

In-vivo skeletal muscle mitochondrial function in Klinefelter syndrome

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ABSTRACT

Klinefelter syndrome (XXY) occurs in 1 in 600 males, resulting in testosterone deficiency and a high prevalence of insulin resistance. Testosterone deficiency in men is a known cause of insulin resistance, and mitochondrial dysfunction is hypothesized to mediate this relationship. The aim of this cross-sectional study was to evaluate muscle mitochondrial function in XXY compared with male controls. Twenty-seven boys with XXY (age 14.7 ± 1.8 years) were compared with 87 controls (age 16.9 ± 0.9). In-vivo calf muscle mitochondrial function was assessed via phosphorus magnetic resonance spectroscopy (^{31}P -MRS) following 90 s of isometric 70% maximal exercise. Multiple linear regression was used to compare ^{31}P -MRS outcomes (ADP and phosphocreatine (PCr) time constants, rate of oxidative phosphorylation (Oxphos), and Q_{\max} or the maximal mitochondrial function relative to mitochondrial density) between groups after adjusting for age differences. There were no statistically significant differences in the mitochondrial outcomes of ADP, Oxphos, PCr, and Q_{\max} between the groups. There were also no differences in a sensitivity analysis within the XXY group by testosterone treatment status. In this study, in-vivo postexercise skeletal muscle mitochondrial function does not appear to be impaired in adolescents with XXY compared with controls and is not significantly different by testosterone treatment status in XXY.

BACKGROUND

Klinefelter syndrome (XXY) is the most common sex chromosome aneuploidy (SCA) in humans and is most commonly in the 47, XXY form.¹ Phenotypically, this additional X chromosome has a wide variety of effects on the affected individuals, but nearly all adults with XXY have testicular insufficiency resulting in hypogonadism and infertility.² Additionally, adults with XXY have a higher risk of metabolic syndrome and type 2 diabetes,^{2–6} and cardiovascular diseases (CVD) are the leading cause of death in men with XXY.^{7–9} Due to the universal testicular dysfunction associated with XXY, it is presumed that hypogonadism contributes to this increased CVD risk; however, the mechanisms are elusive. Prior evidence in male populations without XXY demonstrates that

testosterone deficiency leads to mitochondrial dysfunction, and this mitochondrial dysfunction is the common pathway that contributes to insulin resistance, metabolic syndrome, type 2 diabetes, and CVD risk.¹⁰ Despite the known effect of male hypogonadism on mitochondrial function, there is a paucity of research on mitochondrial function in XXY.

The primary function of mitochondria is to generate energy (ATP) by oxidizing glucose and fatty acids. Mitochondrial function can be assessed with multiple modalities, each having strengths and weaknesses. Phosphorus magnetic resonance spectroscopy (^{31}P -MRS) can be used to assess in-vivo skeletal muscle mitochondrial function, a technique used extensively by our research group.^{11 12} During exercise, phosphocreatine (PCr) decreases and inorganic phosphate (Pi) increases. The return of PCr and Pi to baseline during the recovery phase—PCr and ADP time constants, respectively—reflects the oxidative phosphorylation (Oxphos) capacity of the mitochondria. This technique is physiologically relevant and non-invasive, reflecting skeletal muscle mitochondrial function in the specific muscle being tested.

As mitochondrial impairment has been associated both with hypogonadism and with insulin resistance, we aimed to investigate skeletal muscle mitochondrial function using ^{31}P -MRS in adolescent boys with XXY compared with typical male controls. We hypothesized that in-vivo postexercise mitochondrial function would be lower in XXY than in the controls.

METHODS

This was a cross-sectional study in pubertal boys with non-mosaic 47, XXY karyotype compared with male controls. Boys with XXY were recruited from the eXtraordinary Kids Clinic at Children's Hospital Colorado and through advertisements in a family advocacy group for SCA conditions (Association for X & Y Variations).¹³ Half of the boys with XXY had been on exogenous testosterone for a year or greater (treated), while the testosterone naïve group had not had any exogenous testosterone exposure. Controls were selected from two cohort studies that had performed the same procedures for assessment of in-vivo mitochondrial function: Exploring Perinatal Outcomes in Children



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and SEARCH for Diabetes in Youth.^{14–16} Inclusion criteria for both cases and controls included male sex, age 12–18, pubic hair Tanner stage 2–5 (pubertal), no diagnosis of diabetes mellitus, and no cognitive, psychiatric, or physical impairment preventing them from completing the study. Written parental consent and subject assent was obtained.

The subjects completed the 3-day physical activity recall and the average total daily metabolic equivalent was used to assess habitual physical activity.¹⁷ All assessments were performed after 3 days of no strenuous exercise. Fasting triglycerides and high-density lipoprotein (HDL) cholesterol were measured by standard clinical assays for all subjects, and total testosterone was measured by liquid chromatography tandem mass spectroscopy in the XXY group only. The ratio of fasting triglycerides to HDL was used as a surrogate of insulin resistance.¹⁸

Exercise testing was performed to determine the subjects' maximal volitional contraction (MVC), and MRI scout images of the cross-sectional area of the calf muscles were obtained to verify this measurement using methods previously published.¹¹ As previously described, the subject lays supine with their leg strapped immediately above the knee and across the hip to minimize contribution from the thigh muscles, and the arms crossed to minimize any force from the upper body. The exercise bench had a foot pedal connected to a force transducer box, and the subject was verbally coached to push the foot pedal at 70% MVC for 90 s. A dual-tuned ¹H/³¹P coil was wrapped around the calf to allow measurement of phosphorus spectra at rest, during the 90 s of exercise and after exercise. Fully relaxed spectra were collected for normalization, prior to the exercise protocol. Spectroscopy analysis was conducted as previously described.¹¹ Briefly, all spectra were fit with jMRUI, and exercise spectra were normalized with the relaxed spectra data. Metabolic calculations based on the spectra were performed as previously described, and time constants were calculated with curve fitting (SigmaPlot V.13.0; Systat Software, San Jose, California, USA). Outcome variables included ATP time constant (shorter means better function), rate of Oxphos (higher means better function), PCr time constant (shorter means better function), and Q_{max}, which is a calculation for the maximal mitochondrial function with respect to the mitochondrial density in the muscle cross section (higher means better function).

Descriptive statistics were used to compare the groups. Multiple linear regression was used to compare mitochondrial function outcomes with group, age, body mass index (BMI), and race as independent variables, and violin plots were created to visualize the data. We also conducted a sensitivity analysis based on testosterone treatment status within the XXY group. Finally, we tested for correlations with possible predictors including age, BMI, habitual physical activity, and insulin resistance with the two groups combined. Results were considered significant at a p value of 0.05. Analyses were performed using RStudio V.1.3.1093 and R V.4.0.3, and figures were created with GraphPad Prism V.8.4.0.

RESULTS

Demographics for the 27 boys with XXY and 87 controls are shown in table 1. The XXY group was 2 years younger

Table 1 Descriptive characteristics of study subjects

	XXY (Klinefelter syndrome) (n=27)	Controls (n=87)	P value†
Age (years)	14.7±1.8	16.9±0.9	<0.01*
Race/ethnicity, n (%)			0.02
Non-Hispanic white	21 (78)	52 (60)	
Hispanic	3 (11)	31 (36)	
Black	3 (11)	4 (5)	
Height z-score	1.3±1.1	0.2±0.9	<0.01*
Weight z-score	0.8±1.3	0.5±1.1	0.19
BMI z-score	0.19±1.55	0.37±1.18	0.51
Pubertal stage, n (%)			0.17
Tanner 2	2 (7.4)	1 (1.1)	
Tanner 3	0	1 (1.1)	
Tanner 4	15 (55.6)	32 (36.8)	
Tanner 5	10 (37.0)	53 (60.9)	
Average daily METs	68±20	71±16	0.44
Muscle area (cm ³)	3175 (2949, 3870)	4201 (3571, 5068)	<0.01*
Force (kg)	30.3 (24.7, 34.9)	37.9 (24.3, 32.4)	0.51
Triglyceride:HDL ratio	2.1 (1.6, 2.6)	1.6 (1.2, 2.3)	0.03*
Treatment with exogenous testosterone, n (%)	13 (48.1)	–	

Data presented as mean±SD, median (25%, 75%), or n (%).

*Statistically significant difference at an alpha of 0.05.

†Comparison between XXY and controls by Welch's t-test, Wilcoxon rank-sum test, or χ^2 test.

BMI, body mass index; HDL, high-density lipoprotein; METs, metabolic equivalents.

and less ethnically diverse than the controls, but reported similar habitual physical activity. Approximately half of them were treated with testosterone at the time of the study, but serum testosterone concentrations did not differ (XXY treated 332 ng/dL (278–398) vs XXY untreated 379 ng/dL (290–436), p=0.72). There were no significant differences in mitochondrial function outcomes between the XXY and control groups (figure 1A) or by testosterone treatment status with the XXY group (figure 1B). Furthermore, mitochondrial function outcomes did not correlate with age, BMI, habitual physical activity, or insulin sensitivity in either group (p>0.05 for all).

DISCUSSION

This study is the first to specifically investigate muscle mitochondrial function in youth with XXY, a population known to be at risk of insulin resistance and related disorders. We found no differences in any of the mitochondrial outcomes between boys with XXY and controls. This would imply that mitochondrial function in skeletal muscle post-exercise in adolescent boys with XXY is comparable with their peers and not impaired by the additional X chromosome. In addition, testosterone treatment for 1 year does not significantly affect short duration submaximal postexercise measures of skeletal muscle mitochondria. Due to early identification of XXY in this cohort, hypogonadism was likely promptly treated; therefore, subjects were not exposed to a prolonged period of testosterone deficiency that could impair mitochondrial function.

Few studies have investigated the mitochondria in XXY. Two studies from the 1980s in men aged 16–35 years report abnormal testicular mitochondrial structure on

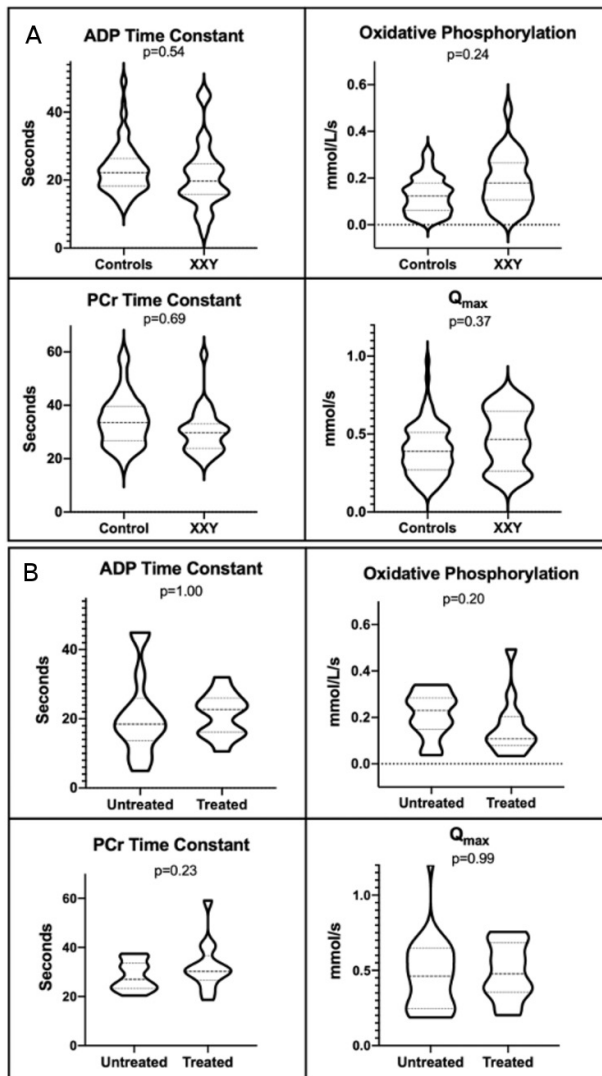


Figure 1 ^{31}P -MRS outcomes were not different in (A) XXY (Klinefelter syndrome) compared with controls or (B) between testosterone-treated and untreated individuals with XXY. The violin plot reflects the minimum, first IQR, median, third IQR, and maximum as the horizontal lines. The width of the violin plot represents the distribution of data points. ^{31}P -MRS, phosphorus magnetic resonance spectroscopy; PCr, phosphocreatine; Q_{max} , maximal mitochondrial function relative to mitochondrial density.

light and electron microscopy, including a lower number of cristae and presence of mitochondrial inclusions.^{19,20} Recently, through next-generation sequencing of mitochondrial DNA, a significant downexpression of nicotinamide adenine dinucleotide hydrogen (NADH):ubiquinone oxidoreductase core subunit 6 was discovered in 10 men with XXY compared with controls, which is a protein involved in complex 1 of the Oxphos chain.²¹ These studies suggest that there would be mitochondrial dysfunction in XXY, although it may be that the changes seen are tissue-specific. The subjects were older and likely untreated and thus had been exposed to hypogonadism for potentially a longer duration. None of these studies assessed for baseline exercise status, which is the most significant driver of skeletal muscle mitochondrial function.

There were several limitations and strengths in this study. This was a clinical sample of youth with XXY who were treated with testosterone when clinically indicated; therefore, chronic hypogonadism was likely not present even within the untreated group, as illustrated by the similar serum testosterone concentrations. Additionally the XXY group was younger than the controls, although muscle mitochondrial function increases during puberty, and if anything this would bias the data toward a difference and our data indicate the opposite.²² Physical activity was low and similar between the groups; thus, any differences due to hormones or genetics are more likely to be detected. Despite these limitations, this novel study used rigorous methods to measure muscle mitochondrial function in XXY adolescents for the first time. Potential future studies include investigating resting energy expenditure as a measure of whole body mitochondrial function, performance of more aerobic protocol to assess fat oxidation rather than an acute, short protocol, and other measures of tissue-specific mitochondrial function to further investigate the relationship between hypogonadism and CVD risk in XXY.

In conclusion, using ^{31}P -MRS to investigate postexercise recovery in the calf muscle, there were no differences in muscle mitochondrial function between adolescents with XXY and controls and testosterone treatment status in XXY did not impact mitochondrial function. Although the skeletal muscle mitochondrial function was found to be similar with this modality, further investigation of mitochondrial function in other tissues or with other exercise perturbations could help determine if mitochondrial dysfunction is an underlying mechanism of the known CVD risk in XXY.

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Competing interests None declared.

Patient consent for publication Not required.

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