Protein Phosphorylation and its critical role in regulating energy dependent glucose induced insulin secretion

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Introduction

Background;

- Type 2 Diabetes mellitus (T2DM) is a metabolic disorder characterized by high blood glucose levels due to insulin resistance and relative insulin deficiency.

- Glucose-induced insulin secretion (GSIS) is a process by which pancreatic ß-cells release insulin in response to elevated blood glucose levels. This process triggers the ß-cells to secrete insulin, which help cells throughout the body absorb glucose and use it for energy.

- GSIS is energy dependent and controlled by several protein Kinases.

- AMP-activated protein kinase (AMPK) is an enzyme that is crucial for cellular energy homeostasis. AMPK is an energy sensor that regulates glucose and lipid metabolism in cells that can lead to T2DM.

- Most studies demonstrated that AMPK activity is downregulated in pancreatic ß-cells in T2D and obesity. It is associated with increased glucose concentration.

- The activation of AMPK leads to the phosphorylation and inhibition of ACC1 and ACC2. This shifts the cell's metabolism away from fatty acid synthesis and towards increased fatty acid oxidation, maintaining energy balance.

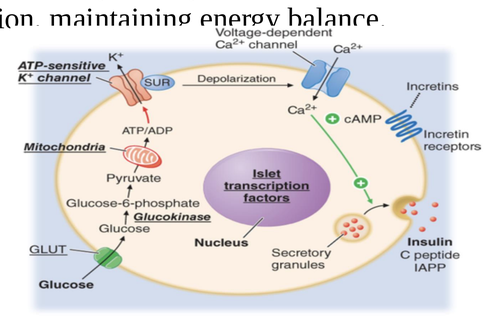

Figure 1. Demonstration of GSIS within the ß-cell [3].

Research Focus;

- The aim of this study was to investigate how INS-1 cultured at chronic and acute glucose concentrations mimicking T2DM have an effect on glucose-induced protein phosphorylation.

- Existing research has shown that chronic high glucose can lead to altered phosphorylation states of various proteins. For instance, hyperglycemia has been associated with decreased activity of AMPK. Hyperglycemia happens when glucose levels are too high resulting in increased circulating insulin, leading to insulin resistance in cells.
- This research could provide insights into the molecular mechanisms underlying beta cell dysfunction in T2DM and how changes in protein phosphorylation happen in response to glucose contribute to GSIS.

Hypothesis;

- INS-1 cells exposed to chronically elevated glucose acquire glucolipotoxicity leading to altered protein phosphorylation regulating lipid metabolism.

Results

The INS-1 was incubated for two days in a high (11G) then switched to low (4G) glucose condition.

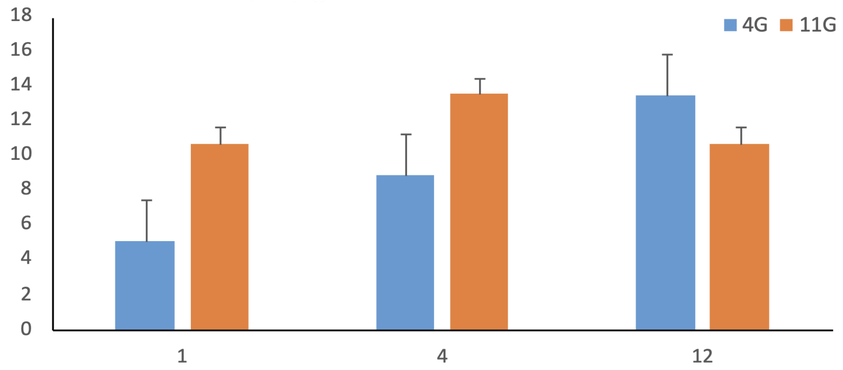

Figure 3. Secretion high and low glucose. On low glucose (4G), there is a 2.5 fold increase throughout varying dosages of glucose basal 1,4 and high 12mM G. Cells cultured in high glucose exhibited elevated basal secretion with little GSIS.

Cells were cultured in chronic glucose (4mM) and (11mM) glucose stimulated with 1 hour acute glucose condition (1mM and 12mM) for the following measure of protein phosphorylation.

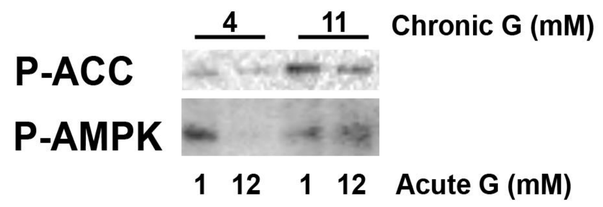

Figure 4. The effect of chronic glucose on phosphorylated proteins. At low glucose (1 mM G), AMPK is phosphorylated and active. Activated AMPK phosphorylates and inhibits ACC. This causes a decrease in malonyl CoA leading to high FA oxidation. At high glucose (12 mM G) AMPK is less phosphorylated and less active leading to decreased pACC. This leads to an increase in malonyl CoA resulting in reduced FA oxidation.

INS-1 cells were cultured in increasing glucose concentrations ranging from 4mM to 15mM. Cells were preincubated in KRB with 1 mM glucose for 1 hr and followed by stimulation with 1mM or 12mM glucose for 5 min before extraction in PAGE sample buffer.

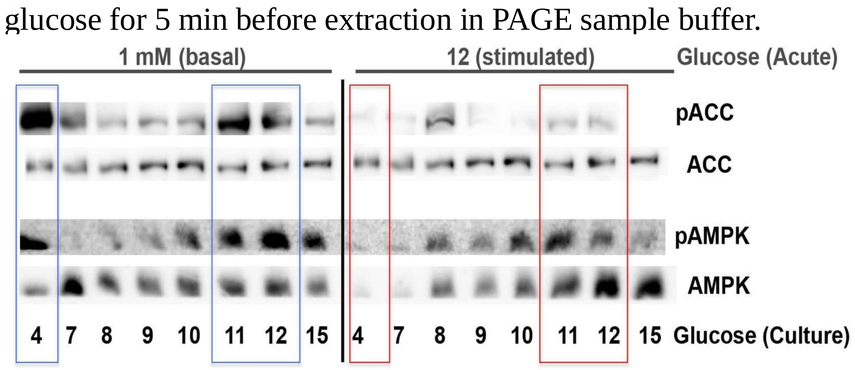

Figure 5. The effect of acute glucose on total and phosphorylated proteins. In cells cultured at 4 mM glucose ACC phosphorylation was much higher in cells incubated at 1mM glucose compared to cells stimulated with 12 mM glucose. At higher glucose culture conditions (11mM and 12mM) pACC was reduced at 1 mM glucose and still observed when cells were stimulated with 12 mM glucose. The blue box is meant for acute low glucose and the red box is meant for acute high glucose exposure.

Discussion

- Chronic elevation of glucose as with excess nutrients leads to glucolipotoxicity, resulting in a left shift in the concentration dependence of GSIS as a result of lipid accumulation in the ß-cell (Fig 3).

- AMPK is phosphorylated and activated at low glucose (Fig. 4). At high glucose AMPK is less phosphorylated and its activity is reduced. ACC phosphorylation is reduced at high glucose increasing its activity and malonyl CoA production resulting in reduced FA oxidation, an important step for producing lipid signals. Chronic exposure to excess nutrients (11mM glucose) impairs signaling leading to glucolipotoxicity and ß-cell dysfunction.

- Cells incubated with chronic excess glucose (11mM) express increased ACC protein compared to cells cultured at 4mM glucose (Fig. 5). Even though the protein is higher in cells cultured at high glucose phosphorylation of ACC is lower when acutely incubated at 1 mM glucose. When stimulated with 12 mM glucose PACC is still present in cells cultured at high glucose compared to the low cultured glucose condition. This could mean that FA oxidation in cells exposed to chronic high glucose is impaired leading to increased intracellular lipids, insulin hypersecretion and increased risk of T2DM.

Conclusions

- Our preliminary results demonstrate that glucose has a direct effect on protein phosphorylation within pancreatic ß-cells. Through this direct effect of protein phosphorylation it can have an impact on insulin secretion.

- Changes in ACC activity can affect insulin secretion as high levels of malonyl-CoA inhibits fatty acid oxidation and can impact glucose metabolism. By regulating ACC, cells can modulate their lipid metabolism, improve insulin resistance, and improve insulin sensitivity. Which make it an exciting enzyme to target for therapeutic solutions for treatment of T2D.

- AMPK is already used in therapeutic practices such as metformin (a common treatment for T2D). It is used to manage blood glucose levels by mimicking effects of AMPK activation. But more research is needed to fully understand the long term effects of targeting AMPK directly.

Methods

INS-1 cells were cultured in RPMI media with 11 mM glucose and switched to 4 mM glucose one day prior to incubating cells in increasing concentrations of glucose (4-15 mM) for 24 hrs. Cells were pre-incubated in the KREBS buffer with 1 mM glucose for 1 hr and subsequently stimulated with 1mM and 12 mM glucose for 5 mins. Later, 25 µl of 1x PAGE was added and collected in Eppendorf tubes that were later boiled for 4 mins 100°C. Protein was separated by gel electrophoresis following by western blot. Membranes were incubated with specific primary and secondary antibodies (ACC and AMPK rabbit, Cell Signaling Technology, USA) and imaged on a Fuji LAS 4000 imaging system.

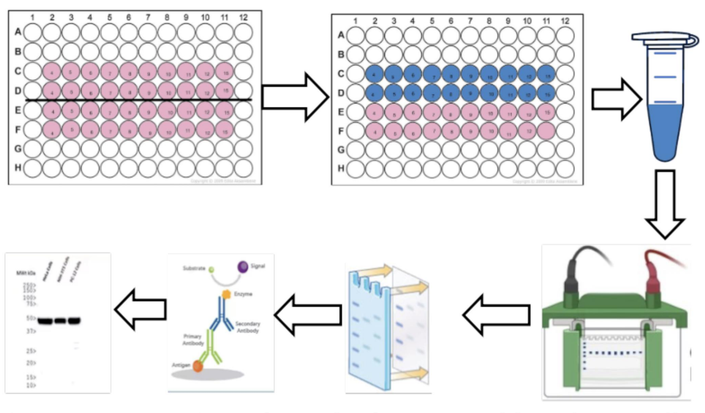

Figure 2. Preparations of samples for western blot. The 96 well cell culture plate was flipped to PAGE solution for wells C and D, then boiled at 100°C for 5m, then the plate was spun for 1m and samples were collected in Eppendorf tubes. These samples were then boiled again for 4m and ran onto the gel. Then gel was transferred to membrane (PVDF) and incubated with primary and secondary antibodies, and was detected with the imaging system.

References

[1]**Erion KA, Berdan CA, Burritt NE, Corkey BE, Deeney JT. Chronic Exposure to Excess Nutrients Left-shifts the Concentration Dependence of Glucose-stimulated Insulin Secretion in Pancreatic β-Cells. J Biol Chem. 2015 Jun 26;290(26):16191-201. doi: 10.1074/jbc.M114.620351. Epub 2015 May 1. PMID: 25934392; PMCID: PMC4481219.**

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[3]**Jameson J, & Fauci A.S., & Kasper D.L., & Hauser S.L., & Longo D.L., & Loscalzo J(Eds.), *Harrison's Principles of Internal Medicine, 20e*. McGraw-Hill Education. https://accessmedicine.mhmedical.com/content.aspx?bookid=2129&sectionid=159213747**

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