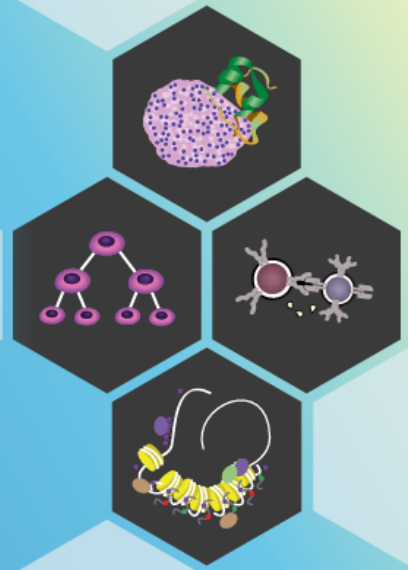
2024 RACHMIEL LEVINE-ARTHUR RIGGS

Diabetes Research Symposium

Targeting Hepatic Mitochondrial Fat Oxidation to Treat MASLD, MASH and Cardiometabolic Disease



Gerald I. Shulman, MD, PhD, MACP, MACE, FRCP

Geroge R. Cowgill Professor of Medicine and Cellular & Molecular Physiology

Co-Director, Yale Diabetes Research Center

Director, Yale Mouse Metabolic Phenotyping Center

Investigator Emeritus, Howard Hughes Medical Institute

Disclaimer

This is a Non-CME Accredited Presentation.

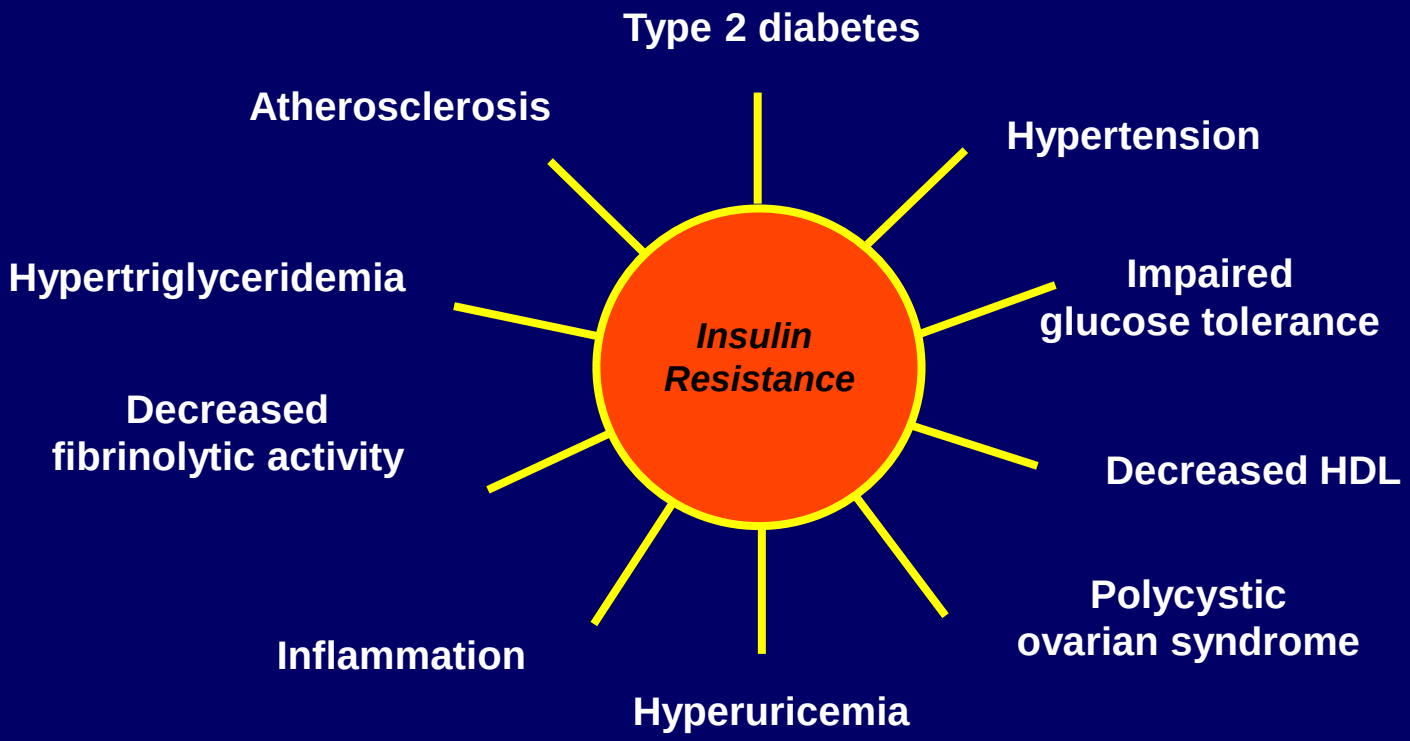
Disclosures

Scientific Advisory Boards: Merck, NovoNordisk, AstraZeneca, Aegerion, iMBP, 89bio, Janssen Research and Development, Ionis, Maze Therapeutics, Levels, Equator Therapeutics, Generian, Bayer, Kriya, Forrest Research Institute, Esperion, Arrowhead Pharmaceuticals

Investigator-Initiated Support: AstraZeneca, Merck, Maze Therapeutics, Esperion, Novo Nordisc

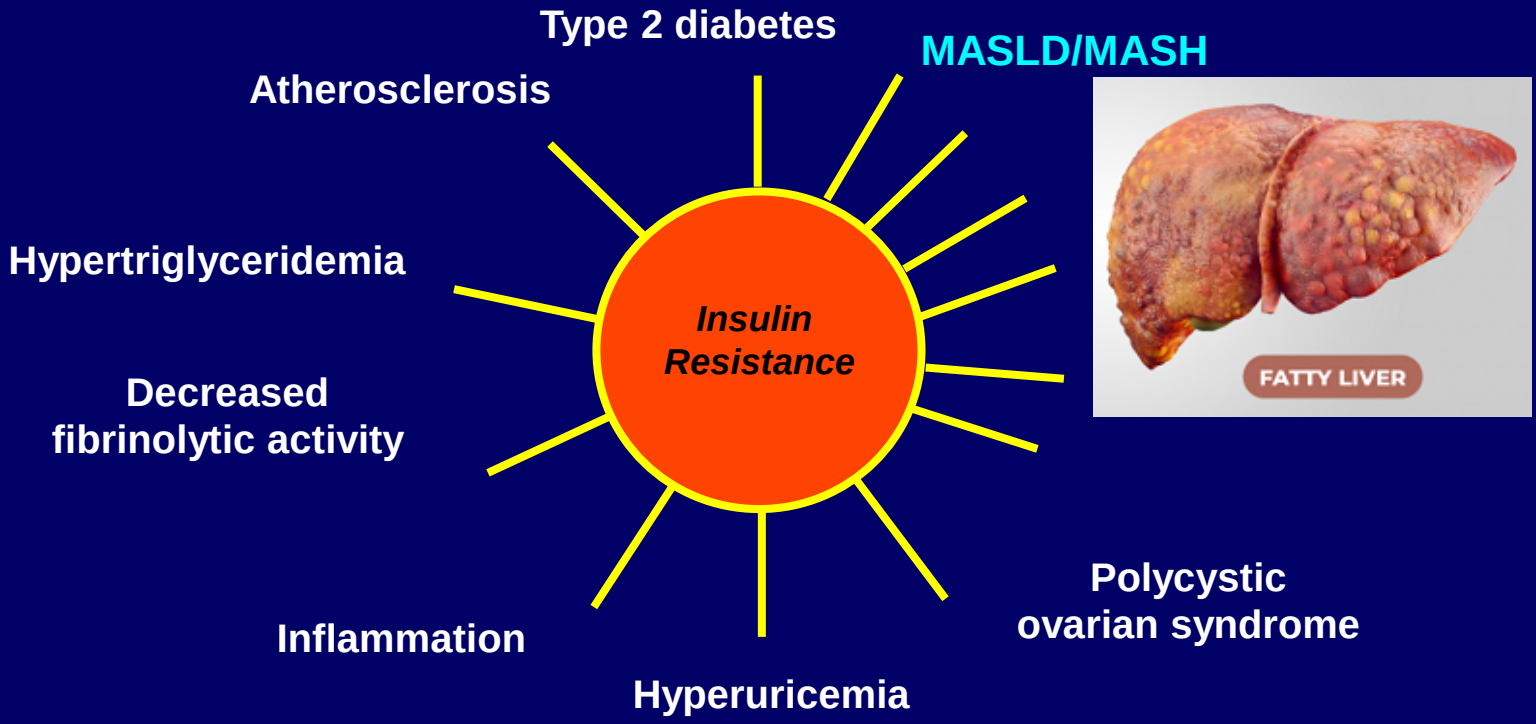
Inventions: GIS is an inventor on Yale patents for liver-targeted mitochondrial uncoupling agents for the treatment of MASLD, MASH, T2D and related metabolic disorders and is a Scientific-Cofounder and Scientific Advisor for OrsoBio.

Insulin Resistance and the Metabolic Syndrome



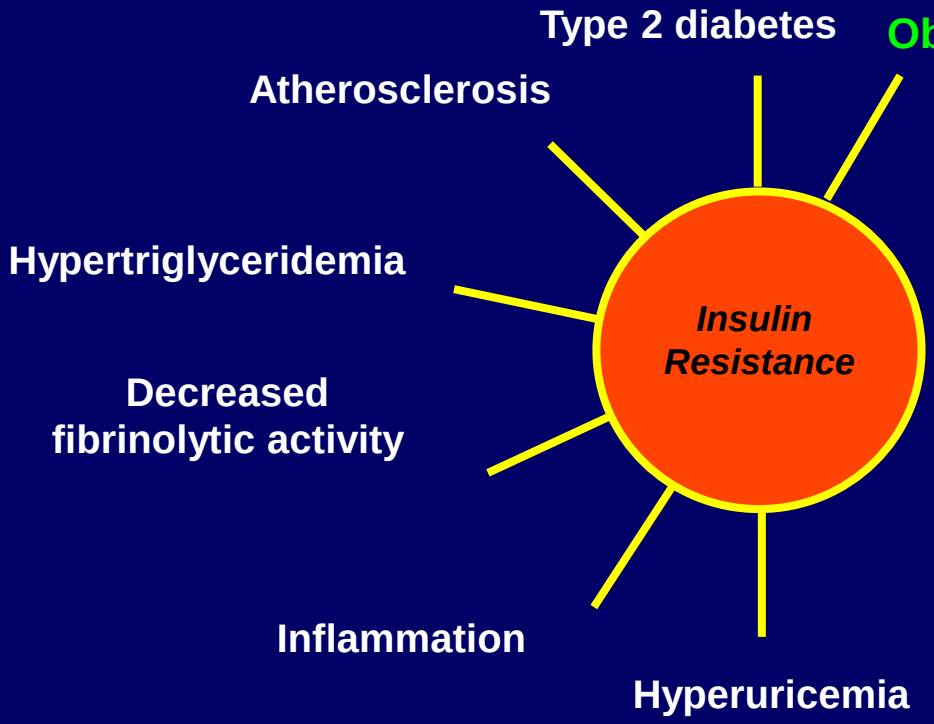
Jerry Reaven, Banting Lecture 1988

Insulin Resistance and Cardiometabolic Disease 2024

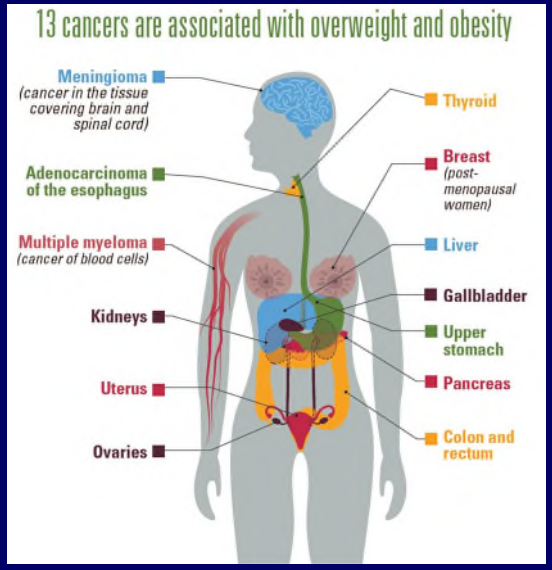


Adapted from Jerry Reaven's Banting Lecture 1988

Insulin Resistance and Cardiometabolic Disease 2024

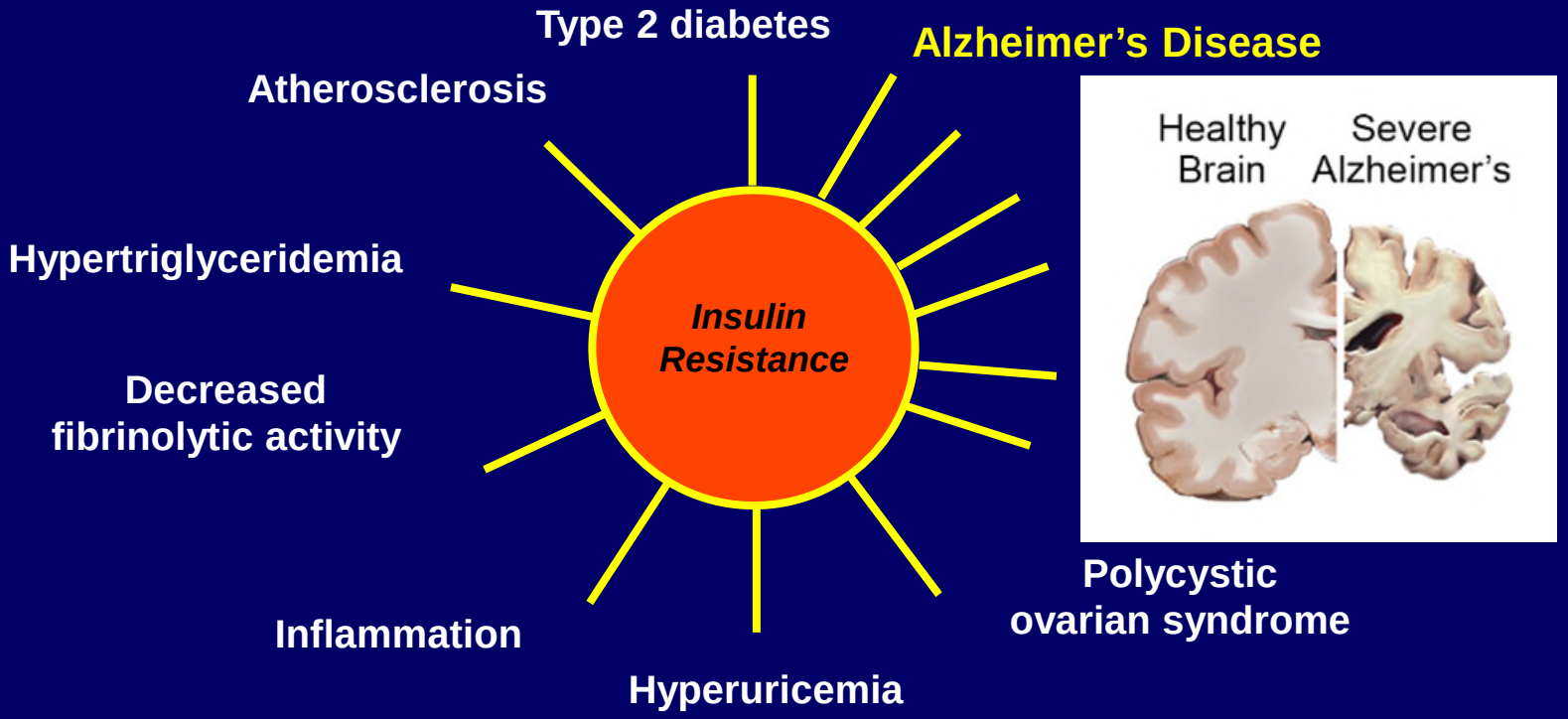


Obesity-Associated Cancers



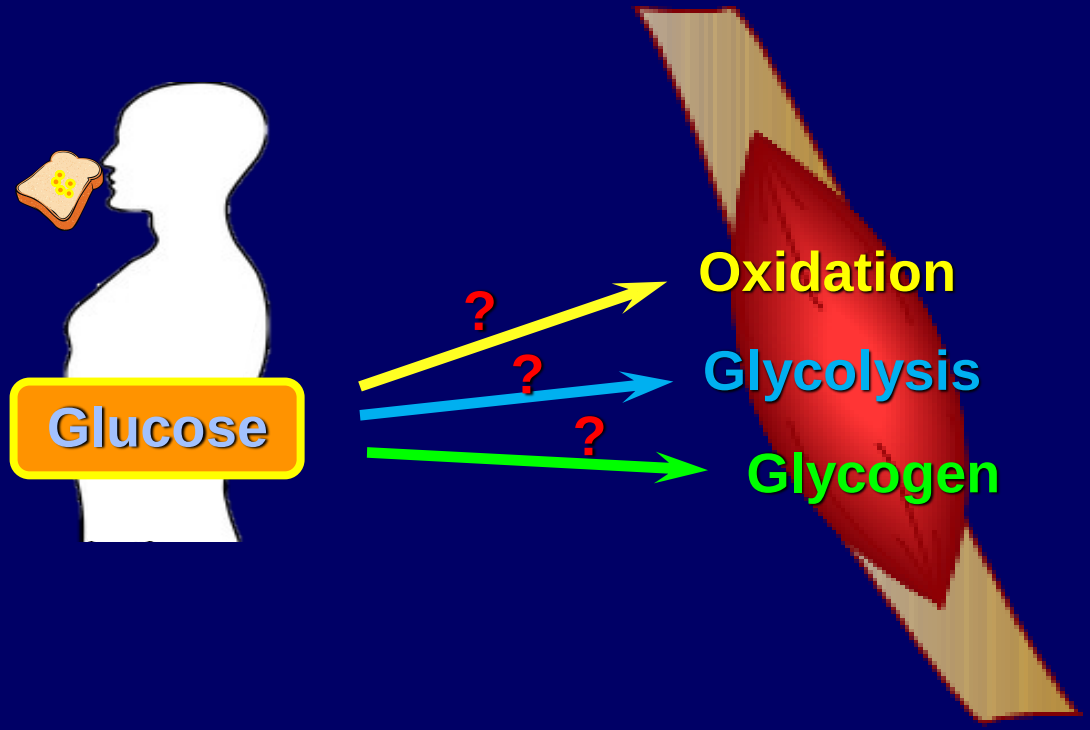
Adapted from Jerry Reaven's Banting Lecture 1988

Insulin Resistance and Cardiometabolic Disease 2024

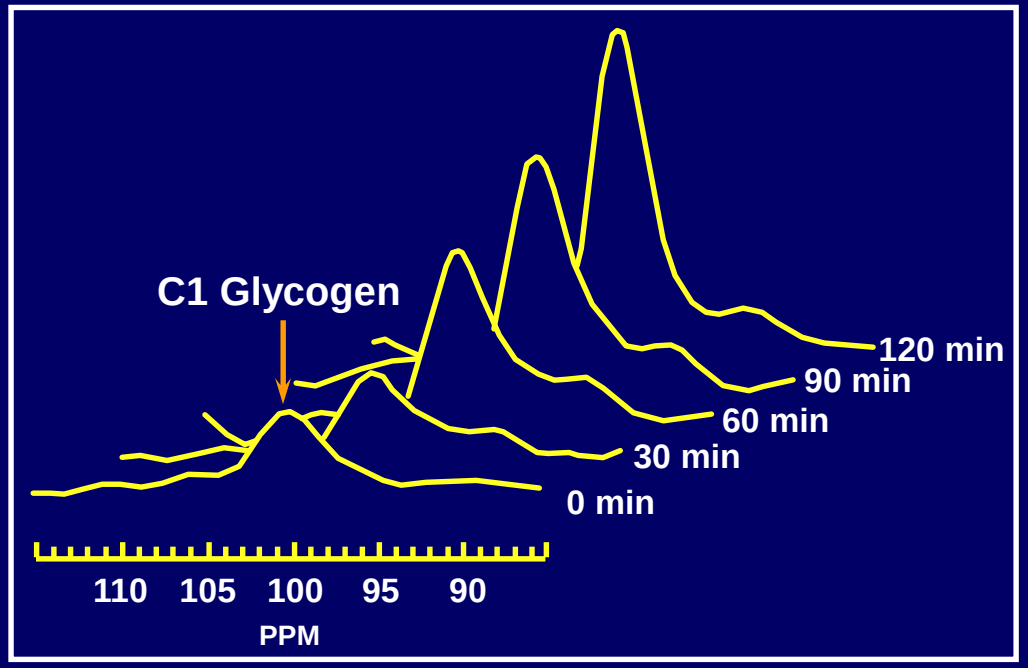


Adapted from Jerry Reaven's Banting Lecture 1988

What causes muscle insulin resistance?

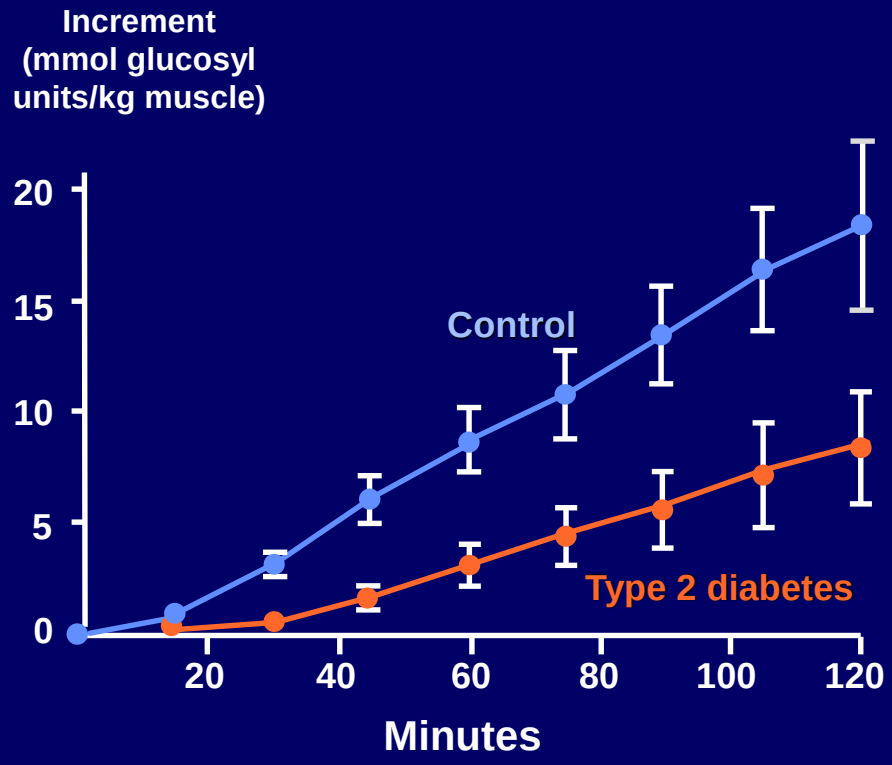


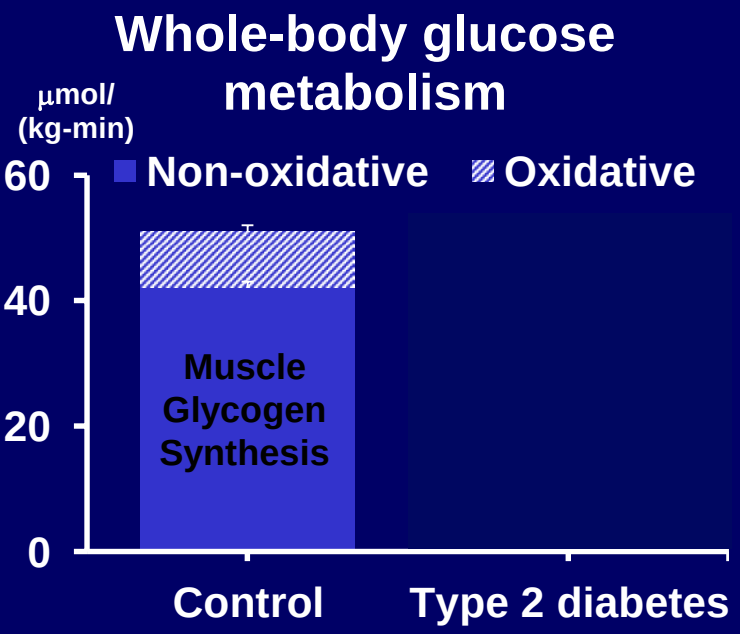
^{13}C NMR spectra of muscle glycogen synthesis in humans



Shulman et al. NEJM 1990

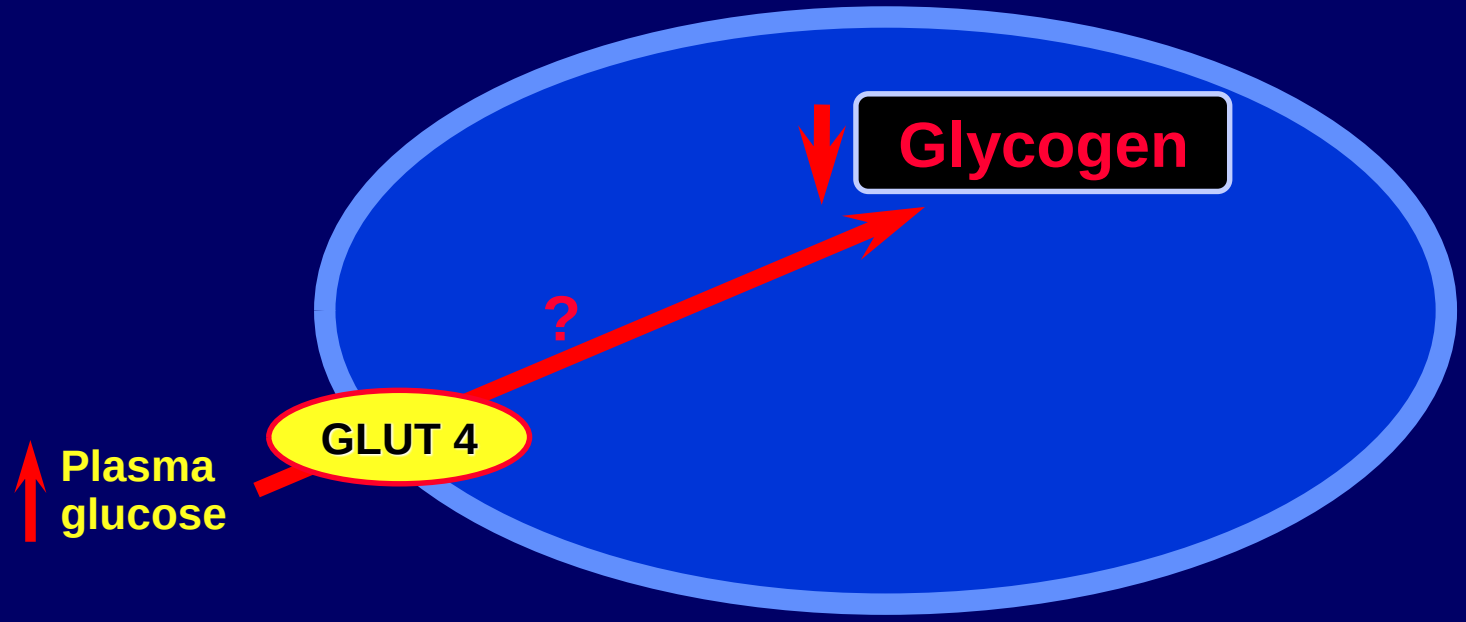
Decreased insulin-stimulated muscle glycogen synthesis is responsible for muscle insulin resistance in type 2 diabetes



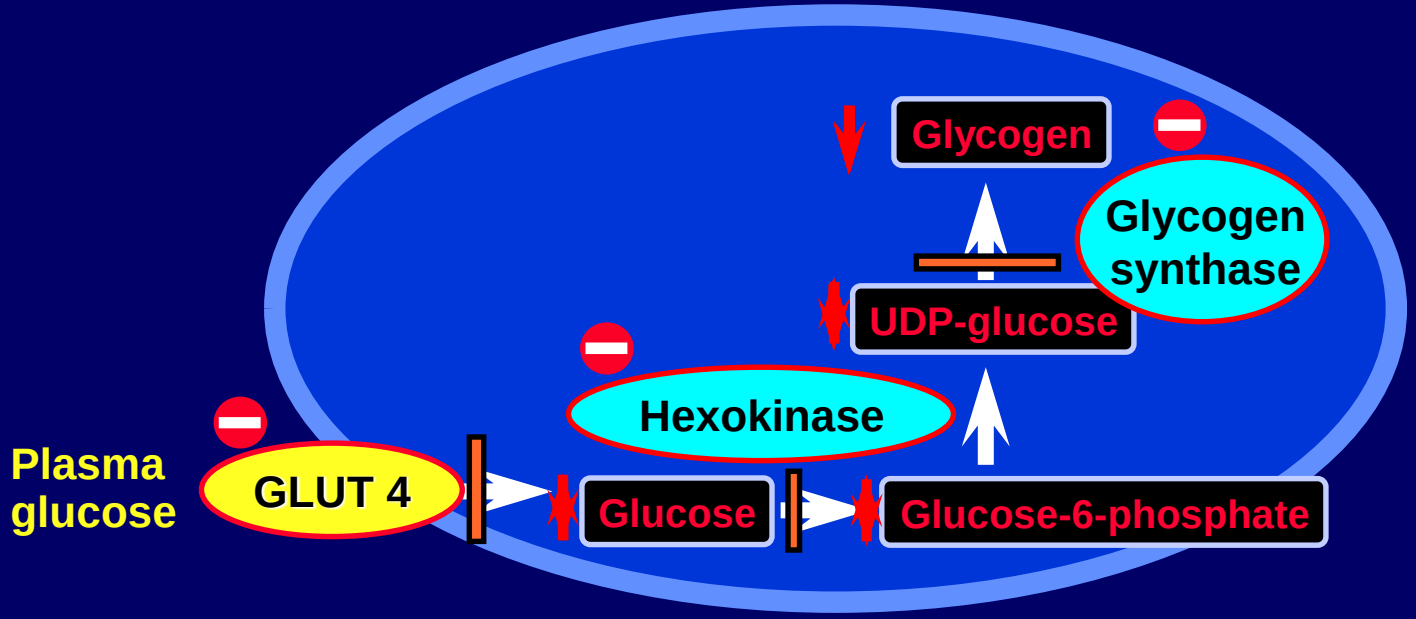


Shulman et al. NEJM 1990

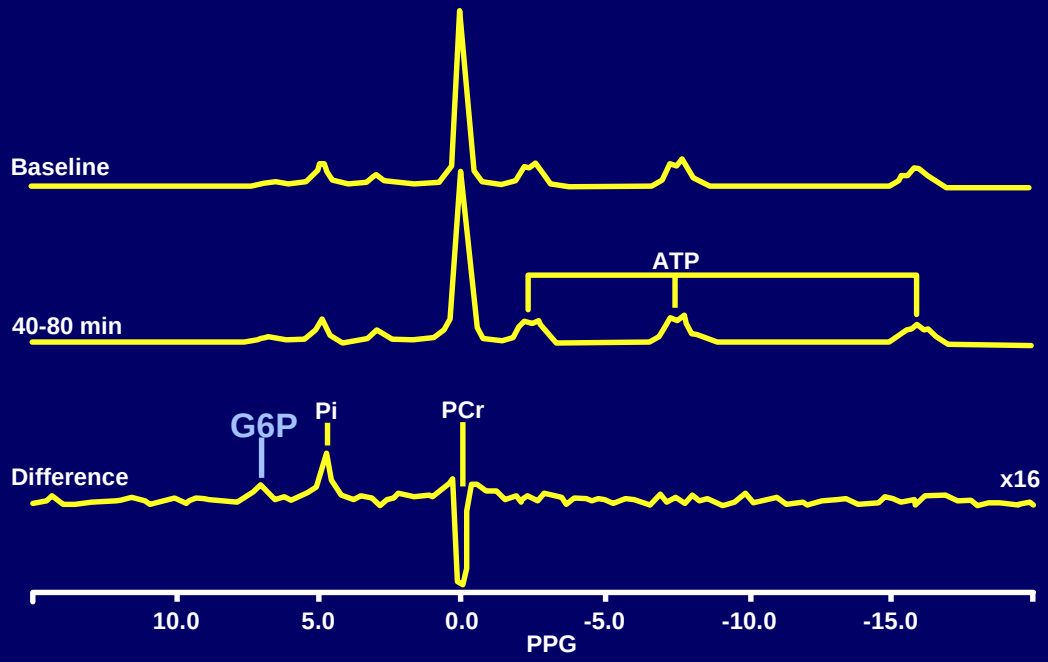
Insulin-Stimulated Muscle Glycogen Synthesis is Impaired in Type 2 Diabetes



Potential Rate-Controlling Steps in Muscle Glucose Glycogen Synthesis

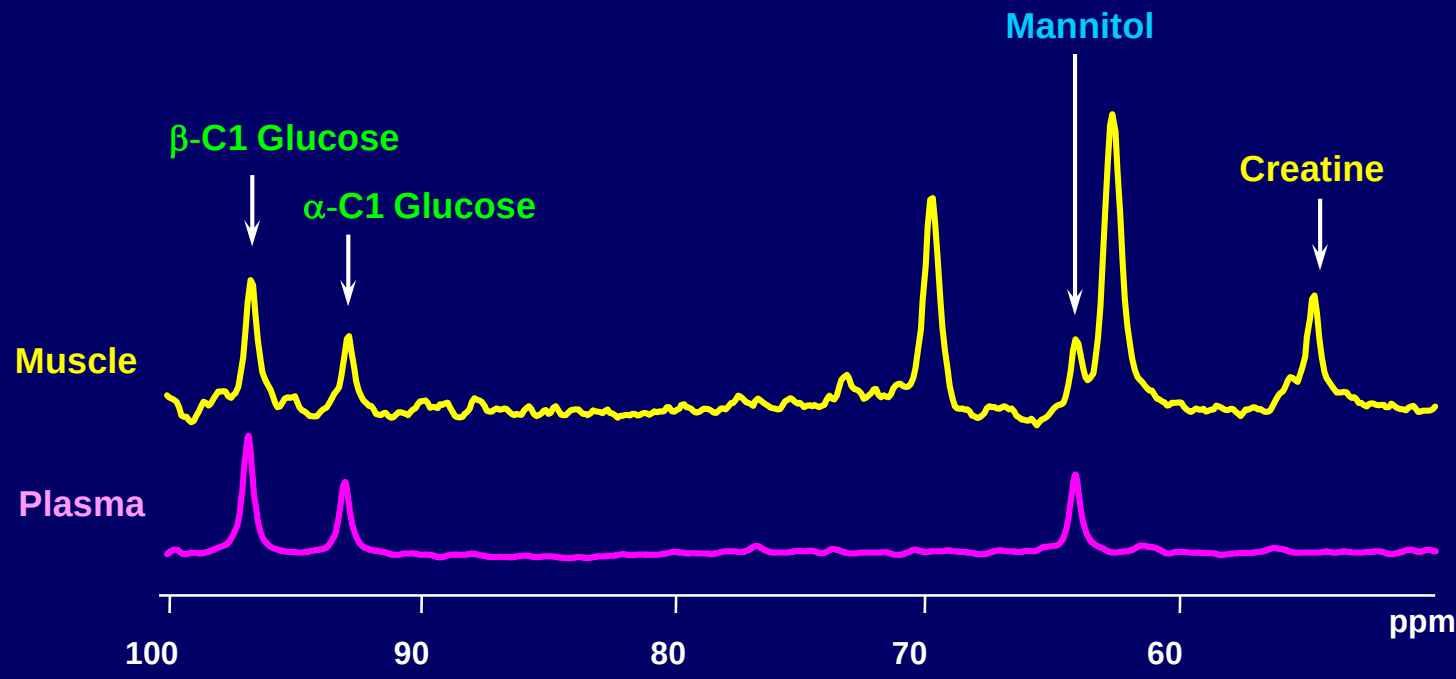


^{31}P NMR spectra of human muscle



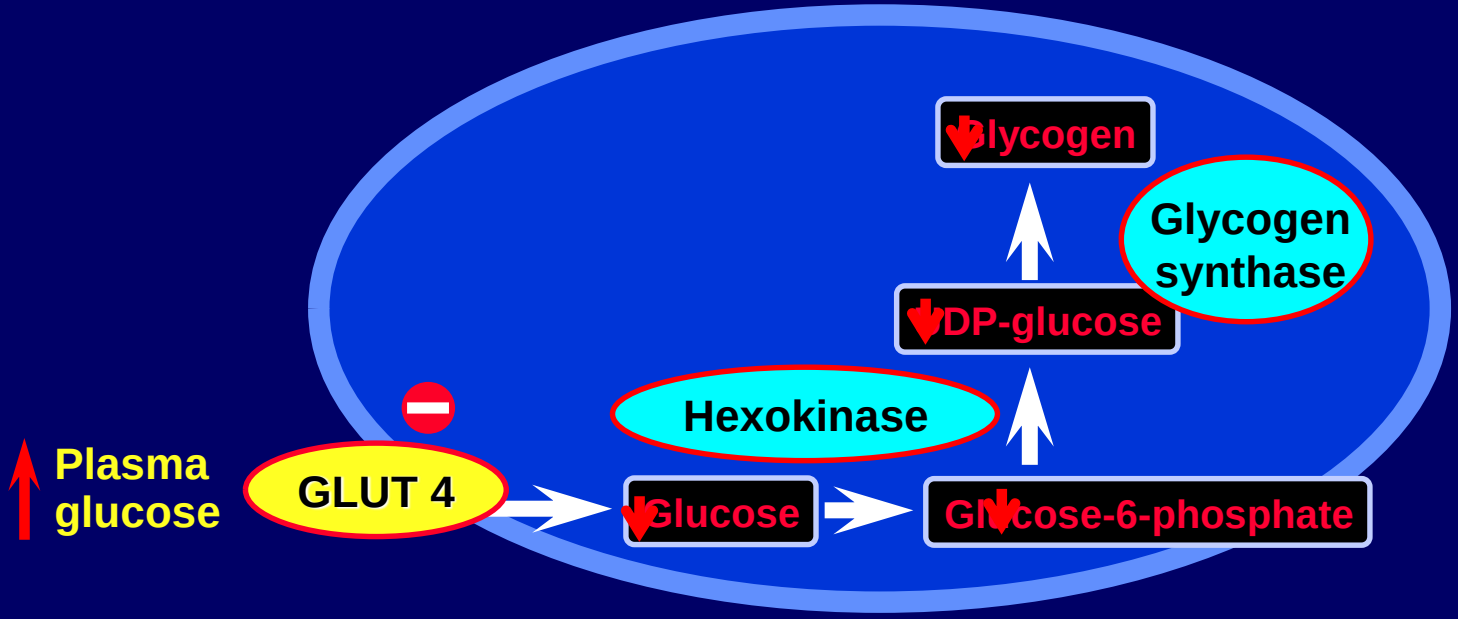
Rothman et al. JCI 1992

^{13}C NMR spectra of human muscle and plasma



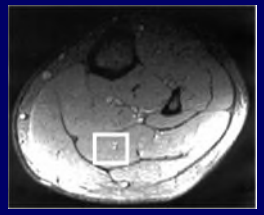
Cline et al. NEJM 1999

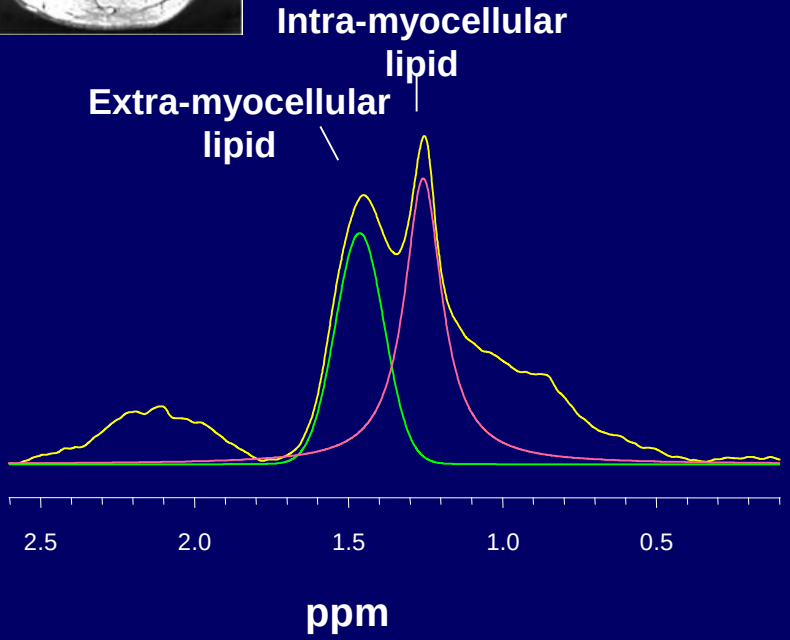
Glucose transport Is rate-controlling for insulin-stimulated muscle glycogen synthesis in T2D

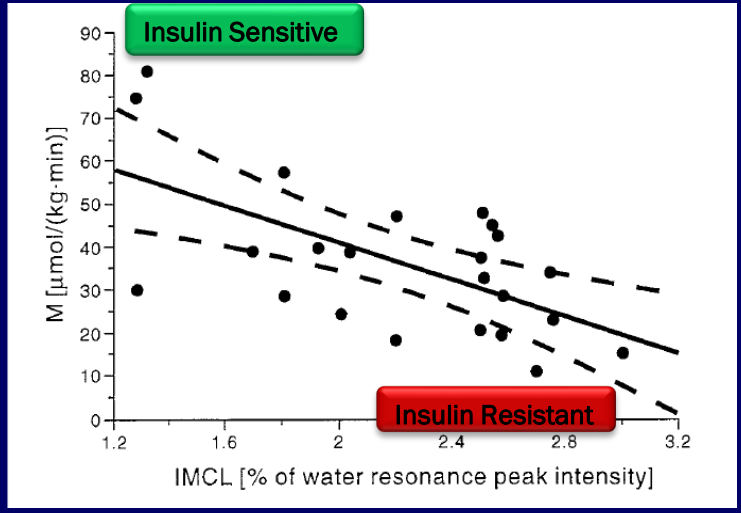


Cline et al. NEJM 1999

Intramyocellular lipid (IMCL) content predicts muscle insulin resistance



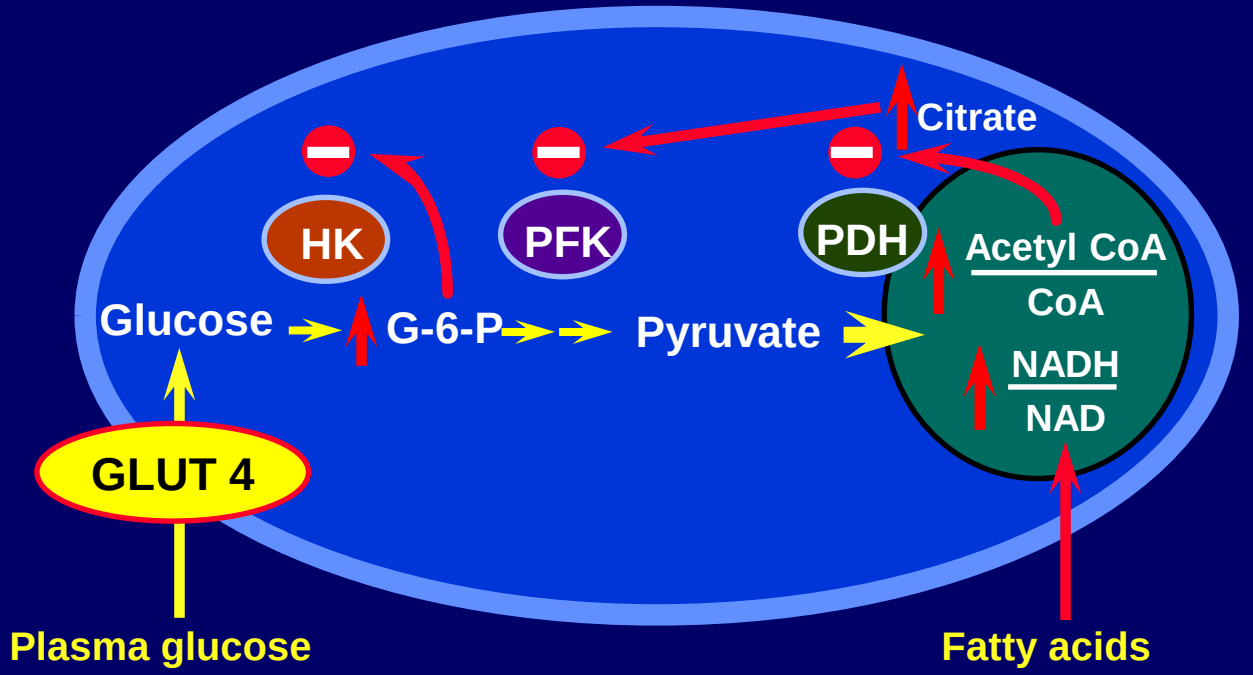




Krssak et al. Diabetologia 1999

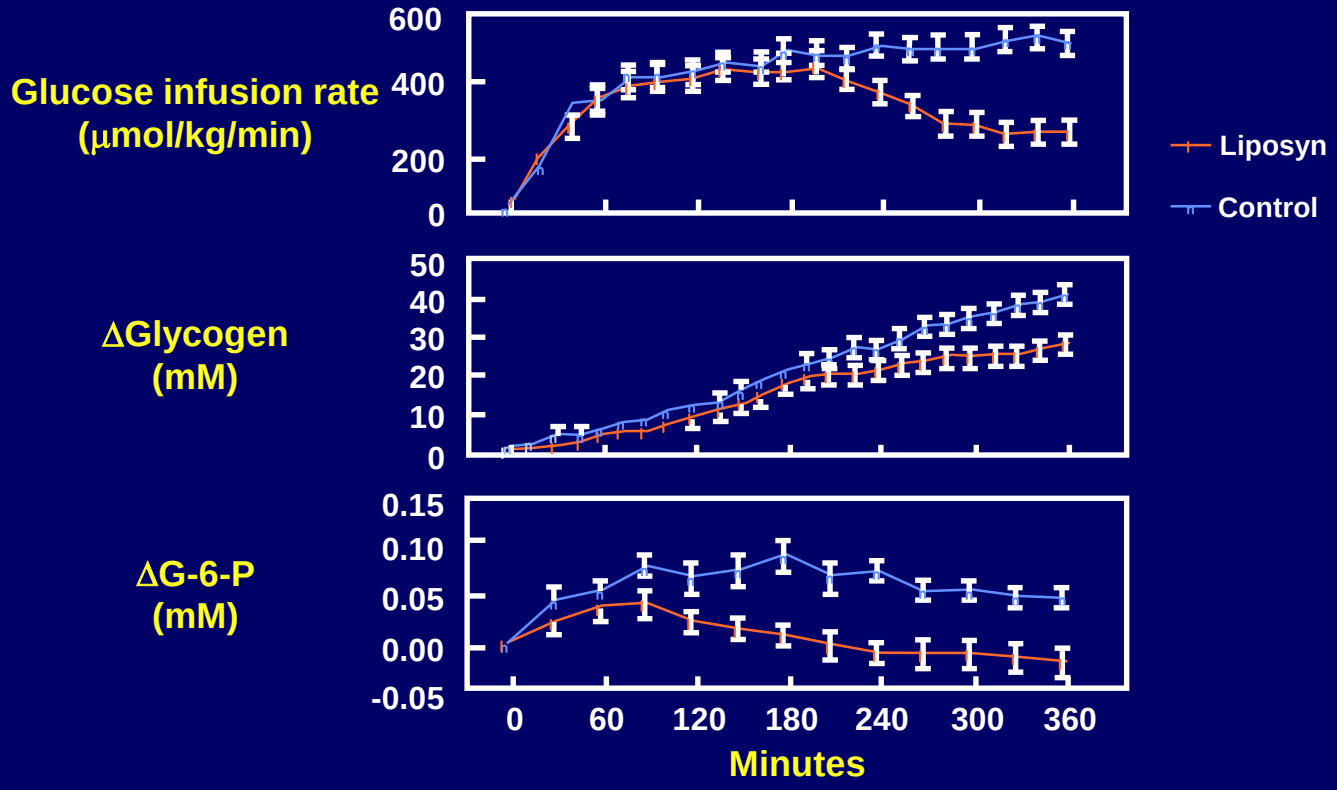
How does ectopic lipid cause muscle insulin resistance?

Randle postulates inhibition of pyruvate dehydrogenase (PDH) activity



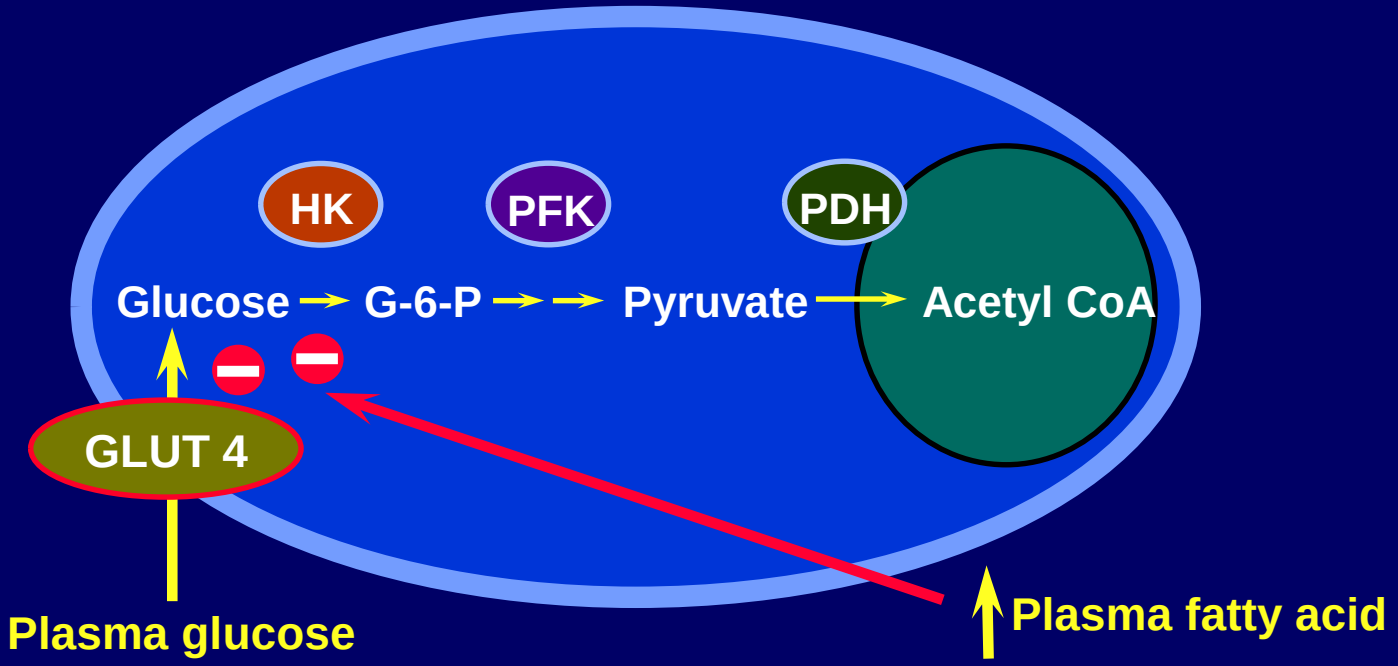
Randle et al. Lancet 1963

Increasing plasma fatty acid concentrations causes a reduction in insulin-stimulated muscle glycogen synthesis and [glucose-6-phosphate]

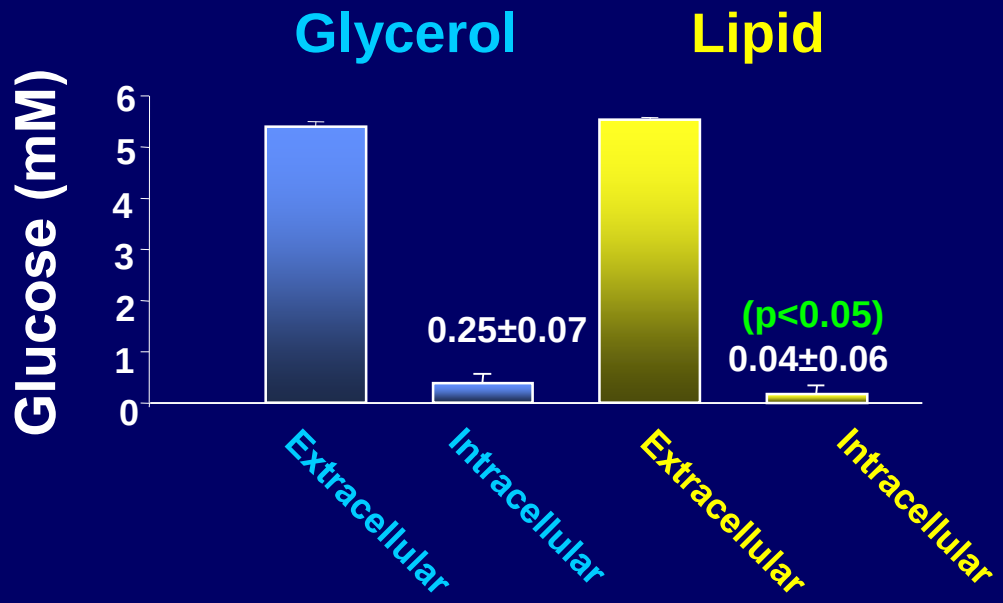


Roden et al. *J Clin Invest.* 1996;97:642-648

Increasing plasma fatty acid concentrations results in a reduction in insulin-stimulated glucose transport/phosphorylation activity

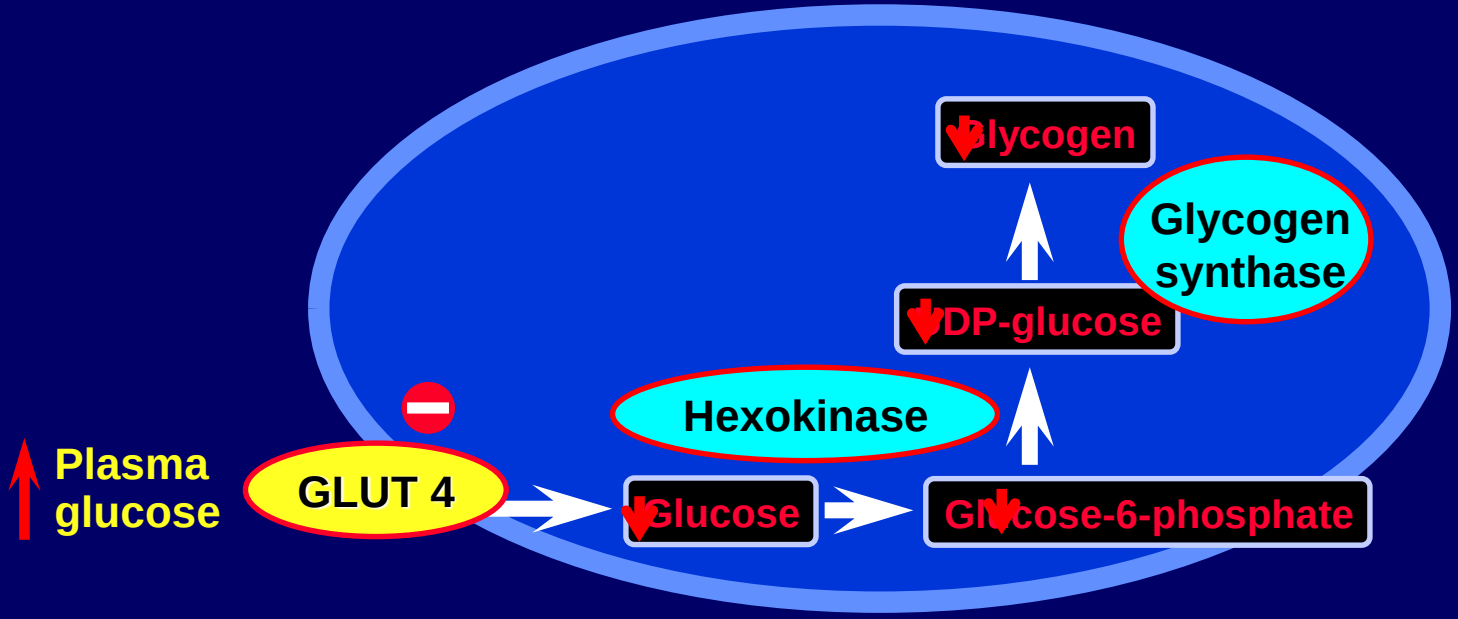


Increasing plasma fatty acid concentrations causes a reduction in intramyocellular glucose concentrations



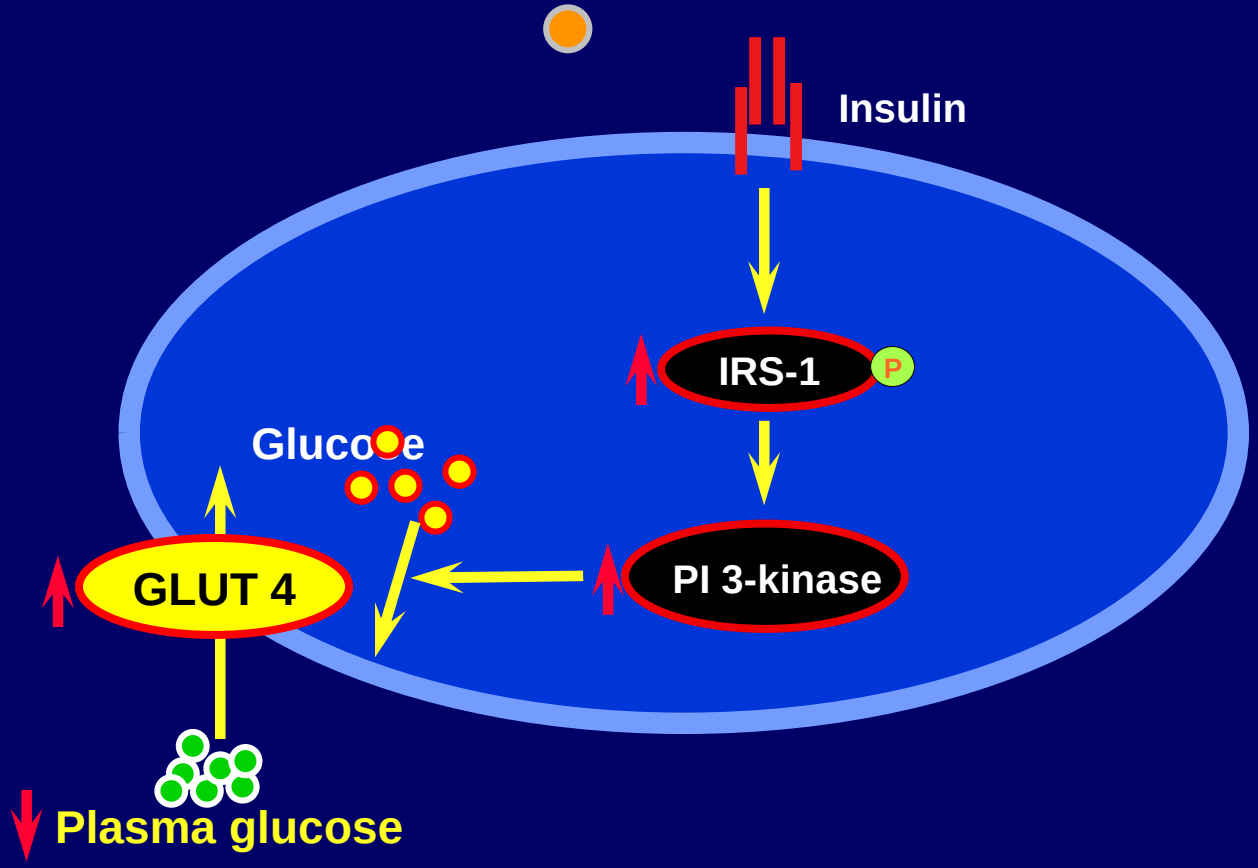
Dresner et al. *J Clin Invest.* 1999;103:253-259

Fatty acids acutely inhibit insulin-stimulated muscle glycogen synthesis by inhibiting glucose transport activity

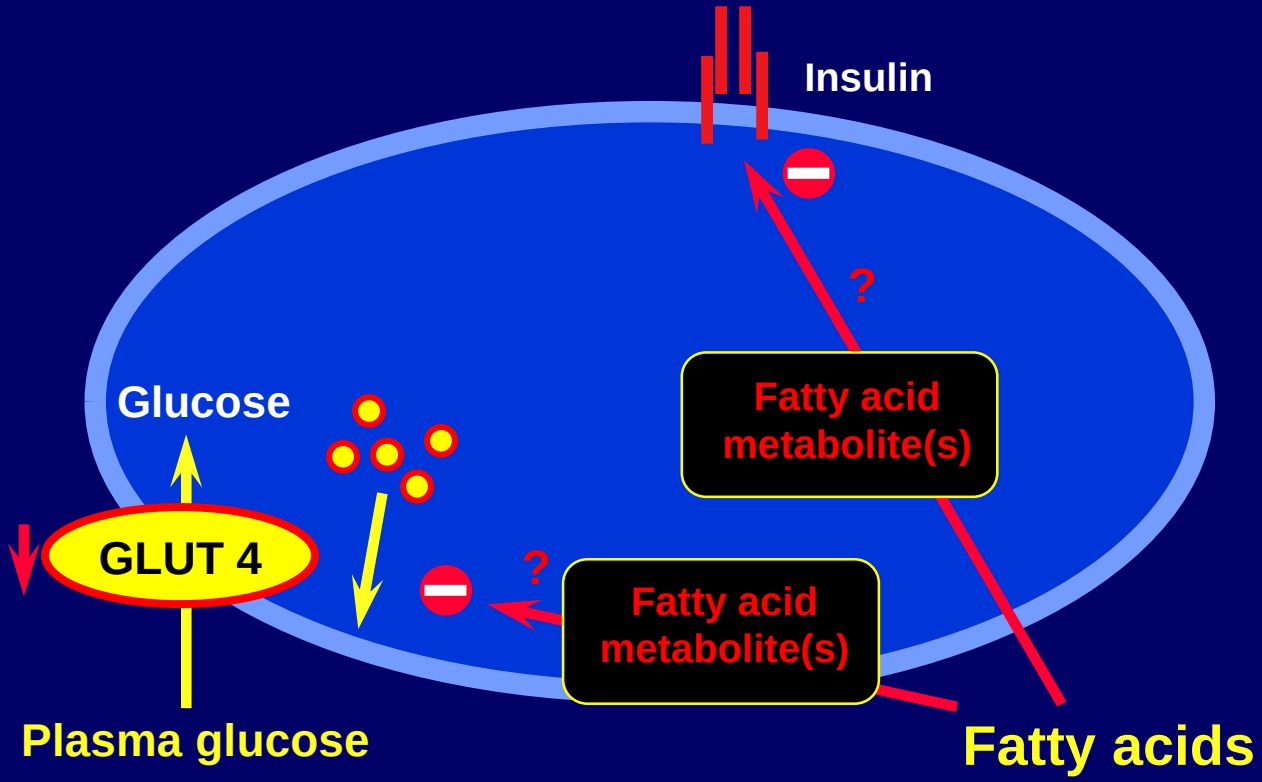


Dresner et al. *J Clin Invest.* 1999;103:253-259

Insulin Action in Skeletal Muscle

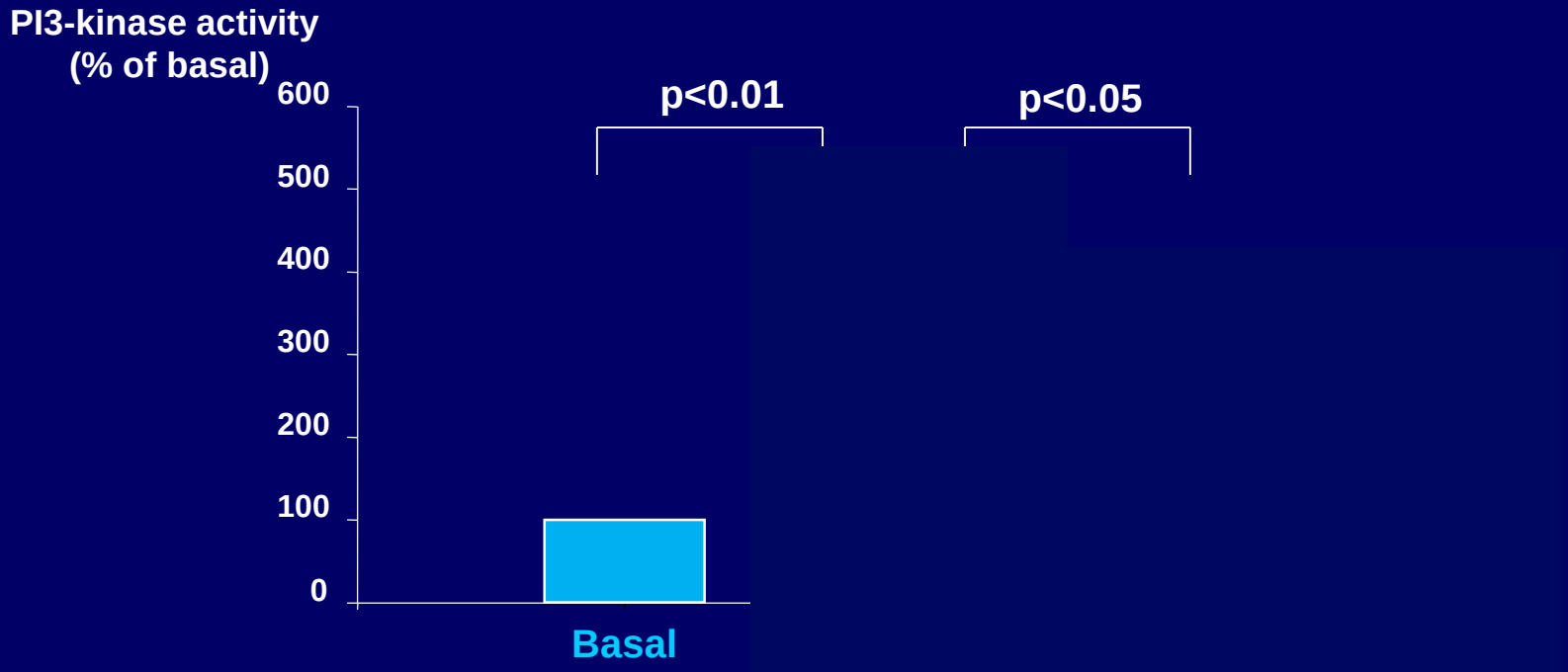


Potential mechanisms by which fatty acids inhibit insulin-stimulated glucose transport activity



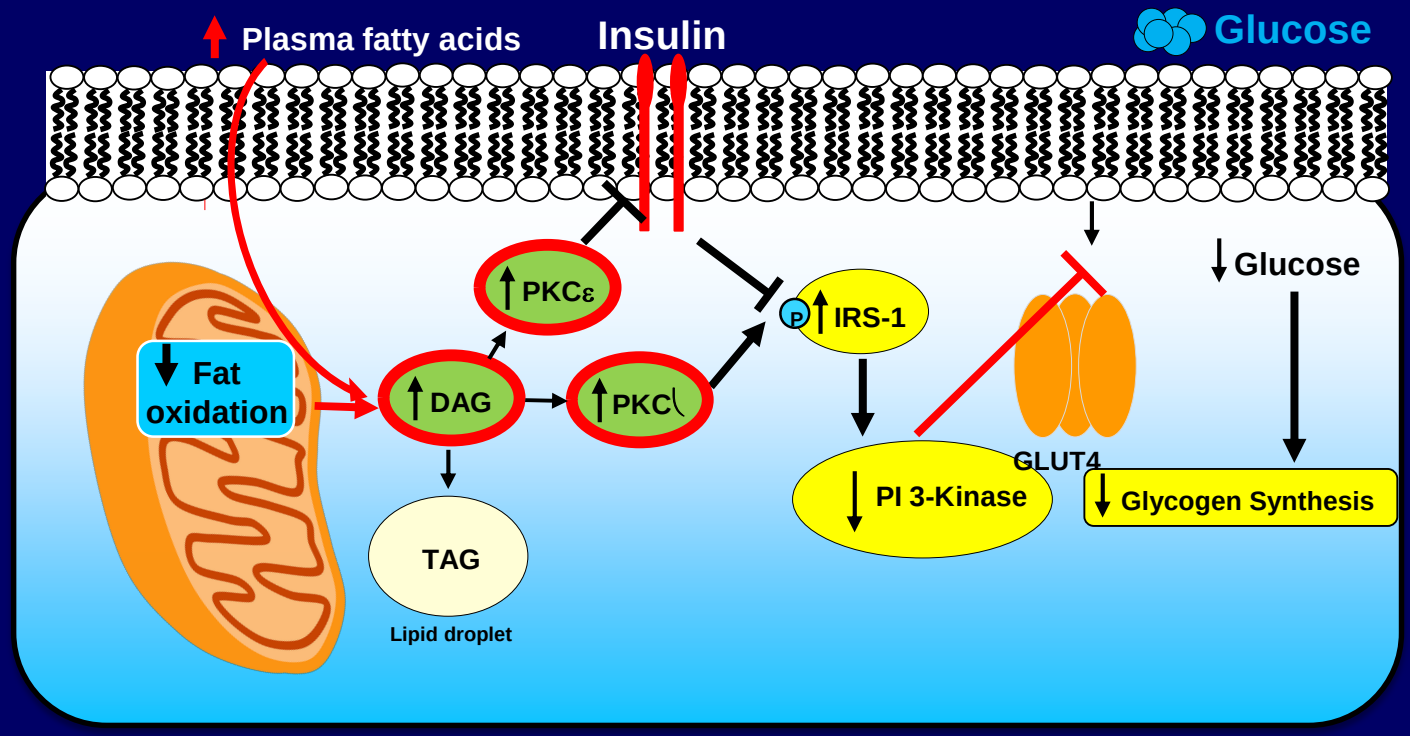
Shulman JCI 2000

Fatty acids inhibit insulin-stimulated PI 3-Kinase activity in human skeletal muscle



Dresner et al. J Clin Invest. 1999

Molecular mechanism of ectopic lipid-induced muscle insulin resistance
Diacylglycerol (DAG)-PKCθ/PKCε-insulin receptor pathway

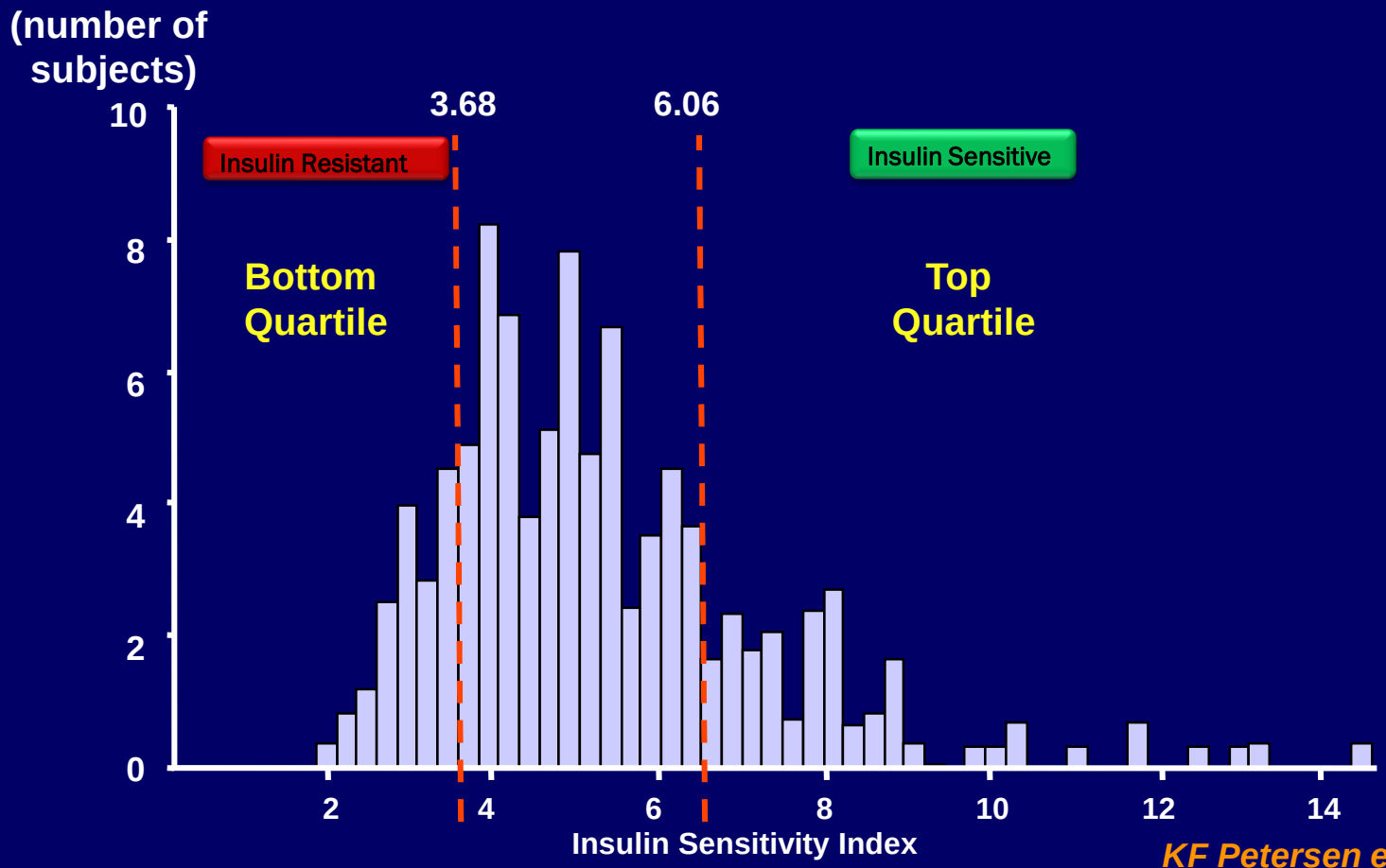


Griffin et al. Diabetes 1999, Yu et al. JBC 2002, Morino et al. JCI 2005, Szendroedi et al. PNAS 2014, Shulman NEJM 2014, Song et al. Cell Met 2020

Role of muscle insulin resistance in the pathogenesis of NAFLD and cardiovascular disease

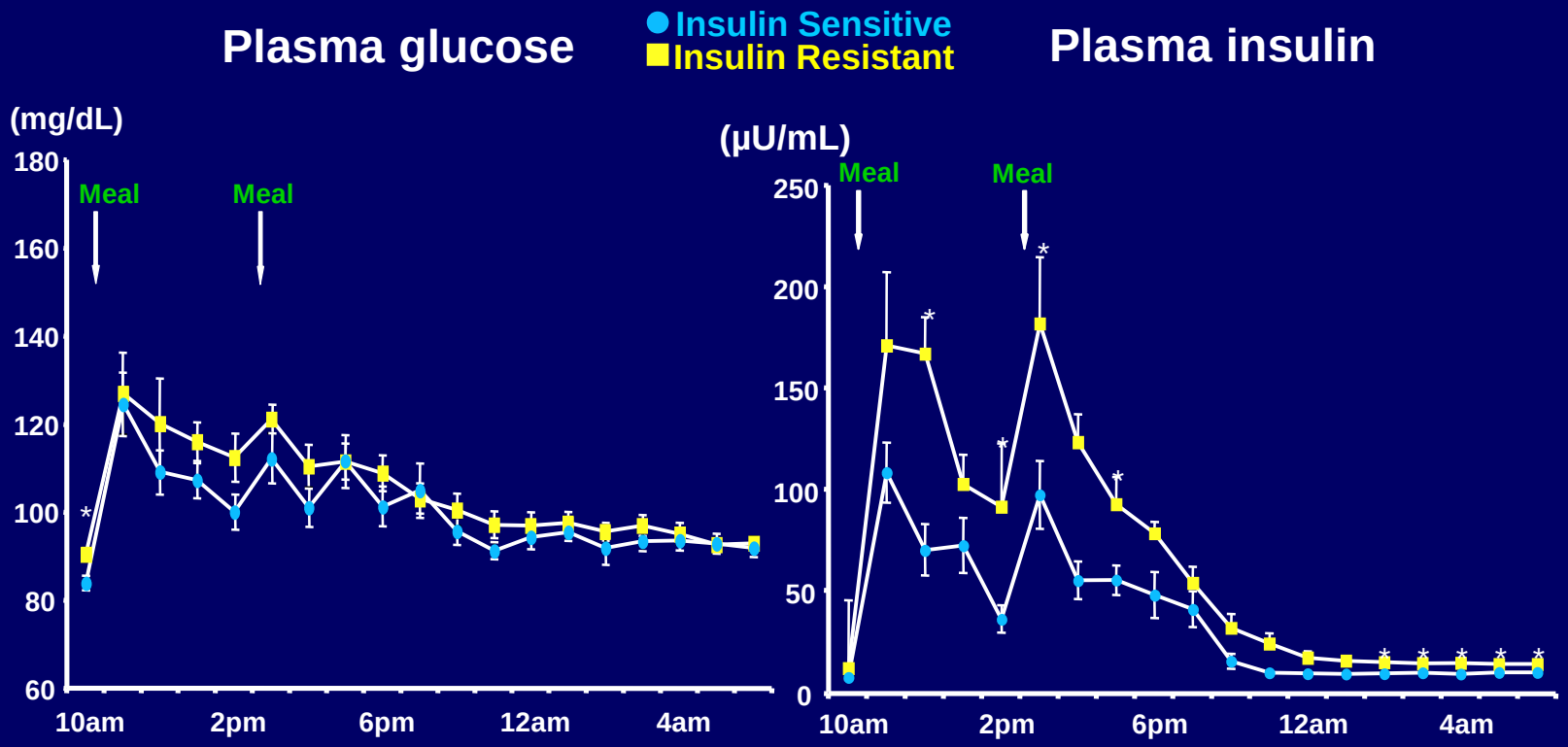
Hypothesis: Muscle insulin resistance promotes NAFLD and atherogenic dyslipidemia by changing the fate of ingested carbohydrate from muscle glycogen to fat.

Distribution of insulin sensitivity index in healthy lean individuals



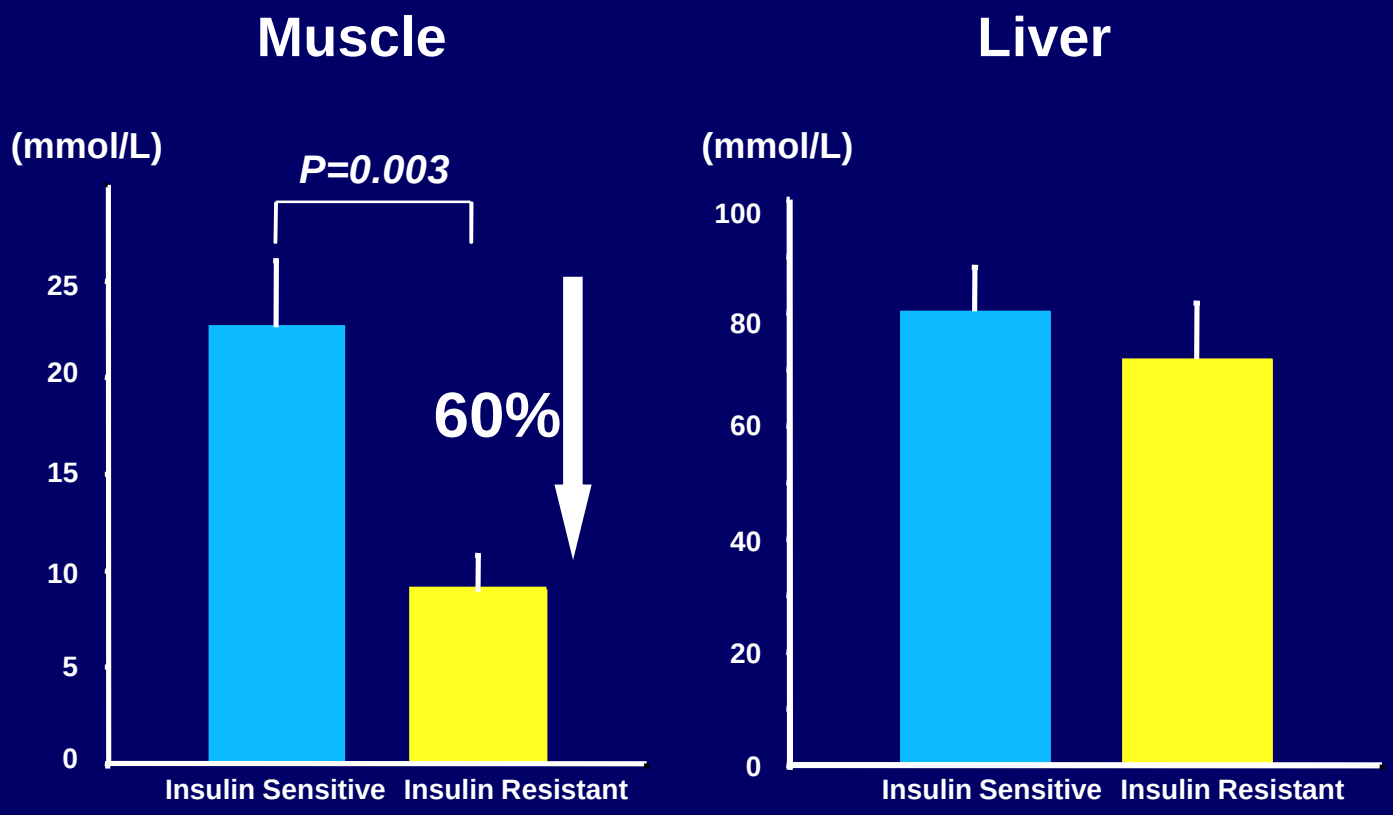
KF Petersen et al. PNAS 2007

Meal tolerance



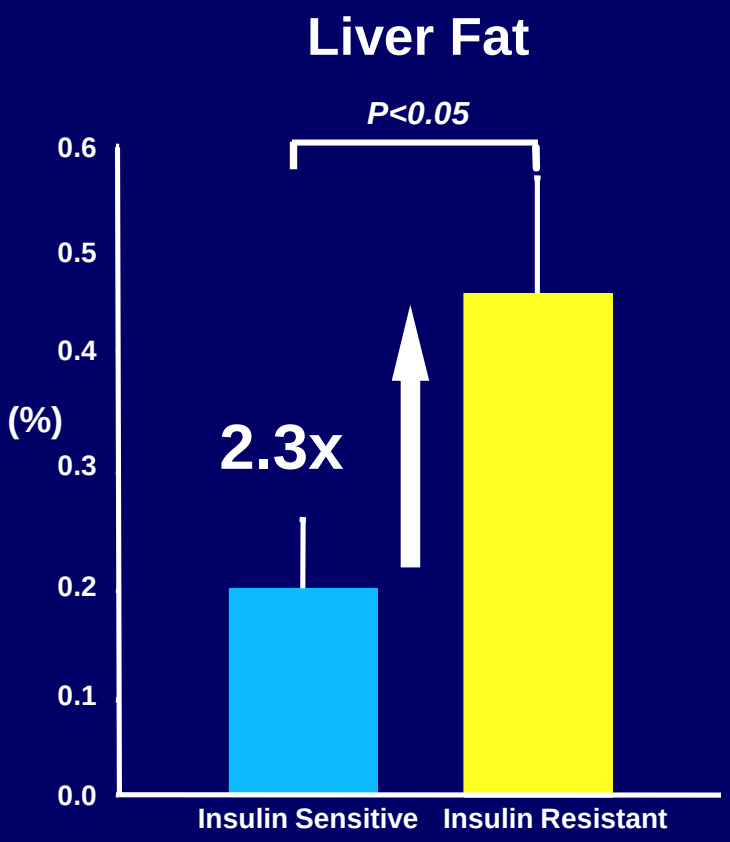
KF Petersen et al. PNAS 2007

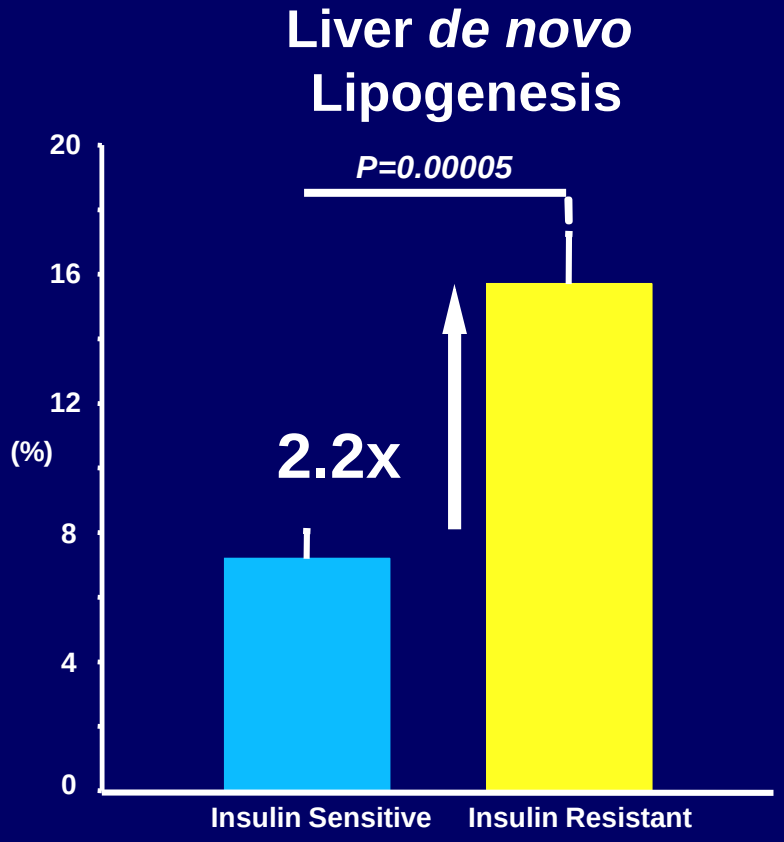
Change in muscle and liver glycogen following carbohydrate ingestion



KF Petersen et al. PNAS 2007

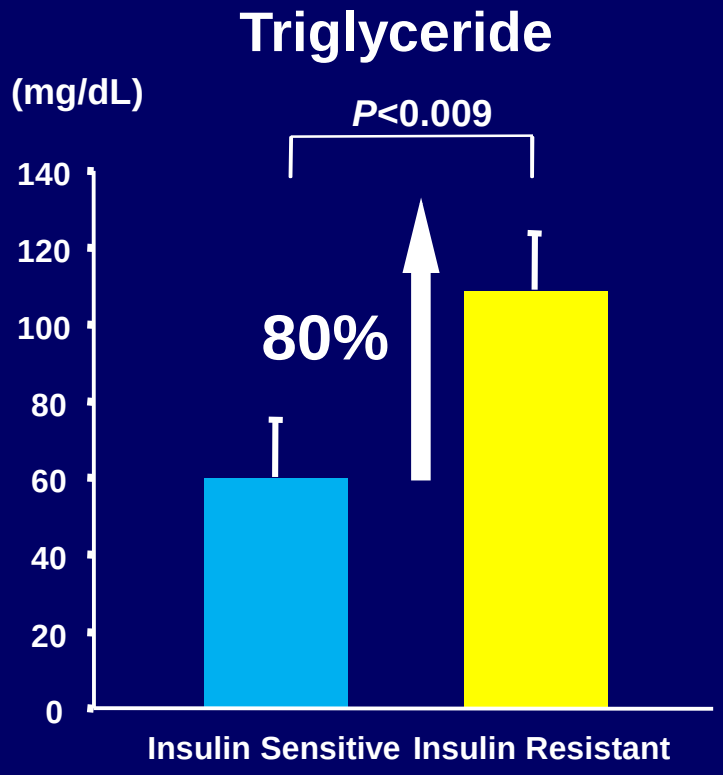
Change in liver fat and hepatic *de novo* lipogenesis following carbohydrate ingestion

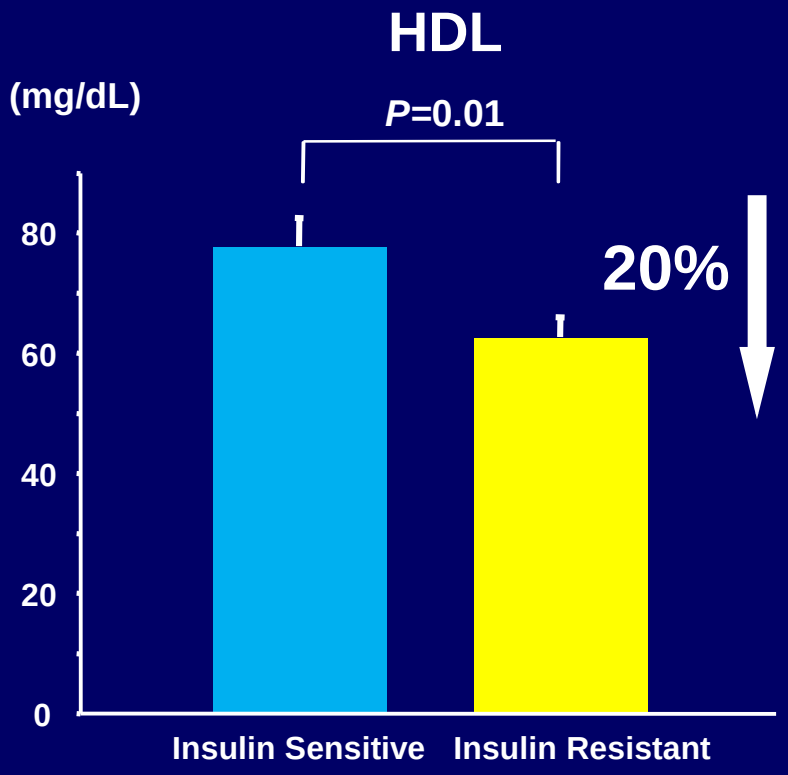




KF Petersen et al. PNAS 2007

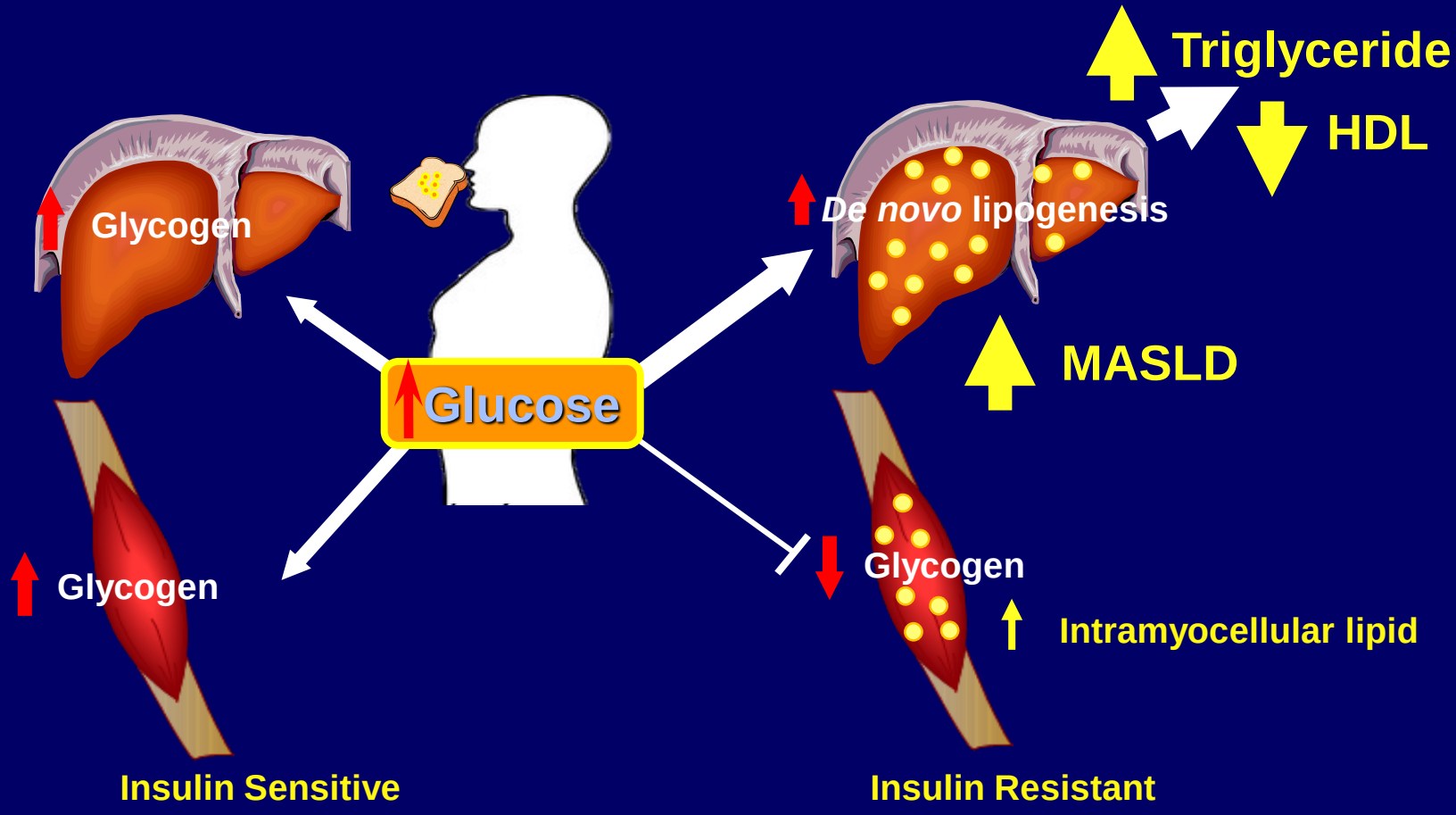
Plasma Lipids



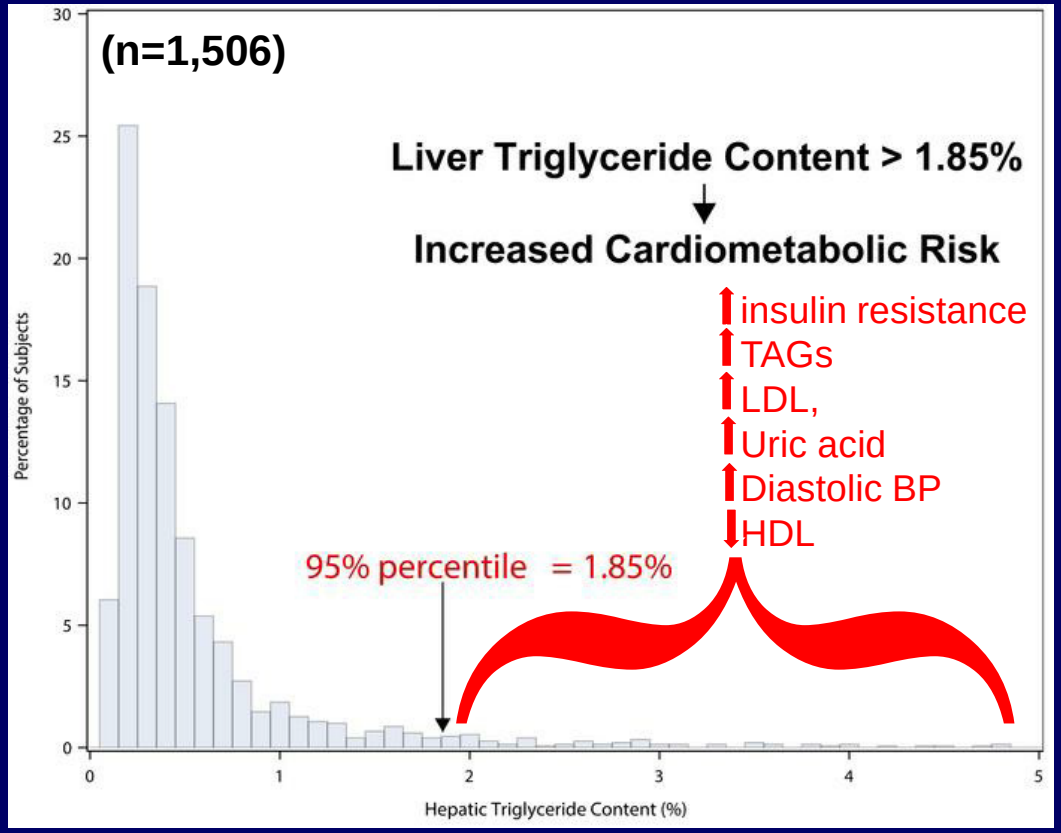


KF Petersen et al. PNAS 2007

Muscle insulin resistance promotes MASLD and atherogenic dyslipidemia by changing the fate of ingested carbohydrate from muscle glycogen to fat

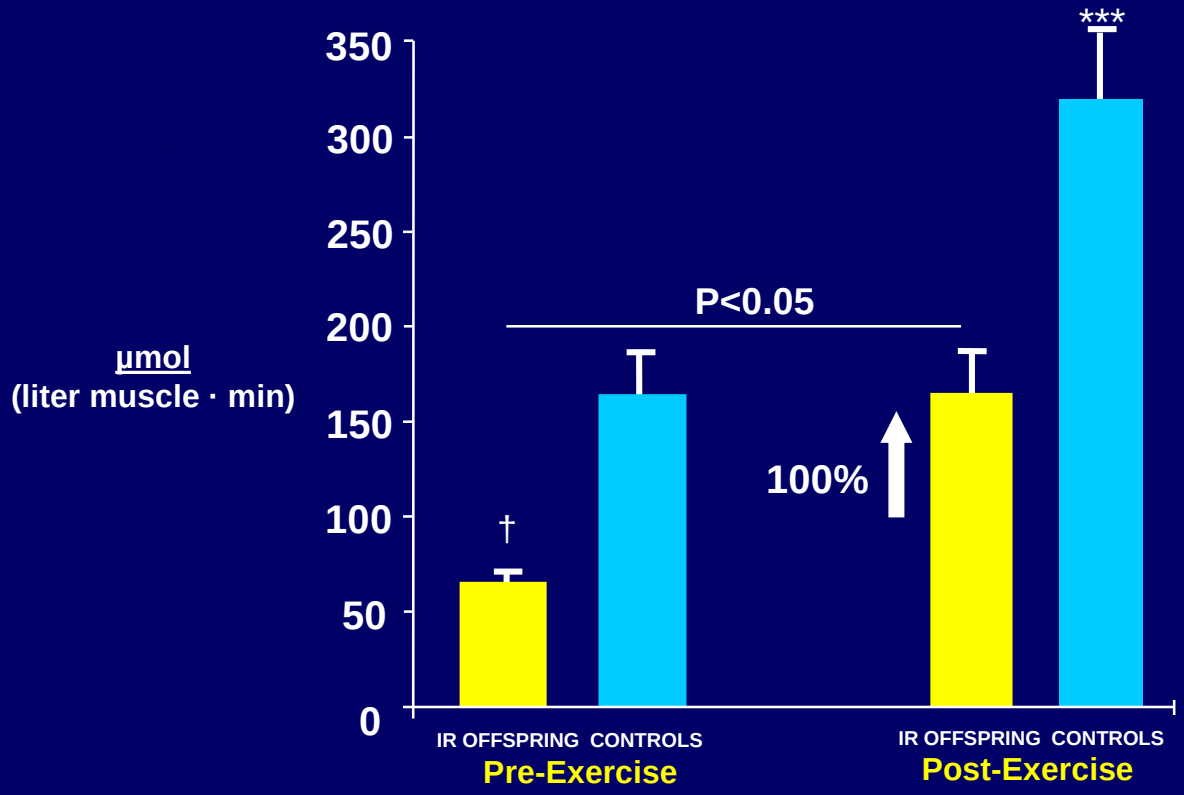


The 95th Percentile Upper Limit of Hepatic Triglyceride Content in Healthy Lean Individuals is 1.85% - not 5.5%



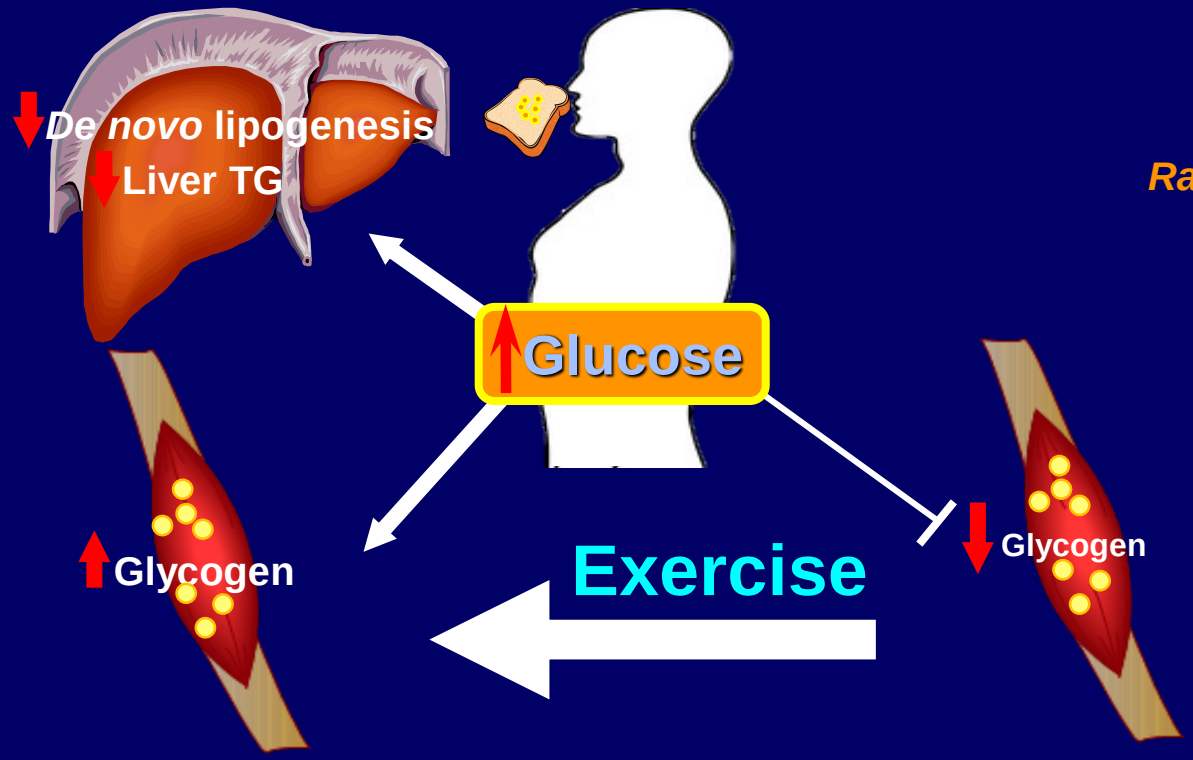
Petersen et al. *JCI Insight 2022*

Effects of a single-bout of exercise on insulin-stimulated muscle glycogen synthesis



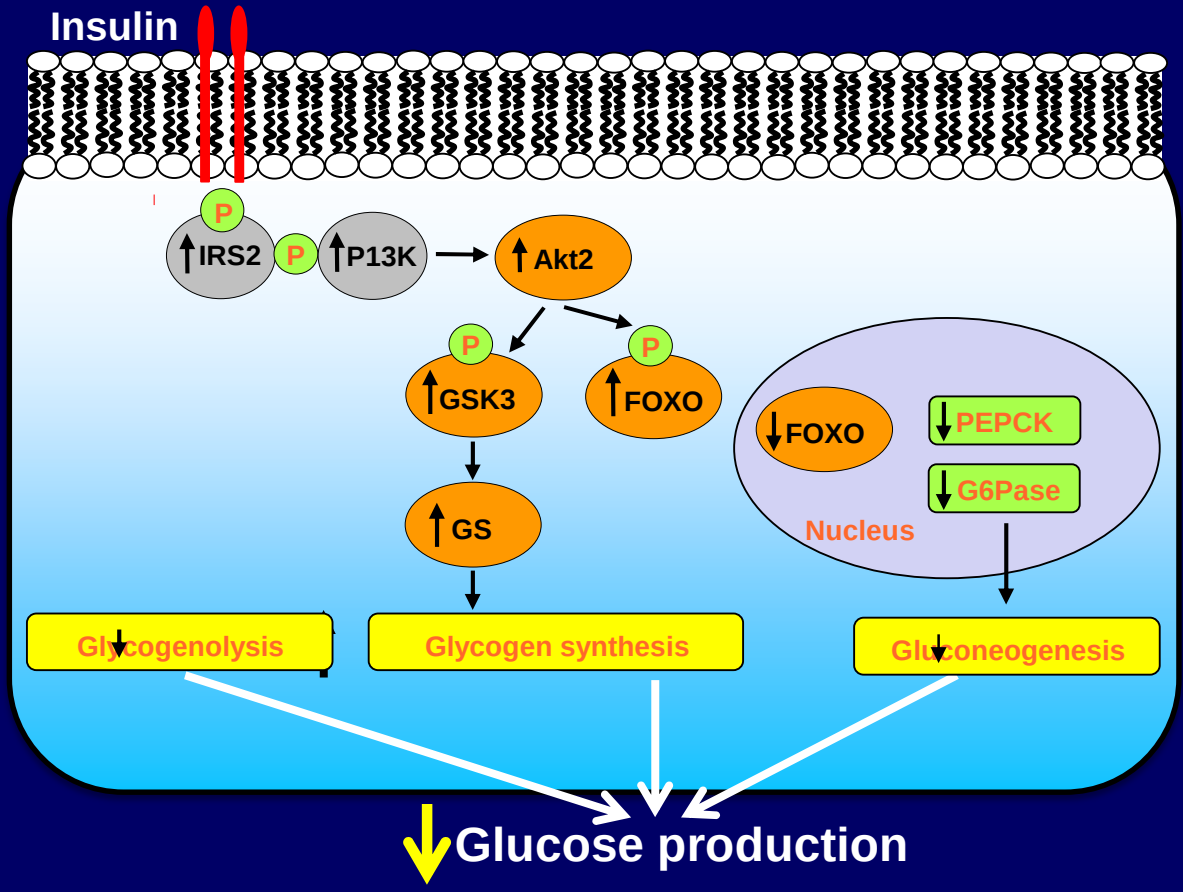
Perseghin et al. NEJM 1996

A single-bout of exercise reverses the abnormal pattern of carbohydrate storage in insulin resistant individuals

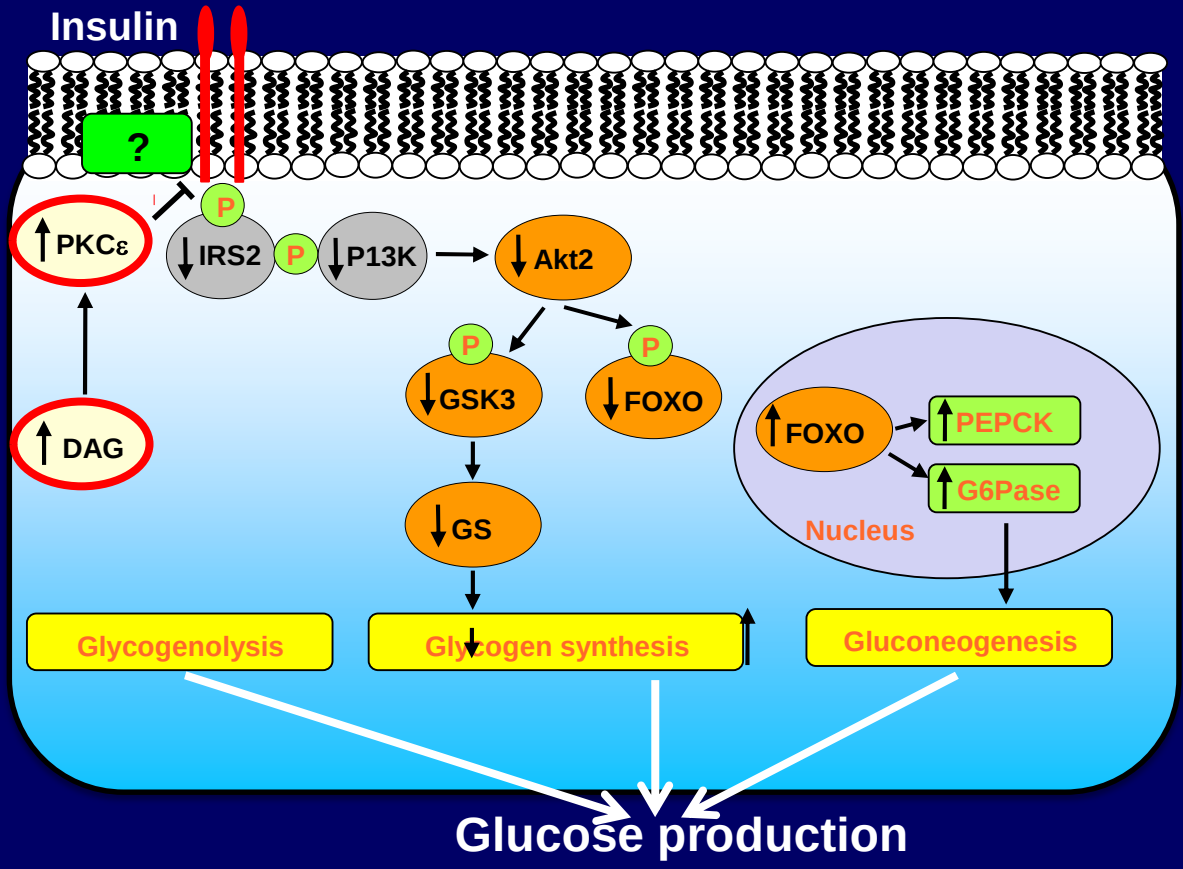


Rabøl et al. PNAS 2011

Insulin signaling in hepatic glucose metabolism

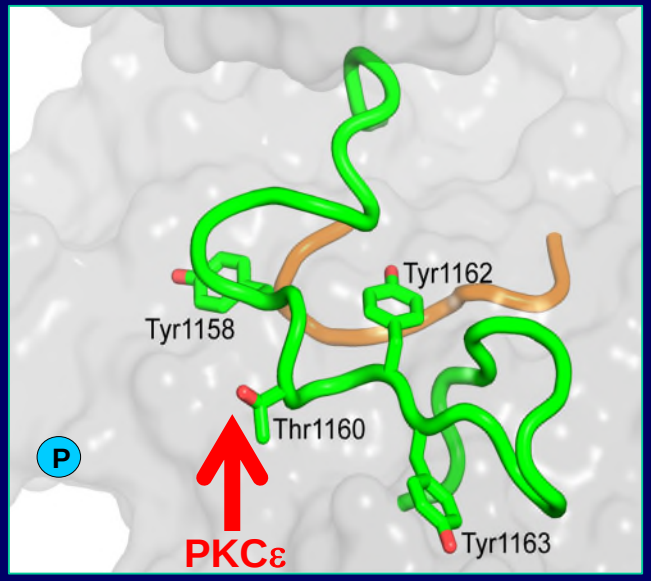


DAG-PKCε-insulin receptor pathway-mediated hepatic insulin resistance



Samuel et al. JBC 2004, Samuel et al. JCI 2007, Samuel and Shulman. Cell 2012

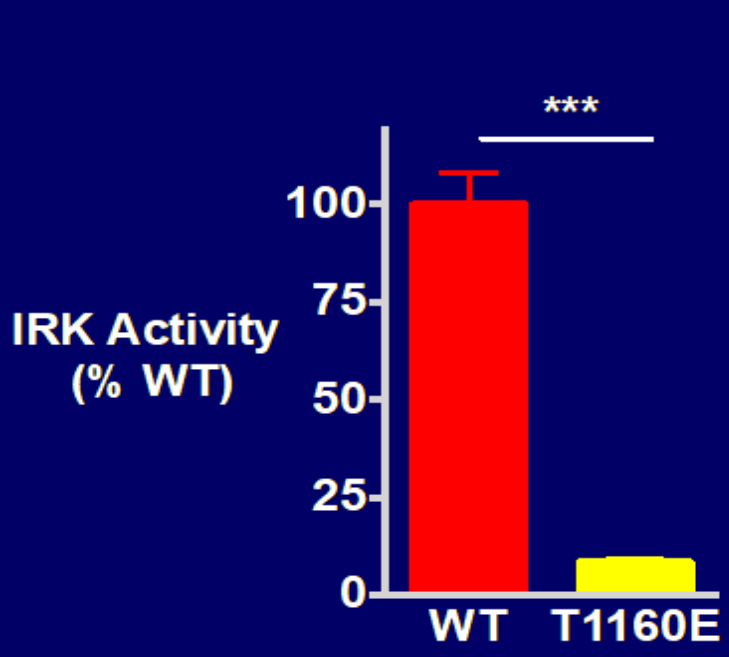
Threonine^{1160} in the insulin receptor catalytic loop is phosphorylated by PKCε and it is evolutionarily conserved from humans to fruit flies

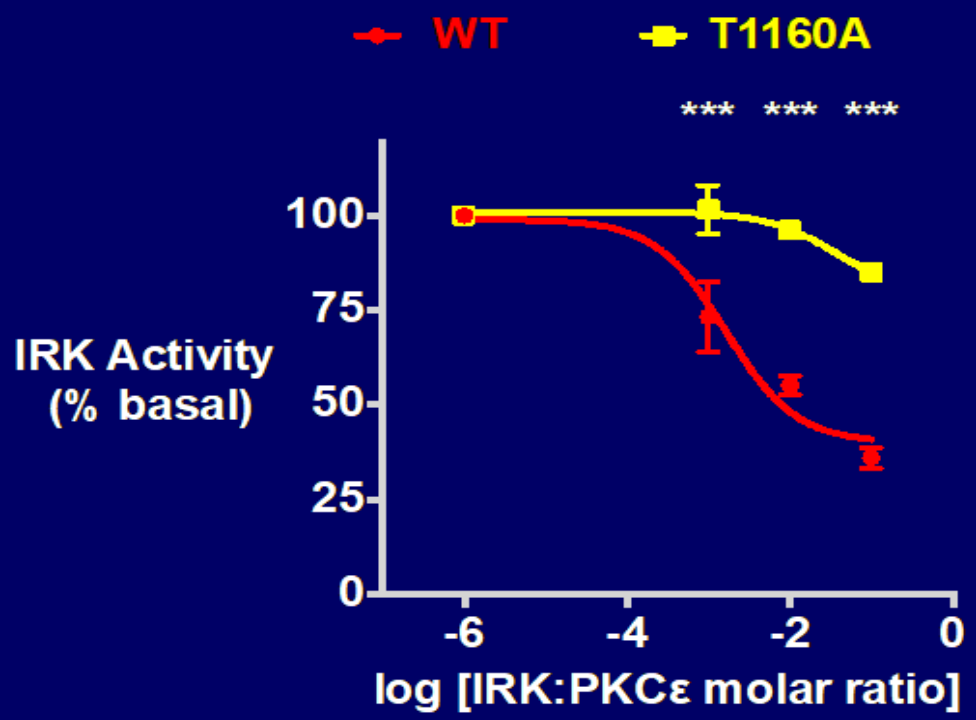


Insulin Receptor Kinase Catalytic Loop

Species	Gene	Sequence Threonine^{1160} ↓
Homo sapiens	*Insr*	DIYETDYYRK
Mus musculus	*Insr*	DIYETDYYRK
Xenopus laevis	*insr*	DIYETDYYRK
Danio rerio	*insra*	DIYETDYYRK
Drosophila melanogaster	*InR*	DIYETDYYRK

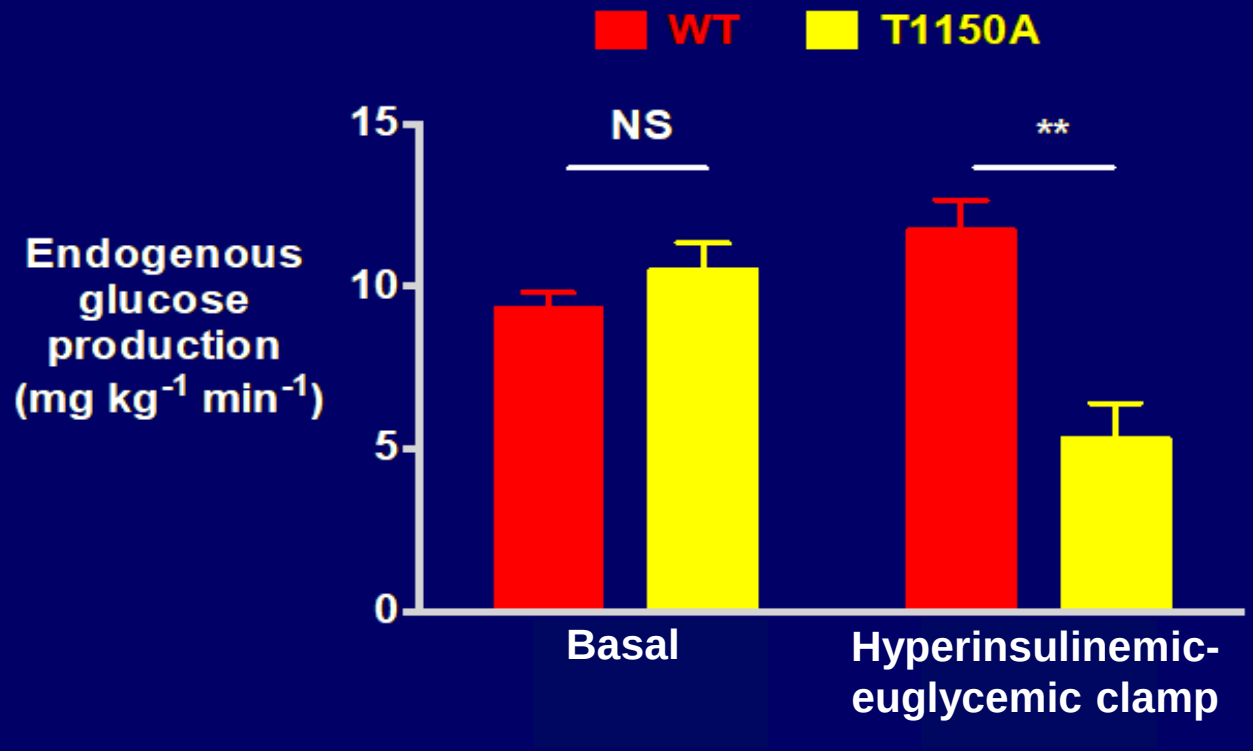
Insulin receptor^{T1160E} is kinase dead
Insulin receptor^{T1160A} is protected from PKCε inhibition





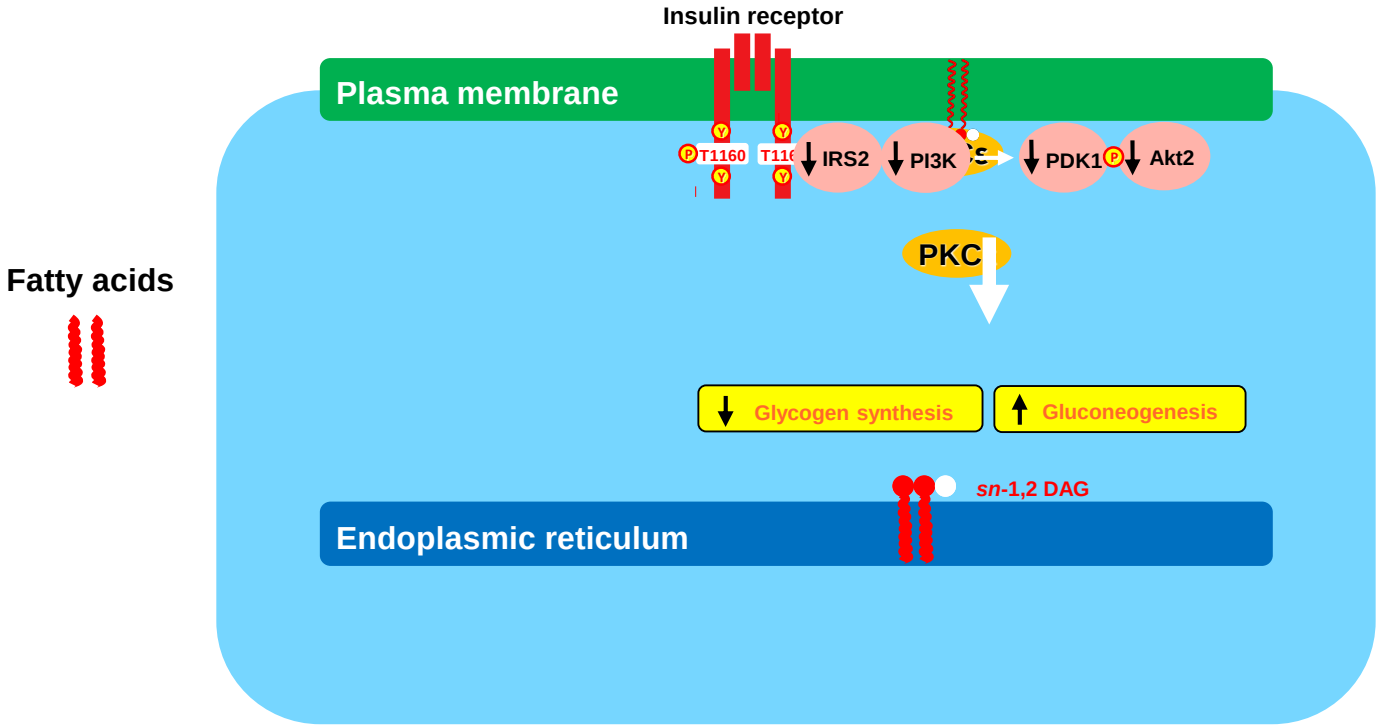
MC Petersen et al. JCI 2016

Insr^{T1150A} *m*ice are protected from high-fat diet induced hepatic insulin resistance



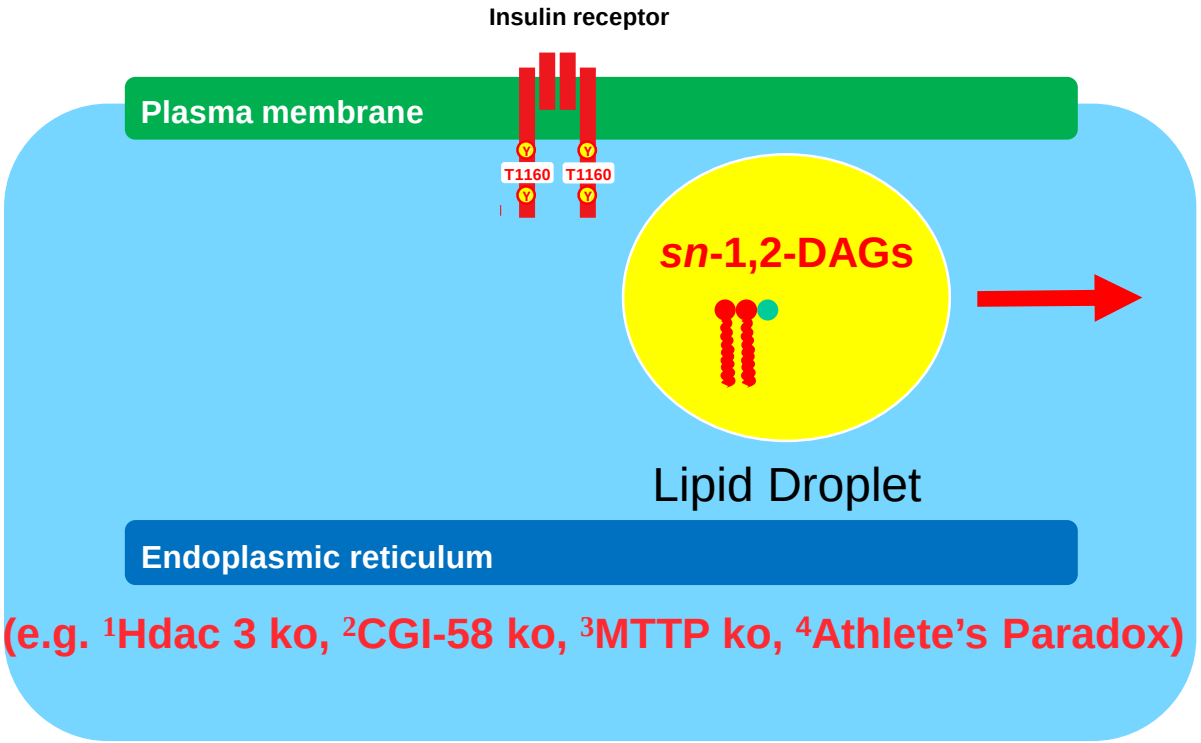
MC Petersen et al. JCI 2016

Molecular mechanism of *sn*-1,2-DAG-PKCε-IRK^{T1160}phosphorylation-induced insulin resistance



Kun et al. ***Cell Metabolism*** 2020

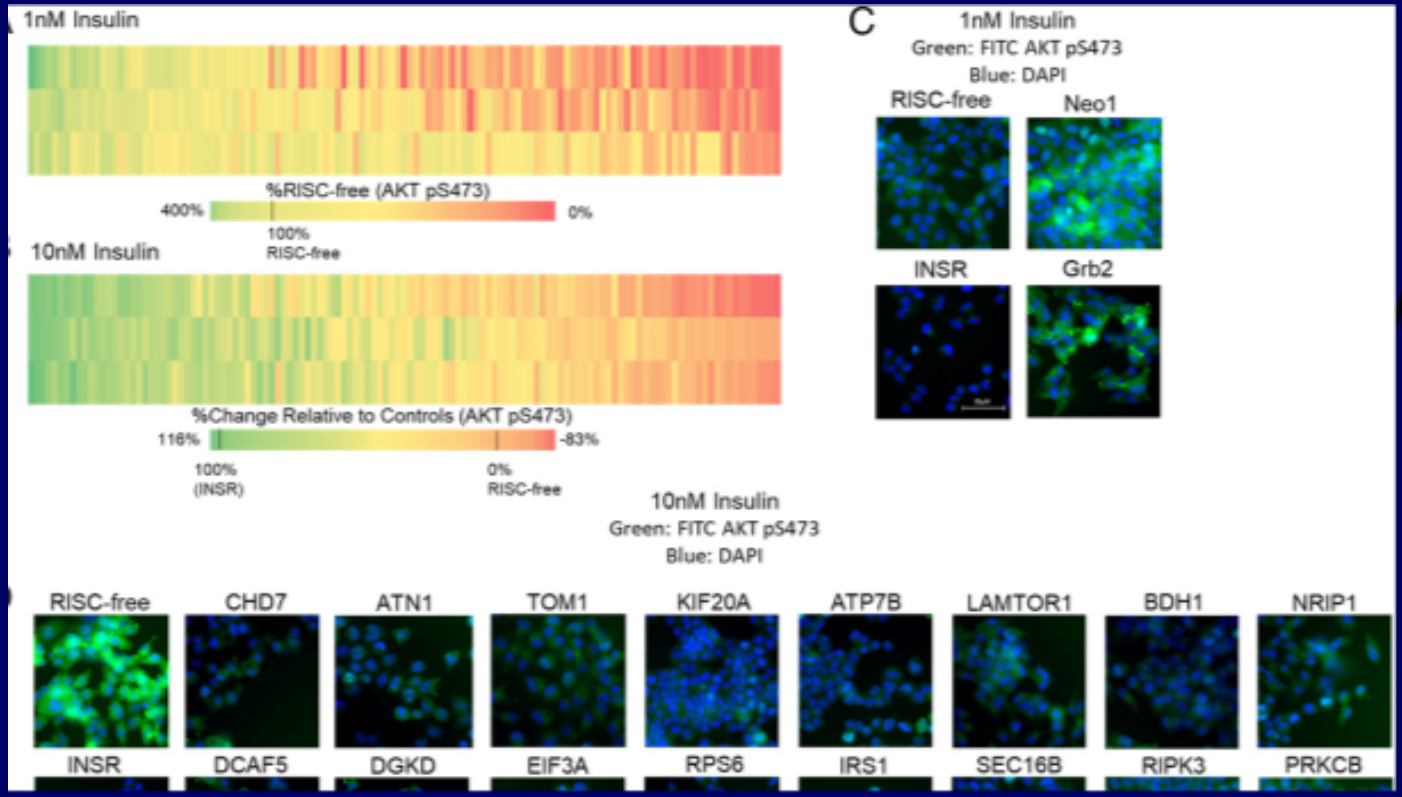
Sequestration of *sn*-1,2-DAGs in lipid droplets do not cause insulin resistance



No Insulin Resistance

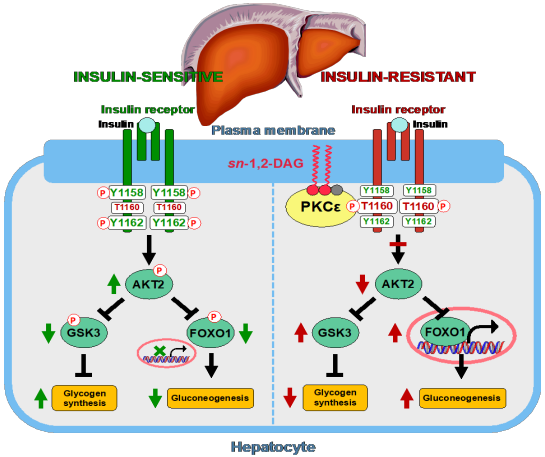
[1]Sun et al. ***Nat Med*** *2012,* [2]Cantley et al. ***PNAS*** *2013,* [3]Abulizi et al. ***J. Lipid Res*** *2020,* [4]Gaspar et al. ***Diabetologia*** *2023*

PKCε contributes to lipid-induced insulin resistance through cross talk with p70S6K and other regulators

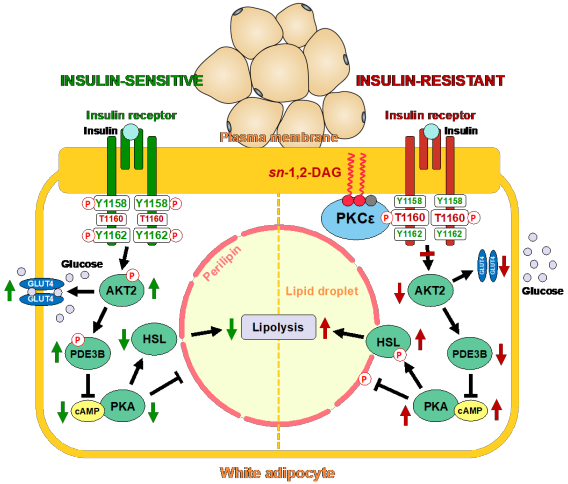


Gassaway et al. PNAS 2018

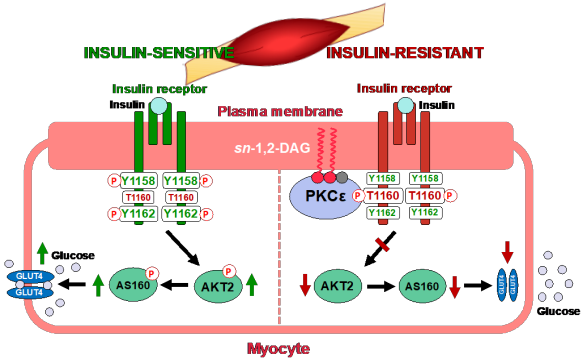
The *sn*-1,2-DAG-PKCε-IRK^{T1160} phosphorylation pathway occurs in many organs



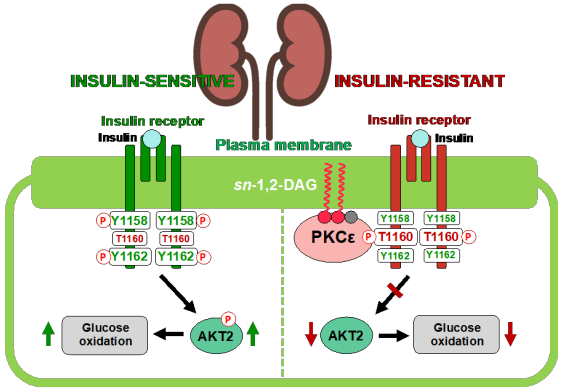
Lyu et al., *Cell Metabolism*, 2020



Lyu et al., *JCI Insight*, 2021

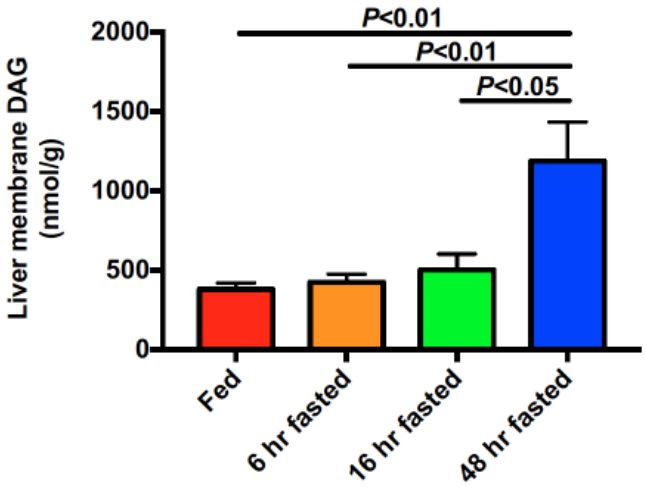


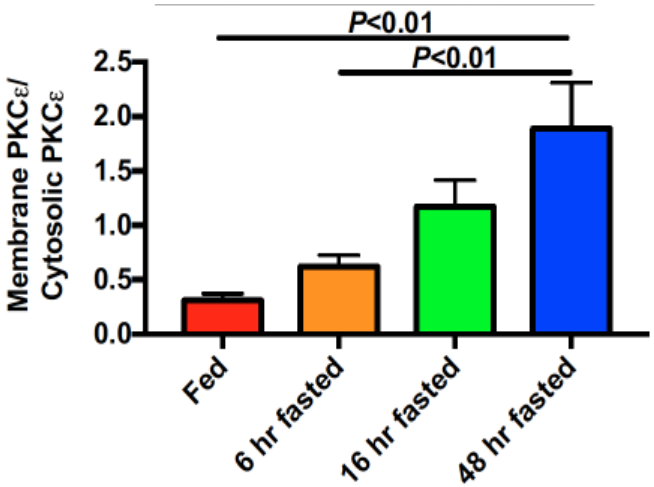
Song et al., *Cell Metabolism*, 2020



Hubbard et al., ADA oral presentation 2021

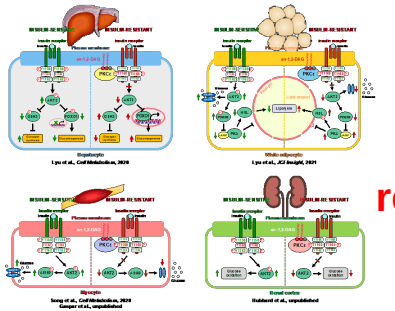
Starvation leads to increased hepatic membrane DAG content and PKCε activation





Perry et al., *Cell*, 2018

The Evolutionary Basis of Insulin Resistance

Starvation → Lipolysis ↑ →  Insulin resistance

→ Refeeding → Plasma glucose level following refeeding ↑

↓

Glucose availability to obligate glucose utilizers (CNS, RBCs, kidney medulla et al.) ↑

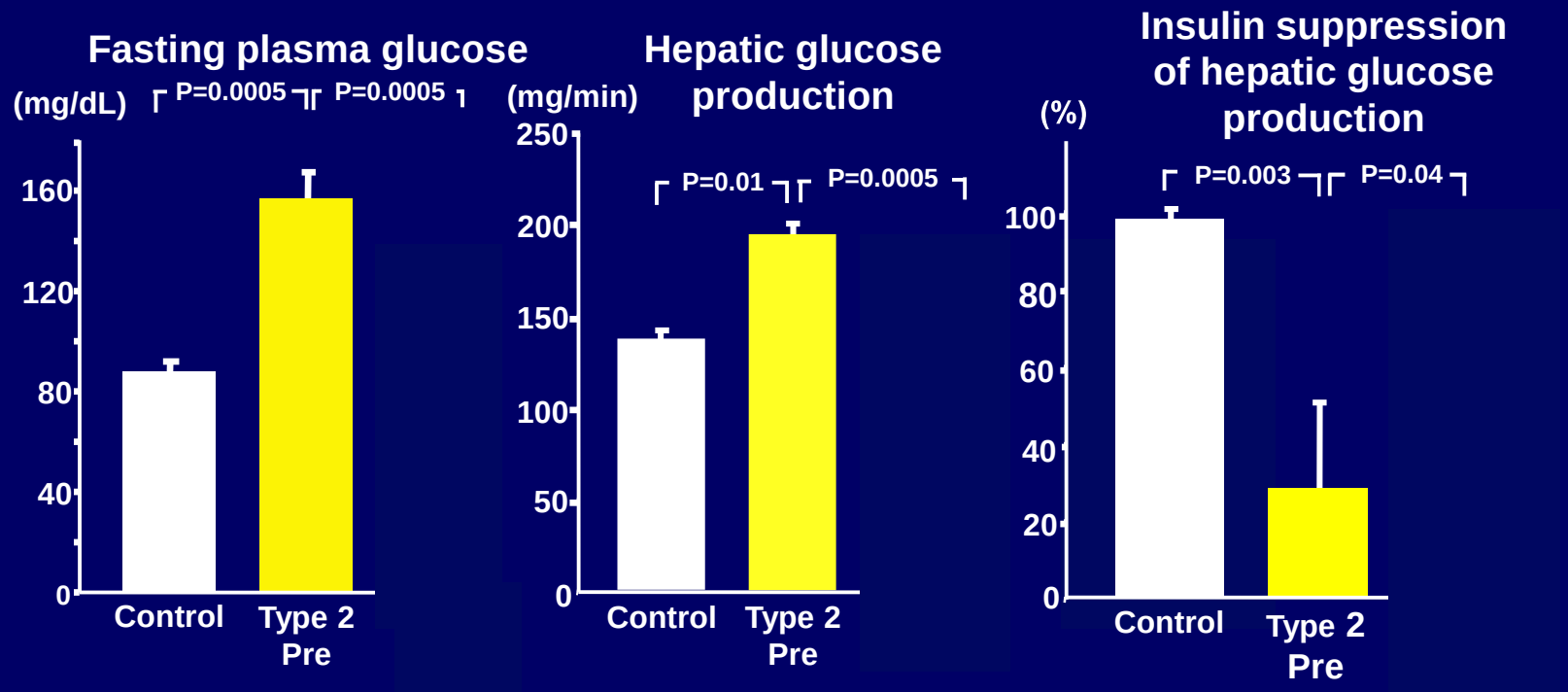
↓

Survival advantage

Effect of modest weight loss on MASLD and T2D

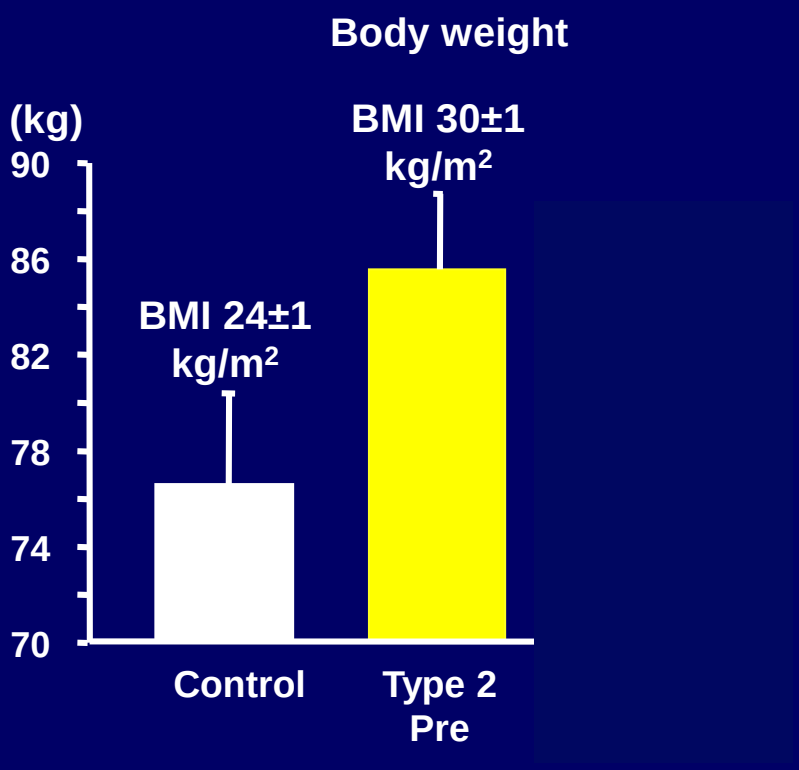
Role of Metabolic Dysfunction-Associated Steatosis Liver Disease (MASLD) in Type 2 Diabetes

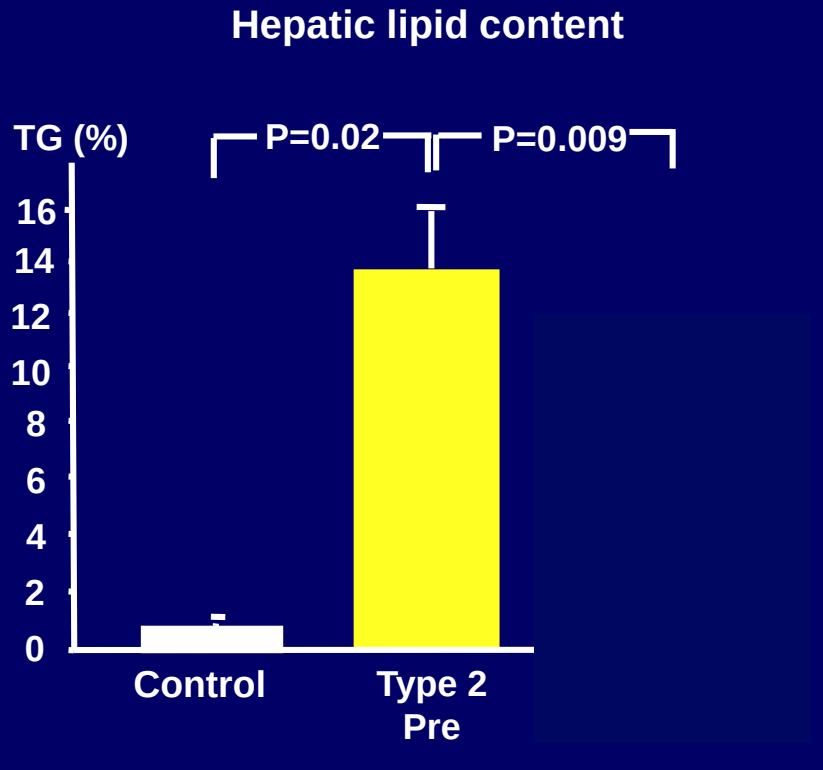
Hepatic glucose metabolism before and after weight loss



KF Petersen et al., Diabetes 2005

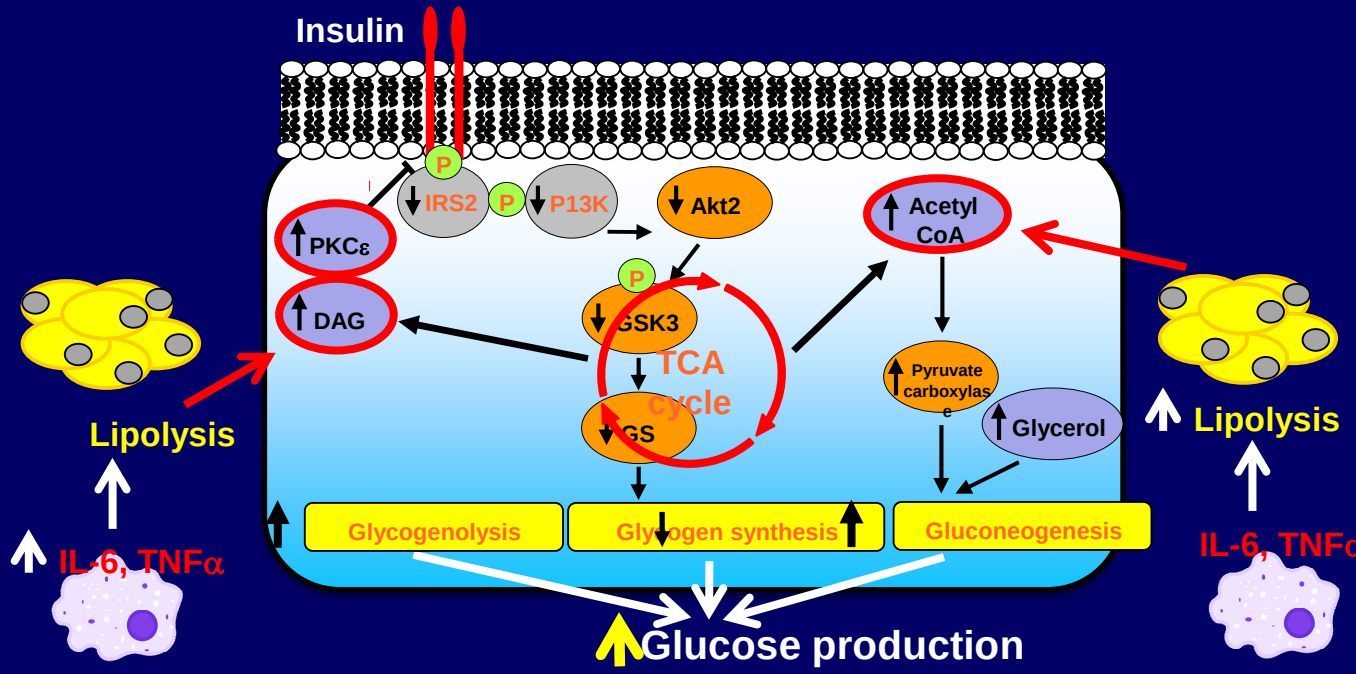
Body weight and hepatic lipid content before and after weight loss





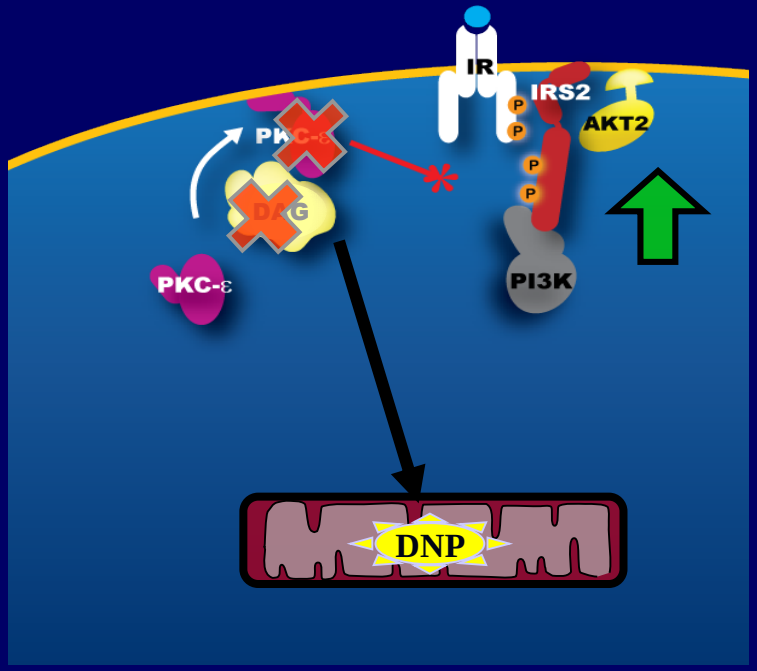
KF Petersen et al. Diabetes 2005

Mechanisms for Dysregulated Hepatic Glucose Metabolism in Type 2 Diabetes



Perry et al. Cell Met 2013, Perry et al. Science 2015, Perry et al. Cell 2015, Samuel and Shulman JCI 2016, Abulizi et al. FASEB J 2017

Dinitrophenol (DNP) corrects MASLD and hepatic insulin resistance

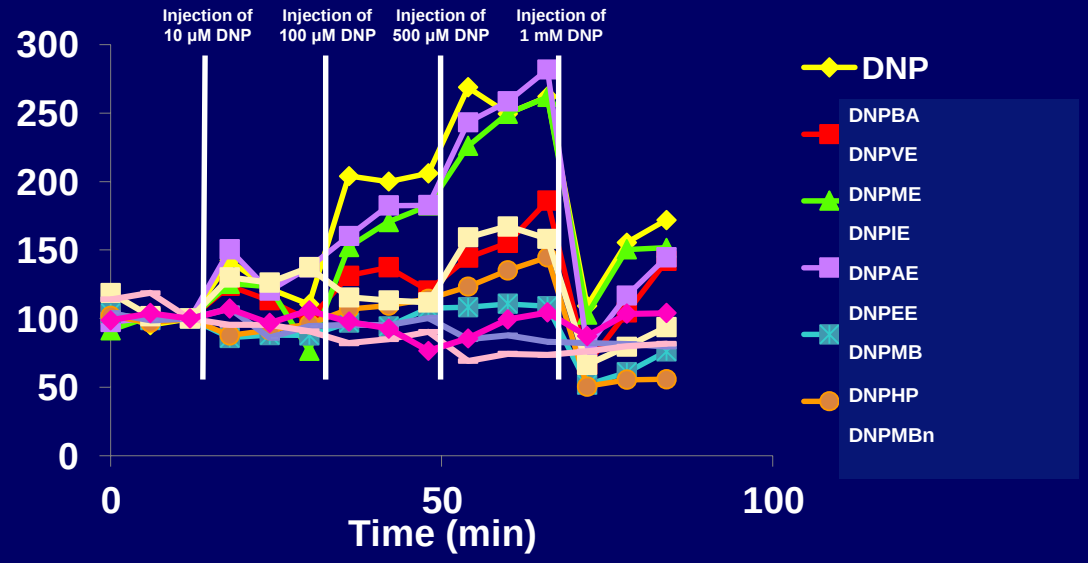


DNP

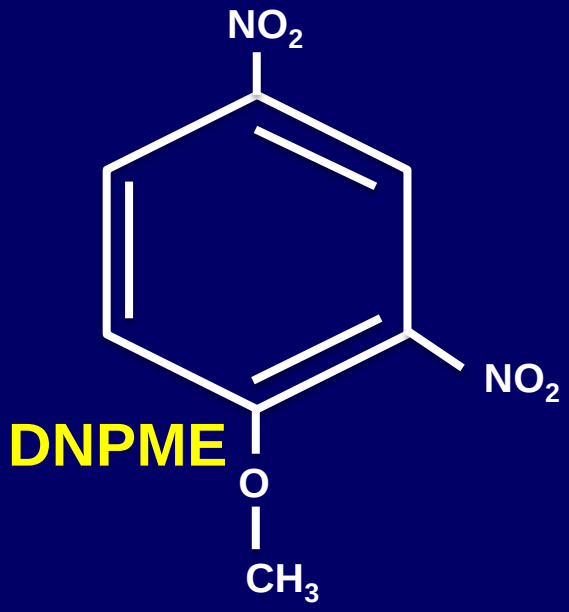
- Increased lipid oxidation
- Reduced hepatic fat content in fat-fed rats
- Prevented PKCε activation
- Improved hepatic insulin sensitivity

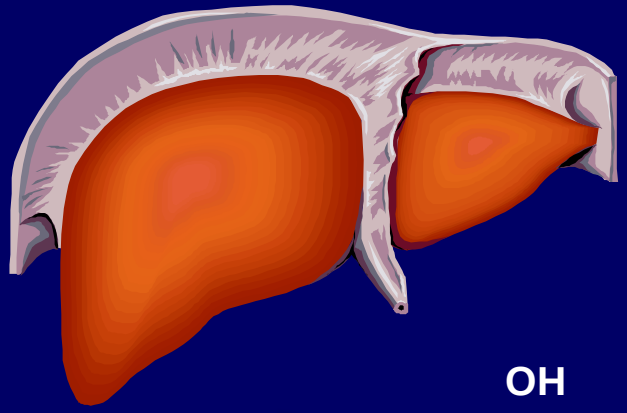
Samuel et. al. JBC 2004

Screening of compounds: Oxygen consumption rate in cultured hepatocytes



DNP-methyl ether (DNPME)





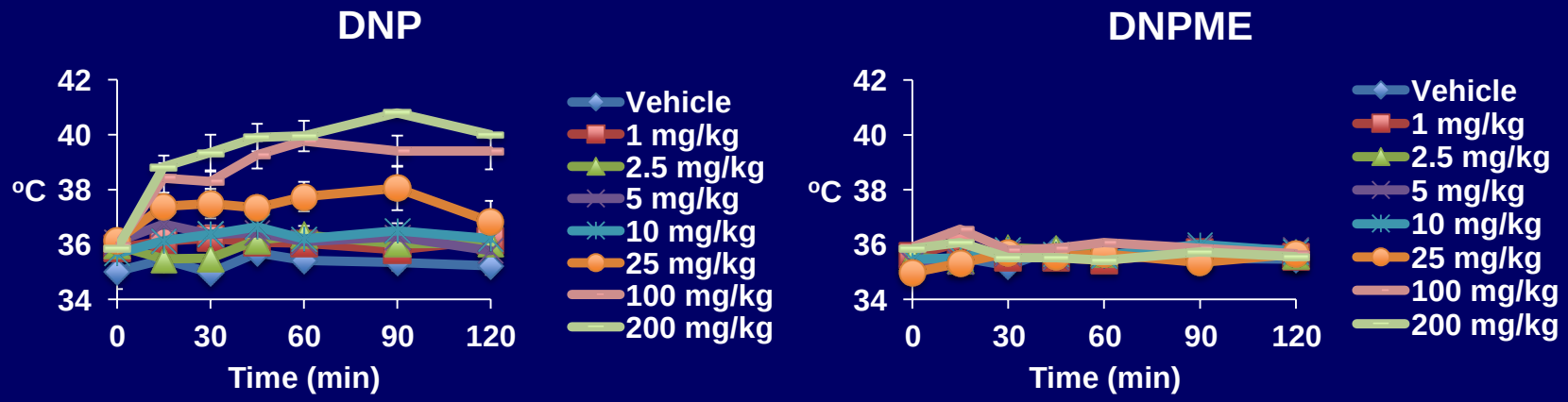
OH

DNP

P450 1A2

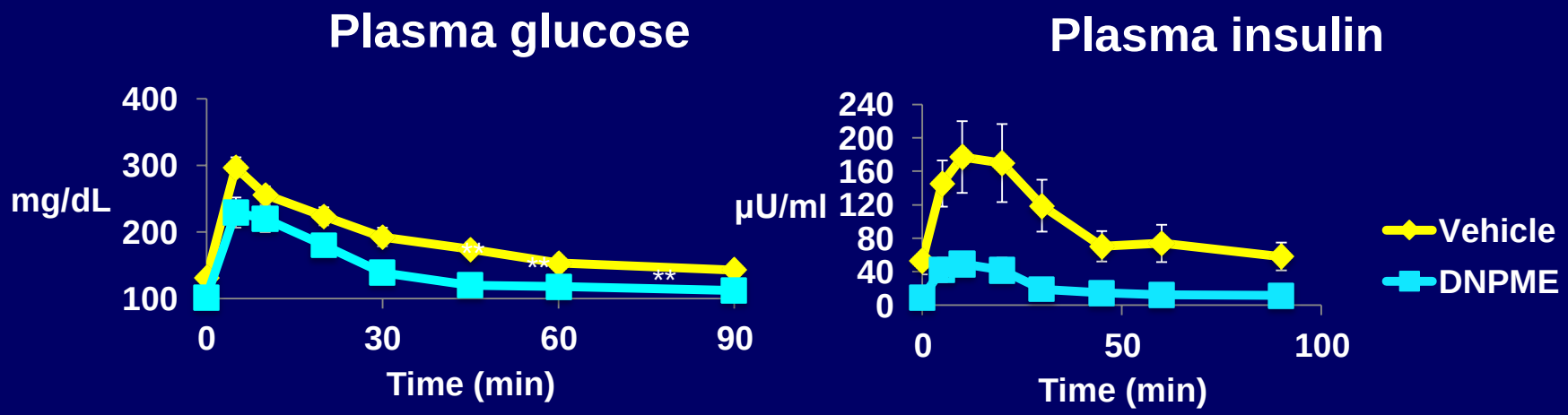
Perry et al. *Cell Metab.* 2013

Dose Response: DNP vs. DNPME
Body temperature



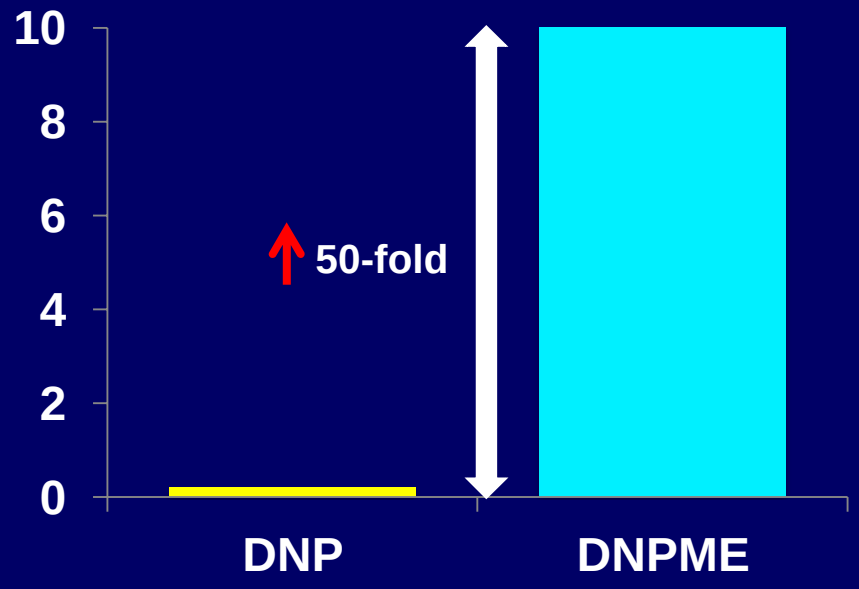
Perry et al. *Cell Metab.* 2013

DNPME Rx Reverses Insulin Resistance in HFD Rats



Perry et al. Cell Metabolism 2013

Toxic/therapeutic dose: DNP vs. DNPME



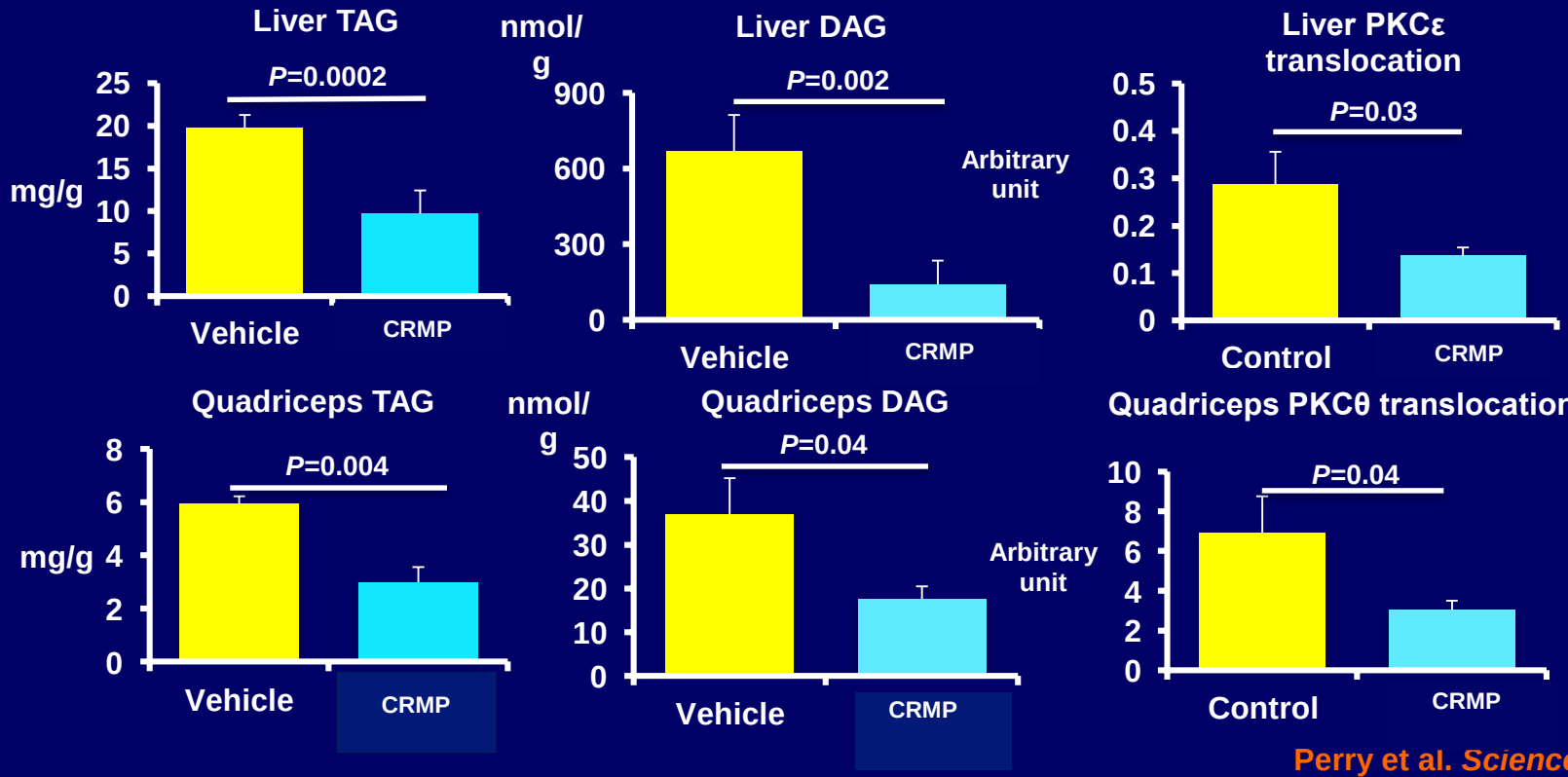
METABOLIC DISEASE

Controlled-release mitochondrial protonophore reverses diabetes and steatohepatitis in rats

Rachel J. Perry,[1,2,3] Dongyan Zhang,[1] Xian-Man Zhang,[2] James L. Boyer,[2,4] Gerald I. Shulman[1,2,3]*

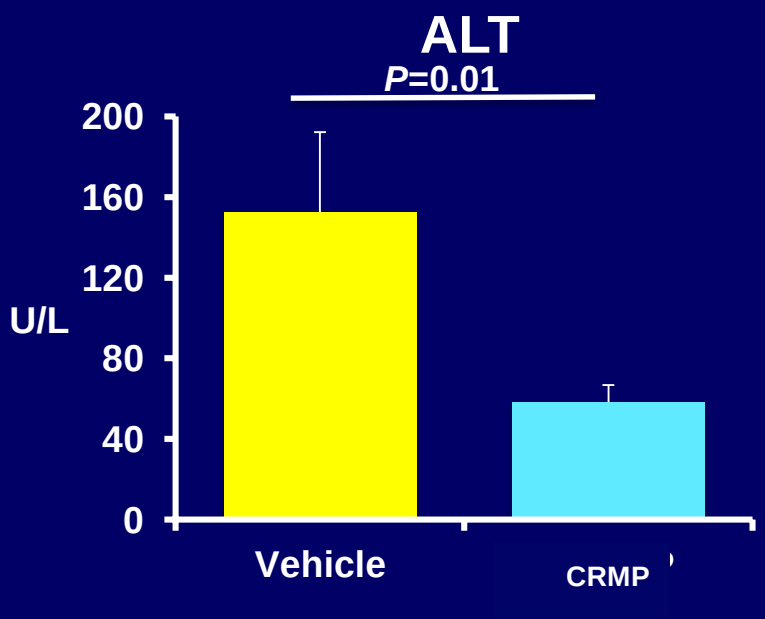
Science 2015

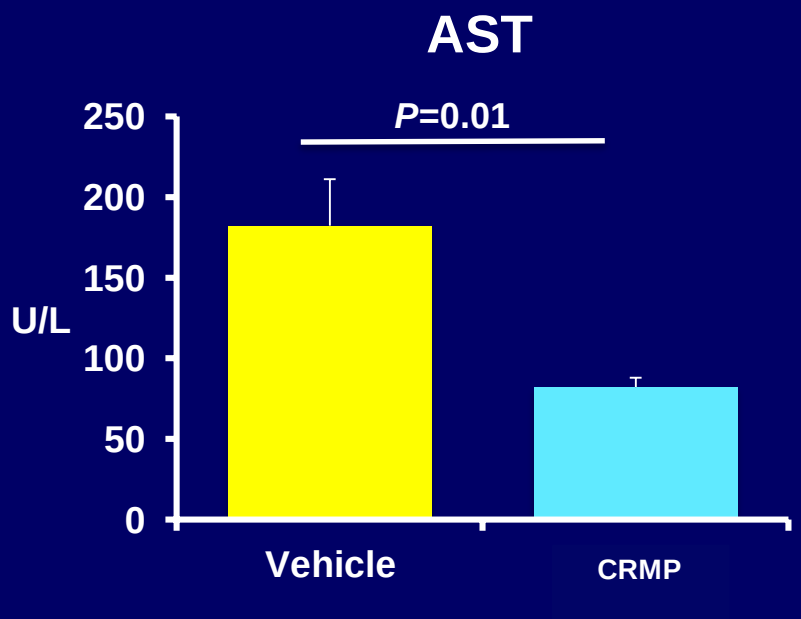
CRMP Rx Reduces Liver and Muscle TAG, DAG content and nPKC activity



Perry et al. *Science* 2015

CRMP Reverses Liver Inflammation in ZDF rats





Perry et al. Science 2015

nature COMMUNICATIONS

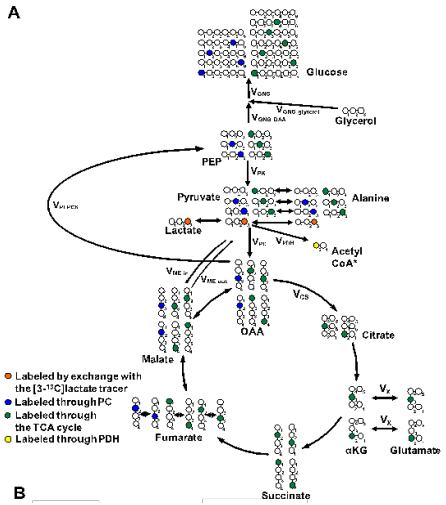
Corrected: Publisher correction

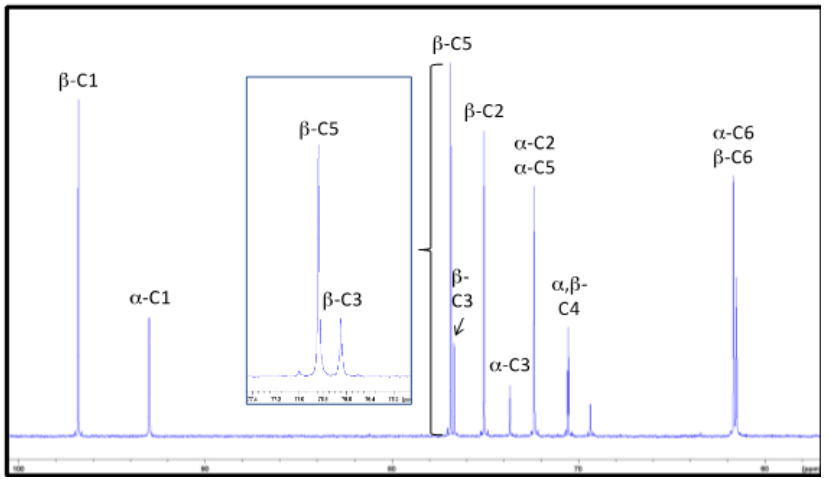
ARTICLE

DOI: 10.1038/s41467-017-01143-w OPEN

Non-invasive assessment of hepatic mitochondrial metabolism by positional isotopomer NMR tracer analysis (PINTA)

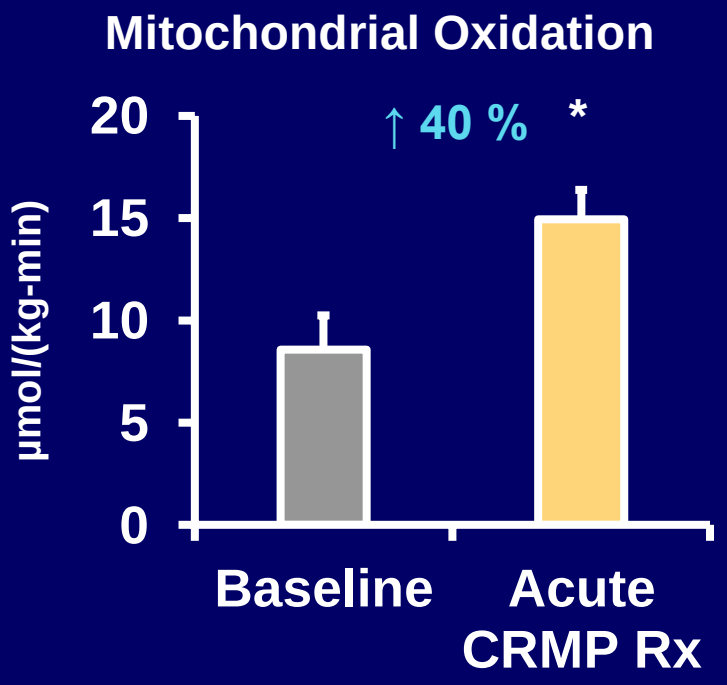
Rachel J. Perry[1], Liang Peng[1], Gary W. Cline[1], Gina M. Butrico[1], Yongliang Wang[1], Xian-Man Zhang[1], Douglas L. Rothman[2,3], Kitt Falk Petersen[1] & Gerald I. Shulman[1,4,5]

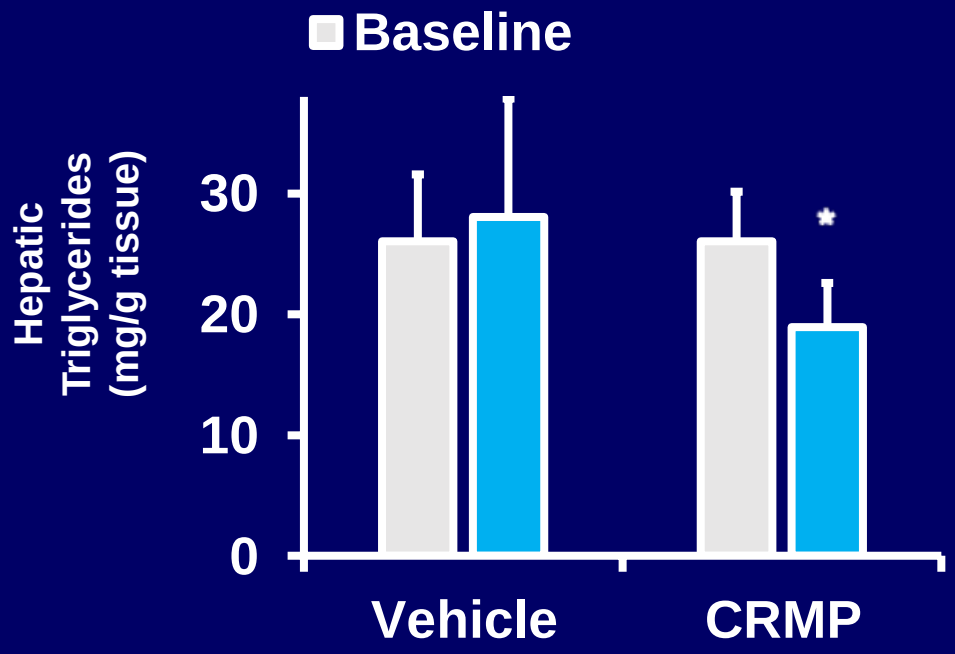




Perry et al. Nature Comm 2017

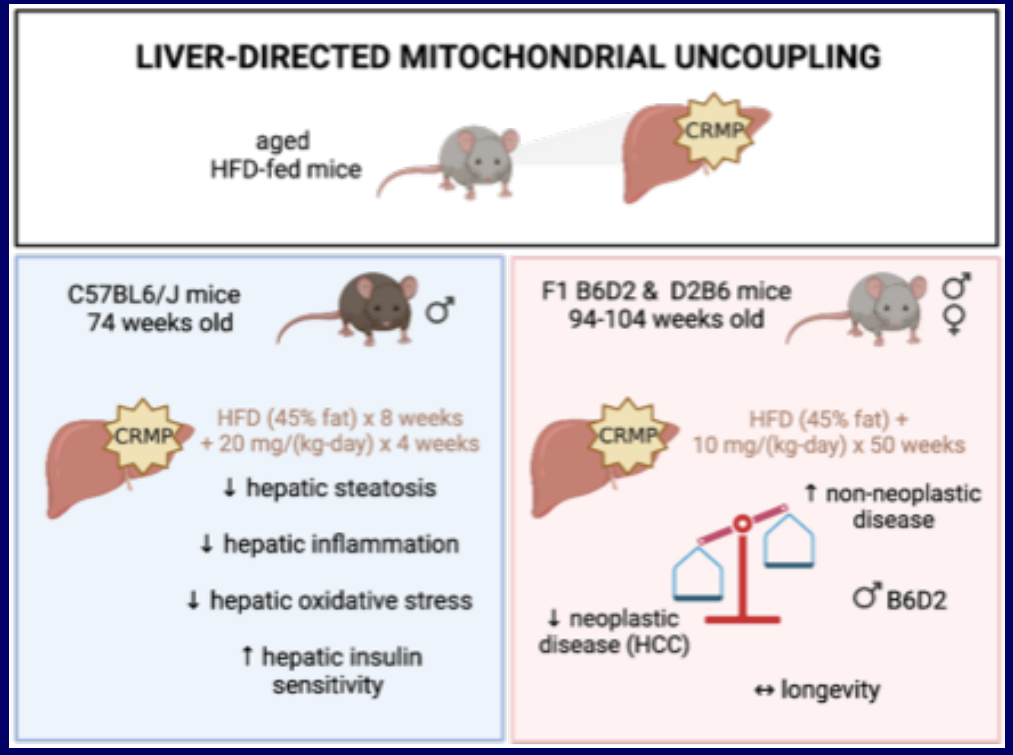
CRMP increases hepatic fat oxidation, reduces liver fat and is safe and well tolerated in non human primates





Goedeke et al. Science Translational Medicine 2019

CRMP Rx Protects Against Age-Related Metabolic Disease and Hepatocellular Carcinoma in HFD fed Mice



Goedeke et al. ***Aging Cell*** *202)*

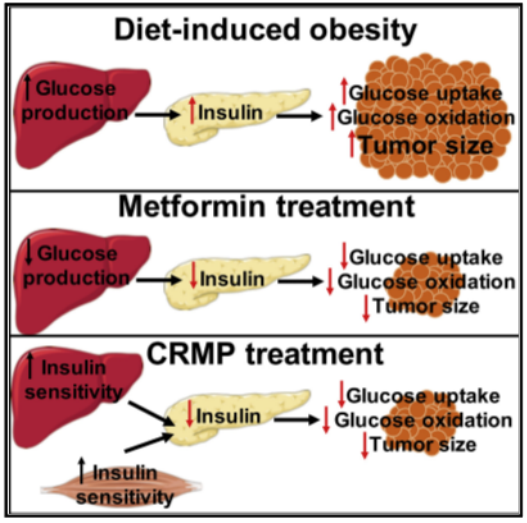
CRMP Reduces Tumor Growth in Murine Models of Colon Cancer

Cell Reports

Report

Uncoupling Hepatic Oxidative Phosphorylation Reduces Tumor Growth in Two Murine Models of Colon Cancer

Graphical Abstract



Authors

Yongliang Wang, Ali R. Nasiri, William E. Damsky, ..., Michael N. Pollak, Gerald I. Shulman, Rachel J. Perry

Correspondence

rachel.perry@yale.edu

In Brief

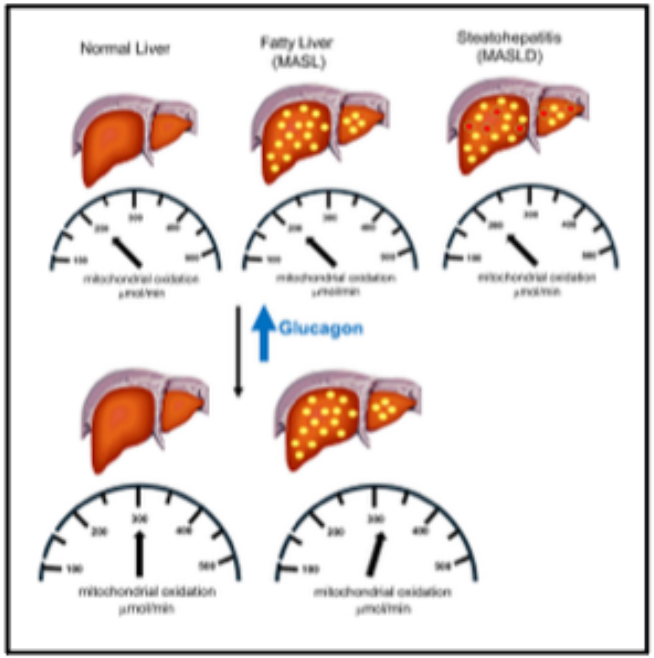
Wang et al. demonstrate that diet-induced hyperinsulinemia increases colon adenocarcinoma tumor glucose uptake and oxidation in mice. They further demonstrate that reversal of hyperinsulinemia by a liver-specific mitochondrial protonophore is sufficient to reverse the obesity-induced acceleration of tumor growth.

Wang et al. Cell Reports 2018

Cell Metabolism
Clinical and Translational Report

Glucagon promotes increased hepatic mitochondrial oxidation and pyruvate carboxylase flux in humans with fatty liver disease

Graphical abstract



Authors

Kitt Falk Petersen, Sylvie Dufour, Wajahat Z. Mehal, Gerald I. Shulman

Correspondence

kitt.petersen@yale.edu (K.F.P.), gerald.shulman@yale.edu (G.I.S.)

In brief

It is unclear whether rates of hepatic mitochondrial oxidation are altered in individuals with MASLD and MASH. Here, Petersen et al. show that rates of hepatic mitochondrial oxidation are not altered in humans with fatty liver or steatohepatitis and that glucagon can increase rates of hepatic mitochondrial oxidation in humans with and without fatty liver by 50%–75%.

Petersen et al. Cell Metabolism 2024

NEWS

PRESS RELEASE Feb 9, 2022

RIVUS PHARMACEUTICALS ANNOUNCES POSITIVE DATA FROM PHASE 2A CLINICAL TRIAL OF LEAD CANDIDATE HU6, DEMONSTRATING FAT REDUCTION AND WEIGHT LOSS IN HIGH BMI PARTICIPANTS

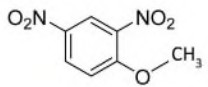
•Met primary endpoint (liver fat reduction), multiple secondary endpoints (whole body, visceral, subcutaneous fat loss)

•Significant fat selective weight loss while preserving muscle mass, without changes in diet or exercise

•Amplified weight and fat loss in patients with elevated HbA1c

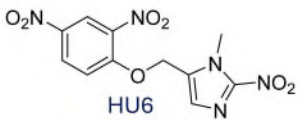
•Improvement in key markers of insulin resistance and inflammation

•Well tolerated across all studied doses



DNPME

Perry *et al.*
Cell Metabolism 2013



HU6

Noureddin *et al.*
Lancet Hepatology/Gastroenterology 2023

Rivus PHARMACEUTICALS

Rivus Pharmaceuticals' Phase 2a HuMAIN Trial Meets Primary Endpoint of Weight Loss and Secondary Endpoints in Patients with Obesity-Related Heart Failure

– Data from the HuMAIN study in patients with obesity-related heart failure with preserved ejection fraction (HFpEF) will be presented in a Late Breaking Clinical Trial Plenary Session at the Heart Failure Society of America Annual Scientific Meeting –

– Enrollment completed in Phase 2 M-ACCEL trial of HU6 in patients with metabolic dysfunction-associated steatohepatitis (MASH) –

– HU6, a novel oral, once-daily Controlled Metabolic Accelerator, is a new class of investigational therapies designed to reduce body fat while preserving muscle –

CHARLOTTESVILLE, Va., and SAN FRANCISCO, Ca., August 13, 2024 – Rivus

1886-LB

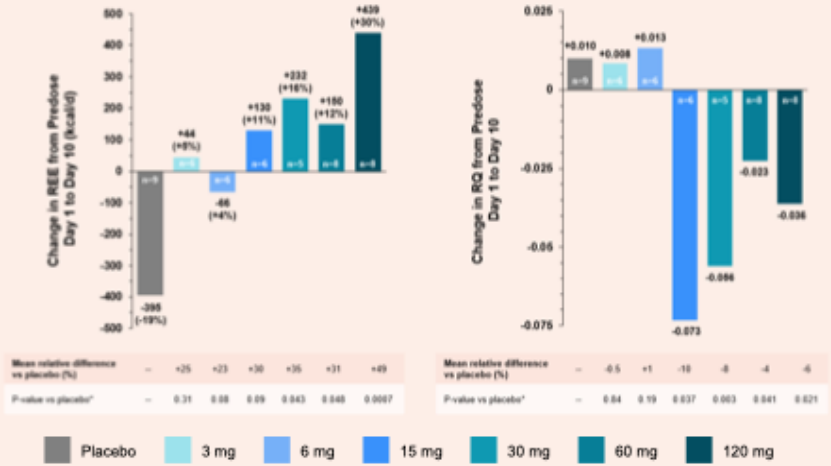
Safety, PK, and preliminary efficacy of the liver-targeted mitochondrial protonophore TLC-6740: A Phase 1 study

Edward J. Gane,[1] Ryan S. Huss,[2] Jane Sur,[2] Eisuke Murakami,[2] Steve Weng,[2] Brian J. Kirby,[2] Gerald I. Shulman,[3] G. Mani Subramanian,[2] Archana Vijayakumar,[2] Robert P. Myers[2]
[1]University of Auckland, New Zealand Clinical Research, Auckland, NZ; [2]OrsoBio, Inc., Menlo Park, CA, USA; [3]Yale University, New Haven, CT, USA

orsobio

TLC-6740 Causes Dose-Dependent Increases in Energy Expenditure and Improvements in Metabolic Parameters

Dose-Dependent ↑ REE & ↓ RQ (↑ Fat Oxidation)



* P-values by t-test for comparison between TLC-6740 groups and placebo with adjustment for baseline REE or RQ.

Relative (%) change from Day 1 to 10†	3 mg: n=8	6 mg: n=8	15 mg: n=8	30 mg: n=8	60 mg: n=8	120 mg: n=8
LDL-C	0.7 (-11.4, 12.8)	-6.2 (-18.3, 6.0)	-8.4 (-20.5, 3.7)	-3.3 (-15.4, 8.8)	**-18.3 (-30.4, -6.2)**	**-22.4 (-34.5, -10.3)**
Total cholesterol	2.0 (-6.6, 10.5)	-6.4 (-14.9, 2.2)	-7.7 (-16.3, 0.8)	-7.0 (-15.5, 1.6)	**-13.6 (-22.1, -5.1)**	**-15.1 (-23.7, -6.6)**
HDL-C	6.0 (-2.8, 14.8)	-2.0 (-10.8, 6.7)	3.4 (-5.3, 12.2)	**-10.1 (-18.9, -1.3)**	-0.3 (-9.1, 8.4)	-4.1 (-12.9, 4.6)
Triglycerides	3.3 (-22.0, 28.5)	-12.2 (-37.4, 13.1)	-23.8 (-49.1, 1.5)	-19.8 (-45.1, 5.5)	-18.7 (-44.0, 6.5)	-9.3 (-34.6, 16.0)
Glucose	-4.5 (-9.5, 0.5)	**-5.6 (-10.6, -0.6)**	3.8 (-1.6, 9.3)	-0.6 (-5.6, 4.4)	-2.0 (-7.0, 3.0)	-4.3 (-9.3, 0.7)
Insulin	-22.4 (-55.7, 10.9)	**-63.7 (-97.0, -30.4)**	-10.0 (-46.4, 26.3)	**-44.6 (-77.9, -11.3)**	**-40.2 (-73.5, -6.9)**	**-47.6 (-80.9, -14.3)**
HOMA-IR	-28.6 (-64.4, 7.2)	**-71.9 (-108, -36.1)**	-4.6 (-43.7, 34.4)	**-47.2 (-83.0, -11.4)**	**-43.8 (-79.6, -8.0)**	**-54.5 (-90.3, -18.7)**
ALT	-7.6 (-31.1, 15.9)	-11.3 (-34.8, 12.2)	-5.0 (-28.5, 18.5)	-1.5 (-25.0, 22.0)	-0.6 (-24.1, 22.9)	-13.2 (-36.7, 10.3)
GGT	-8.3 (-30.6, 14.1)	-0.1 (-22.4, 22.3)	-10.4 (-32.7, 12.0)	-14.8 (-37.2, 7.5)	6.0 (-16.3, 28.4)	-16.2 (-38.5, 6.1)

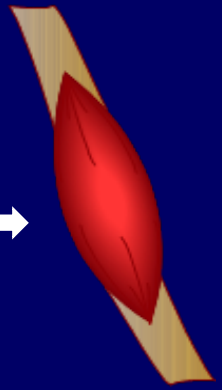
† Mean placebo-adjusted relative (%) change (95% CI) from Day 1 to 10 (statistically significant [p<0.05] changes in bold).

- Increases in REE with TLC-6740 were inversely associated with baseline REE (p<0.0005)

Presented at American Diabetes Association's 84th Scientific Sessions, June 21-24, 2024, Orlando, FL

Liver-Targeted Mitochondrial Uncoupling

↓ Hypertriglyceridemia/
↓ LDL Cholesterol



↑ V_{TCA}, fatty acid oxidation, EE → ↓ VLDL export

↓ Hepatic TAG, DAG, ↓ acetyl CoA

↓ PKCε translocation/inflammation

↑ Hepatic insulin sensitivity

↓ Fasting gluconeogenesis

↓ Intramyocellular TAG, DAG

↓ PKCθ/PKCε translocation

↑ Peripheral insulin sensitivity

Perry et al. Cell Metabolism 2013, Petersen and Shulman, Physiological Reviews 2018, Perry et al. Science 2015, Goedeke and Shulman, Molecular Metabolism 2021, Goedeke, et al. Science Translational Medicine 2023

Collaborators

Yale

Abudukadier Abulizi
Tiago Alves
Sonia Caprio
Gregori Casals
Gary Cline
Jianying Dong
Alan Dresner
Sylvie Dufour
Claire Flannery
Rafael Gasper
Brandon Gassaway
Leigh Goedeke
Sandro Hirabara
Ripu Hundal
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