Metabolomics in Diabetes

Thursday, July 21, 2016

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Division of Molecular and Cellular Pathology

Presenter Disclosure Information

Adam R. Wende, Ph.D.

Metabolomics in Diabetes

FINANCIAL DISCLOSURE:
None

UNLABELED/UNAPPROVED USES DISCLOSURE:
None
Obesity, Metabolic Syndrome, Diabetes, and Heart Failure

From: Roger Unger - UTSW
Maintaining Cardiac Function Through Metabolic Substrate Balance

Glucose  Fatty Acids

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.

Myocardial glucose consumption is reduced in dog and man relative to the elevation in blood glucose concentration. The myocardial usage of ketones is slightly increased in diabetic hearts of patients and significantly elevated in the dog. The main difference concerns the utilization of fatty acids; this is significantly increased in the human heart but is unchanged in the dog. Whether this is due to a species difference or to differences in type and severity of diabetes is not clear. Anesthesia, which was used in the dogs, may have played some part.
Metabolic Substrate Utilization in the Heart

**Table 2. Brief Overview of Myocardial Metabolism in Physiological and Pathophysiological Conditions**

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

Peterson and Gropler 2010 *Circ Cardiovasc Imaging* 3:211

Diabetes and Metabolomics

Metabolomics and Diabetes: Analytical and Computational Approaches.

Sae XM¹, Kamyshny A², Michaelis D³, Hermathur A⁴.

Metabolomics is an integral part for understanding disease processes ... information garnered in the biomarker investigations, future research should shed more light on disease pathogenesis and explore new treatment options.
Changes in Human Heart GLUT Levels

RNA
Human heart failure

Protein
Human heart diabetes

<table>
<thead>
<tr>
<th></th>
<th>GLUT1</th>
<th>GLUT4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonfailing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Failing</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Biopsies obtained during coronary bypass surgery
HL = hyperlipidemia
DM2 = diabetes mellitus type 2

Razeghi … Taegtmeyer 2002 Cardiology 280(41):34786
Glucose Utilization and Rodent Models of Type 1 Diabetes

Protein
Diabetic Mouse Heart

GLUT4

Wild-type

Vehicle
STZ

GLUT4 Protein Level
(arbitrary units)

Vehicle (n = 5)
STZ (n = 6)

Wild-type

Panagia ... Clarke 2005 Am J Physiol 288:H2677

Glucose Uptake
Diabetic Mouse Heart

Total Glucose Uptake
(µmol/g.w)

Vehicle (n = 7)
STZ (n = 6)

Wild-type

Constitutive GLUT4 Expression Prevents Development of Glucose Utilization Defects

Glycolysis

GLOX

Belke ... Severson 2000 Am J Physiol 279:E1104
**Question:** Is the change in cardiac metabolic substrate flexibility adaptive or maladaptive?

**Inducible Cardiomyocyte-Specific GLUT4 Expression (mG4H)**

- **DOX absent = OFF**
  - α-MHC rtTA (MHC-rtTA)
  - TRE mycGLUT4 (TRE-GLUT4)

- **DOX present = ON**
  - α-MHC rtTA (MHC-rtTA)
  - TRE mycGLUT4 (TRE-GLUT4)
mG4H Mice Exhibit Inducible Cardiac-Specific Expression of GLUT4

<table>
<thead>
<tr>
<th>DOX (d)</th>
<th>myc</th>
<th>GLUT4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Con</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Con</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>mG4H</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>mG4H</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>mG4H</td>
<td>8</td>
<td>14</td>
</tr>
</tbody>
</table>

Hrt = Heart  
GC = Gastrocnemius  
Vas = Vastus lateralis  
Sol = Soleus

GLUT4 Induction Increases Basal and Insulin-Stimulated Glucose Uptake

Cardiac Myocytes  
2-DG Uptake

<table>
<thead>
<tr>
<th>PMol mg⁻¹ min⁻¹</th>
<th>Basal</th>
<th>0.1 nM Ins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>mG4H</td>
<td>a</td>
<td></td>
</tr>
</tbody>
</table>

n = 3 – 4  
* P < 0.01 vs. Con-Basal  
* P < 0.001 vs. All

Renata O. Pereira  
Wende … Abel in prep
GLUT4 Induction Increases Glycolysis and Rescues Diabetic Cardiac Glycolytic Defects

$n = 6 – 10$

§ $P < 0.01$ vs. Con

Joseph Tuinei
Wende … Abel in prep

GLUT4 Induction Increases GLOX but Accelerates Diabetic Cardiac GLOX Defects

$n = 6 – 10$

‡ $P < 0.001$ vs. All
* $P < 0.01$ vs. Veh

Joseph Tuinei
Wende … Abel in prep
Conclusion – Part 1

In the context of diabetes, enhancing glucose delivery by expression of GLUT4 accelerates the progression of mitochondrial dysfunction.
**Systems Biology**

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

**Transcriptome**
- NorthernS, 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**
- Southerns, sequencing, GenBank, ENCODE, ChIP-seq, bsDNA-seq, etc.

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**Pathway Analysis of Microarray**

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Threshold</th>
<th>-log(p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amino acid metabolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid metabolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleic acid metabolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrate metabolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal and muscular disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular system development &amp; function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-translational modification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocrine system development &amp; function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocrine system disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gene expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutritional disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein degradation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Oxidative Phosphorylation

GeneSifter using KEGG

Ndufa9 Gene Promoter Structure

KEY

TSS = Transcription start site

= CpG island

= Sp1 RE

http://ecrbrowser.dcode.org
**Systems Biology**

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- Gene expression

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**Adapted from Lewis and Abdel-Haleem 2013 Front Physiol 4:237**
Metabolic Integration: Protein O-GlcNAcylation

Hart... Lagerlof 2011 Annu Rev Biochem 80:825
**GlcNAc Regulation of Sp1**

GlcNAcylation Regulates Ndufa9 Gene Expression

**Transcript Transfection Promoter Activity**

Glucose

- 5.5 mM
- 25 mM

C2C12 Myotubes

\[ n = 3 \]

\[ * \ P < 0.05 \]

Li Wang

Wende … Abel in prep
Conclusion – Part 2

Enhanced glucose delivery regulates oxidative capacity via transcriptional mechanisms including GlcNAcylation of transcription factors.

Mitochondrial Protein O-GlcNAcylation and Neonatal Cardiomyocyte Metabolic Function

Mitochondrial Protein O-GlcNAcylation

Complex I Activity

5.5 mM Glc 30 mM Glc + Adv (-) 30 mM Glc + Adv-OGA

O-GlcNAcylation of NDUFA9

GLUT4 Induction Alters the Cardiac Mitochondrial Glycoproteome

Isolated Mitochondria
2D-PAGE
Pro-Q Emerald

15% SDS-PAGE

pH 3 ↔ pH 10
Con-Veh

pH 3 ↔ pH 10
Con-STZ

Hansjörg Schwertz
Wende, unpublished

Systems Biology

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Obesity, diabetes, heart failure, BHI, etc.

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Adapted from Lewis and Abdel-Haleem 2013 Front Physiol 4:237
**Metabolomic Signatures of Diabetic Heart Disease**

**3D - PCA**

**KEY**
- Con-Veh
- Con-STZ
- mG4H-Veh
- mG4H-STZ

**GC and HPLC - metabolomics**

Studies on Myocardial Metabolism

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

*Birmingham, Alabama*

... lactic acid usage and a slight decline in that of pyruvate. There is no change in utilization of amino acids by the heart in both species. Myocardial glucose consumption is reduced in dog and man relative to the elevation in blood glucose concentration. The myocardial usage of ketones is slightly increased in diabetic hearts of patients and significantly elevated in the dog. The main difference concerns the utilization of fatty acids; this is significantly increased in the human heart but is unchanged in the dog. Whether this is due to a species difference or to differences in type and severity of diabetes is not clear. Anesthesia, which was used in the dogs, may have played some part.

*Ungar ... Bing 1955 Am J Med 18(3):385*
GLUT4 Induction Alters Cardiac Ketone Utilization Genes

**Ketone Synthesis**
- Fatty acids → Acetyl-CoA → AcAc-CoA → HMG-CoA → Acetoacetate → BHB

**Ketone Oxidation**
- BHB → HDH1/Bdh1 → Acetoacetate → SCOT/Oxct1 → Acetyl-CoA → HMGCS2/Hmgcs2 → HMG-CoA → Fatty acids

**RNA - Microarray**

**Input**

**IP – SCOT1**

Manoja Brahma
Conclusion – Part 3

Enhanced cardiac glucose delivery alters metabolic flux through other pathways and regulates the mitochondrial proteome via O-GlcNAcylation.

Systems Biology

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Obesity, diabetes, heart failure, BHI, etc.

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

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

DCCT: Diabetes Control and Complications Trial

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**Epigenetics - Memory**

EDIC: Epidemiology of Diabetes Interventions Trial
Epigenetics: Transgenerational and Drift

Gut and Verdin 2013 Nature 502:489

Epigenetic Code

Fischer 2014 EMBO J 33(9):945:489
Metabolite Signaling to Chromatin

Methylation and Expression

RNA – microarray
Methylation – genome sequencing
Protein – western blot
Other Human/Mouse Comparisons

Mouse Gene Expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Con</th>
<th>Con</th>
<th>mG4H</th>
<th>mG4H</th>
<th>mG4H</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Veh</td>
<td>STZ</td>
<td>Veh</td>
<td>STZ</td>
<td></td>
</tr>
</tbody>
</table>

Mouse DNA Methylation

- Cpt1a

Wende, unpublished
Where Does Glycemic Memory Fit In?

**DOX absent = OFF**

- $\alpha$-MHC rtTA
- MHC-rtTA
- TRE mycGLUT4
- TRE-GLUT4

**DOX present = ON**

- $\alpha$-MHC rtTA
- MHC-rtTA
- TRE mycGLUT4
- TRE-GLUT4

Metabolomics

- Con-Veh
- Con-STZ
- mG4H-STZ
- mG4H-Veh
- mG4H-1wk
- mG4H-2wk

Wende, unpublished
Glucose Cycling Alters Epigenetic Programming

Genomewide bsDNA-seq 5-mCpG

Legend:
0%
100%

Heart, LV

Zymo Research
Wende, unpublished

Background

5-hmC
Wyatt and Cohen 1952 Nature 170(4338):1072
Kriaucioni and Heintz 2009 Science 324(5929):929
Tahiliani … Rao 2009 Science 324(5929):930

http://chemistry.uchicago.edu/faculty/faculty/person/member/chuan-he.html
How does GlcNAc fit in?

Mariappa ... Aalten 2013 EMBO J 32:612

Cellular glucose fluctuations regulates the epigenome via histone modifications and controlling the machinery for DNA methylation.

Conclusion – Part 4
Using combined methylomics, transcriptomics, proteomics, and metabolomics we have begun to define the mechanism of glucotoxicity.