Role of Ectopic Lipid and Inflammation in the Pathogenesis of Type 2 Diabetes

Gerald I. Shulman, M.D., Ph.D., F.A.C.P., M.A.C.E.
Howard Hughes Medical Institute
Yale University School of Medicine
A grim prediction:
By the year:

2050

1:3

Americans will have T2D
Complications of Diabetes

- Proliferative Diabetic Retinopathy
- Diabetic Nephropathy
- Diabetic Foot Gangrene
Global Projections for the Diabetes Epidemic: 2015-2040 (millions)

World 2015 = 415 million
2040 = 642 million

- **Oceania**: 14M
- **North America**: 44M
- **South and Central America**: 30M
- **Europe**: 60M
- **Asia**: 35M
- **Africa**: 14M
- **Mid-East**: 78M
- **Asia**: 153M

IDF Diabetes Atlas
Pathogenesis of Hyperglycemia in Type 2 Diabetes

Liver

Increased glucose production

Glucose

Insulin resistance

Pancreas

Impaired insulin secretion

Muscle
$^{13}$C NMR Spectra of Muscle Glycogen

Incremental Change in Muscle Glycogen

Increment (mmol glucosyl units/kg muscle)

Minutes

Control

Type 2 diabetes

Potential Rate-Controlling Steps in Muscle Glucose Metabolism

**Glycogen Synthase**

**UDP-glucose**

**Hexokinase**

**Glucose-6-phosphate**

**Glucose**

**Plasma glucose**

**GLUT 4**
The diagram shows 31P NMR Spectra of Human Muscle. The baseline spectrum and the spectrum after 40-80 min are compared. The difference spectrum highlights changes in phosphocreatine (PCr), inorganic phosphate (Pi), glucose 6-phosphate (G6P), and ATP. The graph is x16 magnified. Rothman DL et al. *J Clin Invest.* 1992;89:1069-1075.
$^{13}$C NMR Spectra of Muscle and Plasma

Glucose Transport Activity Is Decreased in Type 2 Diabetes

Glucose Transport Activity Is Decreased in Type 2 Diabetes

$^1$H spectrum of the soleus muscle of a lean subject

Randle Mechanism of Lipid-Induced Insulin Resistance

Glucose → G-6-P → Pyruvate

- Glucose
- G-6-P
- Pyruvate

- HK
- PFK
- PDH

- Plasma glucose
- Plasma FFA

- Citrate
- Acetyl CoA
- CoA
- NADH
- NAD

Lancet i:785-789, 1963
Fatty Acids Inhibits Glucose Transport Activity

Glucose $\rightarrow$ G-6-P $\rightarrow$ Pyruvate $\rightarrow$ Acetyl CoA

HK, PFK, PDH

GLUT 4

Plasma glucose $\rightarrow$ Plasma FFA

Potential Mechanisms by which Fatty Acids Inhibit Glucose Transport Activity

- Plasma glucose
- GLUT 4
- Glucose
- IRS-1
- PI 3-kinase
- Fatty Acid

Shulman J Clin Invest. 2000;106;171
Effect of Fatty Acids on PI 3-Kinase Activity

DAG-PKCε hypothesis of lipid-induced muscle insulin resistance

Diacylglycerol (DAG)-PKCε Hypothesis of Non Alcoholic Fatty Liver Disease (NAFLD)-Induced Hepatic Insulin Resistance

Muscle Fatty Acyl CoA (nmol/g)

Liver Fatty Acyl CoA (nmol/g)

Wild-type
Fatless

**Muscle Fatty Acyl CoA (nmol/g)**

- Wild-type
- Fatless
- Fat-Tx Fatless

**Liver Fatty Acyl CoA (nmol/g)**

- Wild-type
- Fatless
- Fat-Tx Fatless

*Kim et al. J. Biol. Chem. 275:8456-8460, 2000*
Lipodystrophy Syndromes

- Paucity of fat
- Insulin resistance
- Hypertriglyceridemia
- Fatty infiltration of liver and other tissues
- Deficiency of adipocyte hormones (e.g. leptin)
Insulin Action: Control vs Lipodystrophy

Muscle Glucose Uptake
mg/(kg LBM-min)

Control
Lipodystrophic

Suppression Glucose Production
%

Control
Lipodystrophic

Fasting Plasma Glucose Concentrations

Plasma Glucose (mg/dl)

Control | Before Rx | After Rx

Effect of Leptin on Insulin Action

Muscle Glucose Uptake

Pre-Leptin

Post-Leptin

mg/(kg LBM-min)

Suppression Glucose Production (%)

Pre-Leptin

Post-Leptin

$^1$H MRS Spectrum of Liver

Coil

Voxel

$,\text{H}_2O$, Lipid

(ppm)
Effect of Leptin on Tissue Triglyceride

Muscle (%)

Liver (%)

MRI of Abdomen

Effect of Leptin on Liver

Before

After
Effect of Leptin on Energy Balance

Energy Intake
kcal/day

Pre-leptin  Post-leptin

Basal Energy Expenditure
(kcal/(kg-hr))

Pre-leptin  Post-leptin

Effect of Leptin Treatment in Lipodystrophy

Before Leptin

1 year on Leptin
Effects of Weight Loss in Obese Type 2 Diabetics
Fasting Plasma Glucose Before and After Wt Loss

Petersen et al, Diabetes 54:603, 2005
Body Weight Before and After Diet

BMI 30±1 kg/m²
BMI 28±1 kg/m²
BMI 24±1 kg/m²

Petersen et al, Diabetes 54:603, 2005
Hepatic Lipid Content

**Petersen et al Diabetes 2005**

Hepatic TG Content (%)

- Control
- Type 2 Pre
- Type 2 Post

P=0.02

P=0.009
Rates of Glucose Production

Petersen et al Diabetes 2005
Insulin Suppression of Glucose Production

Control

Type 2 Pre

Type 2 Post

P=0.003

P=0.04

No change
IL-6
TNF-α
Resistin
RBP-4

Petersen et al Diabetes 2005
Cellular Mechanisms of Insulin Resistance

Defects in Adipocyte Fatty Acid Metabolism

Acquired (aging) or Inherited (IR Offspring) Defects in Mitochondrial Metabolism

Caloric Intake

Shulman *J Clin Invest.* 2000;106;171
Hepatic Sequelae of NAFLD

1 in 3 Americans – NAFLD

\[\downarrow\]

20%¹

NASH

\[\downarrow\]

15%¹

Cirrhosis

\[\downarrow\]

40%²

Hepatocellular carcinoma

¹Bedogni *Hepatol*, ²Page *Clin Liv Dis*
Role of NAFLD in the Pathogenesis of Type 2 Diabetes

- Hepatic insulin resistance
- Increased gluconeogenesis

Fasting and Postprandial Hyperglycemia
Canonical insulin signaling of hepatic glucose metabolism

Insulin

IRS2 → P13K → Akt2

PP1, PDE → GSK3 → FOXO

↓GP (Glycogenolysis) → Glucose production → Glycogen synthesis → Gluconeogenesis

↓FOXO, ↓PEPCK, ↓G6Pase

Nucleus

Regulation of Hepatic Gluconeogenesis

- Lactate
  - NADH/NAD^+
    - Alanine
    - Acetyl CoA
      - Pyruvate
        - Pyruvate Carboxylase
          - OAA
            - TCA cycle
            - NADH/NAD^+
              - Glucagon/Glucocorticoid
                - Insulin (Foxo1-mediated)
                  - PEP
                    - NADH/NAD^+
                      - DHAP
                        - Glycerol
                          - Gluconeogenesis

Transcriptional Substrate Redox Allosteric

Madiraju et al. Nature 2014
$^{13}$C Tracer Labeling Scheme

- [3-13C] Lactate Tracer
- [3-13C] Pyruvate
- [3-13C] Alanine

1st Turn:
- [4-13C] Glutamate
- [2,3-13C] Glutamate

2nd Turn:
- [2,3-13C] Glutamate

Pyruvate kinase
Pyruvate carboxylase
Pyruvate Dehydrogenase

[1,2,5,6-13C] Glucose
[2,3-13C] PEP
[2,3-13C] OAA
[2,3-13C] Fumarate
TCA

Perry et al. Nature Medicine 2014
Indirect Mechanism by which Insulin Acutely Suppresses Hepatic Gluconeogenesis

- Insulin
- Lipolysis
  - Glycerol turnover
  - Fatty acid turnover
  - Acetyl CoA
  - Pyruvate carboxylase activity/flux
  - DHAP

Gluconeogenesis

Perry et al. Cell 2015
DIRECT Effects of Insulin to Stimulate Glycogen Synthesis

INDIRECT Effects of Insulin on Lipolysis to Inhibit Gluconeogenesis

**DIRECT**
- Insulin
- IRS2
- Akt2

**INDIRECT**
- Acetyl CoA
- Pyruvate Carboxylase
- Glycerol

**Glycogen**
- Glycogen Phosphorylase
- Glycogen Synthase

**Gluconeogenesis**
- Glucose production

**Lipolysis**
Mechanism for White Adipocyte Inflammation-Induced Stimulation of Hepatic Gluconeogenesis

Macrophages

JNK

IL-6

Lipolysis

↑ Lipolysis

Fatty acid turnover

Glycerol turnover

↑ Acetyl CoA

↑ Pyruvate carboxylase activity/flux

↑ DHAP

↑ Gluconeogenesis

Perry et al. Cell 2015
Dinitrophenol (DNP) Reverses NAFLD and Hepatic Insulin Resistance

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

Samuel et al. JBC 2004
Brief History of DNP

- 1900s – French munition workers manifest diaphoresis and weight loss when exposed to chemical dust from factory
- 1910s – DNP is first used in humans, without prior safety/efficacy studies
- 1930s – after several reports of DNP’s safety and efficacy, it becomes a popular OTC medication > 100,000 individuals take DNP
- 1938 – FDA removes it from the market after several reports of deaths
- 1940s – Russian soldiers take DNP to stay warm during WWII

Grundlingh et al., J Med Toxicol
Hypothesis

Liver-targeting DNP will increase the therapeutic window and ameliorate hepatic steatosis, insulin resistance and diabetes.
Screening of compounds: Oxygen consumption rate in cultured hepatocytes

Injection of
10 μM DNP
Injection of
100 μM DNP
Injection of
500 μM DNP
Injection of
1 mM DNP

DNP
DNPBA
DNPVE
DNPME
DNPIE
DNPIE
DNPM
d
DNPHP
DNPM

Perry et al. Cell Metabolism 2013
DNP-methyl ether (DNPME)

DNP-\textit{methyl ether} (DNPME)

\textbf{Perry et al.}\textit{ Cell Metabolism} 2013
Dose Response: DNP vs. DNPME
Body temperature

**DNP**

**DNME**

Perry et al. *Cell Metabolism* 2013
DNPME Rx Reverses Insulin Resistance and Hepatic Steatosis in HFD Rats

Plasma glucose

Plasma insulin

Liver TAG

$P=0.04$

Perry et al. Cell Metabolism 2013
Toxic/Therapeutic dose: DNP vs. DNPME
Plasma DNP concentrations

5 mg/kg DNPME or DNP

DNP (μM)

Time (hours)

DNPME injection
DNP injection

Perry et al., Cell Metabolism 2013
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,*
CRMP Rx Increases Hepatic Mitochondrial Fat Oxidation

\[ \uparrow \; 60\%, \; P=0.02 \]

\[ \uparrow \; 70\% \quad P<0.0001 \]

Glucose oxidation
Fatty acid oxidation

Perry et al. Science 2015
Toxic/Effective Dose
DNP vs. CRMP

DNP

CRMP

200

500-fold

0.4
CRMP Rx Reduces Hyperglycemia and Insulin Resistance in High Fat Fed Rats

Perry et al. Science 2015
CRMP Rx Reduces Liver and Muscle TAG, DAG content and nPKC activity

Liver TAG
- Vehicle: 20 mg/g
- CRMP: 15 mg/g
- P = 0.0002

Liver DAG
- Vehicle: 900 nmol/g
- CRMP: 600 nmol/g
- P = 0.002

Liver PKCε translocation
- Control: 0.3 Arbitrary unit
- CRMP: 0.2 Arbitrary unit
- P = 0.03

Quadriceps TAG
- Vehicle: 4 mg/g
- CRMP: 4 mg/g
- P = 0.004

Quadriceps DAG
- Vehicle: 50 nmol/g
- CRMP: 20 nmol/g
- P = 0.04

Quadriceps PKCθ translocation
- Control: 8 Arbitrary unit
- CRMP: 6 Arbitrary unit
- P = 0.04

CRMP Rx reduces liver and muscle TAG, DAG content and nPKC activity as shown in the graphs above. CRMP reduces liver and muscle TAG, DAG content, and nPKC activity compared to the Vehicle control group. The data is from Perry et al. Science 2015.
CRMP Rx Reverses Liver and Muscle Insulin Resistance

**Suppression of Hepatic Glucose Production**

- Control: 20%
- CRMP: 60%

*P*=0.0002

**Muscle Glucose Uptake**

- Control: 40 [nmol/(g-min)]
- CRMP: 80 [nmol/(g-min)]

*P*=0.03

Perry et al. *Science* 2015
CRMP Rx Reduces Hyperglycemia in Diabetic ZDF rats

Perry et al. Science 2015
CRMP Rx Reduces Liver and Muscle Triglyceride in Diabetic ZDF rats

Perry et al. Science 2015
CRMP Rx Reduces Liver Inflammation in Diabetic ZDF Rats

Perry et al. Science 2015
CRMP Rx Reverses Liver Fibrosis in a Methionine-Choline Deficient Diet Rat Model of NASH and Liver Fibrosis

Collagen mRNA

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Vehicle</th>
<th>CRMP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Relative units</strong></td>
<td>0.8</td>
<td>1.2</td>
<td>1</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>N.S.</td>
<td>P&lt;0.01</td>
<td>P&lt;0.00</td>
</tr>
</tbody>
</table>

Fibrosis score

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Vehicle</th>
<th>CRMP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Score</strong></td>
<td>1.6</td>
<td>1.4</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*Perry et al. Science 2015*
Summary
Liver-Targeted Mitochondrial Uncoupling

Increased Fat Oxidation

↓ Hypertriglyceridemia

↓ VLDL export

↓ Hepatic TAG, DAG,
↓ acetyl CoA
↓ PKCε translocation
↓ Gluconeogenesis
↓ Glycogen synthesis

↑ Peripheral insulin sensitivity

↓ Intramyocellular TAG, DAG
↓ PKCθ translocation

Collaborators

Abullzi Abudukadier
Tiago Alves
Douglas Befroy
Andreas Birkenfield
Sonia Caprio
Ralph DeFronzo
Jianying Dong
Yan Chen
Alan Dresner
Sylvie Dufour
Derek Erion
Jonathan Fillmore
Clare Flannery
Brandon Gassway
Matt Gillum
Beat Jucker
Takanori Iwasaki
Rachel Jamison
Mario Kahn
Richard Kibbey
Sheene Kim
Ripu Hundal
Silvio Inzucchi
Yanna Kosover
Mario Kahn
Martin Krssak
Hui-Young Lee
Zhen-xiang Liu
Anila Madiraju
Melissa Marcucci
Katsutaro Morino
Yoshio Nagai
Susanne Neschen
Rachel Perry
Dominik Pesta
Rebecca Pongratz
Max Petersen
Kitt Petersen
Yasmeen Rahimi
Jesse Rinehart
Michael Roden
Raymond Russell
Varman Samuel
Irina Smolgovsky
David Spiegel
Dimitri Tsirigotis
Dirk Weissman
Larry Young
Chunli Yu
Dong Zhang
Xian-man Zhang
Xiao-jian Zhao
Haihong Zong

NIH
Elif Arioglu Oral
Oksana Gavrilova
Philip Gorden
Marc Reitman
Simeon Taylor

Yale-NIH Mouse Metabolic Phenotyping Center
Gary Cline
Joao Paulo Camporez
Michael Jurczak

Yale-Magnetic Resonance Center
Douglas Rothman