Loss of Scaffold Protein AnkB in Brown Adipose Tissue Confers Protection Against Diet Induced Obesity

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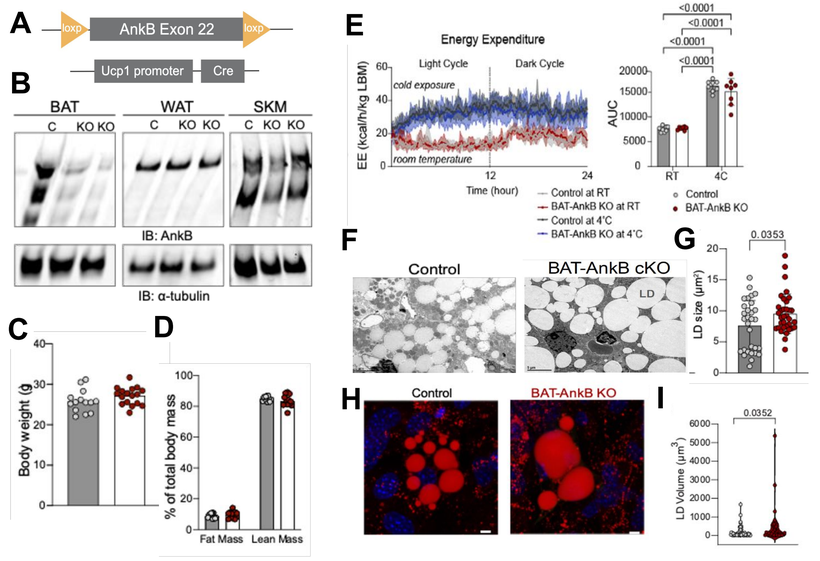
Abstract

Genetic variants of the scaffolding protein ankyrin-B (AnkB), encoded by *ANK2*, have been implicated in obesity and type 2 diabetes. Previous research has shown that AnkB plays a crucial role in facilitating GLUT4 endocytosis in white adipocytes, and its combined deficiency in white (WAT) and brown adipose tissues (BAT) leads to insulin resistance and obesity. To investigate the BAT-specific role of AnkB, we established a mouse model lacking AnkB in (BAT-AnkB KO). In previous work, combined AnkB deficiency in both WAT and BAT was associated with susceptibility to diet-induced obesity. This prompted us to evaluate metabolic health in BAT-AnkB KO and littermate control mice subjected to high-fat diet (HFD) feeding for 18 weeks.

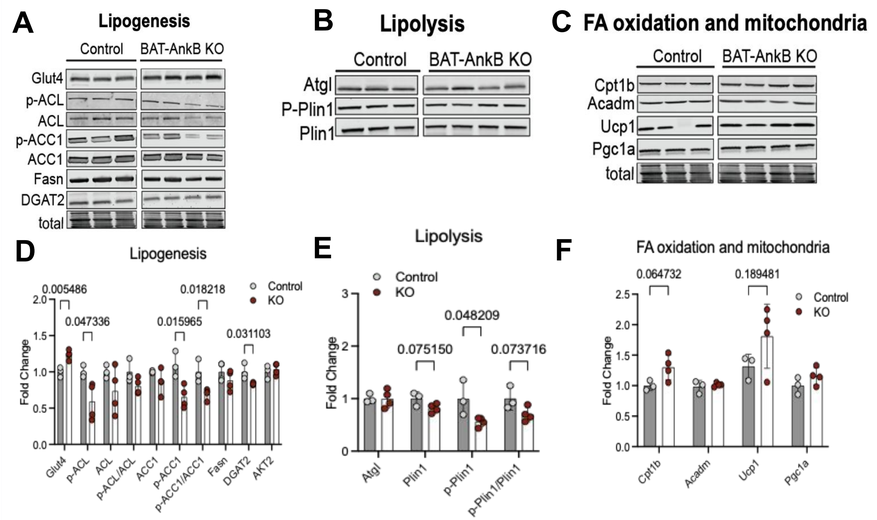
In this study, we explored the consequences of HFD feeding on BAT-AnkB KO mice. Surprisingly, BAT-AnkB KO mice on HFD gained less weight and had lower fat mass compared to control mice on the same diet. Histological analysis also revealed smaller lipid droplet in BAT and WAT of the BAT-AnkB KO mice after HFD. Additionally, BAT-AnkB KO mice maintained a higher percentage of lean mass while on the HFD compared to control mice. These findings suggest that losing AnkB in BAT may protect against diet-induced obesity. Western blot analysis revealed that BAT-AnkB KO mice under HFD conditions exhibited altered lipid metabolism, with decreases in key lipogenesis and lipolysis regulators. Additionally, there was a trending increase in markers of fatty acid oxidation and thermogenesis, including upregulation of Cpt1b and Ucp1, suggesting that loss of AnkB in BAT may cause enhanced fatty acid oxidation. Future studies will directly investigate fatty acid oxidation and other potential compensatory mechanisms causing protection from diet-induced obesity.

AnkB Deficiency in BAT Leads to Enlarged Lipid Droplets



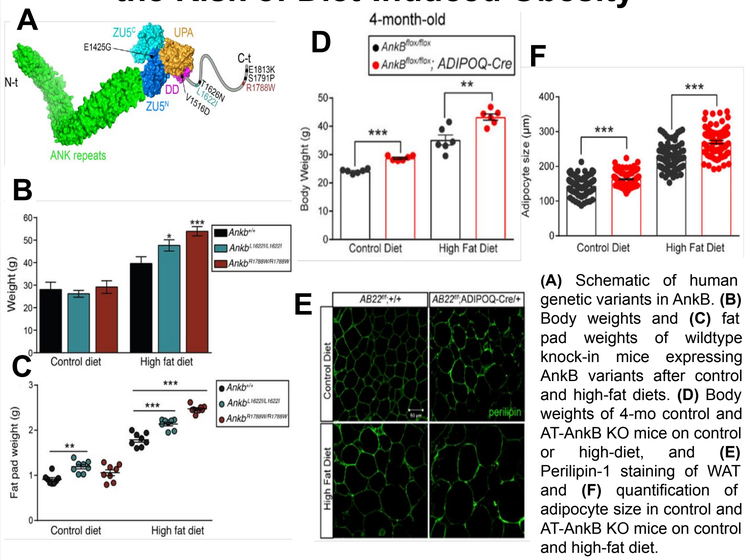
(A) Schematic and **(B)** validation of BAT-AnkB KO mice. **(C)** Body weight and **(D)** Body composition of control and BAT-AnkB KO mice housed at RT. **(E)** Energy expenditure at room temperature (RT) and 4°C in control and BAT-AnkB KO mice. **(F)** Transmission electron microscopy images showing lipid droplet (LD) morphology in BAT from control and BAT-AnkB KO mice. **(G)** LD size in TEM images. **(H)** Immunofluorescence staining of LDs and **(I)** LD volume in brown adipocytes from control and BAT-AnkB KO mice.

Elevated Levels of Cpt1b May Increase FA Oxidation and Promote Resistance to DIO



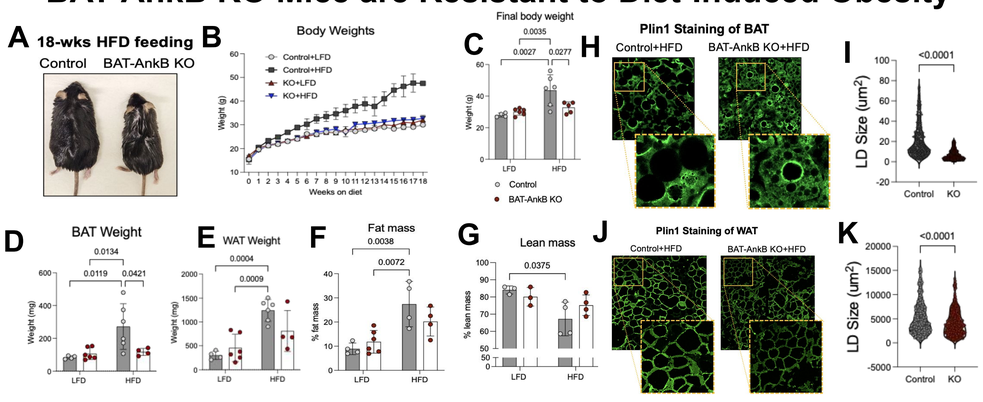
(A) Western blot analysis of proteins involved in lipogenesis, **(B)** lipolysis, and **(C)** fatty acid oxidation and mitochondrial function in BAT from control and BAT-AnkB KO mice. **(D)** Quantification of fold changes in protein expression levels involved in lipogenesis, **(E)** lipolysis, **(F)** fatty acid oxidation and mitochondrial function between control and BAT-AnkB KO mice.

AnkB Deficiency in Adipose Tissue Increases the Risk of Diet-Induced Obesity



(A) Schematic of human genetic variants in AnkB. **(B)** Body weights and **(C)** fat pad weights of wildtype knock-in mice expressing AnkB variants after control and high-fat diets. **(D)** Body weights of 4-mo control and AT-AnkB KO mice on control or high-diet, and **(E)** Perilipin-1 staining of WAT and **(F)** quantification of adipocyte size in control and AT-AnkB KO mice on control and high-fat diet.

BAT-AnkB KO Mice are Resistant to Diet-Induced Obesity



(A) Comparison of physical appearance, **(B)** Growth curve of body weights over 18 weeks of LFD or HFD in control and BAT-AnkB KO mice. **(C)** Final body weight of control and BAT-AnkB KO mice after 18 weeks on LFD or HFD. **(D)** BAT and **(E)** WAT weight of control and BAT-AnkB KO mice after 18 weeks of high-fat diet (HFD) feeding. **(F)** Fat mass percentage and **(G)** lean mass percentage in control and BAT-AnkB KO mice on LFD and HFD. **(H)** Plin1 staining of BAT and **(J)** WAT showing LD morphology in control and BAT-AnkB KO mice on high-fat diet, with a magnified view of lipid droplets. **(I)** Quantification of LD size in Plin1 stained BAT and **(K)** WAT after 18-weeks HFD.

Conclusions

- BAT-AnkB KO mice on a HFD show reduced body weight and fat mass composition compared to controls, indicating that AnkB loss in BAT confers resistance to obesity.
- BAT-AnkB KO mice show reductions in key lipogenesis proteins (Glut4, p-ACL, and p-ACC1) in BAT suggesting impaired fatty acid synthesis, which likely contributes to their resistance to obesity.
- No significant changes in lipolysis proteins, such as Atgl, suggest that the resistance to BAT-AnkB KO mice is not due to increased breakdown of triglycerides into free fatty acids.
- Elevated levels of Cpt1b and trends towards increased Ucp1 expression in BAT of BAT-AnkB KO mice point to enhanced fatty acid oxidation and thermogenesis possibly leading to protection against diet-induced obesity.