Active Vitamin D Enhances Insulin Secretion From the Pancreatic β-Cell

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Introduction

- Approximately 35% of adults living in the US have vitamin D deficiency
- Research shows that low vitamin D is tied to chronic disease and obesity
- The active form of vitamin D is $1,25(OH)_2D_3$ (vitD)
- VitD deficiency may increase the risk of gaining type 2 diabetes (T2D)
- T2D is a chronic disease associated with weight gain and characterized by high blood glucose (> 7mM) due to insulin resistance
- VitD has been reported to stimulate insulin secretion, but the effect is not robust enough and a specific mechanism driving this process has not been elucidated
- I hypothesize that vitD will enhance glucose stimulated insulin secretion (GSIS) from the clonal pancreatic β-cells (INS-1) and also lower lipid accumulation

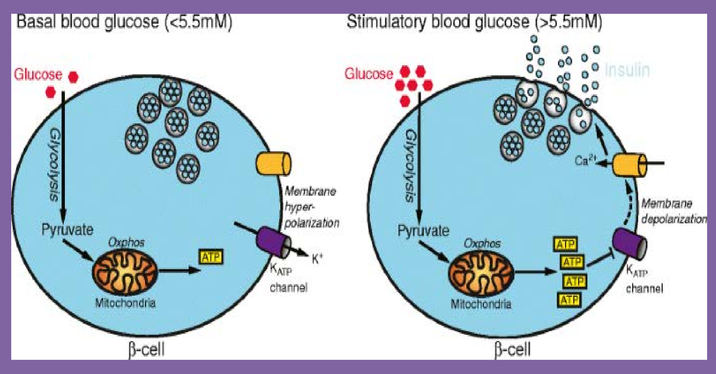


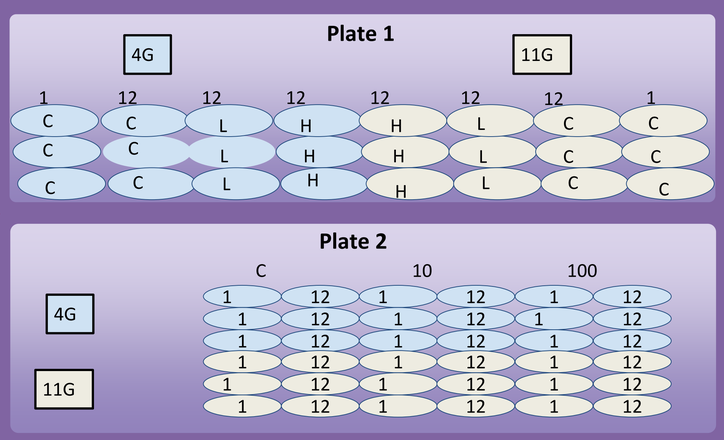
Fig 1. Differences between basal glucose and stimulatory glucose when looking at GSIS

HOW IS INSULIN SECRETED?

- Glucose enters the pancreatic β-cell using the GLUT2 transport membrane
- Glucose is phosphorylated by glucokinase, an enzyme
- Glucose undergoes glycolysis to form pyruvate
- Metabolism of pyruvate takes place and subsequently undergoes cellular respiration in the mitochondria, increasing ATP concentration
- An amplification in ATP closes ATP-sensitive K+ channels, causing membrane depolarization
- Membrane depolarization causes Ca+2 channels to open up
- Ca+2 levels increase, which triggles insulin exocytosis from the pancreatic β-cell

Methods

- INS-1 cells were cultured in 11 mM glucose RPMI media
- Media was switched to 4 and 11 mM glucose with or without VitD (10 and 100 nM) 24 hours prior to the experiment
- Cells were then pre-incubated in Krebs buffer with 1 mM glucose for 1 hour
- Cells were subsequently incubated in 1 mM and 12 mM glucose
- Insulin was measured using an HTRF insulin assay (cis-bio) in a 1536 well plate using a TECAN fluorescence plate reader
- Fluorescence microscopic imaging using Nile red examined triglyceride droplets in INS-1 cells after incubation with 100 nM VitD



Results

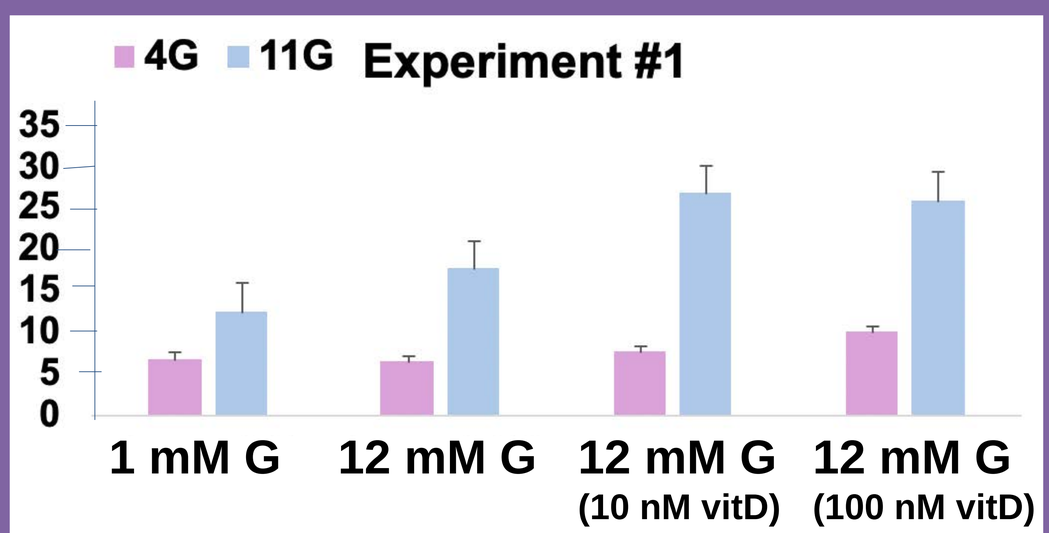


Fig 2. VitD enhanced GSIS from INS-1 cells at high glucose (11 mM) 2-fold at both 10 nM and 100 nM concentrations.

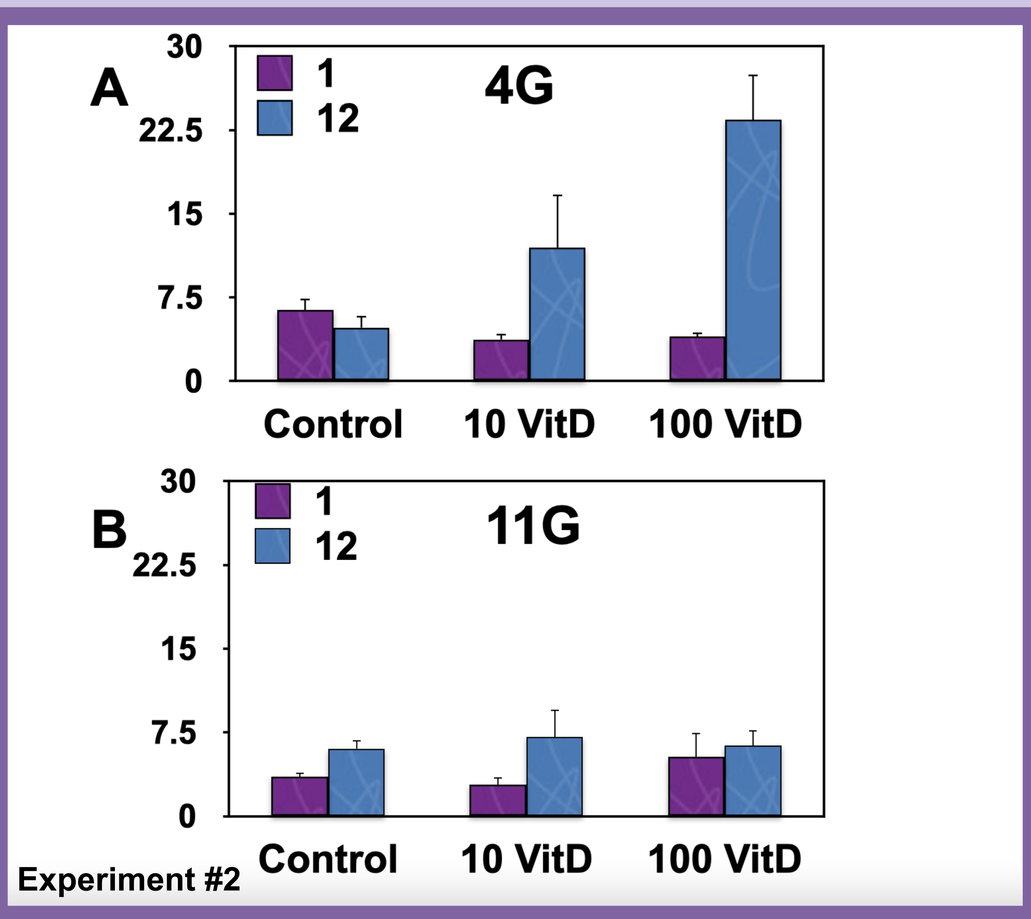


Fig 3. VitD enhanced GSIS from INS-1 cells at low glucose (4 mM) at both 10 nM and 10 nM concentrations. Basal was not affected.

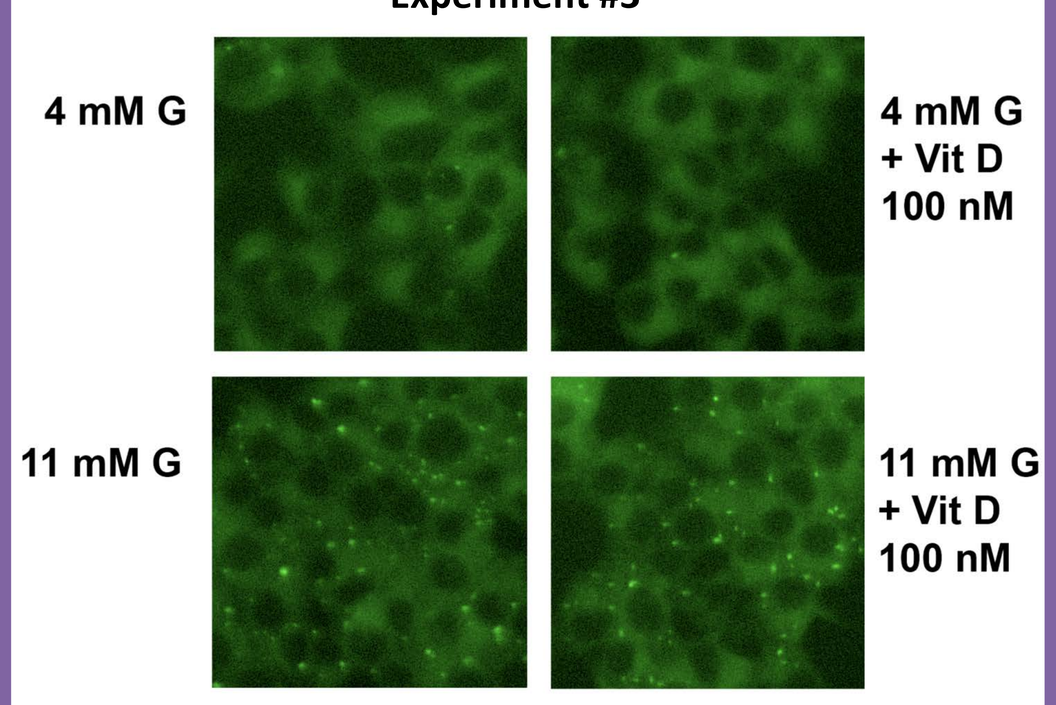


Fig 4. Fluorescence microscopic imaging shows that VitD does not affect lipid accumulation in the pancreatic β*-cell at both high and low glucose after 1 day of incubation*

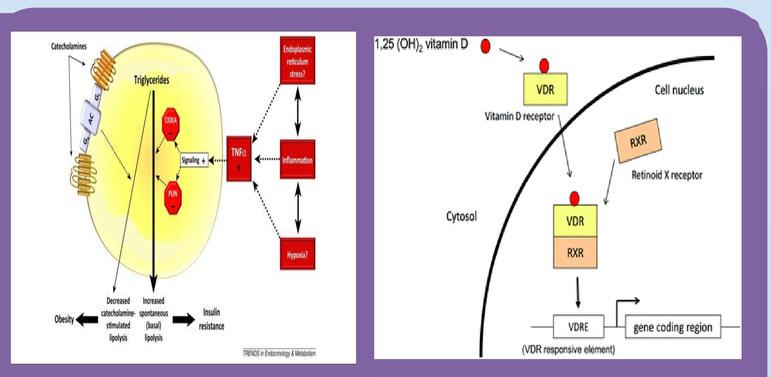


Fig 5. Proposed mechanisms that aid vitamin D in stimulating insulin secretion from INS-1 cells

Discussion

- VitD increased GSIS from INS-1 cells cultured at high and low glucose 2-fold at both 10 nM and 100 nM concentration, but not in the same experiment → could repeat experiment in the future to see if cells will secrete insulin at 4 mM and 11 mM G in the same experiment
- Fluorescence microscopic imaging demonstrated that 100 nM vitD did not alter lipid levels in INS-1 cells cultured at high glucose.
- Future research could investigate the effects that vitamin D has on oscillations while also the left shifting of GSIS from chronic exposure to excess nutrients and how calcium plays a role in it as well
- Scientists in the future could see how incubating the INS-1 cells for a longer or shorter time could impact the amount of insulin secretion in the INS-1 cells after vitD is added to the cells
- If the INS-1 cells were to be incubated for a shorter time and insulin secretion was shown to increase, scientists could conclude that vitamin D receptors (VDR) located in the beta cell could play a role in enhancing the amount of insulin secretion from the pancreatic β-cells without the use of gene transcription
- If the INS-1 cells were to be incubated for a longer time and insulin secretion was shown to be increasing in both high and low concentrations of glucose, scientists could conclude that it may take time for the vitD to kick in before glucolipotoxicity (GLT) can be reversed
- More photos of triglyceride droplets could be taken after incubating cells for a longer period of time and if shown lower lipid accumulation, scientists could conclude that lipolysis, the breakdown of fat, may take place in the pancreatic β-cell. This metabolic process could aid vitamin D in enhancing insulin secretion

Conclusion

VitD enhanced GSIS in the INS-1 cells cultured at both high and low glucose (in different experiments) without affecting lipid accumulation. Therefore, VitD may play a crucial role to increase insulin secretion and lower the risk of developing T2D.

References

1. Sung, C.; Liao M.; Lu, K.; Wu, C. Role of Vitamin D in Insulin Resistance. *J Biomed Biotechnol.* [Online] **2012,** 634195. https://doi.org/10.1155/2012/634195 (accessed August 4, 2024)
2. Arner, P.; Langin, D. Lipolysis in lipid turnover, cancer cachexia, and obesity induced insulin resistance. *Trends in Endocrinology & Metabolism* [Online] **2014,** *5,* 255-262. https://doi.org/10.1016/j.tem.2014.03.002 (accessed August 4, 2024)
3. 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). *Int J Endocrinology Metab.* [Online] **2019,** *1,* e74255. https://doi.org/10.5812/ijem.74255 (accessed August 4, 2024)
4. Holick, M.F.; Mazzei, L.; Menéndez, S.G.; Giménez, V.M.M.; Anouti, F.A.; Manucha, W. Genomic or Non-Genomic? A Question about the Pleiotropic Roles of Vitamin D in Inflammatory Based Diseases. *Nutrients.* [Online] **2023,** *3,* 767. https://doi.org/10.3390/nu15030767 (accessed August 4, 2024)

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