Loss of the Scaffold Protein Ankyrin-B in Brown Adipose is Sufficient to Cause Age-dependent Alterations in Adiposity

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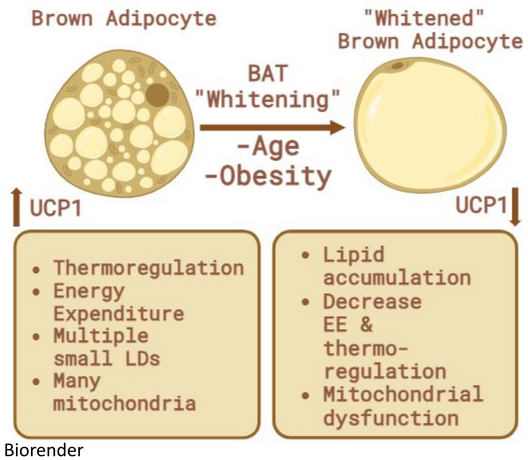
Abstract

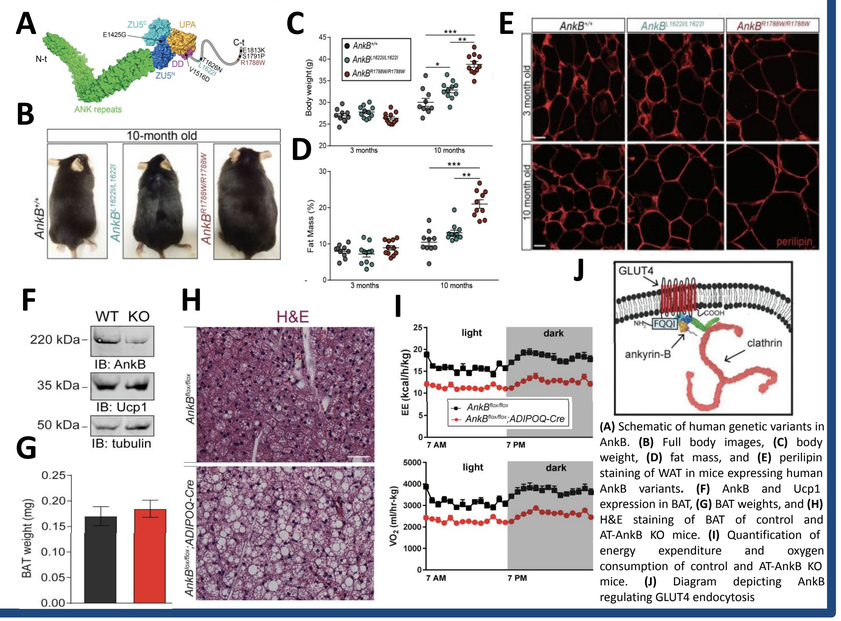
Brown adipose tissue (BAT) is an adipose depot that utilizes fat to generate heat. In individuals with obesity, BAT accumulates excess lipids and becomes less efficient at thermoregulation and energy expenditure, exacerbating metabolic disease. Although unhealthy consumption habits can lead to obesity, genetic mutations can also increase the risk of obesity in humans. Genetic variants in a protein called ankyrin-B (AnkB), encoded by the *ANK2* gene, have been shown to cause age-dependent obesity and type 2 diabetes in humans. Mouse models harboring these variants have AnkB deficiency in adipose tissues. Our previous studies revealed that loss of AnkB in white and brown adipose tissue led to insulin resistance and obesity due to AnkB's role in facilitating GLUT4 endocytosis in white adipocytes. Still, the tissue-specific role of AnkB in BAT and its impact on metabolic health has yet to be uncovered. We investigated the consequences of AnkB deficiency on BAT lipid metabolism and age-related metabolic changes using 4-month-old and 10-month-old mouse models. Histological analysis of mice tissues reveals lipid accumulation in BAT of BAT-AnkB knockout (BAT-AnkB-KO) mice at both 4- and 10-mo mice cohorts with no changes in body weight. Interestingly, 10-month-old BAT-AnkB-KO mice exhibit changes in body composition with an increase in fat mass and a decrease in lean mass, demonstrating age-dependent adiposity. However, despite lipid accumulation in both age groups, young and older BAT-AnkB-KO mice can maintain normal thermogenesis and energy expenditure in response to cold exposure, suggesting that other compensatory mechanisms are at work. Through indirect calorimetry, we also discovered that there is an altered substrate preference from glucose utilization in young 4-month-old BAT-AnkB-KO mice to lipids in 10-month-old BAT-AnkB-KO mice. Glucose tolerance tests revealed that glucose sensitivity is improved in the young mice but is subsequently lost with age. Through insulin tolerance tests, we determined that insulin tolerance isn't impaired, but 10-month-old BAT-AnkB KO mice have hyperinsulinemia. This phenomenon may result from the continuous presence of GLUT4 at the plasma membrane, leading to insulin resistance and the onset of diabetes. Finally, our data reveals that AKT2, the key effector molecule in the insulin signal transduction pathway, is decreased in the 10-month-old BAT-AnkB-KO mice providing further evidence of early insulin resistance. Taken together, these findings indicate the AnkB loss in BAT is sufficient to perturb insulin signaling, glucose homeostasis, and adiposity.

BAT lipid accumulation is associated with impaired BAT function



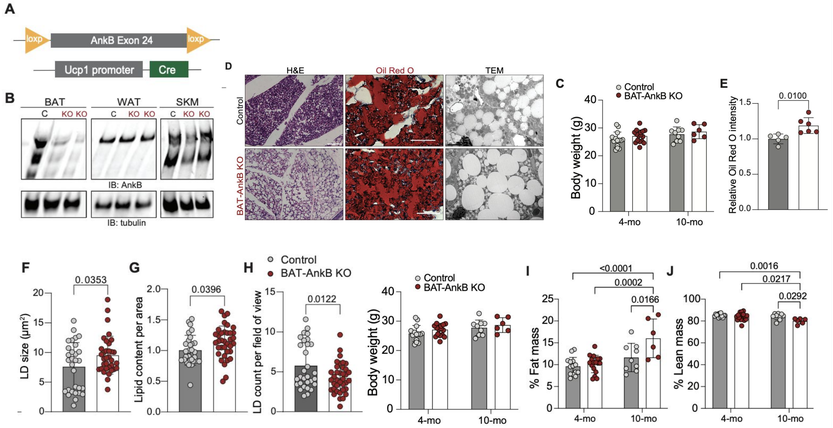
*Made with Biorender

Genetic variants in *Ank2* cause age-dependent obesity and insulin resistance



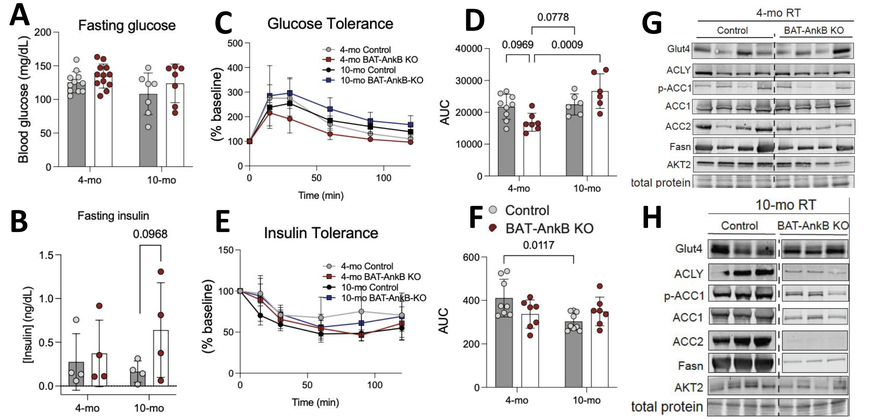
(A) Schematic of human genetic variants in AnkB. **(B)** Full body images, **(C)** body weight, **(D)** fat mass, and **(E)** perilipin staining of WAT in mice expressing human AnkB variants. **(F)** AnkB and Ucp1 expression in BAT, **(G)** BAT weights, and **(H)** H&E staining of BAT of control and AT-AnkB KO mice. **(I)** Quantification of energy expenditure and oxygen consumption of control and AT-AnkB KO mice. **(J)** Diagram depicting AnkB regulating GLUT4 endocytosis

Loss of AnkB in BAT leads to BAT whitening and age-dependent increases in adiposity



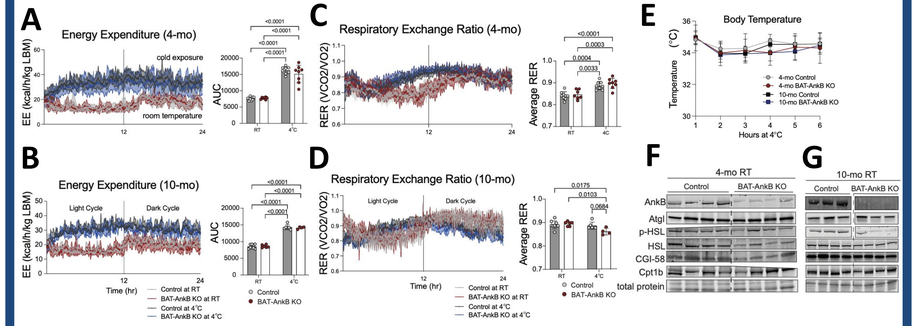
(A) Schematic and **(B)** validation of BAT-AnkB KO mice. **(C)** Body weight, **(D)** H&E staining, ORO staining, TEM images of BAT from control and BAT-AnkB KO. **(E)** Quantification of ORO intensity, **(F)** LD size, **(G)** LD surface area, **(H)** and LD count per field of view in control and BAT-AnkB KO mice. **(I)** Fat mass and **(J)** lean mass percentage from control and BAT-AnkB KO mice housed at RT.

BAT-AnkB KO mice have improved glucose sensitivity at 4-mo that is diminished with age



(A) Plasma glucose levels after fasting for 16 hours and **(B)** plasma insulin levels after 4 hours of fasting in control and BAT-AnkB KO mice housed at RT. **(C)** Blood glucose levels and **(D)** quantification of AUC during 2-hours of a glucose tolerance test. **(E)** Blood glucose levels and **(F)** quantification of AUC during 2-hours of an insulin tolerance test. **(G)** Representative images of western blots from BAT of 4-mo and **(H)** 10-mo control and BAT-AnkB KO mice.

BAT-AnkB KO have no deficits in thermogenesis or EE



(A) Energy expenditure of 4-mo and **(B)** 10-mo control and BAT-AnkB KO mice at RT and 4°C. **(C)** RER of 4-mo and **(D)** 10-mo control and BAT-AnkB KO mice at RT and 4°C. **(E)** Body temperature of 4-mo and 10-mo control and BAT-AnkB KO mice at RT and 4°C. **(F)** Representative image of Western blots of lipolysis and FA oxidation proteins from BAT of 4-mo and **(G)** 10-mo control and BAT-AnkB KO mice.

Conclusions

- 4-mo BAT-AnkB KO mice have BAT lipid accumulation without changes in body weight or composition.
- BAT-AnkB KO mice have age-dependent increases in adiposity that do not impair thermogenesis or EE.
- During a 24-hr cold challenge, 4-mo BAT-AnkB KO mice prefer glucose as a fuel substrate compared to 10-mo BAT-AnkB KO mice that prefer lipids.
- At 4-mo, BAT-AnkB KO mice have improved glucose sensitivity that is diminished with age.
- AKT2 signaling is decreased and insulin levels are slightly elevated in 10-mo BAT-AnkB KO mice which might indicate early signs of insulin resistance.
- Decreases in AKT2 are likely leading to low levels of lipogenesis proteins in 10-mo BAT-AnkB KO mice.