Metabolomics

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Knowledge that will change your world

GBSC 724: Advanced Topics in Metabolomics

Metabolomics in Models of Cardiovascular Disease

Wednesday, March 15, 2017

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Division of Molecular and Cellular Pathology

JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.

NIH National Heart, Lung, and Blood Institute

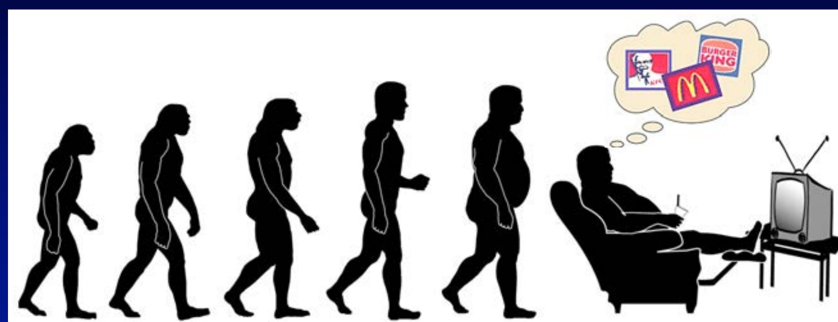
ARW

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)

ARW

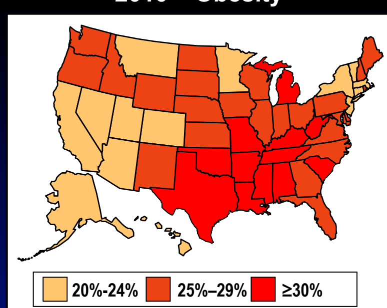
Obesity, Metabolic Syndrome, Diabetes, and Heart Failure



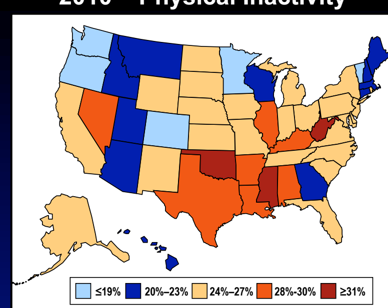
← 2.5 million years → ← 50 years →

From: Roger Unger - UTSW

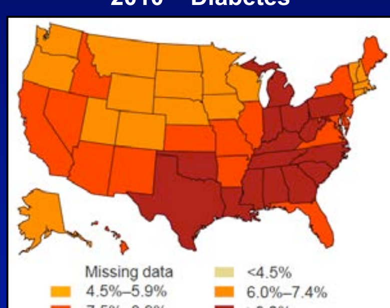
2010 – Obesity



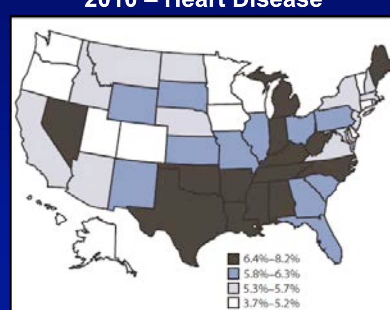
2010 – Physical Inactivity



2010 – Diabetes

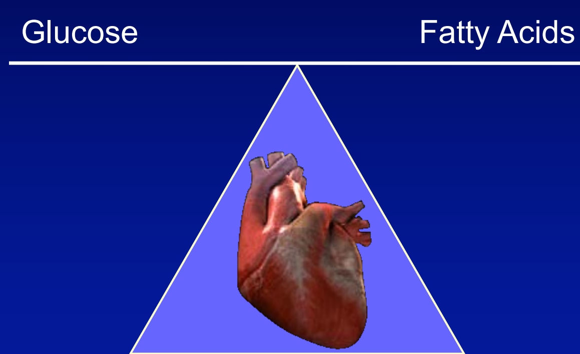


2010 – Heart Disease



www.cdc.gov/diabetes/statistics and www.cdc.gov/mmwr

Maintaining Cardiac Function Through Metabolic Substrate Balance



giphy.com

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.

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.

Ungar … Bing **1955** *Am J Med* 18(3):385

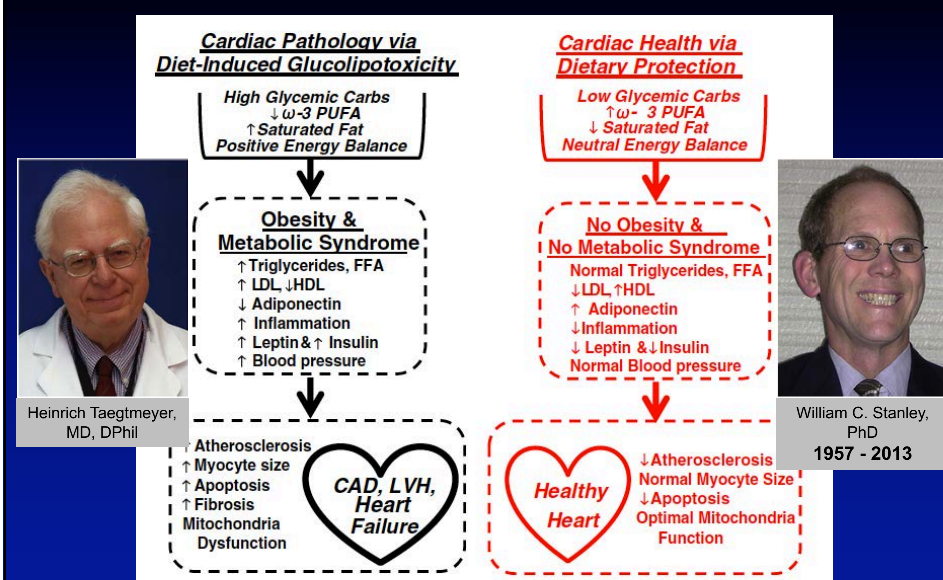
Metabolic Substrate Utilization in the Heart

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

	MVo_2	Glucose Metabolism	Fatty Acid Metabolism
Aging	↑	↑	↓
Female sex	↑	↓	↑
Obesity	↑	—	↑
Diabetes, types 1 and 2	—↑	↓	↑
Hypertension: LV hypertrophy	—	↑	↓
Dilated cardiomyopathy	—	↑	↓
Ischemia	↓	↑	↓

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

Point/Counterpoint - The Right Balance?



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

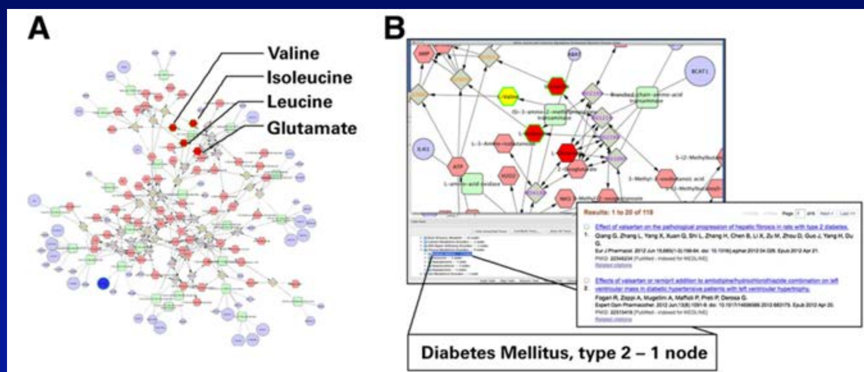
Diabetes and Metabolomics

Diabetes. 2015 Mar;64(3):718-732.

Metabolomics and Diabetes: Analytical and Computational Approaches.

Sas KM[1], Karnovsky A[2], Michailidis G[3], Pennathur S[4].

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.



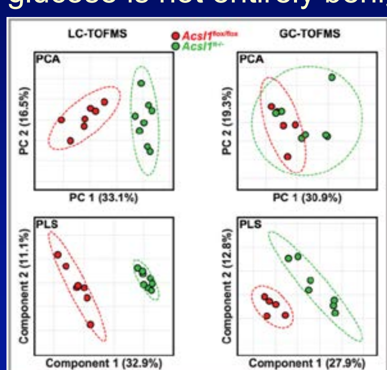
Heart failure and substrate switching

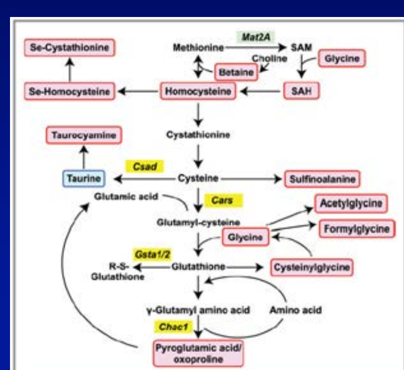
J Am Heart Assoc. 2015 Feb 24;4(2). pii: e001136. doi: 10.1161/JAHA.114.001136.

Cardiac energy dependence on glucose increases metabolites related to glutathione and activates metabolic genes controlled by mechanistic target of rapamycin.

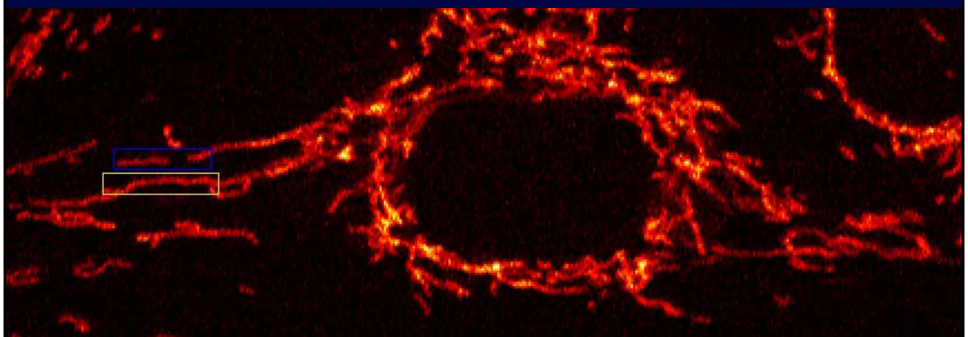
Schisler JC[1], Grevengoed TJ[2], Pascual F[2], Cooper DE[2], Ellis JM[2], Paul DS[2], Willis MS[3], Patterson C[1], Jia W[4], Coleman RA[2].

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.



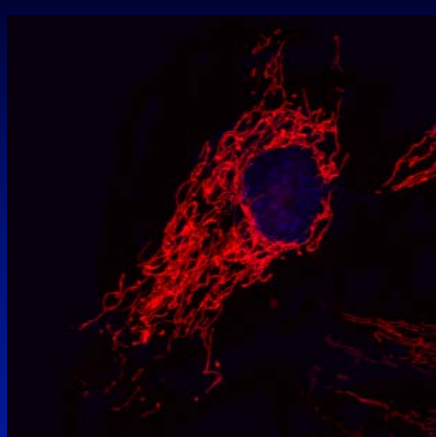
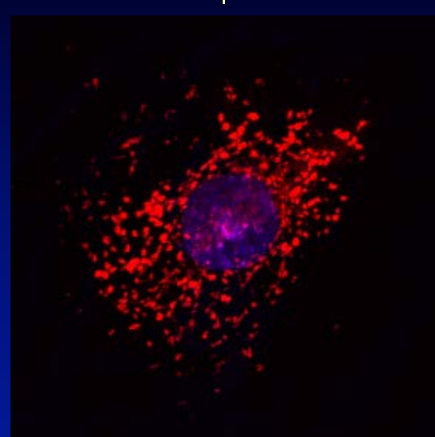


Mitochondria – a Dynamic Network



Fan … Brooks **2010** *Free Radic Biol Med* 49(11):1646

Mitochondria – too much fat

Serum	Serum + 500 µM Palmitate
	

Adapted from Heiko Bugger

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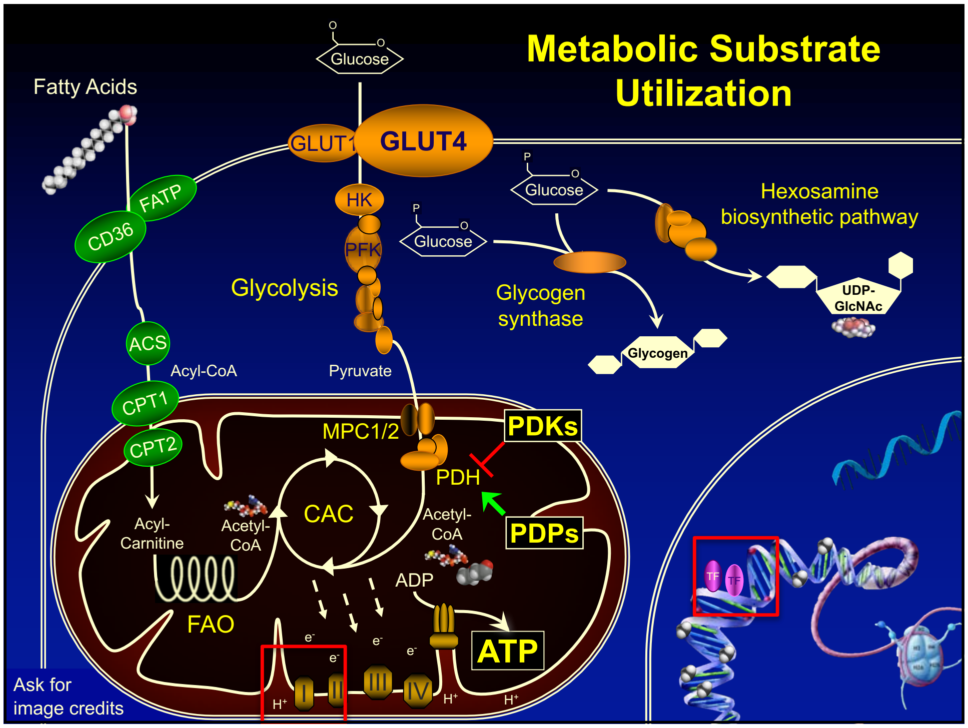
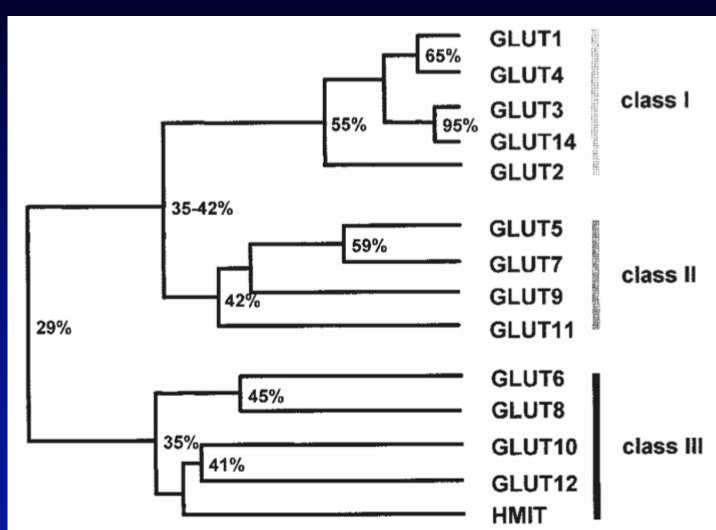


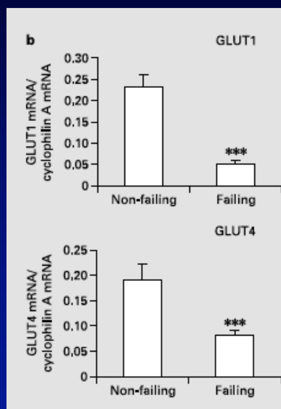
Facilitative Glucose Transporters: GLUTs
“Solute Carrier Family, SLC2A”



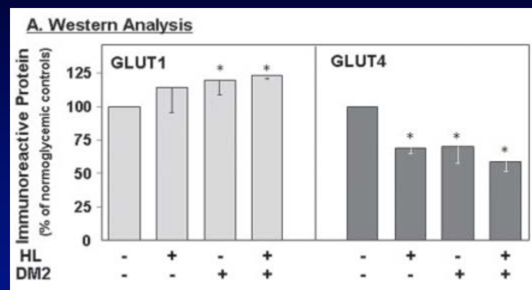
Scheepers … Schurmann **2004** *J Parenter Enteral Nutr* 28:364

Changes in Human Heart GLUT Levels

RNA
Human heart failure



Protein
Human heart diabetes



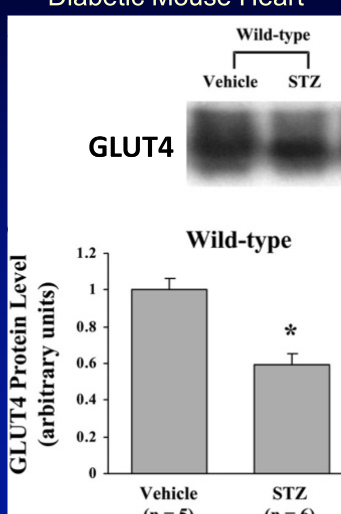
Biopsies obtained during coronary bypass surgery
HL = hyperlipidemia
DM2 = diabetes mellitus type 2

Razeghi … Taegtmeyer **2002** *Cardiology* 280(41):34786

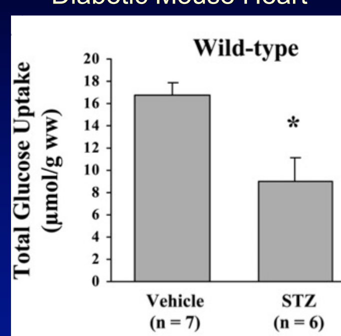
Armoni … Karnieli **2005** *J Biol Chem* 280(41):34786

Glucose Utilization and Rodent Models of Type 1 Diabetes

Protein
Diabetic Mouse Heart

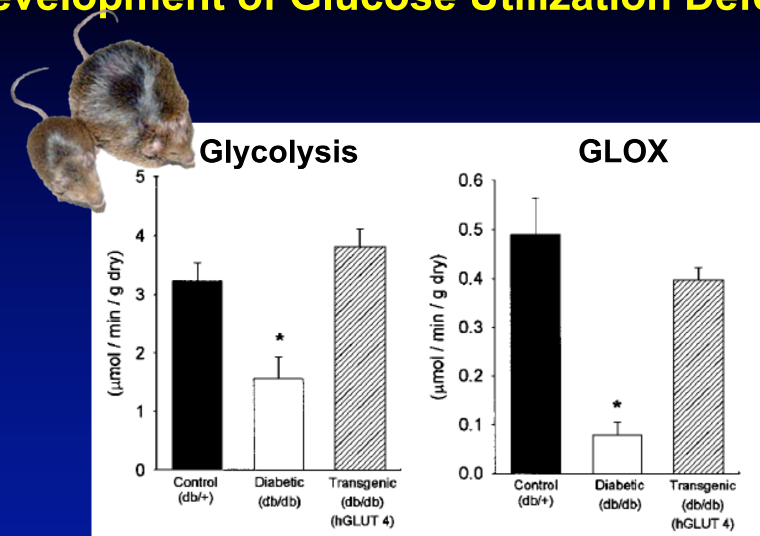


Glucose Uptake
Diabetic Mouse Heart



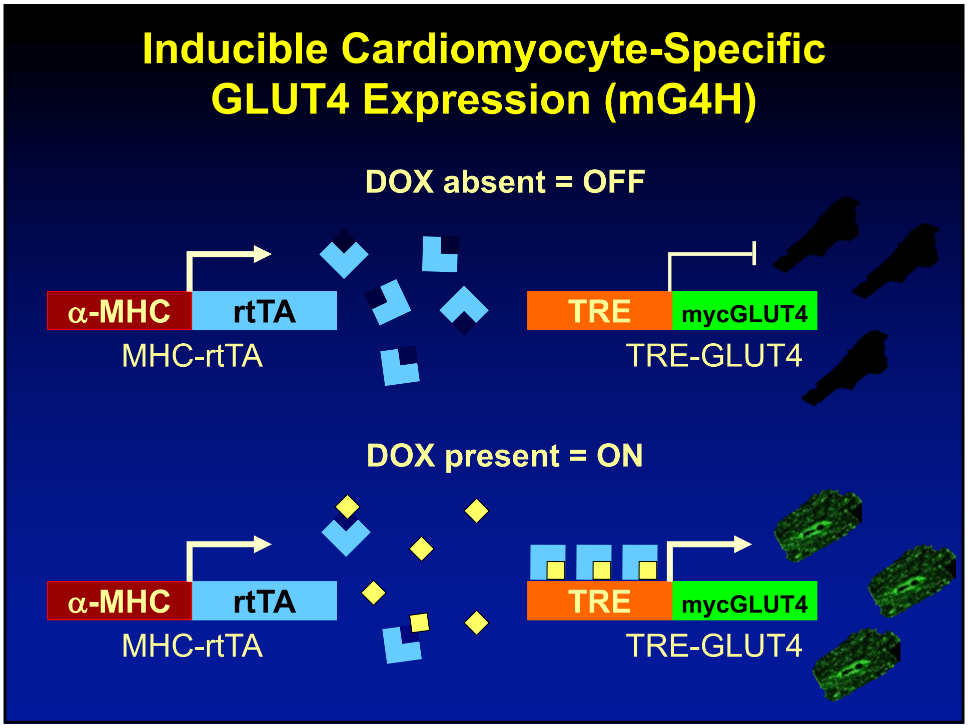
Panagia … Clarke **2005** *Am J Physiol 288:H2677*

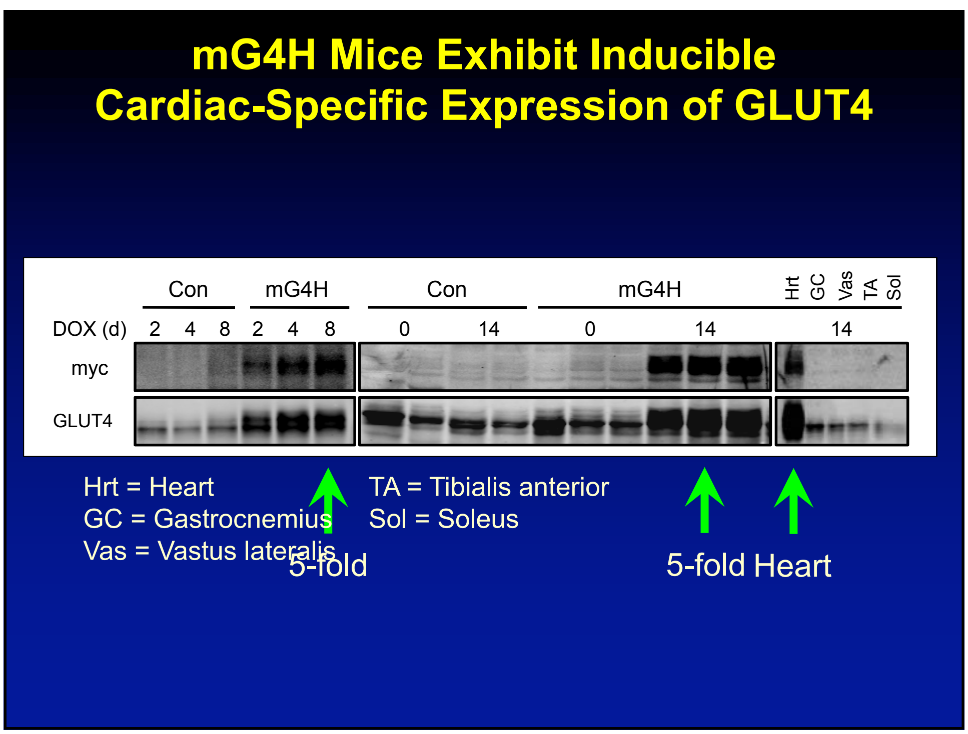
Constitutive GLUT4 Expression Prevents Development of Glucose Utilization Defects



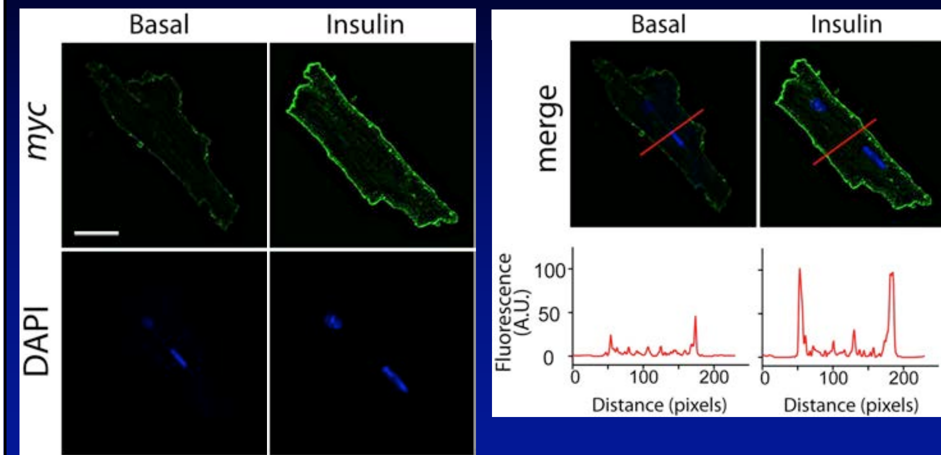
Belke … Severson **2000** *Am J Physiol* 279:E1104

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
Con
mG4H
Con
mG4H
Hrt
GC
Vas
TA
Sol
DOX (d)
2 4 8 2 4 8
0
14
0
14
14
myc
GLUT4
Hrt = Heart
GC = Gastrocnemius
Vas = Vastus lateralis
TA = Tibialis anterior
Sol = Soleus
5-fold
5-fold Heart

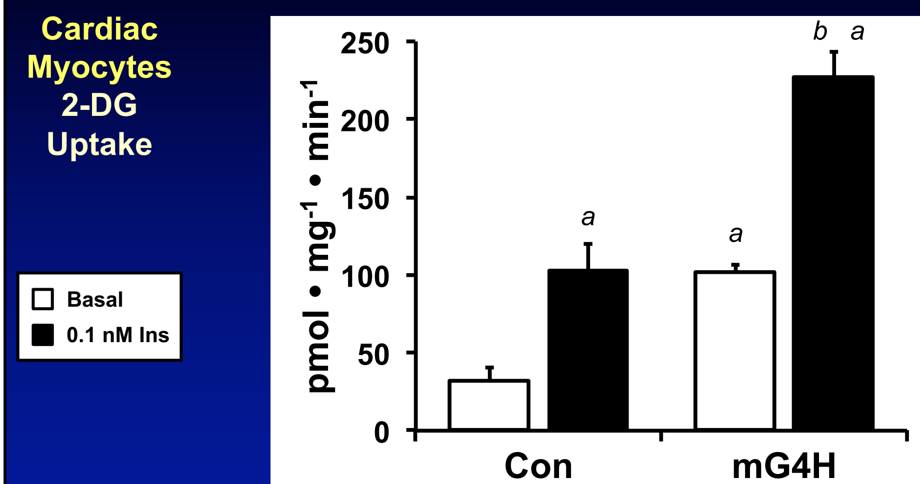
Insulin-induced GLUT4 Vesicle Fusion and Exofacial Myc-Epitope Exposure



Ariel Contreras-Ferrat
Wende … Abel *in prep*

GLUT4 Induction Increases Basal and Insulin-Stimulated Glucose Uptake

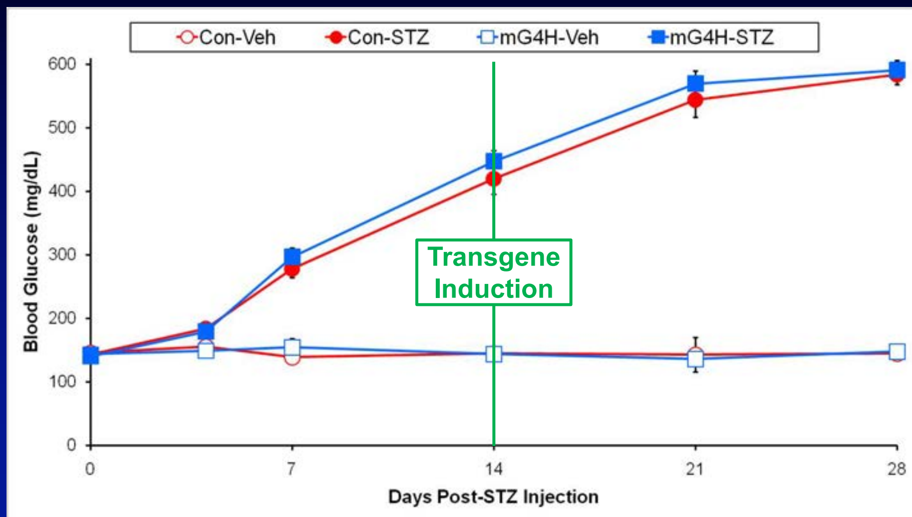
Cardiac Myocytes 2-DG Uptake



n = 3 – 4
[a] P < 0.01 vs. Con-Basal
[b] P < 0.001 vs. All

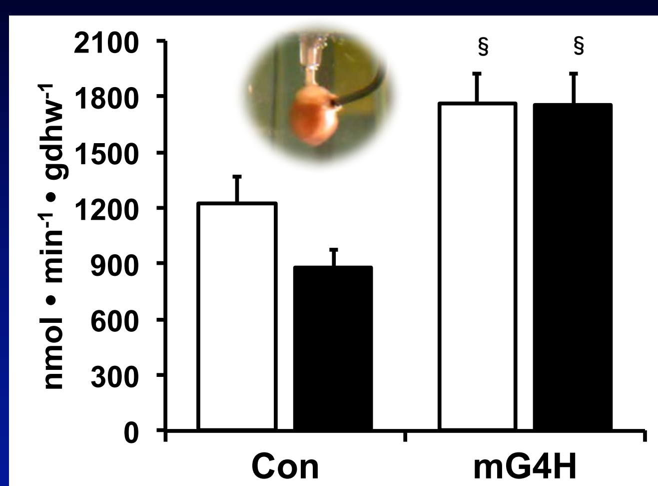
Renata O. Pereira
Wende … Abel *in prep*

Streptozotocin (STZ)-Induced Hyperglycemia is Not Altered by Transgene Induction



GLUT4 Induction Increases Glycolysis and Rescues Diabetic Cardiac Glycolytic Defects

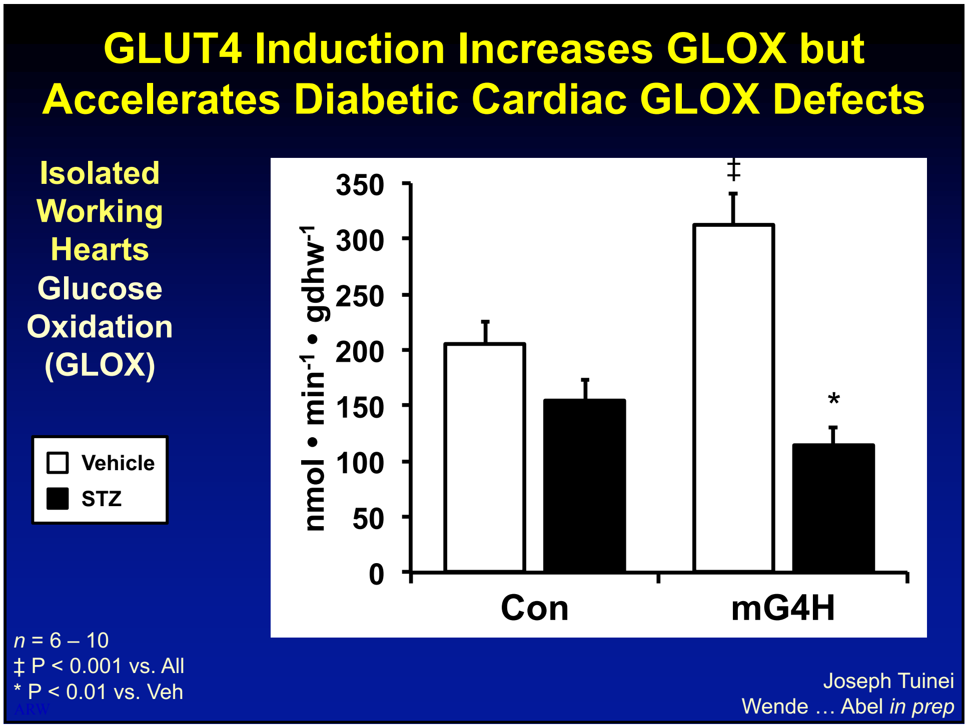
Isolated Working Hearts Glycolysis

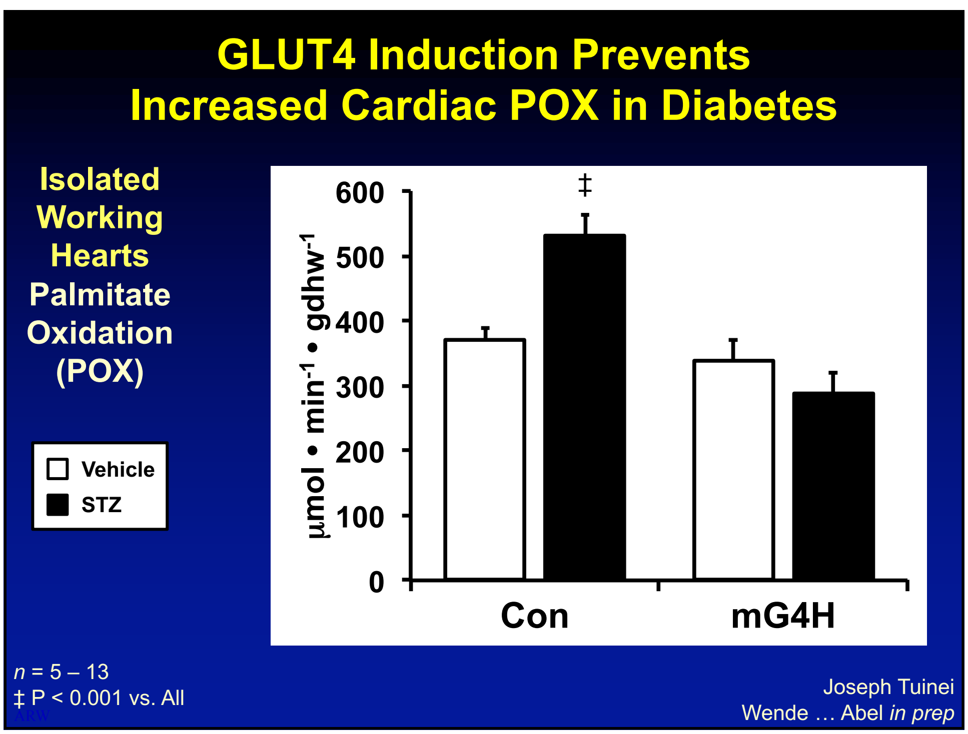


☐ Vehicle
■ STZ

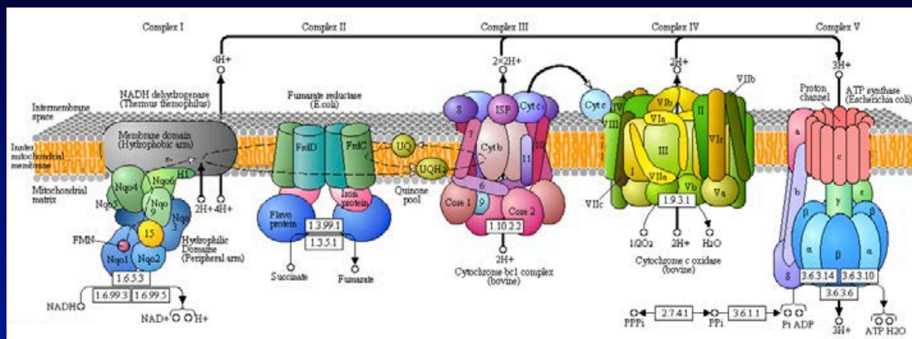
n = 6 – 10
§ P < 0.01 vs. Con

Joseph Tuinei
Wende … Abel *in prep*


GLUT4 Induction Increases GLOX but Accelerates Diabetic Cardiac GLOX Defects
Isolated Working Hearts Glucose Oxidation (GLOX)
Vehicle
STZ
nmol • min-1 • gdhw-1
350
300
250
200
150
100
50
0
‡
*
Con
mG4H
n = 6 – 10
‡ P < 0.001 vs. All
* P < 0.01 vs. Veh
Joseph Tuinei
Wende … Abel in prep

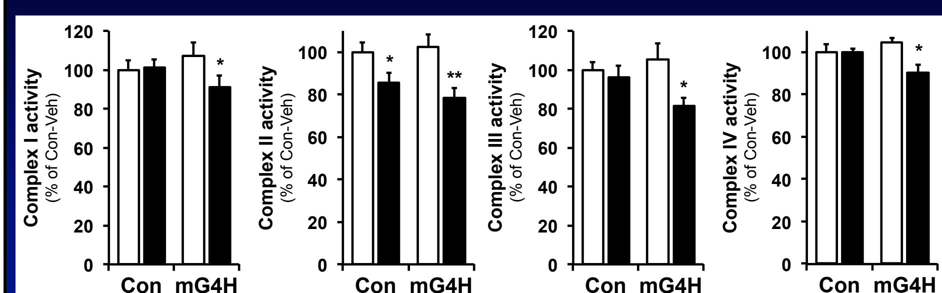

GLUT4 Induction Prevents Increased Cardiac POX in Diabetes
Isolated Working Hearts Palmitate Oxidation (POX)
Vehicle
STZ
μmol • min-1 • gdhw-1
600
500
400
300
200
100
0
‡
Con
mG4H
n = 5 – 13
‡ P < 0.001 vs. All
Joseph Tuinei
Wende … Abel in prep

Oxidative Phosphorylation



www.genome.jp/kegg/pathway.html

GLUT4 Induction Accelerates Development of Mitochondrial Dysfunction



n = 3 – 4
* P < 0.05

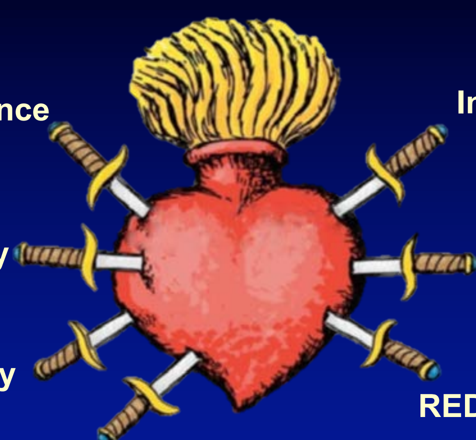
Oleh Khalimonchuk
Wende … Abel *in prep*

Conclusion – Part 1

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

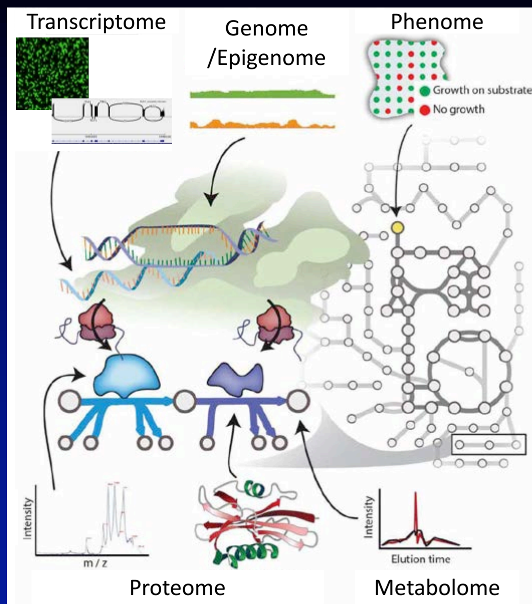
Diabetic Cardiomyopathy

"Death by a Thousand Cuts…"



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

Systems Biology



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

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

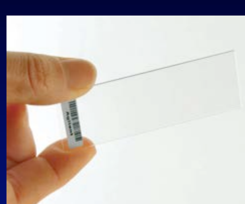
Proteome
Mass spec, western blot, Co-IP, IHC, PTMs, etc.

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

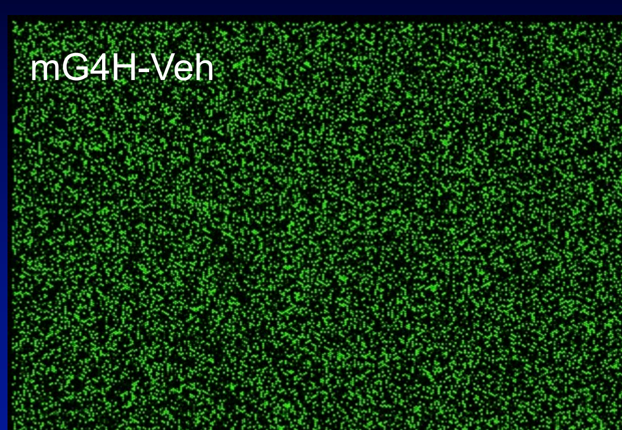
Genome / Epigenome
Southerns, sequencing, GenBank, ENCODE, ChIP-seq, bsDNA-seq, etc.

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

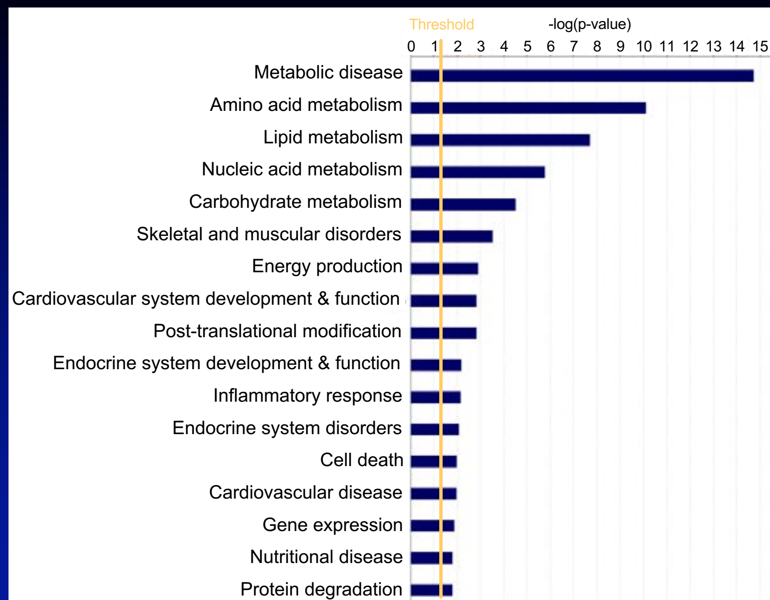
Transcriptomic Analysis Using the Agilent SurePrint G3 60K Microarray



181.9 MB

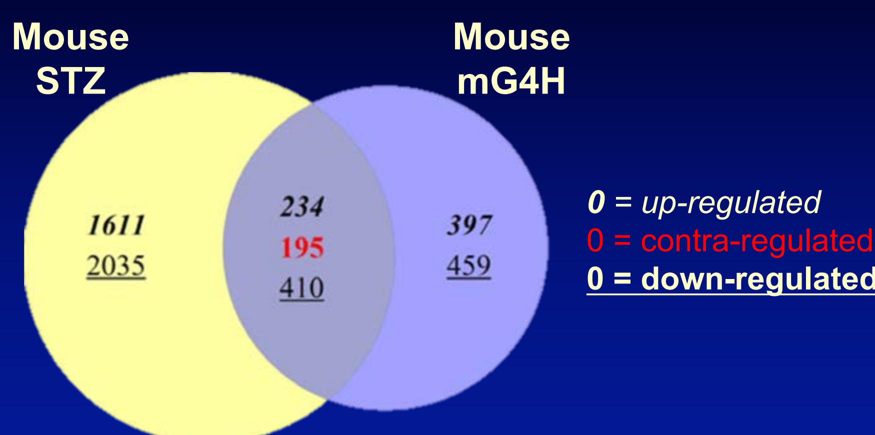


Pathway Analysis of Microarray

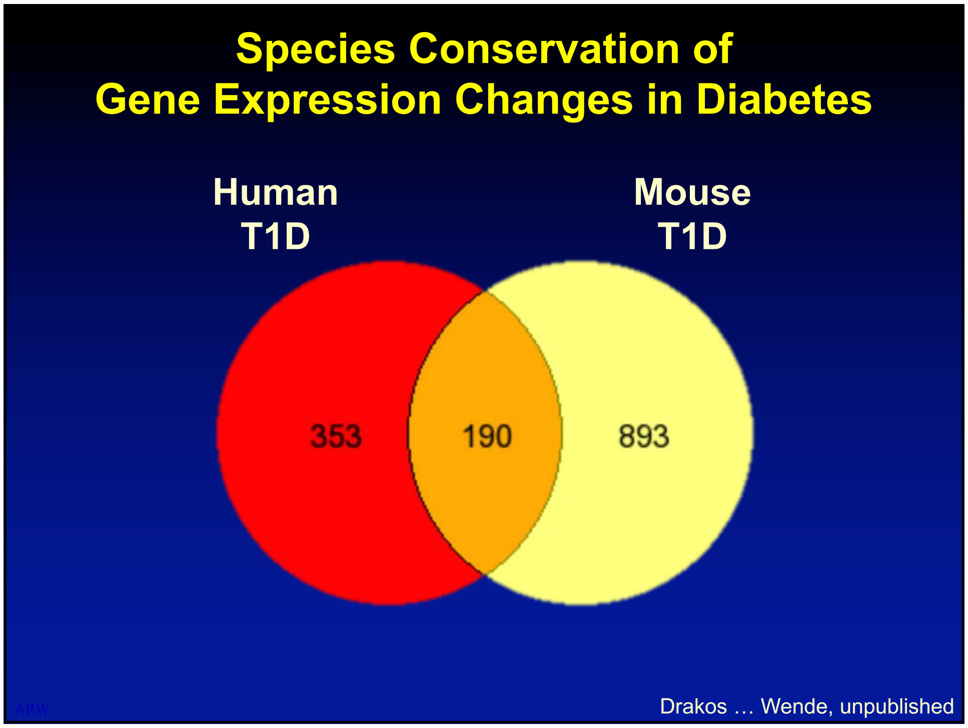


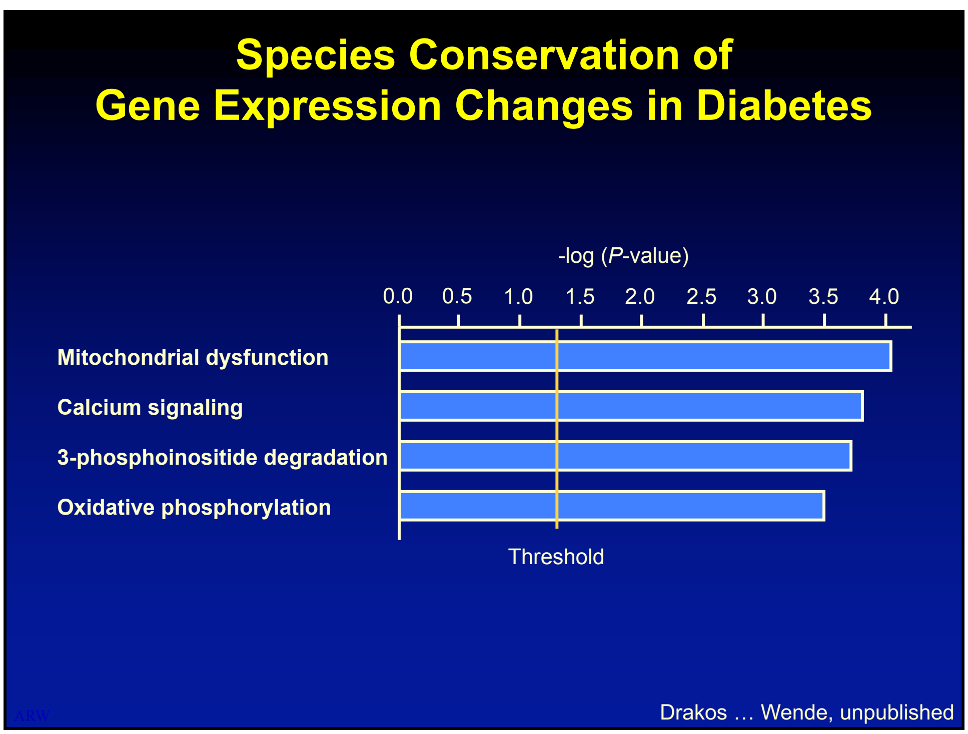
Wende … Abel *in prep*

Glucose Regulated Gene Expression

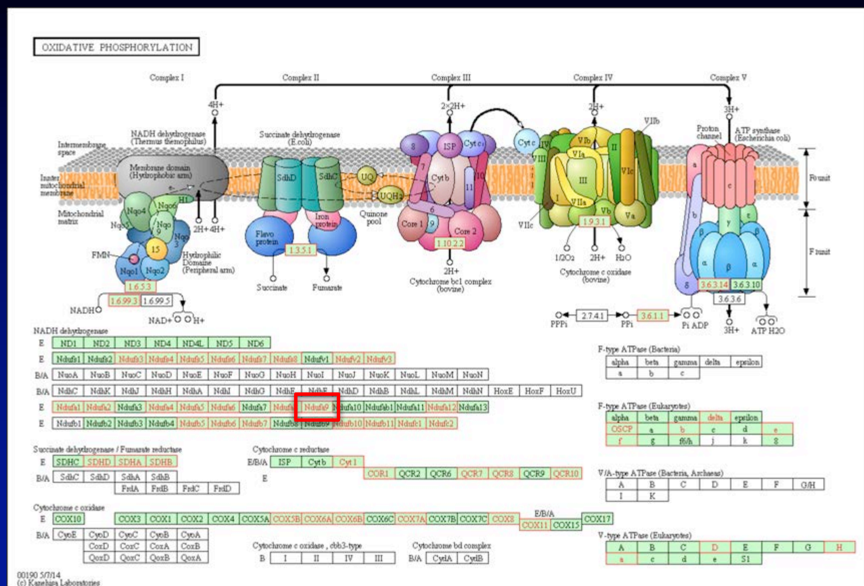


Wende, unpublished


Species Conservation of
Gene Expression Changes in Diabetes
Human
T1D
Mouse
T1D
353
190
893
Drakos … Wende, unpublished

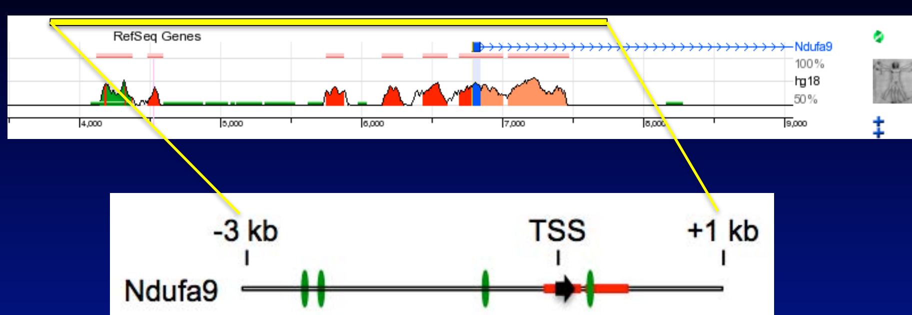

Species Conservation of
Gene Expression Changes in Diabetes
-log (P-value)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Mitochondrial dysfunction
Calcium signaling
3-phosphoinositide degradation
Oxidative phosphorylation
Threshold
Drakos … Wende, unpublished

Oxidative Phosphorylation



GeneSifter using KEGG

Ndufa9 Gene Promoter Structure



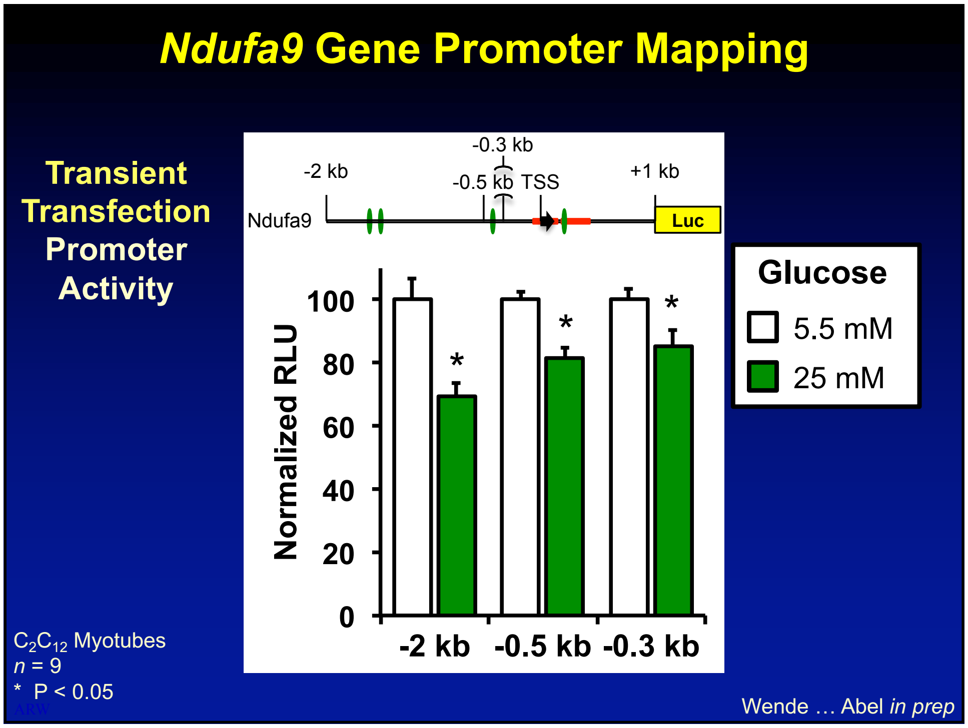
KEY

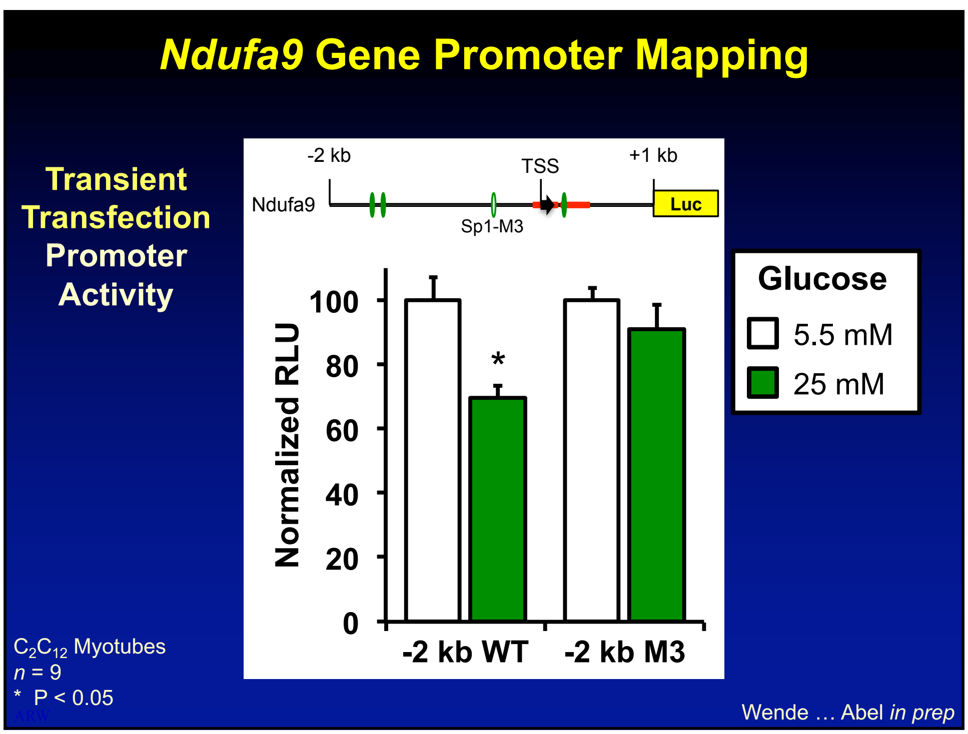
TSS = Transcription start site

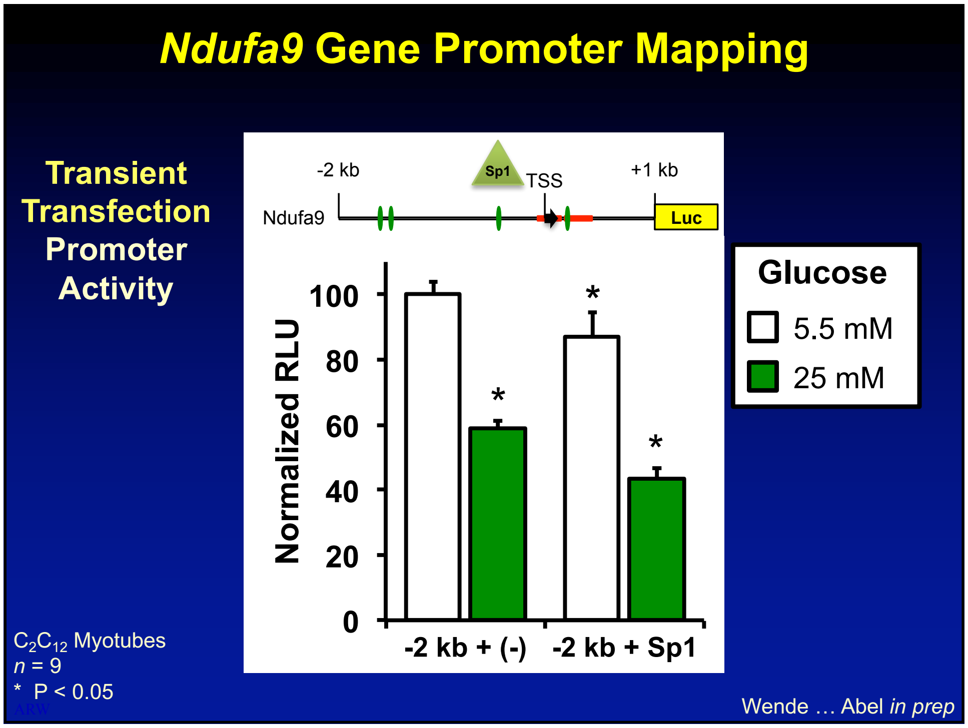
= CpG island

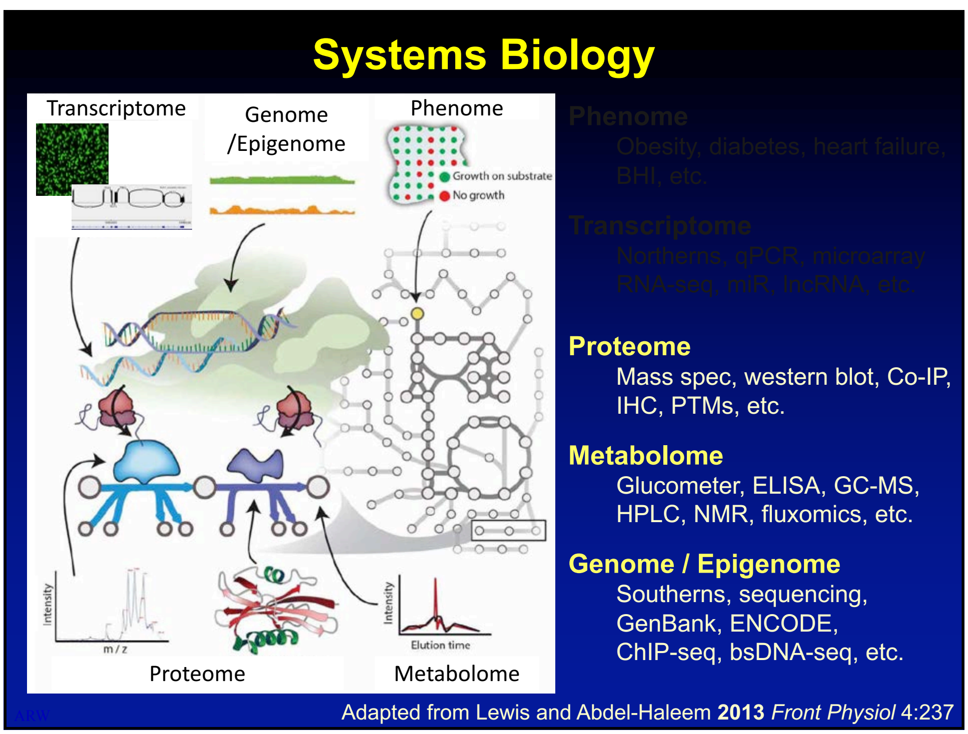
= Sp1 RE

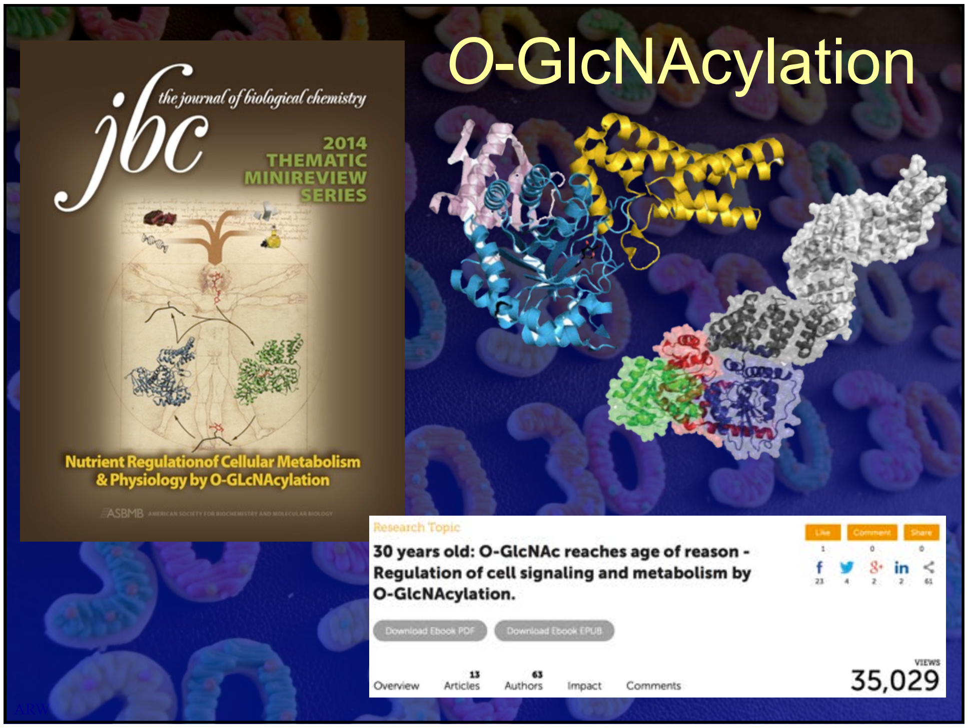
http://ecrbrowser.dcode.org


Ndufa9 Gene Promoter Mapping
Transient Transfection Promoter Activity
-0.3 kb
-2 kb
-0.5 kb
TSS
+1 kb
Ndufa9
Luc
Normalized RLU
100
80
60
40
20
0
*
*
*
-2 kb
-0.5 kb
-0.3 kb
Glucose
5.5 mM
25 mM
C_2C_{12} Myotubes
n = 9
* P < 0.05
Wende … Abel *in prep*


Ndufa9 Gene Promoter Mapping
Transient Transfection Promoter Activity
-2 kb
TSS
+1 kb
Ndufa9
Luc
Sp1-M3
Normalized RLU
100
80
60
40
20
0
*
-2 kb WT
-2 kb M3
Glucose
5.5 mM
25 mM
C_2C_{12} Myotubes
n = 9
* P < 0.05
Wende … Abel *in prep*


Ndufa9 Gene Promoter Mapping
Transient Transfection Promoter Activity
-2 kb
Sp1
TSS
+1 kb
Ndufa9
Luc
Normalized RLU
100
80
60
40
20
0
*
-2 kb + (-)
-2 kb + Sp1
Glucose
5.5 mM
25 mM
C_2C_{12} Myotubes
n = 9
* P < 0.05
Wende … Abel *in prep*


Systems Biology
Transcriptome
Genome /Epigenome
Phenome
Growth on substrate
No growth
Proteome
Metabolome
m / z
Elution time
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.
Adapted from Lewis and Abdel-Haleem **2013** *Front Physiol* 4:237


O-GlcNAcylation
jbc
the journal of biological chemistry
2014 THEMATIC MINIREVIEW SERIES
Nutrient Regulationof Cellular Metabolism & Physiology by O-GLcNAcylation
ASBMB
Research Topic
30 years old: O-GlcNAc reaches age of reason - Regulation of cell signaling and metabolism by O-GlcNAcylation.
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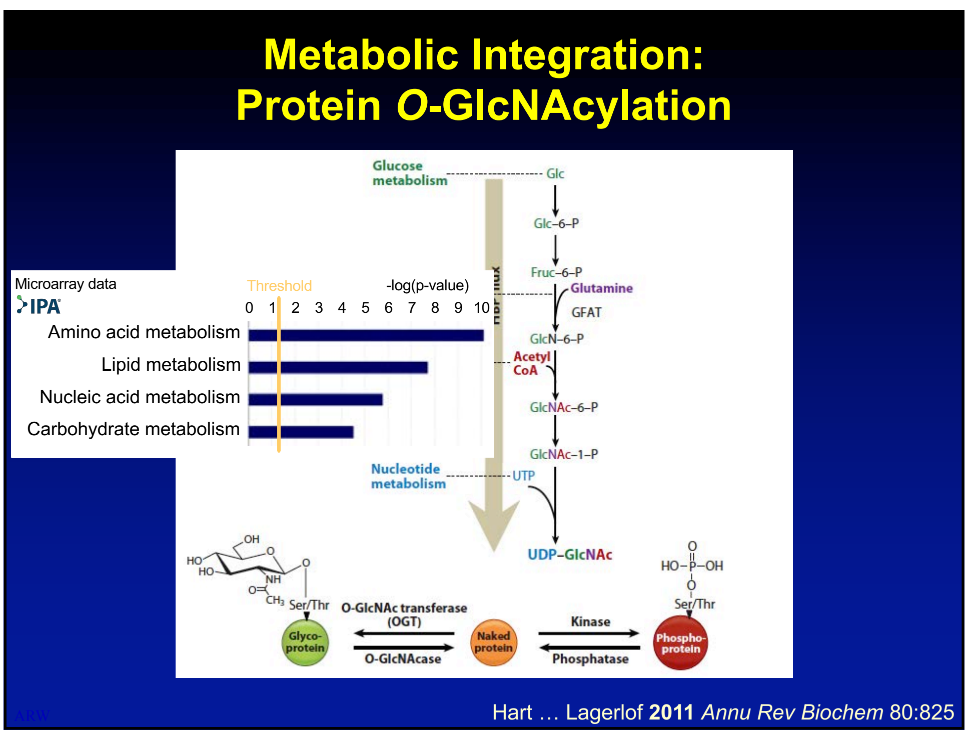
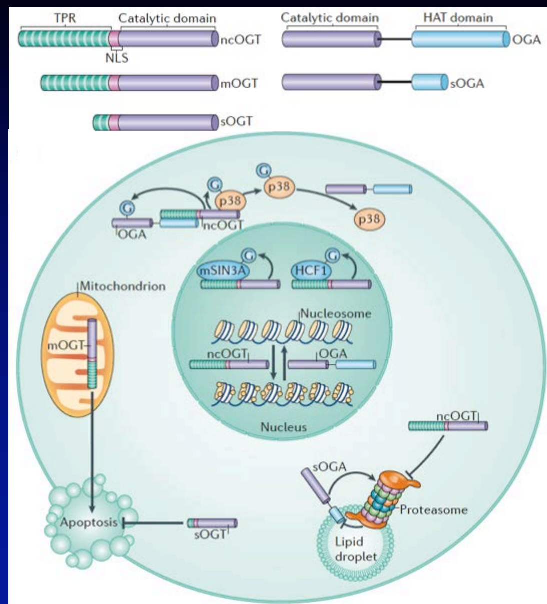
63 Authors
Impact
Comments
VIEWS
35,029

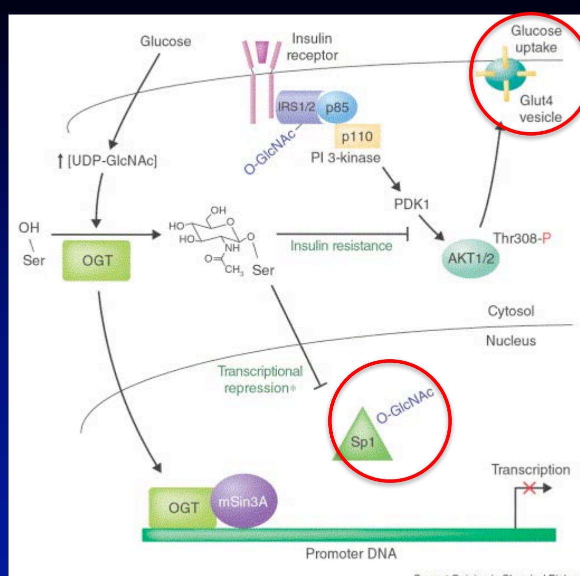

Metabolic Integration:
Protein O-GlcNAcylation
Microarray data
IPA
Threshold
-log(p-value)
0 1 2 3 4 5 6 7 8 9 10
Amino acid metabolism
Lipid metabolism
Nucleic acid metabolism
Carbohydrate metabolism
Glucose metabolism
Glc
Glc–6–P
Fruc–6–P
Glutamine
GFAT
GlcN–6–P
Acetyl CoA
GlcNAc–6–P
GlcNAc–1–P
Nucleotide metabolism
UTP
UDP–GlcNAc
Ser/Thr
O-GlcNAc transferase (OGT)
O-GlcNAcase
Glyco-protein
Naked protein
Kinase
Phosphatase
Phospho-protein
Hart … Lagerlof 2011 Annu Rev Biochem 80:825

O-GlcNAc Cycling



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

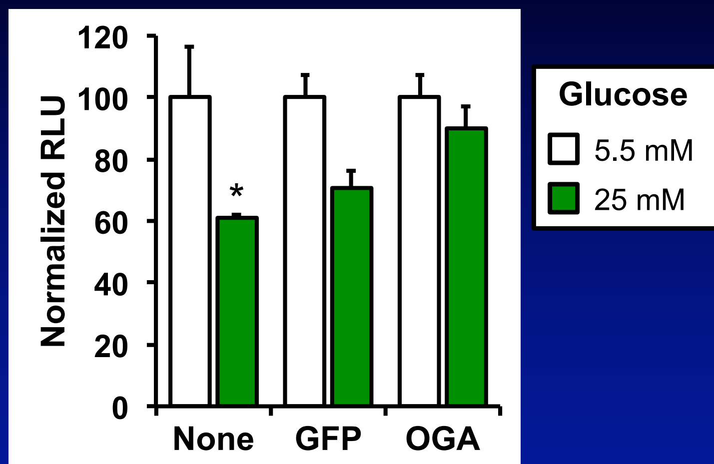
GlcNAc Regulation of Sp1



Vosseller … Hart **2002** *Curr Opin Chem Biol* 6(6):851

GlcNAcylation Regulates *Ndufa9* Gene Expression

Transient Transfection Promoter Activity

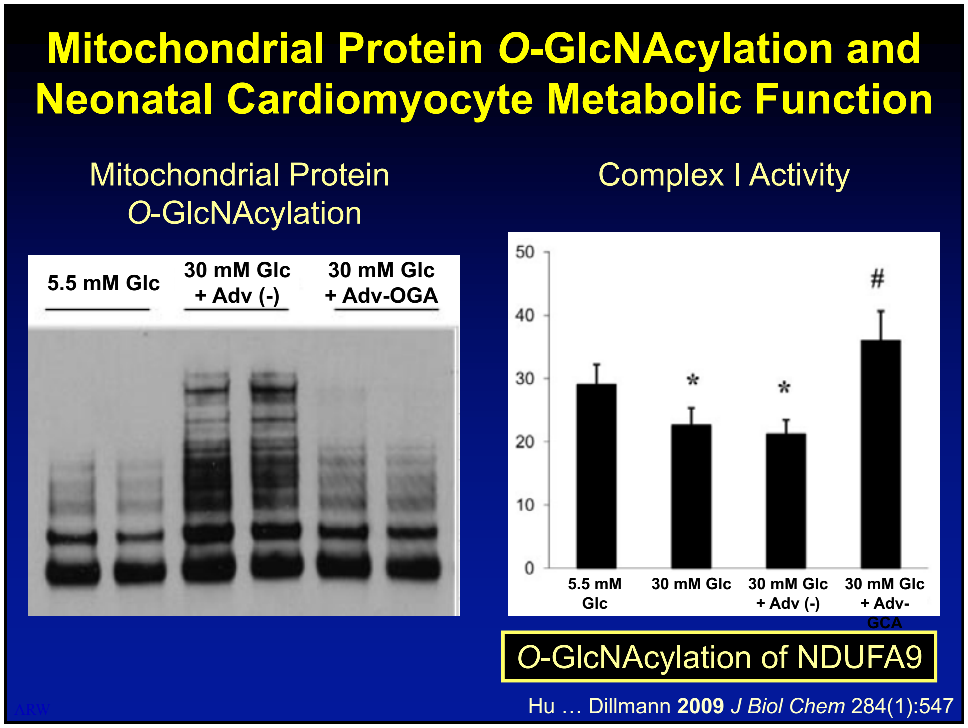


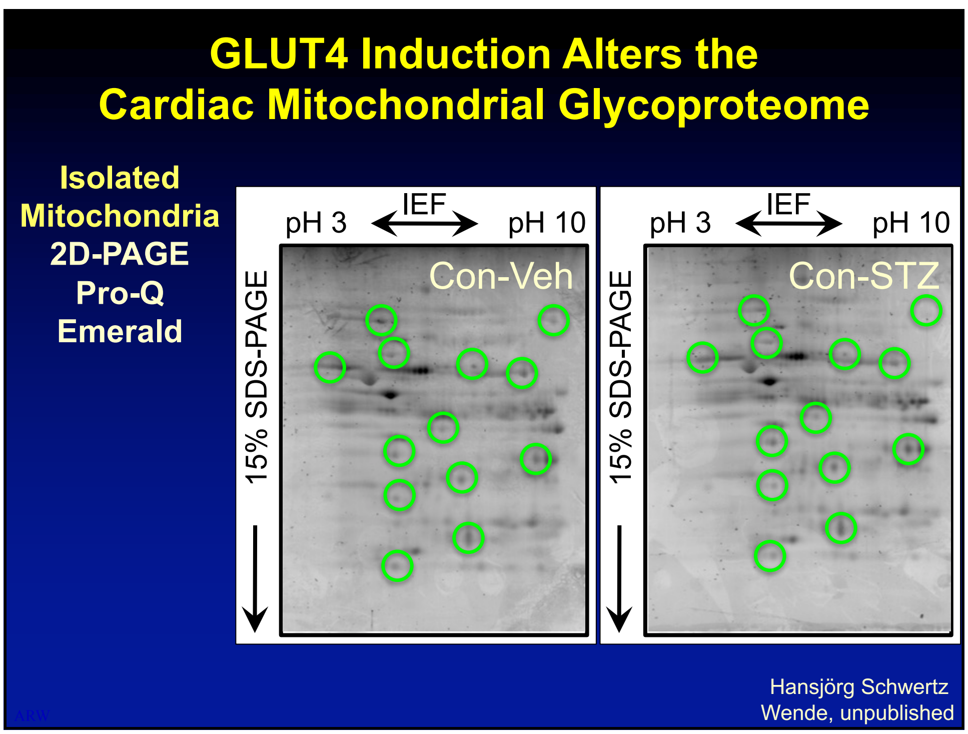
C_2C_{12} Myotubes
n = 3
* $P < 0.05$

Li Wang
Wende … Abel *in prep*

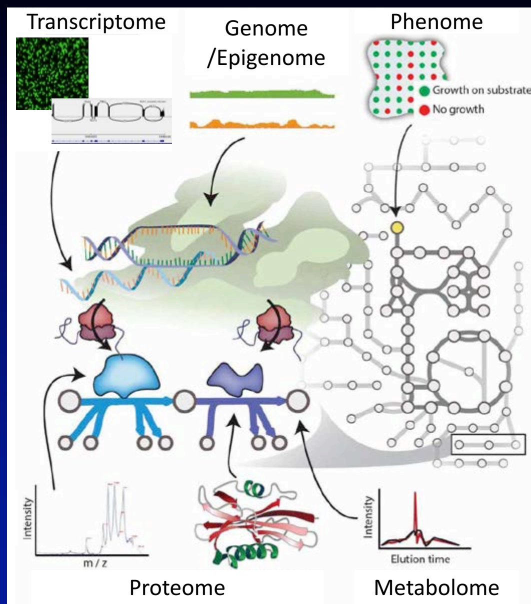
Conclusion – Part 2

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
50
40
30
20
10
0
#
*
*
5.5 mM Glc
30 mM Glc
30 mM Glc + Adv (-)
30 mM Glc + Adv-GCA
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
pH 3
IEF
pH 10
Con-Veh
15% SDS-PAGE
pH 3
IEF
pH 10
Con-STZ
15% SDS-PAGE
Hansjörg Schwertz
Wende, unpublished

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.

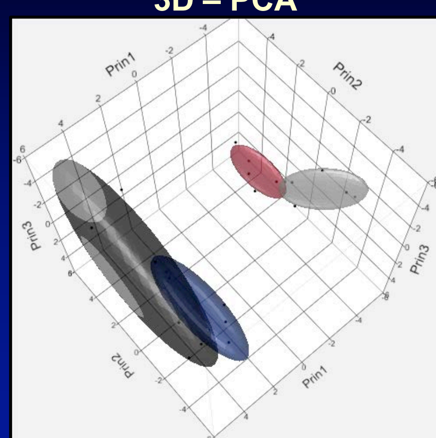
Genome / Epigenome
Southerns, sequencing, GenBank, ENCODE, ChIP-seq, bsDNA-seq, etc.

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

Metabolomic Signatures of Diabetic Heart Disease

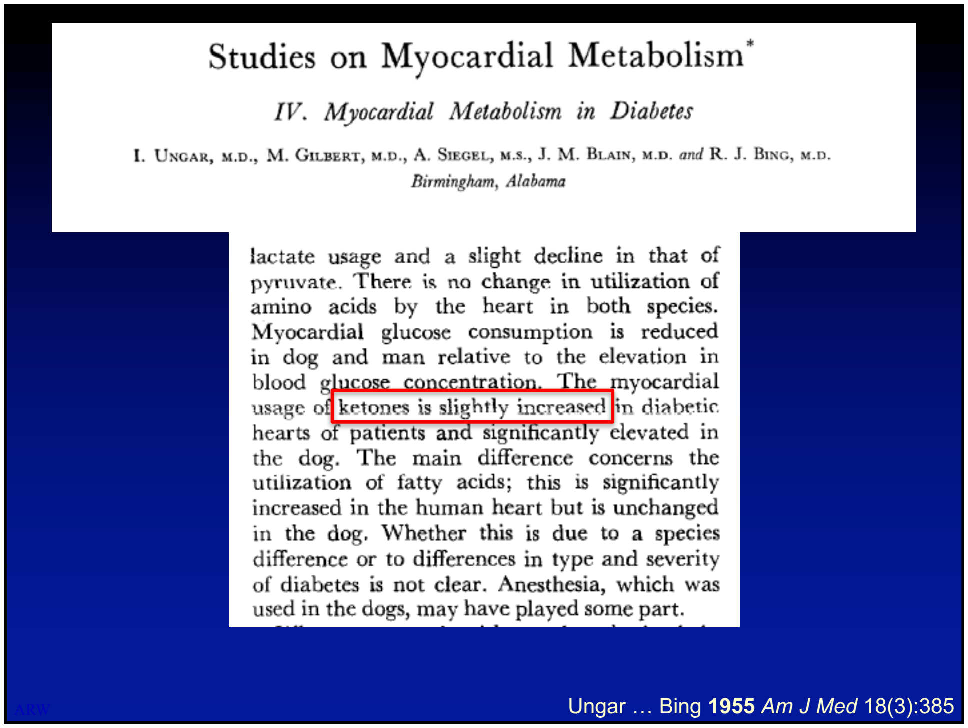
KEY
- Con-Veh
- Con-STZ
- mG4H-Veh
- mG4H-STZ

3D – PCA

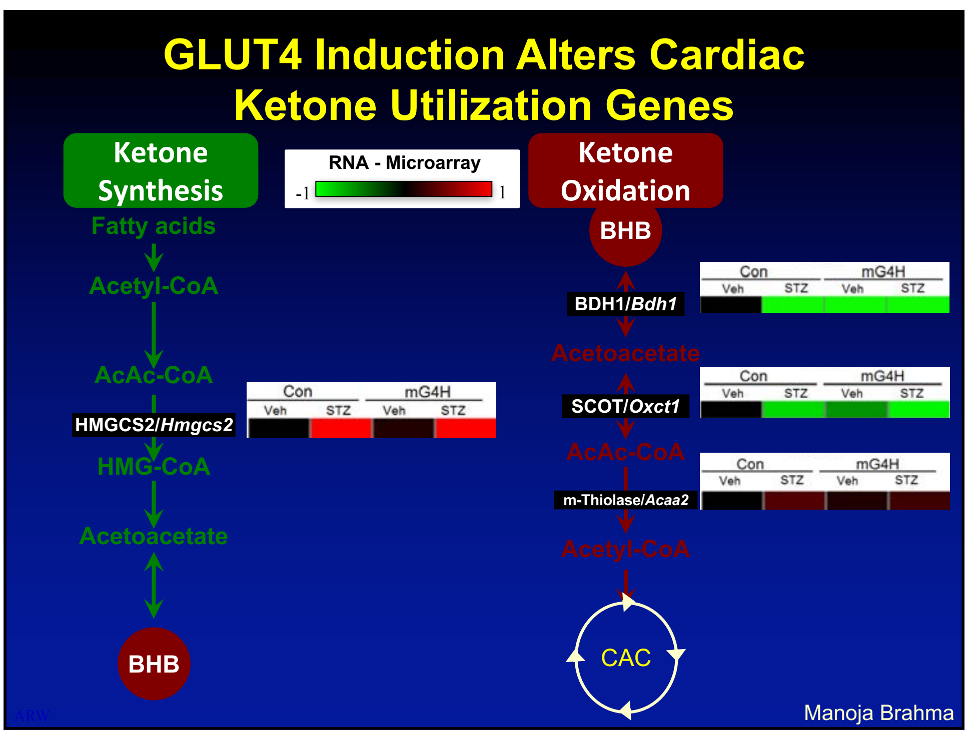


GC and HPLC - metabolomics

James Cox

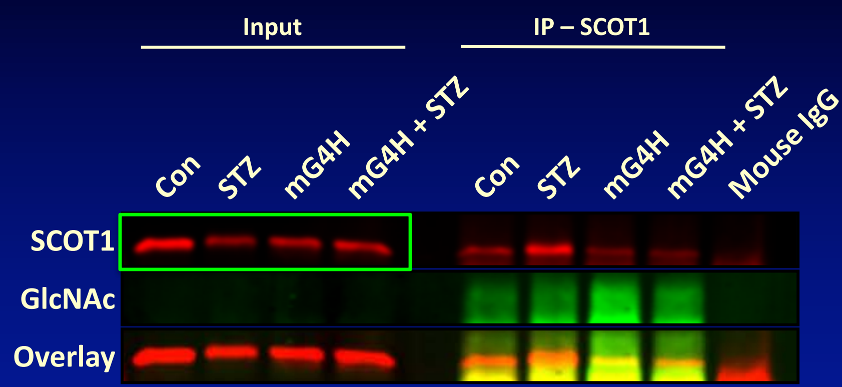


Studies on Myocardial Metabolism*
IV. Myocardial Metabolism in Diabetes
I. UNGAR, M.D., M. GILBERT, M.D., A. SIEGEL, M.S., J. M. BLAIN, M.D. and R. J. BING, M.D.
Birmingham, Alabama
lactate usage and a slight decline in that of pyruvate. There is no change in utilization of amino acids by the heart in 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.
Ungar … Bing 1955 Am J Med 18(3):385



GLUT4 Induction Alters Cardiac Ketone Utilization Genes
Ketone Synthesis
RNA - Microarray
-1
1
Ketone Oxidation
Fatty acids
Acetyl-CoA
AcAc-CoA
HMGCS2/Hmgcs2
HMG-CoA
Acetoacetate
BHB
Con
mG4H
Veh
STZ
Veh
STZ
BHB
BDH1/Bdh1
Acetoacetate
SCOT/Oxct1
AcAc-CoA
m-Thiolase/Acaa2
Acetyl-CoA
CAC
Manoja Brahma

GLUT4 Induction Alters Cardiac Ketone Protein GlcNAcylation

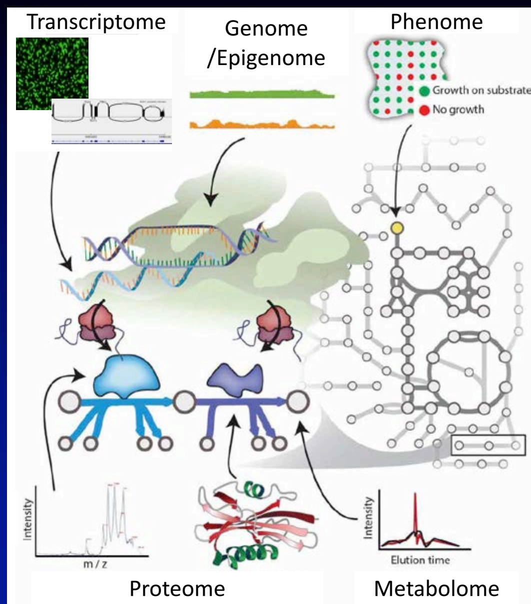


Manoja Brahma

Conclusion – Part 3

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

Systems Biology



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.

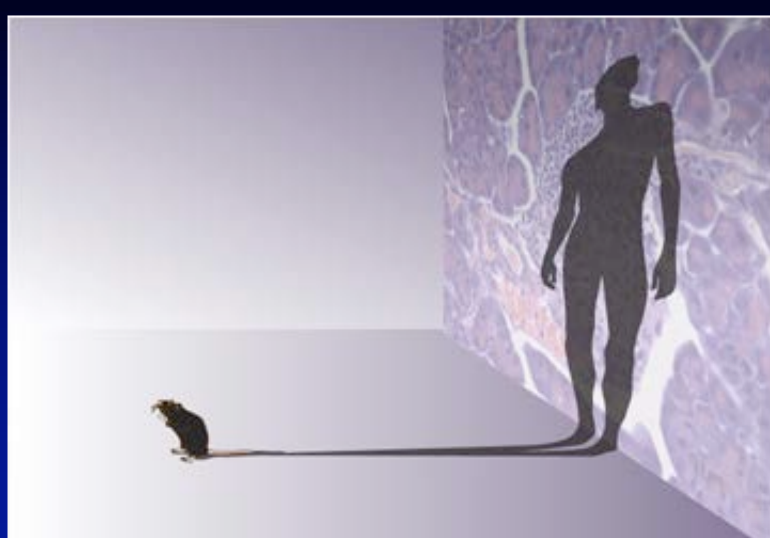
Metabolome
Glucometer, ELISA, GC-MS, HPLC, NMR, fluxomics, etc.

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

ARW

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

From Human to Mouse and Back Again



ARW

Broad Institute Communications

Role of Epigenetics in Gene Expression

Epigenetics - Programming

DCCT: Diabetes Control and Complications Trial

The New England
Journal of Medicine

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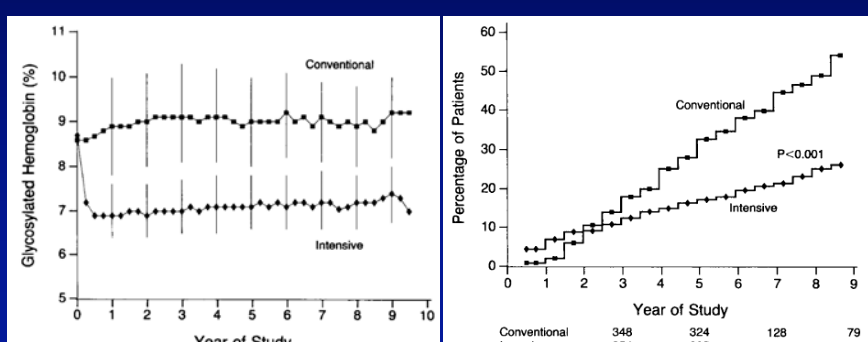
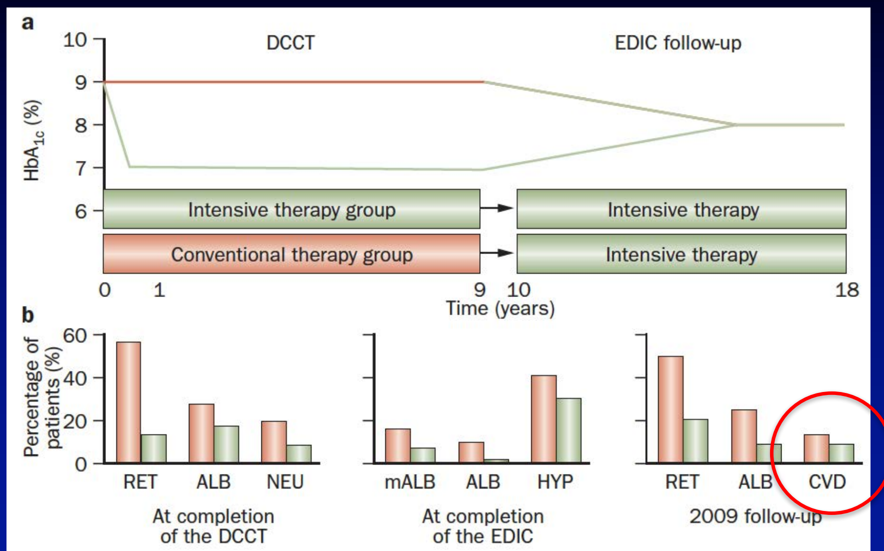
Volume 329 | SEPTEMBER 30, 1993 | Number 14

THE EFFECT OF INTENSIVE TREATMENT OF DIABETES ON THE DEVELOPMENT AND PROGRESSION OF LONG-TERM COMPLICATIONS IN INSULIN-DEPENDENT DIABETES MELLITUS

THE DIABETES CONTROL AND COMPLICATIONS TRIAL RESEARCH GROUP*

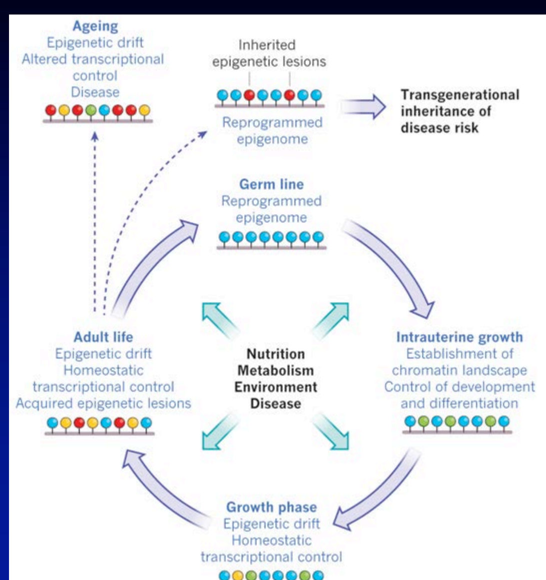


Epigenetics - Memory
EDIC: Epidemiology of Diabetes Interventions Trial



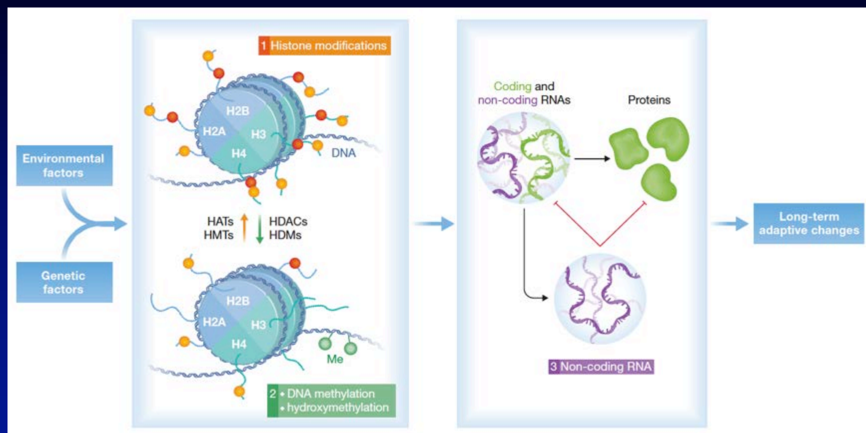
Pirola … El-Osta **2010** *Nat Rev Endocrinol* 6(12):665

Epigenetics: Transgenerational and Drift



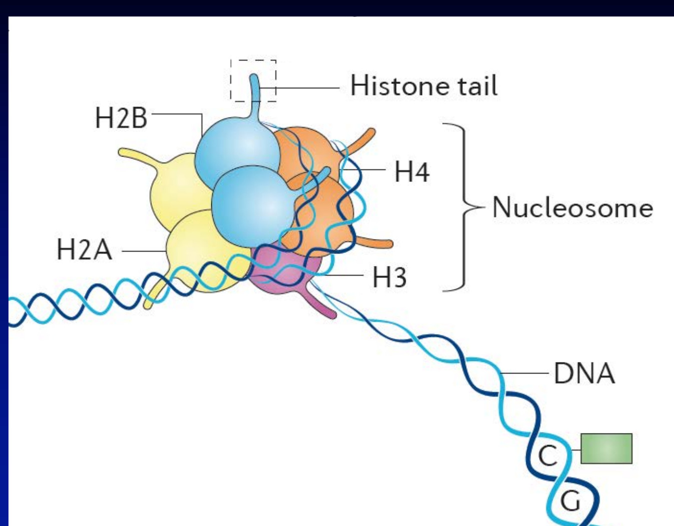
Gut and Verdin **2013** *Nature* 502:489

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?

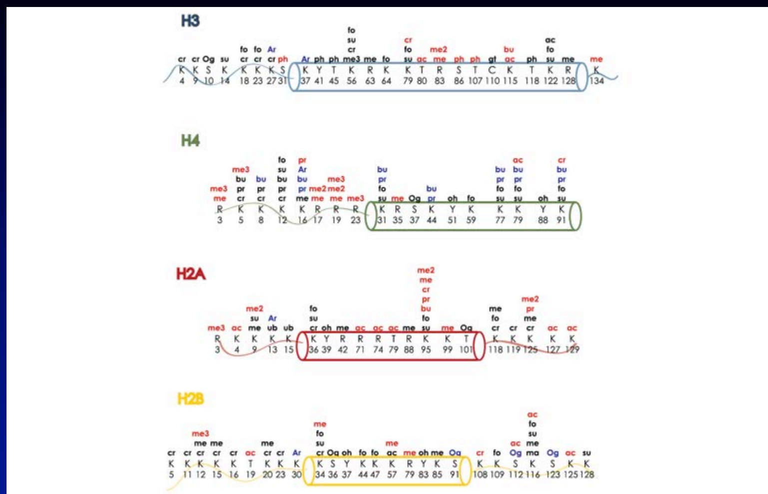
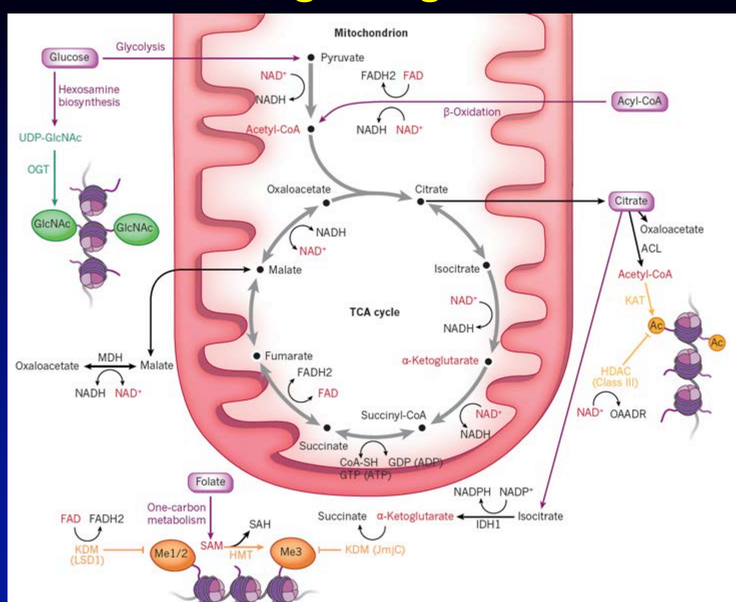


Figure 1 Recently identified modifications on the core histones. Black, modifications found *in vivo* in human; red, modifications found in mouse brain; blue, modifications found *in vitro*. ac, acetylation; Ar, ADP-ribosylation; bu, butyrylation; cr, crotonylation; fo, formylation; gt, glutathionylation; ma, malonylation; me, methylation; Og, O-glcNAcylation; oh, hydroxylation; pr, propionylation; su, succinylation; ph, phosphonylation; ub, ubiquitination.

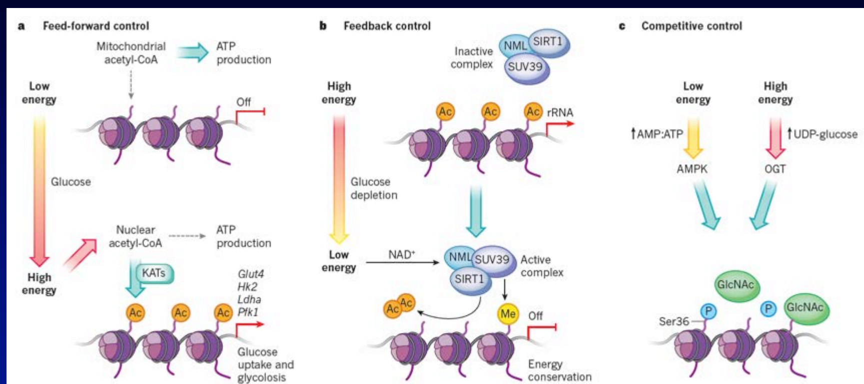
Arnaudo … Garcia **2013** *Epigenetics Chromatin* 6(1):24

Metabolite Signaling to Chromatin



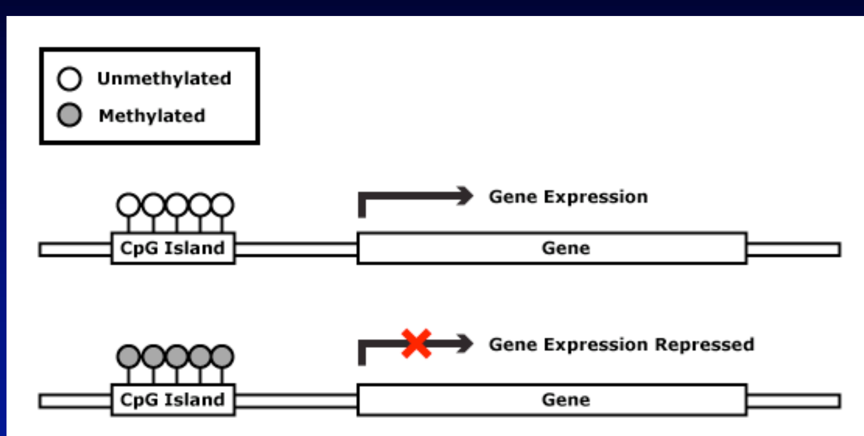
Gut and Verdin **2013** *Nature* 502:489

How does GlcNAc fit in?



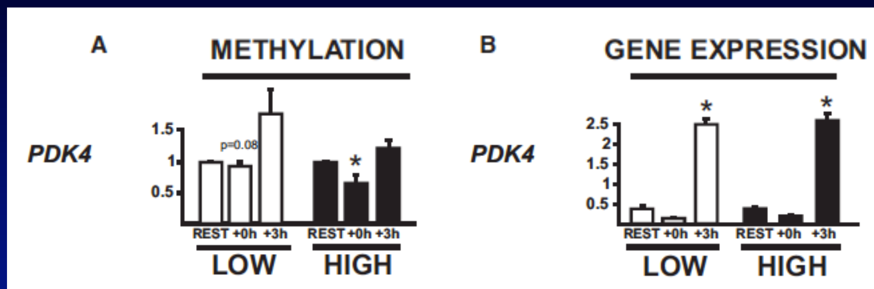
Gut and Verdin **2013** *Nature* 502(7472):489

DNA Methylation 101



ucsf.edu

Exercise Alters DNA Methylation of Key Metabolic Genes



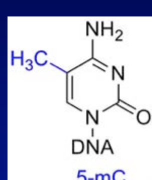
Low = 40% VO_{2peak} High = 80% VO_{2peak}

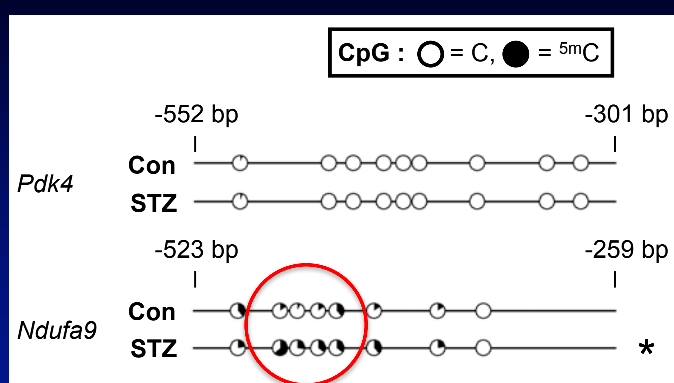
Subjects fasted overnight and then consumed a high carbohydrate diet 4 hr prior to exercise.

Barres and Zierath **2012** *Cell Metab* 15:405

Diabetes Regulated Cardiac DNA Methylation

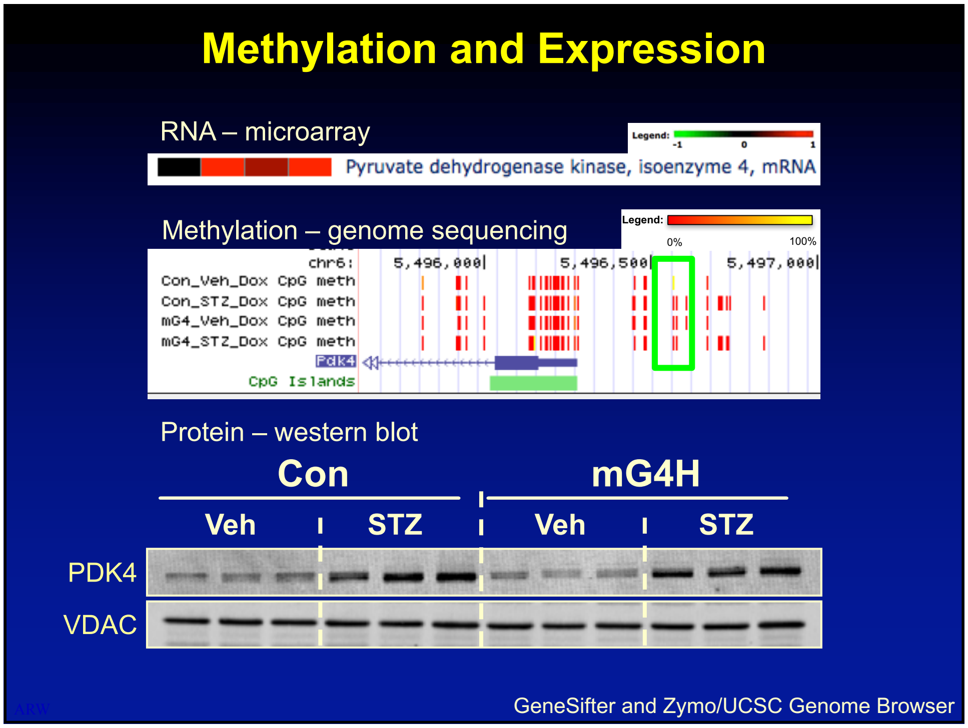
Targeted bsDNA-seq 5-mCpG

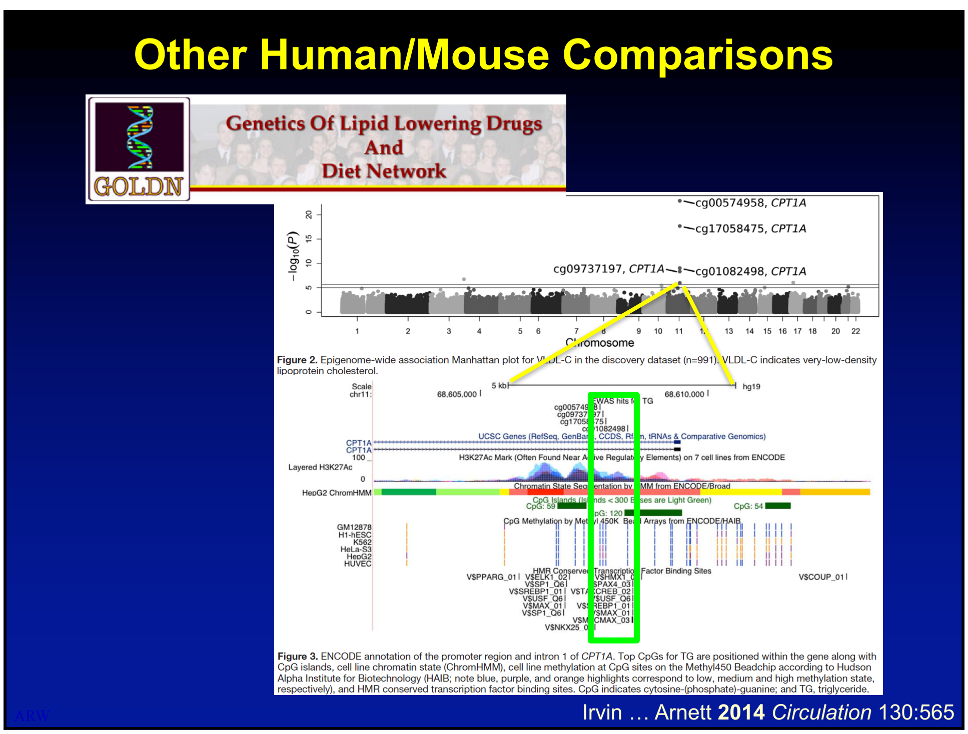


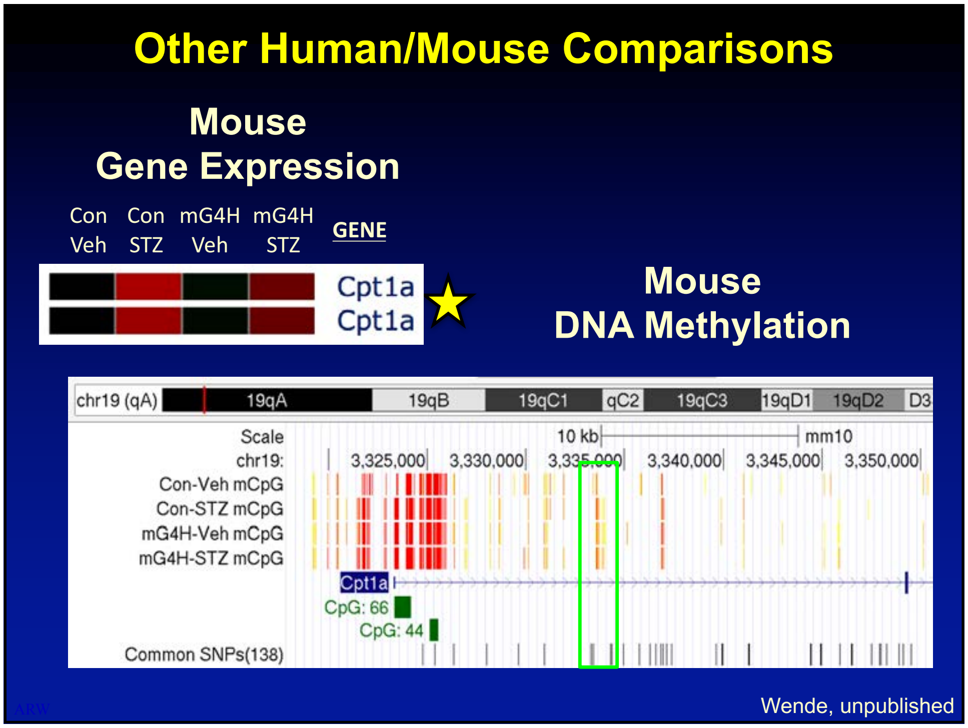


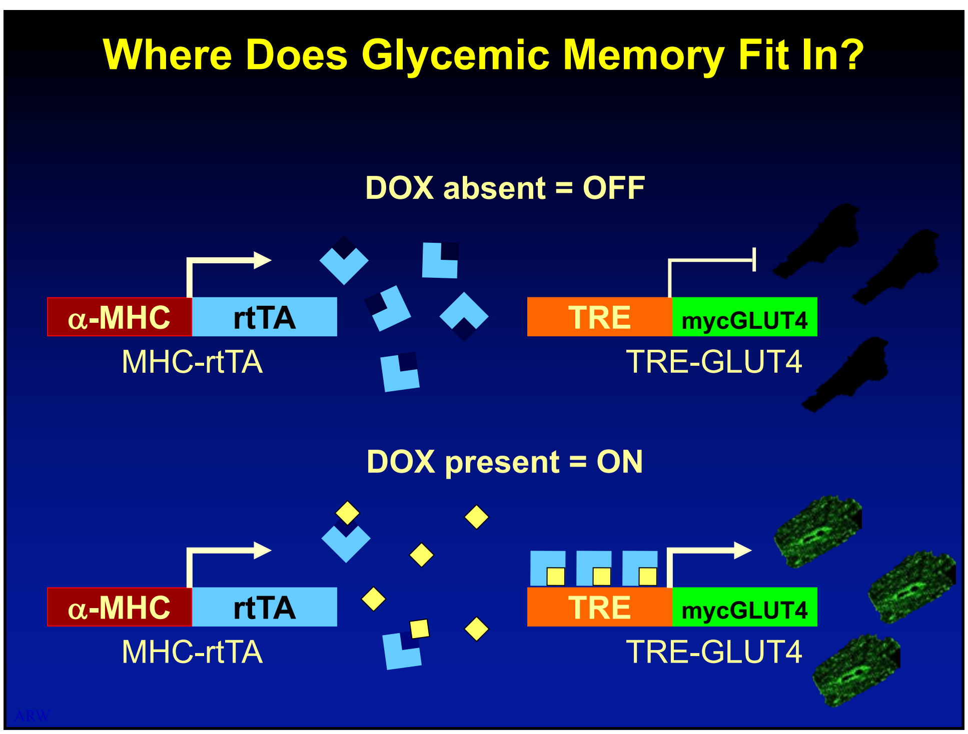
Heart, LV
n = 10
* P < 0.05

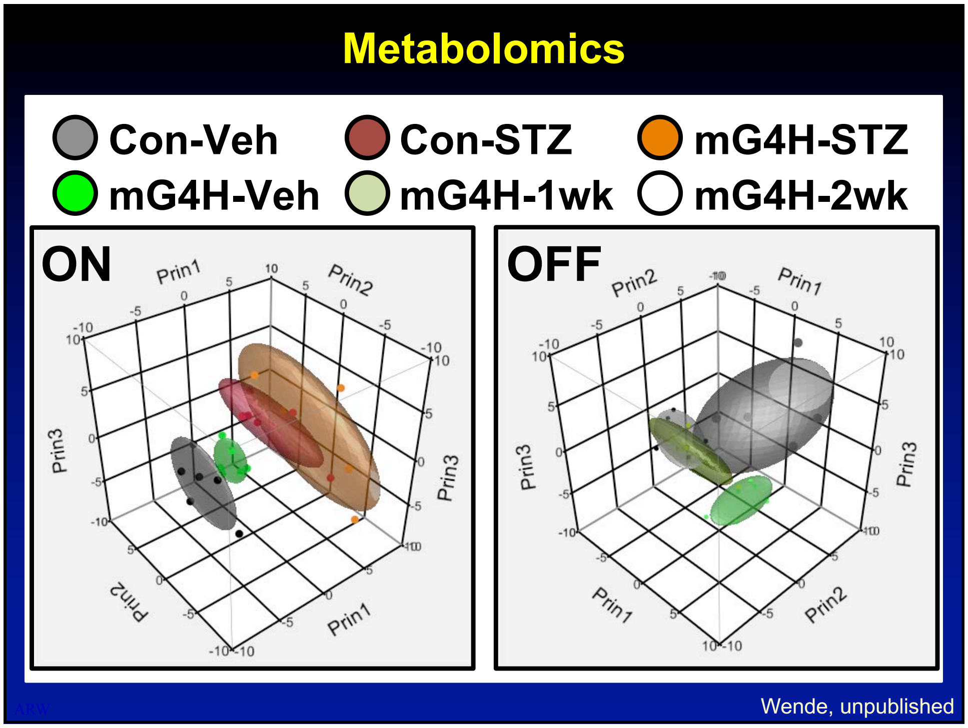
Wende, unpublished

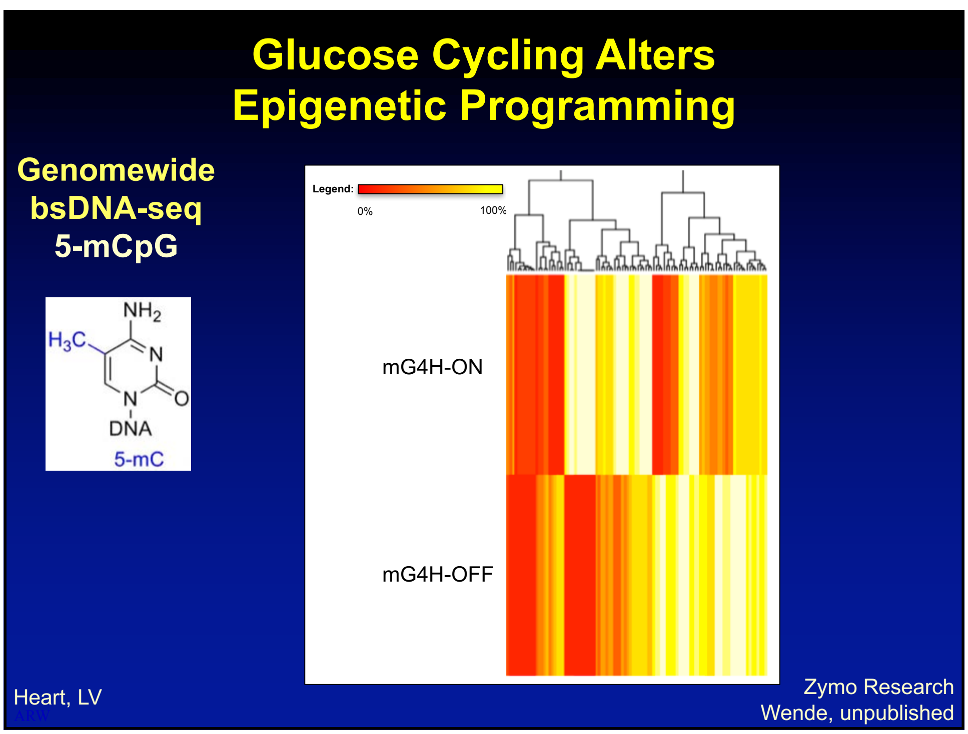

Methylation and Expression
RNA – microarray
Legend:
-1
0
1
Pyruvate dehydrogenase kinase, isoenzyme 4, mRNA
Methylation – genome sequencing
Legend:
0%
100%
chr6:
5,496,000
5,496,500
5,497,000
Con_Veh_Dox CpG meth
Con_STZ_Dox CpG meth
mG4_Veh_Dox CpG meth
mG4_STZ_Dox CpG meth
Pdk4
CpG Islands
Protein – western blot
Con
mG4H
Veh
STZ
Veh
STZ
PDK4
VDAC
GeneSifter and Zymo/UCSC Genome Browser


Other Human/Mouse Comparisons
GOLDN
Genetics Of Lipid Lowering Drugs
And
Diet Network
cg00574958, CPT1A
cg17058475, CPT1A
cg09737197, CPT1A
cg01082498, CPT1A
−log10(P)
Chromosome
Figure 2. Epigenome-wide association Manhattan plot for VLDL-C in the discovery dataset (n=991). VLDL-C indicates very-low-density lipoprotein cholesterol.
UCSC Genes (RefSeq, GenBank, CCDS, Rfam, tRNAs & Comparative Genomics)
H3K27Ac Mark (Often Found Near Active Regulatory Elements) on 7 cell lines from ENCODE
Layered H3K27Ac
HepG2 ChromHMM
CpG Methylation by Methyl 450K Bead Arrays from ENCODE/HAIB
HMR Conserved Transcription Factor Binding Sites
Figure 3. ENCODE annotation of the promoter region and intron 1 of CPT1A. Top CpGs for TG are positioned within the gene along with CpG islands, cell line chromatin state (ChromHMM), cell line methylation at CpG sites on the Methyl450 Beadchip according to Hudson Alpha Institute for Biotechnology (HAIB; note blue, purple, and orange highlights correspond to low, medium and high methylation state, respectively), and HMR conserved transcription factor binding sites. CpG indicates cytosine-(phosphate)-guanine; and TG, triglyceride.
Irvin … Arnett 2014 Circulation 130:565

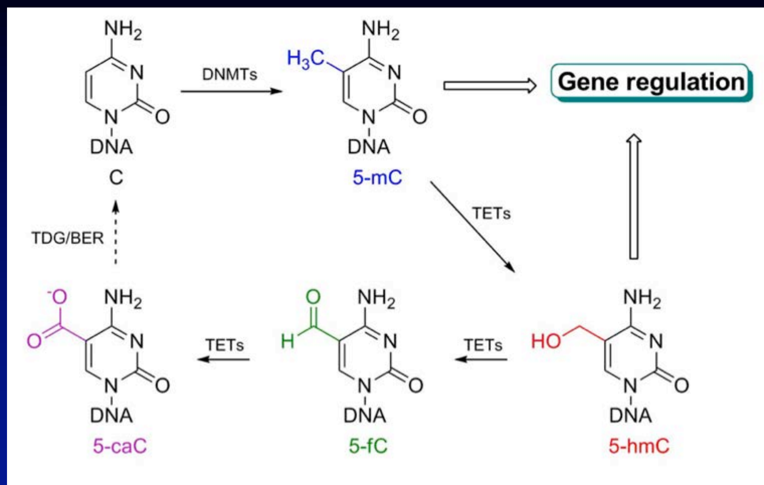

Other Human/Mouse Comparisons
Mouse
Gene Expression
Con Veh
Con STZ
mG4H Veh
mG4H STZ
GENE
Cpt1a
Cpt1a
Mouse
DNA Methylation
chr19 (qA)
19qA
19qB
19qC1
qC2
19qC3
19qD1
19qD2
D3
Scale
10 kb
mm10
chr19:
3,325,000
3,330,000
3,335,000
3,340,000
3,345,000
3,350,000
Con-Veh mCpG
Con-STZ mCpG
mG4H-Veh mCpG
mG4H-STZ mCpG
Cpt1a
CpG: 66
CpG: 44
Common SNPs(138)
Wende, unpublished


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
Con-Veh
Con-STZ
mG4H-STZ
mG4H-Veh
mG4H-1wk
mG4H-2wk
ON
OFF
Prin1
Prin2
Prin3
Wende, unpublished


Glucose Cycling Alters
Epigenetic Programming
Genomewide
bsDNA-seq
5-mCpG
NH2
H3C
N
O
N
DNA
5-mC
Legend:
0%
100%
mG4H-ON
mG4H-OFF
Heart, LV
Zymo Research
Wende, unpublished

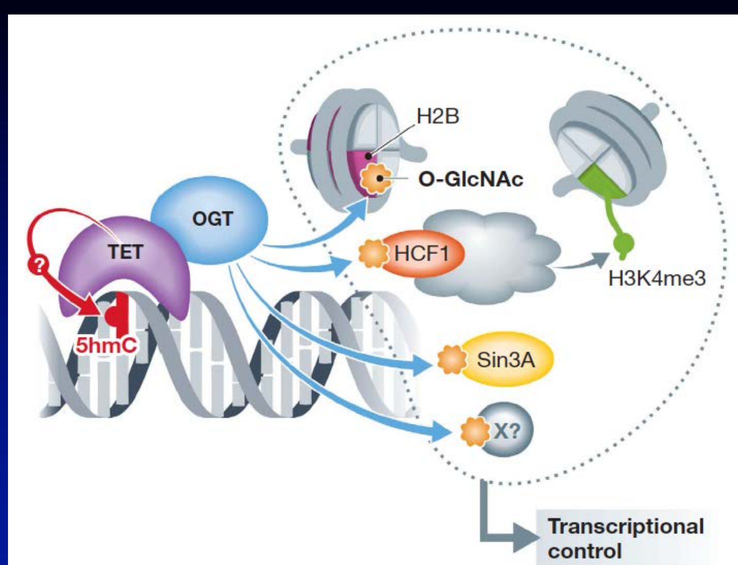
Background



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

Conclusion – Part 4

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

Sugar Gumming Up the Works

giphy.com

Overall Summary

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

Acknowledgements

Wende Lab

Thomas J Bailey – Undergrad
Manoja K. Brahma – Postdoc
Mark C. McCrory – Lab Manager
Brenna G. Nye – Undergrad
Mark Pepin – MSTP
Lamario J Williams – Undergrad

UAB Collaborators

Steve Barnes
John C. Chatham
David K. Crossman
Steve M. Pogwizd
Martin E. Young

E. Dale Abel

John C. Schell
Joseph Tuinei
many others…

Stavros G. Drakos

Nikos A. Diakos

Hansjörg Schwertz

Oleh Khalimonchuk – UNL

Other Colleagues & Mentors past and present

UAB – Knowledge that will change your world
Division of Molecular and Cellular Pathology

NIH National Heart, Lung, and Blood Institute
K99R00 HL111322
R00 HL111322-S1

NIH National Institute of Diabetes and Digestive and Kidney Diseases
U24 DK076169

American Heart Association
AHA 0725064Y

JDRF – Improving lives. Curing type 1 diabetes.
JDRF 51002608