"Why is Eating Too Much Sugar So Toxic?"

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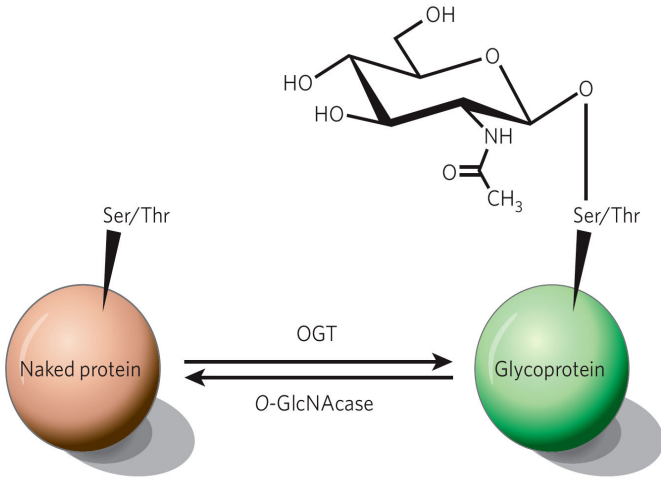
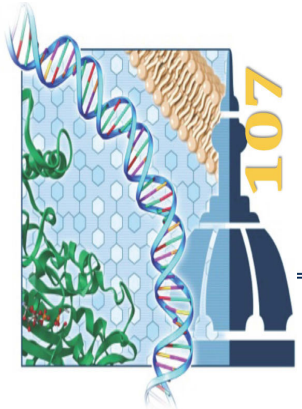
Disclosures: Supported by NIH R01DK61671; P01HL107153; N01-HV-00240; R01CA42486; *Dr. Hart receives a share of royalty received by the university on sales of the CTD 110.6 antibody, which are managed by JHU.*





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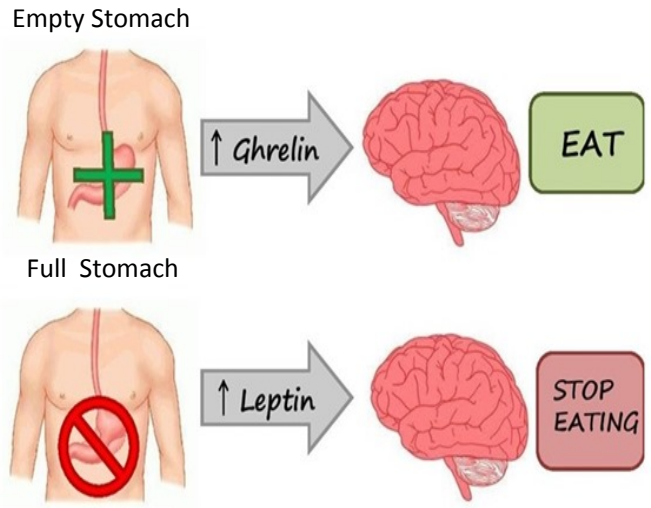
Natl Press Club – May 2016

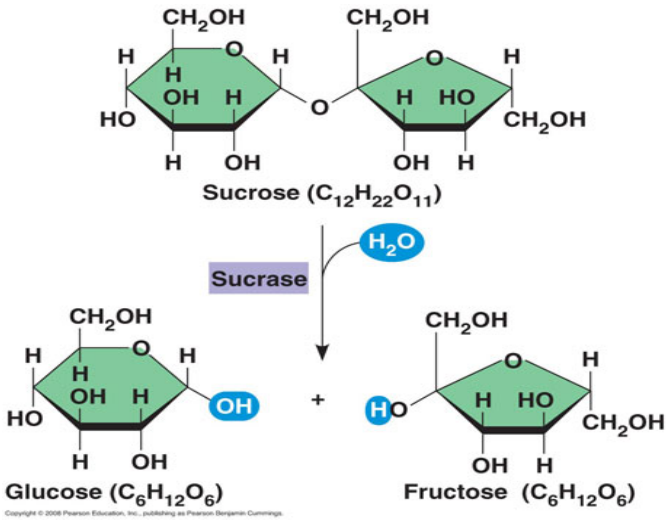
Sugar Toxicity

- Americans consume about **140 lbs. of sugar annually** (57 gallons of soda!).
- One hundred years ago we consumed **~10 lbs**.
- Mostly **sucrose, glucose and fructose.**
- **Fructose**, is particularly toxic - especially High Fructose Corn Syrup – **cheap and sweet**!
  - **ubiquitous** in almost all **processed foods**: soda, fruit juices, cereals, ketchup, jellies, graham crackers, breads, most chocolate milk, and many others – kids eat a LOT of fructose!
- **Eating Sugar Makes Us Hungry -** it interferes with three hormones—**ghrelin, leptin** and dopamine—all of which signal our brain that we have had enough to eat.



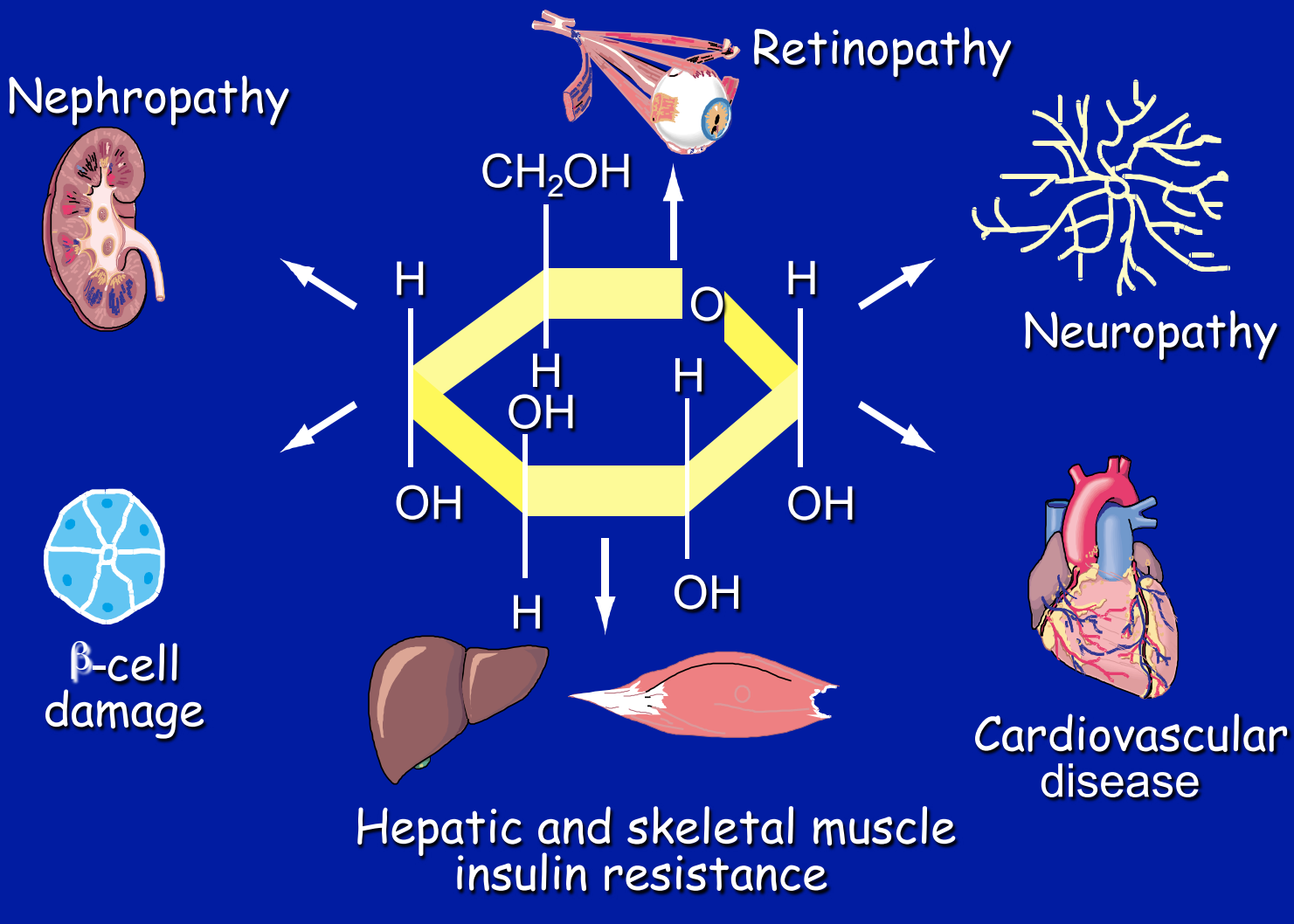
Glucose Stimulates Insulin Signaling to Up-Regulate Leptin Which Tells you to **Stop Eating.**

Fructose is metabolized In the Liver mostly to **Fat** And it **Does NOT stimulate Insulin Signaling to Stop Eating.**



http://www.eatchicchicago.com/blog/2014/01/21/is-fat-or-sugar-making-us-fat/
http://www.joearrigo.com/2012/09/11/the-toxin-that-is-sugar/

Sugar Toxicity: Chronic High Blood Sugar

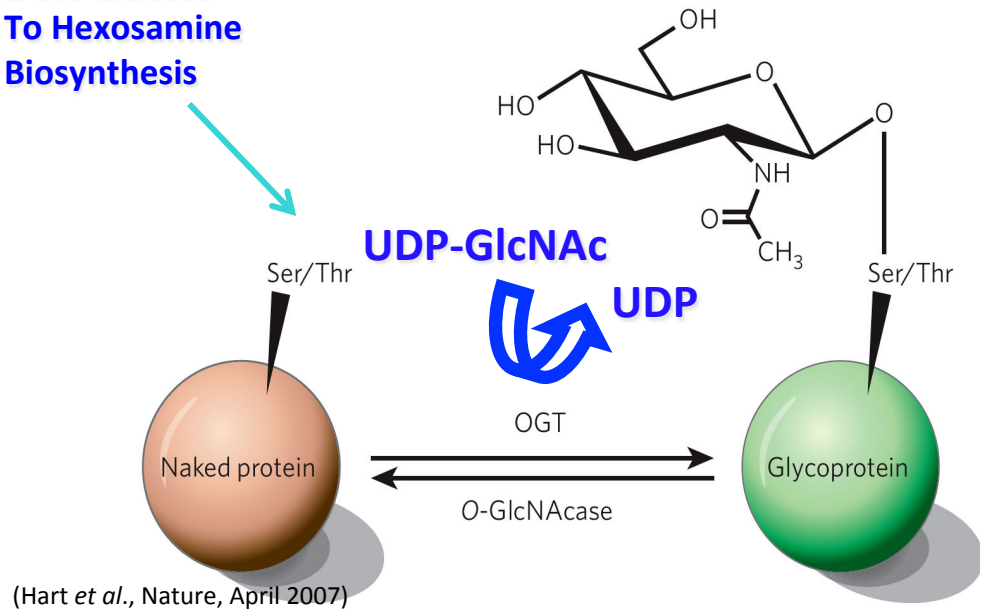


Why is Prolonged High Blood Sugar So Toxic?

Prof. John Betteridge, MD
University College London

Properties of O-GlcNAc.

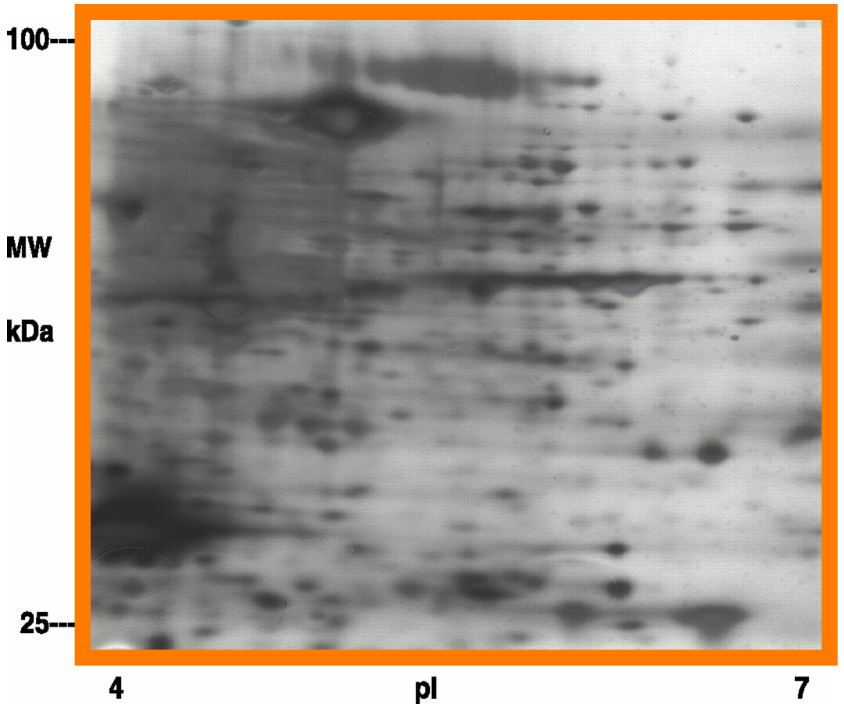
2-5% Glucose To Hexosamine Biosynthesis



(Hart *et al.*, Nature, April 2007)

O-GlcNAc is Abundant on Nuclear & Cytosolic Proteins

Pan >O-GlcNAc Antibody Western Blot - HeLa



- Highly Dynamic **Enzymatic** Modification of Ser and Thr residues by **β-N-acetylglucosamine**
- Localized to the **cytoplasm and nucleus on cell's regulatory proteins.**
- Highly abundant PTM (**>4000 identified proteins**) & Often Reciprocal (**Competitive) with phosphorylation** - Abundance = pancreas islets>>brain>>other tissues>liver.
- Dynamically **cycling** on Ser/Thr residues - **Time scale similar to phosphate.**

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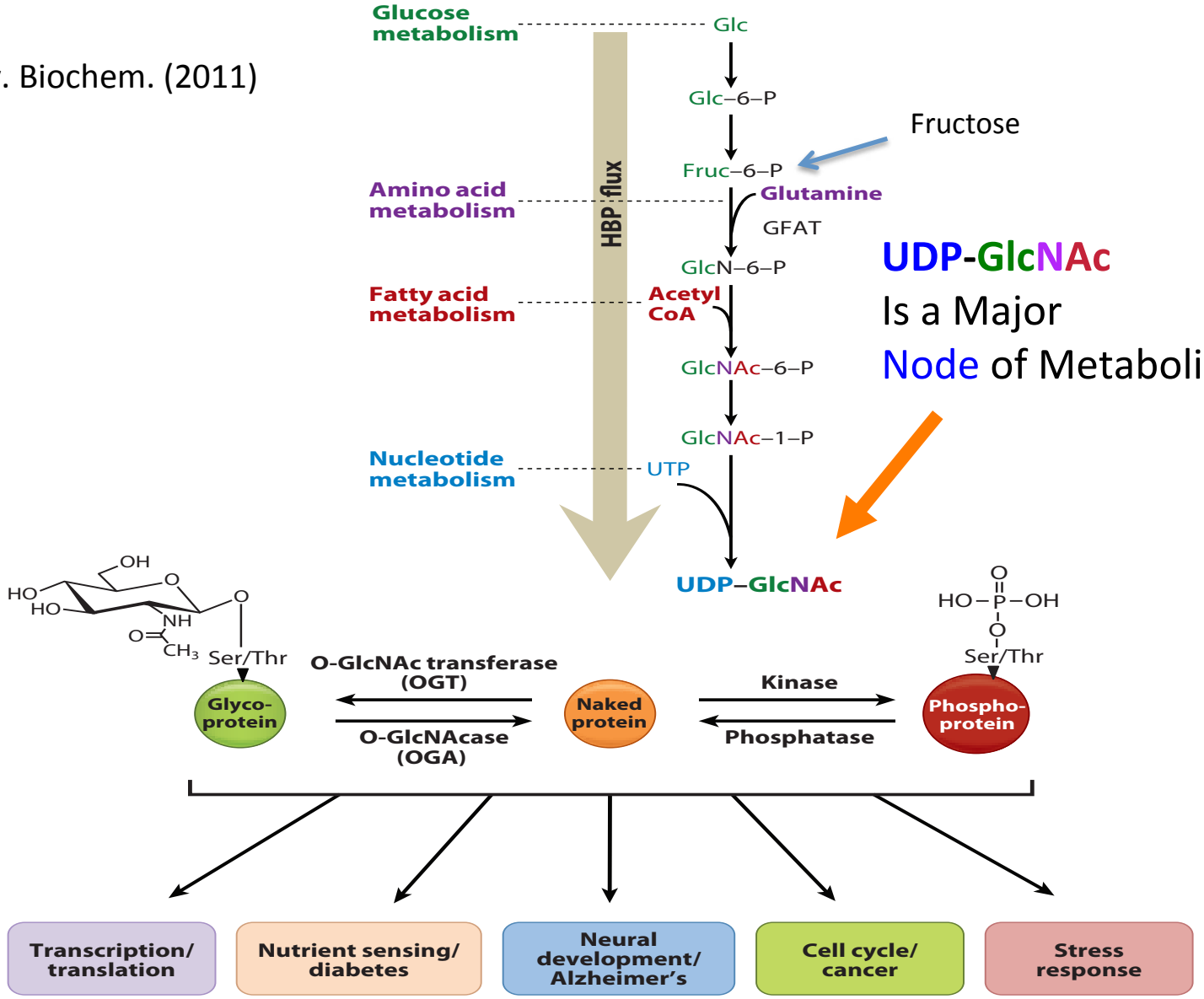
O-GlcNAc Has Extensive Crosstalk with Phosphorylation to Serve As A Nutrient Sensor that Regulates Many Cellular Processes

Ann. Rev. Biochem. (2011)

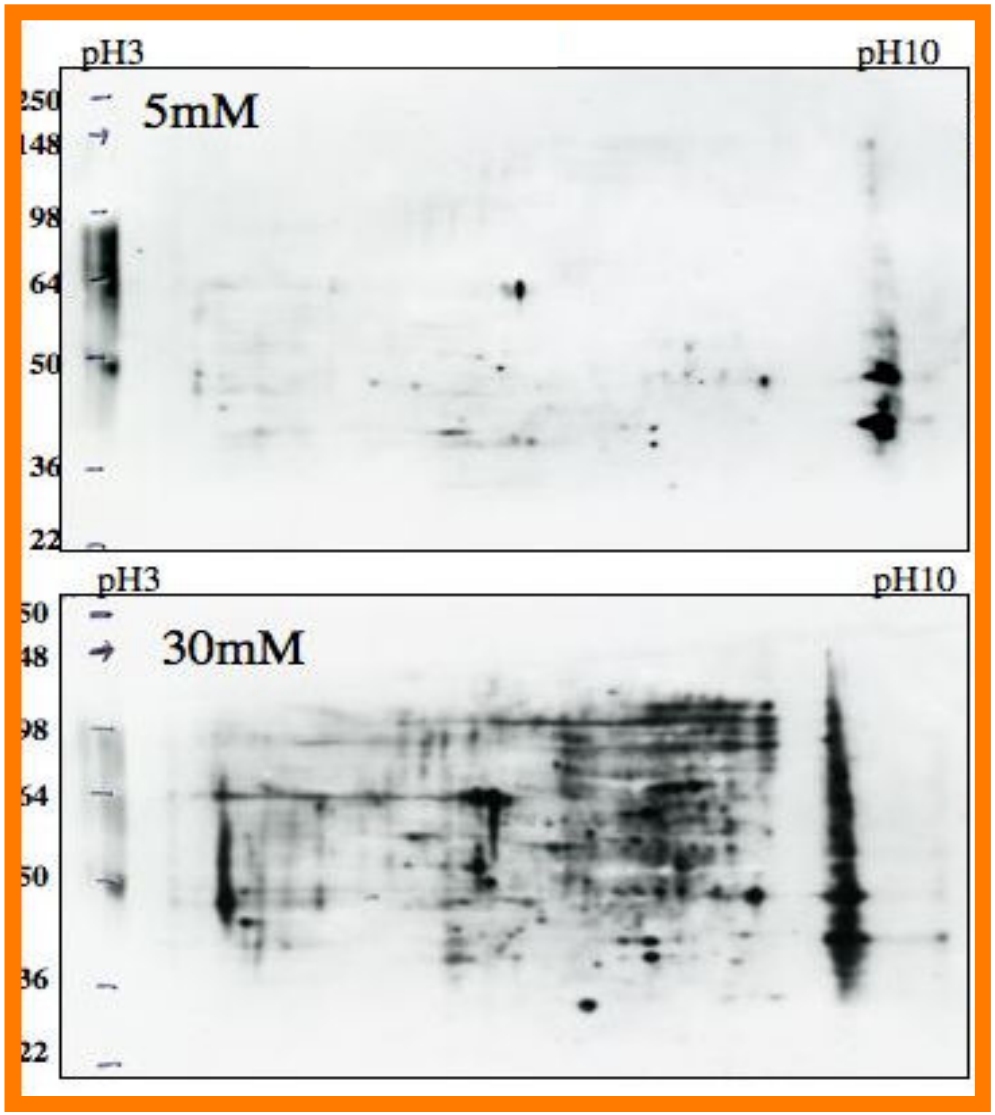


"O-GlcNAc or O-linked N-acetylglucosamine or hexosamine pathway" papers in PubMed = 1862 ~4000 proteins & ~4000 Sites Mapped & Counting!

High Glucose Increases O-GlcNAcylation on Many Proteins

Jurkat Lymphocytes Grown in Media With 5mM or 30mM Glucose

(Coomassie not different)



WB with Pan >O-GlcNAc Antibody:

Steady-State O-GlcNAc Increases on Many Proteins.

High Glucose Increased O-GlcNAc is a Major Mechanism of "Glucose Toxicity"

- Hyperglycemia, hyperlipidemia and hyperinsulinema all increase O-GlcNAcylation of many proteins.

Mechanisms:

- Signaling Molecules & Kinases – Balance with Phosphorylation is Disrupted.
- Transcription Factors and Histones – Altered Promoter Activities. Wrong Genes Expressed.
- Mitochondrial Electron Transport Proteins – ROS Production???...ROS in-turn increases O-GlcNAcylation.
  - Some Examples:

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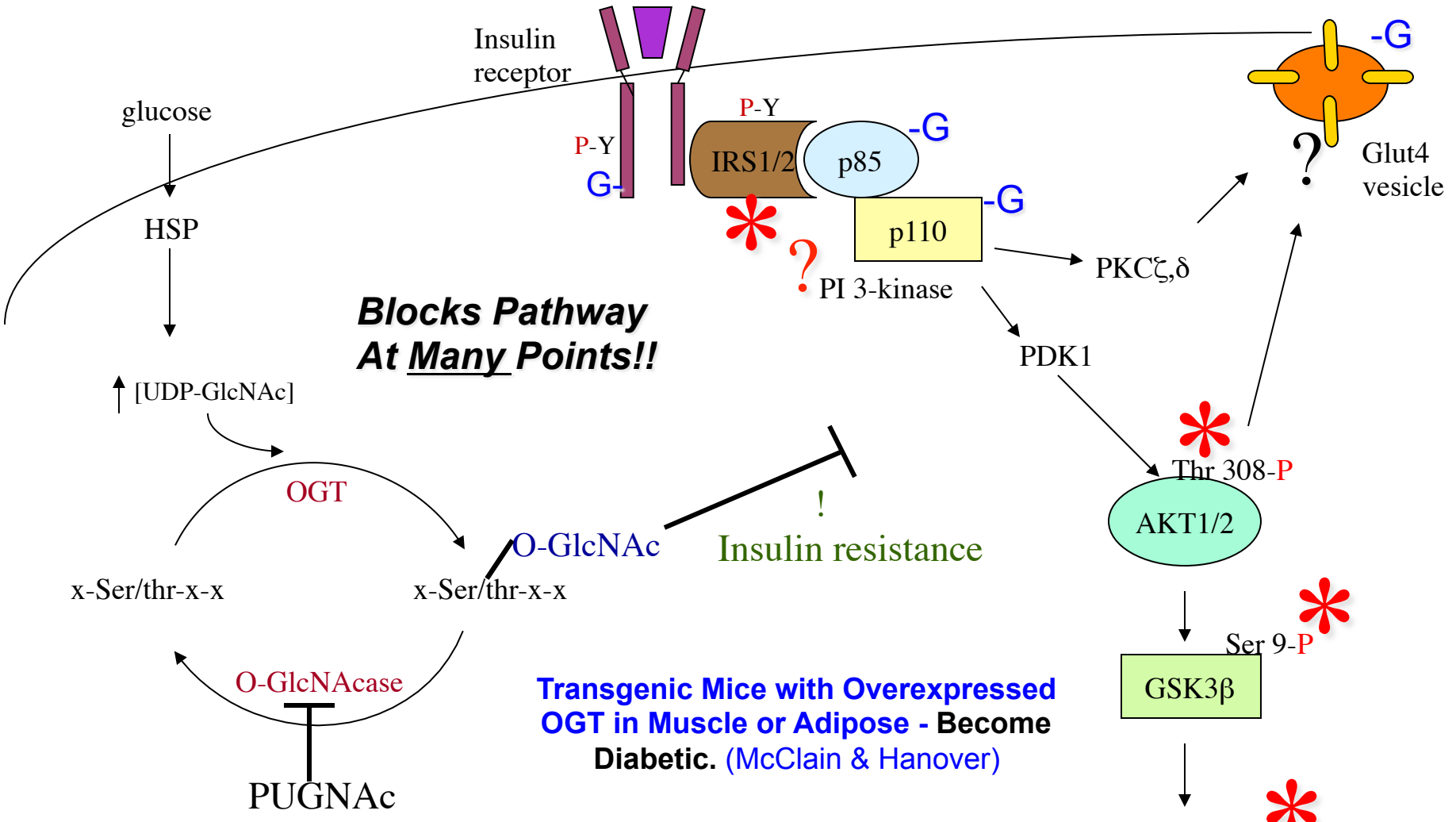
Elevation of O-GlcNAc Blocks Insulin Signaling:

•Blocks AKT phos. at T308 and S9 on GSK3ß

•Inhib. OGase greatly increases OG on ß-catenin and IRS1.

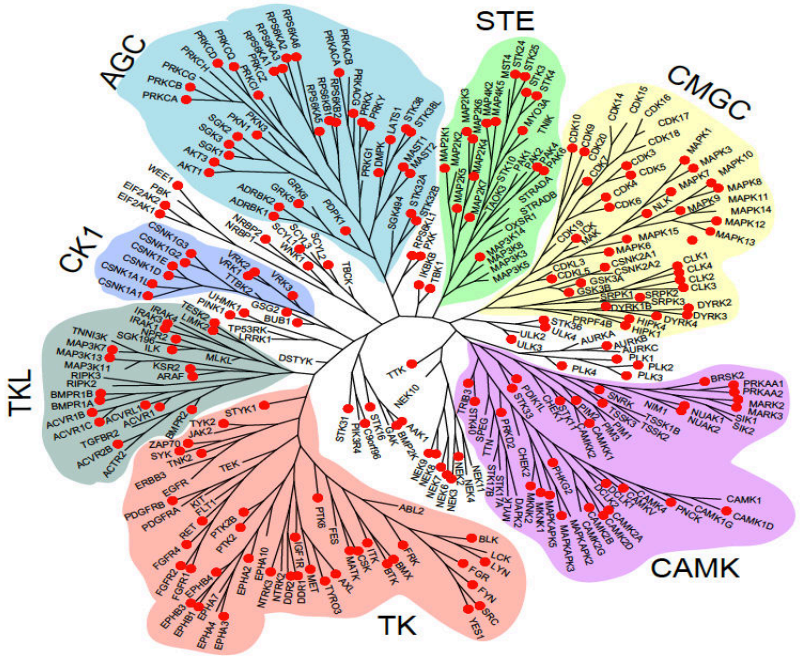


Transgenic Mice with Overexpressed OGT in Muscle or Adipose - Become Diabetic. (McClain & Hanover)

Proc. Nat.Acad.Sci. (2002) 99, 5313-5318.

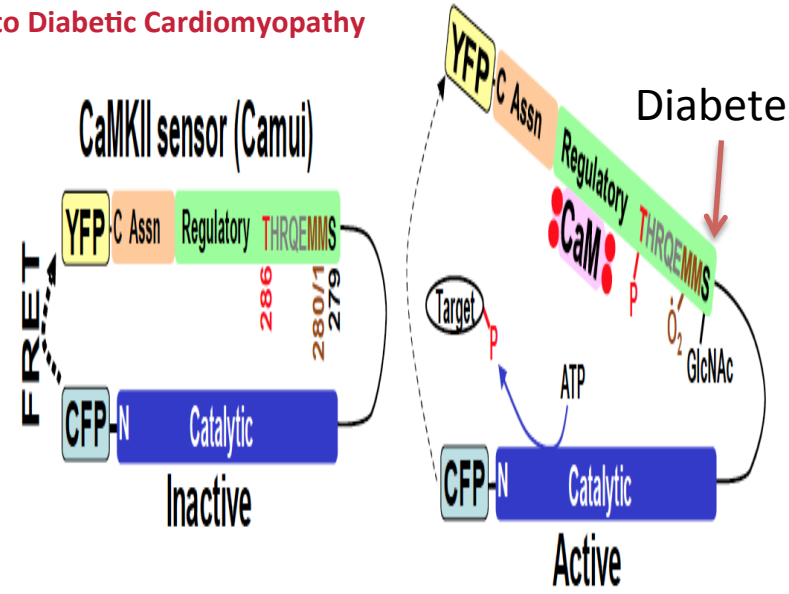
Over one-half of all human protein kinases are dynamically Modified the the Sugar O-GlcNAc.

Family Tree of **Human Kinases**
Modified by O-GlcNAc:



Cardiac Myocytes: CAMKII Becomes Constitutively Active Due to Hyper-O-GlcNAcylation in Diabetes

Key to Diabetic Cardiomyopathy



Contributes to Arrhythmias and Cardiac Problems in Diabetes

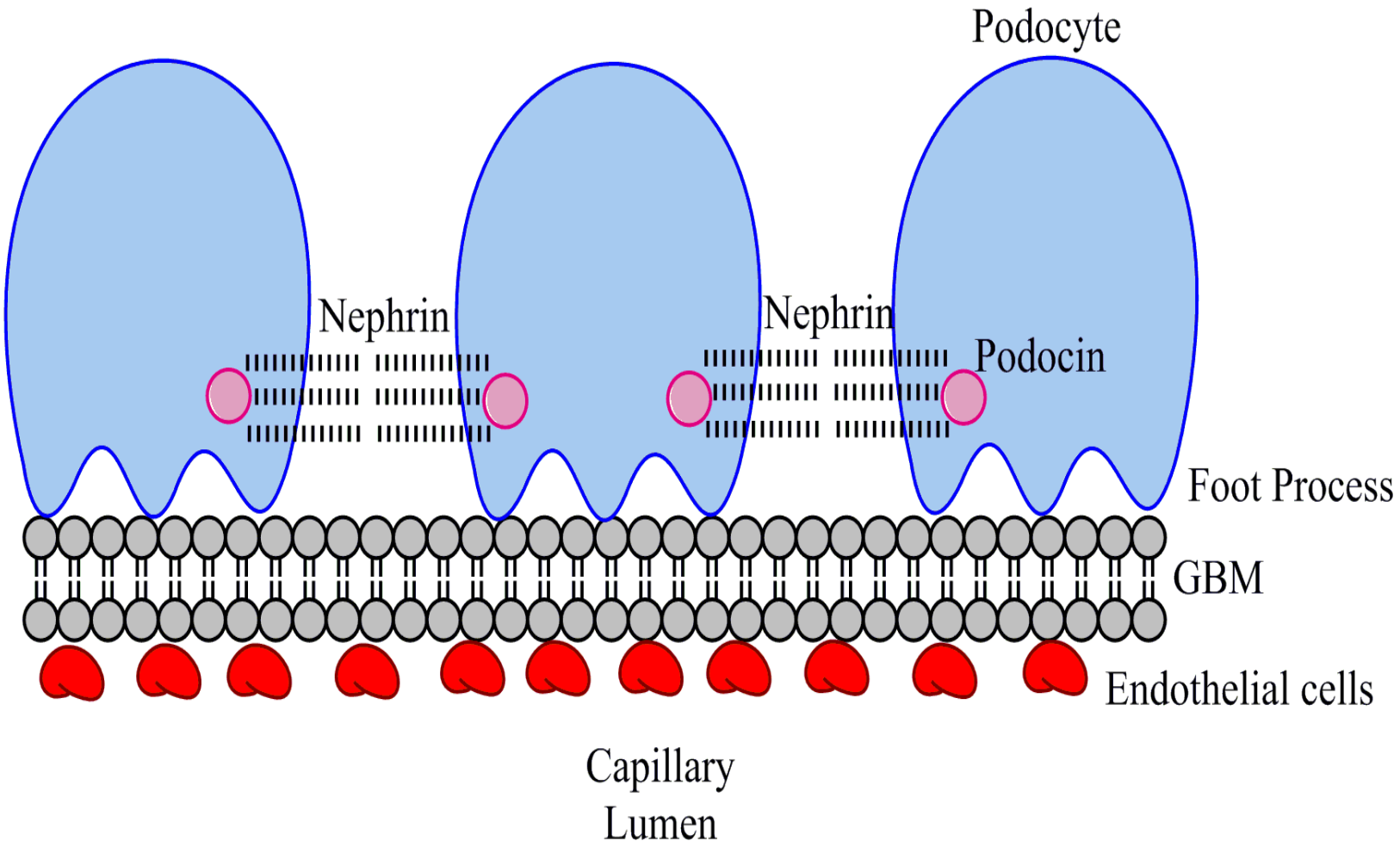
The sugar, **O-GlcNAc Regulates Kinases** Key to Signaling:

- ✓ O-GlcNAc at Active Sites Inhibits CAMKIV - *J. Biol. Chem.* 284, 21327–37.
- ✓ O-GlcNAc Regulates the Substrate Specificity of CKII - Nature Chemical Biology 8(3):262-9.
- ✓ O-GlcNAc Regulates AMPK & AMPK in Muscle (J Biol Chem. 289:10592-606)
- ✓ AKT is regulated by O-GlcNAcylation Am J Physiol Endocrinol Metab.295:E974-80.
- ✓ All PKCs: O-GlcNAc Negatively Regulates Biochim Biophys Acta. 1783:695-712
- ✓ O-GlcNAc Inhibits PFK1 & Glycolysis in Cancer - Increases Flux Through Pentose phosphate pathway. Science 337:975-80

Collaboration with
Donald M. Bers Ph.D.
UC Davis

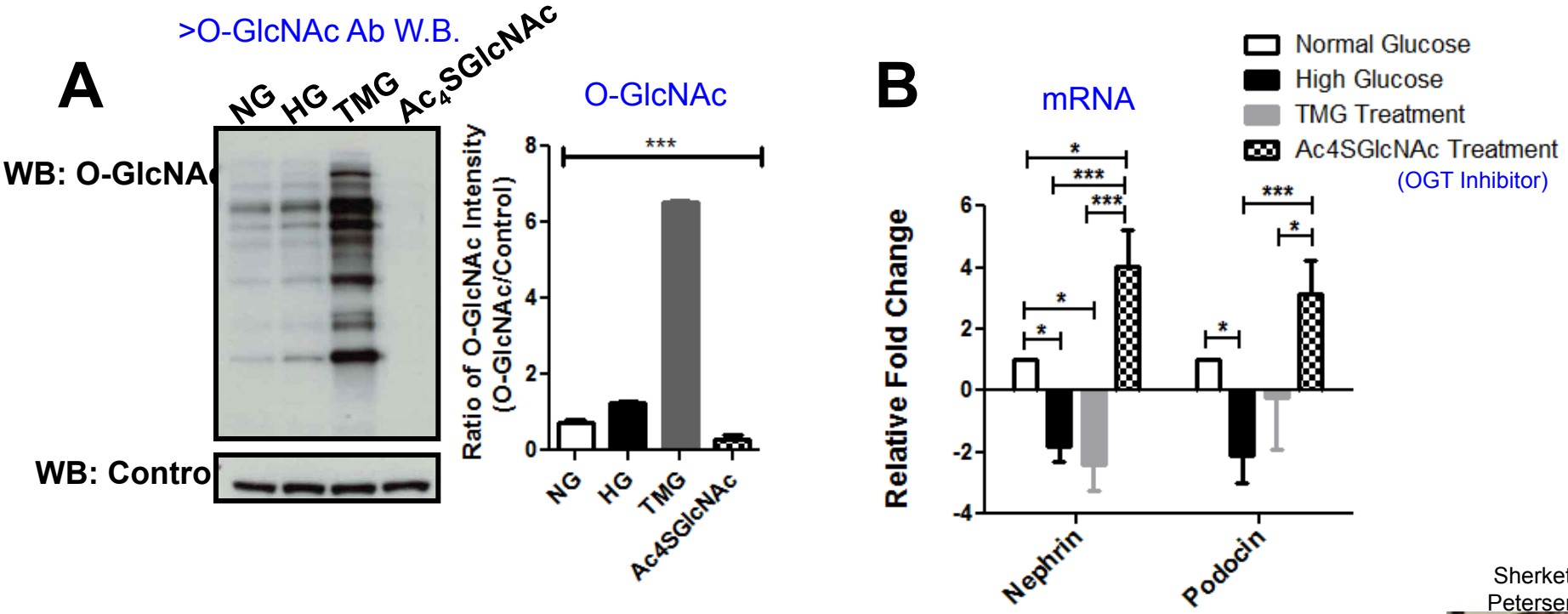
Nature (2013) 502:372-6.

Nephrin & Podocin Proteins Are Key to Kidney Function: Podocyte Filtration Barrier



Sherket Petersen

Increased *O*-GlcNAc Due to High Glucose Blocks the Transcription of Podicin and Nephrin

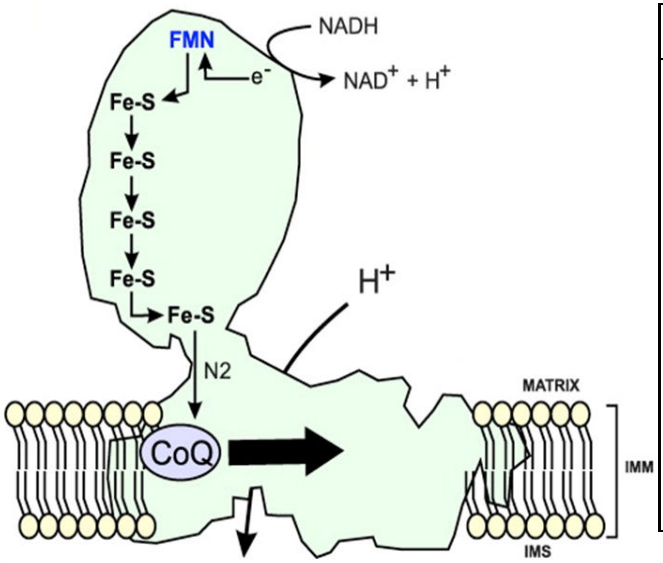


Inhibition of the O-GlcNAc Transferase, **Even in High Glucose** Restores Podicin and Nephrin Expression.

Sherket Petersen

At Least 88 Mitochondria proteins are O-GlcNAcylated:

	Core Subunits
nDNA	ndufv1
	ndufv2
	ndufs1
	ndufs2
	ndufs3
	ndufs7
	ndufv8
mtDNA	nd1
	nd2
	nd3
	nd4
	nd4l
	nd5
	nd6



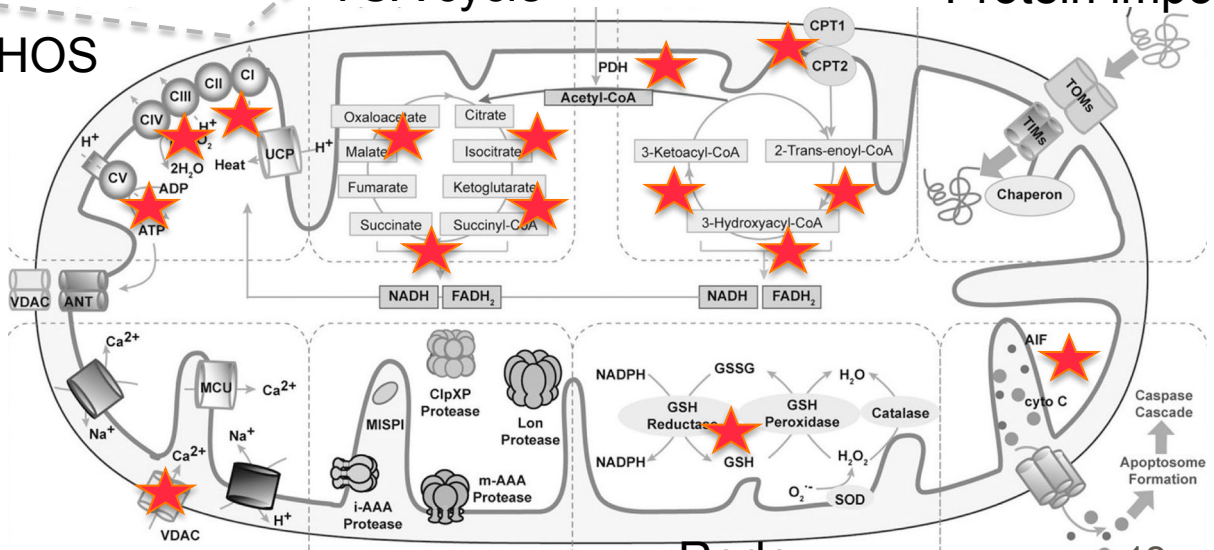
	Accessory Subunits
nDNA	ndufa1 ndufa2
	ndufa3 ndufa4
	ndufa5 ndufa6
	ndufa7 ndufa8
	ndufa9 ndufa10
	ndufa11 ndufs4
	ndufs5 ndufs6
	ndufab1 ndufb1
	ndufb2 ndufb3
	ndufb4 ndufb5
	ndufb6 ndufb7
	ndufb8 ndufb9
	ndufb10 ndufb11
	ndufc1 ndufc2
	ndufv3
	dap13 grim19

Normal Mitochondria:
Elevating O-GlcNAc Improves Mitochondrial Function.

O-GlcNAcylated proteins in complex I (in red color)

OXPHOS

Overview of mitochondrial O-GlcNAcome. (in ★)



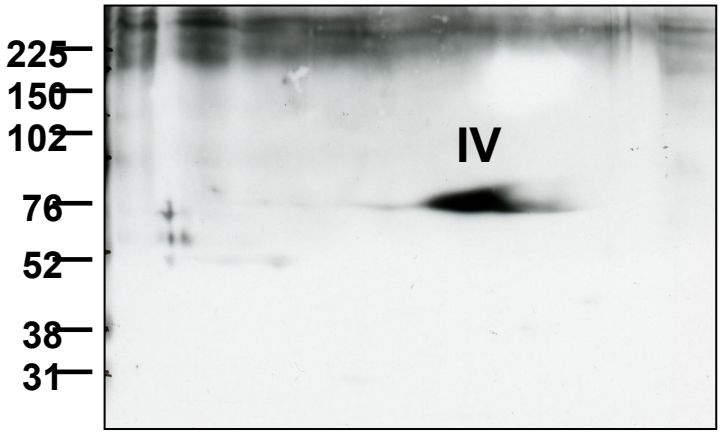
Junfeng Ma

J. Biol. Chem. 290, 29141-29153 (2015).

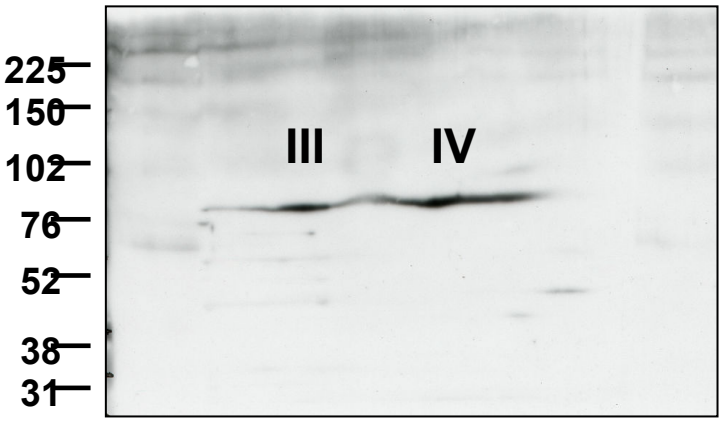
O-GlcNAc Transferase is Mislocalized in Cardiac Mitochondria From Diabetic Rats:

a BN PAGE of mito samples with anti-OGT

Normal



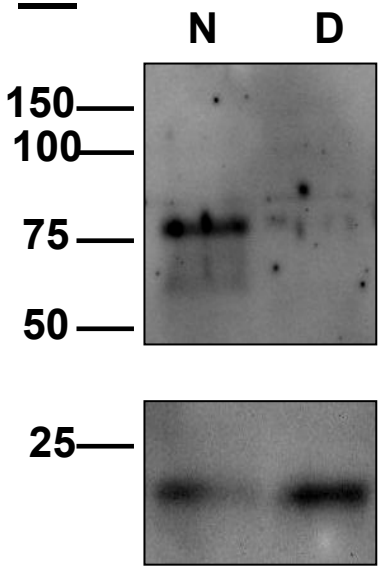
Diabetic



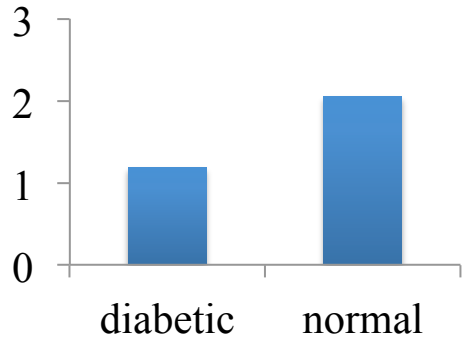
WB: OGT

b IP of complex IV showing OGT interaction

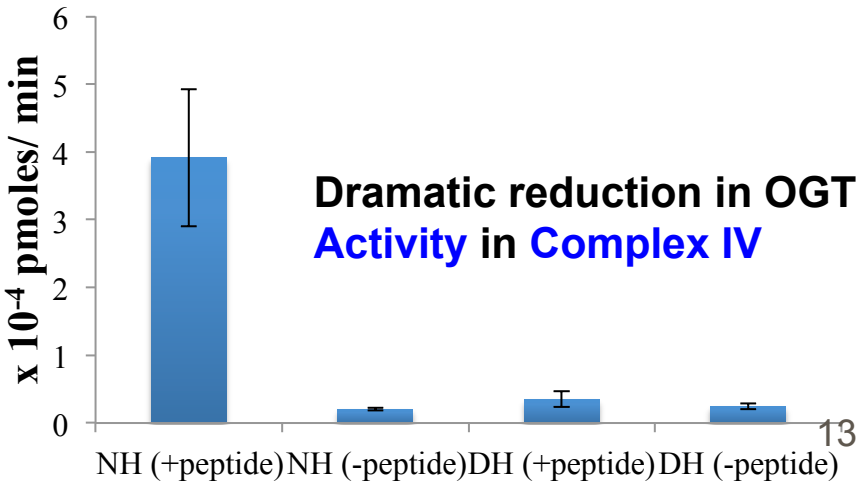
WB:AL03



WB: comIV
oxphos



c OGT activity assay on complex IV IP with antibody conjugated beads

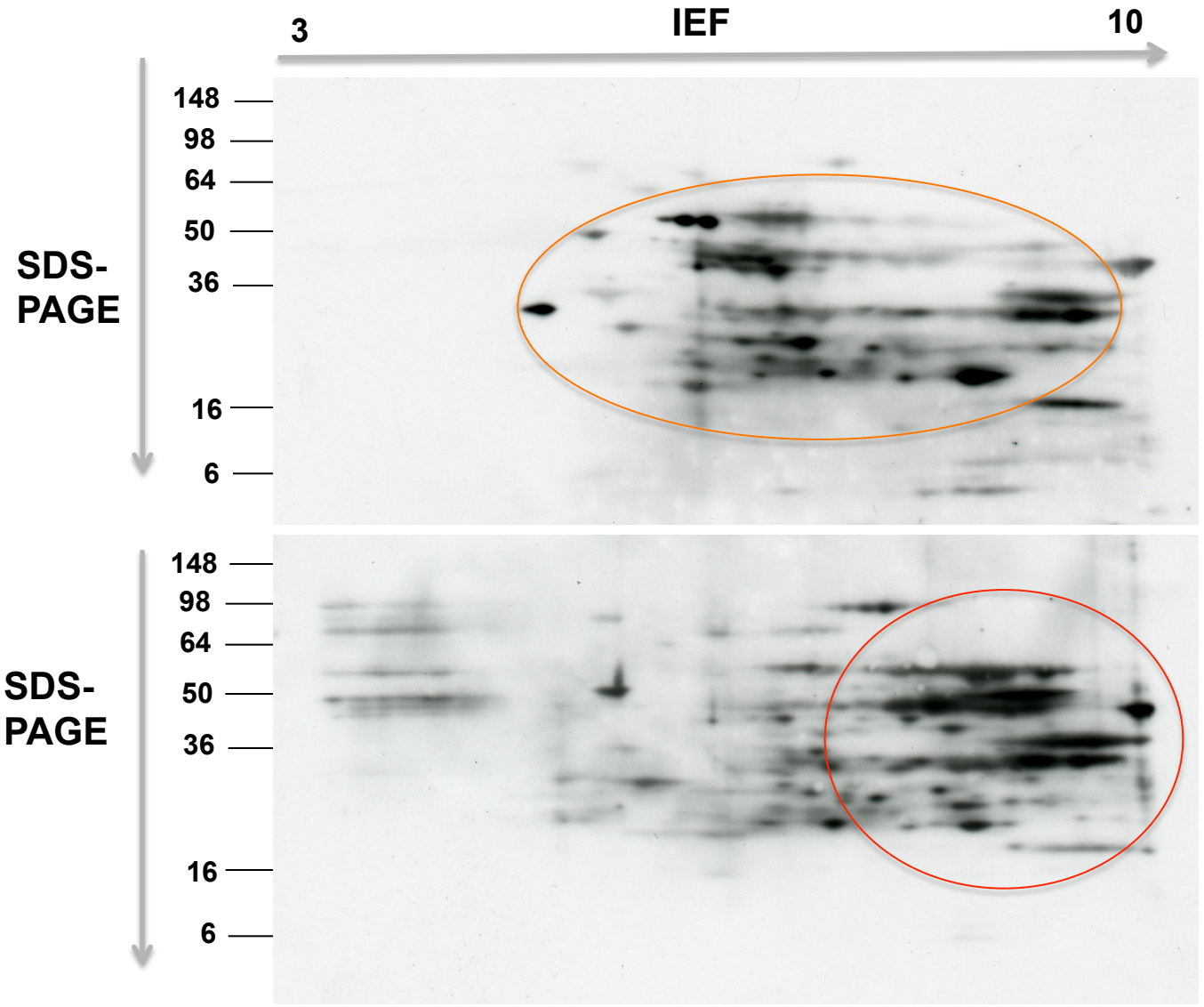


Dramatic reduction in OGT Activity in Complex IV

Partha Banerjee

PNAS 112, 6050-6055 (2015)

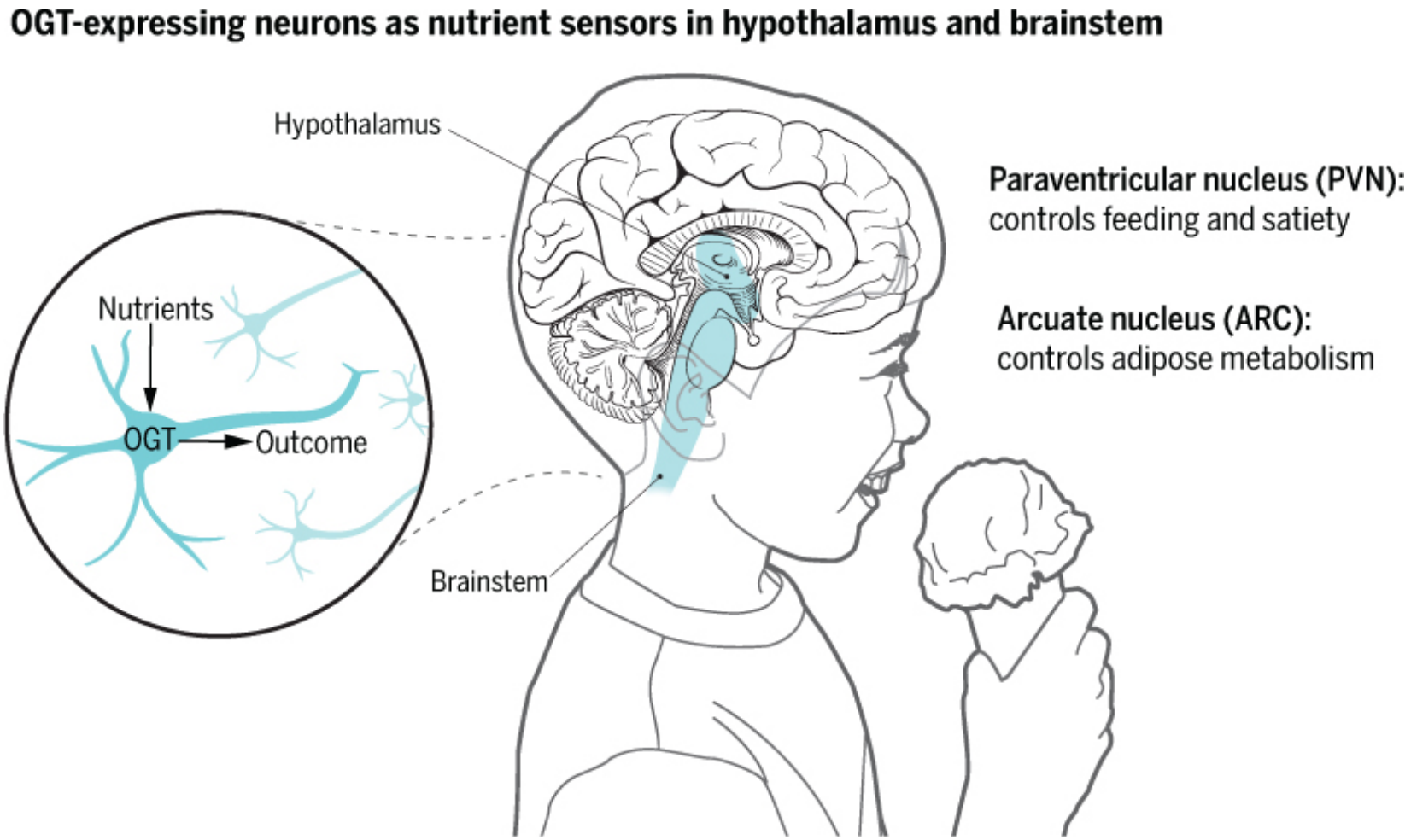
Mitochondrial proteins are O-GlcNAcylated differentially (control vs diabetic rat heart)



Directly Results in Mitochondrial Dysfunction.

PNAS 112, 6050-6055 (2015)

What Happens When You **Knock-Out the Enzyme that Adds O-GlcNAc** to Proteins In the Region of the Brain in Adult Mice that Controls Feeding and Satiety?



Gary J. Schwartz Science 2016;351:1268-1269

~2-3 Weeks Targeted KO is Morbidly Obese & Hyperactive

Mice Missing O-GlcNAc in the PVN Brain Region Can't Stop Eating!

O. Lagerlöf et al., Science 351, 1293 (2016).

Conclusions – O-GlcNAc:

- O-GlcNAc is a Major **Nutrient** Regulatory Post-Translational Modification in all multicellular eukaryotes - Plants & Animals & Viruses (some bacteria).
- O-GlcNAc is **Required for Life at All Levels in Mammals and Plants**.
- **Crosstalk** or Interplay Between **O-GlcNAcylation & Phosphorylation** is Extensive and Involved in **Many** Cellular Processes.
- **O-GlcNAc is Important to Transcription**: is Part of the Histone Code where Most Sites are at Contact Regions with the DNA of the Nucleosome.
- Many Toxic Affects of Hyperglycemia Result From **Dysregulation of the Balance Between O-GlcNAc and Phosphorylation & Dysregulated Transcription** = Glucose Toxicity.
- **Future Drug Targets for Treating Obesity & Diabetes:** 1) Lower O-GlcNAcylation Globally; 2) Lower it Selectively by Targeting the Over 800 specific proteins that Target the O-GlcNAc Transferase to its Substrates.

Supported by NIH grants R33 DK71280 ; R01 HD13563, R01 CA42486, R01 DK61671, NHLBI P01 HL107153 and NHLBI contract N01-HV-28180.

Acknowledgements

Hunt Lab

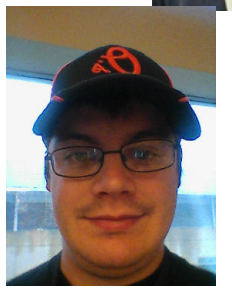
Donald F. Hunt
Namrata D. Udeshi
Univ. Virginia

Heng Zhu
JHMI

Brian Lewis, NIH

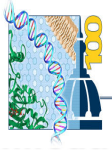
Genaro A. Ramirez-Correa, Weidong Gao, and Anne M. Murphy
Department of Pediatrics/
Division of Cardiology

Brian O'Rourke



Johnathan Neiswinger
JHMI

Rick Huganair



For tools to study O-GlcNAc (eg. antibodies, plasmids, protocols): ***email: gwhart@jhu***