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Blunted Fat Oxidation upon Submaximal Exercise is Partially Compensated by Enhanced Glucose Metabolism in Children, Adolescents and Young Adults with Barth Syndrome

W. Todd Cade^{1,2}, Kathryn L. Bohnert¹, Linda R. Peterson², Bruce W. Patterson², Adam J. Bittel¹, Adewole L. Okunade², Lisa de las Fuentes², Karen Steger-May³, Adil Bashir^{4,5}, George G. Schweitzer², Shaji K. Chacko⁶, Ronald J. Wanders⁷, Christina A. Pacak⁸, Barry J. Byrne⁸, and Dominic N. Reeds²

¹Program in Physical Therapy, 4444 Forest Park Avenue, Washington University School of Medicine, St. Louis, MO ²Department of Medicine, Washington University School of Medicine, St. Louis, MO ³Division of Biostatistics, Washington University School of Medicine, St. Louis, MO ⁴Department of Radiology, Washington University School of Medicine, St. Louis, MO ⁵Department of Electrical and Computer Engineering, Auburn University, Auburn, AL ⁶Department of Pediatrics, Baylor College of Medicine, Houston, TX ⁷Department of Pediatrics, University of Amsterdam, Amsterdam, The Netherlands ⁸Department of Pediatrics, University of Florida, Gainesville, FL

Summary:

Barth syndrome (BTHS) is a rare X-linked condition resulting in abnormal mitochondria, cardioskeletal myopathy, and growth delay however; the effects of BTHS on substrate metabolism regulation and their relationships with tissue function in humans are unknown. We sought to characterize glucose and fat metabolism during rest, submaximal exercise, and post-exercise rest in children, adolescents and young adults with BTHS and unaffected controls and examine their relationships with cardio-skeletal energetics and function. Children/adolescents and young adults with BTHS (n=29) and children/adolescent and young adult control participants (n=28, total n=57) underwent an infusion of 6'6'H₂ glucose and U-¹³C palmitate and indirect calorimetry during rest, 30-minutes of moderate exercise (50% V̇O₂peak) and recovery. Cardiac function, cardioskeletal mitochondrial energetics and exercise capacity were examined via echocardiography, ³¹P magnetic resonance spectroscopy and peak exercise testing, respectively. Glucose turnover rate was significantly higher in individuals with BTHS during rest (33.2 ± 9.8 vs. 27.2 ± 8.1 μmol/kgFFM/

Address correspondence to: W. Todd Cade, PT, PhD, Washington University School of Medicine, Campus Box 8502, 4444 Forest Park Avenue, St. Louis, MO 63108, tcade@wustl.edu, Telephone: (314) 286-1432, Fax: (314) 286-1410.

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Conflict of Interest:

W. Todd Cade declares, Kathryn L. Bohnert, Linda R. Peterson, Bruce W. Patterson, Adam J. Bittel, Adewole L. Okunade, Lisa de las Fuentes, Karen Steger-May, Adil Bashir, George G. Schweitzer, Shaji K. Chacko, Ronald J. Wanders, Christina A. Pacak, Barry J. Byrne, and Dominic N. Reeds declare that they have no conflict of interest.

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.

min, $p<0.01$) **and** exercise (34.7 ± 11.2 vs. 29.5 ± 8.8 $\mu\text{mol/kgFFM/min}$, $p<0.05$) and **tended to be higher** post-exercise (33.7 ± 10.2 vs. 28.8 ± 8.0 $\mu\text{mol/kgFFM/min}$, $p<0.06$) compared to Controls. Increases in total fat (-3.9 ± 7.5 vs. 10.5 ± 8.4 $\mu\text{mol/kgFFM/min}$, $p<0.0001$) and plasma fatty acid oxidation rates (0.0 ± 1.8 vs. 5.1 ± 3.9 $\mu\text{mol/kgFFM/min}$, $p<0.0001$) from rest to exercise were severely blunted in BTHS compared to Controls. Conclusion: An inability to upregulate fat metabolism during moderate intensity exercise appears to be partially compensated by elevations in glucose metabolism. Derangements in fat and glucose metabolism are characteristic of the pathophysiology of BTHS.

Keywords

Barth syndrome; exercise; mitochondria; fatty acid

Introduction

Barth syndrome (BTHS) is a rare, X-linked recessive, multi-system disorder that results in cardiomyopathy, skeletal muscle myopathy, exercise intolerance, growth retardation and cyclic neutropenia (Barth et al 1983; Clarke et al 2013). BTHS is particularly devastating with frequent mortality in infancy and adolescence (Barth et al 2004). Alterations in the tafazzin gene (*TAZ*, located on Xq28) cause BTHS by altering the protein tafazzin and impairing mitochondrial cardiolipin metabolism (Bione et al 1996). Tafazzin, a phospholipid-acyltransferase, remodels cardiolipin to its mature form and mutations in tafazzin result in mitochondrial abnormalities including smaller and fragmented mitochondria (Wang et al 2014), disruptions in supercomplex formation (Xu et al 2016), inner mitochondrial membrane instability (Zhang et al 2002) and reduced respiratory capacity (Wang et al 2014).

Our group recently found impaired skeletal muscle and cardiac mitochondrial energetics in children, adolescents and young adults with BTHS (Bashir et al 2017). However, it is unknown if glucose and fat metabolism is altered and contributes to impaired energetics in these individuals. A prior study by our group suggest that at rest, the rate of insulin-stimulated glucose metabolism is increased but that whole-body fat oxidation is not different between people with BTHS and unaffected individuals (Cade et al 2013). However, to our knowledge, the effect of increasing energy demand (i.e. exercise) on glucose and fat metabolism has not been characterized in BTHS.

Fat oxidation significantly contributes to energy turnover during rest and submaximal exercise. Adenosine triphosphate (ATP) generation through skeletal muscle fatty acid (FA) oxidation is a multi-step process including transport (both cellular and mitochondrial), beta-oxidation, reducing equivalent formation (via TCA cycle) and oxidative-phosphorylation in the electron transport chain (Houten et al 2016). During rest and low to moderate intensity physical activity ($<65\%$ $\text{VO}_{2\text{peak}}$), plasma FA's are the major contributor ($\sim 60\%$) to skeletal muscle FA oxidation with the remainder of FA oxidation coming from circulating VLDL-TG and intramuscular triglycerides (Romijn et al 1993; van Loon et al 2003). During conditions of high energy requirement (i.e., exercise), abnormalities in mitochondrial

function impair energy production from FA's and will require alternative substrates including glucose derived from muscle glycogen or plasma glucose. If these substrates are insufficient, an energy deficit will occur altering both the substrate metabolism and function of the tissue (e.g., heart, skeletal muscle) and impairing whole-body function. We hypothesized that during higher energy-requiring activities (i.e., exercise), fat oxidation would be reduced in children, adolescents and young adults with BTHS compared to healthy controls, and that reduced fat oxidation would be associated with impaired skeletal muscle mitochondrial energetics and exercise tolerance. We further hypothesized that glucose metabolism (turnover) would be elevated during exercise to compensate for impaired fat oxidation. Therefore, the primary objective of the current study was to characterize glucose and fat kinetics during rest, moderate-intensity exercise and post-exercise recovery in children, adolescents and young adults with BTHS. Our secondary objective was to examine the relationships between glucose and fat metabolism, cardiac- and skeletal muscle mitochondrial energetics, cardiac function and exercise tolerance.

Participants

Fifty-seven participants were studied, including 30 children/adolescents (BTHS n=14, Control n=16) and 27 adults (BTHS n=15, Control n=12) between 8 and 36 years of age (Table 1). Participants with BTHS were recruited through the Barth Syndrome Registry located at the University of Florida and controls were recruited through non-affected siblings and local recruitment from the Greater St. Louis community. Inclusion criteria included 1) confirmed diagnosis of BTHS or healthy control, 2) age 8-36 years, 3) sedentary (physically active less than 2x/wk), 4) medically stable and stable on medications for 3 months, and 5) lived in North America, the UK, Europe, or other locations feasible for travel to the US. Control participants were matched for age and Tanner stage, to control for delayed puberty in BTHS. All participants completed the clinical metabolism studies however, skeletal muscle and cardiac ^{31}P -MRS were not performed in n=9 participants due to technical difficulties (one child) and intra-cardiac defibrillator placement (8 adults). Studies were approved by the Human Research Protection Office at Washington University in St. Louis. **All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.** All minor participants provided assent and all participants and/or parents provided written informed consent.

Methods

All participants completed the study procedures at the Washington University Institute for Clinical and Translational Sciences Clinical Research Unit (CRU) over a 2-day period.

Day #1 of Study Visit

Body Composition—Following a medical history and physical examination from the study physician, fat mass (kg and %) and fat-free mass (kg and %) in all participants were determined through air-displacement plethysmography (Bod Pod, Life Measurements Inc., Concord, CA).

Echocardiography—All participants underwent conventional two-dimensional (2-D), pulsed-wave Doppler, and tissue Doppler echocardiography (General Electric Vivid E9; Waukesha, WI, USA). Left ventricular (LV) mass was determined by 2D echocardiography according to recommendations of the American Society of Echocardiography (Lang et al 2005). Left ventricular (LV) end-diastolic and end-systolic volumes were determined using the method of discs and LV ejection fraction was calculated. LV end-diastolic and end-systolic dimensions (LVED and LVES) were measured in the parasternal short-axis view. Fractional shortening was calculated as (LVED-LVES)/LVED. 2D speckle tracking echocardiographic-derived global peak systolic strain was determined from the apical 4 chamber, 2 chamber and apical long axis views and calculated as the average of the basal, mid- and apical segments of all walls.

Cardiac and Skeletal Muscle Mitochondrial Energetics—Cardiac and skeletal muscle mitochondrial energetics was determined by ^{31}P Phosphorus Magnetic Resonance Spectroscopy (^{31}P -MRS) as previously described (Bashir et al 2017).

Graded Exercise Test—Participants perform a graded exercise on a recumbent cycle ergometer (Lode, the Netherlands). Continuous ECG/BP and oxygen consumption (O_2) was recorded during the test to determine peak exercise capacity ($\text{O}_{2\text{peak}}$, ParvoMedics, Sandy, UT). The graded exercise protocol consisted of 1-min incremental stages, beginning with a 10(BTHS) or 20(Control)-watt workload, progressing every minute by 10- or 20-watts until volitional exhaustion or symptom limitation.

Evening Prior to Day #2 of Study Visit:

The evening prior to Day 2 of the study visit, participants consumed a standardized meal containing a total of 12 kcal/kg body wt (55% of total energy from carbohydrates, 30% from fat, and 15% from protein) prepared by the Bionutrition Research Kitchen at the Washington University CRU. At 1900 h, participants ingested a carbohydrate beverage (80 gm carbohydrates, 12.2 gm fat, 17.6 gm protein, Boost; Nestle, Vevey, Switzerland) to ensure adequate muscle and hepatic glycogen stores. Participants were instructed to fast from 2000 h (except water) until the study visit the following morning.

Day #2 of Study Visit:

Substrate Metabolism Kinetics—Participants reported fasted to the Washington University CRU at 0700 h. A catheter was inserted into an antecubital vein and used to administer stable isotope-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 (Jensen and Heiling 1991). Following 30-min of rest, a bolus of ^{13}C -labeled sodium bicarbonate ($1.5 \mu\text{mol/kg}$) and constant intravenous infusions of $[6,6\text{-}^2\text{H}_2]\text{glucose}$ ($0.25 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with a $22.5 \mu\text{mol/kg}$ priming dose) and $[\text{U-}^{13}\text{C}]\text{palmitate}$ ($12 \text{ nmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, unprimed) were initiated during a 210-min (120-min rest, 30-min exercise at 50% of $\text{O}_{2\text{peak}}$, and 60-min post-exercise recovery) study period. The 30-min moderate-intensity exercise protocol to quantify FA oxidation has been successfully used in other mitochondrial and nutrient storage diseases (Jeppesen et al 2009; Orngreen et al 2009). Blood and breath samples were collected in vacutainers before starting

the tracer infusions to quantify background glucose, palmitate, and $^{13}\text{CO}_2$ enrichments, and collected every 10 min during the last 30 min of the rest and post-exercise recovery periods and every 5 min during the last 10 min of the exercise period to quantify hormone levels, substrate levels, and glucose and FA kinetics. Whole-body oxygen consumption (O_2) and carbon dioxide production (CO_2) were measured continuously for 15 min during rest/post-exercise and intermittently (2 min x 3) during exercise using indirect calorimetry (Parvo Medics, Sandy, UT). The study paradigm is depicted in Figure 1. All tracers came from Cambridge Isotope Laboratories (Andover, MA). Palmitate oxidation rate was corrected for incomplete labeled CO_2 recovery by an acetate correction factor during rest and post-exercise recovery as previously determined by our group (Cade et al 2013). We used a previously published acetate recovery factor in healthy young adults for the correction of exercise kinetics (Sidossis et al 1995). We did not individually determine acetate recovery factors for each participant due to participant burden and in particular for children and adolescents (e.g., additional overnight stay, exercise session, catheter insertions).

Sample Analyses

Plasma substrate and hormone concentrations.—Plasma glucose concentration was determined by using an automated glucose analyzer (YSI 2300 STAT PLUS, Yellow Springs Instrument Co., Yellow Springs, OH). Plasma insulin and leptin concentrations were measured by using a chemiluminescent immunometric method (IMMULITEVR, Siemens, Los Angeles, CA). Plasma free fatty acid concentrations were quantified by gas chromatography (HP 5890-II, Hewlett-Packard, Palo Alto, California) (Reeds et al 2006). Plasma palmitate and glucose tracer-to-tracee (TTR) ratios were measured by using selected ion-monitoring electron impact ionization GC-MS (Hewlett-Packard MSD 5973 system) as previously described (Patterson et al 1999).

Glucose and palmitate kinetics.—Plasma glucose and palmitate rate of appearance (R_a) were calculated by dividing each tracer infusion rate by the average TTR obtained during the last 20 min of rest, last 10 min of exercise, and last 20 min of recovery periods. Palmitate rate of disappearance was assumed equal to palmitate R_a during rest. Steele's equation for non-steady-state conditions (Steele 1959) was used to calculate kinetics during exercise and recovery periods.

Substrate oxidation.—Total lipid and carbohydrate oxidation rates were calculated from rates of volume of oxygen consumed (O_2) and CO_2 as described by Frayn (Frayn 1983). Plasma palmitate oxidation rate was determined by dividing breath $^{13}\text{CO}_2$ production ($[\text{^{13}CO}_2 \text{ TTR}/16] \times \text{CO}_2 \text{ production}$) by the plasma palmitate TTR. This value was corrected for $^{13}\text{CO}_2$ recovery as described above (Sidossis et al 1995; Cade et al 2013). Plasma FA oxidation was calculated by dividing palmitate oxidation rate by the proportional contribution of palmitate to total plasma FA concentration. Non-plasma FA oxidation was determined by subtracting plasma FA oxidation from total FA oxidation (as determined by indirect calorimetry). We assumed that intramuscular TG were the primary source of non-plasma FA oxidation and that plasma TG did not significantly contribute to oxidation during exercise (Kiens and Lithell 1989).

Statistics

Based on our preliminary data, the study was primarily powered to detect a $3.8 \pm 6.0 \mu\text{mol/kgFFM/min}$ difference in FA oxidation rate during exercise as a combined group (i.e., both children and adults). Demographics, energetic, cardiac and peak exercise testing variables between groups were compared by independent-sample t-tests and Mann Whitney U tests, depending on normality. Variables measured during rest, exercise, and post-exercise recovery were analyzed with mixed model repeated measures analyses of variance (RM-ANOVA) that included the group (i.e., Control vs. BTHS) by condition interaction. The primary model included all children/adolescent and adult individuals combined. Within the framework of the mixed model, statistical contrasts were used to assess the between-group equality of (a) values during each condition and (b) changes between exercise and post-exercise recovery.

An exploratory RM-ANOVA was performed separately for the children/adolescents and the adults. With one exception, these models are analogous to those performed for the combined group: When the overall model interaction was not significant, the between-group comparison of change between rest and exercise was expressed with the overall interaction p-value (that tests the equality of changes over all three conditions between groups) rather than the statistical contrast p-value (that tests the equality of change between the rest and exercise conditions between groups).

For all RM-ANOVAs, the Kenward-Roger method was used to compute the degrees of freedom, and the Akaike information criterion was used to identify the covariance structure among the repeated measures. Due to abnormally distributed residuals, the following variables were log-transformed prior to analysis: glucose, insulin, glucose turnover, plasma FA concentration, plasma FA R_a , plasma FA Ox, and non-oxidative palmitate disposal. Total glucose oxidation was rank-transformed. Relationships between variable were examined by Spearman Rank Correlation Coefficient analysis. Statistical significance was defined as a p-value of 0.05 or less.

Results

Demographics

Participant demographics are provided in Table 1. All participants were Caucasian except for one African American (AA) adult participant with BTHS, one AA adult control, one adult participant with BTHS who was of Middle Eastern descent, and two Asian adult controls. Participants with BTHS were currently taking: beta blockers (62%), ACE inhibitors (41%), digoxin (48%), granulocyte colony-stimulating factor (31%), anti-depressants (24%) and supplemental amino acids (48%). Age was not different between BTHS vs. Control in either children/adolescent or adult groups. Body weight and body mass index were significantly lower in BTHS vs. Control in both children/adolescents and adults. Absolute fat-free mass (kg) and fat-free mass expressed as percentage of total mass in children/adolescents and adults with BTHS were significantly lower than Controls.

Fasting Hormones and Metabolites

Plasma total and low-density cholesterol, insulin-like growth factor-1 (IGF-1) and serum creatinine concentrations were significantly lower in both children/adolescents and adults with BTHS compared to Controls (Table 1). Plasma cortisol concentration was significantly higher in adults with BTHS vs. Controls and tended to be higher in BTHS children/adolescents vs. Controls (Table 1).

Peak Exercise Testing and Cardiac Function

Peak exercise tolerance (i.e., O_{2peak}), peak work rate and peak heart rate was significantly lower in BTHS vs. Controls. Peak respiratory exchange ratio was significantly higher in BTHS vs. Controls. Resting heart rate was higher and resting systolic blood pressure and ejection fraction were lower in BTHS Compared to controls. Peak global strain tended ($p=0.08$) to be lower in BTHS vs. Controls (Table 2).

Cardiac and Skeletal Muscle Energetics

Cardiac ATP/PCr and skeletal muscle PCr recovery time and maximum oxidation capacity were significantly lower in participants with BTHS compared to Controls (Table 2).

Substrate Kinetics during Rest, Exercise and Post-Exercise Glucose Kinetics

Plasma glucose concentration was not different at rest, during exercise or post-exercise between groups however decreased during exercise to a greater extent in BTHS ($n=38$ at post-exercise due to missing data). Glucose turnover rate per kg of fat-free mass (FFM) was significantly higher in BTHS than Controls during the resting and post-exercise conditions. Glucose turnover rate per FFM increased with exercise in both groups; however, the mean value was higher in BTHS during exercise (Figure 2A, Table 3). During resting and post-exercise conditions, glucose oxidation rate was not different between groups. Exercise increased glucose oxidation rate in both groups; however, the relative increase above resting conditions was blunted in BTHS (Figure 2B, Table 3). Plasma lactate concentration was increased in both BTHS and Controls during exercise compared to rest and post-exercise periods. Further, plasma lactate concentration during exercise was significantly higher in BTHS compared to Controls (Table 3).

Fatty Acid Kinetics

At rest, plasma fatty acid (FA) rate of appearance per kg FFM was higher at rest and post-exercise in BTHS, but not different during exercise compared to Controls (Table 3). Plasma FA rate of appearance increased in Controls with exercise but did not increase in BTHS. Plasma FA concentration was higher at rest and during recovery ($p=0.08$) but not different during exercise in BTHS vs. Controls. Plasma FA concentration decreased with exercise in BTHS but did not change in Controls (Table 3). As expected fat oxidation from both plasma and non-plasma FA's increased in control subjects during exercise. Conversely, exercise did not increase total fat or plasma FA oxidation and suppressed non-plasma FA oxidation in BTHS (Figure 3A-D, Table 3).

Substrate Kinetics in Children/Adolescent vs. Adults

Although the study was not powered *a priori* to assess age group differences between BTHS and Controls (i.e. children/adolescents and adults), separate substrate metabolism data for children/adolescents and adults are presented in Supplemental Tables 1 and 2 respectively. Briefly, total lipid, plasma FA and non-plasma FA oxidation rates were significantly lower during exercise in both children/adolescents and adults with BTHS compared to Controls. In addition, change (i.e. delta) in total fat, plasma FA and non-plasma FA oxidation rates from rest to exercise was blunted in both children/adolescents and adults with BTHS compared to Controls. Glucose turnover rate at rest, during exercise and during recovery was found to be greater only in adults with BTHS compared to Controls. Plasma FA rate of appearance (Ra) at rest and during post-exercise recovery and plasma FA concentration (during all conditions) were only higher in adults with BTHS compared to Controls. Thus, combined group differences in glucose turnover rate, plasma FA rate of appearance and plasma free FA concentration appear to be primarily driven by adult participants with BTHS.

Relationships between Substrate Metabolism, Cardiac and Skeletal Muscle Energetics and Function

With BTHS and controls combined as an aggregate, a blunted ability to increase plasma FA oxidation rate from rest to exercise (i.e. delta) was significantly associated with lower skeletal muscle ($Q_{\max}$ $r=0.69$, <0.0001) and cardiac (PCr/ATP $r=0.56$, <0.02) energetics, lower $O_{2\text{peak}}$ ($r=0.62$, <0.0001), higher glucose turnover rate during exercise ($r=-0.41$, <0.01) and higher plasma lactate during exercise ($r=-0.67$, $p<0.0001$). However, when groups were analyzed independently, the associations did not remain significant. When BTHS was examined independently, lower increase in plasma FA oxidation rate from rest to exercise (i.e. delta) remained significantly associated with a higher glucose turnover rate during exercise ($r=-0.46$, <0.01) and higher work rate during exercise ($r=0.42$, <0.01). These relationships did not exist in controls when analyzed independently.

Discussion

Our findings suggest that BTHS is characterized by a profound inability to increase fat oxidation in response to modest exercise and that energy deficits are partially compensated by enhanced glucose metabolism. This inability to increase fat oxidation during physical activity correlated with impaired skeletal muscle energetics and exercise intolerance. Whether blunted fat oxidation with exercise contributes to tissue level impairments in BTHS, however, is not completely clear. Overall, these data suggest that abnormalities in fat and glucose metabolism contribute to the pathophysiology in BTHS.

Fat oxidation rate was markedly lower during exercise in both children/adolescents and young adults with BTHS irrespective of whether these fats were derived from circulating FA or other sources such as intramyocellular lipid, suggesting that the primary defects lie in FA oxidation rather than cellular uptake. This notion is also supported by a reduction in plasma FA concentration in participants with BTHS suggesting circulating FA uptake into the exercising muscle. Strikingly, the ability to increase fat (including plasma FA and non-plasma FA) oxidation rate with exercise was almost completely absent in individuals with

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BTBS. Studies examining the effect of exercise on fat oxidation in other mitochondrial myopathies have produced variable results. Similar to our findings in BTBS, fatty acid oxidation rate was normal at rest, and while exercise-stimulated oxidation increased, it remained significantly blunted in young and middle-aged individuals with carnitine palmitoyltransferase 2 gene mutations (Orngreen et al 2005) and very long-chain acyl-CoA dehydrogenase deficiency (Orngreen et al 2004) (i.e. long-chain fatty acid oxidation disorders). In addition, in a patient with neutral lipid storage disease, the expected exercise-induced increase in lipid oxidation rate was almost completely absent (Laforet et al 2012). However, other studies conducted in patients with mutations in mitochondrial DNA failed to show impairments in exercise-induced FA oxidation (Jeppesen et al 2009). With chronic heart failure (a condition that share some characteristics with BTBS), lipid oxidation rate during submaximal intensity exercise was higher compared to controls (Riley et al 1990).

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Reducing equivalents generated from fat oxidation enter the electron transport chain at complexes I (NADH) and III (FADH₂) and lead to ATP production (Wang et al 2010). We found that limitations in exercise-stimulated fat oxidation capacity were associated with the degree of impairment of skeletal muscle and cardiac mitochondrial energetics and with exercise intolerance. This finding suggests that these abnormalities in fatty acid oxidation might directly contribute to impairments in cellular energetics in BTBS and thereby impair physical function. However, these associations were only found when both groups were examined together and did not remain significant in stratified analyses thus; this finding must be interpreted with caution. In the current study, we measured both plasma fatty acid oxidation and total fat oxidation using differing methods to dissect whether whole body fatty acid oxidation is reduced or whether there is a reduction merely in the capacity to extract and oxidize circulating free fatty acids. For plasma fatty acid oxidation kinetics, we quantified breath ¹³CO₂ content from labeled palmitate which produced in the TCA cycle where for total fat oxidation kinetics, we quantified VCO₂ production and VO₂ consumption rates which is based on the stoichiometric oxidation of long-chain fatty acids (typically palmitate) (Frayn 1983). While neither method can define the specific mechanistic pathways that are altered, the findings suggest that both beta-oxidation and oxidative phosphorylation are impaired and contribute to reduced cellular energetics in BTBS. These results are further supported by our recent study in BTBS mice that demonstrated significantly improved exercise tolerance and increased O₂ consumption rates in cardiac mitochondria following TAZ gene therapy (Suzuki-Hatano et al 2018).

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The precise molecular mechanisms resulting in the dramatically impaired ability to upregulate fat metabolism with modest exercise in BTBS are unclear. Mechanisms for impairments in fatty acid oxidation in long-chain fatty acid disorders are mostly characterized and include abnormalities in fatty acid degradation in the mitochondria (Knottnerus et al 2018). In BTBS, **alterations** in the tafazzin (TAZ) gene cause both reductions in and structural alterations in mitochondrial cardiolipin (Xu et al 2016). Disruptions in cardiolipin can result in alterations in several mitochondrial components including membrane fluidity, protein import, cristae content, ATP synthase function and ability of mitochondria to undergo fission, fusion and mitophagy (Wang et al 2014; Gaspard and McMaster 2015). In addition, defects in tafazzin result in destabilized mitochondrial

supercomplex formation (McKenzie et al 2006; Acehan et al 2011; Xu et al 2016). BTHS-associated mitochondrial abnormalities are believed to severely blunt mitochondrial respiration (i.e., maximal electron transport chain capacity) (Wang et al 2014) and whole-body oxygen consumption (Spencer et al 2011). Therefore, it might be postulated that the primary defects in BTHS occur at the level of the electron transport chain. In the current study, although fat oxidation rate was severely reduced during exercise, carbohydrate oxidation rate markedly increased during exercise, albeit to a lesser extent than controls. This suggests that impairments in fat oxidation might (also) occur upstream of the electron transport chain (e.g., beta-oxidation, TCA cycle, mitochondrial import, etc.). However, due to impaired immune function (i.e., neutropenia) in patients with BTHS, we were not able to obtain skeletal muscle biopsies to assess specific fatty acid oxidative pathways and thus further study of the mechanisms for impaired fat oxidation in BTHS is needed.

During exercise, long-chain fatty acids are released from adipose tissue (i.e., lipolysis) and delivered to the exercising skeletal muscle for uptake and oxidation. During exercise, lipolytic rate is tightly coupled with skeletal muscle oxidation rate and typically increases 2-3-fold from rest to submaximal exercise (Romijn et al 1993; Klein et al 1994). In the current study, increases in palmitate rate of appearance per fat-free mass with exercise was blunted in BTHS but it is not clear if this contributed to the lower plasma fatty acid oxidation rate in these individuals. Plasma free fatty acid concentration during exercise was similar between BTHS and controls indicating that fatty acid availability was similar between groups and therefore fatty acid availability did not appear to be responsible for reduced oxidation within skeletal muscle. **In addition, although unclear, lower adipose tissue lipolytic rate might suggest lower adipose tissue fatty acid mobilization and have contributed to the higher adipose tissue content (i.e. fat mass) in BTHS individuals.**

Enhanced glucose metabolism appeared to partially compensate for impaired fat oxidation during submaximal exercise in BTHS. This was evidenced by 1) higher glucose turnover rate compared to controls, 2) greater increases in plasma lactate compared to controls (possible evidence of increased glycolysis), 3) greater reductions in plasma glucose concentration compared to controls, and 4) retained ability to increase carbohydrate oxidation with exercise. In addition, we found that glucose turnover rate during exercise was inversely related to the ability to upregulate plasma fatty acid oxidation rate upon exercise in those with BTHS but not in healthy controls. Moreover, in the BTHS mouse model, we recently found increased liver glycogenolysis during exercise compared to wild-type mice (Schweitzer 2018). Together, these findings suggest that during conditions of elevated energy requirements above rest (e.g., modest exercise), individuals with BTHS have increased dependence on glucose metabolism. This is similar to observations made in other mitochondrial diseases; several groups have reported greater glucose turnover and plasma lactate concentration during moderate exercise in individuals with mitochondrial myopathies than in healthy controls (Vissing et al 1996). Also, in patients with mtDNA mutations, plasma lactate production was higher and lactate oxidation was not impaired during exercise compared to healthy controls (Jeppesen et al 2013).

These findings might have clinical implications for individuals with BTHS. Carbohydrates, particularly complex carbohydrates, should be recommended prior to exercise or sustained

physical activity and following exercise. A complex carbohydrate meal or snack would provide a slowly released energy source during exercise and upon post-exercise, a glycogen store (i.e. liver, skeletal muscle) replenishment for future activity needs. Anecdotally, several younger patients with BTHS consume oral cornstarch at nighttime to prevent nocturnal hypoglycemia. These anecdotal reports, along with the current research findings, suggest a significant reliance on glucose metabolism in BTHS. More research is necessary to determine post-prandial carbohydrate metabolism and clinical carbohydrate supplementation recommendations for exercise and other physical activities in BTHS.

Limitations

There were several limitations to our study. First, we quantified fat and glucose kinetics based on a relative-exercise intensity (i.e., O_{2peak}) rather than absolute intensity. We believe this was the most accurate method to assess substrate kinetics although there were large differences in exercise work rate between groups. In addition, we did not quantify acetate recovery factor during exercise in participants, which could have affected the results. We used a predetermined acetate recovery factor for resting conditions from a previous study in adolescents and young adults with BTHS (Cade et al 2013) and used a validated recovery factor during exercise in healthy controls (Sidossis et al 1995). Due to the participant burden of catheter placement, fatigue with exercise and an additional overnight stay (especially in children and adolescents), we did not perform the acetate recovery study. **Although not measured, travel- and procedure-related stress could have elevated plasma epinephrine concentration in participants with BTHS. Moreover, plasma cortisol concentration in adults with BTHS was higher than controls. In addition to affecting fatty acid metabolism (Jensen et al 1996), both epinephrine and cortisol might have influenced glucose metabolism (glycogenolysis and glycogenesis) (Coderre et al 1991; Kolnes et al 2015) in these individuals however, 1-2 days spanned the time between participant arrival and testing which might have somewhat mitigated this effect. Also, more than half of participants with BTHS were taking beta-blocker medication (of those taking beta-blocker approximately 60% were taking non-vasodilating beta-blockers) which has been reported to affect glucose and lipid metabolism (Fonseca 2010). Non-vasodilating beta-blockers appear to have a negative effect on glucose metabolism (Jacob et al 1996) while vasodilating beta-blockers either have a neutral (Bakris et al 2004) or insulin-sensitizing effect (Celik et al 2006). In the current study, beta-blocker medication could have slightly affected fatty acid and glucose metabolism in BTHS participants however, it was unsafe to study these individuals off medication. Unfortunately, our study was not powered to examine metabolic differences between BTHS participants taking vasodilating vs. non-vasodilating beta-blockers and those not taking beta-blockers.** While we were not powered to detect differences in substrate metabolism for individual age groups (i.e., children/adolescents and adults) we were still able to identify large impairments in fat oxidation found as a combined group existed in children/adolescents and adults when analyzed separately. We acknowledge that many statistical tests were performed and that there is an increased likelihood that any single test is significant by chance alone. To this end, we describe the results of this study using the combined weight of evidence across variables rather than any individual test. Lastly, we did not obtain muscle biopsies for the examination of molecular mechanisms of glucose and fat

metabolism due to 1) the increased risk for infection due to the frequent neutropenia in individuals with BTHS and 2) participant burden for children and adolescents.

Conclusion

In summary, we found severely blunted fat oxidation upon submaximal exercise in children/adolescents and young adults with BTHS that was associated with impaired skeletal muscle and cardiac energetics. In addition, elevated glucose metabolism appeared to compensate for fat oxidation defects in BTHS. Abnormalities in lipid and glucose metabolism contribute to the pathophysiology of BTHS and represent key outcome measures to be considered in future evaluations of potential BTHS therapeutics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Synopsis:

A severely blunted ability to upregulate fat metabolism during a modest level of physical activity is a defining pathophysiologic characteristic in children, adolescents and young adults with BTHS.

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Figure 1: Study Schematic for Substrate Kinetics Study.

IC: indirect calorimetry.

Figure 2: Glucose Metabolism during Rest, Exercise, and Post-Exercise.
A. Glucose turnover rate, B. Glucose oxidation rate. BTHS: Barth syndrome, μmol : micromole, FFM: fat free mass, Exs: exercise, PostExs: post-exercise.

Figure 3: Lipid Metabolism during Rest, Exercise and Post-Exercise.

A. Total lipid oxidation rate, B. Plasma FA oxidation rate, C. Non-plasma FA oxidation rate, D. Change in plasma FA oxidation rate from rest to exercise. BTHS: Barth syndrome, μmol : micromole, FFM: fat free mass, Exs: exercise, PostExs: post-exercise.

Table 1:**Participant Demographics**

	Children			Adults		
	Control (n=16)	BTHS (n=14)	p-value	Control (n=12)	BTHS (n=15)	p-value
Age (years)	12 ± 3	13 ± 3	0.50	24 ± 5	26 ± 4	0.26
Height (cm)	158.0 ± 18.3	143.8 ± 14.2	<0.02	176.6 ± 5.3	177.2 ± 7.3	0.80
Weight (kg)	52.5 ± 20.5	34.9 ± 12.4	<0.01	81.1 ± 11.7	68.1 ± 12.8	0.01
BMI	20.2 ± 4.6	16.5 ± 3.5	<0.01	26.1 ± 3.9	21.6 ± 3.4	<0.001
FFM (kg)	41.2 ± 15.4	29.6 ± 11.0	0.03	63.8 ± 6.8	41.1 ± 6.1	<0.001
FFM (%)	82 ± 9	72 ± 9	0.01	80 ± 7	63 ± 12	<0.001
FM (kg)	10.2 ± 8.0	12.8 ± 10.2	0.19	16.8 ± 7.3	26.1 ± 10.7	0.03
FM (%)	18 ± 9	28 ± 9	0.01	20 ± 7	37 ± 12	<0.001
Tanner Stage	2.3 ± 1.1	1.6 ± 1.1	0.44			
WBC (K/cumm)	5.2 ± 1.6	4.4 ± 2.5	0.02	5.8 ± 1.4	3.9 ± 1.2	0.001
RBC (M/cumm)	4.6 ± 0.4	4.2 ± 0.4	0.03	4.8 ± 0.2	4.7 ± 0.4	0.44
Hemoglobin (g/dL)	13.0 ± 1.5	12.3 ± 0.8	0.58	14.4 ± 0.8	14.1 ± 10	0.50
Hematocrit (%)	38.8 ± 4.0	35.6 ± 2.9	0.02	43.1 ± 2.1	41.7 ± 3.2	0.26
PC (K/cumm)	224.4 ± 28.4	215.9 ± 52.7	0.58	200.2 ± 23.7	193.3 ± 59.4	0.71
MCV (fL)	8.7 ± 0.6	9.2 ± 0.8	0.02	8.9 ± 0.9	10.6 ± 1.9	0.01
ANC (K/cumm)	2.8 ± 1.4	1.8 ± 2.2	<0.01	3.3 ± 1.1	1.3 ± 1.0	<0.001
ALC (K/cumm)	1.7 ± 0.4	1.6 ± 0.4	0.42	1.8 ± 0.7	1.6 ± 0.5	0.87
AMC (K/cumm)	0.5 ± 0.2	0.8 ± 0.4	0.01	0.5 ± 0.2	0.8 ± 0.4	0.04
AEC (K/cumm)	0.2 ± 1	0.2 ± 0.1	0.88	0.2 ± 0.1	0.2 ± 0.1	0.76
ABC (K/cumm)	0.0 ± 0.0	0.1 ± 0.1	0.04	0.0 ± 0.0	0.0 ± 0.1	0.21
Albumin (g/dL)	4.1 ± 0.2	3.8 ± 0.2	<0.001	4.4 ± 0.3	3.8 ± 0.3	<0.001
Calcium (mg/dL)	9.2 ± 0.2	9.4 ± 0.2	0.77	9.2 ± 0.3	8.9 ± 0.3	0.05
BUN (mg/dL)	13.1 ± 1.6	12.1 ± 3.0	0.25	16.3 ± 4.0	16.1 ± 4.5	0.87
Bilirubin (mg/dL)	0.3 ± 0.2	0.3 ± 0.2	0.82	0.8 ± 0.4	0.4 ± 0.2	0.01
AST (IU/L)	23.0 ± 4.8	30.8 ± 12.2	0.02	44.3 ± 56.4	23.6 ± 7.0	0.17
ALT (IU/L)	16.7 ± 6.7	25.0 ± 13.8	<0.01	28.8 ± 20.6	24.1 ± 13.5	0.48
Creatinine (mg/dL)	0.5 ± 0.1	0.4 ± 0.1	0.005	1.0 ± 0.1	0.7 ± 0.1	<0.001
CO2 (mmol/L)	21.2 ± 1.5	21.5 ± 2.7	0.69	24.9 ± 1.3	23.5 ± 0.9	0.005
Total Protein (g/dL)	6.5 ± 0.3	6.6 ± 0.4	0.66	6.8 ± 0.4	6.6 ± 0.4	0.26
TG (mg/dL)	64.8 ± 38.7	51.0 ± 21.1	0.25	96.7 ± 53.3	91.5 ± 33.6	0.76
Total Chol (mg/dL)	140.8 ± 20.8	108.7 ± 25.0	0.001	167.3 ± 24.1	126.1 ± 25.2	<0.001
HDL (mg/dL)	53.1 ± 11.1	47.3 ± 15.4	0.24	47.3 ± 11.1	40.0 ± 12.7	0.12
LDL (mg/dL)	74.8 ± 18.1	51.1 ± 16.5	0.001	101.4 ± 23.2	67.7 ± 23.2	0.001
IGF-1 (ng/mL)	229.7 ± 79.5	132.3 ± 82.4	0.002	188.9 ± 36.3	118.4 ± 35.5	<0.001
Cortisol (ug/dL)	8.6 ± 2.5	10.8 ± 5.3	0.26	9.8 ± 2.7	15.3 ± 6.8	0.03

Values are means $\pm$ SD. FFM: fat free mass, FM: fat mass, WBC: white blood cells, RBC: red blood cells, PC: platelet count, MPV: mean corpuscular volume, ANC: absolute neutrophil count, ALC: absolute lymphocyte count, AMC: absolute monocyte count, AEC: absolute eosinophil count, ABC: absolute basophil count, BUN: blood urea nitrogen, AST: aspartate amino transferase, ALT: alanine amino transferase, CO₂: carbon dioxide, TG: triglyceride, Chol: cholesterol, HDL: high-density lipoprotein, LDL: low-density lipoprotein, IGF-1: insulin-like growth factor.

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Table 2:**Peak Exercise and Cardiac Function**

Variables	Control (n=28)	BTHS (n=29)	p-value
Peak Exercise			
VO _{2peak} (ml/kgBW/min)	39.1 ± 9.1 (35.7, 42.5)	12.8 ± 3.4 (11.6, 14.0)	<0.0001
Peak Work Rate (watts)	191.4 ± 70.3 (181.3, 188.5)	50.2 ± 15.1 (44.7, 55.7)	<0.0001
Peak HR (bpm)	184.9 ± 9.7 (24.1, 30.4)	157.0 ± 15.1 (151.5, 162.5)	<0.0001
Peak RER	1.1 ± 0.1 (1.1, 1.1)	1.4 ± 0.2 (1.3, 1.5)	<0.0001
Cardiac and Skeletal Muscle Energetics			
Cardiac ATP/PCr	2.1 ± 0.2 (2.0, 2.2)	1.7 ± 0.3 (1.6, 1.8)	<0.0001
Tau PCr (sec)	30.9 ± 7.1 (28.2, 33.5)	74.3 ± 22.8 (66.0, 82.6)	<0.0001
Qmax linear (mmol/s)	1.1 ± 0.3 (1.0, 1.2)	0.5 ± 0.1 (0.5, 0.5)	<0.0001
Cardiac Function			
Resting HR (bpm)	71.0 ± 13.6 (66.0, 76.0)	80.6 ± 13.0 (75.9, 85.3)	0.01
Resting SBP (mmHg)	123.7 ± 13.8 (118.6, 128.1)	102.0 ± 11.5 (97.8, 106.2)	<0.0001
Resting DBP (mmHg)	68.4 ± 8.1 (65.4, 71.4)	62.2 ± 9.8 (58.6, 65.8)	0.02
LVM (g)	147.3 ± 60.6 (124.8, 169.8)	145.9 ± 78.4 (117.4, 174.4)	0.94
FS (%)	39 ± 2 (38, 40)	34 ± 8 (31, 37)	0.28
EF (%)	65 ± 5 (63, 67)	60 ± 10 (56, 63)	0.03
E/A	1.9 ± 0.4 (1.8, 2.1)	1.9 ± 0.7 (1.7, 2.2)	0.64
Global Strain (%)	-19.7 ± 3.1 (-20.8, -18.6)	-18.1 ± 3.5 (-19.4, -16.8)	0.08

Values are means ± SD. kgBW: kilogram of body weight, HR: heart rate, bpm: beats per minute, RER: respiratory exchange ratio (VCO₂/VO₂), ATP: adenosine triphosphate, PCr: phosphocreatine, Tau PCr: time constant for PCr recovery following exercise, Qmax linear: maximum oxidative capacity calculated by the linear model, SBP: systolic blood pressure, DBP: diastolic blood pressure, LVM: left ventricular mass, FS: fractional shortening, EF: ejection fraction, E/A: early to late filling ratio during diastole.

Table 3:

Substrate Metabolism during Rest, Exercise, and Post-Exercise

Variables	Control (n=28)	BTHS (n=29)	p-value [*]
Rest			
Glucose (mg/dL)	92.9 ± 6.8 (90.3, 95.5)	95.2 ± 11.6 (90.8, 99.6)	0.41
Insulin (μU/ml)	9.8 ± 6.4 (7.4, 12.3)	11.0 ± 8.0 (8.0, 14.1)	0.79
Glucose Turnover (μmol/kgFFM/min)	27.2 ± 8.1 (24.1, 30.4)	33.2 ± 9.8 (29.4, 36.9)	0.01
Total Carb Ox (μmol/kgFFM/min)	9.2 ± 14.1 (3.7, 14.7)	11.4 ± 10.0 (7.6, 15.2)	0.72
Lactate (uM/L)	1.1 ± 0.4 (1.0, 1.3)	1.3 ± 0.5 (1.1, 1.5)	0.001
FFA (mEq/L)	0.37 ± 0.18 (0.30, 0.44)	0.53 ± 0.22 (0.44, 0.61)	0.003
Palmitate R _a (μmol/kgFFM/min)	1.6 ± 0.8 (1.3, 1.9)	2.2 ± 0.9 (1.9, 2.6)	0.01
Plasma FA R _a (μmol/kgFFM/min)	5.9 ± 3.2 (4.6, 7.1)	8.3 ± 3.0 (7.2, 9.4)	0.003
Palmitate Ox (μmol/kgFFM/min)	0.5 ± 0.2 (0.4, 0.6)	0.8 ± 0.6 (0.6, 1.0)	0.04
Plasma FA Ox (μmol/kgFFM/min)	1.9 ± 1.1 (1.5, 2.3)	3.0 ± 2.1 (2.2, 3.8)	0.04
Non-Ox Palm Disp (μmol/kgFFM/min)	1.1 ± 0.6 (0.9, 1.3)	1.4 ± 0.6 (1.2, 1.7)	0.02
Non-Ox FA Disp (μmol/kgFFM/min)	5.1 ± 3.6 (3.6, 6.5)	4.7 ± 3.5 (3.4, 6.1)	0.81
Total Lipid Ox (μmol/kgFFM/min)	7.0 ± 4.1 (5.4, 8.6)	7.7 ± 4.4 (6.0, 9.4)	0.63
Exercise			
Glucose (mg/dL)	88.7 ± 7.7 (85.7, 91.7)	86.5 ± 11.7 (82.1, 90.9)	0.27
Insulin (μU/ml)	7.6 ± 5.4 (5.5, 9.7)	9.3 ± 8.8 (6.0, 12.7)	0.58
Glucose Turnover (μmol/kgFFM/min)	29.5 ± 8.8 (26.1, 32.9)	34.7 ± 11.2 (30.5, 39.0)	0.05
Total Carb Ox (μmol/kgFFM/min)	105.1 ± 44.1 (88.0, 122.2)	66.1 ± 38.2 (51.5, 80.6)	0.02
Lactate (uM/L)	2.3 ± 1.2 (0.8, 1.6)	4.9 ± 2.5 (3.9, 5.8)	<0.0001
FFA (mEq/L)	0.37 ± 0.20 (0.30, 0.45)	0.44 ± 0.21 (0.36, 0.52)	0.22
Palmitate R _a (μmol/kgFFM/min)	2.5 ± 1.3 (2.0, 3.0)	2.2 ± 1.0 (1.9, 2.6)	0.31
Plasma FA R _a (μmol/kgFFM/min)	9.3 ± 5.4 (7.2, 11.4)	8.5 ± 3.9 (7.0, 10.0)	0.94
Palmitate Ox (μmol/kgFFM/min)	1.9 ± 1.1 (1.4, 2.3)	0.8 ± 0.4 (0.6, 0.9)	<0.0001
Plasma FA Ox (μmol/kgFFM/min)	7.0 ± 4.6 (5.2, 8.8)	3.0 ± 1.4 (2.5, 3.5)	<0.0001
Non-Ox Palm Disp (μmol/kgFFM/min)	0.6 ± 0.6 (0.4, 0.8)	1.4 ± 0.7 (1.2, 1.7)	<0.0001
Non-Ox FA Disp (μmol/kgFFM/min)	10.5 ± 6.4 (8.0, 13.0)	0.8 ± 7.9 (-2.2, 3.8)	<0.0001
Total Lipid Ox (μmol/kgFFM/min)	17.5 ± 8.2 (14.3, 20.7)	3.8 ± 8.1 (0.8, 6.9)	<0.0001
Work Rate (watts)	12.6 ± 5.7 (10.6, 14.7)	83.8 ± 35.1 (70.8, 96.7)	<0.0001 ^{**}
Post-Exercise			
Glucose (mg/dL)	85.8 ± 3.8 (83.9, 87.6)	89.4 ± 12.9 (83.8, 94.9)	0.42
Insulin (μU/ml)	6.8 ± 4.2 (5.1, 8.4)	7.9 ± 6.7 (5.4, 10.4)	0.97
Glucose Turnover (μmol/kgFFM/min)	28.8 ± 8.0 (25.7, 31.8)	33.7 ± 10.2 (29.9, 37.6)	0.06
Total Carb Ox (μmol/kgFFM/min)	8.5 ± 9.9 (4.7, 12.4)	7.2 ± 11.6 (2.8, 11.6)	0.42
Lactate (uM/L)	1.1 ± 0.4 (1.0, 1.3)	1.2 ± 0.6 (1.0, 1.4)	0.001
FFA (mEq/L)	0.44 ± 0.19 (0.37, 0.52)	0.53 ± 0.17 (0.47, 0.60)	0.08

Variables	Control (n=28)	BTHS (n=29)	p-value *
Palmitate R _a (μmol/kgFFM/min)	1.8 ± 0.8 (1.5, 2.2)	2.4 ± 0.8 (2.1, 2.7)	0.04
Plasma FA R _a (μmol/kgFFM/min)	6.8 ± 3.5 (5.4, 8.2)	9.0 ± 3.2 (7.8, 10.2)	0.01
Palmitate Ox (μmol/kgFFM/min)	0.8 ± 0.4 (0.6, 1.0)	1.0 ± 0.6 (0.8, 1.2)	0.20
Plasma FA Ox (μmol/kgFFM/min)	2.9 ± 1.7 (2.3, 3.6)	3.7 ± 2.0 (2.9, 4.5)	0.13
Non-Ox Palm Disp (μmol/kgFFM/min)	1.1 ± 0.6 (0.8, 1.3)	1.4 ± 0.5 (1.2, 1.6)	0.02
Non-Ox FA Disp (μmol/kgFFM/min)	3.9 ± 3.4 (2.6, 5.2)	4.2 ± 3.9 (2.7, 5.7)	0.82
Total Lipid Ox (μmol/kgFFM/min)	6.8 ± 3.7 (5.4, 8.2)	7.9 ± 4.9 (6.0, 9.8)	0.49
Delta Exercise-Rest			
Glucose (mg/dL)	-4.2 ± 6.9 (-6.9, -1.5)	-8.7 ± 5.9 (-10.9, -6.4)	0.005
Insulin (μU/ml)	-2.2 ± 2.6 (-3.2, -1.2)	-1.7 ± 3.7 (-3.1, -0.3)	0.58
Glucose Turnover (μmol/kgFFM/min)	2.3 ± 1.4 (1.8, 2.9)	1.6 ± 2.5 (0.6, 2.5)	0.01
Total Carb Ox (μmol/kgFFM/min)	95.9 ± 48.3 (77.2, 114.6)	54.7 ± 35.5 (41.2, 68.2)	0.02
Lactate (uM/L)	1.2 ± 1.2 (0.8, 1.6)	3.6 ± 2.4 (2.7, 4.5)	<0.0001
FFA (mEq/L)	0.01 ± 0.13 (-0.04, 0.06)	-0.09 ± 0.17 (-0.15, -0.02)	0.02
Palmitate R _a (μmol/kgFFM/min)	0.9 ± 0.7 (0.6, 1.2)	0.0 ± 0.7 (-0.3, 0.2)	<0.0001
Plasma FA R _a (μmol/kgFFM/min)	3.4 ± 2.9 (2.3, 4.5)	0.2 ± 2.5 (-0.8, 1.1)	<0.0001
Palmitate Ox (μmol/kgFFM/min)	1.4 ± 1.0 (1.0, 1.7)	0.0 ± 0.5 (-0.2, 0.2)	<0.0001
Plasma FA Ox (μmol/kgFFM/min)	5.1 ± 3.9 (3.6, 6.6)	0.0 ± 1.8 (-0.7, 0.7)	<0.0001
Non-Ox Palm Disp (μmol/kgFFM/min)	-0.5 ± 0.5 (-0.7, -0.3)	0.0 ± 0.7 (-0.3, 0.3)	0.0006
Non-Ox FA Disp (μmol/kgFFM/min)	5.4 ± 7.7 (2.5, 8.4)	-3.9 ± 7.5 (-6.7, -1.0)	<0.0001
Total Lipid Ox (μmol/kgFFM/min)	10.5 ± 8.4 (7.3, 13.8)	-3.9 ± 7.5 (-6.7, -1.0)	<0.0001

Values are means ± SD and 95% confidence intervals for the mean (CIs). mM: millimolar, μU: micro unit, μM: micromolar, kgBW: kilogram of body weight, FFA: plasma free fatty acid concentration, Palm: palmitate, R_a: rate of appearance, Non-Ox Disp: palmitate non-oxidative disposal, FA Ox: fatty acid oxidation rate.

* P-value tests the hypothesis that the specified least squares means (i.e., at each condition and for the change between rest and exercise) are not statistically different for controls as compared to BTHS by a statistical contrast within a mixed model repeated measures analysis of variance

*** P-value independent t-tests.