Unraveling the Role of Hypothyroidism, Lipid Accumulation, and ER Stress in Non-Alcoholic Fatty Liver Disease

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Introduction

The sodium/iodide symporter (NIS) transports iodide (I^-) from the bloodstream into thyroid epithelial cells, constituting the first step in thyroid hormone (TH) synthesis. I^- is then oxidized to iodine and incorporated into thyroglobulin to eventually yield the THs triiodothyronine (T_3) and thyroxine (T_4) (*Fig. 1 A&B*). Insufficient dietary I^- and/or impaired NIS function leads to hypothyroidism, a condition often associated with obesity, which frequently results in the development of non-alcoholic fatty liver disease (NAFLD)[1]. Surprisingly, recent findings using NIS knockout mice fed a high-fat diet (HFD) revealed that these mice develop severe hypothyroidism but maintain a lean phenotype and are protected against NAFLD[2]. This unique mouse model enables us to determine the causes of lipid accumulation and NAFLD in the liver by distinguishing the effects of obesity from the effects of the HFD itself. NAFLD is often linked to endoplasmic reticulum (ER) stress, a phenomenon characterized by the accumulation of unfolded and misfolded proteins in the ER lumen, which triggers the unfolded protein response (UPR), initiated by the activation of three main ER transmembrane sensors (PERK, IRE1α, and ATF6), each of which has its own signaling pathways[3]. The activation of any of these transmembrane sensors aims to reduce ER stress by decreasing the accumulation of unfolded and misfolded proteins. Severely hypothyroid NIS KO mice on a high-fat diet are protected from NAFLD. We investigated whether they are also protected from ER stress.

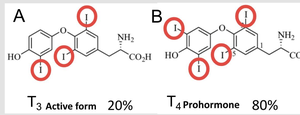

Figure 1: Structure of the thyroid hormones (A) T_3 is the active form, with three iodine atoms, and makes up 20% of the TH secreted into the bloodstream. (B) T_4 is the prohormone, containing four iodine atoms, and constitutes 80% of the TH secreted into the bloodstream.

Methodology

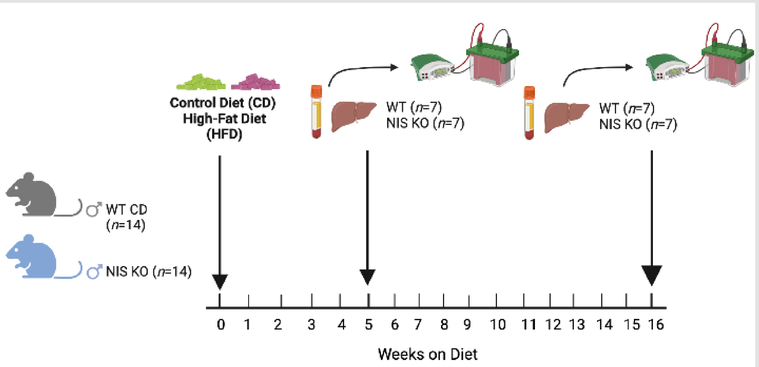

Figure 2: Experimental design
Eight week-old male WT and NIS KO mice were put on either a control diet or a high-fat diet HFD for 5 weeks or 16 weeks. Then, the mice were euthanized and the livers were collected and homogenized to perform western blots.

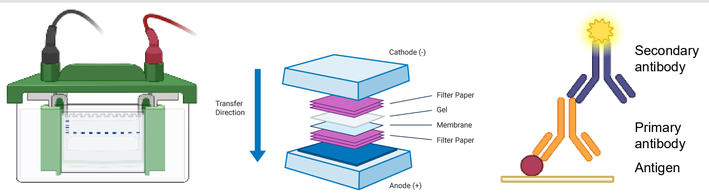

Figure 3. Western blot pipeline
Electrophoresis was conducted to separate proteins by size and then the proteins were transferred from the gel to a nitrocellulose membrane. The nitrocellulose membrane was probed with antibodies for ER stress-related proteins: BiP (binding immunoglobulin protein), total eIF2α (eukaryotic translation initiation factor 2A), total IRE1α (endoribonuclease inositol-requiring enzyme 1α), and CHOP (C/EBP homologous protein). Using the image obtained through chemiluminescence, the expression of ER stress-related proteins from the different cohorts of mice was compared.

Results

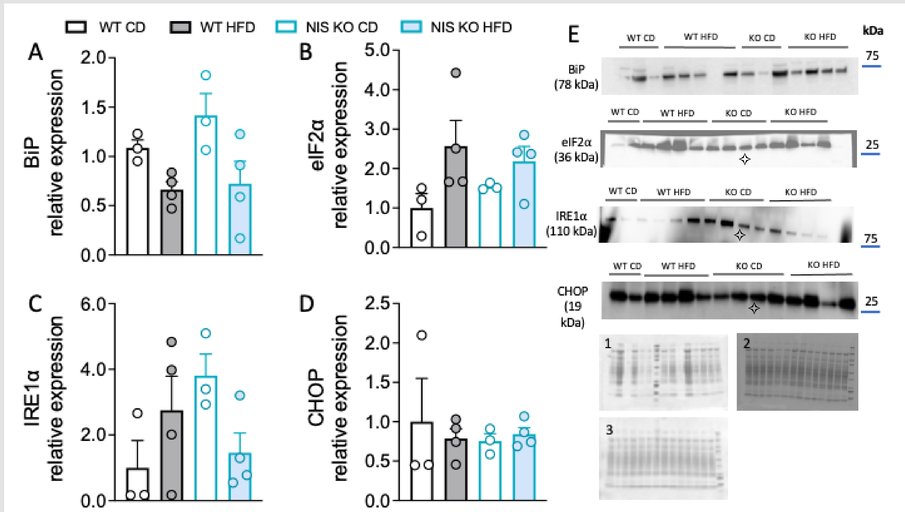

Figure 4. Expression of ER stress-related proteins on NIS KO male livers after 5 Weeks on a HFD. (A) BiP, (B) eIF2α, (C) IRE1α, (D) CHOP, (E) Representative images of western blots and Ponceau staining of (1) BiP, (2) eIF2α & IRE1α, (3) and CHOP. No statistical differences were found across groups. One-way ANOVA, Tukey *post hoc* test.
✧ This sample is WT CD.

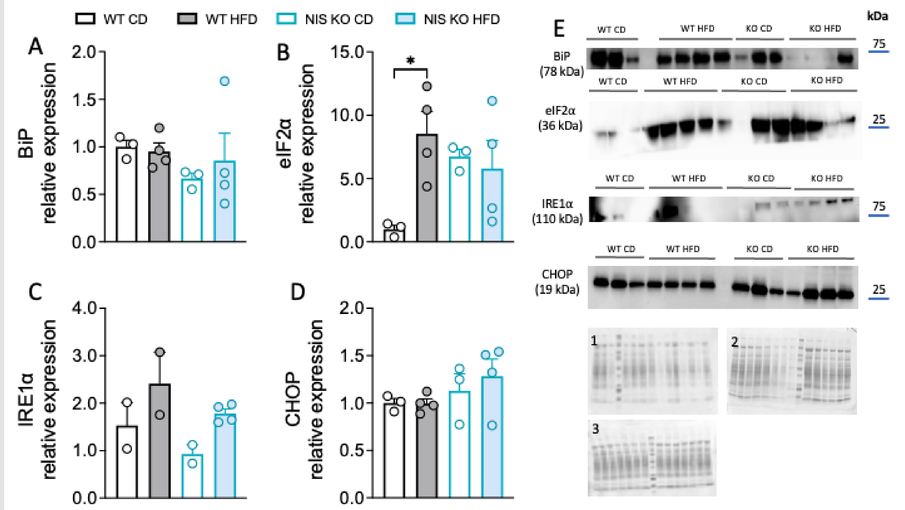

Figure 5: Expression of ER stress-related proteins in the livers of male NIS KO mice after 16 weeks on a HFD. (A) BiP, (B) eIF2α, (C) IRE1α, and (D) CHOP. (E) Representative images of western blots and Ponceau staining of (1) BiP, (2) eIF2α & IRE1α, and (3) CHOP. No statistical differences were found between groups except for eIF2α. *= $p<0.05$. One-way ANOVA, Tukey *post hoc* test.

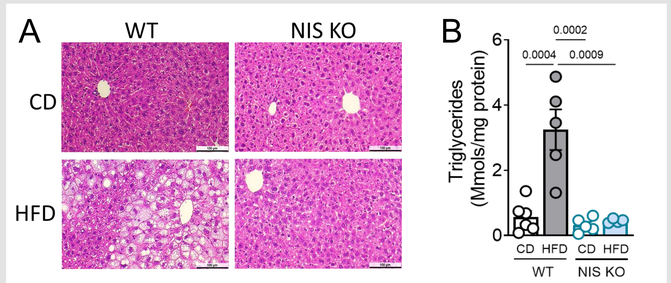

Figure 6. NIS KO fed a HFD are protected against NAFLD (A) Photomicrographs of liver sections from WT and NIS KO mice fed a HFD for 16 weeks. HE 20X. (B) Triglycerides quantitation from (A).

Discussion & Conclusion

Since there were no statistical differences in the expression of ER stress-related proteins after 5 weeks on a HFD even among the WT mice, we could assume that 5 weeks was not enough time for lipids to accumulate to pathological levels and induce ER stress (*Fig. 4*). After 16 weeks, however, eIF2α levels were higher in the WT mice fed a HFD than in those fed the control diet (*Fig. 5B*), which would suggest signs of mild ER stress in the former. Meanwhile, the NIS KO mice did not display any statistical differences in eIF2α, IRE1α, or CHOP levels (*Fig. 5B, C, & D*). Although there was no statistical difference, there was a trend for NIS KO mice on a HFD to express these proteins at levels similar to those of their WT counterparts or slightly higher. This suggests that, although the NIS KO mice did not develop NAFLD, they may not have been protected from ER stress by their hypothyroidism.

Furthermore, there was a trend for the IRE1α levels of the WT HFD and KO HFD mice to increase, but no statistical difference. Since CHOP is activated towards the end of the UPR pathway and no differences were observed, these data suggest that, although there were mild signs of ER stress in the WT mice, there were no signs of severe ER stress that would induce apoptosis.

To ensure that there are no outliers in the data, it will be important, in further investigations, to increase the sample size and keep the mice on the experimental diet for longer to determine whether these animals' levels of ER stress-related proteins will eventually change. It will also be crucial, in further studies, to investigate the activation of proteins on other branches of the UPR pathway, such as ATF6, to deepen our understanding of the conditions under which ER stress occurs.

References

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