# SUN 613 Arv1 KO Mice Are Resistant to Diet-Induced Obesity and Related Glucohomeostatic and Hepatic Impairments

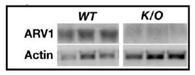
Michael D. Hayward[1,*], Christina Gallo-Ebert[2,*], Hsing-Yin Liu[2,*], Beverly K. Jones[1,*], Jamie Francisco[2,*], Olesia Buiakova[1,*], Joseph T. Nickels Jr.[2,*], [1]Invivotek and [2]Institute of Metabolic Disorders, Genesis Biotechnology Group, Hamilton, NJ. *Potential conflict of interest may exist. Refer to the abstract

## Introduction

The health consequences of obesity include insulin resistance, which can lead to type 2 diabetes, and lipid deposition in the liver leading to hepatic steatosis and nonalcoholic fatty liver disease (NAFLD). The Arv1 (ARE2 [Acyl-CoA cholesterol acyl transferase related enzyme 2] required for viability) gene is involved in a critical step in lipid transport from the endoplasmic reticulum (ER) to the plasma membrane. First identified in *Saccharomyces cerevisiae*, the phylogenetic preservation of this gene is demonstrated by the ability of human Arv1 to rescue the viability defects of a null mutation in the yeast ortholog. Loss of Arv1 in yeast results in accumulation of sterols in the ER and reduced sterol content in the plasma membrane. Considering the functional conservation between yeast and human, we hypothesized that Arv1 plays a critical role in lipid trafficking in mammals and generated a null mutant (KO) mouse that lacks Arv1. This mutant was generated in C57BL/6 stem cells and maintained by crossing to C57BL/6J mice, allowing for subsequent studies in a strain of mice that develops obesity on a high fat diet (HFD).

## Generation of Arv1 KO Mice

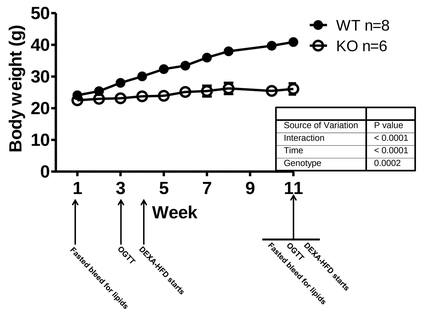
Arv1 KO mice were produced by deletion of exons 5 & 6 through standard homologous recombination techniques.




Elimination of the Arv1 mRNA was confirmed by PCR analysis. Total liver mRNA was extracted and was used as a template for *q*RT-PCR. The expression levels of exons 1, 4, and 5 were determined.

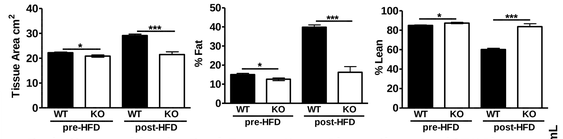
## Body Weights, Body Composition and Adipose Distribution On A High Fat Diet (HFD)

### Body Weights on a HFD



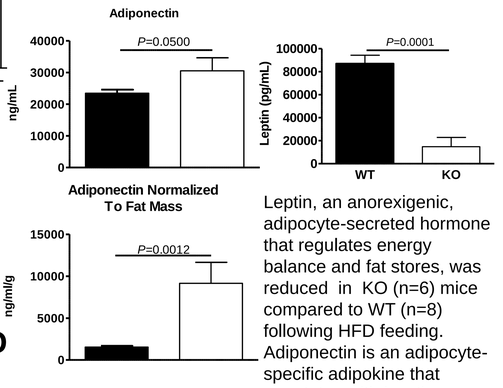

**Longitudinal Body Weights and Diet Challenge**
Arv1 KO mice did not gain weight on the high fat diet while WT mice nearly doubled their body weight.

### Body Composition By DEXA



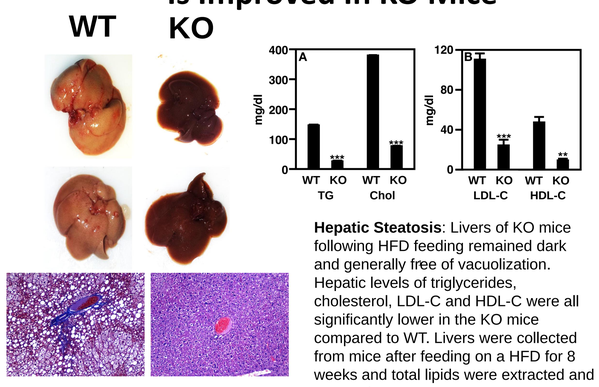

**Body Composition by Dual-Energy X-ray Absorptiometry (DEXA)**
The KO (n=6) mice were smaller than the WT (n=8) mice but also had a lower fat percentage before and especially after the HFD with a corresponding increase in lean mass.

## Leptin Levels Were Significantly Decreased In Arv1 KO Mice, Adiponectin Levels Were Increased




Leptin, an anorexigenic, adipocyte-secreted hormone that regulates energy balance and fat stores, was reduced in KO (n=6) mice compared to WT (n=8) following HFD feeding. Adiponectin is an adipocyte-specific adipokine that correlates with insulin resistance and is reduced in obese individuals. Adiponectin levels were elevated in KO mice compared to WT. The level of adiponectin secreted per total fat mass (from DEXA) was highly elevated in KO animals.

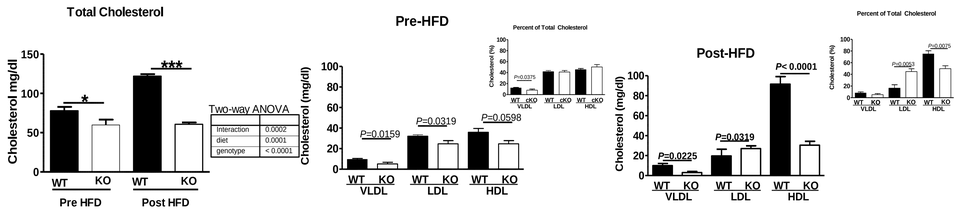
## Hepatic Health Following HFD Feeding Is Improved In KO Mice




**Hepatic Steatosis**: Livers of KO mice following HFD feeding remained dark and generally free of vacuolization. Hepatic levels of triglycerides, cholesterol, LDL-C and HDL-C were all significantly lower in the KO mice compared to WT. Livers were collected from mice after feeding on a HFD for 8 weeks and total lipids were extracted and assayed.

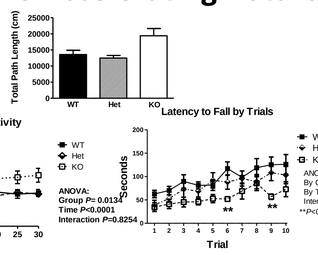
H&E and Trichrome processed tissue were examined using the "Scheuer Classification for Grading and Staging of Chronic Hepatitis

## Cholesterol And Lipoprotein Levels In Response to a HFD




**Serum Cholesterol Levels**
KO (n=6) mice had lower total cholesterol before and following the HFD compared to WT (n=8) mice, with nearly no increase in cholesterol levels following the diet in contrast to the large increase in cholesterol levels in WT mice following HFD. Serum apolipoprotein analysis revealed that KO mice secreted less VLDL than WT mice prior to and post high fat feeding. The LDL-C level of KO mice prior to high fat feeding was also lower than that seen in WT animals. The average level of HDL-C in KO mice was one third of that seen in WT mice post high fat feeding. The percentage distribution of cholesterol within apolipoprotein classes was similar between WT and KO mice before the HFD but following the HFD, KO mice had higher percent LDL-C and lower percent HDL-C compared to the WT mice. Serum cholesterol levels were measured at the times indicated in the HFD body weight chart.

## Arv1 KO Mice Are Hyperactive in the Open Field and Have Impaired Performance on the Accelerating Rotarod



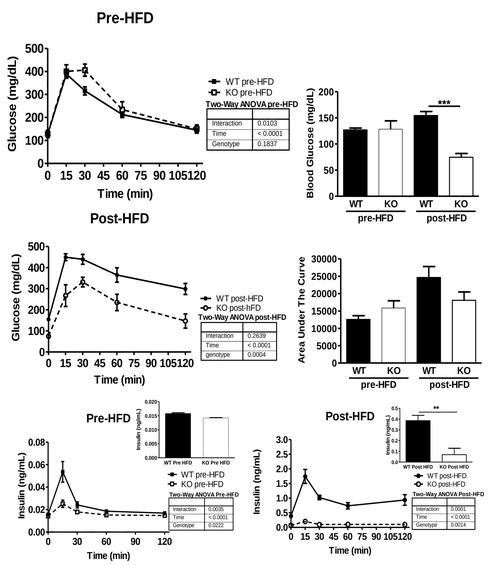




**Open Field** KO mice had increased locomotor activity and traveled a greater distance than Het and WT mice (8 weeks old) throughout the duration of the 30 minute trial. Group size = 7-8

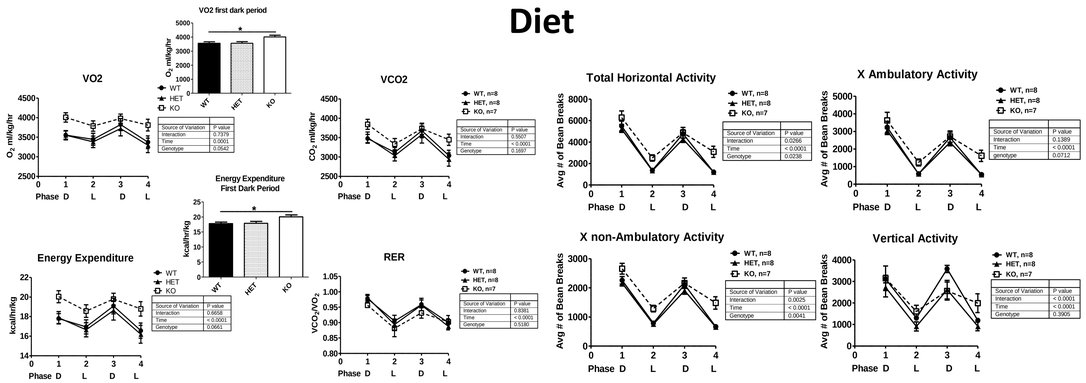
**Rotarod:** KO mice were less capable of maintaining balance on an accelerating rotarod than Het and WT mice (9 weeks old). While The KO mice showed improvement over multiple trials they never achieved the same average duration as the Het and WT mice. Group size = 8

## Improved Oral Glucose Excursion Following HFD




**Oral Glucose Tolerance Test (OGTT)**
Few differences were seen in glucose levels during an OGTT before the HFD treatment but afterwards, the KO (n=6) mice had much lower excursion than the WT (n=8) mice with the final glucose reading nearly returning to baseline levels. Insulin secretion during the OGTT was lower in the KO mice than the WT mice even though baseline insulin levels were similar. After the HFD, hyperinsulinemia detected in WT mice was absent in the KO mice along with much lower levels of insulin secretion during the assay.

## Increased Metabolic Rates And Locomotor Activity on Regular Chow Diet




***Experimental Overview***
Increased metabolic rates in the KO mice compared to Het or WT mice (9-10 weeks old) were detected, with increased $O_2$ and $CO_2$ levels as well as calculated energy expenditure. Increased locomotor activity was also detected over the 48 hrs testing period in the KO mice compared to the Het and WT mice. More of that increased activity appeared to be small movements (non-ambulatory activity) while vertical activity was not significantly different. Group size =8

## Summary

- Targeted mutation of the *Arv-1* gene resulted in multiple neurological effects. These are possibly developmental in nature, given the large demand for cholesterol synthesis in the developing brain and the similarity to other developmentally occurring neurological defects.
- Targeted mutation of the *Arv-1* gene results in mice that are lighter and smaller than their WT counterparts.
  - These mice are also resistant to body weight gain on a HFD
  - The resistance to body weigh gain on a HFD includes lower levels of adiposity
  - Consistent with these phenotypes is lower leptin levels following HFD feeding
- Metabolic rates of the KO mice were significantly increased, which likely contributes to the lower body weight as well as resistance to body weight gain on a HFD.
- Cholesterol levels were lower in the KO mice than the WT mice before and after the HFD challenge
  - Total cholesterol levels did not significantly change in the KO mice following a HFD challenge
  - Apoliprotein levels in the KO mice following the HFD were lower than the WT mice and the profile reflected that of an animal on a regular chow diet
- The resistance to body weight gain on a HFD by the KO mice resulted in improved glucohomeostasis,
  - Improved glucose excursion on a HFD
  - Low levels of insulin secretion before and after a HFD
  - High levels of adiponectin after a HFD
- Livers from KO mice were spared from lipotoxicity associated with HFD feeding
  - Decreased fatty liver appearance
  - Lack of hepatic steatosis, vacuolization
  - Lower lipid levels

## Conclusion: ARV1 function is most likely involved in the initiation and progression of the conditions that make up Metabolic Syndrome. It may represent a novel drug target for the treatment or management of these conditions.