Metabolomics

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

4th Annual Workshop on Metabolomics

Metabolomics in Diabetes

Thursday, July 21, 2016

Adam R. Wende, Ph.D.

Assistant Professor
Division of Molecular and Cellular Pathology

JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.

NIH National Heart, Lung, and Blood Institute

ARW

Presenter Disclosure Information

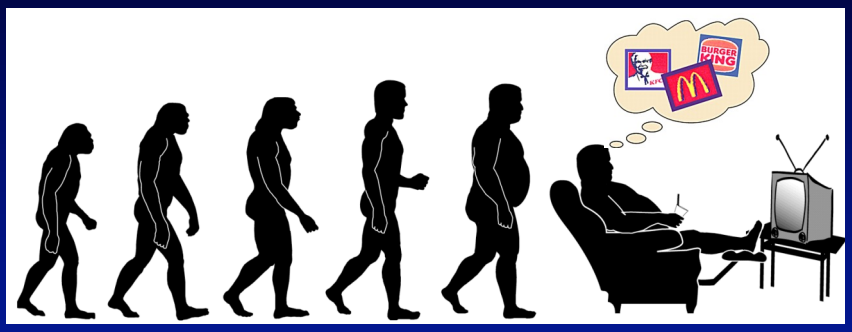
Adam R. Wende, Ph.D.

Metabolomics in Diabetes

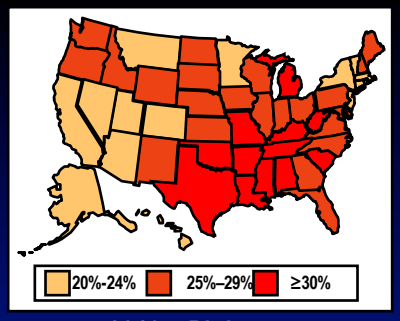
FINANCIAL DISCLOSURE:
None

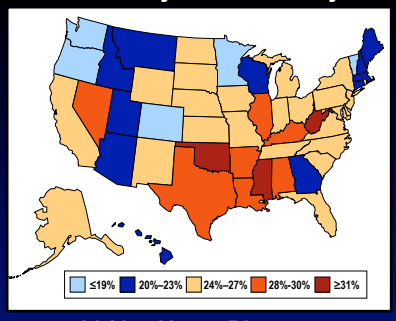
UNLABELED/UNAPPROVED USES DISCLOSURE:
None

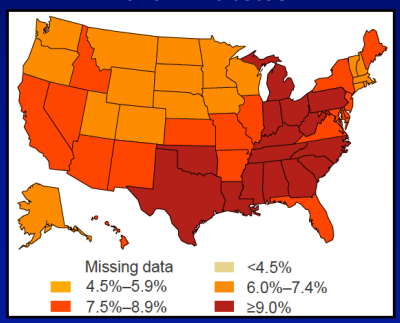
Obesity, Metabolic Syndrome, Diabetes, and Heart Failure

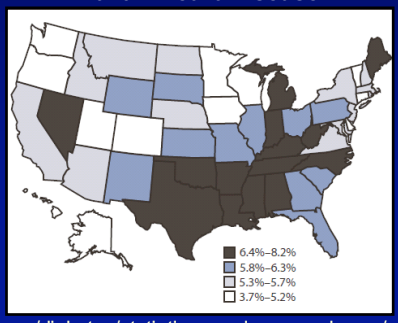


From: Roger Unger - UTSW





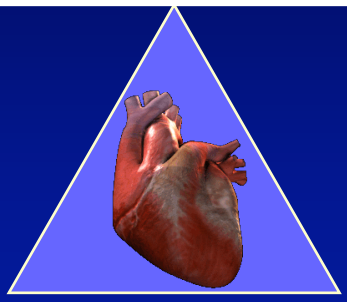




www.cdc.gov/diabetes/statistics and www.cdc.gov/mmwr

Maintaining Cardiac Function Through Metabolic Substrate Balance

Glucose Fatty Acids



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

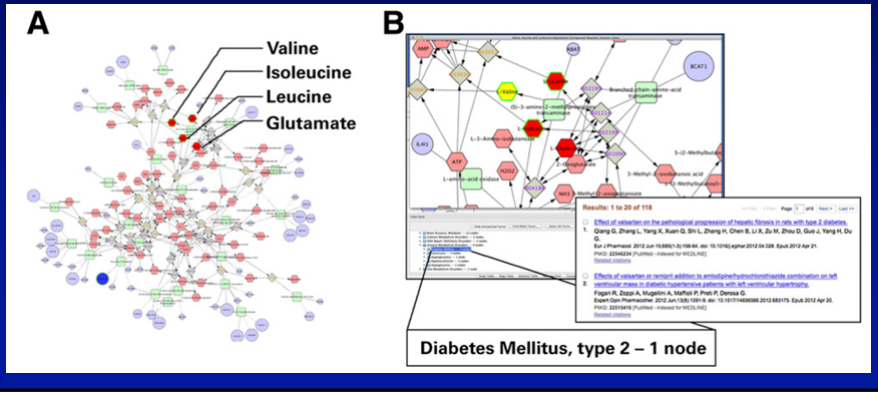
Diabetes and Metabolomics

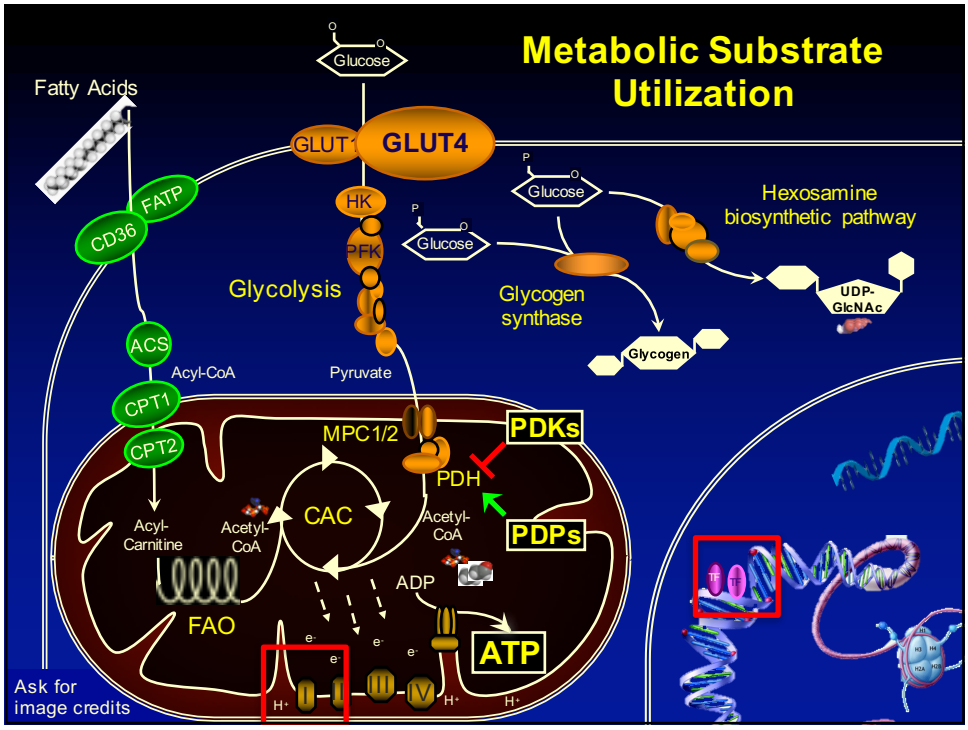
Diabetes. 2015 Mar;64(3):718-732.

Metabolomics and Diabetes: Analytical and Computational Approaches.

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

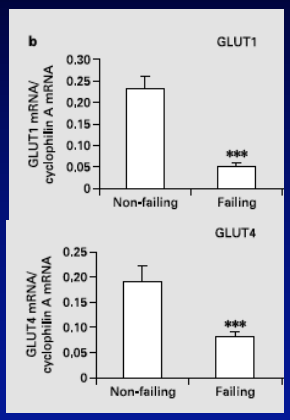
Metabolomics is an integral part for understanding disease processes … information garnered in the biomarker investigations, future research should shed more light on disease pathogenesis and explore new treatment options.



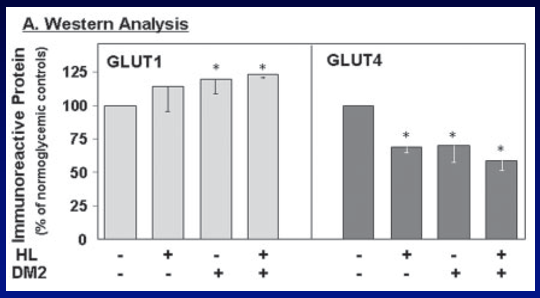


Changes in Human Heart GLUT Levels

RNA
Human heart failure



Protein
Human heart diabetes



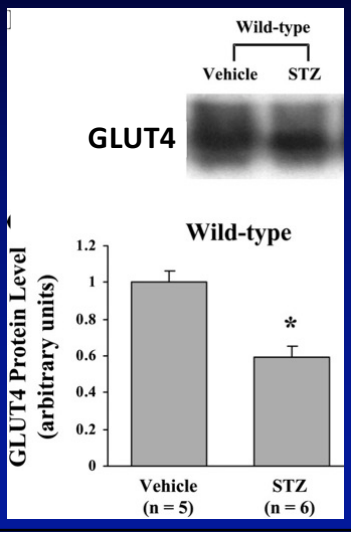
Biopsies obtained during coronary bypass surgery
HL = hyperlipidemia
DM2 = diabetes mellitus type 2

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

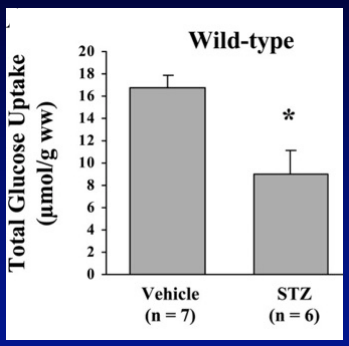
Armoni … Karnieli **2005** *J Biol Chem* 280(41):34786

Glucose Utilization and Rodent Models of Type 1 Diabetes

Protein
Diabetic Mouse Heart

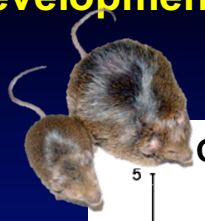


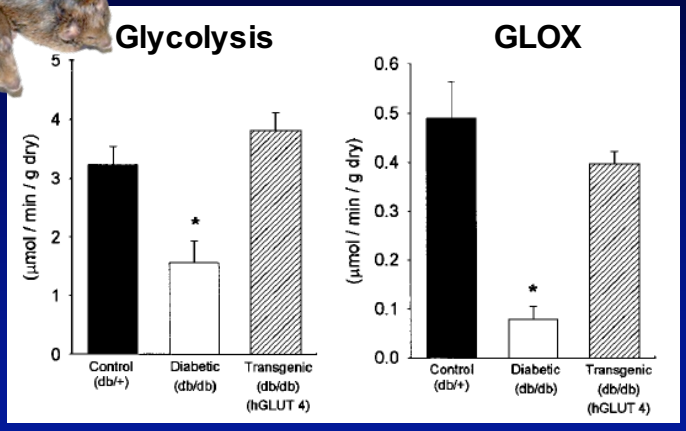
Glucose Uptake
Diabetic Mouse Heart



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

Constitutive GLUT4 Expression Prevents Development of Glucose Utilization Defects

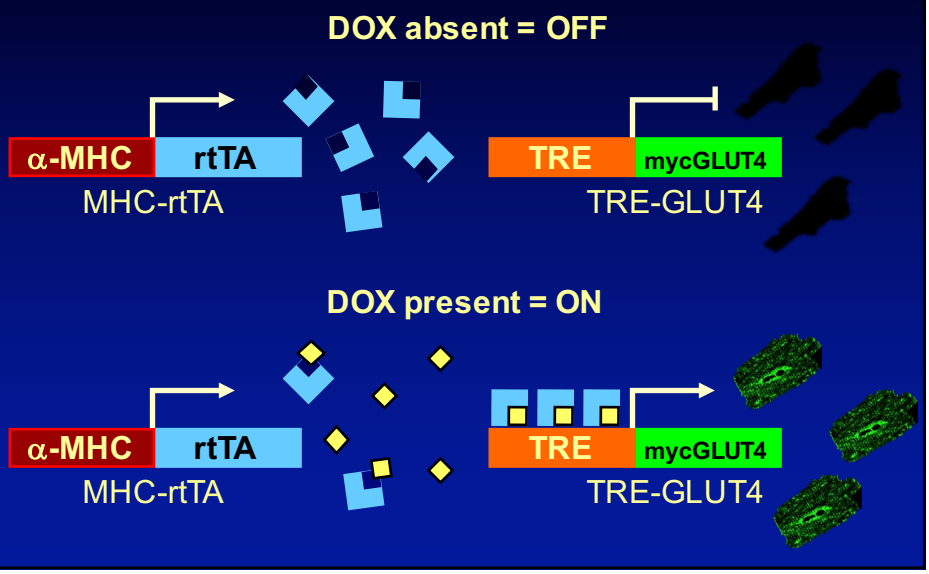




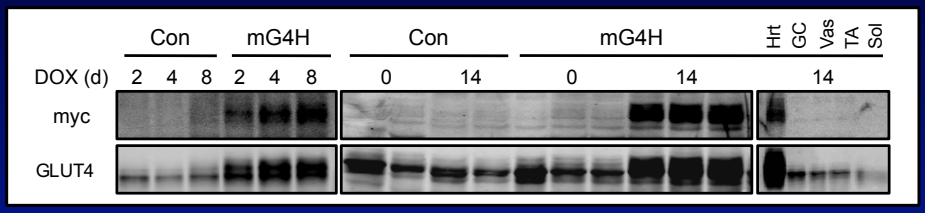
Belke … Severson **2000** *Am J Physiol* 279:E1104

Question: Is the change in cardiac metabolic substrate flexibility adaptive or maladaptive?

Inducible Cardiomyocyte-Specific GLUT4 Expression (mG4H)



mG4H Mice Exhibit Inducible Cardiac-Specific Expression of GLUT4



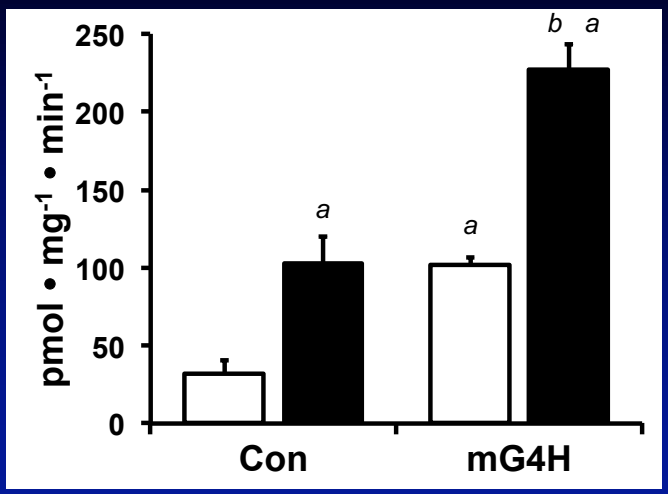
Hrt = Heart
GC = Gastrocnemius
Vas = Vastus lateralis
TA = Tibialis anterior
Sol = Soleus

5-fold

5-fold Heart

GLUT4 Induction Increases Basal and Insulin-Stimulated Glucose Uptake

Cardiac Myocytes 2-DG Uptake

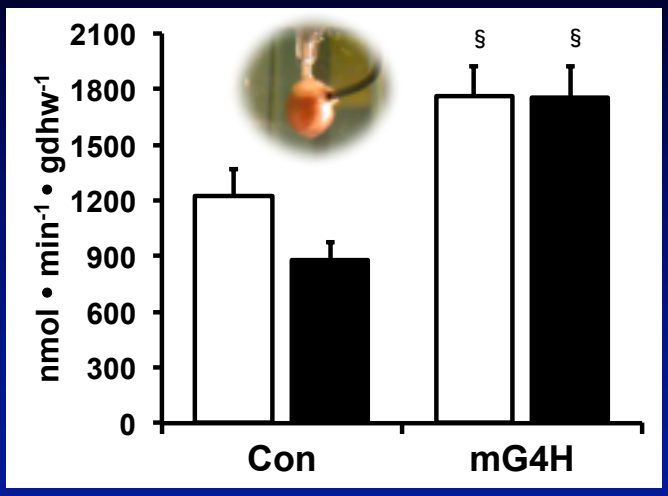


n = 3 – 4
[a] P < 0.01 vs. Con-Basal
[b] P < 0.001 vs. All

Renata O. Pereira
Wende … Abel *in prep*

GLUT4 Induction Increases Glycolysis and Rescues Diabetic Cardiac Glycolytic Defects

Isolated Working Hearts Glycolysis



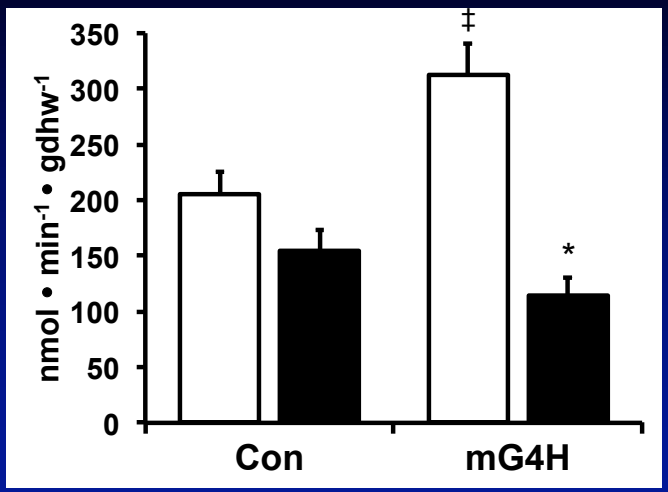
□ Vehicle
■ STZ

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)

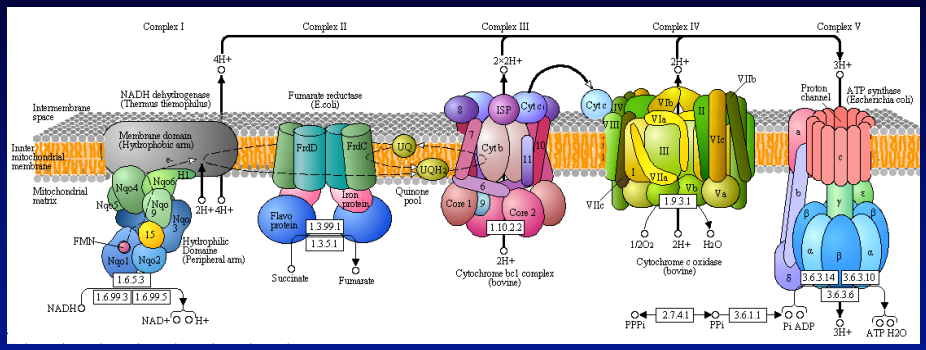


□ Vehicle
■ STZ

n = 6 – 10
‡ P < 0.001 vs. All
* P < 0.01 vs. Veh

Joseph Tuinei
Wende … Abel *in prep*

Oxidative Phosphorylation

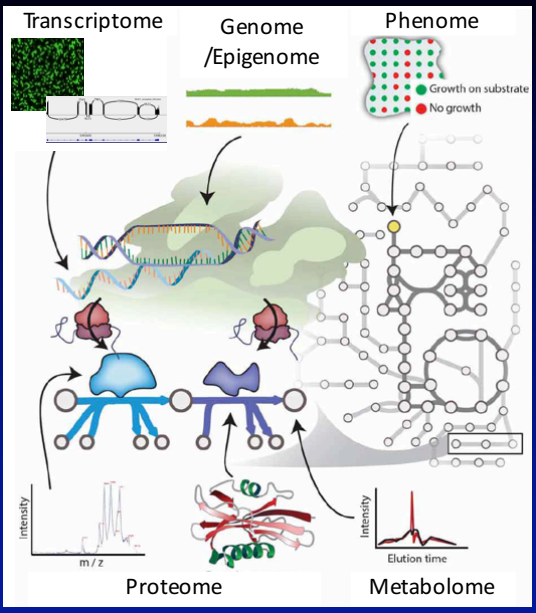


www.genome.jp/kegg/pathway.html

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.

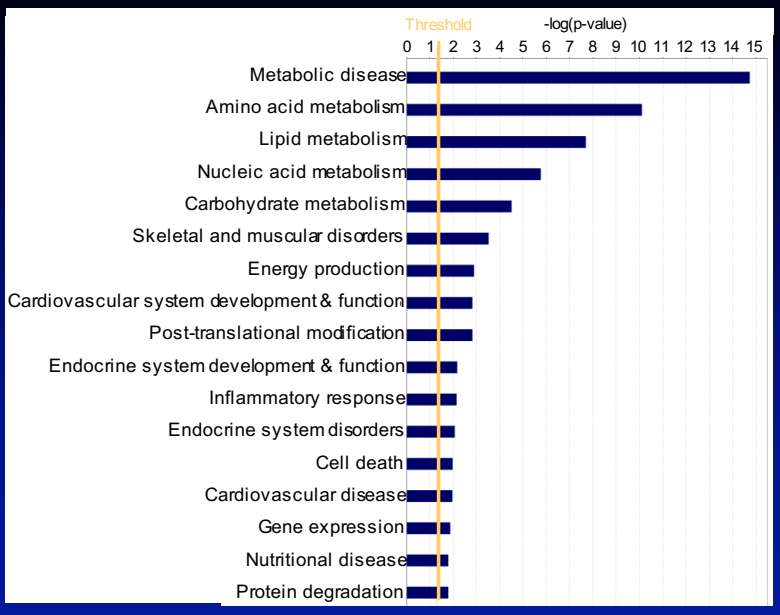
Proteome
Mass spec, western blot, Co-IP, IHC, PTMs, etc.

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

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

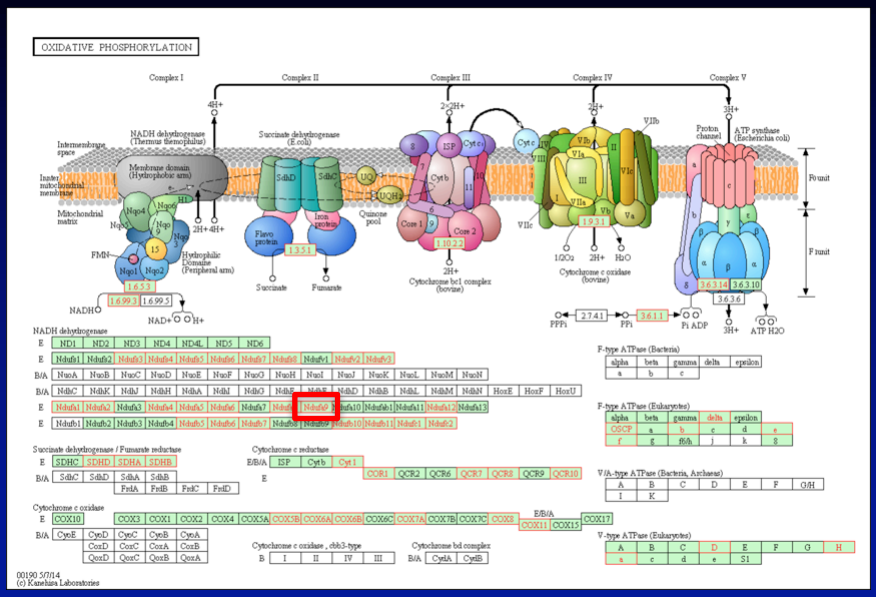
Adapted from Lewis and Abdel-Haleem **2013** *Front Physiol* 4:237

Pathway Analysis of Microarray



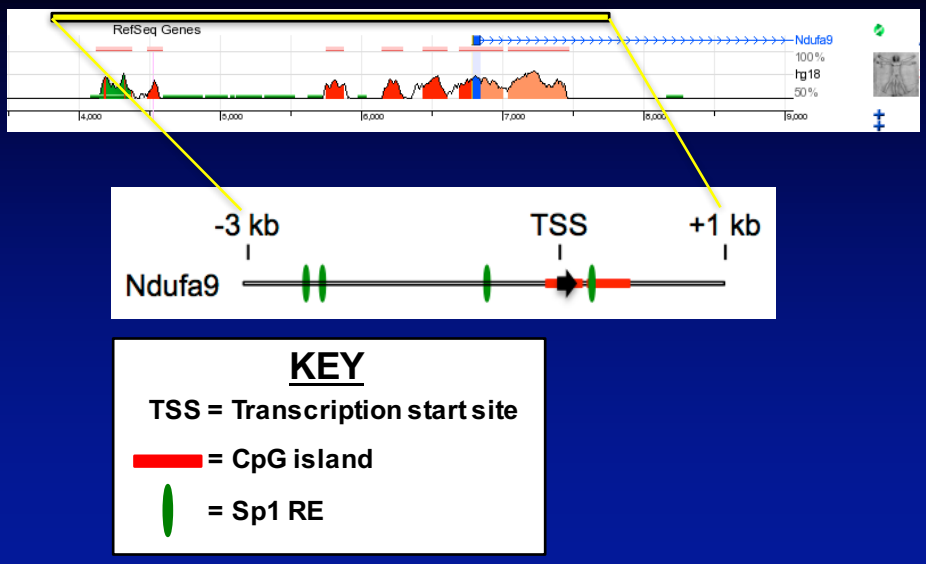
Wende … Abel *in prep*

Oxidative Phosphorylation



GeneSifter using KEGG

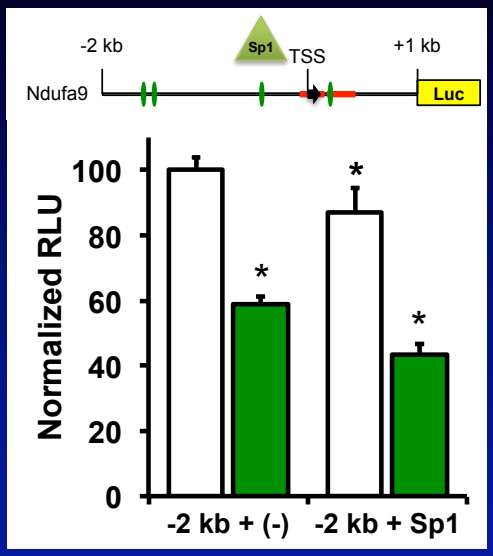
Ndufa9 Gene Promoter Structure



http://ecrbrowser.dcode.org

Ndufa9 Gene Promoter Mapping

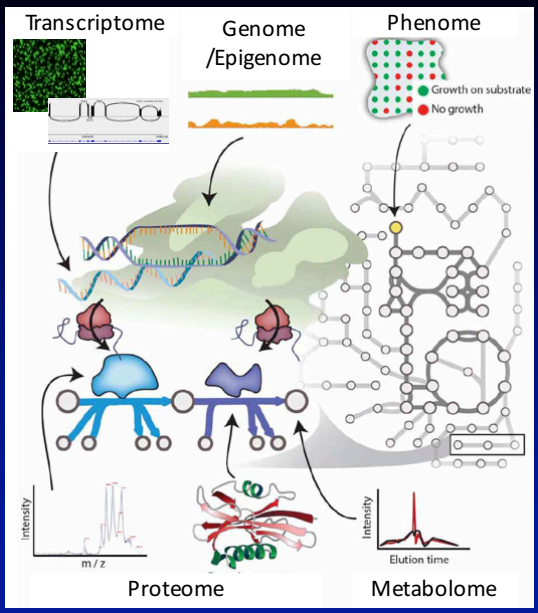
Transient Transfection Promoter Activity



C_2C_{12} Myotubes
n = 9
* P < 0.05

Wende … Abel *in prep*

Systems Biology



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

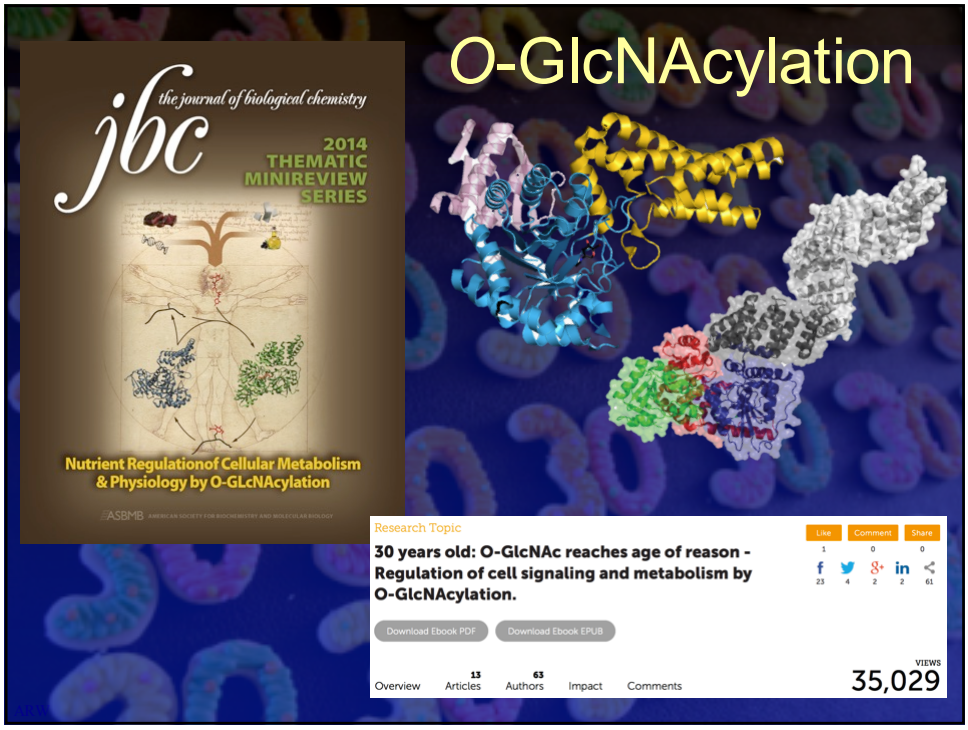
Transcriptome
Northerns, qPCR, microarray RNA-seq, miR, lncRNA, etc.

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

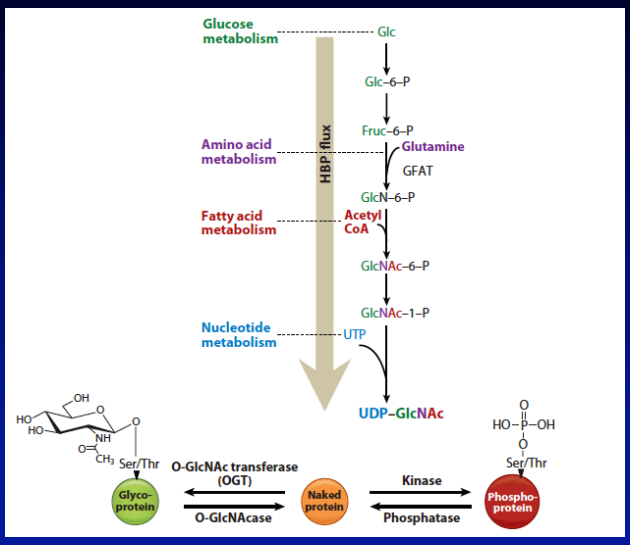
Metabolome
Glucometer, ELISA, GC-MS, HPLC, NMR, fluxomics, etc.

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

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

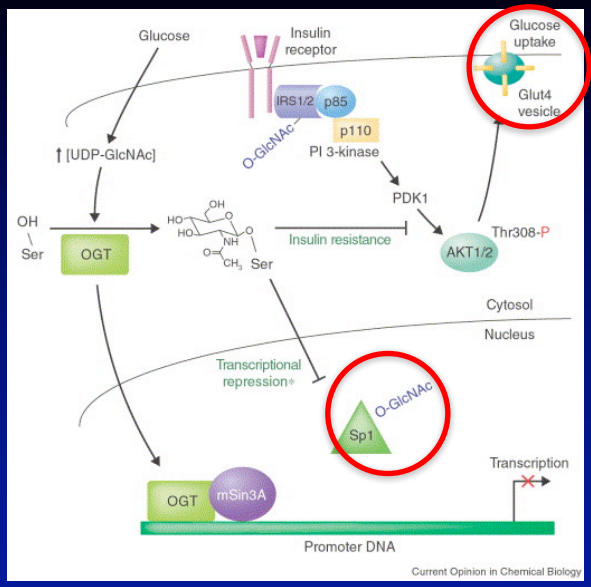


Metabolic Integration: Protein *O*-GlcNAcylation



Hart … Lagerlof **2011** *Annu Rev Biochem* 80:825

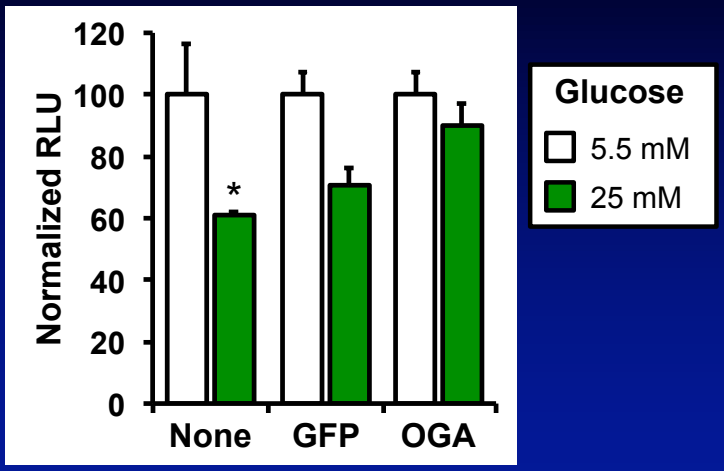
GlcNAc Regulation of Sp1



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

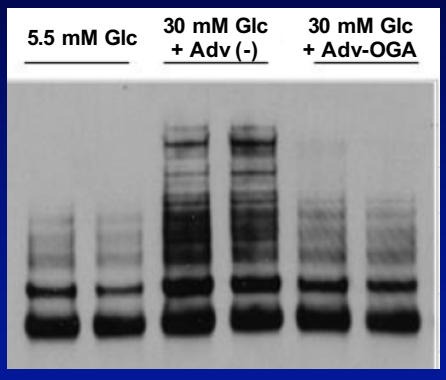
Li Wang
Wende … Abel *in prep*

Conclusion – Part 2

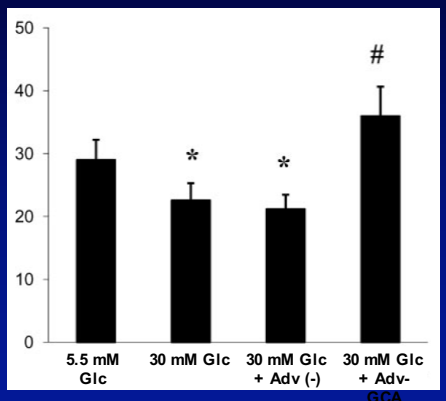
Enhanced glucose delivery regulates oxidative capacity via transcriptional mechanisms including GlcNAcylation of transcription factors.

Mitochondrial Protein *O*-GlcNAcylation and Neonatal Cardiomyocyte Metabolic Function

Mitochondrial Protein *O*-GlcNAcylation



Complex I Activity

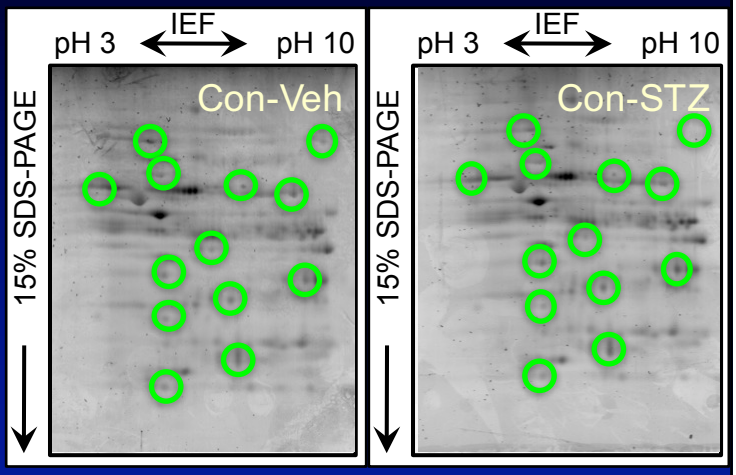


O-GlcNAcylation of NDUFA9

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

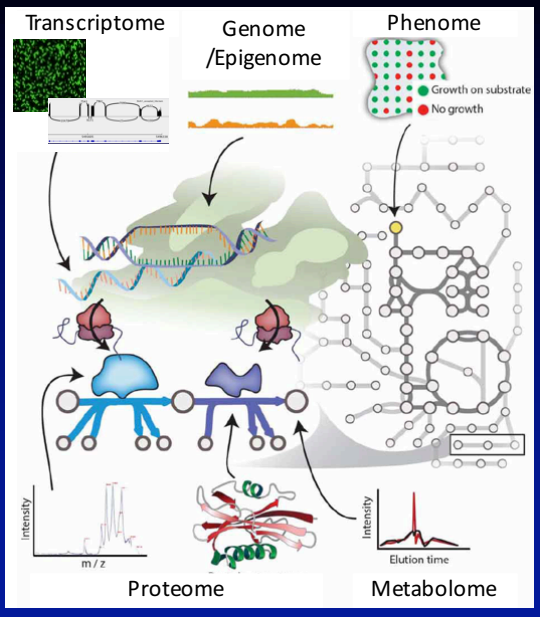
GLUT4 Induction Alters the Cardiac Mitochondrial Glycoproteome

Isolated Mitochondria
2D-PAGE
Pro-Q Emerald



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.

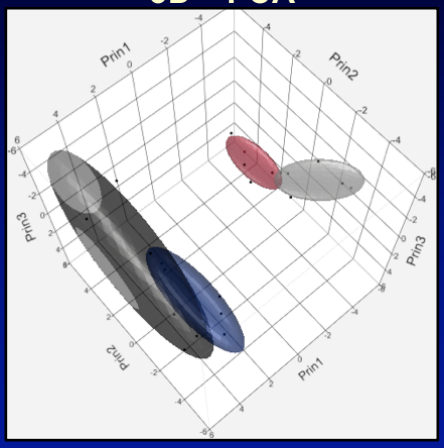
Genome / Epigenome
Southerns, sequencing, GenBank, ENCODE, ChIP-seq, bsDNA-seq, etc.

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

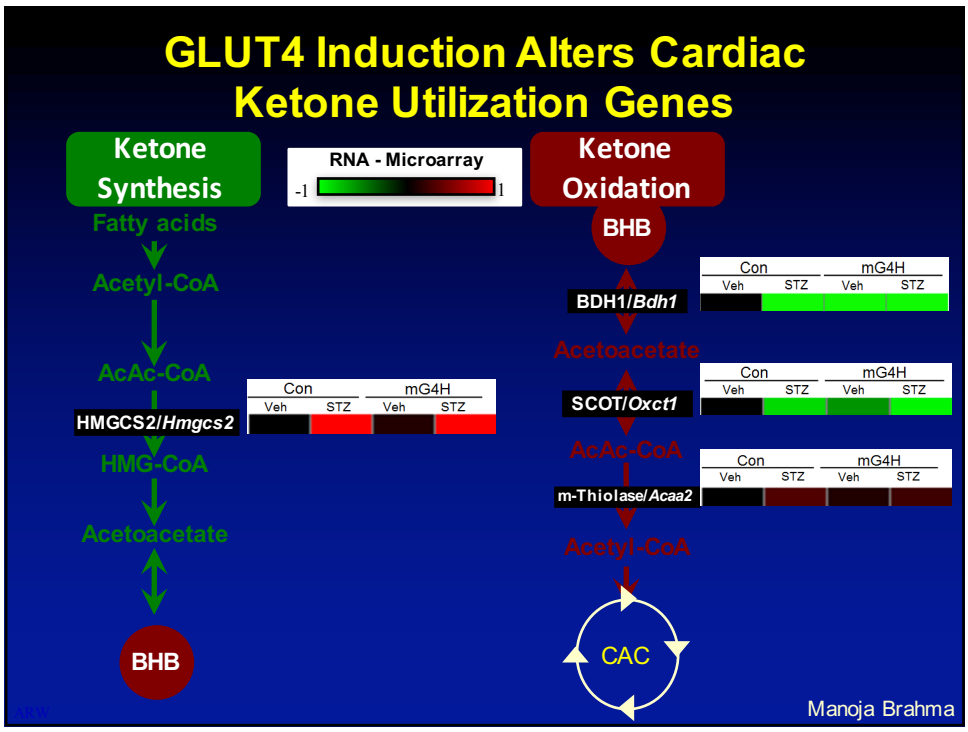
Studies on Myocardial Metabolism*

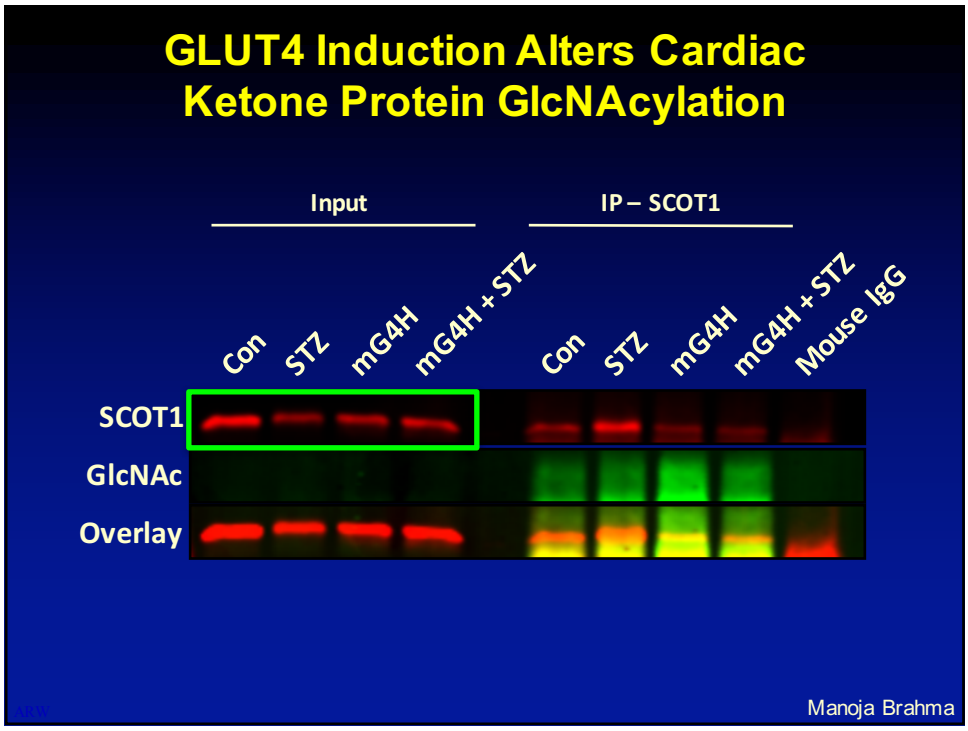
IV. Myocardial Metabolism in Diabetes

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

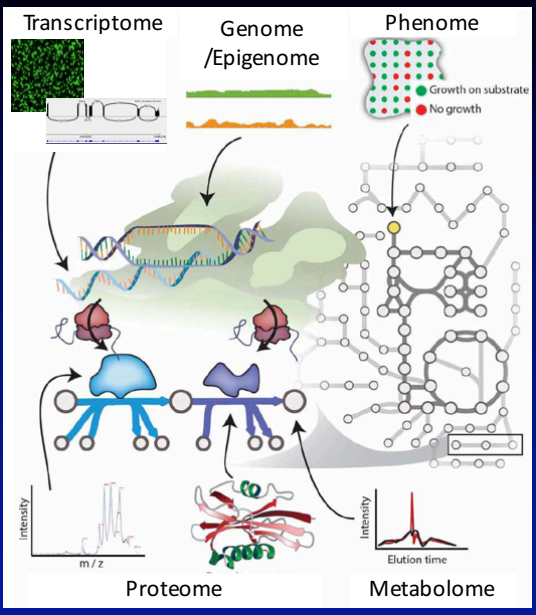

GLUT4 Induction Alters Cardiac Ketone Utilization Genes
Ketone Synthesis
RNA - Microarray
-1
1
Ketone Oxidation
Fatty acids
Acetyl-CoA
AcAc-CoA
HMGCS2/Hmgcs2
HMG-CoA
Acetoacetate
BHB
Con
mG4H
Veh
STZ
BHB
BDH1/Bdh1
Acetoacetate
SCOT/Oxct1
AcAc-CoA
m-Thiolase/Acaa2
Acetyl-CoA
CAC
Manoja Brahma


GLUT4 Induction Alters Cardiac Ketone Protein GlcNAcylation
Input
IP – SCOT1
Con
STZ
mG4H
mG4H + STZ
Con
STZ
mG4H
mG4H + STZ
Mouse IgG
SCOT1
GlcNAc
Overlay
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.

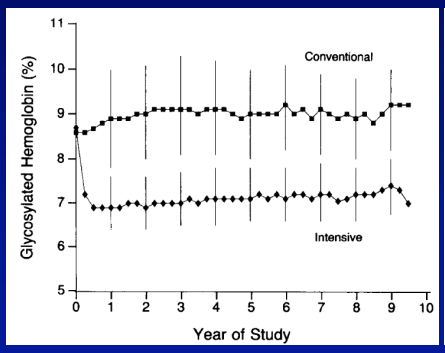
Adapted from Lewis and Abdel-Haleem **2013** *Front Physiol* 4:237

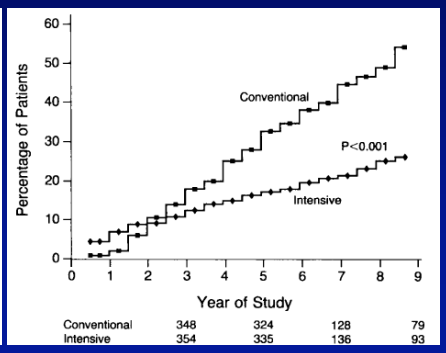
Epigenetics - Programming
DCCT: Diabetes Control and Complications Trial

The New England
Journal of Medicine
©Copyright, 1993, by the Massachusetts Medical Society
Volume 329 SEPTEMBER 30, 1993 Number 14

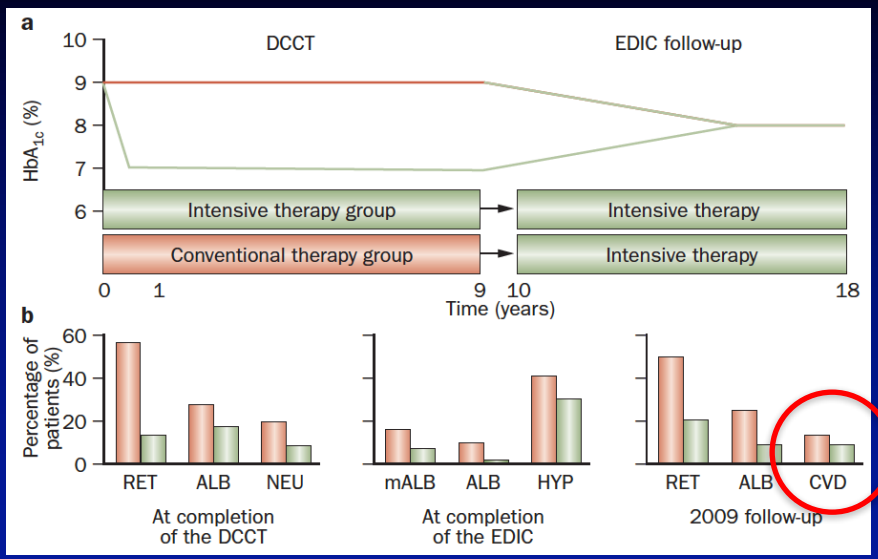
THE EFFECT OF INTENSIVE TREATMENT OF DIABETES ON THE DEVELOPMENT AND PROGRESSION OF LONG-TERM COMPLICATIONS IN INSULIN-DEPENDENT DIABETES MELLITUS

The Diabetes Control and Complications Trial Research Group*



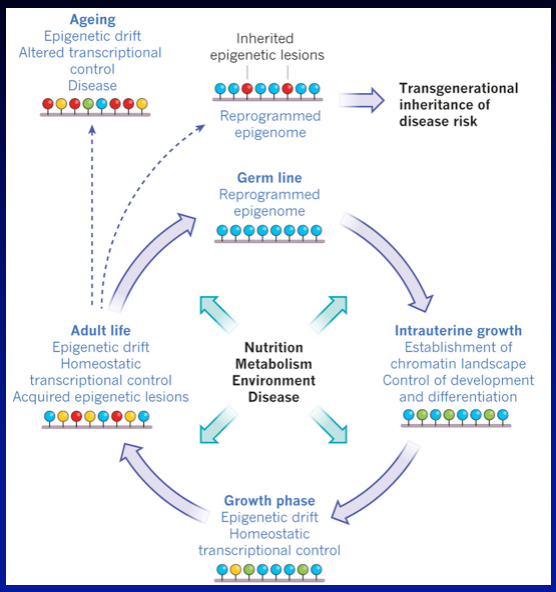


Epigenetics - Memory
EDIC: Epidemiology of Diabetes Interventions Trial



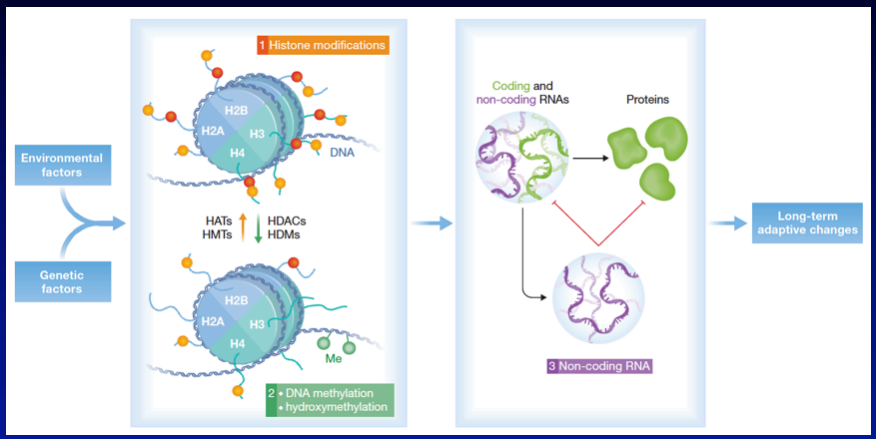
Pirola … El-Osta **2010** *Nat Rev Endocrinol* 6(12):665

Epigenetics: Transgenerational and Drift



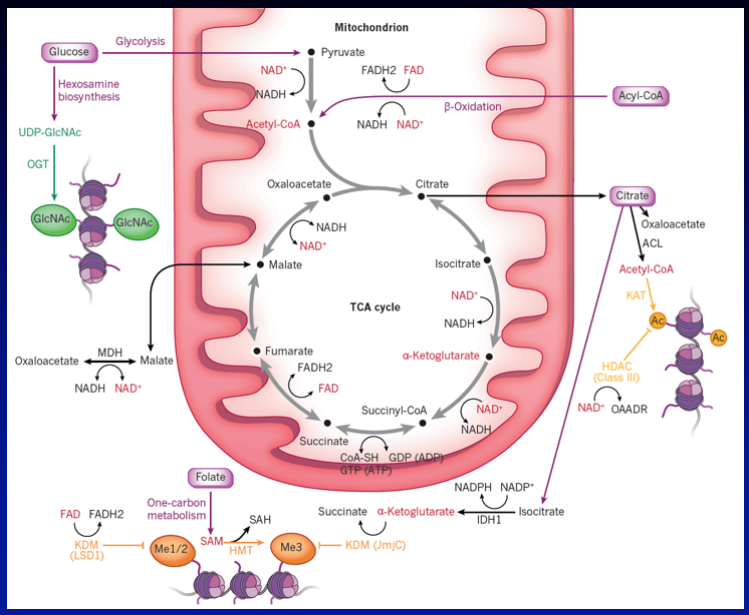
Gut and Verdin **2013** *Nature* 502:489

Epigenetic Code



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

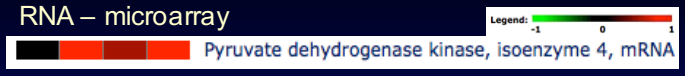
Metabolite Signaling to Chromatin



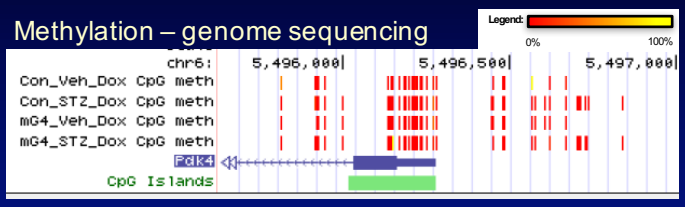
Gut and Verdin **2013** *Nature* 502:489

Methylation and Expression

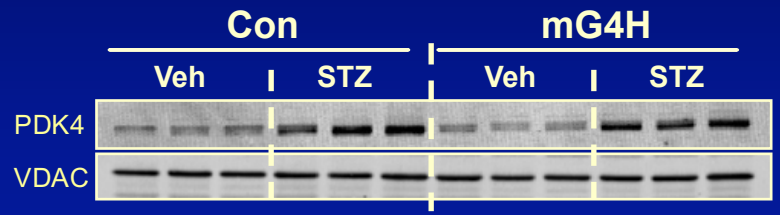
RNA – microarray



Methylation – genome sequencing



Protein – western blot



GeneSifter and Zymo/UCSC Genome Browser

Other Human/Mouse Comparisons

Genetics Of Lipid Lowering Drugs And Diet Network

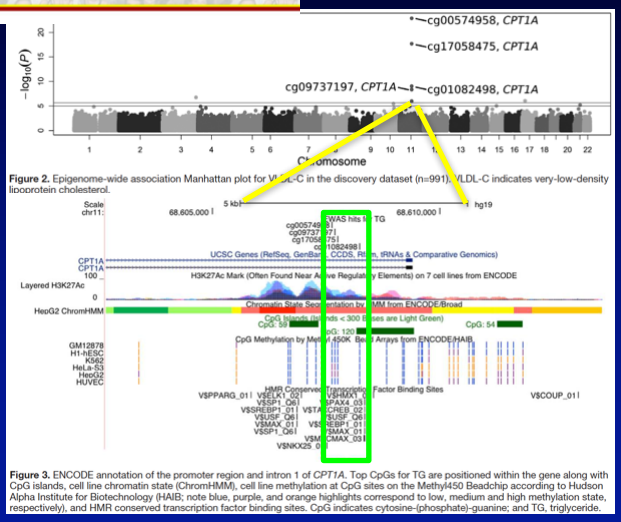


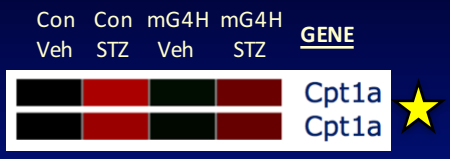
Figure 2. Epigenome-wide association Manhattan plot for VLDL-C in the discovery dataset (n=991). VLDL-C indicates very-low-density lipoprotein cholesterol.

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.

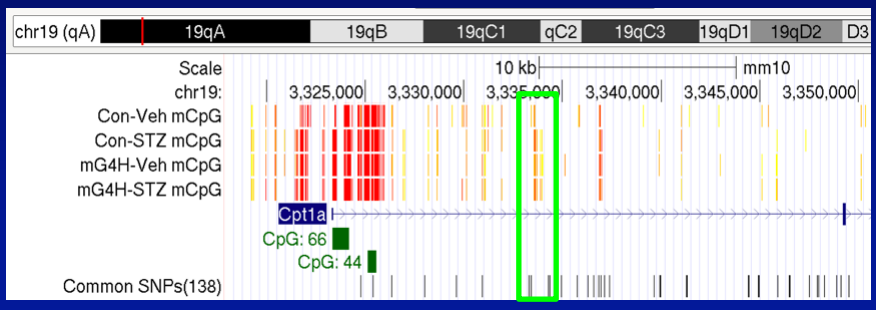
Irvin … Arnett **2014** *Circulation* 130:565

Other Human/Mouse Comparisons

Mouse Gene Expression

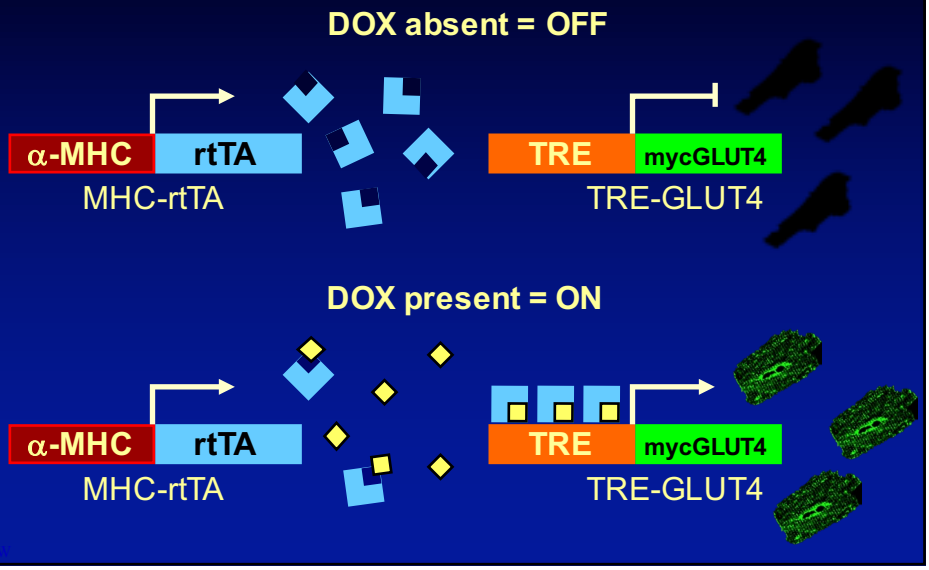


Mouse DNA Methylation

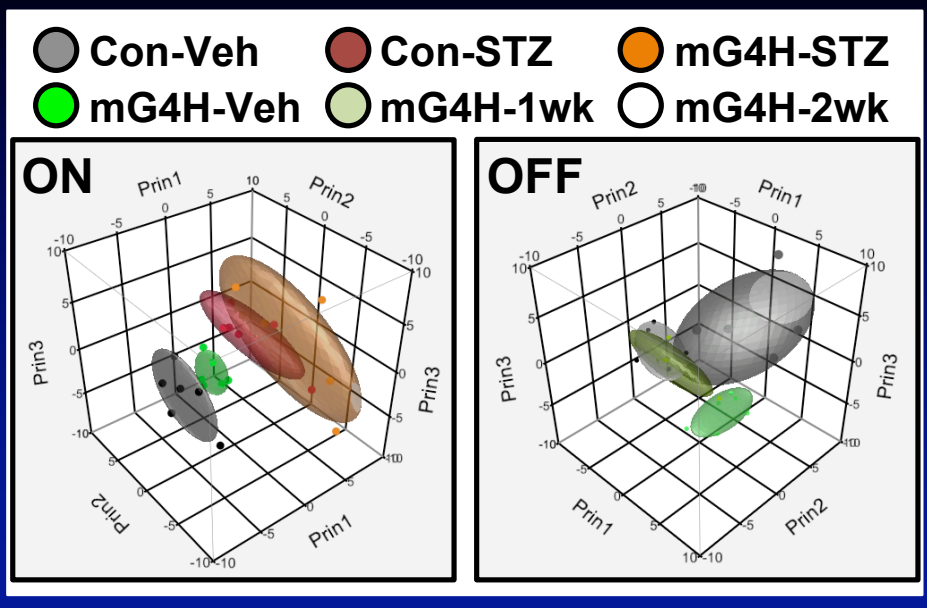


Wende, unpublished

Where Does Glycemic Memory Fit In?



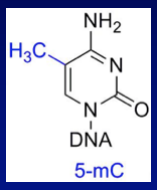
Metabolomics

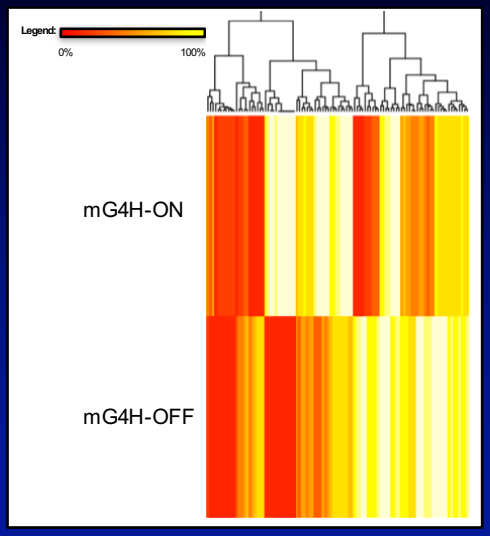


Wende, unpublished

Glucose Cycling Alters Epigenetic Programming

Genomewide bsDNA-seq 5-mCpG

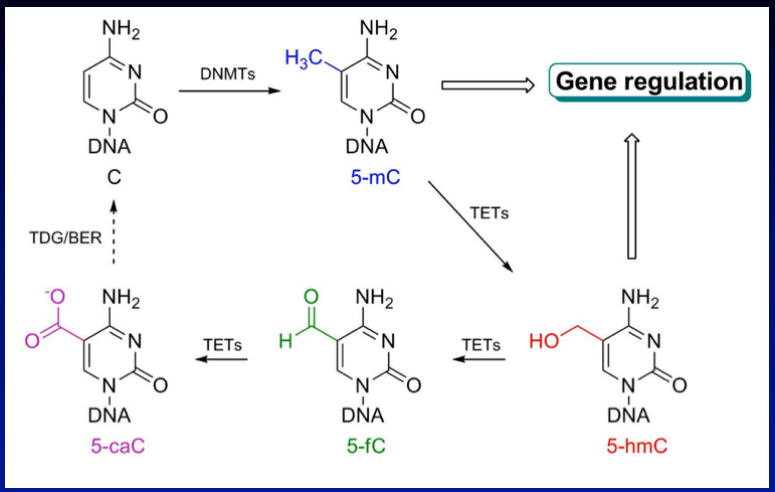




Heart, LV

Zymo Research
Wende, unpublished

Background

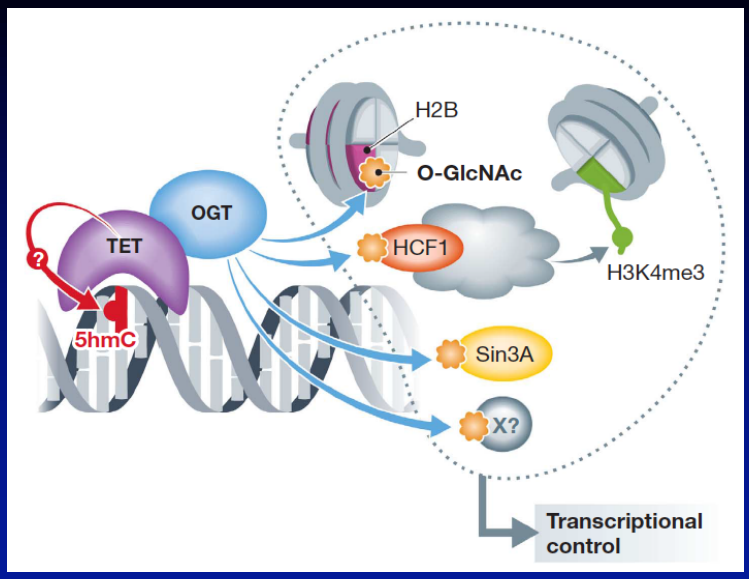


5-hmC

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.

Overall Summary

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

APW

Acknowledgements

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 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

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

American Heart Association.
AHA 0725064Y

JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.
JDRF 51002608