An Examination of Glucolipotoxicity on β-Cell Calcium Sensitivity and DAG Production Through KCl-Stimulated Depolarization

Kelly Yeh[1,2], Aryan Velu[2], Dylan Foss[2], Hung Le[2], Tanisha Sood[2], Anne Kelly[2], Yuvika Bhalla[2], Gulzhan Narmuratova[2], Jude Deeney[2]

Portola High School, Irvine, CA[1], Boston University Chobanian & Avedisian School of Medicine, Boston, MA[2]

Introduction

Type 2 Diabetes (T2D)

- A chronic metabolic disease characterized by insulin resistance and progressive β-cell dysfunction
  - β-cells compensate for insulin resistance through hyperinsulinemia, prolonged stress → dysfunction

Glucolipotoxicity (GLT)

- Chronic exposure to elevated glucose + fatty acids, contributes to T2D
- Associated with altered intracellular signaling + membrane excitability

Insulin Secretion Pathways

- In healthy individuals, pancreatic β-cells secrete insulin in response to elevated blood glucose → **glucose-stimulated insulin secretion (GSIS)** (Figure 1)
  - Glucose metabolism → increased ATP → ATP sensitive potassium (K_{ATP}) channels close → membrane depolarizes → calcium (Ca^{2+}) influx → insulin granule fusion and exocytosis
- Alternative stimuli help isolate downstream steps in this pathway:
  - **Diazoxide** opens K_{ATP} channels → keeps membrane hyperpolarized → prevents insulin secretion
  - **Potassium chloride (KCl)** directly depolarizes β-cells by bypassing K_{ATP} channels, allowing assessment of Ca^{2+}-dependent insulin secretion independent of glucose metabolism
- Changes in PLC pathway may explain why β-cells exposed to GLT show altered Ca^{2+} sensitivity and insulin secretion (Figure 2)

Objective

- Investigate the effects of GLT on Ca^{2+} sensitivity and insulin secretion in pancreatic β-cells via KCl induced insulin secretion

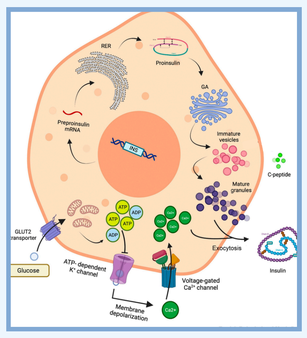


Figure 1. Illustration of GSIS pathway

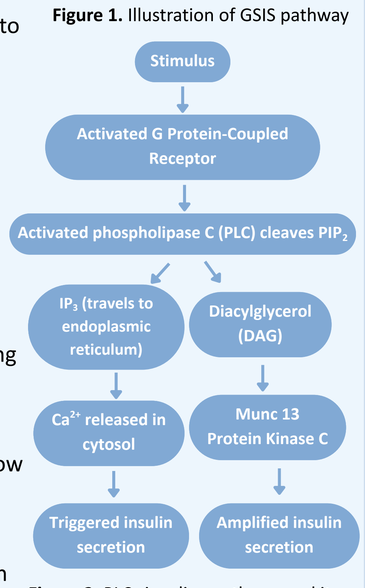


Figure 2. PLC signaling pathway and its effects on insulin

Methods

Cell Culture

- Clonal rat pancreatic β-cells (INS-1) plated in 48-well plates and cultured under two glucose conditions: 4 mM glucose **(control)** and 11 mM glucose **(diabetic GLT model)**

Glucose/KCl Stimulated Insulin Secretion

- All cells pre-incubated twice for 30 minutes with Krebs Ringer Buffer (KRB) containing 1 mM glucose, 2 mM Ca^{2+}, and 0.05% Bovine Serum Albumin (BSA)
- Test solutions (250 µL per well) applied for 1 hour (Figure 3)
  - Baseline glucose (1 mM) vs. stimulatory glucose (12 mM)
  - Diazoxide (400 µM) and KCl (5-40 mM) added to 12 mM glucose KRB
- 60 µL per well transferred to 96-well plate pre-loaded with 60 µL 1% BSA

Insulin Quantification via Homogenous Time-Resolved Fluorescence (HTRF) Assay

- Samples diluted in 0.5% BSA at varying dilutions and 2µL/well added to a 384-well plate
- 2 µL detection antibodies from CisBio HTRF insulin kit added to each well (Figure 4)
- Plates read using Synergy H1 plate reader. Excitation performed at 317 nm, emissions measured at 617 nm and 658 nm
- Insulin values calculated by applying emission ratios to a standard curve (Figure 5)

Calcium Spectrophotometry

- Cells loaded with Fura-2 AM dye, resuspended in buffer, and transferred to Hitachi spectrophotometer; KCl added in 5 mM increments (5–40 mM) to stimulate Ca^{2+} influx
- Measured fluorescence at 340/380 nm excitation, 510 nm emission, calculated ratios to assess intracellular Ca^{2+} across KCl conditions

$$[Ca^{2+}] = \left(\frac{\%\text{Bound}}{\%\text{Free}}\right) \times K_d$$

$[Ca^{2+}]$: Concentration of free calcium ions (µM)
%Bound : Percentage of Fura-2 bound to calcium
%Free : Percentage of unbound (free) Fura-2 dye
K_d : Dissociation constant of Fura-2 for Ca^{2+} (223 nM)

Membrane Lipid Imaging

- Nile Red (lipophilic fluorescent dye) to stain membrane lipids in 4 mM vs. 11 mM cells

Diacylglycerol (DAG) Sensing

- DAG production monitored using Montana Molecular's Green Up DAG Protein Sensor, a fluorescence-based sensor delivered via BacMam (insect virus-based vector for mammalian gene expression). Signals detected on Tecan 200 Pro fluorescence plate reader.

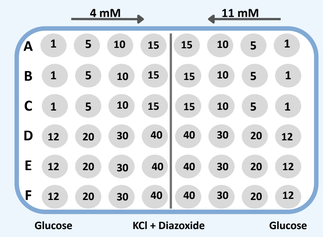


Figure 3. KCl and glucose stimulated secretion plate set up

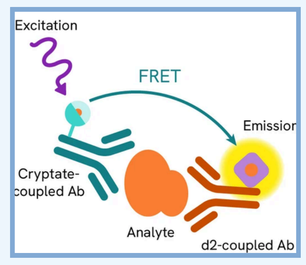
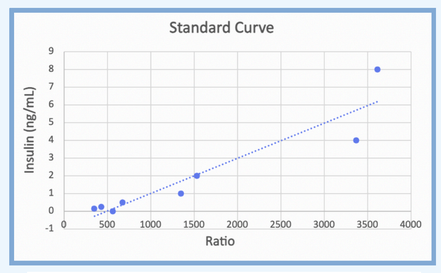


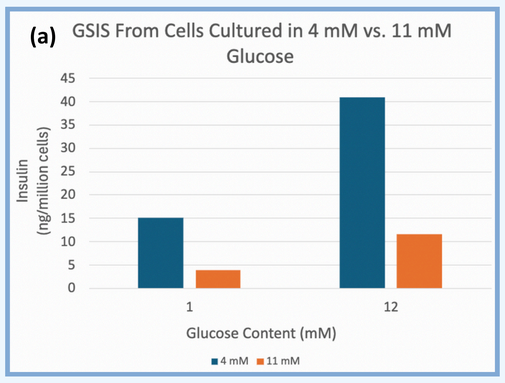
Figure 4. Depiction of HTRF insulin detection assay. Insulin is bound by donor (cryptate) and acceptor (d2) antibodies



$$y = (5 \times 10^{-7})x^2 + 0.0001x - 0.0909$$

Figure 5. Standard curve used to generate HTRF assay values

Results



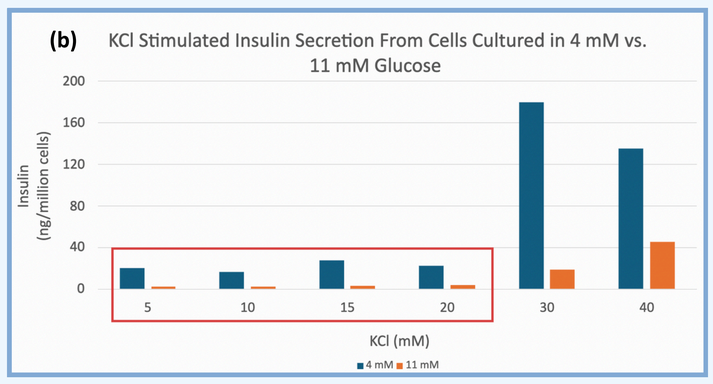
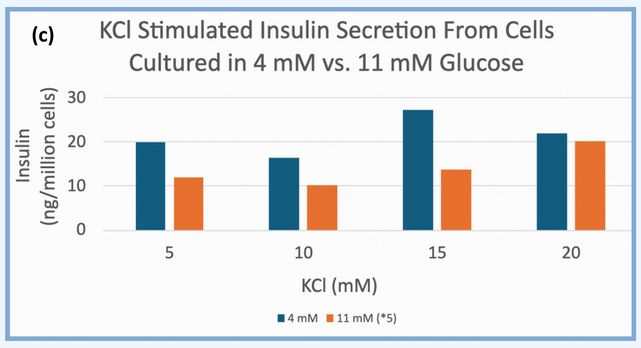


Figure 6. (a) Insulin secretion at 1 mM and 12 mM glucose increases in cells cultured at both 4 mM and 11 mM glucose. **(b)** 4 mM-cultured cells show a jump from 20-30 mM KCl, 11 mM-cultured cells show gradual increase across KCl range.



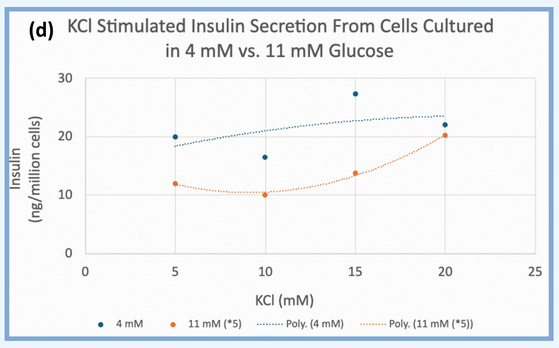


Figure 6. **(c)** Bar graph of insulin secretion from 5–20 mM KCl with insulin values from 11 mM-cultured cells multiplied by 5; secretion from cells cultured in 11 mM is increased between 15-20 mM KCl. Cells cultured in 4 mM do not show an increase over this range. **(d)** Line graph of insulin secretion from 5–20 mM KCl; 4 mM-cultured cells show a clear upward trend, while 11 mM-cultured cells remain relatively flat.

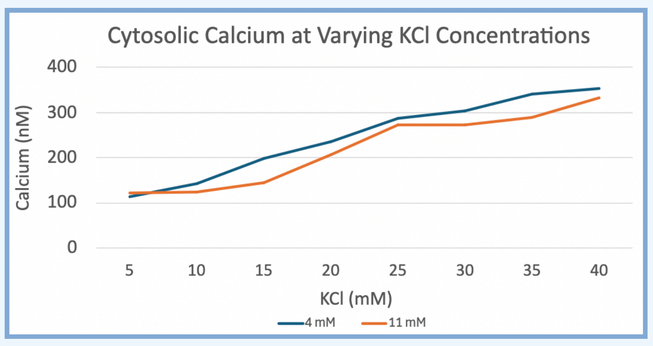


Figure 7. Line graph showing increasing intracellular Ca^{2+} levels across 5-40 mM KCl concentrations in both groups.

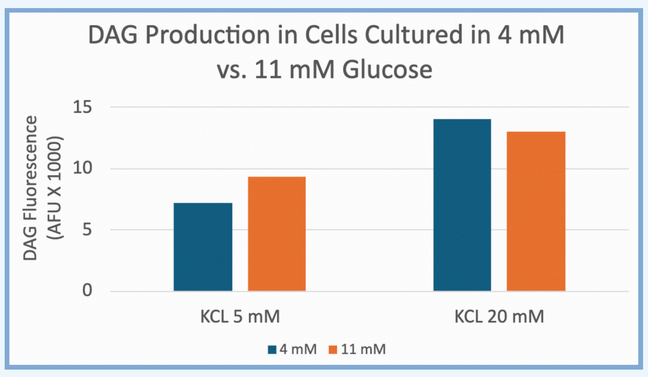


Figure 8. DAG production in INS-1 cells cultured in 4 mM vs 11 mM glucose following KCl stimulation. 11 mM glucose cells showed higher basal DAG levels but a smaller increase with added KCl.

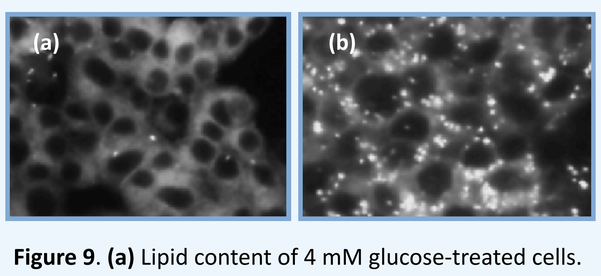


Figure 9. **(a)** Lipid content of 4 mM glucose-treated cells. **(b)** 11 mM glucose-treated cells show increased intracellular lipid droplets.

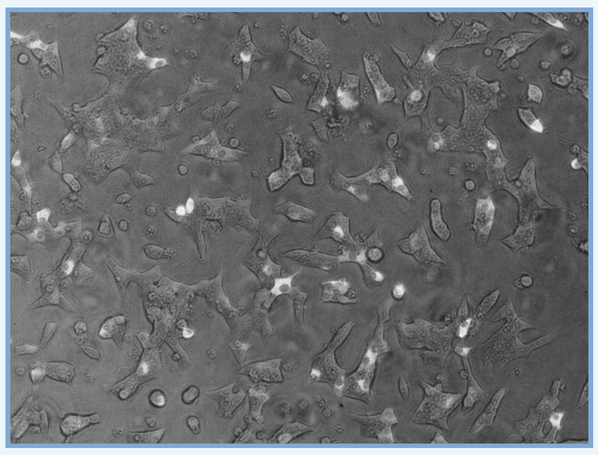


Figure 10. DAG production in 4 mM glucose treated INS-1 cells visualized via fluorescent DAG sensor. Bright cells are an indicator of sensor expression.

Discussion

Significance

- Both 4 mM and 11 mM glucose-cultured INS-1 cells were **glucose responsive**, as both increased insulin secretion from 1 mM to 12 mM glucose in the GSIS assay.
- Focusing on 5–20 mM KCl: After multiplying 11 mM secretion values by 5, a clear **left shift** was observed in their KCl response curve. 4 mM cells had **elevated basal secretion**, but insulin levels fluctuated rather than increasing consistently.
- Cytosolic Ca^{2+} levels rose with KCl in both conditions.
  - Since Ca^{2+} promotes insulin secretion, but only 11 mM cells showed a clear increase in secretion from 5–20 mM KCl, this suggests **increased calcium sensitivity** in 11 mM cells and possible **lowered Ca^{2+} threshold for exocytosis.**
- Elevated basal DAG production and increased DAG response to rising KCl (5–20 mM) were observed in 11 mM cells, mirroring the KCl-insulin secretion pattern.
  - Suggests **stronger PLC pathway activation** causing **increased DAG levels** in 11 mM cells (Figure 8) or **heightened DAG sensitivity** since increase in DAG is slightly blunted in 11 mM cells.
- Nile Red staining revealed **higher intracellular lipid droplets** in 11 mM cells (Figure 9) → Indicates greater lipid in membranes due to impaired fat oxidation from GLT.
  - Could suggest altered PIP_2 levels, affecting synaptotagmin (Ca^{2+} sensor) binding ability and lowering the **Ca^{2+} threshold** for cells cultured in 11 mM glucose.
- Our findings highlight Ca^{2+} signaling and DAG/lipid dynamics as potential therapeutic targets to **preserve β-Cell function** and **delay T2D progression**.

Limitations

- **Insulin content** was not measured. Without content normalization, direct comparisons of insulin secretion may be misleading.
  - 4 mM cells secrete more insulin due to increased insulin content compared to 11 mM cells, which have lower insulin content due to secretion in culture.
- Insulin secretion is representative of a single experiment in triplicate.
  - DAG production levels measured in single experiment.

Future Work

- Examine how elevated **DAG** levels, possibly linked to increased membrane lipid content, affect Ca^{2+} signaling dynamics and insulin secretion in β-cells.
- Extend studies to **primary islets** or **T2D animal models** to explore how increased Ca^{2+} sensitivity contributes to early-stage β-cell hypersecretion.
- Investigate changes in Ca^{2+} channel and regulator expression under GLT to identify molecular causes of increased Ca^{2+} sensitivity.
- Test pharmacological agents such as antioxidants to see if they can **reverse** GLT-induced dysfunction in insulin secretion and Ca^{2+} response.

References

Acknowledgements

We would like to thank Anne Kelly, Yuvika Bhalla, Gulzhan Narmuratova, and Dr. Jude Deeney for their invaluable support and guidance throughout this project. We would also acknowledge the RISE program, Boston University, and the DOM Analytical Instrumentation Core for providing the resources and opportunity to conduct this research.