“Translating biomarkers to metabolic disease mechanisms and targets”

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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”
Translating Molecular Signatures to Biological Mechanisms and Clinical Relevance

Molecular Signatures in Animal Models

Hypothesis

Functional Relevance in Cell Culture Models

Proof of Concept in Animal Models

Human Translation - Clinical Trials (diet, exercise, nutritional supplements, drugs)
“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, Q-Exactiv) 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
Progression to T2D in ZDF fa/fa rats
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 $\Delta$HOMA in WLM subjects

Change in Weight (Baseline – 6 months)
# 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, Nonesterified Fatty Acid</td>
<td>1.19</td>
<td>0.28</td>
<td>0.08 (-0.07, 0.24)</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>

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

- Ln Ctrl (n=13)
- Ln Def (n=9)
- Ob Ctrl (n=14)
- Ob Def (n=15)
Potential Significance of Glycine Depletion

- Acyl-glycine
- Acyl-carnitine
- Carnitine
- Unidentified Product
- BCAA
- GLYCINE
- GLYAT
- Acyl-CoA
- TCA Cycle
- B-Oxidation
BCAA regulate urinary acylglycine pool
Skeletal Muscle Acyl CoAs

Skeletal Muscle Acyl-CoAs (pmol)

Ln CTL  Ln Res  Ob CTL  Ob Res

0 100 200 250

acetyl  propionyl  butyl  isovaleryl  hexanoyl  octanoyl  decanoyl  lauroyl  myristoyl  palmitoyl  oleoyl  stearoyl  arachidonoyl
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?

Shah, Svetkey & Newgard
*Cell Metabolism* 13: 491, 2011
Gut Microbiota from Twins Discordant for Obesity Modulate Metabolism in Mice


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

LoSF/HiFV diet (15 dpc)

Valine, Leucine, and Isoleucine Degradation

Valine, Leucine, and Isoleucine Biosynthesis
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

<table>
<thead>
<tr>
<th></th>
<th>DMSO</th>
<th>BT2</th>
</tr>
</thead>
<tbody>
<tr>
<td>nmol/mg protein</td>
<td>15</td>
<td>10</td>
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</tbody>
</table>

B

Hepatic Triglycerides

<table>
<thead>
<tr>
<th></th>
<th>GFP</th>
<th>PPM1K</th>
</tr>
</thead>
<tbody>
<tr>
<td>nmol/mg protein</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

Graph showing RER (Respiratory Exchange Ratio) with time for Zucker Fatty Vehicle Control and Zucker Fatty BT2 20mg/kg.
How do BDK/PPM1K regulate glucose and lipid metabolism?

**BT2 vs Vehicle**

- **Phosphopeptides**
  - 19 total phosphopeptides
  - 16 phosphopeptides only changed with BT2
  - 3 common phosphopeptides

**PPM1K vs GFP**

- **Phosphopeptides**
  - 8 total phosphopeptides
  - 5 phosphopeptides only changed with PPM1K

**Significant Changes**

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

Phillip White, Paul Grimsrud
ATP citrate lyase produces lipogenic and gluconeogenic substrates.

- Citrate
- Oxaloacetate
- Acetyl CoA
- Malonyl CoA
- Glucose
- Pep
- PEPCK
- ACC
- Lipogenesis
- FA oxidation
BDK/PPM1K: Integrating BCAA, lipid, and glucose metabolism

- **Fatty acids**
  - Malonyl CoA
  - CPT1
  - Fatty acid oxidation

- **Gluconeogenesis**
  - OAA
  - Acetyl CoA
  - ACC2

- **BDK**
  - ACLY
  - BCKDH
  - PPM1K
  - C3 + C5

- **BCAAs**
  - BCAA
  - BCAT
  - BCKA

- **TCA Cycle**
  - Acetyl CoA
  - Citrate
  - Citrate

**Diagram**: A detailed metabolic pathway illustrating the integration of BCAA, lipid, and glucose metabolism involving enzymes such as ACC2, CPT1, ACLY, BCKDH, and BCAT, along with the TCA cycle.
Summary

- BCAA and related metabolites associate with, and are prognostic for insulin resistance and type 2 diabetes

- BCAA supplementation or restriction demonstrates a cause/effect relationship with systemic and muscle insulin resistance

- The microbiome of obese individuals and decreased BCAA catabolism in adipose and liver contribute to increased BCAA levels in obesity

- Activation of BCKDH by inhibition of BDK or overexpression of PPM1K lowers BCAA and BCKA and improves glucose and lipid homeostasis

- BDK and PPM1K may integrate BCAA, glucose and lipid metabolism by regulating ATP citrate lyase phosphorylation state and activity
Our laboratory

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