Metabolomics in Models of Cardiovascular Disease

Wednesday, March 15, 2017

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Assistant Professor
Division of Molecular and Cellular Pathology

Outline

• Define the question and model to determine the connection between metabolism and diabetic heart disease.

• Identify the molecular mechanisms by which glucose directly alters molecular function using systems biology.
  • Transcriptomics
  • Proteomics
  • Metabolomics
  • Epigenetics (e.g. methylomics)
Obesity, Metabolic Syndrome, Diabetes, and Heart Failure

2.5 million years

50 years

2010 – Obesity
2010 – Physical Inactivity
2010 – Diabetes
2010 – Heart Disease

www.cdc.gov/diabetes/statistics and www.cdc.gov/mmwr
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. Baird, 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>( MV_{O_2} )</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>

Peterson and Gropler 2010 Circ Cardiovasc Imaging 3:211

Point/Counterpoint - The Right Balance?

Cardiac Pathology via Diet-Induced Glucolipotoxicity:
- High Glycemic Carbs
- ↑ω-3 PUFA
- ↑Saturated Fat
- Positive Energy Balance
  - Obesity & Metabolic Syndrome
    - ↑Triglycerides, FFA
    - ↑Adiponectin
    - ↑Inflammation
    - ↑Leptin & ↑Insulin
    - ↑Blood pressure
  - Atherosclerosis
    - ↑Myocyte size
    - ↑Apoptosis
    - ↑Fibrosis
    - Mitochondria Dysfunction
  - CAD, LVH, Heart Failure

Cardiac Health via Dietary Protection:
- Low Glycemic Carbs
- ↑ω-3 PUFA
- ↓Saturated Fat
- Neutral Energy Balance
  - No Obesity & No Metabolic Syndrome
    - Normal Triglycerides, FFA
    - ↓LDL, ↑HDL
    - ↓Adiponectin
    - ↓Inflammation
    - ↓Leptin & ↓Insulin
    - Normal Blood pressure
  - Healthy Heart
    - ↓Atherosclerosis
    - Normal Myocyte Size
    - ↓Apoptosis
    - Optimal Mitochondria Function

Taegtmeyer and Stanley 2011 J Mol Cell Cardiol 50(1):2

Heinrich Taegtmeyer, MD, DPhil
William C. Stanley, PhD
1957 - 2013
Diabetes and Metabolomics

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.

Heart failure and substrate switching

The hypertrophy, oxidative stress, and metabolic changes that occur within the heart when glucose supplants FA as a major energy source suggest that substrate switching to glucose is not entirely benign.
Mitochondria – a Dynamic Network


Mitochondria – too much fat

Serum  Serum + 500 μM Palmitate

Adapted from Heiko Bugger
Facilitative Glucose Transporters: GLUTs
“Solute Carrier Family, SLC2A”

Scheepers … Schurmann 2004 J Parenter Enteral Nutr 28:364
Changes in Human Heart GLUT Levels

RNA
Human heart failure

Protein
Human heart diabetes

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

Glucose Uptake
Diabetic Mouse Heart

Panagia … Clarke 2005 Am J Physiol 288:H2677
Constitutive GLUT4 Expression Prevents Development of Glucose Utilization Defects

**Question:** Is the change in cardiac metabolic substrate flexibility adaptive or maladaptive?
Inducible Cardiomyocyte-Specific GLUT4 Expression (mG4H)

DOX absent = OFF

DOX present = ON

mG4H Mice Exhibit Inducible Cardiac-Specific Expression of GLUT4

<table>
<thead>
<tr>
<th>DOX (d)</th>
<th>Con</th>
<th>mG4H</th>
<th>Con</th>
<th>mG4H</th>
<th>Hrt</th>
<th>GC</th>
<th>Vsl</th>
<th>TA</th>
<th>Sol</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4</td>
<td>8</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>0</td>
<td>14</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>8</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>0</td>
<td>14</td>
<td>0</td>
<td>14</td>
</tr>
</tbody>
</table>

Hrt = Heart  
GC = Gastrocnemius  
Vas = Vastus lateralis  
TA = Tibialis anterior  
Sol = Soleus

5-fold  
5-fold Heart
**Insulin-induced GLUT4 Vesicle Fusion and Exofacial Myc-Epitope Exposure**

Ariel Contreras-Ferrat  
Wende ... Abel *in prep*

**Cardiac Myocytes 2-DG Uptake**

GLUT4 Induction Increases Basal and Insulin-Stimulated Glucose Uptake

Cardiac Myocytes 2-DG Uptake

![Graph showing glucose uptake](image)

Renata O. Pereira  
Wende ... Abel *in prep*
Streptozotocin (STZ)-Induced Hyperglycemia is Not Altered by Transgene Induction

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

Isolated Working Hearts Glucose Oxidation (GLOX)

$n = 6 – 10$

* $P < 0.01$ vs. Veh

Joseph Tuinei
Wende … Abel in prep

GLUT4 Induction Prevents Increased Cardiac POX in Diabetes

Isolated Working Hearts Palmitate Oxidation (POX)

$n = 5 – 13$

* $P < 0.001$ vs. All

Joseph Tuinei
Wende … Abel in prep
Oxidative Phosphorylation

www.genome.jp/kegg/pathway.html

GLUT4 Induction Accelerates Development of Mitochondrial Dysfunction

\[ n = 3 - 4 \]

* P < 0.05

Oleh Khalimonchuk
Wende … Abel in prep
In the context of diabetes, enhancing glucose delivery by expression of GLUT4 accelerates the progression of mitochondrial dysfunction.

Diabetic Cardiomyopathy

“Death by a Thousand Cuts…”

Insulin resistance

Inflammation

Lipotoxicity

ER stress

Glucotoxicity

REDOX Imbalance

Mitochondrial dysfunction

Adapted from Wende, Symons, and Abel 2012 Curr Hypertens Rep 14(6):517
**Systems Biology**

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

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**Transcriptomic Analysis Using the Agilent SurePrint G3 60K Microarray**

mG4H-Veh
Pathway Analysis of Microarray

<table>
<thead>
<tr>
<th>Threshold</th>
<th>-log(p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Metabolic disease</td>
</tr>
<tr>
<td>1</td>
<td>Amino acid metabolism</td>
</tr>
<tr>
<td>2</td>
<td>Lipid metabolism</td>
</tr>
<tr>
<td>3</td>
<td>Nucleic acid metabolism</td>
</tr>
<tr>
<td>4</td>
<td>Carbohydrate metabolism</td>
</tr>
<tr>
<td>5</td>
<td>Skeletal and muscular disorders</td>
</tr>
<tr>
<td>6</td>
<td>Energy production</td>
</tr>
<tr>
<td>7</td>
<td>Cardiovascular system development &amp; function</td>
</tr>
<tr>
<td>8</td>
<td>Post-translational modification</td>
</tr>
<tr>
<td>9</td>
<td>Endocrine system development &amp; function</td>
</tr>
<tr>
<td>10</td>
<td>Inflammatory response</td>
</tr>
<tr>
<td>11</td>
<td>Endocrine system disorders</td>
</tr>
<tr>
<td>12</td>
<td>Cell death</td>
</tr>
<tr>
<td>13</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>14</td>
<td>Gene expression</td>
</tr>
<tr>
<td>15</td>
<td>Nutritional disease</td>
</tr>
<tr>
<td></td>
<td>Protein degradation</td>
</tr>
</tbody>
</table>

Glucose Regulated Gene Expression

Mouse STZ

1611
2035

Mouse mG4H

234
195
410
397
459

0 = up-regulated
0 = contra-regulated
0 = down-regulated

Wende, unpublished
Oxidative Phosphorylation

GeneSifter using KEGG

Ndufa9 Gene Promoter Structure

-3 kb  TSS  +1 kb

Ndufa9

KEY

TSS = Transcription start site

= CpG island

= Sp1 RE

http://ecrbrowser.dcode.org
**Ndufa9 Gene Promoter Mapping**

**Transient Transfection Promoter Activity**

- **Glucose**
  - 5.5 mM
  - 25 mM

**Normalized RLU**

-2 kb  -0.5 kb  -0.3 kb

C_{2}C_{12} Myotubes
n = 9
* P < 0.05

Wende ... Abel in prep
**Ndula9 Gene Promoter Mapping**

Transgenic Transfection Promoter Activity

C2C12 Myotubes

\[ n = 9 \]

* \( P < 0.05 \)

**Glucose**

- 5.5 mM
- 25 mM

**Normalized RLU**

-2 kb + (-)
-2 kb + Sp1

Wende … Abel *in prep*

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**Systems Biology**

Transcriptome

- Northerns, qPCR, metatranscriptome
- RNA-seq, microRNA, etc.

Genome / Epigenome

- Southerns, sequencing, GenBank, ENCODE, ChIP-seq, bisulfite DNA-seq, etc.

Phenome

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

Proteome

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

Metabolome

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

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

O-GlcNAc Cycling

Hanover ... Love 2012 Nat Rev Mol Cell Biol 13(5):312

GlcNAc Regulation of Sp1

Vosseller ... Hart 2002 Curr Opin Chem Biol 6(6):851
GlcNAcylation Regulates *Ndufa9* Gene Expression

### Transient Transfection Promoter Activity

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Normalized RLU</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>~75 ± 10</td>
</tr>
<tr>
<td>5.5 mM</td>
<td>~85 ± 10</td>
</tr>
<tr>
<td>25 mM</td>
<td>~100 ± 10</td>
</tr>
</tbody>
</table>

C2C12 Myotubes
n = 3
* P < 0.05

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

Conclusion – Part 2

Li Wang
Wende … Abel *in prep*
Mitochondrial Protein $O$-GlcNAcylation and Neonatal Cardiomyocyte Metabolic Function

Mitochondrial Protein $O$-GlcNAcylation

Complex I Activity

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

O-GlcNAcylation of NDUFA9

Hu ... Dillmann 2009 J Biol Chem 284(1):547

GLUT4 Induction Alters the Cardiac Mitochondrial Glycoproteome

Isolated Mitochondria 2D-PAGE Pro-Q Emerald

Con-Veh Con-STZ

Hansjörg Schwertz Wende, unpublished
**Systems Biology**

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

**Transcriptome**
- Northerns, qPCR, massarray
- RNA-seq, miR, lncRNA, etc.

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

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**Metabolomic Signatures of Diabetic Heart Disease**

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

3D – PCA

GC and HPLC - metabolomics

James Cox
Studies on Myocardial Metabolism

IV. Myocardial Metabolism in Diabetes

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

Birmingham, Alabama

Lactate 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.

GLUT4 Induction Alters Cardiac Ketone Utilization Genes

Ketone Synthesis

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Acetyl-CoA</th>
<th>AcAc-CoA</th>
</tr>
</thead>
</table>

Ketone Oxidation

<table>
<thead>
<tr>
<th>BHB</th>
<th>BHB</th>
<th>AcAc-CoA</th>
</tr>
</thead>
</table>

RNA - Microarray

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

-1 - 1

BHB

Acetate

<table>
<thead>
<tr>
<th>AcAc-CoA</th>
<th>AcAc-CoA</th>
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</thead>
</table>

HMG-CoA

<table>
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<tr>
<th>HMGCS2/Hmgcs2</th>
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HMG-CoA

<table>
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</table>

Acetoacetate

<table>
<thead>
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<th>Acetoacetate</th>
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</thead>
</table>

m-Thiolase/Acaca2

<table>
<thead>
<tr>
<th>m-Thiolase/Acaca2</th>
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Acetyl-CoA

<table>
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<th>Acetyl-CoA</th>
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</thead>
</table>

CAC

<table>
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<tr>
<th>CAC</th>
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</thead>
</table>

Manoja Brahma
GLUT4 Induction Alters Cardiac Ketone Protein GlcNAcylation

Conclusion – Part 3

Enhanced cardiac glucose delivery alters metabolic flux through other pathways and regulates the mitochondrial proteome via O-GlcNAcylation.
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
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Genome / Epigenome
- Southerns, sequencing
- GenBank, ENCODE
- ChIP-seq, bsDNA-seq, etc.

From Human to Mouse and Back Again

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

Broad Institute Communications
Role of Epigenetics in Gene Expression

Epigenetics - Programming
DCCT: Diabetes Control and Complications Trial

Graph showing the impact of intensive treatment on glycated hemoglobin and percentage of patients with complications over the years of study.
Epigenetics - Memory
EDIC: Epidemiology of Diabetes Interventions Trial

Epigenetics: Transgenerational and Drift
Epigenetic Code

Fischer 2014 *EMBO J* 33(9):945-489

Chromatin Regulation

Gräff and Tsai 2013 *Nat Rev Neurosci* 14(2):97
How do metabolites fit in?

Metabolite Signaling to Chromatin
How does GlcNAc fit in?

DNA Methylation 101

unmethylated
methylated

Gene Expression
Gene Expression Repressed
Exercise Alters DNA Methylation of Key Metabolic Genes

Low = 40% VO$_{2peak}$ High = 80% VO$_{2peak}$
Subjects fasted overnight and then consumed a high carbohydrate diet 4 hr prior to exercise.

Barres and Zierath 2012 Cell Metab 15:405

Diabetes Regulated Cardiac DNA Methylation

Targeted bsDNA-seq 5-mCpG

Heart, LV 

\( n = 10 \)

\( * P < 0.05 \)

Wende, unpublished
Methylation and Expression

RNA – microarray
- Pyruvate dehydrogenase kinase, isoenzyme 4, mRNA

Methylation – genome sequencing
- GeneSifter and Zymo/UCSC Genome Browser

Protein – western blot

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>mG4H</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Veh STZ</td>
<td>Veh STZ</td>
</tr>
<tr>
<td>PDK4</td>
<td></td>
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</tr>
<tr>
<td>VDAC</td>
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</tbody>
</table>

Other Human/Mouse Comparisons

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

Mouse Gene Expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Con Veh</th>
<th>mG4H Gen 1</th>
<th>mG4H Gen 2</th>
</tr>
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<tbody>
<tr>
<td>Cpt1a</td>
<td>[Image]</td>
<td>[Image]</td>
<td>[Image]</td>
</tr>
</tbody>
</table>

Mouse DNA Methylation

Where Does Glycemic Memory Fit In?

DOX absent = OFF

α-MHC rtTA

MHC-rtTA

TRE mycGLUT4

TRE-GLUT4

DOX present = ON

α-MHC rtTA

MHC-rtTA

TRE mycGLUT4

TRE-GLUT4
Metabolomics

Legend:

0% 100%

Glucose Cycling Alters Epigenetic Programming

Genomewide bsDNA-seq 5-mCpG

Heart, LV

Zymo Research
Wende, unpublished
Background

5-hmC
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.
Using combined methylomics, transcriptomics, proteomics, and metabolomics we have begun to define the mechanism of glucotoxicity.