My Sweetheart is Broken: Role of Glucose in Diabetic Complications

Saturday, October 15th, 2016

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NIDDK Pilot Award

DiaComp

www.diacomp.org

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NIH National Heart, Lung, and Blood Institute

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Conflict of Interest Disclosure

- **Grants:**

  JDRF 10-2006-261

  NIH NHLBI R00 HL111322

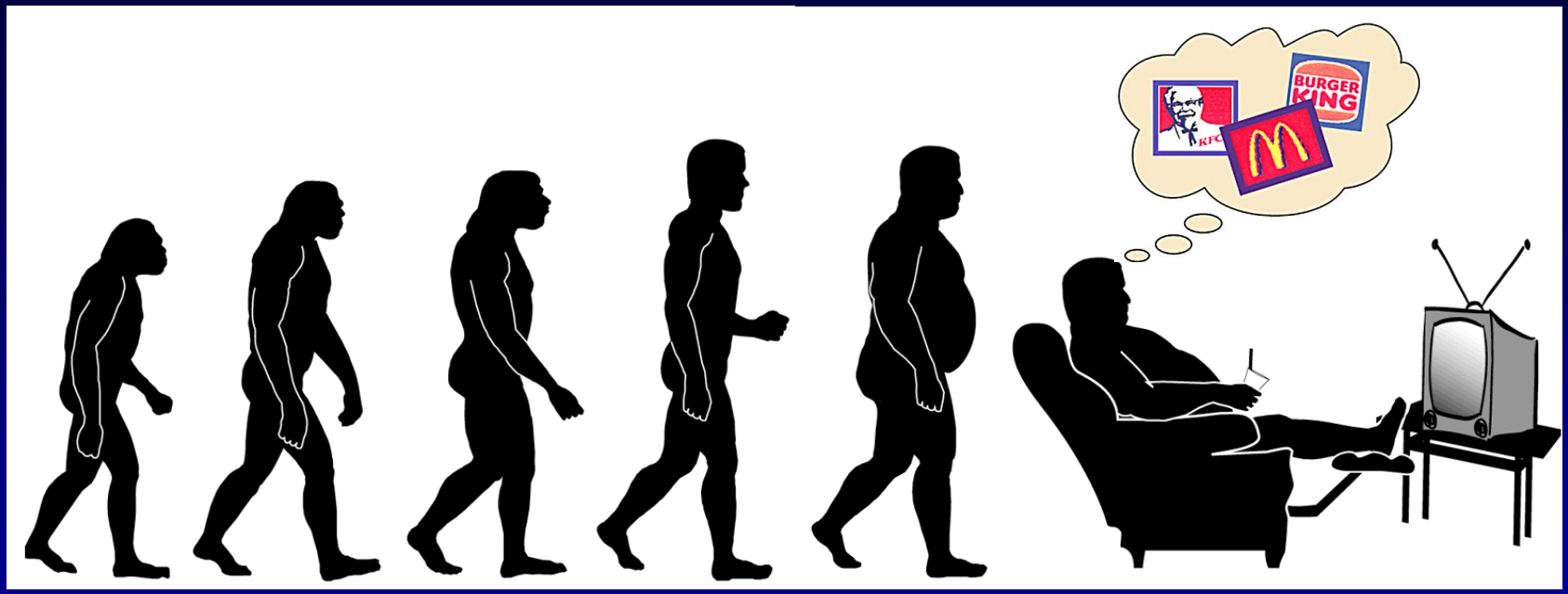
  NIH NHLBI R56 HL133011

  NIH NIDDK U24 DK076169

Outline

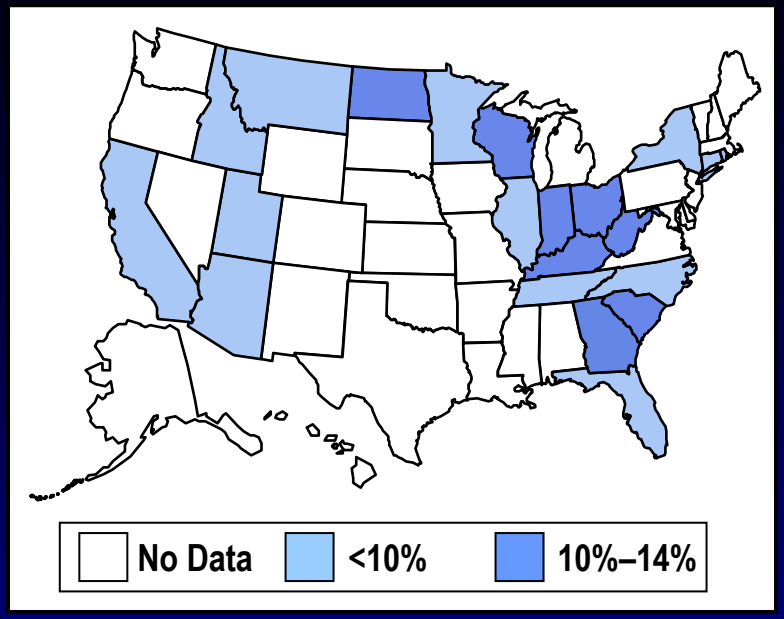
- Define the question and model to determine the connection between diabetes and heart disease.
- Identify the molecular mechanisms by which glucose directly alters molecular function.
- Determine cross species comparisons and additional mechanisms.

Metabolic Origins of Disease



From: Roger Unger - UTSW

1985 – Obesity



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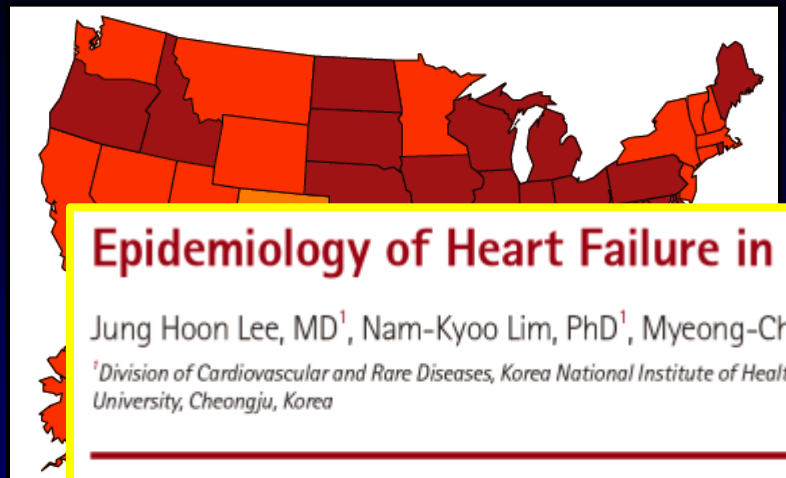
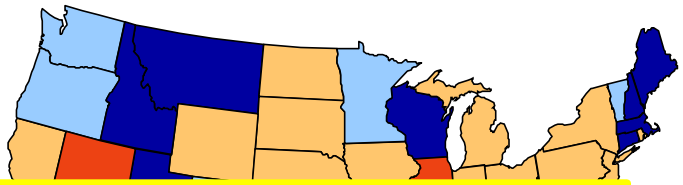
www.cdc.gov/diabetes/statistics and www.cdc.gov/mmwr

2010 – Obesity



2010 – Physical Inactivity



Epidemiology of Heart Failure in Korea: Present and Future

Jung Hoon Lee, MD[1], Nam-Kyoo Lim, PhD[1], Myeong-Chan Cho, MD[2], and Hyun-Young Park, MD[1]

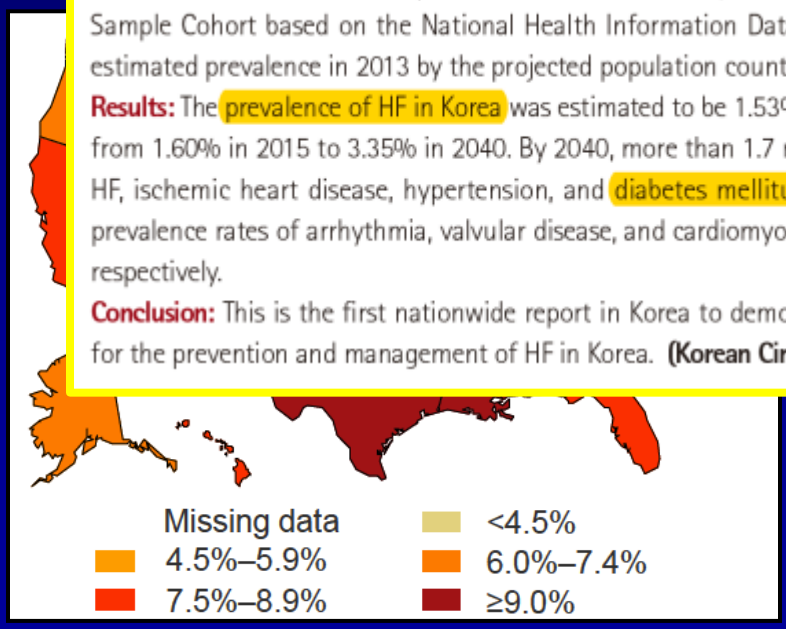
[1]Division of Cardiovascular and Rare Diseases, Korea National Institute of Health, Cheongju, [2]Department of Internal Medicine, College of Medicine, Chungbuk National University, Cheongju, Korea

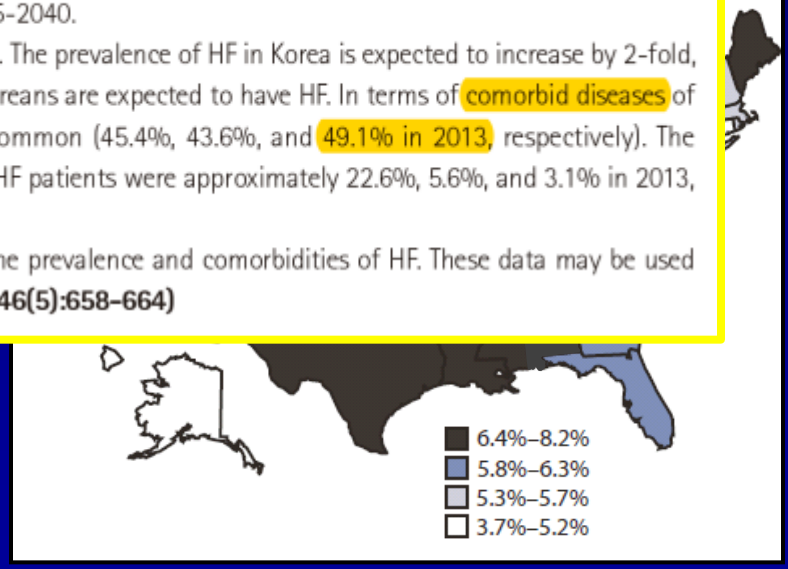
Background and Objectives: Heart failure (HF) is an important healthcare issue because of its high mortality, morbidity, and healthcare costs. The number of HF patients is increasing worldwide as a consequence of aging of the population. However, there are limited studies on the prevalence of HF in Korea. This study aimed to estimate the prevalence of HF, its comorbidities, and the projected population with HF in the future.

Materials and Methods: The prevalence and comorbidity estimates of HF were determined using data from the 2002-2013 National Sample Cohort based on the National Health Information Database. We calculated the projected prevalence of HF by multiplying the estimated prevalence in 2013 by the projected population counts for 2015-2040.

Results: The prevalence of HF in Korea was estimated to be 1.53% in 2013. The prevalence of HF in Korea is expected to increase by 2-fold, from 1.60% in 2015 to 3.35% in 2040. By 2040, more than 1.7 million Koreans are expected to have HF. In terms of comorbid diseases of HF, ischemic heart disease, hypertension, and diabetes mellitus were common (45.4%, 43.6%, and 49.1% in 2013, respectively). The prevalence rates of arrhythmia, valvular disease, and cardiomyopathy in HF patients were approximately 22.6%, 5.6%, and 3.1% in 2013, respectively.

Conclusion: This is the first nationwide report in Korea to demonstrate the prevalence and comorbidities of HF. These data may be used for the prevention and management of HF in Korea. **(Korean Circ J 2016;46(5):658-664)**





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

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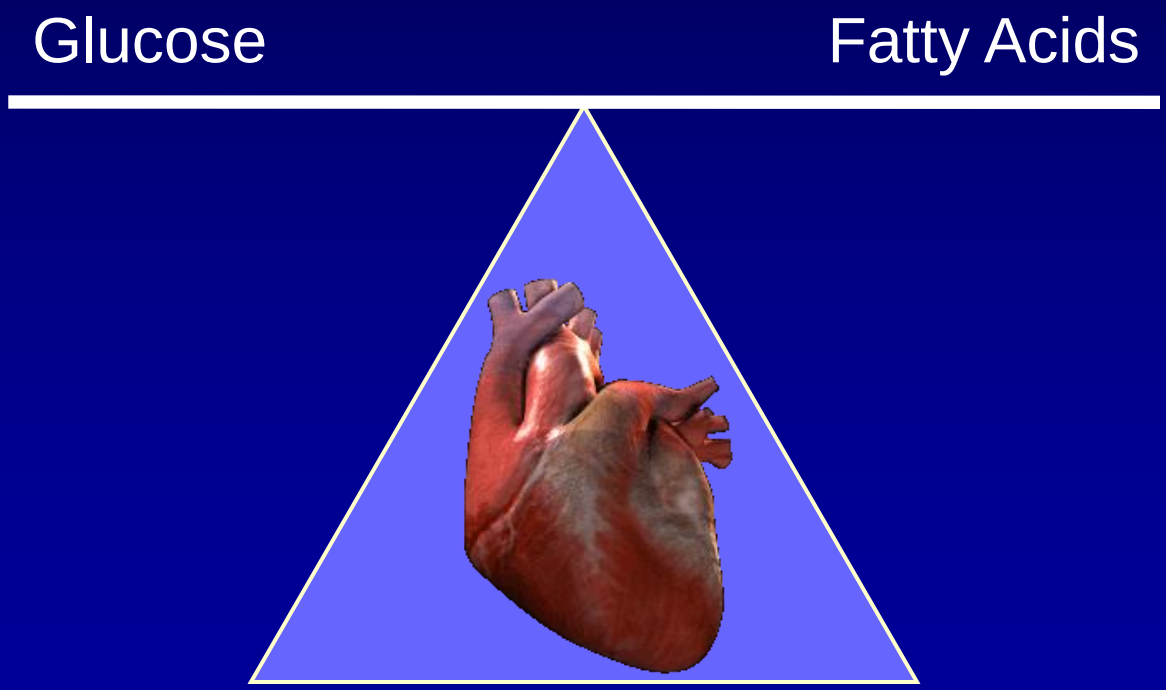
Maintaining Cardiac Function Through Metabolic Substrate Balance



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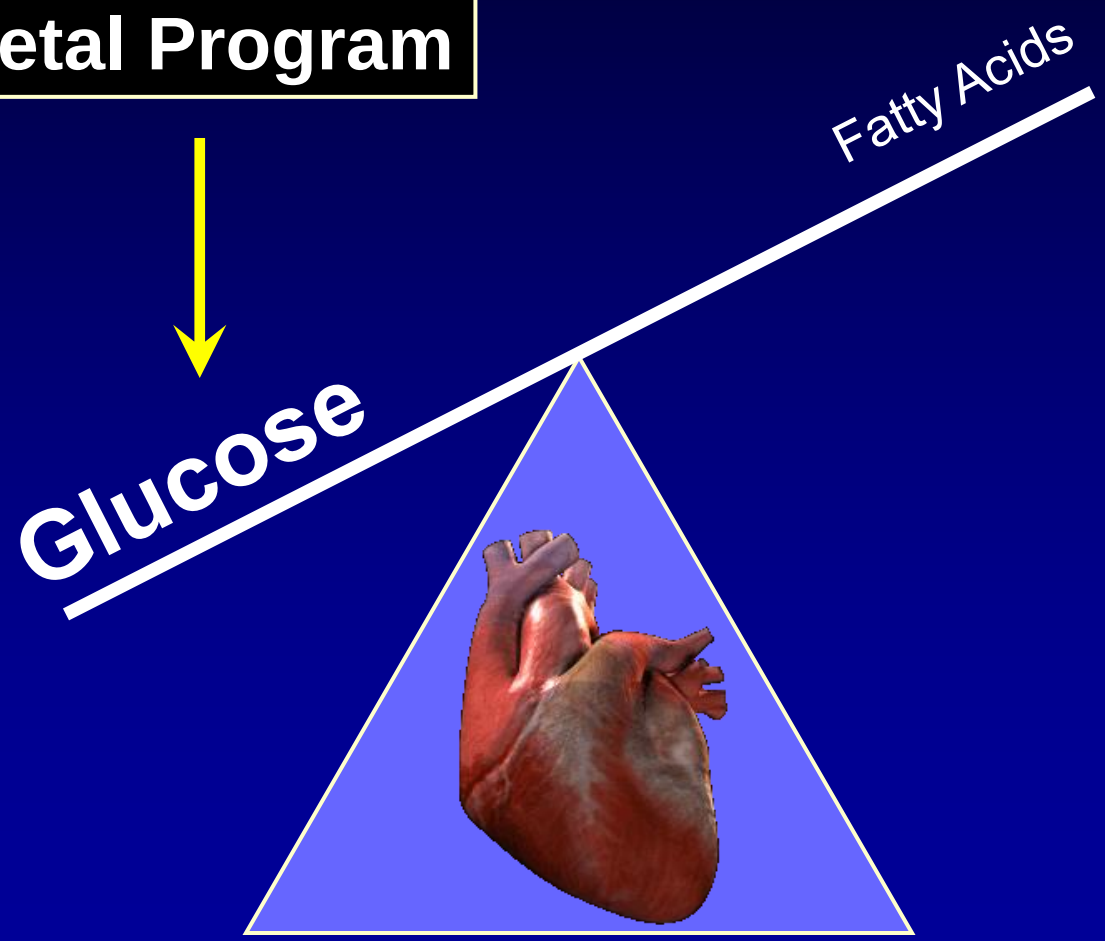
Maintaining Cardiac Function Through Metabolic Substrate Balance

Fetal Program



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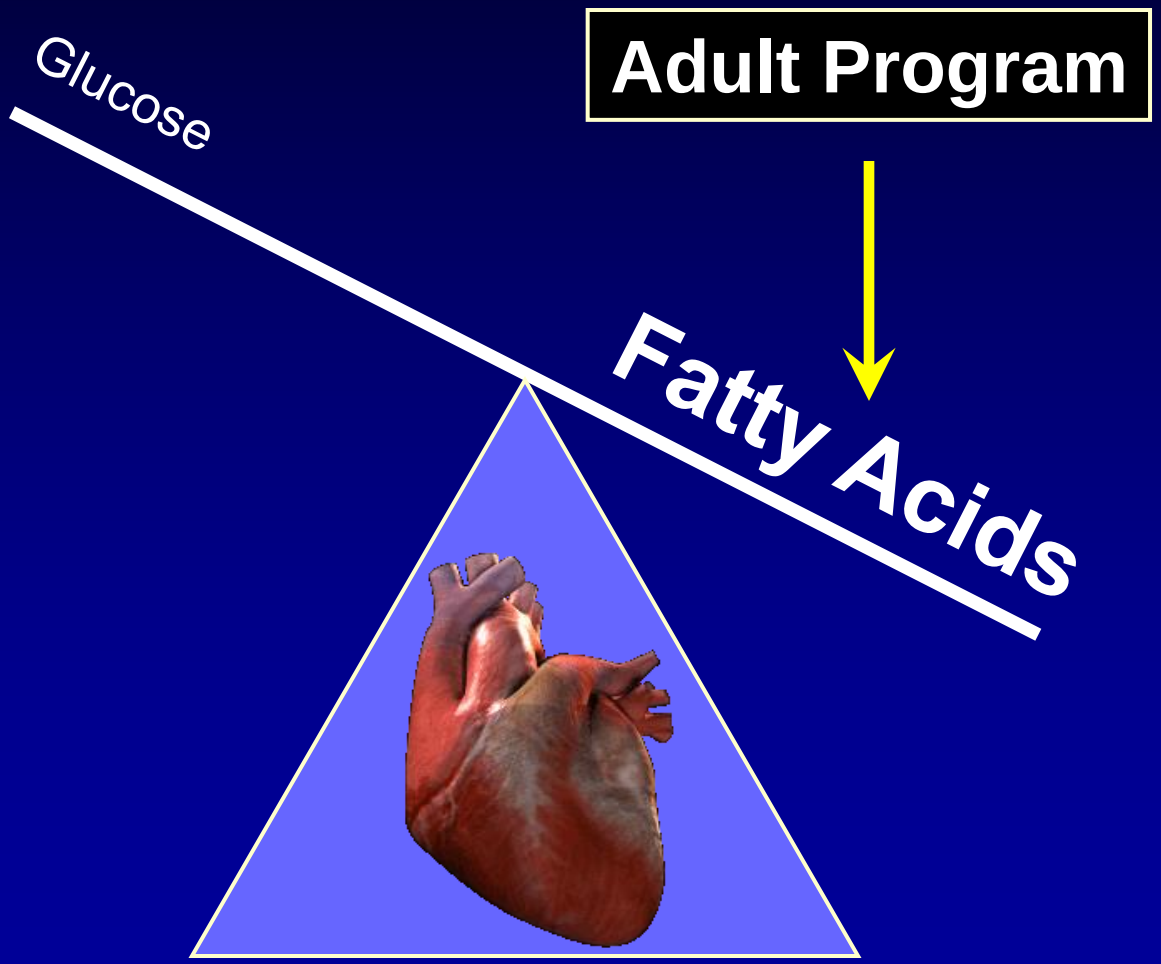
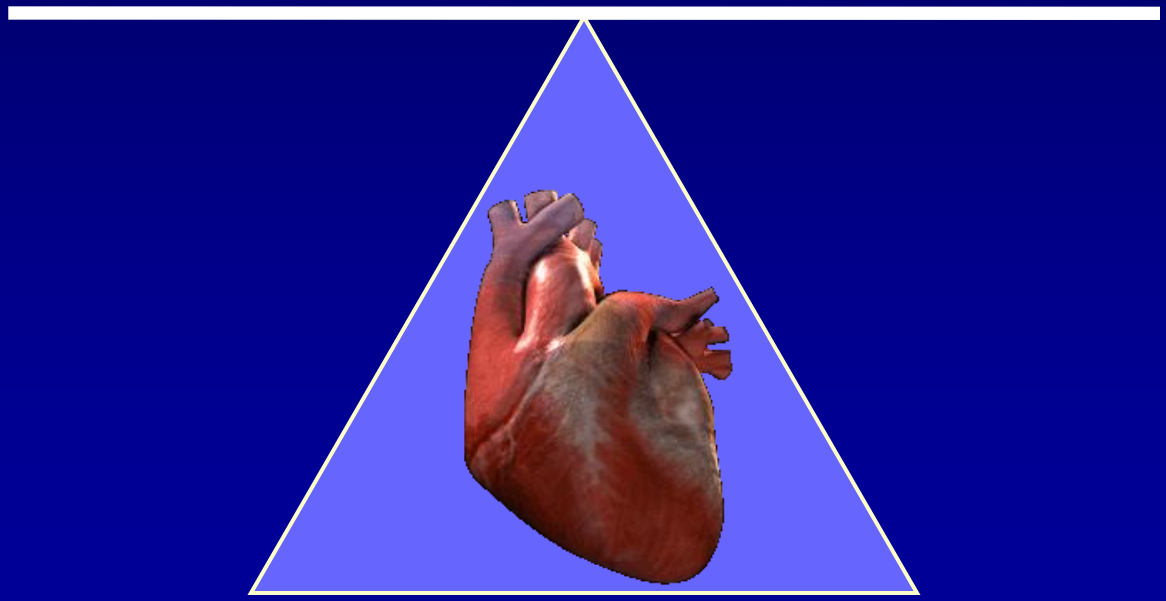
Maintaining Cardiac Function Through Metabolic Substrate Balance



Maintaining Cardiac Function Through Metabolic Substrate Balance

Diabetes



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Maintaining Cardiac Function Through Metabolic Substrate Balance

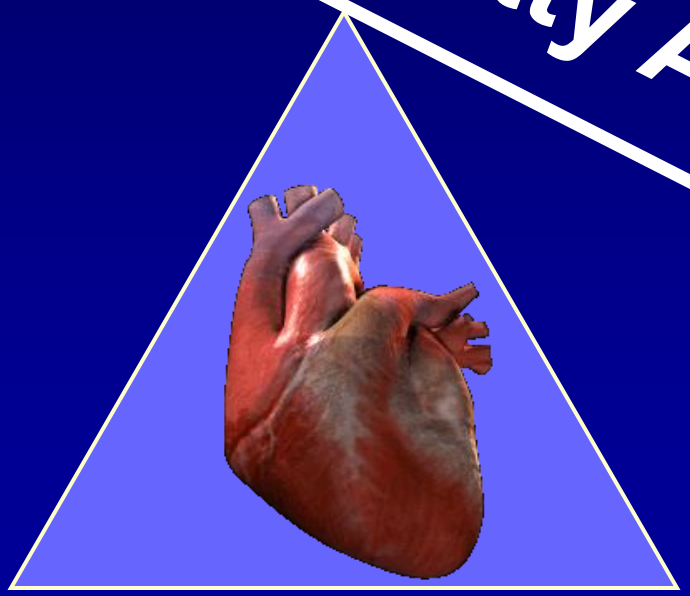
Glucose

Diabetes

Fatty Acids

Fatty Acids

Glucose



Glucose

Fatty Acids

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Maintaining Cardiac Function Through Metabolic Substrate Balance

Glucose

Diabetes

Fatty Acids

Fatty Acids

Glucose

Glucose

Fatty Acids

ARW

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Studies on Myocardial Metabolism*

IV. Myocardial Metabolism in Diabetes

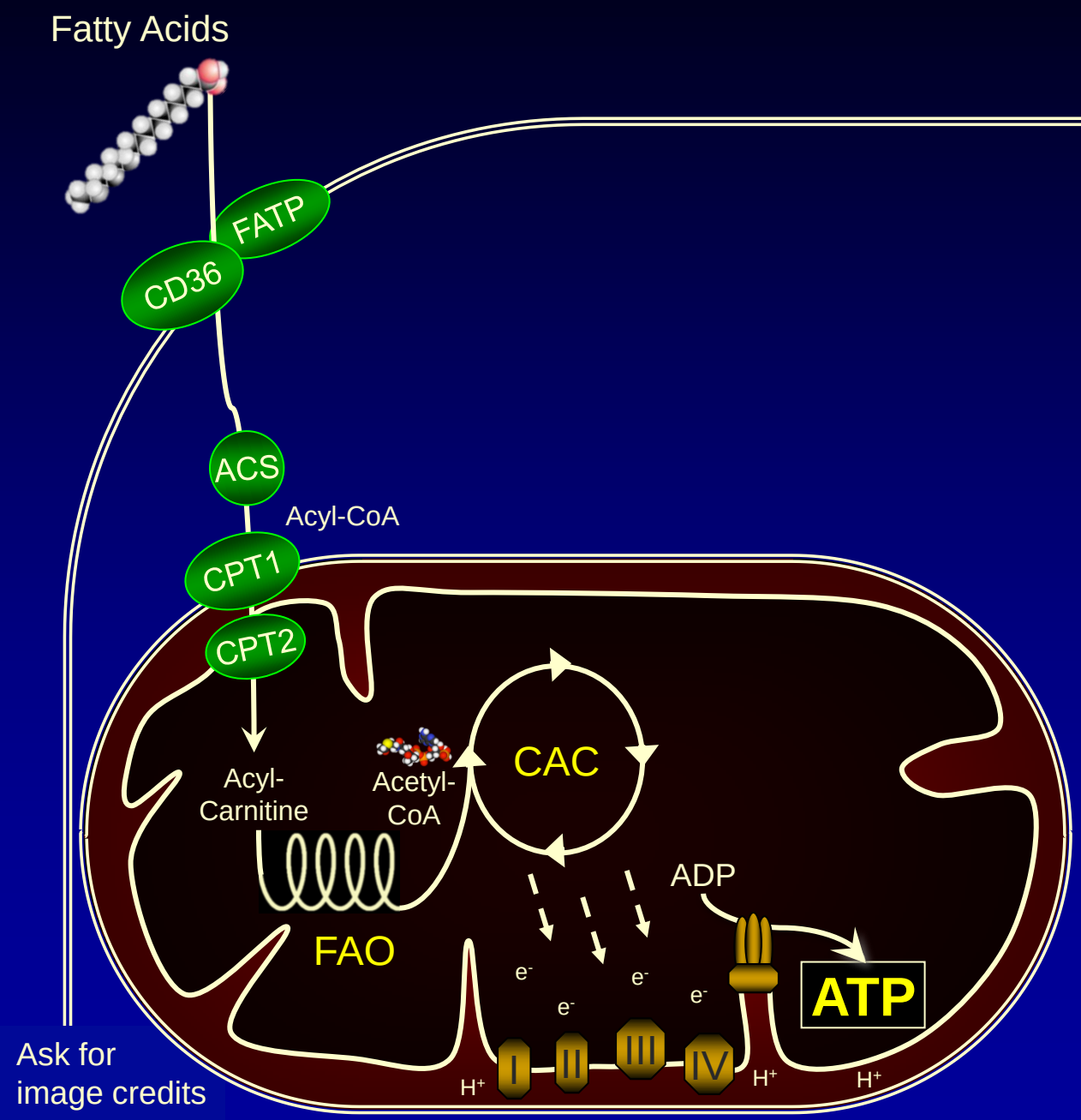
I. Ungar, M.D., M. Gilbert, M.D., A. Siegel, M.S., J. M. Blain, M.D. *and* R. J. Bing, M.D.

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

Wende and Abel **2010** *BBA 1801:311*

Metabolic Substrate Utilization



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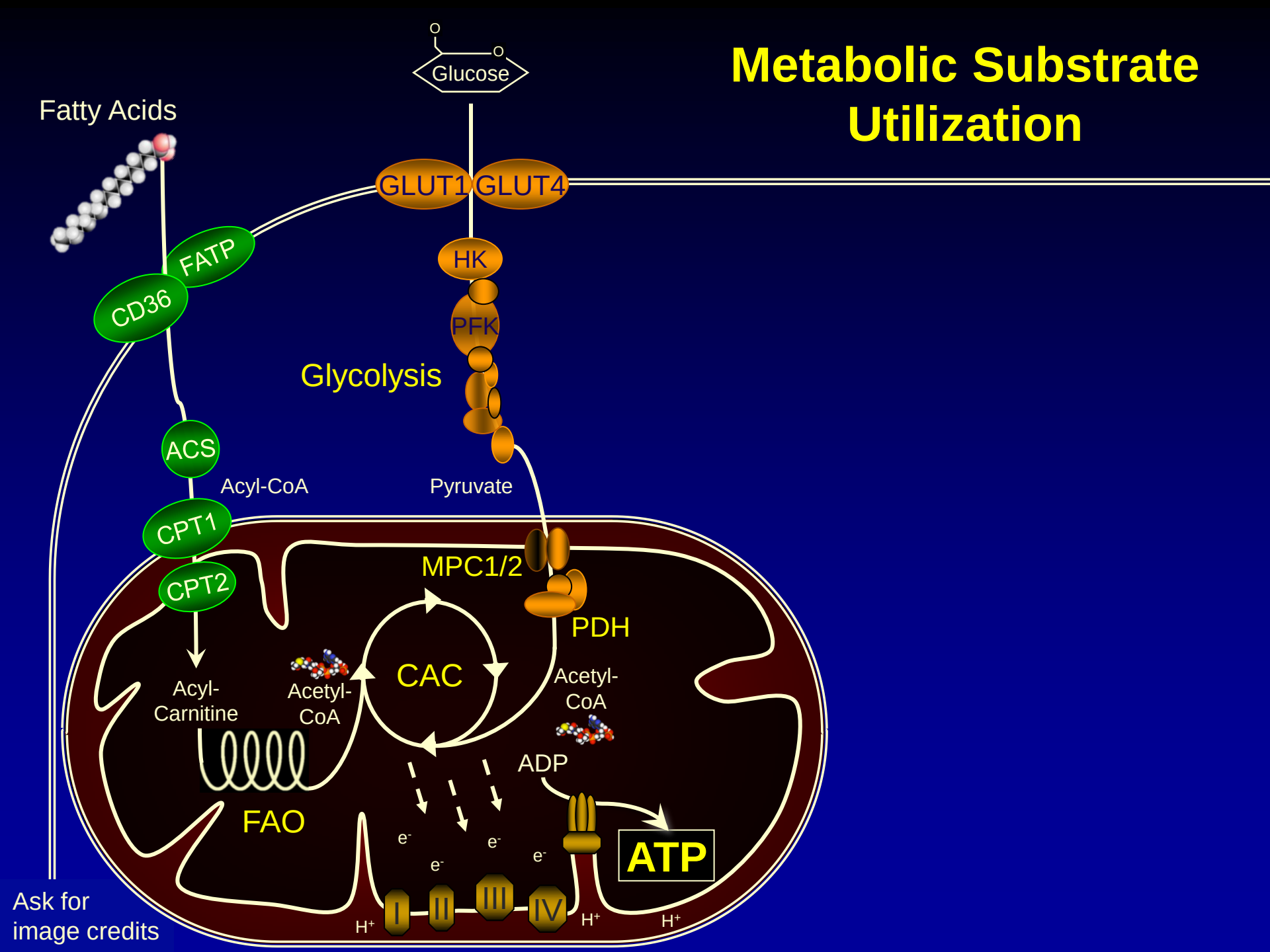
Metabolic Substrate Utilization



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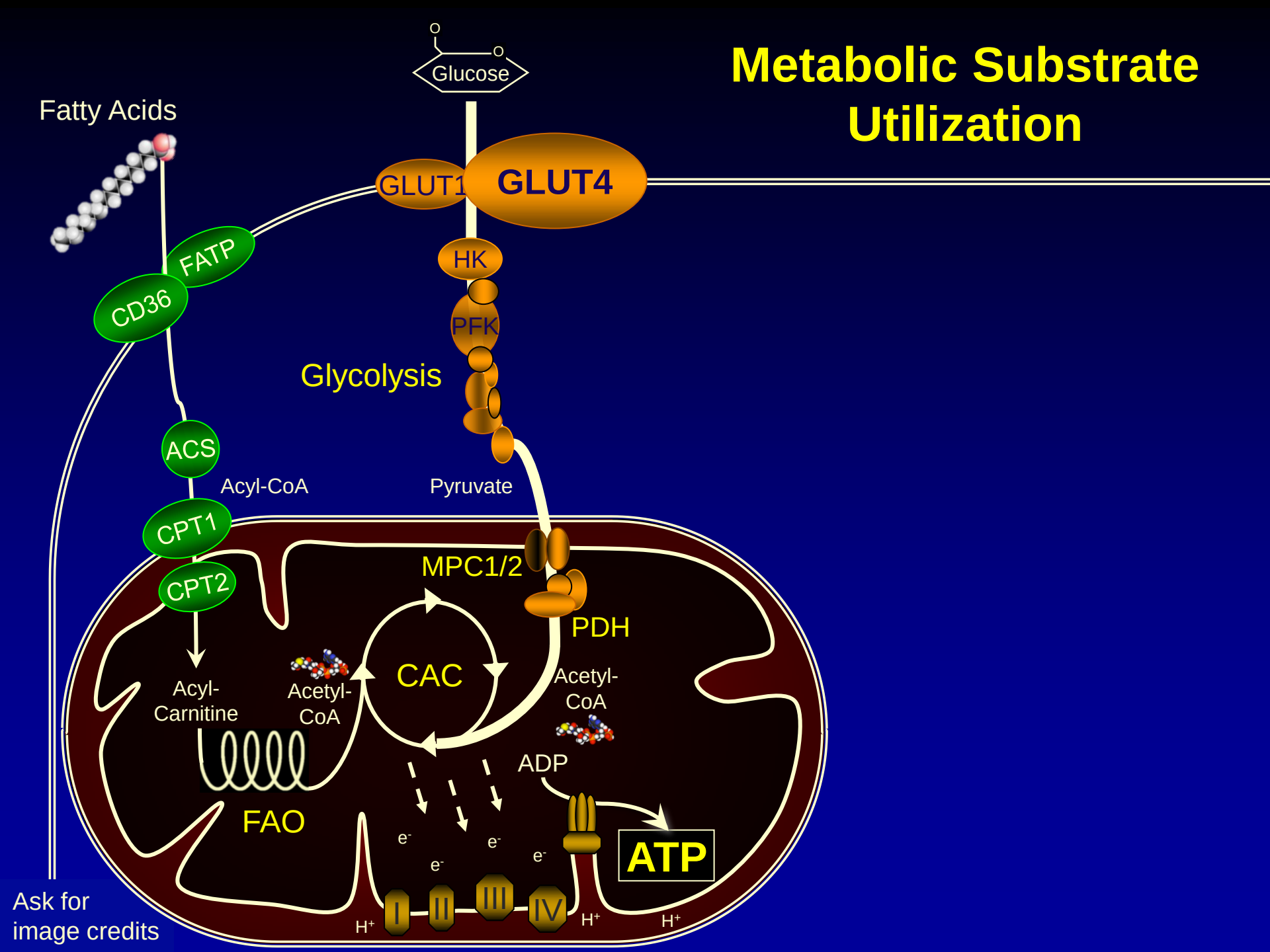
Metabolic Substrate Utilization



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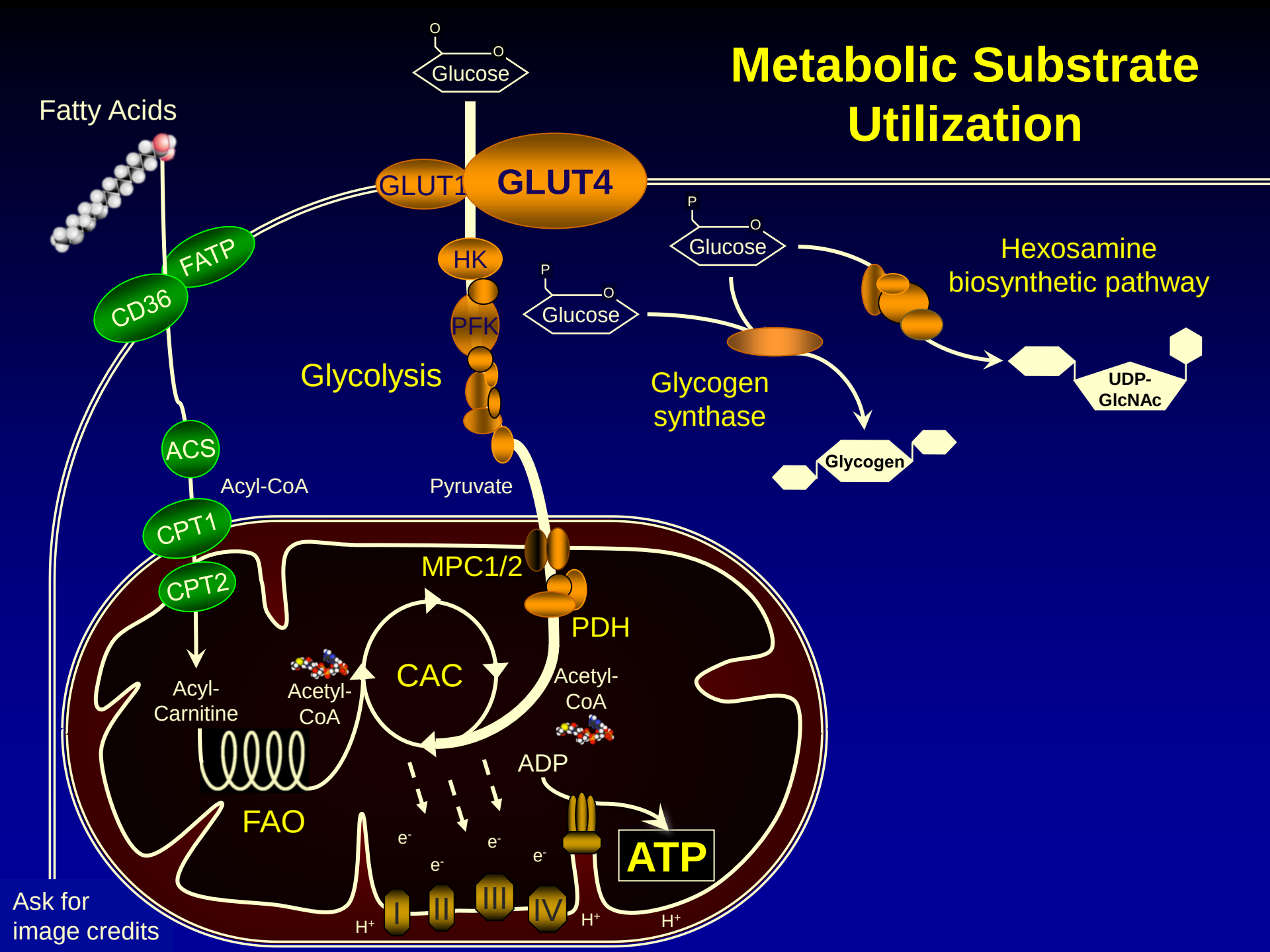
Metabolic Substrate Utilization
Glucose
Fatty Acids
GLUT1
GLUT4
FATP
CD36
HK
PFK
Glycolysis
Glucose
Glucose
Hexosamine biosynthetic pathway
UDP-GlcNAc
Glycogen synthase
Glycogen
ACS
Acyl-CoA
Pyruvate
CPT1
CPT2
MPC1/2
PDH
CAC
Acyl-Carnitine
Acetyl-CoA
Acetyl-CoA
ADP
FAO
e-
e-
e-
e-
ATP
I
II
III
IV
H+
H+
H+
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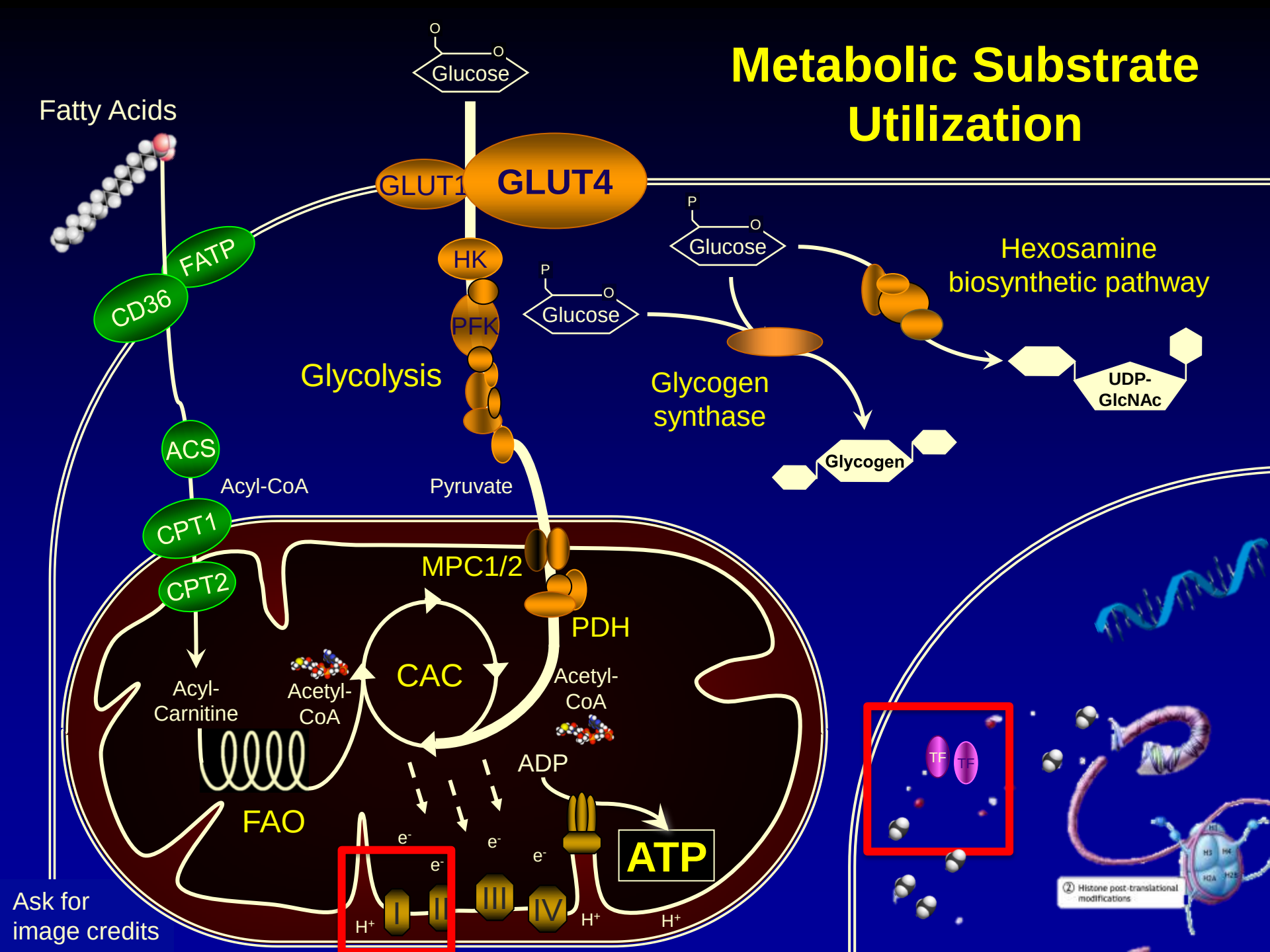
Metabolic Substrate Utilization



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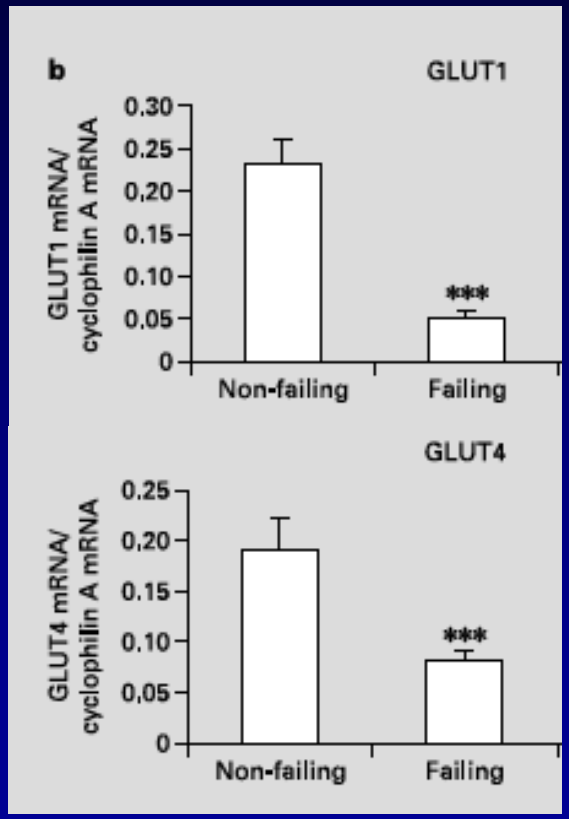
Changes in Human Heart GLUT Levels

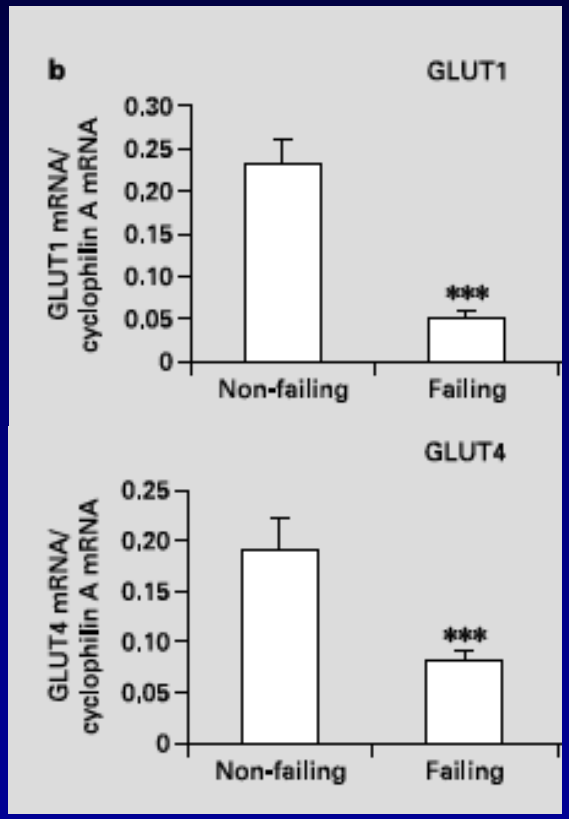
RNA
Human heart failure



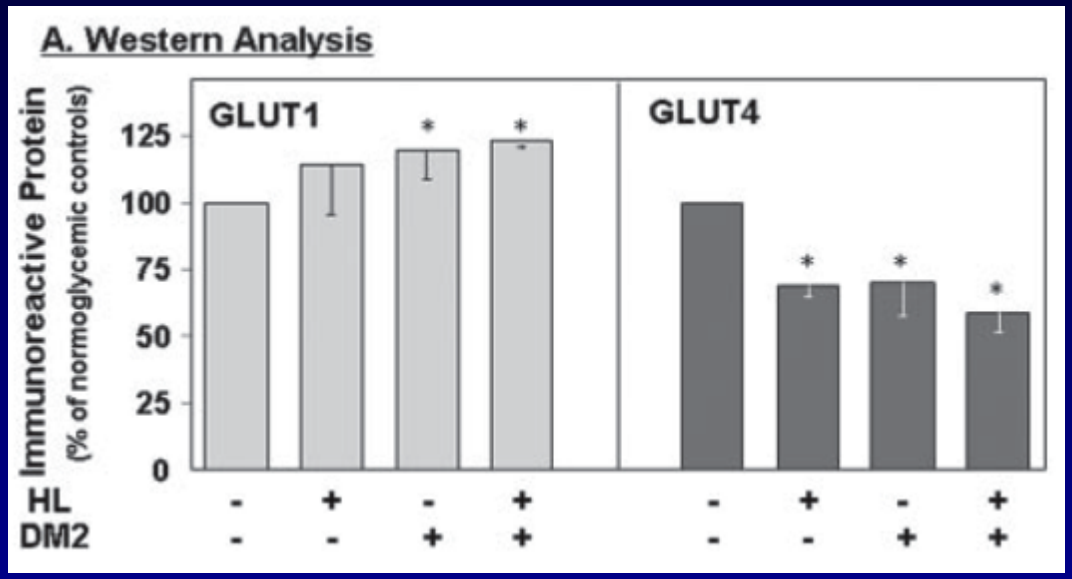
Razeghi … Taegtmeyer **2002** *Cardiology* 280(41):34786

Changes in Human Heart GLUT Levels

RNA
Human heart failure



Protein
Human heart diabetes

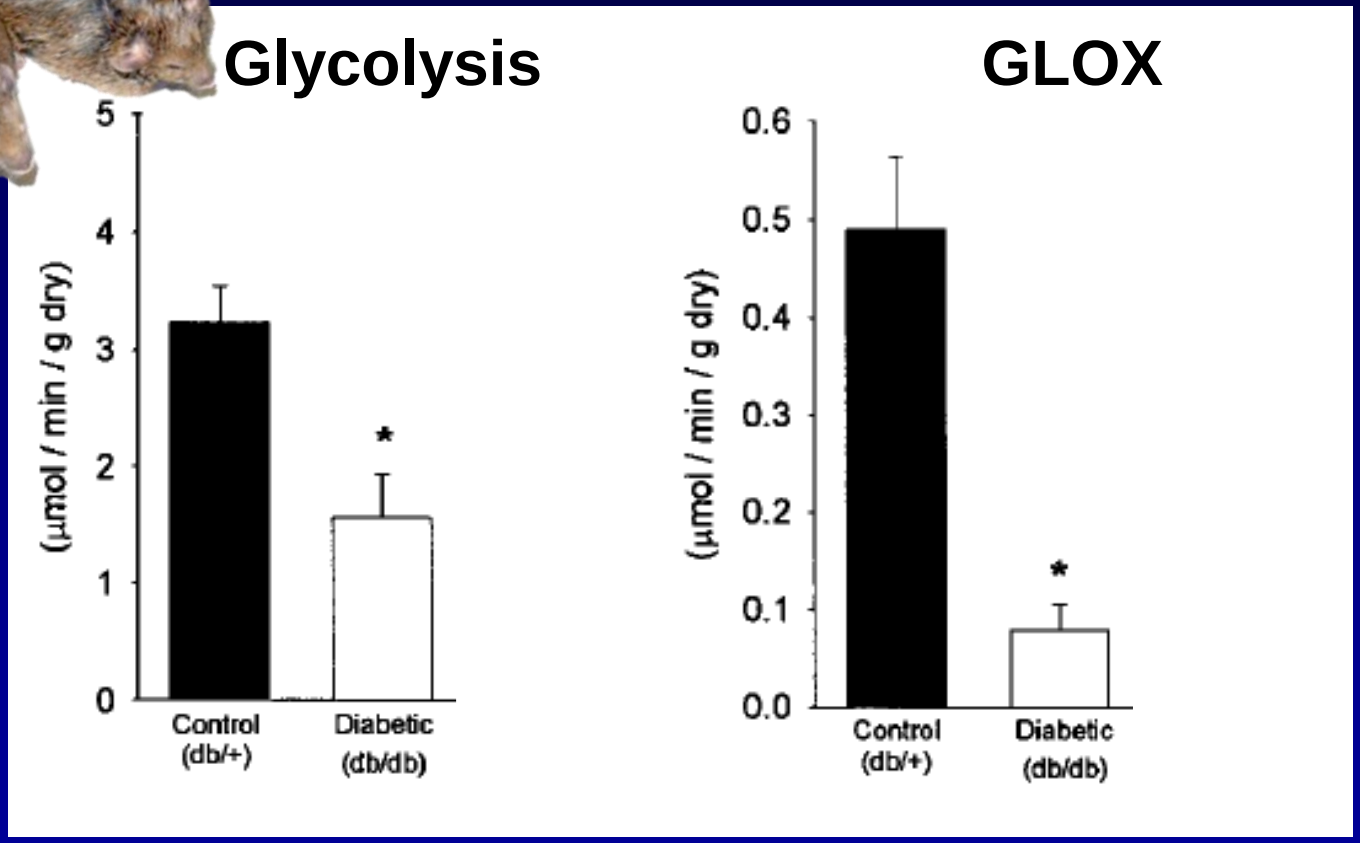


Biopsies obtained during coronary bypass surgery
HL = hyperlipidemia
DM2 = diabetes mellitus type 2

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

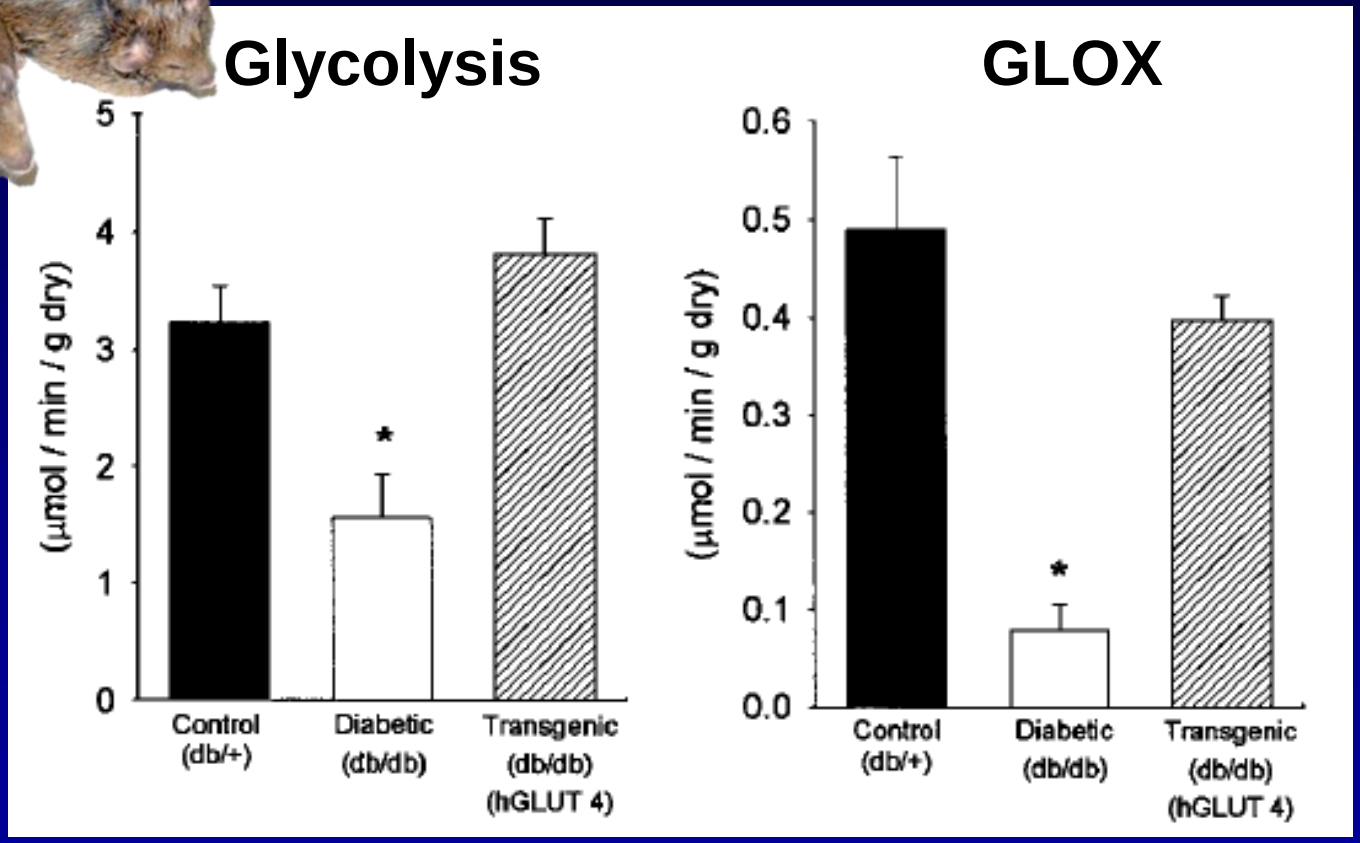
Armoni … Karnieli **2005** *J Biol Chem* 280(41):34786

Constitutive GLUT4 Expression Prevents Development of Glucose Utilization Defects



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

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?

From Human to Mouse and Back Again

Broad Institute Communications

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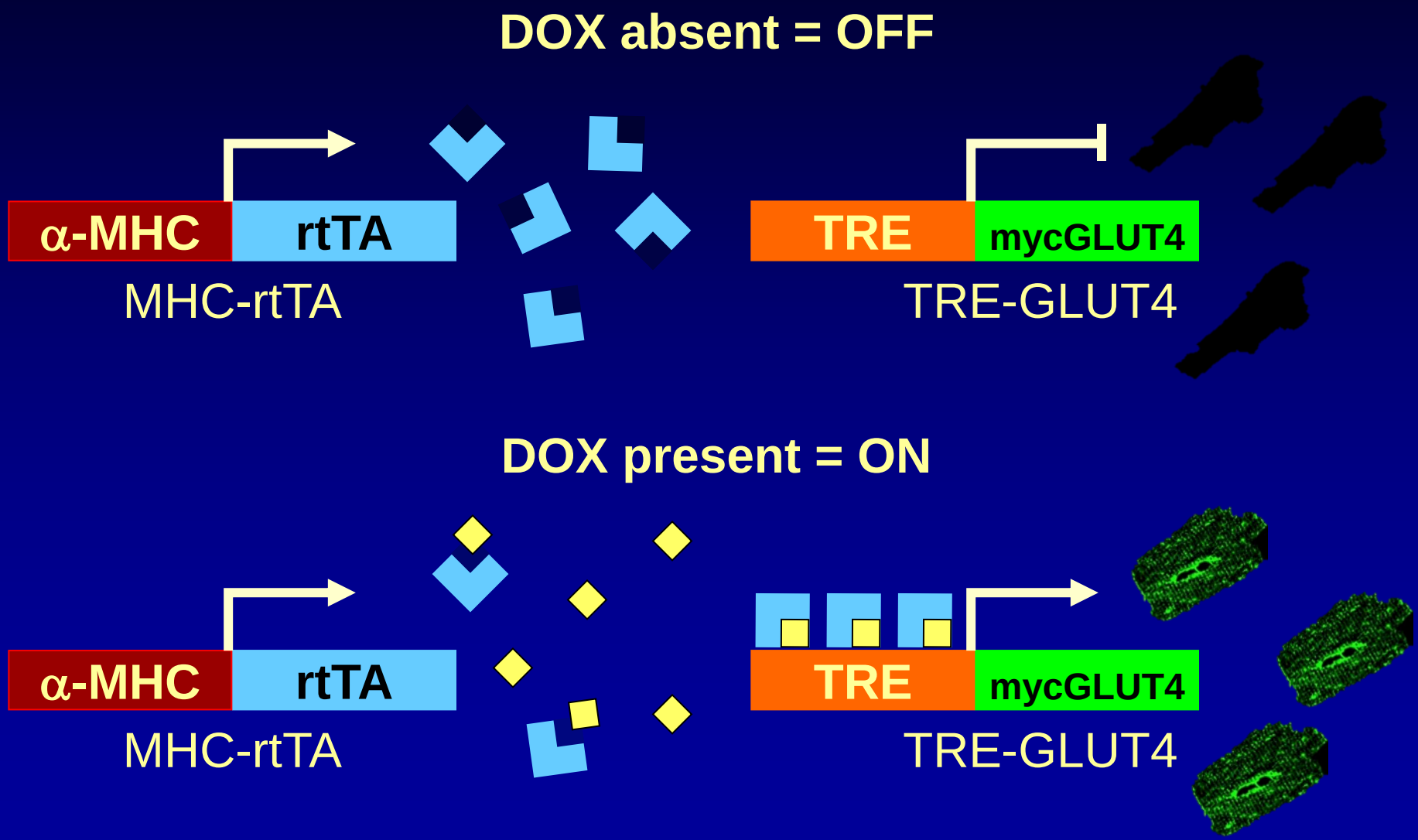
Inducible Cardiomyocyte-Specific GLUT4 Expression (mG4H)



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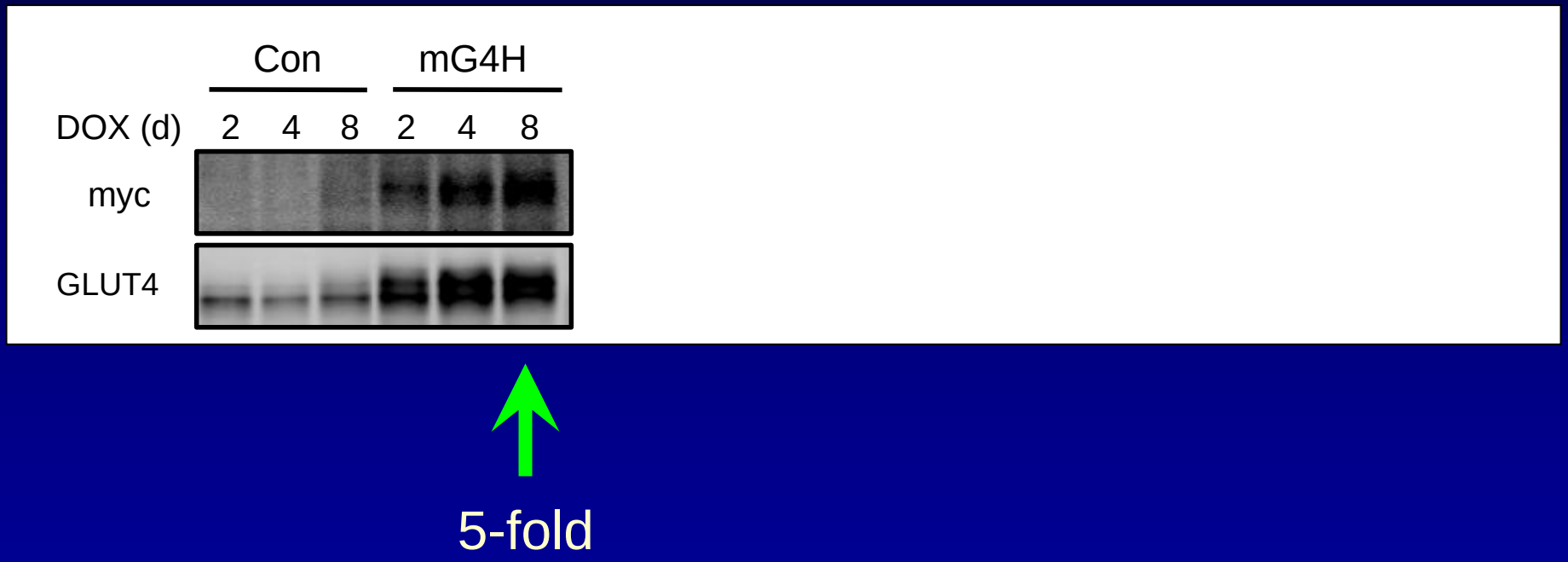
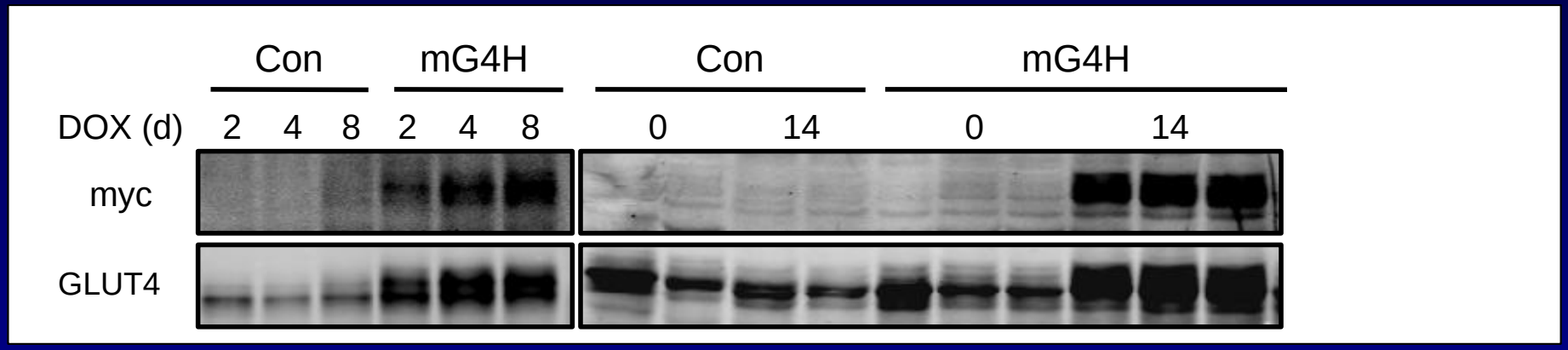
mG4H Mice Exhibit Inducible Cardiac-Specific Expression of GLUT4

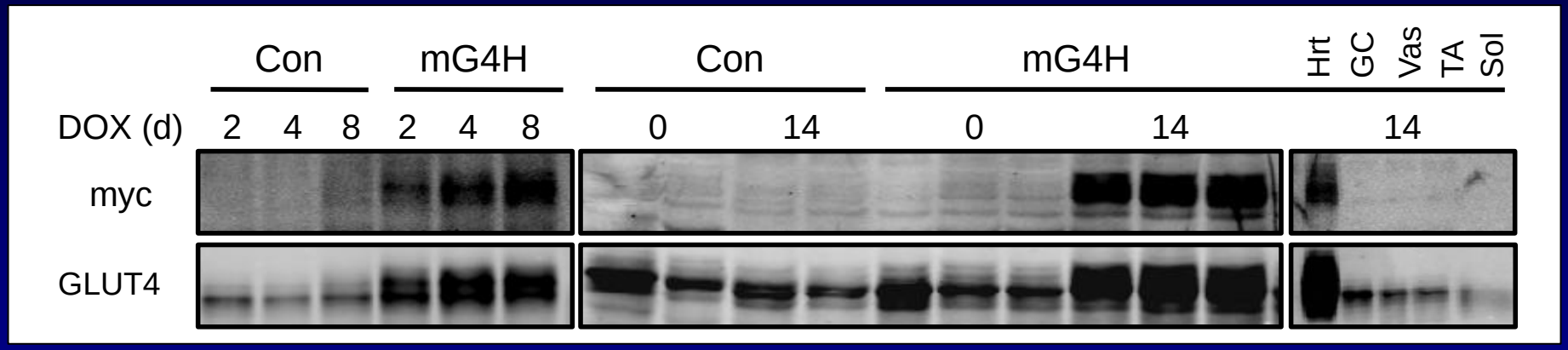


mG4H Mice Exhibit Inducible Cardiac-Specific Expression of GLUT4



5-fold

mG4H Mice Exhibit Inducible Cardiac-Specific Expression of GLUT4

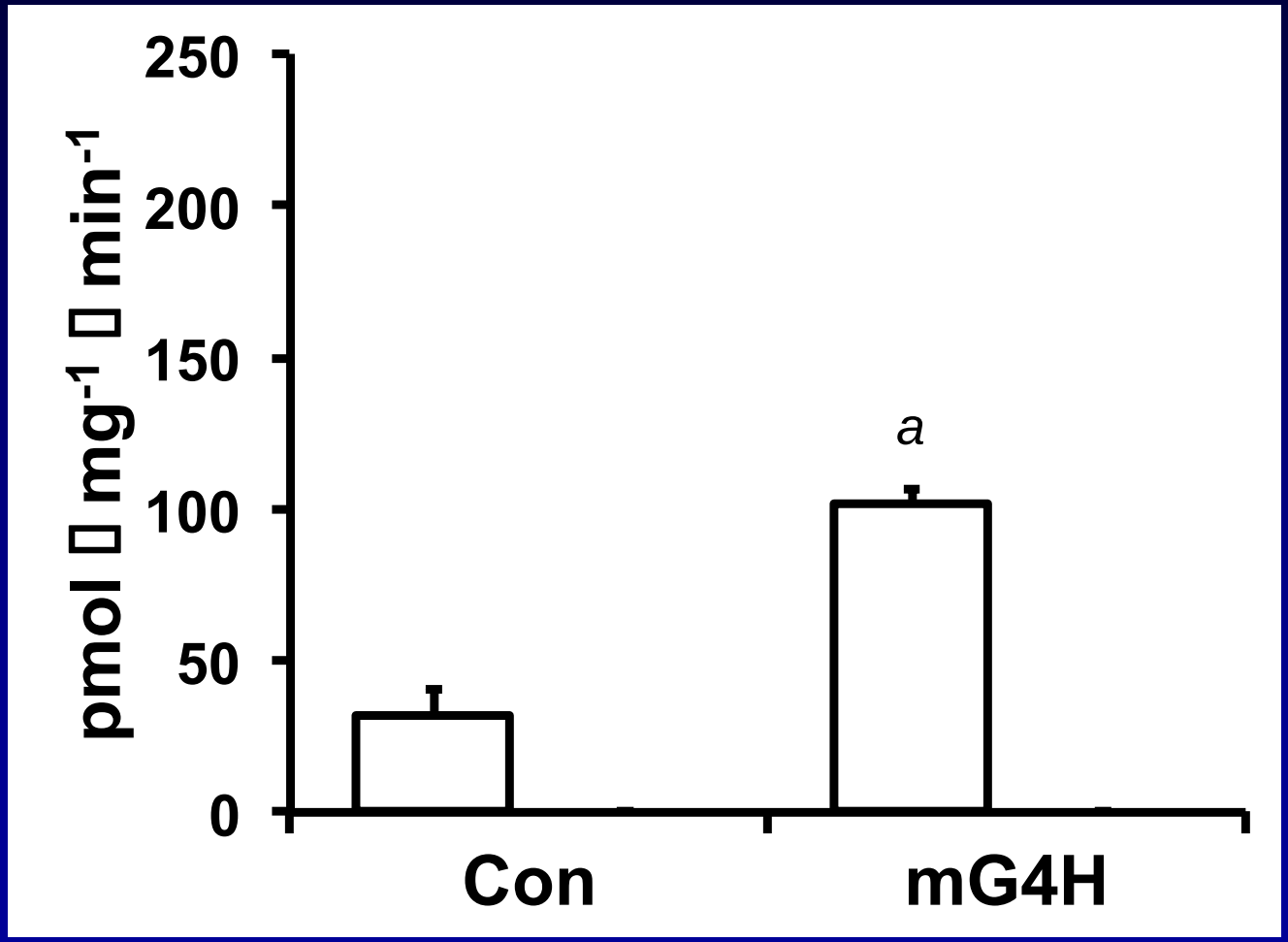


Hrt = Heart
GC = Gastrocnemius
Vas = Vastus lateralis
TA = Tibialis anterior
Sol = Soleus

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

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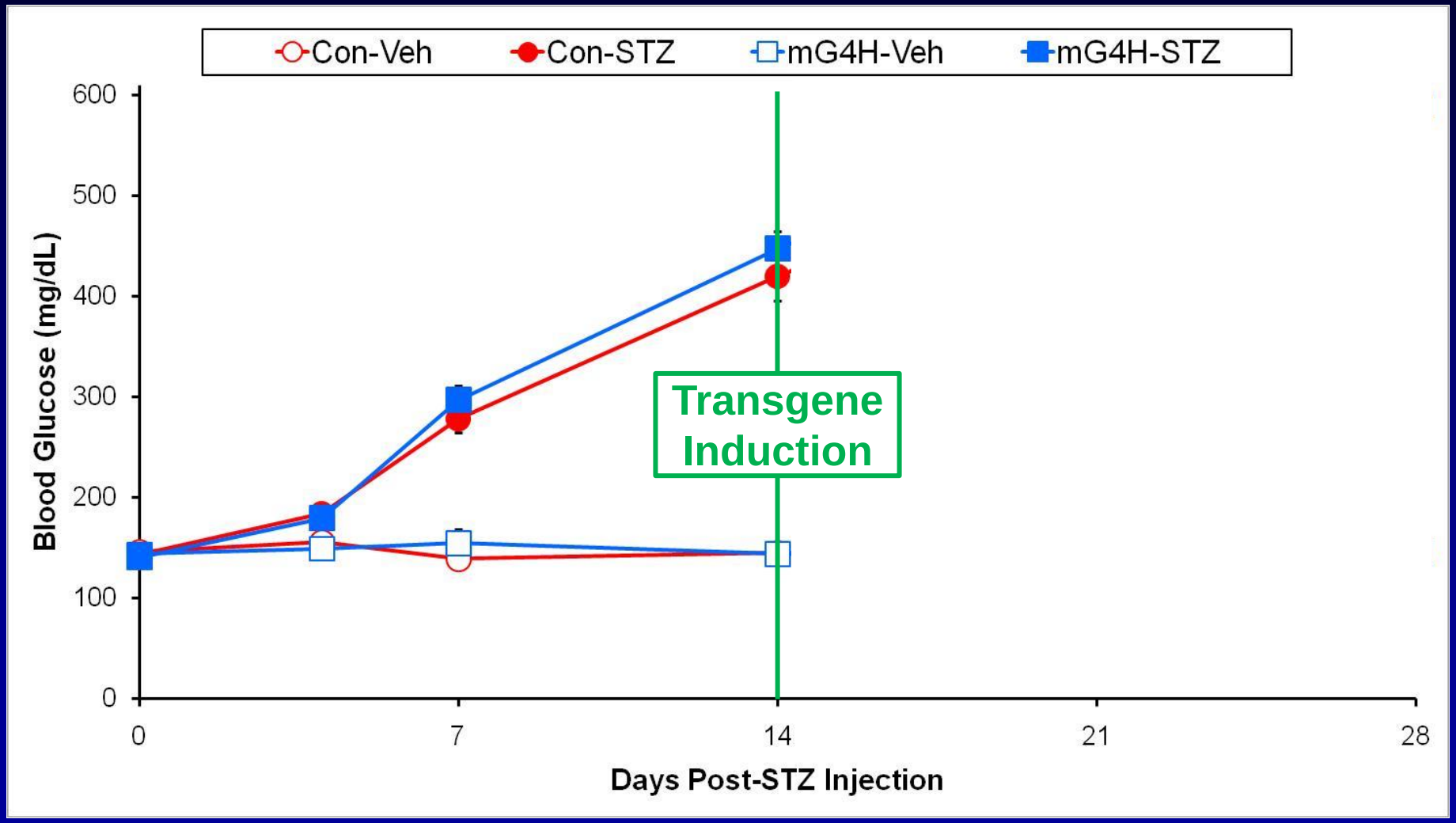
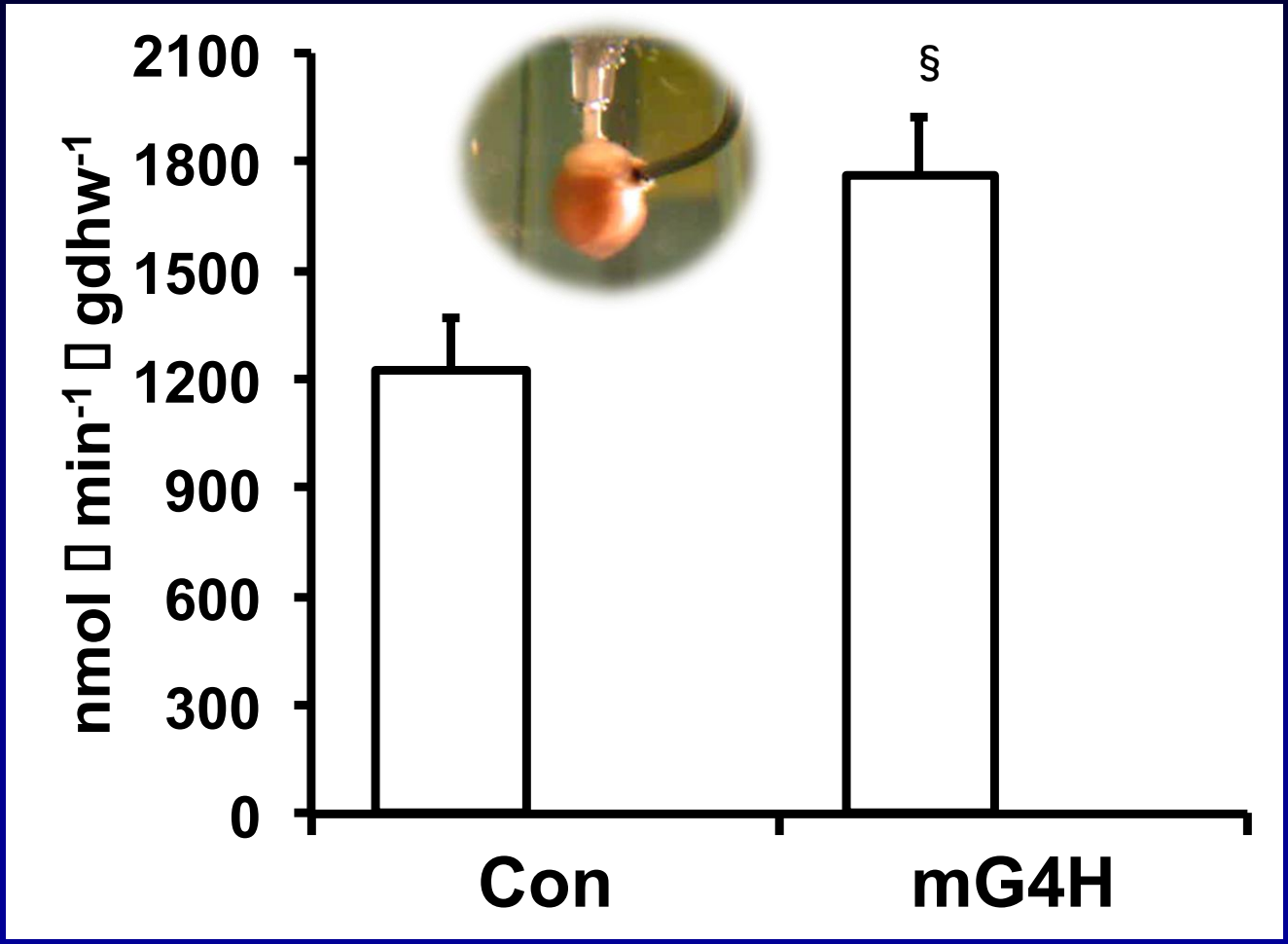
Streptozotocin (STZ)-Induced Hyperglycemia is Not Altered by Transgene Induction



GLUT4 Induction Increases Glycolysis and Rescues Diabetic Cardiac Glycolytic Defects

Isolated Working Hearts Glycolysis



n = 6 – 10

§ P < 0.01 vs. Con

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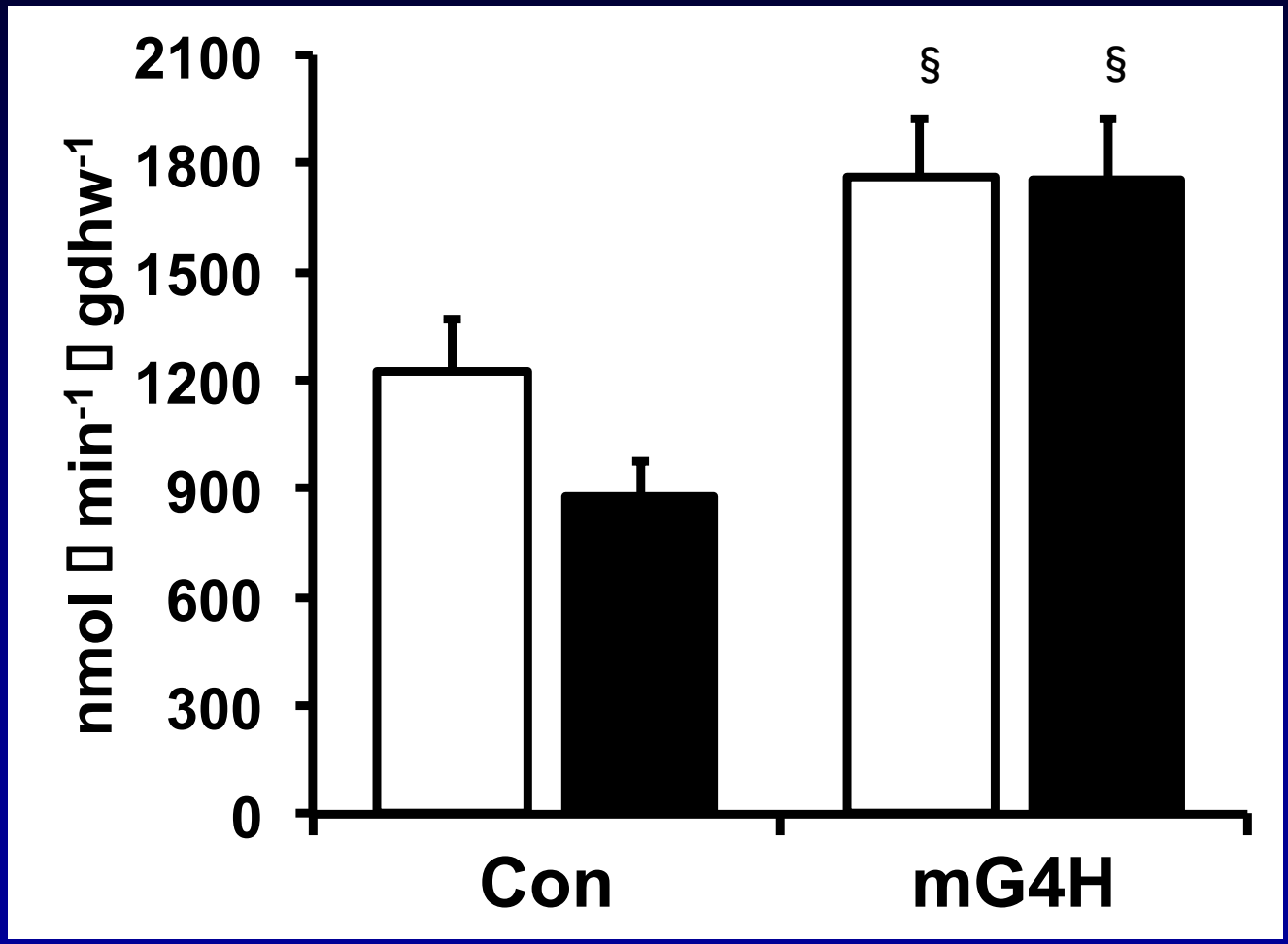
GLUT4 Induction Increases Glycolysis and Rescues Diabetic Cardiac Glycolytic Defects

Isolated Working Hearts Glycolysis



n = 6 – 10

§ P < 0.01 vs. Con

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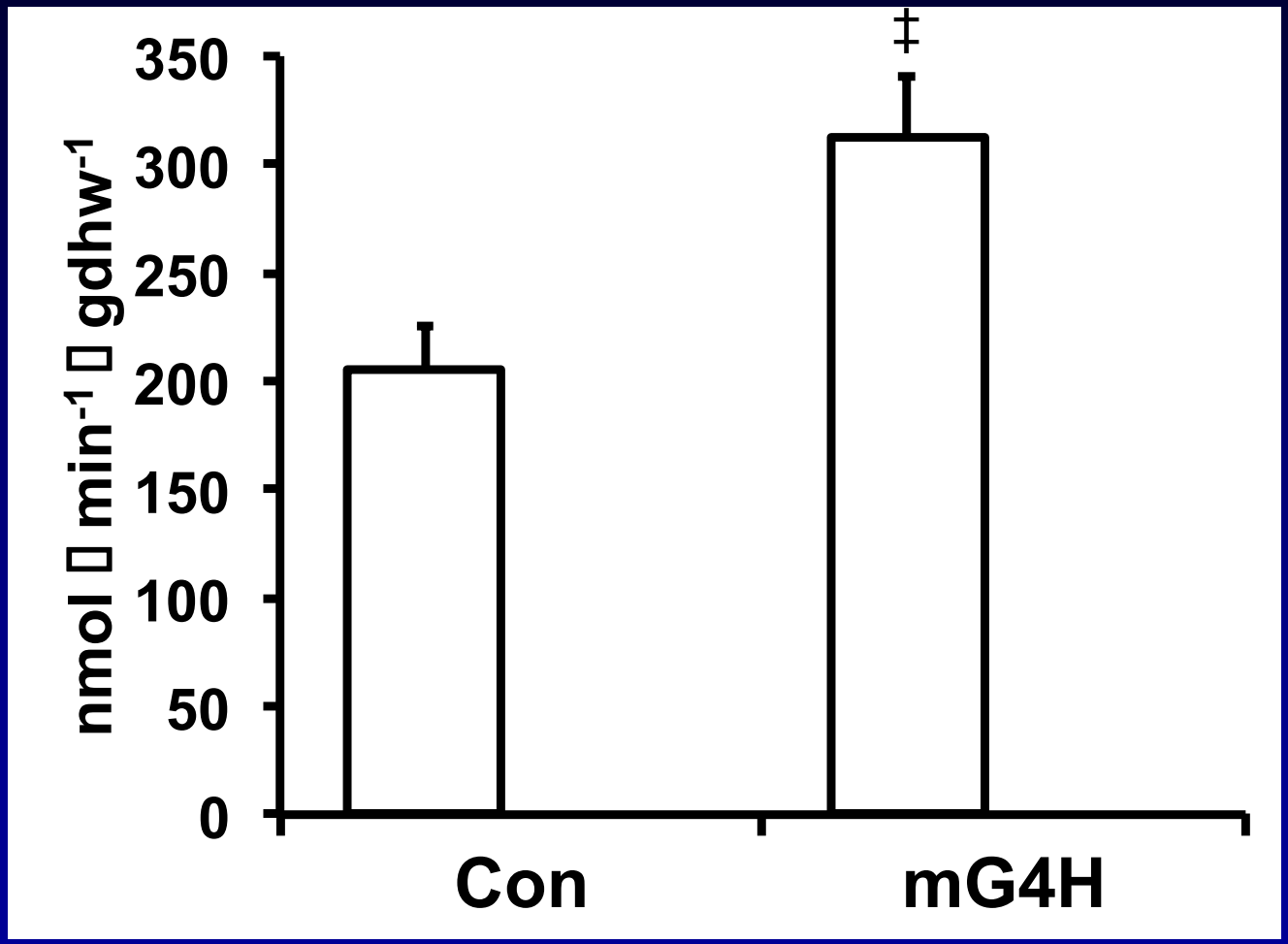
GLUT4 Induction Increases GLOX but Accelerates Diabetic Cardiac GLOX Defects

Isolated Working Hearts Glucose Oxidation (GLOX)



Vehicle
STZ

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

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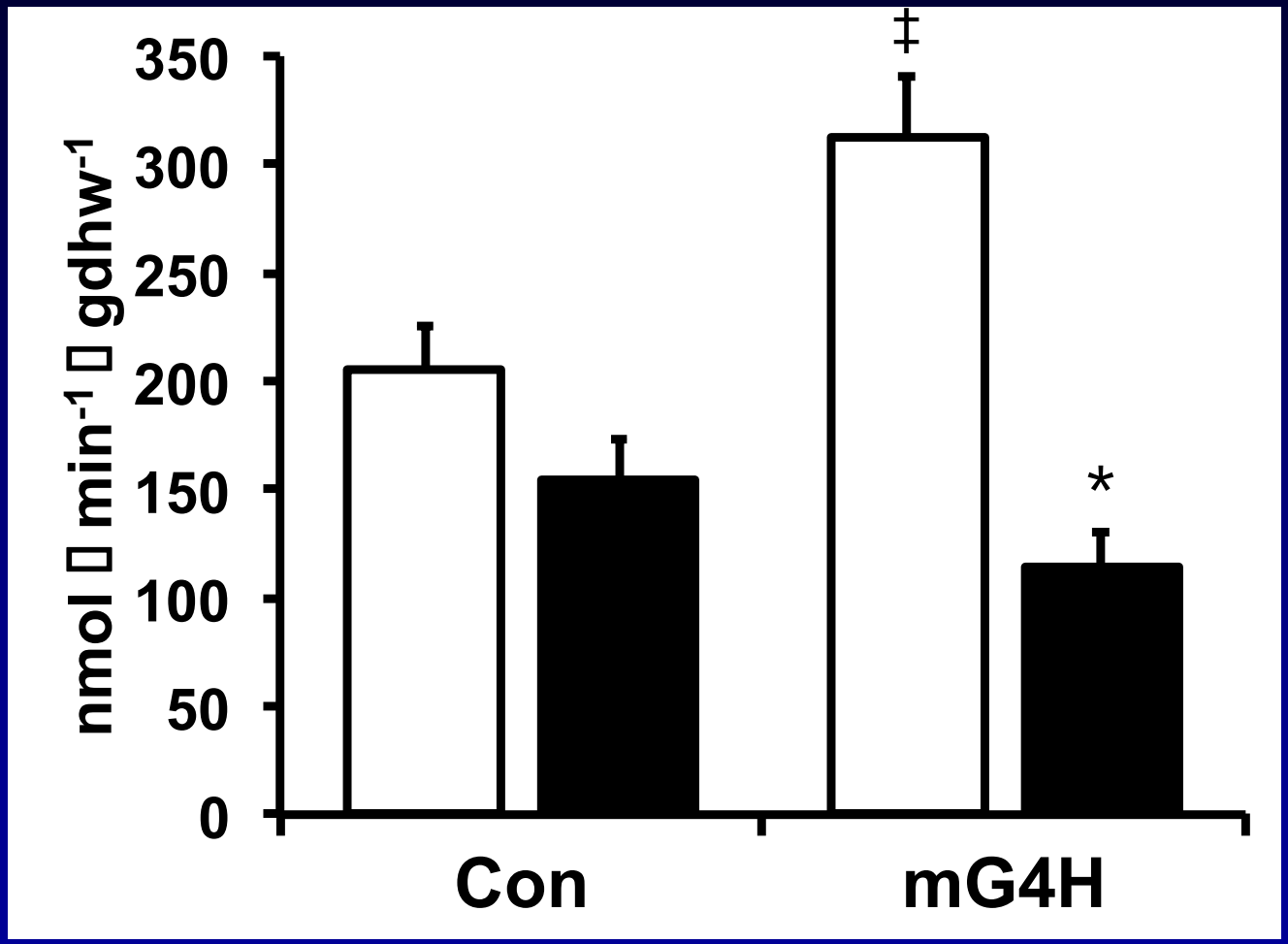
GLUT4 Induction Increases GLOX but Accelerates Diabetic Cardiac GLOX Defects

Isolated Working Hearts Glucose Oxidation (GLOX)



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

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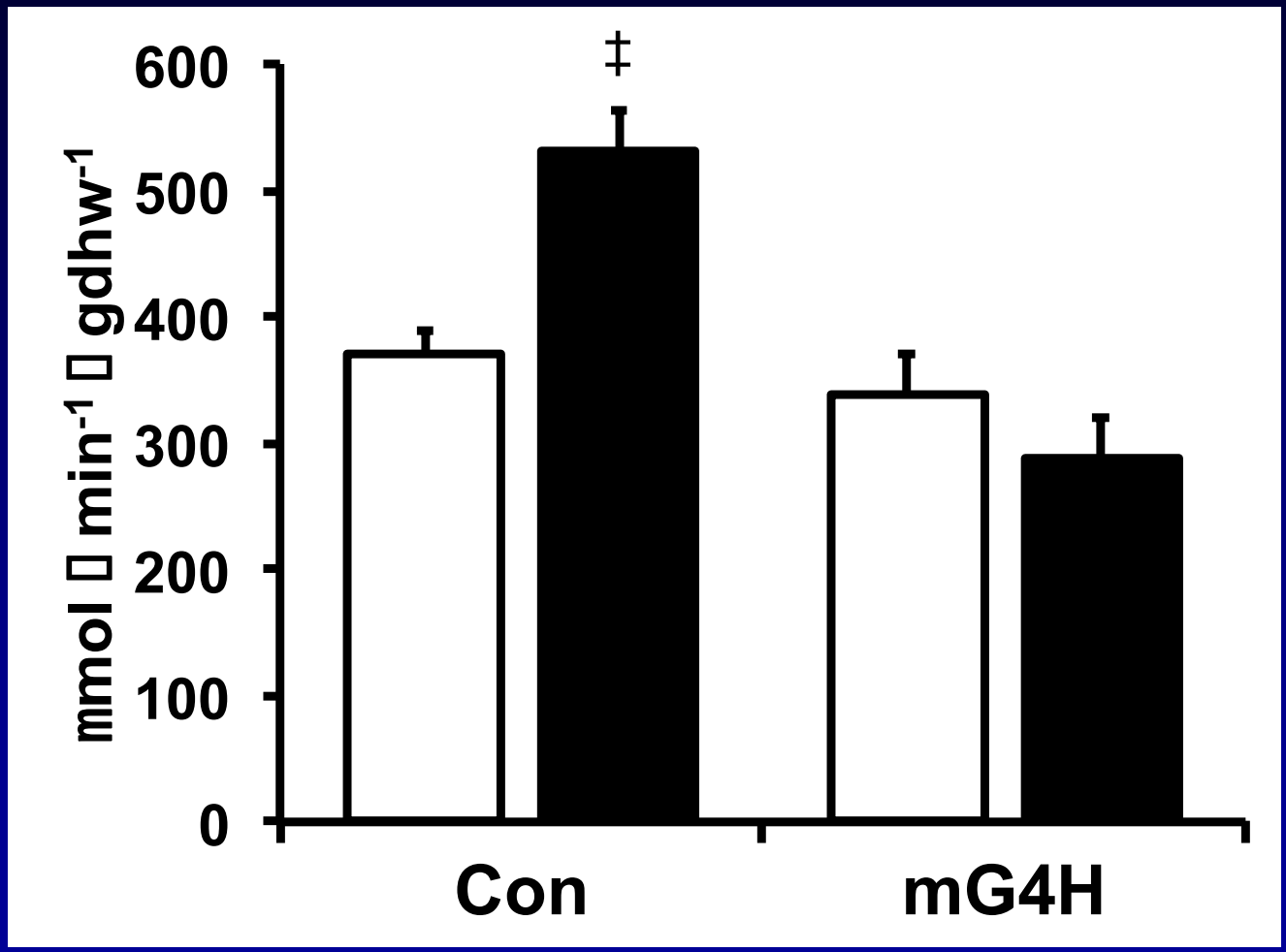
GLUT4 Induction Prevents Increased Cardiac POX in Diabetes

Isolated Working Hearts Palmitate Oxidation (POX)



n = 5 – 13
‡ P < 0.001 vs. All

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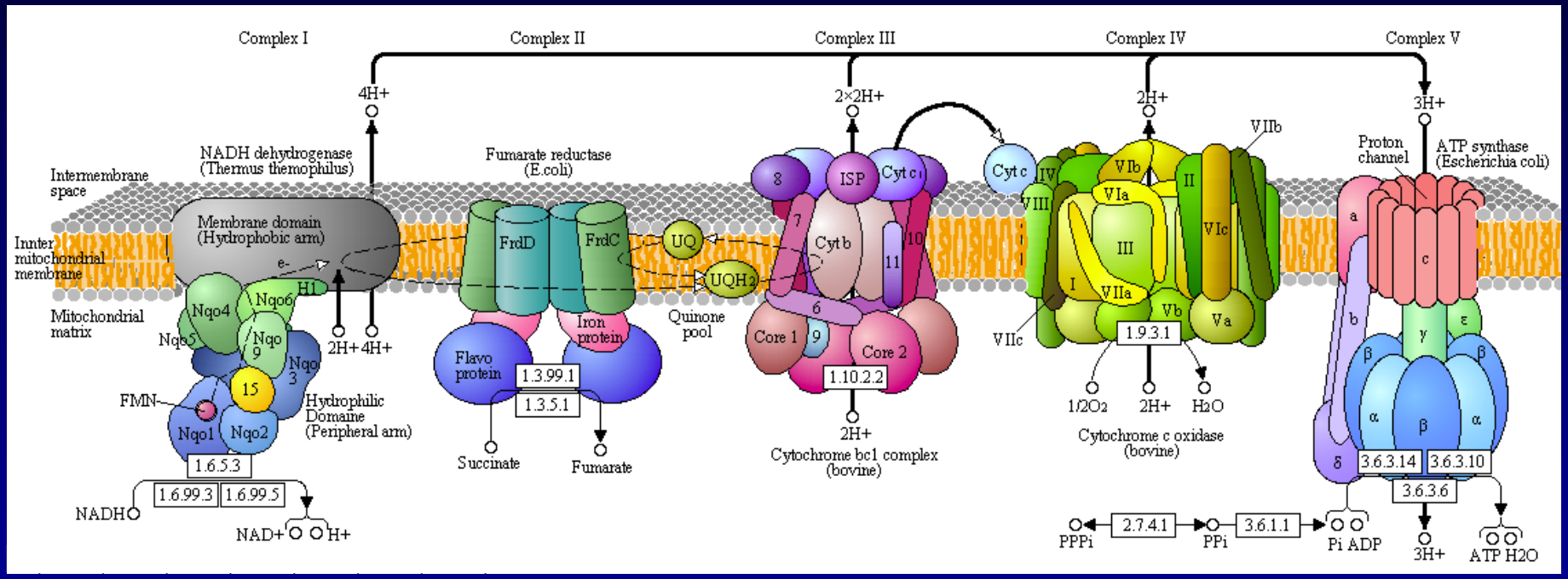
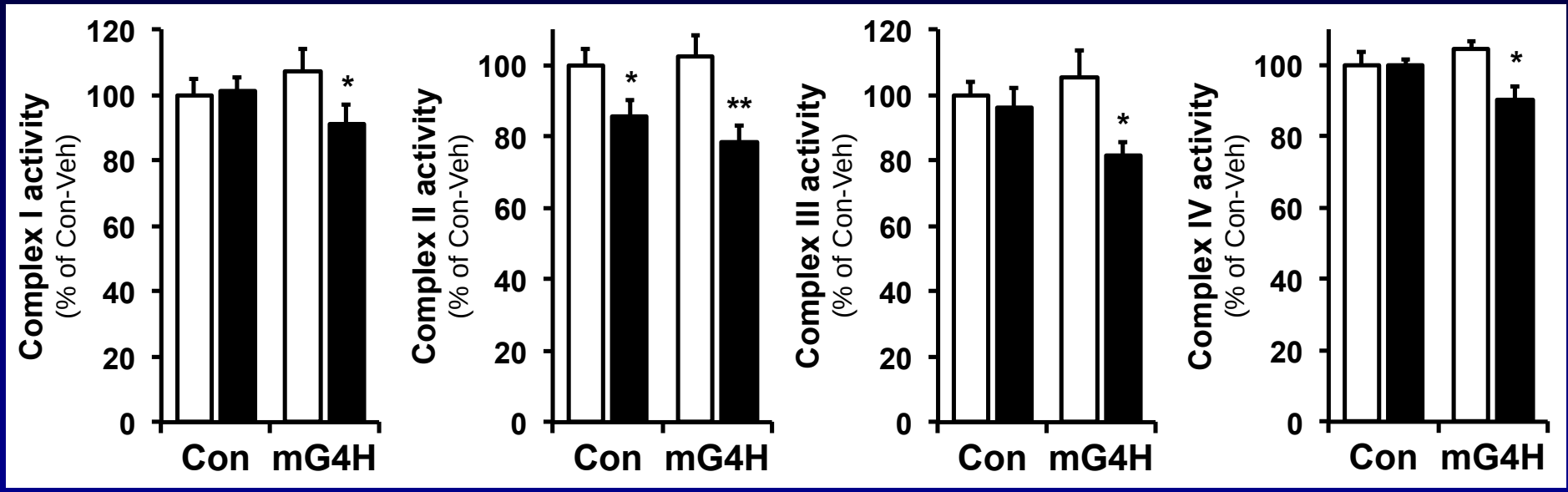
Oxidative Phosphorylation



www.genome.jp/kegg/pathway.html

GLUT4 Induction Accelerates Development of Mitochondrial Dysfunction



n = 3 – 4

* P < 0.05

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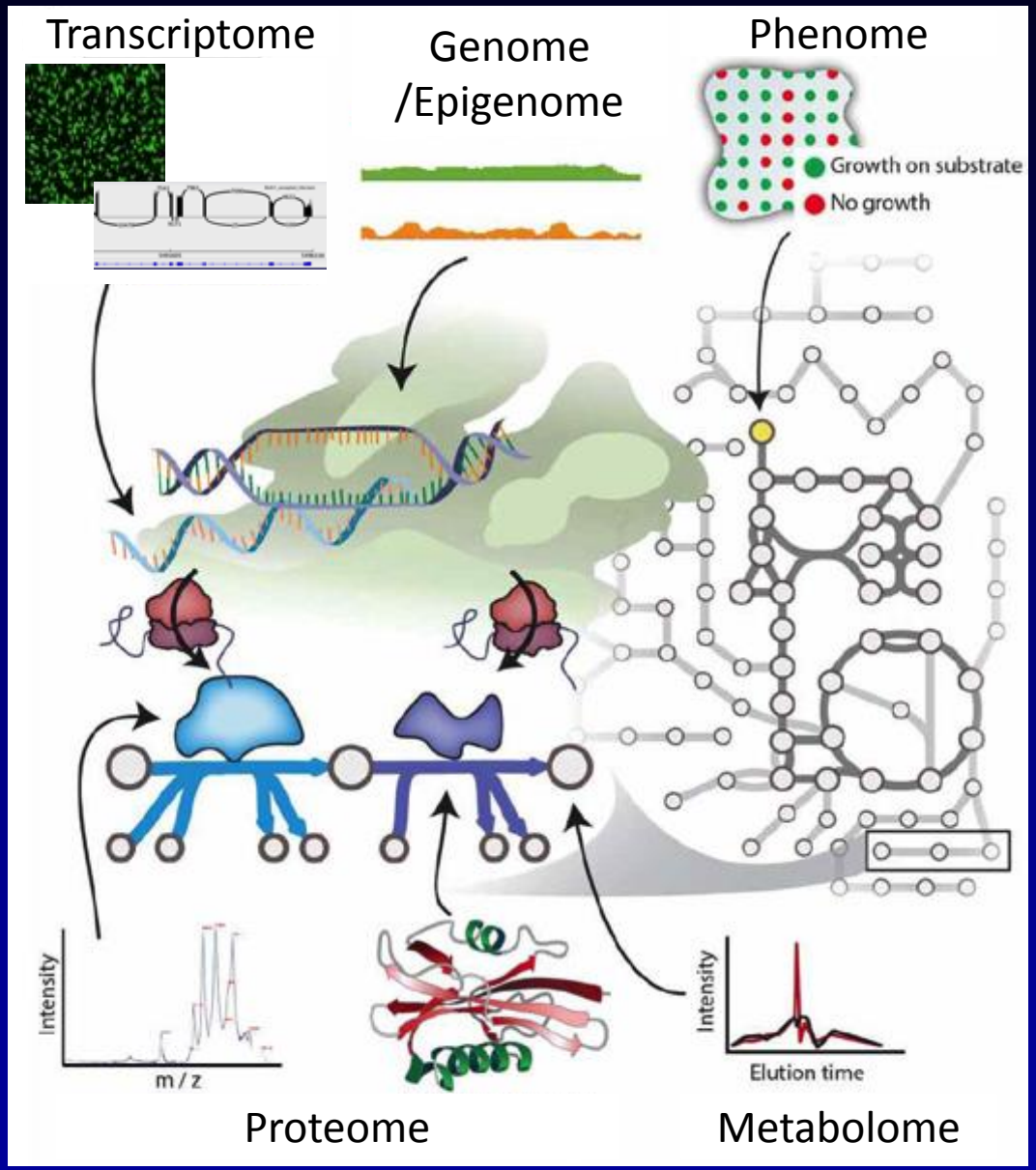
Conclusion – Part 1

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

Hypothesis:

Glucose directly regulates molecular function through non-metabolic pathways

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

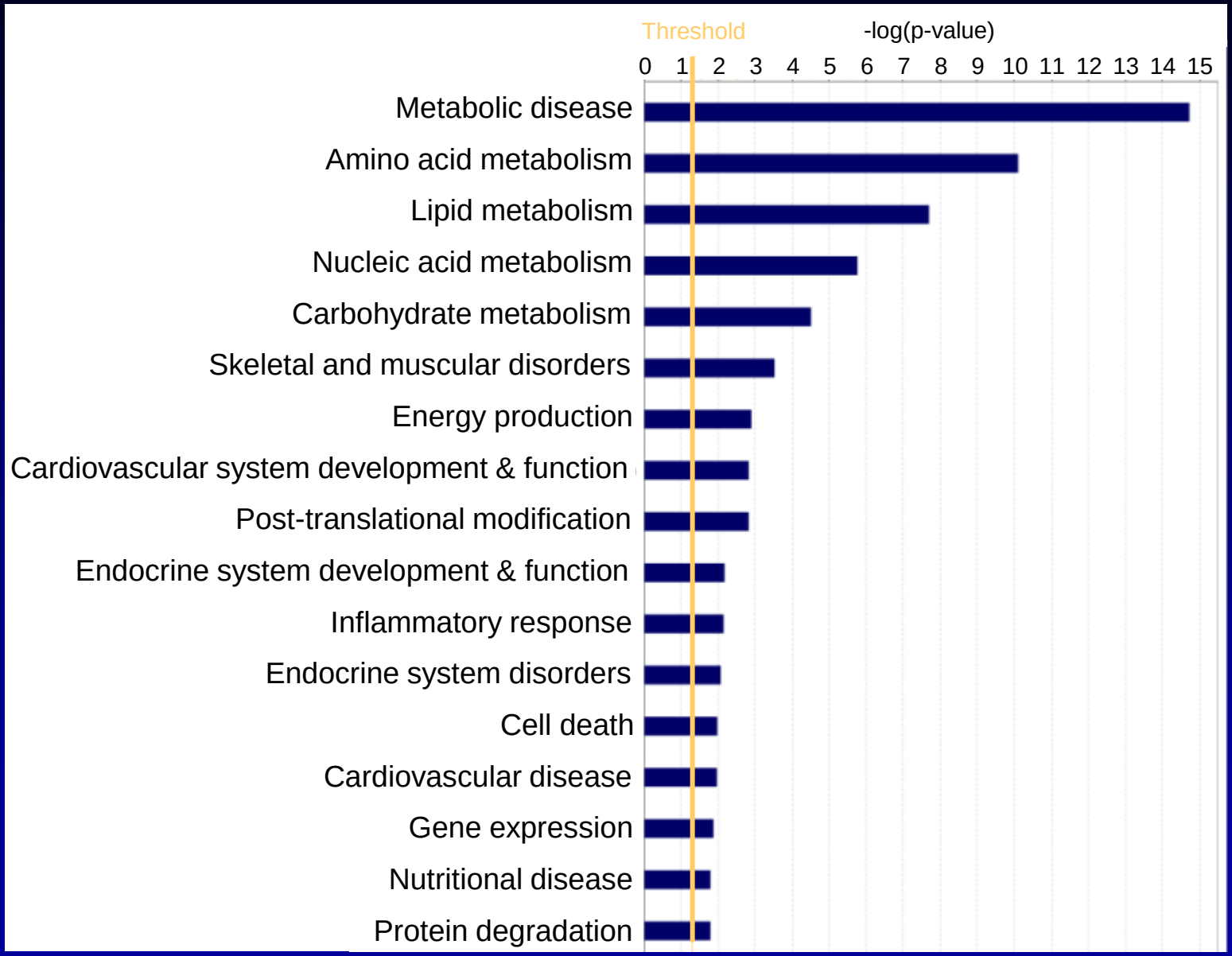
Glucometer, ELISA, GC-MS, HPLC, NMR, fluxomics, etc.

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



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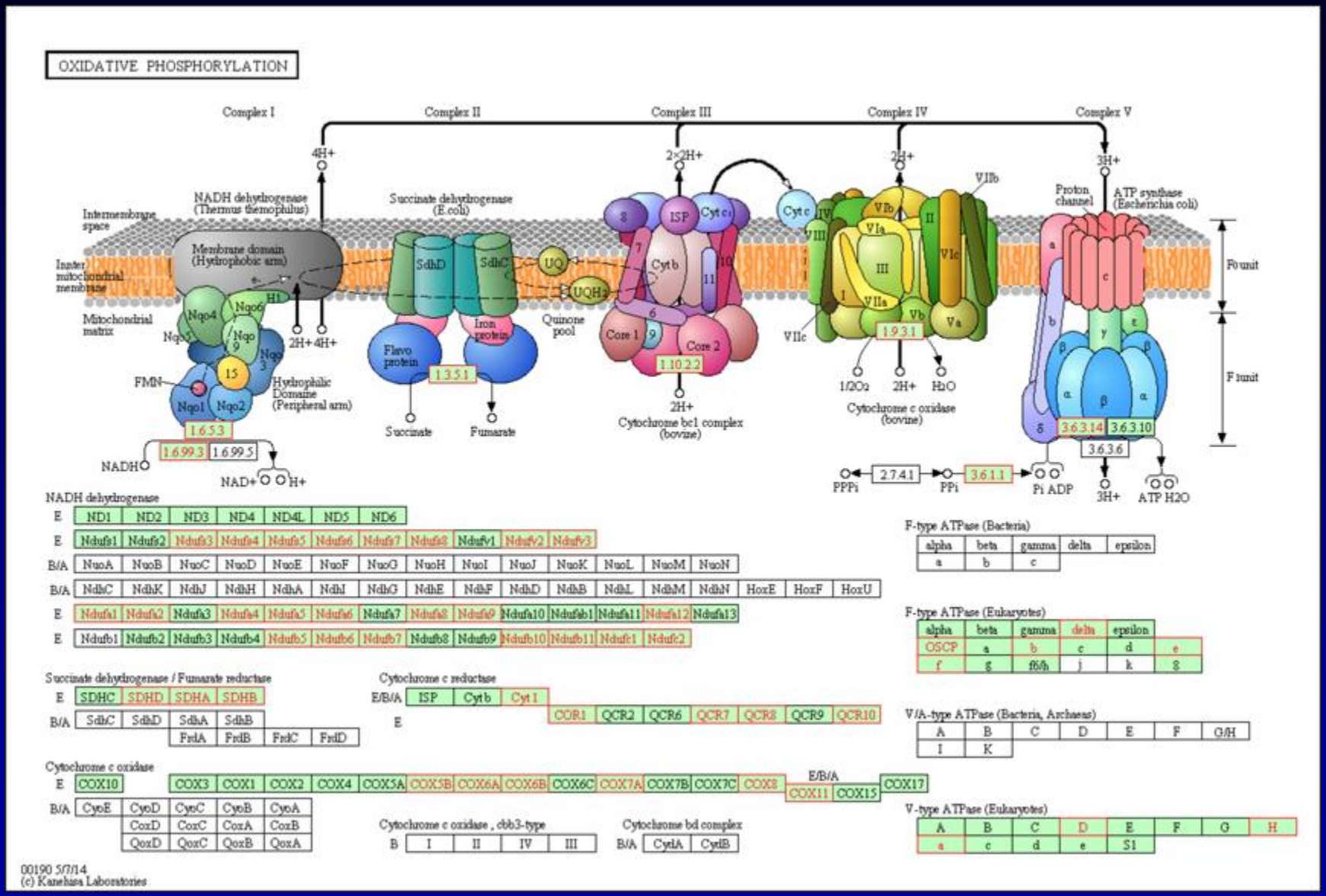
Microarray Data – Oxidative Phosphorylation



GeneSifter using KEGG

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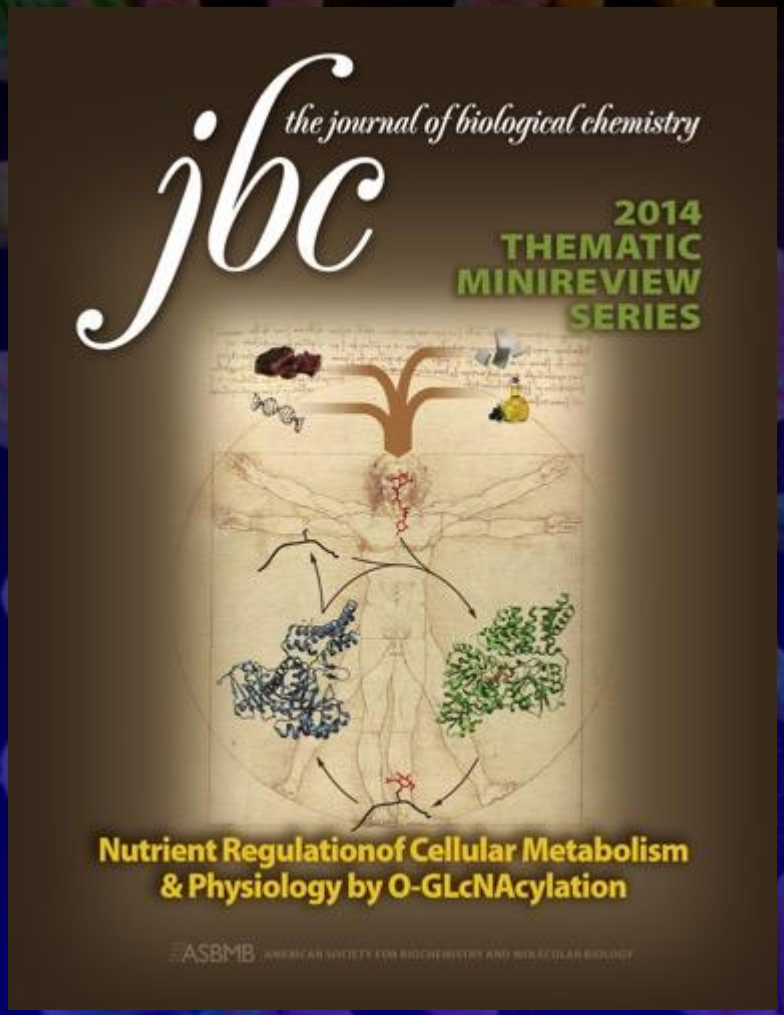
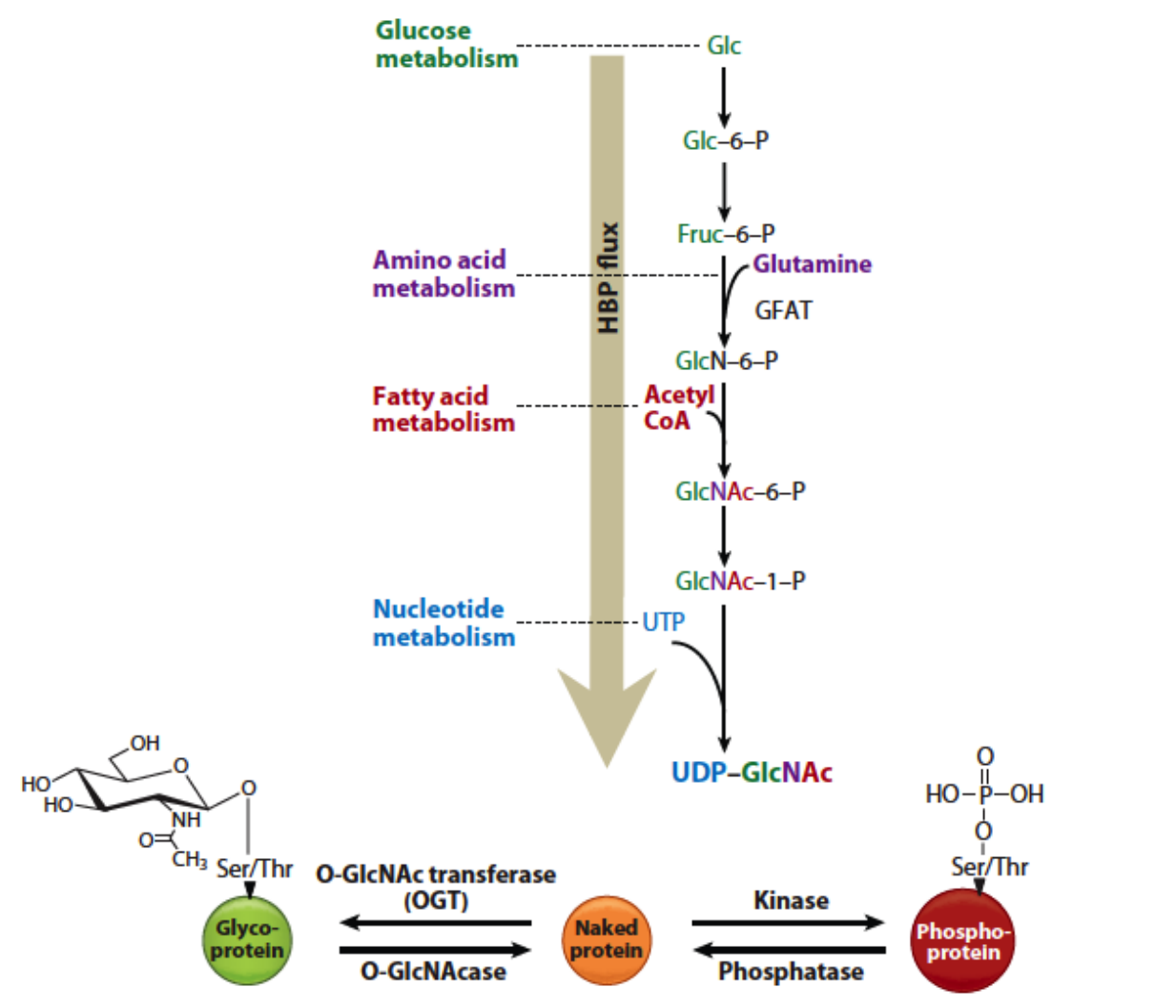
O-GlcNAcylation

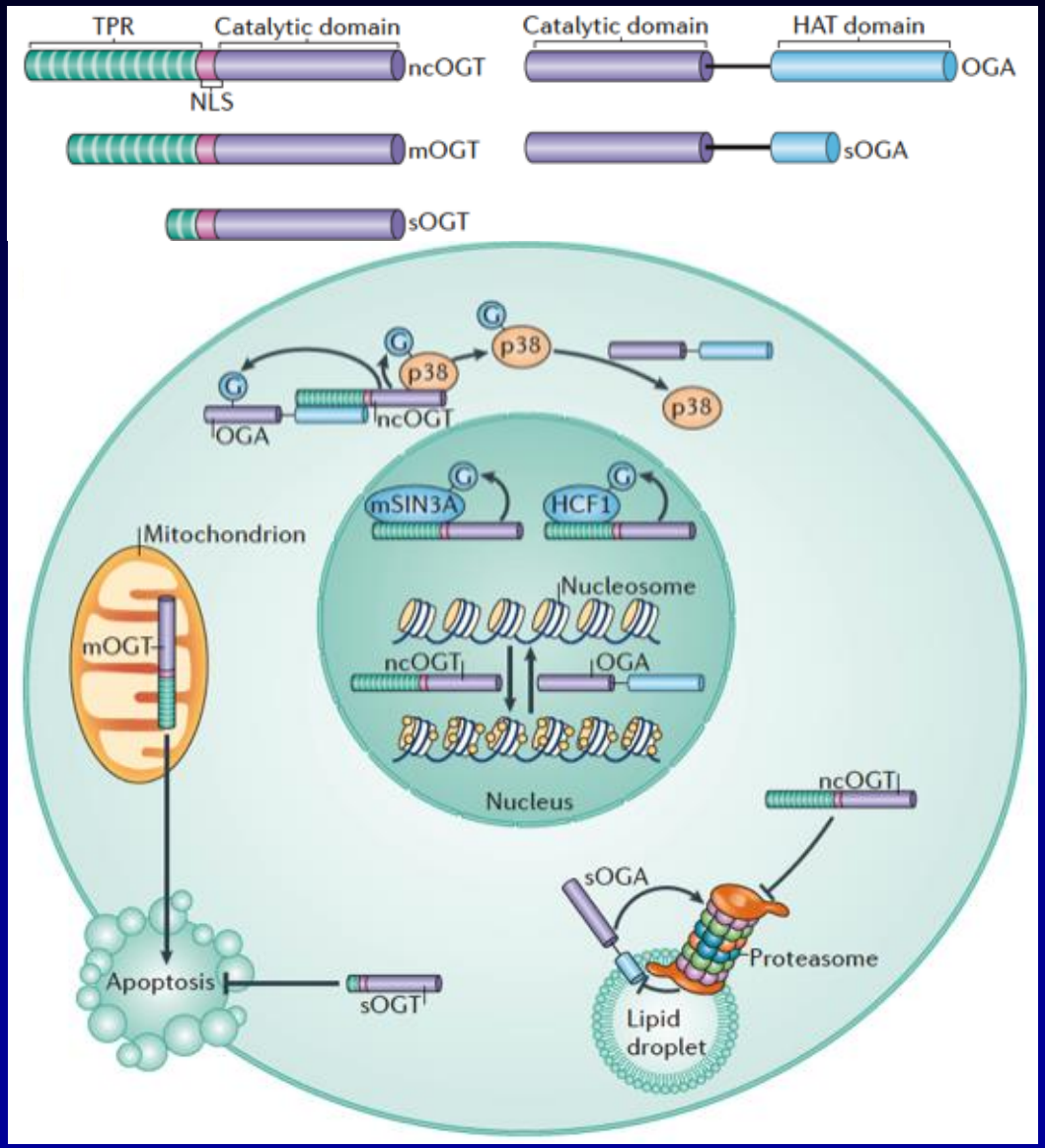


Hexosamine Biosynthesis Pathway / Protein *O*-GlcNAcylation



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

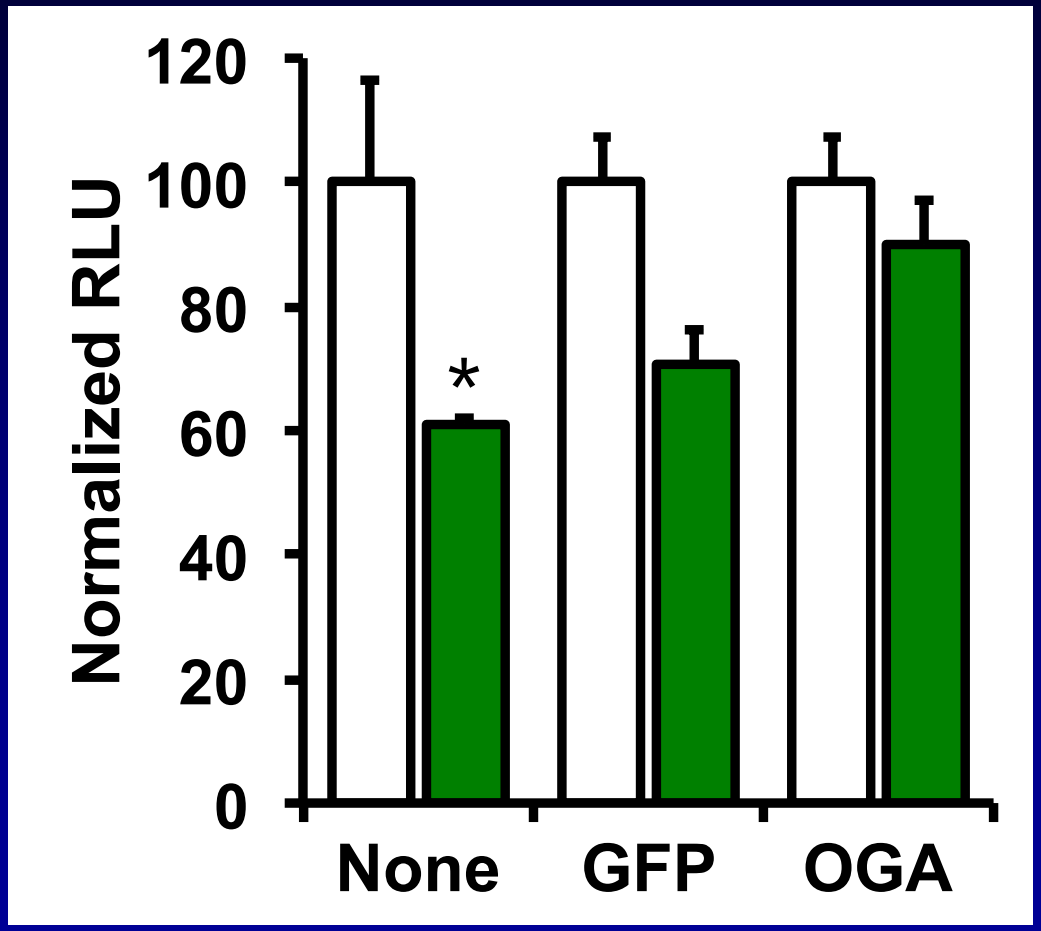
O-GlcNAc Cycling



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

GlcNAcylation Regulates *Ndufa9* Promoter Activity

Transient Transfection Promoter Activity



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

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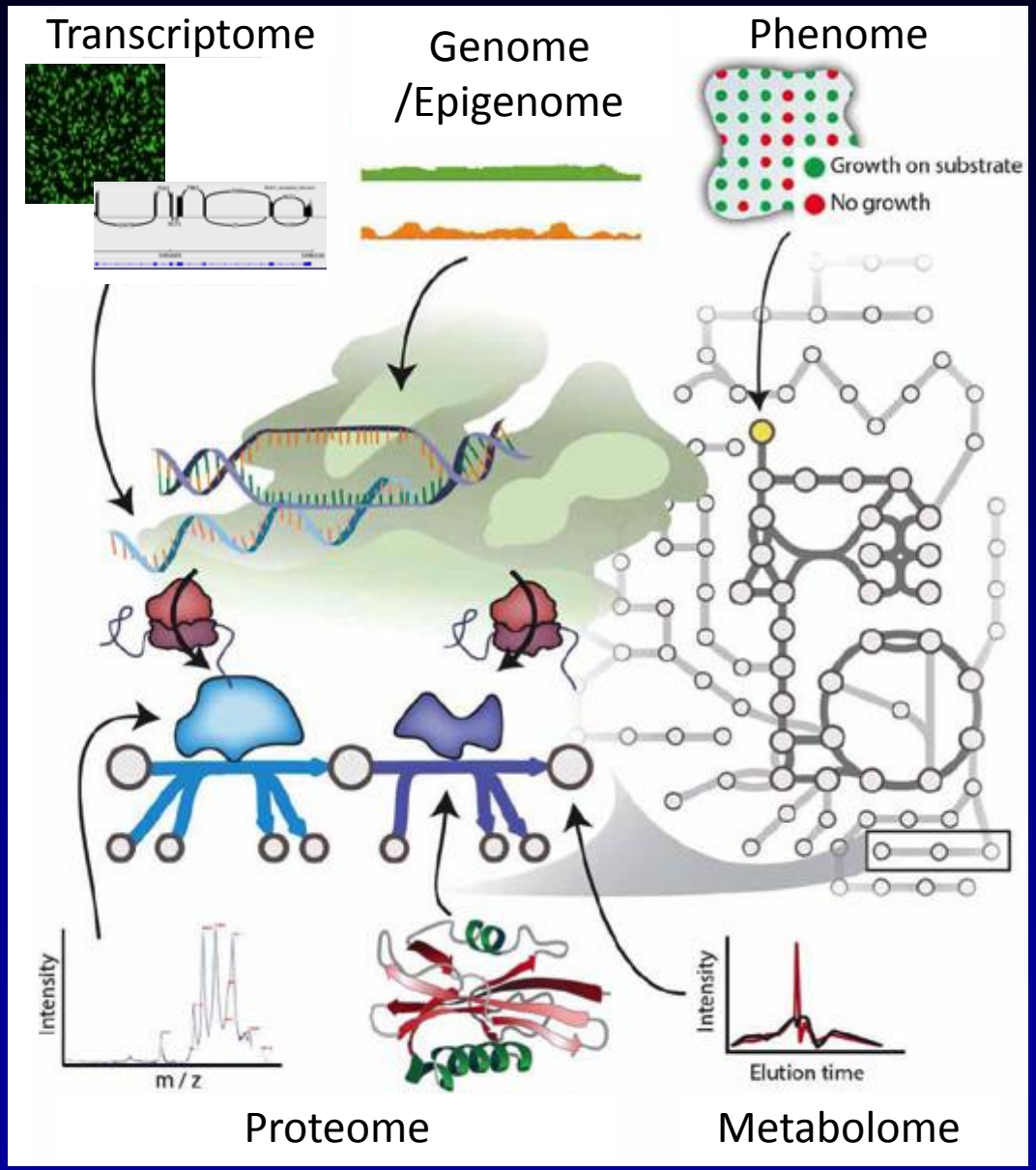
Systems Biology



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.

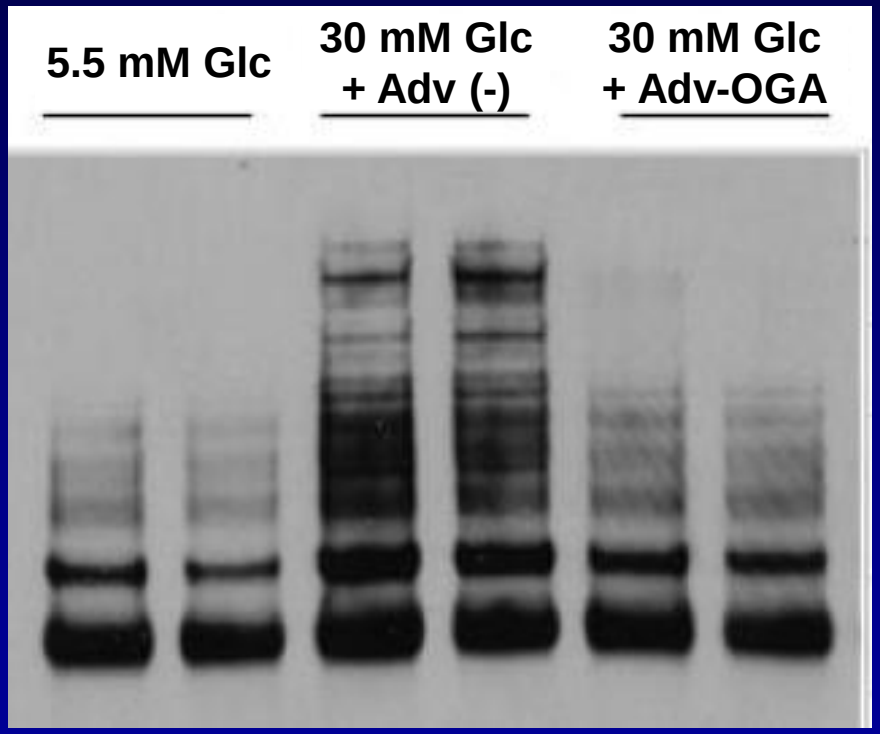
Genome / Epigenome

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

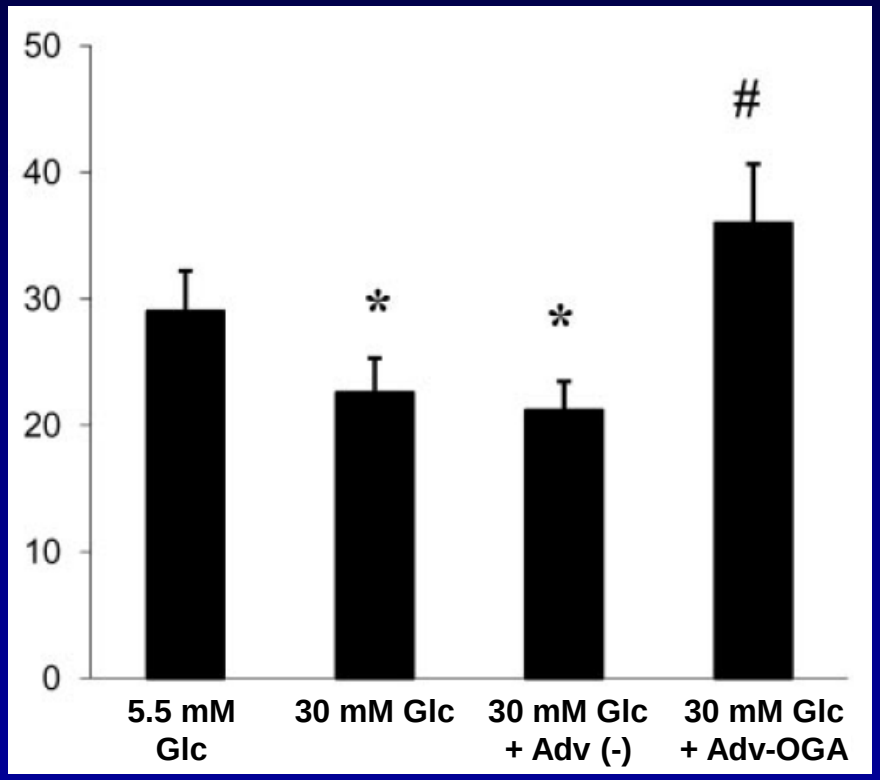
Adapted from Lewis and Abdel-Haleem **2013** *Front Physiol* 4:237

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

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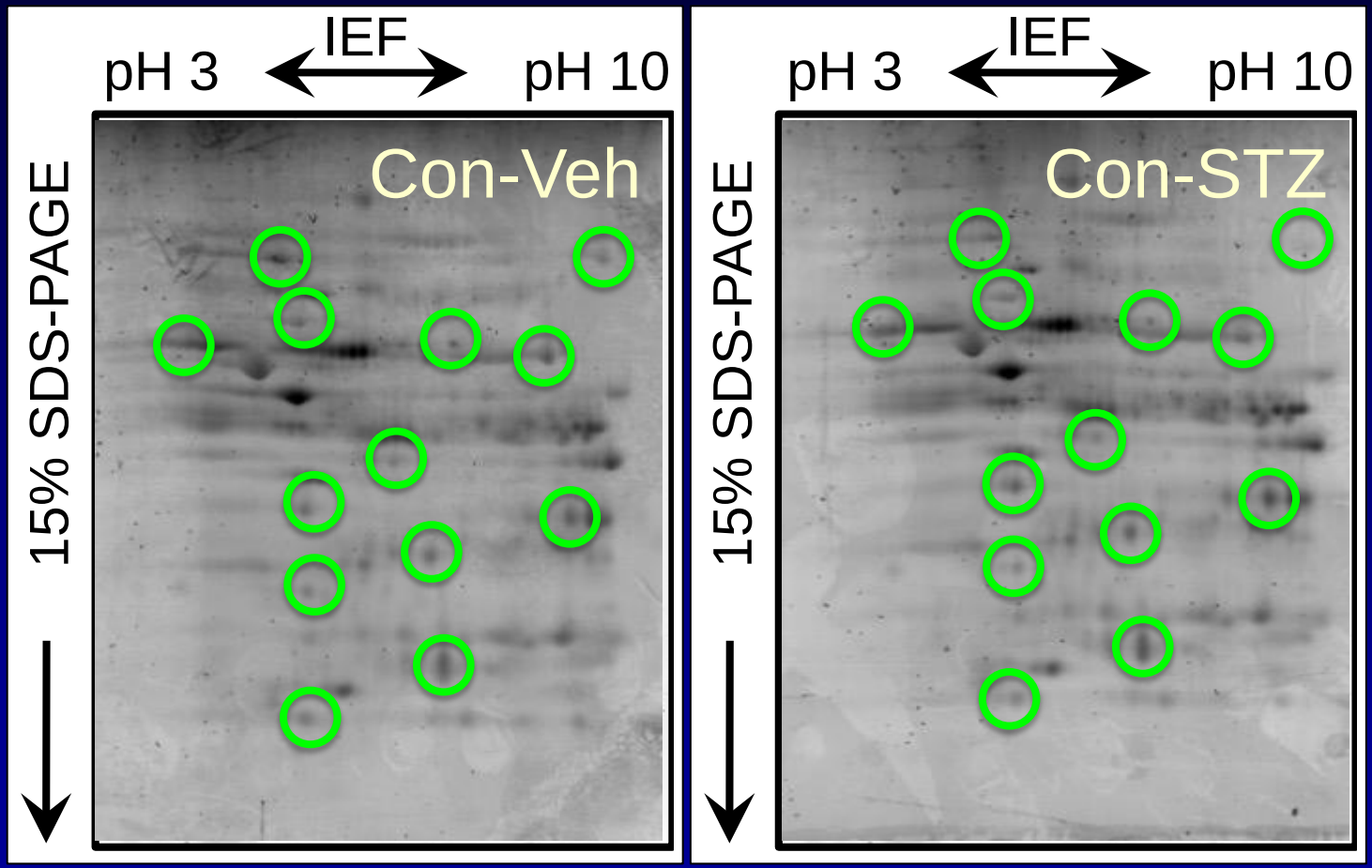
GLUT4 Induction Alters the Cardiac Mitochondrial Glycoproteome

Isolated Mitochondria 2D-PAGE Pro-Q Emerald



GLUT4 Induction Alters the Cardiac Mitochondrial Glycoproteome

Symbol	Description	Gene ID	*O*-GlcNAc Target	LC-MS/MS PTM	Pathway
ATP5A1	ATP synthase F1 complex, α subunit 1	11946	known[1,2]	Ac-	OXPHOS
ATP5H	ATP synthase F0 complex, subunit d	71679	novel	Ac-	OXPHOS
ATP5O	ATP synthase F1 complex, O subunit	28080	novel		OXPHOS
ETFB	Electron transferring flavoprotein, β polypeptide	110826	novel	Ac-	OXPHOS
UQCRC1	Ubiquinol-cytochrome c reductase core protein 1	22273	known[1]		OXPHOS
UQCRFS1	Ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	66694	novel		OXPHOS
MDH2	Malate dehydrogenase 2, NAD (mitochondrial)	17448	novel	Ac-	TCA
OGDH	Oxoglutarate dehydrogenase (lipoamide)	18293	known[3]	P-	TCA
SUCLA2	Succinate-Coenzyme A ligase, ADP-forming, β subunit	20916	novel	Ac-, P-	TCA
DLAT	Dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex)	235339	known[1]		Glycolysis/ TCA
ACADL	Acyl-Coenzyme A dehydrogenase, long-chain	11363	novel	P-	FAO
HADHB	Hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), β subunit	231086	novel	P-	FAO

PTM = post-translational modification; Ac- = acetylation; P- = phosphorylation; OXPHOS = Oxidative phosphorylation; TCA = Tricarboxylic acid cycle; FAO = Fatty acid β-oxidation

[1]Clark et al 2008 J Am Chem Soc 130(35): 11576; Previously identified modification by *O*-GlcNAc in rat brain and HeLa cells.

[2]Teo et al 2010 Nat Chem Biol 6(5):338; Previously identified modification by *O*-GlcNAc in rat liver.

[3]Nandi et al 2006 Anal Chem 78(2):452; Previously identified modification by *O*-GlcNAc in HeLa cells.

dbOGAP http://cbsb.lombardi.georgetown.edu and YinOYang www.cbs.dtu.dk

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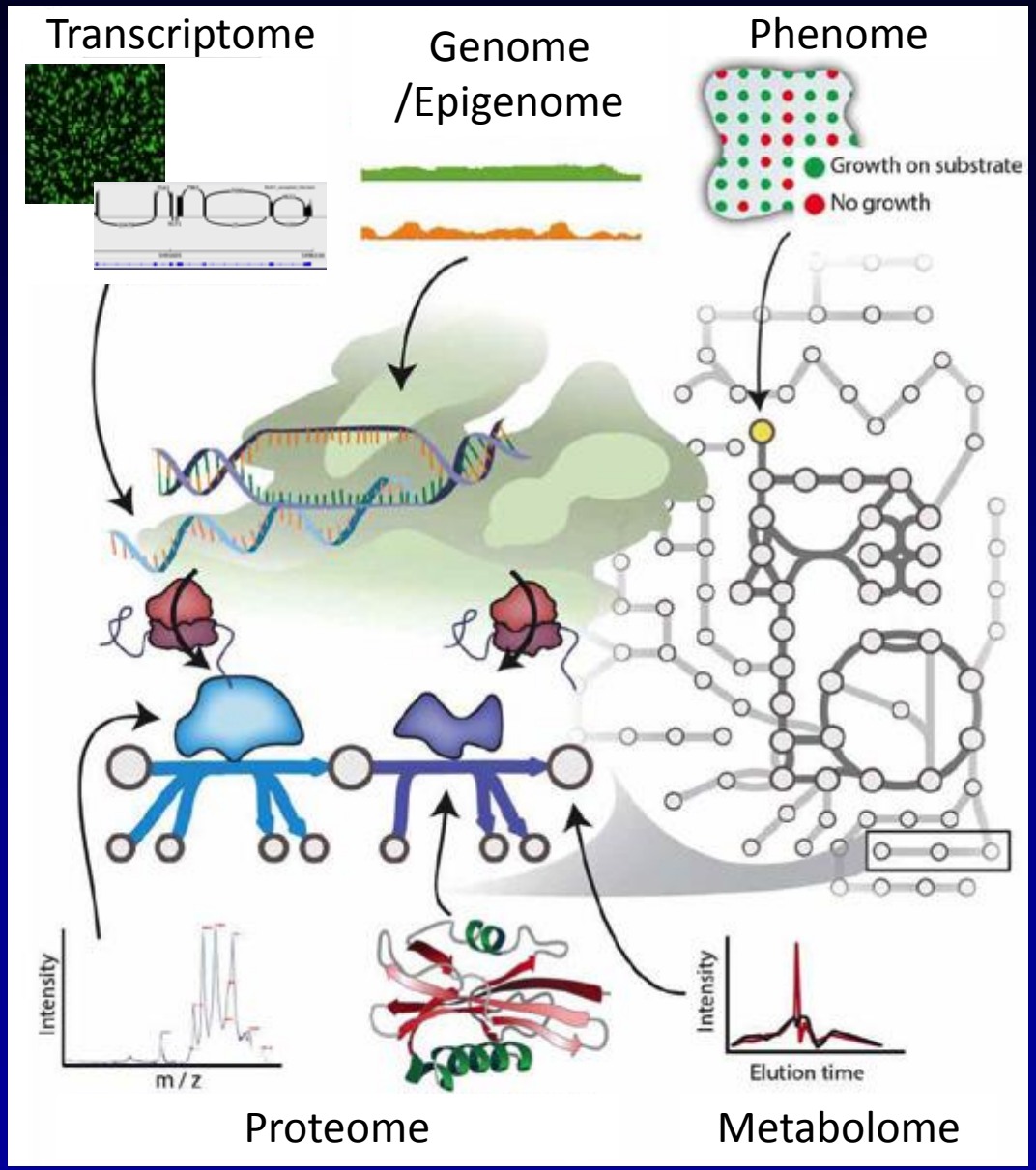
Conclusion – Part 3

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

Systems Biology



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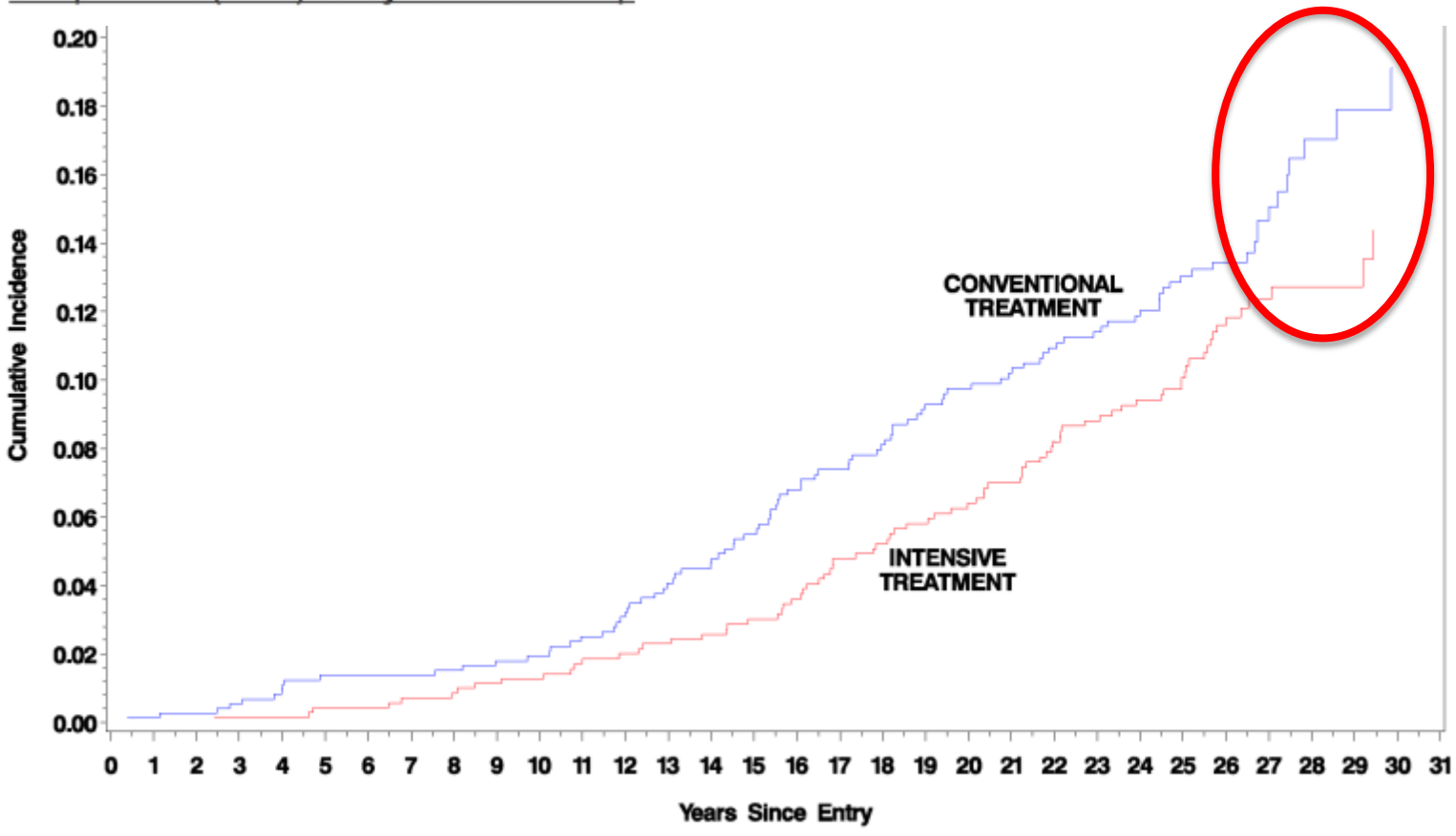
Glycemic Control, Epigenetics, and "Programming" of Cardiovascular Outcomes

Diabetes Care. 2016 May;39(5):686-93. doi: 10.2337/dc15-1990. Epub 2016 Feb 9.

Intensive Diabetes Treatment and Cardiovascular Outcomes in Type 1 Diabetes: The DCCT/EDIC Study 30-Year Follow-up.

Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group.



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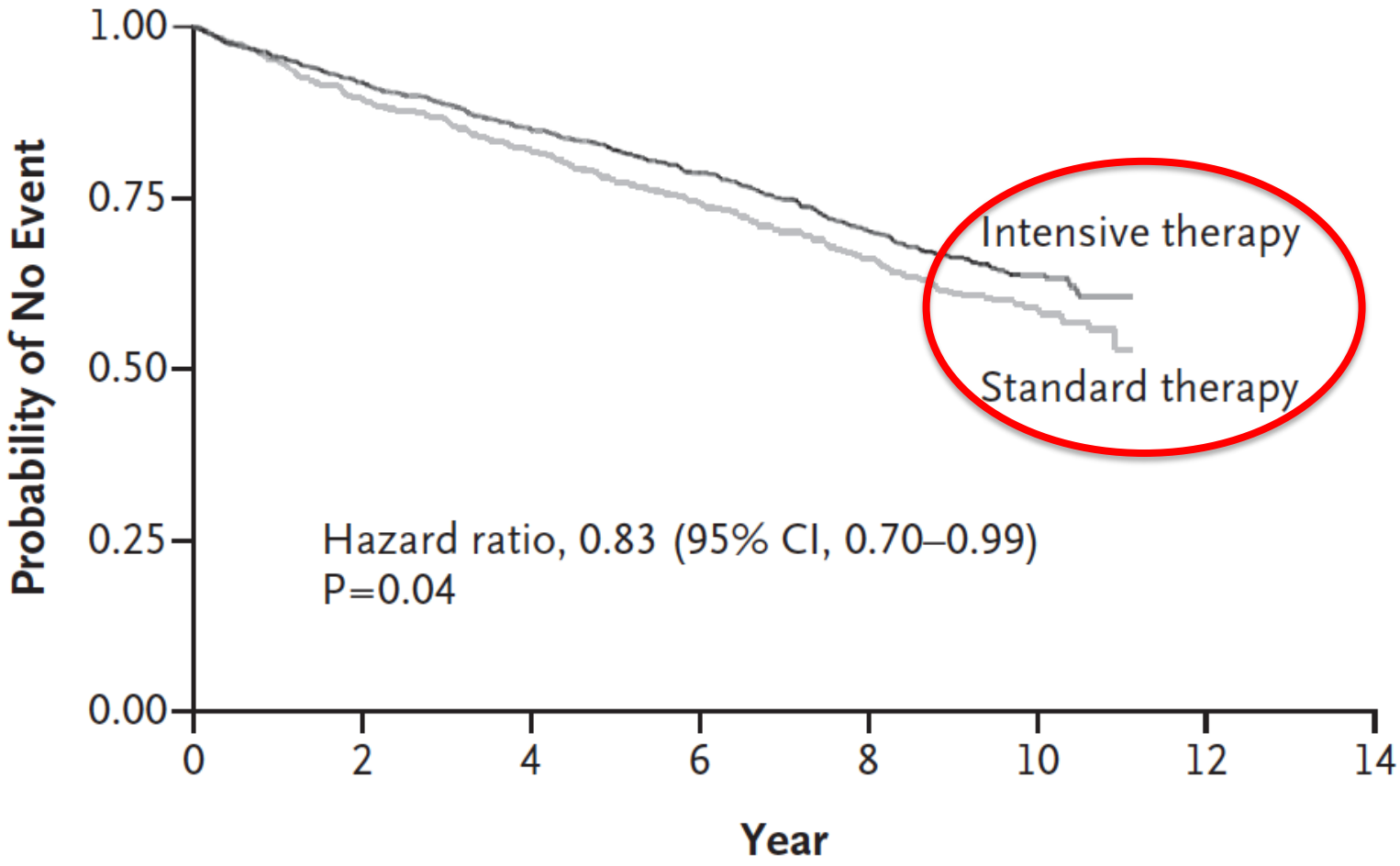
Glycemic Control, Epigenetics, and "Programming" of Cardiovascular Outcomes

N Engl J Med. 2015 Jun 4;372(23):2197-206. doi: 10.1056/NEJMoa1414266.

Follow-up of glycemic control and cardiovascular outcomes in type 2 diabetes.



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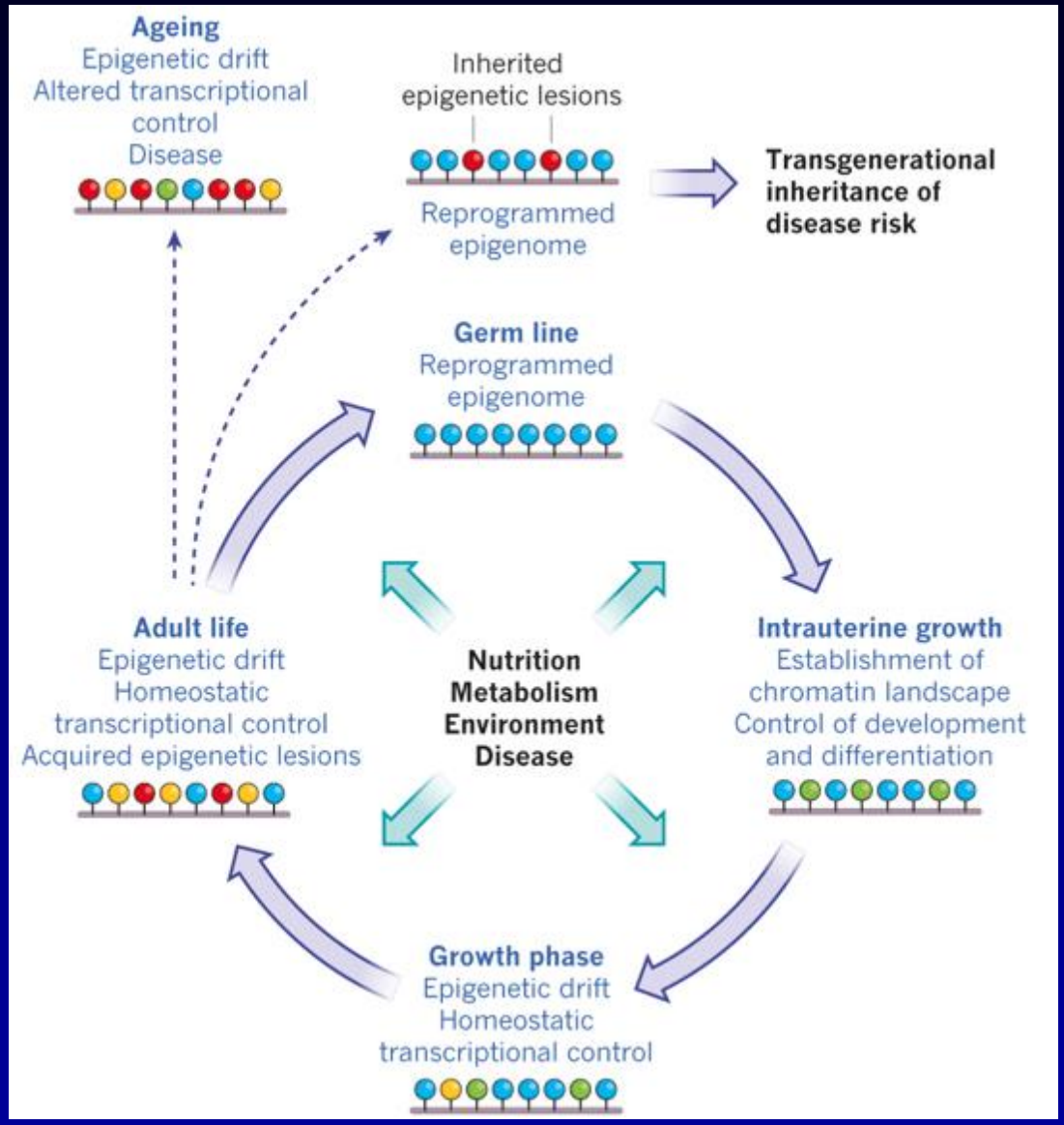
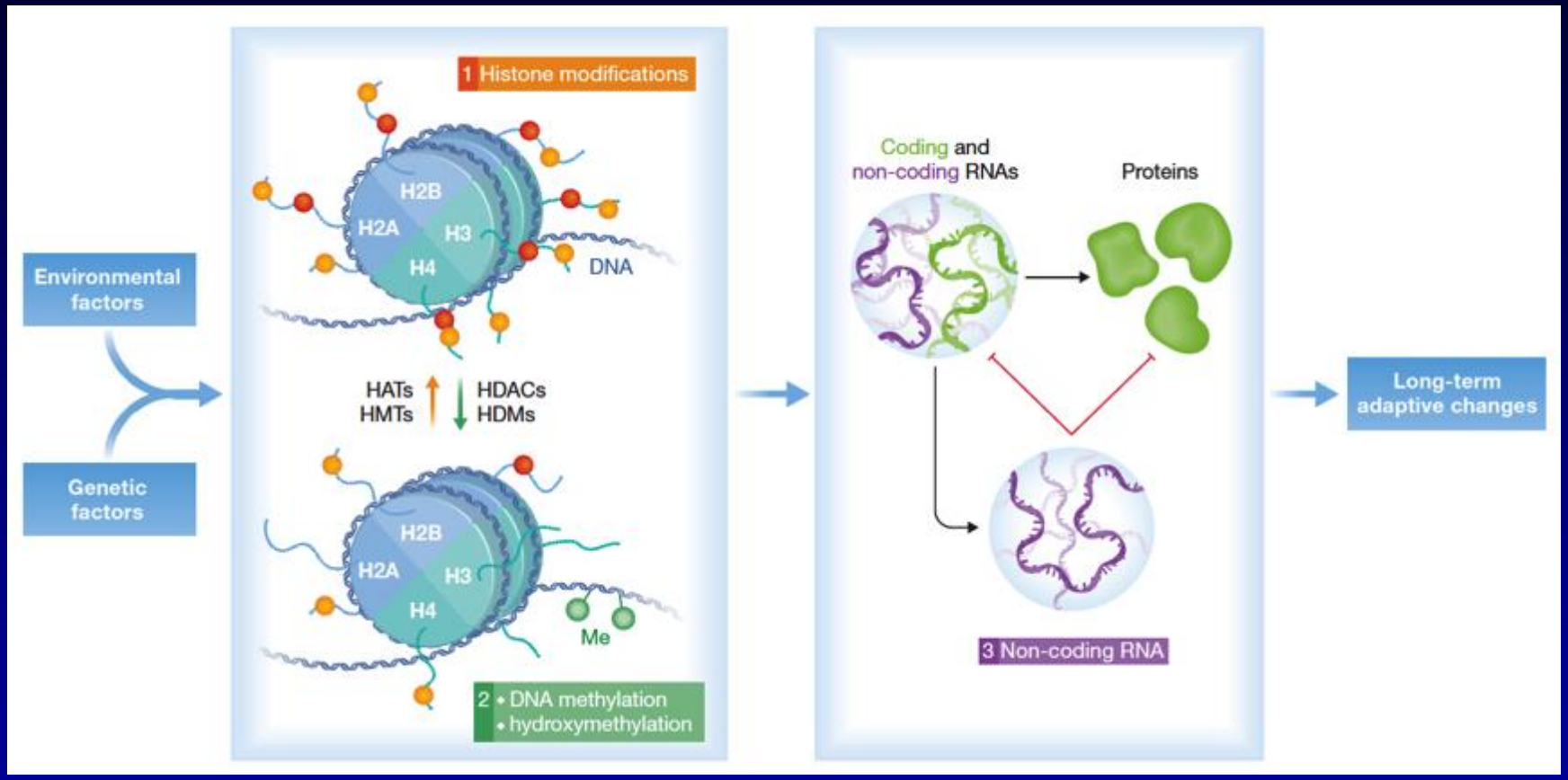
Epigenetics: Transgenerational and Drift



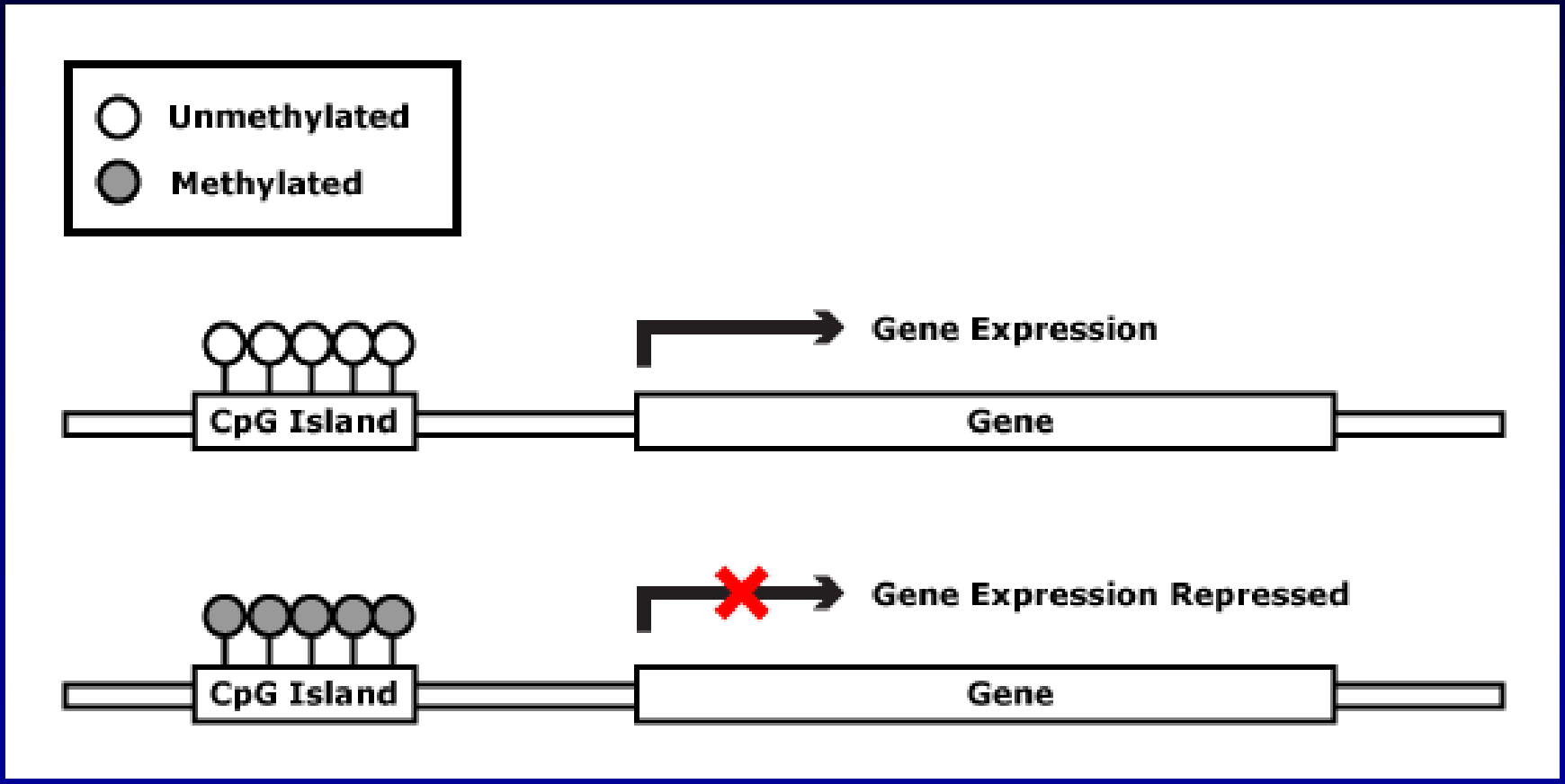
Gut and Verdin ***2013*** *Nature* 502:489

Epigenetic Code



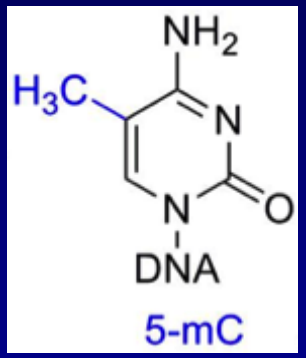
Fischer **2014** *EMBO J* 33(9):945:489

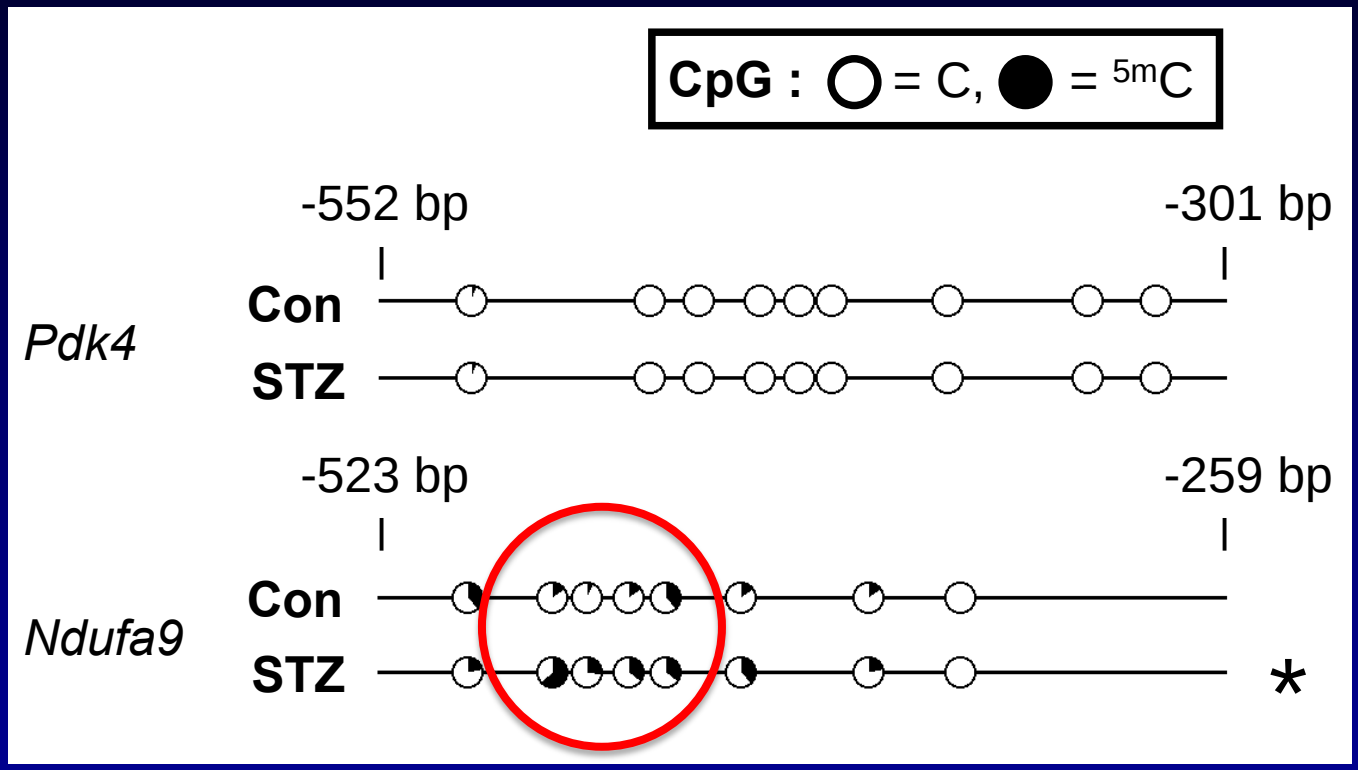
DNA Methylation 101



Diabetes Regulated Cardiac DNA Methylation

Targeted bsDNA-seq 5-mCpG





Heart, LV
n = 10
* P < 0.05

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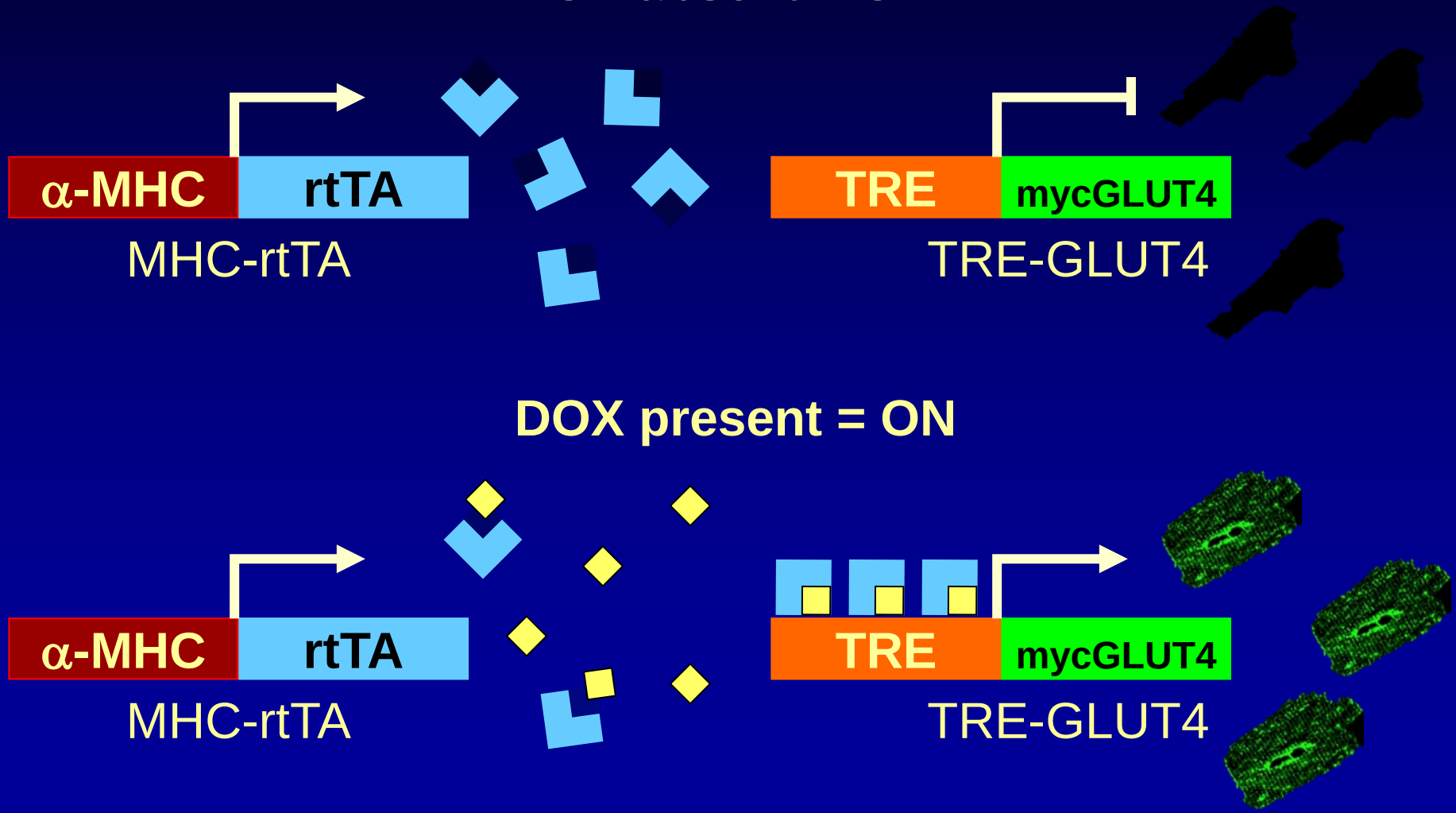
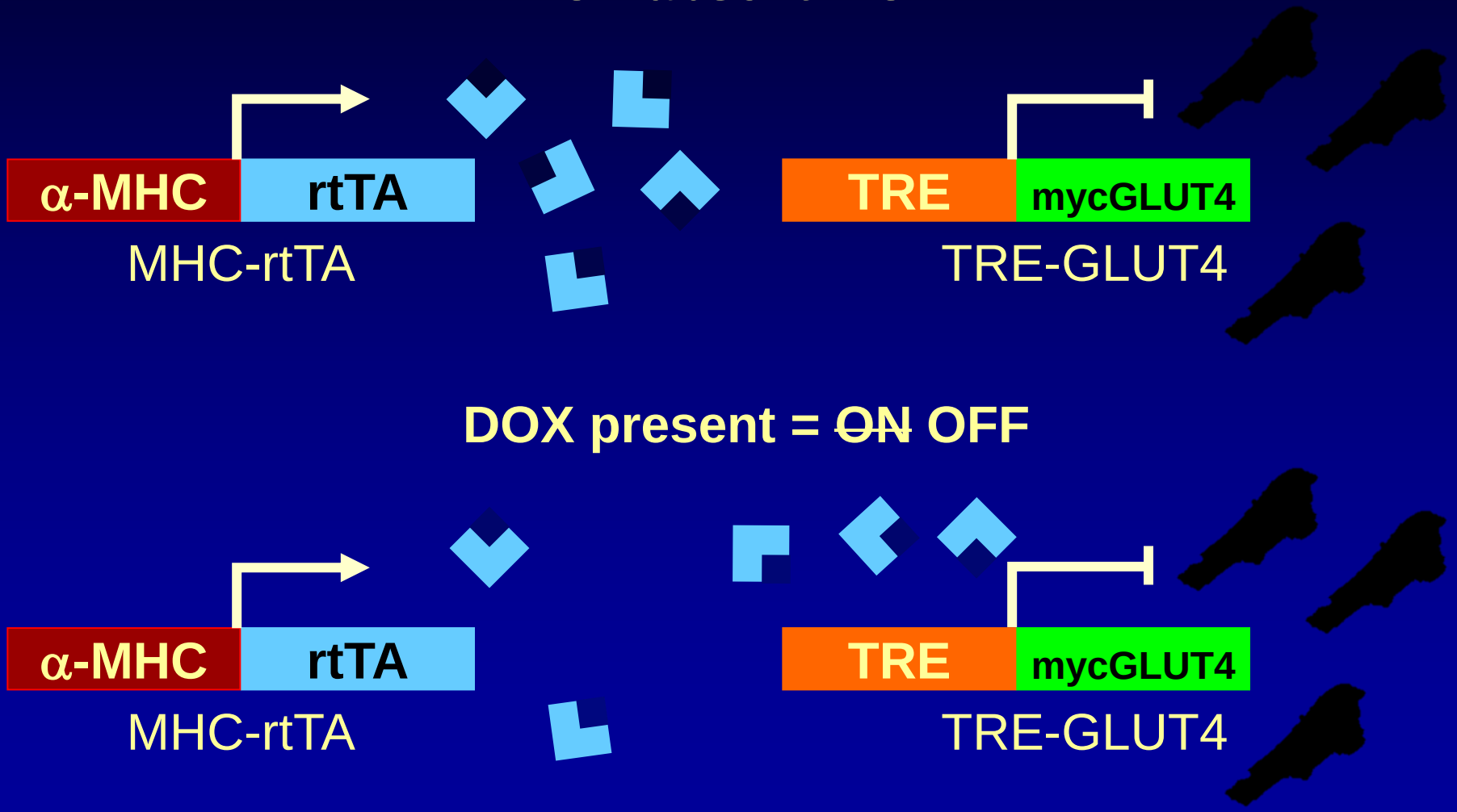
Where Does Glycemic Memory Fit In?

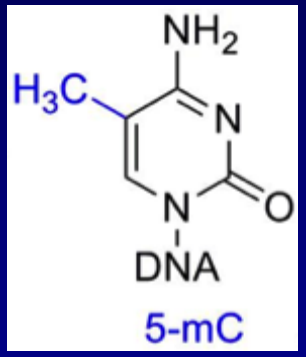


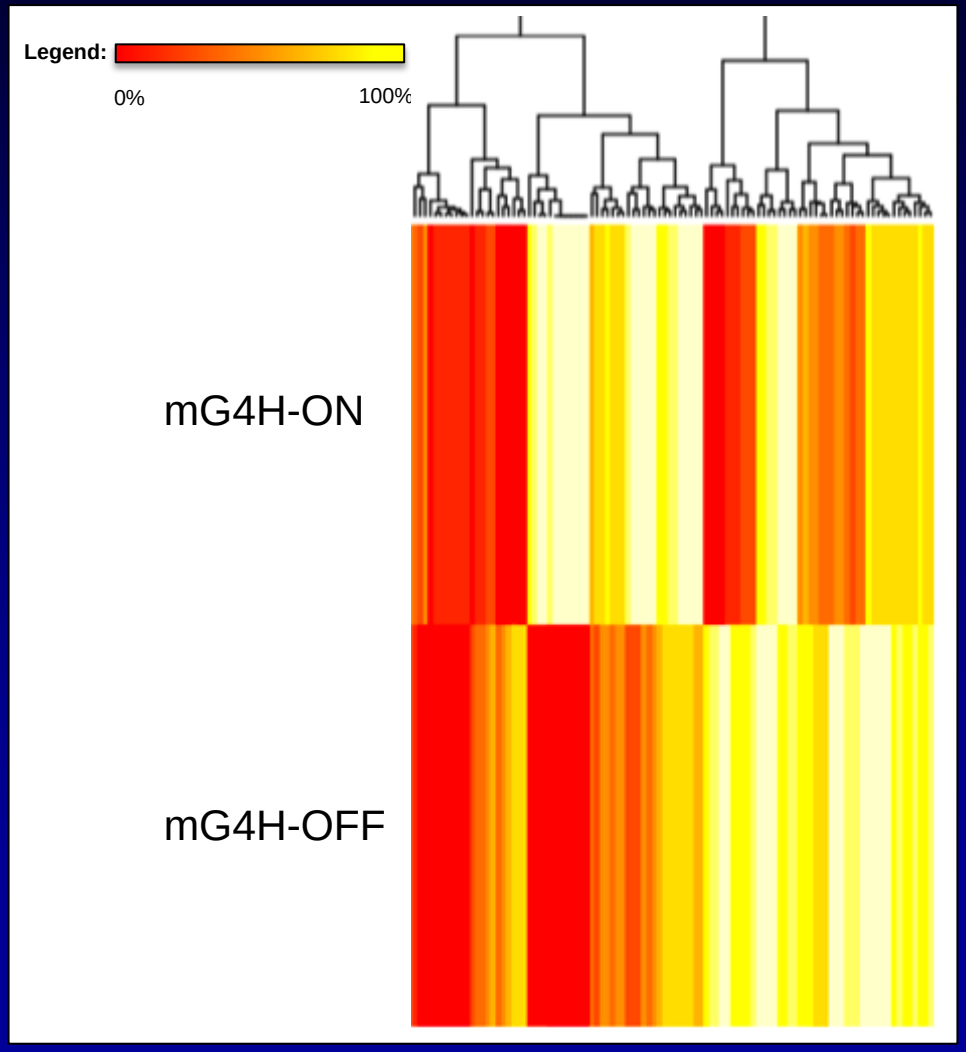
Where Does Glycemic Memory Fit In?



Glucose Cycling Alters Epigenetic Programming

Genomewide bsDNA-seq 5-mCpG





Heart, LV

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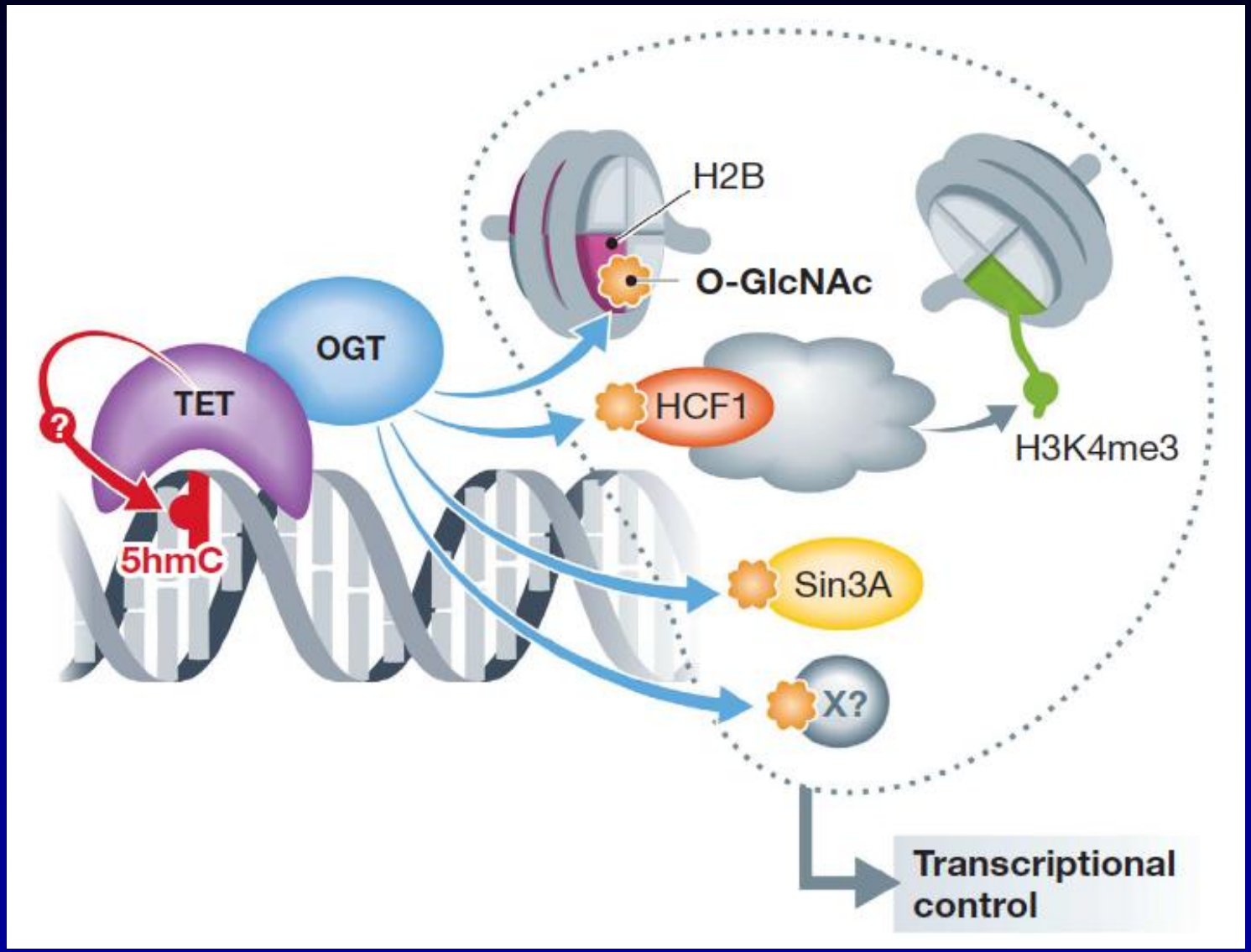
OGT:TET Mediate DNA "Tailoring"



Mariappa … Aalten **2013** *EMBO J* 32:612

From Human to Mouse and Back Again

Broad Institute Communications

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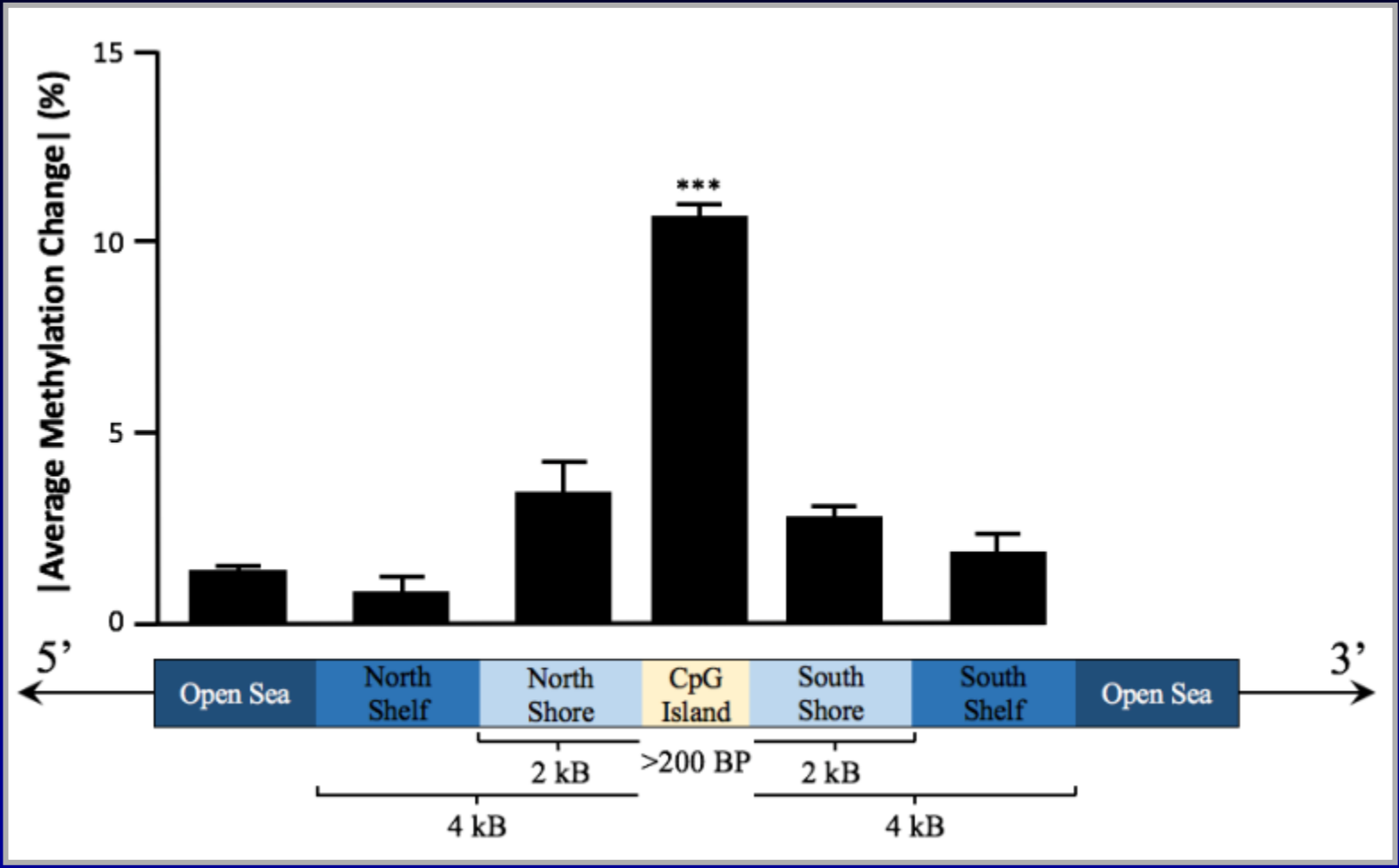
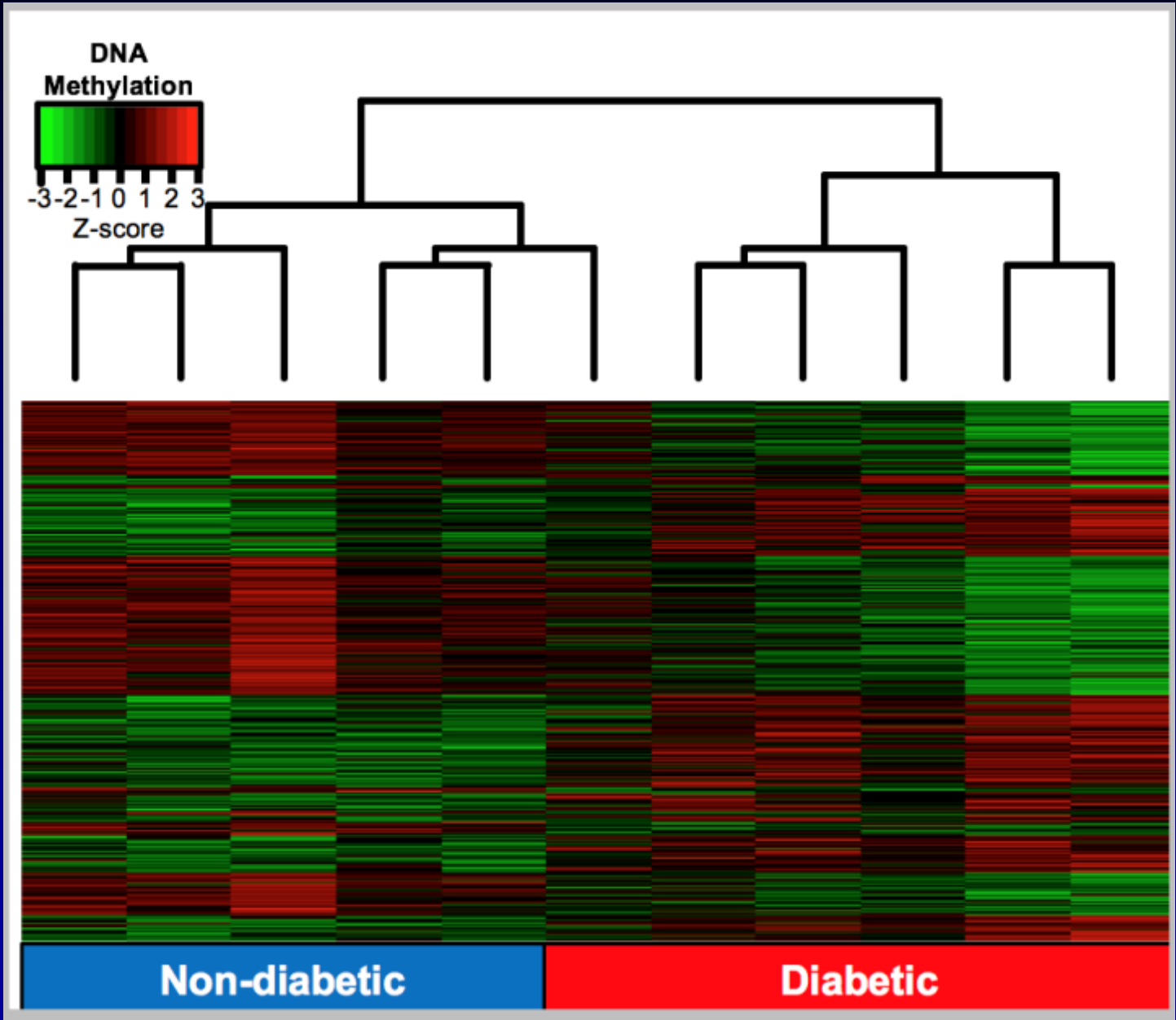
Left Ventricular Assist Device (LVAD)

Image from HeartWare International, Inc.

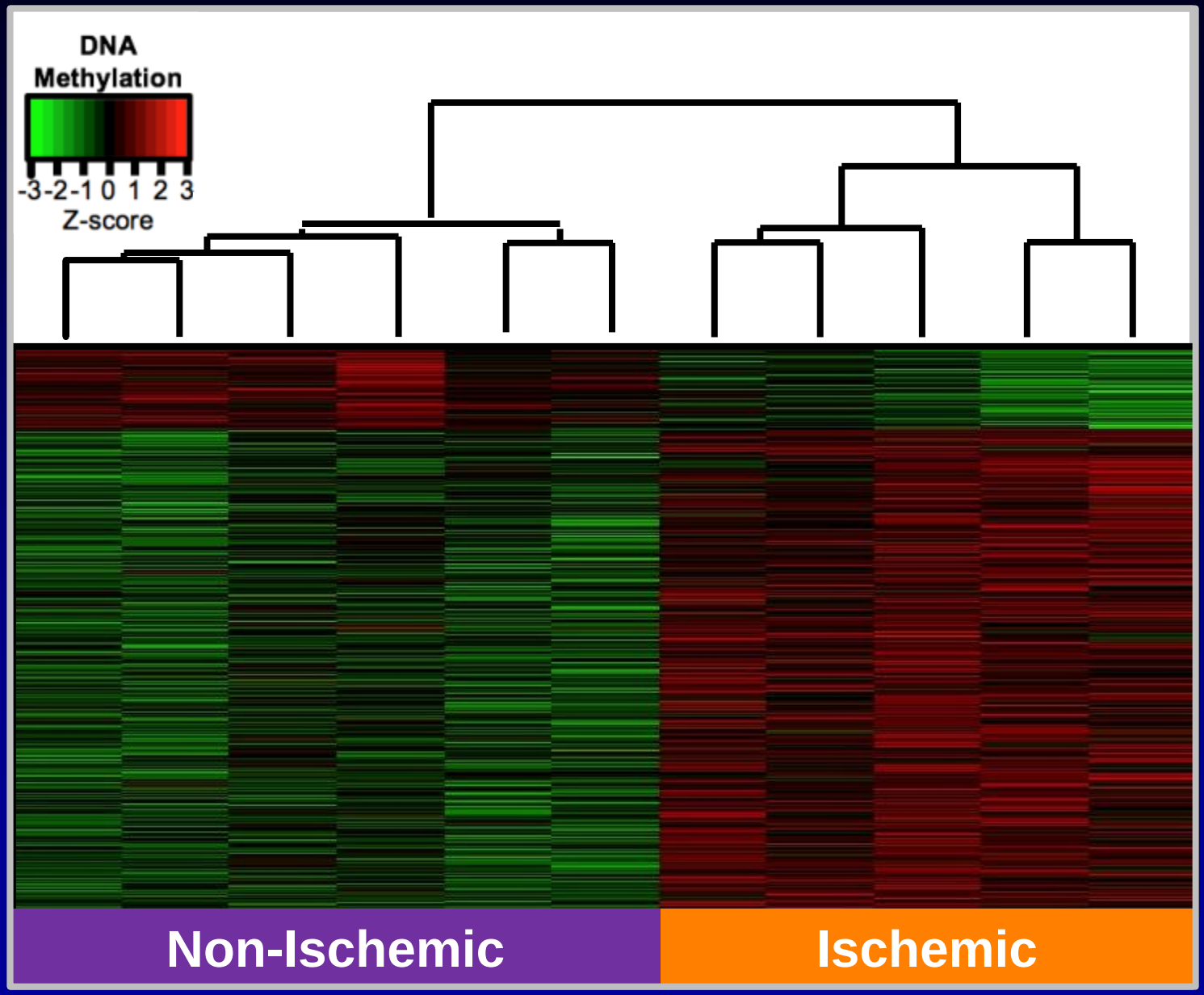
Human Cardiac DNA Methylation



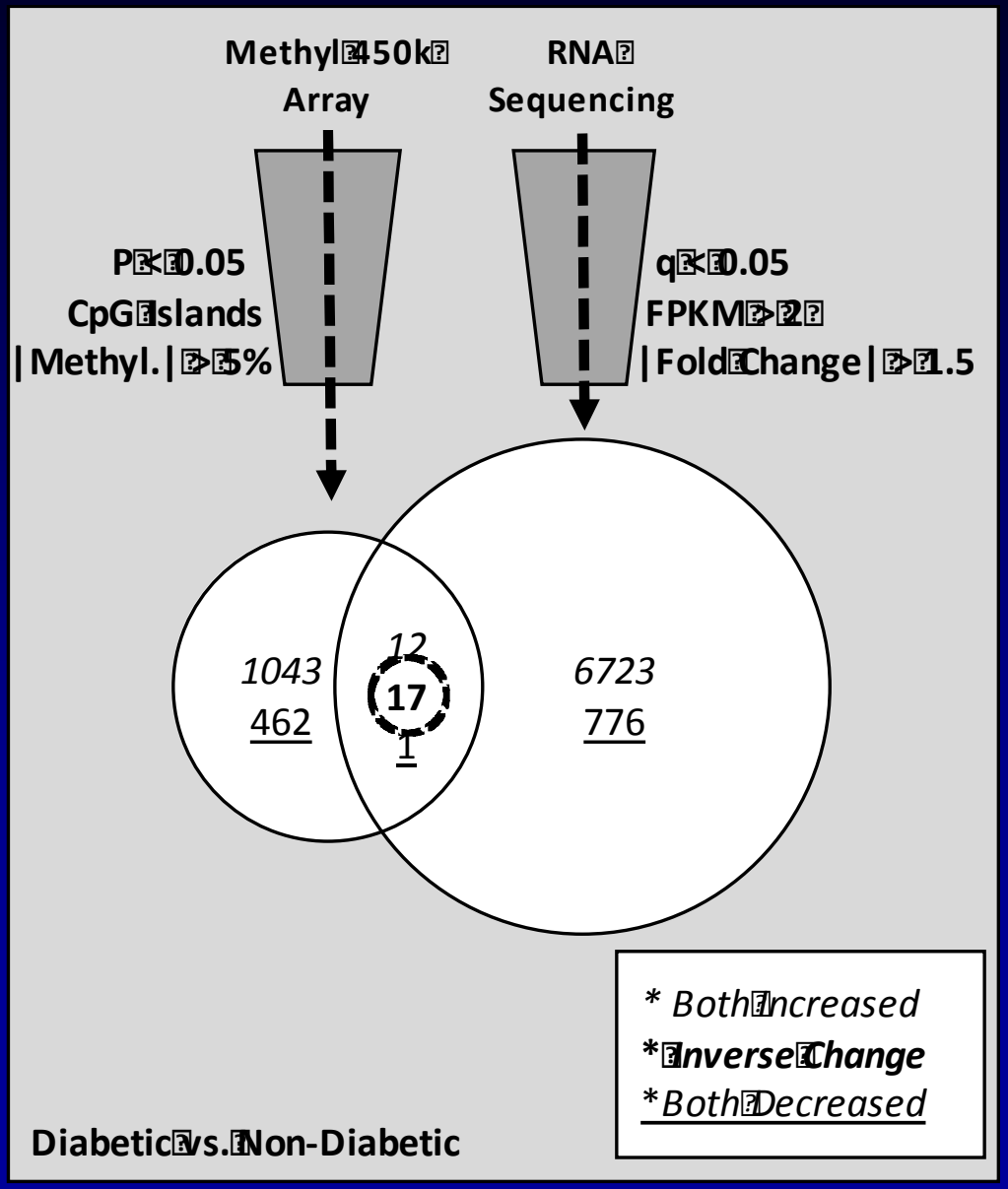
Human Cardiac DNA Methylation – Diabetes



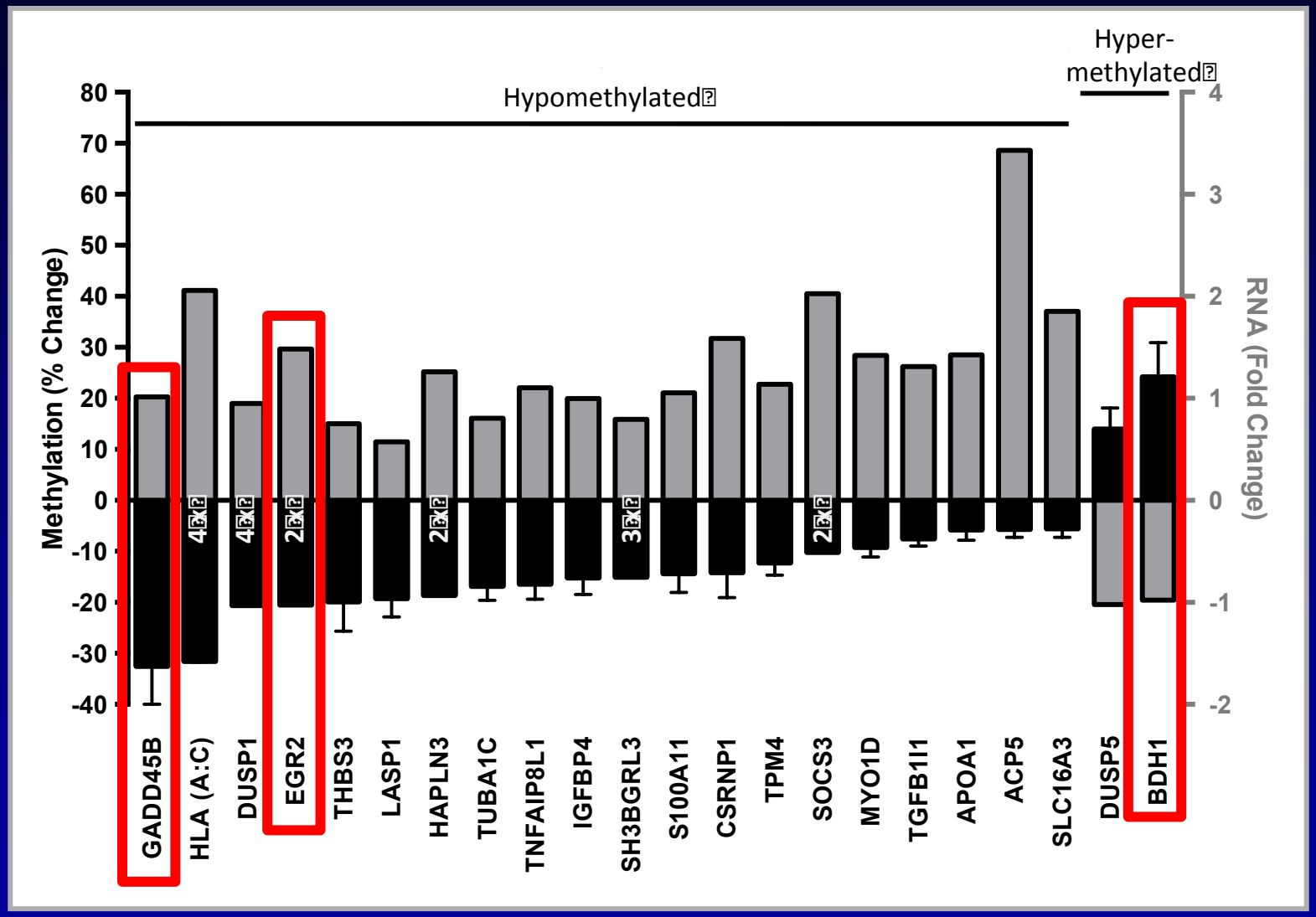
Human Cardiac DNA Methylation – Ischemic



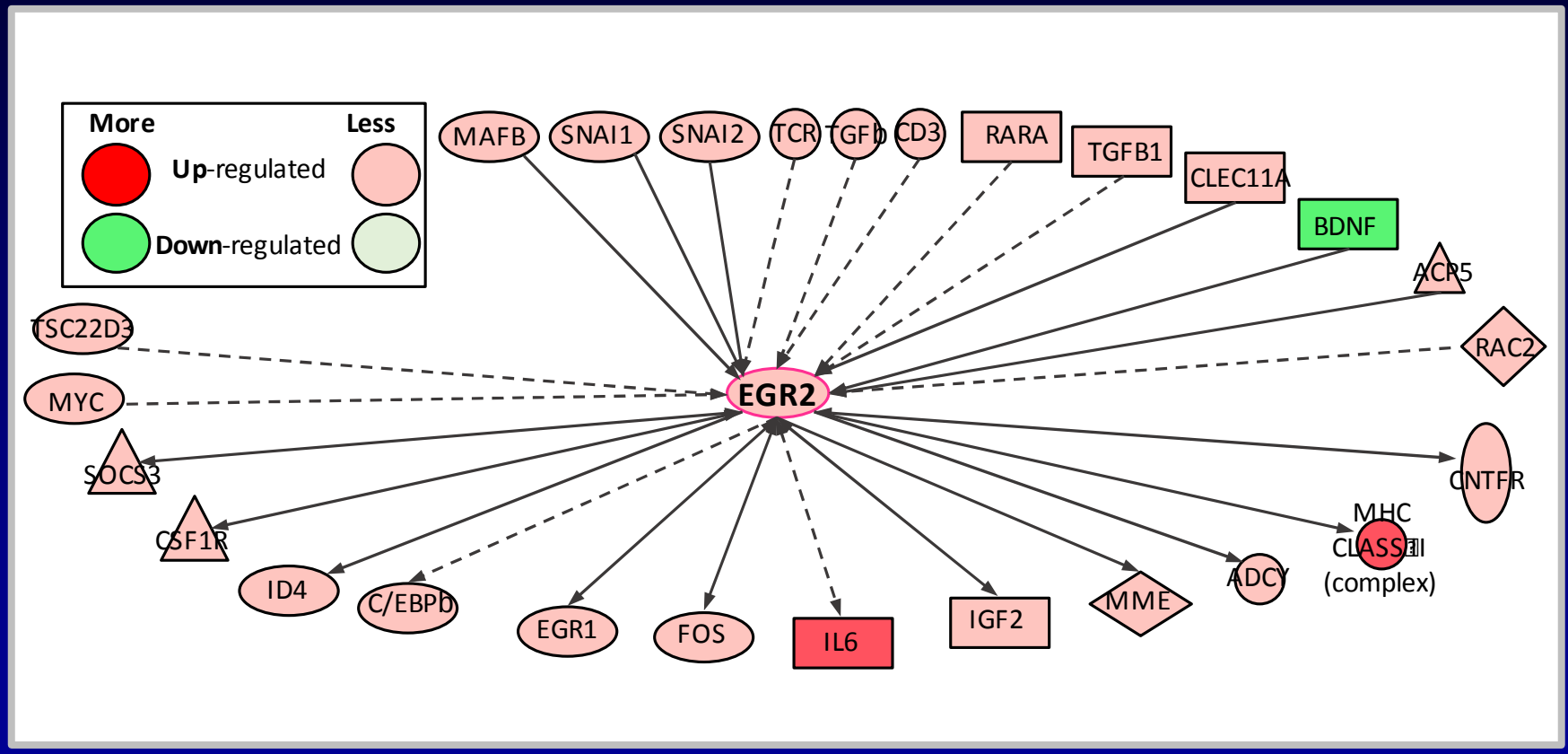
Relationship of Methylation to Gene Expression



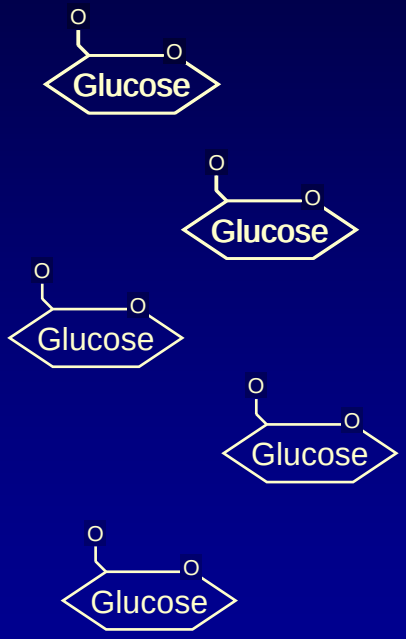
Combined DNA Methylation and Gene Expression in Diabetic Hearts



Other Nodal Pathways



Overall Conclusion: *Diabetes Mellitus*
To pass through; like honey “sweet”



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giphy.com

Summary and Conclusions

Enhanced glucose delivery in diabetes accelerates development of metabolic dysfunction.

The molecular mechanisms of this regulation are controlled at multiple levels including post-translational regulation of transcription factors.

Glucose alters DNA methylation in a manner that persists even after glucose has returned to normal.

There is a distinct cardiac methylome in response to diabetes and heart failure in both rodent models and human patients.

Acknowledgements

Manoja K. Brahma – Postdoc
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Brenna G. Nye – Undergrad
Mark Pepin – MSTP
Lamario J Williams – Undergrad

You? Looking for motivated postdoc

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

E. Dale Abel – U of Iowa

John C. Schell – U of Utah
Joseph Tuinei – U of Utah
many others…

Hansjörg Schwertz – U of Utah

Oleh Khalimonchuk – UNL

Other Colleagues & Mentors

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