LIVER AMPK REGULATES TOTAL BODY LIPID ACCUMULATION ON A LCHF DIET BUT IS DISPENSABLE FOR INSULIN RESISTANCE

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Abstract

Introduction

Methods

* Generating liver-specific AMPKalpha1/2 knockout mice
  + Black 6 mice that harbored homozygous, floxed alleles for both AMPK a1 and a2 were obtained from (\_\_??\_). To produce liver-specific AMPKalpha1/2 knockout mice, at 70 days old these mice were injected through the tail vein with adeno-associated virus (AAV2/8?) expressing either GFP (control) or Cre (treatment) recombinase from a liver-specific TBG promoter (AAV-TBG-GFP or AAV-TBG-CRE).
* Animals
  + Control and KO mice were born in the animal facility and studied at 70 days of age. Animals were housed under control temperature (21C) and lighting [12-h light (0700-1900 h), 12 h dark (1900h to 077 h] with free access to water and normal chow diet (Lab Diet; 2.91 kcal/g; 5% fat, 24% protein, 2.7% sucrose, 32% starch). All procedures were performed in accordance with the principles and guidelines established by the \_\_\_\_\_\_\_\_\_? (Andreelli et al. 2006 - Liver Adenosine Monophosphate-Activated Kinase-\_2…)
* Making Liver lysates
  + After harvesting liver from mice, livers were placed in an eppendorf tube in liquid nitrogen to freeze. Frozen tissue samples were cut using dry ice to 20-50 mg of tissue per sample. 20 uL/mg of RIPA buffer was added to each sample and they were homogenized using Qiagen Tissue Lyser (3 min at 25Hz). Samples were centrifuged at 14 000 RPM at 4C for 10 min. 160 uL of supernatant was removed and 40 uL of reducing agent and 200 uL of sample buffer was added. Samples were heated with loading buffer at 85C for 2 mins and then snap frozen at -80˚ F. Where was this kit from with the RA and SB?
* Western Blots
  + Liver lysate samples were boiled for 3 minutes at 85˚C using a heating block. 10 uL of sample was placed in a 15 well, 4-12% Tris-glycine, 1.0 MM mini protein gel in 1x SDS running buffer at 125 Volts until samples and ladder reach the bottom of the gel.

# Invitrogen™ Novex™ WedgeWell™ 4 to 12%, Tris-Glycine, 1.0 mm, Mini Protein Gel, 15-well

* Body composition measurements using MRI
* Retro-orbital bleeding
* Ketone body analysis
* Insulin tolerance tests
* Sacrifice mice

Results

* Experimental Design
  + Mice were raised on a normal chow diet (Lab Diet; 2.91 kcal/g; 5% fat, 24% protein, 2.7% sucrose, 32% starch). At 70 days old, mice were injected with either AAV-TBG-GFP or AAV-TBG-CRE to produce liver-specific knockouts and controls. The mice continued to consume normal chow for two weeks post injection at which point they were placed on either a ketogenic (KD) (6.4 kcal/g; 85% fat, 15% protein, 0% sucrose, 0% starch) or matched control diet (CD) (3.8 kcal/g; 10% fat, 15 protein, 0% sucrose, 75% starch). One week later, blood samples were taken using retro-orbital bleeding and ketone bodies were analyzed (do I include that here?). Another week later insulin tolerance tests was performed. Two weeks later mice were sacrificed, tissues were collected and ketone bodies were analyzed. Body composition (weight, fat mass and lean mass) and food intake was measured weekly from the start of injections until sacrifice.
* AMPK was effectively knocked out and confirmed using Western Blots

Discussion

Author Contributions

Acknowledgements

References

Figure/Table Legends

* Figure 1. Experimental Design.
  + - Insert Figure 1 from illustrator?
* Figure 2. Western Blots
  + \*\*\*Figure not from illustrator\*\*
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