INVESTIGATING THE EFFECTS OF CROSSTALK BETWEEN ADIPOCYTES AND PANCREATIC B-CELLS ON INSULIN SECRETION

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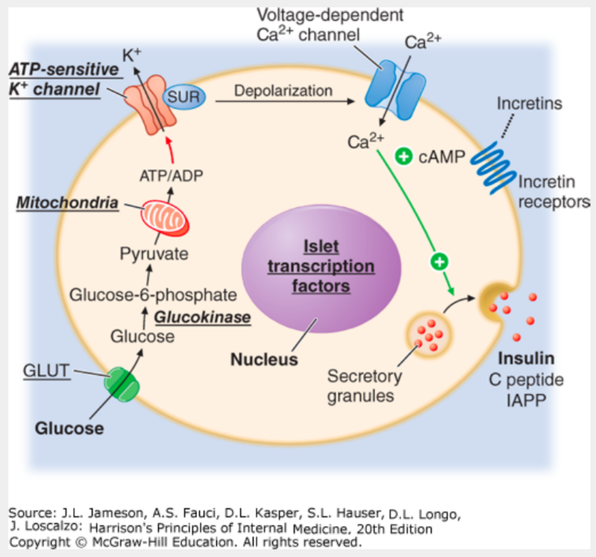
INTRODUCTION

- Diabetes is a chronic condition affecting 37.3 million people in the United States. Furthermore, 96 million people in the US have prediabetes
- Type 2 diabetes occurs when insulin resistance is coupled with deficient insulin levels. Due to the fact that insulin is required to transport glucose into muscle and fat cells for energy, type 2 diabetes leads to hyperglycemia.
- A disease closely linked to type 2 diabetes is obesity. One in every five children and over one in every three adults suffer from obesity in the US. This disorder is characterized by excess levels of body fat; elevated levels of lipid in cells can lead to type 2 diabetes.
- Both diabetes and obesity present a heightened risk for cardiovascular disease, the leading cause of death worldwide.
- Excess nutrients (glucose and fatty acid) lead to glucolipotoxicity (GLT) in pancreatic beta-cells (INS-1), leading to impaired insulin secretion.
- Mechanisms of GSIS can be studied in INS-1 cells cultured at different levels of glucose to mimic normal and diabetic conditions in humans.
- Crosstalk between fat cells and pancreatic beta-cells has been proposed.
- In a previous study done in our lab, conditioned media from primary mouse adipocytes inhibited GSIS. In this experiment, conditioned media was collected overnight after one day of digestion. My study replicates this in more controlled conditions of differentiated preadipocyte cell lines (3T3L1).
- To investigate possible factors released from fat that may influence beta-cell function INS-1 cells were cultured with non-differentiated adipocyte-conditioned media (preadipocyte) and differentiated adipocyte-conditioned media (CM); insulin secretion was observed.



Source: J.L. Jameson, A.S. Fauci, D.L. Kasper, S.L. Hauser, D.L. Longo, J. Loscalzo: Harrison's Principles of Internal Medicine, 20th Edition

METHODOLOGY

- In order to identify a cell passage with sufficient insulin secretion for the experiment, multiple cell passages were cultured in high (12 mM) and low (1 mM) glucose and treated with either 20 mM or 40 mM KCl diazoxide.
- Clonal pancreatic beta-cells (INS-1) were cultured in RPMI 1640 media (11 mM glucose)
- Cells were seeded in multiwell plates and cultured at low (4 mM) and high (11 mM) glucose to reach the same cell confluence on the day of the experiment.
- Cells not part of control groups were treated for either 1 day (overnight) or 2 days with preadipocyte- and adipocyte-conditioned media.
- Cells were pre-incubated in a modified Krebs buffer (1 mM glucose) and underwent two half-hour incubations, changing the buffer in between incubations
- Test solutions were added to cells, with two control groups of 1 mM and 12 mM glucose at both high and low glucose conditions. Cells were incubated for one hour.
- Samples were collected and diluted 1:2 with a 1% bovine serum albumin (BSA) solution. Samples were further diluted as needed with a 0.5% BSA solution.
- Using an HTRF (fluorescence-based) insulin assay kit (PerkinElmer), insulin release and cellular content were measured.
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- To visually track lipid accumulation, Green BODIPY (fluorescent dye) was added to the cells two days after the experiment.

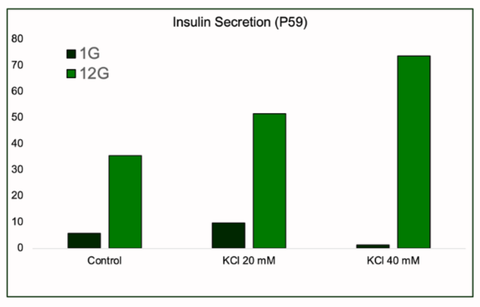

Figure 2. Cell passage 59 showed abundant insulin secretion, with a major increase in secretion between 1 mM and 12 mM glucose.

RESULTS

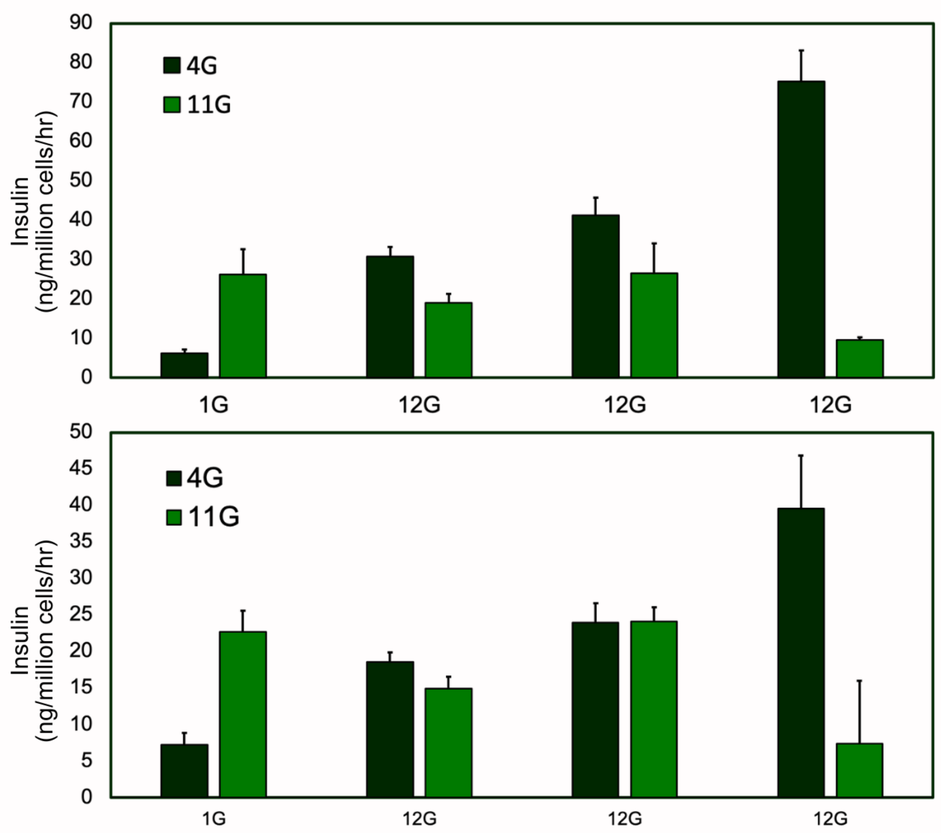

Figure 3. Insulin secretion from INS-1 cells (P73) increased 3-fold when glucose concentration was increased from 1 mM to 12 mM, confirming the validity of the cell passage. In the presence of adipocyte-conditioned media, GSIS was stimulated in cells cultured at 4 mM glucose. No effect was observed in cells cultured at 11 mM glucose. Conditioned media from non-differentiated cells was without effect in both the high and low concentrations of glucose.

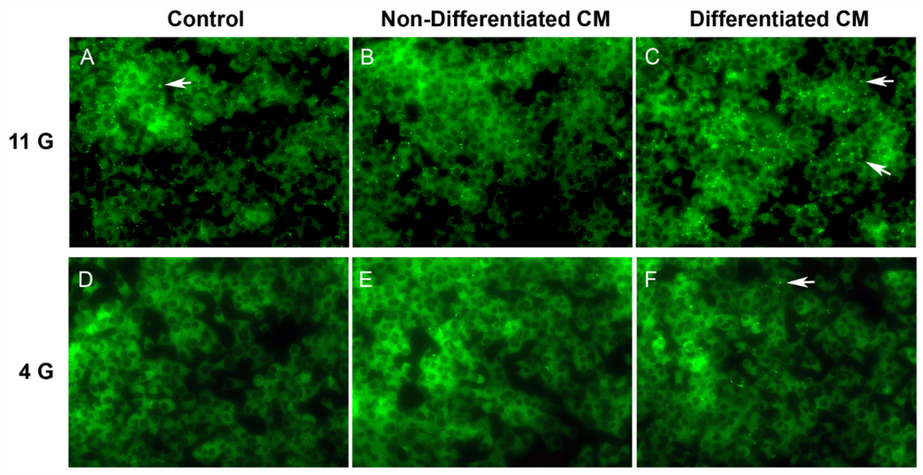

Figure 4. Cells treated with different conditions produced different levels of lipid accumulation. The cells cultured in adipocyte-conditioned media showed the most lipid proliferation, preadipocyte-conditioned cells had less lipid, and the control groups had the least. 11G cells accumulated more lipid than 4G cells.

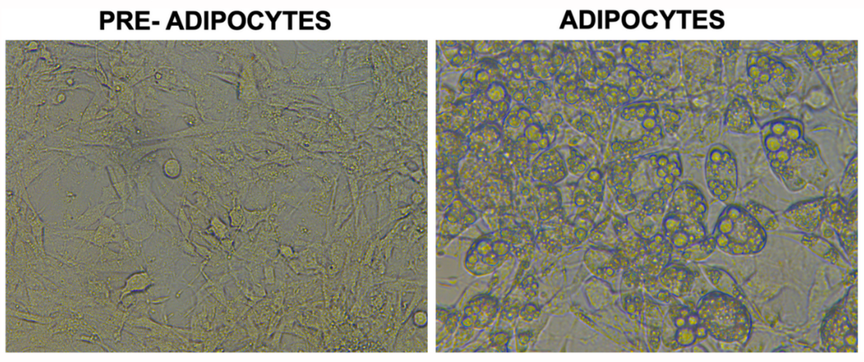

Figure 5. 3t3-L1 cells A) Pre-adipocytes B) Adipocytes

CONCLUSION

- Crosstalk between adipocytes and pancreatic beta-cells potentially contributes to the regulation of GSIS.
- The stimulation in glucose-stimulated insulin secretion is adipocyte-specific, as preadipocyte-conditioned media did not lower levels of insulin secretion.
- There may exist factors that are released from cultured adipocytes that can stimulate insulin secretion that are not present in pre-adipocytes, explaining the observed differences between results from the two conditioned medias.
- More research is needed to specifically identify these adipocyte factors, if they do exist, and their mechanisms.
- These findings contrast the conclusions of our lab's previous research. This may be due to the fact that cell lines are not a good model to replicate primaries. In order to confirm this, primaries would have to be studied for the same amount of time as the cell lines.

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Introduction

Results

Discussion/ Conclusions

Methods

References

Acknowledgements