Investigating the Effects of Active Vitamin D on Insulin Secretion from Clonal Pancreatic Beta Cells (INS-1)

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Introduction

- More than 37 million people have diabetes in the US. Most of those diabetic people (90-95%) have type 2 diabetes.
- Type 2 diabetes is a condition that can lead to health complications, including an increased risk for cardiovascular disease.
- Type 2 diabetes occurs when the pancreas produces insufficient insulin to satisfy the body's requirement because of insulin resistance associated with this disease.
- Vitamin D has been reported to have non-skeletal health benefits.
- In the US, around 35% of the population is vitamin D deficient. Vitamin D deficiency has been linked to increased risk for cardiovascular disease, certain cancers, and type 2 diabetes.
- Wexler's[3] clinical research study reported that patients with pre-type 2 diabetes are less likely to progress to type 2 diabetes if they took 4000 IU vitamin D daily.
- Evidence for vitamin D affecting the regulation of insulin secretion in the pancreas was reported by Bornstedt et al.[4] They propose that the active vitamin D (1,25-dihydroxyvitamin D_3: $1,25(OH)_2D_3$) may be interacting with receptors to cause this increase in insulin secretion.

Hypothesis

The present study aims to compare the effects of $1,25(OH)_2D_3$ treatment on the insulin secretion of rat insulinoma (INS) cells cultured at both low (nondiabetic) and high (diabetic) glucose concentrations.

Methods

- INS-1 cells were grown in RPMI 1640 media (11 mM glucose) and cultured in 4 mM or 11 mM glucose (G) media. Passage 73 (tested for insulin secretion before experimentation) was used.

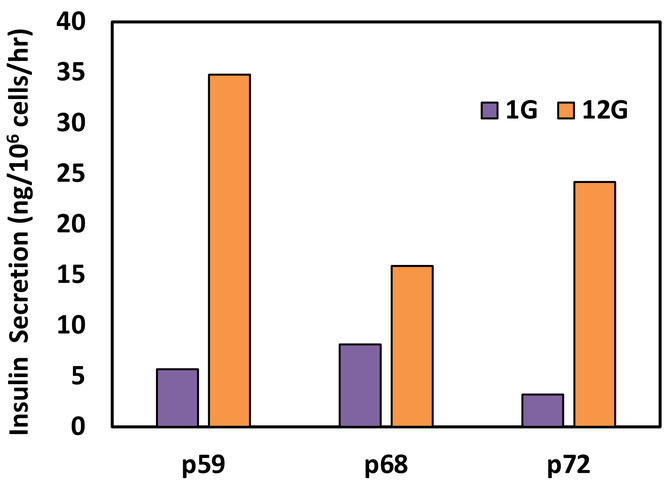


Figure 1: Insulin Secretion in Different Passages of Cells. *We can observe the differences in secretion between three different passages. P72 and p59 had good secretion. Both were deemed useable for the experiment. P68, however, had poor secretion and should not have been used.*

- INS-1 cultured cells were then exposed to varying concentrations of glucose (G).
- Cells were treated for one day with the $1,25(OH)_2D_3$ at 10 nM (10^{-8} M) and 1000 nM (10^{-6} M) and compared to vehicle controls
- Samples for insulin secretion and insulin content were measured using an HTRF (fluorescence-based) insulin assay kit (PerkinElmer).
- Green BODIPY was added to cells, and fluorescence microscopy was used to image stored lipids in the form of triglyceride droplets.

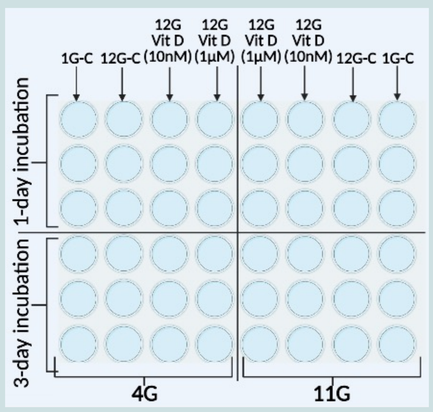
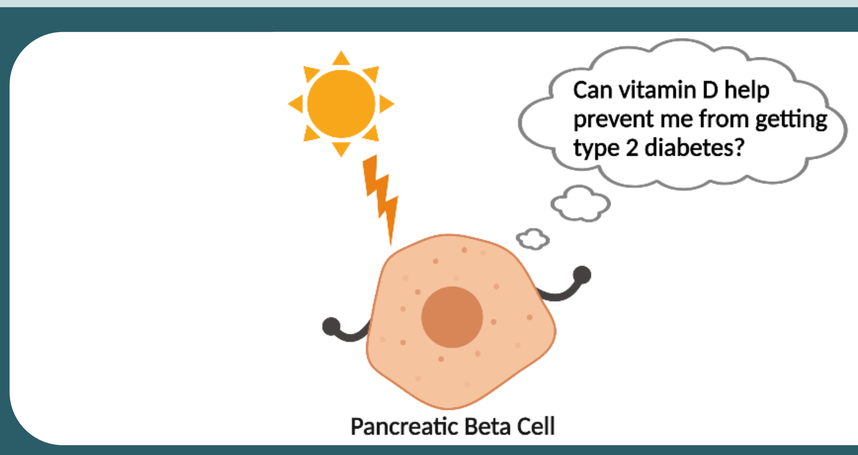


Figure 2. Diagram of how the INS-1 cells (P73) were plated on the 48-well plate.



Results

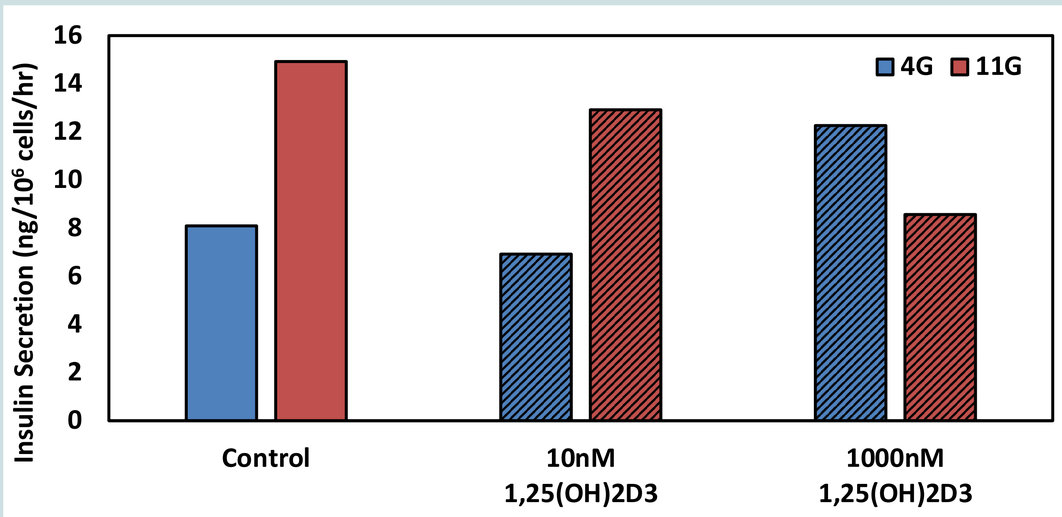


Figure 3. The Effect of $1,25(OH)_2D_3$ Exposure (72hrs) on GSIS. *$1,25(OH)_2D_3$ dose-dependently (1000 nM > 10 nM) decreased GSIS in INS-1 cells cultured in 11 mM glucose (red bars). $1,25(OH)_2D_3$ increased GSIS from cells cultured at 4 mM glucose but only at high (1000 nM) concentration (blue bars).*

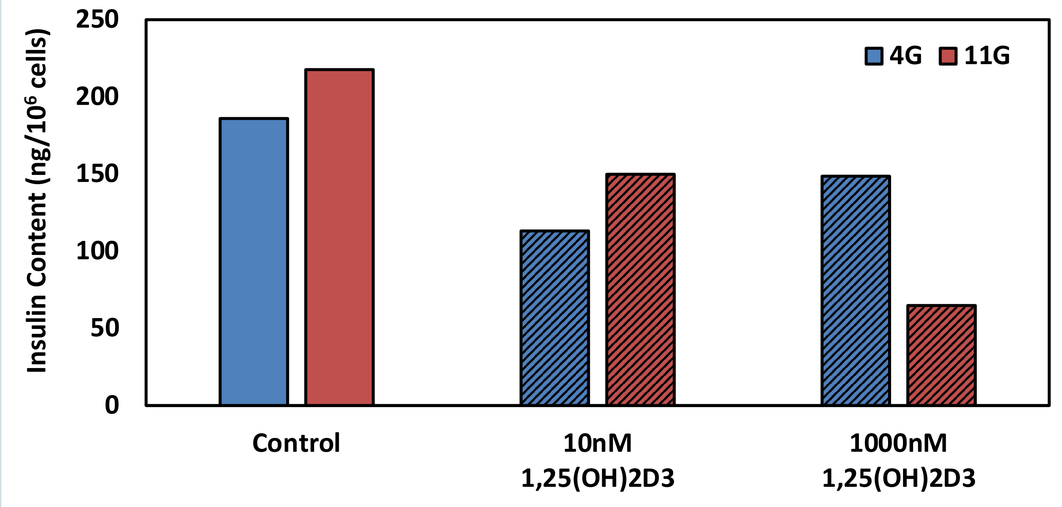


Figure 4. The Effect of $1,25(OH)_2D_3$ Exposure (72hrs) on Insulin Content. *$1,25(OH)_2D_3$ reduced insulin content In INS-1 cells cultured at both 4 mM (blue) and 11 mM glucose (red) cells. This decrease was $1,25(OH)_2D_3$ dose-dependent in cells cultured at 11 mM glucose, while cells cultured in 4 mM glucose were maximally reduced at low $1,25(OH)_2D_3$ concentration.*

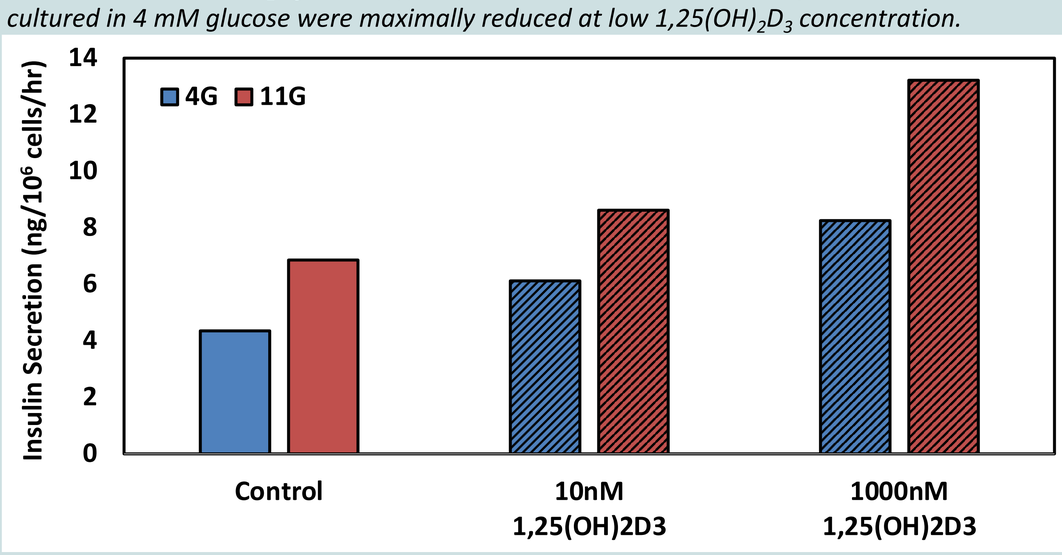


Figure 5. The Effect of $1,25(OH)_2D_3$ Exposure (72hrs) on GSIS (Adjusted for Insulin Content). *When taking into account the insulin content, GSIS increases with exposure to $1,25(OH)_2D_3$ in both 4G (blue) and 11G (red) cells, and it's more pronounced in 1000nM $1,25(OH)_2D_3$*

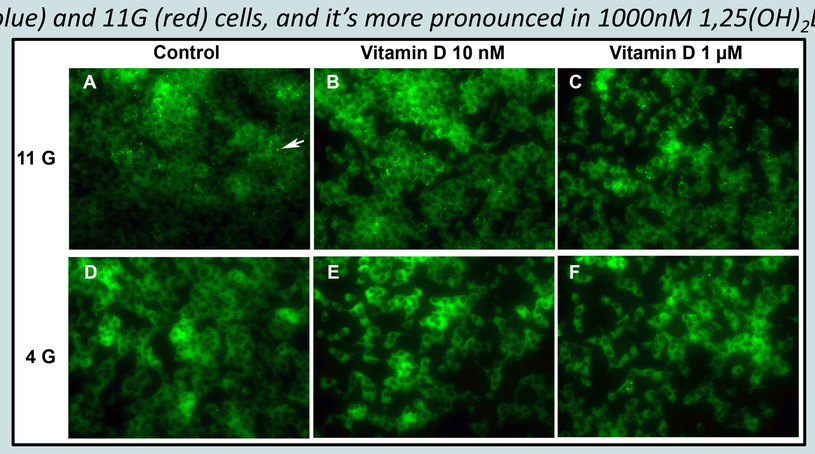


Figure 6. Fluorescence Microscope Images of triglyceride (TG) Droplets in INS-1 Cells Exposed to Different concentrations of $1,25(OH)_2D_3$. *There is no change in TG droplet accumulation between the treatments. There are noticeably fewer cells in the images of the cells treated with $1,25(OH)_2D_3$.*

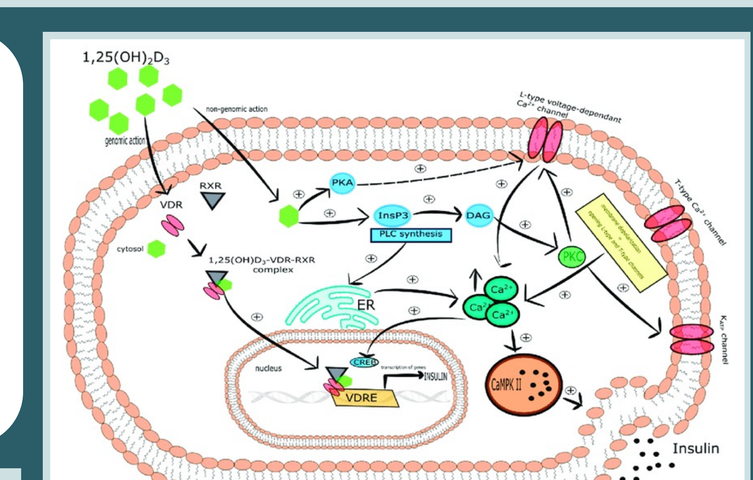


Figure 7. Proposed Mechanism for how Vitamin D can help Prevent Type 2 Diabetes.

Discussion

- $1,25(OH)_2D_3$ increased GSIS from INS-1 cells at 10 and 1000 nM concentrations.
- Decreased cell proliferation can also be observed in the fluorescent images taken for lipid accumulation. Lipid might be expected to increase in slower-growing cells cultured in high glucose, but this was not the case.
- We believe that adding $1,25(OH)_2D_3$ may cause an increase in the activity of the exocytotic machinery. This may cause an initial increase in insulin secretion, which over time reduces the insulin content of INS-1 cells.
- It could be that such a significant loss of insulin content is specific to the clonal INS-1 cells we used in our experiments. In the future, tests on beta-cells from isolated pancreatic islets could be beneficial because of their higher insulin content, significantly more than the cells used in this experiment. We also suspect the beta islets to have stronger synthesis machinery to help prevent content depletion.
- Acute addition or a short pre-incubation in vitamin D could be tested in the future.

Conclusion

My preliminary results demonstrate that the active form of vitamin D appears to increase insulin secretion. This may help explain the observation that severely vitamin D deficient pre-diabetic patients who took 4000 IUs daily for 2.5 years reduced their risk of getting type 2 diabetes by 62%

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References

(1) Wacker, M.; Holick, M. F. Sunlight and Vitamin D. *Dermato-Endocrinology* **2013**, *5* (1), 51–108. https://doi.org/10.4161/derm.24494.

(2) Hossein-nezhad, A.; Holick, M. F. Vitamin D for Health: A Global Perspective. *Mayo Clinic Proceedings* **2013**, *88* (7), 720-755.https://doi.org/10.1016/j.mayocp.2013.05.011.

(3). Wexler, D. J. D2d — No Defense against Diabetes. *New England Journal of Medicine* **2019**, *381* (6), 581–582. https://doi.org/10.1056/nejme1906815.

(4) Bornstedt, M. E.; Gjerlaugsen, N.; Pepaj, M.; Bredahl, M. K. L.; Thorsby, P. M. Vitamin D Increases Glucose Stimulated Insulin Secretion from Insulin Producing Beta Cells (INS1E). *International Journal of Endocrinology and Metabolism* **2019**, *In Press* (In Press). https://doi.org/10.5812/ijem.74255.