Exercise and Substrate Metabolism Studies in Barth Syndrome: Updates and Future Directions

W. Todd Cade, PT, PhD
Physical Therapy & Medicine
Washington University School of Medicine
St. Louis, MO, USA
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School of Medicine

Background

- Tafazzin mutations in BTHS result in abnormal cardiolipin (CL) remodeling, leading to mitochondrial structural abnormalities and impaired mitochondrial function
- Cardiac and skeletal muscle have high levels of CL and therefore are most affected in BTHS
- Clinical complaints: excessive fatigue, physical activity intolerance
- Clinical phenotype: underdeveloped or reduced skeletal musculature
- Amino acid abnormalities (Dr. Kelley)
- Clinically variable presentation

Vreken et al, Biochem Biophys Res Commun., 2000, Houtkooper et al. Biochem Biophys Acta, 2000, Spencer et al. 2006

Exercise Intolerance in BTHS

- Objectives were to:
  - 1) objectify reports of exercise intolerance and fatigue
  - 2) determine if exercise intolerance was mediated by cardiac or skeletal muscle impairments, or both

Methods

- 2008 International Scientific, Medical & Family Conference in Clearwater, FL
- 15 boys with BTHS, 9 controls
- Controls obtained from convenience sample of BTHS siblings, friends, family
- GXT with continuous metabolic (VO_2) measurement, EKG and near infrared spectroscopy (NIRS) of lateral quadriceps
- 2D, Doppler and TD echocardiography performed at baseline and at peak exercise

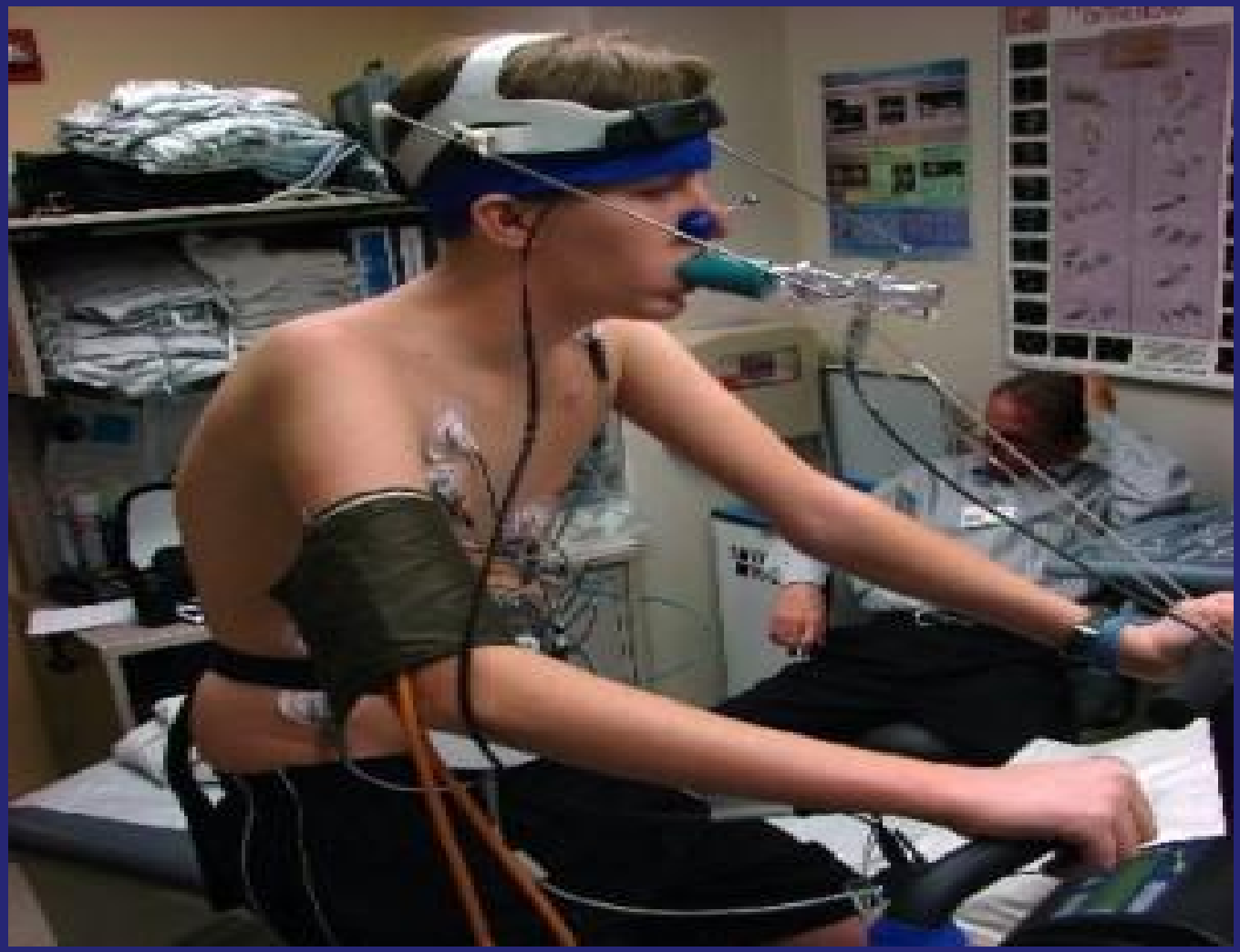


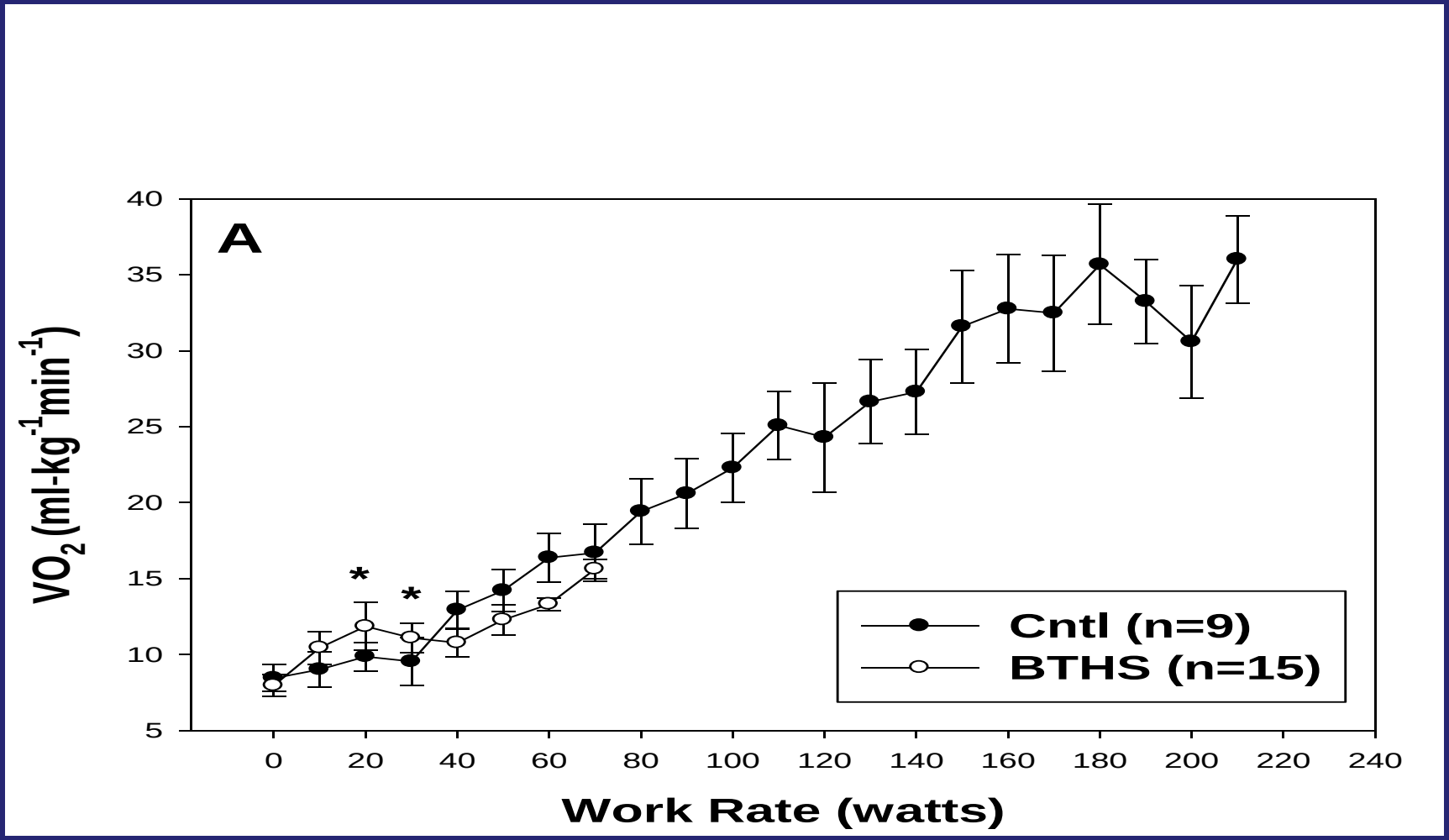
Table 1: Demographics and Medication Profile

Variable	Control (n=9)	BTHS (n=15)
Age (yrs)	13 ± 4	17 ± 5
Height (cm)	158.9 ± 18.3	161.0 ± 20.4
Weight (kg)	53.6 ± 22.0	45.8 ± 18.0
BMI	20.3 ± 5.2	17.0 ± 3.5
ICD (#)	0	3[†]
Medication		**n (%)**
β –Blockers	0	7 (47)
Carvedilol		5 (33)
Atenolol		1 (7)
Metoprolol		1 (7)
Ace inhibitors	0	11 (73)
Enalapril		4 (27)
Captopril		3 (20)
Lisinopril		4 (27)
Digoxin	0	8 (53)
GCSF	0	6(40)
Coenzyme Q	0	3 (20)
Carnitine	0	3 (20)
Others*		

ICD: implantable cardioverter defibrillator, [†]: all subjects with proven ventricular arrhythmia, GCSF: granulocyte colony stimulating factor *: Losartan, Riboflavin, Singulair, iron, thiamine, vitamin C, bactrim: all n=1for BTHS group

Spencer et al. *Am J Physiol Heart Circ Physiol 2011*

Exercise Intolerance in BTHS



*p<0.05 vs. Cntl

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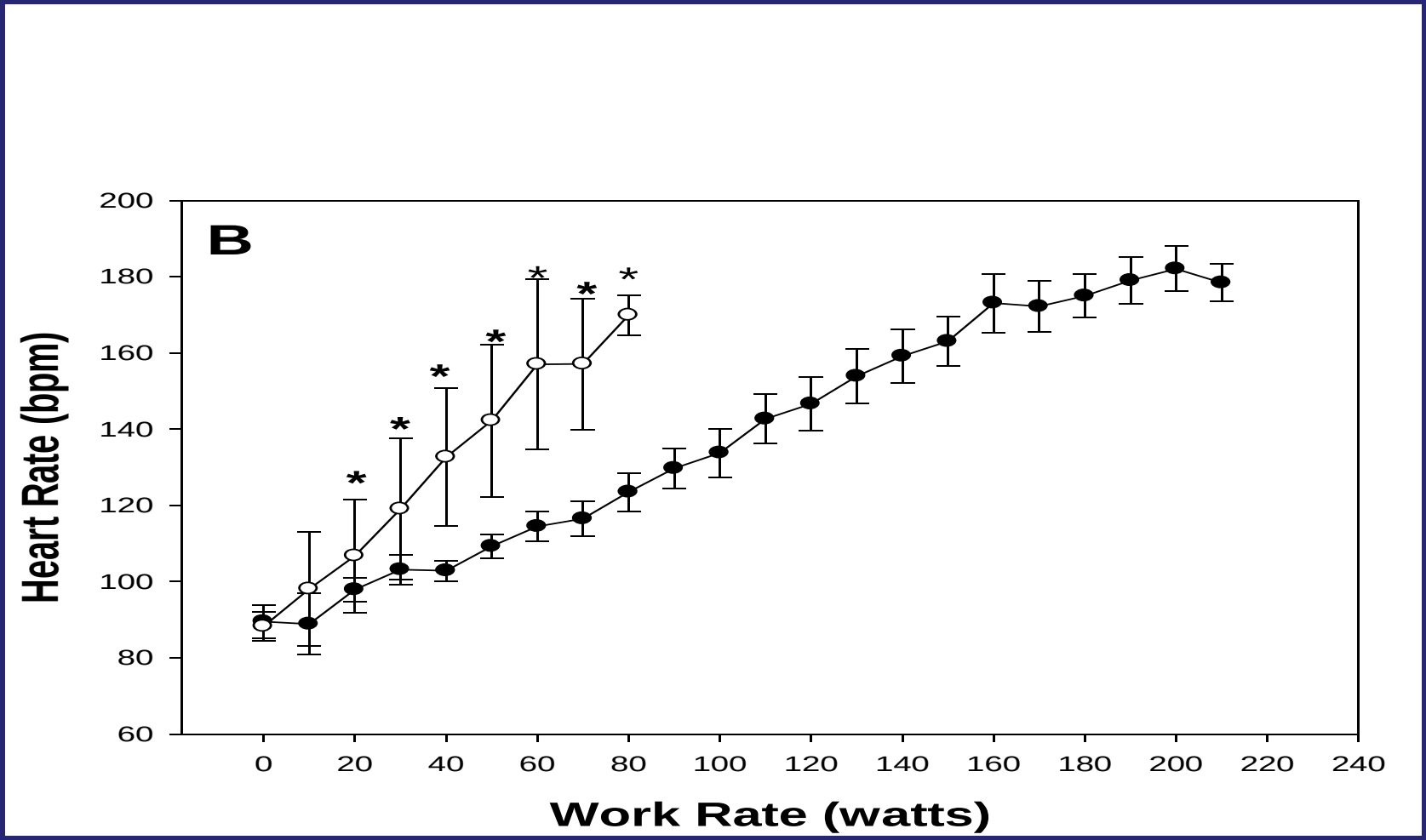
Heart Rate Response During Exercise



*p<0.05 vs. Cntl

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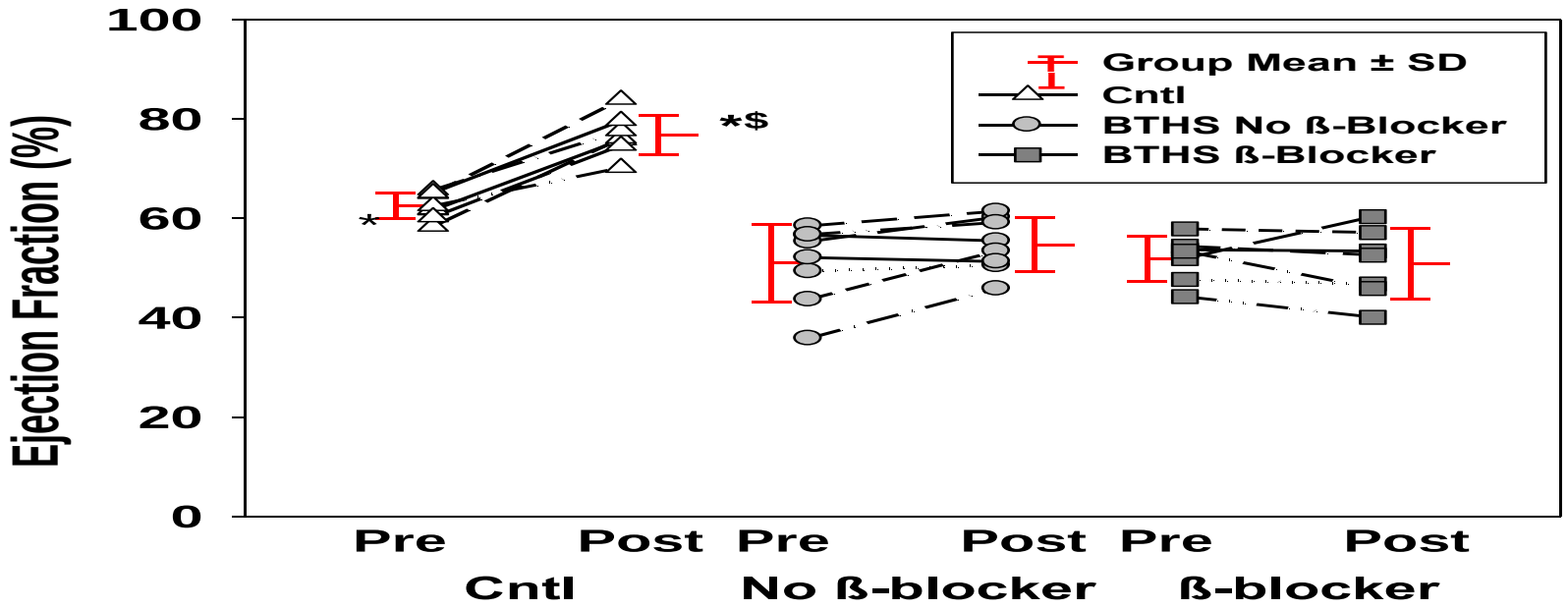
Cardiac Reserve During Exercise



*p<0.05 vs. baseline, $p<0.05 vs BTHS groups

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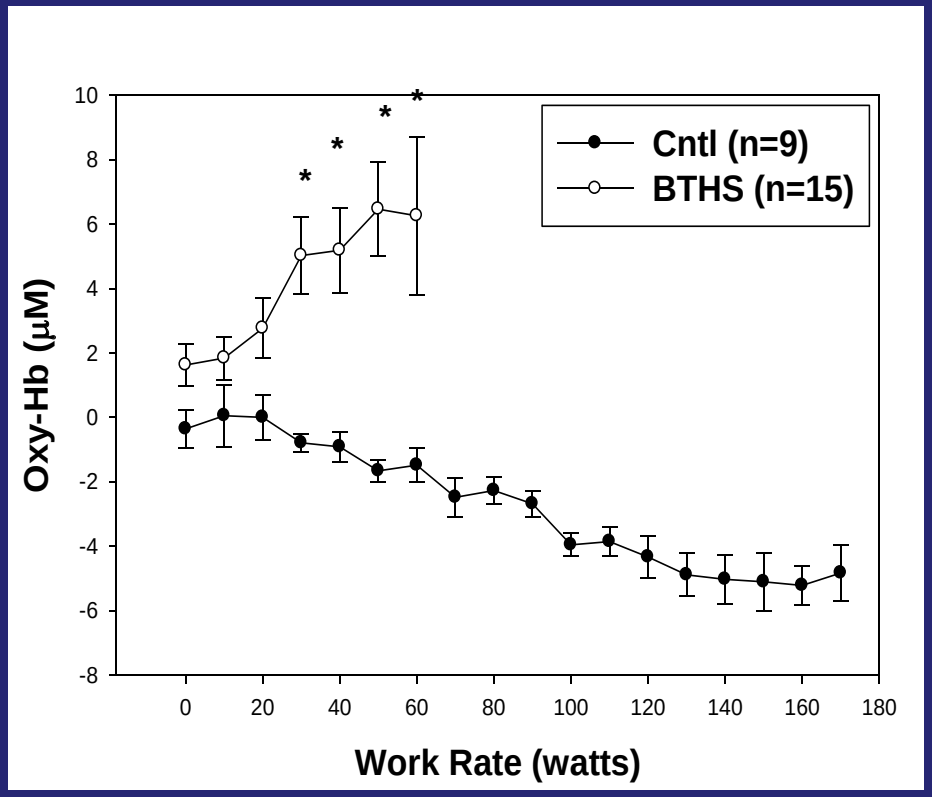
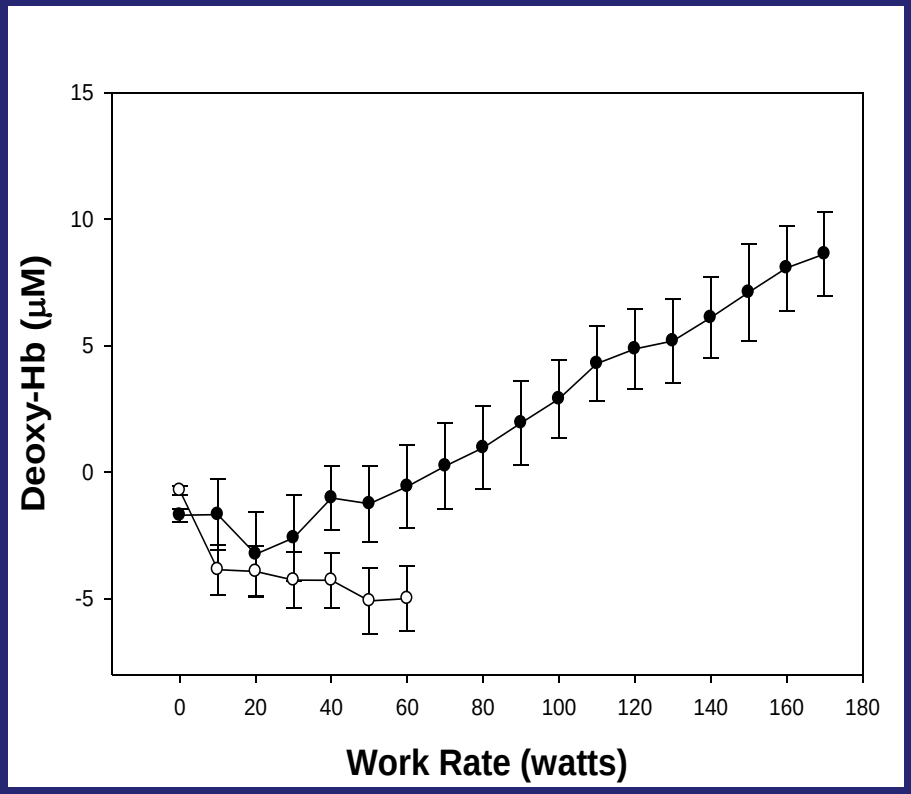
Skeletal Muscle Oxygen Extraction During Exercise (NIRS)





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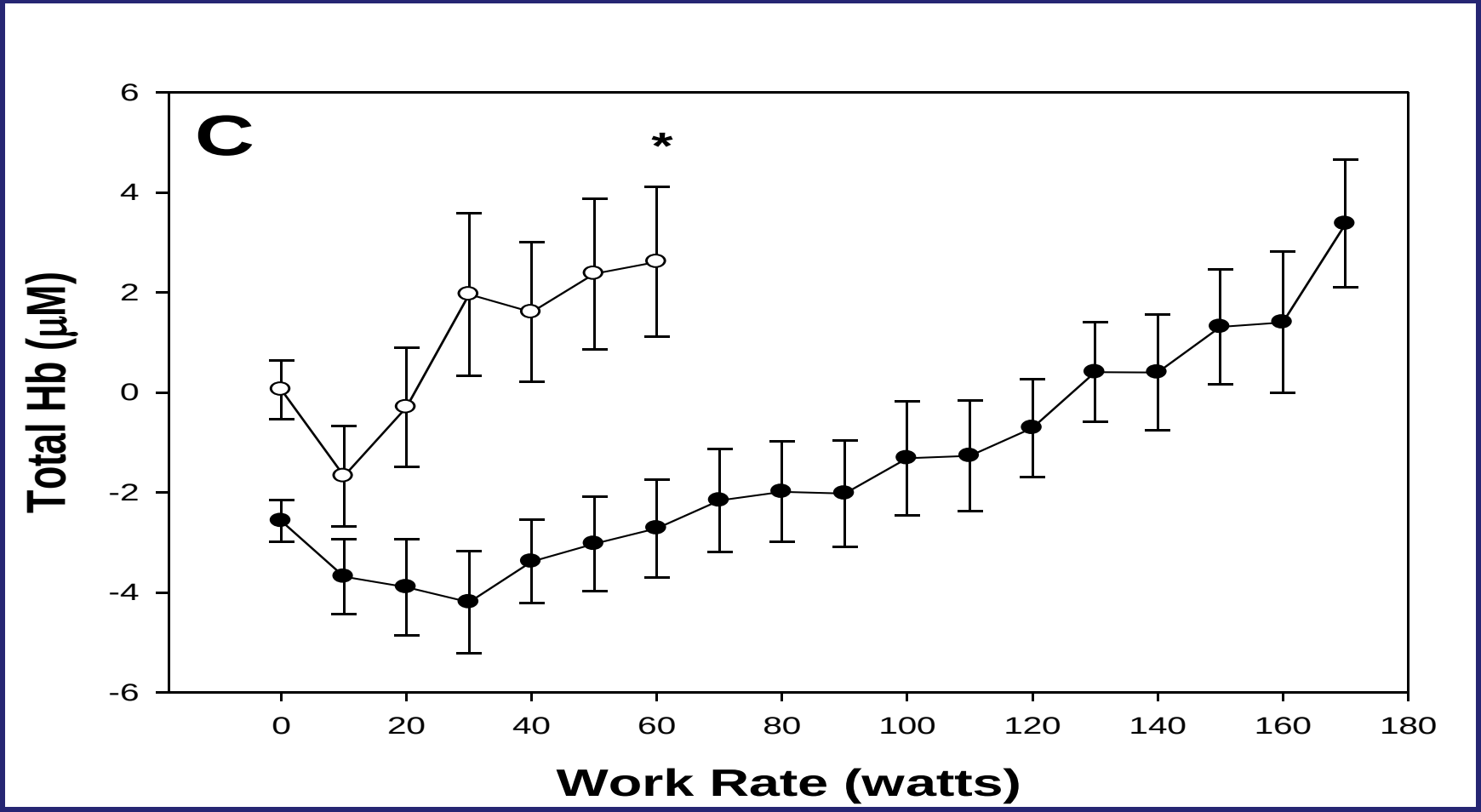
Muscle Blood Volume During Exercise



*p<0.05 vs. Cntl

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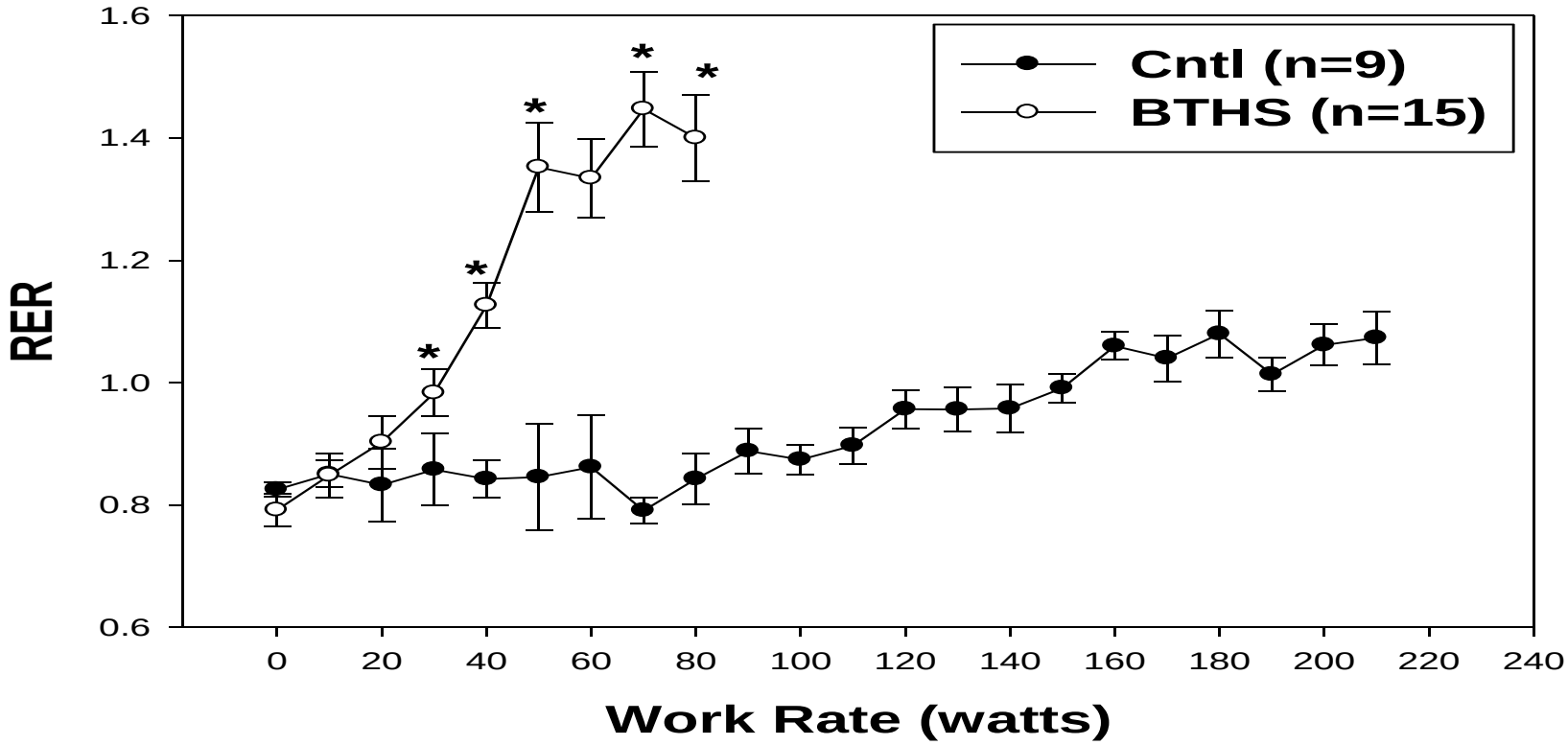
Respiratory Exchange Ratio (VCO_2/VO_2) During Exercise



*p<0.05 vs. Cntl

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Conclusions led to questions

- Severe exercise intolerance in BTHS
- Exercise intolerance mediated by both cardiac fx and skeletal muscle oxygen extraction/utilization impairments
- Compensation by elevated glucose/lactate metabolism
- Consistent with etiology of BTHS
- Could participants with BTHS effectively and safely endurance train (hx of ventricular arrhythmias)?
- If so, would endurance exercise training mediate improvements come from increased cardiac fx, skeletal muscle O_2 extraction or both?
- Would endurance exercise training just increase the # of impaired mitochondria?

Endurance Exercise Training Pilot: In Progress

- Barth Syndrome Foundation grant
- 4 participants 15 years and older
  - Participant #1: 22 yrs, completed
  - Participant #2, 21 yrs, completed
  - Participant #3, 28 yrs, completed
  - Participant #4, 18 yrs, awaiting site IRB approval

Methods

- Pre-exercise testing performed at 2010 BSF conference in Orlando
- Peak exercise testing
  - Exercise tolerance-O_2 consumption on cycle ergometer
  - Muscle O_2 extraction/utilization-NIRS
  - Heart fx- echocardiography
- Aerobic training program on cycle ergometer
  - Goal was 45 min of continuous exercise at moderate intensity (Borg scale) at study completion
  - 3x/wk for 12 wks (36 visits) performed at PT or cardiac rehab site near participant's home
  - QOL assessed by Minnesota Living with Heart Failure Questionnaire
- Post testing performed at Washington University

Exercise Training Summary (n=3)

Participant	Month	Exs Time (min)	Exs HR (bpm)	Exs BP	Ave RPE	Ave Watts
1	1	51.8 ± 9.0	116 ± 5 (58%)	93/54	6.1 ± 0.6	41.7 ± 9.9
1	2	48.8 ± 3.4	114 ± 6 (57%)	92/51	6.2 ± 0.6	41.2 ± 8.2
1	3	47.2 ± 2.9	118 ± 4 (59%)	94/54	6.4 ± 0.2	48.7 ± 3.8
1	Ave	49.2 ± 6.0	116 ± 5 (58%)	114/66	6.2± 0.2	43.4 ± 3.8
2	1	13.8 ± 4.3	136 ± 6 (68%)	114/66	3.4 ± 0.8	15.0 ± 1.3
2	2	21.2 ± 2.9	139 ± 6 (70%)	109/64	4.1 ± 0.1	15.0 ± 1.3
2	3	40.8 ± 10.0	145 ± 4 (73%)	98/77	3.9 ± 0.6	15.0 ± 1.3
2	Ave	25.5 ± 13.5	140 ± 6 (70%)	114/66	3.4 ± 0.8	15.0 ± 1.3
3	1	17.0 ± 1.4	130 ± 4 (68%)	112/64	4.8 ± 0.7	14.9 ± 0.4
3	2	21.6 ± 1.7	131 ± 4 (69%)	109/68	5.4 ± 0.6	14.7 ± 0.0
3	3	22.2 ± 2.6	128 ± 4 (67%)	102/62	5.4 ± 0.6	14.7 ± 0.0
3	Ave	20.2 ± 3.0	130 ± 4 (68%)	107/62	5.2 ± 0.7	14.8 ± 0.2

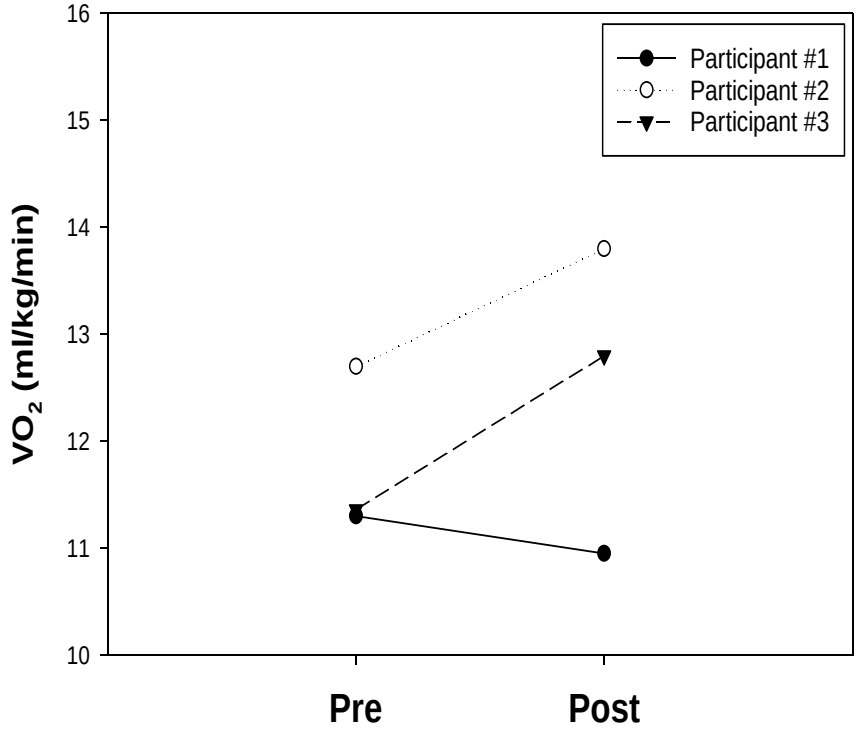
Demographics and Serum Metabolites (n=3)

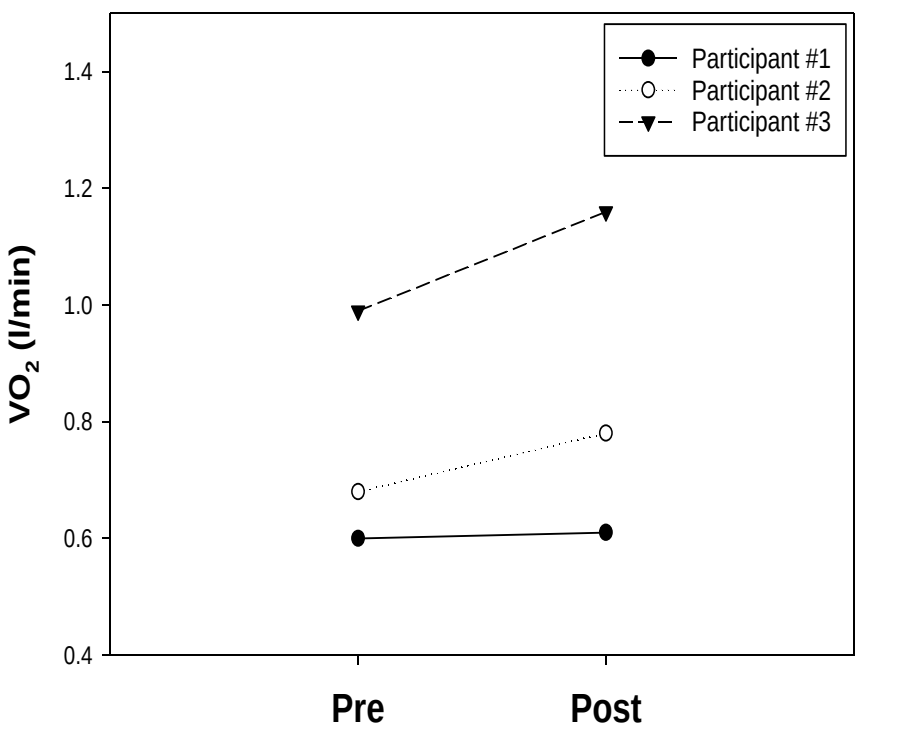
Outcome	Pre	Post	Delta
Weight (kg)	64.9 ± 19.6	67.3 ± 20.0	1.7 ± 1.4
WBC (K/cumm)	3.7 ± 0.6	2.9 ± 0.5	-0.8 ± 0.4
Absolute Neutrophil (K/cumm)	1.3 ± 0.7	0.8 ± 0.3	-0.5 ± 0.6
Neutrophi (%)	33 ± 18	28 ± 6	-4.6 ± 14
Hb (g/dl)	15.1 ± 0.9	15.0 ± 1.5	-0.1± 0.7
HCT (%)	43.0 ± 2.1	42.6 ± 4.9	-0.4 ± 3.0
BNP (pg/ml)	79 ± 102	61 ± 54	-18 ± 50
Pre-Albumin (mg/dl)	18.3 ± 3.1	17.2 ± 4.9	-1.1 ± 2.0

Results-Cardiorespiratory (n=3)

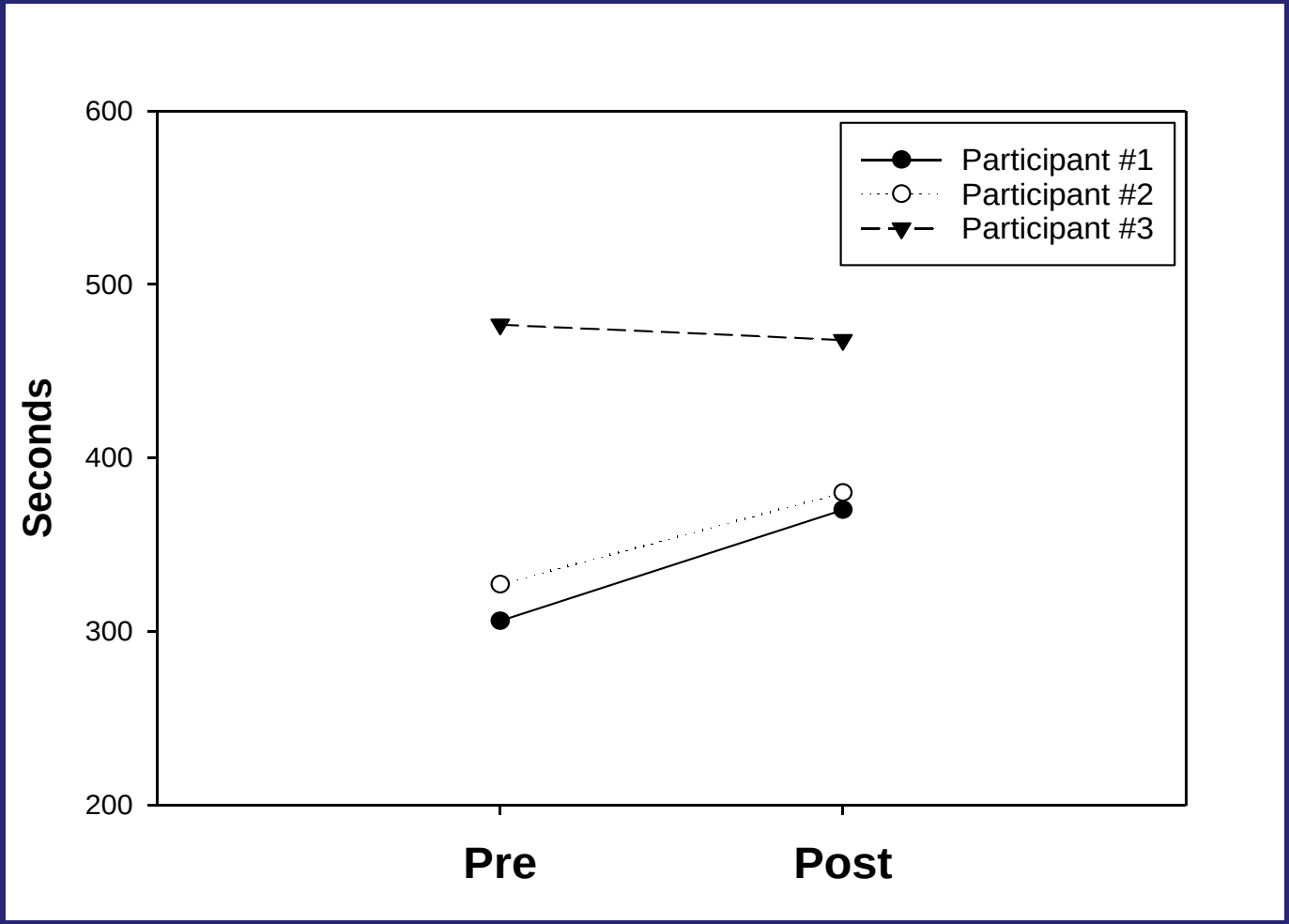
Outcome	Pre	Post	Delta
Peak HR (bpm)	152.3 ± 16.2	163.0 ± 10.4	10.7± 14.8
Peak HR (%)	78 ± 11	83 ± 6	5± 8
Peak RER	1.7 ± 0.3	1.6 ± 0.2	-0.1± 0.7

Peak Oxygen Consumption





Exercise Time



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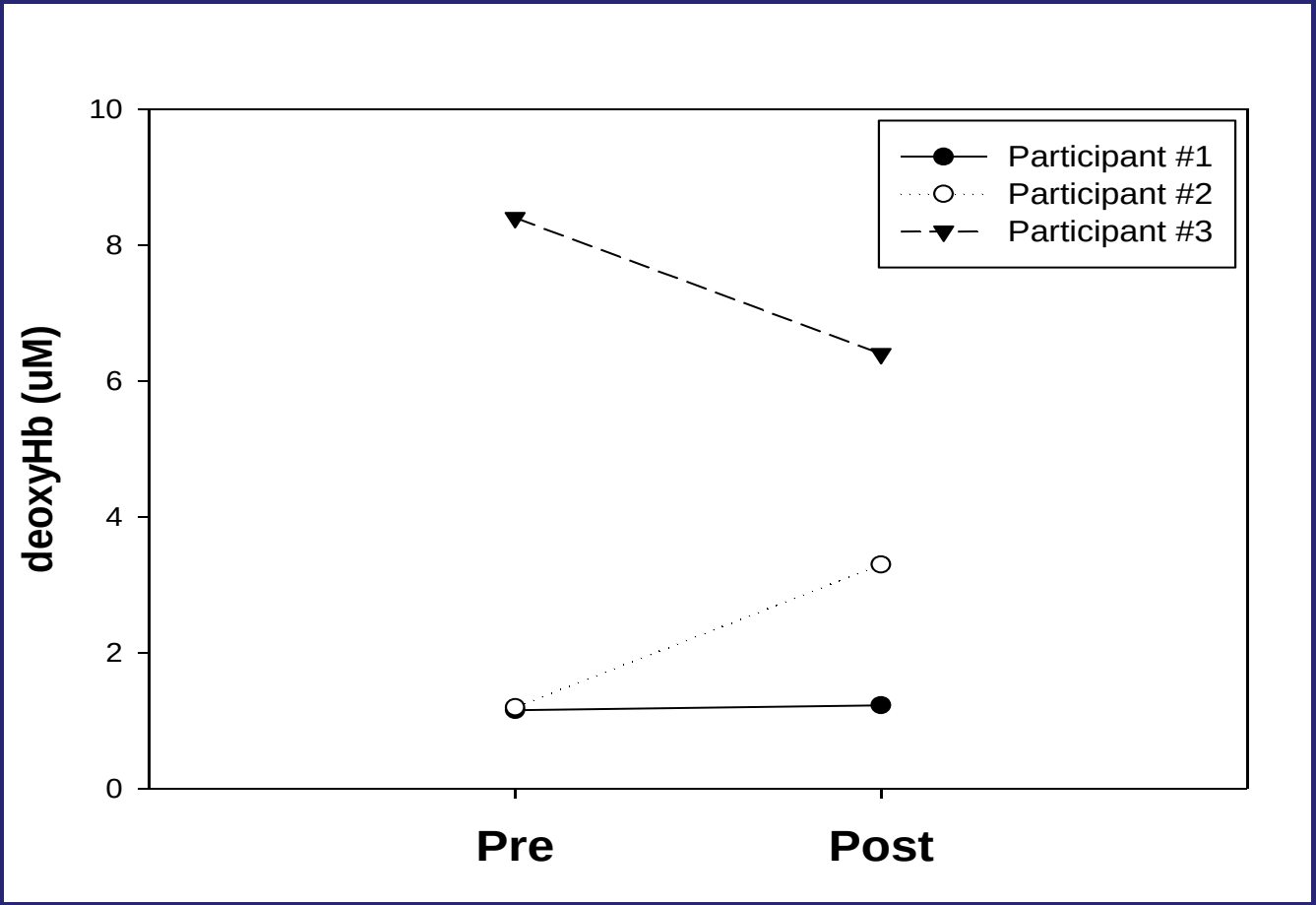
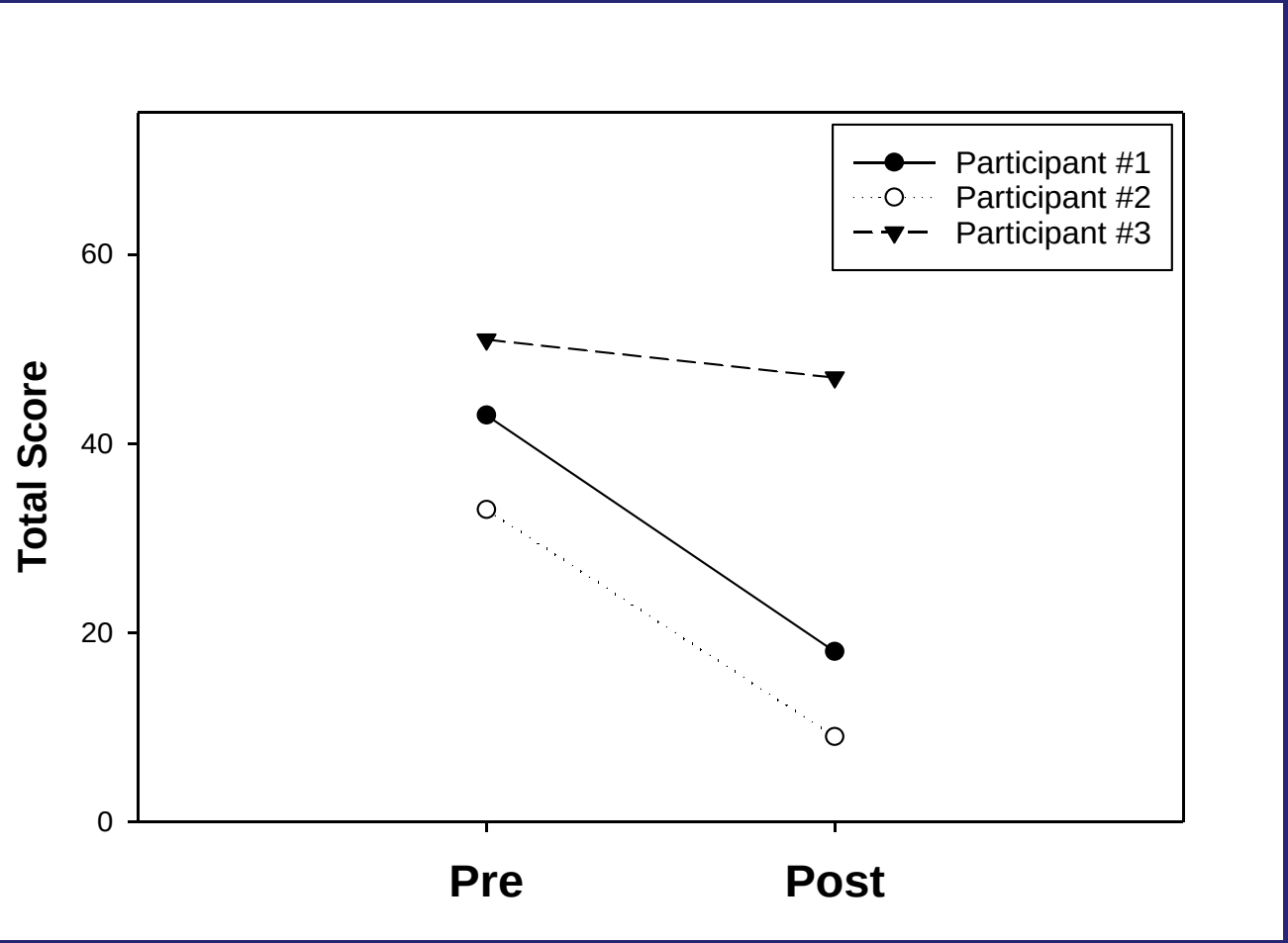
Peak Muscle Oxygen Extraction



Quality of Life Score (MLWHFQ)



Preliminary Conclusions- Exercise Training

- Endurance exercise well-tolerated by participants and safe
- Exercise training ↑'ed exercise tolerance (either exercise time or VO_{2peak}) in all participants (~10-15%)
- ↑ in muscle O_2 extraction appeared to mediate ↑ in VO_2 in Participant #2- no change or slight decrease in other participants
- Although not clear yet (data pending), does not appear exercise training ↑ cardiac function however peak HR ↑ in both participants
- QOL markedly ↑ following exercise training
- Clinical importance-recreation report and house/yard work ↑ but no change in ability to walk/climb stairs
- Would longer training program further ↑ exercise tolerance?
- Resistance training more effective?

Resistance Exercise Training (RET) in BTHS

- Other populations, including non-BTHS heart failure, appear to receive a greater benefit from endurance exercise training (e.g. ~15-25% increase in exercise tolerance) than does BTHS
- The blunted effect of endurance exercise training in BTHS: inherent pathogenesis of BTHS→genetic mitochondrial dysfunction in type I (oxidative>glycolytic capacity) muscle fibers?
- Endurance exercise primarily targets type I muscle fibers where resistance training involves more type II fibers
- RET beneficial in non-BTHS heart failure

Specific Aims

- *In 3 adolescents/young men (ages 15-30 years) with BTHS*:
- Specific Aim #1: To evaluate the safety of 12 weeks of supervised resistance exercise training (RET)
- Specific Aim#2: To evaluate of effectiveness of 12 weeks of supervised RET on left ventricular function, skeletal muscle strength and mass, exercise tolerance, whole-body protein synthesis rate and subjective quality of life.
- Exploratory Aim: To examine arginine metabolism and its response to supervised RET

Methods

- Safety: MM-CK, MB-CK
- Exercise tolerance: GXT with metabolic measurements
- Heart function: resting echocardiography
- Muscle strength: 1 RM, isokinetic dynamometry
- QOL: Minnesota Living with Heart Failure Questionnaire
- Whole body protein metabolism: 1-^{13}C leucine
- Arginine metabolism: $^{15}N_2$ guanidino-arginine, $^{2}H_2$ citrulline stable isotope tracers, mass spectrometry
- Baseline protein and arginine metabolism will be compared to healthy, age matched controls
- To begin August 2012

Results from exercise led to questions regarding substrate metabolism…

- FA metabolic impairments would be consistent with findings of low aerobic capacity/exercise intolerance
- In addition, low skeletal muscle mass may be indicative of amino acid metabolism impairments
- Stable-isotope tracer methodology would be ideal to examine these questions which may shed light on potential contributing mechanisms to cardio-skeletal myopathy in BTHS

What is Known in BTHS re: substrate metabolism?

- Reported CL (Dr. Schlame) and AA (Dr. Kelley) abnormalities
- FA and AA metabolism?
- Alternate fuel use in heart and skeletal muscle appears to cause pathology in other non-BTS pathology
- Myocardial fatty acid (MFA) uptake and oxidation significantly lower and myocardial glucose metabolism greater in adults with idiopathic dilated cardiomyopathy and children with mt disorders
- Whole-body abnormalities in amino acid metabolism in adults with chronic heart failure
- Cardiac cachexia hypothesis

Davila-Roman, et al. JACC, 2002, Bergmann et al., J Inherit Met Dis, 2001, Norrelund et al. J Intern Med 2006, Toth & Matthews, JPEN 2006, Anker & Sharma, Int J Cardiol, 2002

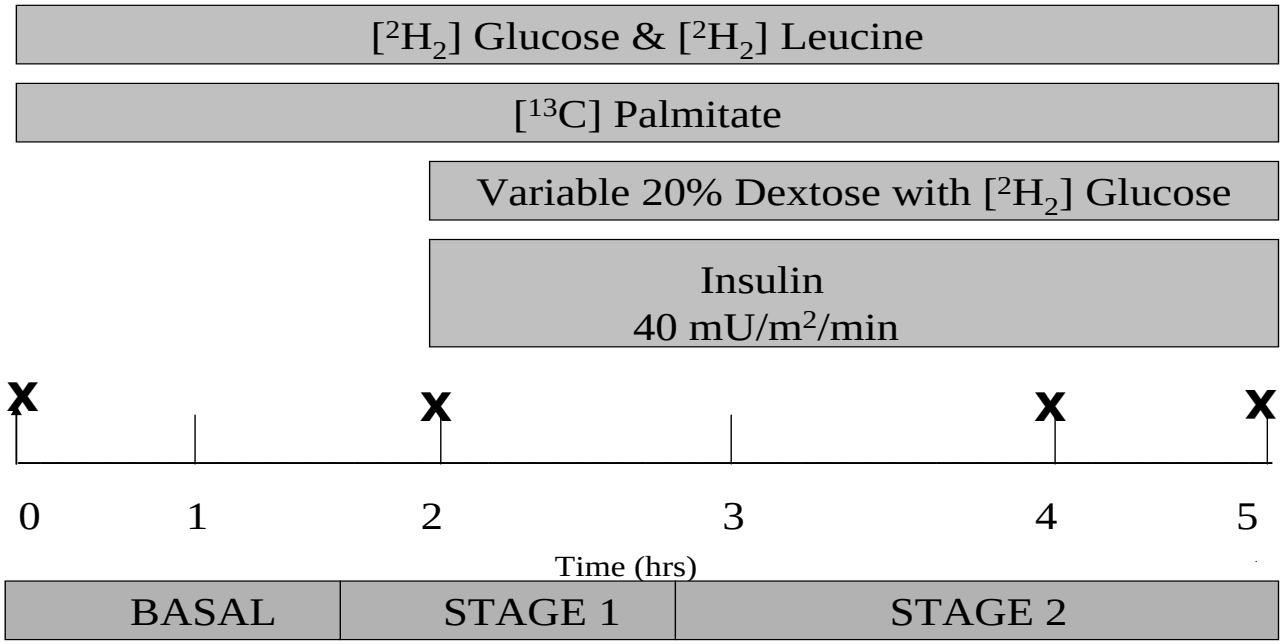
Characterization of Nutrient Metabolism in BTHS

- Question: it is not known whether nutrient metabolism abnormalities mediate or contribute to skeletal and cardiomyopathy in BTHS
- Overall objective: to collect preliminary data on the following hypotheses:
  - 1) Impaired whole-body fatty acid oxidation leads to abnormal cardiac lipid accumulation (i.e. “lipotoxicity”) and decreased left ventricular function in BTHS
  - 2) Elevated whole-body protein degradation rate leads to skeletal muscle wasting and decreased left ventricular function in BTHS

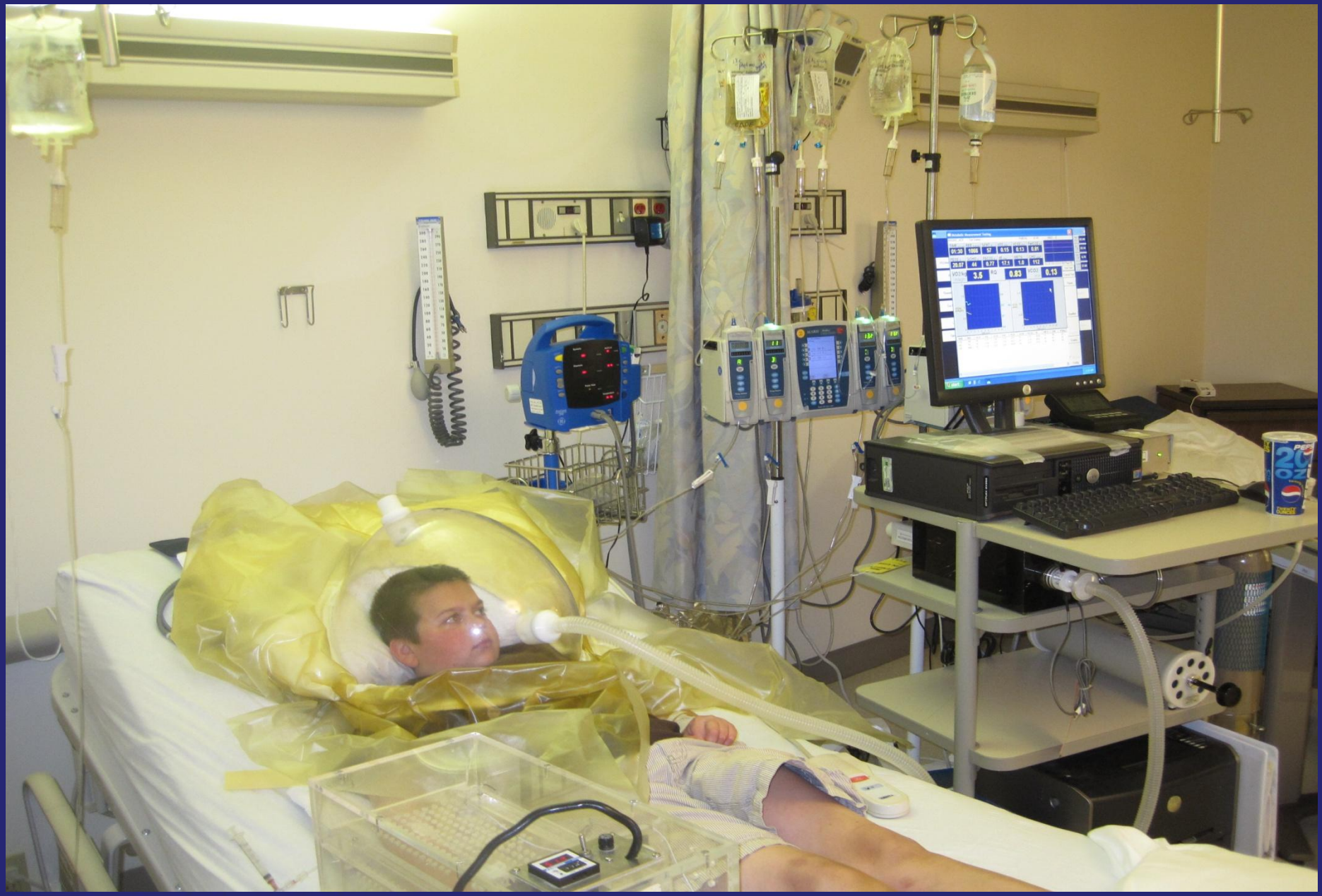
Methodology

- 5 boys/young men with BTHS, 5 age-matched controls 15-25 years
- No ICD, lives in US or Canada
- Willing to travel to St. Louis
- Participants undergo:
  - DEXA
  - ^{13}-C acetate infusion for correction factor
  - 2D, Doppler and TDI echocardiogram with strain analysis
  - ^{1}H-MRS for cardiac lipid content
  - 1-stage hyperinsulinemic clamp with ^{13}-C palmitate, 5’5’5’-d_3 leucine and 6’6’d2 glucose infusion and breath sample collection

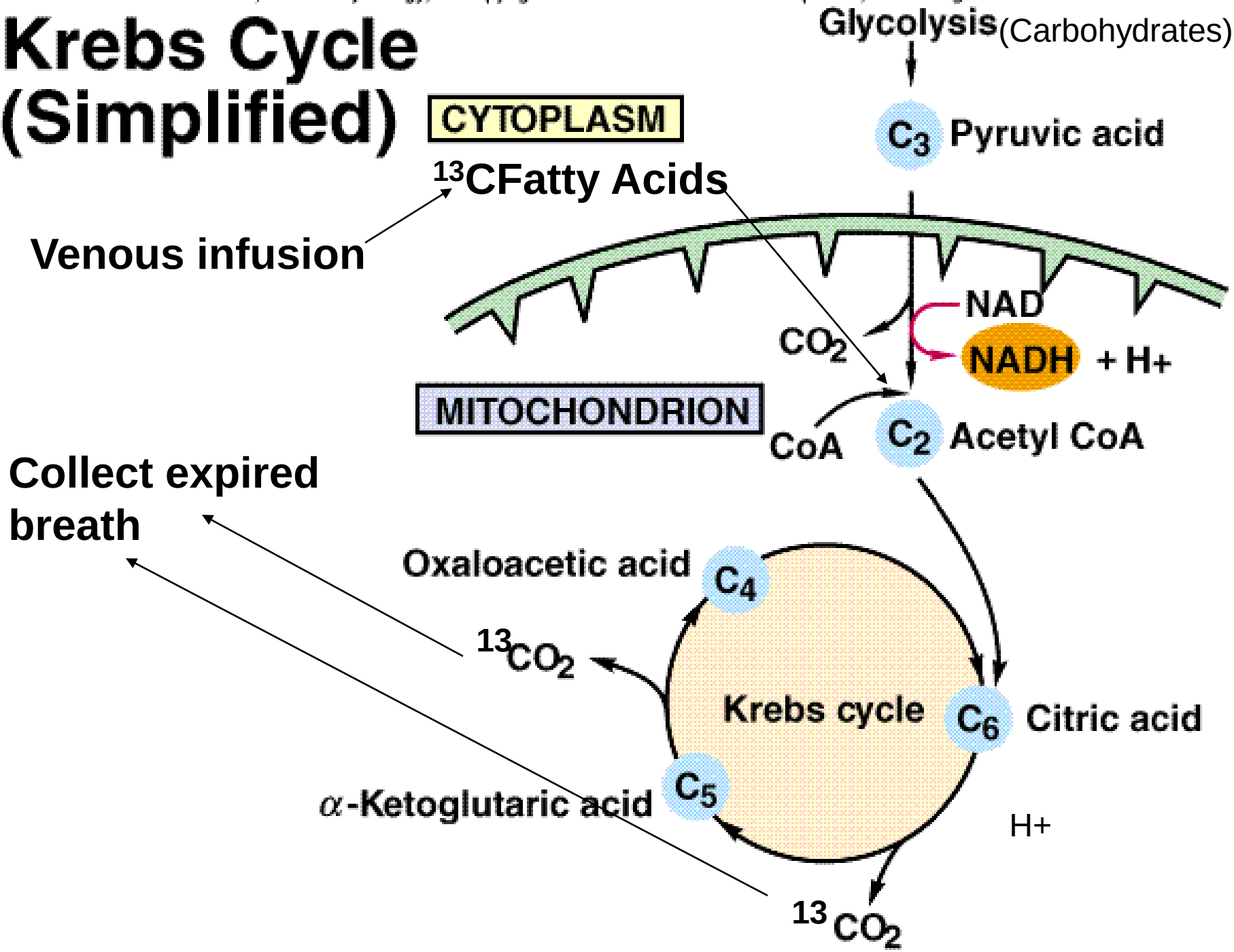
Clamp Procedure

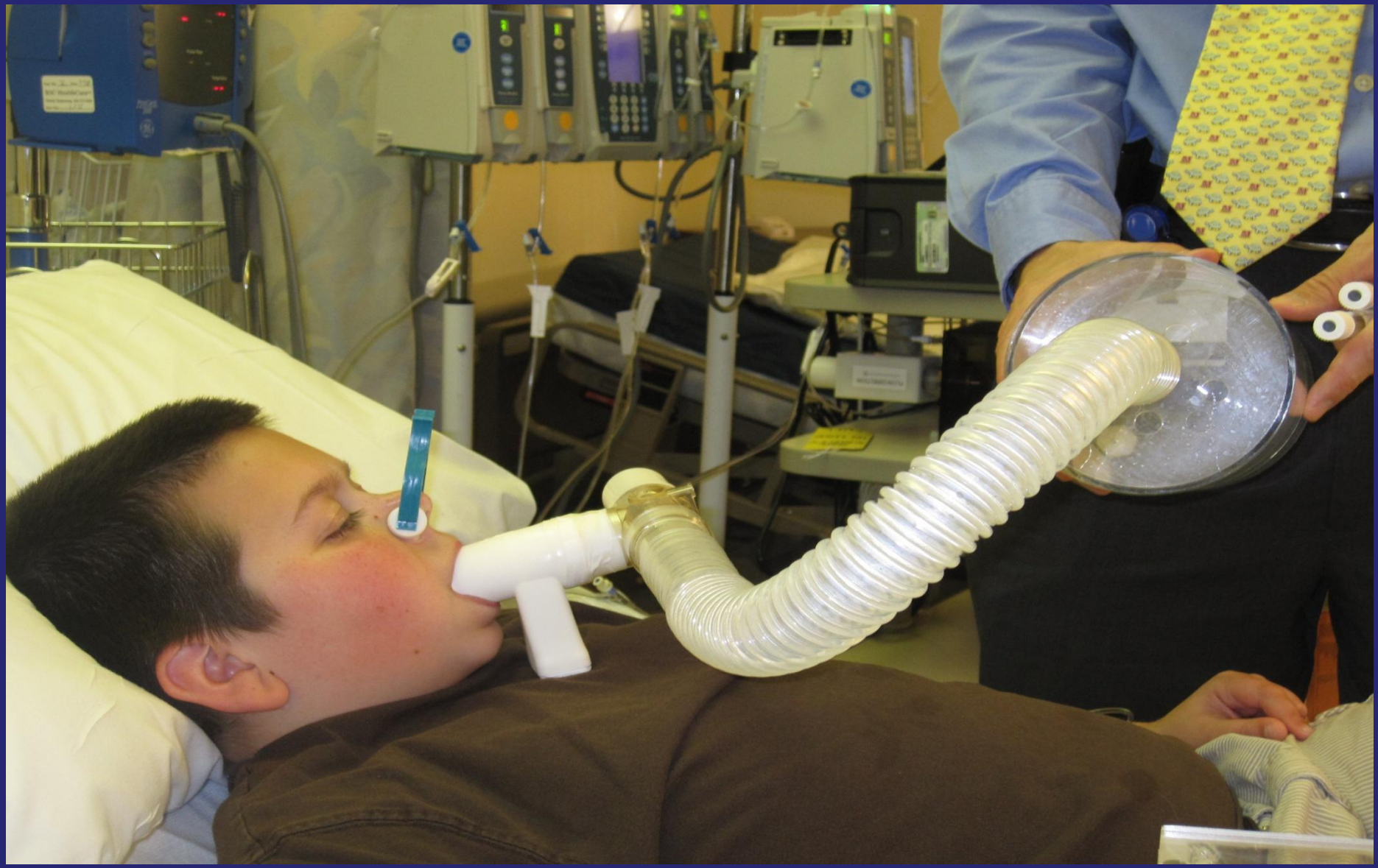


X=Blood (insulin, FFA, glucose, kinetics) and breath samples ($^{13}CO_2$)- every 10 minutes for last 30' of hour



Krebs Cycle (Simplified)





Demographics

	Control (n=5)	BTHS (n=5)	P-value
Age (yrs)	20 ± 4	18 ± 4	NS
ACE (n)	N/A	3	
Beta-blockers (n)	N/A	2	
Height (cm)	176.6 ± 7.5	170.0 ± 18.5	NS
Weight (kg)	65.0 ± 10.6	51.8 ± 8.5	0.06
BMI	21 ± 3	18 ± 3	NS
Hematocrit (%)	38.9 ± 1.8	39.0 ± 2.1	NS
White Blood Count (K/cumm)	7.7 ± 3.1	3.2 ± 1.7	0.03
Neutrophil (%)	62.9 ± 14.9	33.5 ± 15.3	0.02

Cade et al. *J Inherit Metab Dis* 2012

Body Composition & Metabolic Variables

	Control (n=5)	BTHS (n=5)	P-value
Fat free mass (kg)	46.7 ± 5.3	31.4 ± 6.9	0.005
Fat free mass (%)	86 ± 8	71 ± 13	0.06
Fat mass (kg)	8.4 ± 6.4	13.2 ± 5.2	0.24
Total Fat (%)	14 ± 8	29 ± 13	0.06
Serum Glucose (mg/dL)	87.4 ± 7.9	81.0 ± 13.8	NS
Fasting Insulin (μU/mL)	6.8 ± 2.5	9.5 ± 10.8	NS
HDL (mg/dL)	36.0 ± 5.1	35.8 ± 4.2	NS
LDL (mg/dL)	76.8 ± 22.9	79.5 ± 31.7	NS
Total Cholesterol (mg/dL)	130.8 ± 28.5	127.3 ± 36.3	NS
Triglycerides (mg/dL)	90.0 ± 24.4	59.5 ± 21.6	0.11
FFA (nmol/mL)	298 ± 127	816 ± 427	0.03
Lactate (μmol/L)	0.89 ± 0.15	1.54 ± 1.4	NS

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Echo and Hemodynamic Measures

	Control (n=5)	BTHS (n=5)	P-value
HR (bpm)	69 ± 14	80 ± 18	NS
SBP (mmHg)	120 ± 8	100 ± 8	0.01
DBP (mmHg)	70 ± 13	61 ± 14	NS
LVM 2DE	142 ± 16	139 ± 33	NS
EF (%)	58 ± 3	52 ± 9	0.17
LVOT VTI (cm/s)	19.5 ± 3.1	15.7 ± 1.1	0.03
Sm_{Sep} (cm/s)	9.8 ± 2.5	8.0 ± 1.9	0.23
Sm_{Lat} (cm/s)	12.6 ± 1.7	10.0 ± 2.5	0.09
E/A	1.7 ± 0.6	1.8 ± 1.0	NS
Em_{Sep} (cm/s)	14.8 ± 2.3	11.4 ± 1.5	0.02

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Glucose Metabolism Kinetics

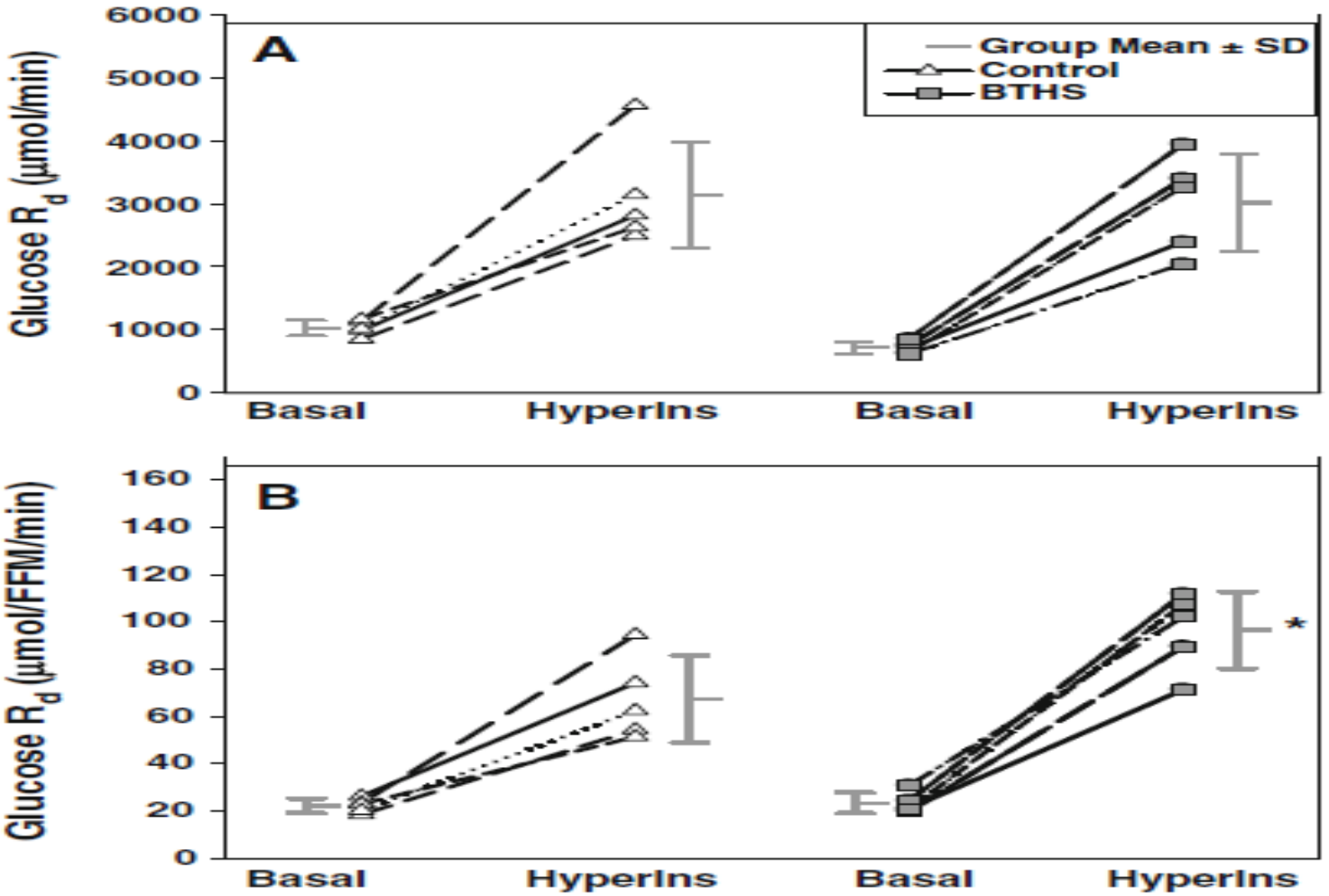

Fig. 1 Glucose rate of disappearance in BTHS and controls during the basal and hyperinsulinemic condition. BTHS: Barth syndrome, R_d: rate of disappearance, HyperIns: hyperinsulinemia, FFM: fat free mass. *: $p<0.05$

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Fatty Acid (Palmitate) Metabolism Kinetics

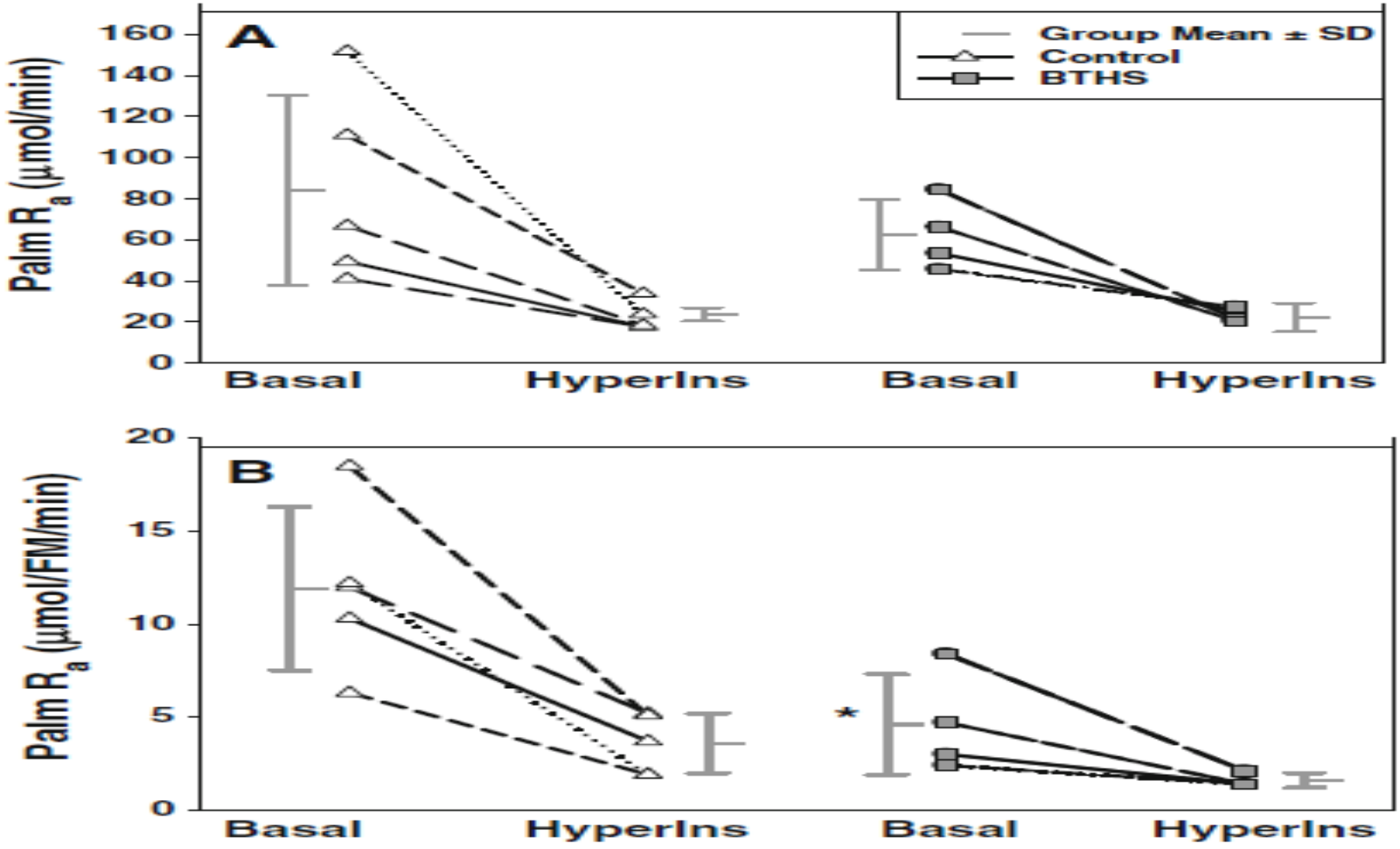

Fig. 2 Palmitate rate of appearance in BTHS and controls during the basal and hyperinsulinemic condition. BTHS: Barth syndrome, R_a: rate of appearance, HyperIns: hyperinsulinemia, FM: fat mass. *: $p<0.05$

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Amino Acid (Leucine) Metabolism Kinetics

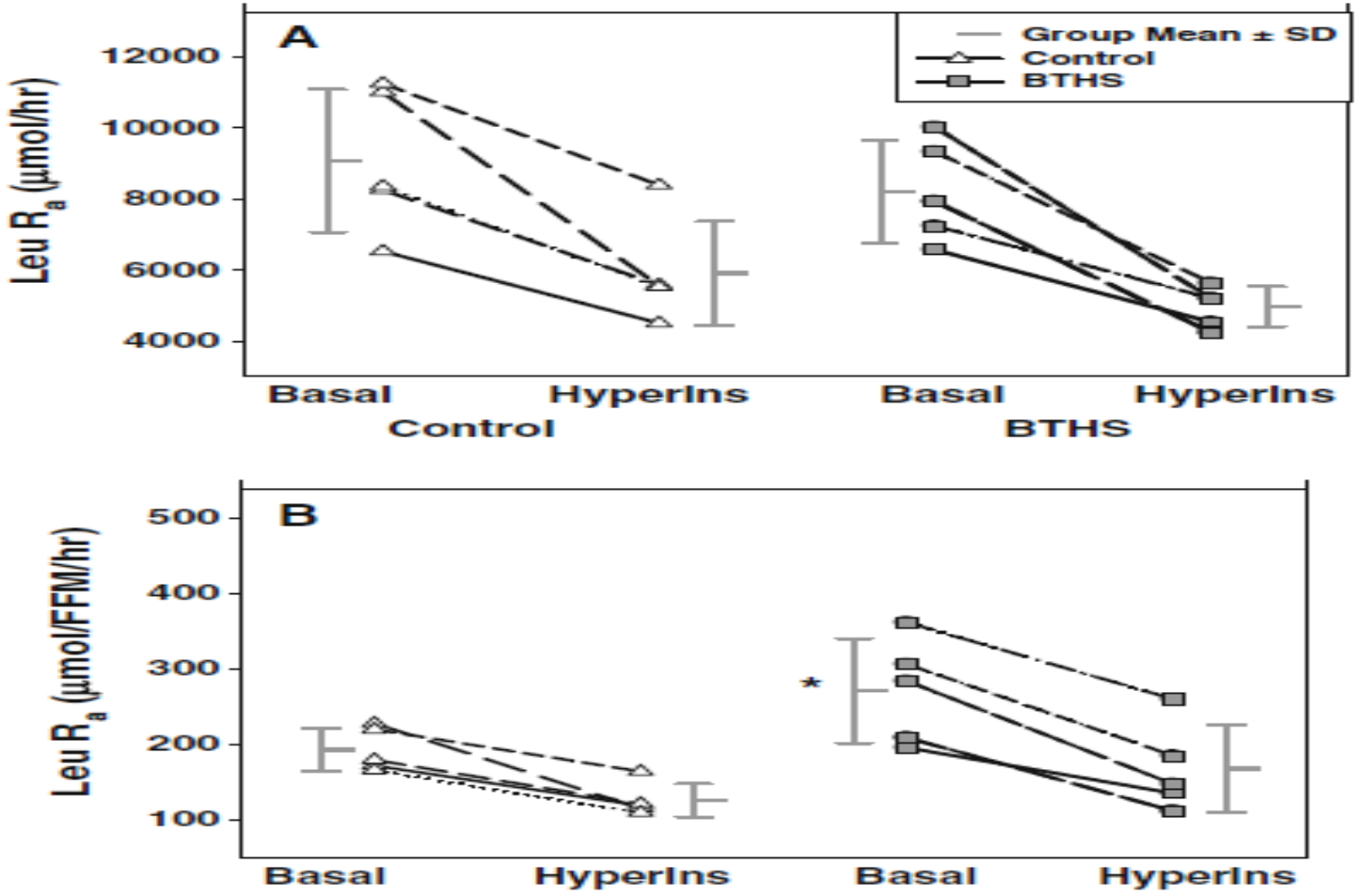

Fig. 4 Leucine rate of appearance in BTHS and controls during the basal and hyperinsulinemic condition. BTHS: Barth syndrome, R_a: rate of appearance, HyperIns: hyperinsulinemia, FFM: fat free mass. *: p=0.09

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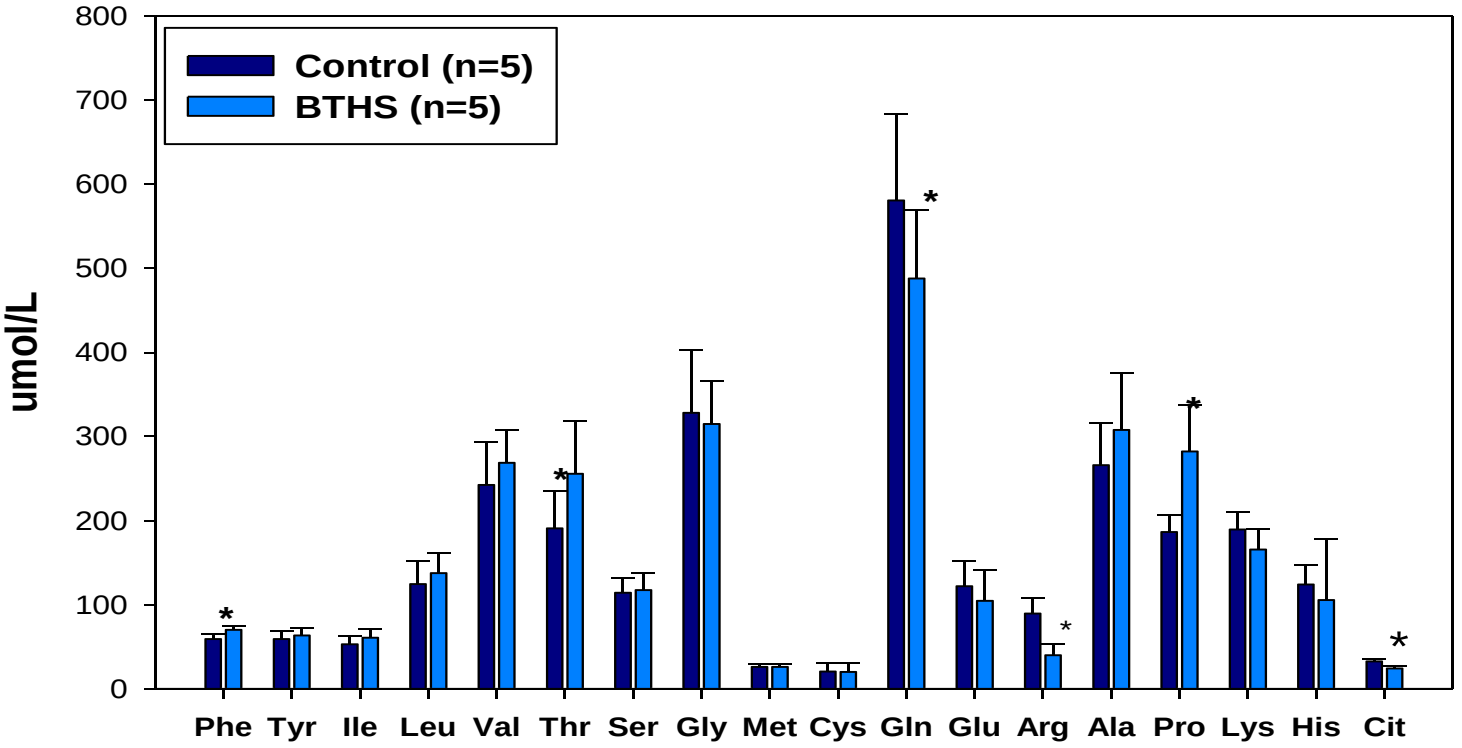
Serum Amino Acid Profile



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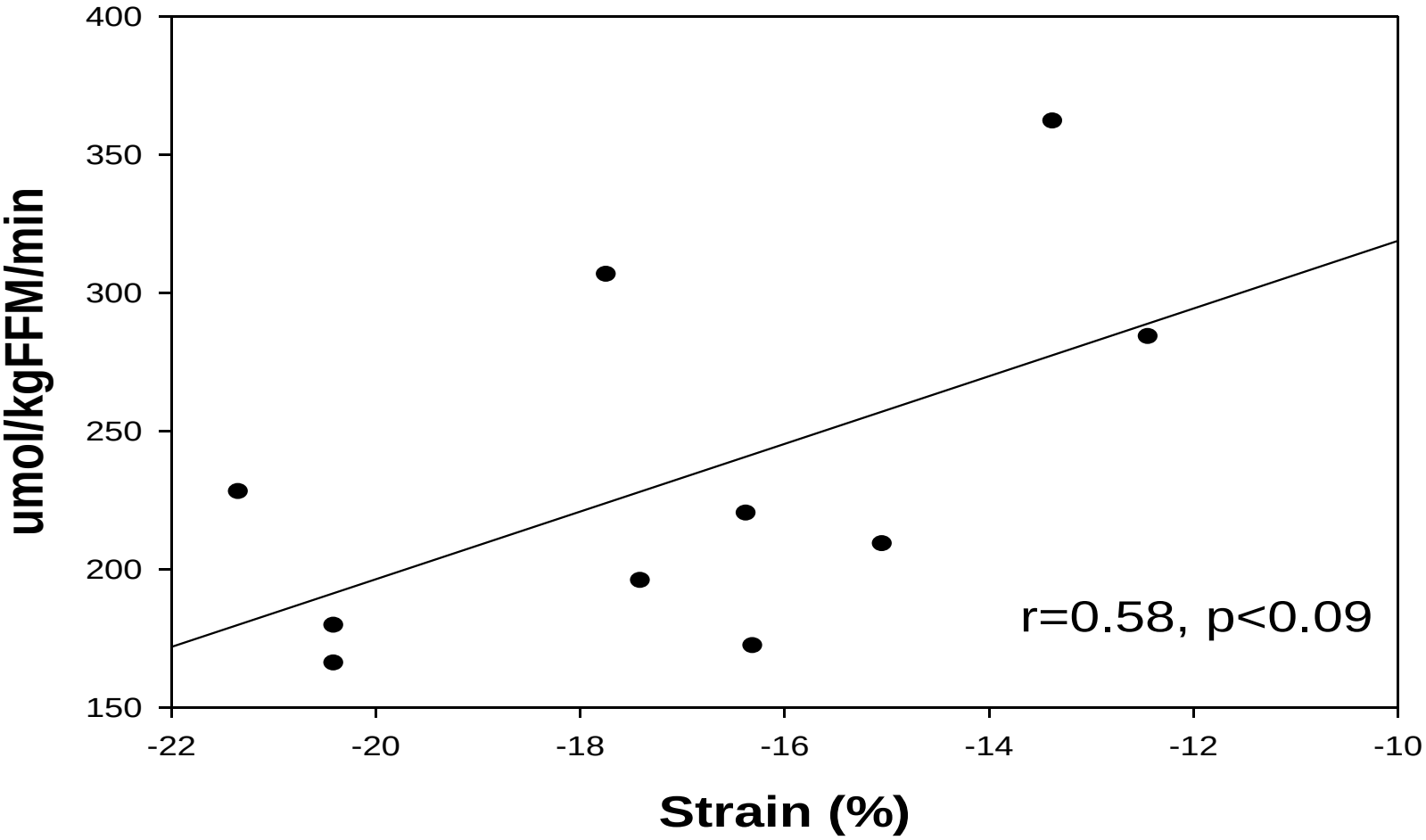
Relationship between Systolic Function and Baseline Protein Breakdown Rate



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Conclusions

- Endogenous glucose production is elevated in BTHS during hyperinsulinemia; suppression from baseline tends to be mildly blunted
- Glucose disposal during hyperinsulinemia increased in BTHS however insulin sensitivity is not increased in BTHS
- Elevated serum FFA level in BTHS but is normally suppressed with hyperinsulinemia
- Adipose tissue lipolytic rate per unit of fat mass is lower in BTHS (inhibition by ↑ serum FFA level?)- does not appear to cause ↑ serum FFA level

Conclusions

- Surprisingly, FA oxidation similar to Cntl at baseline-need mild exercise to stimulate?
- Whole body protein breakdown increased in BTHS during baseline and hyperinsulinemia
- Specific AA's are different in BTHS: Arg, Cit, Gln are lower, and Thr, Phe and Pro are higher in BTHS than controls
- Myocardial lipotoxicity hypothesis not relevant in BTHS but may be in skeletal muscle (Takeda et al. *Eur J Pediatr* 2011)
- Elevated whole-body protein breakdown at rest and during hyperinsulinemia may be associated with lower systolic function
- Provides some evidence for cardiac cachexia hypothesis

Future Directions

- R01 HL107406-01A1 "Heart and Skeletal Muscle Metabolism, Energetics and Function in Barth Syndrome“
- Overall hypothesis: Cardioskeletal FA metabolism is severely impaired which facilitates increased cardioskeletal protein catabolism, AA anaplerosis and enhanced glucose metabolism in order to supply the energy required for normal heart and skeletal muscle function.
- Because AA’s and glucose provide an inherently lower amount of ATP than FA’s to these high energy requiring tissues, an energy deficit occurs.
- We hypothesize that cardioskeletal energetics are impaired which mediates/exacerbates exercise intolerance, fatigue, muscle wasting, and heart failure in BTHS.

Specific Aims

Specific Aim 1: To characterize cardioskeletal nutrient metabolism in children (8-17 yrs, n=15) and adults (18-30 yrs, n=15) with BTHS and compare this to corresponding healthy age, BMI, pubertal level and activity level-matched control children (n=15) and adults (n=15) (total n=60).

Specific Aim 1A: To characterize skeletal muscle FA (oxidation and lipolytic rate), AA (proteolytic and oxidation rate) and glucose (disposal and hepatic production rate) metabolism during rest, low-intensity exercise and post-exercise recovery in participants with BTHS and in controls.

Specific Aim 1B: To characterize myocardial FA, AA (exploratory) and glucose metabolism during rest in adults with BTHS (n=15) and in adult controls (n=15).

Methods: stable and radio-isotope tracers, mass spectrometry, PET imaging

Specific Aims

Specific Aim 2: To examine the relationship between cardioskeletal nutrient metabolism, energetics and function in BTHS in children (n=15) and adults (n=15) with BTHS and in control children (n=15) and adults (n=15).

Methods: Magnetic resonance spectroscopy (31 P MRS), echocardiography, exercise testing

Specific Aims

Exploratory Aim: To explore mechanistic molecular pathways of nutrient metabolism: specifically protein catabolism, oxidative phosphorylation and FA metabolism, in human fibroblasts and myocytes derived from induced pluripotent stem cells obtained from adults and children with BTHS and in adult controls.

Methods: skin biopsy to obtain skin fibroblast→iPSC→skeletal muscle cells, rtPCR, western blot, mitochondrial respiration (Seahorse)

Collaboration with Barry Byrne, MD, PhD, University of Florida

Thanks to:

Study Team:
Kay Bohnert, MS
Linda Peterson, MD
Dominic Reeds, MD
Carolyn Spencer, MD
(Medical University of South Carolina)
Melissa Maisenbacher, MS
(University of Florida)
Barry Byrne, MD, PhD
(University of Florida)
Bob O'Connor, PhD
Alan Waggoner, MS
Rachel Friedman, MS
Kevin Reiersen, BS

Kevin Yarasheski, PhD
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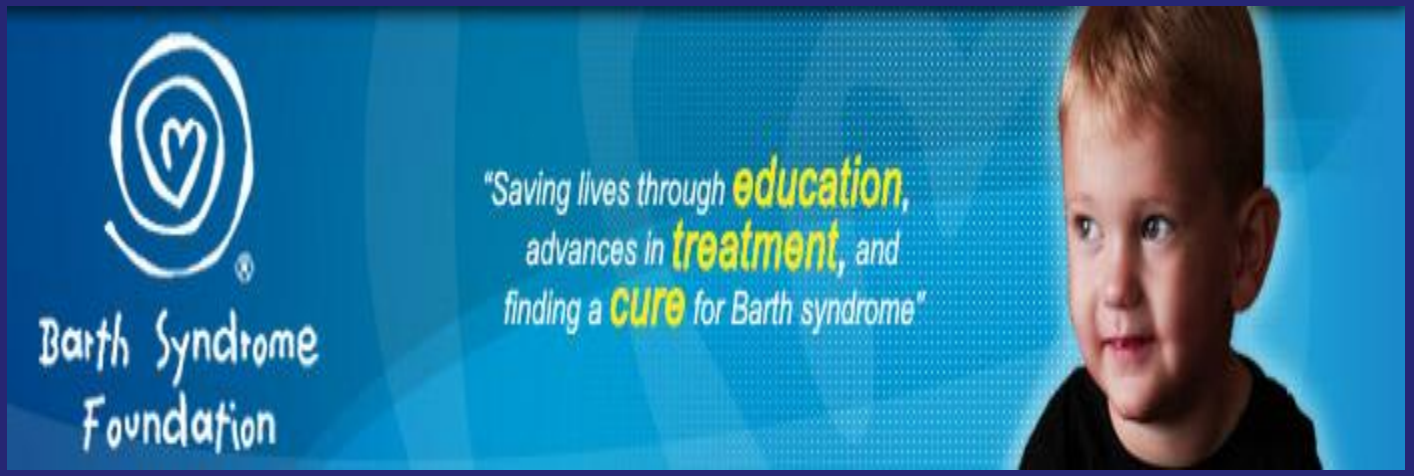

Washington University in St. Louis
School of Medicine

Participants!

“Tafazzi”