourable for CVD development: when compared to unaffected women, women with TS have a higher BMI and an increased hip-to-waist ratio, increased total and visceral fat mass, and decreased skeletal muscle mass. (77, 78) Obesity is an independent risk factor for CVD development and death (see section 1.3.1.3.1). (163) Furthermore, excess visceral adiposity has been shown to be associated with poor cardiovascular outcomes independently of BMI in the general population. (163)

Physical activity is protective against atherosclerotic disease (see section 1.3.1.3.3). Physical activity and fitness levels are lower in girls and women with TS compared to unaffected female peers. (164, 165) In a qualitative study recruiting adolescent girls with TS, physical and psychosocial factors related

to TS, including short stature, visual-spatial defects, and social anxiety, were reported as barriers to physical activity. (166)

1.3.2.5. Type 2 Diabetes Mellitus

Diabetes mellitus is a well-established cardiovascular risk factor in the general population. (167) The approximate prevalence of glucose intolerance and T2DM in TS is 15-50% and 10%, respectively. (4) Studies have shown a strong correlation between age and abnormal glucose metabolism in TS. (99, 168) Interestingly, hypogonadism as well as the traditional risk factors for T2DM development in the general population (obesity, high levels of visceral adiposity) does not seem to be a significant factor in the development of T2DM in TS, especially in the paediatric population. (169, 170) The pathogenesis of T2DM in TS is thought to linked to TS-specific mechanisms of insulin resistance and impaired beta-cell function. (170)

1.3.2.5.1. Insulin resistance

Most studies comparing insulin sensitivity in TS compared to control groups have demonstrated insulin resistance. (170) A study utilising the euglycemic–hyperinsulinemic clamp technique displayed a defect of insulin action in women with TS, whom had normal fasting glucose levels, compared to age-matched controls, which persisted after adjustments for several fat mass parameters. (171) These data, alongside evidence excluding the role of ovarian failure-related hypogonadism, (130) indicate that the presence of an intrinsic defect specific to the TS karyotype may be responsible for insulin resistance. (171)

1.3.2.5.2. Insulin secretion

A genetic mechanism of impaired glucose metabolism in TS has been proposed: it is hypothesised that haploinsufficiency of genes important in beta cell function on the X chromosome could predispose to T2DM development. (169) Decreased pancreatic beta cell function in young girls and adolescents with TS has been linked to decreased insulin secretion, resulting in glucose intolerance. (172) Increased insulin secretion in TS has been reported, however given the hyperbolic association between insulin secretion and sensitivity, it is thought that hyperinsulinaemia is compensatory for insulin resistance. (173)

1.3.2.6. Lipid Disorders

Elevated cholesterol, especially LDL-C, is strongly linked to CVD development in the general population (see section 1.3.1.1). (174)

Some studies of individuals with TS have demonstrated a comparable lipid profile to controls, (165, 175) whereas other studies have demonstrated a proatherogenic profile. (176-179) However, severe elevations in total cholesterol, LDL-C and triglycerides (TG), which meet the cut-off for pharmacological intervention, are rarely reported. (179)

It may be that there is an unknown mechanism specific to TS that predisposes individuals to dyslipidaemia. Alternatively, it could be a consequence of the increased prevalence of obesity and T2DM observed in this population or due to insufficient oestrogen replacement. (4)

1.3.2.7. Vascular function

Differences in vascular function have been demonstrated in TS when compared to the general population. For example, when 35 young women with TS (age 19.4 years, 13.9-27.5) were compared to female individuals of the same age, ethnicity and body-type, arterial stiffness was increased, and this difference persisted over a seven-year period. (180) Decreased aortic elasticity has also been observed, even without aortic dilatation or BAV and/or CoA. (181) Increased arterial stiffness has been demonstrated in different conduit arteries in young normotensive TS patients, with girls as young as nine years of age being affected. (143, 182-185)

Arterial stiffening is thought to proceed clinical atherosclerosis, and FMD is a non-invasive technique used to assess this (see section 1.3.1.2.1). Studies using FMD as a marker of vascular function demonstrated that individuals with TS had comparable or even higher FMD of the brachial artery compared to controls. (186, 187) However, one of these studies showed the presence of a strong inverse correlation between FMD and age in individuals with TS, suggesting an increased tendency of premature derangement of arterial function compared to the general population. (186)

cIMT directly assesses atherosclerosis through analysis of structural arterial changes (see section 1.3.1.2.2). Studies of cIMT, comparing measurements between TS patients and controls, have shown inconsistencies: some studies report comparable results and others report increased cIMT in TS

compared to controls, which suggests that the arterial walls of individuals with TS are pro-atherogenic. (186-190)

1.3.2.8. Pregnancy Risk

Due to reports of poor maternal cardiovascular outcomes during pregnancy, it has been debated if pregnancy is advisable in TS patients at all. With the development of assisted reproductive technologies, invitro fertilisation with oocyte donation or autologous oocytes (via oocyte cryopreservation), reproduction possibilities have radically changed. (4) Spontaneous pregnancies in TS are rare, being reported in only 4.8-5.6% of women and most commonly occurring in those with the 45X0/46XX mosaic karyotype. (191, 192)

Once pregnant, via assisted reproductive technologies or spontaneously, high spontaneous abortion rates have been observed in women with TS. This may be attributed to structural uterine abnormalities and associated reduced uterine perfusion, autoimmune mechanisms, or compromised endometrial receptibility due to haploinsufficiency of relevant genes on the X chromosome. (191, 193, 194) Caesarean section rates are also higher than in the general population, in which short stature causing fetopelvic disproportion is a common contributor. (191, 192, 195, 196) A high rate of chromosomal abnormalities has been demonstrated in live-born infants whose biological mother have TS, which is possibly due to imbalance of gene regulation in the mother transferred to the embryo. (192, 197)

1.3.2.8.1. Aortic Disease

Compared to aged-matched healthy women, the risk of aortic dissection is over 100-fold greater in young and middle-aged women with TS. (198) Pregnancy is a likely additive risk factor for aortic dissection due to the increased blood volume and subsequent increase in cardiac output. (199) The elevated levels of oestrogen and prostaglandin in the third trimester could also contribute to this risk through weakening of the aortic walls. (200)

It is estimated that aortic dissection or aortic rupture occurs in 2.2% of pregnant women with TS. (200) International guidelines recommend cardiovascular imaging within two years of a planned pregnancy or assisted reproduction for all women with TS. (4)

1.3.2.8.2. Hypertensive Disorders

Women with TS who conceive spontaneously or via assisted reproductive technologies are at a high risk of hypertensive disorders of pregnancy: the rate of gestational hypertension and pre-eclampsia is as high as 40.5%. (193)

Hypertensive disorders occur less frequently in TS patients with spontaneous pregnancies compared to those who conceive via oocyte donation. The maternal immunoglobulins response to a genetically foreign embryo could cause abnormal placental development, predisposing to hypertension. (201) Furthermore, it may be that the phenotype of individuals who achieve spontaneous pregnancies is less severe in all body systems, resulting in less cardiovascular and renal abnormalities. However, the rate of hypertensive disorders in spontaneous pregnancies in TS is still double that of the general population, which suggests the presence of risk factors inherent to TS itself. (192)

1.3.2.9. Sports Participation Risk

Women with TS are at increased risk of premature heart disease and death from acquired cardiovascular diseases. (127, 129, 130) International guidelines recommend that clinicians encourage patients to lead a heart-healthy lifestyle from an early age. However, advice regarding participation in competitive sport should take account of the relative aortic diameter, as this is an important risk factor for aortic dissection. (4)

1.3.2.10. Cardiovascular surveillance

From diagnosis, international guidelines for the care of girls and women with TS recommend weight/BMI and BP measurements annually. From 10 years of age, it is recommended that HbA1c with or without fasting glucose is measured annually, with the addition of a lipid profile in adults if at least one cardiovascular risk factor (obesity, hypertension, tobacco use, diabetes, or physical inactivity) is present.

Cardiac imaging is used to screen for congenital cardiac defects and as a surveillance method for aortic dissection. It is recommended that cardiac imaging, commonly including transthoracic echocardiography, CMR imaging or cardiac computed tomography, is performed at diagnosis. If TS is

diagnosed prenatally a foetal echocardiogram should be performed. To measure aortic dilation, ascending aortic size index is used in individuals aged 16 years or over, and TS-specific Z score are used in younger individuals. These measurements determine the adequate frequency of cardiovascular follow-up. (4)

1.4. The Hypothalamic-Pituitary-Adrenal Axis

1.4.1. Glucocorticoid axis

Under basal conditions, glucocorticoids are released in a circadian rhythm with an underlying ultradian rhythm. (202, 203) Additionally, rapid synthesis and secretion of glucocorticoids occurs in response to physiological internal and emotional external stress. (202)

Corticotrophin-releasing hormone (CRH), a 41-amino acid neuropeptide, (204) is the main activator of the hypothalamic-pituitary-adrenal (HPA) axis in both basal and stress-induced glucocorticoid synthesis and secretion. (205) CRH is synthesised in hypophysiotropic neurones located in the paraventricular nucleus of the hypothalamus and released into hypophysial portal vessels projecting into the anterior pituitary gland. CRH regulates the transcription of pro-opiomelanocortin (POMC), which is cleaved to ACTH and stored in vesicles. (206) CRH binds to CRH type 1 receptors on pituitary corticotropes, stimulating in the release of adrenocorticotropic hormone (ACTH) into the systemic circulation through activation of the cyclic adenosine monophosphate pathway. Melanocortin type 2 receptors found in the zona fasciculata cells of the adrenal cortex are the principal target for circulating ACTH. These adrenal parenchymal cells both synthesise and secrete glucocorticoids into the systemic circulation. Circulating glucocorticoids modulate the HPA axis via negative feedback mechanisms (Figure 1.7). (207)

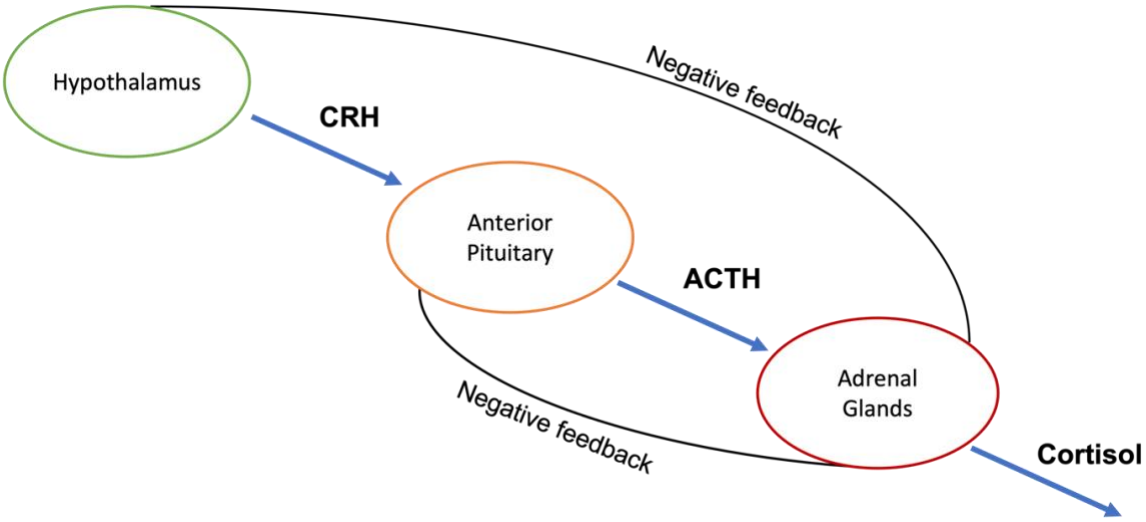


Figure 1.7 | Cortisol negative feedback loop. CRH is released from the hypothalamus, stimulating the release of ACTH from the anterior pituitary into the bloodstream, which promotes the release of cortisol from the adrenal cortex. Circulating cortisol levels modulate the negative feedback mechanisms. CRH: cortisol-releasing hormone; ACTH: adrenocorticotrophin hormone.

1.4.1.1. Glucocorticoid and Mineralocorticoid receptors

Glucocorticoids utilise the similar-structured intracellular nuclear glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) to exert their effects. (206) After binding, translocation to the nucleus occurs, where gene transcription followed by subsequent protein synthesis takes place. (206)

GR is expressed ubiquitously throughout the body and mediates the energy distribution and immune functions of glucocorticoids. (206) In the brain, GR is highly expressed in the hippocampus, amygdala, paraventricular nucleus of the hypothalamus and prefrontal context, whilst MR expression is high in the hippocampus and moderate in the prefrontal cortex and the amygdala. (208) The plasma levels of glucocorticoids are much higher than mineralocorticoids, and the MR has equal binding affinity to glucocorticoids and mineralocorticoids. The MR is protected from the effects of glucocorticoids by inactivation of cortisol to cortisone by 11β-hydroxysteroid dehydrogenase (11βHSD) type 2. At high concentrations of glucocorticoids, the enzyme is saturated, resulting in MR stimulation. (209)

1.4.1.2. Glucocorticoid Negative Feedback

At all levels of the HPA axis, no less than two distinct mechanisms of negative feedback via genomic and non-genomic glucocorticoid regulation have been described. (206, 210-215) These mechanisms

are essential in maintaining homeostasis: overstimulation of the axis would cause prolonged exposure to the actions of glucocorticoids, which can be pathological to multiple body systems, while under secretion, or cortisol deficiency, can be a life-threatening medical emergency. (216, 217)

1.4.1.2.1. The hypothalamus

In response to high levels of circulating glucocorticoids, cortisol mediated rapid inhibition of the HPA axis occurs, typically in under 10 minutes. This non-genomic response, mediated by endocannabinoid signalling, inhibits glucocorticoids locally within the paraventricular nucleus of the hypothalamus. Activation of membranous GR receptors results in the generation of retrograde endocannabinoid release across the synaptic cleft to CB1-receptors and suppression of the release of glutamate from presynaptic excitatory synapses, subsequently limiting the excitation of CRH neurones and supressing the release of CRH. At the same time, through the facilitation of nitric oxide, the frequency of the release of the main inhibitory neurotransmitter, gamma-Aminobutyric acid, is increased. (210) Other non-genomic negative feedback, primarily via MR-mediated pathways, occurs in brain regions regulating paraventricular nucleus output including the hippocampus, pre-frontal cortex and amygdala. (218, 219)

In contrast, the genomic negative feedback response to high levels of circulating glucocorticoids occurs over a couple of hours. Glucocorticoids have been shown to directly inhibit CRH expression in the paraventricular nucleus of the hypothalamus and to suppress the transcription of the ACTH precursor proopiomelanocortin (POMC) in pituitary corticotrope cells, although the underlying mechanisms are not fully understood. (206) Additionally, glucocorticoids have been found to regulate POMC mRNA by decreasing its stability. (215)

Although CRH is largely regarded as the main activator of the glucocorticoid HPA axis, it should be noted that responses have been observed in the absence of CRH. (220) Other hypothalamic neuropeptides, vasopressin and oxytocin, have demonstrated roles in regulating ACTH and glucocorticoid activity in response to stress. The role of these hormones under basal glucocorticoid conditions is unclear. (206)

1.4.1.2.2. The pituitary

At the level of the pituitary, glucocorticoid-driven negative feedback of ACTH has been described. Corticotroph cells of the anterior pituitary are electrically excitable: they have been shown to produce single-spike action potentials as well as pseudo-plateau bursting behaviour. Through raising intracellular Ca2+, bursting-type firing is thought to underlie rapid ACTH release from vesicles. The generation of bursting-type firing is dependent on activation of large-conductance calcium and voltage-activated potassium channels (BK). (221) High levels of circulating glucocorticoid have been shown to reduce bursting behaviour and subsequent ACTH secretion through BK-dependant mechanisms. BK-independent mechanisms have also demonstrated a response to high glucocorticoid levels, with a decreasing frequency of single-spike action potentials. (211, 221)

An additional non-genomic feedback mechanism of regulation of ACTH release from corticotroph cells utilises Annexin A1 (ANXA1). This protein, produced in pituitary folliculostellate cells, inhibits ACTH secretion driven by CRH. (214) Glucocorticoids promote ANXA1 translocation from the cytoplasm to the plasma membrane, rapidly inhibiting ACTH secretion from vesicles. (222)

At the genomic level, glucocorticoids mediate negative feedback by regulating transcription of the promoter POMC region and POMC. (206) These mechanisms involve the binding of GR to negative glucocorticoid response element within the POMC promoter and inhibiting Nur77-induced POMC transcription, respectively. (223, 224)

1.4.1.2.3. The adrenal cortex

It has been hypothesised that glucocorticoids suppress their own steroidogenesis via a local feedback mechanism contained within the adrenal cortex. An intra-adrenal negative feedback loop involving the GR has been described. (206) This genomic mechanism involves inhibition of the transcription and activity of Nur77, a nuclear receptor, thereby repressing the transcription of Steroidogenic acute regulatory protein (StAR). (212) Rapid non-genomic self-inhibition has also been described. (213) The mechanisms are largely unknown, however as in the anterior pituitary, it is thought that ANXA1 has a role. (206)

1.4.2. Cortisol release

1.4.2.1. Diurnal circadian rhythm and awakening response

The cortisol circadian rhythm (Figure 1.8) typically demonstrates low levels during sleep and inactivity, which rise during the night and peak in the morning within the first hour after awakening. During the morning hours cortisol levels decrease rapidly, following the mid-day meal they increase slightly, before declining more slowly through the remainder of the day back to nadir levels at night. (225) The age at which the adult diurnal profile of cortisol is established has been variously reported but is usually by 9 months of age. (226) This rhythm is not made up of smooth hormone changes over 24-hours, but instead due to changes in activity of an underlying ultradian rhythm. (227)

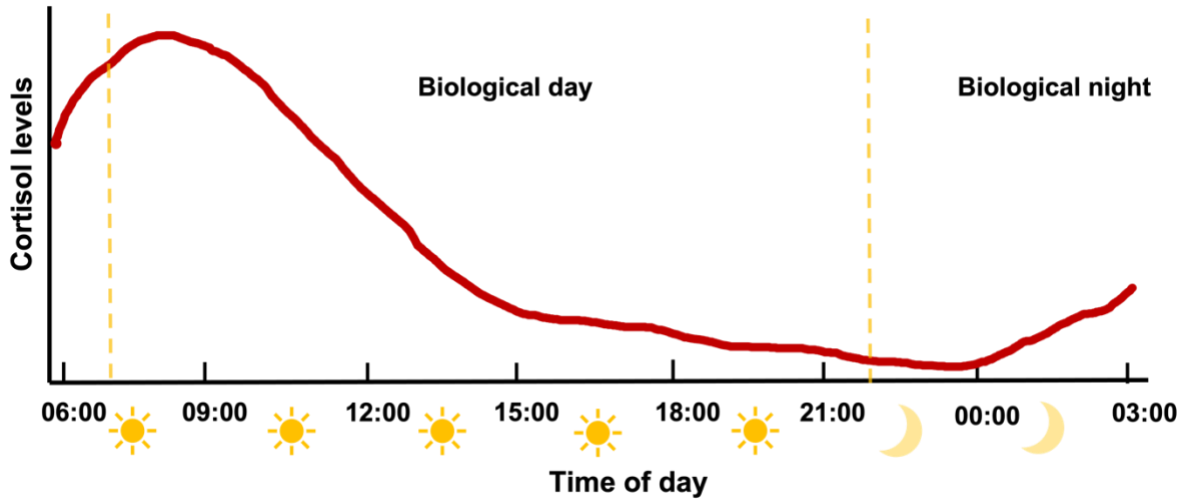


Figure 1.8 | Cortisol diurnal rhythm. Image adapted from Elverson, C. A., et, Al (2005) (225).

The cortisol awakening response refers to the rise in cortisol in the first hour post-awakening. Cortisol concentrations typically peak in the first 30 minutes, rising by approximately 9.3 ± 3.1 nmol/L from awakening levels in healthy adults. (228)

The suprachiasmatic nucleus, a bilateral structure located in the anterior hypothalamus, is the main mammillary circadian pacemaker. It is responsible for many physiological and behavioural processes, including regulation of locomotor activity, core body temperature, and hormone secretion. (229) The suprachiasmatic nucleus determines circadian activity of the HPA axis by modulating the release of CRH from the paraventricular nucleus. (230)

1.4.2.2. Ultradian rhythm

Cortisol secretion occurs at a constant frequency, approximately every 80 minutes. These secretory episodes vary in amplitude allowing for production of the circadian rhythm of cortisol (see section 1.4.2.1). (227)

In rodents, lesions in the suprachiasmatic nucleus of the hypothalamus abolished circadian rhythms, however they had no effect on the pulsatile ultradian rhythm of cortisol, suggesting that the hypothalamus is not the generator of these rapid oscillations. (229) Another rodent study supported that generation of this rhythm is independent of the brain, whereby despite continuous corticotrophin-releasing hormone infusion, ultradian oscillations of cortisol remained. (231) It has been hypothesised that the ultradian cortisol rhythm is an intrinsic property of the HPA axis: theoretical mathematical modelling demonstrated it as a product of the dynamic interaction between a pituitary-hypothalamus positive feedforward pathway and glucocorticoid-mediated negative feedback from the adrenal glands. (232)

1.4.3. Bioavailability of glucocorticoids

In the bloodstream, glucocorticoids are kept biologically inactive by being bound to and carried by plasma proteins. Corticosteroid-binding globulin (CBG) is the main binding protein, binding to >80% of serum glucocorticoids with a high affinity and low capacity. Albumin has a lower affinity and higher capacity than CBG and binds to 10-15% of circulating glucocorticoids. Under basal conditions, only five to 10% of glucocorticoids are unbound or 'free'. (233)

Free glucocorticoids are lipophilic and diffuse through the cell membrane into the cytoplasm. The bioavailability of glucocorticoids is regulated in the cytoplasm by two isoforms of the enzyme 11βHSD. Cortisol is generated from cortisone by 11βHSD type 1, and 11βHSD type 2 catalyses the reverse action. (234) 11βHSD type 2 is highly expressed in mineralocorticoid-responsive tissues, to protect the receptor against glucocorticoid-mediated actions. (235) Biologically active glucocorticoids bind to their receptors in the cytoplasm to exert their physiological effects. (234)

1.4.4. Measurement of cortisol

1.4.4.1. Serum and plasma

Traditional methods of measuring cortisol in serum and plasma measure total and free cortisol. Immunoassays with radioactive or non-isotopic labels are used routinely in most laboratories, although, more recently, mass spectrometric methods are becoming more popular. (236) The free hormone hypothesis predicts that the biological activity of a hormone corresponds to the concentration of unbound, or "free", hormone. (237) Most current assays attempt to displace CGB through pH change or synthetic steroid substitutes to measure "free" cortisol, although in the presence of CGB excess these displacement methods are not always sufficient. (238)

When testing for cortisol excess or deficiency, a random, untimed, serum cortisol measurement is of limited clinical value. Static cortisol testing should be timed to correspond with the physiological diurnal rhythm: If excess or deficiency is suspected, midnight or early morning samples are most useful, respectively. Given the pulsatile nature and diurnal variation of cortisol secretion, its regulation via a feedback system, and response to a variety of stressors, dynamic stimulation and suppression testing is preferable to static testing to confirm diagnoses. (239)

Static early morning and midnight total cortisol serum measurements and dynamic tests of cortisol reserve also have the advantage of well-characterised cuff-off values and are widely available in clinical practice. For the diagnosis of cortisol deficiency, these tests are the only established diagnostic measure. (239) Conversely, the direct measurement of serum-free cortisol, which is thought to be the most predictive of the biological activity of the hormone, is too time consuming and labour intensive for routine clinical use and reference ranges are less well-established. (238)

Serum assays can be affected by exogenous and endogenous steroids since many have a similar structure to cortisol. A midnight serum sample assessing for cortisol excess may be the particularly unreliable, as sleep deprivation can increase cortisol levels and venepuncture can lead to stress-induced elevated cortisol levels. (239, 240)

1.4.4.2. Urine

Unbound cortisol is filtered and partially reabsorbed by the kidneys. Under normal conditions, approximately 2% of cortisol is excreted unchanged in the urine. The 24-hour urinary cortisol collection is reflective of cumulative endogenous cortisol secretion during that time. (238) In states of cortisol excess there is a higher 24-hour renal cortisol clearance because the binding capacity of CBG is exceeded and the proportion of free cortisol is increased. (238)

Urinary cortisol collection is a widely available non-invasive outpatient test. (239) However the test is inconvenient and there is often poor compliance, resulting in an unrepresentative 24-hour collection. (241) Metabolites may also interfere with some assays, leading to falsely high cortisol values. (239) Creatine clearance should be established, and urinary cortisol should be adjusted for accordingly. (242)

1.4.4.3. Saliva

The enzyme 11βHSD type 2 is highly expressed in salivary gland tissue, and as free cortisol diffuses through acinar cells into the gland it is rapidly converted into cortisone. The ratio of cortisol to cortisone is reversed in saliva compared to serum samples. (240) Salivary cortisone is detectable at lower levels than salivary cortisol, for example in the afternoon and evening, and is a superior surrogate marker of serum cortisol. (243) Both salivary cortisol and salivary cortisone have a good correlation with serum and plasma free cortisol throughout the circadian cycle and with different dynamic stress tests. (242) For Cushing's syndrome salivary cortisol has a higher sensitivity and specificity than that of urinary cortisol. (239)

Saliva samples are easier to collect, store and transport than urine samples. They can be collected from home and fitted between normal daily activities. The samples can be collected periodically to characterise the individual's cortisol circadian rhythm. Saliva sampling is especially convenient for midnight samples and overcomes an artificial stress-induced cortisol rise from hospital admission and venepuncture. (239)

Patients taking diuretics or who have conditions such as depression and anxiety where xerostomia is commonly observed may find it difficult to provide samples. To avoid blood contamination of samples, patients should abstain from eating or brushing teeth for an hour before sample collection. Self-sampling "user error" may occur through maladherence to sample collection methodology. (239)

1.4.4.4. Hair

Cortisol collected in blood, urine, and saliva are reflective of the acute status of the HPA axis. Hair cortisol is a valuable tool for describing the chronic status of the HPA axis. Hair cortisol, commonly measured as a 3-cm sample taken from the posterior vertex of the scalp, describes mean cortisol concentrations in the preceding three months. (244)

Hair collection is not physically uncomfortable for the patient, and of all the sampling methods, hair cortisol specimens are the easiest to store and transport. The specimen can be separated into segments allowing for retrospective analysis of cumulative cortisol exposure over each month. (244)

Disadvantages of hair cortisol include that it provides no indication of the HPA axis acute stress response or description of the cortisol circadian rhythm, and this method of measuring cortisol is not accommodating to those without hair in the posterior vertex of their scalp. (244) The impact of the difference in growth rates of hair between different ethnic groups on hair cortisol concentrations is also largely unknown. (245) Hair care, for example, washing frequently or bleaching, is known to significantly impact cortisol levels too. (246)

1.4.4.5. Factors to consider

Past medical history alongside current medication use should be considered when selecting the method of measuring cortisol. Medications such as the oral oestrogen-containing contraceptive pill can increase hepatic synthesis of CBG, resulting in falsely higher total serum cortisol concentrations. (247) Similarly, pregnancy can cause a rise in CBG due to the hyperoestrogenic state. (239) Measurements of free cortisol in serum or saliva are unaffected and therefore recommended in these patients. (248, 249) Transdermal oestrogen treatments have demonstrated a lesser effect on circulating CBG levels, and when assessing the HPA axis via any method, it is likely unnecessary to discontinue this treatment. (247) In conditions where hypoalbuminaemia or low CBG concentrations are commonly observed, for example in severe liver disease or critical illness, salivary measurement of cortisol is preferred. In these conditions, measurement of total serum cortisol underestimates free cortisol concentrations, and if these measurements are used to assess adrenal reserve, there is a risk of overdiagnosis of adrenal insufficiency. (239, 240, 250) In patients with severe renal disease, urinary cortisol levels should be interpreted with caution, as the sensitivity to the diagnosis of Cushing's Syndrome is diminished. (242, 251)

1.4.5. Previous Studies of cortisol in Turner Syndrome

To our knowledge, only two studies have investigated cortisol levels in TS in the past decade. The earliest study demonstrated increased basal cortisol levels and an increased peak cortisol response to ACTH stimulation in 44 young people (mean age 14.6 ± 4 years) with TS. (252) These measurements

were time-point specific, which makes the findings difficult to interpret, since cortisol is subject to diurnal and pulsatile variability and influenced by acute stress. (202, 203) A study of hair cortisol, which reflected mean cortisol concentrations in the preceding three months, attempted to circumvent these factors. Significantly higher measurements in a cohort of women with TS compared to age matched healthy control subjects were observed. This report of chronically high cortisol levels may be clinically meaningful, as increased cortisol exposure in these individuals with TS correlated inversely with height and positively with total cholesterol levels. (253) To our knowledge the circadian profile of cortisol has not been evaluated in the TS population.

1.5. Possible impacts of glucocorticoid excess on cardiovascular health in Turner Syndrome

Patients exposed to glucocorticoid excess have a number of cardiovascular risk factors common in TS, including hypertension, increased visceral fat and reduced muscle mass, and higher prevalence of glucose intolerance and T2DM. (254) Patients with subclinical Cushing's Syndrome are also at an increased risk of cardiovascular events and mortality. (255) In the general population, elevated cortisol levels are positively associated with risk factors, such as insulin resistance and low high-density lipoprotein cholesterol (HDL-C), for CVD development. (256) Despite these similarities (Table 1.1), there are very few data describing cortisol concentrations in patients with TS.

Table 1.1 | Similarities and differences of the phenotype of children with Cushing's Syndrome and Turner Syndrome.

Similarities	Differences
Hypertension	Mechanism of growth failure
Decreased bone mineral content	Mechanism of delayed puberty
Central obesity and visceral obesity	Mechanism of infertility
Reduced muscle mass	
T2DM	
Neurocognitive and psychiatric problems	

Abbreviations – T2DM: Type 2 diabetes mellitus.

In the Sava et al. study of hair cortisol, patients with TS displayed a worse cardiometabolic profile, consisting of higher fasting glucose and TG levels, than the age-matched control subjects. Furthermore, the higher hair cortisol concentrations in this cohort of TS patients were associated with

higher total cholesterol levels, which may indicate that elevated cortisol levels are an additional detrimental contributing factor to poor cardiovascular health in TS. In this study, BP was not measured and there was no assessment of vascular function. (253)

The method of cortisol measurement in the Sava et a. study did not allow for assessment of the circadian profile of cortisol in this cohort. (253) This profile is of interest as it is important in the evolution of cardiovascular and metabolic disease. Disruption of this physiological profile is associated with cardiovascular and metabolic disease in shift workers. (257-259)

The cardiovascular comorbidities associated with excess glucocorticoid exposure usually manifest after prolonged periods. (260) A child and adolescent population has been chosen for this pilot study to assess for pre-clinical markers of atherosclerotic disease in TS.

1.5.1. Aims of the thesis

This pilot study will investigate the acceptability of a study protocol that will describe the circadian profile of salivary cortisol and cortisone in girls with TS, compare these measurements to profiles obtained from 100 healthy children and young people, (243) and describe associations between salivary cortisol and cortisone and markers of evolving cardiovascular risk. This pilot study will generate data to inform the design of a much larger study, powered adequately to determine whether cortisol profiles in girls with TS differ to those in unaffected girls, and how these relate to risk factors for cardiovascular disease. The data generated in this pilot study will be included in the definitive study.

The pilot study protocol is introduced in Chapter 2. Chapter 3 presents the study feasibility data and Chapter 4 presents the study experimental data. Chapter 5 is an audit evaluating how current routine clinical care of girls and adolescents with TS in the Liverpool region compared to the recommendations from the 2016 Cincinnati TS Clinical Practice Guidelines. Chapter 6 is a national paediatric BP survey. Finally, Chapter 7 discusses the strengths and weaknesses of this work, future directions, and implications for clinical practice.

2. The STARRY study

2.1. Study set-up and design

For ease, due to the long study name, it was decided that this study would be called the STARRY Study (a pilot study of Salivary corTisol and cortisone profiles and associations with cArdiovascular Risk profile in paediatric patients with tuRner sYndrome). It was felt that this study name was inviting and memorable. A study logo was designed and was included on all patient-facing documentation (Figure 2.1).

Figure 2. 1 | The STARRY study logo.

2.1.1. Study setting

The STARRY study was conducted on the National Institute for Health and Care Research (NIHR) Clinical Research Facility (CRF), Alder Hey Children's NHS Foundation Trust, Liverpool. Founded in September 2012, it is one of the four paediatric-exclusive NIHR funded research facilities in the United Kingdom. The NIHR Alder Hey CRF team consists of more than 20 full-time staff members, including paediatricians, research nurses, play specialists and trial pharmacists, who work together supporting research projects helping to improve the health and wellbeing of children and young people. In October 2021, a presentation was given to the CRF staff on TS and the rationale behind the STARRY study. The presentation included a discussion about how the study activities would be best executed on the CRF. The STARRY study team members included Miss Jones (LJ), Mr Dliso (SD), Dr Park (JP), Professor Blair (JB), Dr Hawcutt (DH), Dr Shantsila (AS) and Professor Lip (GL).

2.1.2. Study protocol

The STARRY study protocol below was approved by JB, GL and AS.

Aims:

1. To determine the acceptability of the STARRY study protocol to girls with TS and their parents/carers.

Objectives:

1. To describe how many girls with TS, given information about this study, consent to participate and how many decline.
2. To describe the number of girls who gave consent to participate in the study and completed the study protocol.
3. To identify, which of the study procedures were not completed in 25% or more of study participants.

Exploratory analyses

To describe salivary cortisol and cortisone circadian profiles, blood glucose and BP profiles, and potential biochemical and vascular mechanisms to look for evidence that:

(1) Salivary cortisol and cortisone profiles in girls with TS differ to healthy girls following correction for BMI.

(2) Salivary cortisol and cortisone profiles are associated with BP disruption, endothelial dysfunction and biochemical determinants of cardiovascular risk.

2.1.3. Study design materials

All patient-facing documentation, which includes all of the below, excluding the Case Record Forms, was submitted to the Hampshire Research Ethics Committee (REC) for feedback and approval in September 2021.

2.1.3.1. Patient Information Leaflets

JB created a parent information leaflet and age-appropriate patient information leaflets for the study. The following age groups were chosen for the information leaflets: below 8 years, 8-12 years, 13-15 years, 16-18 years. These five leaflets varied in length, using language suitable to each age group with an aim to describe the study in a way which could be easily understood. The leaflets provided information about why they had been chosen to take part in the study, gave some background information on cortisol, described what would happen at each study visit if they chose to participate,

the risks and benefits of participating, and how we would use their information. These information leaflets were shared mostly as paper-copies in clinic or via email if the patient's appointment was held virtually.

2.1.3.2. Consent/Assent Forms

The following consent and assent forms were devised for the study:

- Consent Form for 16-18 years
- Consent Form for a child with Gillick competence*
- Parental Responsibility Consent Form
- Assent Form for 13-15 years
- Assent Form for children aged 5-12 years

*Assessed by a member of the study team

The study was explained in detail at the first study visit (LJ) and the appropriate consent / assent form(s) were signed at the first study visit to provide documentation that the participant clearly understood why we were doing the study and what participating would mean for them (LJ and SD/JP/JB). It gave participants and parents/carers, the opportunity to discuss the study again and to have answers to any outstanding questions that they might have. The original signed forms were stored in the paper site file and electronic copies were uploaded to the electronic site file and the participants' Alder Hey Records. Photocopies of the signed forms were also given to the participant to take home.

2.1.3.3. Study visit Questionnaires

Questionnaires were devised to record participant feedback on study activities. Inspiration was taken from a research pharmacists' feasibility study which assessed the acceptability of different sized placebo tablets in children. Most questions employed a 10-point Likert-style scale (Figure 2.2).

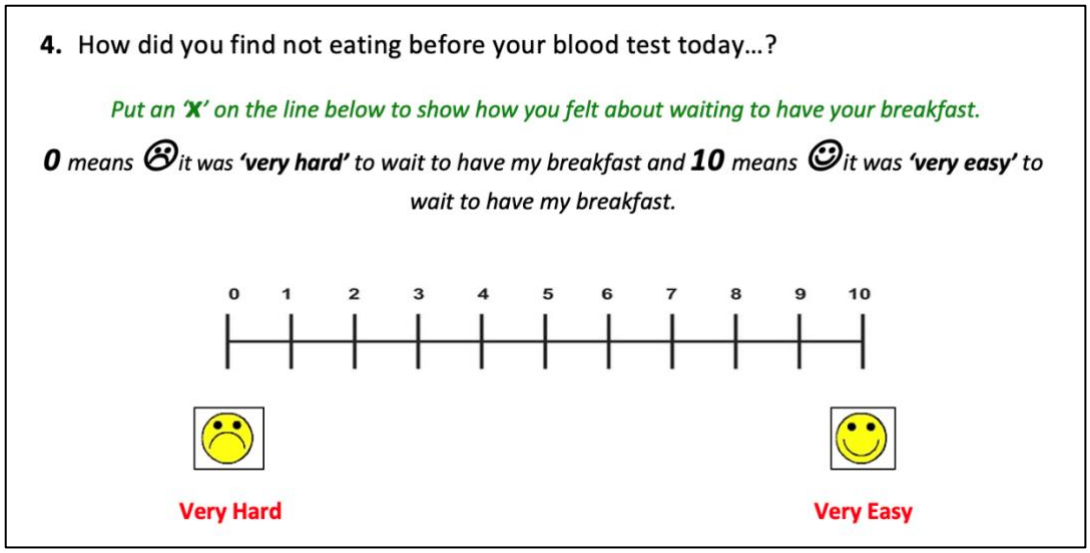


Figure 2.2 | Example Likert-type scale question (Question 4, Participant Questionnaire Visit 1).

Before completing the first participant questionnaire, participants completed a practice questionnaire. This questionnaire had been used previously in Alder Hey sponsored feasibility studies. It provided a worked-example and two opportunities for participants to practice answering questions. This helped to indicate the level of support that the participant would require when answering the study questionnaires, for example needing questions to be read aloud by a member of the study team.

The first participant questionnaire was completed at the end of the first study visit and included questions on the fasted blood test and vascular scanning. The second participant questionnaire was completed at the end of the second study visit and gathered feedback on the study activities completed by the participant at home (wearing the BP monitor and glucose monitor and completing the saliva samples). The parent/carer questionnaire was also completed at the second study visit. This questionnaire included questions on completing saliva samples at home and the number of trips to Alder Hey that the study required.

2.1.3.4. Salivary Sample Record Logbook

A Patient Information Booklet used in another Alder Hey sponsored study, a study of salivary biomarkers (SMILE study), was adapted for the STARRY study. The 5-page booklet included instructions on how to collect and store saliva samples (Figure 2.3), reminders to not eat or brush teeth an hour before sample collection and to wait two hours between samples, and a table to document timings of saliva sample collection.

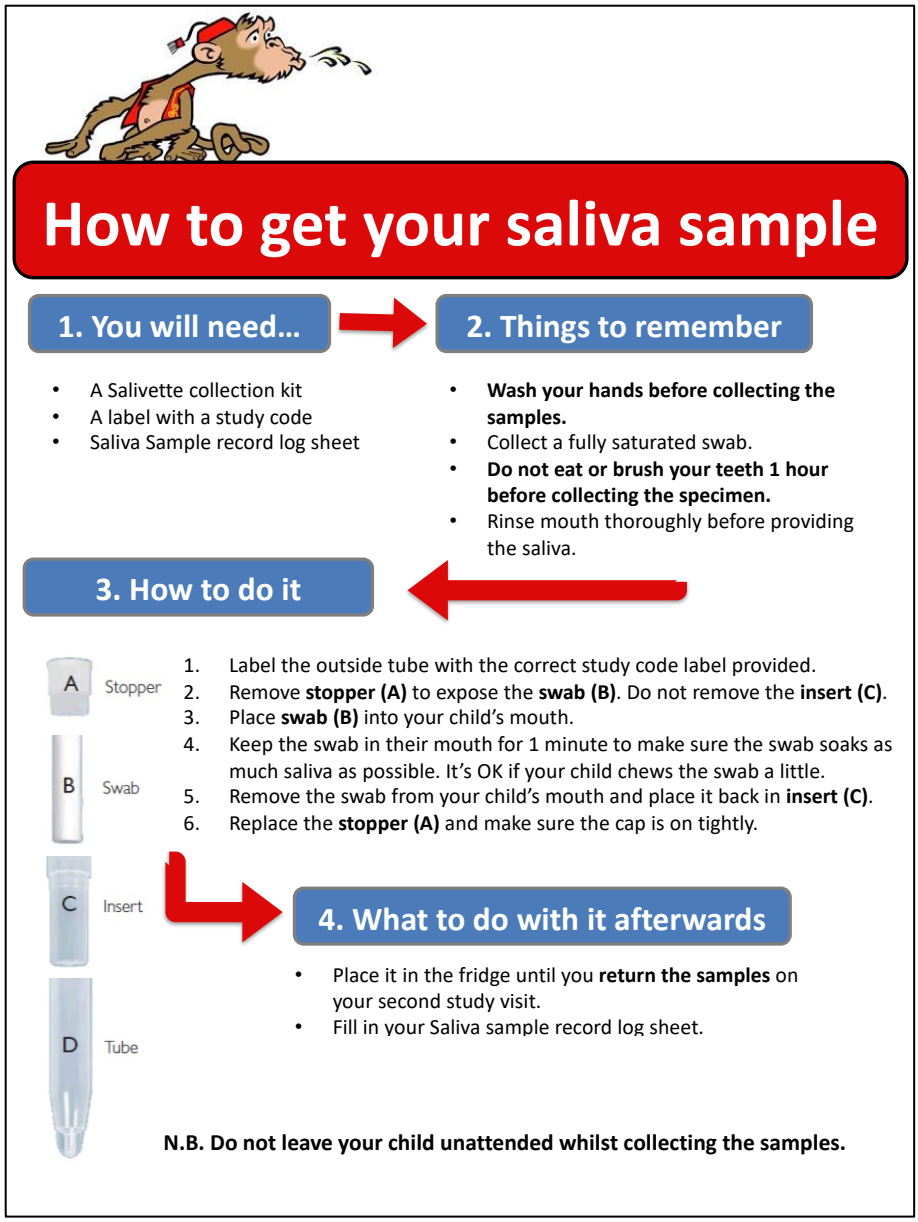


Figure 2.3 | Instructions on how to provide a saliva sample (Page 2, Saliva sample record logbook).

This booklet was collected when the saliva samples were returned to Alder Hey. Timings of sample collection documented in this booklet were referenced against the timings documented on the salivettes/salivette bag labels.

2.1.3.5. Case Record Form

Two case record forms were created for the study team to use at the two study visits. Both forms acted as a checklist and a place for documentation of the date/time of completion of each study activity. The case record form for the first study visit gathered clinical information about the

participant: their date of birth, sex, ethnicity, birth weight, gestational age, antenatal history, family history of CVD, medical history and current medication use. This form also included space to record the participants height, weight, BP readings and date of last menstrual period (if applicable).

2.1.4. Research Ethics Committee

The STARRY study was discussed at a virtual REC meeting held on the 14th of September 2021. The Hampshire REC issued a provisional opinion and gave details of further required action (Figure 2.4).

Ethical Review – Further information required

	Ethical Review - Further Information required	Response from the applicant
1	If the participants or carers will be expected to come for 2 additional visits and no travel expenses will be offered, then please ensure this is clearly highlighted in the PIS.	We have added that we unfortunately cannot offer travel reimbursement for the study visits to the Patient Information Sheets (ages 13-15, 16-18 and Parent Leaflets).
2	The additional participant information provided in the separate information sheets should be combined within the main Participant Information Sheet.	We have combined the main Participant Information Sheet with the Additional Information Sheet to form one document.
3	The Participant Information Sheets should be thoroughly proofread as there are a number of errors and grammatical mistakes. There is also a lack of consistency in the different Information Sheets. It will also be useful to consult a patient group regarding content and design of the Information Sheets.	The Participant Information Sheets have been thoroughly proofread and we have ensured that the information within the different Information Sheets is consistent. We have shared the Participant Information Sheets with the Turner Syndrome Support Society and are awaiting a response from them. We will seek amendments if concerns are raised.
4	One of the main aims of the study is to assess the acceptability of the protocol, but the study does not involve collecting qualitative data. As discussed at the meeting please provide the additional questions/documentation that will be used to collect this data.	We have provided the additional documentation that will be used to collect the data to inform the acceptability of the study protocol. This includes a Practice Questionnaire, Participant Questionnaire Visit 1, Participant Questionnaire Visit 2, Parent/carer Questionnaire and Saliva Sample Logbook.
5	It should be stated in the PIS that this study is being conducted as a part of an MPhil degree by Ms Lily Jones.	A note about this study being conducted as part of an MPhil degree has been added to all Participant Information Sheets.

Assessment - Further information required

Assessment - Further Information Required	Response from the applicant
IRAS A45 states "Paper storage will be off site for ten years". Please clarify where exactly this is and what the arrangements are in terms of access.	The secure archiving facility we utilise is:- Restore 7 Overman Way Agecroft Commerce Park Swinton Manchester M27 8UJ We are able to recall data the next working day.
The PIS states that samples will be stored in Alder Hey for 5 years, IRAS A43 states that personal data will be stored or accessed between 3-6 months after	Identifiable data is stored for 3-6 months after the study is ended then removed.

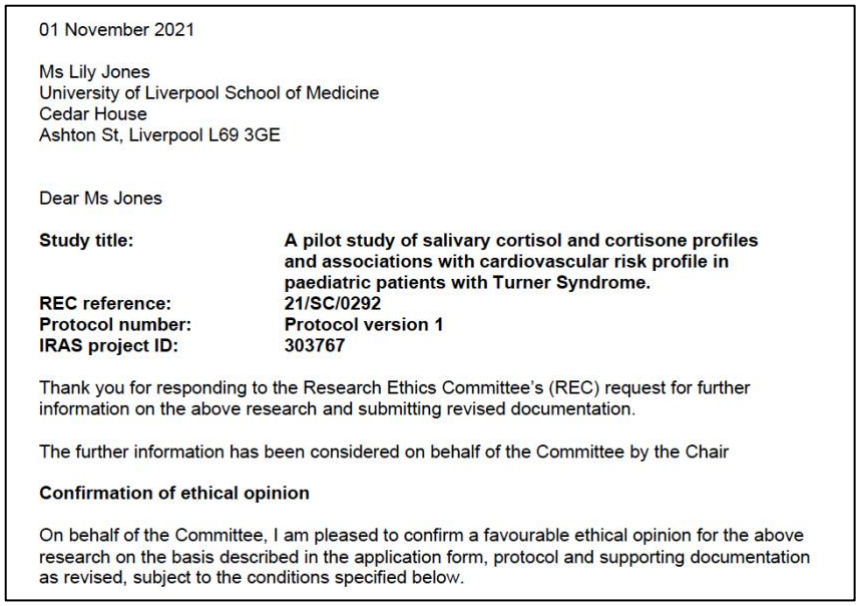
Assessment - Further Information Required	Response from the applicant
the study has ended, IRAS Part B Section 5 question 8 states that samples will be stored in a form that is identifiable to researchers and question 14 of this same section states that the samples will be disposed in accordance with the Human Tissue Authority's Code of Practice at the end of the research. Given these contradictions, please clarify what will happen to the participants samples at the end of the study.	When a participant consents to take part in the study, they are given a study number and this is then utilised on all data and samples. The pseudo anonymised samples are stored for 5 years and then disposed of in accordance with the human tissue act.
In the 13-15year PIS under the heading "what will happen to my information", it states that their data will be kept for 10years after the study which corresponds with information provided in IRAS A45 but this is not stated in the parent or 16-18 PIS.	We have added that data will be kept for 10 years to the 16-18 Participant Information Sheet and Parent/carer Information Sheet.

Figure 2.4 | Health Research Authority and Health Care Research Wales ethical review and assessment submitted for the STARRY study. The left column details the required changes, and the right column is our response.

One of these actions included consulting a patient group regarding the content and design of the Information Leaflets, so we invited the UK Turner Syndrome Support Society (TSSS) to share their thoughts. The TSSS Executive Officer distributed these leaflets to a couple of girls in each age group and their parents, and gave the following feedback:

- *"Both the 13-15 year and the parents' leaflet were easy to read and myself and XXXX understand everything."* – Parent from 13-15 years age category
- *"I read the parents leaflet and thought it was very thorough and well explained. It equipped me with all the knowledge of the study and included information about cortisol which I was able to explain to XXXX when she needed more information."* – Parent from 8-12 years age category

It was reassuring that the study had some interest and that the girls and their parents from the TSSS were able to understand our Information Leaflets. We responded to the committees' comments and a final favourable ethical opinion was issued by the chair of the Hampshire REC on the 1st of November 2021 (Figure 2.5). Health Research Authority and Health Care Research Wales approval was issued on the 3rd of November (Figure 2.6).



01 November 2021

Ms Lily Jones
University of Liverpool School of Medicine
Cedar House
Ashton St, Liverpool L69 3GE

Dear Ms Jones

Study title:	**A pilot study of salivary cortisol and cortisone profiles and associations with cardiovascular risk profile in paediatric patients with Turner Syndrome.**
REC reference:	**21/SC/0292**
Protocol number:	**Protocol version 1**
IRAS project ID:	**303767**

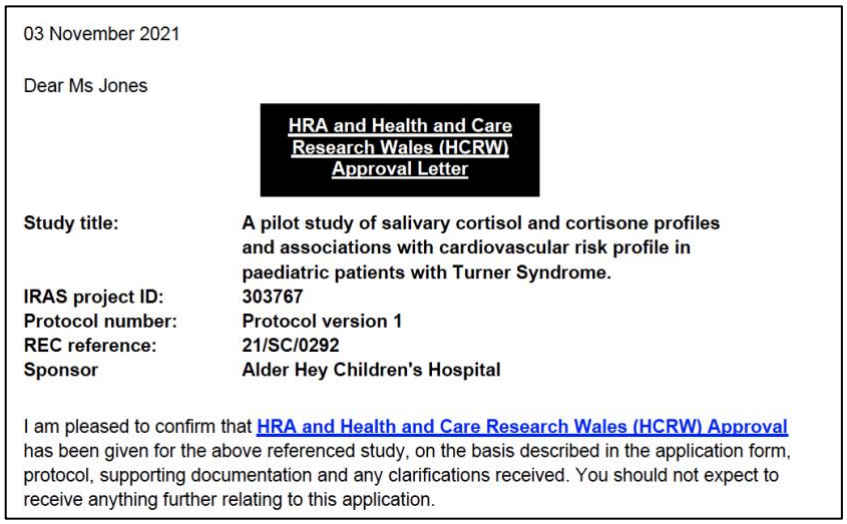
Thank you for responding to the Research Ethics Committee's (REC) request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Figure 2.5 | Research Ethics Committee approval letter for the STARRY study.



03 November 2021

Dear Ms Jones

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**A pilot study of salivary cortisol and cortisone profiles and associations with cardiovascular risk profile in paediatric patients with Turner Syndrome.**
IRAS project ID:	**303767**
Protocol number:	**Protocol version 1**
REC reference:	**21/SC/0292**
Sponsor	**Alder Hey Children's Hospital**

I am pleased to confirm that HRA and Health and Care Research Wales (HCRW) Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Figure 2.6 | Health Research Authority and Health Care Research Wales approval letter for the STARRY study.

It was interesting to observe a second ethics meeting for another Alder Hey sponsored study with a similar study protocol. This meeting was hosted by a different REC, and the requirements from this committee greatly differed.

2.1.5. Study opens to recruitment

Alder Hey, the sponsor of the STARRY study, issued "green light" on the 3rd of December 2021 (Figure 2.7). All study activities could commence from this date onwards. For "green light" to be issued, the

master files had to be created, all study materials to be sourced, and external agreements signed by the relevant organisations.

Good Afternoon,

Confirmation of Sponsor 'Green Light' Activation – Alder Hey

Short Title:	**A pilot study of Salivary corTisol and cortisone profiles and associations with cArdiovascular Risk profile in paediatric patients with tuRner sYndrome (STARRY)**
IRAS:	**303767**
Chief Investigator:	**Professor Jo Blair**

I am pleased to inform you that all conditions have now been satisfied to confirm sponsor 'green light' activation. Please consider this email as confirmation that **you may commence study activities with effect from 3rd December 2021.**

Please file a copy of this email in your Trial Master File as confirmation of sponsor green light activation.

I would like to take this opportunity to wish you every success with the study and please do not hesitate to contact me if I can provide any further assistance.

Kind Regards
Linda

Figure 2.7 | Confirmation email from sponsor of 'Green Light' activation for the STARRY study.

2.1.5.1. Material transfer agreements

Material transfer agreements were signed between Alder Hey NHS Foundation Trust and University Hopsital Southampton NHS Foundation Trust, Cambridge University Hospitals NHS Foundation Trust and Wythenshawe Hospital, for renin, leptin and saliva samples, respectively. All samples were agreed to be transferred to the relevant site when the study closes.

2.1.5.2. Dexcom

Dexcom ®, founded in 1999, is an internationally operating company that develops and manufactures continuous glucose monitoring (CGM) systems for the management of diabetes mellitus. The company supports external research projects, encouraging the use of CGM in study protocols for original diabetes-related research.

Hyperglycaemia as a contributor to cardiovascular risk was a variable considered in the exploratory analysis of the STARRY study. On the 11th of October 2021, we applied through the Dexcom External Research Portal for 2 receivers, 20 transmitters and 8 boxes of sensors. Our request was approved on the 26th of October 2021 and the items were sent free-of-charge to Alder Hey (Figure 2.8).

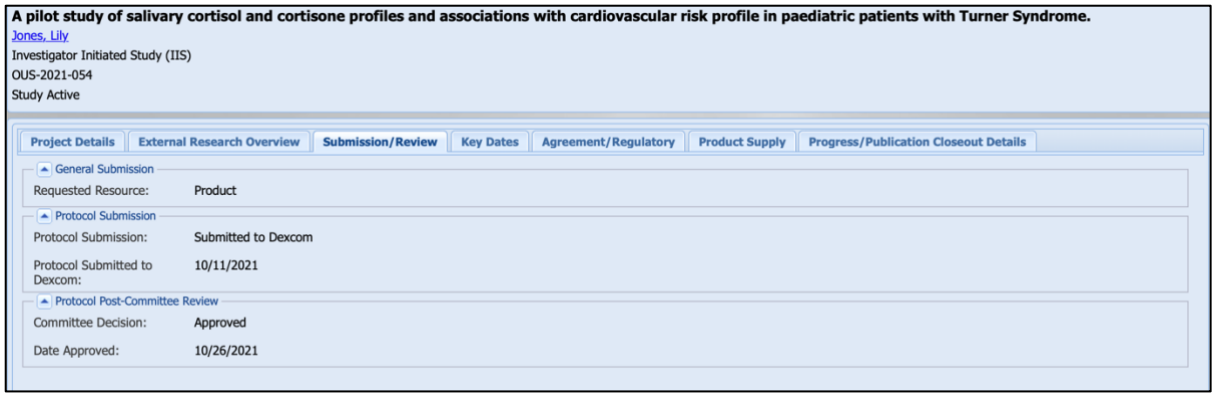


Figure 2.8 | Screenshot of Dexcom's External Research Portal for the STARRY study (April 2022).

Before study materials were dispatched, online training for the navigation of the online platform "Clarity" with a member of the Dexcom team was completed.

As part of the agreement support terms, quarterly study progress reports were sent to Dexcom. These periodic updates allow Dexcom to verify the status of the study and to accommodate ongoing study needs.

2.1.5.3. Site File

An electronic and paper site file were created. These files contain the study protocol, all patient information leaflets, and blank copies of consent/assent forms, visit questionnaires, case record forms and saliva sample record logbooks. They also contain Health Research Authority approval, legal agreements, documentation regarding funding and information about the study team. Signed consent/assent forms were uploaded to the electronic site file. The paper site file, located in the CRF, contains all documentation completed at the study visits, including original copies of consent/assent forms.

2.2. Recruitment

Hospital numbers were provided by JB's secretary for all girls with a known TS diagnosis under regular follow-up at Alder Hey Children's NHS Foundation Trust, Liverpool.

2.2.1. Inclusion/Exclusion Criteria

To be invited to participate in this study, patients were required to fulfil the following inclusion criteria:

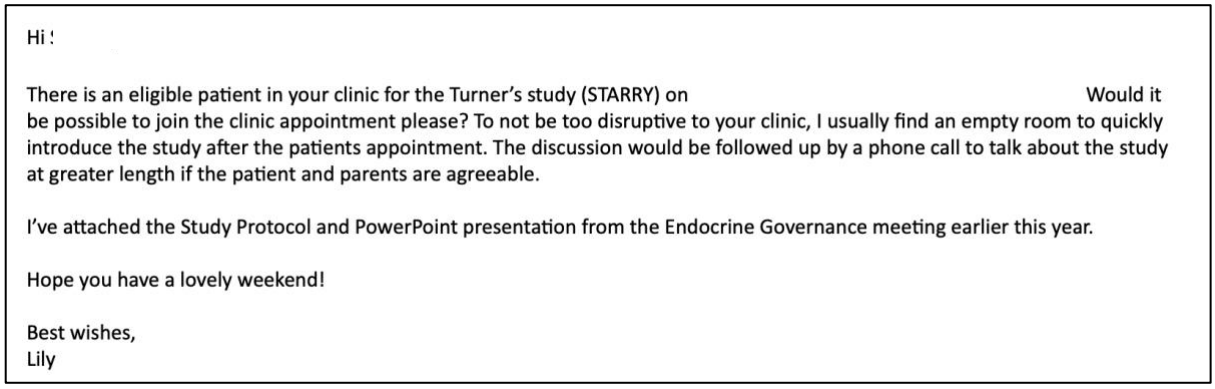
- Aged between five and 18 years old at time of recruitment.
- Have a diagnosis of TS, which has been genetically confirmed via karyotyping.

In addition, the following exclusion criteria were in place:

- Patients with additional diagnoses or treatment likely to influence blood glucose or BP (for example T1DM or chronic kidney disease).
- Patients with oral lesions likely to result in blood contamination of saliva samples.

2.2.2. Approaching patients

Girls with TS are under routine surveillance from a number of medical and surgical specialities and were invited to take part in the study during any outpatient clinic appointments at Alder Hey Children's NHS Foundation Trust, Liverpool. It was assumed that most participants would be recruited from Endocrinology, since this is the speciality where the girls have the most frequent follow-up. A presentation (LJ) was given to the endocrine team introducing the STARRY study at an Endocrine Governance Meeting in October 2021. Before each eligible patient's Endocrinology clinic appointment, the consultant in charge of the patient's care was emailed (LJ) to ask permission to attend the appointment and introduce the study (Figure 2.9). If the patient had an appointment in a different specialty, then the study was introduced via email (JB) to the consultant in charge of the patient's care (Figure 2.10).



Hi !

There is an eligible patient in your clinic for the Turner's study (STARRY) on Would it be possible to join the clinic appointment please? To not be too disruptive to your clinic, I usually find an empty room to quickly introduce the study after the patients appointment. The discussion would be followed up by a phone call to talk about the study at greater length if the patient and parents are agreeable.

I've attached the Study Protocol and PowerPoint presentation from the Endocrine Governance meeting earlier this year.

Hope you have a lovely weekend!

Best wishes,
Lily

Figure 2.9 | Example email to a member of the endocrine team.

Hi

Hope all well.

We are currently recruiting patients with Turner Syndrome to a study of metabolic and cardiovascular health.

We think that who is due to attend your clinic tomorrow, might be eligible to participate.

Please could my MPhil student, Lily (cc'd) join you in clinic to share the Patient Information Leaflets? She would only need a small amount of time to introduce the study, and would make arrangements to call the family in a week or so if they are interested in learning mare about the study.

Many thanks indeed,

Jo

Figure 2.10 | Example email to a consultant who is not a member of the endocrine team.

2.2.3. Follow-up Phone call

If patients showed interest in participating in the study at their clinic visit, a follow-up phone call was arranged for a convenient time the following week. This gave potential participants time to thoroughly read the patient information leaflets and discuss the study with anyone they wanted to. Most patients (85%) booked a phone call.

The follow-up phone call (LJ) was an opportunity to ask any questions about the study that they had after reading the information leaflets. If they wanted to go ahead with the study, a mutually convenient date was decided on for the first study visit. The study team (LJ) would then book the visit into the CRF and, if appropriate, would request the 24-hour ambulatory blood pressure monitoring (ABPM) and a play specialist for the visit.

2.2.4. Confirmation of visit

An email was sent to the participant to confirm the date/time of the visit (Figure 2.11).

Hi

It was lovely to speak to you again this evening. Thank you again for your interest and support with the STARRY study.

For the first study visit on Wednesday the 20th of April please could you attend the Clinical Research Division (1st Floor, below L2 outpatients) at 8:30am. As we discussed, you will need to come to Alder Hey fasted (you can still have water, just no food or caffeine), but we will give you a voucher for the canteen to use as soon as we have done the bloods and scans. Most importantly, I'll buy you a coffee!

We will need the BP monitor to be returned to Alder Hey the following day (Thursday 21th April) please. This is because we don't want to lose any data collected. You don't need to be present for this, someone can just drop it at reception.

The second study visit will be on Wednesday the 27th of April. This doesn't need to be at a specific time, so we can organise this at the first study visit. I will be free all day and the visit should only take 30 mins to remove the glucose monitor and fill out some quick questionnaires, so I can work around what works best for you (after school ect).

I will give you a call after 4pm on Monday the 18th of April to check everything is okay ahead of the first study visit. We will be going through the consent process on the morning of the visit, and as with all research, you can change her mind at any time!

Just some things we discussed:

- The glucose monitor has no alarms, so won't disrupt your exams. You can either leave the device in your bag during the exams or we can write a letter to your school.
- The BP monitor shouldn't affect your exercise or daily activities (the glucose monitor won't too).
- We will be doing height and weight, but you absolutely don't need to see these if you don't want to!

Any questions, please do not hesitate to contact me via this email.

I'm looking forward to seeing you soon!

Best Wishes,
Lily

Figure 2.11 | Example of an email to a participant confirming their first study visit.

A member of the study team (LJ) would call the participant one or two day(s) before their first scheduled study visit. This was to confirm their attendance and to answer any further questions that they had. It was also an opportunity to remind the participant that they should arrive to Alder Hey fasted and to check their COVID-19 status. Following this phone call, equipment was set up in the CRF in preparation for the visit, and if the participants routine TS bloods were due, then they would be requested on the Alder Hey system.

2.3. Study visits

The STARRY study consisted of two study visits to the CRF. An example of each study visit can be found below. The study visits were adapted in accordance with the participants needs, for example, if they were needle-phobic then we would do venepuncture at the start of the visit to relieve anxiety. The date and time of completion of each study activity were recorded in the appropriate case record form throughout the visit.

2.3.1. First study visit

Table 2.1 | Order of first study visit activities.

Category	Activity	Study member
Arrive on CRF at 08:30	Confirm patient has fasted.	LJ
	Meet the CRF team (including play specialist).	LJ, SD, JP/JB
	Discuss study again and decide if happy to procced.	LJ, SD, JP/JB
	Consent/Assent signed (see 2.1.3.2).	LJ, SD, JP/JB
Study Procedures	Demonstration on how to collect saliva samples.	LJ
	First saliva sample collection (Time Point 1).	LJ
	Clinical questions on Case Record Form completed with person with parental responsibility (see 2.1.3.5).	LJ
	3x Left-arm Clinic BP recordings 1 minute apart (Welch Allyn Propaq LT). The mean of the last two readings were taken as the participants clinic BP.	LJ/SD
	Local anaesthetic cream applied for bloods and CGM insertion, if applicable.	LJ/SD
	Height recorded (calibrated stadiometer).	LJ
	Weight and body composition recorded (InBody scales).	LJ
	Flow-mediated dilation of right brachial artery completed **(9.1. Appendix 1).**	SD
	Carotid-intima media thickness of right carotid artery completed **(9.2. Appendix 2**).	SD
	CGM inserted **(9.3. Appendix 3).**	LJ
	Venepuncture[a] completed.	SD
Breakfast	Participants given breakfast voucher.	LJ
Study Procedures	Practice Questionnaire completed (see 2.1.3.3).	LJ
	Participant Questionnaire Visit 1 completed.	LJ
	Participants parent/carer given saliva sample record logbook and 8 labelled salivettes and 8 labelled salivette bags (see 2.1.3.4).	LJ
	Education on saliva sample collection[b] and wearing the CGM[c]. Understanding was checked by asking to recall the information given.	LJ
	24-hour ABPM fitted (Welch Allyn)[d,e].	Cardiology
Participant discharged from CRF	Second study visit to CRF booked.	LJ
	Follow-up phone call to check for problems with collecting the saliva samples or wearing the ABPM or CGM.	LJ

Abbreviations – CRF: clinical research facility; BP: blood pressure; CGM: continuous glucose monitor; ABPM: ambulatory blood pressure monitor; LJ: Miss Jones; SD: Mr Dliso; JP: Dr Park; JB: Professor Blair.

a – The STARRY study anonymised blood tests require a 12.1ml sample for: lipids, urea and electrolytes, fasting glucose, von-Willebrand's factor, insulin, cortisol, leptin, renin. An additional 9ml of blood was collected if the participants annual TS blood tests are due (thyroid function, thyroid peroxidase enzyme, liver function, HbA1c, luteinising hormone, follicle stimulating hormone, oestradiol, anti-Mullerian hormone, vitamin D, bone profile).

b – The participant collected a sample every two waking hours for the rest of the day and a sample between 11pm and midnight. They also collected a sample upon waking the following morning. Samples were stored in the participants fridge and returned to Alder Hey on the second study visit.

c – The participant was reminded to keep the glucose receiver within 6 metres of themselves and to charge the receiver regularly.

d – If the participant was over 10 years old and was expected to tolerate ABPM.

e – The cardiology team provided education on this equipment, for example the participant cannot shower with the monitor. The monitor was to be returned to Alder Hey the following day.

2.3.2. Second study visit

Table 2.2 | Order of first study visit activities.

Category	Activity	Study member
Participant arrives on CRF	Collection of saliva samples and saliva sample record logbook (see 2.1.3.4).	LJ
Study procedures	CGM removed **(9.4. Appendix 4).**	LJ
	Parental questionnaire (see 2.1.3.3).	LJ
	Participant Questionnaire Visit 2 completed.	LJ
Participant discharged from CRF	Thanked for participating in the study.	LJ

Abbreviations – CGM: continuous glucose monitor; CRF: clinical research facility; LJ: Miss Jones.

2.3.3. Play specialist support

The role of a play specialist is to support medical and nursing staff to provide a positive patient experience for children/young people and their parents/carer(s). During difficult experiences in hospital, play specialists can use distraction techniques with the intention of shifting the child's focus away from what is distressing them. The relationship between play specialists and patients are built on trust, and before providing distraction for procedures, the play specialist will spend time with the

patient to get to know them. On medical or surgical wards, play specialists can additionally help to relieve boredom, prepare patients for surgery or procedures and aid child development.

2.3.3.1. Role in the STARRY Study

International guidelines recommend lifelong annual blood tests for patients with TS. (4). All participants were offered the option of play specialist support and anaesthetic cream (Ametop Gel 4% ®). In participants who were anxious about venepuncture, we also adjusted the usual order of study activities: venepuncture was completed first, with the aim of relieving anxiety for the remainder of the study visit.

The play specialist attached to the CRF supported two participants through their first STARRY study visit. Before entering the main CRF ward, both participants and their parent/carer(s) met the play specialist and discussed any worries about the morning ahead. This was also an opportunity for the play specialist to get to know the participant.

The first participant was 14.6 years of age and had an additional diagnosis of ADHD. The participant found it difficult to stay still during the vascular scanning and venepuncture. She engaged well with the play specialist during these study activities, enjoying a 'smell game', and subsequently the study team were able to complete the procedures.

The second participant was 9.01 years of age and had significant developmental delay. She enjoyed getting a sticker after completing each study activity. She was anxious for venepuncture and engaged in 'Where's Wally' as a distraction with the play specialist.

2.4. Statistical analysis

The Statistical Package for the Social Science (SPSS) version 27.0 software for Windows (IBM Corp, Armonk, NY, USA) was used for the statistical analysis. The data distribution was assessed using the Shapiro-Wilk test. Student *t*-test was used for normally distributed data whereas non-normally distributed data was evaluated with the Mann-Whitney-U test. The Pearson's chi square test was applied to continuous variables. A *p*-value of <0.05 was considered statistically significant.

Linear correlation was assessed with the Pearson's correlation coefficient: a coefficient of <0.30 was considered negligible, 0.30-0.50 low correlation, 0.50-0.70 moderate correlation, 0.70-0.90 high correlation and 0.90-1.00 very high correlation.

GraphPad Prism version 8.0 for Windows (GraphPad Software, San Diego, CA, USA) was used to present correlation data and calculate area under the curve (AUC). Microsoft® 365 Subscription Version 16.61 was used to graphically present data from questionnaire responses.

3. The STARRY study – Feasibility results

This chapter aims to describe the feasibility and participant acceptability of the STARRY study protocol.

3.1. Recruitment

As of October 2021, there were 40 patients under regular clinical follow-up in the TS service at Alder Hey Children's Hospital. Seven of these patients were excluded from STARRY study recruitment due to their young age or additional diagnosis of T1DM. Patients aged below five years were excluded from the study as it was believed that they would not reliably tolerate the activities involved in study participation, while those with T1DM were excluded as their study assessments, in particular glucose profiles, will vary, but not in a way that is related to TS. Consultant advice, in reference to complex psychosocial factors such as gender dysphoria and lack of knowledge of their TS diagnosis, excluded four additional patients from recruitment. A patient living in the Isle of Man was excluded from recruitment due to the distance from the study site: we were unable to offer travel reimbursement for the three visits to Alder Hey as part of the STARRY study. This left 28 patients to introduce the STARRY study to (Figure 3.1).

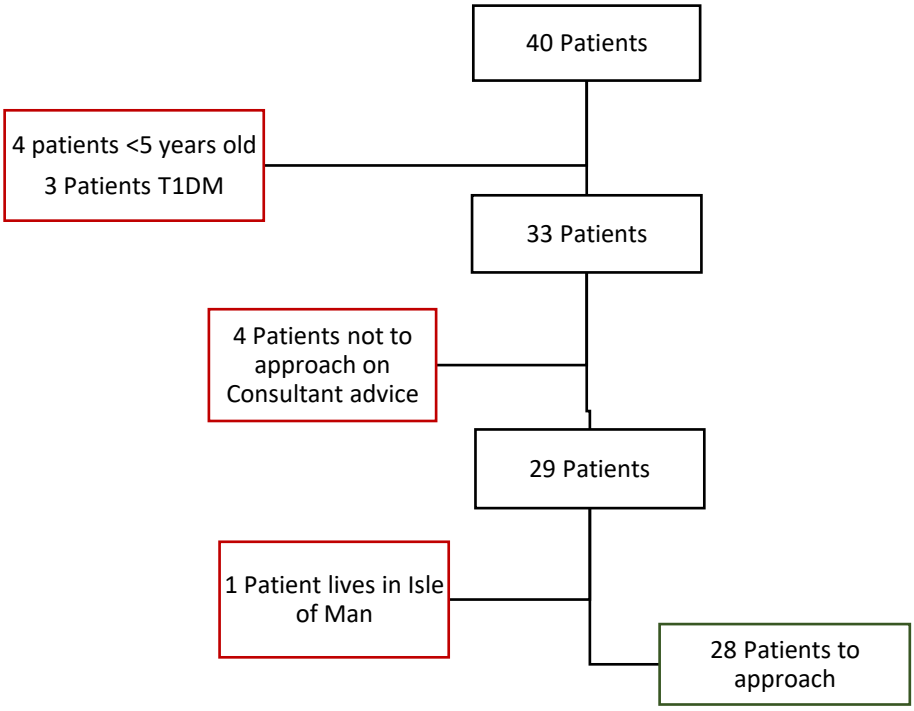


Figure 3.1 | Diagram showing the number of patients who could be approached and introduced to the STARRY study at Alder Hey Children's Hospital. T1DM: type 1 diabetes mellitus.

The recruitment period of the STARRY study lasted five months (between the 15th of December 2021 and the 19th of May 2022). Of the 28 patients identified, 24 had clinic appointments scheduled during the study recruitment period. The remaining four patients had outreach Endocrinology appointments at their local hospital or were last seen in clinic in November 2021, just before the study opened for recruitment. Four patients were not brought to their scheduled clinic appointments. Therefore, 20 patients were able to be identified and provided with information about the STARRY study (Figure 3.2).

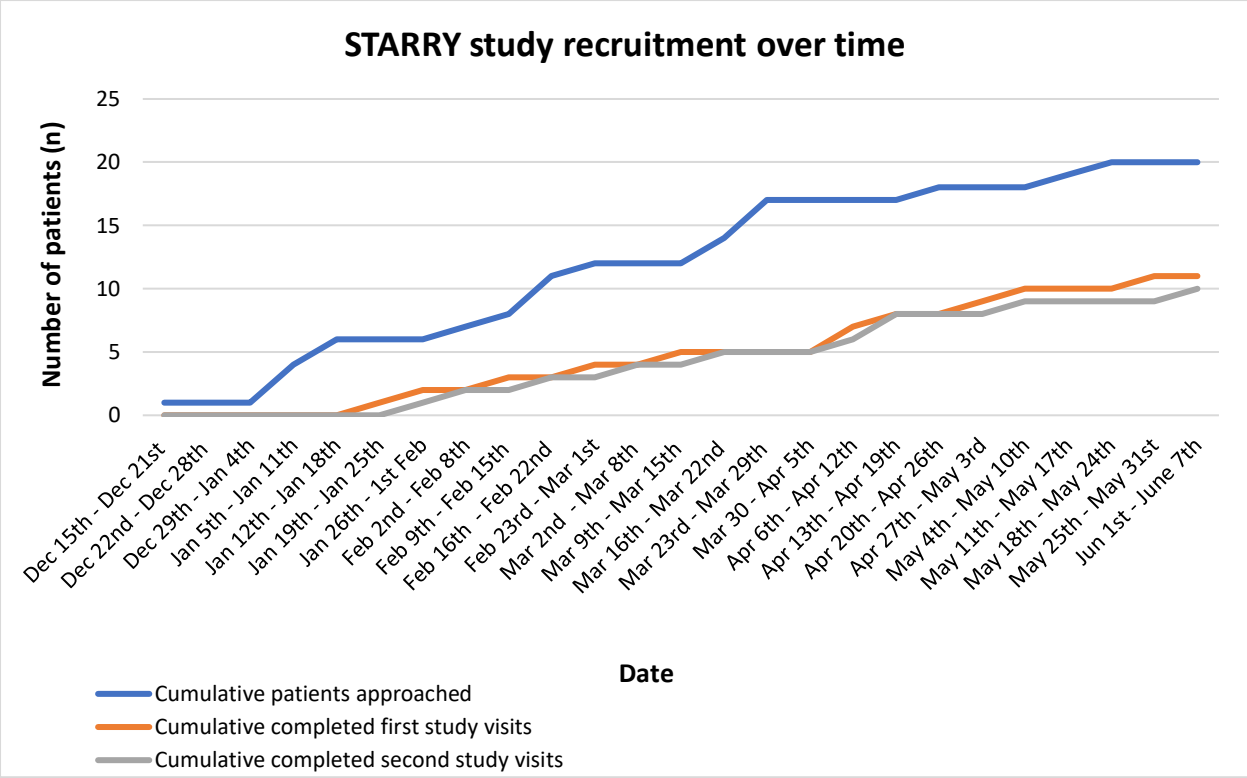


Figure 3. 2 | Graph showing the weekly rate of approaching patients and completing study visits.

Before the Christmas-period, only one patient was introduced to the study. After Christmas, throughout the first three-months of 2022, there were consistently five or six patients' clinic appointments per month for the study team to attend and introduce the study. The rate of clinic appointments then slowed, with only one eligible patient attending clinics in April and two patients in May. It was unusual for more than one patient to be introduced to the study from the same clinic-list: thirteen different healthcare professionals were leading the clinics where the STARRY study was

introduced and had been informed about the study and agreed to recruitment. Most clinic appointments were held during school hours, with few coinciding with Liverpool City Council School Holidays.

3.1.1. Factors affecting recruitment

Endocrinology clinic appointments were the most common place that patients were provided information about the STARRY study (65%) (Table 3.1). This was as anticipated since endocrinology is the speciality where the majority of patients with TS receive the most frequent follow-up in childhood. Of all the clinic appointments where the study was introduced, only two were nurse-led (Table 3.2) and only one appointment was held virtually.

Table 3.1 | Patients recruitment rate from each clinical area.

Clinical area	Number of patients approached	Number of patients enrolled	% Successful recruitment rate
Cardiology Clinic	4	3	75.0
Ear Nose and Throat Clinic	1	0	0.0
Endocrinology Clinic	13	7	53.8
Medical Day-care Unit	1	0	0.0
Nephrology Clinic	1	1	100.0
Total	20	11	55.0

The number of patients approached from each clinical area was too small to explore significant differences between successful recruitment rates. However, the data in Table 3.1 are useful in suggesting that future studies for patients with TS should recruit from all clinical areas, irrespective of the focus of the study.

Table 3.2 | Patients recruitment rate from healthcare professionals leading the appointment.

Healthcare professional	Number of patients approached	Number of patients enrolled	% Successful recruitment rate
Doctor (Consultant)	11	7	63.7
Doctor (Non-Consultant)	7	3	42.9
Nurse	2	1	50.0
Total	20	11	55.0

The job title and experience level of the healthcare professional leading the clinic appointment in which the STARRY study was introduced, did not appear to influence patients' decision to enrol in the study. This strengthens our recommendation that clinical studies involving patients with TS should recruit patients from any clinical area.

In the one virtual clinic appointment (held on the platform NHS Attend Anywhere) where the STARRY study was introduced, the patient did not consent to participate. There were no patients introduced to the study via telephone. Given the absence of data, it is unclear whether approaching patients remotely for study involvement is a worthwhile for both patients and researchers.

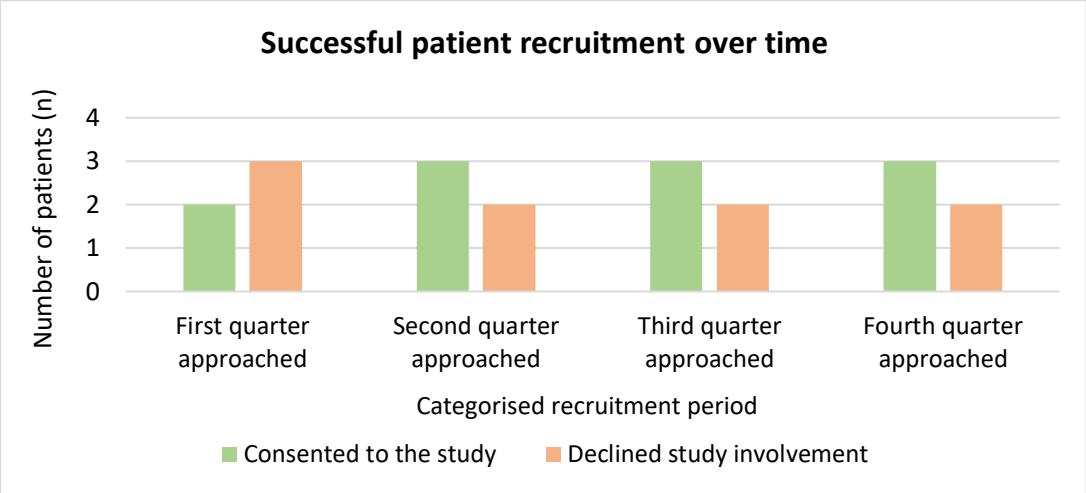


Figure 3.3 | Patient recruitment categorised chronologically.

Figure 3.3 shows the 20 patients approached categorised into chronological quarters (5 patients in each quarter) and the number of patients who consented and declined participation in the STARRY study.

3.1.2. Comparison to other patient groups

Of the 20 patients introduced to the STARRY study, 11 patients consented to participate, and nine patients declined (recruitment rate of 55%).

We compared our recruitment data to two studies operating similar protocols (Table 3.3). Excluding the measurement of body composition and participant questionnaires, the protocols had the same study activities. These studies were for populations of paediatric patients with a diagnosis of adrenal insufficiency.

Table 3.3 | STARRY study recruitment compared to other patient groups.

Cohort of patients	Patients approached, n	Patients enrolled, n	Recruitment rate, %
Turner Syndrome	20	11	55.0
Primary AI	42	26	61.9
Secondary/Tertiary AI	32	20	62.5

Abbreviations – AI: Adrenal insufficiency.

A three-way contingency table chi-square statistic showed no statistical significance between the recruitment rates, *p*-value = 0. 84.

3.2. Recruitment vs Audit data

To establish whether our STARRY study cohort was representative of the whole TS clinic population at Alder Hey Children's Hospital, we compared the data collected at the STARRY study visits to data collected from our audit (see Chapter 5).

3.2.1. Baseline demographics

The patients included in both the STARRY study and audit cohort were older at the time of the STARRY study, as the study visits took place between January and May 2022 and the audit data collection occurred in October 2021. Patients below 5 years of age (n=4) were included in the audit cohort and excluded from STARRY study recruitment. Considering these factors and other psychosocial factors, we hypothesised that there would be a statistically significant difference between the mean age of participants in the two cohorts: that the STARRY study cohort would have a higher mean age. It was assumed that older patients would have a greater understanding of their TS diagnosis and therefore might be more inclined to participate in research. Patients' parent/carer(s) may also believe that their child would better tolerate the study activities if they were older.

We hypothesised that the time in years since the patient's diagnosis of TS would not be statistically significantly different between the two cohorts. We thought that patients' who were recently diagnosed and their parent/carer(s) might be more willing to participate in the STARRY study, as they may see this as an opportunity to find out more about their diagnosis. However, we also considered that newly diagnosed patients and their parent/carer(s) might be overwhelmed with information, and

therefore chose not to participate in research at present. We hypothesised that these opposing factors would result in a negligible difference between the cohorts.

We hypothesised that there would be a statistically significant difference between the mean index of multiple deprivation decile between the STARRY study cohort and the audit cohort: with the STARRY study cohort having a higher mean score. (261, 262) A NIHR study found that deprived areas, where there is the highest burden of disease, have the lowest numbers of involvement in research. (263) The STARRY study may have been inaccessible to low-income families, as the study team could not offer travel reimbursement for study visits. Some working parents might find it difficult to take leave from work to attend the study appointments, and this might be particularly true if time off work resulted in loss of earnings.

Table 3.4 reports the results of our hypotheses.

Table 3.4 | Demographic differences between the STARRY study cohort and audit cohort.

	STARRY study (n=11), Mean (± SD)	**Audit cohort (n=40), Mean (± SD)**	***p*-value**
Age at study	13.8 (2.4) years	11.8 (4.6) years	0.25
Age at TS diagnosis	5.6 (3.6) years	5.1 (4.8) years[a]	0.57
Time since TS diagnosis	8.3 (3.3) years	7.0 (4.7) years	0.17
Index of multiple deprivation decile	3.4 (2.5)	4.4 (2.9)[b]	0.30

Abbreviations – TS: turner syndrome; SD: standard deviation.

a – n=38 patients; b – n=39 patients.

There was no statistically significant difference between the baseline characteristics of the STARRY study cohort and the audit cohort. The implications of these findings are discussed in section 3.5.

3.2.2. Cardiovascular risk factors

We hypothesised that patient and parental worry about cardiovascular health may influence patients' decision on whether to partake in the STARRY study. We therefore expected to find a significantly higher proportion of patients with documentation of metabolic risk factors and structural cardiac abnormalities in the medical records of patients within the STARRY study cohort compared to the audit cohort. Table 3.5 presents our results.

Table 3.5 | Documented cardiovascular risk factors in the STARRY study cohort compared to the audit cohort.

	STARRY study (n=11), n (%)	Audit cohort (n=38[a]), n (%)	*p*-value
(i) Metabolic Factors			
Average BMI SDS	+0.92 ±1.39	+1.19 ± 1.49	0.59
Overweight (264)	2 (18.2)	11 (29.0)	0.47
Obese (264)	4 (36.4)	9 (23.7)	0.40
Weight management advice given in clinic	4 (36.4)	9 (23.7)	0.35
Lipid profile abnormalities	4 (36.4)	6 (15.8)	0.11
Diagnosed hypertension	1 (9.1)	2 (5.3)	0.61
(ii) Structural Cardiac Abnormalities			
Bicuspid aortic valve	2 (18.2)	6 (15.8)	0.80
Coarctation of the aorta	0 (0.0)	2 (5.3)	0.45
Aortic stenosis	1 (9.1)	1 (2.6)	0.32
Elongation of the transverse aorta	0 (0.0)	1 (2.6)	0.60

Abbreviations – BMI: body mass index; SDS: standard deviation score.

a – data was unavailable for two of the 40 patients in the audit cohort.

There was no significant difference between the cardiovascular risk factors observed in the STARRY study cohort and the audit cohort. The implications of these findings are discussed in section 3.6.

3.3. Completion of study protocol

Most study activities and specific aspects of study activities were completed in 75% or more study participants: only saliva sample collection between 11pm and midnight was not completed in 54.54% of participants. A single participant was not able to complete all study procedures. Her partial data are included in our results.

3.3.1. Successfully completed study activities

Study blood tests, measurements of body composition, clinic BP and 24-hour ABPM were successfully completed by all participants.

3.3.1.1. Venepuncture

All blood samples were collected in a maximum of two venepuncture attempts. Clinical blood samples were prioritised in sample collection. For one participant, sample volume was inadequate to measure clotting factor levels.

3.3.1.2. Body Composition

All participants had their height, weight and body composition measured.

3.3.1.3. Clinic Blood Pressure

All participants tolerated three automated clinic BP measurements.

3.3.1.4. Ambulatory Blood Pressure Monitoring

The study team considered whether girls aged between seven and ten years were likely to tolerate a 24-hour ABPM. The study team assumed that participants aged at least ten years were able to tolerate this study activity, although additional factors were considered and discussed with each participants parent/carers(s) ahead of their first study visit. Table 3.6 details the decision made for each participant.

Table 3.6 | Ambulatory blood pressure monitoring decision for each participant.

Age	Additional factors	Decision for ABPM
9.0	Developmental delay	No
11.2	Developmental delay	No
12.4	Developmental delay	No
13.2	-	Yes
13.7	-	Yes
13.8	-	Yes
14.6	ADHD	Yes
15.2	-	Yes

15.8	-	Yes
16.2	-	Yes
17.4	-	Yes

Abbreviations – ABPM: ambulatory blood pressure monitoring; ADHD: attention deficit hyperactivity disorder.

Eight participants had 24-hour ABPM. All participants' data were included in analysis: all participants had >50% successful readings. Participants had the BP machine on for a mean of 24.1 ± 2.8 hours (range 20.4 – 28.5 hours). The mean possible number of readings were 42.3 ± 4.1 (range 37 – 49) and the mean percentage of successful ABPM reading were 83.9 ± 12.2% (range 58.1 – 97.5%).

We hypothesised that older participants would have a greater tolerance to ABPM and therefore have a higher percentage of successful BP readings in their reports. Surprisingly, there was moderate negative correlation between age in years of participants and percentage of successful ABPM readings (Pearson's correlation coefficient -0.550). Figure 3.4 presents these data.

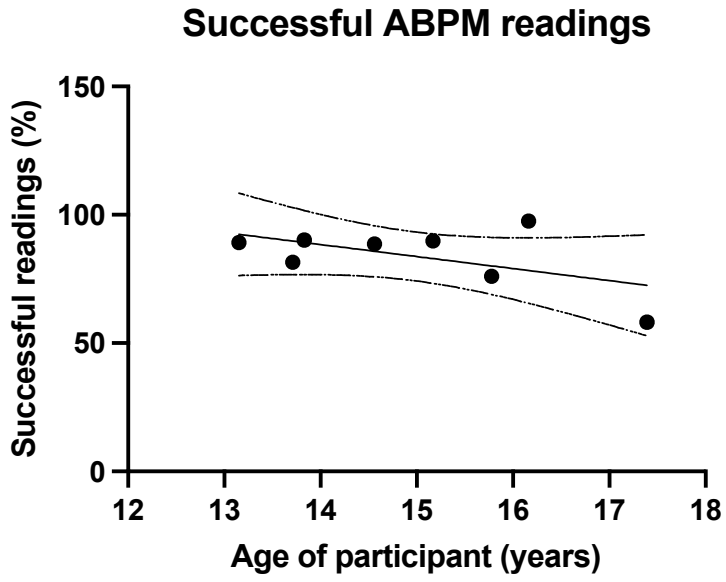


Figure 3.4 | Age of participant and percentage of successful ambulatory blood pressure readings.

3.3.2. Saliva samples

Of the 11 participants, ten collected saliva samples. Participants collected between five and nine samples (mean 8.1 ± 1.3). No participants missed a sample collection between the time-period that they started and ended sample collection. One sample was not analysable due to inadequate volume.

One participant was unable to collect a morning sample. This participant found saliva sample collection difficult, and her parents thought that adding this to her school morning routine would be too stressful. Only five participants (50%) collected a sample between 11pm and midnight.

Adolescent participants tended to take charge of their saliva sample collection at home. In these participants there were more labelling errors, with poor adherence to pre-labelled Time Points. Parental support with the study activity, regardless of the participants age, should be encouraged in future studies.

3.3.3. Vascular ultrasound studies

Ten of the 11 participants completed both vascular ultrasound studies.

3.3.3.1. Flow-mediated dilatation

FMD was analysed after the participants study visits. Two participant's scans were not interpretable and therefore excluded from our analysis. During a different participants scan, the clinician undertaking the scan concluded that the image would not be analysable. This scan was repeated an hour later. A requirement for this scan was that the participant had fasted, so repeating the scan at the second study visit was avoided.

3.3.3.2. Carotid intima-media thickness

cIMT measurements were calculated whilst the participant was completing other study activities on the CRF. Images were not analysable for one participant, and they subsequently had this scan repeated at the end of their first study visit. During the second scan, this participant was still very restless, and it was decided to repeat the scan on their second study visit. Fasting was not a requirement for this scan.

The study team aimed for three 10mm cIMT measurements with a ≥95% success score for each participant. Except from the participant who had difficulty tolerating this scan, where two analysable measurements were collected, this was achieved. The mean success of the reading used for analysis was 98.7 ± 1.5%.

3.3.4. Continuous glucose monitoring

Ten of the 11 participants wore the CGM. Participants had the sensor active for a mean of 6.5 ± 1.0 days (range 4.9 – 8.0 days). A similar study at Alder Hey Children's Hospital demonstrated hypoglycaemia in the first 24-hours of CGM insertion, which was inconsistent with data for remainder of the study period. We therefore excluded the first 24-hours of STARRY study participant data. For comparative purposes, the first 24-hours were excluded from each participant in the raw normative data also. (265)

Glucose readings were taken every 5 minutes. The mean total number of readings for each participant was 1573.9 ± 279.7 (range 1116 – 2019).

3.4. Feasibility of study activities

Formal quantitative feedback on study activities were collected using 10-point Likert-type scales. Participants were required to rate statements about study activities from 1 (disagree/hate) to 10 (agree/love). The study team also collected informal verbal feedback. Ten of the 11 participants completed the study questionnaires.

Before completing feedback on study activities, participants completed a practice questionnaire where they told the study team their favourite food and rated how much they "like eating it" from 1 (I hate it) to 10 (I love it). The mean score was 8.9 ± 1.1 (range 7.0 – 10.0). This questionnaire was used to assess participants reading comprehension skills to decipher how much support they would need to complete the study questionnaires.

Using the defined limit of acceptability of other studies using hedonic and visual analogue scales, the study team used a score of >5 out of the possible 10 as the threshold for an acceptable study activity. (266)

3.4.1. Body composition

The InBody scales showed participants their real-time body composition measurements on an electronic screen. Informal feedback from participants suggested that this was the most eagerly anticipated study activity, with many participants expressing excitement to know their results. The

participant who did not continue with most of the study activities asked if she could *"still use the cool scales"* before leaving the CRF.

One study participant, who had a high BMI, found seeing these measurements quite distressing. After this study activity, her parents told the study team that she has been concerned about her weight for some time and *"had been making herself sick".* Subsequently, the participants clinician was made aware of this and referrals for psychological support were made.

The above situation occurred at one of the first STARRY study visits. Subsequently, an effort was made to ensure that patients would want to see their results. For example, in one patient's clinic appointment where the STARRY study had been introduced, the patient specifically stated that she did not want to know her current weight. Before the body composition study activity, she therefore was given the option to have the electronic screen covered. The participant decided that she was comfortable seeing her results but expressed gratitude on being given the option. She later confided that she had suffered with bulimia in the past and at that time would have found this study activity difficult.

In future studies involving girls and adolescents with TS where body composition is measured, we recommended that this activity is blinded. Overall, it was felt that participants knowing their results in real-time was unhelpful. As with all results, abnormal findings should be relayed to the patients' clinical team for discussion in their next clinic appointment.

3.4.2. Venepuncture

All participants (n=10) recorded that they had memory of a previous blood test. The blood tests as part of the STARRY study differed from the annual TS blood tests in that participants were fasted for this sample. Anecdotally, parents described their daughters to be more irritable without having breakfast. All participants usually eat breakfast. Participants rated how easy they found not eating before their blood test as a mean score of 5.9 ± 3.6. Figure 3.5 shows the distribution of participant scores.

The first study visit commenced at 8:30am. Our questionnaire gave participants the option for their study visit to commence one-hour earlier, with the rationale that participants could have their breakfast earlier. All participants recorded that they were satisfied with the 8:30am appointment

time. Some participants travelled over an hour to Alder Hey for their study visits, and these participants felt that an earlier appointment would mean that they would have to *"wake up too early"*.

Participants rated the ease of having their blood test at the STARRY study visit as a mean score of 7.6 ± 2.2. The distribution of participant scores is shown in Figure 3.5. Given observations about how anxious participants were during and before this study activity, this score was higher than the study team expected. Seven of the ten participants rated their blood test easier or equal to the ease of fasting for the visit. The study team observed that many participants were relieved that they didn't require another blood test for a year.

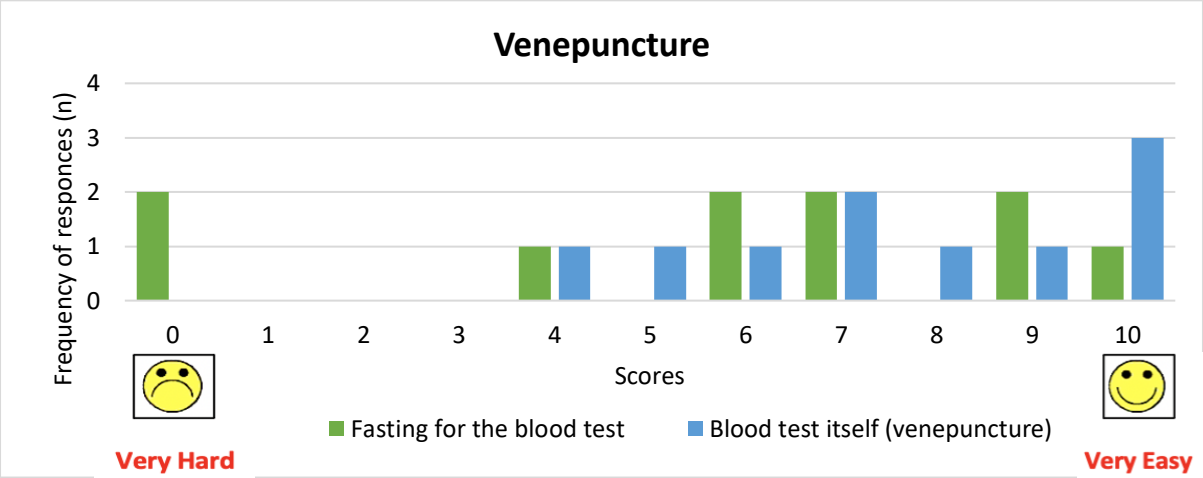


Figure 3.5 | Participant rating on blood tests as a study activity in the STARRY study.

Seven participants (70%) rated fasting as an acceptable part of the STARRY study protocol. Eight participants (80%) rated venepuncture as a suitable study activity. The three participants who rated fasting as unacceptable were different to the two participants who rated venepuncture as unacceptable.

3.4.3. Vascular ultrasound studies

The study team observed that participants with ADHD or developmental delay found the vascular scanning the most challenging. FDM requires the participant to lie still for eight minutes during the scan and to rest for 10 minutes prior the scan. During measurement of cIMT participants are encouraged to stay still and refrain from talking.

Participants rated the ease of having the ultrasounds as a mean score of 7.6 ± 2.7. They rated recommending the study activity to a friend slightly higher, as a mean score of 8.4 ± 1.8. The mean of participants two scores was 8.0 ± 1.9. Figure 3.6 displays the distribution of these scores.

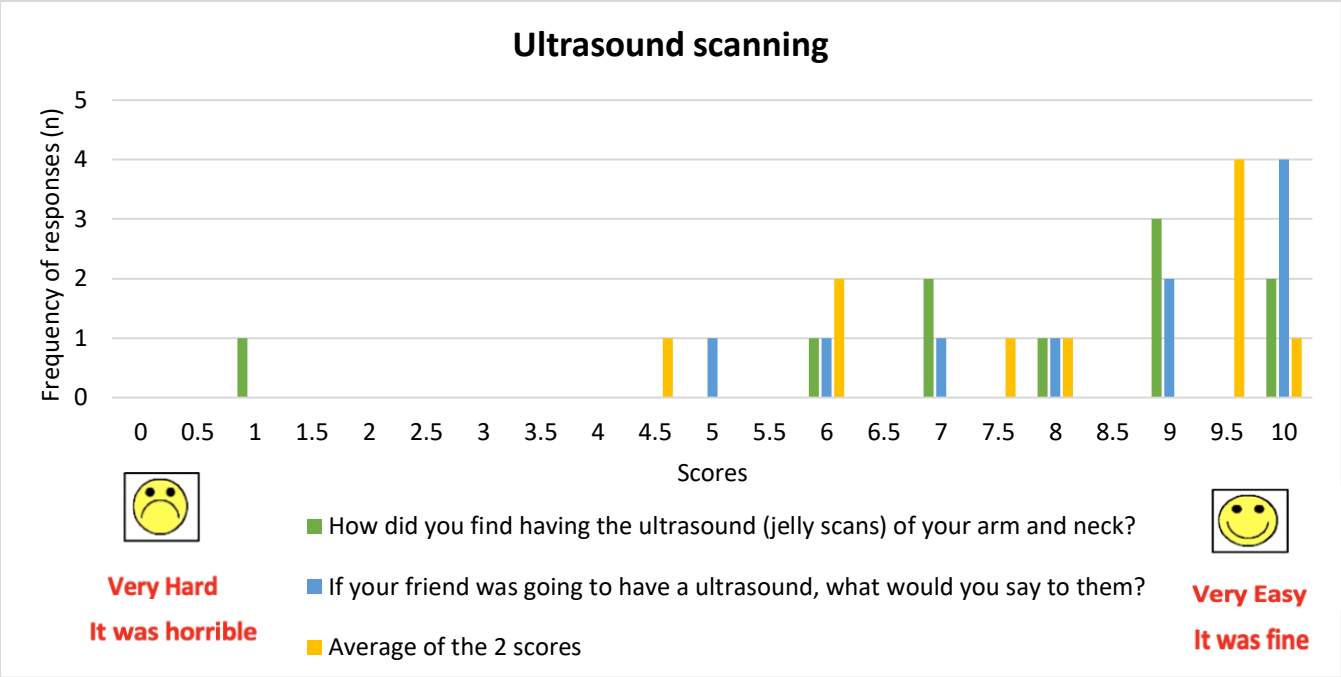


Figure 3.6 | Participant rating on ultrasound scanning as a study activity in the STARRY study.

Four participants provided written feedback on this study activity:

- *"Found it tickly"* (aged 9 years)
- *"Very loud and annoying. I found it hard to sit still"* (aged 12 years)
- *"Was quite difficult (arm one) quite pinchy – had to fast and keep very still – alright though"* (aged 13 years)
- *"It's ticklish*" (aged 14 years)

Using the mean of the participants two scores, nine participants (90%) rated vascular scanning as an acceptable study activity. The participant who rated this as unacceptable gave the following scores: 1 for how they found the scans, 8 for recommending the scans to a friend. Anecdotally, the participants preferred the cIMT scan to the FMD scan as it was quicker and therefore easier to keep still. In future studies where vascular scans are performed, we recommend play specialist support for restless participants.

3.4.4. Continuous glucose monitoring

There was some parent and participant concern of the "unknown" with wearing a CGM, specifically if it would be painful to wear or insert. The knowledge that it involved a small needle was anxiety-provoking for many participants. Despite reassurance from the study team, some participants were also concerned that it would prevent them from doing their daily activities, such as participating in sports or showering.

Most participants were pleasantly surprised with how painless the insertion of the CGM was. Participants rated the ease of wearing the CGM for a week a mean score of 8.8 ± 1.6. They rated recommending the study activity to a friend as a mean score of 9.1 ± 1.5. The mean of participants two scores was 9.0 ± 1.3. Figure 3.7 displays the distribution of these scores.

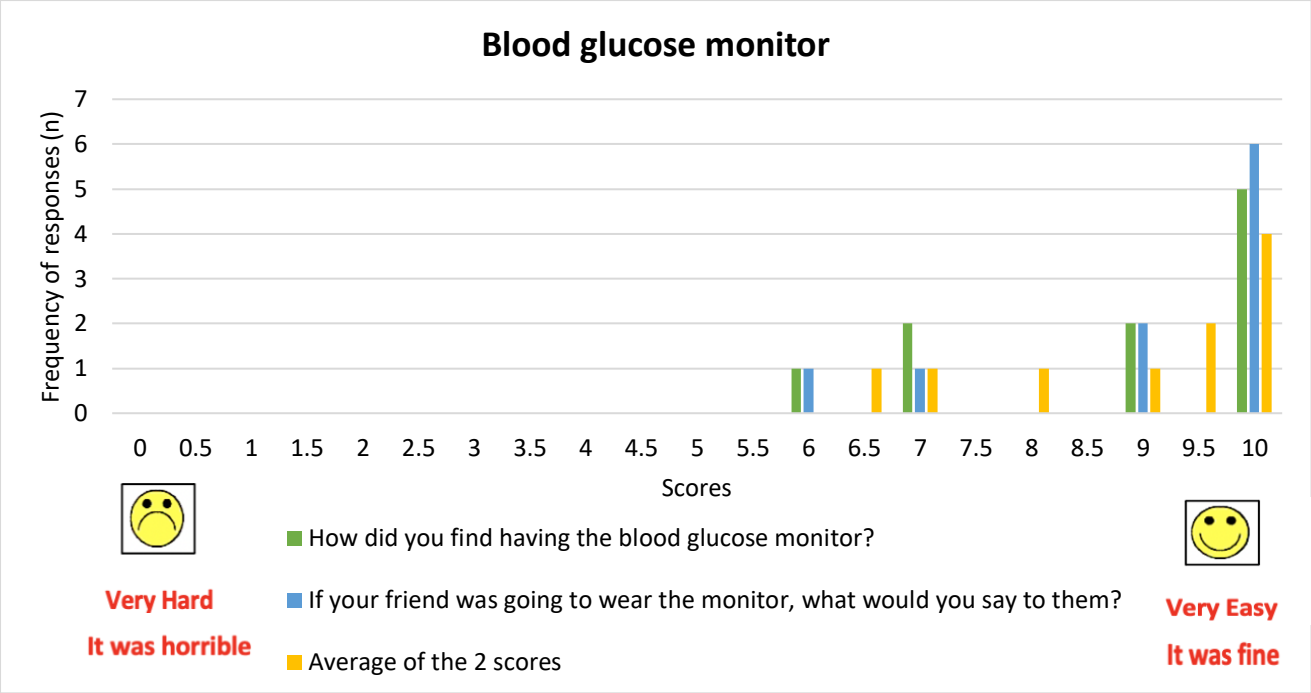


Figure 3.7 | Participant rating on continuous blood glucose monitoring as a study activity in the STARRY study.

All participants (n=10) rated this an acceptable study activity. In future studies utilising CGM, we recommend that a video or extra information booklet, showing the insertion of the GCM and answering common patient concerns is sent to the participant ahead of their first study visit.

3.4.5. Ambulatory blood pressure monitoring

Participants rated the ease of wearing the ABPM in the daytime as a mean score of 6.5 ± 1.3 and in the night-time as a mean score of 6.1 ± 2.4. Figure 3.8 displays the distribution of participant scores.

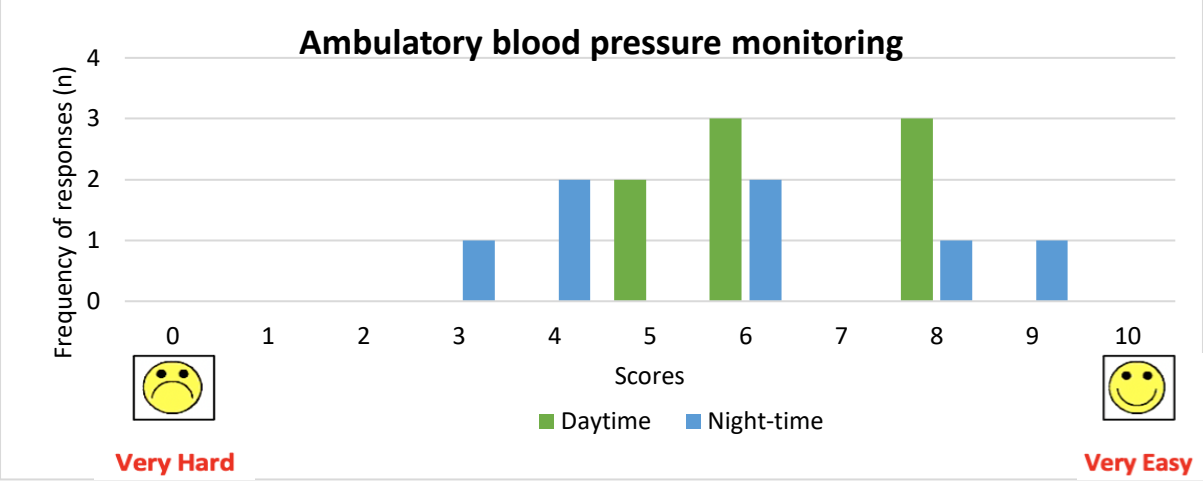


Figure 3.8 | Participant rating on ABPM as a study activity in the STARRY study.

Four participants provided written feedback on this study activity:

- *"It glitched quite a bit at the beginning with errors but otherwise it was okay"* (aged 13 years)
- *"Nearly tripped over the wires (I'm very small). Some pressures awoke me, and others didn't"* (aged 13 years)
- *"It gets easier with time"* (aged 14 years)
- *"Fell off whilst walking twice"* (aged 15 years)

Six participants (75%) rated the ABPM as acceptable in the daytime. Five participants (62.5%) rated ABPM acceptable in the night-time. Two participants rated ABPM unacceptable in both the day and night-time. Anecdotally, participants found this study activity uncomfortable and difficult to tolerate. Some, but not all, participants said wearing the monitor at night affected their sleep.

3.4.6. Saliva sample collection

Seven participants parent/carer(s) (70%) used the saliva sample instruction sheet. There was no significant difference between the mean score that participants parent/carer(s) gave for ease of using the salivette kit from those who used the instruction sheet and those who did not (p-value = 1.00). Parent/carer(s) rated the ease of using the salivette kit a mean score of 9.0 ± 1.9 and the frequency of saliva sample collect as a mean score of 8.4 ± 1.7. The distribution of scores is shown in Figure 3.9.

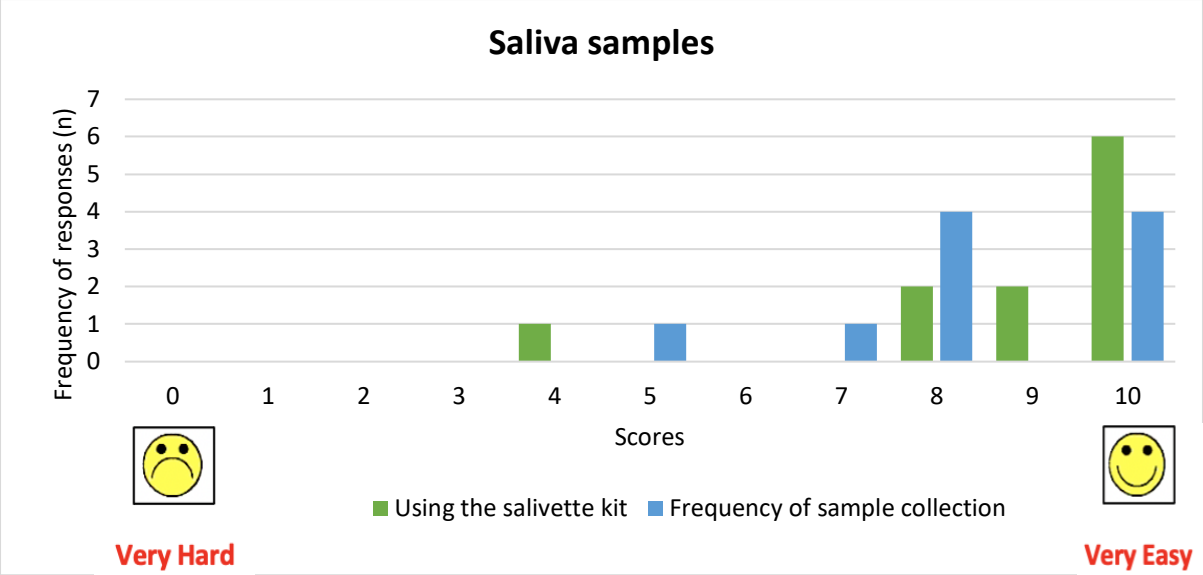


Figure 3.9 | Participant's parent/carer(s) rating of saliva samples as a study activity in the STARRY study.

Five participants (50%) collected samples between 11pm and midnight. Of these participants, one parent/carer (20%) had to wake their daughter for this sample. Of all the parent/carer(s) in the study, five (50%) would wake their daughter to collect a saliva sample for a similar study. Table 3.7 presents the mean age of participants who collected a sample and those who did not, and the mean age of participants whose parents/carer(s) would and would not wake their child. We hypothesised that the mean age would be statistically significantly different between these groups. Table 3.8 presents parent/carer and participant feedback on collection of saliva samples as part of the STARRY study protocol.

Table 3.7 | Parent/carer decision for saliva sample for midnight saliva collection and participant's age.

Question	Mean ± SD (range) age of participants in years		*P*-value
	Yes (n=5)	No (n=5)	
Did your child collect a sample between 11pm and midnight?	15.2 ± 1.5 (13.7 – 17.4)	13.1 ± 2.7 (9.0 – 16.2)	0.16
Would you wake your child?	15.4 ± 1.6 (13.7 – 17.4)	12.9 ± 2.4 (9.0 – 15.2)	0.09

Abbreviations – SD: standard deviation.

Table 3.8 | Parent/carer and participant written feedback on saliva sample collection.

Feedback	Age of participant (years)	Respondent

(i) Waking child for sample between 11pm and midnight		
"Would interrupt sleep pattern"	9	Parent/carer
"It was harder to complete the later saliva samples"	16	Participant
(ii) Using the salivette kit		
"Difficulty with dropping saliva samples"	9	Parent/carer
"Hard getting the sample in and out of the container ?larger container"	15	Parent/carer
(iii) Frequency of sample collection		
"As we were not at home it was difficult to plan meals and activities in the town"	13	Parent/carer
"Got in the way of our activities"	13	Parent/carer
"We may not have monitored the food intake sufficiently well whilst doing the study. Both parents are working and XXX is young to manage the 2-hourly plan for the saliva tests"	13	Parent/carer

Nine parent/carer(s) (90%) rated the use of the salivette kit as acceptable. Nine parent/carer(s) (90%) rated the frequency of sample collection, which was every two waking hours, as acceptable. The same parent/carer rated both aspects of sample collection as unacceptable. This parent/carer(s) child was the youngest study participant and had an additional diagnosis of developmental delay. There was no significant difference between the age of participants who collected a midnight salivary sample and between the age of the participants whose parents/carer(s) would and would not wake their child. There was a tendency, however, for these participants to be older, and with a larger sample size the difference in age may have been statistically significant.

3.4.7. Number of study visits

The mean acceptability rating made by parents/carer(s) of participants who visited Alder Hey three times as part of the starry study was 8.9 ± 1.1. The distribution of scores is shown in Figure 3.10.

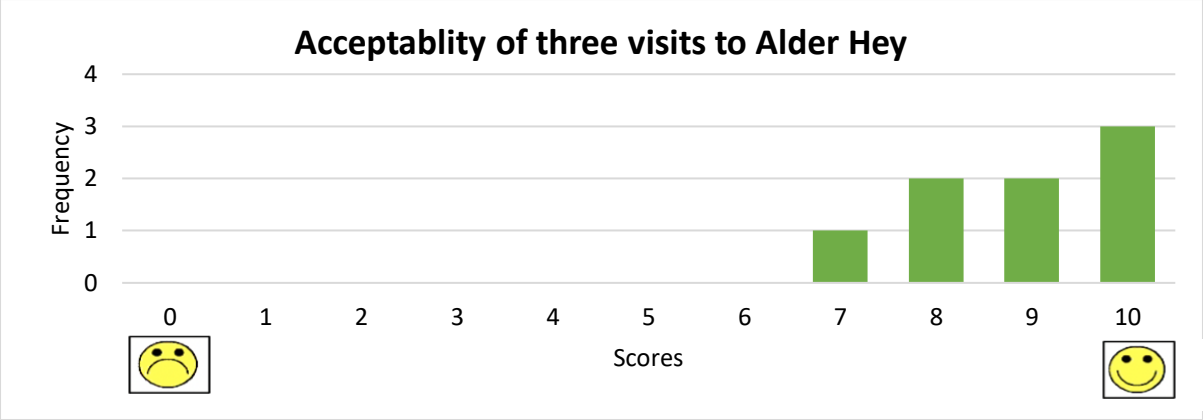


Figure 3.10 | Participant's parent/carer(s) rating of how acceptable three visits to Alder Hey as part of STARRY study was.

All parent/carer(s) (n=8) rated the number of visits to Alder Hey as acceptable and documented that this did not deter them from joining the STARRY study. However, one parent/carer who rated this question a score of 9 noted that *"It helped as my daughter is on school holidays"*.

3.4.8. Participant preference

Participants were asked to rank the ease of completing study activities at home: wearing the CGM, wearing the ABPM and collecting saliva samples. The two participants who did not complete all three activities rated the two "easiest" and "hardest". Responses are shown in Figure 3.11.

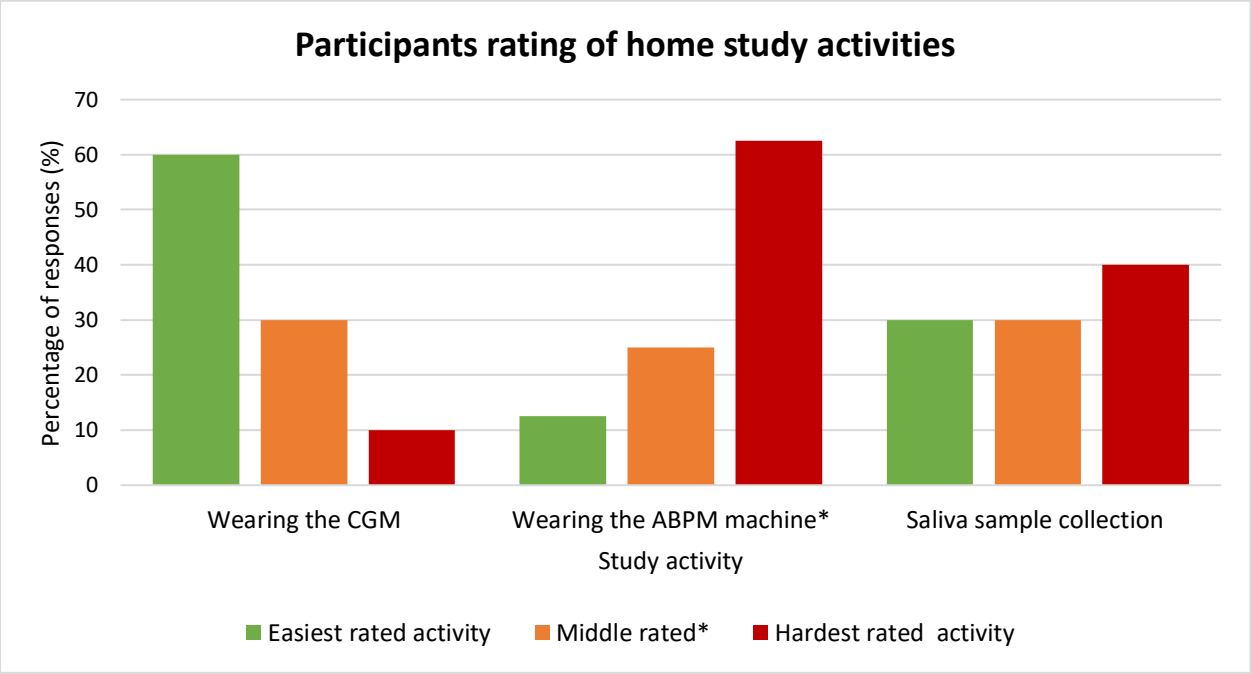


Figure 3.11 | Participant's rating of ease of study activities.

***Only rated by participants completing all three activities.**

Despite the CGM study activity lasting approximately 7-days and the other two activities lasting approximately 24-hours, this was rated the easiest activity by six participants (60%). Of the eight participants who wore the ABPM machine five participants (62.5%) rated this the hardest activity. Saliva sample collection was rated the easiest by three participants (30%) and hardest by four participants (40%).

3.5. Discussion

The STARRY study had a successful recruitment rate comparable to other studies operating a similar protocol. We encourage future studies involving paediatric patients with TS to recruit from all clinical areas, where the patient can be introduced to the study in-person, irrespective of the focus of the study.

Patients declining participation was largely attributed to psychological factors. A wide range of psychological difficulties have been reported in women and girls with TS. These may result from both the physical, psychological and psychiatric impacts of living with TS, but there is also evidence that TS itself may affect patients' risk, and presentation. (59) An awareness and understanding of these challenges common to all girls and adolescents with TS is important to ensure that their care is delivered in a holistic manner. Emotional and cognitive problems should be recognised and addressed in a timely manner to mitigate the risk of lifelong impacts of low mood, anxiety, poor social competence and other recognised associations. International guidelines recommend that neuropsychological and allied behavioural health services are integrated into the care of girls and women with TS. (4) Dedicated, individualised, psychological support is currently not part of the TS service at Alder Hey Children's Hospital.

Despite the small size of the STARRY study cohort, comparison to audit data showed that the baseline characteristics of the cohort did not differ from the overall TS Alder Hey Children's Hospital clinic population. Importantly, the deprivation status was not significantly different between the two cohorts. Individuals from disadvantaged backgrounds, due to a combination of biological, behaviour and psychosocial factors, have an increased burden of acquired cardiovascular disease. (267) The study cohort also had a similar prevalence of metabolic risk factors and congenital cardiac

abnormalities. It is therefore likely that descriptions of cardiovascular disease burden of children and adolescents with TS was not exaggerated in the STARRY study (see Chapter 4).

There is limited literature on measuring acceptability of study activities in paediatric research. With reference to medication, acceptability has been defined as *"an overall ability of the patient and caregiver (defined as 'user') to use a medicinal product as intended (or authorised)."* (268) Likert-style scale methods are typically used in medical research to assess acceptability of products for patients. We combined the two most commonly used scales together, hedonic and visual analogue scales, in the STARRY study. (266) Using scale methods, limited literature reports defined criteria of acceptability. Three studies using hedonic scales in paediatric populations (n=412) defined acceptability as a score between 50%-68% on their respective scales. (269-271) A score of greater than 5 on our 10-point scale defined acceptability of study activities in the STARRY study.

Most participants disliked fasting for their first study visit, and overall, this was rated as the least acceptable aspect of the STARRY study protocol. Without breakfast, parents reported their daughters to be more irritable than usual, and it is plausible that this could have contributed to participant anxiety surrounding the first study visit. This was difficult for the study team to determine, as the study activities after breakfast generally caused participants less worry. Participants were all satisfied with their appointment time, therefore, without removing fasted blood tests (fasting glucose, insulin, lipids) and FMD of the brachial artery from the study protocol, the solution to this problem is unclear. The set-up of CGM could feasibly be moved to after participants have had their breakfast, although this study activity caused a lot of anxiety for participants, so it was felt that it should remain before the break.

Despite the heightened concerns surrounding this study activity, CGM was rated as an acceptable study activity by all participants. Of the study activities completed at home, most participants (60%) rated this as the easiest. Sufficient data was collected for all participants who engaged in this study activity. In future studies, further information detailing what wearing a glucose monitor would mean for the participant, may go some way in reducing participant anxiety.

Despite the high prevalence of needle-phobia among participants, most participants (80%) rated venepuncture an acceptable study activity. It would be interesting to see how many participants would think venepuncture was acceptable if there was not the opportunity to have their annual routine blood tests collected as part of the sample. A large enough sample volume approximately

20ml of blood was collected for most participants (90.9%). We recommend that studies requiring a venous blood sample in paediatric patients with TS should be arranged to coincide with routine annual blood tests.

Nine participants had ABPM as part of the STARRY study protocol. Despite all participants tolerating ABPM to gather sufficient data, anecdotally, participants found this study activity difficult. Over half of participants (62.50%) rated this study activity as the hardest to complete at home. Participants rated the ease of ABPM to be similar in the daytime and night-time.

FMD scans were analysable in all but two participants, and measurements of cIMT were collected for all participants. Nine participants (90%) rated vascular scanning as an acceptable study activity. The only participant who rated this as unacceptable had developmental delay and found staying still especially difficult. Of the vascular scanning, the study team observed that participants found FMD of the brachial artery more difficult to tolerate than measurements of cIMT, as they had to lie still for a longer time-period. FMD of the brachial artery also requires participants to be fasted, while cIMT does not. FMD and cIMT are known to be related: in a middle-aged population free of cardiovascular disease, decreased FMD was associated with cIMT progression over a 6-year period. Endothelial dysfunction, measured via FMD, likely precedes structural atherosclerotic development, partially reflected by increased cIMT. (272) A possibilty to detect atherosclerotic disease at an earlier stage makes FMD clinically advantageous over cIMT.

The ease of use of the salivette kit and the frequency of sample collection was rated as acceptable by nine of parent/carer(s) (90%), and day-time sample collection adherence was good. Despite this, participants and their parent/carer(s) documented difficulties with both using the salivette kit and collecting samples every 2-hours. Only five participants (50%) collected a saliva sample between 11pm and midnight, and only one parent/carer woke their child for this sample. This sample is for the assessment of hypercortisolism and is therefore an important part of the STARRY study protocol. (239) Given the interruption to sleep, future studies should consider the age of their population for acceptable adherence to late-night sample collection.

We recommend that all study activity results, including measures of body composition, should be blinded to the participant. This is especially important for studies involving children and adolescents with TS given the high prevalence of mental health difficulties. (59) Abnormal findings should be

relayed to the participants' clinical team for discussion in their next clinic appointment, unless urgent action is required, for example a very high blood pressure measurement.

Clinicians should be reminded that they can refer their needle-phobic patients for play specialist support or desensitisation therapy at Alder Hey Children's Hospital. We believe that play specialist support would be beneficial at all studies involving children and young people. Two participants aged 15.48 and 11.16 years, despite being very anxious about blood tests, refused play specialist support. Both participants' parents mentioned that their daughters felt "too grown up" or "too old" to benefit from a play specialist's support. An alternative name for this role, for example, a 'procedural anxiety specialist', may encourage more adolescents to engage with this support.

To conclude, most paediatric patients with TS and their parent/carer(s) were interested in the STARRY study. Mental health difficulties were barriers to participation for some patients. All aspects of the STARRY study protocol were acceptable to the majority of participants and their parents/carer(s). Participants had the most difficulty with fasting for the first study visit, wearing the ABPM and collecting a saliva sample between 11pm and midnight. Overall, our data give some confidence that a larger, multicentre study is likely to have an acceptable recruitment rate, and that most study participants will complete the study protocol.

4. The STARRY study experimental results

In this chapter, the experimental data from the STARRY study will be presented. The cardiovascular profile of the cohort is discussed, and comparisons are made to the existing literature.

4.1. Participant information

4.1.1. Baseline Demographics

Eleven patients consented to participate in the STARRY study. The age of participants was normally distributed, and the mean age was 13.8 ± 2.4 years (range 9.0 – 17.4 years). The majority of participants were of White British ethnicity (81.8%). One participant was of Chinese ethnicity and one participant was of other White (Eastern European) ethnicity. Nine participants (81.8%) lived in England and two participants lived in Wales. The median index of multiple deprivation decile, based on data published in 2019 for England and Wales, was 3.0 (IQR 1.0 – 4.5). The index of multiple deprivation deciles (1 – most deprived, 10 – least deprived) are calculated using seven and eight different domains of deprivation in England and Wales, respectively. (261, 262) Four participants (36.4%) lived in the 10% most deprived areas (decile 1) of England.

4.1.2. Antenatal History

The mean gestational age of participants at birth was 38.8 ± 3.0 weeks (median 40.0 weeks). One participant was a twin (twin 2). Using definitions from the World Health Organisation (WHO), one participant was born very preterm (32.0 weeks), and one participant was born moderate-to-late preterm (34.0 weeks): both participants also had a very-low-birth weight (1.0kg and 1.4kg). (273, 274) Additionally, a another participant born at full-term had a low-birth weight (2.0kg). (274) The mean birth weight of the cohort was 2.8 ± 1.0 kg (median 3.1 kg).

The mean birth weight-for-gestational age centile was 39.6 ± 38.6th (median 31.0st centile). Five participants (45.5%) were born with a weight less than the 10th centile for their gestational age, which classified them as small-for-gestational age (SGA) according to the WHO criteria. (274) There was no significant difference between the body composition Z-scores recorded in the STARRY study of participants born SGA and participants born with a normal birth weight (Table 4.1). There was a tendency, however, for participants born SGA to have a healthy BMI, although given the small sample size of the study, further studies are required to determine the significance of this observation.

Table 4.1 | Effect of being small-for-gestational age on current body composition amongst participants.

Z-Score	Small-for-gestational age (n=5) Mean ± SD (range), SD	Normal birthweight (n=6) Mean ± SD (range), SD	P- value
Height	-1.9 ± 0.8 (-2.8 – -1.3)	-2.1 ± 0.6 (-2.6 – -1.1)	0.68
Weight	-0.6 ± 1.8 (-2.0 – 1.6)	-0.4 ± 1.1 (-1.8 – 1.6)	0.54
BMI	0.6 ± 1.6 (-1.0 – 2.0)	1.2 ± 1.3 (-0.8 – 2.9)	0.46

Abbreviations – n: number; SD: standard deviation; BMI: body mass index.

It is traditionally thought that girls with TS grow at a rate expected of the general female population until the age of three years, and being SGA is not widely associated with the condition. (275) However, Wisniewski, A., et. al retrospectively analysed the records of 548 newborn girls diagnosed with TS consecutively between 1990 and 2005 and found a birth weight deficit compared to the reference newborn population. The authors recommended that TS should be routinely considered in a full-term female with a marked weight deficit (<-2 SD or <2.5kg), especially in a first pregnancy. (276)

4.1.2.1. Significant Maternal history

Three participants' mothers had an infection during pregnancy: an unspecified kidney infection, Hepatitis C, and varicella-zoster, respectively. None of the participants were adversely affected in the neonatal period. These infections would not have contributed to their daughters TS karyotype: monosomy X is resultant of a spontaneous event in formation of parental reproductive cells, similarly, a spontaneous event in early foetal development results in mosaicism, and, rarely, inherited partial deletions of the X chromosome can lead to a TS variant karyotype. (4)

Both parents of one participant (9.1%) smoked during the antenatal period. There is evidence that foetal smoke exposure might be implicated in the associations between low-birth weight and cardiovascular disease (CVD) development in later life, although further studies are needed to account for potential cofounders. (277) This participant, however, was born at full-term with a healthy birth weight-for-gestational age on the 84.7th centile.

Two different participants' mothers had preeclampsia during pregnancy. Both participants were subsequently born preterm and had a very-low-birth weight. Increasing evidence suggests in utero exposure to preeclampsia is linked to long-term development of acquired CVD. The mechanism is

likely to be multifactorial; of environmental, genetic and epigenetic nature. (278) This should be considered when interpretating the exploratory STARRY study results.

4.1.3. Turner Syndrome diagnosis

In all participants, the diagnosis of TS was confirmed by genetic analysis. (Table 4.2). (4) Two participants (18.2%) have the classical monosomy X karyotype. Of the participants with mosaic karyotypes, the proportion of abnormal cell lines usually exceeded the proportion of 46,XX cell lines. The mean time from the participants date of TS diagnosis to consenting to the STARRY study was 8.3 ± 3.3 years (median 7.4 years).

Table 4.2 | Chromosomal abnormalities of the study group.

Karyotype	Frequency of STARRY study participants, n	Description
45, XO	2	Monosomy X
88% 45,XO, 12% 46,XX	1	Mosaicism
80% 45,XO, 20% 46,XX	1	
30% 45,XO, 70% 46XX	1	
90% 45,XO, 10% 47,XXX	1	Mosaicism with 'Triple X'
50% 45,XO, 50% 46,X,idic(Xq)	1	Turner Syndrome variant
7% 45,XO, 93% 46,XX, del(q)	1	
46,XX, idic(Xq),del(p)	1	
46,XX, del(Xp11.21)	1	
MISSING	1	

Abbreviations – n: number; X: X chromosome; idic: Isodiscentric; q: long arm of chromosome; del: deletion; p: short arm of chromosome.

STARRY study participants received their diagnosis of TS at a mean age of 5.6 ± 3.6 years (range 0.0 – 10.8 years). This is considerably younger than observed in a study of 781 Danish women alive any time between 1970 and 2001, where the median age of diagnosis was 15.1 years. (2) Interestingly, there was a significant moderate negative correlation (Pearson correlation coefficient -0.638) between the participants index of multiple deprivation decile and age at TS diagnosis in our cohort (Figure 4.1). This correlation remained significant, although not as strong, for the whole clinic population at Alder Hey Children's Hospital (n=38) (Pearson correlation coefficient -0.369) (Figure 4.2). This is an important finding as early diagnosis allows for assessment and timely initiation of medical interventions that can go some way to minimising the physical and psychosocial difficulties associated with short stature and

delayed puberty in individuals with TS. (4) When considering how to advocate for patients living in more deprived areas, it may be useful to determine whether this correlation is specific to the North-West of England, or applicable nationwide.

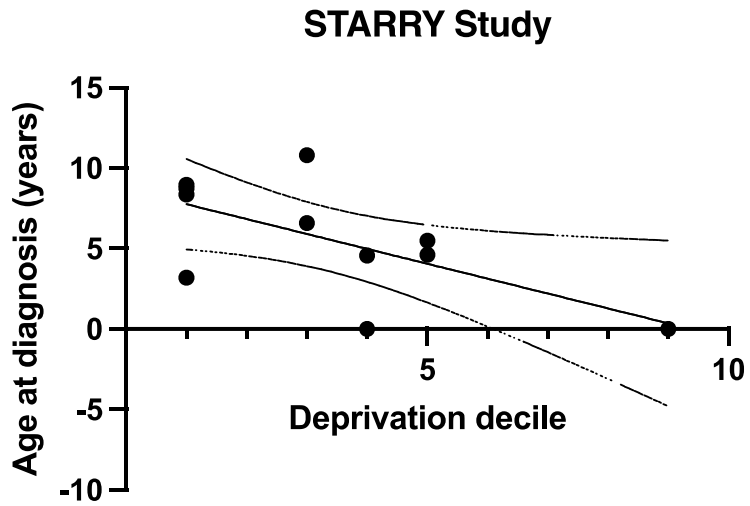


Figure 4.1 | Correlation between Index of multiple deprivation decile and participants age at diagnosis of Turner Syndrome.

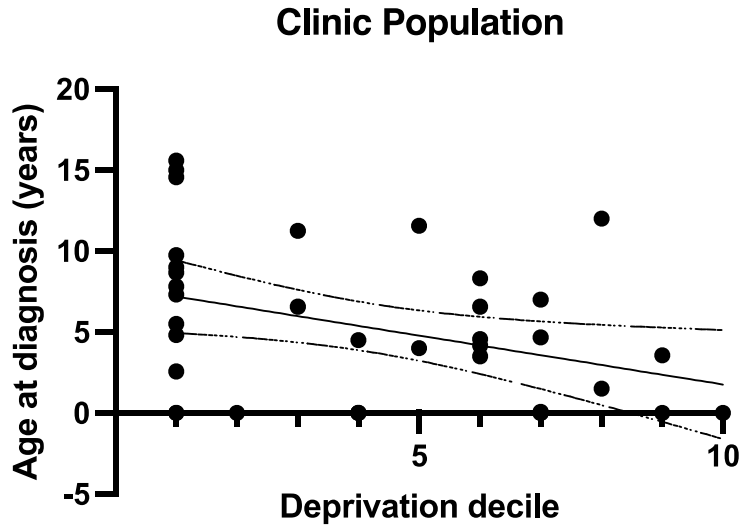


Figure 4.2 | Correlation between Index of multiple deprivation decile and age at diagnosis of Turner Syndrome in the Alder Hey clinic population.

Two participants were diagnosed with TS in the prenatal period via an amniocentesis. Routine antenatal scans identifying intrauterine growth restriction and a frank cystic hygroma prompted investigation of foetal karyotype. Short stature was the indication for assessing all other participants' (n=9) karyotype. As well as presenting with short stature, three participants initially presented with

unexplained weight gain, alopecia areata and aortic stenosis, respectively. The mean age at which the subgroup of participants diagnosed during childhood received their TS diagnosis was 6.8 ± 2.5 years (range 3.2 – 10.8 years). All participants were diagnosed before adolescence.

4.1.4. Prevalence of congenital cardiac abnormalities

Congenital cardiac abnormalities are known to occur in 23-39% of individuals with TS, and most commonly involve the left side of the heart. (131, 132) Data collection from STARRY study participants was consistent with these historical data: left-sided congenital cardiac abnormalities were diagnosed in three participants (27.3%). Table 4.3 demonstrates that it was not uncommon for participants to have more than one cardiac abnormality: two of the three participants had three cardiac abnormalities.

Table 4.3 | The frequency of congenital cardiac abnormalities in our study group.

Cardiac abnormality	Frequency of STARRY study participants, n (%)
Functional bicuspid aortic valve	3 (27.3)
Aortic dilatation	2 (18.2)
Aortic stenosis	1 (9.1)
Aortic regurgitation	1 (9.1)

Abbreviations – n: number.

These congenital abnormalities deserve recognition when interpreting the STARRY study exploratory data. However, they may not be especially important for our study population, since clinical manifestations relating to acquired CVD, such as BAV leading to premature valvular stenosis, typically develop in adulthood. (135) The participant with severe aortic stenosis has undergone a series of procedures since birth (see section 4.1.5.3.) and has documentation in her medical records of *"no significant aortic stenosis"* from an echocardiogram in December 2021.

4.1.5. Additional past medical and surgical history

The phenotype of TS demonstrates marked heterogenicity, with many features showing only a weak association with genotype. (4, 12) Multiple organ systems may be affected. (1)

4.1.5.1. Medical history

Each STARRY study participant has at least one additional diagnosis, which are summarised in Table 4.4. The possible effects of untreated diagnoses on STARRY study exploratory data are discussed in Section 4.1.5.2.

Table 4.4 | Participant diagnoses (excluding those mentioned elsewhere).

	Diagnoses	Frequency of study cohort, n (%)
(i)	**Urogenital**	
	Horseshoe kidney	2 (18.2)
	Unilateral duplex collecting system	1 (9.1)
	Anorectal malformation	1 (9.1)
(ii)	**Autoimmune**	
	Coeliac disease	2 (18.2)
	Eczema	2 (18.2)
	Vitiligo	1 (9.1)
	Alopecia areata[a]	1 (9.1)
	Low immunoglobulins	1 (9.1)
(iii)	**Ear, nose and throat**	
	Recurrent middle ear infections	6 (54.5)
	Speech difficulty	2 (18.2)
	Sensorineural hearing loss	1 (9.1)
	Narrow nasal passage	1 (9.1)
	Long-standing tinnitus	1 (9.1)
(iv)	**Neuropsychiatric**	
	Developmental delay	4 (36.4)
	Anxiety	2 (18.2)
	Attention deficit hyperactivity disorder[a]	2 (18.2)
	Depression	1 (9.1)
	Disordered eating[b]	1 (9.1)
	Dyslexia	1 (9.1)
(v)	**Ophthalmology**	
	Nystagmus[b]	1 (9.1)
	Strabismus[b]	1 (9.1)
	Vision perception difficulties	1 (9.1)
(vi)	**Miscellaneous**	
	Vitamin D inadequacy	2 (18.2)

Hypertension[b]	1 (9.1)
Osteopenia	1 (9.1)
Iron deficiency (without anaemia)	1 (9.1)
Patellofemoral syndrome	1 (9.1)

Abbreviations – n: number.

a – 1 participant is currently being investigated for this diagnosis; b – now resolved.

4.1.5.2. The possible effects of (untreated) participant diagnoses on exploratory analysis

4.1.5.2.1. Urinary tract abnormalities

The prevalence of congenital malformations of the urinary tract in patients with TS is approximately 30-40%, with horseshoe kidney and duplex collecting systems being the most common abnormalities. (47, 48) Indeed, three participants (27.3%) in the STARRY study cohort had one of these diagnoses.

These abnormalities have a limited, but possible, role in the aetiology of hypertension in TS. Horseshoe kidney and duplex collecting systems can result in hydronephrosis and vesicoureteral reflux, which can cause renal scarring, resulting in secondary hypertension. (4, 279) However it is important to note that a study of 62 girls and young women with TS showed that the presence of a renal abnormality had no significant impact on BP. (280)

One participant, with a horseshoe kidney, had a DMSA scan in October 2021 which showed no definite focal abnormalities, and similarly no hydronephrosis was identified from an abdominal ultrasound in 2018. The two other participants with renal abnormalities had documentation of abdominal ultrasounds consistent with a horseshoe kidney and a unilateral duplex collecting system in 2017, however were otherwise normal. Therefore, given the absence of renal scarring, it is not necessary to account for these participants urological diagnoses in analysis of cardiovascular function.

4.1.5.2.1. Anxiety

Two participants (18.2%) had an anxiety disorder diagnosis.

Compared to depressive illness, there is limited literature and consensus on HPA axis function in patients with an anxiety disorders. (281) A study of 1,427 participants (774 with current anxiety) demonstrated an association between significantly elevated awakening salivary cortisol levels in participants with current anxiety compared to healthy controls, which persisted after adjustment for

sociodemographic, race, sampling, and health factors. However, this study found no association with anxiety status and evening cortisol levels. (282) The findings from this study deserve consideration when assessing participants salivary cortisol values.

Meta-analysis of 46 cohort studies concluded that anxiety disorders were associated with an increased risk of cardiovascular events: coronary heart disease (41%), stroke (71%), heart failure (35%). (283) One recent study suggested that even subclinical levels of anxiety should be considered as a cardiovascular risk factor. (284) Celano et al. described behaviour factors, such as reduced treatment adherence and unhealthy dietary habits, and physiological factors, such as autonomic and platelet dysfunction, to contribute to the adverse relationship between anxiety and acquired CVD. (285) For these reasons, participants' additional diagnosis of anxiety should be considered when interpretating the STARRY study exploratory data.

4.1.5.3. Surgical history

Six participants have undergone a surgical procedure. From the participants medical records, it did not appear that they were treated with glucocorticoids, which eliminates the possibility of glucocorticoid-induced adrenal insufficiency. (286) Participants with a surgical history should have a similar HPA axis to participants without a surgical history.

Two participants had an adenotonsillectomy and insertion of grommets for recurrent otitis media with effusion. A different participant had a right myringoplasty for a perforated ear drum. Another participant had Kestenbaum surgery for the management of nystagmus.

One participant with severe aortic stenosis had a surgical aortic valvotomy. Severe aortic valve restenosis occurred and catheter balloon aortic valvotomy was performed. Another participant had a posterior sagittal anorectoplasty for an anterior anus. She was subsequently troubled by rectal bleeding and had a mucosal rectal prolapse repaired.

4.1.6. Participants current medication usage

Short stature and premature ovarian failure are the most consistent features of TS. (4, 14) With an aim to increase their final adult height, nine participants had completed or were being treated with human growth hormone (hGH) at the time of the study (Table 4.5). The mean age of commencing hGH treatment was 7.7 ± 2.7 years.

Table 4.5 | Human growth hormone treatment usage in the STARRY study cohort.

Growth Hormone	Frequency of cohort, n (%)	Additional information
On treatment	7 (63.6)	
Completed treatment	2 (18.2)	• Final adult height reached[a,b]
Treatment not indicated	2 (18.2)	• Congenital cardiac abnormality[c]
		• Discontinued after 4.5 years due to excessive weight gain

Abbreviations – n: number.

a – 150.1 centimetres (cm); b – 149.8 cm; c – Mosaic karyotype (30% 45,XO, 70% 46,XX), height Z-score -2.64 SD, aortic stenosis.

As a consequence of ovarian dysgenesis, most girls with TS require oestrogen for pubertal induction. (4) Three participants (27.3%) were prescribed oestrogen-only transdermal hormonal replacement for pubertal induction. Three participants (27.3%) were prescribed oestrogen and progesterone HRT: cyclical combined, continuous combined pill, continuous combined transdermal patch. The average age of artificial pubertal induction was 12.3 ± 0.5 years. Two participants (18.2%) with a mosaic karyotype (88% 45,XO and 12% 46,XX and 30% 45,XO, 70% 46,XX) and did not require HRT at present as they had spontaneous thelarche and had regular menstrual bleeds. These participants had menarche at 11.5 and 12.8 years, respectively. Three participants, aged 9.0, 11.2 and 13.2 years, had not commenced HRT or experienced spontaneous thelarche.

Participants additional medications can be found in Table 4.6.

Table 4.6 | STARRY study participants medications (excluding those mentioned elsewhere).

Medication	Frequency of cohort, n (%)	Indication
Oxandrolone	2 (18.2)	Poor growth velocity[a]
Cholecalciferol	2 (18.2)	Low vitamin D levels
Tranexamic acid[b]	1 (9.1)	Heavy menstrual bleeding
Methylphenidate	1 (9.1)	Attention deficit hyperactivity disorder
Fluoxetine	1 (9.1)	Depression
Skin emollients	1 (9.1)	Eczema

Abbreviations – n: number.

a – current height Z-score -2.0 SD & -2.4 SD; b – spontaneous menarche (karyotype – 88% 45,XO, 12% 46,XX).

4.1.6.1. Considerations for assessment of participants cardiovascular health

4.1.6.1.1. Human Growth Hormone therapy

hGH has likely beneficial effects on the cardiovascular system in TS. Studies have demonstrated a decreased prevalence of arterial hypertension, improvements in lipid profiles and body composition and decreased BMI in women treated with hGH in childhood compared to women who were not treated. (287-290) The negative impact of hGH therapy on glucose metabolism in TS has been widely debated, although most long-term data have been reassuring. (289, 291) A study of 102 girls (76 hGH-treated) suggested that the beneficial effects of hGH on body composition may outweigh the negative effects of hGH-induced insulin resistance. (290) Independent of hGH-therapy, it is thought that inherent TS-specific mechanisms of insulin resistance and impaired beta-cell function put patients at greater risk of T2DM. (170) Insulin resistance in the STARRY study cohort will be discussed in section 4.2.4.

4.1.6.1.2. Hormone replacement therapy

There has been considerable debate regarding the optimal protocol for the induction of puberty in girls with TS, reflecting the lack of robust evidence in this area. It is possible that early induction of puberty, using very small doses of oestrogen, may have a beneficial effect on cardiovascular health. Long-term HRT has demonstrated lowered BP, improved lipid profile, decreased visceral adiposity and normalised coagulation in women with TS. (4) The optimal route of administration, dose and type of oestrogen and progesterone replacement therapy for cardiovascular health in TS is largely unknown. (1) All STARRY study participants were either below the expected age of thelarche of general population, had entered puberty spontaneously or were receiving adequate HRT. It was therefore assumed that participants would not be at an increased risk of CVD development compared to the general female population as a consequence of oestrogen deficiency.

The participant receiving HRT via the continuous combined pill may be at greater risk of hypertension compared to participants taking transdermal oestrogen regimens: there is a much greater induction of renin substrate by the ethinyl radical of artificial oestrogens. (292) Studies have also shown that

girls with TS treated with transdermal natural regimens have a more physiological hormonal profile than those treated with oral natural oestrogens. (23)

4.1.6.1.3. Methylphenidate

There is conflicting evidence on the short-term cardiovascular safety of methylphenidate. Three large cohort studies concluded that there was no increased risk of serious cardiovascular events. (293-295) More recently, a large self-controlled case series analysis of 1224 children and young people (≤17 years of age) demonstrated an increased risk of arrythmias, especially in children with congenital cardiac abnormalities, during the first two-months of treatment with methylphenidate. This study also demonstrated an increased risk of myocardial infarction after a week of treatment initiation, persisting for two-months. (296) The participant taking this medication has been doing so for longer than two-months, and despite having congenital cardiac abnormalities, therefore was not in the high risk-period at the time of the study.

4.1.6.2. Considerations for assessment of participants cortisol levels

4.1.6.2.1. Cholecalciferol

In a study of 15 healthy volunteers, possibly due to a reduction in 11β-HSD1 activity, vitamin D supplementation decreased urinary cortisol and cortisol: cortisone ratio. (297) The relevance of this to the STARRY study analysis is unclear: saliva was the method of measuring cortisol and cortisone levels, and the two participants on cholecalciferol therapy should have physiological Vitamin D levels.

4.1.6.2.2. Methylphenidate

A systemic review, including nine case-control studies, identified that salivary cortisol levels in untreated children with ADHD are significantly lower than in healthy controls. The authors hypothesised that patients with ADHD have an impaired ability to regulate the HPA axis stress response, with an elevated threshold for stressors. (298)

A study of 45 children showed that one-month of treatment with methylphenidate significantly increased in salivary cortisol levels, so that there was no longer a significant difference to the healthy control group levels. (299) Methylphenidate increases cortisol levels by triggering central nervous system-mediated dopamine release. (298) The participant taking methylphenidate for combined

inattentive-hyperactive ADHD had been doing so for over one-month and therefore, for the purpose of our exploratory analysis, should have normalised salivary cortisol levels.

4.1.6.2.3. Fluoxetine

The participant waiting for an ADHD assessment, who may therefore have low salivary cortisol levels, also has comorbid depression, for which she takes low-dose (10.0mg) fluoxetine medication. In a study of 25 patients with major depressive disorder (35.5 ± 7.5 years) salivary cortisol levels significantly decreased after 6 weeks of fluoxetine therapy (20.0 – 40.0mg), although these cortisol levels were still higher than in the control group (14.5 ng/dl compared to 7.9 ng/dl). (300) The data from this patient should therefore be interpreted with caution, and it may be appropriate to consider the use of these medications as exclusion criteria in future studies.

4.1.7. Family History of cardiovascular disease

The Framingham Offspring Study defines a cardiovascular event as: coronary death, non-fatal myocardial infarction or coronary insufficiency, angina pectoris, stroke and intermittent claudication. (301) The frequency of the discussed cardiovascular events in the study populations' family members are shown in Table 4.7.

Table 4.7 | Cardiac history in participants family members.

Cardiac History	Relation to participant	Frequency of cohort, n
(i) First degree relative[a]		
Coronary artery disease/angina pectoris	Father	1
(ii) Second degree relative[b]		
Atherothrombotic stoke	Grandfather	3
	Grandmother	1
Coronary artery disease/angina pectoris	Grandfather	5
Myocardial infarction (fatal and non-fatal)	Grandfather	2

Abbreviations – n: number.

a – parent, full-sibling, child; b – aunt, uncle, grandparent, grandchild, niece, nephew, half-sibling.

Seven participants (63.6%) had at least one second-degree family member with a history of a cardiovascular event. Of these seven participants, one participant had a first-degree family member with a history of acquired CVD. The Framingham Offspring Study demonstrated that, after accounting

for additional risk factors, offspring of at least one parent with premature CVD (father <55 years of age, mother <65 years of age) was associated with a 70% increased cardiovascular risk for women (aged 44.3 ± 9.1 years) followed over eight-years. (301) The implications from the Framingham Offspring Study cannot easy be applied to the STARRY study cohort, where the mean age of participants was 13.8 ± 2.4 years. It is unclear whether the participant with a first-degree relative with a history of CVD is at an additionally increased cardiovascular risk, as the age at which the participants' father was when had a cardiovascular event is unknown.

4.2. Biochemical results

4.2.1. Renal Function

Table 4.8 presents information on participants electrolyte, urea and creatine levels. Reference ranges were sourced from the Alder Hey Biochemistry Laboratory Handbook. (302)

Table 4.8 | Participants renal function tests.

Electrolyte	Mean ± SD (range), mmol/L	Age in years, (n in cohort)	Reference range, mmol/L
Sodium	140.0 ± 1.3 (139.0 – 143.0)	All (11)	135.0 – 145.0
Potassium	3.9 ± 0.3 (3.4[a] – 4.3)	All (11)	3.5 – 5.5
Chloride	106.7 ± 1.2 (105.0 – 109.0)	All (11)	100.0 – 110.0
Bicarbonate	24.2 ± 1.3 (23.0 – 26.0)	All (11)	18.0 – 29.0
Anion gap	13.0 ± 1.1 (11.0 – 15.0)	All (11)	4.0 – 16.0
Calcium	2.4 ± 0.1 (2.3 – 2.5)	2-16 (10)	2.15 – 2.74
	2.30[b]	>16 (1)	2.25 – 2.74
Phosphate	1.6 ± 0.4 (1.3 – 1.9)	8 – 12 (2)	0.97 – 1.94
	1.3 ± 0.2 (1.0 – 1.6)	12 – 16 (8)	0.81 – 1.51
	1.3[b]	>16 (1)	0.74 – 1.55
Magnesium	0.8 ± 0.1 (0.8 – 0.9)	All (11)	0.7 – 1.0
Urea	21.3 ± 4.5 (2.1[a] – 6.2)	All (11)	2.3 – 6.4
Creatinine	48.0[b]	8 – <10 (1)	35.0 – 66.0[c]
	39.0[b]	10 – <12 (1)	38.0 – 71.0[c]
	44.3 ± 2.2 (42.0 – 47.0)	12 – <14 (4)	40.0 – 80.0[c]
	42.3 ± 8.1 (33.0[a] – 47.0)	14 – 16 (3)	47.0 – 88.0[c]

	50.0 ± 9.9 (43.0[a] – 57.0)	>16 (2)	49.0 – 81.0[c]

Abbreviations – SD: standard deviation; n: number; mmol/L: millimoles per litre.

a – low result; b – one participant; c – micromoles per litre.

One participant had mild hypokalaemia, which did not warrant further investigation. All other participants had normal electrolyte levels. Regarding cardiovascular health, electrolyte disturbances can precipitate cardiac arrhythmias. (303)

Two participants had a low creatine level (33.0 mmol/L and 43.0 mmol/L), and a different participant had a low urea level (2.1 mmol/L). These low levels may be variations of normal, however, it is plausible that the participant with the creatine level of 33.0 mmol/L may be attributed to her reduced muscle mass (percentage appendicular muscle mass was below the 2nd centile for the participant's age and gender). (304, 305) Creatine levels may be reduced secondary to hyperthyroidism, although all participants had normal thyroid function tests. (306) Advanced liver disease can also contribute to low serum creatine and urea levels, however all participants also had normal aspartate transaminase and alanine transaminase levels for their age. (304, 307) These abnormal renal biochemical findings did not warrant further investigation or additional consideration in the STARRY study exploratory analysis.

4.2.2. Alkaline Phosphatase

Three participants had elevated serum alkaline phosphatase (ALP) levels. These participants were aged 15.2, 15.8 and 17.4 years, and all were being treated with hGH. Before commencing hGH therapy, bone age was documented in two of these participants medical records: delayed bone age compared to their chronological age (1.7 years and 2.5 years) was observed. It may be more appropriate to interpret these values against bone age opposed to chronological age. Indeed, accounting for bone age delay, both participants serum ALP levels would be classified as within normal limits.

Several studies have shown an association between elevated serum ALP levels and coronary atherosclerosis. (308, 309) These studies are likely of limited relevance to these participants, as it is expected that their ALP levels will fall to normal levels for their age and gender after completion of hGH treatment.

4.2.3. Metabolic variables

4.2.3.1. Lipid profile and fasting glucose

Reference ranges were sourced from the Alder Hey Biochemistry Laboratory Handbook. (302) A triglyceride-to-HDL-C ratio of ≥2.0 was used as a cut-off for monitoring lipid profiles based on data from an outpatient population of 884 white children and adolescents from Italy. Independent of cofounding factors, participants with a ratio above this threshold had at least a two-fold higher risk of elevated alanine transaminase levels and concentric left ventricular hypertrophy compared to those with a ratio below this. (310) Table 4.9 presents participants data from their metabolic blood tests.

Table 4.9 | Participants metabolic blood test results.

Biochemical variables	Mean ± SD (range), mmol/L	Age in years (n in cohort)	Reference range, mmol/L	Frequency of low readings, n (%)	Frequency of high readings, n (%)
Fasting glucose	4.6 ± 0.4 (4.0 – 5.5)	All (11)	2.6 – 6.1	-	-
HbA1c	31.5 ± 1.4 (30.0 – 34.0)	All (10)[a]	20.0 – 42.0	-	-
TG	0.5[b]	0-9 (1)	<0.85	-	-
	0.7 ± 0.2 (0.5 – 1.0)	10-19 (10)	<1.02	-	-
Total cholesterol	4.0 ± 0.9 (2.8 – 5.6)	2-14 (7)	3.1 – 5.4	1 (9.1)	1 (9.1)
	4.1 ± 0.9 (3.2 – 5.3)	Adult (4)	3.1 – 6.5	-	-
LDL-C	2.6 ± 0.7 (1.3 – 3.8)	All (11)	<2.85		2 (18.2)
HDL-C	1.4 ± 0.4 (1.0 – 2.1)	All (11)	>1.17	3 (27.3)	
TG-to-HDL-C ratio	0.5 ± 0.2 (0.3 – 0.9)[c]	All (11)	≤2.0		-

Abbreviations – mmol/L: millimoles per litre; SD: standard deviation; n: number; TG: triglycerides; LDL-C: low-density lipoprotein cholesterol; HLD-C: high-density lipoprotein cholesterol.
a – missing one participant's data (insufficient sample); b – one participant; c – no units.

All participants had a fasting glucose and HbA1c blood sample level within normal limits. In the absence of clinical features, such as polyuria, polydipsia and unintentional weight loss, further investigations for the diagnosis of diabetes mellitus (type 1 or type 2) were not warranted in any participant. (311)

Triglyceride levels were normal in all participants, and no participant had a triglyceride-to-HDL-C ratio of ≥2.0. However, five participants (45.5%) had a proatherogenic lipid profile: either a high LDL-C level or a low HDL-C level. No participant had both abnormal LDL-C and abnormal HDL-C levels. One participant with an elevated HLD-C level also had an elevated total cholesterol level. Similarly, one participant with a low HDL-C level had a low total cholesterol level.

Dietary improvements and increased physical activity can help to raise low HLD-C levels. (312, 313) Interestingly, one participant with a low HLD-C level had a healthy weight (BMI Z-score -0.2 SD). (264) The two participants with elevated LDL-C levels did not reach the cut-off for pharmacological intervention. (314) Both of these participants were overweight, but not obese (BMI Z-score of +1.4 SD and +1.5 SD). (264)

Table 4.10 presents the difference between age, BMI Z-scores or percentage body fat mass of participants with a normal and a proatherogenic lipid profile. There was a tendency towards higher BMI Z-scores in participants with a proatherogenic lipid profile, although this difference between the two groups was not significant, likely due to the small size of our cohort.

Table 4.10 | Abnormal lipid profile and other cardiovascular risk factors.

Variable	Proatherogenic lipid profile, n=5 Mean ± SD (range)	Normal lipid profile, n=6 Mean ± SD (range)	*P*-value
Age, years	13.8 ± 1.9 (11.2 – 16.2)	13.2 ± 2.7 (9.0 – 17.4)	0.67
BMI Z-score, SD	+1.5 ± 1.1 (-0.2 – +2.9)	+0.4 ± 1.5 (-1.0 – +2.4)	0.22
BFM, %	31.9 ± 8.9 (22.6 – 46.1)	27.8 ± 8.3 (17.0 – 41.4)	0.45

Abbreviations – n: number; SD: standard deviation; BMI: body mass index; BFM: body fat mass.

4.2.3.2. Leptin

There is an absence of paediatric reference values for serum leptin concentrations. Hamann T.V. et al cautions against directly comparing leptin concentrations across studies due to sources of variation (preanalytical, analytical and post analytical). (315) Indeed, the two large studies of healthy weight Danish schoolchildren establishing age-and-sex-specific reference percentiles lacked consistency. (315, 316) Table 4.11 categorises STARRY study participants data (n=11) according to the two studies.

Table 4.11 | Leptin data compared to reference percentiles.

Study Population		Number of STARRY participants in each category, n (%)					
Cohort size, n	Characteristics, median (range)	<5th	5th – 25th	>25th – 50th	>50th – 75th	>75th – 95th.	>95th

Lausten-Thomsen, U., et. al (2016) (316)	1196 (730 girls)	Age: 11.9 (6 – 18) years; BMI: 0.34 (-1.65 – +1.65) SDS	0 (0)	5 (45.5)	1 (9.1)	1 (9.1)	2 (18.2)	2 (18.2)
Hammann, T.V., et. al (2022) (315)	853 (437 girls)	Age: 10.0 (7 – 17) years; BMI: +0.04[a] ± 1.06 SDS	0 (0)	0 (0)	0 (0)	5 (45.5)	2 (18.2)	4 (36.4)

Abbreviations – n: number; BMI: body mass index; SDS: standard deviation score.

a – mean; b – standard deviation.

Participants data is consistent with the Lausten-Thomsen U., et. al reference population, with six participants (54.5%) with levels below and five participants (45.5%) with levels above the 50th centile. (316) Whereas, when compared to the Hammann T.V. et. al reference population, participant data is skewed towards the higher centiles, with no participants levels falling below the 50th centile. (315) In this reference population, leptin Z-scores positively correlated with BMI-Z scores. It is plausible that the difference in mean BMI Z-score between the cohorts (approximately +1 SD greater in STARRY study participants), contributed to the higher levels observed. Further studies with age-sex-and-BMI-matched control groups are required to further investigate leptin concentrations in paediatric patients with TS.

In STARRY study participants there was a strong statistically significant positive correlation between fasting serum leptin and percentage body fat mass (Pearson's Correlation coefficient +0.905) (Figure 4.3).

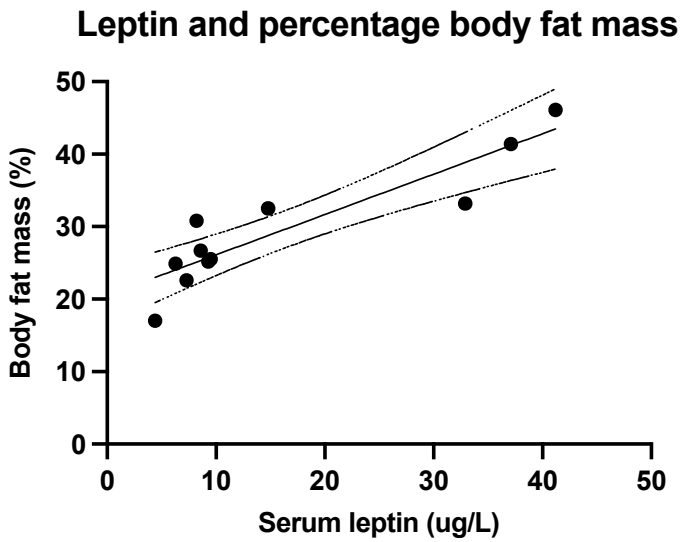


Figure 4.3 | Correlation between participants serum 9am leptin samples and their percentage body fat mass.

4.2.4. Insulin resistance

The homeostasis model assessment (HOMA) and fasting glucose to insulin (G:I) ratio can be used to calculate insulin resistance. Both methods were used to calculate insulin resistance for each STARRY study participant.

4.2.4.1. HOMA-IR

The HOMA formula utilises fasting glucose and insulin concentrations: HOMA-IR = Insulin (mU/L) x glucose (mmol/L) / 22.5. (317) The mean insulin level for participants was 8.8 ± 4.4 mU/L and average HOMA-IR was 1.9 ± 1.4.

There is limited literature on HOMA-IR cut-offs for insulin resistance to identify metabolic syndrome in children and adolescents. Table 4.12 presents four studies and the number of STARRY study participants who were above each respective threshold.

Table 4.12 | Insulin resistance in the STARRY study participants compared to reference data.

Study	Background information	Definition of IR, HOMA-IR	Frequency of STARRY study participants with IR, n (%)

Lee, J. M., et. al (2006) (318)	1,169 US adolescents (564 female) with a BMI <85th centile, aged 12 –19 years. >+2 SD above mean HOMA-IR for healthy weight adolescents with normal fasting glucose.	>4.39	0 (0)
Keskin, M., et. al (2005) (319)	57 Turkish pubertal obese (BMI >95th centile) adolescents (30 female), average age 12.0 ± 2.9 years. Cut-off point for diagnosis of IR yielded a sensitivity of 76% and specificity of 66%.	>3.16	1 (9.09)
Yin, J., et. al (2013) (320)	1037 Chinese children and adolescents (595 female) aged 6 – 18 years without metabolic syndrome. 95th percentile for HOMA-IR.	>3.0	3 (27.3)
Shashaj. B., et. al (2016) (321)	808 Caucasian Italian children and adolescents (374 female) with a BMI <85th centile, aged 2 – 17 years. 75th percentile for HOMA-IR (IR yielded a sensitivity of 47.4% and specificity of 84.9%).	>1.68	6 (54.5)

Abbreviations: IR: insulin resistance; BMI: body mass index; SD: standard deviation; n: number; US: United States of America.

Lee, J. M., et. al and Yin, J., et. al similarly defined their cut-off for HOMA-IR using the 95th HOMA-IR percentile (>+2 SD above the mean) for their healthy weight population. Interestingly, the HOMA-IR cut-off for the Chinese population was lower than the United States population by 1.39. (318, 320) Aside from increased body weight, increased prevalence of insulin resistance is associated with diets high in saturated fats, low levels of physical activity, chronic stress and chronic sleep deprivation. (322) Indeed, a self-reported questionnaire comparing body composition and lifestyle habits of 2,488 healthy weight Chinese men and women residing in North America and the Peoples Republic of China showed significant differences: participants residing in China had a diet lower in fat, spent more time doing vigorous physical activity and sleeping and had a leaner body composition. (323) It is possible that the lifestyle habits between healthy weight populations living on the two continents could provide an explanation for the differences in HOMA-IR cut-offs.

Obesity is known to affect HOMA-IR values. Shashaj. B., et. al defined HOMA-IR values above 1.68 as "non-physiological" for healthy weight children and adolescents, and values above 3.42 for those who

were overweight or obese. These thresholds were derived from the 75th percentile of HOMA-IR values of the populations: the 90th percentile was 2.33 and 4.98 for healthy weight and overweight and obese children and adolescents, respectively. (321) Using STARRY study participants BMI Z-scores and the WHO classification, (264) two healthy weight and no overweight and obese participants had a HOMA-IR above the 90th percentile according to this reference population. The two healthy weight participants, BMI Z-score -0.5 and +0.3 SD, had an HOMA-IR of 3.16 and 3.07, respectively. One of these participants had a low muscle-to-fat ratio (see section 4.3.4).

The participant with the highest HOMA-IR in the STARRY study of 3.22 was obese (BMI Z-score +2.9 SD) and pubertal. This participant had a number of other cardiovascular risk factors, for example, an abnormal lipid profile, a high ambulatory arterial stiffness index (AASI) and masked hypertension. Interestingly, this was the only participant to never have commenced hGH therapy, which is known to increase insulin resistance during treatment. (170)

4.2.4.2. Fasting glucose to insulin ratio

A low ratio of fasting glucose (mg/dL) to insulin (mU/L) (G:I) can depict insulin resistance. The cut-off G:I ratio value of <4.5 mg/10 $^{-4}$U (95% sensitivity, 84% specificity) for insulin resistance was derived from a study of 40 women (aged 18 – 40 years) with polycystic ovarian syndrome when compared to 15 control subjects. (324) This reference range is used in the Alder Hey Biochemistry Laboratory Handbook. (302)

The average G:I ratio of STARRY study participants was 13.8 ± 10.8 mg/10 $^{-4}$U (range 5.5 – 35.6 mg/10 $^{-4}$U). No participant had a G:I ratio indicative of insulin resistance (<4.5 mg/10 $^{-4}$U).

4.2.5. Cardiovascular variables

Of the ten participants with measured plasma clotting factor levels, four participants (40.0%) had levels exceeding our reference ranges. (325, 326) Two of these four participants had isolated abnormal values. No participant had plasma levels indicative of von Willebrand disease (vWD). (326) Four participants had inflammatory marker levels exceeding our reference range. (327) One participant had both raised inflammatory and procoagulant markers. Table 4.13 presents these data.

Table 4.13 | Participants pro-coagulant and inflammatory markers.

	Mean ± SD (range)	Reference range	Frequency of low readings, n (%)	Frequency of high readings, n (%)
(i) Procoagulant markers (n=10)				
Factor VIII %	145.1 ± 56.3 (88.7 – 240.8)	50.0 – 150.0	0	3 (30.0)[a,b,c]
vWF ACT %	121.6 ± 55.6 (65.3 – 217.8)	50.0 – 150.0	0	3 (30.0)[a,b,d]
vWF AG %	103.5 ± 32.5 (64.1 – 153.5)	50.0 – 150.0	0	1 (10.0)[a]
(ii) Inflammatory markers (n=10)				
Fibrinogen g/L	2.8 ± 0.4 (2.3 – 3.5)	1.8 – 3.5	0	1 (10.0)[e]
ESR mm in 1 hour	6.3 ± 3.9 (2.0 – 18.0)*	0.0 – 8.0	0	3 (27.3)[b,f,g]

Abbreviations – SD: standard deviation; n: number; VWF: von Willebrand Factor; ACT: activity; AG: antigen; ESR: erythrocyte sedimentation rate; g/L: grams per litre.

a, b, c, d, e, f, g – different participants.

***11 participants.**

4.2.5.1. Renin

The mean plasma renin value for participants was 20.2 ± 8.3 milliunits per litre (mU/L) (range 9.2 – 30.2 mU/L). Reference ranges were provided from the processing laboratory (University Hospital Southampton Trust). Four participants (36.4%) had a plasma renin value below the age-appropriate reference range. No participants had values above this range.

4.2.6. Cortisol

The mean 9am serum cortisol value for participants was 300.1 ± 166.8 (median 282.5) nanomoles per litre (nmol/L) (range 110.0 – 672.0 nmol/L). This value was not dissimilar from a study of 19 healthy female paediatric volunteers from the Liverpool region, where the mean 9am serum cortisol was 280.2 ± 83.6 (median 301.0) nmol/L (range 156 – 419 nmol/L). (243) In fact, there was no significant difference between these two values (p=0.67).

The reference range for 9am serum cortisol sourced from the Alder Hey Biochemistry Laboratory Handbook is 140.0 – 500.0 nmol/L. (302) One participant had a cortisol value below this range (110.0 nmol/L), although due to unforeseeable delays this participant had their blood drawn at approximately 10:30am, when cortisol levels are physiologically starting to fall. One participant had a cortisol value above this range (672.0 nmol/L). This participant was needle-phobic, and it is possible

that this value was a result of stress-induced elevation. Neither participant's abnormal serum cortisol value warranted further investigation.

4.2.6.1. Corelation to salivary cortisol and cortisone

Ten participants had both a 9am serum cortisol value and 9am salivary biomarker (cortisol and cortisone) values. There was a strong positive corelation between participants serum cortisol and salivary cortisol (Pearson correlation coefficient +0.795) (Figure 4.4). The correlation between serum cortisol and salivary cortisone was strong, although weaker than with salivary cortisol (Pearson correlation coefficient +0.760) (Figure 4.5). The correlation between serum cortisol and salivary biomarkers was not strengthened using the sum of salivary cortisol and salivary cortisone (Pearson correlation coefficient +0.780).

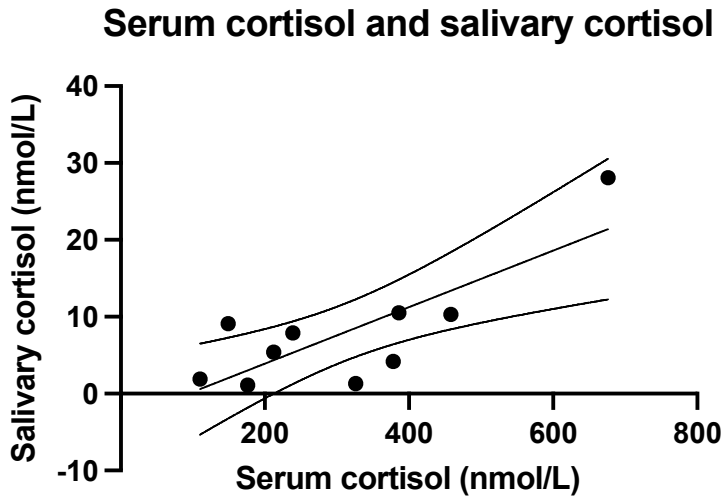


Figure 4.4 | The correlation between 9am serum cortisol and 9am salivary cortisol.

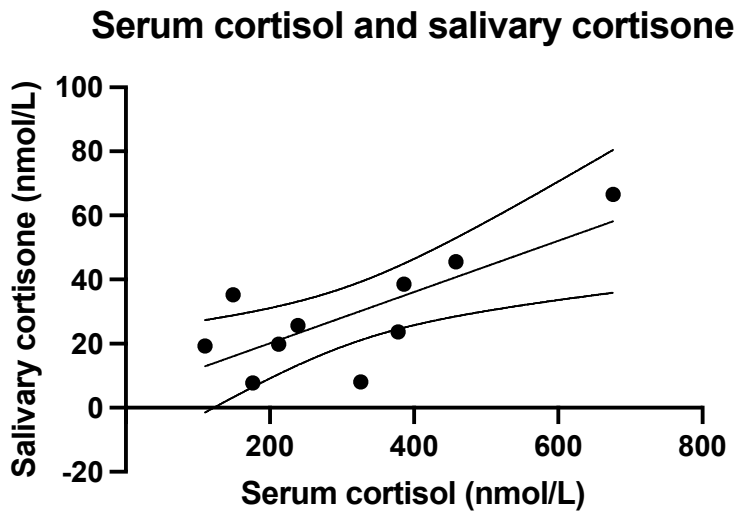


Figure 4.5 | The correlation between 9am serum cortisol and 9am salivary cortisone.

4.3. Body composition

Height, weight and BMI Z-scores were calculated using iGrow software. iGrow is installed in over 20 Hospitals and Community Trusts throughout the UK and uses UK data behind the chart's plotting. InBody scales calculated participants waist-to-hip (WTH) ratio, total body mass, body fat mass (BFM) and appendicular skeletal muscle mass (SMMa) in kilograms (kg). Percentages of BFM and SMMa out of total body mass were calculated. Muscle-to-fat ratio was derived by dividing SMMa (kg) by BFM (kg).

WHO definitions of obesity were used to classify participants BMI Z-score: thinness <-2 SD, overweight >+1 SD, obesity >+2 SD. (264) The WHO defined a WTH ratio of >0.85 as a substantially increased metabolic risk for women. (328) Reference centile curves for Caucasian children aged 5-18 years in the UK were used for bioimpedance variables. (305, 329) For percentage BFM reference data classified ≤2nd centile for age and gender as "under-fat", ≥85th centile as "over-fat" and ≥95th centile as "obese". (329) We similarly used the ≤2nd centile for age and gender to define "under-muscular". Reference data derived the lower limit of normal for muscle-to-fat ratio (MFR) as the mean minus 2 SDs for children in the middle fifth of the BMI range. For girls aged 5 – 10 years this was 1.1 and for girls aged between 11 and 18 years this was 0.8. (305)

Table 4.14 presents these data.

Table 4.14 | Participants body composition.

	Mean ± SD (range)	Under, n (%)	Healthy, n (%)	Over, n (%)	
				Overweight, n (%)	Obese, n (%)
I. General					
Height Z-score, SD	-2.0 ± 0.7 (-2.9 – -1.1)				
Weight Z-score, SD	-0.3 ± 1.4 (-2.0 – +1.6)				
BMI Z-score, SD	+0.9 ± 1.4 (-1.0 – +2.9)	-	5 (45.5)	2 (18.2)	4 (36.4)
II. Central adiposity					
WHR	0.8 ± 0.1 (0.7 – 0.9)		9 (81.8)	2 (18.2)	
III. Bioimpedance variables					
BFM, %	29.6 ± 8.4 (17.0 – 46.1)	-	6 (54.5)	1 (9.1)	4 (36.4)
SMMa, %	26.2 ± 2.9 (20.7 – 28.5)	2 (18.2)	9 (81.8)		
MFR	1.0 ± 0.3 (0.5 – 1.7)	7 (63.6)	4 (36.4)		

Abbreviations: BFM: body fat mass; SMMa: Appendicular skeletal muscle mass; WHR: waist-to-hip ratio. MFR: muscle-to-fat ratio.

4.3.1. Height

The height z-score for participants age and gender was normally distributed, with a mean of -2.0 ± 0.7 SD (range -1.1 – -2.9 SD). Short stature is defined as a height of 2 or more SD below the mean for chronological age and gender, and six participants (54.5%) fit this definition. (330) This is as expected, as short stature is a near universal feature of TS. (4)

Participants height Z-score had a negligible positive correlation with participants weight Z-score (Pearson correlation coefficient +0.281), shown in Figure 4.6. This suggests that height Z-scores are not a strong predictor of weight and metabolic risk in TS, although further studies are needed to confirm this.

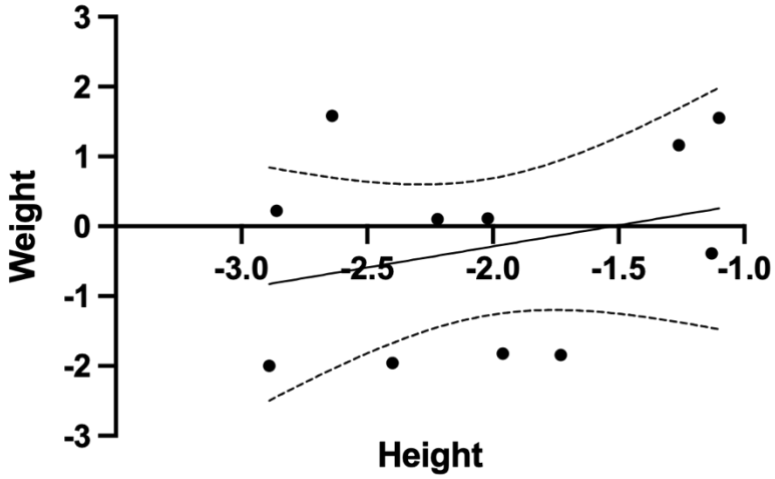


Figure 4.6 | Correlation between participants height and weight Z -scores.

4.3.2. Weight

The median BMI Z-Score was +1.4 SD (IQR -0.4 – +2.0 SD). The WHO defines a BMI Z-score of <-2 SD as thin, >+1 SD as overweight and >2+ SD as obese. Over half of participants (54.5%) in our cohort were classified as overweight or obese. None of the participants were underweight. (264)

4.3.3. Metabolic Syndrome

The two participants with the highest BMI Z-scores (+2.4 SD and +2.9 SD) had a WTH ratio indicative of excess abdominal adiposity (0.92 and 0.89). (328) Unfortunately, waist circumference (WC) was not measured in participants, so defining metabolic syndrome was difficult. However, assuming the two participants with a high WTH ratio had a WC ≥90th percentile for their age and gender, they did not meet the International Diabetes Federation (IDF) definition of metabolic syndrome in children and adolescents (Table 4.15). (331)

Table 4.15 | International Diabetes Federation Metabolic Syndrome definition for adolescents aged 10 – 16 years.

Participant ID	WC ≥90th percentile[a]	TG ≥1.7 mmol/L	HDL-C <1.03 mmol/L	Systolic BP ≥130 or diastolic BP ≥85 mm Hg	FPG ≥5.6 mmol/L
301	Yes	No	Yes	No	No
305	Yes	No	No	No	No

Abbreviations – ID: identifier; WC: waist circumference; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; BP: blood pressure; FPG: fasting plasma glucose.
a – accounting for age and gender.

Due to the characteristic short stature seen in TS, (4) the IDF definition of metabolic syndrome might not be appropriate for this population. Increased WC is an integral part of the definition, and these are defined by percentiles using age and sex, but not height. (331) The same WC in two individuals of the same age and different heights would pose an increased metabolic risk in the shorter individual. Furthermore, participant 301 had an awake systolic BP ≥95th percentile for her height on ABPM, (332) however this was lower than the IDF cut-off of 130 mmHg (123 mmHg). Given a high systolic BP would have resulted in a diagnosis of metabolic syndrome for this participant, her metabolic risk may be underestimated. (331)

4.3.4. Muscle and Fat ratio

Muscle and fat tissue have opposing effects on insulin sensitivity and energy disposable, (333, 334) and the balance between the two tissues may more accurately predict metabolic risk than BMI. (335) A low MFR is resultant of low SMMa and high BFM. All participants with a BMI Z-score of over +1.0 SD (overweight range) had a low MFR. One healthy weight participant (BMI Z-score -0.5 SD) also had a low MFR. (264, 305) There was a high prevalence of low MFR in the STARRY study cohort (63.6%). (305)

Just under half of participants (45.5%) had a %BFM over the normal range based on their age and gender. (329) All obese participants (BMI Z-score ≥+2 SD) had a %BFM in the obese range. One overweight participant had a %BFM in the overweight range. All other participants %BFM was in the healthy range. (264, 329) A high BFM and a low SMMa, hence a low MFR may confer a higher metabolic risk than high %BFM alone. (305) All participants with a high BFM had a low MFR. Two participants with a healthy %BFM also had a low MFR. (264, 305)

4.4. Continuous glucose monitoring

Continuous glucose monitoring (CGM) raw data from a healthy nondiabetic population was sourced. (265) Using a random number generator, the 10 STARRY study participants were matched to control subjects for gender, age and ethnicity. Table 4.16 presents data from the two groups.

Table 4.16 | Participants glucose data compared to matched controls subjects.

	STARRY participants	Control subjects	*P*-value
(I) Overall glucose distribution and variability, mean ± SD (range)			
Mean mmol/L	6.1 ± 0.4 (5.4 – 6.8)	5.5 ± 0.4 (4.9 – 5.9)	0.030[a]
S.D mmol/L	1.0 ± 0.2 (0.8 – 1.5)	0.8 ± 0.2 (0.6 – 1.1)	0.20
Coefficient of variation %	15.7 ± 3.8 (13.0 – 25.8)	15.3 ± 2.8 (11.9 – 18.8)	0.79
Number of readings	1627.2 ± 297.5 (1116.0 – 2019.0)	2069.0 ± 215.4 (1625.0 – 2345.0)	0.001[a]
(iii) Percentage of time spent with glucose readings in each category, median (IQR)			
>10.00 mmol/L	0.1 (0.0 – 0.4)	0.0 (0.0 – 0.1)	0.22
>8.89 mmol/L	1.2 (0.4 – 2.9)	0.0 (0.0 – 0.5)	0.023[a]
>7.78 mmol/L	5.4 (3.9 – 8.8)	1.1 (0.4 – 2.3)	0.002[a]
3.39 – 7.78 mmol/L	94.2 (90.1 – 95.8)	96.8 (91.9 – 98.7)	0.11
<3.39 mmol/L	0.2 (0.00 – 1.0)	1.4 (0.5 – 3.7)	0.06
<3.33 mmol/L	0.1 (0.00 – 0.2)	0.3 (0.0 – 0.5)	0.28
<3.00 mmol/L	0.0 (0.0 – 0.0)	0.1 (0.0 – 0.4)	0.28

Abbreviations – mmol/L: millimoles per litre; SD: standard deviation; IQR: interquartile range.

a – significant difference between the two groups.

The participants in the Shah, V. N. et. Al study were wearing the CGM for up to 10 days, three days more than participants in the STARRY study. (265) This resulted in a significant difference in the mean number of glucose readings between the two cohorts, however it was thought that these data were still comparable.

The mean of participants mean glucose readings was significantly different between the two groups, with higher readings in the STARRY study participants. Indeed, STARRY study participants spent a significantly greater percentage of time spent with glucose readings >7.78 mmol/L and >8.89 mmol/L than the age, gender and ethnicity matched control subjects overall. However, there was no significant difference between the percentage of time spent with clinically significant hypoglycaemia (<3.00 mmol/L) and hyperglycaemia (>10 mmol/L) between the two groups. (336) The current consensus for target glucose ranges for patients with T1DM is set between 3.39 – 10.00 mmol/L. The STARRY study cohort spent a median of 2.7% less time in this range compared to the control subjects. (265)

The difference in mean glucose readings between the two groups may not be immediately clinically significant, however, the finding that glucose readings were approximately 10% elevated in STARRY study participants compared to the control subjects overall deserves recognition. (265)

The coefficient of variation (CV), which is a metric for glucose variability, was not significantly different between the two groups. The threshold for unstable glucose homeostasis is defined a CV ≥36% in patients with diabetes mellitus. (337) None of the STARRY study participants had a CV above this threshold.

4.4.1. Participant 304

Participant 304 had a mean glucose of 5.8 mmol/L and a CV of 25.8%. Her sensor was active for 6.8 days. The percentage of time spent in each glucose category compared to guidance from the International Consensus Report from the Advanced Technologies & Treatments for Diabetes Congress can be found in Table 4.17. (338)

Table 4.17 | Participant 304's glucose data.

Glucose value, mmol/L	**<3.00**	**<3.33**	**<3.39**	**3.39 – 7.78**	**>7.78**	**>8.89**	**>10.00**
Time spent in each category %	3.0[a]	3.9	8.0[a]	83.2	8.8	4.2	1.6
Target time for adults with T1DM and T2DM %	<1.0	-	<4.0		>70.0		<25

Abbreviations – mmol/L: millimoles per litre; T1DM: type 1 diabetes mellitus; T2DM: type 2 diabetes mellitus.

a – result exceeding target range.

In view of her abnormal CGM profile, this participant is being brought back to the CRF for a repeat CGM. This CGM will be unblinded and have a hypoglycaemia alarm, which when sounds, the patient will complete finger prick blood glucose tests.

Participant 304 was asymptomatic for hypoglycaemia during the period of CGM as part of the STARRY study protocol. Her relevant 9am fasting STARRY study bloods tests values were as follows: cortisol 378.0 mmol/L, glucose 4.1 mmol/L, HbA1c 32.0 mmol/L, insulin <14.4 qmol/L.

4.4.2. Comparison to fasting glucose and HbA1c

There was a moderate positive correlation between mean plasma fasting glucose measurement by CGM and participants fasting glucose readings (Pearson's correlation coefficient +0.404). Figure 4.7 presents these data. The low correlation between these variables suggests that plasma fasting glucose does not accurately predict CGM values. The clinical relevance of this deserves consideration.

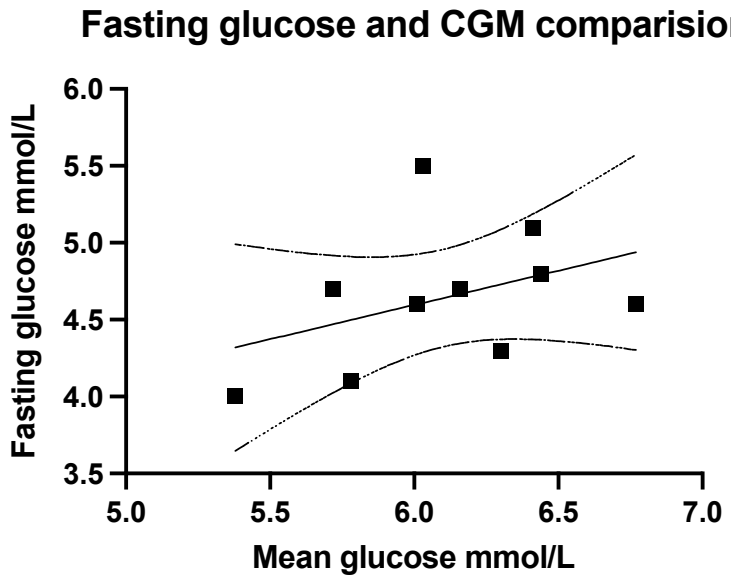


Figure 4.7 | Correlation between participants mean glucose from CGM and plasma fasting glucose.

Interestingly, the correlation between mean glucose measurement by CGM and participants' HbA1c readings was less significant than with fasting plasma glucose (Pearson's correlation coefficient +0.340) (Figure 4.8). This was unexpected, and may be reflective of our small sample size, as HbA1c is representative of the cumulative preceding three-month glycaemic history. (339) However, our data is supportive of the Shah, V. N. et. al study findings, in which our control subject data was sourced, where HbA1c failed to reflect true glycaemic status (Pearson's correlation coefficient +0.270). (265)

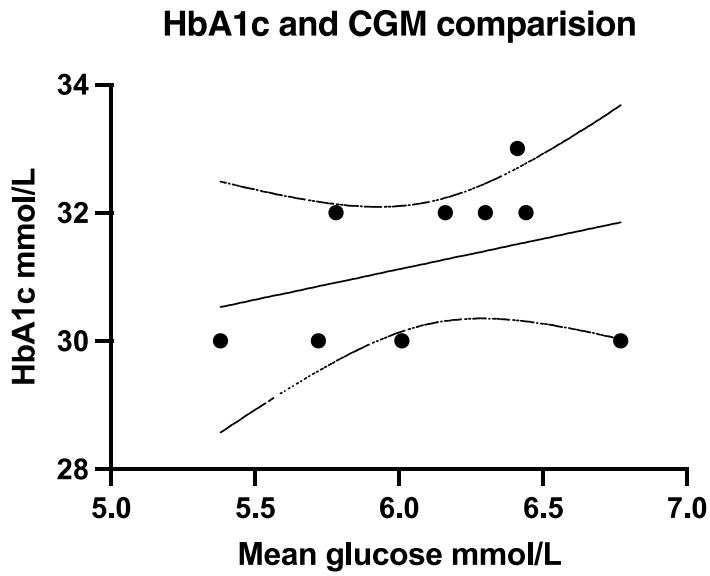


Figure 4.8 | Correlation between participants mean glucose from CGM and HbA1c.

4.5. Blood pressure

The 2016 European Society of Hypertension guidelines for the management of high BP in children and adolescents were used to classify participants BP values (Table 4.18). (332) Clinic BP was measured against age, gender and height values. Combined age, gender and height centiles for ABPM were not available. Given that short stature is a near-universal feature of TS, (4) two separate height and age ABPM centiles were calculated for the participants gender. All participants (n=11) had measurement of clinic BP and eight participants had ABPM.

Table 4.18 | Participants blood pressure percentiles.

	BP centile, n (%)		
	<90th	**≥90th – <95th**	**≥95th**
I. Clinic BP for age, gender and height (n=11)			
Systolic	5 (45.5)	2 (18.2)	4 (36.4)
Diastolic	6 (54.5)	3 (27.3)	2 (18.2)
II. ABPM for age and gender (n=8)			
Overall systolic	8 (100.0)	-	-
Systolic daytime	8 (100.0)	-	-
Systolic night-time	8 (100.0)	-	-
Overall diastolic	8 (100.0)	-	-

Diastolic daytime	8 (100.0)	-	-
Diastolic night-time	6 (75.0)	-	2 (25.0)
I. ABPM for height and gender (n=8)			
Overall systolic	8 (100.0)	-	-
Systolic daytime	7 (87.5)	-	1 (12.5)
Systolic night-time	8 (100.0)	-	-
Overall diastolic	6 (75.0)	2 (25.0)	-
Diastolic daytime	8 (100.0)	-	-
Diastolic night-time	6 (75.0)	-	2 (25.0)

Abbreviations – n: number.

Four participants (36.4%) had a systolic and/or diastolic clinic BP above or equal to the 95th centile for their age, gender and height. Two of these participants had isolated systolic hypertension. A further three participants had pressures above or equal to the 90th centile (63.6% of participants had a clinic BP ≥90th centile). (332)

ABPM showed that three participants (37.5%) had a systolic and/or diastolic clinic BP above or equal to the 95th centile for height and/or gender. (332) Table 4.19 presents the participants BP phenotypes according to clinic BP and ABPM. (340, 341)

Table 4.19 | Blood pressure phenotypes.

Study ID	Clinic BP ≥95th centile	ABPM BP ≥95th centile	Non-Dipping?	BP Phenotype
301	N	Y	Y	Masked hypertension with non-dipping pattern
302	Y	Y	Y	Hypertension with non-dipping pattern
303	N	N	Y	Sustained normotension with non-dipping pattern
304	N	N	Y	Sustained normotension with non-dipping pattern
305	N	-	-	Incident normotension
306	N	-	-	Incident normotension
307	N	N	Y	Sustained normotension with non-dipping pattern
308	N	N	N	Sustained normotension with extreme-dipping pattern
309	Y	Y	Y	Hypertension with non-dipping pattern
310	Y	-	-	Incident hypertension
311	Y	N	Y	White-coat hypertension with non-dipping pattern

Abbreviations – ID: identifier; BP: blood pressure; N: no; Y: yes.

4.5.1. The clinical utility of ABPM in Turner Syndrome

ABPM is useful in confirming the nature of high clinic BP readings. For example, as participant 310 did not have ABPM it is impossible to know whether her incident hypertension is white-coat or sustained. (340) Two participants had a clinic BP ≥95th centile for their age, height and gender (n=1, isolated systolic hypertension), and on ABPM had a normal systolic BP and isolated diastolic nocturnal hypertension. (332) A scientific statement from the American Heart Association concluded that isolated abnormalities in sleep BP on ABPM should be given the same weighting as awake BP abnormalities. (342) ABPM in these participants was able to confirm that their incident clinic hypertension was sustained and deserved further investigation.

ABPM also identified one participant with masked isolated systolic hypertension. This participant had a clinic systolic BP above the 90th percentile, but an ABPM with a systolic awake BP above the 95th percentile for her height. (332) This participant was therefore referred to nephrology for further investigation.

ABPM identified one participant with white-coat clinic hypertension (See section 4.5.1.1.). It also allowed for participants BP circadian rhythm to be evaluated (see section 4.5.2). (156)

4.5.1.1. Participant 311 – White-coat hypertension

Participant 310 had ABPM for 23 hours with 90.2% successful readings. Her average overall BP was 108/63 mmHg, which was below the 50th centile for her age and at the 50th centile for her height. Her daytime BP (111/64 mmHg) and night-time BP (103/59 mmHg) were also in the normal range for her age and height. (332) Table 4.20 presents her three most recent clinic BP measurements.

Table 4.20 | Participant 311's recent clinic BP measurements.

Date	Age (years)	Setting	BP reading (mmHg)	Percentile[a]
May 2022	13	CRF	133/87	99th
			141/87	99th
			123/78	95th
May 2022	13	Cardiology clinic	126/74	95th
November 2018	10	Cardiology clinic	119/81	95th

Abbreviations – BP: blood pressure; mmHg: millimetre of mercury; CRF: clinical research facility.

a – for age, height and gender.

During her clinic BP measurements on the CRF, the study team observed that the participant was anxious. A large retrospective cohort study of 1,392 patients with TS concluded that they are at a greater risk of receiving a psychiatric diagnosis compared to the general population, and the authors suggested that it likely that anxiety disorders are largely underdiagnosed. (59) In patients with TS, ABPM can be useful in detecting white-coat hypertension and preventing over-treatment of high clinic BP measurements.

4.5.2. Blood pressure circadian rhythm

Table 4.21 presents the mean BP dipping for the STARRY study participants.

Table 4.21 | Blood pressure circadian rhythm.

Dipping	Mean ± SD (range) %	Reference range (156) %	Frequency of participants below the reference range (%)
Systolic	8.6 ± 3.1 (3.9 – 13.0)		5 (62.5)
Diastolic	10.9 ± 5.6 (7.5 –23.7)	10.0 – 20.0	5 (62.5)
Mean arterial pressure	8.0 ± 3.9 (4.7 – 16.7)		7 (87.5)

Abbreviations – SD: standard deviation.

No participant had a conventional BP circadian rhythm. Three participants (37.5%) had non-dipping systolic and diastolic BP. Four participants (50.0%) had isolated systolic or diastolic non-dipping BP. The one participant (12.5%) without non-dipping mean arterial pressure had extreme-dipping diastolic BP. (156)

4.5.3. Heart rate

From the ABPM reports, the mean heart rate of participants was 90.4 ± 8.5 beats per minute (bpm) (range 79.0 – 99.0 bpm). The mean wake heart rate was 92.9 ± 8.2 bpm (81.0 – 105.0 bpm) and the mean sleep heart rate was 80.43 ± 10.33 bpm (69.0 – 95.0 bpm).

Using heart rate cut-offs derived from centile charts from a systematic review of 69 studies including over 140,000 healthy children's heart rate data, all participants (n=8) had an awake heart rate above

the median for their age and gender. Two participants had a heart rate above the 90th centile and one participant had a heart rate above the 99th centile. The STARRY study participants therefore had skewed higher heart rate values compared to the reference population. (343)

Mean heart rate had weak positive correlation with overall systolic BP (Pearson's correlation coefficient +0.436). Figure 4.7 presents this data.

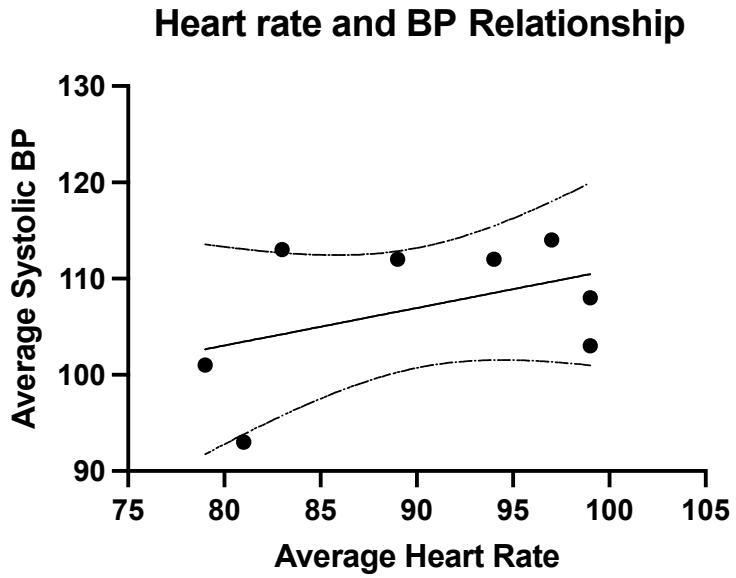


Figure 4.9 | The relationship between participants heart rate and blood pressure.

4.5.4. Ambulatory arterial stiffness index

Ambulatory arterial stiffness index (AASI), calculated by 1 minus the slope of diastolic and systolic pressure during 24-hour ABPM, is a measure of arterial stiffness. (344) Normal AASI values likely to be <0.50 at 20 years. (344) Two participants had AASI of over this value (0.62 and 0.63). The mean AASI of the cohort was 0.4 ± 0.2 (range 0.1 – 0.6).

It is important to note that AASI depends on nocturnal dipping, and a lack of nocturnal dipping can overestimate this parameter of vascular stiffness. (345, 346) Given that non-dipping is highly prevalent in TS, (144) ASSI values for the assessment of vascular stiffness should be interpreted with caution in this population.

4.5.5. Pulse Pressure

Pulse pressure (PP) is the difference between systolic and diastolic pressures. Normal PP is therefore 40 mmHg. The mean PP for the STARRY study cohort calculated from ABPM was consistent with this: 41.0 ± 7.2 mmHg (range 30.0 – 53.0 mmHg). No participant had a narrowed PP (<25% of systolic pressure) or a wide PP of >100 mmHg, (347) which is a marker of increased arterial stiffness. (348)

4.6. Vascular scanning

4.6.1. Carotid intima-media thickness

For their age and gender, five participants (50.0%) had a cIMT between the 50th and 75th percentile and five participants (50.0%) had a cIMT between the 75th and 90th percentile. The STARRY study participants therefore had cIMT values skewed towards the higher range compared to data from 249 healthy Caucasian adolescents. (349)

Using the same normative dataset, Table 4.22 compares the mean cIMT values from the STARRY study participants categorised into two age groups. (349) There was no significant difference between the two groups, however, the younger participants in the STARRY study tended to have higher cIMT values, compared to the age-matched controls. The small sample size of the study limits meaningful conclusions being derived from these data.

Table 4.22 | Carotid intima-media thickness data compared to control group.

	STARRY study participants[a]	**Control subjects (349)**	***p*-value**
I. Age 10.0 – 13.9 years			
Number of participants, n	4	61	-
Mean (SD), mm	4.1 (0.05)	3.8 (0.03)	0.052
II. Age 14.0 – 16.9 years			
Number of participants, n	4	34	-
Mean (SD), mm	4.0 (0.00)	3.9 (0.05)	0.69

Abbreviations – n: number; mm: millimetres; SD: standard deviation.

a – two participants were excluded due to age: One participant was 9 years of age, and another participant was 17 years of age.

4.6.2. Flow-mediated dilatation of the brachial artery

The mean FMD for the cohort (n=8) was 9.4 ± 3.1 (range 5.3 – 13.3). Data from 978 healthy volunteers (448 females) indicated that normal FMD is between 7.7–8.2% (95% CI) for children and adolescents aged 6–16 years. (350) One participant had FMD in this range: three participants were below 7.7% and four participants were above 8.2%.

4.7. Saliva Samples

4.7.1. Circadian rhythm

The ten STARRY study participants who collected saliva samples were matched (1:1) for sex-and-age to healthy control subjects from the Liverpool region. The healthy control population were given the same instructions for sample collection and samples were processed at the same laboratory. (243) Table 4.23 presents the data from the two groups.

Table 4.23 | Salivary cortisol and cortisone in STARRY study participants compared to healthy controls.

		STARRY participants	Healthy controls	*P*-Value
(i)	**Baseline demographics, mean ± SD (range)**			
	Age, years	14.1 ± 2.3 (9.0 – 17.4)	14.1 ± 2.4 (9.1 – 17.5)	
	BMI Z-score, SD	0.9 ± 1.5 (-1.0 – 2.9)	1.5 ± 1.1 (-0.7 – 2.9)[a]	
(i)	**Salivary cortisol nmol/L, median (IQR)**			
	Waking sample	5.4 (3.7 – 8.4)	10.5 (6.92 – 12.41)	0.32
	Mean of 2hrs – 12hrs sample	3.9 (2.4 – 4.2)	2.0 (1.5 – 3.9)	0.70
	Mean of waking – 2hrs samples	4.6 (4.2 – 6.0)[b]	3.0 (2.5 – 5.6)	0.54
	Sum of 2hrs – 12hrs samples	22.5 (13.9 – 25.0)	11.9 (9.2 – 23.2)	0.44
	Sum of waking – 12hrs samples	32.1 (27.0 – 36.1)[b]	21.0 (15.0 – 39.2)	0.21
(ii)	**Salivary cortisone nmol/L, median (IQR)**			
	Waking sample	23.1 (20.8 – 33.0)	32.4 (19.8 – 38.1)	0.36
	Mean of 2hrs – 12hrs samples	16.8 (12.5 – 22.1)	15.2 (13.7 – 17.0)	0.25
	Mean of waking – 2hrs samples	19.7 (15.9 – 22.7)	16.0 (14.4 – 20.6)	0.50
	Sum of 2hrs – 12hrs samples	101.0 (74.8 – 132.8)	91.4 (82.2 – 101.8)	0.23
	Sum of waking – 12hrs samples	120.5 (100.3 – 155.7)[b]	111.9 (100.5 – 144.3)	0.91
(iii)	**Ratio of salivary cortisone to cortisol**			

Waking sample	4.7 (2.9 – 5.9) [b]	3.2 (2.7 – 4.2)	0.55
Mean of 2hrs – 12hrs samples	4.6 (3.6 – 5.9)	8.3 (4.9 – 9.1)	0.02
Mean of waking – 12hrs samples	4.1 (3.6 – 5.9)[b]	5.1 (3.7 – 6.9)	0.45

Abbreviations – SD: standard deviation; BMI: body mass index; SDS: standard deviation score; nmol/L: nanomoles per litre; IQR: interquartile range; hrs: hours.

a – missing data for 2 healthy subjects; b – missing data from 1 STARRY study participant.

The mean BMI Z-score of the control population was 0.6 SD greater than that of the STARRY study cohort. Titman. A., et al found that an increase in BMI of one SD was associated with a 23% (95% CI: 4-48%) increase in total cortisol values throughout the day, whereas BMI had no significant association with cortisone concentrations. The age and sex matched healthy control subjects were sourced from Titman. A.et al's raw dataset. (243) Therefore, compared to STARRY study participants, the sum and mean 12-hour cortisol concentrations of the control subjects should be elevated by approximately 13%.

The salivary cortisol and cortisone awakening response was not significantly different between the two groups. There was also no significant difference of the sum and mean salivary cortisol and cortisone concentrations, with and without the waking sample, between the two groups. The non-significant difference between the two groups for mean 12-hour cortisol concentrations persisted after correcting for BMI (p=0.277). The ratio of salivary cortisone / cortisol was not significantly different between the two groups.

The profile of salivary cortisol (Figure 4.10), salivary cortisone (Figure 4.12) and the ratio of salivary cortisol and cortisone (Figure 4.14) were compared between the two groups. Data are presented as median and error (95%CI).

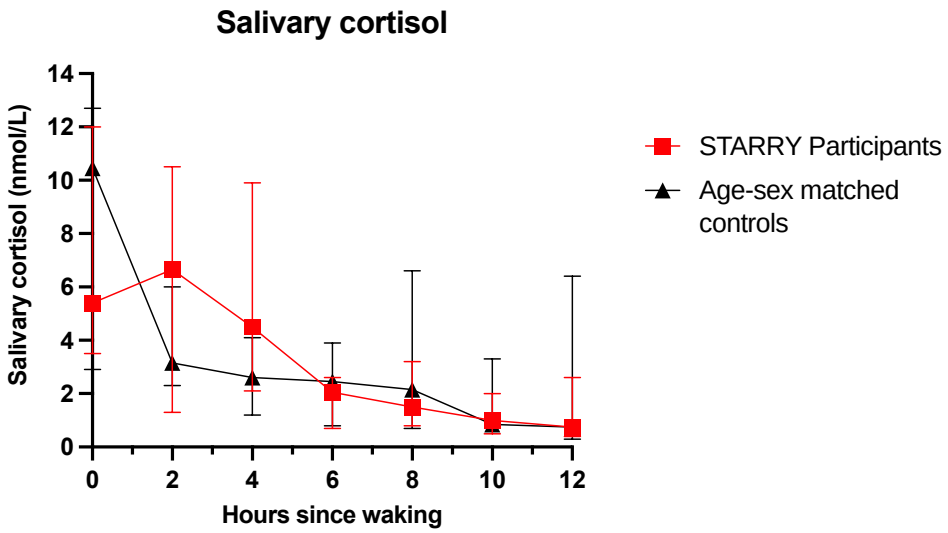


Figure 4.10 | Salivary cortisol circadian profile.

Compared to the healthy control subjects, the cortisol circadian rhythm of STARRY study participants appeared to be blunted (Figure 4.10): the morning peak is less pronounced and cortisol levels remain elevated throughout the morning, declining to levels reported in healthy control subjects in the afternoon samples.

The mean total area under the curve for salivary cortisol was statistically significantly greater in STARRY study participants (mean 49.7 nmol/L; 95%CI [18.3-81.1 nmol/L]) compared to the control group (42.3 nmol/L; 95%CI [16.6-67.9 nmol/L]; p=0.005) (Figure 4.11). With adjustment for BMI the mean total area under the curve for STARRY study participants would be 56.2 nmol/L, and the significant difference between the two groups would be greater.

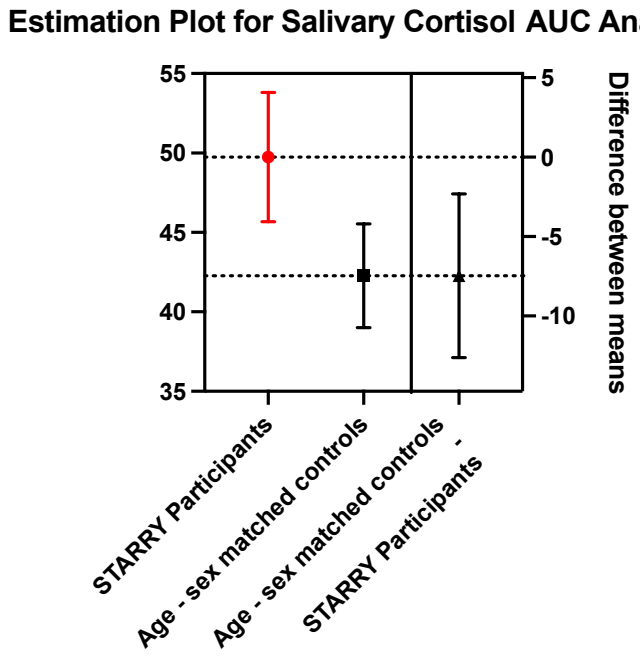


Figure 4.11 | Mean area under the curve analysis for salivary cortisol.

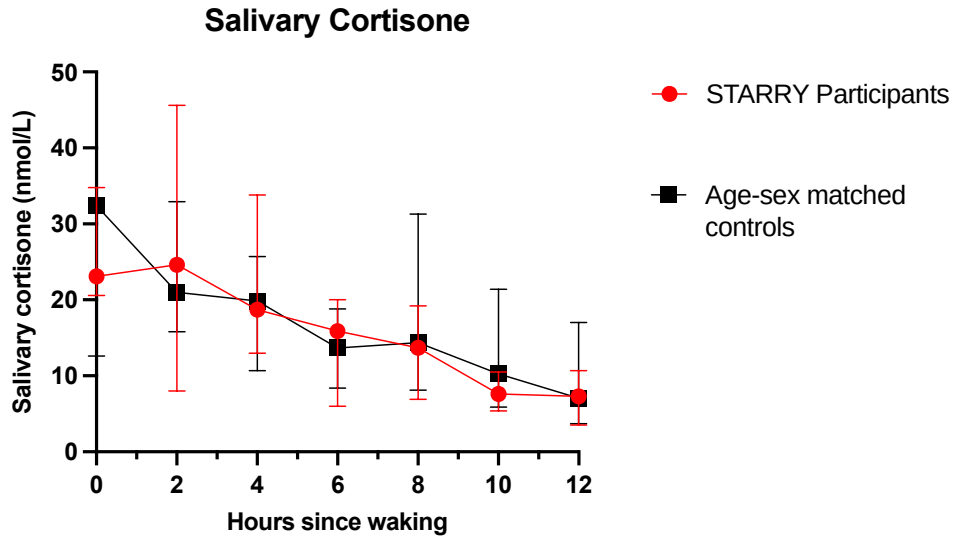


Figure 4.12 | Salivary cortisone circadian profile.

The circadian salivary cortisone profiles of the two groups were similar (Figure 4.12). No statistically significant difference was observed for the salivary cortisone concentration from the awakening sample between groups (Table 4.23). Likewise, there was no significant difference in the slope of change in cortisone concentrations between the awakening and the 2-hour samples when comparing the study participants (+0.75) to the healthy control subjects (-3.88) (p=0.30). The gradient of salivary

cortisone between two-and-12-hours was also not significantly different between the STARRY participants (-1.58) and the healthy control subjects (-1.18) (p=0.31).

The mean total area under the curve for salivary cortisone was greater in STARRY study participants (mean 222.3 nmol/L; 95%CI [142.6 to 302.1 nmol/L] compared to the control group (215.4 nmol/L; 95%CI [147.1 to 283.6 nmol/L], however this difference did not reach statistical significance (p=0.31) (Figure 4.13).

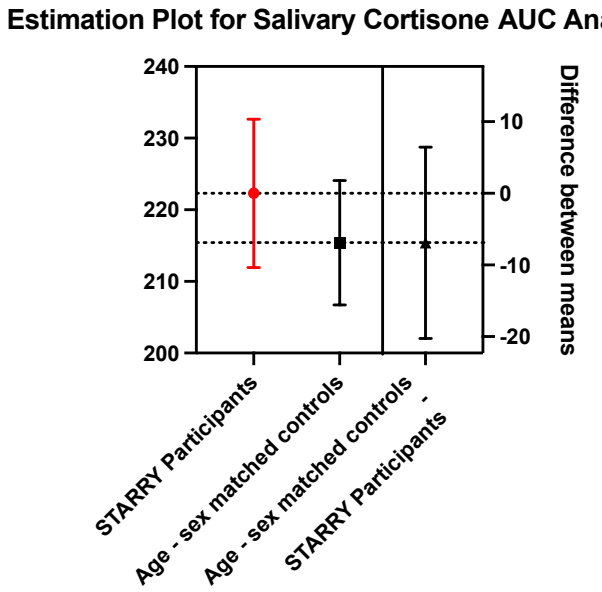


Figure 4.13 | Mean area under the curve analysis for salivary cortisone.

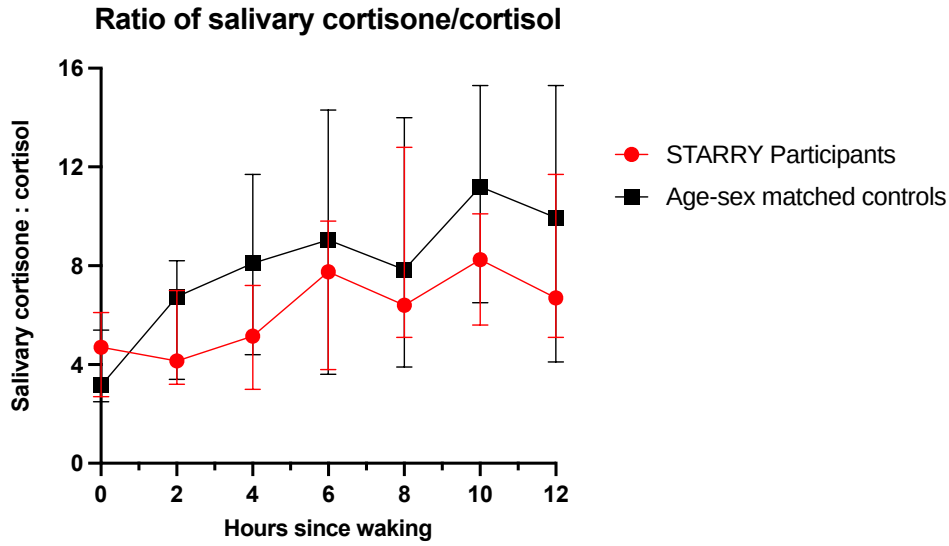


Figure 4.14 | Ratio of salivary cortisone to salivary cortisol.

Figure 4.14 demonstrates that both groups displayed a circadian rhythm in the ratio of salivary cortisone / cortisol, with cortisone concentrations increasing relative to cortisol concentrations throughout the day. The circadian rhythm in STARRY participants, however, appears flatter than the control subjects. Excluding the sample taken within 30-minutes of waking, the median ratio of salivary cortisone / cortisol was also consistently lower in the STARRY study cohort compared to the control cohort.

4.7.2. Measurement of hypercortisolism

Detectable late-night salivary cortisol concentrations are indicative of hypercortisolism. (351) Five STARRY study participants collected this sample. Two participants had undetectable levels (<0.3 nmol/L) and three participants had the following detectable levels: 3.5, 0.9 and 0.6 nmol/L.

All five participants had detectable late-night salivary cortisone levels (mean 6.5 ± 5.6 nmol/L). A retrospective study of 54 adults investigated for the diagnosis of Cushing's Syndrome defined late-night salivary cortisone levels >14.5 nmol/L to have a sensitivity of 95.2% and specificity of 100% for diagnosis. (352) One STARRY study participant had a salivary cortisone level above this threshold (16.0 nmol/L). Further biochemical testing for Cushing's Syndrome in this participant was not warranted. (351)

4.7.3. Correlation to cardiovascular variables

Participants mean 12-hour salivary cortisol concentrations were correlated to BMI Z-score, %BFM, MFR, fasting glucose, mean CGM, TG, LDL-C, HLD-C, cIMT and clinic systolic and diastolic BP. Only clinic diastolic BP showed a statistically significant correlation with mean cortisol values (Pearson's correlation coefficient +0.662). Figure 4.15 displays this correlation.

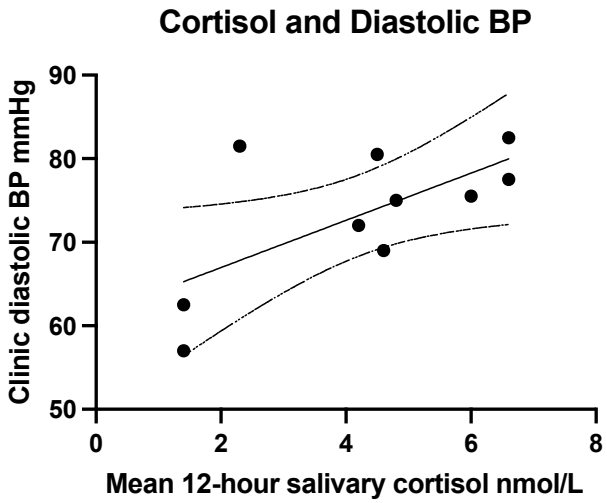


Figure 4.15 | Correlation between mean 12-hour salivary cortisol concentrations and average clinic diastolic BP.

4.8. Discussion

The STARRY study presents data from a small population of children and adolescents with TS who, in majority, were of White British ethnicity and living in the North-West of England in areas with high levels of deprivation. (262) Participants' karyotypes had a high proportion of 45,XO cell lines, and the prevalence of congenital cardiac abnormalities was consistent with previous reports in the literature. (4)

4.8.1. Metabolic biomarkers

Elevated cholesterol, especially LDL-C, is strongly linked to CVD development in the general population. (174) There was a high prevalence of lipid profile abnormalities in STARRY study participants. (302) Previous studies of women and girls with TS have shown inconsistencies: some have demonstrated a comparable lipid profile to controls, (165, 175) whereas others have demonstrated a proatherogenic profile. (176-179) However, there is consensus that lipid abnormalities which meet the cut-off for pharmacological intervention are rarely reported in adult women with TS, and this was also true of our cohort. (179) Proatherogenic lipid profiles in the STARRY study tended to be observed in the participants with higher BMI Z-scores, which supports the hypothesis that the increased prevalence of lipid abnormalities observed in TS are a consequence of the increased prevalence of obesity and T2DM in this population. (4) However, it may be that there is an unknown mechanism specific to TS that also predisposes individuals to dyslipidaemia. Supportive

of this hypothesis, a healthy weight STARRY study participant had an isolated low HDL-C, (264, 302) although further, larger studies are needed for further investigation.

High levels of leptin in obese individuals are a possible contributor in accelerated atherosclerosis: recent evidence suggested that cholesterol uptake by macrophages, resulting in increased foam cell formation, is stimulated by leptin. (110) Paediatric reference ranges for leptin concentrations are inconsistent, (315, 316) and combined with our small sample size and the absence of a control group, it was difficult to make meaningful conclusions from our data. It was apparent, however, that leptin concentrations positively correlated with percentage body fat mass in our cohort. Studies evaluating leptin concentrations in women with TS compared to control subjects have generated contrasting results: some demonstrating lower leptin concentrations than expected for the degree of adiposity, (353) and others demonstrating higher leptin concentrations which positively correlated with fat mass. (77)

4.8.2. Type 2 Diabetes Mellitus and Insulin Resistance

T2DM is a well-established CVD risk factor in the general population. (167) The approximate prevalence of glucose intolerance and T2DM in patients of all age-ranges with TS is 15-50% and 10%, respectively. (4) A diagnosis of diabetes mellitus excluded patients from STARRY study recruitment. No participant was identified as requiring further investigation for diabetes mellitus from venous study blood samples. (302)

A meta-analysis of 65 studies, including more than 500,000 individuals, concluded that increased HOMA-IR, a surrogate marker of insulin resistance, is associated with an increased risk of incident CVD in individuals without diabetes mellitus. (354) Threshold HOMA-IR levels for defining insulin resistance in healthy and obese non-diabetic paediatric populations are inconsistent. (318-321) Our data supports most studies comparing insulin sensitivity in women with TS to control groups, in which an increased prevalence of insulin resistance in TS has been observed. (170, 171) Interestingly, ovarian failure-related hypogonadism as well as the traditional risk factors for T2DM development in the general population (obesity, high levels of visceral adiposity) does not seem to be a significant factor in the development of T2DM in TS, especially in the paediatric population. (130, 169, 170) These data indicate that the presence of an intrinsic defect specific to the TS karyotype may be responsible for insulin resistance. (171) Indeed, two of the three participants with insulin resistance in our cohort had a BMI within the healthy-range and a healthy %BFM. (264, 329)

4.8.3. Cardiac biomarkers

Greater than half of participants had plasma clotting factor levels, vWF and Factor VIII (FVIII), and/or inflammatory marker levels, fibrinogen and ESR, associated with a heightened risk of CVD development in the general adult population. (325-327) Plasma VWF levels above 150% in adults are associated with increased prevalence of deep venous thrombosis, cerebrovascular disease, peripheral vascular disease and ischaemic heart disease. (326, 355) VWF is considered a mediator of acquired cardiovascular events and marker of risk associated with atherosclerosis. (356) Inactive VWF circulates in the plasma bound with the coagulation cofactor FVIII. (326) In the adult population, high circulating levels of FVIII (>150%) also present a strong risk for thrombosis, especially venous thrombosis. (325, 357) Elevated levels of fibrinogen and prolonged ESR have also been associated with coronary artery disease. (358, 359) Our data are consistent with a study of 60 women with TS, in which a subset of women had an unfavourable haemostatic environment. (360) To our knowledge, our study is the first to evaluate these measures in a paediatric TS population. In our cohort, high plasma clotting factor levels were observed independently of specific CVD risk factors, whereas high inflammatory levels were only observed in the participants with a BMI in the obese range. (264, 325-327)

It is important to note that vWF, FVIII and fibrinogen are acute phase proteins. Acute stress, for example anxiety at the time of venepuncture, increases the level of acute phase proteins. (361) There was a high prevalence of needle-phobia within our cohort, which may have resulted in falsely elevated clotting factors/ inflammatory markers in some study participants.

In renovascular hypertension, plasma renin activity (PRA) values are elevated and can therefore be used as a marker of disease. (362) Plasma renin values in our cohort were either below or within reference range. Studies reporting PRA values in TS have generated contrasting results. The high baseline sympathetic tone observed in TS would support high PRA values, (363) which has been reported on one adolescent cohort, with more than 50% of patients having values above the normal range. (280) However, normal PRA activity has also been reported in adult and paediatric TS populations. (148, 182) Supine and upright PRA values have been reported to be higher in paediatric patients with TS compared to healthy control subjects, and to be higher in hypertensive TS patients than in normotensive TS patients. (364)

In the general population, elevated cortisol levels are positively associated with risk factors for CVD development, such as insulin resistance and low HDL-C. (256) There was no evidence of an increase in

9am cortisol concentrations in our TS participants, contrast to a study of 44 young people with TS, which demonstrated increased basal cortisol levels and an increased peak cortisol response to ACTH stimulation. (252) Measurements of cortisol at 9am are of limited clinical value as they are unlikely to be accurately timed to the cortisol awakening response: the salivary cortisol and cortisone profiles in the study protocol are likely to provide more meaningful data. (351)

4.8.4. Body composition

Obesity is an independent risk factor for CVD development and death in the general population. (163) (163) Previous studies have demonstrated that body habitus in TS is unfavourable for CVD development: when compared to unaffected women, women with TS have a higher BMI and an increased WHR, increased total and visceral fat mass, and decreased skeletal muscle mass. (77, 78) Our pilot bioimpedance data demonstrates that the unfavourable body habitus is also present in a paediatric TS population.

4.8.5. Continuous glucose monitoring

A meta-analysis of 29 studies concluded that non-diabetic hyperglycaemia is a risk factor for CVD development in the general population. (365) CGM data demonstrated that, compared to the age, sex and ethnicity matched control subjects, the mean of STARRY study participants mean glucose levels was 10% elevated. To our knowledge this is the first study to report CGM data in TS. A larger cross-sectional study could confirm if elevated mean glucose levels are observed lifelong in TS, and determine the cumulative effect of this on cardiovascular health. (366)

All STARRY study participants had a normal CV of glucose, which is important as it has been suggested that wide fluctuations in glucose concentrations and postprandial hyperglycaemia are an independent risk factor for atherosclerosis in patients with T2DM. (367, 368)

4.8.6. Hypertension

Hypertension is one of the most important risk factors for acquired CVDs in the general population. (140-143) Observational studies have described hypertension in 21-40% of girls and adolescents with TS, (99, 144, 145) and there was a similar prevalence observed in our cohort. (332) The aetiology of hypertension in TS is largely unknown. The low, but significant correlation between heart rate and systolic BP in our cohort is supportive of the hypothesis that increased sympathetic tone in TS has a

role in the pathogenesis of hypertension in TS. (135) Our data were consistent with previous reports of increased baseline sympathetic tone in women with TS: compared to our reference population, STARRY study participants had heart rate values skewed towards the higher range. (147, 343)

A non-dipping BP profile, in which there is loss of the protective mechanism of reduced pressure load on the arterial walls at night, is an independent predictor of cardiovascular risk in the general population. (160, 161) Most STARRY study participants demonstrated this adverse BP circadian profile. (156) A previous study of 75 girls with TS demonstrated a lower (57%), but still increased prevalence of 'non-dipping' compared to the general population. (144) All STARRY study participants had an unconventional BP circadian rhythm, including one participant who was was an 'extreme-dipper'. (156) Compared to the 'non-dipping' BP phenotype, the prognostic cardiovascular significance of 'extreme dipping' is much debated in the literature, especially in young normotensive individuals. (369)

Determined from ABPM, AASI has shown to predict cardiovascular mortality independently of other CVD risk factors in the general adult population. (370) A previous study showed a significantly elevated AASI in women with TS compared to controls. Interestingly, despite this study population having an average age of 19-years senior to the STARRY study cohort, the mean AASI value was lower (0.36 compared to 0.41), which suggests that the STARRY study participants are at an even greater cardiovascular risk. (371)

4.8.7. Atherosclerotic changes

cIMT increases as atherosclerosis becomes more advanced, and there is extensive data to support the association between increased cIMT with cardiovascular and cerebrovascular events in the general population. (107, 108) STARRY study participants cIMT values were skewed towards the higher range compared to the healthy reference population. (349) Studies of cIMT, comparing measurements between TS patients and controls in paediatric and adult populations, have shown inconsistencies: some studies report comparable results and others report increased cIMT in TS compared to controls, which suggests that the arterial walls of individuals with TS are pro-atherogenic. (186-190)

A diminished arterial vasodilator response is observed in the early stages of atherogenesis. (104) There was large variability in STARRY study participants FMD of the brachial artery, with low, normal and elevated values observed. (350) Previous studies have demonstrated that adolescents and women with TS had comparable or even higher FMD of the brachial artery compared to controls. (186, 187)

However, one of these studies showed the presence of a strong inverse correlation between FMD and age in individuals with TS, suggesting an increased tendency of premature derangement of arterial function compared to the general population. (186)

4.8.8. Salivary cortisol and cortisone

For analysis of salivary cortisol and cortisone data, study participants were matched (1:1) for age and sex to a healthy cohort from the Liverpool-region. (243) Mean total area under the curve analysis, even without adjustment for BMI, showed that STARRY study participants were exposed to statistically significant greater total cortisol concentrations over the day compared to the healthy control subjects. Similarly, chronically elevated cortisol levels have been demonstrated in a cohort of adult women with TS. Compared to age matched healthy control subjects, these women had statically significant higher measurements of hair cortisol, which correlated inversely with height and positively with total cholesterol levels, possibly indicating that chronically elevated cortisol levels are an additional detrimental contributing factor to poor cardiovascular health in TS. (253) In our study, diastolic clinic BP was the only cardiovascular variable that correlated with mean salivary cortisol concentrations with statistical significance. This should be interpreted with caution, as BP is influenced by age and height, and raw values opposed to centiles were used for this correlation.

Disruption of the physiological cortisol profile is associated with cardiovascular and metabolic disease in shift workers. (257-259) To our knowledge, our pilot study is the first study to evaluate the circadian profile of salivary cortisol and cortisone in TS. Our pilot data described a blunted circadian cortisol profile in paediatric patients with TS.

As demonstrated in previous studies, (243, 372) a circadian rhythm in the ratio of salivary cortisone / cortisol was observed in STARRY study participants. Compared to the healthy control subjects, however, the median of this ratio was lower at all time points following the waking sample. The activity of the 11βHSD enzyme in TS is an area of further study.

Cortisol is known to be elevated during stress. There was a high prevalence of needle-phobia within the STARRY study cohort. The salivary cortisol value 2 hours after waking coincided with venepuncture and this may have contributed to the elevated concentrations observed at this time point. However, the healthy control subjects study protocol similarly involved venepuncture at this time. The salivary cortisol concentrations also remained higher than control subjects for the subsequent time point (4

hours after waking), where there was no obvious external stressors present, providing further evidence that these were true elevations.

Further information on the clinical background, cardiovascular risk profile and cortisol values for each study participant can be found in Appendix 5.

4.8.9. Conclusion

To conclude, this pilot data describes an adverse cardiovascular risk profile in paediatric patients with TS. Risk factors for CVD development were observed more commonly in participants with a higher BMI, although were also present in those with a healthy weight. Compared to healthy controls, participants also demonstrated a disrupted salivary cortisol circadian rhythm and increased cortisol exposure. Further adequately powered studies are required to validate these novel cortisol findings and investigate the impact on the cardiovascular profile of paediatric patients with TS.

5. Audit comparing clinical care of girls with Turner Syndrome at Alder Hey to the 2017 International Guidelines

5.2. Abstract

5.2.1. Background

Clinicians from a variety of medical specialities provide clinical care for girls and adolescents with TS at Alder Hey Children's Hospital. International clinical practice guidelines are used to inform standard care. (4) Clinical audits compare current clinical practice against standard care to identify the areas of the clinical service promoting good practice, and similarly areas of the service where improvement is required. Clinical audits are important for improving patient care in a systematic and collaborative way.

5.2.2. Aims

The primary aim of the audit was to evaluate how current routine clinical care of girls and adolescents with TS in the Liverpool region compared to the recommendations from the 2016 Cincinnati TS Clinical Practice Guidelines. (4) The secondary aim was to compare the Alder Hey cohort to published data (genotypic and phenotypic) of young people with TS.

5.2.3. Methods

Clinical data from the medical records of the 40 girls and adolescent females with TS under the care of a Consultant Endocrinologist at Alder Hey Children's Hospital, Liverpool, UK, a tertiary paediatric hospital, were collected retrospectively in October 2021.

This audit comprised part of a larger, regional audit, which will also collect data from the clinical services for adult women with TS. The audit was registered with the Clinical Audit Department at Alder Hey Children's NHS Foundation Trust (Alder Hey audit department registration number 6456). Patient data were anonymised and were accessed by the study team only. All data collection occurred in October 2021 and the report was finalised in May 2022.

5.3. Baseline demographics

The mean age of the patients in our clinic population was 11.8 ± 4.6 years (range 2.4 – 17.7 years). The mean index of multiple deprivation decile was 4.4 ± 2.9 (median 4.0) for patients living in England and Wales (excluding one patient living in the Isle of Man), and 35.9% of patients live in the 10-20% most deprived areas of their country. (261, 262) All patients included have had their TS diagnosis genetically confirmed via karyotyping. (4)

5.3.1. Chromosome abnormalities

Due to the retrospective nature of data collection, not all karyotypes could be found in the patients' medical records. Karyotypes were found for 34 out of 40 patients, shown in Table 5.1. Local hospitals were contacted for genetic information on the patients (n=6) who were missing documentation of their karyotype from their Alder Hey medical records.

Table 5.1 | Chromosomal abnormalities of the study group (n=34) compared to the known international prevalence in the Turner Syndrome population.

Karyotype	Frequency of patients in our clinic population (%)	% Approximate known prevalence (4)	Description
45,X	9 (26)	40-50	Monosomy X
45,X/46,XX	7 (21)	15-25	Mosaicism
Mosaic (non-specified)	4 (12)		
45,X/46,XY	2 (6)	10-12	Mixed gonadal dysgenesis
46,XX, del(p22.3)	1 (3)		Deletion Xp22.3
45,X/46,X,r(X)	3 (9)		Ring X chromosome
45,X/47,XXX	1 (3)	3	Mosaicism with 'Triple X'
45,X/46,XX/47,XXX	1 (3)		
45,X/46,X,i(Xq)	1 (3)	10[a]	Isochromosome Xq
45,X/46,X,idic(Xq)	1 (3)		Isodicentric Xq
46,XX, idic(Xq),del(p)	1 (3)	Rare	Various
46,XX, del(q)	1 (3)		
46,XX, del(p11.21)	1 (3)		
45,X/46,X, idic(Y)	1 (3)		

Abbreviations – X: X chromosome; del: deletion; p: short arm of chromosome; r: ring; q: long arm of chromosome; i: Isochromosome; idic: Isodiscentric; Y: Y chromosome.

a – inconsistent data, percentage should be viewed with caution.

When comparing information from the international guidelines about the type and frequency of chromosome abnormalities in TS, our cohort were relatively representative. Monosomy X and mosaicism were similarly the most common karyotypes found, although, the frequency of mosaicism exceeded that of monosomy X in our cohort. There were also a higher prevalence of rarer karyotypes in our patients. (4)

Although at an individual level, patients genotype may not predict their clinical phenotype, some TS patients who share a genotype have demonstrated similar clinical outcomes. For example, the guidelines recognise that a ring X chromosome can sometimes be associated with variable intellectual disability, and the presence of Y chromosomal material is associated with a heightened risk of gonadoblastoma. (4) Having genetic information more readily available, which would have been performed to confirm the diagnosis of TS, could therefore help clinicians to provide more individualised patient care.

5.3.2. Age at diagnosis

The date that each patient received their diagnosis of TS could be found in 38 medical records of the clinic population. The age that the individuals were at this time were categorised, shown in Figure 5.1. The mean age of diagnosis was 5.1 ± 4.8 years ranging from a pre-natal diagnosis to 15.5 years (median 5.5 years). For the two patients where a date of diagnosis could not be found in the case notes, TS was listed in their past medical history in their first available clinical letters when they were 3 years old. Therefore, the mean age at the time of diagnosis of TS in this cohort would be lower if these data were included.

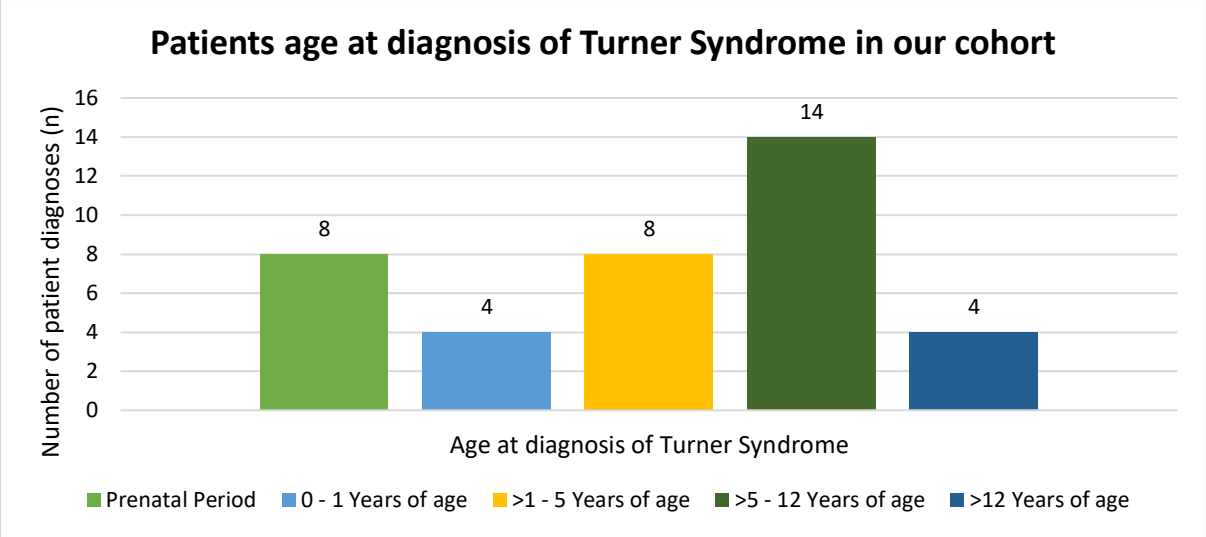


Figure 5.1 | Bar Chart showing the age at diagnosis of Turner Syndrome within our clinic population.

In 2018, data obtained from the medical records of 67 girls and adolescents with TS at Alder Hey Children's Hospital concluded that diagnostic delay in TS was a problem that needs to be addressed. (18) Our 2021 data shows that improvements have been made, with the mean age at diagnosis of TS for patients at Alder Hey Children's Hospital decreasing by 9.2 months. Figure 5.2 presents the percentage of girls diagnosed in each age category in 2021 and 2018, respectively.

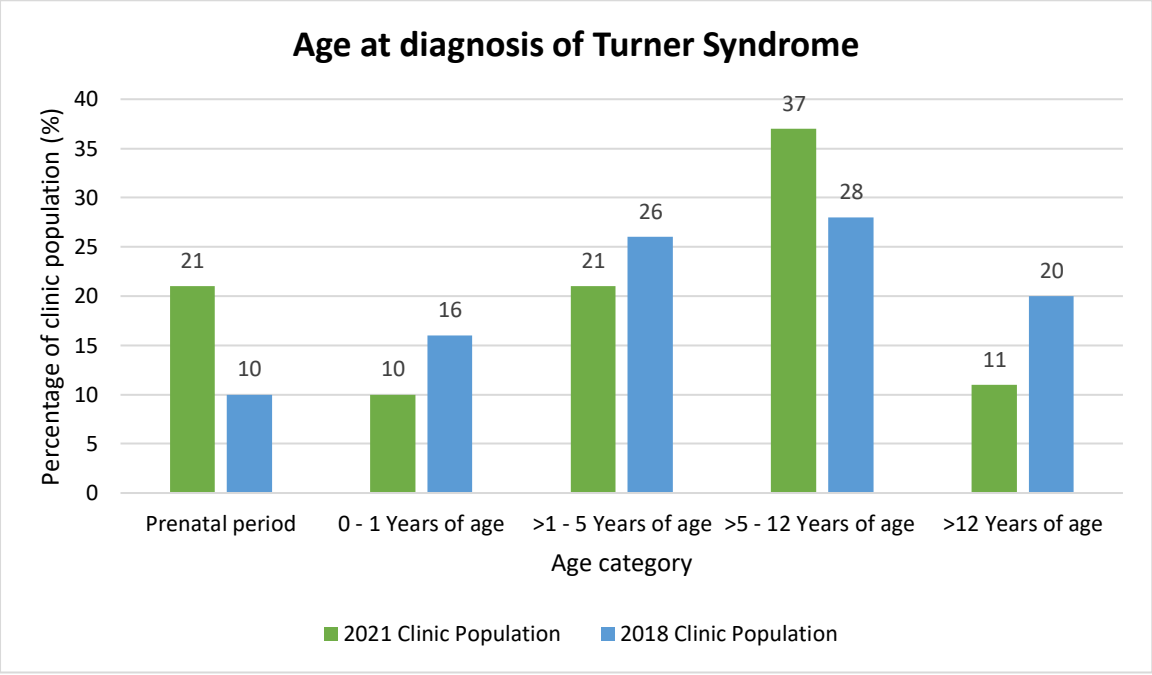


Figure 5.2 | Paired Bar Chart that shows the percentage of girls diagnosed with Turner Syndrome at different ages at Alder Hey Children's NHS Foundation Trust within the 2021 and 2018 clinic population.

5.3.2.1. Prenatal diagnosis

Eight patients in our cohort were diagnosed with TS in the prenatal period through chorionic villus sampling or amniocentesis. All patients had their diagnosis confirmed soon after birth. Amniocentesis was only indicated once for increased maternal age. For four participants, investigation of foetal karyotype was prompted after routine antenatal scans detected foetal abnormalities: increased nuchal thickness, frank cystic hygroma, hypoplastic aortic arch and horseshoe kidney. Genetic testing for an autosomal recessive condition was the indication for one patient, and investigation of intrauterine growth restriction was the reason that an amniocentesis was performed for another patient. Unfortunately, the reason for screening two patients could not be identified in our records.

Guidelines recommend that a foetal echocardiogram should be performed if TS is highly suspected or confirmed in the antenatal period. (4) There was no documentation of cardiology involvement during pregnancy for any of our patients diagnosed in this period.

5.3.2.2. Diagnosis in newborn/infant period (birth to 1 year of age)

In our cohort, four girls were diagnosed with TS between birth and 1 year of age. The mean age at diagnosis in this time-period was 0.02 ± 0.4 years. Karyotyping was indicated in two patients who had ambiguous genitalia at birth. Congenital heart abnormalities, including CoA and BAV, were the indication for testing one patient after birth. The presence of persistent oedematous feet was the reason for screening one patient at one month old.

5.3.2.3. Diagnosis in childhood (1 to 12 years old)

In total, 22 patients were diagnosed with TS in childhood in our cohort. Short stature, presenting either as the main complaint or identified incidentally in an unrelated consultation, was the main reason for screening in this age group. When short stature was noted incidentally, the consultation was often for a condition known to occur more commonly in girls with TS, such as recurrent otitis media or aortic stenosis. The indication for genetic testing in childhood is shown in Figure 5.3.

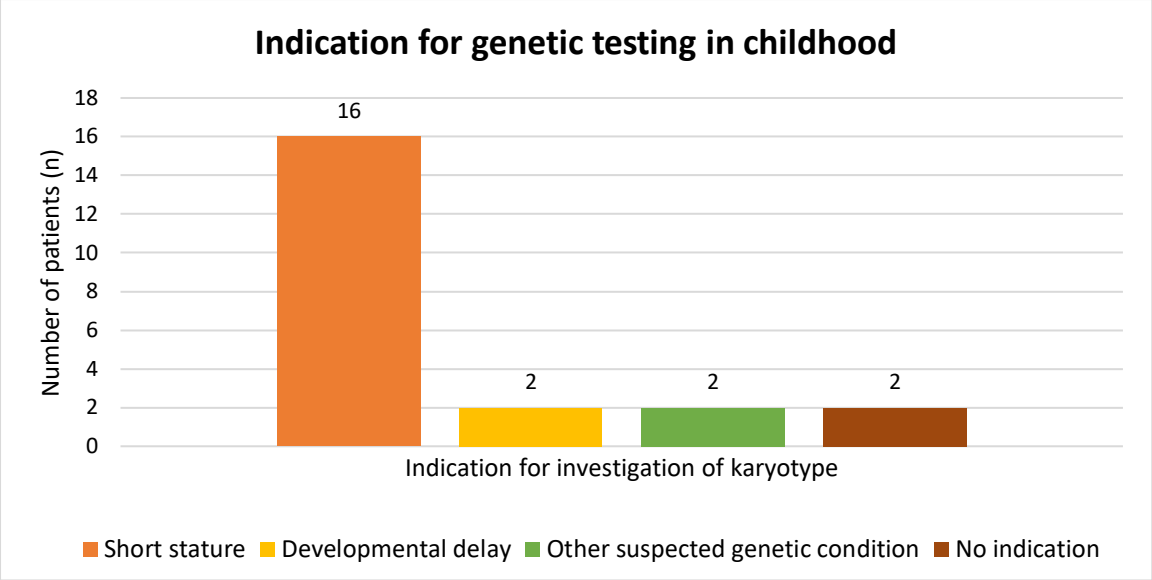


Figure 5.3 | Bar chart showing the indications for genetic testing for patients (n=22) in the group of girls in our clinic population who were diagnosed during childhood.

Of these girls, the mean age at diagnosis was 6.2 ± 2.8 years, which is reassuring as guidelines recommended that hGH therapy is commenced between four and six years of age for girls to receive the greatest benefit from treatment. (4)

5.3.2.4. Diagnosis in adolescence (12 to 18 years old)

Four patients in our clinic population were diagnosed in adolescence, with a mean age of 14.3 ± 1.6 years. The indication for genetic testing in adolescence is shown in Figure 5.4.

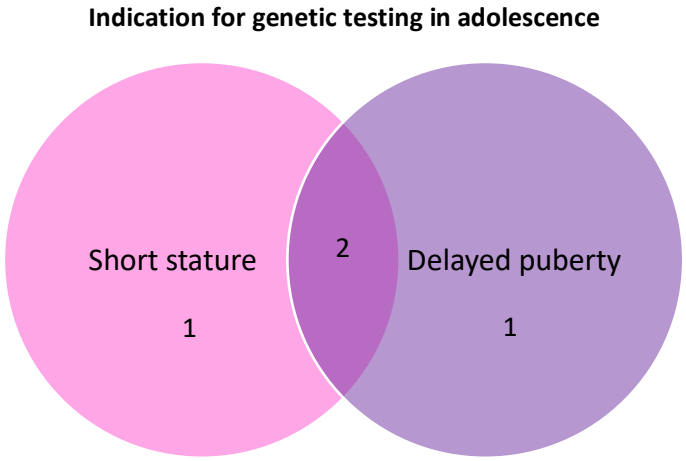


Figure 5.4 | Venn diagram showing the indications for genetic testing for patients in the clinic population diagnosed during adolescence.

It is reassuring that a lower percentage of girls were diagnosed in this age group (9% decrease) at Alder Hey Children's Hospital compared to the 2018 data. This is important as guidelines recommend that hGH therapy is commenced before 12 – 13 years of age and oestrogen replacement therapy is started between the ages of 11 and 12 years. (4)

5.3.3. Other diagnoses

Additional diagnoses found in patient Alder Hey medical records are shown in Table 5.2.

Table 5.2 | Frequency of additional diagnoses in the clinic population (n=40) compared to the known international prevalence in the Turner Syndrome population.

Condition	Prevalence in clinic population (%)	% Approximate known prevalence (4)
ADHD	3 (7.5)	25
Anxiety	4 (10)	-
ASD	1 (2.5)	-
Coeliac disease	3 (7.5)	8
Cow's milk protein allergy	1 (2.5)	-
Developmental delay + Learning difficulties	9 (22.5)	40[a]
Headaches	4 (10)	-
Hearing problems	10 (25)	30
Horseshoe kidney	5 (12.5)	10
Lymphoedema	2 (5)	-
Obstructive sleep apnoea	2 (5)	-
Thyroid disease	3 (7.5)	15-30
Type 1 Diabetes Mellitus	3 (7.5)	?
Upper airway obstruction	2 (5)	-

Abbreviations – ADHD: attention deficit hyperactivity disorder; ASD: autism spectrum disorder.
a – inconsistent data, percentage should be viewed with caution.

Our cohort had fewer additional diagnoses compared to the known approximate prevalence in TS. (4) The lack of psychological care integrated into the TS service at Alder Hey Children's Hospital may account for lower prevalence of neuropsychological diagnoses in our clinic population. Guidelines recommend annual developmental and behavioural screenings until adulthood and

neuropsychological assessments at key transition stages in schooling. (4) Similarly, the lower prevalence of thyroid disease may be as result of poor adherence to the guideline's recommendation of annual thyroid function monitoring (see section 5.7.1). (4) In contrast, the prevalence of hearing problems in our clinic population is similar to the known approximate prevalence in TS, and this may be reflective of good adherence to guideline recommendations. (4) Guidelines recommended audiometric evaluation every three years during childhood and 82.5% of our clinic population receive this care annually.

5.4. Cardiac Investigations at diagnosis

Information on cardiac history from diagnosis could be found for 38 patients in our clinic population. For the two patients where no cardiac information was available, Alder Hey Children's Hospital was not their local hospital. In one of these patients, it was documented that they were seeing cardiology services elsewhere.

For nine of these 38 patients there was little cardiac history available, limited to the documentation of "cardiac assessment normal", for example. Factors such as diagnosis made abroad or a recent been transfer of care from another hospital are possible reasons for this. We have assumed that investigations were not performed if there were no documentation of them in the medical records available to us.

5.4.1. Cardiac Imaging at diagnosis

As recommended by the guidelines, all patients in our clinic population received a transthoracic echocardiogram at diagnosis (Figure 5.5). In the guidelines it is also recommended that adolescents have a CMR at diagnosis, and if this cannot be tolerated then a computerised tomography (CT) thorax is an appropriate alternative. (4) In our clinic population, only one of the four patients diagnosed in adolescence had a CMR. Five patients in our cohort have received a CMR in their lifetime and no patients received the alternative CT Thorax scan. Three of these patients who had a CMR at diagnosis had congenital heart disease: CoA and/or BAV. One patient had a CMR after concerns over increasing stretching of the aortic root, in preparation for her transfer to the adult services.

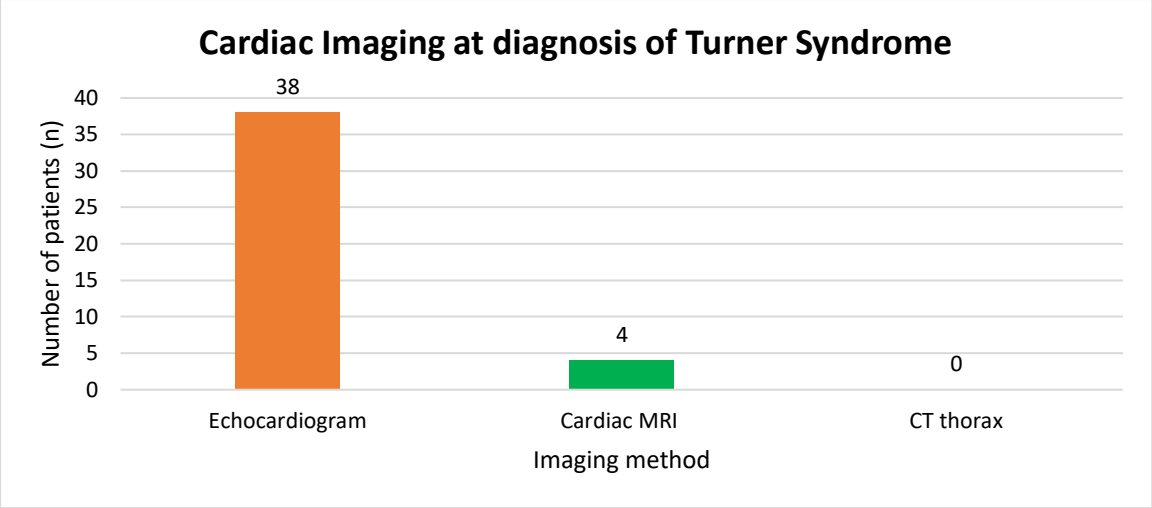


Figure 5.5 | Bar chart that shows the number of patients in our clinic population who had each cardiac imaging investigation at diagnosis (n=38). MRI: magnetic resonance imaging; CT: computerised tomography.

5.4.2. Electrocardiogram at diagnosis

In our clinic population, 24 out of the 38 patients had an electrocardiogram at diagnosis. It was documented that the corrected QT formula (QTc) was used in eight of these patients. Therefore, fewer than a quarter of patients received the standard of care recommended by guidelines, as is recommended that a QTc interval value is calculated for every patient (Figure 5.6). If this value is found to be prolonged then 24-hour Holter monitoring and exercise testing should be considered. (4) In our cohort one patient had cardiac exercise testing, but this was for investigation of unexplained tiredness. No patients had 24-hour Holter monitoring.

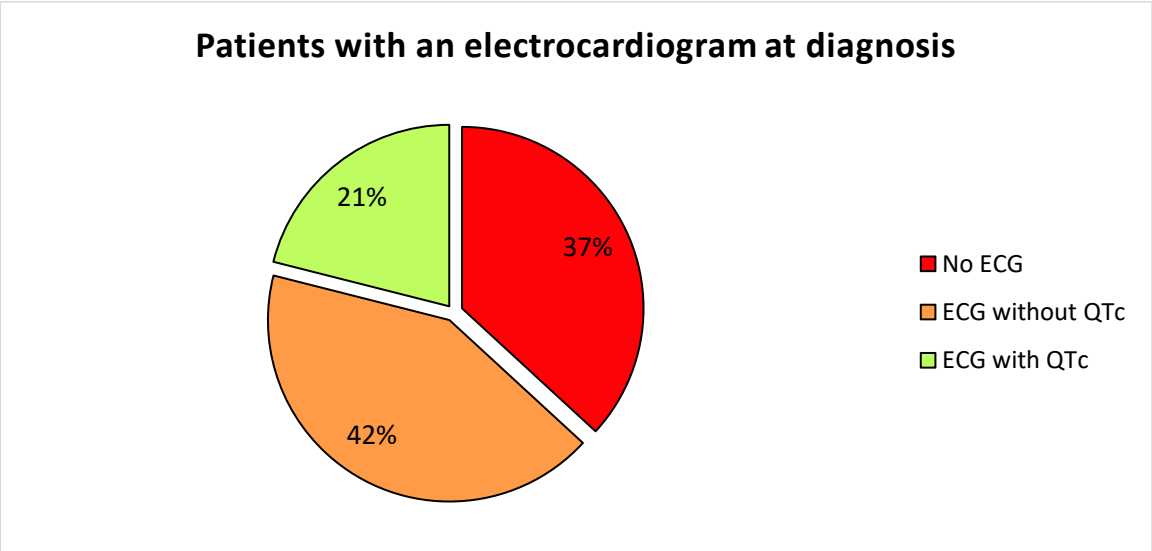


Figure 5.6 | Pie chart that shows the percentage of patients who had an ECG with and without a QTc at diagnosis (n=38). ECG: electrocardiogram; QTc: corrected QT interval.

5.4.3. Structural heart abnormalities

Compared to the data from the guidelines, the prevalence of structural heart abnormalities in our clinic population is relatively representative of what is known about the TS population internationally (Table 5.3). (4)

Table 5.3 | Structural cardiac abnormalities in our clinic population (n=38) compared to the known international prevalence in the Turner Syndrome population.

Cardiac abnormality	Frequency of diagnoses in our clinic population (%)	Approximate known prevalence in Turner's population (%) (4)
Aortic Stenosis	1 (2.6)	NK
Bicuspid Aortic Valve	6 (15.8)	14-34
Elongation of the transverse aorta	1 (2.6)	NK
Coarctation of the aorta	3 (7.9)	7-14

Abbreviations – NK: not known.

In our clinic population there was no documentation of girls undergoing an aneurysm repair or aortic valve replacement.

5.4.4. Aortic Dissection surveillance

Based on the guidelines, there are currently nine patients at Alder Hey Children's Hospital who should have a CMR or transthoracic echocardiogram every 1-2 years reported by a paediatric cardiologist, due to being classified as "moderate risk" of aortic dissection (Table 5.4). (4) All other patients, if their TS-specific Z-score is ≤ 3, are classed as "low risk" and should therefore have imaging every 5 years.

Table 5.4 | Reasons for the girls being "moderate risk" in our clinic population (n=9).

Cardiac abnormality	Frequency of girls diagnosed
Bicuspid aortic valve	6
Coarctation of the aorta	3[a]
Hypertension	2

a – two of the three patients with coarctation of the aorta also had a bicuspid aortic valve.

If these patients who are at a "moderate risk" have a TS-specific Z-score > 3 then they should be considered "high risk" and have monitoring every 6 months to 1 year by a paediatric cardiologist. Documentation of TS-specific Z-scores was only found in one patient's Alder Hey medical records. TS-specific Z-scores are used in the guidelines to not only advise on the frequency of cardiac follow-up, but for advice on sports participation and, most importantly, when to intervene medically or surgically. (4)

Reassuringly, assuming all nine patients are at "moderate risk" of aortic dissection, all but one patient (with a diagnosis of hypertension) are due to be seen by a paediatric cardiologist within the timeframe recommended by the guidelines (within 2 years). (4)

5.4.4.1. Counselling on cardiac events

Guidelines recommend that women with aortic dilatation and/or a BAV should be counselled on the acute symptoms of aortic dissection. (4) There was no documentation of such counselling in the seven patients' medical notes that this would apply to.

There was no documentation of patients being counselled on the cardiovascular risks of pregnancy. In fact, a discussion about pregnancy between the clinician and patient was only documented in three patient records. All discussions were in joint endocrine and gynaecology clinics and were surrounding fertility preservation. This is interesting as in a qualitative study of 97 girls and women with TS, infertility was described as the most painful and prevalent challenge associated with a diagnosis of TS. (28) The young age of the clinic population (mean age of 11.8 years) could be a factor in the lack of discussion of this topic.

5.5. Blood pressure

Guidelines recommend that BP should be taken at every clinic visit for girls and adolescents with TS. (4) In our clinic population, 30 patients (75%) had at least one BP measurement in their Alder Hey medical records.

5.5.1. Recording blood pressure

Of the patients who have documentation of a single BP measurement in their medical records (n= 30), over half (60%) did not have their BP taken annually. To account for the COVID-19 pandemic, where many routine appointments were held virtually, we took measured annual measurements as at least two consecutive measurements in the past three years.

It was hypothesised that the younger patients were less likely to have annual BP measurements. The study group had an average age of 11.8 (± 4.6) years and the average age of the patients who had their BP taken annually was 14.0 (± 4.1) years. The difference between the mean ages were not considered statistically significant (p=0.82).

5.5.2. Hypertension

Two girls are currently taking antihypertensive medications, Lisinopril 2.5mg and Amlodipine 2.5mg. They were diagnosed with hypertension at age 15.6 years and 11.8 years, respectively. One of these patients also has a diagnosis of T1DM. Aside from these two patients diagnosed with hypertension, 12 other patients had documentation of BP readings above the 95th percentile for their gender, age and height. In our clinic population, 47% of the 30 patients had at least one elevated BP reading documented in their medical notes. (373)

5.5.3. Challenges in the identification of hypertension in children and young people with Turner Syndrome

In our cohort, 11 patients had their most recent BP reading documented as ≥95th percentile for their gender, age and height in their medical notes, (373) however none of the patients had been investigated further for hypertension.

To try and explain this finding, for each patient we calculated their true (T) BP percentile and compared this to a hypothetical (H) BP percentile based on a height at the 50th percentile for their age and gender. We defined a systolic and/or diastolic BP value ≥95th BP percentile as an indication for further investigation of hypertension. (373) In our cohort, 55.6% of BP values warranting investigation using T BP percentiles would not warrant investigation using H BP percentiles (Table 5.5).

Table 5.5 | Comparison of true and hypothetical blood pressure centiles in observed cases.

Age (years)	Blood pressure (mmHg)	tBP ≥95th centile	hBP ≥95th centile

2	102/56	Y	N
5	111/72	Y	Y
6	109/73	Y	N
10	119/81	Y	Y
14	126/78	Y	Y
15	124/72	Y	N
15	126/73	Y	N
15	128/75	Y	Y
16	124/84	Y	Y
16	126/67	Y	N
16	127/83	Y	N

Abbreviations – mmHg: milimiters of mercury; tBP: blood pressure for measured height SDS; hBP: blood pressure centile for the patients age and gender with a height 0SDS; Y: yes; N: no.

We concluded a contributing factor may have been that the classical short stature seen in individuals with TS may not have been accounted for when noting patients BP values.

In paediatric patients with TS, the use of BP percentile calculators, where age, height, and gender are mandatory variables, should be encouraged in clinical practice. If height is not available, estimating it at the 50th will identify some, but not all, hypertensive patients with TS. These data were presented at Royal College of Paediatrics and Child Health Conference in June 2022 **(Appendix 6).**

5.5.4. PowerApp

In collaboration with the Alder Hey Innovation Team we are working to develop a BP App to be used in clinic. An Endocrine Research Fellow presented the idea to the Innovation Team and the correspondence can be seen in Figure 5.7.

It was good to meet you last week and go through your requirements for an app. I've made some notes based on our discussion:

- You would like an app that captures the following information:
  - AH number
  - Age
  - Height
  - Gender
  - Systolic and Diastolic measurements
- This will then calculate the centile blood pressure in whole numbers with a RAG rating in accordance with the Hypertensive guidelines you provided, but roughly 50 is green, 90 is amber and 95 is red
- This app can be accessed by any clinician that has involvement with patients that have chromosomal/short stature diagnoses (are there any more chronic conditions that we need to list here?) across multiple specialties. We mentioned Endocrine, Renal, Gastro, Cardiology, Diabetes, Gynae.
- You were going to discuss with the wider team how the information was to be flagged
- You would like a BI dashboard – can you confirm what data you would like to see on this?
- Potentially using RPA to log the individual data into Meditech

Figure 5.7 | Correspondence with the Alder Hey innovation team.

This app will serve as a useful reminder to clinicians to regularly take BP and help to identify which patients warrant further investigation.

5.6. Weight management

It is recommended that weight/BMI should be recorded at every visit in childhood. (4) Data on BMI SDS was available for 38 out of the 40 patients in our clinic population. Using each patient's latest documented BMI SDS, the average BMI SDS was +1.2 (± 1.5). The range of BMI SDS were from -1.6 to +5.5. The WHO interpretation of BMI cut-offs for our cohort are shown in Figure 5.8. (264)

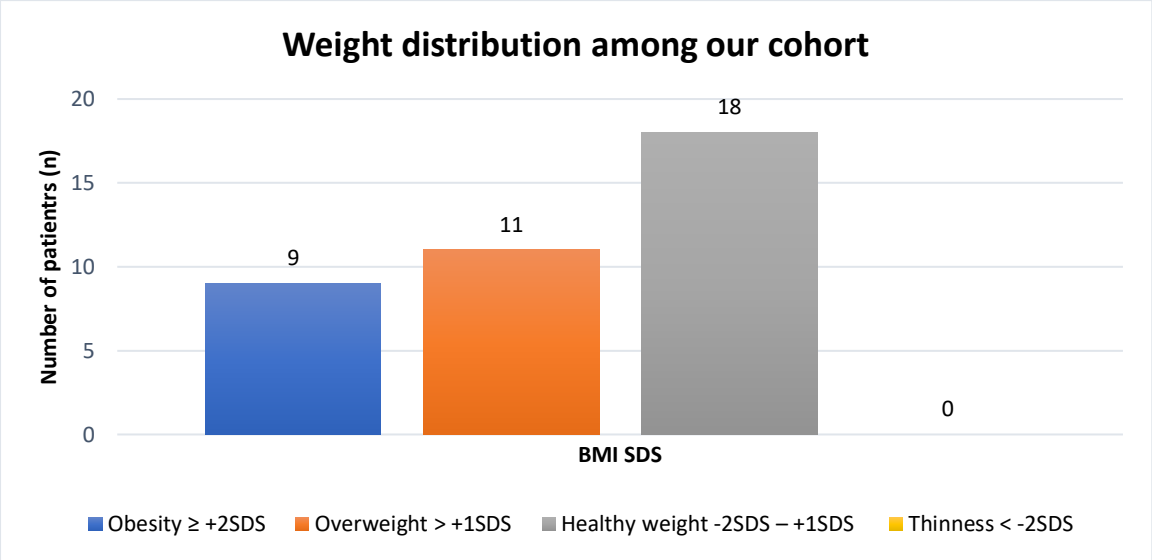


Figure 5.8 | Body mass index classification (World Health Organisation) of patients in our clinic population (n=38). BMI: body mass index; SDS: standard deviation score.

5.6.1. Lifestyle advice

Guidelines suggest that counselling on healthy nutrition and physical activity should start in early childhood to prevent the development of obesity and the associated medical complications. (4) In our clinic population there was documentation of lifestyle advice in nine patients Alder Hey medical records (Table 5.6).

Table 5.6 | Age and reason for lifestyle advice given to the girls in our clinic population.

Age when advice was given (years)	Reason for lifestyle advice
7	Large weight increase since last clinic visit
10	Weight gain from being less active during the COVID-19 lockdown
10	Excessive weight gain in recent clinic visits
13	Large weight increase since last clinic visit
14	Weight gain from being less active during the COVID-19 lockdown
14	Low weight and vegan diet
15	High BMI at diagnosis of Turner Syndrome[a]
15	Large weight increase since last clinic visit[a]
16	At diagnosis – mentioned past struggle to maintain a healthy weight

a – an additional diagnosis of type 1 diabetes mellitus.

In the clinic population, exercise advice was largely given in response to weight gain. Guidelines suggest that this advice should be given as a prophylactic measure. (4)

5.6.2. Healthy Living Fact Sheet

We produced a healthy living fact sheet **(9.7. Appendix 7)** in collaboration with the Turner Syndrome Support Society (TSSS) UK and weight management service at Alder Hey Children's hospital. This fact sheet includes the cardiovascular benefits of maintaining a healthy weight, eating a nutrient-dense diet, and having adequate sleep. The advice provided in the fact sheet aims to help and encourage patients to make heart-healthy lifestyle choices.

This fact sheet will be available electronically on the TSSS website. Funding from a grant from the Society of Endocrinology means that hard copies will be available to be used in clinics across the United Kingdom.

5.7. Routine Blood Tests

Data regarding blood test results could be retrieved from the Alder Hey medical records of 38 out of 40 patients in our clinic population. One patient, where this information was not available, had documentation of blood tests being requested from their GP surgery. The other patient has returned to the service after 5 years of disengaging and has not had blood tests since then, as she was unaware of her TS diagnosis. We excluded three patients with additional diagnoses of T1DM, as their annual TS blood tests coincided with their annual diabetic review blood tests. We also excluded a patient who was having additional adalimumab monitoring blood tests. Therefore 34 patients were included in this analysis.

5.7.1. Thyroid function tests

It is recommended that free T4 and thyroid-stimulating hormone are measured annually in all patients with TS. (4) All patients in our clinic population had a at least one thyroid function test result documented in their medical records. However, for four patients (12%), there was no documentation of thyroid function tests in the last three years. We recognise that disruption from the COVID-19 pandemic may have impacted this result.

Of the 34 patients, 31 patients had been diagnosed with TS for over 2 years. Only 61% of patients of these 31 patients had at least one time point in their medical records where they had the results of thyroid function tests documented for two consecutive years.

5.7.2. Liver Function Tests

Guidelines recommend annual liver function test monitoring (aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase and alkaline phosphatase) throughout the lifetime of all TS patients starting at 10 years of age. (4) In our cohort, 19 patients were aged 11 years or older, all of whom should have received at least one liver function test. Considering the patients age and the date of their TS diagnosis, we calculated the number of blood tests each patient should have received. For

example, a patient who is aged 12.5 years, who was diagnosed with TS at birth, should have had at least two bloods tests. We categorised the percentage of completed recommended liver function tests for each patient into groups, shown in Figure 5.9.

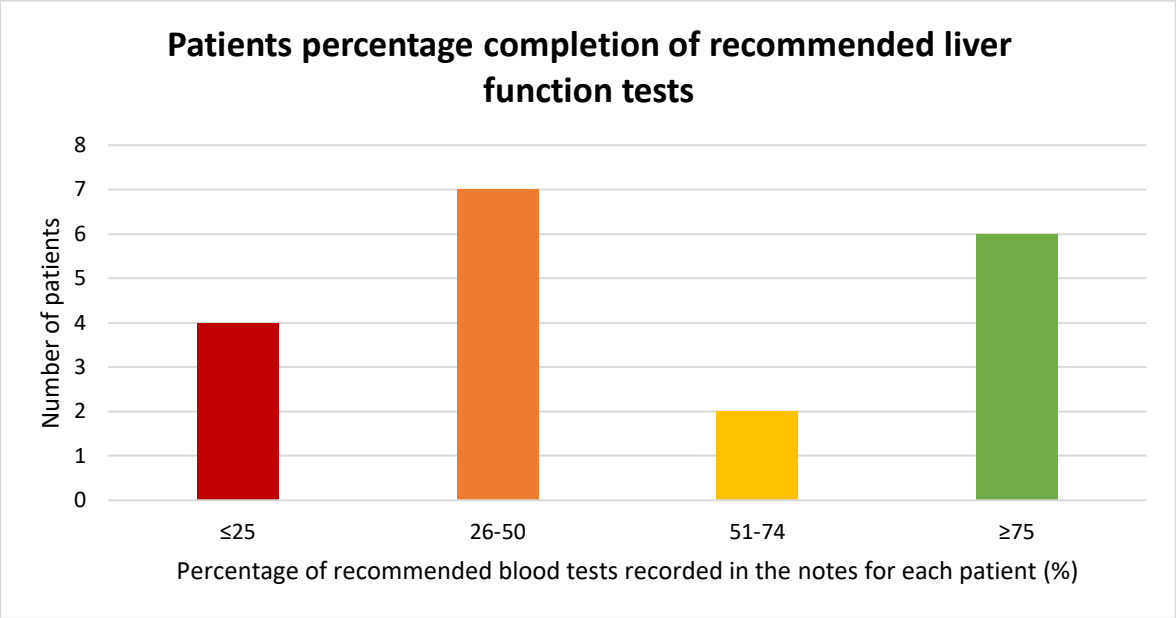


Figure 5.9 | Bar chart showing the number of patients (n=19) who were in each category of percentage completed recommended liver function surveillance blood tests in the clinic population.

The median percentage of completed recommended blood tests was 50% (IQR 33.3-90%). The mode percentage was 100%.

5.7.3. Testing for hyperglycaemia

Guidelines recommend that HbA1c with or without fasting plasma glucose should be repeated annually in girls with TS aged 10 years and over. (4) We used the same method as in 5.7.2 to calculate the percentage of completed recommended HbA1c tests for each patient. Figure 5.10 presents these data.

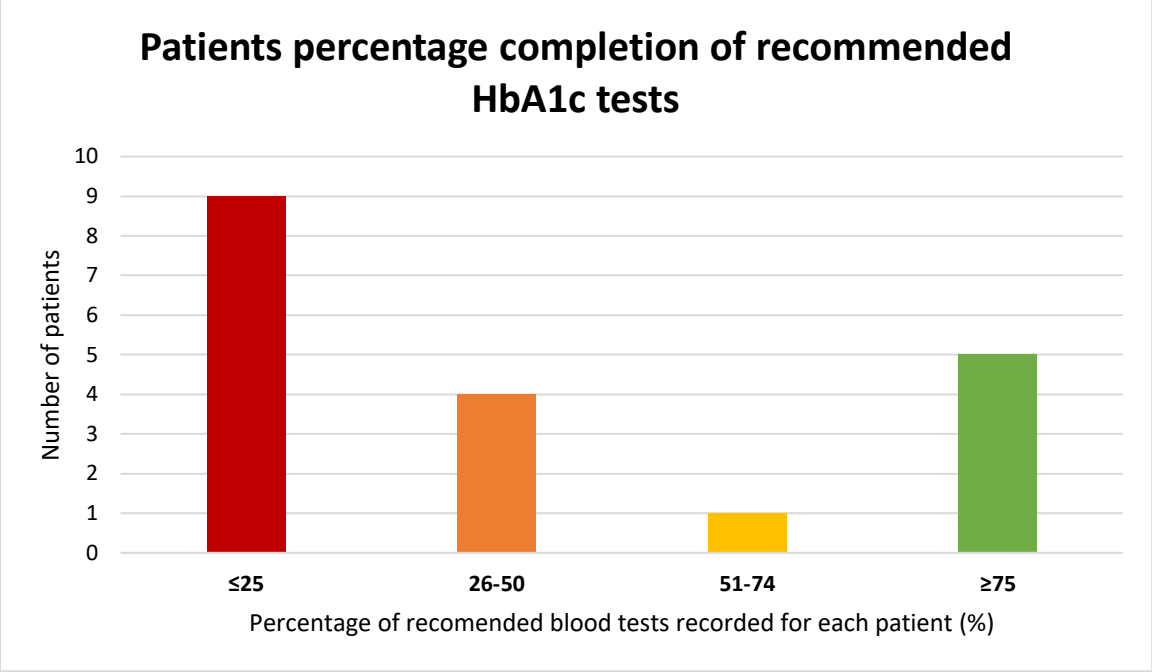


Figure 5.10 | Bar chart showing the number of patients (n=19) who were in each category of percentage completed recommended HbA1c blood tests in the clinic population.

The median percentage of completed recommended blood tests was 33.9% (IQR 0-70%). The mode percentage was 0%.

5.7.4. Lipid Profiles

Guidelines recommend that a lipid profile should be performed in individuals who have at least one risk factor for cardiovascular disease starting at age 18 years. (4)

In our clinic population of 34 patients, 15 patients (44%) had documentation of results of at least 1 lipid profile in their Alder Hey medical records as part of their annual review bloods. The youngest patient to have these tests was aged six years. Six patients had at least one abnormal reading (Table 5.7).

Table 5.7 | Lipid abnormalities documented in the clinic population (n=15).

Lipid abnormality	Frequency in the clinic population
High triglycerides	4[a]
Low HDL-C	2[a]
High LDL-C	2[a]

Abbreviations – HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. a – two patients had two abnormalities on their lipid profile.

5.8. Key Findings

Our key findings from this audit are summarised in Table 5.8.

Table 5.8 | Key Findings from evaluating how current routine clinical care of girls and adolescents with TS in the Liverpool region compared to the recommendations from the 2016 Cincinnati TS Clinical Practice Guidelines (4).

Category	Findings
Baseline demographics	• Having genetic information more readily available could help to provide more individualised patient care. • Patients at Alder Hey Children's Hospital are being diagnosed earlier than they were in 2018.
Cardiac Investigations at diagnosis	• A foetal echocardiogram should be performed in patients diagnosed antenatally. • All patients are routinely receiving a transthoracic echocardiogram, but adolescents should have another form of cardiac imaging at diagnosis. • TS-specific z-scores of aortic dimensions should be documented in patient notes. • The corrected QT formula should be applied to the patients ECG at diagnosis. • Most patients are receiving the appropriately timed follow-up with a cardiologist. • Patients with risk factors for aortic dissection should be counselled on the acute symptoms.
Blood Pressure	• BP should be taken at each patient contact. Measurements should be compared to age and height matched BP percentile charts.
Weight Management	• Healthy living advice should be given as a preventative measure, opposed to after the development of obesity.
Routine Blood Tests	• Thyroid function tests should be performed annually. • HbA1c and Liver function tests should be performed annually starting from age 10 years. • Lipid profiles are not recommended in patients under 18 years old.

Abbreviations – TS: Turner Syndrome; ECG: electrocardiogram; BP: blood pressure.

5.9. Discussion

This audit evaluates how current routine clinical care of girls and adolescents with TS at Alder Hey Children's Hospital compares to the recommendations from the 2016 Cincinnati TS Clinical Practice Guidelines. (4) Areas of the TS service that are performing well are highlighted, as are areas which require improvement.

There were many missing data in this audit, which could be attributed to clinical care of girls and adolescents with TS at Alder Hey Children's Hospital being delivered by multiple healthcare professionals from a variety of medical specialities. There were inconsistencies between patients' engagement with the service, and between engagement in different areas of the service by the same patient. For example, some patients were disengaging with the Cardiology service, however, were seeing their Endocrinology and Audiology services at appropriately timed intervals. A multidisciplinary approach to clinic visits could circumvent these factors and help to standardise care. Patient engagement with the overall service may improve with this approach, as there would be less hospital visits for the same number of appointments. This could also encourage patients who are receiving part of their care at external hospitals to transfer all TS services to Alder Hey Children's Hospital, which may improve communication between different clinical areas.

As recommended by the guidelines, it is positive that endocrinologists are consistently checking that patients were receiving regular audiometric evaluation. It is also positive that the average age of diagnosis of TS has decreased at Alder Hey Children's Hospital since 2018. However, it is unclear whether this is improvement is by chance, opposed to changes in clinical practice, as it may be a result of the higher proportion of patients diagnosed in the prenatal period. Crucially, a lower proportion of patients were diagnosed during adolescence than in 2018. This is important, as a diagnosis before this period allows for assessment and timely initiation of medical interventions that go some way to minimising the physical and psychosocial difficulties associated with short stature and delayed puberty. Early diagnosis can also allow for timely screening for conditions associated with TS and detection of subclinical disease. (4)

Although most patients were receiving the appropriately timed follow-up with a cardiologist and cardiology investigations at diagnosis were consistently undertaken, they were not documented to

the standards of the guidelines. For example, for many patients the QTc formula was not applied to their ECG and TS-specific z-scores of aortic dimensions from cardiac imaging were rarely documented. (4) In cardiology and endocrinology clinic appointments, patients also had documentation of BP measurements; however, it did not appear that measurements were regularly compared to age and height matched BP percentile charts. This suggests that there is capacity within the TS service at Alder Hey Children's Hospital to perform the recommended cardiac investigations, which is promising, and that small changes to clinical practice have the potential to improve clinical care for patients.

Similarly, patients were receiving appropriately timed follow-up with their endocrinologist, and given that this was at least one appointment annually, it would have been expected that more patients received the guidelines recommended frequency of blood tests. The high prevalence of anxiety in TS may impact patient engagement with venepuncture. (4) Play specialist support should be encouraged for patients who are known to be needle phobic.

There was an overall lack of documentation of patient counselling across the TS service: symptoms of aortic dissection, fertility and pregnancy advice, and weight management advice. These conversations may be difficult for patients and their families, and they may benefit from additional psychological support. Guidelines recommend that neuropsychological and allied behavioural health services are integrated into the care of girls and women with TS. This area is currently lacking from the TS service at Alder Hey Children's Hospital. If it is feasible to add this support, clinicians may feel more comfortable having these discussions. This would also improve the TS service by presenting an opportunity for patients to receive the guideline-recommended behavioural and developmental screenings and neuropsychological assessments. (4)

To conclude, a change to the TS service with the implementation of multidisciplinary clinics with endocrinology, gynaecology, cardiology, audiology, and psychology present, could improve compliance with the guidelines. (4) The next clinical audit of the TS service at Alder Hey Children's Hospital should be untaken in 2024.

6. National paediatric blood pressure survey

6.1. Abstract

6.1.1. Background

In 1997 a survey identified a general lack of standardisation of BP measurement techniques and little consensus on the criteria for diagnosing hypertension amongst paediatricians. We have conducted a updated online survey in 2021, to compare clinical practice between the two time periods.

6.1.2. Methods

A national descriptive survey was approved by the General and Adolescent Paediatric Research in the United Kingdom & Ireland (GAPR-UKI) committee and then circulated to consultant-grade general paediatricians.

6.1.3. Results

125 analysable replies from 34 different sites were received and compared with the 1997 data. 106 (84.8%) reported clinic nurse involvement in BP measurement, more than twice the rate reported previously (40.6%). Most paediatricians (53.6%) now rely on BP recording systems, whereas the mercury sphygmomanometer was favoured previously (82.7%). If assessing BP manually (n=89), most (79.8%) now use Korotkoff phase V as the auscultatory endpoint for diastolic BP (Korotkoff phase IV was previously used (52.1%)). For a diagnosis of hypertension, the criteria (≥95th centile for gender, age and height) were constant, and 100% of paediatricians diagnosed it using systolic BP, but only 43 (34.4%) would do so using diastolic BP, a decrease from 79.4% previously. Ambulatory BP Monitoring was six-times more available than in 1997 (81.6% compared with 13.6%). Similar to previous findings, only 12 (9.6%) paediatricians would manage hypertensive patients themselves, however 82 (72.6%) would keep general paediatric input.

6.1.4. Conclusions

There have been important changes in the assessment of BP in children and adolescents, including increased nurse involvement and greater use of technology. However, fewer paediatricians are

responding to high diastolic pressures than twenty years ago. The clinical implications of this change, which is against international guidance, deserves consideration.

6.2. Introduction

A previous study performed just over twenty years ago, suggested a lack of standardisation of BP measurement techniques and little consensus on the criteria for diagnosing hypertension amongst paediatricians in the United Kingdom and Ireland. (374) Updated clinical practice guidelines on the diagnosis and management of hypertension in paediatric patients have since been published (between the years 2004 and 2021). (332, 375-378) Whilst these updated guidelines have continued to promote routine BP measurement (Figure 6.1), there are some inconsistencies in the categorisation of BP status, (379, 380) and the introduction of fixed cut-offs at 13-years of age in the 2017 American Academy of Paediatrics guidance has been particularly controversial. (381)

Key recommendations from clinical practice guidelines

- From 3 years of age, children in a medical setting should have routine blood pressure measurement.
- The auscultatory method is recommended and should be used to confirm a diagnosis of hypertension if oscillometric methods have been used previously.
- Using the auscultatory method, Korotkoff phase I and V sounds should be used to identify systolic blood pressure and diastolic blood pressure, respectively.
- Hypertension is defined as systolic blood pressure and/or diastolic blood pressure persistently at least 95th percentile for age, sex and height on three separate clinic visits.
- 24-hour ambulatory blood pressure monitoring should confirm hypertension before commencing antihypertensive drug treatment.

Figure 6.1 | Key recommendations for blood pressure measurement and management in the paediatric population.

The implementation of evidence-based guidelines into daily practice is a recognised challenge. (382) Unsurprisingly, adherence to paediatric hypertension guidelines in a recent multi-centre study, including a variety of communities across the United States, was sub-optimal. (383)

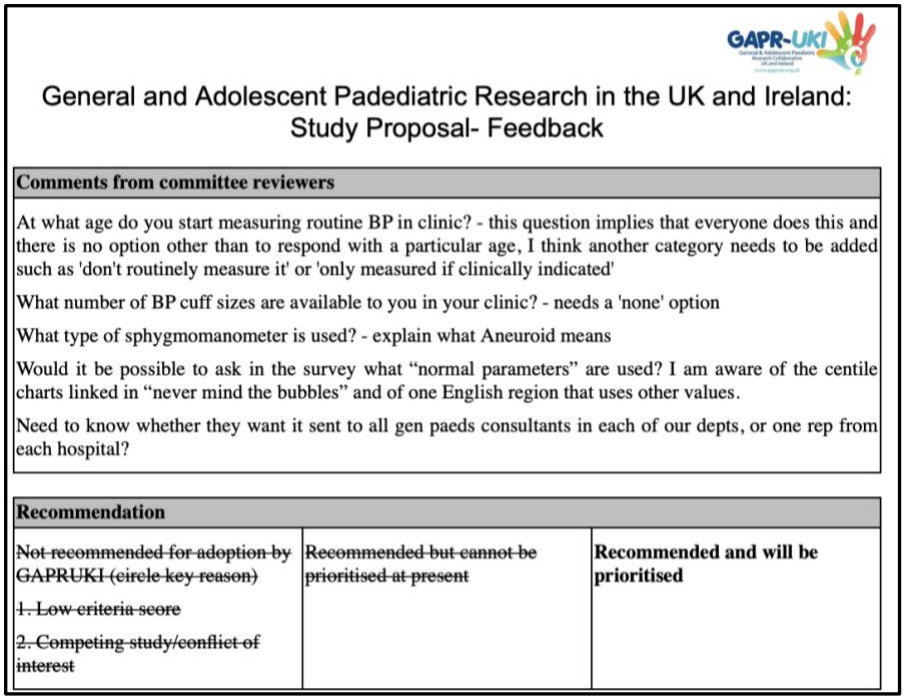
For the facilitation of BP measurement in paediatric clinics, there should be availability of a wide selection of cuff sizes to allow for large variation in upper arm size. The measurement of BP in the

paediatric population, especially in restless young or anxious children, can be technically challenging. (384) These environmental and human factors, combined with height, age and sex specific centile charts to constitute abnormal BP values, can make measurement and interpretation of BP more difficult in paediatric compared to adult patients.

To further investigate current clinical practice and interpretation of BP measurement and treatment of hypertension in children and adolescents in the United Kingdom and Ireland, we conducted an online survey. This was distributed via the General and Adolescent Paediatric Research in the United Kingdom & Ireland (GAPR-UKI) network, an organisation established in June 2016 to facilitate multi-centre paediatric research. This survey was consistent with the survey sent twenty years previously, to enable evaluation of how clinical practice has changed between the two time periods. (374) Our aim is to evaluate whether progress has been made in achieving a consistent approach to the measurement and interpretation of BP measurements in children and adolescents.

6.3. Methods

Modelled on the postal survey from twenty years ago, (374) a Microsoft Form was devised to facilitate our survey. This survey and a completed GAPR-UKI Study Proposal Form were sent to the GAPR-UKI committee for review in October 2021. Our proposal was accepted, and following agreed revisions (Figure 6.2), the survey link and an accompanying body of text outlining the rationale behind the study was sent via email to all GAPR-UKI mailing list members in November 2021.



General and Adolescent Padediatric Research in the UK and Ireland:
Study Proposal- Feedback

Comments from committee reviewers
At what age do you start measuring routine BP in clinic? - this question implies that everyone does this and there is no option other than to respond with a particular age, I think another category needs to be added such as 'don't routinely measure it' or 'only measured if clinically indicated'
What number of BP cuff sizes are available to you in your clinic? - needs a 'none' option
What type of sphygmomanometer is used? - explain what Aneuroid means
Would it be possible to ask in the survey what "normal parameters" are used? I am aware of the centile charts linked in "never mind the bubbles" and of one English region that uses other values.
Need to know whether they want it sent to all gen paeds consultants in each of our depts, or one rep from each hospital?

Recommendation		
~~Not recommended for adoption by GAPRUKI (circle key reason)~~ ~~1. Low criteria score~~ ~~2. Competing study/conflict of interest~~	~~Recommended but cannot be prioritised at present~~	**Recommended and will be prioritised**

Figure 6.2 | Comments from the GAPR-UKI Committee on our study proposal.

Our email invited members of GAPR-UKI to participate and distribute the survey to their consultant-grade General Paediatric colleagues (Figure 6.3). Reminder emails were sent to all GAPR-UKI mailing list members, regardless of response, due to the anonymity of the questionnaire, a week after initial posting and a week before the survey deadline. It was advertised to take approximately 10-15 minutes to complete and consisted of 17 required questions, and dependant on selected responses, a maximum of four further optional follow-up questions. The survey was due to be live for 3 weeks, however to improve participation we extended the study period, and the survey was live for 8 weeks in total. All data were automatically entered into Microsoft Excel for analysis. The survey was anonymous, however, to ensure that our data was representative of practice across the United Kingdom and Ireland, participants were required to disclose their NHS Trust employer. Ethical approval was not required.

Dear Consultant Paediatrician,

We would like to ask you to complete a questionnaire about paediatric blood pressure, which should take between 10-15 minutes. The aim is to update our knowledge on the practice and interpretation of blood pressure measurement and treatment of hypertension in children and young people. A previous study was performed just over twenty years ago, and we hope to compare our knowledge, understanding and clinical practice between the two time periods. We are looking for as many perspectives as possible, not just a single respondent per site, so would also appreciate if you can share this between your consultant general paediatric colleagues.

The link is attached here:
https://forms.office.com/Pages/ResponsePage.aspx?id=MVElUymxEECG4UdL_X6AdvarU4L71h5MmeMs5R8Y0oVURUFONUVTVVhVMEVaR1ExT01aSlA3Q0laSy4u

The deadline for completion is **4th December 2021**.

Thank you, for your time.

Kind regards
Dr Julie Park, Miss Lily Jones, Dr Dan Hawcutt, Professor Greg Lip, Dr Helen Shantsila and Professor Jo Blair

Figure 6.3 |Email sent to GAPR-UKI members.

6.4. Results

We received responses from 34 GAPR-UKI sites. In 22 sites where more than one response was received, it was assumed that consultants sent the survey onto their colleagues. Response rate varied from one to ten per site. Unfortunately, the number of GAPR-UKI sites is unknown and therefore a site response rate could not be established. The absolute response rate in the 1997 survey was 47.1%. (374)

Participants who were not consultant-grade or were not working in the UK currently were excluded, leaving 127 respondents. Of these, two respondents said that BP measurement was not relevant to their clinical practice, so their replies were excluded too. There were 125 analysable replies (Figure 6.4). The absolute number of responses was 683 in the 1997 survey. (374) On average, the questionnaire took participants 07:05 minutes to complete.

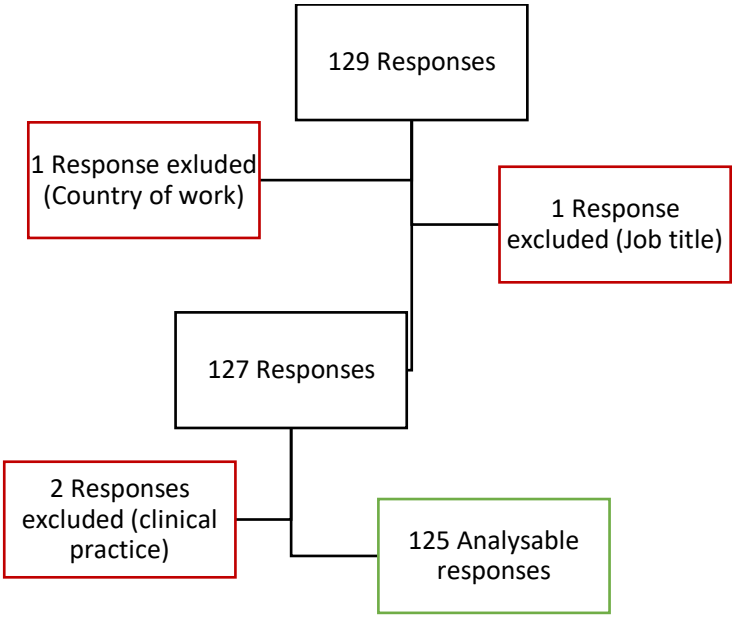


Figure 6.4 |Diagram showing inclusion and exclusion of survey responses.

The timeline of survey responses is shown in Figure 6.5. Half of responses were received by the 1st of December 2021. Responses were sporadic throughout the study period.

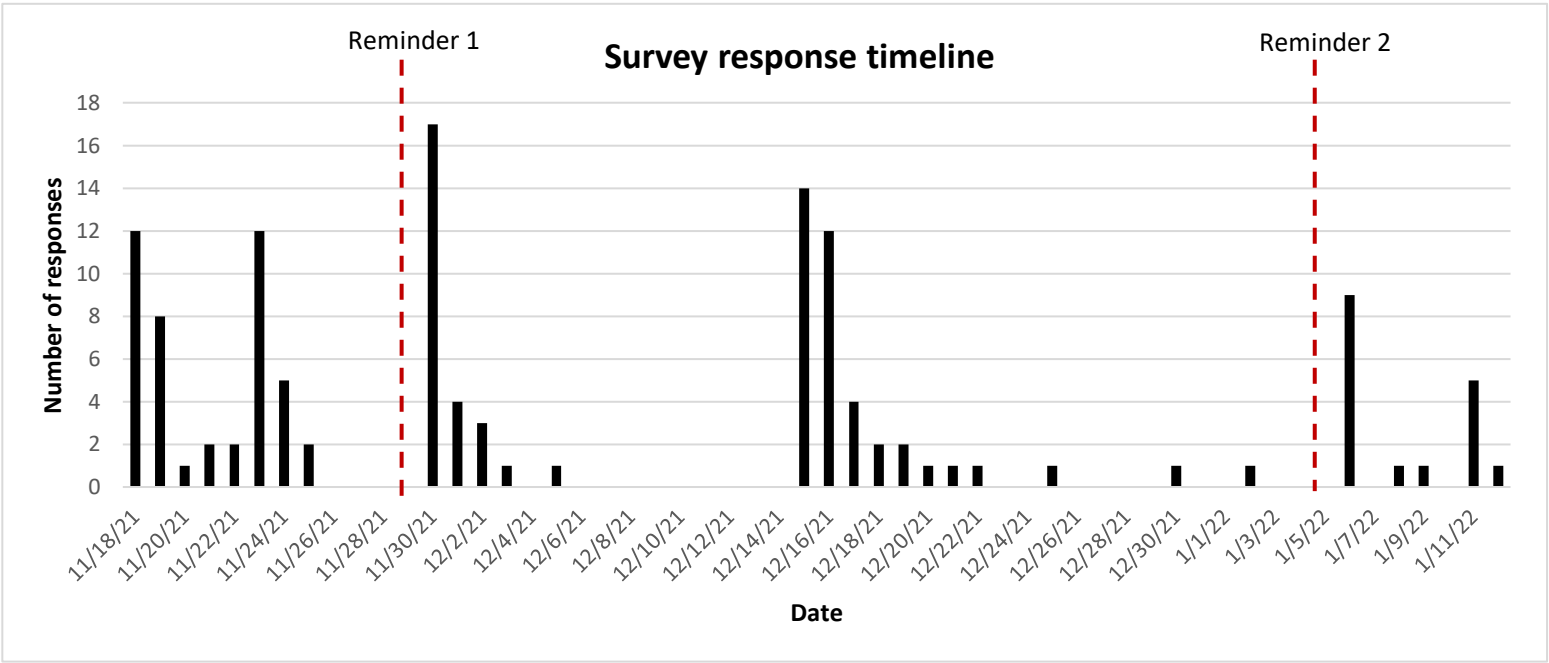


Figure 6.5 | Bar chart showing the number of survey responses between November 2021 and January 2022.

In both surveys not all respondents answered every question, and the percentages reported reflect the number of responses received for each individual question. Although designed using the survey

from twenty years ago, (374) modifications before general mailing meant that not all questions had identical response options.

6.4.1. Blood Pressure Measurement

In the 2021 survey, 114 (91.2%) responded that the circumstances behind routinely measuring BP was dependant entirely on the clinical presentation. Of these, 110 (96.5%) would measure at any age, including from birth, if clinically indicated. Compared to the 1997 data, more paediatricians reported that they would measure BP at any age (Table 6.1). Of the 456 (68.6%) respondents to this question in 1997, 256 (56.1%) would not routinely measure BP in children below the age of three years.

Table 6.1 | Age of screening, identity of measurer and patient posture in paediatric practice.

		1997 survey, n (%)	2021 survey, n (%)
(i)	**Age when routine BP measurement performed in an outpatient**		
	From birth	118 (17.7)	13 (10.4)
	From 1 years	82 (12.3)	0 (0.0)
	From 3 years	153 (20.0)	2 (1.6)
	From 7 years	80 (12.0)	1 (0.8)
	From 13 years	23 (3.5)	0 (0.0)
	Never	209 (31.4)	-
	Other	-	3 (2.4)
	At any age if clinically indicated	-	106 (84.8)
(ii)	**Posture for BP measurement in outpatient clinic**		
	Seated	407 (60.0)	87 (69.6)
	Supine	124 (18.3)	8 (6.4)
	No preference to position	147 (21.7)	20 (16.0)
	Both seated and supine in the same patient	-	10 (8.0)
(iii)	**Who measures BP**		
	Paediatrician	358 (59.4)	19 (15.2)
	Clinic nurse	54 (9.0)	62 (49.6)
	Either	190 (31.6)	44 (35.2)

Abbreviations – n: number; BP: blood pressure.

In the clinic, only 19 (15.2%) paediatricians reported consistently measuring BP themselves, whilst 44 (35.2%) reported measurements were taken by either themselves or nurse. Clinic nurse involvement in BP measurement was reported by 106 (84.8%) paediatricians, a percentage more than double the rate of nursing involvement reported in 1997 (Table 6.1). The chosen posture for BP measurement was reported as seated in 87 (69.6%), supine in eight (6.4%), and 20 (16.0%) had no preference. There were 10 (8.0%) respondents who preferred to routinely measure both seated and supine BP in the same patient. These data are consistent with the data from 1997 (Table 6.1).

One paediatrician reported no access to BP cuffs in clinic, whilst most (96.0%) reported access to at least three different cuff sizes. These results are similar to those reported in 1997 (93.0%) (Table 6.2). In 2021, most paediatricians (53.6%) relied on automatic or semi-automatic BP recording systems, 24 (19.2%) used an aneroid sphygmomanometer and 2 (1.6%) a mercury sphygmomanometer. All three types were used interchangeably by 32 (25.6%) paediatricians. This greatly differed from 1997 data, where the mercury sphygmomanometer was used most frequently (82.7%) (Table 6.2). For further investigation of suspected hypertension, 102 (81.6%) of paediatricians had access to ABPM. ABPM was reported to be six times more available than in 1997 (Table 6.2).

Table 6.2 | Equipment available for BP measurement in children and young people.

		1997 survey, n (%)	2021 survey, n (%)
(i)	**Number of BP cuff sizes available**		
	0	-	1 (0.8)
	1	8 (1.4)	0 (0)
	2	32 (5.6)	4 (3.2)
	3	343 (32.5)	39 (31.2)
	4 or more	343 (60.5)	81 (64.8)
(ii)	**Type of sphygmomanometer used**		
	Mercury sphygmomanometer	565 (82.7)	2 (1.6)
	Aneroid	36 (5.3)	24 (19.2)
	Automatic or semi-automatic	64 (9.4)	67 (53.6)
	All types	18 (2.6)	32 (25.6)
(iii)	**Ambulatory BP monitoring available**		

Yes	93 (13.6)	102 (81.6)

Abbreviations – n: number; BP: blood pressure.

6.4.2. Diastolic end-points

If paediatricians were measuring BP manually (n=89), the most favoured (79.8%) auscultatory end-point for measuring diastolic BP was the disappearance of sounds (Korotkoff phase V). This differed from the 1997 data, where majority used the muffling of sounds (Korotkoff phase IV), although there was considerable variation in these data (Table 6.3).

Table 6.3 |Manual BP measurement.

	1997 survey, n (%)	2021 survey, n (%)
(i) Diastolic end point measurement in outpatient clinic		
Phase 4 (muffling of sounds)	348 (52.1)	16 (18.0)
Phase 5 (disappearance)	213 (31.9)	71 (79.8)
Both phase 4 and 5	106 (15.9)	2 (2.2)
No reply / Not applicable	16 (-)	36 (-)

Abbreviations – n: number.

6.4.3. The clinical diagnosis of hypertension

All paediatricians used systolic BP for a diagnosis of hypertension: 26 (20.8%) would not take diastolic readings into account, 56 (44.8%) required both systolic and diastolic pressures to be raised and 43 (34.4%) responded to either raised systolic or diastolic pressures. Only 43 (34.4%) paediatricians responded to a raised diastolic pressure alone, which contrasts to 465 (79.4%) who reported to do so in the 1997 data (Table 6.4).

Table 6.4 | The clinical diagnosis of hypertension in children and adolescents.

	1997 survey, n (%)	2021 survey, n (%)
(I) Reporting hypertension		
Systolic alone	105 (17.9)	26 (20.8)

Diastolic alone	79 (13.5)	0 (0.0)
Both systolic and diastolic	16 (2.7)	56 (44.8)
Either systolic or diastolic	386 (65.9)	43 (34.4)
(II) BP centile to diagnose hypertension		
90th	76 (12.9)	12 (9.6)
95th	246 (41.8)	85 (68.0)
99th	-	23 (18.4)
Other	-	5 (4.0)
>95th	267 (45.3)	-

Abbreviations – n: number.

Readings at the 95th percentile in relation to gender, age and height were used to diagnose hypertension by 85 (68.0%) of paediatricians. Pressures at the 90th percentile were used by 12 (9.6%) paediatricians and 23 (18.4%) used the 99th percentile for diagnosis. In the 1997 survey, 513 (86.3%) paediatricians made a diagnosis based on measurements at or greater than the 95th percentile. This percentage was lower (77.6%) in our 2021 survey (Table 6.4).

Most paediatricians (55.2%) relied on raised serial BP measurements over a morning, afternoon, or day for a diagnosis of hypertension. Of the paediatricians who responded with visit numbers (n=56), 45 (80.4%) would require at least three visits where BP was raised before treating or referring their patient for management of hypertension.

6.4.4. The management of the hypertensive child and adolescent

Routinely measuring BP in the legs of a hypertensive child or adolescent was reported by 17 (13.6%) paediatricians, and a further 84 (67.2%) reported that they would do so if clinically indicated. In contrast, 49 (39.2%) paediatricians reported that they routinely measure BP in both arms and 68 (54.4%) would do so if clinically indicated in a child or adolescent with hypertension. In a hypertensive child or adolescent, fewer paediatricians reported never measuring BP in both arms (6.4%) compared to the legs (19.2%), which differed to responses in 1997 (Table 6.5).

Table 6.5 | The clinical management of hypertension in children and adolescents.

	1997 survey, n (%)	**2021 survey, n (%)**

(I)	**Measuring leg BP in a hypertensive child / adolescent**		
	Yes, routinely	204 (30.3)	17 (13.6)
	No	173 (25.7)	24 (19.2)
	Yes, if clinically indicated	296 (44.0)	84 (67.2)
(II)	**Measuring BP in both arms in a hypertensive child / adolescent**		
	Yes, routinely	299 (43.9)	49 (39.2)
	No	225 (33.0)	8 (6.4)
	Yes, if clinically indicated	157 (23.1)	68 (54.4)
(III)	**Medical management**		
	Manage	76 (11.4)	12 (9.6)
	Refer	591 (88.6)	113 (90.4)

Abbreviations – n: number; BP: blood pressure.

Only 12 (9.6%) paediatricians, of which six (50%) had a specialist interest in either paediatric cardiology or nephrology, would manage these patients themselves. The remainder of paediatricians would refer to specialists, although 82 (72.6%) would continue to see the child or adolescent in the general paediatric clinic. Nephrology was the preferred specialty for referral for 65 (58.0%) paediatricians. This was consistent with findings from 1997 (Table 6.5).

6.5. Discussion

Expert guidelines recommendations influence clinician's attitudes and clinical practice. The two main guidelines for BP measurement and management in children and adolescents are the European Society of Hypertension (ESH) and the American Academy of Paediatrics (AAP) guidelines, which were recently updated in 2016 and 2017, respectively. (332, 375)

Our survey shows that for routine BP measurement, most respondents answered in concordance with current guideline recommendations and will measure BP at any age if clinically indicated. Both guidelines recommend routine BP measurement from the age of three years, and only in younger children where risk factors for hypertension are present. (332, 375) In otherwise healthy individuals, the ESH guideline recommends biennial BP measurement, (332) whereas the AAP guideline recommends annual measurement. (375)

The majority of the survey responders would measure BP in the seated position, using an oscillometric device, as per both guidelines' recommendations. It should be noted that the device model needs to be validated for the paediatric population. (332, 375) In keeping with technological advances of the past two decades, when compared to the 1997 data, wider availability of ABPM for the investigation of hypertension, and more common routine use of automatic or semi-automatic BP recording system were reported. (374) For a diagnosis of hypertension, however, both guidelines still recommend auscultatory-confirmed measurement. (332, 375)

It is concerning that 28.8% of respondents from the present survey reported that measuring of diastolic end-point was not applicable to their clinical practice, as this suggests that they are not confirming BP measurement via auscultatory methods. Of those who specified a favoured diastolic end-point, the majority (79.4%) used Korotkoff phase V, which is endorsed by both guidelines. (332, 375) At the time of mailing of the 1997 survey, recommendations were changing and there was considerable confusion on the correct auscultatory end-point for the measurement of diastolic BP, (385-387) which was reflected in the survey responses. (374)

Both guidelines recommend defining hypertension as a systolic or diastolic BP of at least 95th percentile for age, gender and height measured clinically on three separate occasions. (332, 375) The data from 1997 suggests closer adherence to these recommendations. (374) The present survey responses suggests paediatricians have a higher threshold for the diagnosis of hypertension than previously, and are much less likely to respond to high diastolic readings. The clinical significance of this change in practice deserves further consideration. Most general paediatricians in both the present and 1997 survey would refer their hypertensive patients to the appropriate specialists, which is also recommended by the ESH guidelines. (332, 374)

The present survey is likely to have encountered similar limitations of the postal survey sent twenty years previously. (374) As with all self-reported clinical practice surveys, the possibility of reported practice differing from actual practice cannot be discounted. Furthermore, the option of "when clinically indicated" was heavily favoured when included as a possible response in survey questions. This is somewhat problematic, as it gives no indication of the clinical circumstances that inform decision making, and it is likely that these differ between paediatricians. The possibility that the clinical practice of the responders differed from the non-responders also cannot be excluded. The survey was sent via the GAPR-UKI network, so responders were likely to have an interest in paediatric and adolescent research, leading to over-representation of research-workers among our respondents. The

clinical practice of clinicians in the North West of England was also over-represented, although there was at least one respondent employed by every regional team in NHS England and respondents from Scotland, Wales and Northern Ireland.

Our questionnaire was delivered on Microsoft Forms, an online platform that clinicians should be familiar with, and efforts were made to make the questionnaire clear and concise. Despite these factors, and our two reminder emails, we received considerably fewer analysable replies compared to the postal questionnaire twenty years previously. (374) However, although becoming more prevalent during the past two decades, (388) hypertension is still an uncommon clinical problem encountered in general paediatric practice. Accounting for this, we feel our sample size is suitable to assess the usual current clinical practice of consultant-grade general paediatricians in the United Kingdom.

From comparing responses from the present questionnaire to those collected twenty years previously, it is evident that BP measurement using auscultatory methods are more standardised now, however paediatricians are more likely to rely on oscillometric technology. It is important to note that paediatric BP reference data were derived from data collected from manual readings, and these might not be directly applicable to measurements made using oscillometric technology. There is greater availability of BP equipment and technology, yet disparity remains in the classification of paediatric hypertension and fewer paediatricians are responding to high diastolic pressures than twenty years ago. Given that hypertension is rare in paediatric practice, it is reassuring that appropriate specialist input is sought when required. However, an increase in hypertension and a decline in the cardiovascular health of the paediatric population might be anticipated in line with the high rates of childhood obesity. Overall, in the past two decades some progress has been made in improving availability and use of equipment to measure BP, for detection of hypertension in children and adolescents. However, fewer paediatricians are responding to diastolic measurements >95th centile for age, sex and height than twenty years ago, and the clinical implications of this change deserves consideration.

These data were presented at Royal College of Paediatrics and Child Health Conference in June 2022 **(9.7. Appendix 7).**

7. Discussion

The main body of this thesis describes the feasibility and acceptability of a study protocol investigating salivary cortisol and cortisone profiles and associations with markers of evolving cardiovascular risk in paediatric patients with TS. The rationale of this pilot study, its set-up from ethical review to participant discharge from the CRF, and the participant data collected at study visits, is described in detail. Additionally, evaluation of a regional paediatric TS service, and a national descriptive clinical practice survey on paediatric BP measurement, interpretation and management, were discussed.

7.1. Limitations

There are limitations to this thesis which should be discussed, with perhaps the most significant being the low number of participants enrolled in our pilot study (Chapter 3 and Chapter 4). Although our study cohort appeared to have similar characteristics to both the TS clinic population at Alder Hey Children's Hospital and the existing literature, the possibility that our small population was not representative of the overall paediatric TS population cannot be excluded. Due to our small sample size, it is possible that type II statistical error (failure to reject a false null hypothesis) also occurred in our results. Importantly, our pilot data was not adequately powered for accurate conclusions to be drawn, and therefore the findings documented in Chapter 3 and Chapter 4 should be viewed as descriptive only.

Secondly, there was a lack of control group data for the majority of study investigations (Chapter 4). Only CGM and salivary biomarker data had a sex and age matched (1:1) control group. For descriptive analysis of the remaining study variables, a variety of normative reference values and percentiles, of mixed applicability to our population group, were sourced. For example, for some variables, such as AASI, we were unable to source paediatric reference values and referenced against an adult population. Ideally, our study protocol would be completed by age, sex and ethnicity matched healthy volunteers from the Liverpool-region, and comparisons would be made to these data.

We were also unable to account for protentional confounders in our experimental study data (Chapter 4). For example, there was a high prevalence of obesity in our study population, however most normative percentiles used to describe our cardiovascular data were derived from healthy weight cohorts and we were unable to match the healthy control groups for BMI Z-score. Additional diagnoses, such as anxiety disorder, and external factors, such as needle phobia, also likely influenced

participants salivary biomarker data and some measures of cardiovascular health. Identification of confounders and appropriate adjustment for these would reduce bias in our results.

There were many missing data in our audit evaluating how current routine clinical care of girls and adolescents with TS at Alder Hey Children's Hospital compared to the recommendations from the 2016 Cincinnati TS Clinical Practice Guidelines (Chapter 5). There was documentation of some patients receiving part of their care at their local hospitals, however due to access restrictions, any investigations performed elsewhere were not included in our results. Also, one only investigator extracted data from patients' medical records, and the possibility of human-error during this task cannot be excluded.

Our clinical practice survey (Chapter 6) had a lower number of responders compared to the 1997 survey. An absolute response rate for our survey could not be established, as the number of GAPR-UKI sites is unknown and survey responders were also encouraged to forward the survey onto their colleagues. As with all self-reported clinical practice surveys, the possibility that the clinical practice of the responders differed from the non-responders, and reported practice differed from actual practice, cannot be discounted. The clinical practice of clinicians in the North West of England was also over-represented and it is likely that there was over-representation of research-workers among our respondents.

7.2. Areas for further research

Our recruitment data (Chapter 3) suggests that future studies for paediatric patients with TS should recruit from all clinical areas, irrespective of the focus of the study.

To our knowledge our pilot study (Chapter 4) was the first to describe elevated CGM profiles, an unfavourable haemostatic environment for CVD development, a disrupted salivary cortisol circadian rhythm and increased total cortisol exposure in paediatric patients with TS when compared to matched control groups or reference populations. These novel findings and their clinical consequences deserve further investigation in larger, adequately powered studies.

The findings from Chapter 7 demonstrate that clinicians are more likely to rely on oscillometric technology opposed to auscultatory methods for the measurement of BP in clinic. There is therefore

the need for development of paediatric age-sex-and-height-specific clinic BP reference ranges derived from data collected from oscillometric technology.

7.3. Implications for clinical practice

Our pilot study feasibility data (Chapter 3) highlighted that the name 'Play Specialist' may discourage older children and adolescents from engaging and benefiting from available support. An alternative name for the role, for example a 'procedural anxiety specialist', may be more appropriate. Clinicians at Alder Hey Children's Hospital should also be reminded that they can refer their needle-phobic patients for play specialist support or desensitisation therapy.

We concluded in Chapter 4 that implementation of multidisciplinary clinics within the TS service at Alder Hey Children's Hospital may improve compliance with the 2016 Cincinnati TS Clinical Practice Guidelines, through improvements in overall patient engagement and communication between clinical specialties. Additionally, the presence of psychology services at this clinic would allow for the recommended integration of neuropsychological and allied behavioural health services into patient's care, which is currently lacking from the TS service at Alder Hey Children's Hospital. It was evident when analysing the pilot study feasibility data (Chapter 3) that this clinic population would benefit from dedicated, individualised, psychological support.

Given the high prevalence of cardiovascular risk factors observed in this pilot study (Chapter 4) and audit (Chapter 5), clinicians should encourage their paediatric patients with TS to make heart-healthy lifestyle choices. The Healthy Living Factsheet (Appendix 07) is a resource which can be used in clinic.

7.4. Conclusion

To conclude, our pilot data supports the existing literature, demonstrating an adverse cardiovascular risk profile in paediatric patients with TS. This study also presents novel findings, including the description of a non-physiological circadian profile of salivary cortisol. Further adequately powered studies are required for validation and investigation of the impact of this finding on the cardiovascular profile of paediatric patients with TS. Our acceptability and feasibility data give some confidence that a larger, multicentre study is likely to have an acceptable recruitment rate, and that most study participants will complete the study protocol.

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9. Appendices

9.1. Appendix 1

Appendix 1: Standard Operating Procedure for Flow-Mediated Dilation of the Brachial artery (STARRY study)

Purpose:
To establish guidelines for the procedure of measuring brachial artery flow-mediated dilation.

Definitions:
FMD – Flow Mediated Dilatation
BP – Blood pressure
USS – Ultrasound
DPP – Debut Professional Programme
TGC – Time Gain Compensation
CIMT – Carotid Intima-Media Thickness

Procedure:

Whilst patient is having height, weight, body composition measurement:

1. Collect clean equipment (USS scanner, laptop, gel)
2. Connect Avio monitor to laptop with DPP
3. Press Pre-set and Arterial mode on USS machine
4. Wash hands

Once patient has returned:

5. Confirm with participant that they have fasted (including no caffeine)
6. Explain procedure and gain informal consent again
7. Enter anonymised unique participant identifier into USS machine
8. Confirm the participants seated systolic BP reading with a member of the study team (measured earlier)
9. Ask the participant to lie in a comfortable position on the bed (lying on their back)
10. Ask the participant to rest for 10 minutes
11. Ensure that the participants right arm is comfortable and supported
12. Place BP cuff on right forearm, midway between the wrist and elbow
13. Palpate the participants brachial artery
14. Place gel on USS probe
15. Ask a member of the study team to dim the lights
16. Ensure that you are in comfortable position before you start scanning
17. Use red area of USS probe to locate brachial artery – Grey line should be at the top
18. Press Colour doppler on USS machine
19. Set the depth on USS machine
20. Lightly compress vessel with USS probe to confirm presence of artery
21. Set the focus on USS machine
22. Remove colour doppler on USS machine
23. Turn on the pulse wave (x2) on USS machine
24. Turn down volume on the USS machine
25. Turn duplex on
26. Move the blue arrow on USS machine so that it is not interfering with the measurable area of the artery
27. Ensure that the arrow is pointing towards the artery (270 degrees) → Angle adjust 60
28. Press spectral invert on USS machine
29. Move baseline on USS machine
30. Change the scale on USS machine
31. Ensure the gain of the image is OK
32. Adjust TGC on USS machine for contrast and clarity of image
33. Check that the participant is comfortable
34. Check you are happy with the image of your artery
35. Press record on DPP

36. Wait 1 minute, holding USS probe on identified artery
37. Inflate BP cuff to 50 mm Hg above the earlier systolic BP reading
38. Leave cuff inflated for 5 minutes, maintain brachial artery image
39. Check on participant regularly and give countdowns (i.e. half way)
40. Deflate BP cuff at the end of the 5 minute period
41. Keep recording the brachial artery for a further 2 minutes
42. Stop recording on DPP (total time recording should be 8 minutes. Leave a 5 second grace period before stopping the recording).
43. Remove USS probe and BP cuff from participant's arm
44. Provide the participant with tissues to remove any excess gel
45. Thank participant and make them aware that it is the end of the FMD procedure
46. Ask a member of the study team to turn on the lights
47. Press end exam on USS on machine
48. Export data
49. Prepare for CIMT

9.2. Appendix 2

Appendix 2: Standard Operating Procedure for Carotid intima-media thickness test (STARRY study)

Purpose:
To establish guidelines for the procedure of measuring the intima-media thickness of the carotid artery.

Definitions:
CIMT – Carotid Intima-Media Thickness
USS – Ultrasound
DPP – Debut Professional Programme

Procedure:

Once patient is ready:
1. Press Pre-set and Carotid mode on USS machine
2. Explain procedure and gain informal consent again
3. Enter anonymised unique participant identifier into USS machine
4. Ask participant to lie on their back and turn their head to their left
5. Place gel on USS probe
6. Ask a member of the study team to dim the lights
7. Ensure that you are in comfortable position before you start scanning
8. Use red area of USS probe to locate brachial artery – Grey line should be at the top
9. Press Colour doppler on USS machine
10. Set the depth on USS machine
11. Lightly compress vessel with USS probe to confirm presence of artery
12. Set focus on USS machine
13. Turn Colour doppler on USS machine off
14. Both top and bottom intima should be visible on the image (if possible)
15. The carotid blub should be visible
16. Press Acquire to take more images (3 images)
17. Provide the participant with tissues to remove any excess gel
18. Thank participant and make them aware that it is the end of the procedure
19. Ask a member of the study team to turn on lights
20. End exam on USS machine to save carotid images

Once patient has left for breakfast:
21. Export data for both scans before turning off machine
22. Clean and put away used equipment
23. Wash hands

Data analysis:

24. Search patient
25. Click on study
26. Press open study
27. Click on image
28. Open selected image on QLAB
29. Press IMT
30. Select Right - Mid - CCA
31. Find area of interest (approximately 0.5cm from the carotid bulb)
32. Measure a 10mm interval
33. Ensure that success is 95% or above (can reduce to measuring 8mm if needed)
34. Press Acquire to save to study images
35. Press X to get to the next image
36. Repeat steps 28-35 twice more
37. Calculate an average from these measurements
38. Record on secure STARRY study spreadsheet

9.3. Appendix 3

Appendix 3: Standard Operating Procedure for Continuous Glucose Monitor Insertion (STARRY study)

Purpose:
To establish guidelines for the procedure of inserting a continuous glucose monitor

Definitions:
CGM – Continuous Glucose Monitor

Procedure:

Whilst vascular scanning is taking place:

1. Collect clean equipment (alcohol wipe, charged receiver, transmitter, sensor, bag)
2. Login to Dexcom CLARITY for Clinics Account
3. Wash hands

Once patient is ready:

4. Explain procedure and gain informal consent again
5. Enter anonymised unique patient identifier via 'Add patient' on the CLARITY Account
6. Ask the participant to lie supine, in a comfortable position, on the bed
7. Remove the anaesthetic cream
8. Confirm which side the abdomen the participant would prefer the sensor
9. Clean the area where the sensor will be placed with an alcohol wipe (avoid scars, hair, tattoos, bony areas, irritation, and waist band area)
10. Turn on the receiver (press and hold for 2 seconds)
11. When prompted, enter the date and time into the receiver
12. Go through the Alarms and Alerts screens of the receiver with the participant
13. Enter the unique four-digit sensor code on the display screen of the receiver
14. Enter the serial number located on the bottom of the transmitter (and the box that it came in) into the display screen of the receiver
15. Keep the box of the transmitter and note when it expires (Reusable for 3 months)
16. Open applicator pack used during display setup
17. Remove both labels from applicator; do not touch adhesive
18. Move applicator to the clean sensor site and place adhesive on skin
19. Fold and break off safety guard of the applicator
20. Press button on the applicator to insert the sensor
21. Discard applicator
22. Insert transmitter tab into slot on the sensor patch
23. Snap transmitter firmly into place
24. Rub around the patch 3 times

25. Thank participant and make them aware that it is the end of the insertion of the CGM
26. Use the display screen to start the pairing of the transmitter to the receiver
27. Once communication is confirmed, use the display screen to start the sensor to begin the 2-hour sensor warmup
28. Connect the receiver via a USB to the laptop and click "Manage receiver" and then "Connect"
29. Blind the receiver
30. Give the participant a charger for the receiver

Once the participant leaves for breakfast:

31. Clean and put away used equipment
32. Wash hands

9.4. Appendix 4

Appendix 4: Standard Operating Procedure for Continuous Glucose Monitor Removal (STARRY study)

Purpose:
To establish guidelines for the procedure of removing the continuous glucose monitor

Procedure:

Before patient arrives:

1. Collect adhesive remover, tissues and laptop
2. Login to Dexcom CLARITY for Clinics Account
3. Wash hands

Once patient has arrived:

4. Ask the participant to lie supine, in a comfortable position, on the bed
5. Add adhesive remover to the edges of the patch
6. Peel the patch off like a plaster
7. Provide participant with tissues to wipe excess adhesive remover
8. Bend and break the transmitter-holder to release the transmitter
9. Slide the transmitter straight out if the transmitter-holder
10. Collect receiver and charger from the participant
11. Thank participant for their time and engagement with the study
12. Wash hands

Once patient leaves:

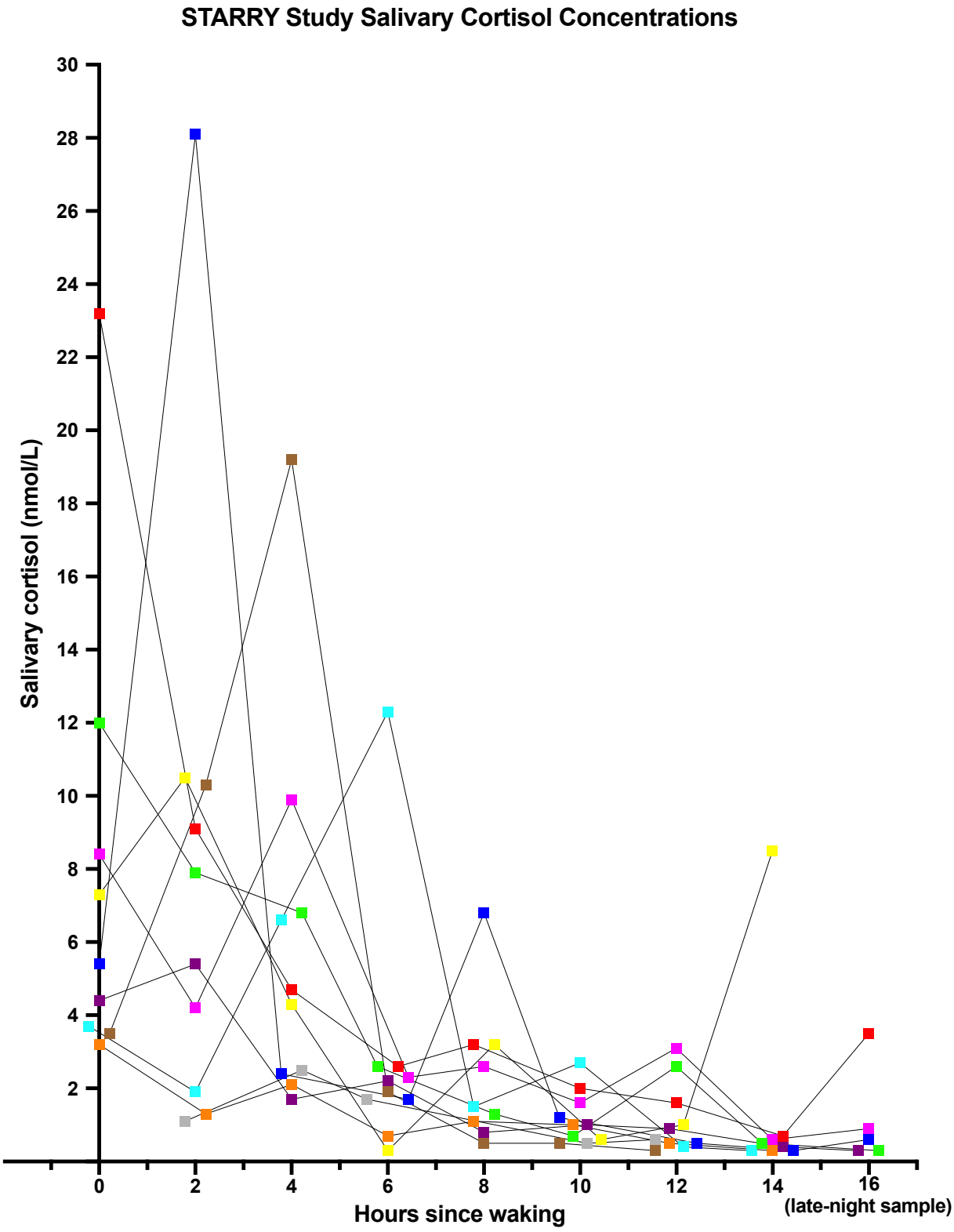
13. Connect the receiver via a USB to the laptop and click "Manage receiver" and then "Connect"
14. Unblind the receiver
15. Click on the participants "Name" in the Patient List
16. Click "Upload Data"
17. Click "Export" to download the participant's data
18. Logout of Dexcom CLARITY for Clinics Account

9.5. Appendix 5

Study ID	306	310	305	301	307	308	311	302	304	309	303
i. Baseline Characteristics											
Age (years)	9.01	11.16	12.37	14.56	13.15	13.71	13.83	15.17	15.78	16.16	17.39
Karyotype	TS variant	Monosomy X	Mosaic	Mosaic	Mosaic (XXX)	TS Variant	TS Variant	Mosaic	TS Variant	Monosomy X	MISSING
Ethnicity	WB	WB	WB	WB	Chinese	WB	WB	WB	WB	EE	WB
IMD decile[a]	4	9	5	1	4	5	3	3	1	1	1
ii. Relevant History											
Antenatal history					Preeclampsia	Preeclampsia				Smoker	Twin
SGA			Yes		Yes	Yes		Yes			Yes
CVD in FDR						Yes					
Congenital CVD				Yes	Yes						Yes
Diagnoses				Anxiety							Anxiety
Medications										Oral HRT	
iii. Cardiovascular variables											
Proatherogenic Lipid profile		Yes		Yes		Yes		Yes		Yes	
Insulin resistance				Yes			Yes				Yes
↑ Clotting factors		Yes	Yes				Yes	-	Yes		
↑ Inflammatory markers	Yes		Yes	Yes		Yes					
↑ Leptin (≥85th centile)	Yes	Yes	Yes	Yes		Yes	Yes				
BMI status[b,c]	Obese	Overweight	Obese	Obese		Obese				Overweight	
↑ Waist-to-hip ratio (>0.85)			Yes	Yes							
↑ % BFM (≥85th centile)	Yes		Yes	Yes		Yes				Yes	
↓ SMMa (≤2nd centile)			Yes	Yes							
↓ MFR (<0.80[d])	Yes	Yes	Yes	Yes		Yes				Yes	Yes
↑ Renin											
Clinic HTN (≥95th centile)		Yes					Yes	Yes		Yes	
Ambulatory HTN (≥95th centile)	-	-	-	Yes				Yes		Yes	
↑ Heart rate (≥90th centile)	-	-	-				Yes	Yes	Yes		
↑ AASI (≥0.50)	-	-	-	Yes			Yes				
↑ cIMT (≥75th centile)	Yes	-	Yes			Yes					Yes
↓ FMD (<7.7%)				-		-		Yes	Yes	Yes	
Abnormal CGM		-							Yes		
i. Cortisol values											
↑ 9am serum cortisol							Yes				
↑ Late-night salivary cortisol	-	-	-	-	-		Yes		Yes		Yes
Mean cortisol[e] nmol/L	1.4[f]	-	6.0	1.4	4.2	4.8	6.6	2.3	4.6	4.5	6.6
Cortisol circadian rhythm colour (see graph below)		-									

Abbreviations – ID: identifier; TS: Turner syndrome; WB: White British; EE: Eastern European; IMD: index of multiple deprivation; SGA: small-for-gestational age; CVD: cardiovascular disease; HRT: hormone replacement therapy; FDR: first-degree relative; BMI: body mass index; BFM: body fat mass; SMMa: appendicular skeletal muscle mass; MFR: muscle-to-fat ratio; HTN: hypertension; AASI: ambulatory arterial stiffness index; cIMT: carotid intima-media thickness; FMD: flow-mediated dilatation; CGM: continuous glucose monitoring; nmol/L: nanomoles per litre.

a – 1: most deprived; b – Obese: ≥+2 BMI Z-score; c – Overweight: ≥+1 BMI Z-score; d – <1.10 participant 306; e – from waking to 12-hours; f – waking sample missing.


STARRY Study Salivary Cortisol Concentrations
Salivary cortisol (nmol/L)
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
2
4
6
8
10
12
14
16
Hours since waking
(late-night sample)

9.6. Appendix 6

Important factors to consider when reporting blood pressure in children and young people with Turner Syndrome

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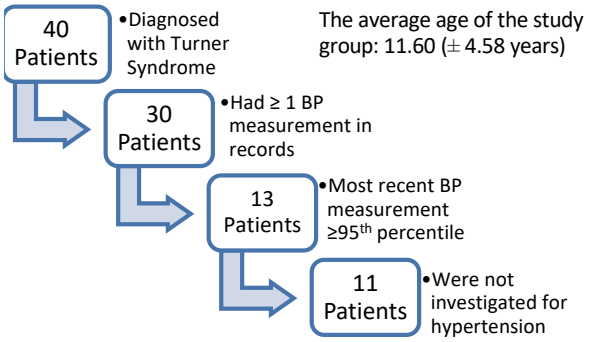
Aims:

- Hypertension can develop at a young age in Turner Syndrome, and International Guidelines recommend that blood pressure (BP) is measured at every clinic visit during childhood. [1]
- Measurements of BP and clinical interpretation were audited to assess compliance with these guidelines.



Methods

1. The most recent evaluable BP measurement was identified in the medical records of all girls diagnosed with TS in a tertiary centre in October 2021.
2. BP percentiles were calculated using gender, age, and height matched BP percentile charts. [2] True (T) BP percentile was compared to a hypothetical (H) BP percentile based on an age expected height at the 50th percentile.
3. We defined a systolic and/or diastolic BP value ≥95th BP percentile as an indication for further investigation.

Results

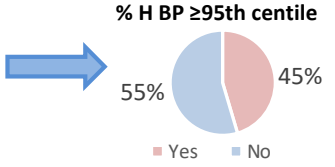
Evaluation of BP in clinic resulted in referral and subsequent diagnosis for hypertension in two girls; however, T BP percentiles suggested an additional 11 patients in our cohort warranted further investigation.

Table 1: Comparison of true and hypothetical blood pressure centiles in observed cases

Age (years)	Blood pressure (mmHg)	tBP ≥95th centile	hBP ≥95th centile
2	102/56	Y	N
5	111/72	Y	Y
6	109/73	Y	N
10	119/81	Y	Y
14	126/78	Y	Y
15	124/72	Y	N
15	126/73	Y	N
15	128/75	Y	Y
16	124/84	Y	Y
16	126/67	Y	N
16	127/83	Y	N

tBP: blood pressure for measured height SDS; hBP: blood pressure centile for girl with height 0SDS; Y : Yes; N : No.

Even H BP percentiles indicated that the BP values of 5/11 patients should be investigated further.

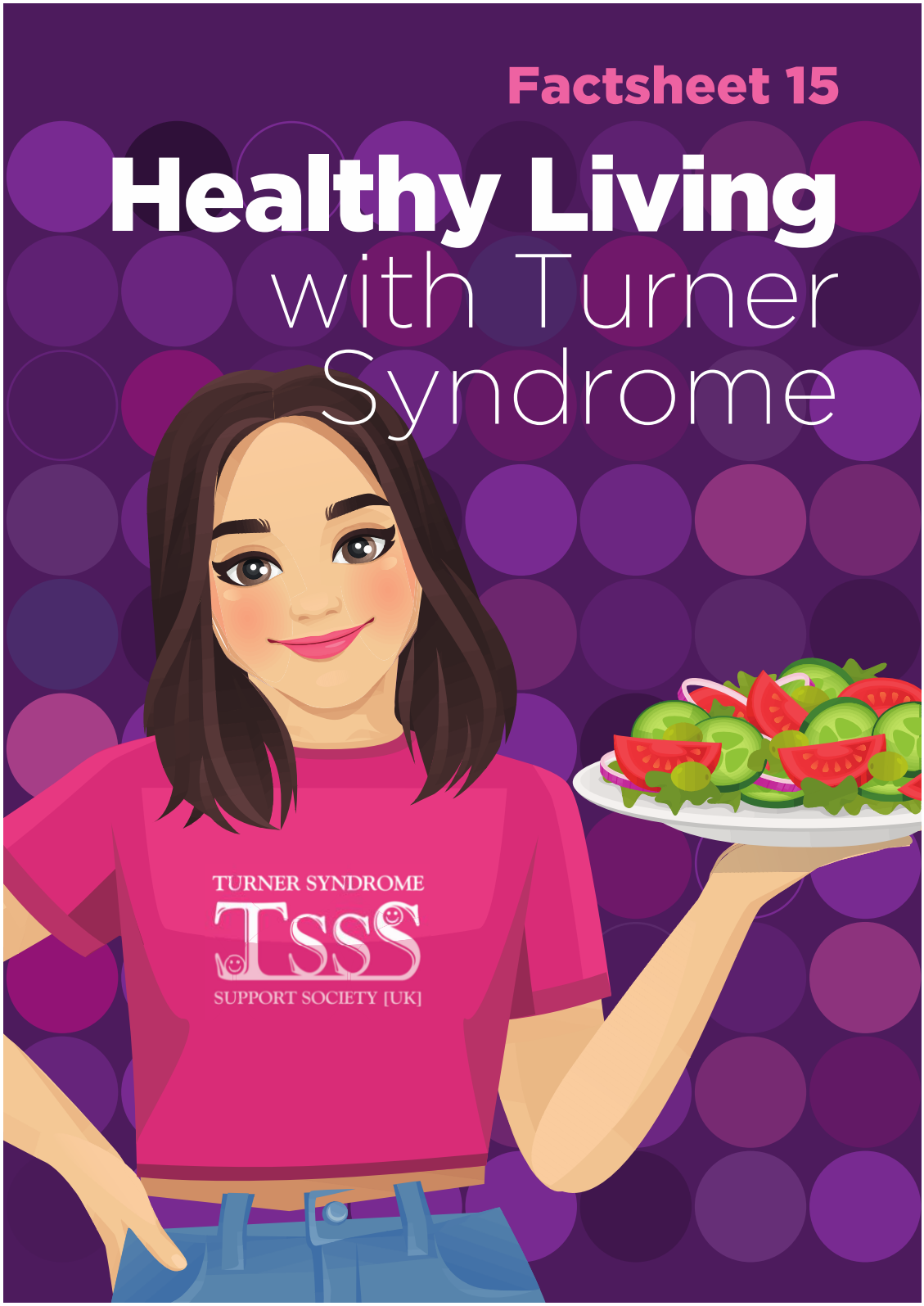


Conclusion

In paediatric patients with Turner Syndrome the use of BP percentile calculators where age, height, and gender are mandatory variables, should be encouraged. If height is not available, estimating it at 50th percentile will identify some (but not all) hypertensive girls with Turner Syndrome.

1.Gravholt CH, Andersen NH, Conway GS, Dekkers OM, Geffner ME, Klein KO, et al. Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting. European Journal of Endocrinology. 2017;177(3):G1-G70.
2.Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll AE, Daniels SR, et al. Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents. Pediatrics. 2017;140(3).

9.7. Appendix 7



Maintaining a healthy weight and eating a balanced diet are important for health and overall wellbeing for everyone. Research shows that an unhealthy diet, low levels of physical activity and increased BMI (Body Mass Index – how heavy we are compared to how tall we are) increase our risk of developing cardiovascular (heart and blood vessel) disease. Women with Turner Syndrome (TS) are more likely to develop a problem with their heart and blood vessels compared with women without TS, so a healthy lifestyle is especially important for girls and ladies with TS.

Acquired Cardiovascular Disease

(heart and blood vessel disease that develops during your lifetime)

Girls and ladies with TS should have regular heart check-ups (See **Factsheet 13: Heart Health in Turner Syndrome**).

Coronary artery disease occurs when fatty material collects in the blood vessels that supply blood to the heart (your coronary arteries). The coronary arteries become narrow, and they cannot carry as much blood to the heart. This means that the heart receives less oxygen, causing symptoms such as chest pain (angina) and breathlessness. A piece of fatty material may break off and cause a blood clot to form and block the coronary artery, cutting off the blood and oxygen supply to the heart. This is known as a heart attack.

There are many risk factors that make it more likely that people will develop coronary artery disease. These include high blood pressure, high cholesterol, being diabetic, your ethnicity, and a family history of heart attacks. There are ways to reduce your risk of coronary heart disease by living a healthy lifestyle, for example, doing plenty of exercise, having a healthy and nutritious diet and refraining from smoking. In this fact sheet we have provided tips to help you lead a healthy lifestyle. We hope you find these useful.

Weight Management

Some girls and ladies with TS struggle to maintain a healthy weight. Our weight increases if we eat food that contains more energy (calories) than the amount of energy we use up during the day.

We recognise that girls and ladies with TS often find it even more difficult to keep a healthy weight than their sisters and friends. This can be extremely frustrating!

We have given some tips on how to lead a healthy lifestyle below, and we hope these are useful. However, losing weight can be difficult, and extra support from healthcare professionals can be helpful if you are finding it hard to make progress.

Physical Activity

What are the benefits of exercise?

Increased fitness fevel
Improves how well your lungs work, increasing stamina and reducing breathlessness.

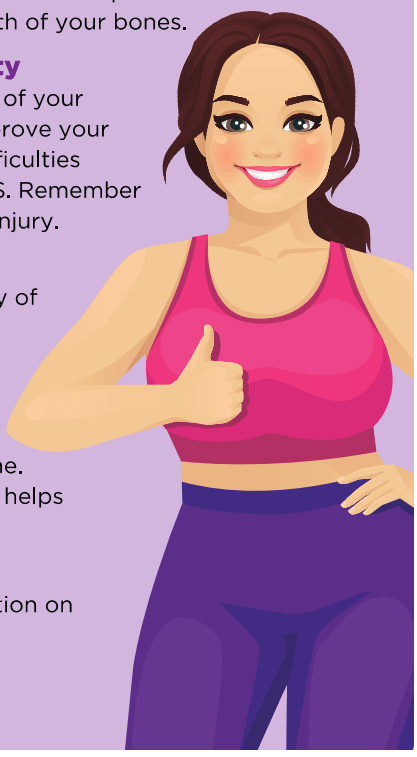
Decreased chance of illness
Boosts your immune system so you are less likely to get coughs, colds and other illnesses.

Increased confidence and self-esteem
Helps you to achieve goals and feel more confident about what you can achieve.

Contributes to maintaining a healthy weight
Helps you to have a healthy appetite and burn energy, helping you to reach or maintain a healthy weight.

Makes you feel good
Reduces levels of stress hormones and increases "feel-good chemicals" (endorphins) in the brain. This can help to reduce stress and improve your mood, helping to improve your overall mental and physical wellbeing.

Improves bone health
Regular weight-bearing exercise, such as jogging, can help to increase your bone strength. This is important because the risk of developing osteoporosis (weak bones) is higher in women with TS. Remember, your hormone replacement therapy (HRT), is also important in protecting the health of your bones.



Improves balance, co-ordination and flexibility
Exercise, especially exercise that includes coordination of your movements such as dancing, can help develop and improve your spatial abilities. Coordination and spatial awareness difficulties (see **Factsheet 5: Spatial awareness**) are common in TS. Remember to exercise in a safe environment to reduce the risk of injury.

Improves sleep
Promotes relaxation, improving the amount and quality of your sleep.

Increases social skills
Taking part in team sports or group exercise is a great way to make new friends. You'll develop greater empathy and improve your social skills at the same time. Physical activity also improves your confidence, which helps in social situations.

Improves concentration and learning
Relieving stress by exercising can help with concentration on important work and when learning new skills.

Frequently asked questions:

How much exercise should I be doing?

Higher levels of weekly physical activity are recommended for individuals who are at an increased risk of developing cardiovascular disease. Ladies with TS fall into this category. National guidelines recommend that adult women in this category should do:

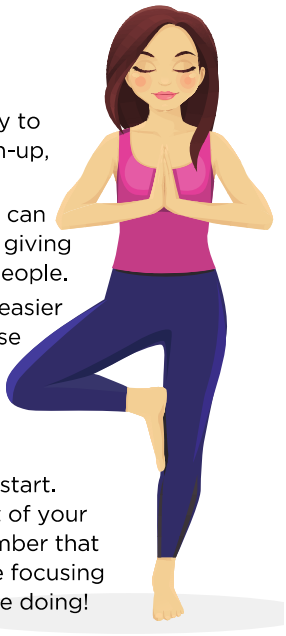
- at least 150 minutes of moderate intensity aerobic activity every week or
- 75 minutes of vigorous intensity aerobic activity or a mix of moderate and vigorous aerobic activity every week

Intensity of aerobic physical activity	Examples
Light	Normal walking, general housework, bowling, stretching
Moderate	Brisk walking, gentle cycling, swimming, low impact dance, swimming
Vigorous	Jogging, skipping, fast cycling, high impact aerobics, playing competitive sports, circuit weight training, heavy housework (e.g., moving furniture)

The guidance for ALL children and young people is as follows:

Age group	Recommended physical activity
Infants <1 year	**Mobile:** interactive floor-based activity, e.g. crawling **Not Mobile:** at least 30 minutes of tummy time each day
1 – 5 years	**Minimum of 180 minutes** of variety of physical activity including indoor/outdoor play each day These 180 minutes should include **60 minutes of moderate – vigorous** intensity activity
5 – 18 years	**Moderate – vigorous** intensity for average of **60 minutes per day across the week** Variety of types & intensities to develop movement skills, muscular fitness, and bone strength **Minimise** sedentary behaviours

How can I start being more active?



Walking is excellent way to keep physically active. It is free and easy to build up. Walking with friends and family can be a nice way to catch-up, whilst probably forgetting that you are being active!

Group exercise such as dance, yoga and Zumba classes are fun and can help to keep you motivated to keep going when you might feel like giving up. They have the additional benefits of meeting new like-minded people.

It is important to remember that physical activity tends to become easier with time. This promotes a rewarding cycle in which you can increase physical activity levels as the activity becomes easier to do.

What if I'm worried about exercising in front of people?

It is normal to feel self-conscious when exercising, especially at the start. Free exercise videos online, which can be completed in the comfort of your home, can help to increase your confidence. It's important to remember that when exercising in a group environment, the people around you are focusing on their own exercise, and probably not thinking about what you are doing!

What if I'm worried about exercising because of my heart?

Always talk to your doctor if you are worried about this. Overall, international guidelines for girls and women with TS recognise the benefits of safe levels exercise on improving heart health and reducing risk of heart disease.

A free cardiac alert card can be posted to you by the TSSS. It might help you to feel safe if you keep this on yourself when exercising.

Our exercise checklist:

60 minutes per day!
Aim for an average of at least 60 minutes of moderate or vigorous intensity physical activity a day. It doesn't matter how 'intense' the exercise is – if your heart rate is increased and you're out of breath – great!

Switch it up!
You should aim to do 2 types of physical activity each week: aerobic exercise and exercise to strengthen your muscles and bones.

Variety
Try a variety of types and intensities to develop movement skills, muscles and bones.

Spread it out!
Break up long periods of not moving with some activity.

Do what you enjoy
Most importantly – find something you enjoy as you will want to exercise and move more, don't force yourself to run if you don't enjoy it – there's lots of other aerobic activities you can try instead!

Dietary Advice

Why is a Healthy Diet important?

Foods and fluid contain essential nutrients which can help prevent infections, reduce inflammation, keep your heart and lungs healthy and keep you feeling as fit and strong as possible!

Support your immune system

Fruit and vegetables have vitamins and minerals that support your immune system. This can help you fight-off infections. You should aim to eat 5 portions of a variety of fruit and vegetables a day. They should make up a bit less than half of your overall diet.

Gives you energy

Starchy carbohydrates, such as potatoes, bread, rice, pasta and breakfast cereals, give your body energy for breathing, exercising and everyday tasks. Choosing high fibre wholegrain varieties will make you feel fuller for longer, help protect your heart and keep your bowels moving. Carbohydrates should make up the same amount of your overall diet as fruit and vegetables.

Strong muscles

Protein, such as lean meats and fish, helps keep your muscles strong. This includes chest muscles which help your ribs expand as you breathe. Protein is also important for your immune system and should make up about 10% of your overall diet.

Strong bones

Dairy foods (milk) **and their alternatives** (soya drinks) are a good source of protein, vitamins, and minerals. This includes calcium and vitamin D which helps keep bones strong and healthy. Lower fat and lower sugar options are better for your health. As with protein, they should make up about 10% of your overall diet.

Fighting infections

Oils contain a range of vitamins, such as vitamin A and E, which are important for fighting infections. Vegetable oils are often lower in saturated fat and a better choice. Oils and spreads should be consumed in small quantities.

How do I know if I'm eating well?

The amount of energy each person needs is different, it depends on height, activity levels, rate of growth and lots of other factors. The energy we need each day for our body to function is called the Basal Metabolic Rate. We measure this energy in calories.

Any nutritious food changes you make, however small, can make your intake healthier.

It is important to create a healthy relationship with food. Focus on enjoyment, healthy eating should not be a chore! Eating a varied and balanced diet provides the body with optimal nutrition and can help you to maintain a healthy weight.

Sleep

Did you know?

About ⅓ of our lives are spent sleeping.

Why is sleep important?

Sleep is essential - it should be considered as important as eating, drinking and breathing. It is a time where the mind and body can rest and recover from the stresses and strains of the day. Research has shown that without adequate sleep the brain cannot function properly, resulting in concentration and memory problems. Sleep has a large impact on our wellbeing.

Research has demonstrated sleep and health to be **linked**. This is true of both physical and mental health. For example, depression and anxiety have been associated with poor sleep.

What is good sleep hygiene?

Good sleep hygiene includes a bedroom environment and daily routine which promotes consistent, uninterrupted sleep. Limiting caffeine, nicotine, alcohol and bright light before bed can help you to sleep better.

Our top tips: :

Be consistent: Aim to go to bed and wake up at the same time each day. This includes weekends!

Calm environment: Try and make your bedroom a relaxing space that is quiet and dark. Room temperature is also super important so make sure it is comfortable for you. Studies suggest that bedroom temperature of around 65°F (18°C) promotes the best night sleep.

Unplug before bed: Aim use screens (mobile phone, laptop, TV) as little as possible for at least one hour before bed. The screen light from devices can disrupt our natural body clock and staying alert on games, phones or watching videos will keep your mind active, keeping your awake.

Limit caffiene and stimulants: Try and limit your caffeine intake at least 6 hours before bed. This includes food and drinks that contain caffeine such as chocolate, coffee, non-herbal teas and fizzy drinks.

Get regular exercise: Research shows that a regular exercise routine can help improve the quality of your sleep. Try to keep being physically active during the daytime to help you fall asleep more easily at night-time. Ideally try to exercise at least 3 hours before your bedtime.

Some good websites:

https://www.nhs.uk/live-well/eat-well/

https://www.nhs.uk/better-health/lose-weight/

Other useful websites are available on the weblinks tab:
https://tss.org.uk/links/useful-websites

This factsheet was written by Miss Lily Jones, Psychologist, Miss Ellie Clarke, Physician Associate, Prof Jo Blair, Paediatric Endocrinologist, Alderhey in Liverpool and reviewed by Dr E Orchard, Cardiologist & Dr H Turner, Endocrinologist, E Weingart, Cardiac Nurse Specialist, Oxford.

Ref: Clinical Practice Guidelines, European Journal of Endocrinology (2017) 177, G1–G70 **www.tss.org.uk**.

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TURNER SYNDROME
TSSS
SUPPORT SOCIETY [UK]

Charity Reg No. 1080507
Scottish Charity No. SC037932

Further information about Turner Syndrome can be obtained from: Arlene Smyth - Executive Officer
Turner Syndrome Support Society (TSSS)
12 Simpson Court • 11 South Avenue
Clydebank Business Park • Clydebank G81 2NR
Helpline **0300 111 7520**
Email **turner.syndrome@tss.org.uk**
Twitter: @**turnerSyndSoc**
Facebook: **www.facebook.com/TSSSUK**

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NIHR | Alder Hey Clinical Research Facility

Alder Hey Children's NHS Foundation Trust

UNIVERSITY OF LIVERPOOL

Measuring blood pressure in children and adolescents: 20 years of change

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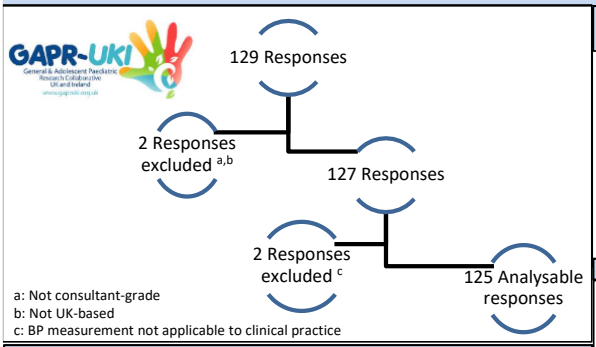
Aim

- In 1997 a survey identified a general lack of standardisation of blood pressure (BP) measurement techniques and little consensus on the criteria for diagnosing hypertension amongst paediatricians. (1)
- We have conducted a new online survey in 2021, to compare clinical practice between the two time periods.



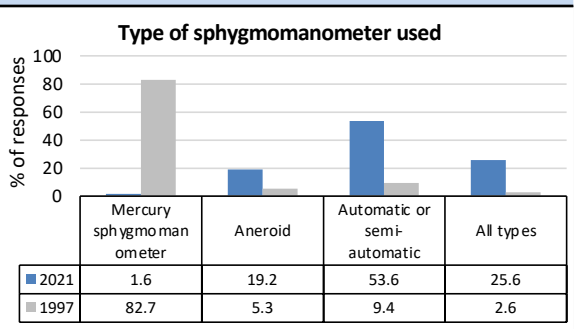
a: Not consultant-grade
b: Not UK-based
c: BP measurement not applicable to clinical practice

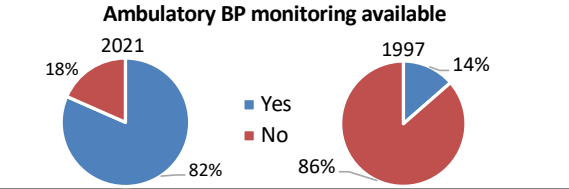
Methods

A national descriptive survey was approved by the General and Adolescent Paediatric Research in the United Kingdom & Ireland (GAPR-UKI) committee and then circulated to consultant-grade general paediatricians.

Results

	2021 survey, n (%)	1997 survey, n (%)
(i) Age when routine BP measurement performed in an outpatient		
From birth	13 (10.4)	118 (17.7)
From 1 years	0 (0.0)	82 (12.3)
From 3 years	2 (1.6)	153 (20.0)
From 7 years	1 (0.8)	80 (12.0)
From 13 years	0 (0.0)	23 (3.5)
Never	-	209 (31.4)
Other	3 (2.4)	-
At any age if clinically indicated	106 (84.8)	-
(i) Posture for BP measurement in outpatient clinic		
Seated	87 (69.6)	407 (60.0)
Supine	8 (6.4)	124 (18.3)
No preference to position	20 (16.0)	147 (21.7)
Both seated and supine in the same patient	10 (8.0)	-
(i) Diastolic end point measurement in outpatient clinic		
Phase 4 (muffling of sounds)	16 (18.0)	348 (52.1)
Phase 5 (disappearance)	71 (79.8)	213 (31.9)
Both phase 4 and 5	2 (2.2)	106 (15.9)
No reply / Not applicable	36 (-)	16 (-)
(i) Who measures BP		
Paediatrician	19 (15.2)	358 (59.4)
Clinic nurse	62 (49.6)	54 (9.0)
Either	44 (35.2)	190 (31.6)
(i) Reporting hypertension		
Systolic alone	26 (20.8)	105 (17.9)
Diastolic alone	0 (0.0)	79 (13.5)
Both systolic and diastolic	56 (44.8)	16 (2.7)
Either systolic or diastolic	43 (34.4)	386 (65.9)
(i) BP centile to diagnose hypertension		
90th	12 (9.6)	76 (12.9)
95th	85 (68.0)	246 (41.8)
99th	23 (18.4)	-
Other	5 (4.0)	-
>95th	-	267 (45.3)





Conclusion

There have been important changes in the assessment of BP in children and adolescents, including increased nurse involvement and greater use of technology. However, fewer paediatricians are responding to high diastolic pressures than twenty years ago. The clinical implications of this change, which goes against international guidance, (2) deserves consideration.

1. Lip GY, Beevers M, Beevers DG, Dillon MJ. The measurement of blood pressure and the detection of hypertension in children and adolescents. J Hum Hypertens. 2001;15(6):419-23.
2. Lurbe E, Agabiti-Rosei E, Cruickshank JK, Dominiczak A, Erdine S, Hirth A, et al. 2016 European Society of Hypertension guidelines for the management of high blood pressure in children and adolescents. J Hypertens. 2016;34(10):1887-920.