Lipid Signaling Is Required For Glucose Stimulated Insulin Secretion in the Pancreatic β Cell: Type II Diabetes Mellitus

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Introduction

- According to the CDC,1 in 10 Americans have Diabetes, 90-95% of which have Type 2 Diabetes Mellitus (T2DM)
- T2DM is a chronic metabolic disorder that is characterized by dysregulation of blood glucose homeostasis, which could be an indicator of impaired insulin secretory function in the pancreatic β Cell
- Chronic exposure to excess nutrients (glucose and lipids) can lead to Glucolipotoxicity (GLT): alteration of enzyme activity, insulin gene downregulation, glucose hypersensitivity and left shift in Glucose Stimulated Insulin Secretion (GSIS)
- Previously found: Fatty Acid (FA) stimulates GSIS, has regulatory roles in triggering and amplification mechanism through the intracellular form of FA, Long Chain Fatty Acyl-CoA (LC-CoA)
- This study aims to further elucidate the role of lipid signaling in GSIS

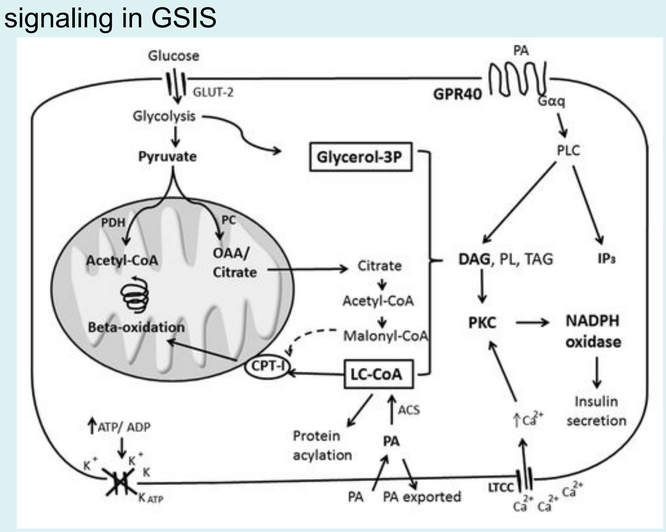

Figure 1: *GSIS pathway regarding KATP channel closure, membrane depolarization, LC-CoA accumulation, DAG and PKC activity.* Graciano, M. F., Valle, M. M., Curi, R., & Carpinelli, A. R. (2013). Evidence for the involvement of GPR40 and NADPH oxidase in palmitic acid-induced superoxide production and insulin secretion. *Islets*, *5*(4), 139–148. https://doi.org/10.4161/isl.25459

- High Glucose metabolism induces increased production of Malonyl-CoA, an inhibitor of Carnitine palmitoyltransferase I (CPT-1). CPT-1 is a receptor needed for β oxidation. Inhibition leads to accumulation of LC-CoA in cytosol and re-esterification of complex lipids. Notable lipid droplet formation can be found.
- LC-CoA can mediate the exocytotic machinery of GSIS by acylating granule fusion proteins (SNARE) such as, synaptosomal-associated protein-25 (SNAP-25) and synaptotagmin, which may increase Ca^{2+} sensitivity in the docking of secretory insulin granules to the cell membrane
- Influx of exogenous fatty acid may provide available substrates for formation of Diacylglycerol (DAG), a lipid messenger involved in activating Protein Kinase C (PKC), an enzyme that could regulate the amplification function of insulin secretion
- Exogenous Fatty Acid can also amplify calcium influx and insulin secretion by facilitating the activity of Phospholipase C in the cleavage of PIP2 into DAG and Inositol triphosphate (IP3) by attaching with the GPR40 (FFAR1) receptor. DAG is directly involved in exocytotic machinery and IP3 activates the release of Ca^{2+} from the endoplasmic reticulum

Results

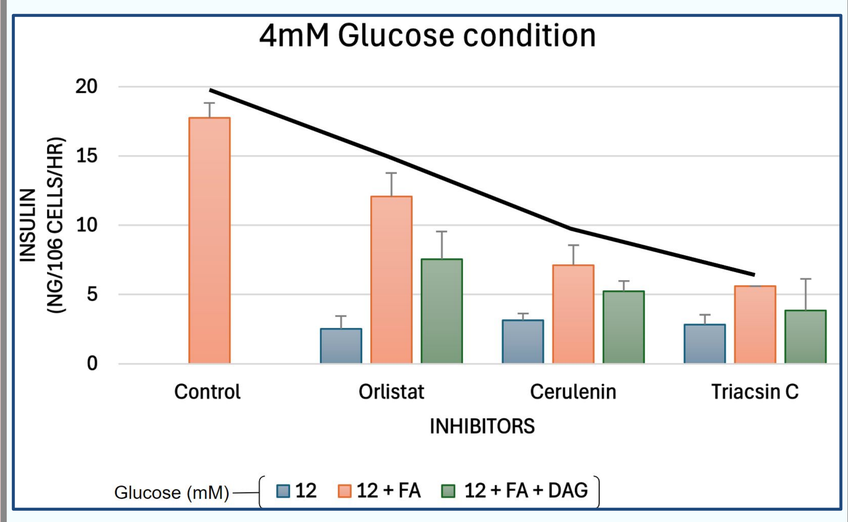

Figure 3: *Pancreatic β-Cells cultured in 4mM Glucose. Step down recovery cascade after addition of FA can be observed. Re-addition of FA partially recovered GSIS under Orlistat. GSIS recovered less in Cerulenin, and even less in Triacsin C.*

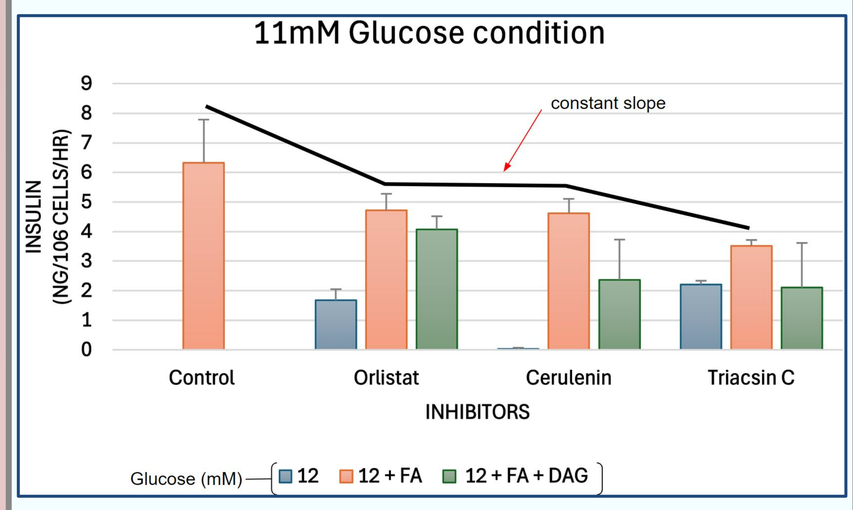

Figure 4: *Pancreatic β-Cells cultured in 11mM Glucose. There is a Step down recovery cascade, but a flat slope can be observed between orlistat and cerulenin. Re-addition of FA partially recovered GSIS under Orlistat. Cerulenin seemed to have the same amount of recovery as Orlistat. Less recovery in Triacsin C.*

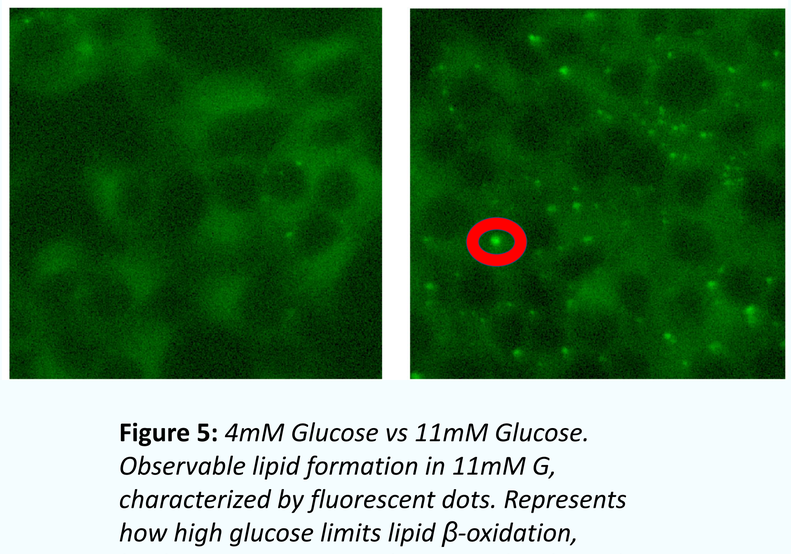

Figure 5: *4mM Glucose vs 11mM Glucose. Observable lipid formation in 11mM G, characterized by fluorescent dots. Represents how high glucose limits lipid β-oxidation, leading to excess cytosolic LC-CoA levels, and thus increased complex lipid formation.*

Discussion

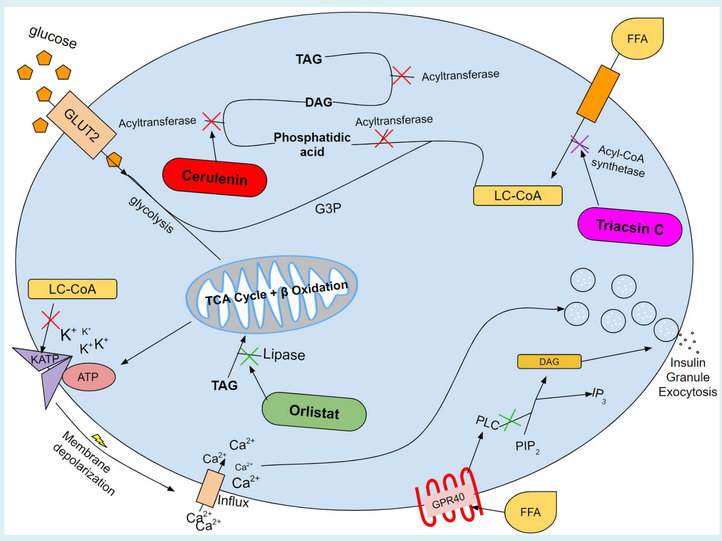

Figure 6: *Inhibitory mechanisms of Orlistat, Cerulenin, and Triacsin C in the context of lipid activity*

- Three inhibitors will be used to assess the role of lipid signaling in three distinct ways. The effects of each inhibitor will tell us what is and what is not required in lipid signaling
- Orlistat is a non-specific lipase inhibitor. In the context of the Pancreatic β Cell, Orlistat inhibits the de esterification of complex lipid moieties such as Triglycerides and diglycerides, preventing lipolysis
- Cerulenin binds to various acyltransferases, inhibiting complex lipid biosynthesis, protein acylation of exocytotic proteins, and may inhibit the action of acyl-CoA on the KATP channel.
- Triacsin C is an inhibitor that binds to acyl-CoA synthetase, a notable enzyme that facilitates the conversion of exogenous fatty acids in LC-CoA. This effectively reduces LC-CoA levels in the cytosol and prevents de novo synthesis of complex glycerolipids.
- In 4mM Glucose condition, the effect of Orlistat (Lipase inhibitor) was partially reversed after addition of FA, indicating that a lack of FA product due to inhibited lipase was replenished through addition of extra FA. Cerulenin led to less recovery than Orlistat, which could indicate that acyltransferases have a potentiative ability for lipid signaling, and is therefore required in Lipid controlled amplification of GSIS. Triacsin C recovered GSIS even less, which could indicate that the conversion of FA into LC-CoA through acyl-CoA synthetase was inhibited, and as a result, the lack of LC-CoA did not provide enough substrate for acyltransferases to induce complex lipid formation (Fig.3).
- In the 11mM Glucose condition, Orlistat and Cerulenin seemed to reach the same recovery capacity. A possible reason is that high glucose conditions lead to ample lipid formation through the use of acyl-transferase. So, inhibiting acyl-transferases in this condition does not have much of an effect because lipid products have already been synthesized beforehand (Fig.4).

Conclusion

It has been observed that the use of lipid signaling in GSIS is highly required for triggering and amplification. It can be concluded that the lipid signaling pathway in GSIS is mediated by the levels of LC-CoA in the cytosol, and it requires CoA and protein acylation for successful GSIS. It also leads slightly altered pathways between 4mM Glucose (physiologic) and 11mM Glucose (hyperglycaemic) conditions. Meaning, Gluclipotoxic cells (11mM Glucose) are less sensitive to exogenous FA in the presence of inhibitors. Future Directions could be to analyze insulin content and explore exocytotic machinery of GSIS.

Methods

- INS-1 (832/13) Clonal Pancreatic β-Cells were cultured at 37° in RPMI 1640 media (11mM glucose) and switched to either 4mM or 11mM glucose conditioned media.
- Cells were washed and incubated twice with a modified Krebs-Ringer Bicarbonate Buffer (KREBS) and 0.05% Bovine Serum Albumin.
- Glucose (1 mM or 12 mM), Free Fatty Acid (FFA) (200 μM, 6:1 molar ratio to BSA), and Diacylglycerol (100 μM), were incubated for 1 hour in the presence of inhibitors (i) including, Orlistat (lipase i) (200 μM), Cerulenin (acyltransferase i)(200 μM), Triacsin C (acyl-CoA synthetase i) (50 μM). Sample size for each was n=3.
- Secreted Insulin was transferred to a 1536 microplate and was added with Anti-Insulin antibody using a Cis-Bio HTRF Insulin Kit. Microplate was incubated 24-48 hours. Insulin levels were measured with a Tecan Infinite M1000 Pro plate reader.

<u>HTRF Insulin Kit: fluorescence resonance energy transfer (FRET)</u>

- Time delay of 50 to 100 μs before measurement to remove autofluorescence and background noise
- Two antibodies (donor: Europium cryptate, acceptor: XL665 near-infrared)
- Close proximity of both antibodies during insulin binding induces energy transfer and fluorescence at wavelength 665nm

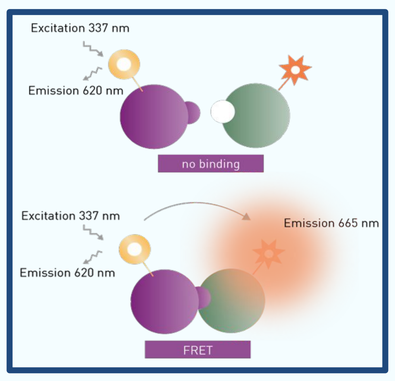

Figure 2*: HTRF mechanism (donor and acceptor interaction). Energy transfer of fluorophore antibodies induces accurate signal of Insulin[2].* Pusterla, T. (2020). *HTRF (Homogeneous time-resolved fluorescence).* Bmglabtech. https://www.bmglabtech.com/en/blog/htrf/

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