3rd Annual Workshop on Metabolomics

Integration of Biology and the Metabolome: Breaking Barriers Across Disciplines

Monday, June 15, 2015

Adam R. Wende, Ph.D.

Assistant Professor
Molecular and Cellular Pathology
Department of Pathology
Defining the Problem

2.5 million years

50 years

From: Roger Unger - UTSW
2010 – Obesity

2010 – Diabetes

2010 – Physical Inactivity

2010 – Heart Disease

www.cdc.gov/diabetes/statistics and www.cdc.gov/mmwr
Table 2. Brief Overview of Myocardial Metabolism in Physiological and Pathophysiological Conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>MVO₂</th>
<th>Glucose Metabolism</th>
<th>Fatty Acid Metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aging</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Female sex</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Obesity</td>
<td>↑</td>
<td>—</td>
<td>↑</td>
</tr>
<tr>
<td>Diabetes, types 1 and 2</td>
<td>—↑</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Hypertension: LV hypertrophy</td>
<td>—</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>—</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Ischemia</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
</tbody>
</table>
Cardiac Metabolic Substrate Utilization

Studies on Myocardial Metabolism*

IV. Myocardial Metabolism in Diabetes

I. Ungar, M.D., M. Gilbert, M.D., A. Siegel, M.S., J. M. Blain, M.D. and R. J. Bing, M.D.

* From the Department of Medicine and Physiology, University of Alabama Medical Center, Birmingham, Ala.

Work supported by the U. S. Public Health Service Grant No. H-1129(CS), The Life Insurance Medical Research Fund and the American Heart Association.

UAB founded in 1969

Ungar ... Bing 1955 Am J Med 18(3):385
Metabolic Substrate Utilization

- Fatty Acids
- Glycolysis
  - Glycolysis
  - Acyl-CoA
  - Pyruvate
- FAO
  - CPT1
  - CPT2
  - GLUT1
  - GLUT4
  - CAC
  - Acetyl-CoA
  - Acetyl-CoA
  - MSC1/2
  - PDH
  - PDKs
  - PDPs
  - ATP
  - ADP
- Glycogen synthase
- Hexosamine biosynthetic pathway
  - UDP-GlcNAc
  - Acetyl-CoA
- Glucose
  - PFK
  - HK
  - PDH
  - PDKs
  - PDPs

Ask for image credits
Defining the Mechanism

Barallobre-Barreiro … Mayr 2013 Rev Esp Cardiol 66:657

Metabolomics

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Knowledge that will change your world
Model Development

DOX absent = OFF

α-MHC  rtTA
MHC-rtTA

TRE  mycGLUT4
TRE-GLUT4

DOX present = ON

α-MHC  rtTA
MHC-rtTA

TRE  mycGLUT4
TRE-GLUT4
GLUT4 Induction Increases Glycolysis and Rescues Diabetic Cardiac Glycolytic Defects

$n = 6 – 10$
§ $P < 0.01$ vs. Con
GLUT4 Induction Increases GLOX but Accelerates Diabetic Cardiac GLOX Defects

$n = 6 – 10$
§ $P < 0.01$ vs. Con
Oxidative Phosphorylation

[Diagram of Oxidative Phosphorylation with enzymes and reactions]

www.genome.jp/kegg/pathway.html

Metabolomics

UAB - The University of Alabama at Birmingham
Knowledge that will change your world
GLUT4 Induction Accelerates Development of Mitochondrial Dysfunction

$n = 3 – 4$
* $P < 0.05$

Oleh Khalimonchuk
Wende … Abel in prep
Systems Biology of the Diabetic Heart

Phenome
Obesity, diabetes, heart failure, BHI, etc.

Transcriptome
Northern, qPCR, microarray, RNA-seq, miR, lncRNA, etc.

Proteome
Mass spec, western blot, Co-IP, IHC, PTMs, etc.

Metabolome
Glucometer, ELISA, GC-MS, HPLC, NMR, fluxomics, etc.

Genome / Epigenome
Southern, sequencing, GenBank, ENCODE, ChIP-seq, bsDNA-seq, etc.

Adapted from Lewis and Abdel-Haleem 2013 Front Physiol 4:237

Metabolomics
THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Knowledge that will change your world
Metabolism, Bringing the System Together

Hart ... Lagerlof 2011 Annu Rev Biochem 80:825
Metabolite Modification of the Proteome

Isolated Mitochondria
2D-PAGE
Pro-Q Emerald

15% SDS-PAGE

pH 3  IEF  pH 10

mG4H-Veh

Metabolomics

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Knowledge that will change your world
Metabolite Modification of the Transcriptome

mG4H-Veh

181.9 MB
### pathway analysis of Microarray

<table>
<thead>
<tr>
<th>Pathway</th>
<th>List</th>
<th>Genes</th>
<th>Z-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic pathways</td>
<td>194</td>
<td>1085</td>
<td>5.42</td>
</tr>
<tr>
<td>Focal adhesion</td>
<td>35</td>
<td>190</td>
<td>2.30</td>
</tr>
<tr>
<td>Insulin signaling pathway</td>
<td>27</td>
<td>135</td>
<td>2.49</td>
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<tr>
<td>Axon guidance</td>
<td>25</td>
<td>127</td>
<td>2.30</td>
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<tr>
<td>PPAR signaling pathway</td>
<td>22</td>
<td>76</td>
<td>4.20</td>
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<tr>
<td>Chagas disease</td>
<td>21</td>
<td>102</td>
<td>2.33</td>
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<tr>
<td>Hematopoietic cell lineage</td>
<td>21</td>
<td>83</td>
<td>3.39</td>
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<tr>
<td>Peroxisome</td>
<td>21</td>
<td>78</td>
<td>3.72</td>
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<tr>
<td>Dilated cardiomyopathy</td>
<td>20</td>
<td>86</td>
<td>2.88</td>
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<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>20</td>
<td>83</td>
<td>3.06</td>
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<tr>
<td>Arrhythmogenic right ventricular cardiomyopathy</td>
<td>19</td>
<td>71</td>
<td>3.50</td>
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<tr>
<td>ECM-receptor interaction</td>
<td>19</td>
<td>79</td>
<td>2.97</td>
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<tr>
<td>Glycolysis / Gluconeogenesis</td>
<td>19</td>
<td>68</td>
<td>3.72</td>
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<tr>
<td>Pyrimidine metabolism</td>
<td>19</td>
<td>95</td>
<td>2.08</td>
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<tr>
<td>Valine, leucine and isoleucine degradation</td>
<td>18</td>
<td>47</td>
<td>5.21</td>
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<tr>
<td>Metabolism of xenobiotics by cytochrome P450</td>
<td>16</td>
<td>64</td>
<td>2.90</td>
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<tr>
<td>Leishmaniasis</td>
<td>15</td>
<td>64</td>
<td>2.53</td>
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<tr>
<td>Glutathione metabolism</td>
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<td>53</td>
<td>2.95</td>
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<tr>
<td>p53 signaling pathway</td>
<td>14</td>
<td>66</td>
<td>2.02</td>
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<td>Arginine and proline metabolism</td>
<td>13</td>
<td>52</td>
<td>2.61</td>
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<td>Graft-versus-host disease</td>
<td>13</td>
<td>49</td>
<td>2.85</td>
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<tr>
<td>Lysine degradation</td>
<td>13</td>
<td>41</td>
<td>3.60</td>
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<tr>
<td>Type II diabetes mellitus</td>
<td>13</td>
<td>47</td>
<td>3.03</td>
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<tr>
<td>Fatty acid metabolism</td>
<td>12</td>
<td>46</td>
<td>2.68</td>
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<tr>
<td>Type I diabetes mellitus</td>
<td>12</td>
<td>53</td>
<td>2.12</td>
</tr>
</tbody>
</table>
From Human to Mouse and Back Again…
Epigenetics - Memory

EDIC: Epidemiology of Diabetes Interventions Trial

Pirola ... El-Osta 2010 Nat Rev Endocrinol 6(12):665
Metabolite Signaling to Epigenetics

Gut and Verdin 2013 Nature 502:489
How does GlcNAc fit in?

Mariappa ... Aalten 2013 EMBO J 32:612
Metabolite Modification of the Methylome

Legend:
0% 100%

RNA
DNA

Abat
Con Veh STZ mG4H Veh STZ

Bdh1
Con Veh STZ mG4H Veh STZ
Human/Mouse Comparisons

Figure 2. Epigenome-wide association Manhattan plot for VLDL-C in the discovery dataset (n=991). VLDL-C indicates very-low-density lipoprotein cholesterol.

Irvin … Arnett 2014 Circulation 130:565
Human/Mouse Comparisons

Mouse

Gene Expression

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>Veh</th>
<th>Con</th>
<th>STZ</th>
<th>mG4H</th>
<th>mG4H</th>
</tr>
</thead>
</table>

Mouse DNA Methylation

 chr19 (qA) | 19qA | 19qB | 19qC1 | qC2 | 19qC3 | 19qD1 | 19qD2 | D3 |

Scale

chr19:
Con-Veh mCpG
Con-STZ mCpG
mG4H-Veh mCpG
mG4H-STZ mCpG

Common SNPs(138)

CpG: 66
CpG: 44

Wende, unpublished
Metabolite Modification of the Metabolome
Metabolite Modification of the Metabolome
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Farah D. Lubin – Epigenetics

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Molecular & Cellular Pathology
Redox Biology
Diabetes Center
Cardiovascular Center

JDRF 51002608

National Heart Lung and Blood Institute
R00 HL111322

Metabolomics