Heart Failure and Mitochondrial Function

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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

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
LC-MS Metabolomics Analysis

Load frozen biopsies into homogenization vials.

Homogenization

Raw metabolite extract

Cation exchange SPE

Functional Derivitization

Spike with Isotope labeled Standards

10 ng/mL Succinate

LOD = 2 fg

4 BNMA Derivitization

150 fold increase in sensitivity!

4 BNMA Derivitization

Organic Acids (TCA intermediates)

Carbodiimide Coupling

R₁Reagent

N=C=N

R₂

Derivatization

Reagent

H₂N–R₃

Organic Acid

(TCA Intermediate)

Activated Carboxylic Acid

Acylcarnitines
Substrate Metabolism in the Mitochondria

The TCA Cycle is central to substrate metabolism.
KL2 Timeline

Q1
- Recruit and conduct study #1 (EAA supplementation)
- ACTS meeting
- Mock Study Section
- NIA program officer

Q2
- Drafting specific aims
- IRB for studies #2 and #3
- IRB approved

Q3
- Form K25 mentor team
- Check-in with NIA
- changed to NHLBI

Q4
- KL2 Year 1
- Analysis (Study #1)
- Manuscript #1 submission
- IRB submission
- K submission
- Due (10/12)

Training Activities
Research Activities
KL2 Timeline

Q1: Recruit and conduct study #2 (HF) & #3 (new biopsy tool)
   - Scored (not funded)

Q2: Manuscript #2 submission
   - ACTS meeting program officer
   - Keystone Conference
   - K resubmission Due (7/12)

Q3: Manuscripts accepted

Q4: Analysis (Study #2 & #3)
   - Isotope Tracer Course
   - Scored (no change)

KL2 Year 2

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

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

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?

Previous work:
Plasma TG decrease with chronic EAA supplementation

Borsheim et al, Nutrition, 2010

Effect greatest in highest plasma TG subjects

<table>
<thead>
<tr>
<th>TG concentration at baseline (mmol/l)</th>
<th>Average change in TG concentration (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.56-1.12</td>
<td>-0.10</td>
</tr>
<tr>
<td>1.13-1.68</td>
<td>-0.30</td>
</tr>
<tr>
<td>&gt;1.69</td>
<td>-0.60</td>
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* and # indicate significance levels.
Essential Amino Acid Supplementation (EAAS)

<table>
<thead>
<tr>
<th>EAAS mixture</th>
<th>3.26% Histidine</th>
<th>4.65% Phenylalanine</th>
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<tbody>
<tr>
<td></td>
<td>8.57% Isoleucine</td>
<td>9.57% Threonine</td>
</tr>
<tr>
<td></td>
<td><strong>35.88% Leucine</strong></td>
<td>7.44% Valine</td>
</tr>
<tr>
<td></td>
<td>17.0% Lysine</td>
<td>9.97% Arginine</td>
</tr>
<tr>
<td></td>
<td>3.59% Methionine</td>
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**Dose**

Challenge: 22 g over 3.5 hours (drink)

Chronic: 22 g a day for 8 weeks

High leucine improves net protein synthesis.
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

<table>
<thead>
<tr>
<th>Subject Gender (F/M)</th>
<th>Age (years)</th>
<th>BMI</th>
<th>Plasma TG Week 0 (mmol/l)</th>
<th>Plasma TG Week 8 (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(4/2)</td>
<td>69 ± 4</td>
<td>35 ± 9</td>
<td>2.3 ± 0.4</td>
<td>1.8 ± 0.3*</td>
</tr>
</tbody>
</table>

* p < 0.05
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: Mitochondria

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

![Diagram of acylcarnitines and BCAAs]
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).
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
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)]

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*Arslan et al. Tex Heart Inst J, 2007*
Metabolic Remodeling in Heart Failure

Cardiac metabolism exhibits decreased reliance on fatty acids in HF.

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

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

Doenst et al, Circulation Research, 2013

Metabolic Remodeling in Heart Failure

- 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):

- **Older HF**
- **Older Healthy**
- **Young Healthy**

**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

Time

O2 Flux
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.
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