Destabilization of β Cell FIT2 by saturated fatty acids alter lipid droplet numbers and contribute to ER stress and diabetes

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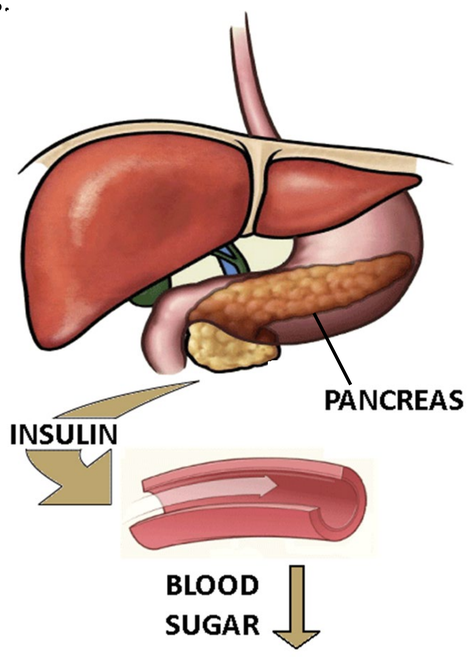
Introduction

Type 2 diabetes mellitus (T2D) is a major public health problem, for which an unhealthy diet is a major risk factor. This paper presents a novel mechanism for how saturated fat contributes to diabetes.

Saturated fatty acids (SFAs) trigger endoplasmic reticulum (ER) stress, cell dysfunction, and apoptosis of pancreatic β cells which produce insulin. Lipid droplets (LDs) sequester toxic free fatty acids (FFAs) produced in insulin resistance conditions, meaning that limitations in LD build-up results in β-cell dysfunction and hence, increased T2D susceptibility.



Methodology

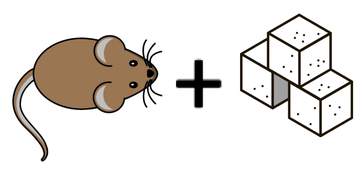
Male mice were used in all experiments and housed in a 12-h light-dark cycle facility with food and water available ad libitum. MIN6 cells were cultured as previously described[1]. Mouse pancreatic islets were isolated by perfusing the pancreas through the common bile duct with collagenase, as previously described[2]. β cell-specific FIT2 knockout mice (βFIT2KO, KO) were generated using the Cre-lox recombination system. Mice with floxed FIT2 were bred with mice expressing Cre-recombinase, under control of rat insulin promoter.

Experiment 1:

To recapitulate β cell vulnerability to SFAs, clonal MIN6 cells were exposed to either oleate (300 mM) or palmitate (300 mM) and lipid droplet regulatory proteins (including FIT2) were tested. Experiments were then performed on FIT2-downregulated mice to observe the effect of FIT2 downregulation on the levels of lipid droplet regulatory proteins

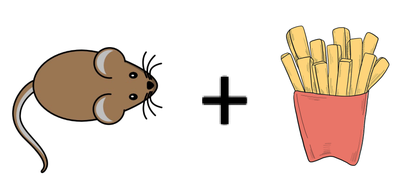
Experiment 2:

An intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (ITT) was performed for both floxed control mice and βFIT2KO mice at 12-wk-old to assess glucose intolerance



Experiment 3:

5-wk-old βFIT2KO mice and their corresponding, floxed littermates (FIT2fl/fl) were metabolically challenged with a high–saturated fat, high-sucrose–containing diet (western diet) for a further 25 wks. Plasma glucose concentrations, insulin and ceramide levels were measured



Results

Experiment 1:

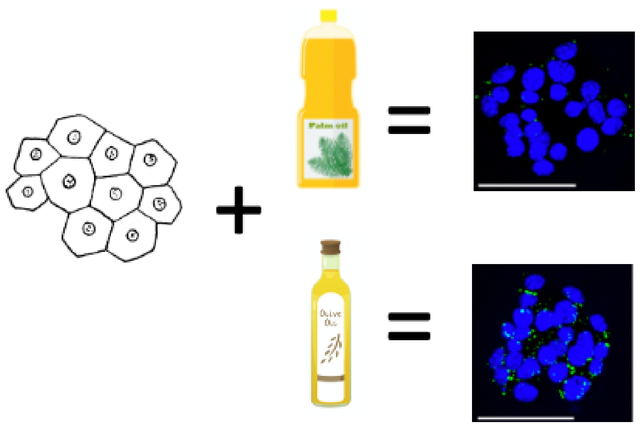
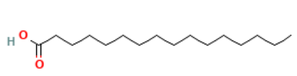
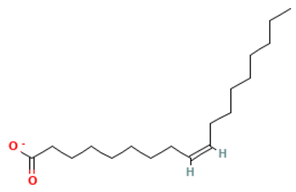


Fig 1: Exposure of MIN6 cells to oleate significantly increased LD numbers, whereas exposure to palmitate did not

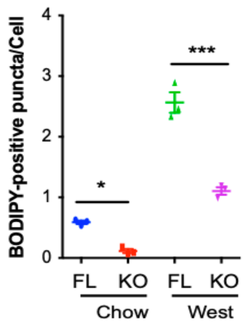


Palmitate (SFA)

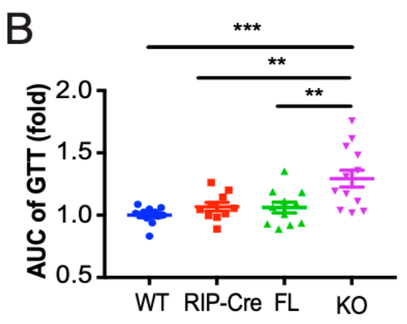


Oleate (Non-SFA)

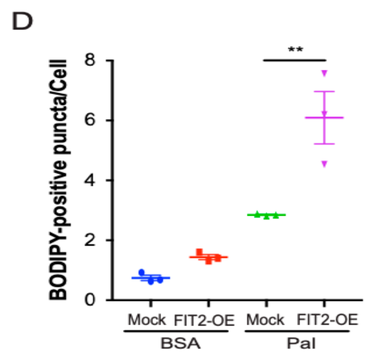
Experiments 2 and 3:



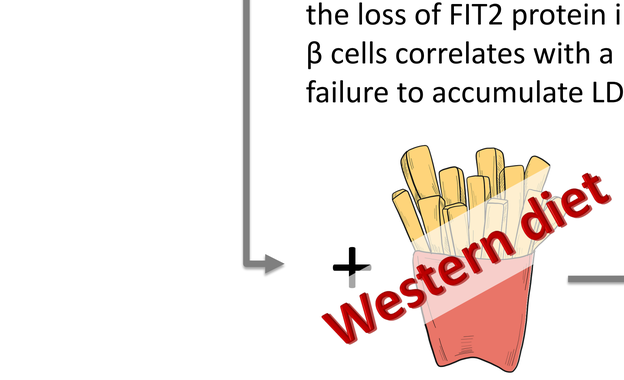
FIT2 downregulation in βFIT2KO mice significantly reduced LD numbers in both chow fed mice and Western-diet fed mice compared to floxed control mice, suggesting that the loss of FIT2 protein in β cells correlates with a failure to accumulate LDs.

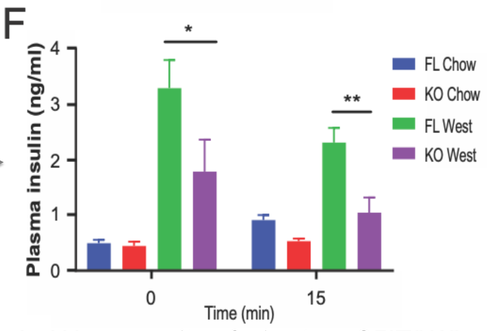


βFIT2KO mice displayed a mild, but significant glucose intolerance compared with wild-type and floxed control mice, as well as the mice with the Cre transgene alone, suggesting a link between FIT2 downregulation and impaired glucose tolerance in mice.



Partial restoration of FIT2 in FIT2-OE cells exposed to palmitate led to a significant increase in the number of LDs, suggesting β cell LDs can be formed following palmitate exposure provided that FIT2 is preserved.

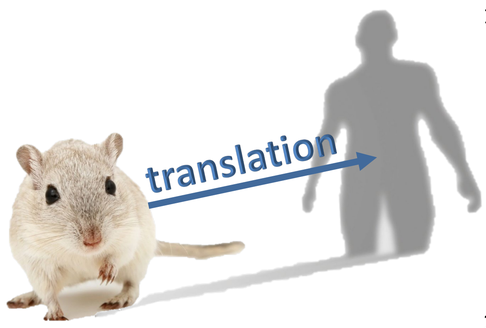




In Western diet fed mice, βFIT2KO mice displayed modest but significantly lower fasting plasma insulin levels. This significant difference is exacerbated following a glucose challenge test, showing that the loss of FIT2 in β cells significantly compromised compensatory hyperinsulinemia. This resulted in significantly elevated, fed state glucose levels of western diet fed βFIT2KO mice compared to the floxed control counterparts

Conclusion

Partial restoration of FIT2 levels rescues lipid droplet biogenesis and mitigates palmitate-mediated effects in MIN6 cells.
If artificially supplementing FIT2 can mitigate palmitate-mediated reduction in lipid droplet numbers in humans, this mechanism could be useful in alleviating ER stress and diabetes risk, particularly in individuals who are not able to reduce their saturated fatty acid intake.



Considering the huge burden of diabetes worldwide, this could be of great clinical value in its prevention.

References

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2. Li, D.-S., Yuan, Y.-H., Tu, H.-J., Liang, Q.-L., & Dai, L.-J. (2009). A protocol for islet isolation from Mouse Pancreas. Nature Protocols, 4(11), 1649–1652. https://doi.org/10.1038/nprot.2009.150