"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



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 nontargeted 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 Svetkey, Newgard, et al. *Cell Metabolism* 9: 311, 2009

Summary: BCAA and related metabolites.....

- Are strongly associated with insulin resistance and type 2 diabetes (Newgard, et al. *Cell Metabolism* 9: 311, 2009; Huffman, et al. *Diabetes Care* 32: 1678, 2009; Tai, E., et al. *Diabetologia* 53: 757, 2010)
- Are prognostic for intervention outcomes (Shah, Svetkey, et al, Diabetologia 55: 321, 2011)
- Are prognostic for development of type 2 diabetes (Wang, et al. Nature Med. 17: 448, 2011; Palmer, et al. JCEM 100: E463, 2015)
- Are highly responsive to the most efficacious intervention methods (Laferrere, Svetkey, Newgard, et al. Science Trans. Med 3: 80r2e, 2011; Hsiao, et al. Am. J. Physiol. 300: E164, 2010; Khoo, et al. Ann. Surgery 259: 687, 2014; Glynn, et al. Diabetologia 58: 2324, 2015)

Poor association of weight loss and AHOMA in WLM subjects



Change in Weight (Baseline – 6 months)

Factor Univariates for HOMA-Change Model

Entry Variable	Factor name	F val	P-val	Effect Size (95% CI)
F1	Medium Chain Acylcarnitines	0.08	0.78	-0.02 (-0.17, 0.13)
F2	Medium Chain Dicarboxyl-acylcarnitines	1.96	0.16	-0.11 (-0.26, 0.04)
F3	Branched-Chain Amino Acids (BCAA)	47.82	<.0001	-0.51 (-0.66, -0.37)
F4	C2, C4-OH, C16:1, Total Ketones, 3-OH Butyrate, Nonesterified Fatty Acid	1.19	0.28	0.08(-0.07, 0.24)
F5	C18:1-OH/C16:1-DC, C18-OH/C16-DC, C20, C20:1-OH/C18:1-DC, C20-OH/C18-DC	0.32	0.57	-0.04 (-0.20, 0.11)

Shah, et al. Diabetologia 55: 321, 2012

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

White, P., Lapworth, A., et al. Molecular Metabolism 5: 538, 2016

Circulating Amino Acids, 9 weeks on Diet



White, P., Lapworth, A., et al. Molecular Metabolism 5: 538, 2016

BCAA Restriction Improves Insulin Sensitivity in Obese Rats—Hyperinsulinemic Clamp



White, P., Lapworth, A., et al. Molecular Metabolism 5: 538, 2016

BCAA Restriction Enhances Muscle Glucose Uptake and Glycogen Synthesis



MUSCLE



Potential Significance of Glycine Depletion



BCAA regulate urinary acylglycine pool



Skeletal Muscle Acyl CoAs



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)

White, P., Lapworth, A., et al. Molecular Metabolism 5: 538, 2016

What causes BCAA to rise in human metabolic diseases?



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,¹ Nicholas W. Griffin,¹ Vincent Lombard,⁴ Bernard Henrissat,^{4,5} James R. Bain,^{6,7,8} Michael J. Muehlbauer,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



Α



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



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



How do BDK/PPM1K regulate glucose and lipid metabolism? BT2 vs Vehicle PPM1K vs GFP

Phosphopeptides

Phosphopeptides



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

Phillip White, Paul Grimsrud



BDK/PPM1K: Integrating BCAA, lipid, and glucose metabolism



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

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