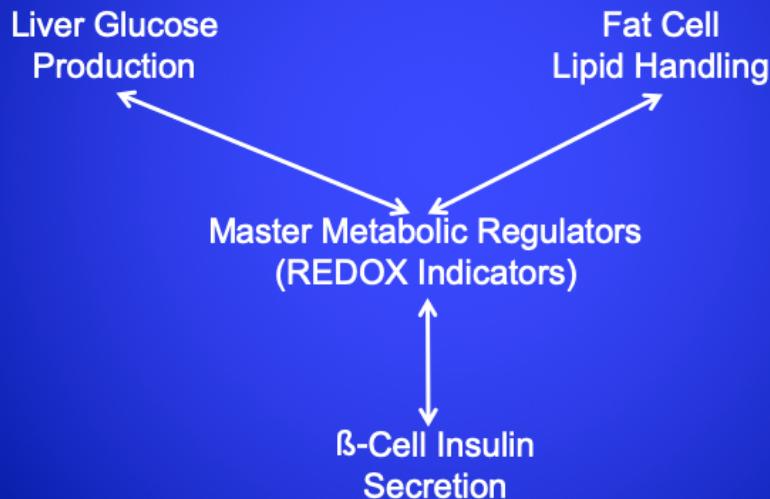


*The Redox Communication
Network as a Master Regulator
of Metabolism*

International Summer Institute on
Network Physiology-2019

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Obesity Research Center
Boston University

Circulating Redox as a Master Regulator of Metabolism



Model of metabolic master regulation. It is important that all tissues in the body know the metabolic status at all times.

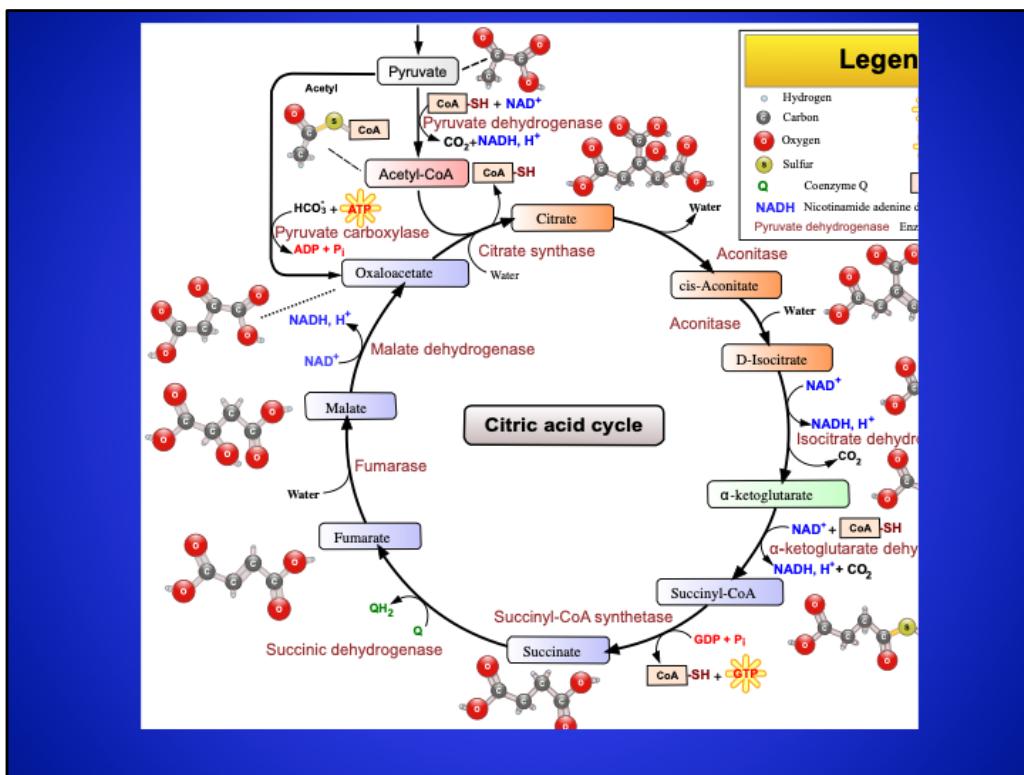
How is Energy State Communicated?

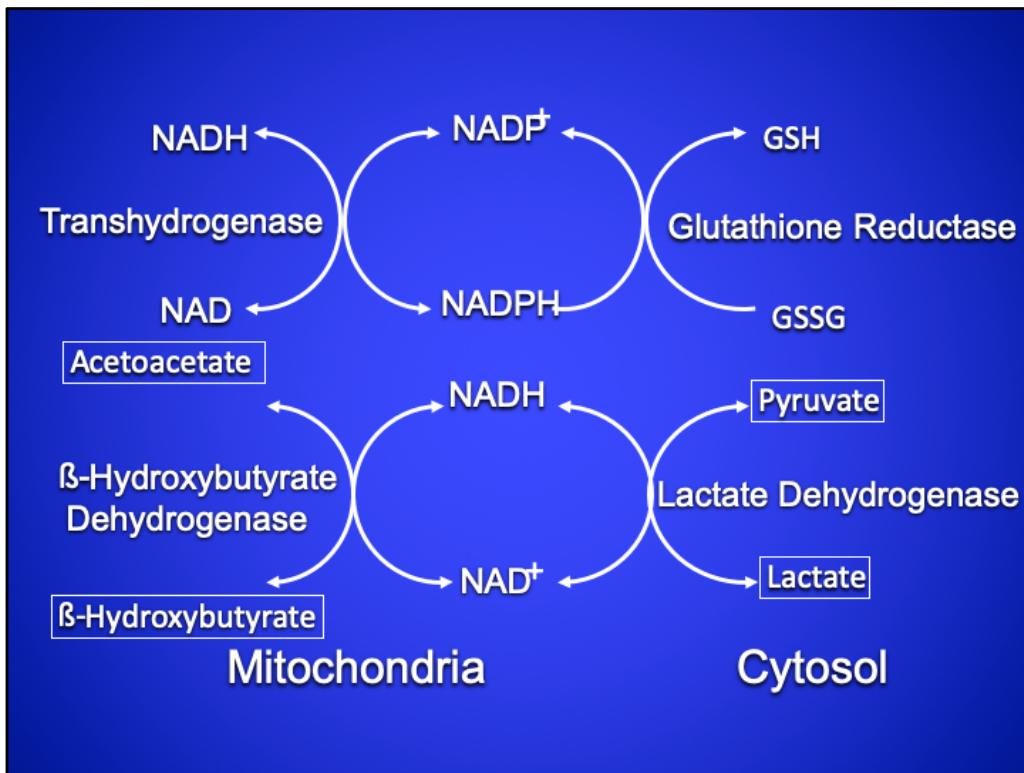
- Information from shared co-factors: pyridine nucleotides, adenine nucleotides, CoA esters and ROS
- And Mitochondrial metabolism
- Via communication to circulating metabolites

Information Derived from Shared Co-factors within cells

- Pyridine nucleotides: NAD(P) and NAD(P)H
- Adenine nucleotides: ATP, ADP, AMP
- Coenzyme A derivatives: CoASH, acetyl CoA, LC-CoA, etc
- Participate in numerous equilibrium reactions

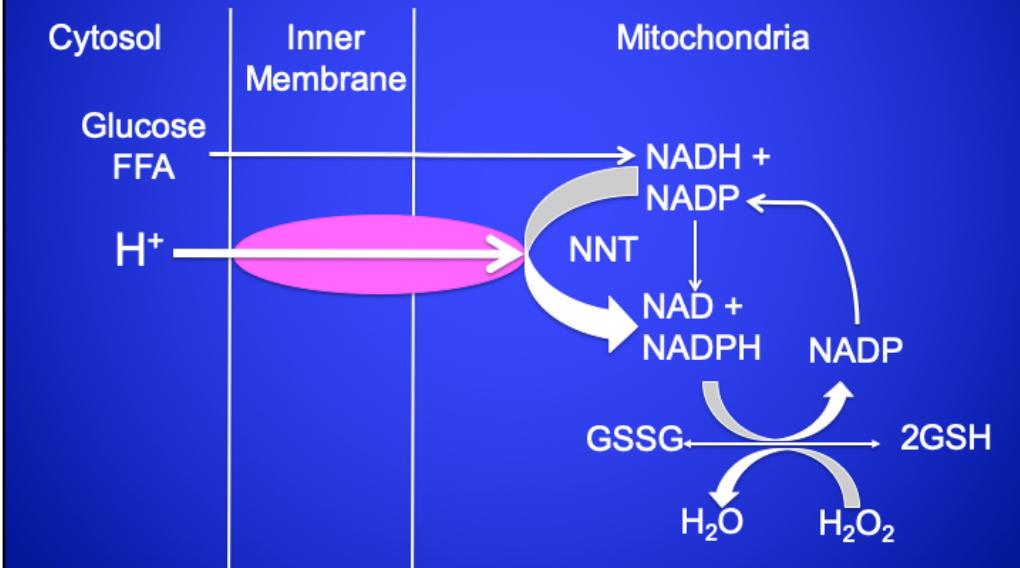
The common currency for enzyme reactions





Several examples of reactions using shared cofactors.

NNT a ROS-Scavenging Enzyme Driven by the Proton Gradient



Nicotinamide Nucleotide Transhydrogenase (NNT) provides an important link between pyridine nucleotide generation by glucose and FFA, reactive oxygen species (ROS) and the thiol redox state (eg, GSH). ROS is a shorthand for all reactive oxygen species including peroxide. Tools are not available to measure the variety of ROS in real time, hence the lack of specificity.

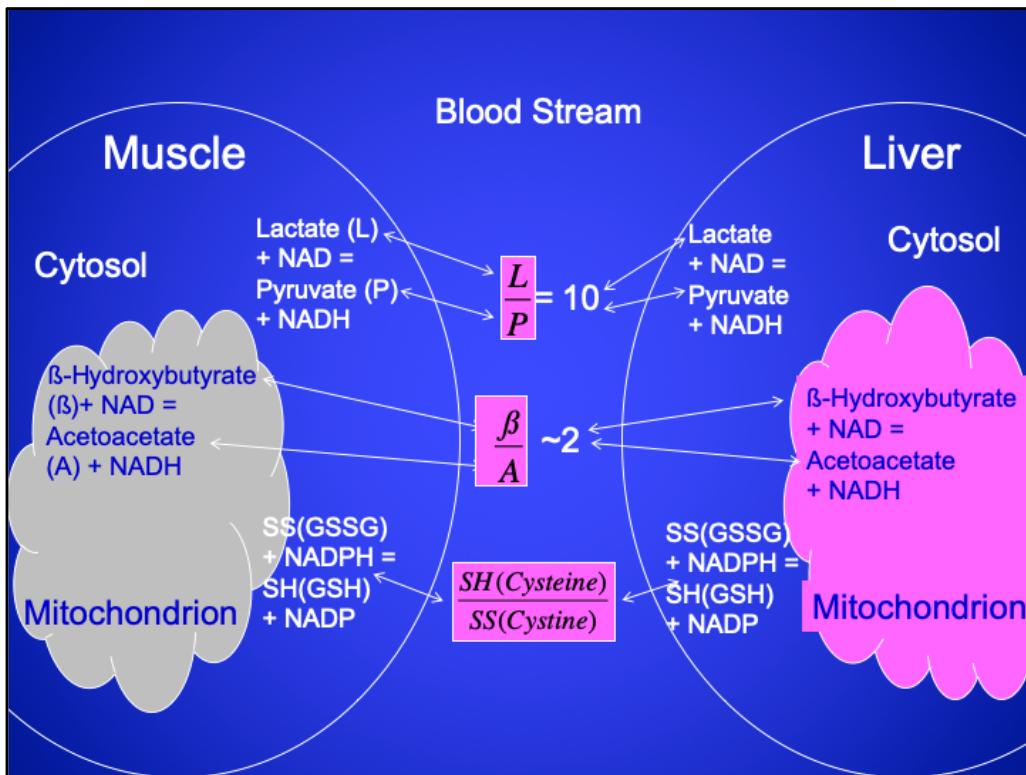


Illustration of how the intracellular redox state is communicated to the blood stream to regulate the redox network. Specific substrate-product pairs (like pyruvate and lactate or acetoacetate and β -hydroxybutyrate) are in equilibrium with NAD and NADH in their respective compartments. In addition, there are membrane transporters that allow these metabolites to enter and leave the cell rapidly, according to their concentration gradient, moving from higher to lower concentration compartments.

When does Redox Change?

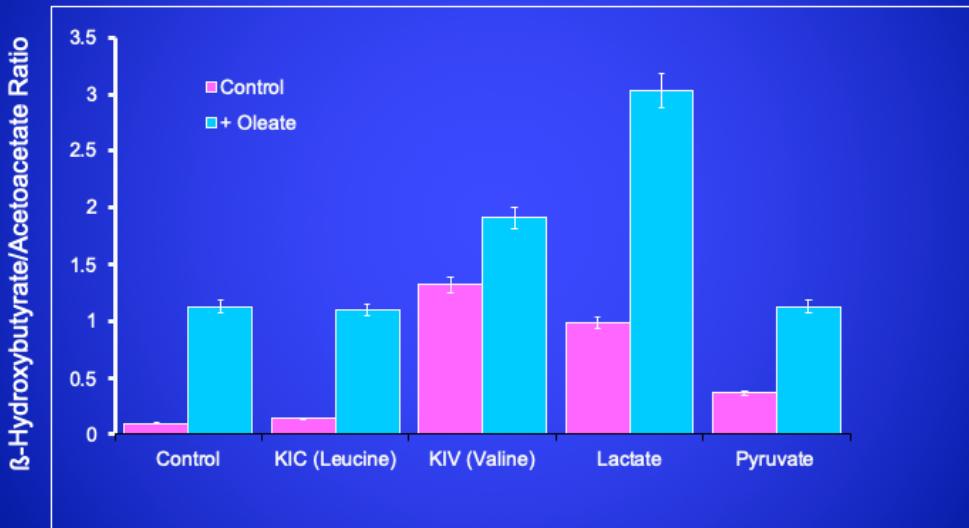
Human Fasting

Hours	β OHB	Acoc	β/A	FFA
Meal	0.06	0.03	2.0	0.66
3	0.07	0.03	2.3	0.71
6	1.21	0.41	3.0	1.25
24	5.78	1.27	4.6	1.55

Cahill Annu. Rev. Nutr. 2006 26:1

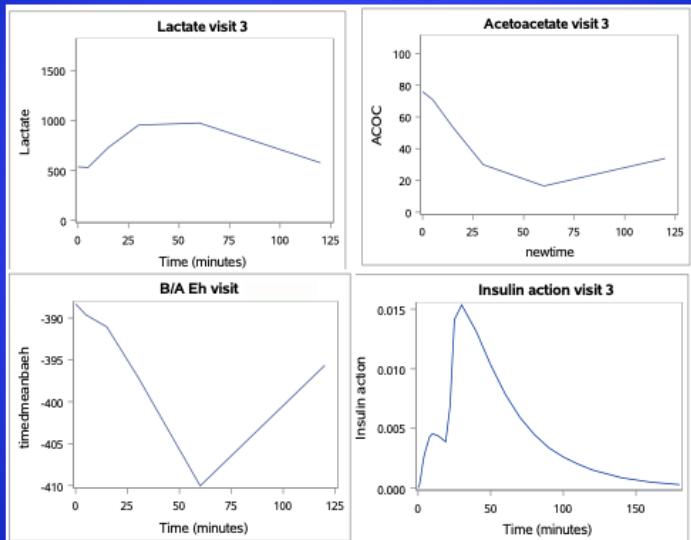
The ketones, acetoacetate and β -hydroxybutyrate, are formed from fat so their total amount correlates with the circulating FFA. However, the ratio can be high or low depending on the state of fuel supply.

Regulation of Hepatic Mitochondrial Redox State by Fuels



These increases in redox in response to fuels occur within hepatocytes and can be communicated throughout the organism through the metabolites that circulate: β -hydroxybutyrate and acetoacetate. Note that only the FFA oleate can form ketones but the addition of other fuels that do not form ketones can change their ratio.

Blood Metabolites in Patient fed Glucose



Thomas et al unpublished

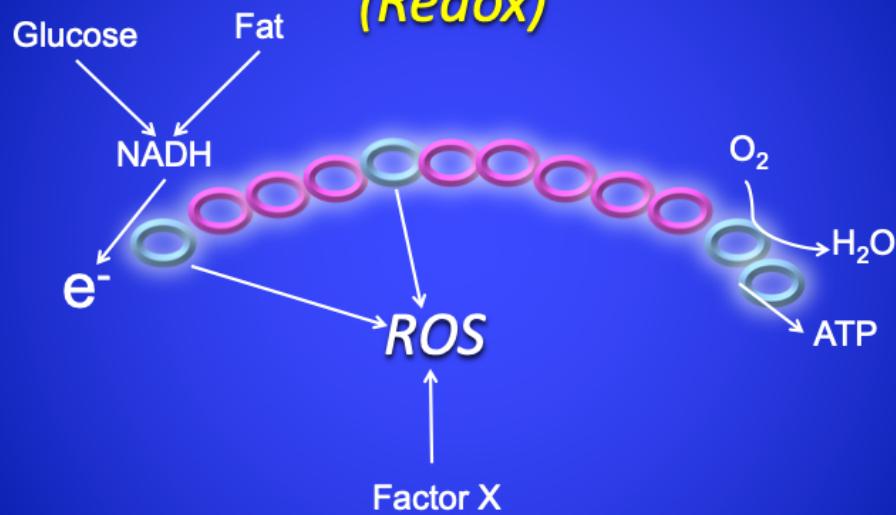
These are changes in blood redox metabolites and insulin action in response to glucose feeding are readily measured.. This is an illustration of data obtained from a single patient from our clinic. Lactate rises because excess glucose is metabolized to lactate. Acetoacetate falls because glucose rather than FFA is being used as energy source and the B/A ratio decreases transiently as glucose is being metabolized.

Circulating Redox Changes

- Fasting
- Lean vs obese or high fat diet
- Dean Jones: blood thiol redox in diabetes, aging and cancer
- Response to fuels
- Lean and obese human subjects undergoing glucose tolerance test (collaboration with Human Metabolism Core directed by Nawfal Isfan)

*How do Redox Changes Induce
ROS Changes?*

ROS are Produced at High NADH (Redox)



ROS is produced in the mitochondrial electron transport chain and is a signal of plenty. This occurs when substrate availability is high (high NADH) but ATP needs have been fulfilled. Factor X implies that external environmental influences can also lead to ROS generation.

H₂O₂ Production Rates in Intact Organ

Perfused liver data were obtained by methanol titration of catalase Compound I. Data from Oshino et al (1973).

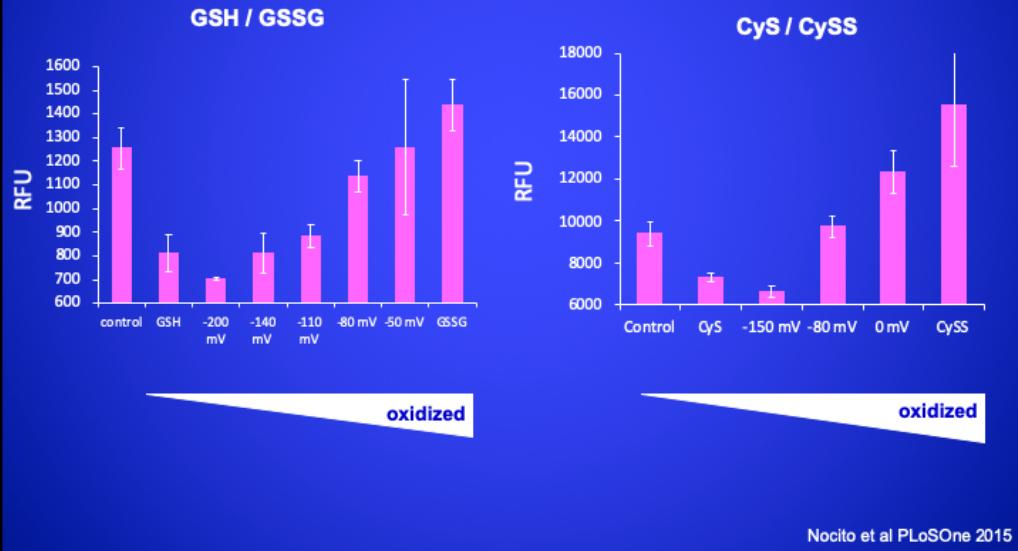
Substrate or inhibitor	Production Rate nmol H ₂ O ₂ /min per g liver
L-Lactate, 2 mM; pyruvate, 0.3 mM	49
+ antimycin, 8 µM	75
+ octanoate, 0.3 mM	170
+ oleate, 0.1 mM	66

Oshino et al (1973) *Arch. Biochem. Biophys.* **154**, 117-131

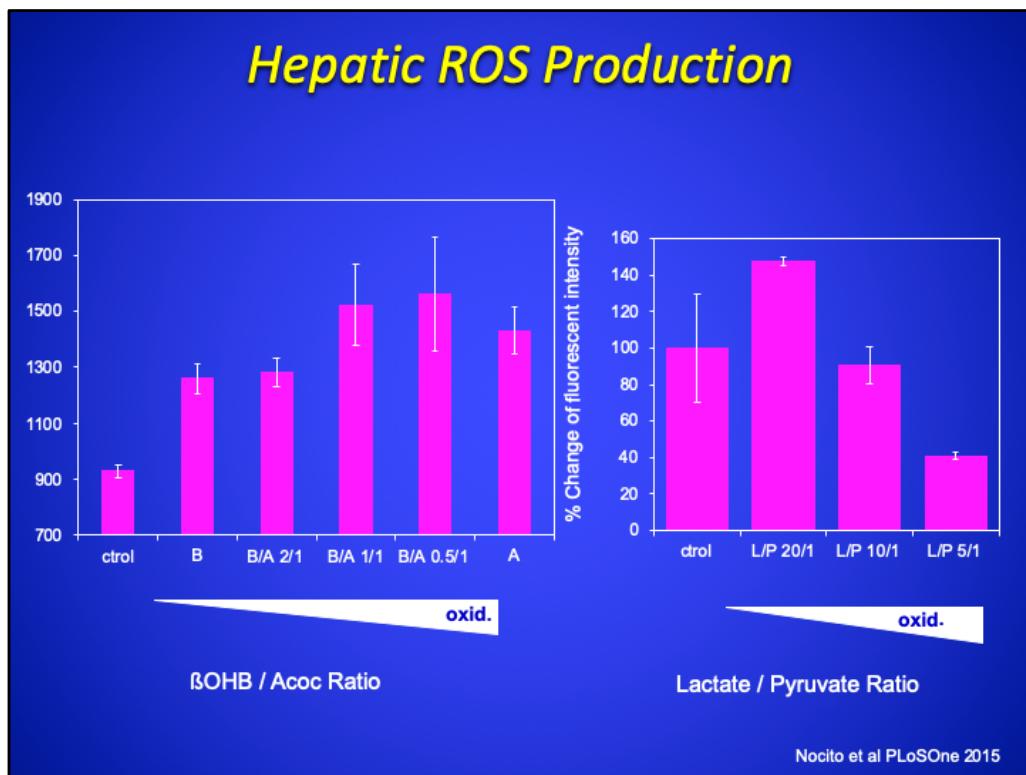
This old data illustrates that excess fuel, lactate plus pyruvate and FFA stimulate ROS production. Antimycin A inhibits the electron transport chain after the ROS generating step, forcing entering electrons to form ROS.

**Intracellular Fuels Impact
Intracellular Redox.
How do External Changes in Redox
Affect Intracellular Redox and
Function?**

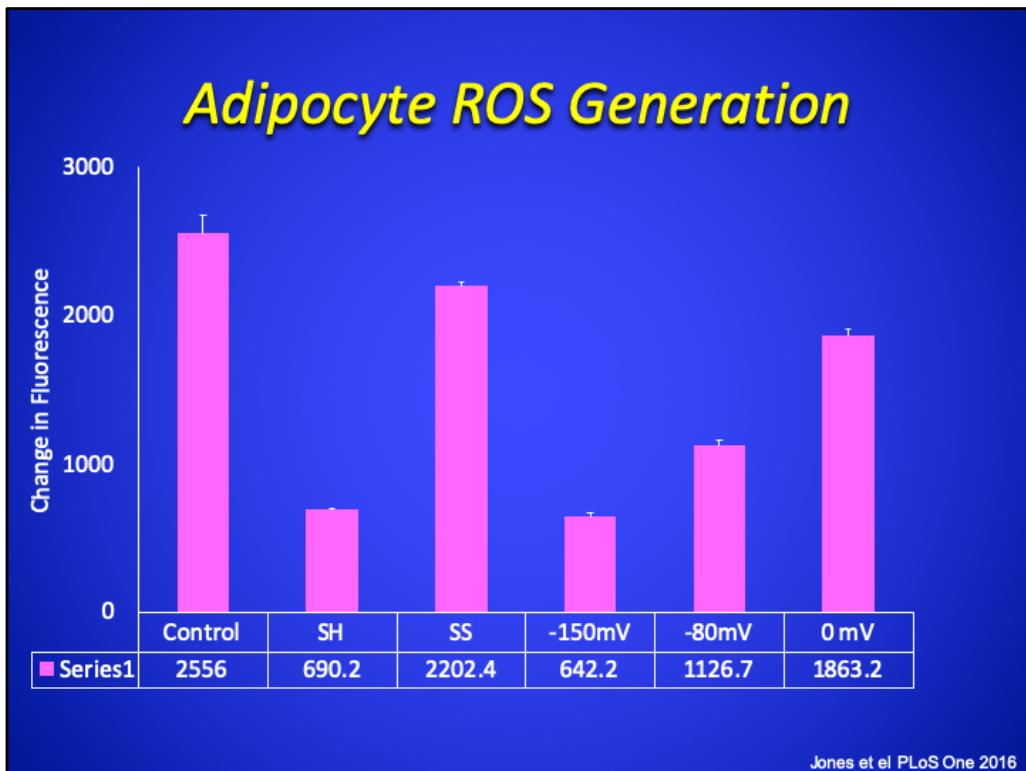
ROS Production in Hepatocytes



Changes in ROS production occur in response to extracellular thiol couples over a physiological range of electrochemical potentials.



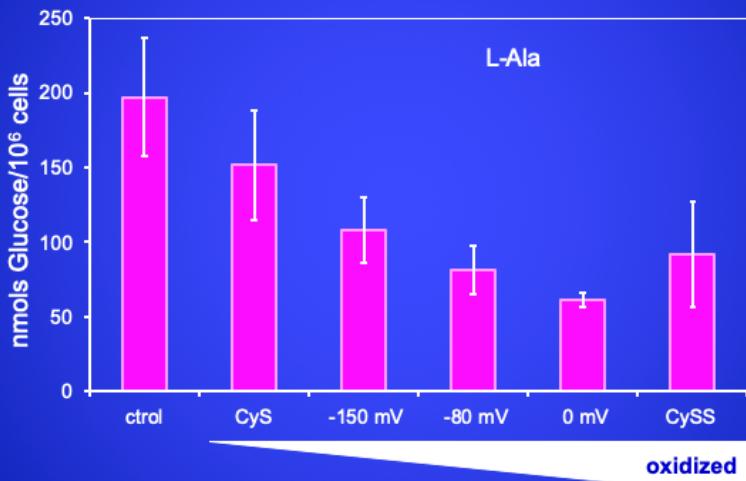
Changes in ROS production occur in response to pyridine nucleotide couples over a physiological range of electrochemical potentials also. Note that changing the cytosolic redox state has the opposite effect to the thiol and mitochondrial redox states, probably due to the ability of pyruvate to enter the mitochondria and increase NADH.



Similar responses to variations in extracellular redox potential occur in many cell types including fat cells.

*Yes External Redox Can Impact
Cellular ROS Production.
Do Changes in Redox or ROS Alter
Function?*

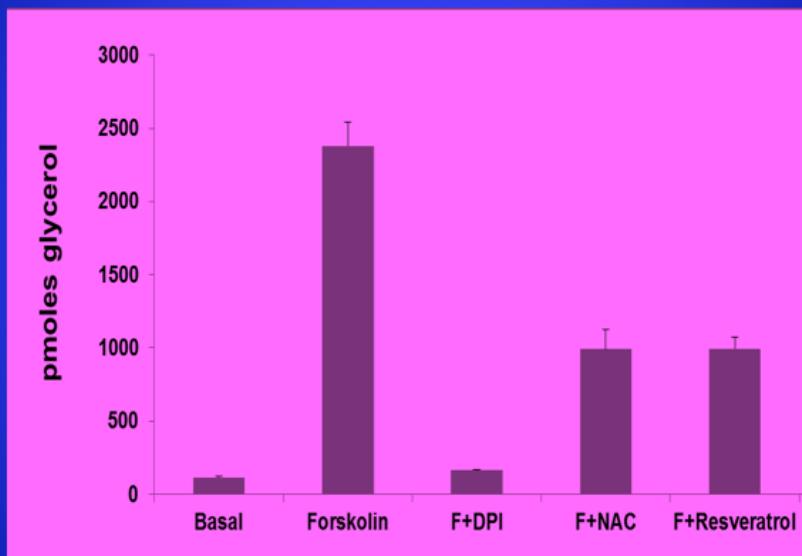
Hepatic Glucose Production



Nocito et al PLoSOne 2015

Changes in gluconeogenesis occur in response to thiol couples over a physiological range of electrochemical potentials. Since high ROS that accompanies the more oxidized state is a signal of fuel excess, it is logical that glucose production by the liver should be inhibited.

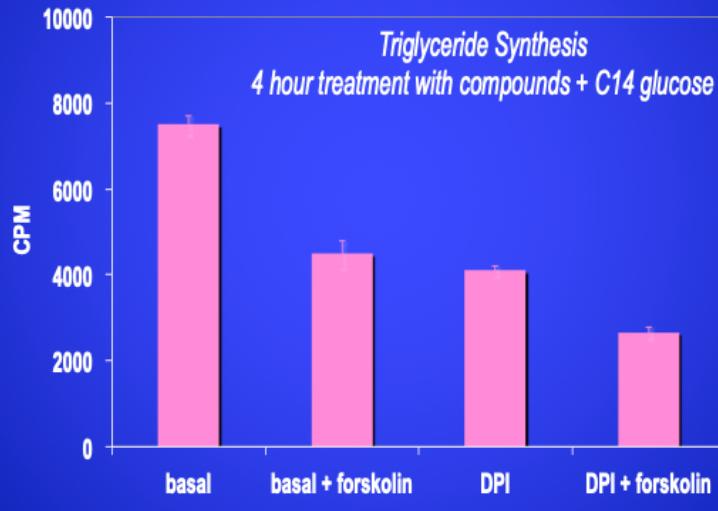
Adipocyte Lipolysis



Jones et al PLoS One 2016

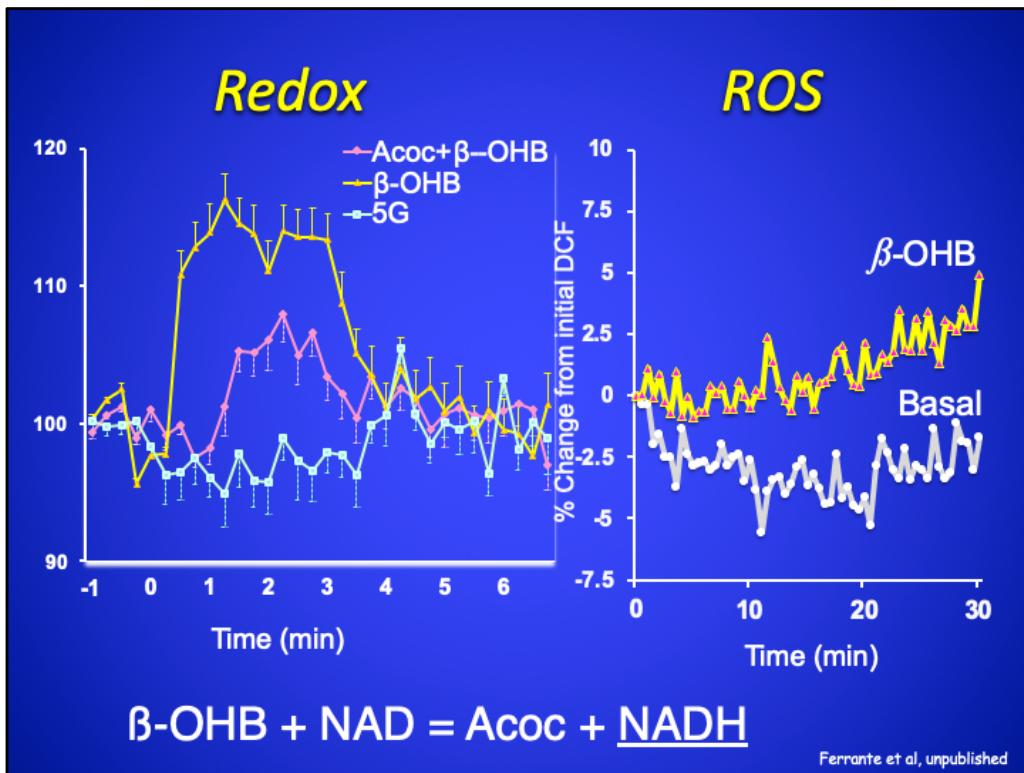
Lipolysis is stimulated by the drug forskolin. Addition of DPI, NAC or resveratrol, ROS scavengers, inhibits lipolysis.

ROS Required for Basal Triglyceride Synthesis



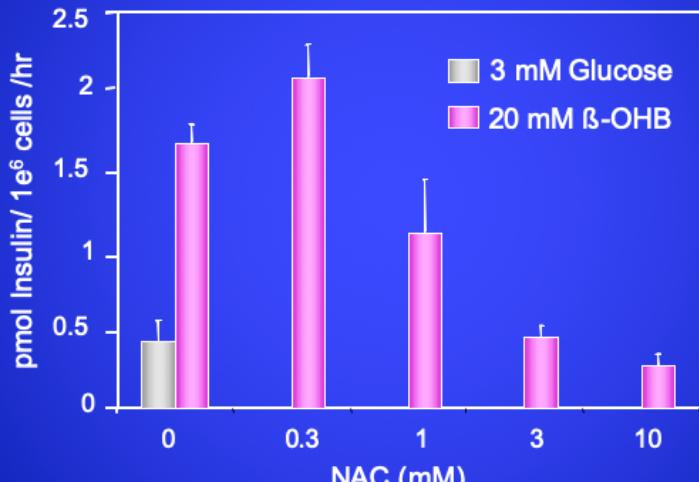
Krawczyk et al, 2012 PLoSone

Triglyceride synthesis occurs rapidly in adipocytes. Forskolin that stimulates lipolysis diminishes triglyceride synthesis. ROS removal with the ROS scavenger, DPI inhibits lipid synthesis in fat cells whose main function is to store fat by this pathway.



Circulating metabolites like $\beta\text{-OHB}$ and Acetoacetate can enter cells and cause changes in redox and ROS in β -cells.

Effect of β -OHB and ROS Removal on Insulin Secretion from INS-1 Cells



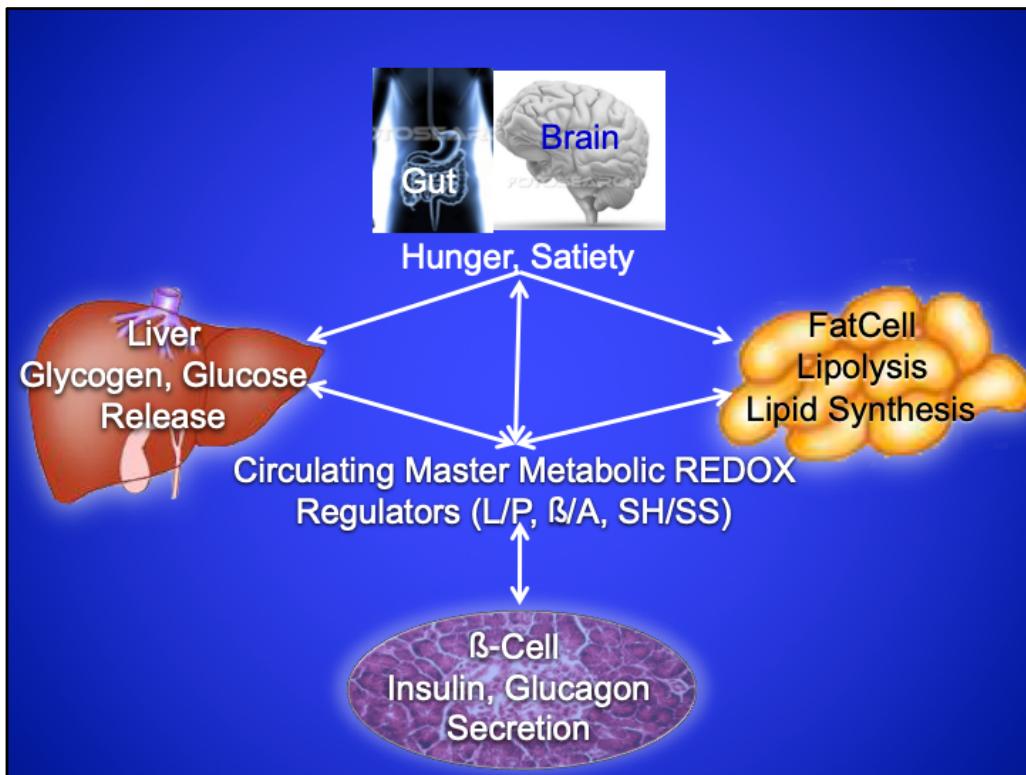
Saadeh et al, PLoS One 2012

A consequence of the entry of circulating metabolites like β -OHB is stimulation of insulin secretion in β -cells. Scavenging ROS with N-acetylcysteine inhibits insulin secretion.

Summary and Implications

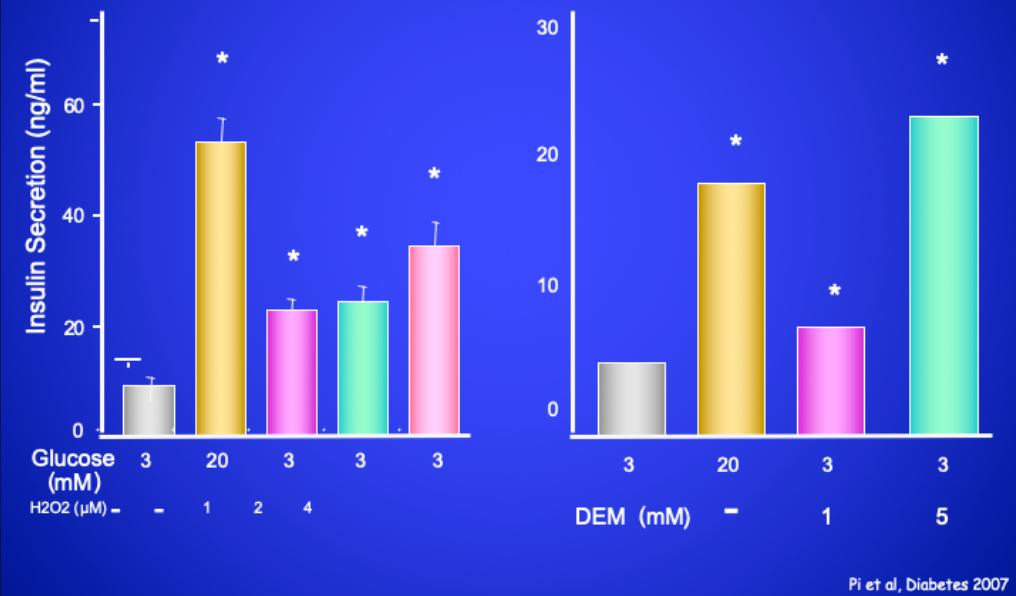
1. Fuels and exogenous agents that change redox can generate ROS in many organs
2. Redox couples that are transported to cells via the circulation and can interact with all organs
3. ROS and redox changes impact function in an organ-specific manner
4. Environmental agents, known to cause oxidative stress, can also increase ROS and insulin secretion in the absence of a stimulatory fuel

Exogenous agents can mimic the effect of fuels.

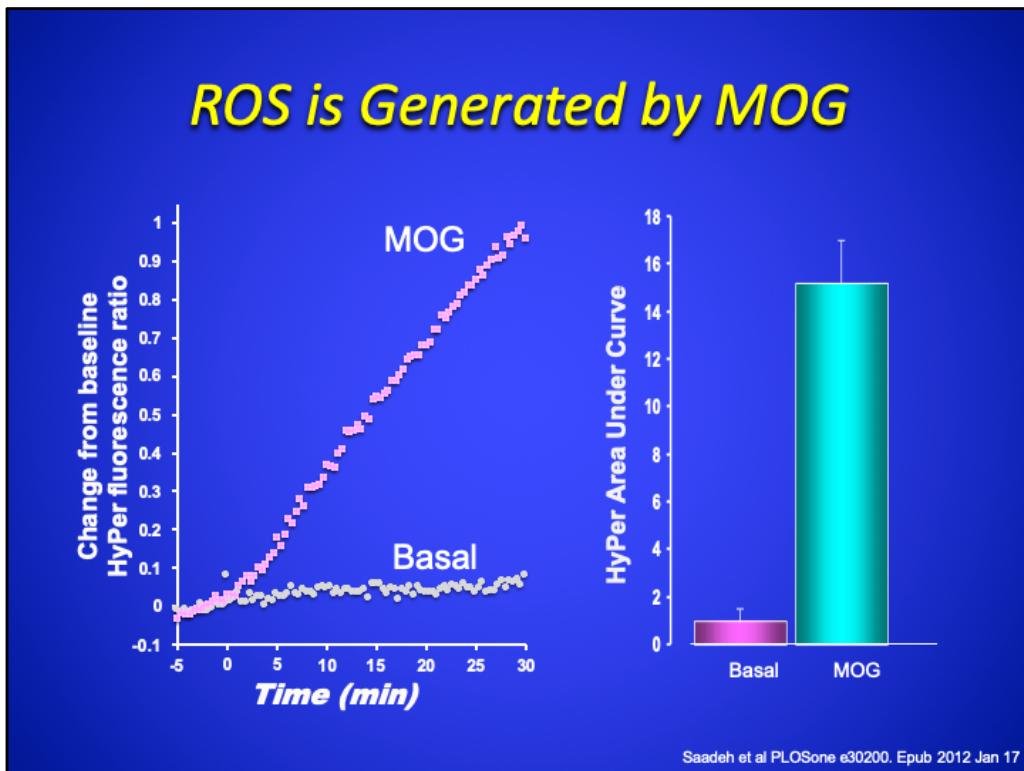


*Illustration of some Exogenous
Compounds that Induce ROS?*

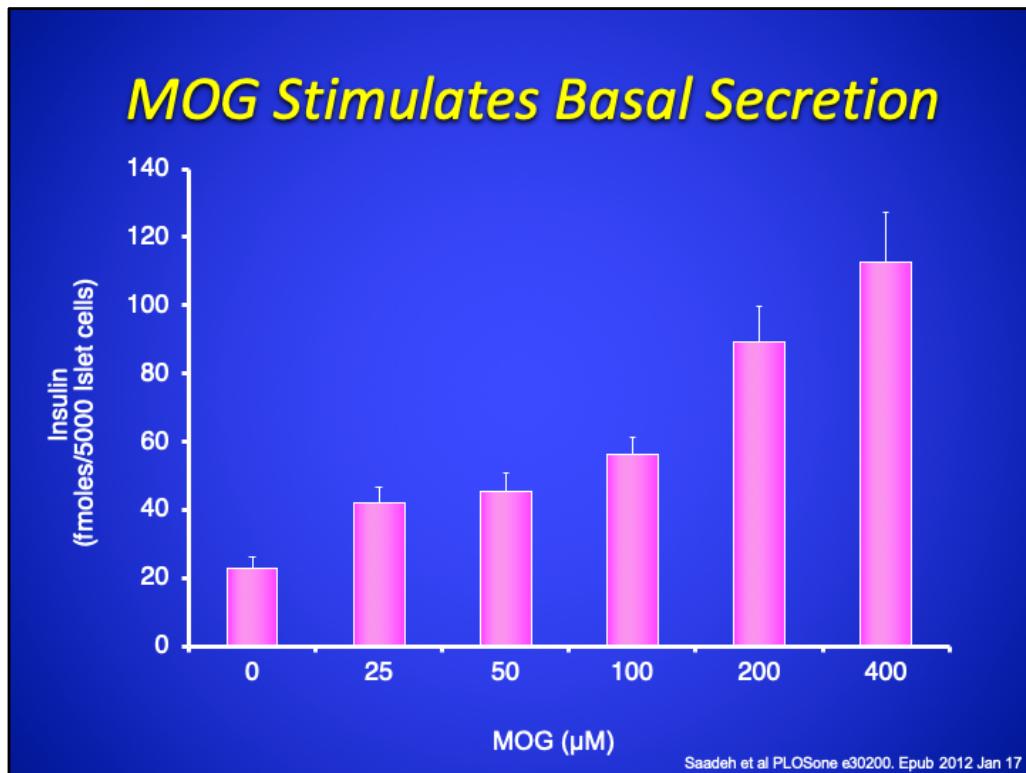
H₂O₂ Increases Insulin Secretion in INS-1 Cells



Increases in hydrogen peroxide stimulate insulin secretion like excess fuel but in the absence of a stimulatory fuel. H₂O₂ can be added outside the cell or generated within the cell by DEM.

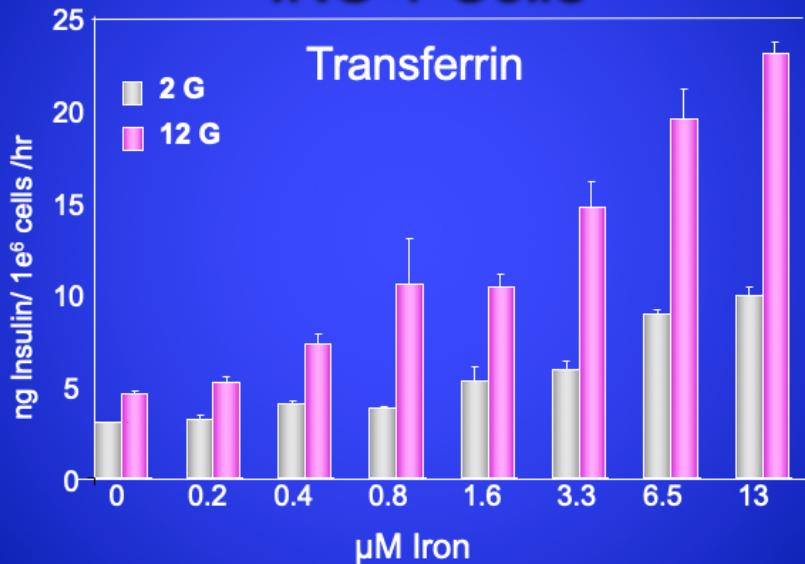


MOG, mono-oleoyl glyceride is a natural product and a common additive to most dairy products that serves as an emulsifier (to prevent cream from separating) and preservative. ROS is generated when it is added to an unstimulated β -cell.



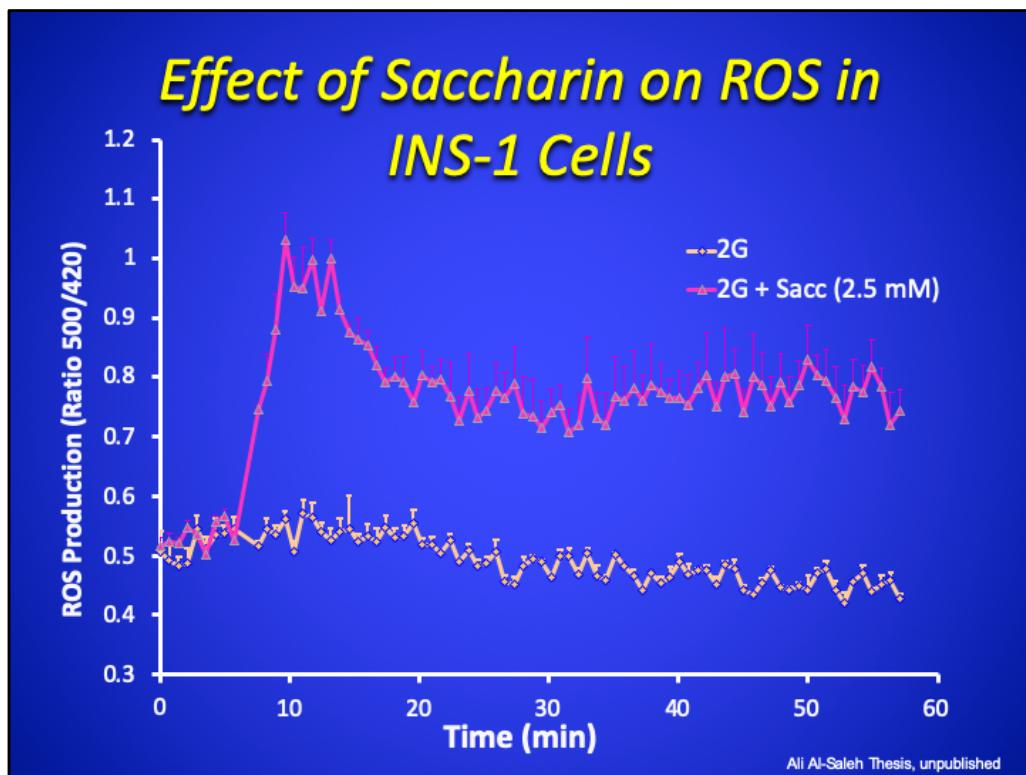
At concentrations that increase ROS, MOG stimulates insulin secretion inappropriately at low glucose.

Iron Induces Insulin Secretion in INS-1 Cells



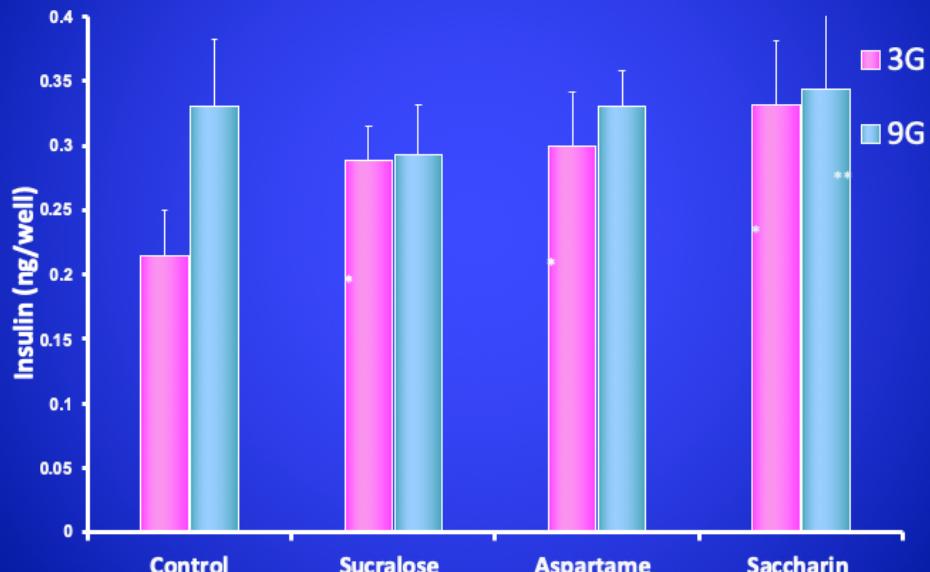
Ferrante, Deeney, Unpublished.

Iron exposure induces Insulin Secretion in clonal β -cells & dissociated mouse islets due to ROS generation. Lean red meats contain more iron than other meats.



Artificial sweeteners increase ROS

Artificial Sweeteners Affect Insulin Secretion in Dissociated Rat Islets

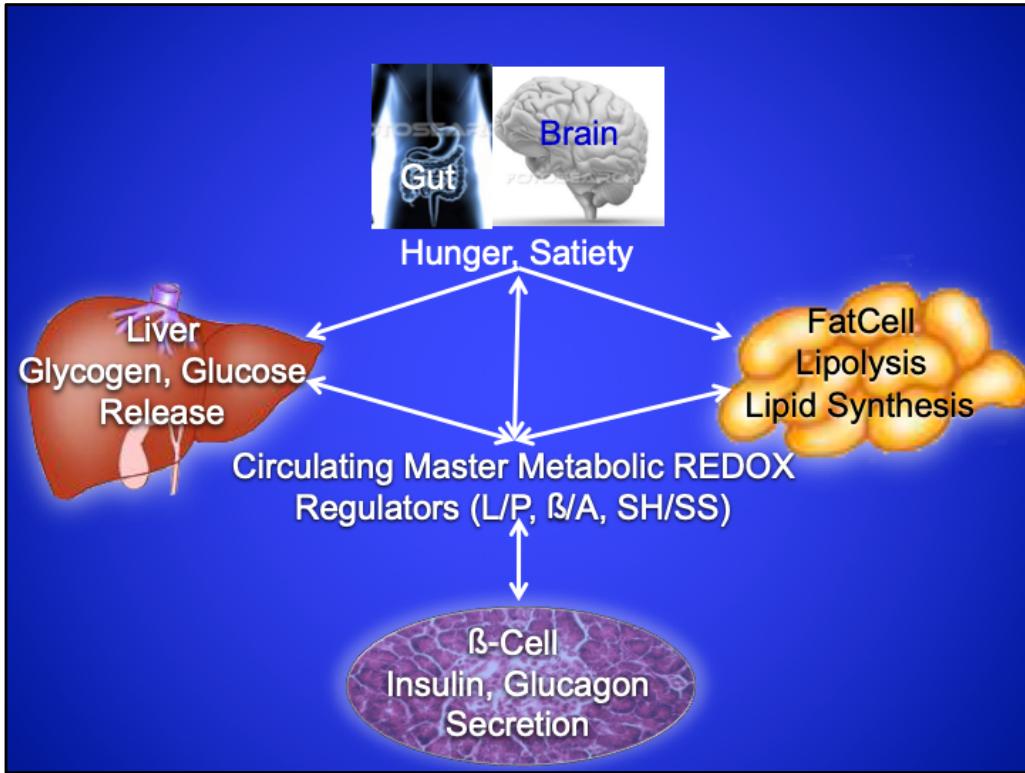


Ali Al-Saleh Thesis, unpublished

All sweeteners tested generate ROS and increase insulin secretion at low non-stimulatory glucose.

Agents that Cause Insulin Secretion in the Absence of a Stimulatory Fuel by Generating ROS

- MOG, a lipid food emulsifier and preservative
- Saccharin, an artificial sweetener
- Iron, an essential mineral
- Bisphenol A, contained in plastics



Much has been learned about the individual processes that comprise metabolic regulation, however, there has been little focus on how the complex network interacts and the manner in which moment to moment energy is continuously maintained. This new discipline will develop tools and approaches to better understand complex interactions.



Thank You