Skeletal Muscle Abnormalities in Girls and Adolescents With Turner Syndrome

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Context: Turner syndrome (TS) is a chromosomal disorder occurring in approximately 1 in 2500 live births. Individuals with TS report lower levels of physical activity than healthy control (HC) subjects. Cardiorespiratory limitations may contribute to the observed reduction in physical activity.

Objective: The objective of this study was to compare muscle metabolism of patients with TS vs HC subjects before and after exercise using exercise testing, magnetic resonance imaging, and magnetic resonance spectroscopy techniques.

Design: We hypothesized that girls and adolescents with TS would have muscle metabolic abnormalities not present in the HC population.

Setting: The research was conducted at the Hospital for Sick Children in Toronto, Ontario, Canada.

Participants: Fifteen participants with TS were age-, activity-, and body mass index Z-score–matched with 16 HC subjects.

Main Outcome Measures: ^{31}P magnetic resonance spectroscopy was used to characterize muscle metabolism at rest and after 30 seconds of high-intensity exercise, 60 seconds of moderate-intensity exercise, and 5 minutes of low-intensity exercise.

Results: While achieving the same workloads, participants with TS exhibited a greater difference between rest and end-exercise pH compared with HC subjects after 30 seconds (TS, 0.29 $\pm$ 0.04; HC, 0.21 $\pm$ 0.08; $P = .03$) and 90 seconds (TS, 0.47 $\pm$ 0.22; HC, 0.32 $\pm$ 0.13; $P = .02$) of exercise. During the 5-minute exercise test, similar workloads were achieved between groups; however, ATP production was greater in participants with TS vs the HC subjects via all 3 bioenergetic pathways (total ATP: TS, 0.90 $\pm$ 0.34; HC, 0.60 $\pm$ 0.25; $P = .01$).

Conclusions: The results of this study suggest that patients with TS exhibit greater anaerobic stress during exercise than HC subjects, which may lead to symptoms of increased muscle fatigue with short bursts of activity. Recovery metabolism after exercise appears to be similar between participants with TS and HC subjects, which is suggestive of normal mitochondrial metabolism and oxygen transport. **(*J Clin Endocrinol Metab* 98: 2521–2527, 2013)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received November 27, 2012. Accepted March 25, 2013.
First Published Online April 3, 2013

Abbreviations: BMI, body mass index; CK, creatine kinase; HC, healthy control; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; Pi, inorganic phosphate; Pcr, phosphocreatine; TS, Turner syndrome; Vo_{2peak}, volume of oxygen at peak exercise.

Turner syndrome (TS) is a chromosomal disorder occurring in approximately 1 in 2500 live births (1), most often as a 45,X monosomy or 45,X/46,XX mosaicism (2). Girls and women with TS characteristically display short stature, gonadal dysgenesis and premature ovarian failure, congenital cardiovascular and/or renal abnormalities, and numerous acquired abnormalities in cardiovascular, metabolic, and hepatic function (3). In fact, a 3-fold increase in mortality due to cardiovascular disease and diabetes has been reported (4). An atherogenic lipid profile is characteristic of TS (5), as is increased circulation of inflammatory markers such as IL-6 and C-reactive protein (3, 6). A decreased insulin secretory response to glucose is also observed, possibly as a result of a pancreatic β-cell defect (2, 7), although some authors have observed normal insulin secretion profiles in patients with TS (8).

Individuals with TS also have increased central adiposity and visceral fat stores, which are associated with insulin resistance (3, 9). However, in TS it is unclear whether increased central adiposity arises from hormonal insufficiency (decreased estrogen) or from other factors related to haploinsufficiency of the X chromosome (5, 10). In a recent study, patients with TS undergoing hormone replacement therapy continued to demonstrate increased central adipose stores and impaired glucose tolerance relative to those of healthy control (HC) subjects (10). The ineffectiveness of hormone replacement therapy to improve features of the metabolic syndrome in patients with TS indicates that alternative methods to treat these symptoms are essential. Interventions to increase habitual physical activity may be effective because individuals with patients with TS report lower levels of physical activity than HC subjects (3, 11). Cardiorespiratory limitations may contribute to the observed reduction in physical activity levels. Women with TS may have increased thoracic stiffness, which limits respiratory function (12). Furthermore, cardiac abnormalities associated with increased risk of adverse cardiac events are increased in the population with TS (1). Other factors contributing to exercise capacity, such as impaired muscle metabolism, have not been studied in this population. Understanding the inherent differences in muscle metabolism and the effects they exert on exercise tolerance in the population with TS will help us better develop targeted physical activity interventions for patients with TS.

Accordingly, the objective of this study was to compare the metabolic profiles of patients with TS vs HC subjects before and after exercise using traditional exercise testing and advanced magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) techniques. Recently, ^{31}P-MRS has been used in vivo to assess muscle metabolism (13, 14), including the function of creatine kinase (CK), oxidative phosphorylation, and anaerobic glycolysis pathways during exercise and recovery (15). We hypothesized that girls and adolescents with TS would exhibit differences in muscle metabolism not present in the HC population.

Materials and Methods

Participants

We recruited 15 girls and adolescents with TS, aged 10 to 18 years, from an endocrinology clinic at the Hospital for Sick Children. Criteria for inclusion were a confirmed diagnosis of TS and no congenital heart disease. Exclusion criteria included a known history of type 1 or type 2 diabetes mellitus, use of medications that would alter lipid levels or adiposity, or the presence of a known respiratory condition. Use of estrogen, GH, or thyroid hormone, which are commonly required in girls with TS, were not exclusion criteria, but participants must have adequate hormone replacement with L-thyroxine or have been taking estrogen or GH for at least 1 year. Participants were assessed for pubertal stage using the Tanner method. HC participants were 16 girls and adolescents, who were age-, activity-, and body mass index (BMI) Z-score–matched with the participants with TS and not receiving medication. All participants and/or their parents signed informed consent forms at the time of their clinic visit. The study was approved through the research ethics board at the Hospital for Sick Children. All tests were performed in the Pediatric Research Exercise Laboratory and MRI suites at the Hospital for Sick Children.

Parameters of assessment

Height and weight were measured using a standard scale and wall-mounted stadiometer. BMI was calculated as weight (kilograms) divided by height (square meters), and BMI Z-scores were calculated according to the Centers for Disease Control and Protection standards (16). Blood pressure was measured using as the average of 3 readings with an automated DINAMAP sphygmomanometer (Critikon, Tampa, Florida) with an age-appropriate cuff and recorded along with resting heart rate. Fat-free mass, fat mass, and percentage of body fat were calculated from skin fold measurements using the Slaughter-Lohmann method (17). Insulin was measured by chemiluminescence using the Immulite 2500 (Siemens, Munich, Germany; range of assay, 15–2165 pmol/L; intra- and interassay coefficients of variation, $<7.6\%$). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated based on fasting insulin and glucose values (18):

$$\text{HOMA-IR} = [\text{glucose (millimoles per liter)} \times \text{insulin(milliunits per liter)}]/22.5$$

Fasting triglycerides (TGs), total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and apolipoproteins were measured by standard enzymatic methods. LDL cholesterol was calculated using the formula:

$$\text{LDL cholesterol} = \text{total cholesterol} - \text{TGs}/2.2 + \text{HDL cholesterol}$$

Pulmonary function (VMax20 Pulmonary Spirometry Instrument; SensorMedics, Yorba Linda, California) was determined according to standard spirometric techniques (19). Participants performed an incremental cycling test to determine peak aerobic capacity (Vo_{2peak}). For the aerobic cycle ergometer test, the participants performed the incremental test on an electrically braked cycle ergometer (Rodby Electronik AB, Enhorna, Sweden). One-minute work increments were according to Godfrey et al (20), taking into account sex, height, and habitual physical activity level. Participants with a height of <120 cm had an initial work rate of 10 W with 10-W increments until they could no longer maintain a cycling cadence of 60 rpm. Those with a height of 120 to 150 cm and >150 cm started with 15 and 20 W, respectively, and increased by 15 and 20 W, respectively. If participants were sedentary or highly active, adjustments were made to the protocol, aiming for approximately 8 to 12 minutes of cycling for all participants. Oxygen consumption, carbon dioxide production, tidal volume, and the respiratory exchange ratio were measured continuously online through an automated exercise-testing program developed for use in our laboratory. The Habitual Activity Estimation Scale questionnaire (21), previously validated in both healthy children and those with chronic illness, was used as an estimation of activity levels.

MRI and MRS tests

Data for MRI and ^{31}P-MRS were collected on a General Electric Twin Speed EXCITE III 12.0 1.5-T imaging and spectroscopy system (GE Healthcare, Milwaukee, Wisconsin). Exercise, with the participant laying supine in the MRI machine, was completed on a calibrated nonmagnetic up-down ergometer (Lode; AEI Technologies, Pittsburgh, Pennsylvania). The lower extremities of the participants were at the center of the magnet bore of the MRI scanner. The nondominant leg was chosen for testing and to reduce movement during exercise; the leg was fastened to the cycle ergometer at the knee and ankle. Motion due to movement of muscle in relation to the coil was minimized by securing the coil in a fixed position midway between the hip and the knee with Velcro straps. The ergometer automatically controlled power output by adjusting resistance in relationship to the participants' freely chosen movement frequency. In this way, exercise was controlled for power output, because the relative work rate is an important factor in the determination of the metabolic pathways used for ATP generation during exercise and recovery. Watts and repetitions per minute of the ergometer were recorded every 5 seconds.

Magnetic resonance values were recorded from the vastus lateralis muscle. The average value of 8 consecutive measurements of the thigh determined muscle cross-sectional area. Data collection consisted of MRI followed by calibration and subsequent ^{31}P-MRS spectroscopy. For spectroscopy measurements, sequential ^{31}P-MRS spectra were obtained under partially saturated conditions with the following parameters: spin-echo sequence, hard pulse, 30° flip angle, repetition time 1250 ms, 3500-Hz spectral width, 1024 data points, 2 NEX (total acquisition time = 8 seconds per spectrum). Spectral analyses were performed using commercial software (SAGE 7 Dev2005.3, GE Healthcare). Resting metabolite data values were based on the average of 8 scans at rest.

A curve using nonlinear least squares analysis, based on Gaussian line shapes, was used to calculate the areas under the inorganic phosphate (Pi), phosphocreatine (PCr), and β-ATP peaks (22). These metabolites were measured because they are important in energy metabolism. Pi is used for phosphorylation in many chemical reactions, PCr is a stored form of energy, and ATP is a key energy substrate for reactions in the human body. Tissue pH can provide insights into anaerobic glycolytic metabolism because of the production of lactic acid in this pathway during high intensity exercise. ^{31}P metabolite concentrations were calculated by normalizing total muscle phosphate to 41.3 $\text{mmol} \cdot \text{L}^{-1}$ (23). The chemical shift difference between PCr and Pi was used to calculate pH values (24). Calculated pH was corrected for changes in cytosolic $[Mg^{2+}]$ using the chemical shift of β-ATP determined by PCr resonance (25). An index of aerobic metabolic function was therefore determined, based on the recovery rate of PCr calculated during recovery after each exercise bout using an exponential curve fit.

MRS exercise protocols

To reproduce the different activities typical of children, 3 different exercise protocols were used. A 30-second interval of maximal exercise was performed to study the physiological response to brief intense activity. The average wattage produced during the 30-second bout was used and recorded to establish later exercise intensity. After 5 minutes of recovery, 90 seconds of intense exercise was performed at a consistent work rate 85% of the mean work rate during the 30-second bout. After recovery, participants performed 10 bouts of 30 seconds of exercise with a 15-second break in between at 65% of the watts produced during the 30-second bout. The 30-second protocol was selected because energy is primarily provided though high-energy phosphate and anaerobic glycolytic metabolism, whereas the 90-second exercise bouts require contributions from the aerobic oxidative and anaerobic glycolytic pathways, and the 10 bouts of 30 seconds of exercise at 65% intensity relied primarily on aerobic metabolism. ATP production by each of the 3 bioenergetic pathways was calculated from data obtained during the 10-second exercise bouts as described previously (14). The equations used to calculate ATP production rates were as follows:

(1) ATP production rate via CK pathway $= -(\Delta PCr/\Delta t)_{exercise}$
(2) ATP production rate via oxidative phosphorylation pathway $= (\Delta PCr/\Delta t)_{recovery}$
(3) ATP production rate via anaerobic glycolysis pathway $= 3/2 \times (-(24.3\ [Pi] \times (\Delta pH/\Delta t) - (0.85 - \Phi) \times (\times/\Delta t))_{exercise}$, where Φ is calculated from $\phi = 1/(1 + 10^{(6.75-pH)})$

Statistical analysis

Summary statistics were compared between the TS and HC groups using the unpaired *t* test or Wilcoxon signed rank test. We hypothesized that girls with TS would exhibit altered muscle metabolism during exercise as evidenced by a longer PCr recovery rate, indicative of mitochondrial dysfunction in skeletal muscle. An a priori sample size of 16 participants per group was calculated for determination for half-time PCr recovery, with a minimum detectable difference of 9 and SD of 9; power was set at 0.8 and $\alpha = .05$. For secondary outcomes, including changes in pH after exercise (resting pH − end-exercise pH) values from HC children collected with similar protocols and equipment, we calculated that 12 participants per group would be sufficient to detect an effect size difference of 0.2, with an SD of 0.15 in pH change, at a significance of $\alpha = .05$ and power of 0.8 (26).

Results

Participants

Baseline characteristics for all participants are shown in Table 1. All HC participants and 15 participants with TS completed the entire experiment. One participant with TS could not complete the MRI protocol because of feelings of claustrophobia. Seven girls with TS had the 45,X karyotype, 4 had 45,X/46,XX mosaicism, and the rest had various other complex karyotypes. Patients with TS were treated with hormone therapy as follows: 8 were receiving estrogen alone or combined estrogen/progesterone; 7 were not receiving hormones for pubertal induction; 5 were treated with GH; and the remaining 10 had completed linear growth and were not receiving GH (2 were GH naive and 8 were treated previously). IGF-1 levels in those receiving GH treatment, measured as part of their clinical care, were in the normal range for age and sex (mean 402 ± 156 μg/L) (27).

Ten girls with TS and 9 HC participants had achieved Tanner stage 3 or higher. There were no statistically significant differences in clinical pubertal status between the groups. Habitual activity levels of both groups were the same, with no difference in intensity of physical activity levels (very active or somewhat active) between the TS and HC groups. Mean BMI Z-score was not significantly different between participants with TS and HC participants (Table 1). Weight-for-age Z-score was not significantly different between the groups (TS 0.01 ± 1.1 vs HC 0.16 ± 0.75; not significant); however, height for age was different (TS 1.1 ± 1.3 vs HC 0.49 ± 1.1; $P = .01$).

Girls with TS receiving GH had lower absolute BMI levels (19.8 ± 3.8 kg/m^2 with GH vs 24.7 ± 5.0 kg/m^2 without GH; $P = .04$); however, BMI Z-scores were not significantly different. Exercise parameters on the incremental cycling test were similar between participants with TS and HC subjects (Table 1). In addition, there were no significant differences in exercise or magnetic resonance parameters in girls with TS receiving GH vs those not taking GH (data not shown). However, absolute Vo_{2peak} (1.3 ± 0.4 L · min^{-1} no estrogen vs 1.7 ± 0.2 with estrogen; $P = .01$), ventilation at peak exercise (50.4 ± 12.6 L · min^{-1} no estrogen vs 74.4 ± 9.8 with estrogen; $P = .01$), and maximum voluntary ventilation (62.0 ± 9.8 L · min^{-1} no estrogen vs 83.9 ± 15.2 with ES; $P = .01$) were all higher in girls with TS receiving estrogen therapy than in those who were not receiving estrogen.

Table 1. Descriptive Characteristics of Participants

	HC (n = 16)	TS (n = 15)
Age, y	12.8 ± 3.4	13.9 ± 2.6
Height, m	1.5 ± 0.2	1.5 ± 0.1
Weight, kg	45.2 ± 3.9	50.2 ± 3.9
Systolic blood pressure, mm Hg	103 ± 7.9	115 ± 2.1[a]
Diastolic blood pressure, mm Hg	58 ± 6.8	67 ± 11.1[a]
Mean blood pressure, mm Hg	74 ± 5.5	82 ± 13.6[a]
Resting heart rate, beats · min^{-1}	66 ± 9.7	87 ± 17.4[a]
BMI	18.7 ± 3.4	23.1 ± 5.3[a]
Z-score BMI	0.26 ± 0.99	0.20 ± 0.93
LBM, kg	34.3 ± 8.9	34.1 ± 7.4
Body fat, %	27.0 ± 5.9	28.8 ± 8.0
Vo_{2peak}, mL · min · kg^{-1}	35.0 ± 4.5	33.2 ± 9.0
Power at peak exercise, % predicted	90.0 ± 13.7	97.6 ± 17.8
Heart rate at peak exercise, beats · min^{-1}	186.9 ± 6.1	189.9 ± 12.6
Respiratory exchange ratio	1.22 ± 0.09	1.18 ± 0.09
Anaerobic threshold, % Vo_{2peak}	78 ± 17	87 ± 11
HWDVA, h	2.0 ± 1.3	1.5 ± 1.2
HWDTA, h	4.7 ± 1.9	3.7 ± 1.5
HWEVA, h	1.9 ± 2.4	2.0 ± 2.4
HWETA, h	5.5 ± 2.6	6.0 ± 2.9

Abbreviations: HWDTA, Habitual Activity Estimation Scale questionnaire weekday total activity; HWDVA, Habitual Activity Estimation Scale questionnaire weekday very active; HWETA, Habitual Activity Estimation Scale questionnaire weekend total activity; HWEVA, Habitual Activity Estimation Scale questionnaire weekend very active; LBM, lean body mass. Data are means ± SD.

[a] $P < .05$ vs HS subjects.

^{31}P-MRS resting measures results

Resting values of ^{31}P-MRS variables were obtained, and the average of 16 resting spectra were averaged. Group means are shown in Table 2. There were no differences in resting PCr, total ATP, pH, or the ratio of Pi to PCr. Significantly lower resting Pi values were seen in the TS group (HC 3.3 ± 0.6 mmol/L vs TS 2.7 ± 0.4 mmol/L; $P = .01$).

^{31}P-MRS After 30 and 90 Seconds of Exercise

^{31}P-MRS spectra were collected immediately after and before the 30- and 90-second exercise bouts (Table 3). There were no significant differences in the spectra collected other than the decrease in pH from resting levels after exercise at 30 seconds (HC 0.21 ± 0.08 vs TS 0.29 ± 0.04; $P = .03$) and 90 seconds (HC 0.32 ± 0.13 vs TS 0.47 ± 0.22; $P = .02$). Further, postexercise half-time of PCr recovery was significantly longer in patients with TS

Table 2. ^{31}P-MRS Variables at Rest

	HC	TS
PCr, mmol · L^{-1})	28.7 ± 1.2	29.0 ± 1.4
Pi, mmol · L^{-1}	3.3 ± 0.6	2.8 ± 0.4[a]
SumATP, mmol · L^{-1}	9.4 ± 1.0	9.3 ± 1.1
pH	7.1 ± 0.6	7.1 ± 0.07
Pi-to-PCr ratio	0.1 ± 0.03	0.1 ± 0.02

Abbreviation: SumATP, total adenosine triphosphate.

[a] $P < .05$ vs HC subjects.

Table 3. ^{31}P-MRS Variables after 30 and 90 Seconds of Exercise

	HC	TS
Change in pH after exercise (−[rest pH − end-exercise pH])		
30 s	−0.21 ± 0.08	−0.29 ± 0.04[a]
90 s	−0.32 ± 0.13	−0.47 ± 0.22[a]
5 min	−0.14 ± 0.09	−0.24 ± 0.17
Pi-to-PCr ratio		
30 s	0.51 ± 0.14	0.52 ± 0.08
90 s	0.68 ± 0.43	0.84 ± 0.33
5 min	0.51 ± 0.21	0.71 ± 0.32
Halftime of PCr recovery, s		
30 s	16.8 ± 6.1	24.2 ± 9.5[a]
90 s	21.7 ± 8.6	19.9 ± 10.4
5 min	21.4 ± 7.7	24.8 ± 15.9
Work during exercise trial, W		
30 s	15.9 ± 5.4	14.8 ± 2.5
90 s	12.6 ± 3.2	12.3 ± 2.4
5 min	9.4 ± 3.1	9.9 ± 2.5

[a] $P < .05$ vs HC subjects.

for the 30-second test only. TS and HC groups achieved the same workloads during 30 and 90 seconds of exercise.

^{31}P-MRS Results During 5 Minutes of Exercise

^{31}P-MRS results were collected immediately before exercise, after each of 10 30-second exercise bouts, and during recovery (Table 4). Participants with TS and HC participants achieved similar workloads during 10 30-second exercise bouts. ATP production by each of the 3 bioenergetic pathways was significantly different between the 2 groups (CK: HC 0.08 ± 0.03 vs TS 0.11 ± 0.03, $P = .02$; oxidative: HC 0.17 ± 0.05 vs TS 0.23 ± 0.06, $P = .01$; and glycolytic: HC 0.35 ± 0.16 vs TS 0.56 ± 0.27, $P = .01$). Total ATP produced between the TS and HC groups was also significantly different (HC 0.60 ± 0.25 vs TS 0.90 ± 0.34, $P = .01$). There were no differences in Pi-to-PCr ratio, PCr recovery, or the change in pH after exercise.

Discussion

This is the first study to evaluate muscle metabolism during exercise in TS. Our results suggest differences in the metabolic demands of exercise between patients with TS and HC girls and adolescents who are matched for age, BMI Z-score, and fitness level. Participants with TS exhibited a significantly higher end-exercise pH after 30- and 90-second exercise bouts vs HC subjects, while experiencing a comparable workload (same Pi-to-PCr ratio), which is suggestive of increased anaerobic glycolysis and lactic acid production during exercise. This difference, however, did not persist after the 5-minute exercise test. Contrary to our original hypothesis, there was no significant difference detected in PCr recovery rates between the TS and HC groups except after the 30-second exercise test; therefore, it is unlikely that there are impairments in oxygen transport or mitochondrial phosphorylation. This conclusion is supported by the observation of similar levels of peak aerobic power during traditional cycle ergometry between the TS and HC groups.

Table 4. ^{31}P-MRS Variables During 5 Minutes of Exercise

	HC	TS
ATP produced (CK), mmol · L^{-1}	0.08 ± 0.03	0.11 ± 0.03[a]
ATP produced (oxidative), mmol · L^{-1}	0.17 ± 0.05	0.23 ± 0.06[a]
ATP produced (glycolytic), mmol · L^{-1}	0.35 ± 0.16	0.56 ± 0.27[a]
Total ATP, mmol · L^{-1}	0.60 ± 0.25	0.90 ± 0.34[a]

[a] $P < .05$ vs HC subjects.

^{31}P-MRS is a useful method for determining muscle metabolic characteristics at rest and during exercise that cannot be detected using traditional cycle ergometry. The levels of muscle metabolites at rest such as ATP, Pi, and PCr have not yet been reported in the population with TS. We found that HC participants and participants with TS had similar Pi-to-PCr ratios and ATP, pH, and PCr levels, but participants with TS had lower resting Pi levels than HC subjects. Reduced resting Pi levels have been observed previously in research on metabolic syndrome and are suggested to be a major characteristic of the metabolic syndrome due to either reduced reabsorption or altered distribution between cellular compartments (28, 29). The participants with TS did have higher blood pressures and lipid levels, so these features of the metabolic syndrome and lower Pi in the TS group is consistent with other literature on this topic.

To compare the workloads experienced for each group, muscle metabolites were used as an analog for metabolic effort. The Pi-to-PCr ratio was used to identify metabolic effort. A higher Pi-to-PCr ratio indicates an increased workload, because more Pi would be present due to increased ATP hydrolysis. We know that both groups achieved similar workloads during the exercise tests not only mechanically (percentage of predicted workload in watts) but also metabolically because of our ^{31}P-MRS measurements of the Pi-to-PCr ratio. We observed lower end-exercise pH in the TS group, which may manifest as a greater level of muscle pain and discomfort with activity and is often described as muscle "burning." Therefore, we interpret the greater change in end-exercise pH to be representative of a greater contribution of anaerobic glycolysis to energy production during 30- and 90-second bouts of exercise in this population. This is important from a functional perspective, because the nature of children's play often involves repeated starting and stopping, and the exercise is often of a relatively high intensity. High-

energy phosphate and anaerobic glycolytic metabolism are the primary energy systems involved in fueling short bursts of high-intensity exercise, and compromising or overtaxing these systems in patients with TS could have a negative impact on their ability to participate in physical activities. A limitation of this study is that ratings of perceived exertion were not collected with the exercise testing, as this may have provided greater insight into the functional impact of the alterations in muscle metabolism observed in the participants with TS.

Patients with TS generated more ATP via all 3 biosynthetic pathways during the 5-minute exercise test. This result suggests that exercise at a similar moderate intensity requires more energy to perform for patients with TS than for HC subjects, which may be indicative of some metabolic inefficiency. However, the PCr recovery time constants after 90 seconds and 5 minutes of exercise were not significantly different, and this variable has been used as an indicator of mitochondrial function. Our observation that patients with TS had a slower recovery of PCr after 30 seconds of exercise is of note, given that this test was designed to stress the high-energy phosphate system. It is possible that with the longer exercise durations of the 90-second and 5-minute tests the aerobic oxidative system was activated more effectively than after 30 seconds of exercise in the TS group. Given the increasing contributions of aerobic oxidation and mitochondrial function to exercise of longer durations, in this case 90 seconds and 5 minutes vs 30 seconds, we suggest that participants with TS do not have a mitochondrial defect that would affect oxidation. Furthermore, it is possible that the differences in the pH response to the 30- and 90-second exercise bouts are the result of a previously described increase in the size of type IIa fibers with unchanged capillary blood flow in patients with TS, indicating impaired metabolic processes due to reduced oxygen supply in the muscle (30). Exercise, therefore, may be more metabolically taxing for patients with TS because of the increased size (with normal capillary blood flow) of their muscle fibers.

In addition to the factors listed above, other potential mediators of our findings may include subtle differences in cardiac function. Although our participants did not have a history of congenital heart disease, it is possible that other alterations in cardiac function exist in this population. For example, we detected significantly higher blood pressure and resting heart rate but did not see a difference in maximal heart rate achieved during the incremental cycling test. This may be indicative of altered resting sympathetic to parasympathetic nervous system balance as a cause for metabolic inefficiency during exercise in patients with TS (31). Further work is indicated in this area. The results of the current study provide a mechanistic rationale for physical activity and exercise and training interventions in individuals with TS. Overall exercise recommendations encourage increasing levels of habitual physical activity to promote health and well-being, rather than prescriptive exercise programs that may not be enjoyable or sustainable for the individual. With this caveat in mind, specific benefits may be realized by focusing on exercise to improve exercise tolerance in individuals with TS. Because it appears that exercise is more metabolically demanding for girls with TS, as evidenced by increased ATP production through all 3 bioenergetic pathways, exercise designed to increase aerobic and anaerobic capacity would be beneficial (32). Examples of training protocols designed to incorporate both systems include interval protocols in which higher intensity exercise is alternated with periods of low to moderate exercise, games such as soccer, or fitness classes such as spinning.

There are several limitations to this study. The primary limitation is the small sample size. Although we were able to recruit enough participants to approach the required sample size based on previous power calculations, our results should be confirmed in a larger cohort. It is also possible that hormone replacement therapy (GH and estrogen) could have affected the results. However, this is unlikely in the case of GH because this hormone has been shown to have positive effects on exercise performance, which is not what we identified in our participants with TS. Furthermore, those receiving estrogen replacement therapy at physiological doses could be considered comparable to HC participants who have endogenous estrogen production at same age. In addition, this study was limited to girls without congenital heart disease, which affects approximately 25% of the population with TS. More severe muscle and bioenergetics dysfunction may certainly be present in this group and are worthy of further study.

This is the first study to examine the complex relationship between exercise and metabolic function in TS using ^{31}P-MRS and demonstrates that participants with TS experience a difference in the metabolic demands of exercise compared with girls without TS. It has been well documented that women with TS exhibit many characteristics of the metabolic syndrome: insulin resistance, increased central adiposity with elevated visceral fat stores, and hypertension (9, 33, 34). These symptoms are known to impair exercise performance, and indeed, metabolic syndrome has also been associated with defects in oxidative phosphorylation. ^{31}P-MRS revealed a reduced level of resting Pi in girls and adolescents with TS, characteristic of the metabolic syndrome. We therefore suggest that compared with BMI-, age-, and fitness-matched HC subjects, patients with TS experience exercise intolerance due to differences in the function of bioenergetic pathways.

Acknowledgments

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This work was supported by Physician Services Inc and the Adrian and Rita Hudson Foundation and a SickKids Restracomp Fellowship Grant.

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Disclosure Summary: The authors have nothing to disclose.

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