Glucolipotoxicity Impairs Calcium-Dependent Fatty Acid Amplification of Insulin Secretion via Impaired Lipid Signaling Pathways in Pancreatic β-Cells

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Introduction

- Type 2 Diabetes Mellitus(T2D) is a chronic metabolic disease characterized by **hyperinsulinemia, insulin resistance, and progressive β-cell dysfunction**
- Exposure to excess nutrients leads to intracellular lipid accumulation and glucolipotoxicity(GLT)
  - GLT is associated with intracellular lipid accumulation and a **left shift in glucose-stimulated insulin secretion(GSIS)**
  - Insulin secretion elevated at basal glucose levels and inhibited at maximal levels of GSIS
- **Two distinct pathways** regulate insulin secretion: The triggering pathway and the amplification pathway

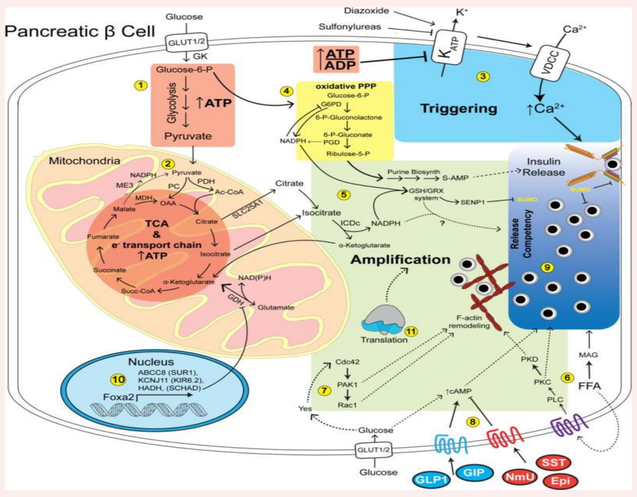

Figure 1: Overview of glucose-stimulated triggering and amplification pathways of insulin secretion. Glucose metabolism increases ATP production, which closes KATP channels and enables calcium influx to trigger exocytosis. Amplification occurs when lipid-derived signals(e.g, PIP2 cleavage to DAG via PLC) enhance SNARE protein function via PKC. Kalwat and Cobb. Pharmacol Ther. 2017 May 18;179:17-30

- GLT disrupts the amplification pathway by **impairing lipid signaling** and altering calcium sensitivity
- Our study aims to isolate this mechanism by **bypassing glucose metabolism** with diazoxide and directly depolarizing cells with KCL to induce calcium influx.
- Acute stimulation of FA is used to test whether FA and FA derived signals inhibit or enhance secretion. Lipids profiled using **Thin Layer Chromatography**

Results

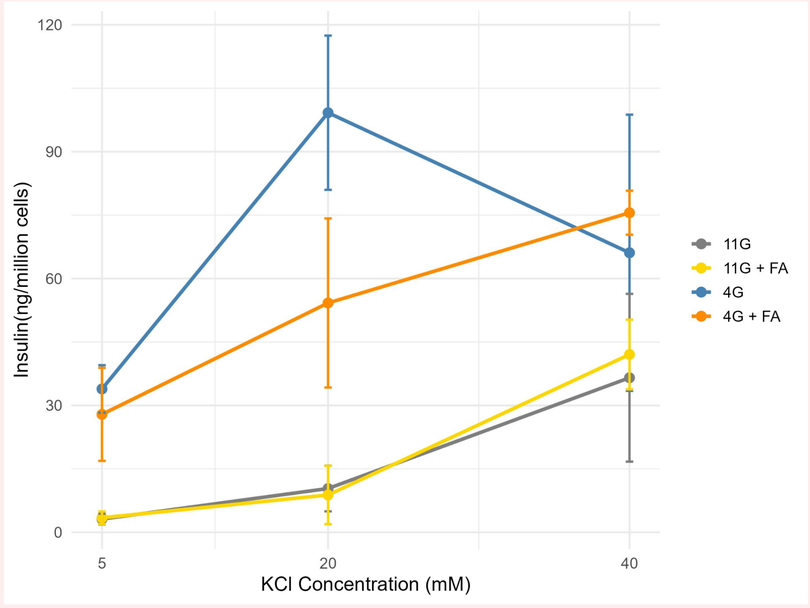

Figure 4: KCL-Induced Insulin Secretion in INS-1 cells. In 4 mM glucose conditions, insulin secretion increased at 20 mM KCL but declined at 40 mM KCL. With FA stimulation, secretion followed a more linear increase. In contrast, cells cultured in 11 mM glucose exhibited reduced secretion overall, and FA failed to enhance secretion at all levels

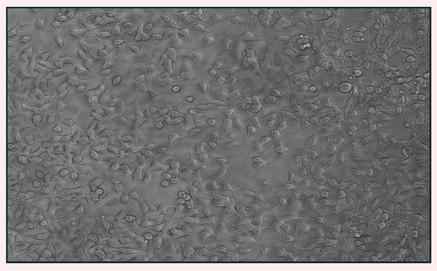

Figure 5: 11 mM glucose cells

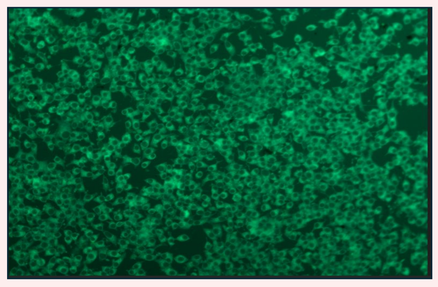

Figure 6: 11 mM glucose cells stimulated with BODIPY FA

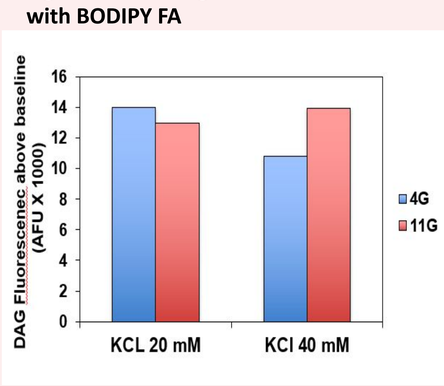

Figure 7: DAG fluorescence at 20 mM and 40 mM KCL for 4G and 11G cells

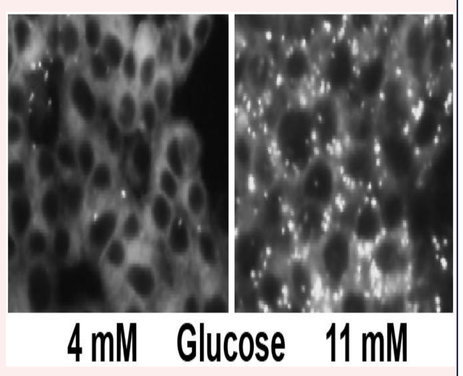

Figure 8: 4 mM Glucose and 11 mM Glucose cells.

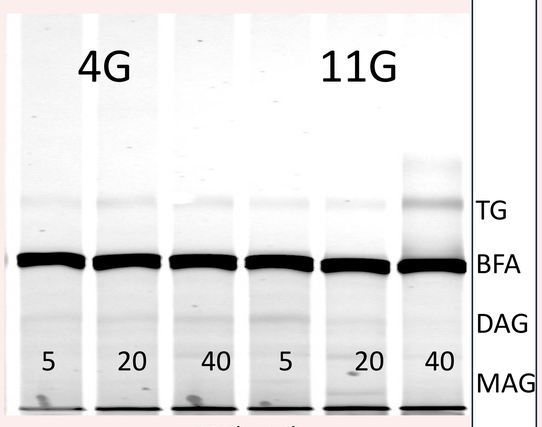

Figure 9: Thin Layer Chromatography depicting different lipids in 4 and 11 mM glucose cells stimulated with BODIPY FA. Lipid bands are monoacylglycerol(MAG, bottom), diacylglycerol(DAG, middle), and triglycerides(TG, top).

Discussion

Conclusions:

- Insulin secretion peaked at 20 mM KCL and dropped at 40 mM KCL in 4G cells
  - When intracellular calcium is maximal, **amplification signalling can be disrupted**
- In 4G cells with FA, **stimulation enhanced secretion**
  - Consistent with findings that FA-derived signals can amplify secretion downstream of Ca influx
- Insulin secretion lower across all levels of calcium and FA **failed to enhance secretion** in 11 G cells
  - Chronic DAG elevation can lead to **negative feedback inhibition** - Persistent DAG leads to continuous PKC activation, altering SNARE protein phosphorylation
  - Leads to **lack of secretion**
- Decreasing DAG but rising triglycerides at high KCL in 11G cells may promote increased secretion
  - Supports lipid cycling where cells convert **excess DAG to TG** as a protective adaptation to prevent toxicity
- Under GLT conditions, FA-induced amplification was blunted due to **disrupted lipid signaling, lipid accumulation, and desensitized exocytotic machinery**

Limitations:

- FRET-based assay measures secreted insulin but we did not measure **changes in insulin content**
- TLC analysis provides lipid profiles but lacks **real-time visualization** of DAG/TG dynamics and does not distinguish between newly synthesized lipid and incorporation of BFA through lipid cycling
- Unclear whether hypothesized changes in DAG are due to **lipid-derived signalling or stored molecules**

Future Directions:

- Track changes in PKC activity and **SNARE protein phosphorylation**
  - Links accumulation to desensitization of exocytotic machinery
- Test whether restoring DAG homeostasis **enhances Insulin secretion**
  - Exploring DAG kinases(DGKS) as primary regulators of DAG

Methodology

Insulin Secretion:

- INS-1 cells were cultured in **RPMI 1640 media (11 mM glucose)** for 4 days.
  - Half were switched to 4 mM glucose media 3 days before the experiment
  - Used to mimic both normal and diabetic conditions
- Cells pre-incubated in **0.05% BSA KREBS buffer**(2x 30 min) then treated with a 12 mM glucose solution with 400 µM Diazoxide containing KCL (5,20,40 mM) ± **150 µM BODIPY FA complex to BSA** for 60 minutes with BSA used as a control
- Insulin secretion measured via **a HTRF assay** in a 384-well plate using a TECAN M1000 Plate Reader

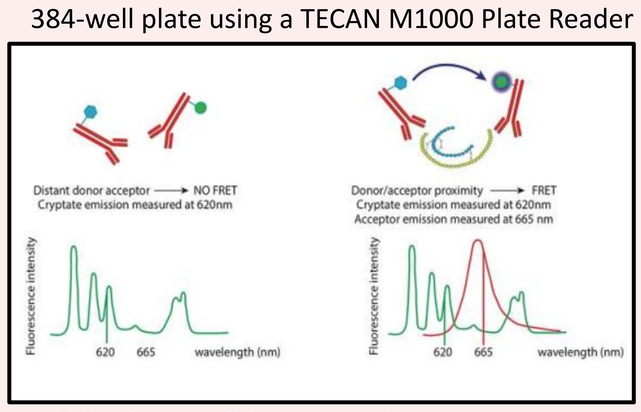

Figure 2: Mechanism of Fluorescence Resonance Energy Transfer(FRET) insulin assay. Farino et al. PLoS One. 2016 Feb 5;11(2):e0148684

Thin Layer Chromatography:

- After secretion, cells with FA addition were **extracted with methanol** for lipid extraction
  - **Chloroform** added in a 2:1 ratio
- PBS buffer added to separate phases
  - Organic layer was extracted and dried
  - Resulting lipids resuspended in **100 µL chloroform-methanol**
- Samples **spotted on silica plates** and run via TLC.
- Solvent used for mobile phase was 100 mL hexane, diethyl ether, and acetic acid (60: 38.5: 1.5)

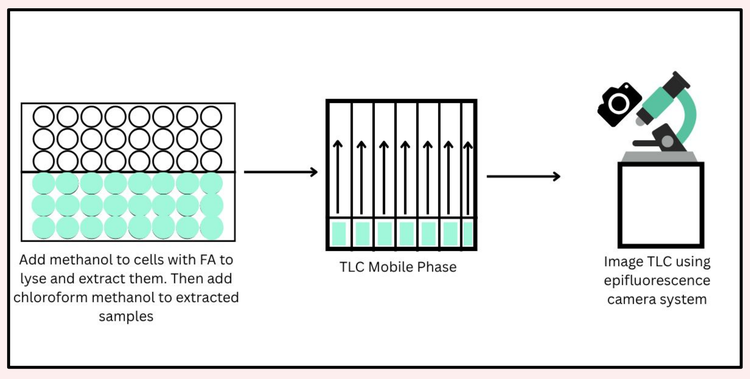

Figure 3: Workflow diagram of Thin Layer Chromatography

References

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