Skeletal muscle microvascular function in girls with Turner syndrome

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Background: Exercise intolerance is prevalent in individuals with Turner Syndrome (TS). We recently demonstrated that girls with TS have normal aerobic but altered skeletal muscle anaerobic metabolism compared to healthy controls (HC). The purpose of this study was to compare peripheral skeletal muscle microvascular function in girls with TS to HC after exercise. We hypothesized that girls with TS would have similar muscle blood-oxygen level-dependent (BOLD) magnetic resonance imaging (MRI) signal responses during recovery from exercise compared to HC.

Methods: Thirteen TS participants and 8 HC completed testing. BOLD MRI was used to measure skeletal muscle microvascular response during 60 second recovery, following 60 s of exercise at 65% of maximal workload. Exercise and recovery were repeated four times, and the BOLD signal time course was fit to a four-parameter sigmoid function.

Results: Participants were 13.7 ± 3.1 years old and weighed 47.9 ± 14.6 kg. The mean change in BOLD signal intensity following exercise at the end of recovery, the mean response time of the function/the washout of deoxyhemoglobin, and the mean half-time of recovery were similar between the TS and HC groups.

Conclusions: Our results demonstrate that compared to HC, peripheral skeletal muscle microvascular function following exercise in girls with TS is not impaired. General significance: This study supports the idea that the aerobic energy pathway is not impaired in children with TS in response to submaximal exercise. Other mechanisms are likely responsible for exercise intolerance in TS; this needs to be further investigated.

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1. Introduction

Turner syndrome (TS) is a relatively common chromosomal disorder that is characterized by a partial or complete deficiency of the X chromosome [1]. TS is associated with multiple medical issues including impaired growth, ovarian insufficiency, structural cardiovascular abnormalities, abnormal fat mass accrual, as well as an increased risk of diabetes mellitus, an unfavorable metabolic profile, hypertension, and impaired endothelial function [1–4].

Participation in exercise is associated with an improved metabolic profile, a lower incidence of diabetes, reduced hypertension, decreased fat mass, and improved cardiovascular health [5], and therefore may be a useful intervention to reduce morbidity in TS. Despite the benefits of exercise, TS patients participate in lower levels of physical activity (including daily activity, leisure activity and sports) and experience a reduced capacity to exercise (i.e., a lower maximal oxygen uptake, VO2 max) compared to healthy controls [3].

It is unclear why individuals with TS have a reduced capacity for exercise, and it is likely multi-factorial in etiology. Factors that might contribute to exercise intolerance in TS include reduced respiratory function due to increased thoracic stiffness [6] and congenital cardiovascular abnormalities [6,7]. Furthermore, recent data from our group demonstrate that compared to healthy controls, girls with TS have normal aerobic but altered skeletal muscle anaerobic metabolism, resulting in an increased metabolic cost to exercise at a given relative workload [8].
Girls with TS also exhibit impaired endothelial function as measured at the fingertip by peripheral arterial tonometry [2]. What is unknown is whether impaired endothelial function is apparent in larger peripheral muscles, potentially resulting in a disruption of blood flow, oxygen delivery and impaired microvascular function in children with TS during exercise. Overall, it remains to be determined if exercise intolerance in patients with TS arises as a result of impaired oxygen delivery to muscle, in addition to changes in intra-skeletal muscle metabolism (i.e., metabolite utilization) as previously reported [8].

To investigate exercise effects on the microvasculature, we used blood-oxygen level-dependent (BOLD) magnetic resonance imaging (MRI). This technique takes advantage of the magnetic susceptibility difference between oxygenated hemoglobin (diamagnetic) and deoxygenated hemoglobin (paramagnetic) to result in differences in MR signal contrast. Previous studies have utilized ischemia–hyperemia protocols (i.e., a cuff to occlude blood supply to the leg) to perturb muscle metabolism, and have assessed the post-oclusion recovery in BOLD signal to provide insight into peripheral microvascular function in healthy adults and adults with chronic disease [9–12]. BOLD MRI can also be used in combination with an exercise stress to examine skeletal muscle microvascular function [9,12–20]. Skeletal microvascular function in response to an exercise stress as measured by the BOLD signal has not been reported in any clinical pediatric population.

Therefore, the purpose of the current study was to compare peripheral skeletal muscle microvascular function in children and adolescents with TS to that of healthy controls (HC) during the recovery period after exercise using the same cohort of participants from our previously published phosphorous magnetic resonance spectroscopy (31P-MRS) study [8]. We hypothesized that girls with TS would have a similar muscle BOLD signal response during exercise recovery compared to HC, based on our previous data demonstrating normal aerobic metabolism following exercise.

2. Materials and methods

2.1. Participants

The current study represents analysis of further data acquired in a study evaluating muscle 31P metabolism in exercising girls with TS, and methods have been previously published [8]. In brief, we recruited girls and adolescents age 10–18 years with TS from an endocrinology clinic at The Hospital for Sick Children. Inclusion criteria included: a confirmed diagnosis of TS and no congenital heart disease. Exclusion criteria included: history of type 1 or type 2 diabetes mellitus, impaired insulin sensitivity (i.e., “pre-diabetes”), use of medications that would alter lipid levels or adiposity (such as metformin, lipid-lowering agents, insulin and steroids or immunosuppressive agents), known cholesterol abnormalities, or presence of a known respiratory condition or structural cardiovascular abnormality [8]. The use of hormone therapy (i.e., estrogen/progesterone, or growth hormone) was not itself an exclusion criteria for girls with TS, however, they had to have been taking hormone therapy for at least 1 year. HCs were not taking any medications and had no history of chronic disease or illness. All participants and/or their parents signed informed consent, and the Research Ethics Board at The Hospital for Sick Children approved the study. All study tests were conducted at The Hospital for Sick Children.

2.2. Demographic characteristics and exercise capacity

Detailed methods used to assess height, weight, blood pressure, body composition (i.e., skin fold measurements), exercise capacity (i.e., an incremental cycling test to determine peak aerobic capacity (VO2peak)), and the administration of the Habitual Activity Estimation Scale (HAES) have been previously published [8].

2.3. MRI measures

The participants ate a non-standardized lunch of their choice. After lunch, participants completed exercise capacity testing prior to MRI testing. All MRI measures were obtained in the afternoon (between noon and 4 pm). Our BOLD MRI acquisition and image analysis protocol has been recently published [21].

2.3.1. Exercise protocol

Participants completed exercise using the non-dominant leg, on a calibrated MRI-compatible up-down ergometer (Lode AEI Technologies, Groningen, The Netherlands) while lying supine. To determine a starting value for the workload during the exercise test, participants initially performed a 30 second maximal exercise using the ergometer before being imaged. During imaging, participants completed 4 cycles of exercise (quadricep extensions) at 65% of maximal workload for 1 min, with 1 min of rest between each bout of exercise. To minimize motion during MR imaging, the leg was secured to the ergometer at the ankle, knee, and upper thigh. The ergometer automatically controlled power output by adjusting resistance in relationship to the participants’ freely chosen movement frequency. Data were collected at baseline, during exercise and recovery from each bout.

2.3.2. MR imaging protocol

MR images were collected at the MR suite at The Hospital for Sick Children using a 1.5 T Twin Speed EXCITETM III 12.0 MR scanner (GE Healthcare, Milwaukee WI). Images were acquired from the quadricep muscle of the non-dominant leg using an MPFLEX receive-only single element surface coil. Axial T2*–weighted BOLD images (gradient echo–planar-imaging (GE–EPI), 200 mm field of view, 10 mm slice thickness, 1 slice, 90° flip angle, TE/TR = 40 ms/250 ms, 2400 time points, 10 min total) were continuously collected from the mid-quadricep region.

2.3.3. Data analysis

To assess BOLD signal changes during exercise recovery, the functional images were first motion corrected using the FMRIB Software Library, FSL (FMRIB Analysis Group) [22,23]. Regions of interest covering 88 mm2 were chosen from the vastus medialis muscle of each subject using the analysis of functional neuro-images (AFNI) software (National Institute of Mental Health) [24]. The vastus medialis muscle is part of the quadriceps muscle group, which was used to contract against the resistance of the ergometer.

We evaluated the mean BOLD signal in each region of interest at rest, and then after each of the four exercise bouts (described below) for a given participant. Matlab (The Mathworks, Natick, MA) was used to fit the recovery data using the Trust-Region fitting algorithm and the curve fitting toolbox. The BOLD curve from each recovery was individually fitted to a four-parameter sigmoid function with the equation:

\[
S(t) = S_0 + \frac{\kappa}{1 + e^{(t - t_0)/\alpha}}
\]

where \(S(t)\) is BOLD signal intensity at time \(t\), \(S_0\) is the baseline BOLD signal intensity, \(\kappa\) is the range of BOLD signal intensity recovery (from lowest to highest signal), \(\alpha\) represents the BOLD signal intensity response time and washout of deoxyhemoglobin, and \(\beta\) is the half-time of recovery in seconds (Fig. 1). Clinically, \(\alpha\) represents the recovery response time (i.e. the higher the modeled value for \(\alpha\), the slower the recovery, derived from the slope); \(S_0\) is the smallest BOLD signal intensity following exercise at the beginning of recovery (i.e., lowest oxygenation level); \(\kappa\) is the change in BOLD signal intensity following exercise at the end of recovery (i.e., change in oxygenation level); and \(\beta\) indicates the time-course of the BOLD signal to reach the half-point of recovery.
2.4. Statistical analysis

We compared demographic characteristics, and BOLD signal curve parameters including: \( \kappa \), \( \beta \), and \( \alpha \) between TS and control groups using independent samples t-tests, or Wilcoxon–Mann–Whitney tests as appropriate. We conducted Pearson correlations to investigate test–retest reliability of our variables between recoveries (to examine consistency of the analyzed BOLD signal recovery curves), and to investigate associations between maximal exercise capacity, habitual physical activity, and modeled BOLD variables. We used repeated measures ANOVA to investigate whether there were changes in variables over time (i.e., over each recovery 1–4; to examine whether post-exercise BOLD signal recovery changes with increasing number of exercise bouts). All statistical analyses were performed with STATA version 11.1 (StataCorp, Texas, USA), no adjustments for multiple comparisons were made, and \( p < 0.05 \) was considered statistically significant.

3. Results

3.1. Participants

Demographic characteristics for all participants are shown in Table 1. BOLD time curves were collected in 15 participants with TS, and in 8 HC participants. As we previously reported [8], in our population 7 girls with TS had 45X karyotype, 4 had 46XX, 45X mosaicism, and the rest of the TS girls had other varying complex karyotypes. Eight TS girls were being actively treated with hormone therapy (either estrogen alone or combined estrogen/progesterone), and 5 were being treated with growth hormone. Of the 15 TS girls, 2 did not have useful BOLD signal recovery curves due to excessive motion, present still after attempted motion correction. Therefore, the data analysis was completed on 13 TS girls, and 8 HCs. Participants on average were 13.7 ± 3.1 years old, weighed 47.9 ± 14.6 kg, had a mean body mass index (BMI) of 21.3 ± 4.6 kg/m², and had a mean body fat of 28.4%.

Demographic characteristics, Turner syndrome vs. Controls. Results are expressed as mean ± standard deviation. HC = healthy controls, TS = turner syndrome.

3.2. BOLD MRI data

As per the BOLD MRI exercise protocol, 4 post-exercise recovery curves were collected, per subject. The mean power achieved during the BOLD imaging exercise protocol was similar between the HC and TS groups (10.3 ± 3.3 and 10.1 ± 2.7 W, \( p = 0.91 \)). On average, from 2 to 3 recovery curves per participant could be analyzed. Other curves had to be excluded due to uncorrectable motion contamination. There was no difference in the number of curves analyzed per participant in the TS group vs. the HC group (\( p = 0.58 \)). Overall, a total of 56 recovery curves were included in the data analysis. The test–retest reliability between each recovery curve and the mean of all recovery curves was acceptable (\( r \geq 0.70 \)). For example, the mean BOLD signal change (\( \% \)) of all curves had a reliability of \( r = 0.74 \) with recovery curve 1, \( r = 0.88 \) with recovery curve 2, \( r = 0.76 \) with recovery curve 3, and \( r = 0.78 \) with recovery curve 4. There were no differences in any of the modeled BOLD parameters over time (recoveries 1 to 4, \( p > 0.05 \) for all).

The sigmoidal-modeled BOLD signal parameters from each recovery curve, and the mean of all curves are shown in Table 2a and 2b respectively. Overall, the sigmoid function fit the data well with a mean \( r^2 \) of 0.90 ± 0.08, and the \( r^2 \) did not differ between the analysis of TS participants vs. HC participants (\( r^2 = 0.89 ± 0.06 \) and 0.91 ± 0.10 respectively, \( p = 0.61 \) (Fig. 2)). Parameter estimates from the sigmoid curve were then used to characterize the BOLD response post-exercise (Fig. 1). The sigmoidal-modeled BOLD signal parameters from each recovery curve, and the mean of all curves are shown in Table 2a and 2b respectively. Overall, the sigmoid function fit the data well with a mean \( r^2 \) of 0.90 ± 0.08, and the \( r^2 \) did not differ between the analysis of TS participants vs. HC participants (\( r^2 = 0.89 ± 0.06 \) and 0.91 ± 0.10 respectively, \( p = 0.61 \) (Fig. 2)). Parameter estimates from the sigmoid curve were then used to characterize the BOLD response post-exercise (Fig. 1). The
3.3. BOLD MRI correlations

We examined correlations between habitual daily activity, VO₂ peak, and modeled BOLD signal parameters in all participants. The mean response time and washout of deoxyhemoglobin (\(\alpha\)) and the mean half-time of recovery (\(\beta\)) were both negatively correlated with weekend vigorous activity levels (\(r = -0.45, p = 0.04; r = -0.61, p = 0.003\), respectively), and VO₂ peak (\(r = -0.58, p = 0.01; r = -0.73, p < 0.001\), respectively). The mean half-time of recovery (\(\beta\)) was also negatively correlated with weekend total activity levels (\(r = -0.46, p = 0.04\)). There were no other significant observations.

4. Discussion

This is the first study to evaluate peripheral skeletal muscle BOLD MRI signal response to exercise in children with TS. We found that compared to HC, children with TS exhibited similar skeletal muscle oxygenation saturation during a 60 second recovery phase following exercise.

For example, mean change in BOLD signal intensity (i.e., the signal change between the lowest and highest oxygenation levels following exercise; \(\kappa\)) was similar between the TS and HC groups, rather than a blunted change in \(\kappa\) in the TS group, which would indicate an impaired response of skeletal muscle microvascular function following exercise.

Neither the time for the BOLD signal to reach the half-point of recovery post-exercise (\(\beta\)) nor the signal intensity response time or deoxyhemoglobin washout (\(\alpha\)) were different between TS and HC groups. If skeletal muscle oxygenation saturation was impaired in the TS group, the \(\alpha\) and the \(\beta\) (seconds) would be higher compared to those of HCs, implying a reduced rate of oxygen perfusion into the skeletal muscles and a delay in aerobic recovery response post-exercise, respectively. This concept is further supported by our observation that a higher VO₂ peak was correlated with a lower \(\alpha\) and \(\beta\) time suggesting that having a higher maximal oxygen uptake is associated with an increased rate of skeletal muscle oxygen perfusion and a quicker aerobic recovery response post-exercise (i.e., a more efficient aerobic system). Therefore, in agreement with our hypothesis, we did not observe any differences between TS and HC in skeletal muscle recovery post-exercise. In our cohort of children/adolescents with TS, reduced oxygenation saturation and disrupted peripheral microvasculature are likely not mechanisms responsible for exercise intolerance.

Our current data is consistent with our recent muscle metabolism data (as measured by ³¹P-MRS) demonstrating that the anaerobic energy system, rather than the oxygen dependent aerobic system, is impaired in the same population of children with TS compared to healthy controls [8]. Wells et al. reported that TS participants exhibited a significantly higher end-exercise pH after 30 and 90 s of exercise vs. controls, while experiencing a comparable workload, which is suggestive of increased anaerobic glycolysis and lactic acid production during exercise. We concluded that children with TS experience greater anaerobic stress during exercise compared to healthy controls, and suggested that it is unlikely that there are impairments in oxygen transport or mitochondrial metabolism in our group of children with TS [8]. The current BOLD data suggests that oxygen delivery and tissue perfusion are likely not impaired in our group of children with TS — rather, exercise intolerance in TS might be a result of an intrinsic defect in muscle bioenergetic metabolism.

The findings of the current study are also supported by a previous study that examined muscle fiber and capillarization in 10 adult women with TS compared to 14 healthy controls. Following a muscle biopsy of the vastus lateralis, women with TS had an increased size of type Ila muscle fibers compared to those of healthy controls, while the sizes of the type I and IIX fibers were similar between groups [25]. Type I muscle fibers are most efficient at utilizing oxygen to generate energy for continuous muscle contractions over time, and are important in aerobic endurance exercise; therefore muscle fibers that are associated with aerobic metabolism were not affected in TS. Furthermore, the women with TS did not have any differences in the capillary density of any of the muscle fiber types compared to controls [25], suggesting that skeletal muscle vasculature is not impaired in TS.

Cardiovascular disease and vascular abnormalities are common in individuals with TS. Indeed, a previous study reported that compared to healthy controls, girls (aged 10–18 years) with TS exhibited impaired endothelial function as measured at the fingertip by peripheral arterial tonometry [2]. However, based on our current findings, altered endothelial function does not appear to translate to impaired blood flow and oxygenation saturation in large skeletal muscles post-exercise. Rather, the previously reported reduced endothelial function may explain a higher risk for vascular disease, and act as a surrogate marker to assess cardiovascular risk rather than overall reduced peripheral vascular function.

To date, only one study has examined changes in BOLD signal in response to exercise in a clinical population [18]. This study evaluated microvacular function in adult men and women with type 1 or type 2 diabetes and compared them to healthy controls. Following maximal

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**Table 2a**

Coefficients obtained from fitting BOLD signal intensity recovery following exercise to a four-parameter sigmoid curve. Results are expressed as mean ± standard deviation. HC = healthy controls, TS = Turner syndrome.

| Recovery 1 | HC (n = 7) | TS (n = 7) | p value |
|------------|------------|------------|---------|
| \(\kappa\) | 0.16 ± 0.08 | 0.13 ± 0.05 | 0.55 |
| \(\alpha\) (s) | 9.39 ± 6.61 | 8.98 ± 4.60 | 0.90 |
| \(\beta\) (s) | 21.95 ± 10.17 | 20.95 ± 7.55 | 0.84 |

**Recovery 2**

| HC (n = 5) | TS (n = 10) | p value |
|------------|------------|---------|
| \(\kappa\) | 0.16 ± 0.08 | 0.17 ± 0.06 | 0.83 |
| \(\alpha\) (s) | 9.54 ± 5.15 | 8.46 ± 4.37 | 0.08 |
| \(\beta\) (s) | 25.29 ± 7.20 | 20.75 ± 7.92 | 0.30 |

**Recovery 3**

| HC (n = 5) | TS (n = 9) | p value |
|------------|------------|---------|
| \(\kappa\) | 0.15 ± 0.06 | 0.22 ± 0.08 | 0.13 |
| \(\alpha\) (s) | 9.13 ± 4.68 | 12.21 ± 7.38 | 0.42 |
| \(\beta\) (s) | 19.98 ± 5.48 | 24.72 ± 5.57 | 0.15 |

**Recovery 4**

| HC (n = 6) | TS (n = 7) | p value |
|------------|------------|---------|
| \(\kappa\) | 0.13 ± 0.06 | 0.14 ± 0.05 | 0.80 |
| \(\alpha\) (s) | 9.23 ± 6.44 | 8.59 ± 2.55 | 0.81 |
| \(\beta\) (s) | 19.09 ± 5.44 | 17.45 ± 5.33 | 0.60 |

**Abbreviations:** \(\kappa\), the range of BOLD signal intensity recovery (from lowest to highest signal); \(\alpha\), the BOLD signal intensity response time and washout of deoxyhemoglobin; \(\beta\), the half-time of recovery.

**Table 2b**

Mean coefficients obtained from fitting BOLD signal intensity recovery following exercise to a four-parameter sigmoid curve. Results are expressed as mean ± standard deviation. HC = healthy controls, TS = Turner syndrome.

| Mean of all recovery curves | HC (n = 8) | TS (n = 13) | p value |
|-----------------------------|------------|------------|---------|
| \(\kappa\) | 0.14 ± 0.06 | 0.17 ± 0.04 | 0.15 |
| \(\alpha\) (s) | 8.13 ± 5.0 | 9.54 ± 4.11 | 0.83 |
| \(\beta\) (s) | 19.46 ± 7.31 | 19.30 ± 6.37 | 0.99 |

**Abbreviations:** \(\kappa\), the range of BOLD signal intensity recovery (from lowest to highest signal); \(\alpha\), the BOLD signal intensity response time and washout of deoxyhemoglobin; \(\beta\), the half-time of recovery.
isometric ankle dorsiflexion, BOLD response was measured in the dorsiflexors. There were no differences in peripheral microvascular function between healthy controls and those with diabetes following exercise [18]. We are the first study to use a similar methodology to explore microvascular function in a pediatric clinical cohort.

Interestingly, we did not observe a reduced exercise capacity (i.e., VO2 peak) in children and adolescents with TS compared to healthy controls, and with the exception of vigorous weekday activity, girls with TS participated in similar amounts of physical activity as healthy controls. Since our cohort of girls with TS had similar microvascular function as healthy controls, we would actually expect that maximal oxygen consumption (i.e., the efficiency of the muscles to receive oxygen and utilize oxygen for energy production) would be similar to controls. An unaffected ability to participate in exercise may contribute to an increased ability to engage in daily physical activity explaining why our children with TS contributed to, or was a result of, unimpaired peripheral skeletal muscle microvascular response to exercise in children with TS. Our results suggest that oxygen saturation following exercise is likely not impaired in our small group of children with TS (who do not exhibit a reduced exercise capacity) compared to healthy controls. Future studies in larger cohorts of girls with TS should be conducted. In combination with the recently published data by our group in the same population [8], this study supports the concept that the aerobic energy pathway is most likely not impaired in children with TS in response to submaximal exercise.

5. Conclusions

In conclusion, this is the first study to use BOLD MRI to evaluate peripheral skeletal muscle microvascular response to exercise in children with TS. Our results suggest that oxygen saturation following exercise is likely not impaired in our small group of children with TS (who do not exhibit a reduced exercise capacity) compared to healthy controls. Future studies in larger cohorts of girls with TS should be conducted. In combination with the recently published data by our group in the same population [8], this study supports the concept that the aerobic energy pathway is most likely not impaired in children with TS in response to submaximal exercise.

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