

*Network of Glucose-Induced Signals for
Insulin Secretion*

International Summer Institute on
Network Physiology-2019

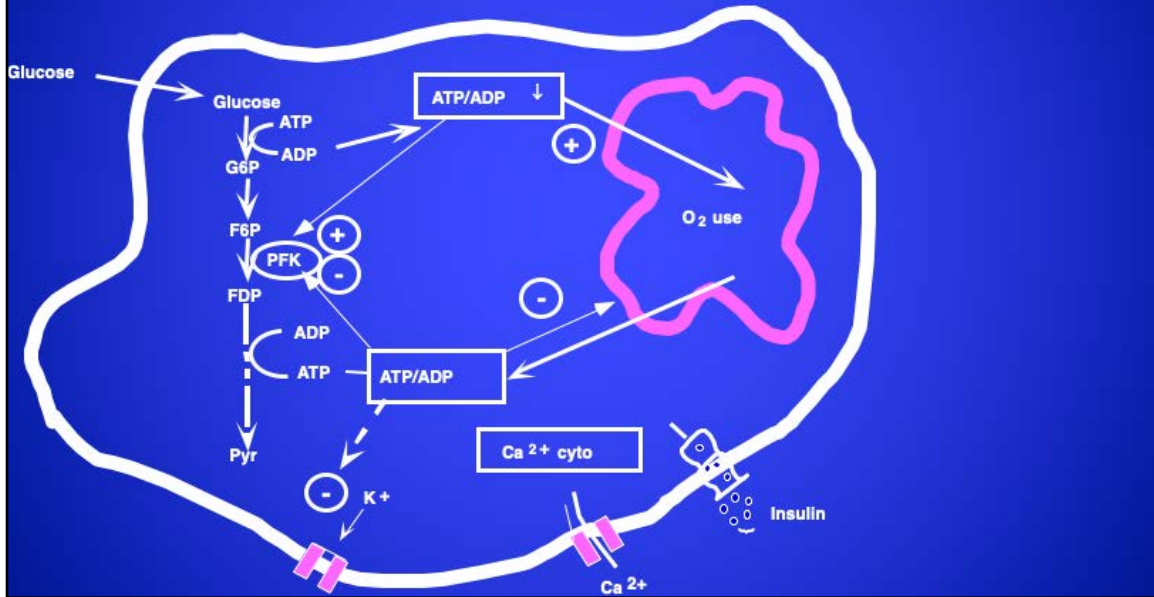
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Pancreatic β -Cells within Islets of Langerhans Respond to Nutrients

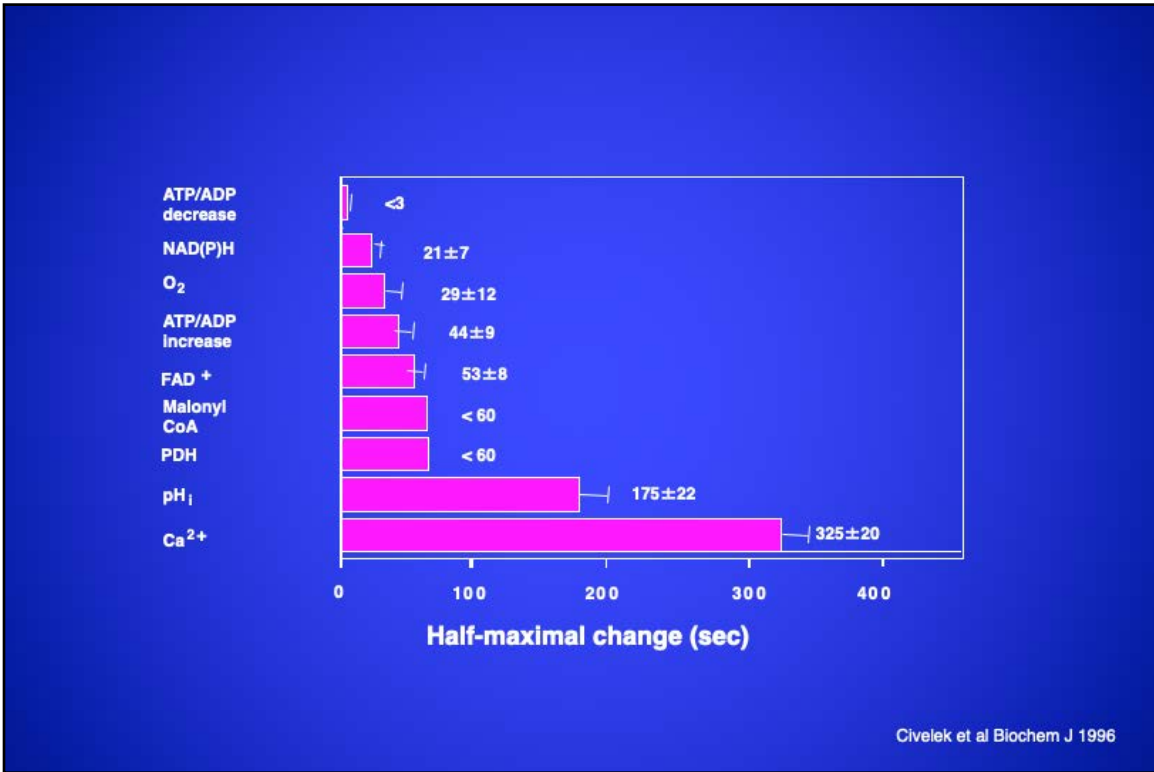
- β -Cells function to secrete insulin in response to food intake
- Insulin functions to store ingested fuels appropriately
- Signals that initiate and maintain secretion are generated by fuel metabolism in β -cells
- β -Cells are most sensitive to glucose but also sense fats and amino acids

The question we asked is how does metabolism of a simple fuel like glucose stimulate the complex pathway of insulin secretion.

Glucose Metabolism Changes Ca^{2+}

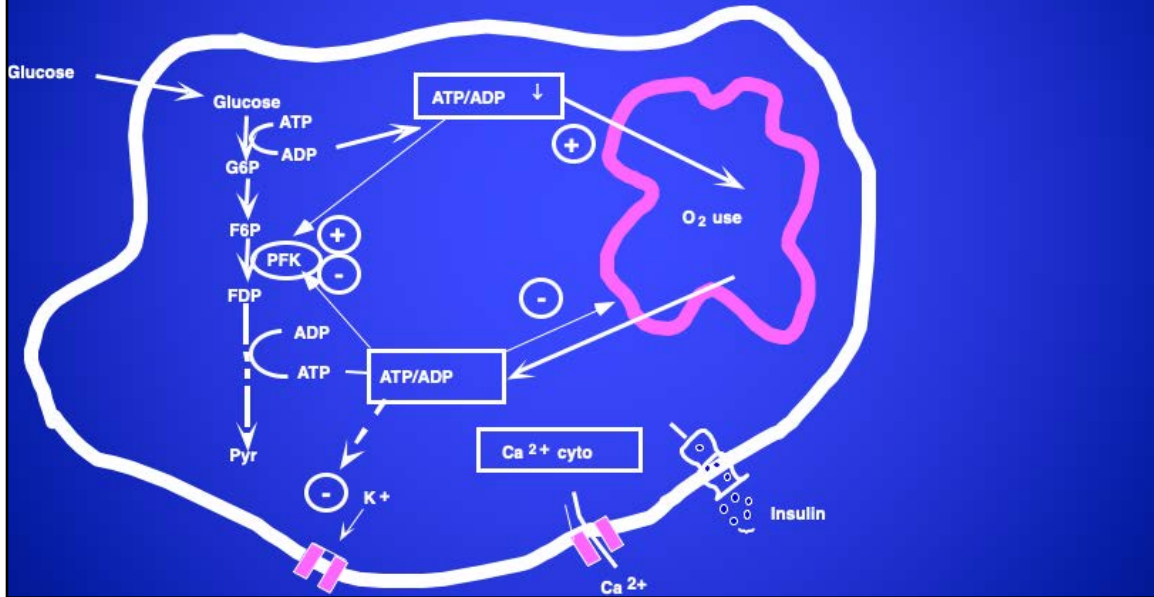


The consensus model of insulin secretion is based on evidence that glucose metabolism in β -cells uniquely, leads to opening of voltage sensitive calcium channels via changes in the membrane potential induced by a rise in the ATP/ADP ratio that closes the ATP-sensitive K^+ channel (K_{ATP}),



Insulin secretion coincides with the rise in Ca²⁺. This slide provides validation for the model by showing that the predicted changes occur with an appropriate time course. Malonyl CoA also changes early. This is the main link between glucose and fat as ATP source.

Glucose Metabolism Changes Ca^{2+}



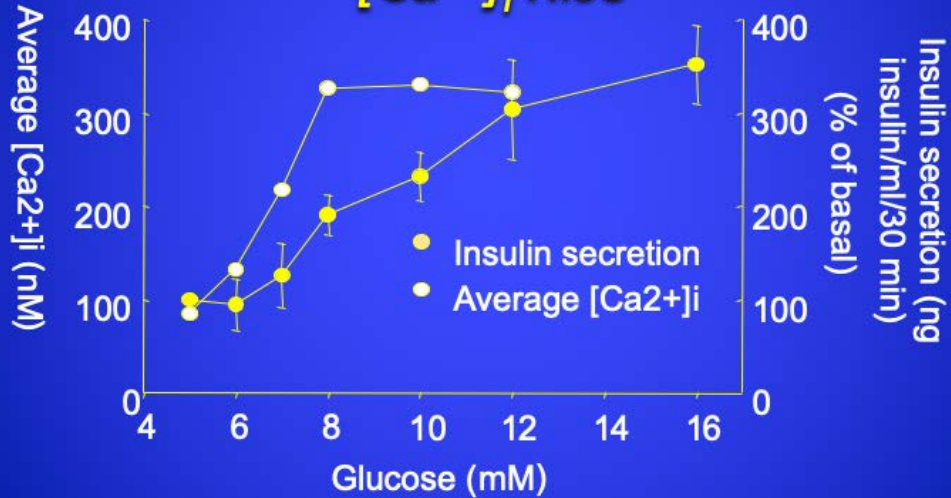
The time course of changes in metabolites is consistent with a metabolism-induced event.

***Glucose Metabolism Raises
Cytosolic Ca^{2+} and Provides
an Important Signal for
Insulin Secretion***

Is it the only one?

This is one signal. Is it the one and only?

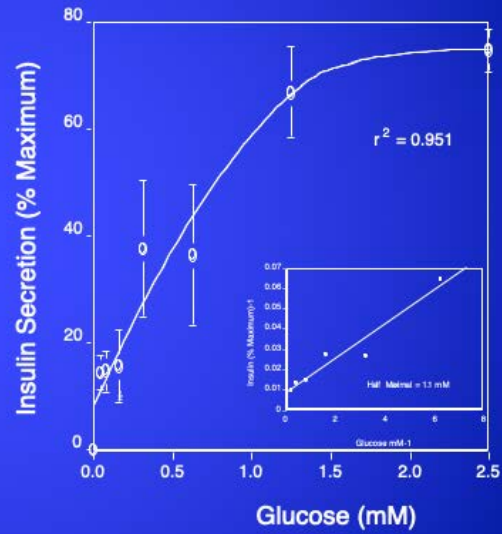
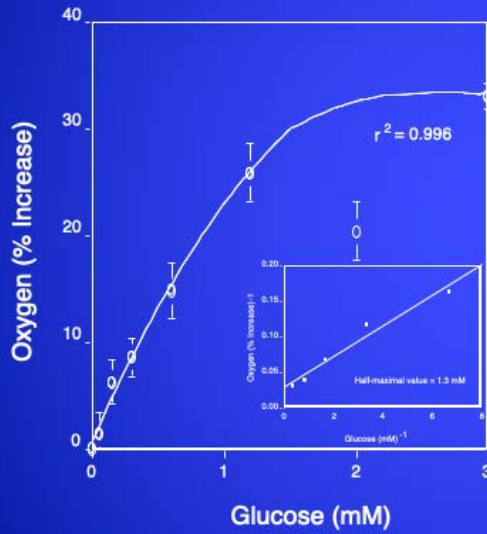
Lack of Correlation Between Magnitude of Insulin Secretion and Average Amplitude of $[Ca^{2+}]_i$ Rise



Heart et al Am J Physiol 2006

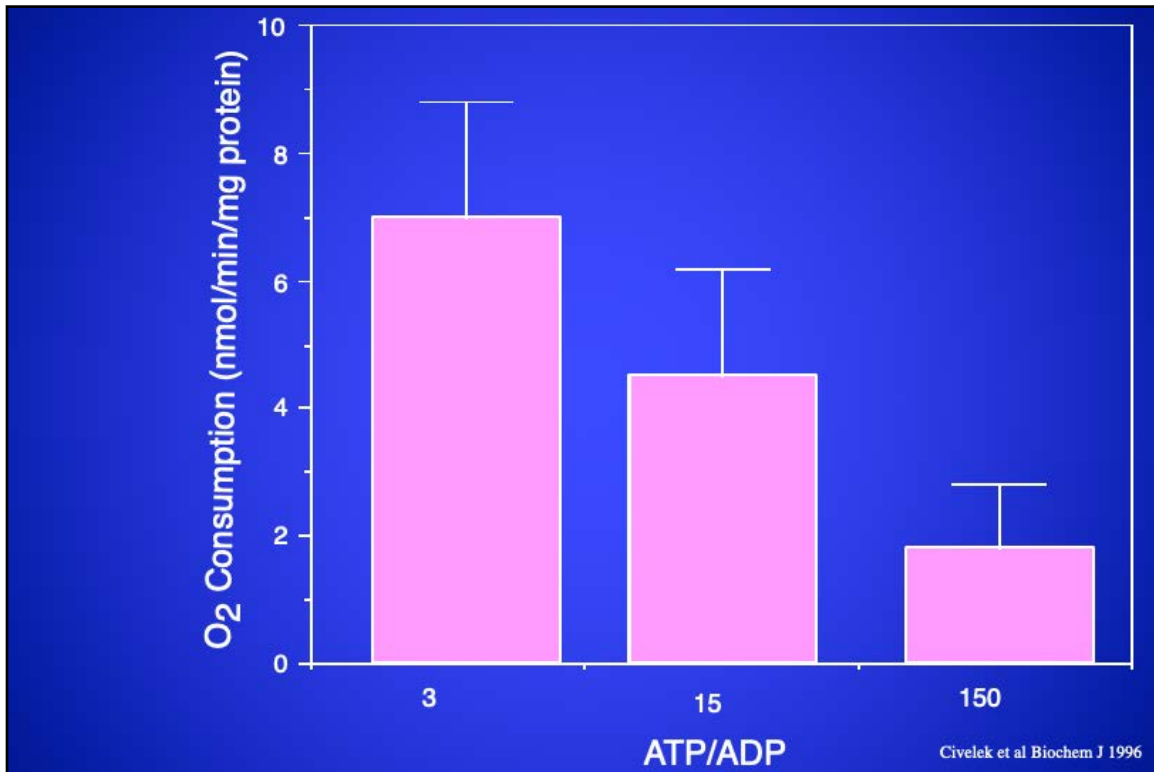
Although a rise in Ca^{2+} is needed for secretion, it is not sufficient to explain the normal range of responses to glucose.

Glucose Half-Maximal = 1.2



Porterfield et al Diabetes 2000

O₂ consumption is a measure of glucose oxidation in the mitochondria.. This slide shows that there is a very nice correlation between glucose induced secretion and oxygen consumption in clonal β -cells. The half-max is the same for both.

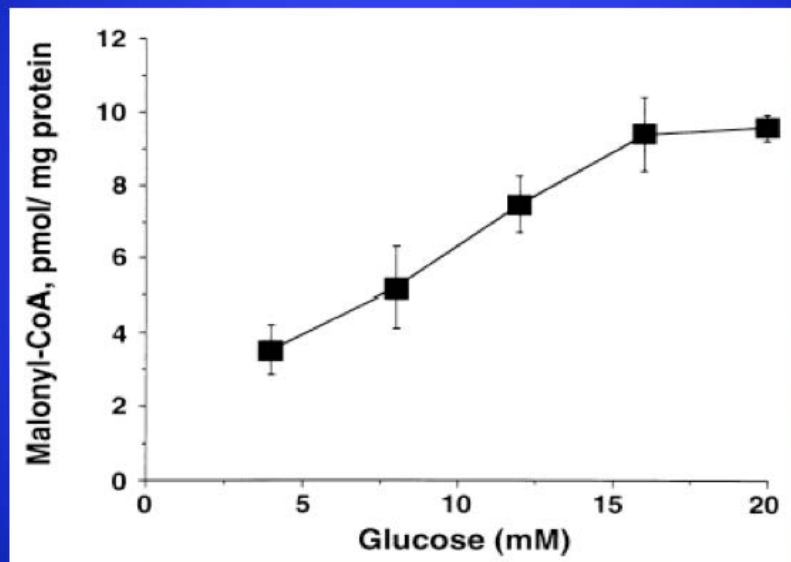


It is well-established that insulin secretion increases the ATP/ADP ratio. This slide points out that an increase in the ATP/ADP RATIO actually decreases oxygen consumption. This apparent quandary can be solved in two possible ways.

First, the ATP/ADP ratio oscillates and the troughs in the ratio are responsible for the increase in oxygen consumption.

Second, factors other than the ATP/ADP ratio are responsible for the increase in respiration, such as calcium, FFA or LC-CoA.

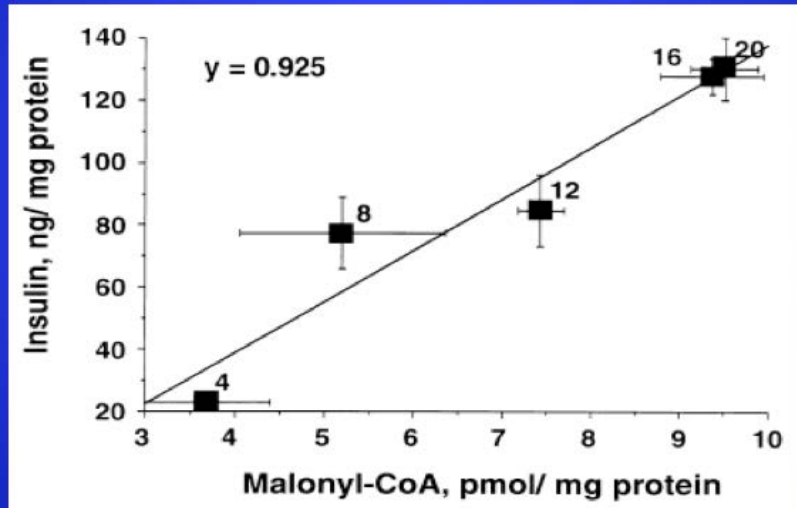
Glucose Increases Malonyl CoA



Parfitt S., Athanasiadis T., Prentki et al J Biol Chem 1992
Cohen B. *Biochem Biophys Res Commun* 1992

Malonyl CoA links glucose metabolism and fat metabolism. It is produced from glucose and inhibits fat from entering the mitochondria and consequently increasing in the cytosol..

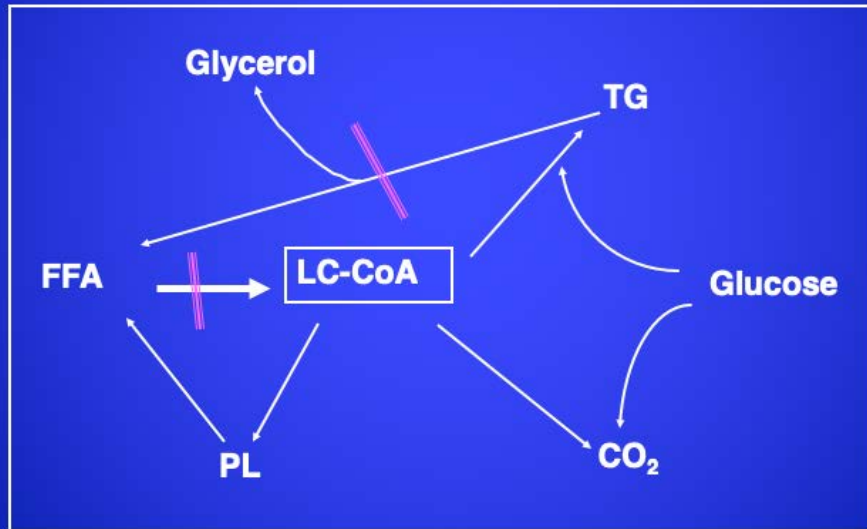
Correlation between Malonyl CoA and Insulin Secretion



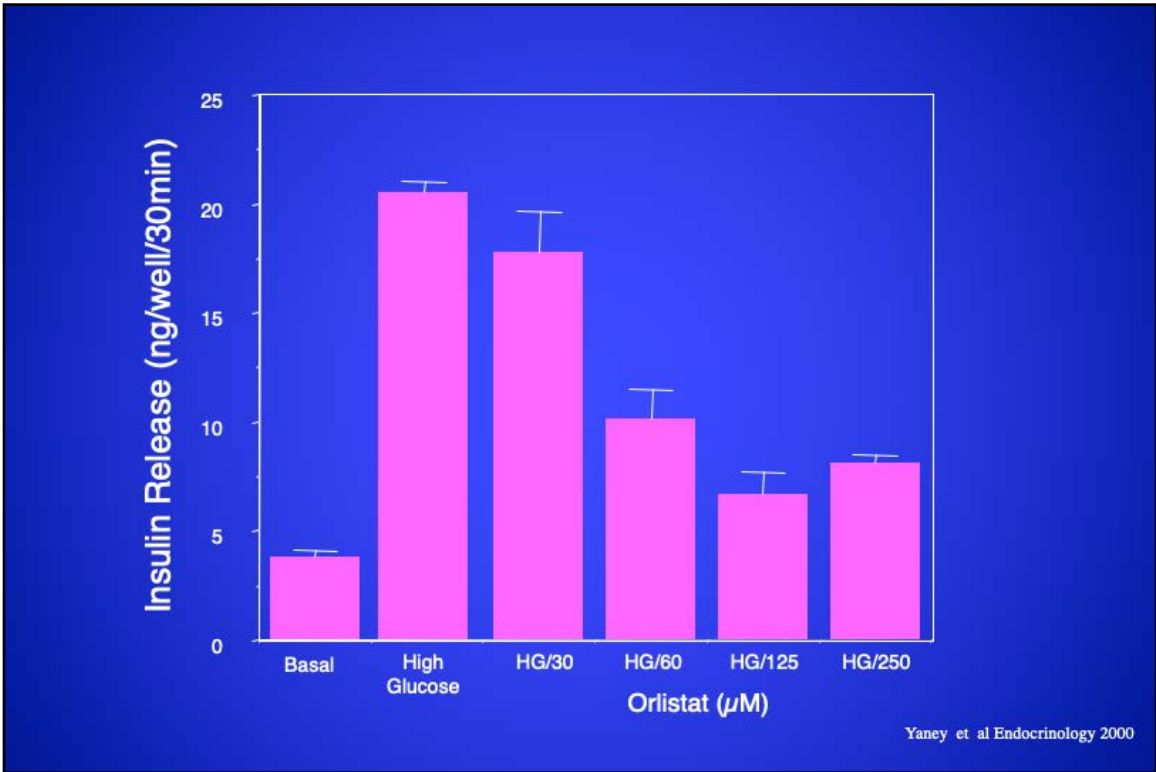
Farfari, S., Assimakopoulos-Dimitriou, E., Farfari et al Diabetes 2000

Malonyl CoA content correlates with insulin secretion

Effect of Inhibiting LC-CoA Production

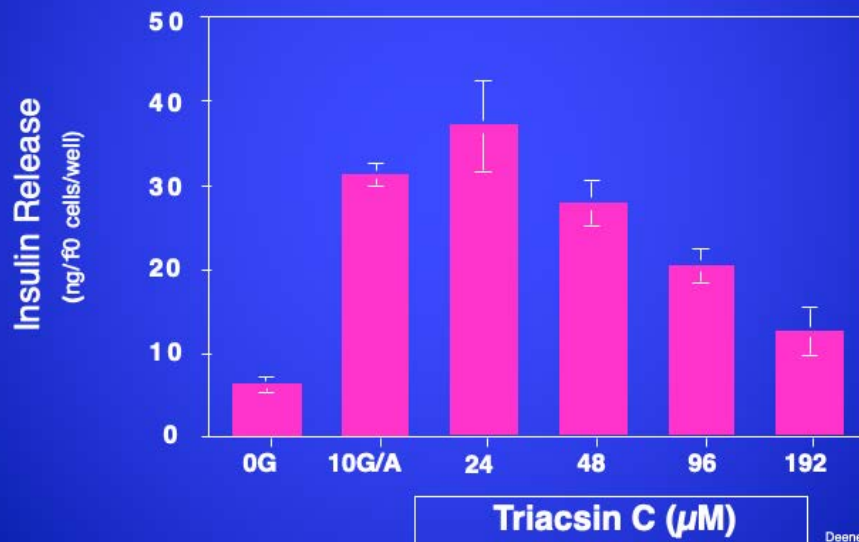


Triacsin C inhibits activation of FFA to LC-CoA. Orlistat inhibits TG breakdown to FFA. If these events are important inhibiting them should effect secretion.



This shows that, as predicted, orlistat (the triglyceride lipase inhibitor) also blocks glucose-induced secretion in a concentration dependent manner.

Inhibiting LC-CoA Inhibits Secretion

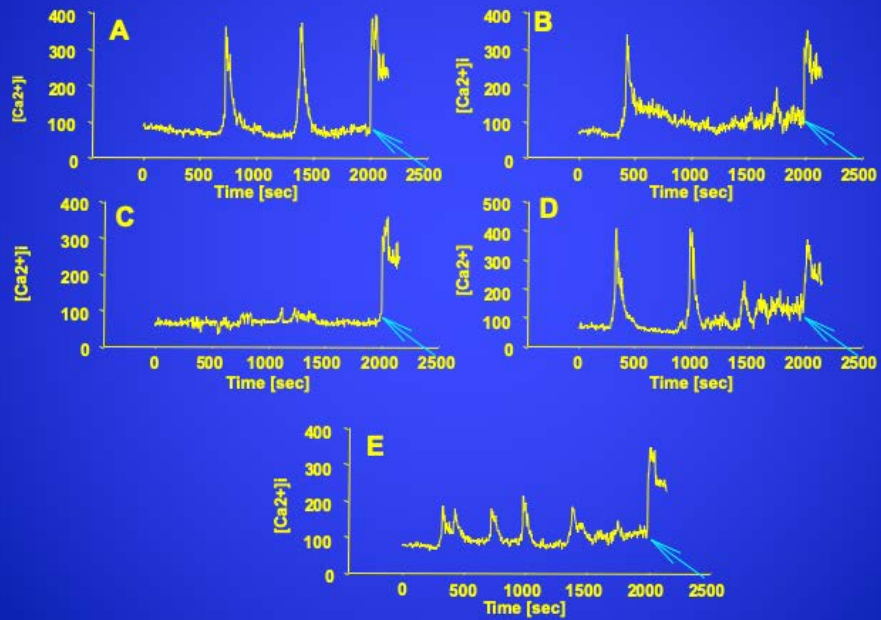


Inhibiting activation of FFA to LC-CoA with triacsin C also inhibits insulin secretion.

Important Players So Far

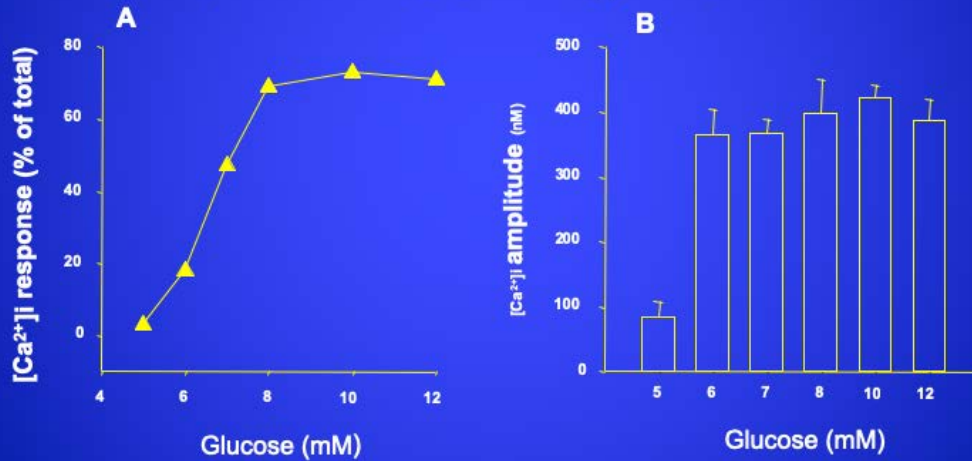
- The ATP/ADP ratio
- Ca^{2+}
- O_2 consumption
- LC-CoA/malonyl CoA
- Are there important patterns to consider?

$[Ca^{2+}]_i$ Responses in Single Islet Cells



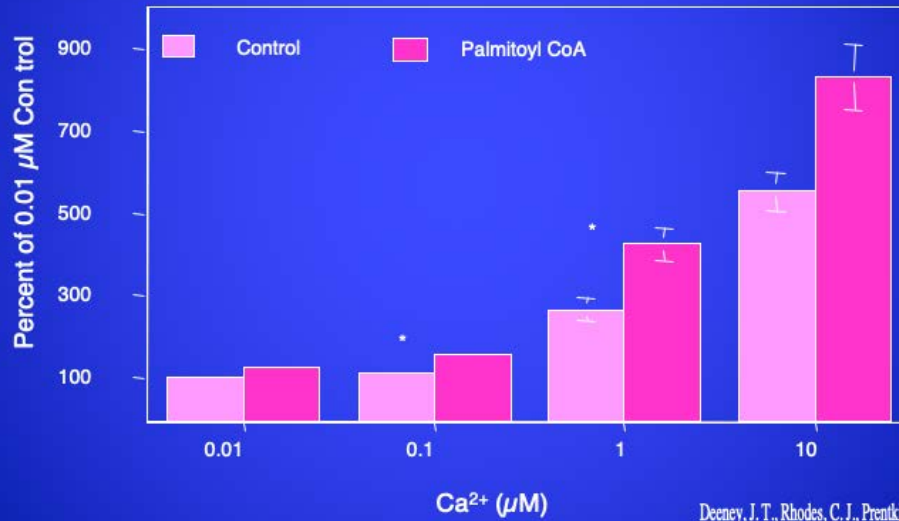
Most single β -cells respond to glucose with an oscillatory Ca^{2+} response with similar amplitude.

Increasing Glucose Implicates Recruitment of Cells rather than Increase in Average Amplitude of $[Ca^{2+}]_i$ Response.



Analyzing hundreds of β -cells yields an increasing integrated Ca^{2+} response but no change in the average amplitude of Ca^{2+} oscillations. Thus the Ca^{2+} response is oscillatory and due to recruitment of more cells as glucose increases.

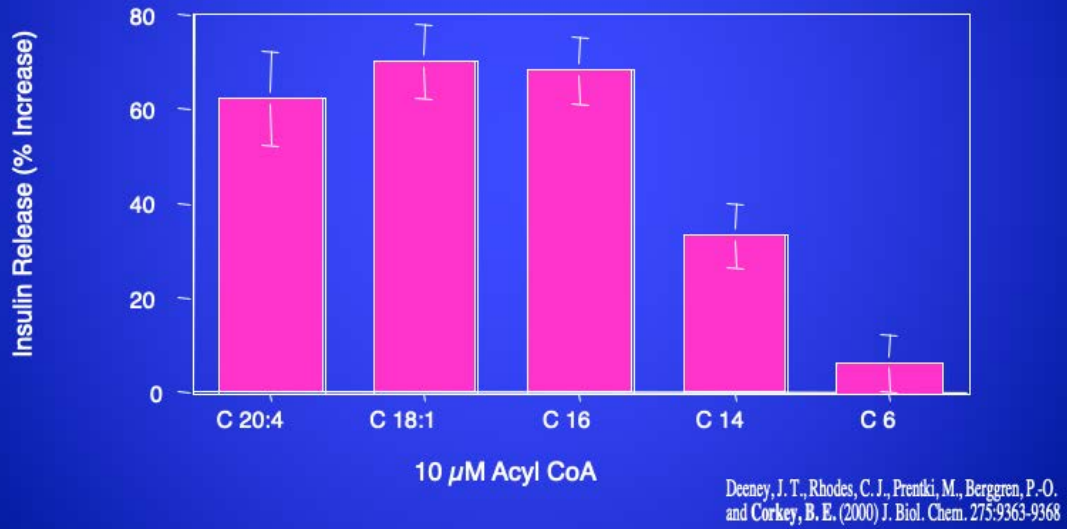
Both Ca^{2+} and LC-CoA Stimulate Exocytosis in Permeabilized β -Cells



Deeney, J. T., Rhodes, C. J., Prentki, M., Berggren, P.-O. and Corkey, B. E. (2000) *J. Biol. Chem.* 275:9363-9368

Both Ca^{2+} and LC-CoA directly stimulate exocytosis but both together are more effective. Cells are permeabilized by inducing small pores with a detergent.

Exocytosis from Permeabilized β -Cells



The most effective acyl CoAs that stimulate exocytosis are long-chain

What Signals are Essential?

What Signals are Sufficient?

- Increased cytosolic Ca^{2+}
- Increased LC-CoA derived from FFA
- Increased redox
- Increased ROS production
- Changes in the ATP/ADP ratio
- All are sufficient to cause secretion
- None induce the normal pattern!

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.

Model Predicts Oscillatory Changes

- Glucose uses ATP, produces ADP and lowers the ATP/ADP ratio in the first 2 steps.
- Glycolysis and oxidative phosphorylation are stimulated by decreases and inhibited by increases in the ATP/ADP ratio
- This regulation causes oscillations in metabolism
- The change in ATP has opposite effects on Ca^{2+} and O_2

Mechanism for Oscillations

- Phosphofructokinase is inhibited by ATP and activated by its *PRODUCT*.
- This causes an overshoot in ATP production and inhibition of PFK.
- Glucokinase, the first step and glucose sensor of β -cells maintains a steady rate of glucose phosphorylation and ATP use.
- Oscillations in ATP/ADP impact many steps.

Phosphofructokinase exerts the strongest rate control for glycolysis because it is highly regulated.

Insulin Oscillations

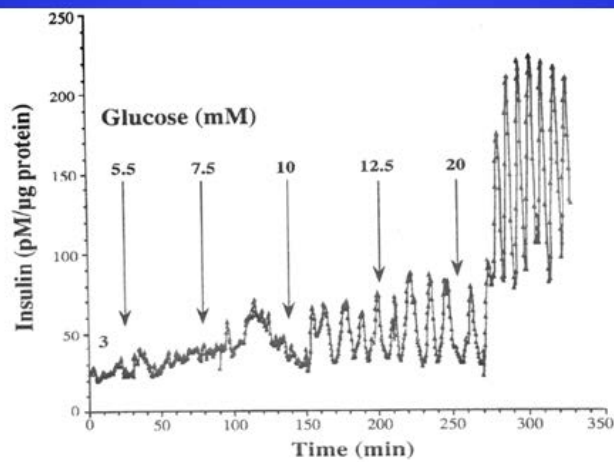
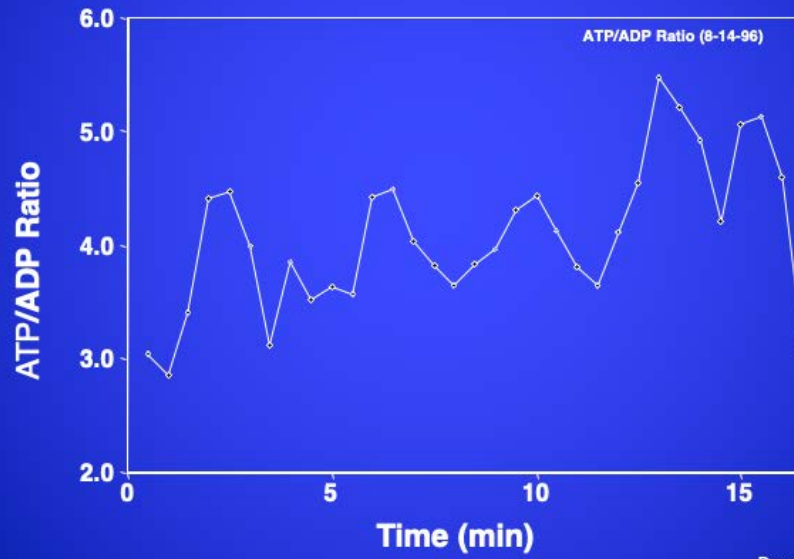


Fig. 1. Effect of glucose concentration on insulin secretion. Islets were initially perfused with 3 mM glucose. Glucose concentration was increased in steps at times indicated by arrows.

Cunningham et al Am J Physiol 1996

Increasing glucose increases insulin secretion and the amplitude of oscillations.

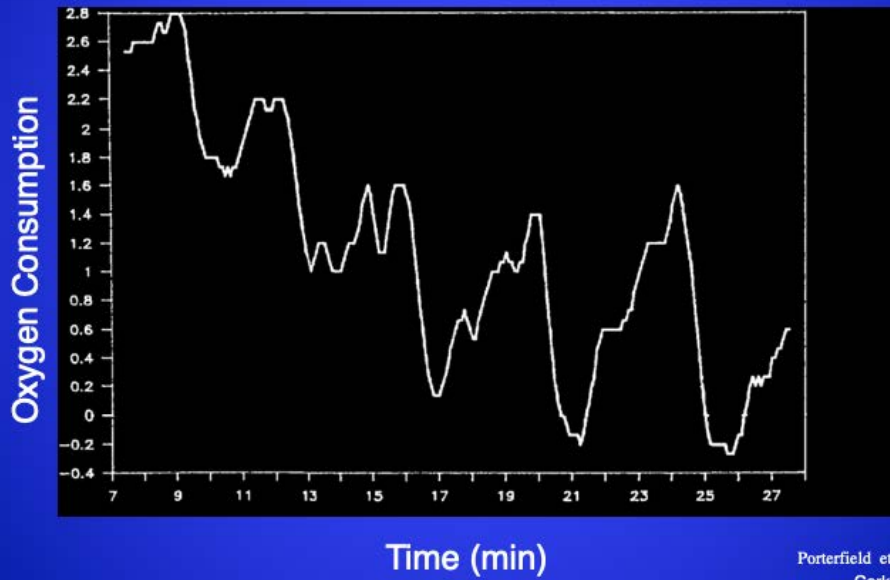
ATP/ADP Oscillations



Deeney et al J Biol Chem 2001

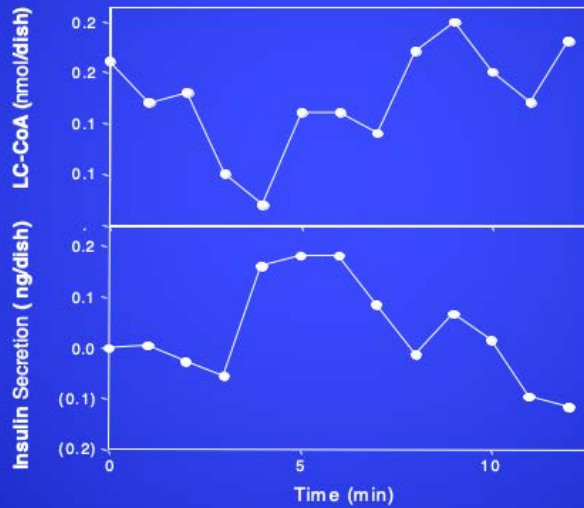
The ATP/ADP ratio oscillates in response to stimulatory glucose

Oxygen Oscillations



O₂ consumption oscillates in response to stimulatory glucose

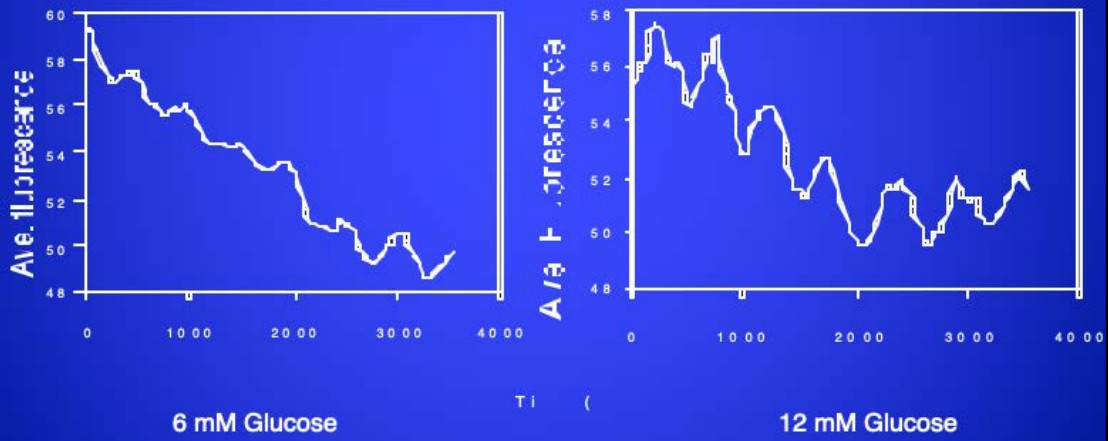
LC-CoA and Insulin Oscillations



Daeney, Richard, Sorhede-Winzell and Corkay, unpublished

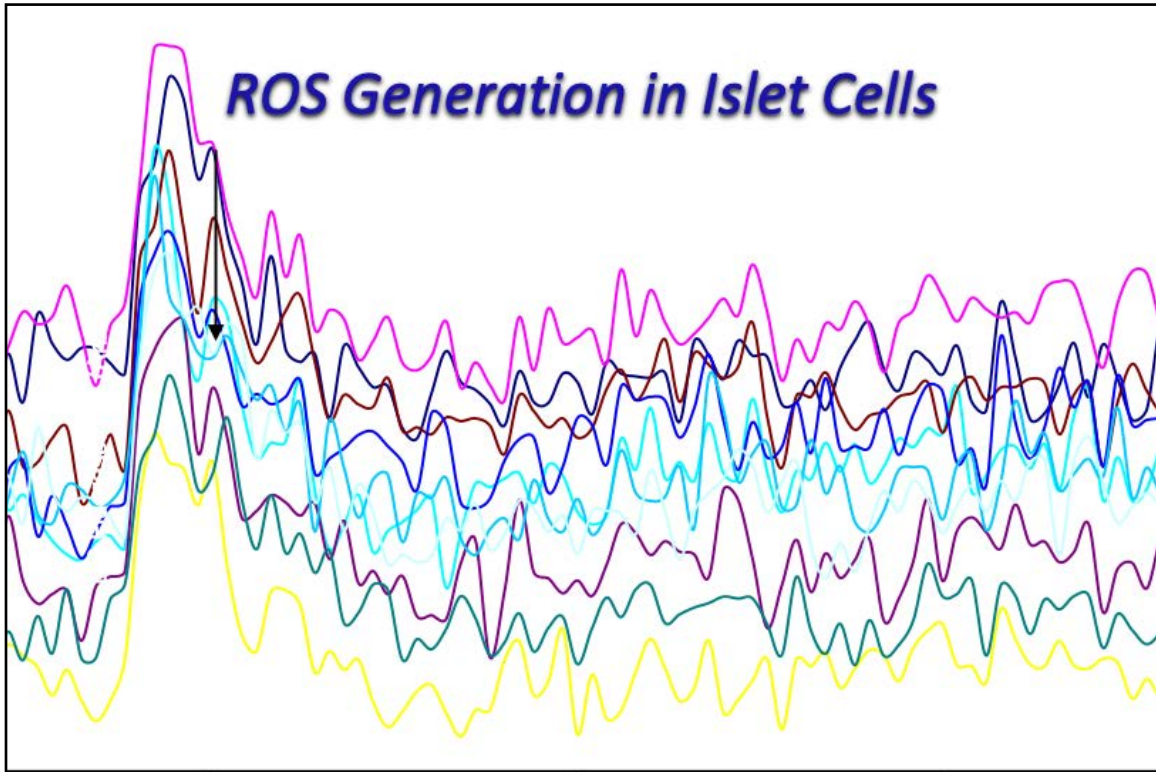
These data indicate that LC-CoA oscillates but out of phase with insulin.

Islet Mitochondrial Membrane Potential Oscillates



Katzman and Shirihai, unpublished data

Mitochondrial membrane potential oscillates with a variable amplitude depending on the strength of stimulation.



ROS is produced in β -cell mitochondria in an oscillatory manner. An initial overshoot is rapidly compensated. This is an illustration of multiple cells in a single experiment over a time period of 30 min.

*Glucose-Induced
Oscillations Provide an
Additional Metabolic
Signal for Insulin
Secretion*

It is important to consider time in assessing metabolic regulation since metabolism changes very rapidly.

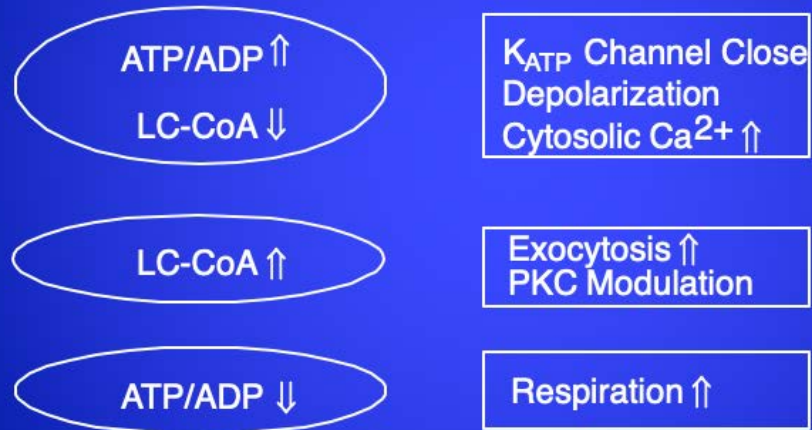
Summary of Oscillatory Processes

- Insulin secretion
- Oxygen consumption
- ATP/ADP ratio
- Redox state (NAD(P)H)
- Cytosolic Ca^{2+}
- Membrane potential
- ROS production
- Probably all metabolites

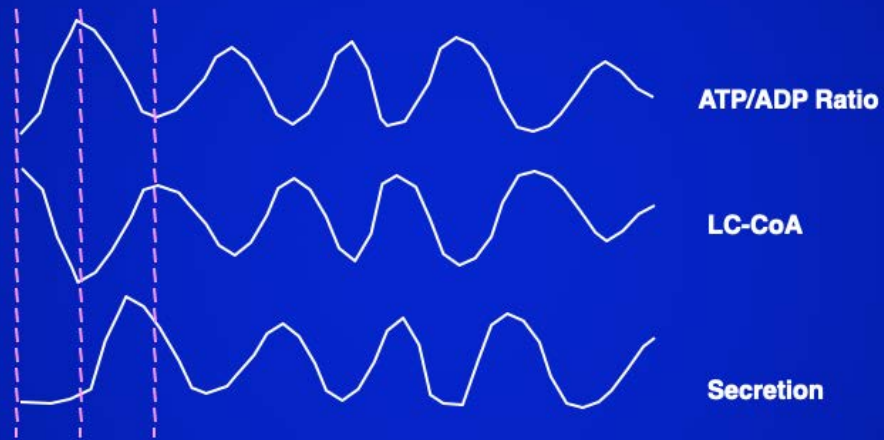
Advantages of an Oscillatory Network

- Greater range of signals with higher peaks and lower troughs
- Continuous control
- Prevent desensitization to insulin in the periphery
- Diabetics and relatives lose oscillatory secretion

Model of Co-Regulation by the ATP/ADP and LC-CoA Network



Putative Insulin Secretion Network



Thank You