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ORIGINAL ARTICLE

Low myocardial glucose uptake in Turner syndrome is unaffected by growth hormone: a randomized, placebo-controlled FDG-PET study

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Summary

Background An unfavourable cardiovascular and metabolic phenotype causes threefold excess mortality in Turner syndrome (TS), and perturbed cardiac substrate metabolism is increasingly recognized as a common component of cardiovascular and metabolic diseases. We therefore hypothesized that myocardial glucose uptake (MGU) is reduced in TS and that growth hormone (GH) treatment improves MGU. To this end, this controlled trial elucidates MGU in TS and the impact of 6 months of growth hormone treatment on MGU.

Methods and Results Women with TS ($n = 9$) were examined at baseline, sequentially treated with either Norditropin® SimpleXx or placebo and re-examined after 6 months. MGU and myocardial blood flow (MBF) were measured using 2-deoxy-2-[18F]fluoro-D-glucose positron emission tomography (FDG-PET) during a hyperinsulinaemic euglycaemic clamp (at baseline and 6 months). Blood pressure measurement, blood sampling, echocardiography and dual energy X-ray absorptiometry scan were also performed. Age-matched female controls ($n = 9$) were examined once. Baseline MGU was reduced in TS (0·24 ± 0·08 vs. 0·36 ± 0·13 μmol/g/min in controls; $P = 0·036$) despite similar insulin sensitivity (whole body glucose uptake (M-value): 9·69 ± 1·86 vs. 9·86 ± 2·58 mg/(min*kg) in controls; $P = 0·9$). Six months of GH carried no impact on MGU (0·25 ± 0·08 vs. 0·26 ± 0·12 μmol/g/min in the placebo group; $P = 0·8$). Plasma glucose, low-density cholesterol and triglycerides increased, while M-value and exercise capacity decreased during 6 months of GH treatment.

Conclusion MGU is reduced in TS despite normal insulin sensitivity. GH treatment does not alter MGU despite decreased whole body insulin sensitivity. A perturbed cardiac glucose uptake appears to be a feature of TS.

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Introduction

Women with Turner syndrome (karyotype 45,X) characteristically display growth retardation with reduced final height and hypogonadism with infertility requiring hormone replacement therapy. The syndrome is accompanied by an unfavourable cardiovascular and metabolic phenotype inflicting a threefold excess mortality in Turner syndrome (TS).[1] Interestingly, myocardial substrate metabolism influences cardiac function in general and the response of the heart to ischaemia in particular.[2,3] However, no previous study has assessed myocardial substrate metabolism in TS and its relation to the metabolic profile. The metabolic profile seen in TS could, in part, be explained by a disturbance of the growth hormone (GH) Insulin-like-growth factor-1 axis with low free insulin-like-growth factor-1 and decreased insulin-like-growth factor binding protein-3.[4] Hence, the question arises whether the GH salutary effects on body composition, with a decrease in body fat and an increase in muscle mass,[5] outweigh the insulin resistance effect of GH treatment with the potential to improve myocardial substrate metabolism. Studies in young adults with TS following cessation of GH therapy indicate a beneficial effect of GH on diastolic blood pressure[6] and lipid profile,[7,8] whereas results concerning insulin sensitivity are equivocal.[7–9] No study has evaluated the metabolic effects of GH treatment in TS after menarche on myocardial glucose uptake (MGU). Notably, adults with GH deficiency unrelated to TS also suffer from an increased incidence of cardiovascular disease,[10] and GH treatment improves low-density lipids, diastolic blood pressure and intima media thickness,[11] with seemingly positive impact on left ventricular mass,[12] but variable results concerning the metabolic syndrome[11]. We therefore hypothesized MGU to be perturbed in TS in adulthood with GH treatment positively influencing the myocardial metabolism as assessed with

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two-deoxy-2-[18F]fluoro-D-glucose positron emission tomography (FDG-PET) before and after 6 months of GH treatment.

Subjects and methods

Subjects

Eleven women with karyotypically verified TS were recruited through the Danish National Society of Turner Syndrome Contact Group and an endocrine outpatient clinic. Two women were excluded due to incomplete FDG-PET studies, leaving nine participants with TS (aged 31·0 ± 6·51 years. (Additional information in Table S1). These women were examined at baseline followed by sequential randomization to Norditropin® SimpleXx or placebo with re-examination after 6 months. Thirteen healthy age-matched women were recruited by advertisement to serve as baseline controls. FDG-PET failed in three and one dropped out of the study, leaving nine healthy controls (aged 30·4 ± 7·73 years). Exclusion criteria included as follows: BMI >35 kg/m^2, untreated thyroid disorders, significant concurrent acute or chronic disease, hypertension, symptom-giving cardiac disorders and/or glucocorticoid treatment. Screening for diabetes was performed using fasting glucose and haemoglobin A1C. All participants underwent echocardiography to confirm normal left ventricular function and the absence of structural heart disease (aortic coarctation, bicuspid aortic valve and thoracic aortic dilation). None of the controls, but all participants with TS received hormone replacement therapy (17β-oestradiol or oestradiol valerate in combination with a gestagen) at study enrolment and all but one had previously received GH treatment (Table S1).

All participants provided informed consent. The study was conducted according to the Good Clinical Practice guidelines and the Declaration of Helsinki with regulatory approval form Danish Health and Medicines Authority as well as the local ethics committee and registered at ClinicalTrials.gov (NCT00420654).

Study design

Following baseline examination, participants with TS were randomised to placebo ($n = 4$) or Norditropin® SimpleXx ($n = 5$) for a 6-month period in a double-blind set-up starting with Norditropin 0·5 mg per day, which was increased fortnightly to a target dose of 1·25 mg per day irrespective of insulin-like-growth factor-1 levels (Figure S1). We chose this dose as an intermediate dose between the supraphysiologic dose normally given to adolescent TS for growth promotion, and the lower dose given to GH-deficient women.

Power calculations

No previous publication has addressed the issue of MGU in Turner syndrome, and hence, power calculation could not be performed. Power calculation for change in lean muscle mass was used to determine sample size. The criteria used were as follows: level of significance=0·05, risk of type-II error=0·20 and change in lean muscle mass (LBM) =4 ± 2·8 kg. Calculations were carried out assuming an unpaired comparison. The resulting minimal sample size was eight.

Myocardial glucose uptake and perfusion

The MGU was quantified by fitting tissue and blood-pool (from arterialized blood samples) time-activity curves to a three-compartment model for 18F-FDG. We used iFit (www.liver.DK/ifit.html) for kinetic modelling. The lumped constant is the conversion factor between the net uptakes of 18F-FDG and glucose, which depends on the uni-directional clearances and the phosphorylation rates. In this study, we used a variable lumped constant, calculated individually, as the lumped constant is not a true constant, but may vary depending on the metabolic environment of the heart.[13] The rate of MGU was obtained by multiplying K* (the net glucose uptake rate of FDG) by the plasma glucose concentration (glc)p divided by the lumped constant: MGU= K* x (glc)p/LC. Myocardial blood flow was quantified by PET in 2D mode (model ECAT EXACT HR47, Siemens Medical, Knoxville, USA) using intravenous ^{13}N-ammonia as perfusion tracer. An attenuation correction scan (20 min) was performed. For each perfusion scan, 740 mBq of ^{13}N-ammonia diluted in 10 ml saline was injected over 30 s. At the time of injection, acquisition of a dynamic sequence of images (12 frames per 10 s) was commenced to obtain time-activity curves from the blood pool and from the myocardium. The method has been described in details previously.[14] Myocardial blood flow (MBF) was calculated by nonlinear fit of the first 120 s of tissue and blood-pool time-activity curves to a standard one-tissue compartment model. Omitting metabolically trapped ^{13}N-ammonia, this model has previously been shown to give good estimates of myocardial blood flow.[15] The input function measured in left ventricular blood pool was corrected for spillover of activity from the myocardium to the left ventricular blood pool using the following equation: MBFkorr = (K1*1/(1−Vp))/1·04. MBF is reported as absolute values (ml/g/min).

Skeletal glucose uptake

Skeletal glucose uptake was quantified in the brachioradialis muscle contralateral to the tracer injection site. The last six frames were summed to acquire high-resolution images. ROIs were drawn in approximately 10 planes to obtain better statistics and minimize noise. Images were not resliced. A lumped constant value of 1·2 was used to derive SGU as follows: SGU= K* x (glc)p/LC. Skeletal glucose uptake was quantified by fitting tissue and blood pool (from arterial blood samples) and time-activity curves to a three-compartment model. We used iFit (www.liver.DK/ifit.html) for kinetic modelling. All images were reconstructed with filtered back projection.

Hyperinsulinaemic euglycaemic clamp

Insulin (Actrapid®, Novo Nordisk, Bagsværd, Denmark) was infused at 1·0 mU/kg/min. Plasma glucose was clamped at 5 mmol/l by adjusting the infusion rate of 20% glucose according

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to the plasma glucose measurements every 5 min. Steady state was maintained for the duration of the PET scan. Whole body glucose uptake (M-value) was calculated during the time frame 125–155 min. The FDG-PET scan was initiated after 155 min of insulin infusion (Figure S2).

Blood samples, cardiovascular and metabolic indices

Plasma glucose was measured in duplicate immediately after sampling on a Beckman Glucoanalyser (Beckman Instruments, Palo Alto, CA, USA). Serum samples were frozen immediately and stored at −80 °C. Insulin was analysed using a commercial time-resolved immunofluorometric assay (AutoDELFIA Insulin kit, PerkinElmer, Turku, Finland) with coefficient of variation (CV) below 4%. Serum NEFAs were analysed by a commercial kit (Wako Chemicals, Neuss, Germany) with CV<6%. Plasma lipids and triglycerides were measured using an automated commercially available system (Aeroset, Abbott Diagnostics, North Chicago, IL, USA). Serum IGF-I and serum insulin-like growth factor binding protein-3 (IGFBP-3) was measured in-house with an IDS-iSYS Multi-Discipline Automated Analyzer (Immunodiagnostic Systems Ltd., Boldon, England) with CV<7% for both and a limit of detection of 4·4 and 50 ng/ml, respectively. Total body fat, leg fat, fat percentage and fat-free mass were measured by DEXA (QDR-2000, Hologic, Bedford, MA, USA). Ambulatory blood pressures (SpaceLabs 91207, Washington, USA) were recorded over 24 h with oscillometric measurement every 20 min. The rate pressure product was calculated as the heart rate [beats per minute] x the systolic blood pressure [mmHg]. A 6-min submaximal exercise test was performed on a bicycle ergometer (Monark Ergometric 829 E, Monark exercise AB, Varberg, Sweden) using a workload of 300–1200 kpm/min yielding VO2-max [ml O2/min * kg].[16]

Statistical analysis

Statistical computations were performed using Stata Statistical Software: Release 12.1 (StataCorp LP, College Station, TX, USA). Normality was assessed by QQ plots of absolute or log-transformed values. Baseline comparisons between the pooled TS group and controls were performed using a two-tailed Student's independent t-test (given as mean ± standard deviation (SD) or if for transformed values as median with range) or Mann–Whitney U-test (median with range) as appropriate. Paired data were analysed by Students paired t-test if assumptions were met as assessed by Bland–Altman plot, QQ-plot, and plotting of the postvalues against the prevalues and if assumptions were not met by the Wilcoxon-signed rank test.

Results

Baseline: Turner syndrome versus controls

The MGU was lower in TS than in control subjects (0·24 ± 0·08 vs. 0·36 ± 0·13 μmol/g/min; $P = 0·036$) (Fig. 1). Adjusting the MGU for the rate pressure product (0·24 [0·17–0·47] vs. 0·45 [0·30–0·94] μmol/g/mmHg; $P = 0·01$) and the myocardial blood flow (0·23 ± 0·08 vs. 0·39 ± 0·14 μmol/ml/mmHg; $P = 0·01$) did not change this finding (Table 1). Whole body glucose uptake normalized to body weight (M-value: 9·69 ± 1·86 vs. 9·86 ± 2·58 mg/(min*kg); $P = 0·9$) and lean muscle mass (15·4 ± 2·99 vs 14·5 ± 3·10 mg/(min*kg); $P = 0·6$) were similar in TS and controls. BMI, body fat, lean body mass and metabolic parameters were also comparable between TS and controls (Table 2), while the rate pressure product (8034 vs. 6270 mmHg/min; $P = 0·03$) and night-time diastolic blood pressure ($P = 0·03$) were higher in TS. Skeletal muscle glucose uptake was similar in the two groups, as were free fatty acids and serum insulin during the clamp (Figure S3).

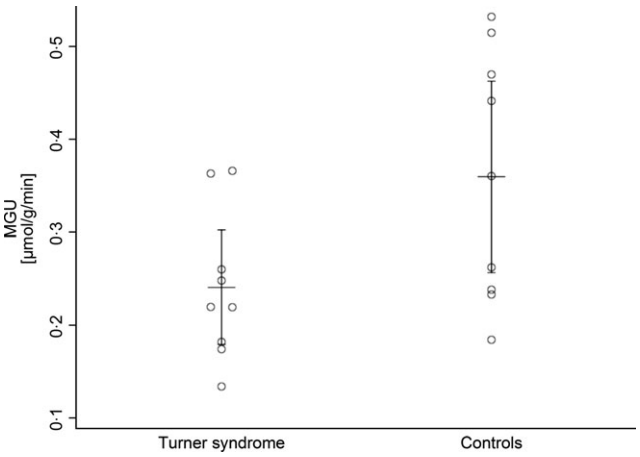

Fig. 1 Depicts myocardial glucose uptake (MGU) in Turner syndrome vs. controls (0·24 ± 0·08 vs. 0·36 ± 0·13 [μmol/ml/mmHg]; $P = 0·036$) at baseline. Horizontal lines are means. Confidence intervals are represented as spikes.

Impact of growth hormone treatment – Baseline compared to 6-month follow-up

There was no difference in MGU, MBF or skeletal muscle glucose uptake between participants treated with GH and placebo (Table S2). During GH treatment, insulin-like growth factor 1 (186 ± 109 vs. 303 ± 122; $P = 0·006$), plasma glucose (4·79 ± 0·50 vs 5·02 ± 0·58; P-value=0·03), low-density cholesterol (2·56 ± 0·63 vs. 2·96 ± 0·56; $P = 0·02$) and triglycerides (0·80 [0·60–1·00] vs. 1·00 [0·70–1·30]; $P = 0·04$) increased, while the M-value (9·86 [8·57–10·84] vs. 7·83 [1·48–8·88]; $P = 0·04$) and exercise capacity (49·9 ± 12·3 vs. 41·9 ± 9·46; $P = 0·01$) decreased. Levels of MGU (0·25 ± 0·08 vs. 0·26 ± 0·12 μmol/g/min; $P = 0·8$) (Fig. 2a and Table 3), skeletal muscle glucose uptake (0·097 ± 0·033 vs. 0·078 ± 0·070 μmol/g/min]; $P = 0·6$) or MBF (0·77 ± 0·10 vs. 0·72 ± 0·14 ml/g/min; $P = 0·2$) (Fig. 2b) did not change, and normalization to MBF and/or the rate pressure product did not alter these findings (Fig. 2c).

Discussion

The MGU was reduced in TS when compared with healthy age-matched female controls despite comparable insulin sensitivity.

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Table 1. Baseline FDG-PET and Clamp data on the pooled Turner syndrome group versus controls

	TS (n = 9)	Controls (n = 9)	P-value
Myocardial glucose uptake (μmol/g/min)	0·24 ± 0·08	0·36 ± 0·13	**0·036**
Myocardial glucose uptake/Rate pressure product (μmol/g/mmHg)	0·24 (0·17–0·47)	0·45 (0·30–0·94)	**0·01**
Skeletal muscle glucose uptake (μmol/g/min)	0·092 ± 0·029	0·087 ± 0·044	0·8
Myocardial blood flow (ml/g/min)	0·81 (0·64–1·66)	0·56 (0·46–0·89)	**0·04**
Myocardial blood flow/Rate pressure product (ml/g/mmHg)	1·01 (0·68–2·16)	0·88 (0·79–1·13)	0·2
Myocardial glucose uptake/(Rate pressure product corrected Myocardial blood flow) (μmol/ml/mmHg)	0·23 ± 0·08	0·39 ± 0·14	**0·01**
Free fatty acids (μmol/l)	253 ± 124	227 ± 110	0·6
Insulin (μmol/l)	333 ± 53·8	335 ± 60·1	1·0
M-value (mg/(min*kg))	9·69 ± 1·86	9·86 ± 2·58	0·9
Glucose infusion rate/Lean muscle mass (mg/(min*kg))	15·4 ± 2·99	14·5 ± 3·10	0·6
Rate pressure product (mmHg/min)	8034 (5922–10264)	6270 (5463–9891)	**0·03**

Free fatty acids and insulin were measured and averaged during the steady state period of the clamp at baseline. P-values are obtained by Students t-test.
P-values < 0.05 are given in bold.

Table 2. Baseline descriptives on the pooled Turner syndrome group versus controls

	TS (n = 9)	Controls (n = 9)	P-value
Age (years)	31·0 ± 6·51	30·4 ± 7·73	0·8
Weight (kg)	55·0 ± 8·61	66·0 ± 6·25	**0·007**
Height (cm)	152·3 ± 5·40	166·4 ± 4·88	**<0·0001**
BMI (kg/m^2)	23·7 ± 3·71	24·0 ± 3·35	0·9
Lean muscle mass (kg)	34·4 ± 39·8	44·3 ± 22·1	**<0·0001**
Lean muscle mass (%)	65·0 ± 7·50	69·0 ± 7·74	0·3
Fat mass (kg)	17·3 ± 64·1	18·2 ± 67·8	0·8
Fat mass (%)	31·5 ± 8·05	27·4 ± 8·11	0·3
Daytime systolic blood pressure (mmHg)	114·0 (100–154)	118·0 (107–135)	1·0†
Daytime diastolic blood pressure (mmHg)	78·0 (63–104)	74·0 (60–91)	0·4
Night-time systolic blood pressure (mmHg)	99·0 (83–137)	99·0 (87–113)	0·6
Night-time diastolic blood pressure (mmHg)	65·0 (53–91)	53·0 (52–75)	**0·03†**
Insulin baseline (pmol/l)	39·0 ± 27·7	24·4 ± 8·55	0·2
Low-density lipids (mmol/l)	2·62 ± 0·58	2·30 ± 0·49	0·2
High-density lipids (mmol/l)	1·62 ± 0·26	1·44 ± 0·31	0·2
Triglycerides (mmol/l)	0·81 ± 0·27	0·68 ± 0·20	0·3
Glucose (mmol/l)	5·05 (3·9–5·7)	4·90 (4·6–5·6)	0·5†
Insulin-like growth factor 1 (μg/l)	177 ± 80·6	204 ± 65·3	0·5
Insulin-like growth factor binding protein 3 (ng/ml)	3655 ± 824	3963 ± 474	0·4
Thyroid-stimulating hormone (10^{-3} IE/l)	3·02 ± 1·75	2·15 ± 0·56	0·2

P-values are obtained by Students T-test unless otherwise stated. †Mann–Whitney U-test.
P-values < 0.05 are given in bold.

Treatment with GH during the short term left MGU unchanged despite significant and previously well-described metabolic changes. These are novel and intriguing findings because they suggest the presence of a fixed, raised cardiac insulin resistance in TS that may represent a previously unrecognized cardiovascular trait.

An in-depth understanding of the cardiovascular system is a crucial step towards an improved disease prophylaxis in TS where cardiovascular mortality is increased due to both congenital and acquired cardiovascular disease. In other cohorts, a low MGU has been linked with ischaemic heart disease,[2] but not with hypertension or left ventricular hypertrophy, which are general features even in young women with TS.[17,18] In animal studies, an increase in MGU protects the myocardium against ischaemic injury and attenuates postischaemic dysfunction.[19] Hence, the reduced MGU in TS may indicate an attenuated defence mechanism against ischaemia and may suggest that myocardial insulin resistance may play a role in the well-documented increased morbidity and mortality due to premature ischaemic heart disease in these women.[20] The reduced MGU in TS could also indicate a shift in the normal balance between fatty acids and glucose utilization for energy production, with

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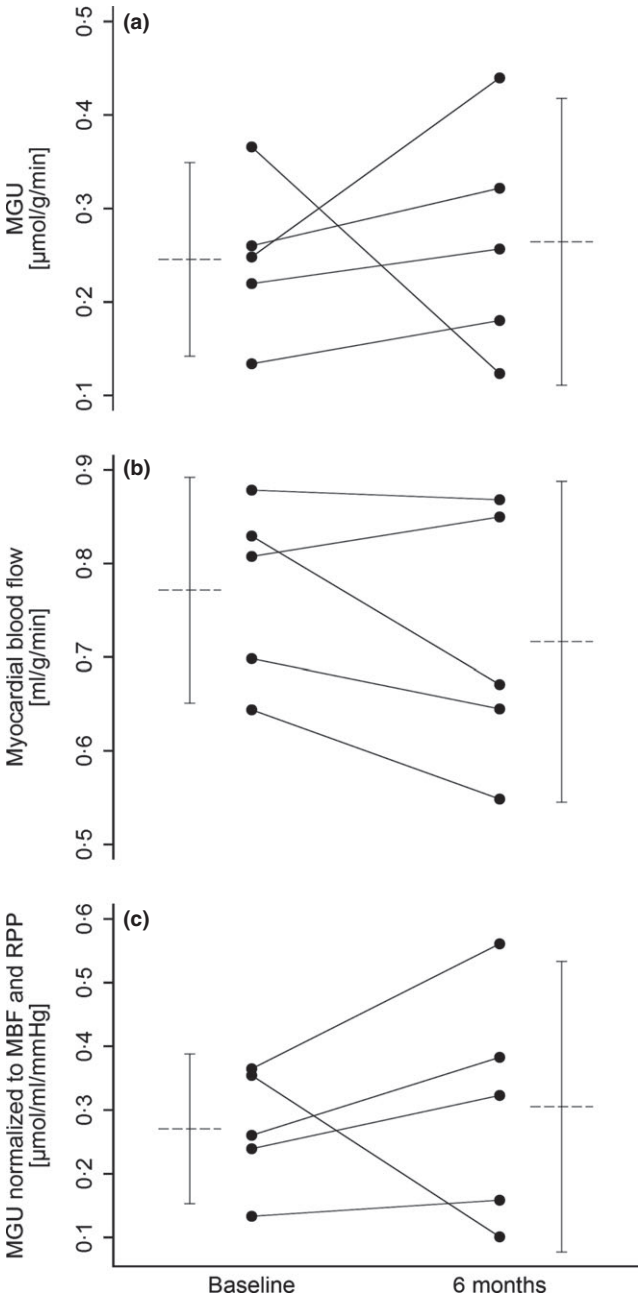

Fig. 2 Depicts changes in the Turner subgroup before and after 6 months of growth hormone treatment. Dotted lines are means. Confidence intervals are represented as spikes. (a) Myocardial glucose uptake (MGU) (0·25 ± 0·08 vs. 0·26 ± 0·12 ml/g/min; $P = 0·8$). (b) Myocardial blood flow (0·77 ± 0·10 vs. 0·72 ± 0·14 ml/g/min; $P = 0·2$). (c) Myocardial glucose uptake normalized to the myocardial blood flow (MBF) and the rate pressure product (RPP) (0·27 ± 0·09 vs. 0·31 ± 0·18 µmol/ml/mmHg; $P = 0·7$).

such changes generally seen in women as well as in insulin deficiency, manifest diabetes and obesity.[3] Intriguingly, neither of these factors was present within the studied young women, consistent with a congenital myocardial anomaly or the presence of an unidentified acquired disease mechanism.

In addition to cardiovascular disease advancing age may also affect MGU. Accelerated ageing is often proposed as a driver of cardiovascular risk in TS. However, with advancing age an increase in glucose and a decrease in fatty acid oxidation would be expected, not explaining the decreased MGU found in the present study.[3]

Six months of GH treatment had no effect on MGU despite evidence of an increased whole body insulin resistance with declining M-values alongside increasing low-density lipids and triglycerides. The lower MGU and possibly increased cardiac metabolism of fatty acids may impair the metabolic profile and increase free radical formation with resulting apoptosis induced by lipo-toxicity. Animal models have linked lipo-toxicity with systolic and diastolic left ventricular dysfunction;[21] a finding that is well aligned with a recent study in type 2 diabetics[22] and echocardiographic findings in TS.[23] However, the present study cohort did not show evidence of such findings despite a low MGU. This could be due to a slow progression towards left ventricular dysfunction which cannot yet be visualized by echocardiography. A prospective re-evaluation at a later age in combination with an evaluation of the fatty acid cardiac metabolism would shed important light on this issue.

Previous studies indicate that changes in MGU may develop in the absence of whole body insulin resistance as a consequence of β-adrenergic stimulation. *In vitro* studies show that acute β-adrenergic stimulation increases MGU, whereas chronic stimulation decreases MGU.[24] A perturbed sympatho-vagal balance is present in TS, and speculatively, this feature could to some extent explain the documented lower MGU.[25] During the 6 months of GH treatment, MGU rose in all but one TS woman. We scrutinized the results and found that this one individual had 45,X monosomy and was diagnosed at age 15. She was treated with growth hormone from diagnosis until age 17 and was 29 years of age at study baseline. No apparent source of the variation could be identified, but interestingly, the same degree of variation has been observed in GH trials on healthy young men.[26]

Contrary to our findings, previous studies, have described a beneficial effect of GH on diastolic blood pressure[6] and lipid profile in a younger age group.[7,8] Our present study is small and therefore may lack power in this context. Furthermore, we cannot rule out that an increase in triglycerides and low-density lipids may not be counterbalanced by the increase in high-density lipids. However, available evidence suggests that treatment with supraphysiologic doses of GH for years during childhood and adolescence does not seem to adversely affect the entire cardiovascular system in TS, although long-term data are still dearly needed.[27]

Insulin sensitivity was similar in TS and controls, as previously indicated in well-matched groups of TS and controls.[28] This is contrary to older studies in which insulin resistance was a consistent finding that was probably due to poor matching of TS and controls on body composition.[29] However, we documented an isolated myocardial insulin resistance in TS. Interestingly, glucose transporter proteins (GLUT-1 and GLUT-4) are responsible for glucose uptake in both skeletal muscle and myocardium, but glucose metabolism in the two tissues differ in two aspects that may at least in part explain the isolated myocardial

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Table 3. Descriptives for the Turner syndrome subgroup treated with growth hormone and comparison at baseline and 6-month follow-up

	Baseline (n = 5)	Follow-up (n = 5)	P-value
Weight (kg)	56·4 (39·8–63·9)	56·1 (41·1–66·4)	0·08†
BMI (kg/m^2)	23·3 (17·2–27·8)	23·2 (17·8–28·8)	0·08†
Lean muscle mass (kg)	35·7 (29·1–38·9)	37·1 (32·5–37·8)	0·1†
Lean muscle mass (%)	67·0 ± 9·58	67·6 ± 10·6	0·7
Fat mass (kg)	14·0 (8·16–25·3)	15·7 (6·13–26·6	0·4†
Fat mass (%)	25·8 (19·6–40·9)	28·4 (15·2–40·7)	1·0†
24-h systolic BP (mmHg)	109·0 ± 7·97	107·6 ± 8·56	0·4
24-h diastolic BP (mmHg)	76·0 (60–89)	73·0 (61–77)	0·2†
Insulin (pmol/l)	34·6 ± 33·5	50·8 ± 28·8	0·07
Low-density lipids (mmol/l)	2·56 ± 0·63	2·96 ± 0·56	**0·02**
High-density lipids (mmol/l)	1·56 ± 0·29	1·74 ± 0·44	0·3
Triglycerides (mmol/l)	0·80 (0·60–1·00)	1·00 (0·70–1·30)	**0·04**†
Glucose (mmol/l)	4·79 ± 0·50	5·02 ± 0·58	**0·03**
Thyroid-stimulating hormone (10^{-3} IE/l)	1·76 (1·01–2·39)	2·68 (0·85–3·67)	0·08†
Insulin-like growth factor 1 (μg/l)	186 ± 109	303 ± 122	**0·006**
Insulin-like growth factor binding protein 3 (ng/ml)	3645 ± 1043	4770 ± 1009	**0·02**
VO2-max (ml O2/min * kg)	49·9 ± 12·3	41·9 ± 9·46	**0·01**
Myocardial glucose uptake (μmol/g/min)	0·25 ± 0·08	0·26 ± 0·12	0·8
Myocardial glucose uptake/Rate pressure product corrected (μmol/g/mmHg)	0·30 ± 0·13	0·36 ± 0·19	0·6
Skeletal muscle glucose uptake (μmol/g/min)	0·097 ± 0·03	0·077 ± 0·07	0·6
Myocardial blood flow (ml/g/min)	0·77 ± 0·10	0·72 ± 0·14	0·2
Myocardial blood flow/(Rate pressure product corrected (ml/g/mmHg)	0·93 ± 0·17	0·96 ± 0·23	0·7
Myocardial glucose uptake/(Rate pressure product corrected myocardial blood flow (μmol/ml/mmHg)	0·27 ± 0·09	0·31 ± 0·18	0·7
Rate pressure product (mmHg/min)	8511 ± 1713	7662 ± 1643	0·1
Free fatty acids during clamp (μmol/l)	212 ± 100	210 ± 57	0·9
Insulin during clamp (μmol/l)	323 ± 89·1	325 ± 62·2	1·0
M-value	9·86 (8·57–10·84)	7·83 (1·48–8·88)	**0·04**†
Glucose infusion rate/Lean muscle mass (mg/(min*kg))	14·8 (13·9–17·6)	11·1 (2·02–15·7)	**0·04**†

P-values are obtained by Students paired t-test unless otherwise stated. †Wilcoxon-signed rank test.
P-values < 0.05 are given in bold.

insulin resistance seen in TS. Firstly, glycogen storages increase in the heart and decrease in skeletal muscle in the fasting state or diabetes. Secondly, insulin-stimulated glucose uptake is at least 10-fold higher in myocardium than skeletal muscle.[30] The impaired MGU in TS supports previous studies, which have similarly demonstrated that myocardial insulin resistance is not an inherent consequence of increased whole body insulin resistance as induced by GH[26] or diabetes.[31] It is therefore highly plausible that the reduced MGU in TS represent an intrinsic abnormality of the cardiac myocytes. However, we cannot exclude that myocardial insulin resistance merely precedes whole body insulin resistance as this is a possible scenario with the risk of type 2 diabetes being quadrupled in TS.[32]

The second part of the study assessed if myocardial insulin resistance is a part of the general metabolic phenotype in TS and if GH treatment could improve MGU with the potential to alter the cardiovascular outcomes. In healthy males, short-term GH treatment previously did not alter myocardial insulin resistance despite an increased whole body insulin resistance.[26] The salutary effects of GH on body composition could outweigh the anti-insulin effect, which could in turn increase MGU. However, MGU remained stable during 6 months of GH treatment, contrary to whole body insulin resistance, which increased as indicated by a significantly lower M-value. This finding is supported by a previous study assessing the M-value in adolescents with TS during GH treatment, but contrary to other studies which used inferior indices of insulin sensitivity like fasting glucose, insulin and standard oral glucose tolerance test.[5,9]

Limitations

The small sample size needs to be taken into consideration when considering the study implications. Also, longer duration of GH treatment could have revealed a difference between the placebo and GH-treated group due to changes in body composition and exercise capacity secondarily affecting the MGU. The included cohort had no structural heart disease, and the findings may not be valid for the TS subgroup with congenital cardiovascular anomalies. We cannot rule out that oestrogen replacement therapy may have influenced the results, although oestrogen has been shown only to increase myocardial fatty acid utilization and not MGU.[33]

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Conclusion

The MGU is reduced in TS despite normal insulin sensitivity, and treatment with GH leaves MGU unchanged despite increasing whole body insulin resistance. Cardiac insulin resistance may be a feature of TS.

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