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## Short Communication

## Arginine kinetics are altered in a pilot sample of adolescents and young adults with Barth syndrome

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## ABSTRACT

Barth syndrome (BTHS) is a rare, X-linked cardiomyopathy that is characterized by abnormalities in glucose and lipid metabolism, with less known regarding amino acid metabolism. This pilot study characterized whole-body arginine kinetics and found lower arginine rate of appearance into plasma ( $0.69 \pm 0.09$  vs.  $0.88 \pm 0.06$   $\mu\text{mol/kgFFM/min}$ ,  $p < 0.01$ ) and arginine non-oxidative disposal rate ( $0.64 \pm 0.11$  vs.  $0.80 \pm 0.03$   $\mu\text{mol/kgFFM/min}$ ,  $p < 0.02$ ) in adolescents and young adults with BTHS compared to Controls. This study provides a foundation for more in-depth studies on how arginine and potentially other amino acid abnormalities contribute to the pathology and clinical manifestations of BTHS.

## 1.1. Introduction

Barth syndrome (BTHS) is a rare, X-linked cardioskeletal myopathy caused by recessive mutations in the tafazzin (TAZ) gene [1] resulting in metabolic disturbances including an increased monolysocardiolipin to cardiolipin ratio, 3-methylglutaconic aciduria [2], hypocholesterolemia [3]. Although dysregulated cardiac [4] and skeletal muscle [5] glucose and fatty acid metabolism and energetics [6] have been reported, much less in known about amino acid metabolism in BTHS.

Low plasma arginine concentration in BTHS [7–9] has received considerable clinical attention and a proportion of patients have been treated with oral L-arginine and L-citrulline amino acid supplementation [10]. However, other features of arginine metabolism, including arginine kinetics, are not known in BTHS. Therefore, the objective of this pilot study was to characterize whole-body arginine kinetics in a pilot sample of adolescents and young adults with BTHS.

## 1.2. Methods

## 1.2.1. Participants

Nine ( $n = 9$ ) participants:  $n = 5$  individuals with BTHS, and  $n = 4$  healthy non-affected controls matched for age, height, weight, body-mass index (BMI), and activity level participated. All participants were considered sedentary defined as participation in routine exercise  $\leq 2 \times$ /week. Participants with BTHS were recruited from the Barth Syndrome Foundation Registry located at the University of Florida. Control participants were recruited from the Volunteers for Health at Washington University School of Medicine and the surrounding St. Louis community. Studies were approved by the Human Studies Committee at Washington University in St. Louis and all participants and parents (of adolescents) provided written informed consent.

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### 1.2.2. Body composition

Each participant underwent whole-body dual energy x-ray absorptiometry (DXA) scans (Lunar iDXA; GE Healthcare Lunar, Madison, WI) to determine regional and composite fat-free mass (FFM) and fat mass (kg) and percentage.

### 1.2.3. Arginine kinetics

The evening prior to the study visit, participants consumed a standardized meal and ingested a carbohydrate beverage as previously described [5]. Participants reported fasted to the Washington University Clinical Research Unit the following day at 0700 h. A catheter was inserted into an antecubital vein and used to administer stable isotopically-labeled tracers. A second catheter was inserted into a hand vein on the contralateral arm; the hand was heated (55 °C) using a thermostatically controlled box to obtain arterialized venous blood samples [11]. Following 30-min of rest, a constant intravenous infusion of [U-<sup>13</sup>C<sub>6</sub>]arginine (Cambridge Isotope Laboratories, Andover, MA; 0.04 μmol/kg fat-free mass (FFM)/min) was initiated and maintained for a 360-min study period [12]. Blood and breath samples were collected in vacutainers before starting the tracer infusions to quantify background arginine and <sup>13</sup>CO<sub>2</sub> enrichments, and collected every 30 min during the last 120 min of the study to quantify hormone levels, substrate levels, and arginine kinetics [13]. Whole-body oxygen consumption ( $\dot{V}O_2$ ) and carbon dioxide production ( $\dot{V}CO_2$ ) was measured continuously for 15 min at 300 min of the study using indirect calorimetry (Parvo Medics, Sandy, UT).

## 1.3. Sample analyses

### 1.3.1. Plasma substrate and hormone concentrations

Plasma glucose and insulin concentrations were determined as previously described [5]. Plasma arginine quantification was performed at St. Louis Children's Hospital via tandem mass spectrometric analysis. For kinetic analysis, plasma amino acids were converted into their 5-dimethylamino-1-naphthalene sulfonamide derivatives, and arginine tracer-to-tracee (TTR) ratio was measured by selected reaction monitoring on a triple quadrupole mass spectrometer using precursor ion *m/z* 408 to product ion 391 for arginine and precursor ion *m/z* 414 to product ion 397 for <sup>13</sup>C<sub>6</sub>-arginine [14]. The chromatographic separation was achieved with a SynergiTM Max-RP column (150 × 2 mm, 4 μm; Phenomenex, Torrance, CA, USA) with a security guard cartridge system (Phenomenex). The isotope analysis was carried out using a TSQ Vantage triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a heated electrospray ionization (HESI) source.

### 1.3.2. Arginine kinetics

Plasma arginine rate of appearance (Ra) into plasma was calculated by dividing the tracer infusion rate by the average TTR obtained during the last 120 min of the study period. Plasma arginine oxidation rate was determined by dividing breath <sup>13</sup>CO<sub>2</sub> production ([<sup>13</sup>CO<sub>2</sub> TTR/6] ×  $\dot{V}CO_2$  production) by the plasma arginine TTR. Non-oxidative disposal rate was determined by subtracting arginine oxidation rate from arginine Ra [13]. Arginine incorporation into glucose was determined by measuring <sup>13</sup>C-glucose enrichment with gas chromatography-combustion-isotope ratio mass spectrometry (Thermo Finnigan Delta+XL GC-C-IRMS) coupled with a 6890 GC (Agilent Technologies).

## 1.4. Statistical analysis

All data were evaluated for normality. Independent *t*-tests or Mann-

Whitney *U* test were used to compare differences between groups depending on normality. A two-tailed *P*-value of ≤0.05 was considered statistically significant.

## 1.5. Results

### 1.5.1. Demographics and body composition

Participants did not differ in age, height, or total body mass but individuals with BTHS had significantly lower FFM (kg) than Control (Table 1).

### 1.5.2. Arginine kinetics and amino acid concentration

Arginine Ra into plasma and arginine non-oxidative disposal rate were lower in BTHS vs. Control. Arginine oxidation rate, metabolic clearance rate, and arginine metabolite incorporation into glucose were not different between groups.

## 1.6. Discussion

The primary finding of our study was that arginine rate of appearance was lower in adolescents and young adults with BTHS, even when normalized for lower fat-free mass and is the first evidence of dysregulated arginine kinetics in BTHS. These data suggest that reduced arginine rate of appearance might contribute to lower plasma arginine concentration in BTHS however; these data need to be confirmed in a larger, more expansive study. In addition, non-oxidative disposal was also lower in those with BTHS compared to healthy controls. Lastly, we also found that arginine incorporation into glucose was not different between those with and without BTHS. We had previously shown that cardiac and skeletal muscle glucose utilization is upregulated in BTHS [4,5,7]. On the basis of these observations, we hypothesized that a greater amount of arginine might be incorporated into glucose (i.e. higher gluconeogenesis) in those with BTHS; however, this does not appear to be the case in this small sample. A limitation of this study was the small sample size thus larger and more in-depth studies of arginine metabolism in BTHS would help clarify the role of arginine kinetics in low plasma arginine concentration and potentially other clinical manifestations of BTHS.

In conclusion, arginine kinetics were dysregulated in a small pilot sample of adolescents and young adults with BTHS. This study provides

**Table 1**  
Demographics and arginine kinetics.

	BTHS	Control	<i>P</i> value
<b>Demographics</b>			
Age	25.0 ± 7.5	29.8 ± 6.0	0.34
Height (cm)	179.6 ± 6.7	182.9 ± 1.8	0.38
BMI	19.8 ± 5.4	24.1 ± 2.7	0.41
Total mass (kg)	61.5 ± 19.9	76.6 ± 9.9	0.21
FFM (kg)	42.1 ± 9.8	59.7 ± 4.9	0.01
FFM (%)	71 ± 10	78 ± 4	0.19
Fat mass (kg)	19.4 ± 11.1	16.9 ± 5.2	0.69
Fat mass (%)	29 ± 10	22 ± 4	0.19
Insulin (μU/mL)	11.5 ± 3.9	3.8 ± 2.7	<0.01
Glucose (mg/dL)	90.8 ± 9.7	87.6 ± 2.5	0.47
<b>Arginine kinetics</b>			
Arginine concentration (μmol/L)	78.4 ± 20.2	94.5 ± 28.8	0.19
Arg Ra (μmol/kgFFM/min)	0.69 ± 0.09	0.88 ± 0.06	<0.01
Arg Ox (μmol/kgFFM/min)	0.05 ± 0.04	0.08 ± 0.05	0.19
Arg Non-Ox Disp (μmol/kgFFM/min)	0.64 ± 0.11	0.80 ± 0.03	<0.03
MCR (L/kgFFM/min)	0.009 ± 0.003	0.010 ± 0.002	0.77
Arg/Glu enrichment (%)	0.06 ± 0.01	0.05 ± 0.03	0.85

FFM: fat free mass, Ra: rate of appearance into plasma, Ox: arginine oxidation rate, MCR: metabolic clearance rate, Arg: arginine, Glu: glucose.

a foundation for more in-depth studies on how arginine and potentially other amino acid abnormalities contribute to the pathology and clinical manifestations of BTHS.

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### Details of ethics approval

Studies were approved by the Human Research Protection Office at Washington University in St. Louis.

### Patient consent statement

All minor participants provided assent and all participants and/or parents provided written informed consent.

### Declaration of Competing Interest

W. Todd Cade, Kathryn L. Bohnert, Bruce W. Patterson, Adam J. Bittel, Shaji K. Chacko, Christina A. Pacak, Barry J. Byrne, Hilary J. Vernon, and Dominic N. Reeds declare that they have no conflict of interest.

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