JQ1 Epigenetic Modulation of Pancreatic β-Cells Promotes Fatty Acid Oxidation via Increased Phosphorylation of Acetyl-CoA Carboxylase and Partially Reverses the Effects of Glucolipotoxicity

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Introduction

- **Type 2 Diabetes mellitus (T2D):**
  - Affect more than 38.5 million people in the U.S.A.
  - Characterized by insulin resistance and hyperglycemia in blood
  - Insulin resistance can be traced back to hyperinsulinemia
- **Pancreatic beta cells**
  - Responsible for secreting insulin in the presence of glucose (glucose-induced insulin secretion, or GSIS)
  - Fatty acid amplifies insulin secretion via production of **long chain (LC) acyl CoA** & **phosphatidylinositol 4, 5-biphosphate (PIP2)**

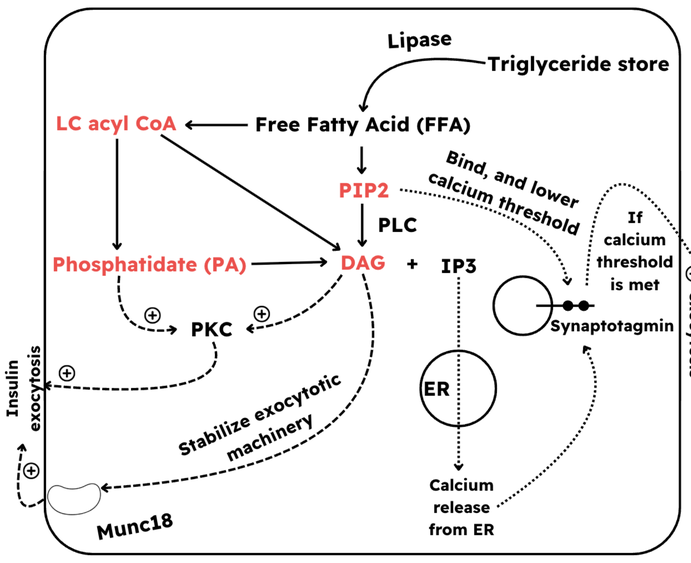


Fig. 1. The hypothesized pathway of LC acyl CoA and PIP2 amplifying GSIS

- **Obesity** is heavily linked with T2D
  - **Glucolipotoxicity (GLT)**: when nutrient is in excess, lipids accumulate in cells, disrupting insulin secretion
  - **Left-shift effect:** basal hypersecretion, hypersensitivity to early glucose change (e.g. 1 mM to 4 mM), decreased maximal GSIS
  - A healthy lifestyle, by removing excess nutrients, can reverse T2D
- **Drugs:**
  - **Sulfonylurea:** use to increase calcium-stimulated insulin secretion
  - **Metformin**: alleviate insulin resistance
  - **Thiazolidinediones**: alleviate insulin resistance
- **JQ1**, an inhibitor of BET (Brd2, Brd4) proteins, is shown to promote FA oxidation in beta cells

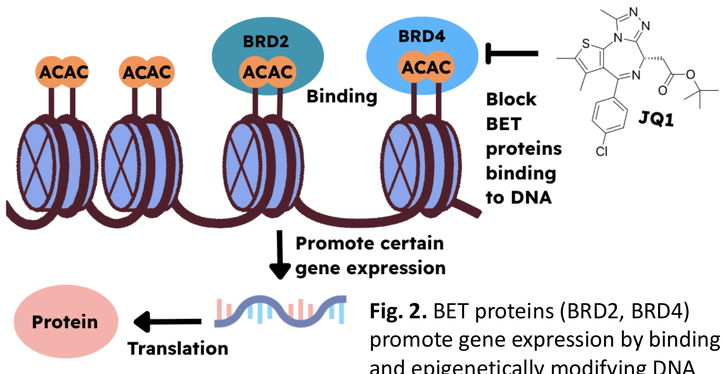


Fig. 2. BET proteins (BRD2, BRD4) promote gene expression by binding to and epigenetically modifying DNA

- **Objective**
  - Elucidate the mechanism by which JQ1 promotes FA oxidation
  - Identify potential therapeutic target for drug development
  - Identify signs of reversal of glucolipotoxicity

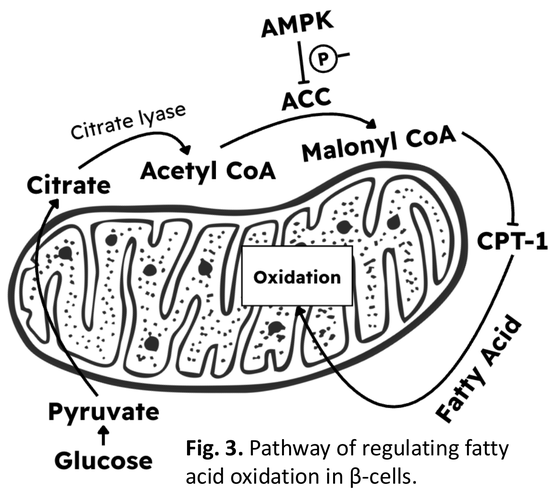


Fig. 3. Pathway of regulating fatty acid oxidation in β-cells.

Results

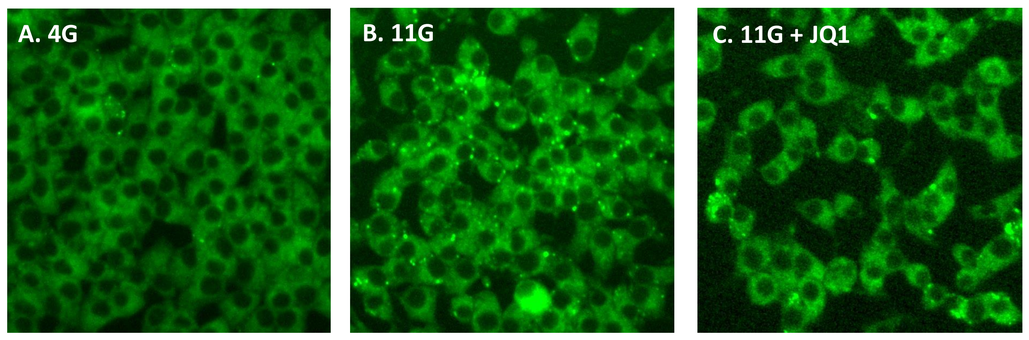


Fig. 7. INS-1 β-cells treated with JQ1 exhibit lower intracellular lipid. (A) Image is of INS-1 β-cells cultured in 4 mM glucose and stained with BODIPY dye. Lipid droplets appear as bright neon-green dots in the cells. (B) β-cells cultured in 11mM glucose. (C) β-cells cultured in 11 mM glucose and treated with JQ1 for three days.

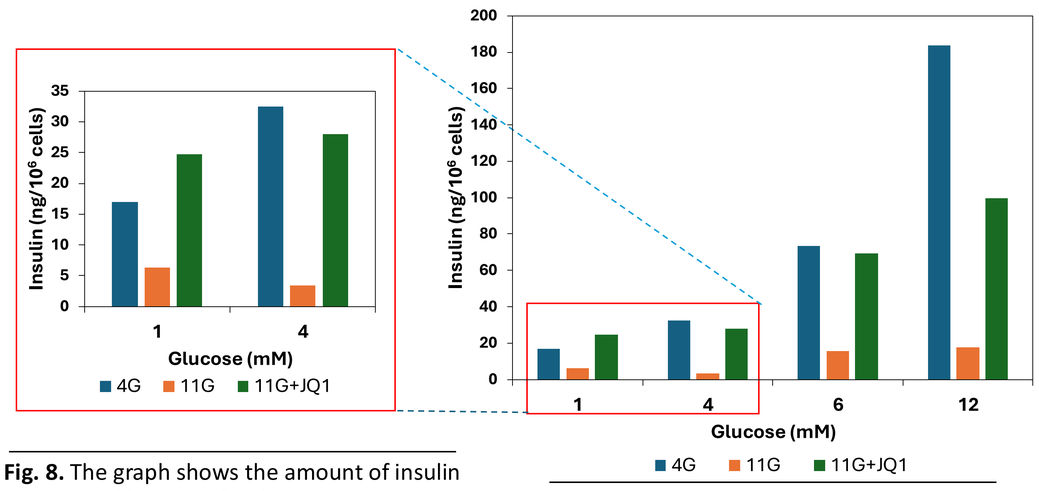


Fig. 8. The graph shows the amount of insulin secreted by INS-1 β-cells in response to different glucose concentration. Cells treated with JQ1 reverse the "left shift" effect in the potentially glucolipotoxic 4G cells.

Fig. 9. INS-1 11G β cells treated with JQ1 show delayed maximal GSIS (happen at 12 mM) compared to untreated 11G cells.

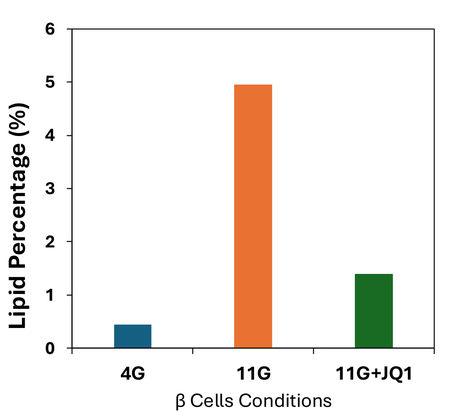


Fig. 10. JQ1-treated INS-1 β cells exhibit lower lipid percentage (lipid area/cell area) compared to untreated 11G cells.

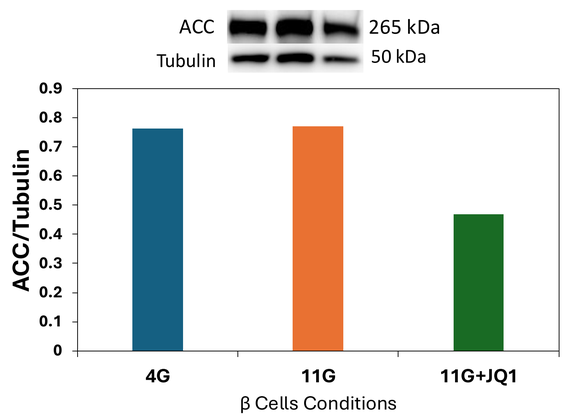


Fig. 11. JQ1-treated 11G β cells show lower ACC compared to 4G and 11G untreated cells.

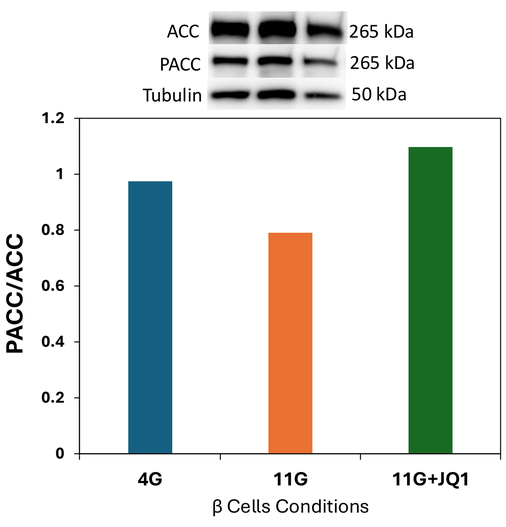


Fig. 12. 4G and JQ1-treated 11G-cultured INS-1 β cells exhibit a higher ratio of PACC:ACC compared to untreated 11G-cultured cells

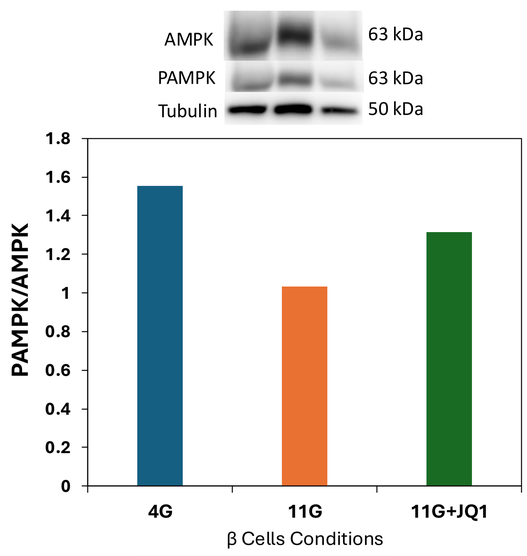


Fig. 13. 4G and JQ1-treated 11G-cultured INS-1 β cells exhibit a higher ratio of PAMPK:AMPK compared to untreated 11G-cultured cells.

Discussion

- JQ1 increases the level of phosphorylation of ACC and AMPK in 11G-cultured beta cells
- Phosphorylated AMPK is active and phosphorylates ACC, which inhibits the production of malonyl CoA and promotes FA oxidation
- JQ1 lowers the level of total ACC, suggesting lower Acaca gene expression
- By increasing FA oxidation, beta cells are "primed" to use lipids as main fuel rather than the excess glucose in 11 mM media
- By increasing the activity of CPT-1, excess glucose stops to be turned into lipid storage in the cells
- JQ1 is suggested to reverse the "left shift" effects, by preventing the increase in insulin secretion from 1mM to 4 mM glucose and delaying maximal GSIS
- We assume that JQ1, by increasing fatty acid oxidation, lower the global intracellular lipid concentration, thereby also decreasing the level of LC-CoA, DAG, PIP2, and PA. Such premise requires further extensive research
- By decreasing lipid-derived metabolic actors that stimulate insulin secretion, the demand for insulin exocytosis is alleviated, thereby partially reversing GLT
  - Ameliorate basal hypersecretion
  - With reduced overstimulation, insulin is able to accumulate within the cells for future GSIS

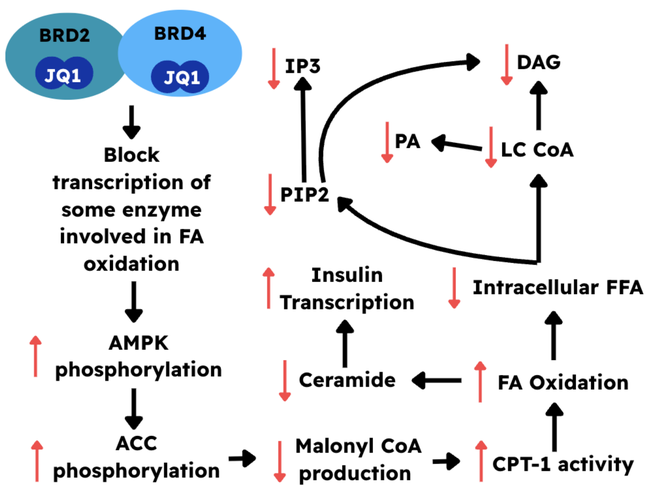


Fig. 14. Proposed mechanism of how JQ1 promotes FA oxidation and reverses glucolipotoxic effects (by lowering lipids that metabolically regulate insulin)

- JQ1 increases the transcription of insulin
  - Past research indicates that inhibition of Brd4 potently enhances insulin transcription
  - Inhibition of Brd2 is suggested to increase FA oxidation, which lowers the level of ceramide. Ceramide is a known inhibitor of insulin transcription

Future Work

- Express fluorescent DAG, PIP2, and calcium sensor in JQ1-treated 11G-cultured beta cells to measure their concentration and distribution
- Investigate the DAG production oscillation in INS-1 beta cells treated with JQ1 when 5 mM concentration of KCl is incrementally added
- Investigate how JQ1-treated 11G cultured cells respond to incrementally increased dose of KCl (calcium level)
- Investigate the interplay of JQ1' potential anti-inflammatory effects (from inhibiting Brd2) and ability to increase FA oxidation and how it can therapeutically "alleviate" T2D

Methods

- Pancreatic β-cells (INS-1) were cultured at: 4mM glucose (low), 11 mM glucose (high), and 11 mM glucose (high) with treatment of 400 nM JQ1 for 3 days. The media used is RPMI1640, with 1:1000 dilution of beta-mercaptoethanol
- Insulin secretion: β-cells are first pre-incubated in 1X Krebs solution buffer with 2 mM calcium, 1 mM glucose, and 0.05% bovine serum albumin (BSA) and then incubated in test solution of 1, 4, 6, 12 mM glucose. After sampled, they are measured using time-resolved fluorescence insulin assay (CisBio).
- Imaging: Intracellular lipid droplets were stained with BODIPY 493/503 dye (1 µL/mL). Using a LAS X Widefield Systems fluorescence microscope, with 20X magnification DFC450C camera and imaging software (Leica Microsystems). images were taken to visualize the concentration and distribution of lipid droplets in beta cells. Using ImageJ, lipid area and cell area are calculated.
- Western blotting: Two wells of each condition of INS-1 beta cells were used to sample proteins (Fig. 4). Western blotting is performed to measure the ratio of phosphorylated ACC to ACC. A FUJIFILM luminescent image analyzer is then used to detect the signal from the antibody. ImageJ is used to calculate the ratio.

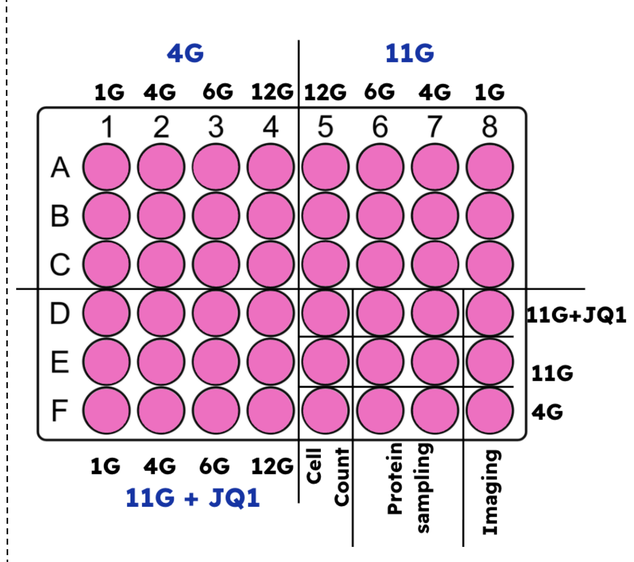
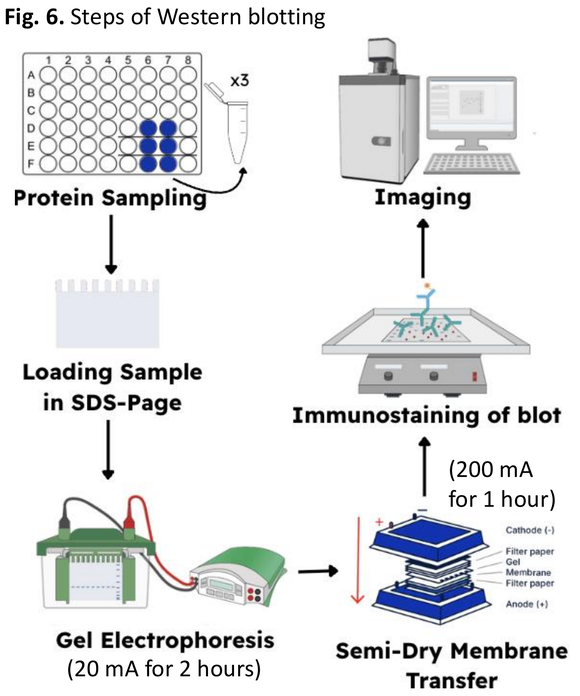


Fig. 4. The illustration above shows the setup for a 48-well plate for cell culturing.

Fig. 6. Steps of Western blotting



Reference

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