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

What next?

Excellent tool for hypothesis generation
Metabolomics

Genomics

DNA

RNA

Transcriptomics

Proteomics

Protein

Metabolites
Load frozen biopsies into homogenization vials.

Homogenization

Raw metabolite extract

Cation exchange SPE

Functional Derivitization

Organic Acids (TCA intermediates)

Acylcarnitines

Spike with Isotope labeled Standards

4 BNMA Derivitization

150 fold increase in sensitivity!

10 ng/mL Succinate

LOD = 2 fg

4BNMA Derivative MRM

LOD = 300 fg

Ion Pairing MRM

Carbodiimide Coupling

有机酸 (TCA中间体)

4 BNMA衍生化

150倍灵敏度的提高！
The TCA Cycle is central to substrate metabolism

Substrate Metabolism in the Mitochondria

Carbohydrates
- Glucose
- Pyruvate

Fats

Fatty Acids

Proteins
- Amino Acids
- Acylcarnitines
- β oxidation
- TCA Cycle
- Oxaloacetate
- Citrate
- Malate
- Fumarate
- Succinate
- Succinyl-CoA
- α-Ketoglutarate
KL2 Timeline

- Recruit and conduct study #1 (EAA supplementation)
- Form K25 mentor team
- Drafting specific aims
- ACTS meeting
  - Mock Study Section
  - NIA program officer
- Q1
- Q2
- Q3
- Q4
- Analysis (Study #1)
- IRB for studies #2 and #3
  - Check-in with NIA changed to NHLBI
  - IRB approved
- K submission
  - Due (10/12)
- Manuscript #1 submission

KL2 Year 1

Training Activities

Research Activities
Recruit and conduct study #2 (HF) & #3 (new biopsy tool)  

K resubmission scored (not funded)  

ACTS meeting program officer  

Manuscript #2 submission  

Keystone Conference  

Manuscripts accepted  

Analysis (Study #2 & #3)  

Q1  

Q2  

Q3  

Q4  

Q1  

Q2  

Q3  

Q4  

Regulatory Science Training  

Scored (not funded)  

Scored (no change)  

Isotope Tracer Course  

KL2 Timeline  

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.

Previous work:
Plasma TG decrease with chronic EAA supplementation

Effect greatest in highest plasma TG subjects

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)

<table>
<thead>
<tr>
<th>EAAS mixture</th>
<th>3.26% Histidine</th>
<th>4.65% Phenylalanine</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>8.57% Isoleucine</td>
<td>9.57% Threonine</td>
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<tr>
<td></td>
<td>35.88% Leucine</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>
<td></td>
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<table>
<thead>
<tr>
<th>Dose</th>
<th>Challenge: 22 g over 3.5 hours (drink)</th>
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<tr>
<td></td>
<td>Chronic: 22 g a day for 8 weeks</td>
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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>
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<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>
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</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
What did we learn?

Metabolites Measured: Mitochondria

FFA → CPT

Acyl-carntines → β-oxidation

Acyl-CoAs

Organic Acids

Pyruvate → CoA → Acetyl CoA → TCA Cycle

Citrate, Isocitrate, α-Ketoglutarate, Succinyl-CoA, Succinate

I, II, III, IV, V
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).
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

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

Romijn, J Appl. Physiol. 1994
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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