AACE Meeting
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Duke Molecular Physiology Institute
Departments of Pharmacology & Cancer Biology and Medicine
Duke University Medical Center

Disclosures, Christopher B. Newgard

PhD Scientist, not involved in prescribing medications

Scientific Advisory Boards, 2016

Eli Lilly
Pfizer
Merck
MedImmune
Janssen

Sponsored Research Agreements related to this presentation

Eli Lilly (current)
Pfizer (completed)
**Duke Molecular Physiology Institute**

Mission statement: “To use integrated multi-omics and physiologic profiles of chronic human diseases to develop new disease detection strategies, novel therapies, and insights into disease mechanisms”

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**“Retro-translation” of Omics Signatures to Biological Mechanisms**

- Molecular Signatures in Animal Models
- Molecular Signatures in Human Studies

- Hypothesis
- Functional Relevance in Cell Culture Models
- Proof of Concept in Animal Models
- Human Translation - Clinical Trials (diet, exercise, nutritional supplements, drugs)
Evolving Metabolomics Core Lab, Stedman Center and DMPI

"Targeted" MS Methods, Static Profiling

- GC/MS and MS/MS for "targeted" analysis. Approx. 300 metabolites in 7 modules (free fatty acids, acyl CoAs, acyl carnitines, organic acids, amino acids/urea cycle, purines/nucleotides, ceramides/sphingolipids)—Olga Ilkayeva, Bob Stevens

"Non-Targeted" MS Methods, Static Profiling

- ~1200 compound spectral library co-developed by James Bain and Mike Muehlbauer (with Agilent and Oliver Fiehn) for non-targeted GC/MS
- LC-MS/MS (Q-TOF) for non-targeted analysis of thousands of metabolites/sample

Metabolic Flux Analysis

- Stable isotope tracer enrichment analyses by GC/MS and LC-MS/MS analyses—Guofang Zhang, Scott Crown

Association of a BCAA-Related PCA Factor with Insulin Resistance in Humans

*PCA factor 1 comprised of Val, Leu/Ile, Glx, C3AC, C5AC, Phe, Tyr

Summary: BCAA and related metabolites.....


Poor association of weight loss and ΔHOMA in WLM subjects

![Poor association of weight loss and ΔHOMA in WLM subjects](image-url)
## Factor Univariates for HOMA-Change Model

<table>
<thead>
<tr>
<th>Entry Variable</th>
<th>Factor name</th>
<th>F val</th>
<th>P-val</th>
<th>Effect Size (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Medium Chain Acylcarnitines</td>
<td>0.08</td>
<td>0.78</td>
<td>-0.02 (-0.17, 0.13)</td>
</tr>
<tr>
<td>F2</td>
<td>Medium Chain Dicarboxyl-acylcarnitines</td>
<td>1.96</td>
<td>0.16</td>
<td>-0.11 (-0.26, 0.04)</td>
</tr>
<tr>
<td>F3</td>
<td>Branched-Chain Amino Acids (BCAA)</td>
<td>47.82</td>
<td>&lt;.0001</td>
<td>-0.51 (-0.66, -0.37)</td>
</tr>
<tr>
<td>F4</td>
<td>C2, C4-OH, C16:1, Total Ketones, 3-OH Butyrate,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nonesterified Fatty Acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F5</td>
<td>C18:1-OH/C16:1-DC, C18-OH/C16-DC, C20, C20:1-OH/C18:1-DC, C20-OH/C18-DC</td>
<td>0.32</td>
<td>0.57</td>
<td>-0.04 (-0.20, 0.11)</td>
</tr>
</tbody>
</table>


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### Metabolite profiles and the risk of developing diabetes

Thomas J Wang1,3, Martin G Larson3,4, Ramachandran S Vasan3,5, Susan Cheng2,6, Eugene P Rhee2,7,8, Elizabeth McCabe5, Gregory D Lewis1,2,8, Caroline S Fox3,8,10, Paul F Jacques1, Céline Fernandez12, Christopher J O’Donnell1,3,8, Stephen A Carr1, Vamsi K Moorthy1,3,14, Jose C Florez1,17, Amanda Souza1, Olle Melander10, Clary B Chish1 & Robert E Gerza1,2

Emerging technologies allow the high-throughput profiling of metabolic status from a blood specimen (metabolomics). We investigated whether metabolite profiles could predict the development of diabetes. Among 2,422 normoglycemic individuals followed for 12 years, 201 developed diabetes. Amino acids, amines and other polar metabolites were profiled in baseline specimens by liquid chromatography-tandem mass spectrometry (LC-MS). Cases and controls were matched for age, body mass index and fasting glucose. Five branched-chain and aromatic amino acids had highly significant associations with future diabetes: isoleucine, leucine, valine, tyrosine and phenylalanine. A combination of three amino acids predicted future diabetes (with a more than fivefold higher risk for individuals in top quartile). The results were replicated in an independent, prospective cohort. These findings underscore the potential key role of amino acid metabolism early in the pathogenesis of diabetes and suggest that amino acid profiles could aid in diabetes risk assessment.
Summary: BCAA and related metabolites


Does this mean that BCAA restriction might improve insulin sensitivity?

- Feed Zucker-obese or Zucker-lean rats on standard chow, or standard chow with 45% depletion of BCAA in diet (not growth limiting)

- Assess insulin sensitivity and metabolic profiles after 10 weeks of feeding

**Circulating Amino Acids, 9 weeks on Diet**


**BCAA Restriction Improves Insulin Sensitivity in Obese Rats—Hyperinsulinemic Clamp**

BCAA Restriction Enhances Muscle Glucose Uptake and Glycogen Synthesis

MUSCLE

Muscle Amino Acids
**Potential Significance of Glycine Depletion**

- Acyl-glycine
- Acyl-carnitine
- Carnitine
- CPT/CrAT
- Acyl-carnitine
- Acyl-CoA
- TCA Cycle
- B-Oxidation
- GLYCINE
- GLYAT
- Unidentified Product
- BCAA

**BCAA regulate urinary acylglycine pool**

- Bar chart showing the mmol/mol creatinine of acetylglycine with different groups: LN CTL FED, LN DEF FED, Ob CTL FED, Ob DEF FED.
Summary of key findings, BCAA restriction study

- BCAA restriction in Zucker obese rats enhances insulin sensitivity and glucose disposal
- BCAA restriction relieves accumulation of excess acyl-CoAs in skeletal muscle
- BCAA restriction normalizes muscle glycine levels and increases excretion of acylglycine metabolites in urine. **Mechanism for relief of substrate overload?**
- BCAA restriction in Zucker obese rats lowers RER (increases fat oxidation)

What causes BCAA to rise in human metabolic diseases?

Gut microbiome → Essential amino acids → Genetics

oxidation → Branched Chain Amino Acids → Aromatic Amino Acids → protein

Diagnostic Read-Out

Shah, Svetkey & Newgard
Cell Metabolism 13: 491, 2011

Gut Microbiota from Twins Discordant for Obesity Modulate Metabolism in Mice

Vanessa K. Ridaura,1 Jeremiah J. Faith,1 Federico E. Rey,1 Jiye Cheng,1 Alexis E. Duncan,2,3 Andrew L. Kau,2 Nicholas W. Griffin,1 Vincent Lombard,4 Bernard Henrissat,4,5 James R. Bain,6,7,8 Michael J. Muehlabauer,6 Olga Ilkayeva,6 Clay F. Semenkovich,9 Katsuhiko Funai,9 David K. Hayashi,10 Barbara J. Lyle,11 Margaret C. Martini,11 Luke K. Ursell,12 Jose C. Clemente,12 William Van Treuren,12 William A. Walters,13 Rob Knight,12,14,15 Christopher B. Newgard,6,7,8 Andrew C. Heath,2 Jeffrey I. Gordon1*

Science 341, 1241214, 2013
Transplantation of the Obese Microbiome Increases Plasma BCAA AND Muscle Acylcarnitine Levels

Valine, Leucine, and Isoleucine Biosynthesis

Valine, Leucine, and Isoleucine Degradation

Working Model of Perturbed BCAA Homeostasis

Newgard, CB. Cell Metabolism 15:606, 2012
Activation of BCKDH Improves Glucose Homeostasis in Zucker-obese rats

Systemic, Chemical agent, BT2 (D. Chuang lab)

Liver-specific molecular activator (PPM1K Adenovirus, P. White)

BT2 or PPM1K Lower RER and Hepatic Triglycerides in Zucker-obese Rats

A

Hepatic Triglycerides

B

Hepatic Triglycerides

0 5 10 15 20

nmol/mg protein

nmol/mg protein

DMCO BT2 GFP PPM1K

DMCO BT2 GFP PPM1K

Zucker Fatty Vehicle Control Zucker Fatty BT2 20mg/kg

0.7 0.75 0.8 0.85 0.9 0.95 1

RER

0 20

20mg
**How do BDK/PPM1K regulate glucose and lipid metabolism?**

**BT2 vs Vehicle**

- **Phosphopeptides**
  - Unchanged: 19
  - Changed (1.5fold P<0.05): 4906

**PPM1K vs GFP**

- **Phosphopeptides**
  - Unchanged: 8
  - Changed (1.5fold P<0.05): 4762

16 phosphopeptides only changed with BT2

3 common phosphopeptides

5 phosphopeptides only changed with PPM1K

-1.82 fold P<0.0096  
**Serine 455 of ATP citrate lyase**  
-1.94 fold P<0.0011

**Phillip White, Paul Grimsrud**

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**ACL produces lipogenic and gluconeogenic substrates**

- **Citrate**
- **Glucose**
- **Pep**
- **PEPCK**
- **Oxaloacetate**
- **Acetyl CoA**
- **ACC**
- **Malonyl CoA**
- **FA oxidation**
- **Lipogenesis**

**Phosphorylation events**

- Serine 455 of ATP citrate lyase:
  - 1.94 fold P<0.0011
Hypothesis: The BCAA/aromatic amino acid metabolic signature provides a clue to mechanism of obesity-associated behavioral disorders

TRANSPORT OF LNAA THROUGH THE BLOOD BRAIN BARRIER

Serotonin

Dopamine

Norepinephrine

Brain

Blood Brain Barrier
BCAA supplementation of energy-dense diets reduces Trp and Tyr Levels in frontal cortex


ANOVA, BCAA, p < 0.002

BCAA supplementation of energy-dense diets causes anxious behavior (elevated maze test)


ANOVA, BCAA, p < 0.002
Fluoxetine (Prozac) Does Not Reverse BCAA-induced Anxious Behavior......


......but Tryptophan Does

Trp supplementation normalizes kynurenic acid levels in frontal cortex

BCAA and related metabolites
(Val, Leu/Ile, Glx, C3AC, C5AC, Phe, Tyr)....


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Our laboratory

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Phillip White           Rob McGarrah
Amanda Lapworth         Jonathan Haldeman
Sam Stephens            Lu Zhang
Lisa Poppe              Danhong Lu
Mette Valentin Jensen   Brett Peterson
Tom Becker (Faculty)    Jenniffer Moss (Faculty)
Hans Hohmeier (Faculty) Larry Moss (Faculty)

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Guofang Zhang (Faculty), Olda Ilkayeva (Core Director), Mike Muehlbauer,
Jessica Gooding, Scott Crown, Tabitha Martin, Haijing Song

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Beth Hauser, Mihai Podgoreeanu, Lillian Lien, Andrea Haqq, Blandine LaFererre,
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Jeff Gordon, Vanessa Ridaura, Michael Patnode, Washington Univ., St. Louis
Amanda Lapworth, Julia Brosnan, Jeff Trimmer, Pfizer Research, Cambridge

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