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Skeletal muscle microvascular function in girls with Turner syndrome

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ABSTRACT

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.

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, VO_2 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].

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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-occlusion 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 (^{31}P-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 ^{31}P 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 (VO_{2peak})), 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 EXCITE^{TM} 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 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 mm^2 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 quadricep 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:

$$S(t) = S_0 + \frac{\kappa}{1 + e^{(\beta - t)/\alpha}}$$

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