Heart Failure and Mitochondrial Function

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November 14th , 2017

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Translational Research Institute

Overview of Presentation

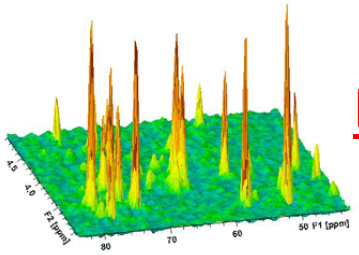
1) Introduction to myself and my KL2 project
2) Timeline of training and research during KL2
3) Research update
4) Plans ahead

Background:

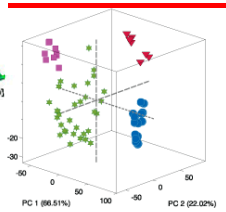
Ph.D. and postdoc Analytical Chemistry

- Metabolomics Method Development
- Statistical Analysis

National Institute of Standards and Technology
U.S. Department of Commerce



Metabolites



Metabolomics

Excellent tool for hypothesis generation

What next?

Career Goal:

Research in aging using metabolomics techniques in clinical research.

Training Goal:

Acquire skills necessary for clinical research

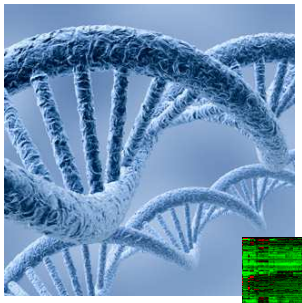
1) Regulatory Science
   - -IRB submission
   - -Informed Consent
   - -Trial design
2) Isotope Tracer Methodology
3) NIH Grant Submission

Research Goal:

Collect preliminary data for K25 research grant

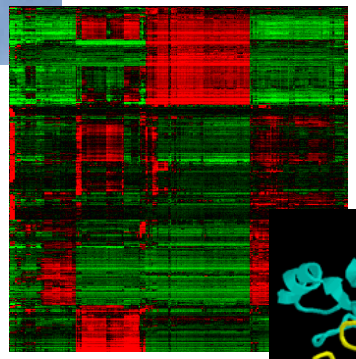
1) Validate methods in skeletal muscle
2) Demonstrate ability to conduct clinical research
3) Characterize metabolic signature of heart failure

Metabolomics



DNA

Genomics

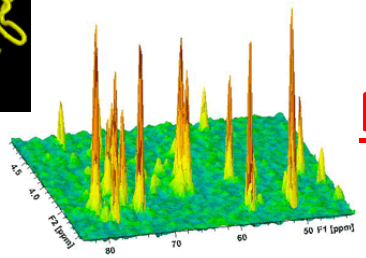


RNA

Transcriptomics

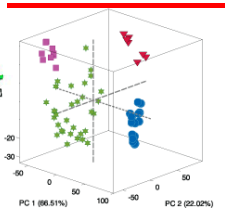
Protein

Proteomics



Metabolites

Metabolomics

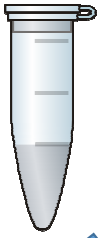


LC-MS Metabolomics Analysis

Load frozen biopsies into homogenization vials.

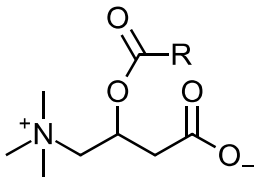
Homogenization

Raw metabolite extract



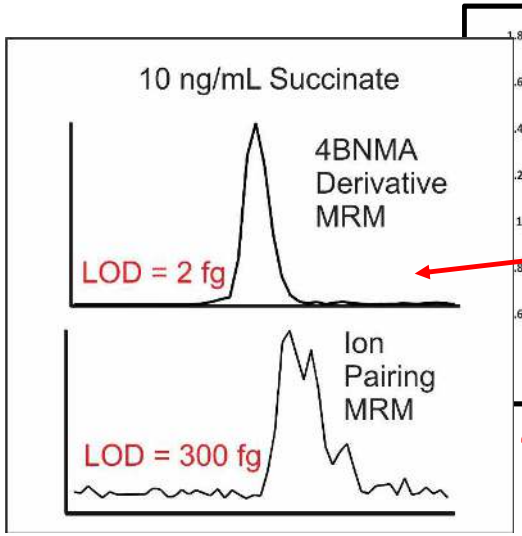
Cation exchange SPE

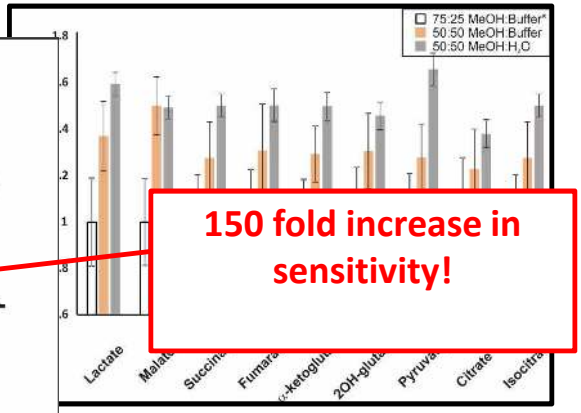
Acylcarnitines



Spike with Isotope labeled Standards

Functional Derivitization



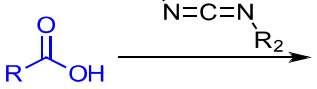


150 fold increase in sensitivity!

4 BNMA Derivitization

Organic Acids (TCA intermediates)

Carbodiimide Coupling

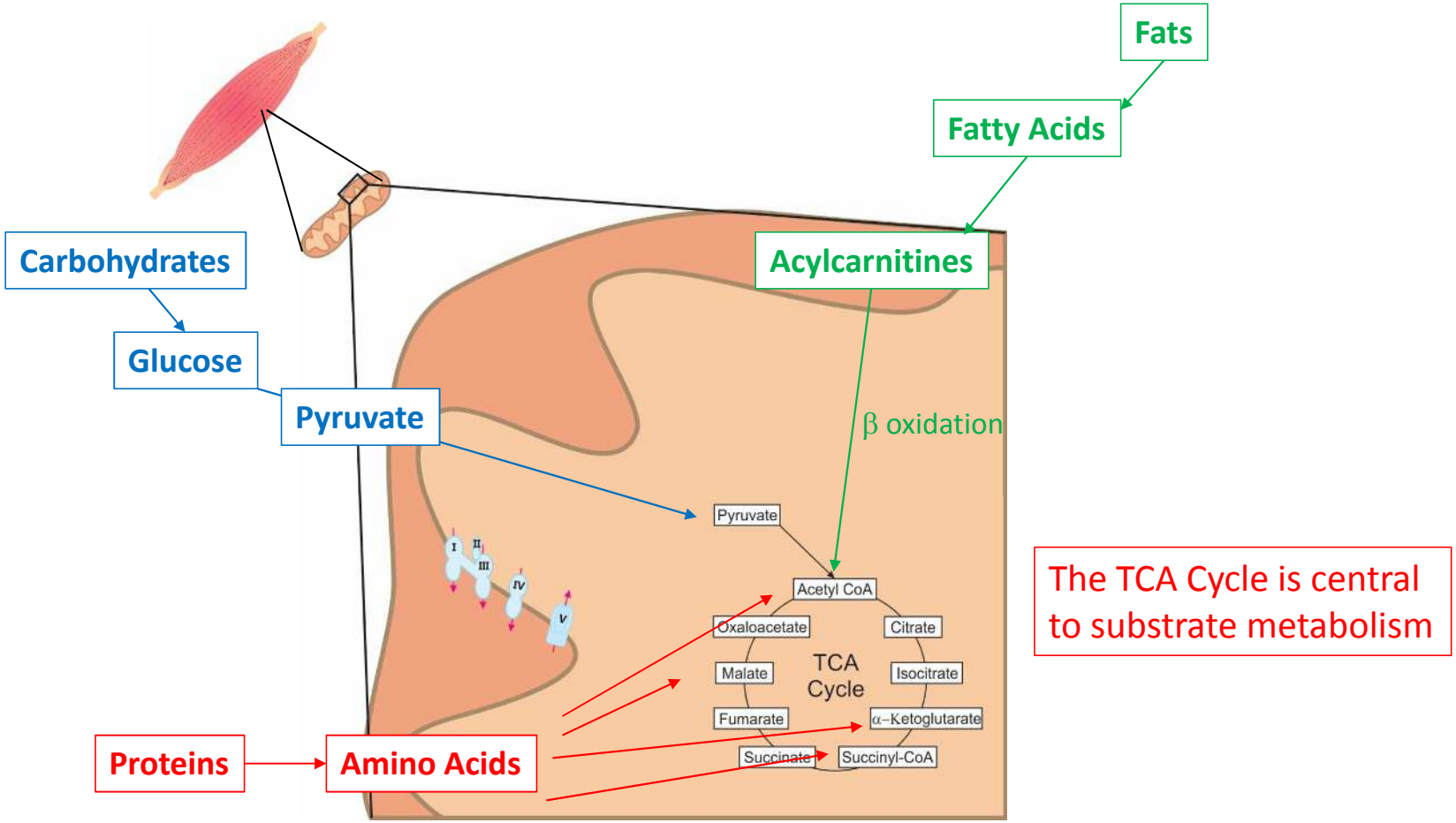


Derivatization Reagent H_2N-R_3

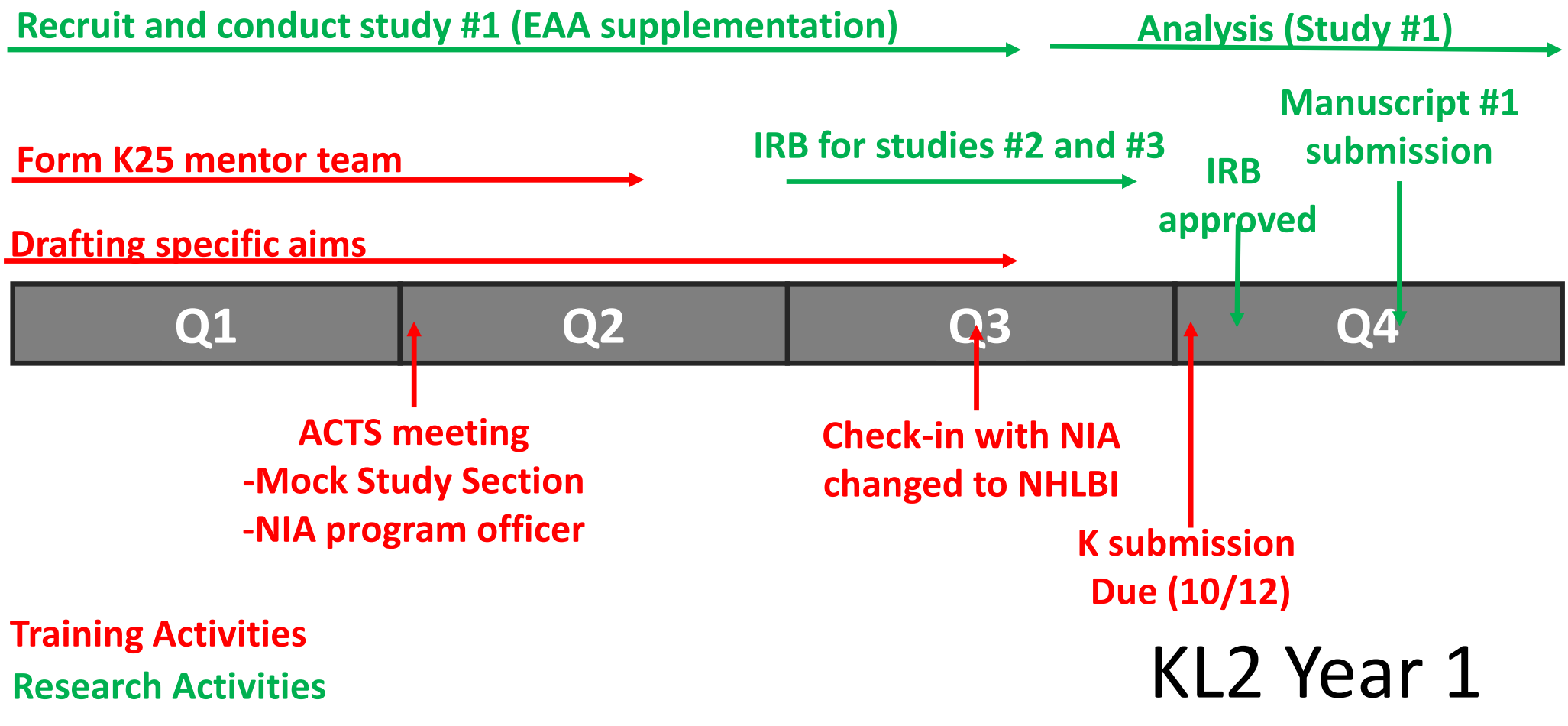
Organic Acid (TCA Intermediate)

Activated Carboxylic Acid

Substrate Metabolism in the Mitochondria

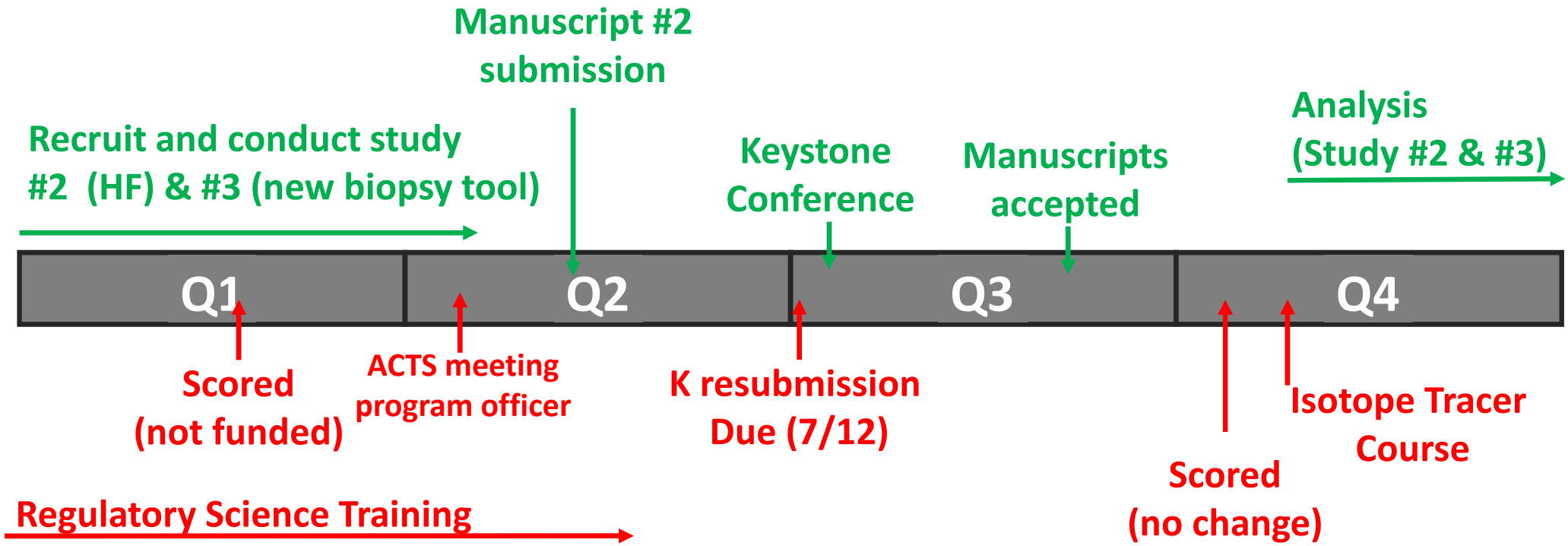


KL2 Timeline



KL2 Year 1

KL2 Timeline



Training Activities

Research Activities

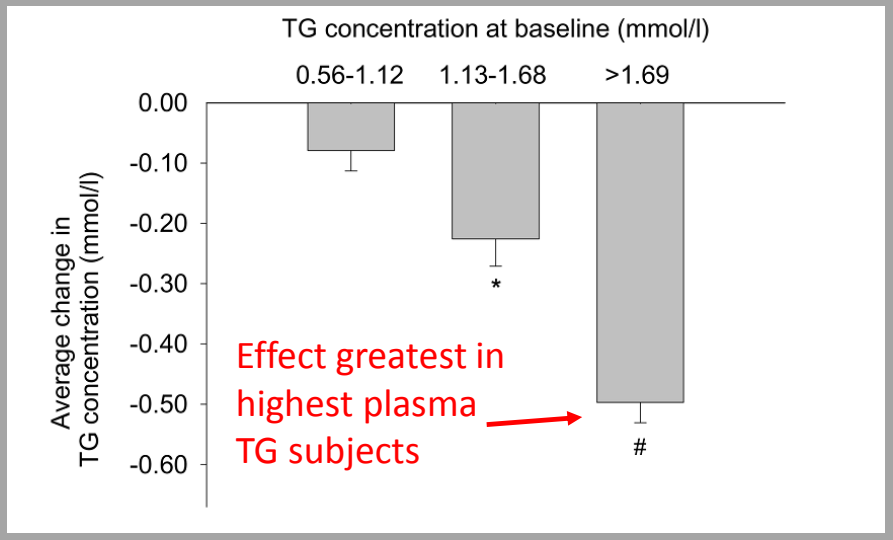
KL2 Year 2

Project #1: Essential Amino Acids (EAA) and Plasma TG

Plasma triglycerides (TG) are an independent risk factor for coronary heart disease.

Previous work:

Plasma TG decrease with chronic EAA supplementation



Borsheim *et al*, *Nutrition*, 2010

Overall goal

What are the effects of EAA supplementation on regional lipid metabolism?

My goal

What are the impacts of EAA supplementation on mitochondrial substrate metabolism?

Essential Amino Acid Supplementation (EAAS)

EAAS mixture

3.26% Histidine
8.57% Isoleucine
35.88% Leucine
17.0% Lysine
3.59% Methionine
4.65% Phenylalanine
9.57% Threonine
7.44% Valine
9.97% Arginine

Dose

Challenge: 22 g over 3.5 hours (drink)

Chronic: 22 g a day for 8 weeks

Subject Information

Inclusion Criteria

Women and men age 50-75
Fasting plasma TG between 130-500 mg/dl

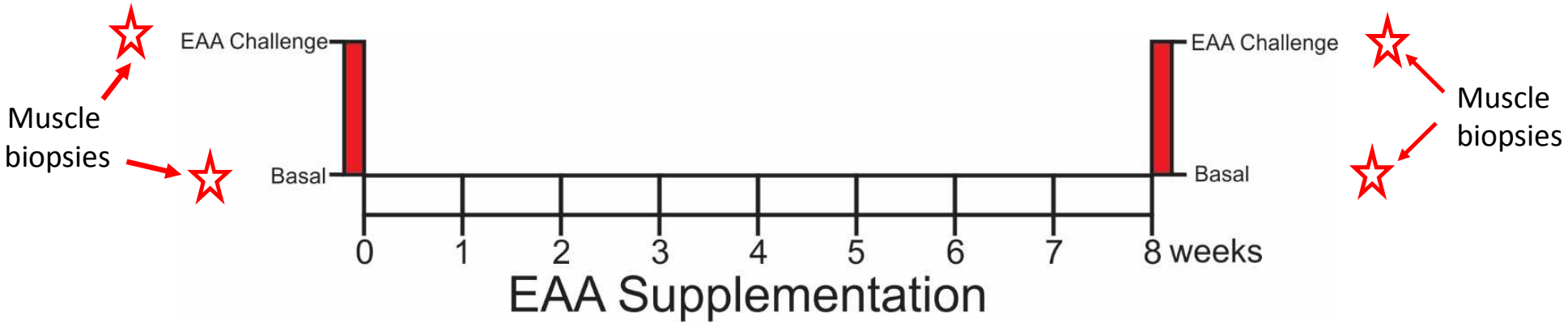
Exclusion Criteria

Use of lipid altering agents
Diabetes
Kidney or liver disease
Bleeding disorders
Anemia
Endocrine disease
Hepatitis or HIV
Alcohol Abuse
Drug Abuse

Subject Gender (F/M)	Age (years)	BMI	Plasma TG Week 0 (mmol/l)	Plasma TG Week 8 (mmol/l)
(4/2)	69 ± 4	35 ± 9	2.3 ± 0.4	1.8 ± 0.3*

* $p < 0.05$

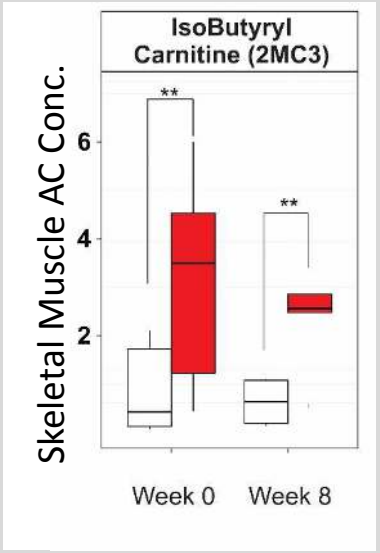
Study Design



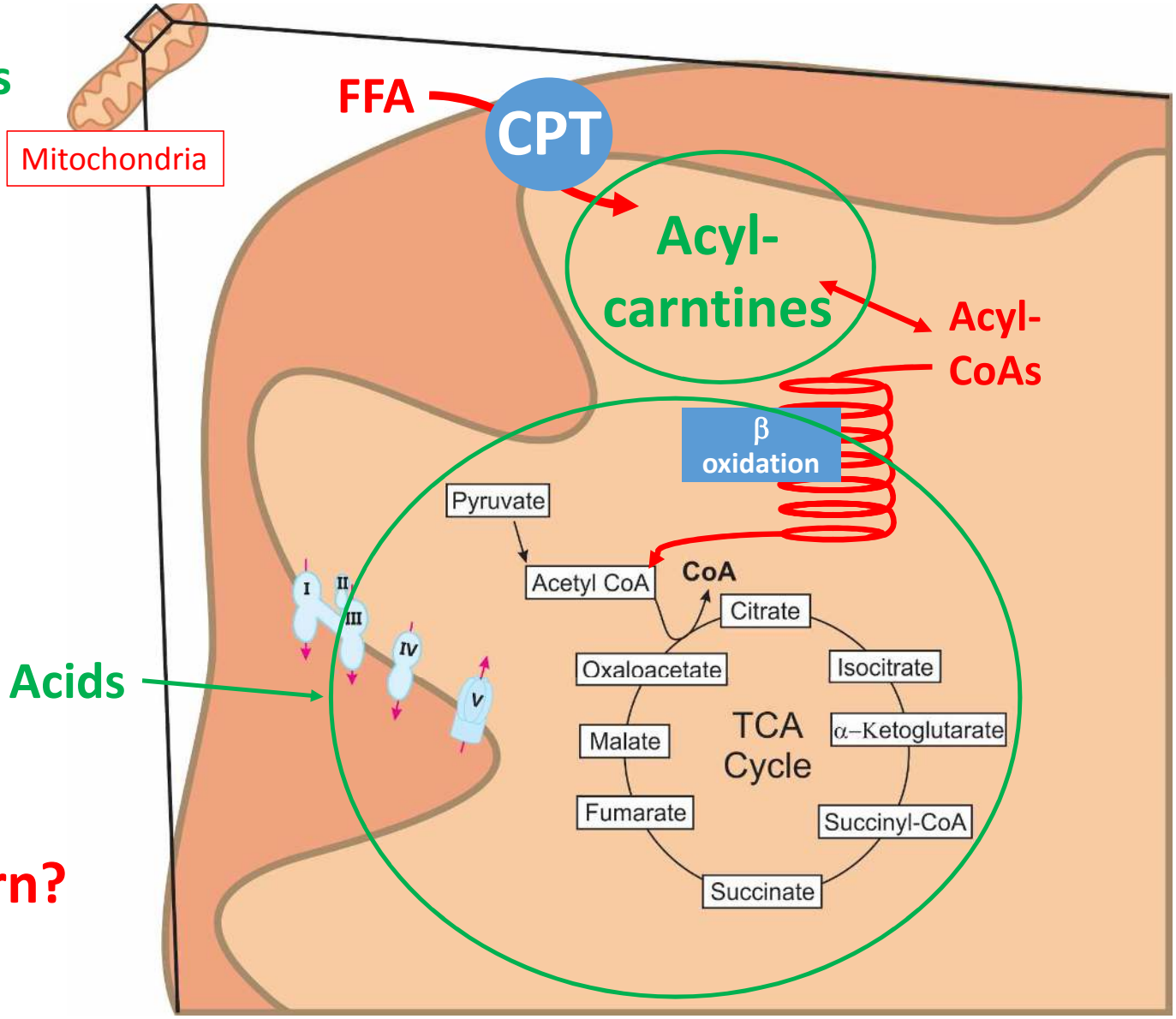
Targeted metabolite measurements in skeletal muscle biopsies collected.

Evaluated three responses by paired t-tests

1) Changes in basal concentrations in response to EAA
2) Response to acute challenge of EAA
3) Change of response to acute challenge of EAA



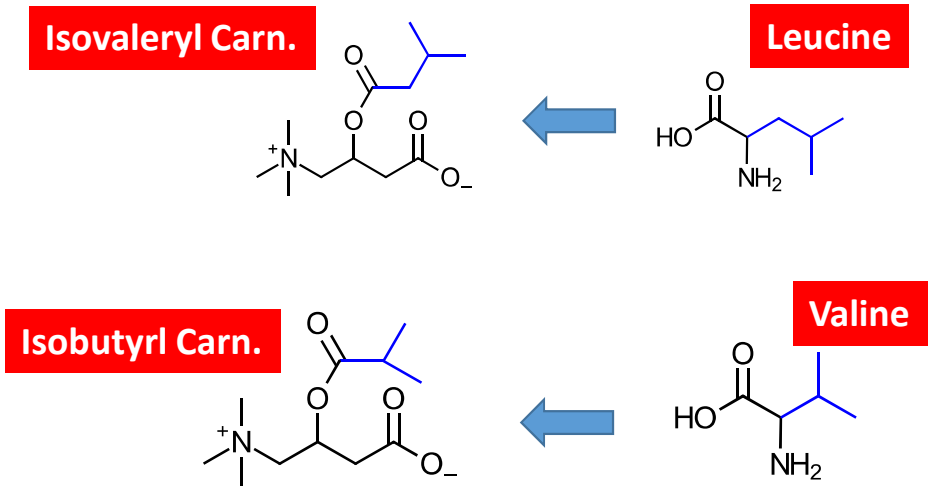
Metabolites Measured:

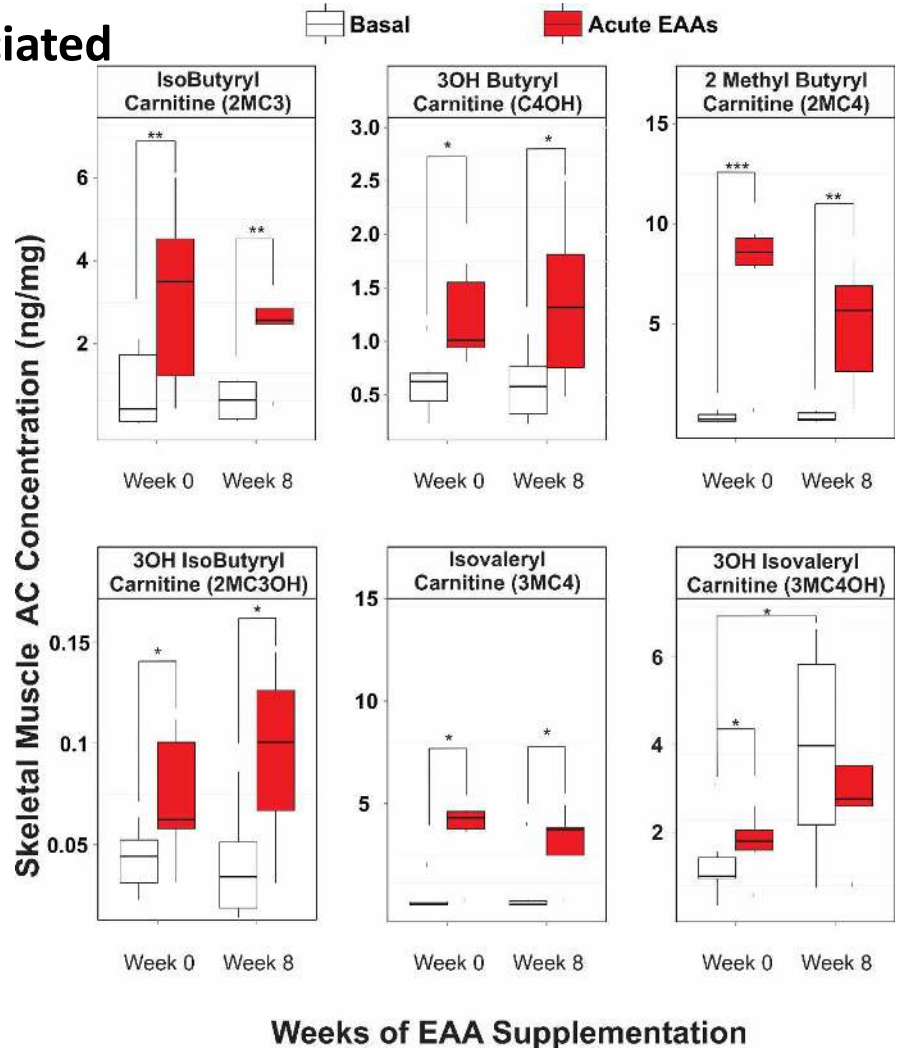


Organic Acids

What did we learn?

1a) There is a large increase in acylcarnitines associated With oxidation of BCAAs in response to EAA challenge.

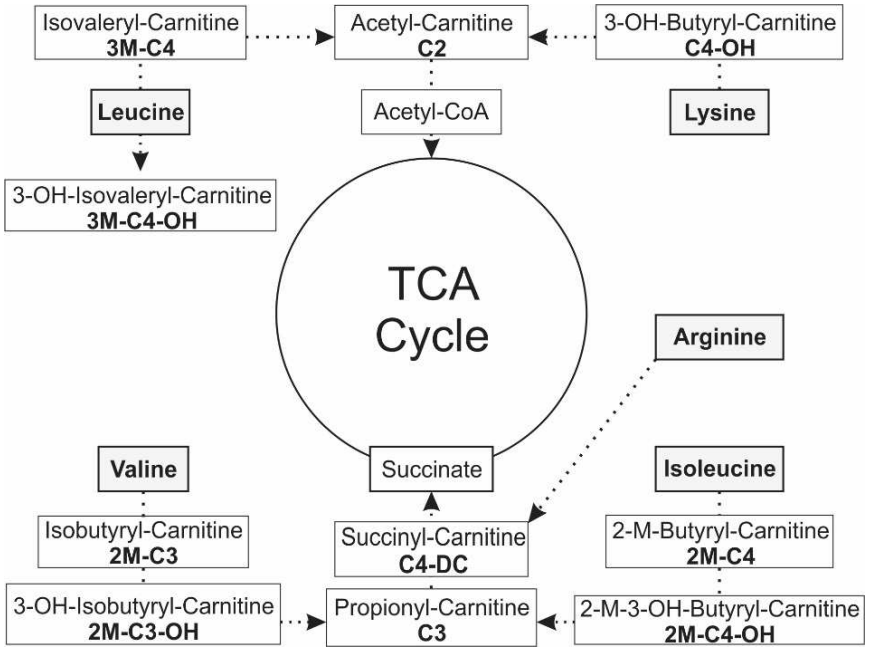


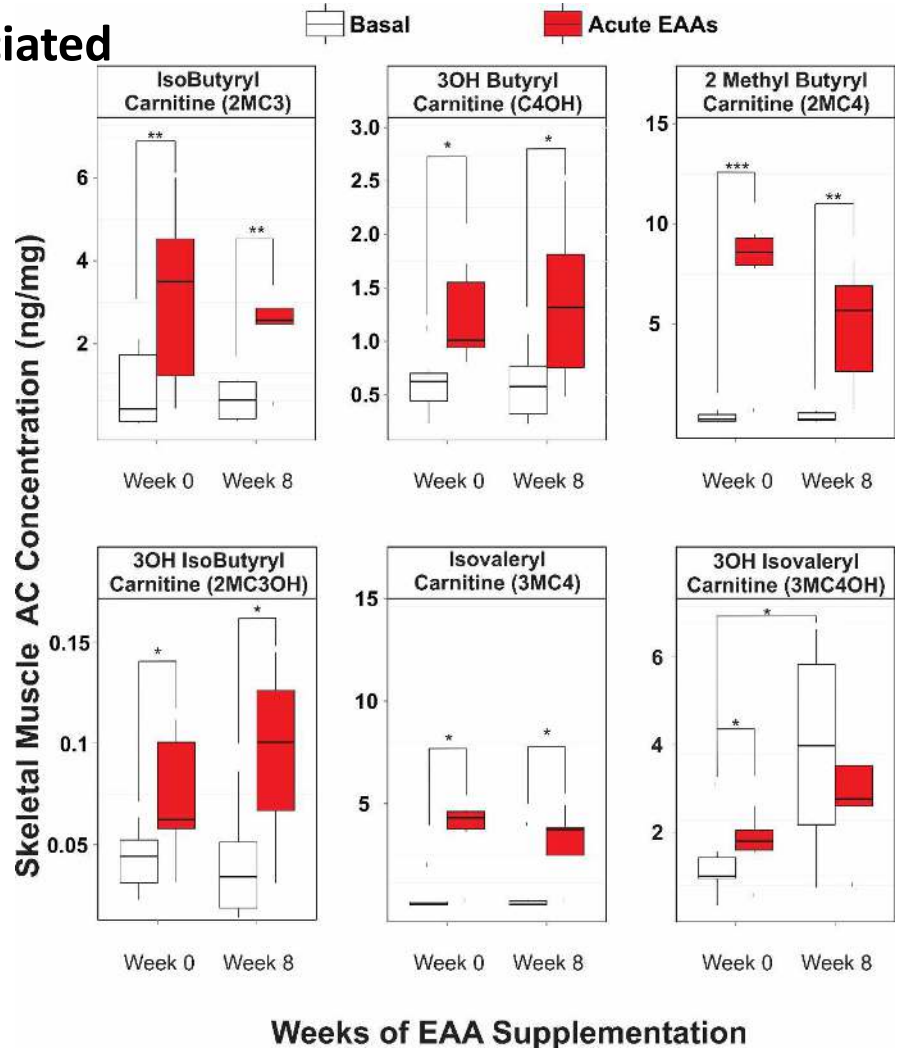


* p <0.05, ** p < 0.01, *** p < 0.005

1a) There is a large increase in acylcarnitines associated With oxidation of BCAAs in response to EAA challenge.

1b) This change is largely consistent with one exception (3MC4OH).

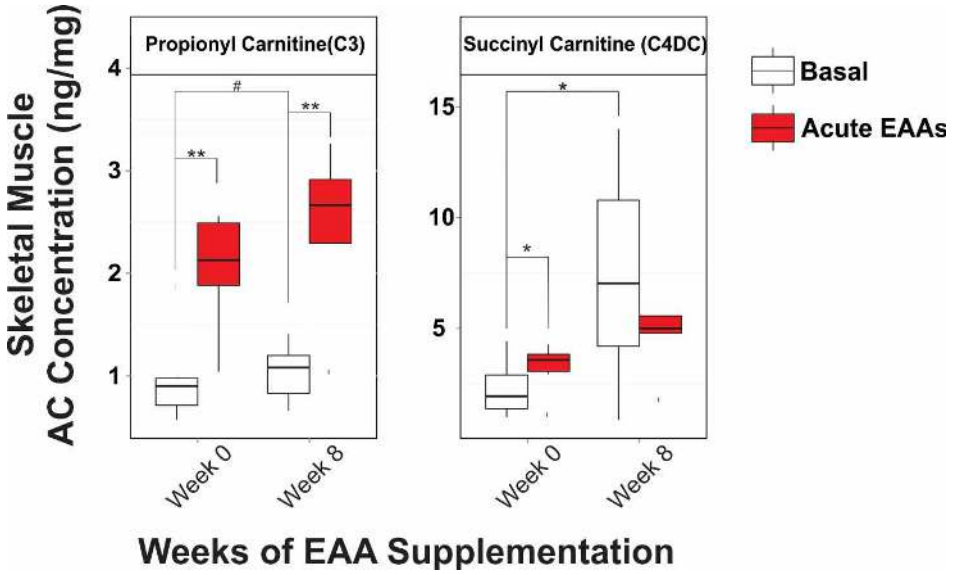


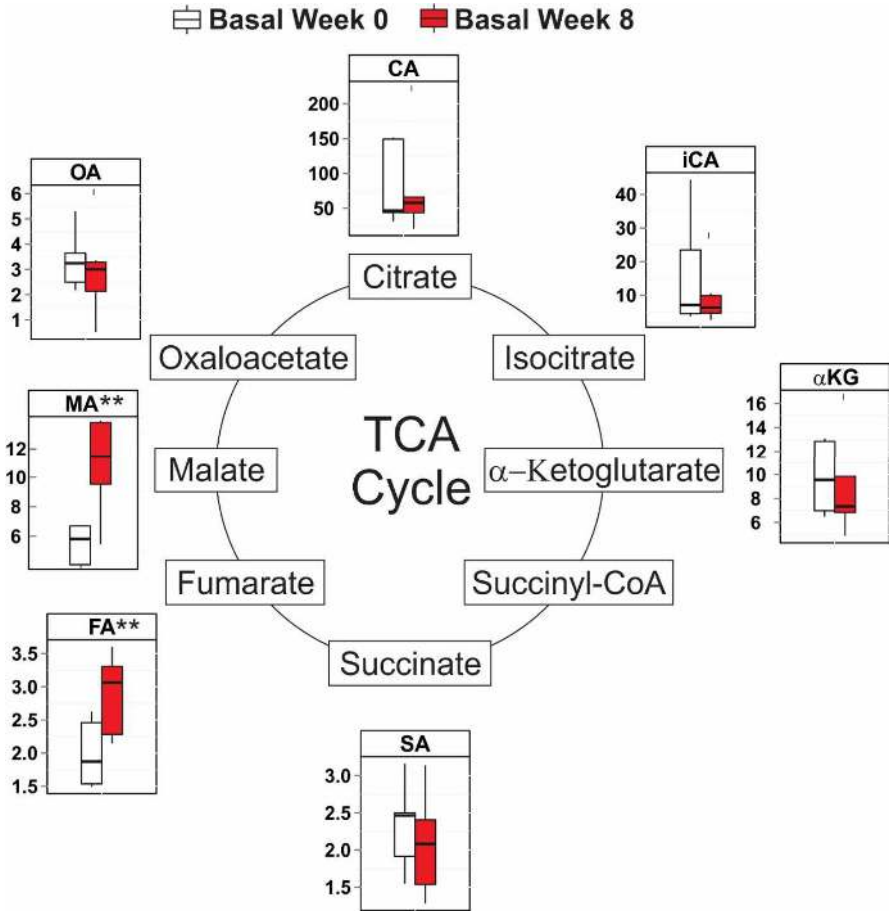


* p <0.05, ** p < 0.01, *** p < 0.005

2a) We see evidence that chronic EAA supplementation increases anaplerosis (replenishes TCA pool)

i) accumulation of late state TCA intermediates
ii) accumulation of anaplerotic acylcarnitines



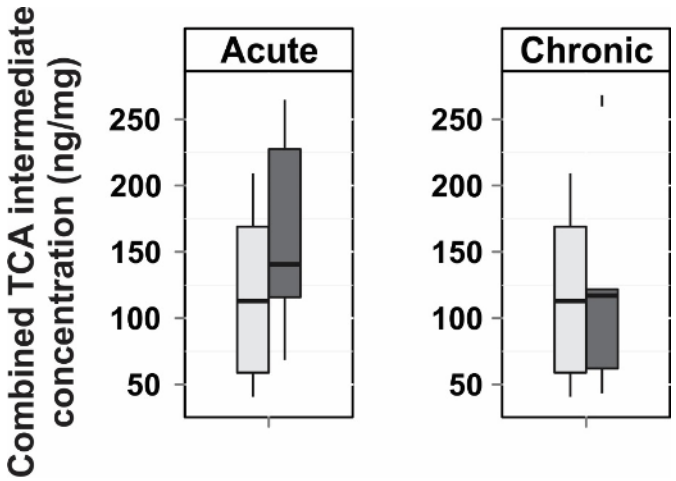


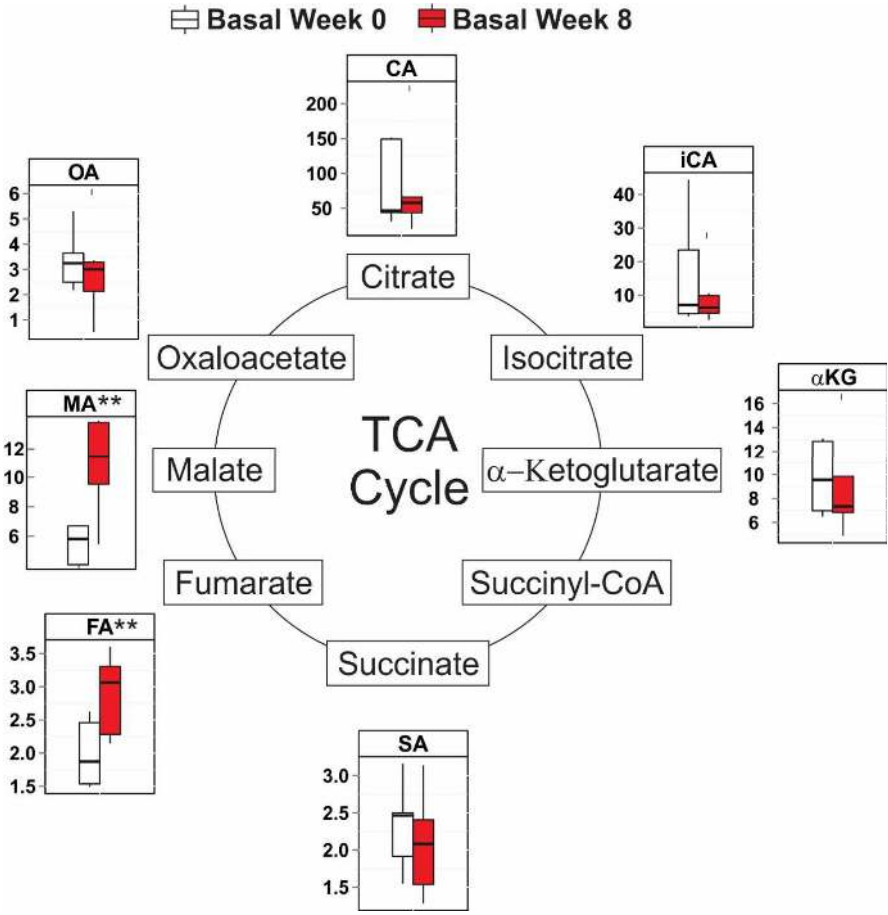
** p < 0.01

2a) We see evidence that chronic EAA supplementation increases anaplerosis (replenishes TCA pool)

i) accumulation of late state TCA intermediates
ii) accumulation of anaplerotic acylcarnitines

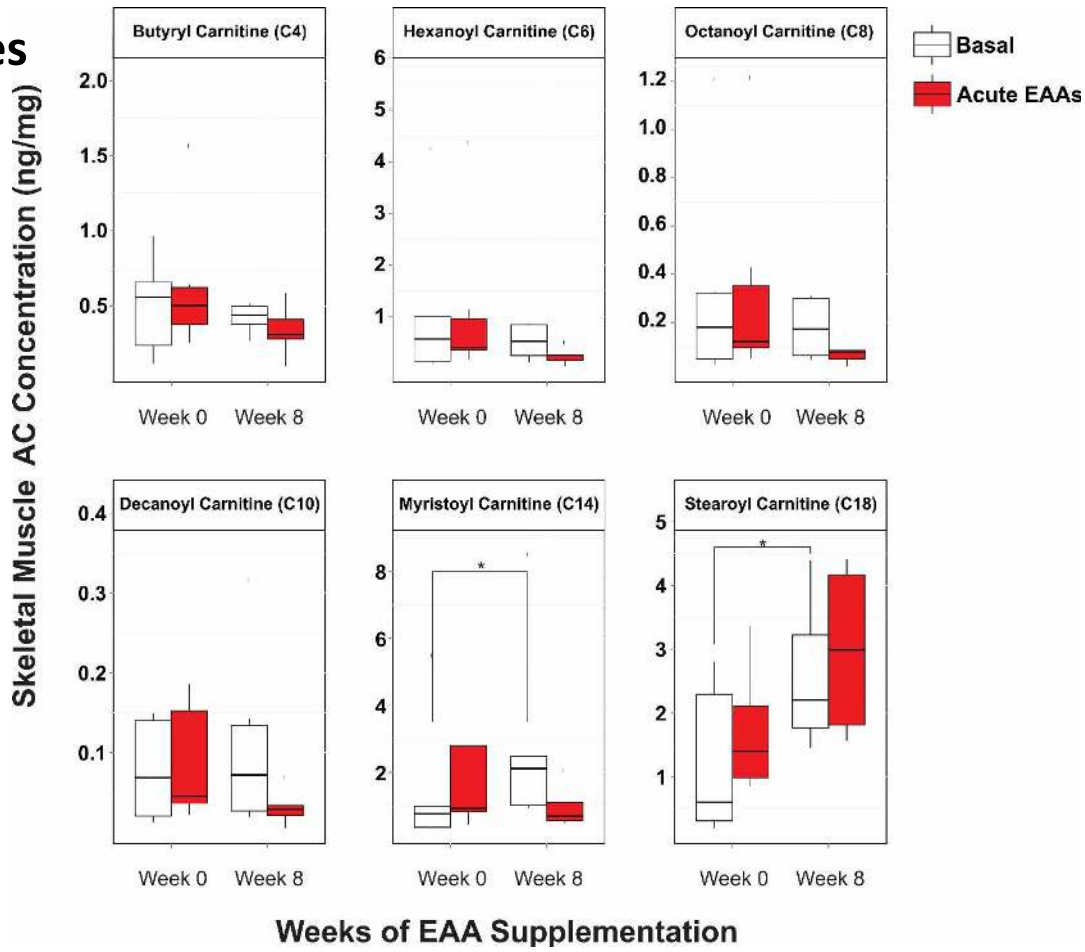
2b) TCA pool size does not change





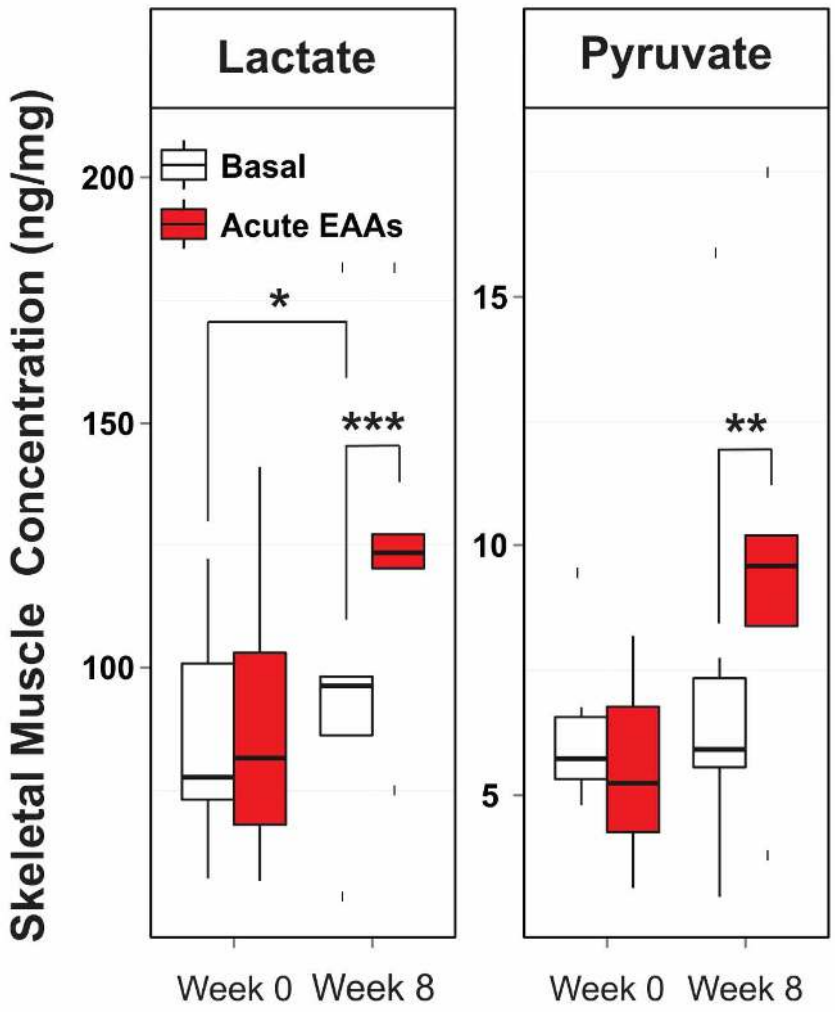
** p < 0.01

3) Long, but not medium, chain acylcarnitines accumulate in skeletal muscle with chronic EAA supplementation.



3a) Lactate accumulates in skeletal muscle with chronic EAA supplementation.

3b) Lactate and pyruvate increase in response to EAA challenge only after chronic period.

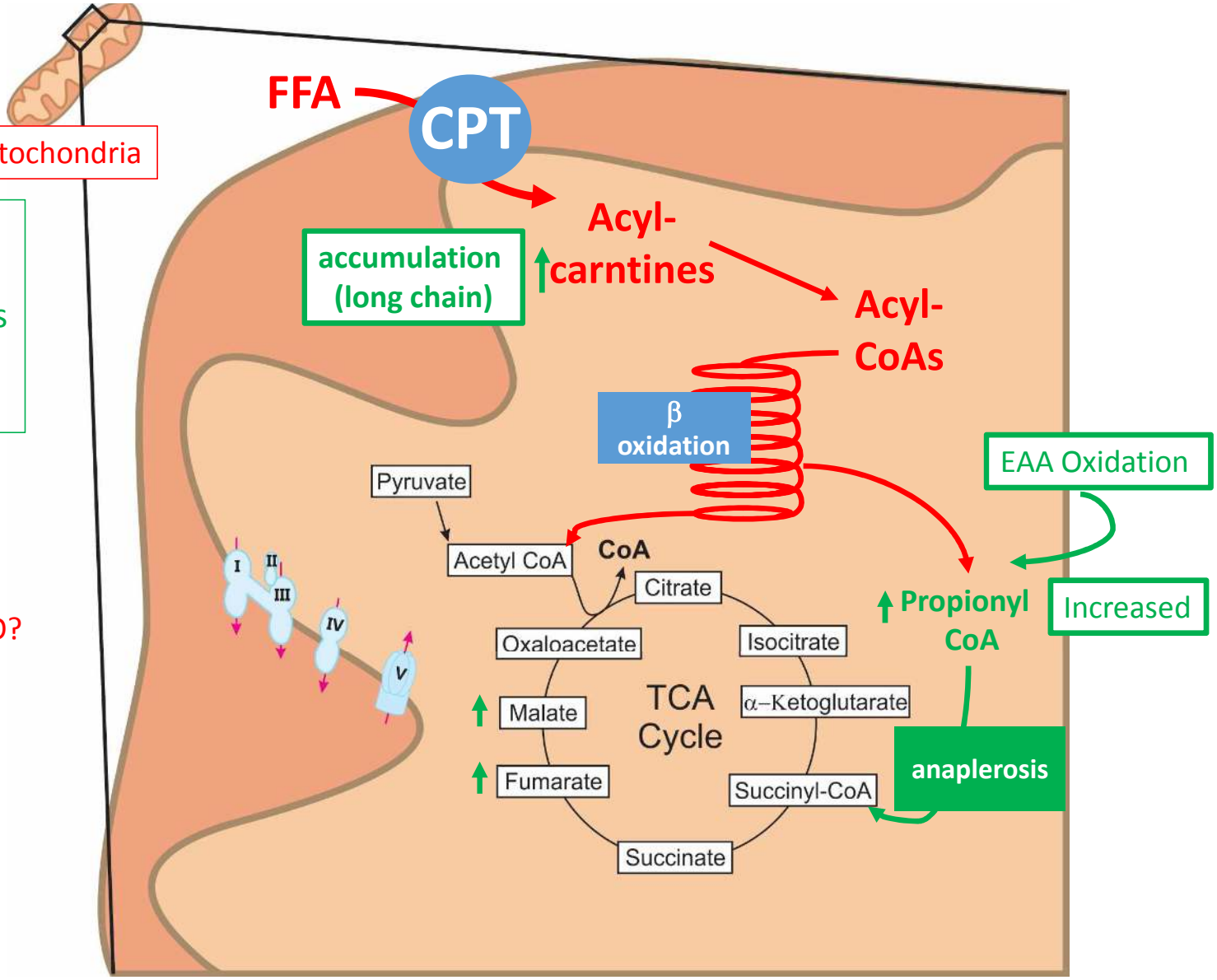


Effects of Chronic EAA Supplementation

Summary:
Increased accumulation of:
- Late state TCA intermediates
- Anaplerotic acylcarnitines
- Long chain acylcarnitines

Does EAA oxidation "box out" FAO?

Does TCA pool size limit FAO?

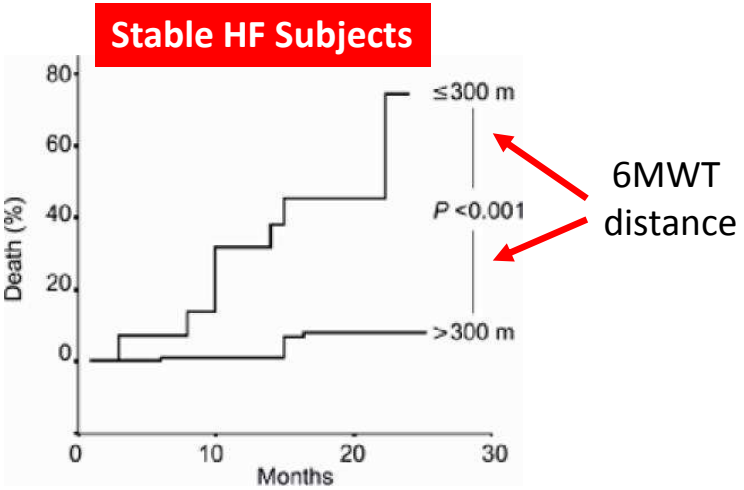


Project #2: Characterize the Metabolic Fingerprint of HF in Skeletal Muscle

Heart Failure (HF) – Condition in which the heart is unable to supply sufficient blood.

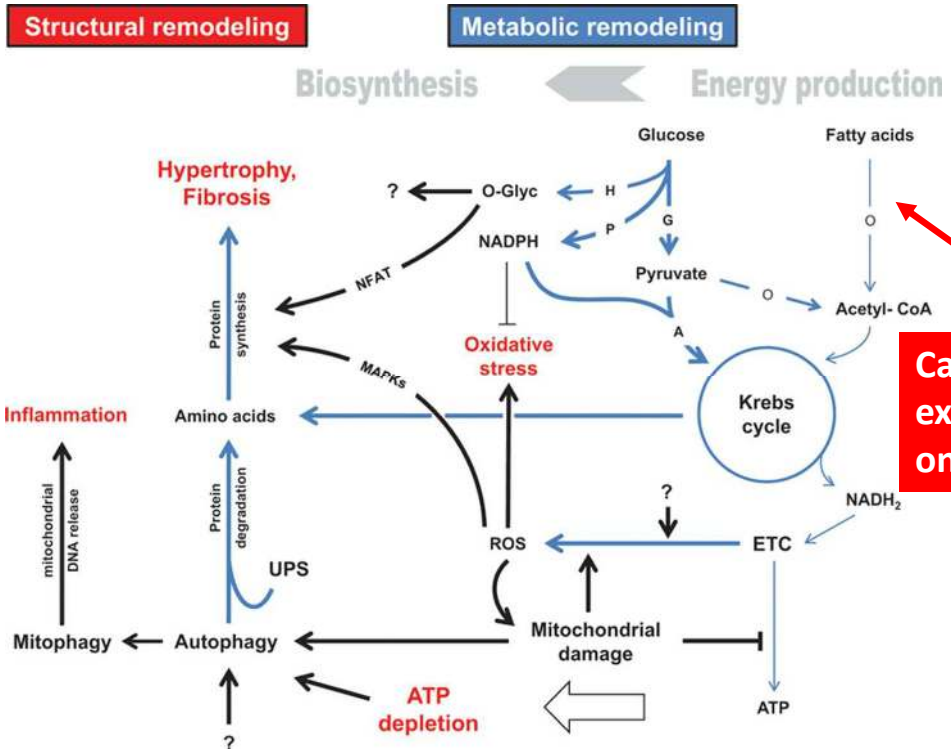
- Effects 5-10% of population over 65.
- 50% risk of death within year of diagnosis.
- Largest source of hospital readmission for Medicare patients.

Exercise intolerance is a hallmark of HF and the is predictive of mortality [as measured by the six minute walk test (6MWT)]



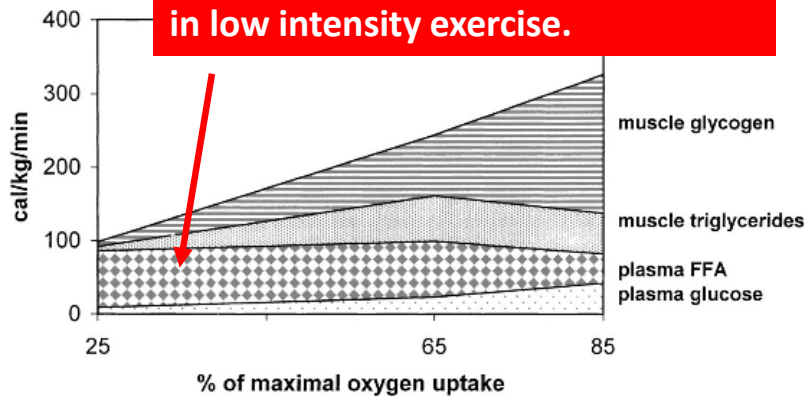
Arslan et al. Tex Heart Inst J, 2007

Metabolic Remodeling in Heart Failure



Doenst et al, Circulation Research, 2013

FFA is the primary source of energy in low intensity exercise.



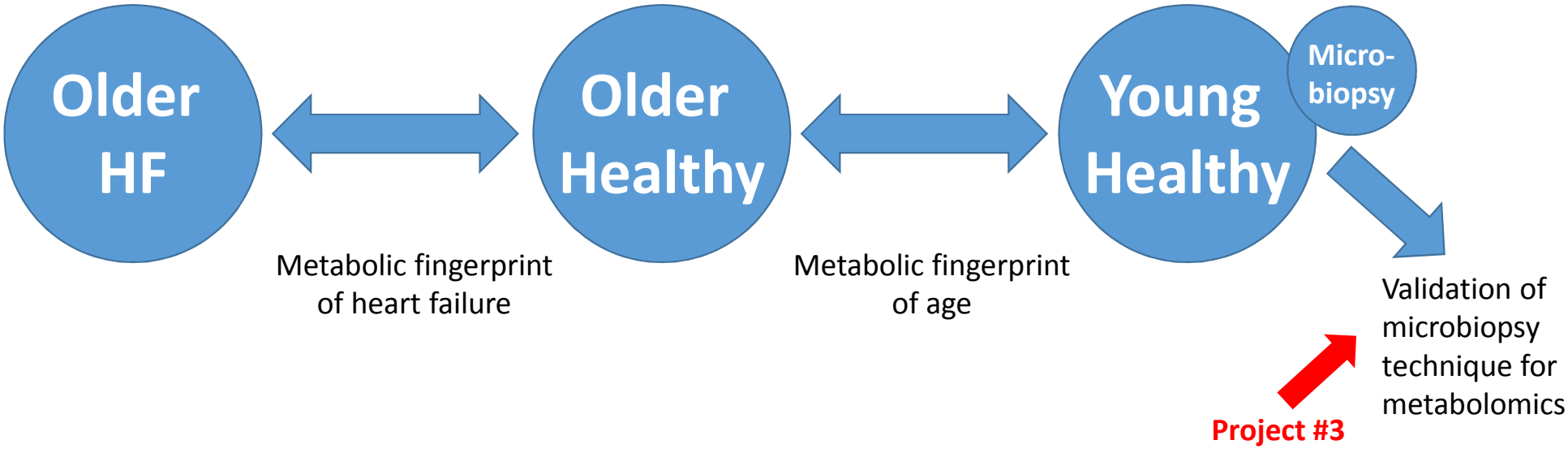
Romijn, *J Apld. Physiol.* 1994

Cardiac metabolism exhibits decreased reliance on fatty acids in HF.

Could reduced fatty acid oxidative capacity contribute to exercise intolerance in HF?

Study Design

Collect fasted muscle biopsies from three groups of subjects (n = 30):

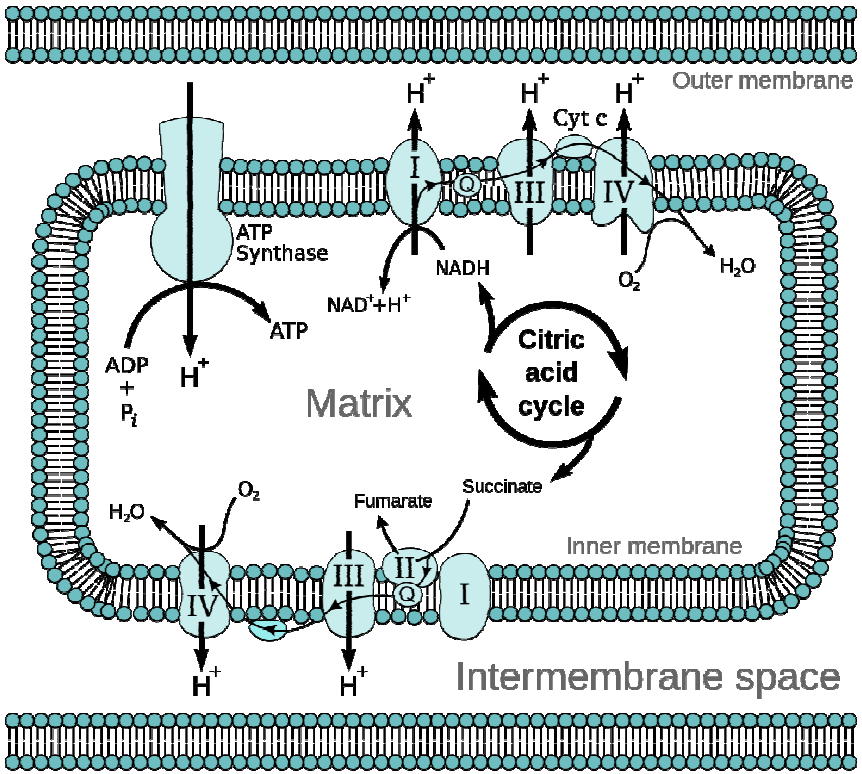


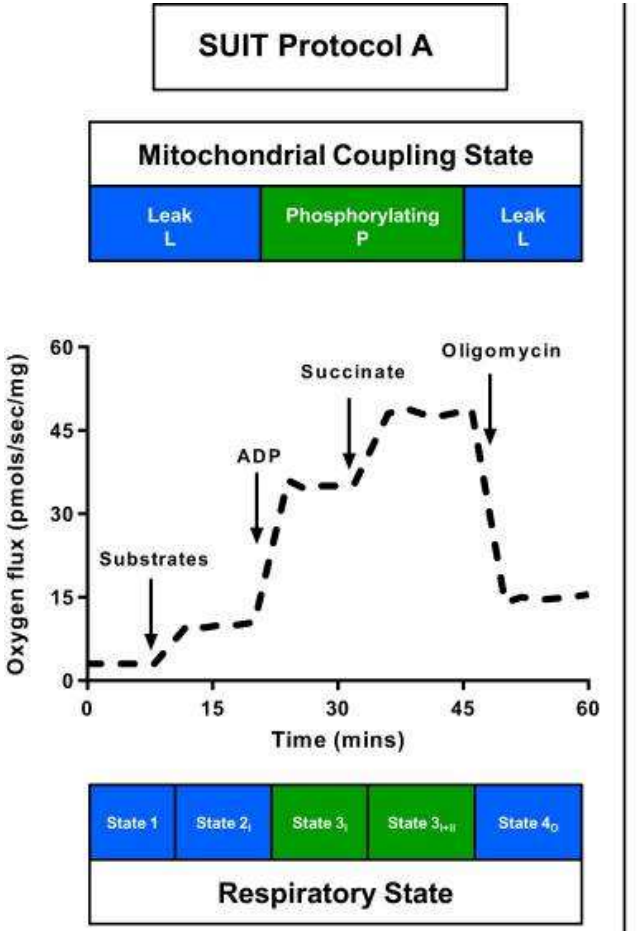
Analysis to be conducted :

1) High resolution respirometry (HRR)
2) Targeted metabolomics ← Currently underway

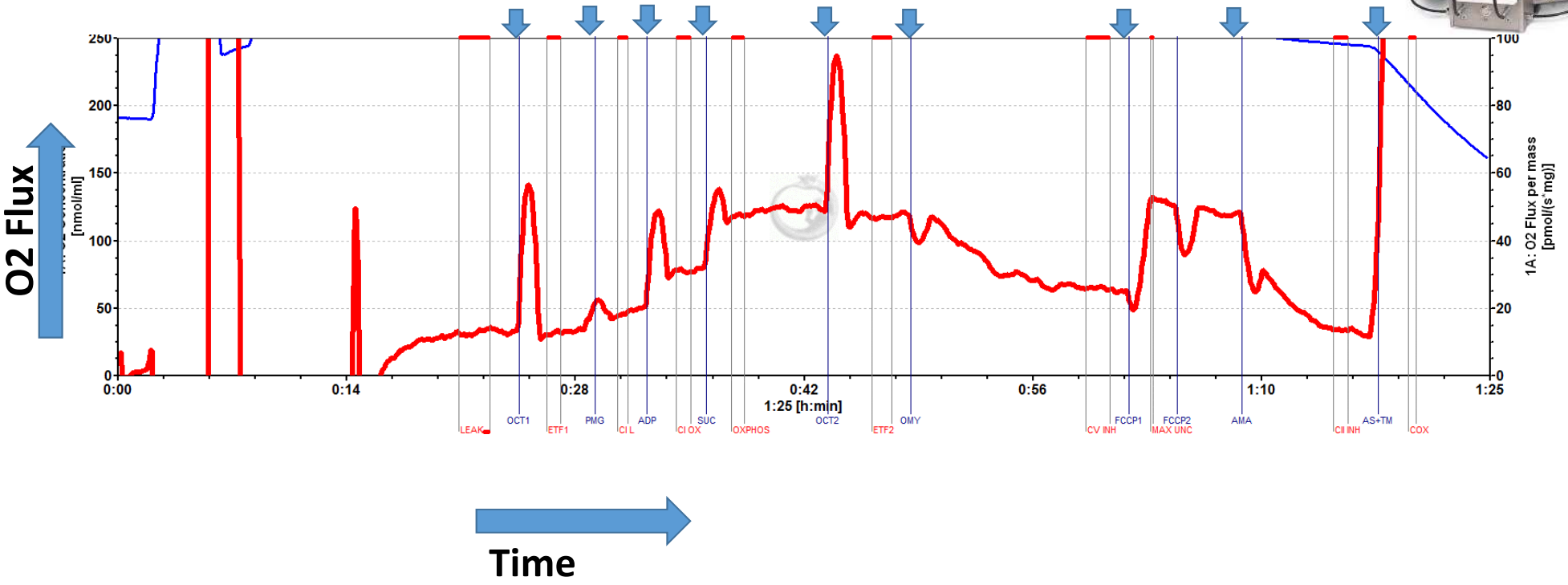
HRR conducted on permeabilized skeletal muscle fibers.

(1)Older Heart Failure (10) (65-85)
(2)Older Healthy (10) (65-85)
(3)Younger Healthy (10) (25-45)
(4)Matched Younger Healthy Microbiopsy (9)





HRR Data


Substrates or inhibitors
O2 Flux
[nmol/ml]
1A: O2 Flux per mass
[pmol/(s*mg)]
0:00
0:14
0:28
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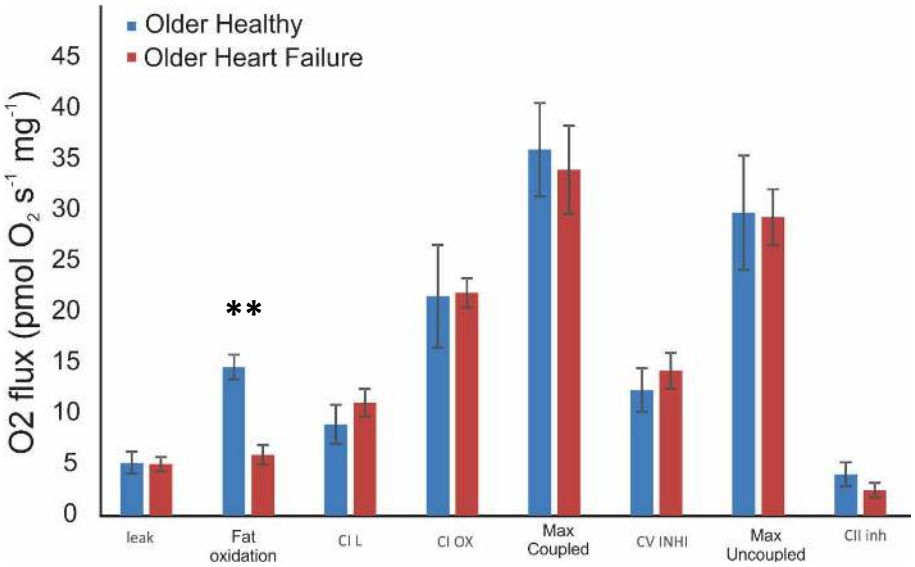
1:25 [h:min]
0:56
1:10
1:25
LEAK
OCT1
ETF1
PMG
CI L
ADP
CI OX
SUC
OXPHOS
OCT2
ETF2
OMY
CV INH
FCCP1
MAX UNC
FCCP2
AMA
CII INH
AS+TM
COX
Time

HRR of HF vs. Healthy Older Adults



HF subject skeletal muscle has reduced fatty acid oxidation potential

** $p < 0.01$

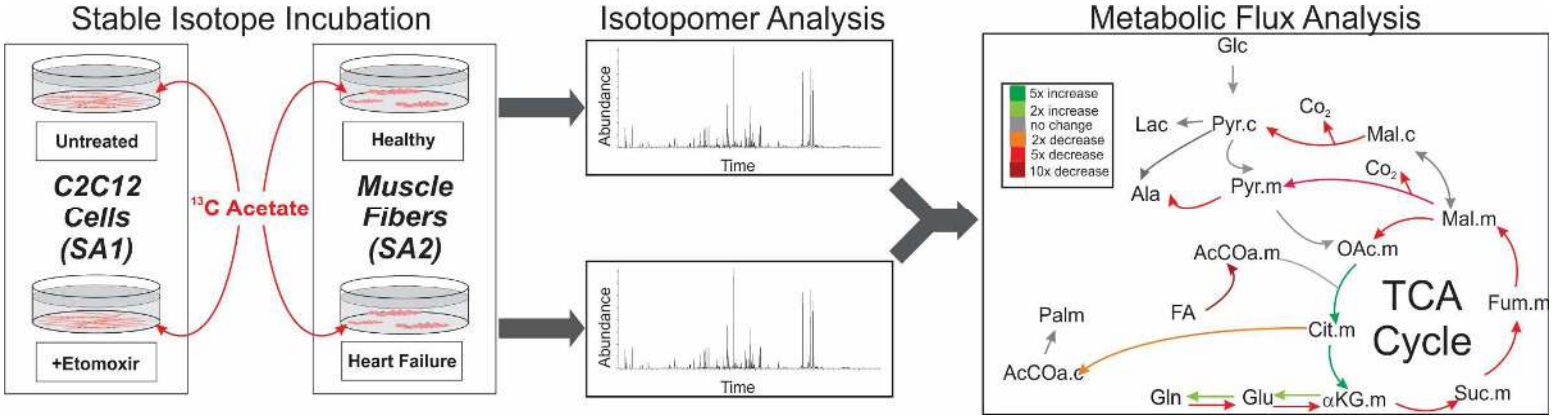
Ongoing and future work

Ongoing:

- Metabolic “fingerprint” of HF in skeletal muscle
- Validation of microbiopsy tool for metabolomics studies
- Relationship of BMI, musculoskeletal performance, functional capacity and ejection fraction in HF

Future:

- Develop metabolic flux analysis (MFA) platform for human skeletal muscle studies.



Acknowledgements

Co-investigators:
Nick Hurren, PhD
Eugenia Carvalho, PhD
Il Young Kim, PhD

Study Nurse:
Scott Schutzler

Study Coordinator:
Cosby Lasley

Mentoring Team:
Robert Wolfe, PhD
Jeanne Wei, MD PhD
Elisabet Borsheim, PhD
Sean Adams, PhD
Gohar Azhar, MD

Funding:

TRI KL2 Mentored Career Development Award

Claude Pepper Pilot Award

TRI Support:
Mary Aitken, MD, MPH
Pedro Delgado, MD

Nia Indelicato
Amy Jo Jenkins

Other KL2 Scholars!

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