Mice lacking ARV1 have reduced signs of metabolic syndrome and non-alcoholic fatty liver disease

Christina Gallo-Ebert1, Jamie Francisco1, Hsing-Yin Liu1, Riley Draper2, Kinnari Modi1, Michael D. Hayward3, Beverly K. Jones3, Olesia Buiakova3, Virginia McDonough2, and Joseph T. Nickels, Jr.1,4*

1The Institute of Metabolic Disorders, Genesis Biotechnology Group, Hamilton, NJ 08691
2Hope College, Holland, MI 49423
3Invivotek, Genesis Biotechnology Group, Hamilton, NJ 08691
4Rutgers Center for Lipid Research, New Jersey Institute for Food, Nutrition, and Health, Rutgers University, New Brunswick, New Jersey 08901

Running Title: ARV1 regulates MetS syndrome

*To whom correspondence should be addressed: Joseph T. Nickels, Jr., Institute of Metabolic Disorders, Genesis Biotechnology Group, 1000 Waterview Drive, Hamilton, NJ 08691, Telephone: (609) 786-2870, Fax: (609) 587-1827, E-mail: jnickels@venenumbiodesign.com

Keywords: metabolic syndrome, non-alcoholic fatty liver disease, glucose, insulin, lipid

ABSTRACT
MetS syndrome (MetS) is a term used to characterize individuals having at least three of the following diseases: obesity, dyslipidemia, hyperglycemia, insulin resistance, hypertension, and non-alcoholic fatty liver disease (NAFLD). It is widespread and the number of individuals with MetS is increasing. However, the events leading to the manifestation of MetS are not well understood. Here, we show that loss of mARV1 in mice results in resistance to acquiring diseases associated with MetS. Arv1-/- animals fed a high fat diet were resistant to diet-induced obesity, had lower blood cholesterol and triglycerides levels, and retained glucose tolerance and insulin sensitivity. Livers showed no gross morphological changes, contained lower levels of cholesterol, triglycerides, and fatty acids, and showed little signs of NAFLD. Knockout animals had elevated levels of liver FXR protein and its target, SHP. They also had decreased levels of CYP7α1, CYP8β1, and mature SREBP1 protein, evidence suggesting that liver FXR signaling was activated. Strengthening this hypothesis was the fact that PPARα protein was elevated, along with its target, FGF21. Arv1-/- animals excreted more fecal cholesterol, free fatty acids, and bile acids. Their small intestines had 1) changes in bile acid composition, 2) an increase in the level of the intestinal FXR antagonist, tauromuricholic acid, and 3) showed signs of attenuated FXR signaling. Overall, we believe that ARV1 function is deleterious when consuming a high fat diet. We further hypothesize that ARV1 is critical for initiating events required for the progression of diseases associated with MetS and NAFLD.

Approximately 20-30% of the world’s population has pathologies that are associated with MetS. MetS is a term used for a set of diseases that includes obesity, type 2 diabetes (T2D), hyperlipidemia, hypertension, insulin resistance, and NAFLD (1). Health consequences include premature death due to T2D-related cardiovascular disease and stroke, and obesity-related increases in...
the levels of lipids associated with atherosclerosis and certain cancers (2,3). It is estimated that by the year 2030, 60% of individuals will be characterized as having MetS (4).

Obesity is thought to be the main driver for progressing the severity of MetS (5). However, ~1/3 of obese individuals are metabolically healthy, so the etiology is not completely clear-cut, and suggests a genetic predisposition to the disease (6). There is a debate as to whether NAFLD precedes the onset of MetS, or requires the presence of one or more of its diseases to progress (7,8). NAFLD is prevalent among the world’s population at least in part due to the emergence of a western diet that is high in saturated fats and refined sugars (9). It can progress to NASH -> fibrosis -> cirrhosis -> hepatic cancer (10). NAFLD is thought to be due to the deposition of fatty acids from adipose to the liver and subsequent accumulation of triglycerides (11). There is data that suggests insulin resistance precedes NAFLD and is required for the manifestation of the disease (10), as it causes a dysregulation of lipogenic and lipolytic activities in the liver and adipose tissue, respectively (12). Thus, while the diseases associated with MetS are known, their relationships to the onset and progression of NALD are not well understood.

ARV1 (ARE1 ARE2 required for viability 1) was first identified in S. cerevisiae using a screen searching for genes required for viability in the absence of sterol esterification (13). S. cerevisiae cells lacking ARV1 have defects in PL (phospholipid), SL (sphingolipid), GPI (glycosylphosphatidylinositol) and sterol syntheses, and lack the ability to localize sterol and mobilize PIP2 (phosphatidylinositol 4,5 bisphosphate) (13-16). arv1 deficient cells are hypersensitive to the PS (phosphatidylserine) binding agent, papuamide-B, suggesting a mislocalization of PS to the plasma membrane (17). They contain fragmented vacuoles and a disrupted organelle phenotype (17), which most likely activates the unfolded protein response (18). Null cells are hypersensitive to fatty acid supplementation (19), suggesting that Arv1 also regulates fatty acid metabolism. Thus, yeast Arv1 has a major role in regulating lipid synthesis, metabolism, and homeostasis, and is needed to rescue cells when these processes become perturbed.

There is evidence that ARV1 regulates lipid distribution and metabolism in mammals. Knockdown of Arv1 in HepG2 cells results in the accumulation of cholesterol in the ER and a reduction in the plasma membrane, suggesting defective sterol transport in the liver (20). Mice treated with ASOs (anti-sense oligonucleotides) to Arv1 have increases in plasma and liver bile acid levels that contain hydrophilic bile acid salts (20). The same authors observed that early loss of Arv1 caused increases in plasma PL, cholesterol, and LDL-C (low density apolipoprotein-cholesterol) levels, and a decrease in HDL-C (high density apolipoprotein-cholesterol) levels (20). Interestingly, human ARV1 possesses in vitro lipid binding activity, as it binds sterol intermediates (21), several fatty acid species (22), and specific phospholipids and cholesterol (H-Y Liu, C. Gallo-Ebert, K. Modi, J. L. Cunningham, and J.T. Nickels, manuscript in preparation).

Recent mouse model studies have presented data exploring the neurological and metabolic phenotypes of NKO (neuronal knockout) and whole body Arv1/− animals (23,24). Palmer et al., (24) found that NKO Arv1/− animals suffered from seizures and epileptic encephalopathy. Lagor et al., (23) discovered that Arv1/− animals displayed multiple beneficial metabolic changes, including lack of weight gain, improved glucose tolerance, elevated adiponectin secretion, increased energy expenditure, and a decrease in WAT (white adipose tissue). These studies strongly suggest that Arv1 plays a critical role in maintaining brain function and metabolic homeostasis. Here, we asked how Arv1/− animals responded to a high fat diet. Our results indicate that ARV1 function is needed for progressing diseases associated with MetS.

**RESULTS**

Arv1/− mice lack any detectable ARV1 protein - C57BL/6J derived ES cells were used to generate Arv1/− animals. They were maintained on the identical C57BL/6J background, making backcrossing not necessary. To generate knockout animals, we excised exon 5 from the Arv1 gene locus (Fig. 1A). To test for proper deletion, Arv1 mRNA transcripts were quantified by qRT-PCR, and ARV1 protein levels were determined by western analysis. Tissues analyzed were liver,
brain, SI (small intestine), lung, kidney, and adipose.

The Arv1 gene encodes a mRNA containing 6 exons (271 AA; 31kDa) (Fig. 1B & C), which expresses a protein that in our hands migrates at 31 kDa by western analysis (Fig. 2). To determine if Arv1/+ animals expressed any mRNA transcripts, we designed primers able to quantify the levels of exon 1, exon 4, intragenic regions between exons 4 and 5, exon 5, and the contiguous junction between exons 4 and 5 (Fig. 1B). We found that Arv1/+ animals expressed a mRNA in liver that contained exons 1 through 4, along with a portion of downstream intragenic sequences between exons 4 and 5 (Fig. 1D; I-5). However, we did not detect a mRNA expressing exon 5 (Fig. 1D; 6 & 7) or sequences coding for the contiguous junction between exons 4 and 5 (Fig. 1D; 8). On the other hand, WT animals expressed a mRNA containing exons 1 through 5 (Fig. 1B). Presumably, this WT mRNA is a full-length transcript that contains exon 6 and downstream sequences containing the 3’UTR and polyA tail. These sequences would be absent in the truncated Arv1 transcript detected in Arv1/- animals. All other tissues analyzed gave identical results (not shown).

Based on the qRT-PCR data, Arv1/+ animals could be expressing a truncated form of the ARV1 protein (Fig. 1C). We addressed this question by determining the levels of ARV1 protein in several tissues using western analysis. Arv1/+ mice did not express either a full-length or truncated form of ARV1 in any tissue tested, with the exception of one animal, which expressed a low level of full-length ARV1 in the SI (Fig. 2; Fig. S1). Western analysis using SIs from all other knockout animals used in our studies did not show any full-length or truncated ARV1 protein expression (not shown). WT animals expressed ARV1 protein in the liver and SI, but we could not detect the protein in brain, lung, adipose, or kidney tissue (Fig. 2; Fig. S1). Densitometry values correlated with band intensities for each organ (Fig. 2A).

Thus, Arv1/+ animals express a stable truncated Arv1 mRNA containing exons 1 through 4, which is not translated into a truncated form of the ARV1 protein. Based on these results, we conclude that Arv1/+ mice are devoid of any form of ARV1 protein.

In all of our studies, we tested WT, Arv1+/+, and Arv1/-/ animals. We only presented data from Arv1/-/ animals when they deviated from what we observed for WT animals.

Arv1/-/ mice have a reduced survival rate, and display neurological and behavioral deficits – Lagor and colleagues (23,24) have reported that Arv1-/- whole body and nestin-Cre mediated neuronal knockouts (NKO) of Arv1 had reduced survival rates. We tested for this phenotype in our Arv1/-/ animals by recording survival of a group of 16 males of each genotype beginning at 8 weeks of age until over 20 weeks of age. The first death on the NC was recorded at 12 weeks of age and by 18 weeks of age, 60% of the Arv1-/- animals were dead (Fig. 3; WT, closed circles; KO, open circles). The survival rate on the HFD was similar, with 60% of the mice dead by 17 weeks of age.

During the survival study, we observed that a subset of adult Arv1/+ males suffered from seizures. In the case of the Arv1/-/ males tested, one animal had to be euthanized because of the severity of the event. Palmer et al., (24) have also reported that NKO animals suffered seizures.

We next determined if Arv1/+ animals displayed other neurological deficits. We compared the motor coordination between WT and Arv1/+ animals using a rota rod test (25). During this test, animals are placed on an accelerated rotating horizontal rod, and the mice must coordinate paw movement to remain on the rod. Ten sequential trials were performed, and latency before the mice fell off of the rod was measured.

WT animals increased their length of time on the rod as the number of trials increased, indicating they adapted by learning how to remain on the rod. Arv1/+ animals fell off the rod sooner than WT animals, but had the same increase in time as WT animals (Fig. 4A; trials 3 & 6; WT, closed circles, KO, open circles), demonstrating an impaired locomotor coordination but retaining some ability to improve performance. However, later trials revealed there was a significant decrease in time on the rod compared to WT animals (Fig. 4A; trials 9 & 10; WT, closed circles, KO, open circles). Thus, Arv1/+ animals display impaired motor coordination.

To complement the rotarod studies, we determined the activity levels of Arv1/+ animals using an open field test (26). Arv1/+ animals had a
higher level of activity than their WT littermates at all time points monitored (Fig. 4B; WT, closed circles; KO, open circles). Moreover, the total path length traveled by knockouts was nearly double that of WT animals (Fig. 4C; WT, closed circles; KO, open circles).

Finally, we tested for male and female fertility, as it has been noted that Arv1/− mice have lower fertility rates (23). Homozygous male and female Arv1/− mice were mated to ICR (Institute of Cancer Research) mice, and we quantified 1) successful mating by the appearance of a mating plug, 2) the average litter size, and 3) the number of successful litters. ICR mice have been used to examine fertility as they display excellent reproduction rates (27,28).

During mating, male mice deposit a mating (copulation/sperm) plug into the female genital tract, which is a gelatinous substance containing lipids and sperm (29). The mating plug solidifies and closes the vaginal tract, which helps in sperm retention. We found that female Arv1/− mice retained mating plugs with a frequency that was similar to their WT littermates (Fig. 5A; WT, black bars; KO, white bars). These females had litter sizes that were similar to WT (Fig. 5B; WT, closed circles; KO, open circles). Moreover, they produced similar numbers of litters (Fig. 5C; WT, black bars; KO, white bars). Male Arv1/− mice were as fertile as their WT littermates under all conditions tested (Fig. 5D-F, WT, closed circles; KO, open circles; WT, black bars; KO, white bars). Upon further examination, we found that all pups produced by female Arv1/− animals died within 4 days due to maternal neglect.

Loss of mARV1 reduces high fat diet-induced obesity - Our Arv1/− mice are maintained on the C57BL/6J mouse background, which is a diet-induced obesity (DIO) sensitive strain. It has been shown that Arv1/− mice display a lean phenotype (23). To test if our Arv1/− animals shared this phenotype, we fed them a HFD (high fat diet) and determined its effects on weight gain. Animals were fed either NC (normal chow) or a HFD, and body weights were measured weekly for 15 weeks (Fig. 6). When fed a NC diet, both groups of animals more or less maintained their body weight throughout the study (Fig. 7A; WT, closed circles; KO, open circles). When fed a HFD, WT mice nearly doubled their body weight over the 15 weeks, while Arv1/− mice fed the same diet showed only a slight weight gain that was not statistically different from mutant animals fed NC (Fig. 7A; WT, closed boxes; KO, open boxes). WT animals fed a HFD increased their body weight by 160%, while the percentage of weight gained for Arv1/− mice was minimal. We point out that Arv1/− animals did not lose weight during the entire time of the study whether fed NC or a HFD.

One possibility for this lack of weight gain is that Arv1/− animals consumed less food. Food consumption was determined over a 72 hr time period for animals on NC or a HFD. Arv1/− animals consumed more chow whether on NC or a HFD diet (Fig. 7B; WT, closed circles; KO, open circles). Arv1/− animals fed a HFD consumed ~5 times more food per kg body weights than their WT littermates (Fig. 7B; WT, closed circles; KO, open circles). Thus, Arv1/− animals consume more food but do not gain weight when fed NC or a HFD.

To explore what was the cause of the increased food consumption, we assayed for the levels of several hormones that are known to regulate eating behavior. Ghrelin is secreted by the small intestine and regulates the hunger response by acting as a neuropeptide activating ghrelin receptors in the hypothalamus. There are two forms of ghrelin that differ in acylation status (30). Leptin also regulates hunger and is secreted by adipose tissue, where it functions in the hypothalamus by binding to leptin receptors (31). Leptin acts as an anorexigenic hormone, while ghrelin is orexigenic.

Both acylated and deacylated ghrelin levels were increased in 8 weeks old Arv1/− animals compared to WT littermates (Fig. 7C; WT, closed circles; KO, open circles). WT animals secreted high levels of leptin in response to being fed a HFD (7.6 ng/ml - ~42 ng/ml) (Fig. 7D; WT, closed circles; KO, open circles). In contrast, leptin levels were dramatically decreased in Arv1/− mice, whether they were fed NC or a HFD (Fig. 7D; WT, closed circles; KO, open circles). Arv1/− mice fed a HFD secreted ~13-fold less leptin than WT animals.

We next measured adiponectin levels. Adiponectin is an adipocyte-specific adipokine, whose levels correlates with insulin resistance, and is reduced in obese individuals (32,33). Arv1/− animals had elevated adiponectin levels compared to WT mice, whether they consumed NC or a HFD.
ARV1 regulates MetS syndrome

(Fig. 7E; WT, closed circles; KO, open circles). Arv1+/ animals secreted ~9-fold more adiponectin per total fat mass when fed a HFD (Fig. 7E).

Thus, Arv1-/ animals have elevated levels of ghrelin and decreased levels of leptin compared to WT animals. These changes may be causing increased food consumption. However, Arv1+/ animals have high adiponectin levels that are characteristic of a lean phenotype (34).

A higher metabolic rate could help in maintaining the resistance to diet-induced obesity observed for Arv1+/ animals. Several tests were performed to measure metabolic status. Metabolic rate measurements were taken prior to and after starting a HFD. Measurements were taken every 12 hr for 2 days. Prior to the initiation of the HFD, there were minimal differences in metabolic rates of Arv1+/ mice and WT animals, (Fig. 8A-C; WT, closed circles; KO, open circles). However, drastic differences were observed when animals were fed a HFD, as Arv1+/ mice had elevated metabolic rates for all parameters tested (Fig. 8A-C; WT, closed boxes; KO, open boxes). Analysis of the respiratory exchange ratio of both cohorts indicated they metabolized mainly carbohydrate when fed NC, whereas both fats and carbohydrates were used on a HFD (Fig. 8D; WT, closed boxes; KO, open boxes).

Thus far, our data strongly suggest that, although Arv1+/ animals consume more food due to elevated ghrelin levels and reduced leptin levels, they are resistant to becoming obese, in part due to a higher metabolic rate and increase in energy expenditure.

Blood cholesterol and triglycerides levels are reduced in Arv1+/ mice - We next examined if there were changes in various blood lipid levels due to the lack of diet-induced weight gain. Cholesterol (Chol), TAGs (triacylglycerides), and apolipoprotein levels were determined. Arv1+/ mice fed either NC or a HFD had reductions in blood cholesterol levels when compared to WT mice (Fig. 9A, (NC, 20%; HFD, 60%); WT, closed circles; hetero, closed diamonds; KO, open circles). Heterozygous Arv1+/ mice fed a HFD had an increase in cholesterol relative to WT animals when fed a HFD (Fig. 9A; WT, closed circles; hetero, closed diamonds; KO, open circles). The reason for this observed elevation in blood cholesterol in heterozygotes is unknown. The levels of TAGs observed for animals fed NC were similar for all groups (Fig. 9B; WT, closed circles; hetero, closed diamonds; KO, open circles). There was a slight trend in reduced levels of TAGs observed in Arv1+/ animals. On the other hand, Arv1+/ animals on a HFD had a 25% decrease in TAGs compared to WT animals (Fig. 9B; WT, closed circles; hetero, closed diamonds; KO, open circles) and 40% (Fig. 9D) when fed NC and a HFD, respectively (Fig. 9C; WT, closed circles; hetero, closed diamonds; KO, open circles). Heterozygous mice had normal HDL levels on NC (Fig. 9C; WT, closed circles; hetero, closed diamonds; KO, open circles), but elevated levels when fed a HFD (Fig. 9D; WT, closed circles; hetero, closed diamonds; KO, open circles). VLDL and LDL levels were unchanged.

Taken in sum, our data shows that loss of ARV1 correlates with reduced blood cholesterol and HDL levels regardless of diet, and reduced TAGs levels in animals fed a HFD.

Arv1+/ animals are leaner and have less fat deposition - We used DEXA scanning to analyze total fat and lean mass. Arv1+/ animals had a reduced fat mass when compared to WT mice (Fig. 10A; WT, closed circles; KO, open circles). The difference was much more pronounced in Arv1+/ animals fed a HFD (Fig. 10A; WT, closed circles; KO, open circles). HFD fed Arv1+/ animals were leaner then WT mice, but their levels of lean mass were decreased by ~30% (not shown).

Multiple fat depots were dissected, and fat distribution and mass were determined for animals fed a HFD. The levels of fat mass per gram body weight in Arv1+/ animals were reduced in all fat depots (Fig. 10B; WT, closed circles; KO, open circles). Decreases ranged from 3-fold in mesenteric fat to 7.5-fold in epididymal fat. Interestingly, Arv1+/ animals contained less brown fat mass and brown fat mass per grams body weight (Fig. 10C & D; WT, closed circles; KO, open circles). Why Arv1+/ animals have less brown fat is unknown. They do display higher metabolic rates then WT animals and are unable to gain weight on a HFD.

Arv1+/ mice are more glucose tolerant and have increased insulin sensitivity - The C57BL/6J mouse strain is used as a model for examining how obesity affects the onset of diabetes (35,36).
As our Arv1⁻/⁻ animals showed resistance to diet-induced obesity, we looked at whether there was a correlation between lack of weight gain and reduced signs of pre-diabetes.

We first performed an OGTT (oral glucose tolerance test) to see if changes in diet affected glucose tolerance. Glucose excursion rates were similar between WT and Arv1⁻/⁻ animals fed NC (Fig. 11A; WT, closed circles; KO, open circles). However, Arv1⁻/⁻ animals had significant decreases in fasting blood glucose levels (Fig. 11B; WT, closed circles; KO, open circles; NC). Moreover, Arv1⁻/⁻ animals fed a HFD had increased glucose excursion rates (Fig. 11A; WT, closed boxes; KO, open boxes) and lower fasting blood glucose levels than WT animals (Fig. 11B; WT, closed circles; KO, open circles; HFD). HFD ending blood glucose levels in Arv1⁻/⁻ animals returned to levels that were seen prior to the initiation of the study, whereas WT levels remained high (Fig. 11A; WT, closed boxes; KO, open boxes; 0 vs. 120 min time points). The fact that WT mice fed a HFD had elevated blood glucose levels at the end of the OGTT further validates the glucose intolerance phenotype associated with the DIO model (36). The data also indicates that Arv1⁻/⁻ animals are resistant to becoming glucose intolerant when fed a HFD.

We determined insulin secretion during the OGTT. Insulin levels in WT and Arv1⁻/⁻ animals fed NC peaked coincident with glucose levels (first-phase insulin response) and decreased thereafter (Fig. 11C; WT, closed circles; KO, open circles). However, Arv1⁻/⁻ animals secreted less insulin than did WT mice (Fig. 11C; WT, closed circles; KO, open circles), and had lower baseline fasting insulin levels (Fig. 11D; WT, closed circles; KO, open circles).

When fed a HFD, WT mice secreted higher levels of insulin during the first-phase insulin response compared to WT animals fed NC (~9 ng/ml vs. 2.2 ng/ml) (Fig. 11C; closed boxes vs. open boxes). On the other hand, Arv1⁻/⁻ animals fed a HFD secreted drastically less insulin than WT animals, (Fig. 11C; WT, closed boxes; KO, open boxes), with levels being equivalent to those observed on NC (Fig. 11C; 1.3 vs. 0.9 ng/ml). Overall, there was ~9-fold reduction in first-phase insulin response secretion in Arv1⁻/⁻ animals compared to WT animals fed a HFD (Fig. 11C). The fasting insulin levels of Arv1⁻/⁻ animals fed a HFD were again reduced compared to WT animals (Fig. 11D; WT, closed circles; KO, open circles; (2.8 pg/ml vs. 0.25 pg/ml)).

Finally, an ITT (insulin tolerance test) was performed on animals fed NC or a HFD. WT and Arv1⁻/⁻ animals responded with normal sensitivity to the low insulin dose (0.5 U/kg) with a small decrease in blood glucose levels (Fig. 11E; WT, closed circles; KO, open circles). WT animals fed a HFD also had a reduction in glucose levels using a much higher insulin dose (1 U/kg), and recovered as seen by an increase in their glucose levels (Fig. 11E; WT, closed boxes). On the other hand, Arv1⁻/⁻ animals fed a HFD were still highly insulin sensitive, requiring glucose supplementation by 60 minutes, at which time they were removed from the study (Fig. 11E; KO, open boxes).

Arv1⁻/⁻ mice have reduced signs of NAFLD - Insulin resistance is tightly associated with the appearance of NAFLD and leads to the induction of lipogenesis in the liver (10). Accumulation of liver TAGs, cholesterol, and fatty acids contributes to, or is the reason for, the onset of fatty liver (37-39). Because our Arv1⁻/⁻ animals remained insulin sensitive when fed a HFD, we explored if the insulin sensitivity phenotype correlated with reduced signs of NAFLD.

We first assayed for the rate of lipogenesis in the livers of animals fed a western diet (WD) by measuring the levels of TAGs, cholesterol, LDL, HDL, and FFAs (free fatty acids). Arv1⁻/⁻ animals had reduced lipid levels compared to WT animals (Fig. 12A & B; TAGs (8-fold); Chol (5-fold); LDL (5-fold); HDL (6-fold); FFAs (8-fold); WT, closed circles; KO, open circles).

Visualization of the gross morphology of livers from WT animals revealed that they were enlarged and had a pale color consistent with a high fat content (Fig. 12C). Arv1⁻/⁻ livers were normal in size and deep pink in color (Fig. 12C). Histological staining of WT livers indicated the presence of micro- and macrosteatosis, hepatocyte ballooning, and the presence of Mallory bodies. (Fig. 12D; arrow, portal vein). WT livers had a standard inflammation grade of 3 and a fibrotic stage of 1 (n=6) (40). Arv1⁻/⁻ livers displayed reduced histological signs of hepatic steatosis, and all had a standard inflammation grade of 2 and a fibrotic stage of 0 (n=6).
Finally, we examined hepatic TAGs export to begin to understand the physiology underlying the reduction of lipid production and attenuation of NAFLD. Animals were challenged with a bolus of olive oil in the presence of Pluronic F127, an endothelial lipase inhibitor, and blood TAGs levels were determined at the indicated times. Arv1/− animals had a significant decrease in TAG production compared to WT animals (Fig. 12E; WT, closed circles; KO, open circles).

Finally, serum clinical chemistries were analyzed on HFD fed animals. Alkaline phosphatase levels were increased in Arv1/− mice (WT=85.50 ± 5.74 IU/L vs KO=262.00 ± 17.09 IU/L, \( p<0.001 \)), suggesting an altered hepatobiliary function. Blood Urea Nitrogen was also elevated (WT=19.00 ± 0.91 mg/dL vs KO= 26.75 ± 1.65 mg/dL, \( p<0.001 \)). Typically, lactate dehydrogenase is elevated in animals fed a HFD, which is consistent with WT animal measurements (886.63 ± 127.61 IU/L). The lactate dehydrogenase levels in Arv1/− animals were lower than that seen in NC fed male C57BL/6 mice (483.50 ± 98.18 IU/L); for example, see [http://www.taconic.com/phenotypic-data/automated-clinical-chemistry-analysis/index.html](http://www.taconic.com/phenotypic-data/automated-clinical-chemistry-analysis/index.html). Alanine aminotransferase, aspartate aminotransferase, and \( \gamma \)-glutamyl transferase levels in Arv1/− animals were similar to values observed for WT animals.

Arv1/− mice accumulate stearic acid and have decreased SCD1 expression in the liver – Total free fatty acid levels were reduced in the livers of Arv1/− animals. Thus, we determined the fatty acid composition of Arv1/− livers using GC/MS. Arv1/− animals accumulated stearic acid (18:0) and arachidonic acid (20:0), and had reduced levels of oleic acid (18:1\( \Delta 9 \)), whether fed NC or a HFD (Fig. 13A & B; WT, closed circles; KO, open circles).

Stearic acid is the precursor for the synthesis of oleic acid and is a substrate for the stearoyl-CoA desaturase 1, SCD1. SCD1 activity is the rate-limiting step in the production of oleic acid by adding a double bond to stearic acid, which leads to the production of monounsaturated fatty acids.

As SCD1 activity modulates the level of stearic acid, we determined its mRNA expression and protein levels. SCD1 mRNA expression was drastically reduced in Arv1/− animals compared to WT animals (data not shown). The reduction in mRNA expression directly correlated with reduced SCD1 protein level (Fig. 13C & D; WT, closed circles; KO, open circles; Fig. S2).

FXR protein levels are increased in the livers of Arv1/− mice - It was shown previously that mice treated with ASOs to ARV1 showed signs of liver FXR (farnesol X receptor) signaling activation (20). FXR signaling results in both an inhibition of SREBP1-c (sterol response element binding protein 1c)-dependent transcription and maturation, leading to reductions in the expressions of FAS and ACC1, and activation of PPARα (peroxisome proliferator-activating receptor α)-dependent signaling resulting in increases in FGF21 (fibroblast growth factor 21) levels and fatty acid β-oxidation.

Based on these previous results, we examined the status of FXR signaling in the livers of Arv1/− animals. We first measured the protein levels of FXR, and its downstream target, SHP (small heterodimer protein). FXR protein levels were induced (Fig. 14A & B; WT, closed circles; KO, open circles; Fig. S3) and this correlated with a concomitant increase in SHP (Fig. 14C & D; WT, closed circles; KO, open circles; Fig. S3). These increases were not seen in WT animals.

We next examined the levels of the SHP downstream targets, CYP7a1 and CYP8b1. Both CYP7a1 and CYP8b1 are required for the synthesis of bile acids and their expressions are reduced upon FXR activation. We found that the protein levels of both CYP7a1 and CYP8b1 were reduced in the livers of Arv1/− animals when compared to those levels seen in WT livers (Fig. 14E-H; WT, closed circles; KO, open circles; Fig. S4). Moreover, mature SREBP1 protein levels were reduced (Fig. 14I & J; WT, closed circles; KO, open circles; Fig. S4).

PPARα levels are elevated in the livers of Arv1/− mice – We next looked at the status of PPARα signaling by determining FGF21 protein levels. Arv1/− animals displayed increases in PPARα (Fig. 15A & B; WT, closed circles; KO, open circles; Fig. S5) and FGF21 (Fig. 15C & D; WT, closed circles; KO, open circles; Fig. S5) protein levels, when compared to levels seen in WT animals.

Overall, our results strongly suggest that liver FXR and PPARα signaling pathways are activated in Arv1/− animals, with activation likely...
contributing to a reduction in liver lipogenesis and reduced signs of NAFLD.

Arv1/ mice have an altered bile acid composition in the SI - Liver cholesterol is a substrate for bile acid synthesis. Bile acids are secreted into the SI for fecal excretion and/or reabsorbed by the portal circulatory system. Any changes in the flux of bile acid transport and/or metabolism could affect liver cholesterol homeostasis. We found that the CYP7A1 and CYP8B1 enzymes required for the synthesis of bile acids were down regulated. It is feasible that this may cause defects in bile acid composition in Arv1/- animals. Thus, we used GC/MS to determine the intestinal bile acid composition of Arv1/- animals.

We first determined the lipid content in the feces in order to determine whether there were global changes in overall lipid levels. Arv1/- animals had increased levels of fecal cholesterol, fatty acids, and total bile acids compared to WT animals (Fig. 16A-C; WT, closed circles; KO, open circles). Analysis of bile acid composition in the SI showed that knockouts had a larger bile acid pool (Fig. 16D; WT, closed circles; KO, open circles) that was more hydrophobic (Fig. 16E; WT, closed circles; KO, open circles). Changes were observed in the levels of TMC (taumuricholic acid), TUDC (tauroursodeoxycholic acid), TC taurocholic acid), and TDC (taurodeoxycholic acid) (Fig. 16F).

The levels of intestinal FXR are induced but this does not lead to increased SHP protein - TMC is a known antagonist of FXR signaling. Thus, we asked whether there was a relationship between TMC accumulation and decreased FXR effects in the SI of Arv1/- animals. Interestingly, FXR protein levels were induced when compared to WT animals (Fig. 17A & B; WT, closed circles; KO, open circles; Fig. S6). However, FXR accumulation did not result in the accumulation of SHP (Fig. 17C & D; WT, closed circles; KO, open circles; Fig. S6).

DISCUSSION

Arv1/- animals fed a HFD 1) were resistant to diet-induced obesity, (i) although they consumed more chow, (ii) had higher levels of ghrelin (iii), and lower levels of leptin, 2) had increased energy expenditure, 3) had reduced levels of blood cholesterol and TAGs, 4) retained glucose tolerance and insulin sensitivity, 5) had reduced fat deposition in several fat depots, 6) had reduced levels of liver cholesterol, TAGs, and fatty acids, 7) excreted high levels of fecal cholesterol, TAGs, bile acids, and fatty acids, 8) had an altered bile acid composition in the SI, and accumulated the FXR antagonist, TMC, 9) show signs of activated FXR and PPARα signaling in the liver and lack of FXR signaling in the SI, and 10) showed little signs of NAFLD. Our results strongly suggest that complete loss of Arv1 protects mice from acquiring MetS and NAFLD in response to a HFD challenge. While it is still up for debate as to what is the function of ARV1 (13-15,18,19,41), evidence is accumulating that suggests it’s involvement in maintaining proper sterol distribution (16,20,42,43). As was shown previously (23,24), our Arv1/- animals had reduced survival rates and suffered from seizures, which occasionally resulted in animals being euthanized. In addition to impaired locomotor coordination, Arv1/- mice were more active than WT mice in an open field, suggesting a generalized neurological defect. Finally, we found no fertility defects, although pups born to Arv1/- females all died due to maternal neglect.

Genetic linkage studies have associated SNPs in the hARV1 locus with prostate cancer (44), while other SNPs have been identified in searches for loci regulating 1) LDL-C and ApoB levels (45), 2) obesity, 3) diabetes, 4) plasma HDL levels, 5) body weight, 6) blood pressure, and 7) fat response to dietary restriction (http://rgd.mcw.edu/rgdweb/search/qtls.html?term =Arv1%5Bgene%5D&speciesType=3). It is interesting that several of these parameters are perturbed in Arv1/- animals.

There is also a neurological link between specific mutations in the cds of ARV1 and epileptic encephalopathy (24,46). The mutations identified result in a single amino acid change (Gln189Arg), or a near complete deletion of the AHD (Lys59-Asn98) (24,46). MRI brain imaging of infants with this disorder shows the appearance of brainstem lesions (24). Mouse model studies using a neuronal knockout of mARV1 found that these mice suffered from seizures and had reduced survival rates (24). Our preliminary histology results looking at brain sections of WT and Arv1/-
ARV1 regulates MetS syndrome

Animals post HFD revealed no differences in gross morphology (white and gray matter) and neuron numbers (C. Gallo-Ebert and J.T. Nickels, unpublished data). As loss of ARV1 has now been linked to several disease states, it will be important to uncover the exact molecular function of ARV1 and how this relates to the regulation of lipid homeostasis, neurological function, and possibly cancer progression.

The lack of weight gain of Arv1-/− animals fed a HFD is highly interesting in light of increased food consumption, elevated levels of ghrelin, and lower levels of leptin. The opposite correlation has been seen in obese individuals, where leptin levels are increased and ghrelin levels decreased (32,33). These individuals are considered leptin resistant (47). Obesity has also been associated with reduced adiponectin secretion (48). Our Arv1-/− animals secreted more adiponectin per gram of fat mass and had a higher adiponectin: leptin ratio (~9:1) then WT animals. A high ratio seems to be critical for establishing good “metabolic” health (49); an adiponectin:leptin ratio > 1.0 is considered advantageous. Adiponectin activates PPARα signaling through binding to the adiponectin receptor, AdipoR2 (50). FXR signaling also activates PPARα. PPARα activation increases FGF21 levels. FGF21 improves energy production by increasing the utilization of fatty acids (51) and is a potent inhibitor of hepatic steatosis (52). Our results showed that FGF21 levels were elevated in Arv1-/− animals. Thus, FGF21 elevation may be involved in the generation of the beneficial phenotypes seen in knockout animals. We do point out that elevated FGF21 levels are seen during insulin resistance (53). However, this elevation is thought to be a compensatory mechanism that is activated in response to hyperglycemia, giving rise to a FGF21 resistance phenotype (54).

Arv1-/− animals showed alterations in bile acid composition in the SI. Of particular interest was the increase seen in TMC. TMC is a potent FXR antagonist and its accumulation decreases FXR signaling in the intestine (55-57). Intestinal FXR knockout animals have been shown to be resistant to diet-induced obesity, insulin resistance, and NAFLD (58-60). It has also been shown that loss of intestinal FXR activity leads to induced GLP-1 (glucagon-like peptide 1) production, which leads to improved glucose metabolism (61). We saw a larger bile acid pool with a high hydrophobicity index in the SI of Arv1-/− animals. One possible explanation for the presence of this increased pool size may be related to the status of FXR signaling in the SI. FXR activation in the SI induces the transport of intestinal bile acids into the portal vein for recycling back to the liver (62). The accumulation of TMC in Arv1-/− animals would presumably cause FXR inhibition, which would lead to a reduction in bile acid recycling back to the liver, thus a larger bile acid pool. Studies using FXR knockout animals have shown that their total bile acid pool size is increased compared to WT animals (63). It is interesting that Arv1 ASO treatment in mice activates the FXR pathway in the liver (20). We saw the same in the livers of Arv1-/− animals. This may suggest that Arv1-/− animals have opposing FXR signaling activities, where it exists in an activated state in the liver, while being inhibited in the SI.

Our Arv1-/− animals also excreted higher levels of fecal cholesterol and fatty acid, but less TAGs. Our hypothesis is that increased cholesterol hydrophobicity index causing less fat solubilization. In the case of the lower TAGs excretion, loss of Arv1 may result in MGAT (monoacylglycerol acyltransferase) and/or DGAT (diacylglycerol acyltransferase) inhibition (64). In this scenario, pancreatic lipase would convert TAGs to monoacylglycerol and FFAs (65), which are substrates for intestinal TAG resynthesis by MGAT and DGAT. Inhibition of either MGAT and/or DGAT may explain why we see an increase in fecal excretion of FFA. It is important to point out that MAG and DAG are signaling molecules that regulate multiple metabolic-related events including fat metabolism in the liver, insulin sensitivity in muscle, and appetite (64).

Drug discovery efforts targeting MGAT inhibition are ongoing for treating metabolic disorders, based on the fact that MGAT2 deficient mice are resistant to diet-induced obesity, NAFLD, hyperlipidemia, and glucose intolerance (66-69). DGAT1 deficient mice are also resistant to high fat diet-induced obesity (70,71), while ASO silencing of DGAT2 results in reduced liver TAGs levels (72). Targeting DGAT1 has met with some difficulty due to severe gastrointestinal side effects (73-75), while targeting DGAT2 has been gaining traction (76). Whether it is feasible...
ARV1 regulates MetS syndrome

to target ARV1 inhibition for treating MetS awaits further investigation into defining its molecular function.

Lipodystrophy is associated with a deficiency in adipose tissue mass (77). Arvl/animals are lipodystrophic, as they have severely reduced fat stores including brown fat. However, these animals show little signs of the hepatic steatosis, insulin resistance, T2D, and hypertriglyceridemia associated with this phenotype (78,79). They are, however, hyperphagic, but this may be caused by increased energy expenditure. Adipose tissue deficiency leads to the reduced secretion of leptin and adiponectin. While Arvl/animals do secrete less leptin, they secrete elevated levels of adiponectin. Thus, Arvl/animals may represent a unique type of lipodystrophy model that is resistant to acquiring multiple metabolic pathologies.

Finally, our Arvl/animals had reduced levels of HDL whether fed NC or a HFD. Diets high in fat can cause HDL levels to rise in the mouse (80-82), and we observed this in WT animals. In addition to dietary modulation, genetic mouse models have shown that overexpression of human CETP (cholesterol ester transfer protein) (83), and reductions in ApoA-I (apolipoprotein A-I) (84) or ApoA-II (apolipoprotein A-II) levels (85,86) can cause reductions in HDL levels. Interestingly, the C57BL/6J mouse strain used to generate our Arvl/animals carries the Ath-I susceptibility allele that causes reductions in HDL levels when animals are fed an atherogenic diet (87,88). So differences in dietary content and genetic background can greatly influence HDL metabolism. Interestingly, although HDL levels were reduced in Arvl/animals, their aortas showed less fatty infiltrate and retained plasticity (H-Y Liu, C. Gallo-Ebert and J.T. Nickels, unpublished data).

Labor et al., have recently (23) examined the metabolic effects brought about by complete loss of ARV1. Their Arvl/Arvl mice did not gain weight, had improved glucose tolerance, secreted elevated adiponectin levels, had a high metabolic rate, and had less WAT. A subset of the data presented in this study was obtained using animals fed NC. Here, we examined how Arvl/animals responded metabolically to being fed a HFD. We point out that the KO line described in our paper was generated using a different strain of ES cells and gene targeting strategy as compared to the Arvl KO mice previously described (23,24), indicating the observed phenotypes are robust and indeed related to the inactivation of the Arvl gene. Thus, the current study describes the contribution of Arvl to the DIO model phenotypes. Moreover, the model used was congenic to the C57BL/6J strain, the standard mouse strain used for the DIO models commercially available.

Overall, our results strongly suggest that FXR signaling is activated in the livers of Arvl/animals, which leads to resistance to acquiring diseases associated with MetS and NAFLD under condition of excess fat consumption (Fig. 18). First, knockout livers had decreased SREBP1-c maturation, possibly leading to the decreases in fatty acid and triglyceride levels observed. Second, liver CYP7α1 and CYP8β1 expressions were attenuated, and these decreases were accompanied by changes in intestinal bile acid composition. In particular, we observed the accumulation of the FXR antagonist, TMC. Interestingly, Cyp7α1/mice are protected from diet-induced onset of MetS disorders, have an up regulated alternative bile acid synthetic pathway, and show a moderate increase in TMC (89). TMC accumulation may be the reason for the lack of intestinal FXR signaling observed. Beneficial metabolic effects have been seen when both FXR signaling is inhibited in the intestines but activated in the liver (55,90).

Third, PPARα protein levels were increased and there was a concomitant increase in its target, FGF21. Elevated FGF21 levels are thought to attenuate the severity of NAFLD progression (91). Finally, and more speculative, Arvl/animals may harbor a malabsorption defect that causes a decrease in dietary fat intake. Arvl/animals excrete more cholesterol, bile acids, and free fatty acids, and they have an indirect correlation between increased excretion, and lower cholesterol and triglycerides blood levels.

In conclusion, we hypothesize that ARV1 plays a critical role in the development of MetS and NAFLD during conditions of lipid toxicity, possibly by preventing the activation of liver FXR signaling. Beyond the scope of this work, studies are underway looking at the metabolic phenotypes of Arvl/ Fxr/animals fed a HFD diet. The availability of liver and intestinal-specific Fxr knockouts will precisely define the relationship
between ARV1 function and regulation of FXR signaling.

EXPERIMENTAL PROCEDURES

Generation of Arv1/- mice - GenOway (Jean Jaures Lyon, France) generated whole body Arv1/- knockout mice using the Cre-Lox system. Briefly, a homologous recombination targeting construct with a floxed exon 5 and exon 6 was used to produce a stable conditional ARV1 knockout in C57BL/6J ES cells. Recombinant ES cells were then injected into C57BL/6 albino (B6(Cg)-Tyr<sup>e2J</sup>) recipient blastocysts, followed by implantation into pseudo-pregnant albino C56BL/6 females for the purpose of generating chimera offspring (floxed mice). Upon breeding with C57BL/6 Cre deleter mice, exons 5 and 6 of Arv1 flanked by loxP sites were excised, resulting in constitutive whole body Arv1/- knockout mice. Mice were bred to the same C57BL/6J strain in order to maintain congenicity. Heterozygous parents were crossed to produce homozygous Arv1/- mice. The Invivotek IACUC approved all studies.

Total RNA Isolation and Quantitative Real-time PCR - Total RNA was extracted with TRIzol reagent (Invitrogen). cDNA was synthesized from total RNA using RT Easy First Strand Kit (Qiagen). qRT-PCR was carried out using a Stratagene MX3005P (Stratagene). Relative mRNA levels were normalized to levels of GAPDH. The data are representative of 5 independent experiments done in triplicate.

Protein extraction and western analysis - Soon after the mice were euthanized, tissues were dissected and cleaned of adhering fat and soft tissues. They were washed in ice cold PBS to remove blood from tissues, snap frozen in liquid nitrogen, and stored at -80°C until further processing. Tissue lysates were prepared by homogenization in RIPA buffer containing phosphatase and protease inhibitors. Tissue debris was removed by centrifugation, and protein concentration was determined using the Bradford Assay (Bio-Rad).

For western analysis, we used Ponceau S staining to determine if the same amount of total protein was loaded and transferred (92,93). Cell lysates to be analyzed were resuspended in protein sample buffer and incubated at 95°C for 10 min. All samples were subjected to 10% SDS-PAGE. Resolved proteins were transferred onto a nitrocellulose membrane. The immunoblot membranes were then blocked for 1 hour with 10% milk and washed once with TBST (Tris-buffered saline, 0.1% Tween 20). (n=8).

The membranes were incubated with primary antibody overnight, at the appropriate dilution. After 5 washes with TBST for 10 minutes each, membranes were incubated with appropriate secondary antibody for 1 hour. After 5 washes with TBST for 10 min each of the membranes were immersed in chemiluminescent agent and exposed for 2 to 5 minutes; β-actin (Abcam) was also used as a loading control at 1:1,000 dilution (Figs. S1-S6).

ARV1 protein levels were determined by chemo-luminescence. Anti-ARV1 polyclonal antibodies were from Abgent (#AP10655a) and used at a 1:500 dilution; Anti-SCD1 polyclonal antibodies were from Abcam (#ab39969) and used at a 1:500 dilution, anti-FXR polyclonal antibodies were from Novus Biologicals (#NB00-153) and used at a 1:500 dilution; anti-SHP polyclonal antibodies were from Abcam (#ab656596) and were used at a 1:500 dilution, anti-CYP7α1 polyclonal antibodies were Abcam (#ab65596) and were used at a 1:1,000 dilution. anti-CYP8β1 polyclonal antibodies were from Santa Cruz (sc-365513) and were used at a 1:250 dilution. We consistently observed a lot-to-lot variability in the specificity of the Abgent anti-ARV1 antibodies. To circumvent this problem, several lots were tested and 1 mg of the lot with the highest specificity was purchased and used. The immunoblots shown are representative of three independent westerns (n=8).

Survival studies – The age of death was recorded for all males used in the HFD and regular diet branches. Data were graphed using survival curve and analyzed using the Mantel-Cox test (GraphPad Prism 5).

Rotarod studies - Individually housed male WT and Arv1/- mice aged 9-14 weeks were used for this experiment. Mice were acclimated to the testing room for at least 30 minutes. The assay was carried out using four EzRod test chambers. For the accelerating Rotarod paradigm, mice were given 10 trials with the maximum duration of 3 min and a 30-sec inter-trial interval (ITI). Each
mouse was placed on the EZRod machine and the latency to fall was recorded for all trials. If the mouse fell or 3 min elapsed, the mouse was left in the bottom of EzRod test chamber for 30 sec before starting the next trial.

The latency to fall was compared between the three groups by analysis of variance (ANOVA) with repeated measures using Bonferroni corrected post hoc analysis for pair wise comparisons (GraphPad Prism 5).

**Open field test** - Individually housed male WT, and Arv1-/- animals aged 10-14 weeks were used for this experiment. The assay was performed in a custom-made open field apparatus. Each chamber is a 50 by 50 cm square. The experiment is recorded and tracked by a tracking system obtained from Viewpoint.

The time and the path length in the center of the open field were determined. The center of the open field was defined as a 13.5 x 13.5 cm square in the geometric center of the arena. The percent of path in the center was calculated as:

\[
\text{Path length in the center} \times 100\% / \text{total path length}
\]

For each mouse the total path length and path length for 30 min at 5 min intervals were determined as measures of locomotor activity. Each chamber was cleaned between individual testing.

Path length for 30 min at 5 min intervals were analyzed by a two-way ANOVA with time as a within subjects (repeated measures) factor and genotype as a between groups factor using GraphPad Prism 5.0. All other parameters were compared using a one-way ANOVA (GraphPad Prism).

**Fertility Assay** - Male Fertility: Sexually mature males (~3 months old) were paired with two Institute of Cancer Research (ICR) females (6 weeks old) on Day 1.

Female Fertility: Sexually mature females (6-7 weeks old) were paired with an ICR male (8 weeks old) on Day 1. The presence of a vaginal plug was checked every day for 6 days then the male was removed. Females were checked daily and size and birth dates of litters were recorded. Litter size was analyzed by a one-way ANOVA. Mann-Whitney was used to analyze the number of days, and Chi-Square was used to analyze the plug/no plug and litter/no litter data (GraphPad Prism 5).

**Longitudinal Body Weights and Diet Challenge** - Individually housed male Arv1-/- and WT mice were maintained on a 12 hr Light:Dark cycle with food and water fed ad libitum unless otherwise noted. Mice were initially maintained on a regular chow diet (Purina rodent diet #5053, W. F. Fisher and Son, Bound Brook, NJ) and baseline body weights were established when the mice were 8 weeks old. At age 12 weeks mice were either left on normal chow or switched to a high fat diet (HFD), (60% fat, D12492, Research Diets, New Brunswick, NJ) and body weights were collected over an 11 week period (Fig. 6). The mice were divided into three branches with 8 males in each. Branch A was fed NC for the entire study. Branch B animals were given normal chow for the first 4 weeks and then switched to a HFD for 11 weeks. Branch C mice were sacrificed at the beginning of the study. In some studies a “western diet” (WD, RD12079B Research Diets, New Brunswick, NJ) was used as the high fat diet. Body weights were analyzed by a two-way ANOVA with genotype as a between group factor and the time course as a within subject factor (repeated measures).

**Adiponectin and leptin determination** - Blood was collected from the retro-orbital sinus under isoflurane anesthesia from mice fasted overnight. The blood was allowed to coagulate for 30 minutes at room temperature and centrifuged at 1000 X g for 15 min at 4°C to collect serum. The serum was immediately frozen and stored at -80°C. Adiponectin concentrations were determined from serum samples by electrochemiluminescence (MA2400 Mouse Adiponectin kit K152BXC, Meso Scale Discovery). Serum leptin concentrations were determined by electrochemiluminescence according to the manufacturer’s recommendations (MS2400 Mouse Leptin Kit, K152BYC-2 Meso Scale Discovery). Data were analyzed by an unpaired t-test.

**Ghrelin determination** - Blood samples were collected in tubes containing EDTA (0.5 mg/ml final, 1:40 dilution) and PHMB (0.4 mM final, 1:100 dilution) to prevent degradation of acylated ghrelin by proteases. Samples were centrifuged at 4,000 rpm for 10 minutes at 4°C and supernatants were transferred to separate tubes. 1N HCl was added at a 1:10 dilution. Samples were frozen at -80°C and Acyl (AG) and de-acyl (DAG) ghrelin
concentrations in plasma were evaluated by specific EIA (SPIbio bertin pharma); A05118 for the de-acylated form; A05117 for the acylated form.

Adipose Distribution - Adipose tissue dissections were performed on 18-21 week old males following 11 weeks on a HFD. Adipose tissue from the inguinal fat pad (subcutaneous) and the visceral fat from the epididymal, renal (retroperitoneal) and mesenteric fat depots. The data were analyzed by an unpaired t-test for each adipose depot individually.

Metabolic studies - Metabolic studies were performed using the Comprehensive Cage Monitoring System (Columbus Instruments, Columbus, OH). This system allows for the long-term automated, non-invasive simultaneous collection of 9 measurements. The experimental window lasted 48 hours, beginning and ending at the start of the dark phase of the light cycle. During that time the following end-points were measured:
- Heat - kcal/kg/hr (calculated by the Oxymax Software using O₂ consumption, CO₂ production and body weight)
- VO₂ - oxygen consumption in ml/kg/hr (calculated by the Oxymax software using O₂ differential and body weight)
- VCO₂ - carbon dioxide production in ml/kg/hr (calculated by the Oxymax software using CO₂ differential and body weight)
- RER - respiratory exchange ratio (VCO₂/ VO₂) (calculated by the Oxymax software)

Oxygen and carbon dioxide concentrations were sequentially measured. The time required for a single round of gas measurements in all of the chambers was defined as an interval. The interval lengths may have varied slightly depending on the environmental parameters of the room (temperature and humidity) and the physiological parameters of the experimental mice. On average, each interval was 48 minutes.

For statistical analysis, all data were averaged into individual days. Data were analyzed by a two-way ANOVA. The Bonferroni adjusted post-hoc procedure was used for pair-wise comparisons. The body weights of the animals were measured before placing the mice in the CCMS chambers and when they were removed after 48 hours; the change in body weight was used as a covariate in the ANOVA analysis of VO₂, VCO₂, and energy expenditure, as these measures use body weight as a constant in their calculation.

Serum Cholesterol Levels - Serum cholesterol levels were measured in WT and Arv1/− male mice after an overnight fast (16 hr) at the time points shown (Fig. 6). Blood was collected from the retro-orbital sinus, and cholesterol concentrations were determined enzymatically from serum (Thermo DMA, Arlington, TX). An unpaired t-test was used to analyze differences between the genotypes.

Triglyceride assay - Triglyceride levels were measured after an overnight fast (16 hr) (Fig. 6). Whole blood was collected via the retro-orbital sinus and triglycerides were assessed using a handheld meter (CardioChek, PTS Inc. Indianapolis, IN) with test strips specific for triglyceride measurements. An unpaired t-test was used to analyze differences between the genotypes.

Body Composition by DEXA - DEXA was performed at the indicated times (Fig. 6). The body composition was assessed using the PIXImus2 X-ray unit (GE Lunar Corporation, Madison, WI) excluding the head region. An unpaired t-test was used to analyze any differences between the genotypes for each measurement.

Oral Glucose Tolerance Test and Insulin Measurements - OGTT was performed after an overnight fast (~16 hr) (Fig. 6). Blood glucose levels were measured using a One-touch Ultra 2 (Lifescan, Johnson & Johnson) glucometer at baseline, just before the mice received 2 g/kg body weight of 100 mg/ml glucose delivered by oral gavage. Blood glucose was subsequently measured 15, 30, 60, and 120 minutes after the administration of glucose. Parallel samples for measuring plasma insulin levels were also collected and assayed by electrochemiluminescence (MA2400 Mouse/Rat insulin kit K152BZC, Meso Scale Discovery).

Glucose and insulin results were analyzed by a two-way ANOVA with genotype as a between group factor and the time course as a within subject factor (repeated measures). The pre-gavage blood glucose and insulin levels were used as the baseline values for calculating the AUC for each subject. Baseline blood glucose and plasma insulin levels and AUC data were also analyzed by an unpaired t-test comparing results from the diets separately.
**Insulin tolerance test** - Prior to testing, the mice were fasted for 4 hours. Blood was obtained from a tail cut (by removing the distal 2 mm of the tail) and was assessed for baseline glucose levels using a One-touch Ultra 2 (Lifescan, Johnson & Johnson) glucometer. The mice then received the indicated concentration of normal insulin (Novolin R) by intraperitoneal injection. At 15, 30, 60, 90 and 120 minutes after the administration of insulin, dried blood and tissue were quickly removed from the tail wound to measure the blood glucose concentration.

**Lipid extraction** - 100 mg of liver, small intestine tissues, or feces, was homogenized using a mortar and pestle. The resulting homogenate was extracted with 18 volumes of a mixture of hexane/2-propanol (3:2) for 1 minute in 15 ml conical tubes. Samples were vortexed for 20 minutes at low speed (2,000 rpm), and subsequently centrifuged at 5,000 X g for 4 minutes at 4°C. The supernatant was transferred to a glass conical tube and subsequently washed with 1/5 volume of 0.9% NaCl. The sample was centrifuged at 5,000 X g for 4 minutes at 4°C, and the aqueous phase was removed. The lower phase was dried down under a stream of nitrogen and resuspended in isopropanol until use (94).

**Apolipoprotein analysis** - HDL, LDL and VLDL (very low density apolipoprotein) levels were measured using LipoPrint LDL system (LipoPrint LDL Subfraction kit, No. 48-7002; Quantimetrix, Redondo Beach, CA) according to the manufacturer's instructions. The results from the lipoprint system are directly quantified as a relative percent from the LipoPrint system and the quantity of total cholesterol measured in the previous total cholesterol assay (% of total cholesterol x total cholesterol).

**Hepatic triglyceride export assay** - Mice fed NC were fasted for 4 hours. They were then dosed with 500 mpk of Pluronic F127 (Polaxamer 404) by ip injection. 5ml/kg of olive oil was given to each animal. Blood was then obtained from the tail vein at the indicated times. TAG levels were determined using the L-Type Triglyceride M Microtiter Procedure (WAKO).

**Histology** - Tissues were fixed in 10% neutral-buffered formalin, processed for 12 hr using Thermo Scientific Excelsior ES, embedded in paraffin blocks, and cut on 4 microns using a Sakura SRM200 microtome. Leica manufacture protocols were used for H&E staining. All stains were performed on a Leica ST5020 automated stainer. H&E processed tissues mounted on glass slides were examined using a Nikon Elipse 50i Clinical Microscope. Tissues were examined under 4X, 10X and 40X magnification. Pictures were taken with an INFINITY 1-2CB microscope camera. The chronic hepatitis assessment scheme used for this study was the “Scheuer Classification for Grading and Staging of Chronic Hepatitis” (95).

**Serum Clinical Chemistries** - Serum was collected by a cardiac bleed from 18-21 week old Arv1-/- (n=4) and WT (n=8) mice following 11 weeks on a HFD and analyzed on an ACE Alera (Alfa Wasswerman) for multiple analytes according to manufacturer's instructions.

**Lipid level assays** - 20 µl of sample, calibrator, and control were added to 150 µl of reconstituted reagent CC3 in a micro plate. The reaction was mixed well by repeated pipetting. The plate was incubated at 37°C for 5 minutes. 30 µl of reagent CC2 was mixed in the reaction. The increase in absorbance after 5 minutes at 37°C was measured using plate reader at 540nm. BA concentration= [((sample A540Blank A540)/(calibrator A540Blank A540]] X calibrator concentration.

Samples were brought up in assay buffer. 2 µl of Acyl CoA synthesis reagent was added to each sample and mixed. Samples were incubated at 37°C for 30 minutes. A Reaction mix containing assay buffer (44 µl), fatty acid probe (2 µl), enzyme mix (2 µl), and enhancer (2 µl) was added. Samples were mixed and incubated for 30 minutes at 37°C in the dark. OD₅₇₀ was measured in a micro plate reader. FFA concentration = FA/Sv (mM), where FA is the fatty acid amount in the well obtained from the standard curve and Sv is the sample volume added to the sample well. Samples were brought up in assay buffer. 2 µl of Acyl CoA Synthesis reagent was added to each sample and mixed. Samples were incubated at 37°C for 30 minutes. Reaction mix containing assay buffer (44 µl), fatty acid probe (2 µl), enzyme mix (2 µl), and enhancer (2 µl) was added. Samples were mixed and incubated for 30 minutes at 37°C in the dark. OD₅₇₀ was measured in a micro plate reader. FFA concentration = FA/Sv (mM), where FA is the fatty acid amount in the well obtained from the standard curve and Sv is the sample volume added to the sample well.
well obtained from the standard curve and $S_v$ is the sample volume added to the sample well.

*Liver fatty acid analysis* - Fatty acids were extracted from frozen, homogenized tissue, and were saponified by a modified Bligh-Dyer method (96). Fatty acids were converted to methyl esters by treatment with boron trifluoride, and analyzed by gas chromatography on a Hewlett-Packard model 6890 gas chromatograph using a 30 m HP-5 column. Instrument conditions were as previously described (97). Identification of fatty acid species was by comparing the retention times to those of authentic standards (Nu-Chek Prep) or by GC-mass spectrograph analysis.

*Small intestine bile acid analysis* - Bile acid analysis was performed as described (98).

**Acknowledgements:** We are grateful for the financial support of Genesis Biotechnology Group. We acknowledge the histology work performed by Dr. Jing Jing Yang and Mariya Nestor at the Institute of Biomarker Research. We acknowledge the Cohen Laboratory for analyzing bile acid composition. We thank Pedro Martins and Elka Devash for the graphic design in figure 18. We appreciate the discussions with our colleagues at the Institute of Metabolic Disorders, Inivovetek, Oncoveda, Femeris, and Genesis Biotechnology Group. We appreciate the intellectual conversations with Drs. Eli Mordechai and Martin Adelson. We acknowledge the many members of Invivotek team for their expertise.

**Conflict of interest:** The authors declare no conflicts of interest with the contents of the article.

**Author contributions:** JTN wrote the manuscript. JTN, OB, CG-E, VM, MDH, and BKJ were involved in the design of the experiments and interpretation of the data. CG-E, JF, H-YL, RD, KM, and VM performed the experiments described in the manuscript.

**Abbreviations:** ACC1, acetyl-CoA carboxylase; AHD, ARV1 homology domain; ApoA-I, apolipoprotein AI; ApoA-II, apolipoprotein AII; ASO, anti-sense oligonucleotides; cds, coding sequence; CETP, cholesterol ester transfer protein; Chol, cholesterol; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; DEXA, Dual-Energy X-ray Absorptiometry; DIO, diet-induced obesity; ER, endoplasmic reticulum; FAS, fatty acid synthase; FGF21, fibroblastic growth factor 21; FFAs, free fatty acids; FXR, farnesol X receptor; GC, gas chromatography; GC, glycocholic acid; GPl, glycoprophosphatidylinositol; GUDC, glycoursodeoxycholic acid; HDLC, high-density lipoprotein cholesterol; HFD, high fat diet; ICR, Institute of Cancer Research; ITI, inter-trial intervals; ITT, insulin tolerance test; KO, knockout; LDL-C, low-density lipoprotein cholesterol; LDLR-C, low-density lipoprotein receptor; MAG, monoacylglycerol; MAT1, monoacylglycerol acyltransferase 1; MS, mass spectrometry; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NC, normal chow; NKO, nestin-Cre ARV1 neuronal knockout; OLTT, oral lipid tolerance test; PPARA, peroxisome proliferator-activated receptor a; PIP2, phosphatidylinositol 4,5, bisphosphate; PL, phospholipid; PS, phosphatidyserine; SI, small intestine; SL, sphingolipid; SREBP, sterol response element binding protein; TAGs, triglycerides; TBST, T2D, type II diabetes; Tris-buffered saline, 0.1% Tween 20; TC, taurocholic acid; TCDC, taurochenodeoxycholic acid; TDC, taurodeoxycholic acid; TMC, tauromuricholic acid; ts, temperature sensitivity; TUDC, taurosodeoxycholic acid; VLDL, very low-density lipoprotein cholesterol; WAT, white adipose tissue; WT, wild type.
REFERENCES

1. Dietrich, P., and Hellerbrand, C. (2014) Non-alcoholic fatty liver disease, obesity and the metabolic syndrome. *Best Pract Res Clin Gastroenterol* **28**, 637-653

2. Fulop, T., Tessier, D., and Carpentier, A. (2006) The metabolic syndrome. *Pathologie-biologie* **54**, 375-386

3. Kaur, J. (2014) A comprehensive review on metabolic syndrome. *Cardiology research and practice* **2014**, 943162

4. Byrne, C. D., and Targher, G. (2015) NAFLD: a multisystem disease. *Journal of hepatology* **62**, S47-64

5. Alberti, K. G., Zimmet, P., and Shaw, J. (2006) Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabetic medicine: a journal of the British Diabetic Association* **23**, 469-480

6. Ruderman, N., Chisholm, D., Pi-Sunyer, X., and Schneider, S. (1998) The metabolically obese, normal-weight individual revisited. *Diabetes* **47**, 699-713

7. Than, N. N., and Newsome, P. N. (2015) A concise review of non-alcoholic fatty liver disease. *Atherosclerosis* **239**, 192-202

8. Targher, G., Marchesini, G., and Byrne, C. D. (2016) Risk of type 2 diabetes in patients with non-alcoholic fatty liver disease: Causal association or epiphenomenon? *Diabetes & metabolism* **42**, 142-156

9. Loomba, R., and Sanyal, A. J. (2013) The global NAFLD epidemic. *Nature reviews. Gastroenterology & hepatology* **10**, 686-690

10. Samuel, V. T., and Shulman, G. I. (2017) Nonalcoholic Fatty Liver Disease as a Nexus of Metabolic and Hepatic Diseases. *Cell metabolism*

11. Gluchowski, N. L., Becuwe, M., Walther, T. C., and Farese, R. V., Jr. (2017) Lipid droplets and liver disease: from basic biology to clinical implications. *Nature reviews. Gastroenterology & hepatology* **14**, 343-355

12. Saltiel, A. R., and Kahn, C. R. (2001) Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* **414**, 799-806

13. Tinkelenberg, A. H., Y.Liu, F.Alcantara, S.Khan,Z.Guo, M.Bard, and S.L. Sturley. (2000) Mutations in yeast ARV1 alter intracellular sterol distribution and are complemented by human ARV1. *J Biol. Chem* **275**, 40667-44070.

14. Swain, E., Stukey, J., McDonough, V., Germann, M., Liu, Y., Sturley, S. L., and Nickels, J. T., Jr. (2002) Yeast cells lacking the ARV1 gene harbor defects in sphingolipid metabolism. Complementation by human ARV1. *The Journal of biological chemistry* **277**, 36152-36160

15. Kajiwara, K., Watanabe, R., Pichler, H., Ihara, K., Murakami, S., Riezman, H., and Funato, K. (2008) Yeast ARV1 Is Required for Efficient Delivery of an Early GPI Intermediate to the First Mannosyltransferase during GPI Assembly and Controls Lipid Flow from the Endoplasmic Reticulum. *Mol Biol Cell* **19**, 2069-2082

16. Villasmil, M. L., Ansbach, A., and Nickels, J. T., Jr. (2011) The putative lipid transporter, Arv1, is required for activating pheromone-induced MAP kinase signaling in Saccharomyces cerevisiae. *Genetics* **187**, 455-465

17. Georgiev, A. G., Johansen, J., Ramanathan, V. D., Sere, Y. Y., Beh, C. T., and Menon, A. K. (2013) Arv1 Regulates PM and ER Membrane Structure and Homeostasis But is Dispensable for Intracellular Sterol Transport. *Traffic* **14**, 912-921

18. Schechtman, C., Henneberry, A., Seiman, T., Tinkelenberg, A., Lee, E., Fazlollahi, M. M., AB, Bussemaker, H., Tabas, I., and Sturley, S. (2011) Loss of subcellular lipid transport due to ARV1 deficiency disrupts organelle homeostasis and activates the unfolded protein respons. *J. Biol. Chem.* **286**, 11951-11959
ARV1 regulates MetS syndrome

19. Ruggles, K. V., Garbarino, J., Liu, Y., Moon, J., Schneider, K., Henneberry, A., Billheimer, J., Millar, J. S., Marchadier, D., Valasek, M. A., Joblin-Mills, A., Gulati, S., Munkacsi, A. B., Repa, J. J., Rader, D., and Sturley, S. L. (2014) A functional, genome-wide evaluation of liposensitive yeast identifies the "ARE2 required for viability" (ARV1) gene product as a major component of eukaryotic fatty acid resistance. The Journal of biological chemistry 289, 4417-4431

20. Tong, F., Billheimer, J., Shechtman, C. F., Liu, Y., Crooke, R., Graham, M., Cohen, D. E., Sturley, S. L., and Rader, D. J. (2010) Decreased expression of ARV1 results in cholesterol retention in the endoplasmic reticulum and abnormal bile acid metabolism. The Journal of biological chemistry 285, 33632-33641

21. Hulce, J. J., Cognetta, A. B., Niphakis, M. J., Tully, S. E., and Cravatt, B. F. (2013) Proteome-wide mapping of cholesterol-interacting proteins in mammalian cells. Nat Methods 10, 259-264

22. Niphakis, M. J., Lum, K. M., Cognetta, A. B., 3rd, Correia, B. E., Ichu, T. A., Olucha, J., Brown, S. J., Kundu, S., Piscitelli, F., Rosen, H., and Cravatt, B. F. (2015) A Global Map of Lipid-Binding Proteins and Their Ligandability in Cells. Cell 161, 1668-1680

23. Lagor, W. R., Tong, F., Jarrett, K. E., Lin, W., Conlon, D. M., Smith, M., Wang, M. Y., Yenilmez, B. O., McCoy, M. G., Fields, D. W., O'Neill, S. M., Gupta, R., Kumaravel, A., Redon, V., Ahima, R. S., Sturley, S. L., Billheimer, J. T., and Rader, D. J. (2015) Deletion of murine Arv1 results in a lean phenotype with increased energy expenditure. Nutr Diabetes 5, e181

24. Palmer, E. E., Jarrett, K. E., Sachdev, R. K., Al Zahrani, F., Hashem, M. O., Ibrahim, N., Sampaio, H., Kandula, T., Macintosh, R., Gupta, R., Conlon, D. M., Billheimer, J. T., Rader, D. J., Funato, K., Walkey, C. J., Lee, C. S., Loo, C., Brammah, S., Elakis, G., Zhu, Y., Buckley, M., Kirk, E. P., Bye, A., Alkuraya, F. S., Roscioli, T., and Lagor, W. R. (2016) Neuronal deficiency of ARV1 causes an autosomal recessive epileptic encephalopathy. Human molecular genetics 25, 3042-3054

25. Deacon, R. M. (2013) Measuring motor coordination in mice. Journal of visualized experiments : JoVE, e2609

26. Wall, P. M., and Messier, C. (2000) Concurrent modulation of anxiety and memory. Behavioural brain research 109, 229-241

27. Warita, K., Sugawara, T., Yue, Z. P., Tsukahara, S., Mutoh, K., Hasegawa, Y., Kitagawa, H., Mori, C., and Hoshi, N. (2006) Progression of the dose-related effects of estrogenic endocrine disruptors, an important factor in declining fertility, differs between the hypothalamo-pituitary axis and reproductive organs of male mice. The Journal of veterinary medical science 68, 1257-1267

28. Wang, H. Y., Li, Y. H., Sun, L., Gao, X., You, L., Wang, Y., Ma, J. L., and Chen, Z. J. (2013) Allotransplantation of cryopreserved prepubertal mouse ovaries restored puberty and fertility without affecting methylation profile of Snrpn-DMR. Fertility and sterility 99, 241-247

29. Khan, A., Bellefontaine, N., and deCatanzaro, D. (2008) Onset of sexual maturation in female mice as measured in behavior and fertility: Interactions of exposure to males, phytoestrogen content of diet, and ano-genital distance. Physiology & behavior 93, 588-594

30. Klok, M. D., Jakobsdottir, S., and Drent, M. L. (2007) The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. Obes Rev 8, 21-34

31. Flak, J. N., and Myers, M. G., Jr. (2016) Minireview: CNS Mechanisms of Leptin Action. Mol Endocrinol 30, 3-12

32. Balsan, G. A., Vieira, J. L., Oliveira, A. M., and Portal, V. L. (2015) Relationship between adiponectin, obesity and insulin resistance. Rev Assoc Med Bras 61, 72-80

33. Fasshauer, M., and Bluher, M. (2015) Adipokines in health and disease. Trends Pharmacol Sci 36, 461-470

34. Aguilar-Salinas, C. A., Garcia, E. G., Robles, L., Riano, D., Ruiz-Gomez, D. G., Garcia-Ulloa, A. C., Melgarejo, M. A., Zamora, M., Guillen-Pineda, L. E., Mehta, R., Canizales-Quinteros, S., Tusie Luna, M. T., and Gomez-Perez, F. J. (2008) High adiponectin concentrations are associated
ARV1 regulates MetS syndrome with the metabolically healthy obese phenotype. The Journal of clinical endocrinology and metabolism 93, 4075-4079

35. Fazio, S., and Linton, M. F. (2001) Mouse models of hyperlipidemia and atherosclerosis. Frontiers in bioscience : a journal and virtual library 6, D515-525

36. Fontaine, D. A., and Davis, D. B. (2016) Attention to background strain is essential for metabolic research: C57BL/6 and the International Knockout Mouse Consortium. Diabetes 65, 25-33

37. Ioannou, G. N. (2016) The Role of Cholesterol in the Pathogenesis of NASH. Trends in endocrinology and metabolism: TEM 27, 84-95

38. Koo, S. H. (2013) Nonalcoholic fatty liver disease: molecular mechanisms for the hepatic steatosis. Clinical and molecular hepatology 19, 210-215

39. Liu, W., Baker, R. D., Bhatia, T., Zhu, L., and Baker, S. S. (2016) Pathogenesis of nonalcoholic steatohepatitis. Cellular and molecular life sciences : CMLS 73, 1969-1987

40. Scheurer, P. J. (1991) Classification of chronic viral hepatitis: a need for reassessment. Journal of hepatology 13, 372-374

41. Georgiev, A. G., Johansen, J., Ramanathan, V. D., Sere, Y. Y., Beh, C. T., and Menon, A. K. (2013) Arv1 regulates PM and ER membrane structure and homeostasis but is dispensable for intracellular sterol transport. Traffic

42. Gallo-Ebert, C., McCourt, P. C., Donigan, M., Villasmil, M. L., Chen, W., Pandya, D., Franco, J., Romano, D., Chadwick, S. G., Gygax, S. E., and Nickels, J. T., Jr. (2012) Arv1 lipid transporter function is conserved between pathogenic and nonpathogenic fungi. Fungal Genet Biol 49, 101-113

43. Villasmil, M. L., and Nickels, J. T., Jr. (2011) Determination of the membrane topology of Arv1 and the requirement of the ER luminal region for Arv1 function inSaccharomyces cerevisiae. FEMS Yeast Res 11, 524-527

44. Berry, R., Schaid, D. J., Smith, J. R., French, A. J., Schroeder, J. J., McDonnell, S. K., Peterson, B. J., Wang, Z. Y., Carpten, J. D., Roberts, S. G., Tester, D. J., Blute, M. L., Trent, J. M., and Thibodeau, S. N. (2000) Linkage analyses at the chromosome 1 loci 1q24-25 (HPC1), 1q42.2-43 (PCAP), and 1p36 (CAPB) in families with hereditary prostate cancer. American journal of human genetics 66, 539-546

45. Feitosa, M. F., Borecki, I. B., Rankinen, T., Rice, T., Despres, J. P., Chagnon, Y. C., Gagnon, J., Leon, A. S., Skinner, J. S., Bouchard, C., Province, M. A., and Rao, D. C. (2005) Evidence of QTLs on chromosomes 1q42 and 8q24 for LDL-cholesterol and apoB levels in the HERITAGE family study. Journal of lipid research 46, 281-286

46. Alazami, A. M., Patel, N., Shamseldin, H. E., Anazi, S., Al-Dosari, M. S., Alzahrani, F., Hijazi, H., Alshammari, M., Alahmash, M. A., Faqeih, E., Alhashem, A., Bashiri, F. A., Al-Owain, M., Kentab, A. Y., Sogaty, S., Al Tala, S., Temsah, M. H., Tulbah, M., Aljelaify, R. F., Alshahwan, S. A., Seidahmed, M. Z., Alhadid, A. A., Alhalaan, H., AlQallaf, F., Kurdi, W., Alfadhel, M., Babay, Z., Alsogheer, M., Kaya, N., Al-Hassan, Z. N., Abdel-Salam, G. M., Al-Sanna, N., Al Mutairi, F., El Khashab, H. Y., Bohlega, S., Jia, X., Nguyen, H. C., Hammami, R., Adly, N., Mohamed, J. Y., Abdulwahab, F., Ibrahim, N., Naim, E. A., Al-Younes, B., Meyer, B. F., Hashem, M., Shaheen, R., Xiong, Y., Abouelhoda, M., Aldeeri, A. A., Monies, D. M., and Alkuraya, F. S. (2015) Accelerating novel candidate gene discovery in neurogenetic disorders via whole-exome sequencing of prescreened multiplex consanguineous families. Cell reports 10, 148-161

47. Crujeiras, A. B., Carreira, M. C., Cabia, B., Andrade, S., Amil, M., and Casanueva, F. F. (2015) Leptin resistance in obesity: An epigenetic landscape. Life sciences 140, 57-63

48. Padmalayam, I., and Suto, M. (2013) Role of adiponectin in the metabolic syndrome: current perspectives on its modulation as a treatment strategy. Curr Pharm Des 19, 5755-5763

18
49. Lopez-Jaramillo, P., Gomez-Arbelaez, D., Lopez-Lopez, J., Lopez-Lopez, C., Martinez-Ortega, J., Gomez-Rodriguez, A., and Triana-Cubillos, S. (2014) The role of leptin/adiponectin ratio in metabolic syndrome and diabetes. *Horm Mol Biol Clin Investig* **18**, 37-45

50. Yoon, M. J., Lee, G. Y., Chung, J. J., Ahn, Y. H., Hong, S. H., and Kim, J. B. (2006) Adiponectin increases fatty acid oxidation in skeletal muscle cells by sequential activation of AMP-activated protein kinase, p38 mitogen-activated protein kinase, and peroxisome proliferator-activated receptor alpha. *Diabetes* **55**, 2562-2570

51. Mai, K., Andres, J., Biedasek, K., Weichert, J., Bobbert, T., Sabath, M., Meins, S., Reinecke, F., Mohlig, M., Weickert, M. O., Clemenz, M., Pfeiffer, A. F., Kintscher, U., Spuler, S., and Spranger, J. (2009) Free fatty acids link metabolism and regulation of the insulin-sensitizing fibroblast growth factor-21. *Diabetes* **58**, 1532-1538

52. Liu, J., Xu, Y., Hu, Y., and Wang, G. (2015) The role of fibroblast growth factor 21 in the pathogenesis of non-alcoholic fatty liver disease and implications for therapy. *Metabolism* **64**, 380-390

53. Chavez, A. O., Molina-Carrion, M., Abdul-Ghani, M. A., Folli, F., Defronzo, R. A., and Tripathy, D. (2009) Circulating fibroblast growth factor-21 is elevated in impaired glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance. *Diabetes care* **32**, 1542-1546

54. Emanuelli, B., Vienberg, S. G., Smyth, G., Cheng, C., Stanford, K. I., Arumugam, M., Michael, M. D., Adams, A. C., Kharitonenkov, A., and Kahn, C. R. (2014) Interplay between FGF21 and insulin action in the liver regulates metabolism. *The Journal of clinical investigation* **124**, 515 - 527

55. Xie, C., Jiang, C., Shi, J., Gao, X., Sun, D., Sun, L., Wang, T., Takahashi, S., Anitha, M., Krausz, K. W., Patterson, A. D., and Gonzalez, F. J. (2017) An Intestinal Farnesoid X Receptor-Ceramide Signaling Axis Modulates Hepatic Gluconeogenesis in Mice. *Diabetes* **66**, 613-626

56. Qi, Y., Jiang, C., Cheng, J., Krausz, K. W., Li, T., Ferrell, J. M., Gonzalez, F. J., and Chiang, J. Y. (2015) Bile acid signaling in lipid metabolism: metabonomic and lipidomic analysis of lipid and bile acid markers linked to anti-obesity and anti-diabetes in mice. *Biochimica et biophysica acta* **1851**, 19-29

57. Sayin, S. I., Wahlstrom, A., Felin, J., Jantti, S., Marschall, H. U., Bamberg, K., Angelin, B., Hyotylainen, T., Oresic, M., and Backhed, F. (2013) Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell metabolism* **17**, 225-235

58. de Boer, J. F., Schoneville, M., Boesjes, M., Wolters, H., Bloks, V. W., Bos, T., van Dijk, T. H., Jurdzinski, A., Boverhof, R., Wolters, J. C., Kuivenhoven, J. A., van Deursen, J. M., Oude Elferink, R. P., Moschetta, A., Kremoser, C., Vink, B. J., Kuipers, F., and Groen, A. K. (2017) Intestinal Farnesoid X Receptor Controls Transintestinal Cholesterol Excretion in Mice. *Gastroenterology* **152**, 1126-1138 e1126

59. Parseus, A., Sommer, N., Sommer, F., Caesar, R., Molinaro, A., Stahlman, M., Greiner, T. U., Perkins, R., and Backhed, F. (2017) Microbiota-induced obesity requires farnesoid X receptor. *Gut* **66**, 429-437

60. Sun, R., Yang, N., Kong, B., Cao, B., Feng, D., Yu, X., Ge, C., Huang, J., Shen, J., Wang, P., Feng, S., Fei, F., Guo, J., He, J., Aa, N., Chen, Q., Pan, Y., Schumacher, J. D., Yang, C. S., Guo, G. L., Aa, J., and Wang, G. (2017) Orally Administered Berberine Modulates Hepatic Lipid Metabolism by Altering Microbial Bile Acid Metabolism and the Intestinal FXR Signaling Pathway. *Molecular pharmacology* **91**, 110-122

61. Trabelsi, M. S., Daoudi, M., Prawitt, J., Ducastel, S., Touche, V., Sayin, S. I., Perino, A., Brighton, C. A., Sebti, Y., Kluza, J., Briand, O., Dehondt, H., Vallez, E., Dorchies, E., Baud, G., Spinelli, V., Hennuyer, N., Caron, S., Bantubungi, K., Caiazzo, R., Reimann, F., Marchetti, P., Lefebvre, P., Backhed, F., Gribble, F. M., Schoonjans, K., Pattou, F., Tailleux, A., Staels, B., and

---

ARV1 regulates MetS syndrome
Lestavel, S. (2015) Farnesoid X receptor inhibits glucagon-like peptide-1 production by enteroendocrine L cells. *Nature communications* 6, 7629

62. Dawson, P. A. (2011) Role of the intestinal bile acid transporters in bile acid and drug disposition. *Handbook of experimental pharmacology*, 169-203

63. Kok, T., Hulzebos, C. V., Wolters, H., Havinga, R., Agellon, L. B., Stellaard, F., Shan, B., Schwarz, M., and Kuipers, F. (2003) Enterohepatic circulation of bile salts in farnesoid X receptor-deficient mice: efficient intestinal bile salt absorption in the absence of ileal bile acid-binding protein. *The Journal of biological chemistry* 278, 41930-41937

64. Shi, Y., and Cheng, D. (2009) Beyond triglyceride synthesis: the dynamic functional roles of MGAT and DGAT enzymes in energy metabolism. *American journal of physiology. Endocrinology and metabolism* 297, E10-18

65. Brownlee, I. A., Forster, D. J., Wilcox, M. D., Dettmar, P. W., Seal, C. J., and Pearson, J. P. (2010) Physiological parameters governing the action of pancreatic lipase. *Nutrition research reviews* 23, 146-154

66. Ohshiro, T., and Tomoda, H. (2015) Acyltransferase inhibitors: a patent review (2010-present). *Expert opinion on therapeutic patents* 25, 145-158

67. Nelson, D. W., Gao, Y., Spencer, N. M., Banh, T., and Yen, C. L. (2011) Deficiency of MGAT2 increases energy expenditure without high-fat feeding and protects genetically obese mice from excessive weight gain. *Journal of lipid research* 52, 1723-1732

68. Yen, C. L., Cheong, M. L., Grueter, C., Zhou, P., Moriwaki, J., Wong, J. S., Hubbard, B., Marmor, S., and Farese, R. V., Jr. (2009) Deficiency of the intestinal enzyme acyl CoA:monoaoylglycerol acyltransferase-2 protects mice from metabolic disorders induced by high-fat feeding. *Nature medicine* 15, 442-446

69. Banh, T., Nelson, D. W., Gao, Y., Huang, T. N., Yen, M. I., and Yen, C. L. (2015) Adult-onset deficiency of acyl CoA:monoaoylglycerol acyltransferase 2 protects mice from diet-induced obesity and glucose intolerance. *Journal of lipid research* 56, 379-389

70. Smith, S. J., Cases, S., Jensen, D. R., Chen, H. C., Sande, E., Tow, B., Sanan, D. A., Raber, J., Eckel, R. H., and Farese, R. V., Jr. (2000) Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. *Nature genetics* 25, 87-90

71. Chen, H. C., Smith, S. J., Ladha, Z., Jensen, D. R., Ferreira, L. D., Pulawa, L. K., McGuire, J. G., Pitas, R. E., Eckel, R. H., and Farese, R. V., Jr. (2002) Increased insulin and leptin sensitivity in mice lacking acyl CoA:diacylglycerol acyltransferase 1. *The Journal of clinical investigation* 109, 1049-1055

72. Liu, Y., Millar, J. S., Cromley, D. A., Graham, M., Crooke, R., Billheimer, J. T., and Rader, D. J. (2008) Knockdown of acyl-CoA:diacylglycerol acyltransferase 2 with antisense oligonucleotide reduces VLDL TG and ApoB secretion in mice. *Biochimica et biophysica acta* 1781, 97-104

73. Gluchowski, N. L., Chitraju, C., Picoraro, J. A., Mejhert, N., Pinto, S., Xin, W., Kamin, D. S., Winter, H. S., Chung, W. K., Walther, T. C., and Farese, R. V., Jr. (2017) Identification and characterization of a novel DGAT1 missense mutation associated with congenital diarrhea. *Journal of lipid research* 58, 1230-1237

74. Denison, H., Nilsson, C., Lofgren, L., Himmelmann, A., Martensson, G., Knutsson, M., Al-Shurbaji, A., Tornqvist, H., and Eriksson, J. W. (2014) Diacylglycerol acyltransferase 1 inhibition with AZD7687 alters lipid handling and hormone secretion in the gut with intolerable side effects: a randomized clinical trial. *Diabetes, obesity & metabolism* 16, 334-343

75. DeVita, R. J., and Pinto, S. (2013) Current status of the research and development of diacylglycerol O-acyltransferase 1 (DGAT1) inhibitors. *Journal of medicinal chemistry* 56, 9820-9825

76. Imbriglio, J. E., Shen, D. M., Liang, R., Marby, K., You, M., Youm, H. W., Feng, Z., London, C., Xiong, Y., Tata, J., Verras, A., Garcia-Calvo, M., Song, X., Addona, G. H., McLaren, D. G., He, T., Murphy, B., Metzger, D. E., Salturo, G., Deckman, D., Chen, Q., Jin, X., Stout, S. J., Wang, S. P., Wilsie, L., Palyha, O., Han, S., Hubbard, B. K., Previs, S. F., Pinto, S., and Taggart, A.
(2015) Discovery and Pharmacology of a Novel Class of Diacylglycerol Acyltransferase 2 Inhibitors. *Journal of medicinal chemistry* 58, 9345-9353

77. Garg, A. (2004) Acquired and inherited lipodystrophies. *The New England journal of medicine* 350, 1220-1234

78. Savage, D. B. (2009) Mouse models of inherited lipodystrophy. *Disease models & mechanisms* 2, 554-562

79. Huang-Doran, I., Sleigh, A., Rochford, J. J., O'Rahilly, S., and Savage, D. B. (2010) Lipodystrophy: metabolic insights from a rare disorder. *The Journal of endocrinology* 207, 245-255

80. Hayek, T., Ito, Y., Azrolan, N., Verdevry, R. B., Aalto-Setala, K., Walsh, A., and Breslow, J. L. (1993) Dietary fat increases high density lipoprotein (HDL) levels both by increasing the transport rates and decreasing the fractional catabolic rates of HDL cholesterol ester and apolipoprotein (Apo) A-I. Presentation of a new animal model and mechanistic studies in human Apo A-I transgenic and control mice. *The Journal of clinical investigation* 91, 1665-1671

81. Escola-Gil, J. C., Llaverias, G., Julve, J., Jauhiainen, M., Mendez-Gonzalez, J., and Blanco-Vaca, F. (2011) The cholesterol content of Western diets plays a major role in the paradoxical increase in high-density lipoprotein cholesterol and upregulates the macrophage reverse cholesterol transport pathway. *Arteriosclerosis, thrombosis, and vascular biology* 31, 2493-2499

82. Yang, Z. H., Miyahara, H., Takeo, J., and Katayama, M. (2012) Diet high in fat and sucrose induces rapid onset of obesity-related metabolic syndrome partly through rapid response of genes involved in lipogenesis, insulin signalling and inflammation in mice. *Diabetology & metabolic syndrome* 4, 32

83. Agellon, L. B., Walsh, A., Hayek, T., Moulin, P., Jiang, X. C., Shelanski, S. A., Breslow, J. L., and Tall, A. R. (1991) Reduced high density lipoprotein cholesterol in human cholesteryl ester transfer protein transgenic mice. *The Journal of biological chemistry* 266, 10796-10801

84. Williamson, R., Lee, D., Hagaman, J., and Maeda, N. (1992) Marked reduction of high density lipoprotein cholesterol in mice genetically modified to lack apolipoprotein A-I. *Proceedings of the National Academy of Sciences of the United States of America* 89, 7134-7138

85. Mehrabian, M., Qiao, J. H., Hyman, R., Ruddle, D., Laughton, C., and Lusis, A. J. (1993) Influence of the apoA-II gene locus on HDL levels and fatty streak development in mice. *Arteriosclerosis and thrombosis : a journal of vascular biology* 13, 1-10

86. Imaizumi, S., Navab, M., Morgantini, C., Charles-Schoeman, C., Su, F., Gao, F., Kwon, M., Ganapathy, E., Meriwether, D., Farias-Eisner, R., Fogelman, A. M., and Reddy, S. T. (2011) Dysfunctional high-density lipoprotein and the potential of apolipoprotein A-I mimetic peptides to normalize the composition and function of lipoproteins. *Circulation journal : official journal of the Japanese Circulation Society* 75, 1533-1538

87. Paigen, B., Mitchell, D., Holmes, P. A., and Albee, D. (1987) Genetic analysis of strains C57BL/6J and BALB/cJ for Ath-1, a gene determining atherosclerosis susceptibility in mice. *Biochemical genetics* 25, 881-892

88. Ishida, B. Y., Blanche, P. J., Nichols, A. V., Yashar, M., and Paigen, B. (1991) Effects of atherogenic diet consumption on lipoproteins in mouse strains C57BL/6 and C3H. *Journal of lipid research* 32, 559-568

89. Ferrell, J. M., Boehme, S., Li, F., and Chiang, J. Y. (2016) Cholesterol 7alpha-hydroxylase-deficient mice are protected from high-fat/high-cholesterol diet-induced metabolic disorders. *Journal of lipid research* 57, 1144-1154

90. Gonzalez, F. J., Jiang, C., Xie, C., and Patterson, A. D. (2017) Intestinal Farnesoid X Receptor Signaling Modulates Metabolic Disease. *Digestive diseases* 35, 178-184

91. Woo, Y. C., Xu, A., Wang, Y., and Lam, K. S. (2013) Fibroblast growth factor 21 as an emerging metabolic regulator: clinical perspectives. *Clin Endocrinol (Oxf)* 78, 489-496

92. Ni, D., Xu, P., and Gallagher, S. (2017) Immunoblotting and Immunodetection. *Current protocols in protein science* 88, 10 10 11-10 37
ARV1 regulates MetS syndrome

93. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry* 72, 248-254

94. Eder, K., Reichlmayr-Lais, A. M., and Kirchgessner, M. (1993) Studies on the extraction of phospholipids from erythrocyte membranes in the rat. *Clinica chimica acta; international journal of clinical chemistry* 219, 93-104

95. Theise, N. D. (2007) Liver biopsy assessment in chronic viral hepatitis: a personal, practical approach. *Mod Pathol* 20 Suppl 1, S3-14

96. Bligh, E. G., and Dyer, W. J. (1959) A rapid method of total lipid extraction and purification. *Canadian journal of biochemistry and physiology* 37, 911-917

97. McDonough, V. M., and Roth, T. M. (2004) Growth temperature affects accumulation of exogenous fatty acids and fatty acid composition in Schizosaccharomyces pombe. *Antonie van Leeuwenhoek* 86, 349-354

98. Rossi, S. S., Converse, J. L., and Hofmann, A. F. (1987) High pressure liquid chromatographic analysis of conjugated bile acids in human bile: simultaneous resolution of sulfated and unsulfated lithocholyl amidates and the common conjugated bile acids. *Journal of lipid research* 28, 589-595

**FIGURE LEGENDS**

Fig. 1. *Arv1*/- animals lack expression of a full-length mRNA containing exon 5. A, arrangement of the *Arv1* gene locus. *numbers indicate exons, lines indicate introns.* B, arrangement of the *Arv1* hnRNA. *boxes indicate exons, lines indicate introns.* C, organization of the full-length *Arv1* mRNA. *numbers indicate exons.* D, *ARV1* mRNA expression levels in the liver. *ARV1* mRNA expression was determined using qRT-PCR. Numbers in figure D indicate what primers were used as indicated in B. *WT, black bars; Arv1-/-, white bars.* ***p<0001; n=8.*

Fig. 2. *Arv1*/- animals do not express ARV1 protein. Total protein was extracted from the indicated tissues. Proteins were resolved by SDS-PAGE and ARV1 protein levels were determined by western analysis using anti-ARV1 polyclonal antibodies (*Abgent #AP10655a*). ARV1 protein runs as a 31kDa protein (*arrow*). Ponceau S staining and the Bradford method were used in order to load the same amount of protein in each well of all gels (92,93). Wild type levels of ARV1 protein density in each tissue was set at a relative value of 1, and ARV1 levels in other tissues was compared to wild type. SI, *small intestines.* ***p<0001.*

Fig. 3. *Arv1*/- animals have reduced survival rates. Male *WT* and *Arv1*/- animals were fed either NC (NC) or a HFD (HFD). They were monitored daily and the age at the time of death was recorded. On the NC there was a significant difference in survival (*Log-rank P=0.0006*) and also on the HFD (*Log-rank P=0.0202*); *n=16*. *WT, closed circles; Arv1-/-, open circles.*

Fig. 4. *Arv1*/- animals display neurological deficits. Male *WT* and *Arv1*/- animals were between the ages of 9-14 weeks and were individually housed prior to initiating the study. Mice were acclimated to the testing room for at least 30 minutes. A, The assay was carried out using EzRod test chambers. For the accelerating rotarod study, mice were given 10 trials with a maximum duration of 3 min with a 30-sec ITI. The time of latency to fall was recorded for all trials. *WT, closed circles; Arv1-/-, open circles.* B & C, the assay was performed as described in the “EXPERIMENTAL PROCEDURES”. The time and the path length in the center of the open field were determined. Total path length and path length for 30 min at 5 min intervals were determined as measures of locomotor activity. *WT, n=14; Arv1-/-, n=15.* *P<0.01; ***p<0001.*
Fig. 5. Male and female Arv1-/ animals are fertile. A-C, sexually mature females (6-7 weeks) were paired with an ICR male (8 weeks). D-F, sexually mature males (~3 months) were paired with two ICR females (6 weeks). Presence of a vaginal plug was checked every day for 6 days then the male was removed. Females were checked daily and size and birth dates of litters were recorded. A & D & C & F, WT, black bars; Arv1 / , white bars; +, success; -, no success. B & E, WT, closed circles; Arv1 / , open circles.

Fig. 6. Workflow chart. The diagram indicates the day an experiment was performed and the age of the mice.

Fig. 7. Arv1 / animals are resistant to diet-induced obesity. A, body weights of individually housed male Arv1 / and WT mice over 15 weeks, the HFD was added on week 5. WT, closed circles; Arv1 / open circles (NC); WT, closed boxes; Arv1 / open circles (HFD). B, the amount of food intake/kg body weight of individually housed male WT and Arv1 / animals were determined over a 24 hr period (WT, closed circles; KO, open circles). C, Acyl and de-acyl ghrelin concentrations in plasma were determined (WT, closed circles; KO, open circles). D, Non-fasted leptin concentrations were determined (WT, closed circles; KO, open circles). E, Fasted adiponectin concentrations were determined from serum samples (WT, closed circles; KO, open circles). Panel A, (NC & HFD), WT, n=10; Arv1 /, n=8. Panel B, (NC), WT, n=8; Arv1 /, n=6; (HFD), WT, n=7; Arv1 /, n=6. Panel C (NC), WT, n=8; Arv1 /, n=10; (HFD), WT, n=8; Arv1 /, n=10. Panel D, (NC), WT, n=6; Arv1 /, n=5; (HFD), WT, n=8; Arv1 /, n=7. Panel E, (NC), WT, n=7; Arv1 /, n=6; (HFD), WT, n=8; Arv1 /, n=6. *p<0.01; ***p<0.0001.

Fig. 8. Arv1 / animals have increased energy expenditure. A-D, metabolic studies were performed using the Comprehensive Cage Monitoring System (Columbus Instruments, Columbus, OH). Parameters were measured for 48 hr, beginning and ending at the start of the dark phase of the light cycle. A, energy expenditure. B, oxygen consumption. C, carbon dioxide production. D, respiratory exchange ratio. All statistical data were calculated as described in “EXPERIMENTAL PROCEDURES” (NC), WT, closed circles; Arv1 /, open circles; (HFD), WT, closed boxes; Arv1 /, open boxes. WT, n=8; Arv1 /, n=6. *p<0.01; ***p<0.0001.

Fig. 9. Arv1 / animals have reduced blood cholesterol and triglyceride levels. Cholesterol, triglycerides, and blood apolipoprotein levels were determine for animals on the NC the third week of the study and again after 8 weeks on a HFD. A, Serum cholesterol levels in mice were measured after an overnight fast. Cholesterol concentrations were determined enzymatically from serum (Thermo DMA, Arlington, TX). B, Serum triglyceride levels were also measured after an overnight fast. Whole blood was collected and triglycerides were assessed on a hand held meter (CardioChek, PTS Inc. Indianapolis, IN). C & D, apolipoprotein levels. The LipoPrint LDL system was used for calculations according to the manufacturer’s instructions. WT, closed circles; hetero, closed triangles; Arv1 /, Arv1 /, open circles. Panel A, (NC), WT, n=16; Arv1 /, n=16; Arv1 /, n=12; (HFD), WT, n=15; Arv1 /, n=16; Arv1 /, n=9 (cholesterol). Panel B, (NC), WT, n=16; Arv1 /, n=16; Arv1 /, n=8; (HFD), WT, n=16; Arv1 /, n=16; Arv1 /, n=14 (triglyceride). Panel C, (NC), WT, n=12; Arv1 /, n=7; Arv1 /, n=7 (VLDL). (NC), WT, n=15; Arv1 /, n=16; Arv1 /, n=12 (LDL). (NC), WT, n=16; Arv1 /, n=16; Arv1 /, n=12 (HDL). Panel D; (HFD), WT, n=12; Arv1 /, n=12; Arv1 /, n=8 (VLDL). (HFD), WT, n=15; Arv1 /, n=14; Arv1 /, n=8 (LDL). (HFD), WT, n=15; Arv1 /, n=15; Arv1 /, n=8 (HDL). *p<0.01; **p<0.001; ***p<0.0001.

Fig. 10. Arv1 / animals remain lean on a high fat diet. DEXA and dissected fat pads were used to measure adiposity in NC and HFD fed male mice. A, total fat mass. Values represent the sum of all fat depots. B, adipose tissue dissections were performed on a NC fed group of mice ~10 weeks of age. Adipose tissue was dissected from the inguinal fat pad (subcutaneous fat), epididymal, renal (retroperitoneal), and mesenteric fat pads (visceral fat). Individual values were then divided by weight of
an animal at the time of dissection. Values are represented as mg of fat mass per gram of weight. C & D, total brown adipose tissue was dissected from the suprascapular area of the back from NC fed group of mice ~10 weeks of age. Total brown fat mass was calculated by weighing the tissue extracted. Total brown adipose mass per gram of tissue was calculated by dividing total brown adipose mass by weight. Panel A, (NC), WT, n=7; Arv1/-, n=6; (HFD), WT, n=8; Arv1/-, n=6. Panel B, WT, n=7; Arv1/-, n=8 (MES). WT, n=7; Arv1/-, n=10 (REN). WT, n=6; Arv1/-, n=7 (EPI). WT, n=7; Arv1/-, n=7 (ING). Panel C, WT, n=8; Arv1/-, n=8. Panel D, WT, n=8; Arv1/-, n=8. **p<0.001; ***p<0.0001.

Fig. 11. Arv1/- animals fed a high fat diet remain glucose tolerant and insulin sensitive. A, male mice were subjected to an OGTT following 9 weeks on a HFD or age-matched mice fed a NC. A & B, mice were fasted for 16 hr prior to the initiation of the study. C & D, male mice that were subjected to an OGTT test prior to and post HFD feeding were used for determining insulin levels. Basal insulin levels were determined in mice fasted for 16 hr. Insulin levels measured during the OGTT presented in panel A and B. E, Mice were fasted for 4 hr. NC fed animals were given 0.5 U/kg of insulin by intraperitoneal injection. HFD fed animals were given 1.0 U/kg of insulin by intraperitoneal injection. At the indicated times blood glucose values were obtained. A, C, & E, (NC), WT, closed circles; Arv1/-, open circles; (HFD), WT, closed boxes; Arv1/-, open boxes. B & D, WT, closed circles; Arv1/-, open circles. Panel A, C, & E; (NC), WT, n=12; Arv1/-, n=6; (HFD), WT, n=11; Arv1/-, n=6. Panel B, (NC), WT, n=12; Arv1/-, n=6; (HFD), WT, n=11; Arv1/-, n=6. Panel D, (NC), WT, n=10; Arv1/-, n=8; (HFD), WT, n=14; Arv1/-, n=6. **p<0.001; ***p<0.0001.

Fig. 12. Arv1/- animals fed a high fat diet have reduced signs of NAFLD. Liver tissue was extracted from euthanized animals using standard sterile surgical techniques. A & B, lipid levels were determined using standard commercial assay kits and calculated per grams of liver. C, WT and Arv1/- animal livers are shown. D, tissues were embedded in paraffin blocks, and cut on 4 microns. H&E processed tissues were examined using a Nikon Elipse 50i Clinical Microscope. The chronic hepatitis assessment scheme was the “Scheuer Classification for Grading and Staging of Chronic Hepatitis” (95). The green arrows in the panels in D indicate the portal vein. E, Mice were fasted for 4 hr. Hepatic triglyceride export was measured in NC fed 10 week-old WT and Arv1/- mice measured following ip Pluronic F-127 injection (500 mpk). A & B & E, WT, closed circles; Arv1/-, open circles. Panel A, WT, n=5; Arv1/-, n=5 (triglyceride). WT, n=5; Arv1/-, n=6 (cholesterol). WT, n=6; Arv1/-, n=6 (LDL). WT, n=6; Arv1/-, n=6 (HDL). Panel B, WT, n=6; Arv1/-, n=6. ***p<0.0001.

Fig. 13. Arv1/- animals have reduced levels of oleic acid and reduced SCD1 activity in the liver. Animals were either fed NC or a HFD. A, fatty acid levels in the livers of animals fed NC. B, fatty acid levels in the livers of animals fed HFD. C, SCD1 protein levels. D, densitometry of panel C. Protein identification was determined using anti-SCD1 polyclonal antibodies. Ponceau S staining and the Bradford method were used in order to load the same amount of protein in SDS-PAGE each well (92,93). Panels A, WT, closed circles; Arv1/-, open circles. Panel D, WT, black bars; KO, white bars. WT, n=6; Arv1/-, n=6. **p<0.001; ***p<0.0001. (n=8).

Fig. 14. FXR signaling is activated in the livers of Arv1/- animals. WT and Arv1/- animals were fed a HFD. At the end of the study, total protein was extracted and was resolved by SDS-PAGE. Protein identification was determined by western analysis using polyclonal antibodies against the indicated proteins. Ponceau S staining and the Bradford method were used in order to load the same amount of protein in each well (92,93). Protein levels were determined using densitometry. Panels B, D, F, H, and I, WT, black bars; Arv1/-, white bars. *p<0.01; **p<0.001; ***p<0.0001. (n=8).
**Fig. 15.** PPARα signaling is activated in the livers of Arv1/- animals. WT and Arv1/- animals were fed a HFD. At the end of the study, total protein was extracted and was resolved by SDS-PAGE. Protein identification was determined by western analysis using anti-FXR and anti-SHP polyclonal antibodies. Ponceau S staining and the Bradford method were used in order to load the same amount of protein in each well (92,93). Protein levels were determined using densitometry. Panels B & D, WT, black bars; Arv1/-, white bars. *p<0.01. (n=8).

**Fig. 16.** Arv1/- animals have elevated fecal lipid levels and an altered bile acid intermediate composition. A-C, fecal lipids were extracted from male animals using hexane/2-propanol (3:2) (94). Lipid levels were determined using commercially available assay kits. D-F, total bile acid pools and compositions in intestines were determined using a previously published protocol (98). panels A-D; WT, closed circles; Arv1/-, open circles. Panels A-E, WT, n=6; Arv1/-, n=6. Panel F, WT, n=4; Arv1/-, n=4. *p<0.01; **p<0.001; ***p<0.0001.

**Fig. 17.** FXR signaling is not activated in the small intestines of Arv1/- animals. WT and Arv1/- animals were fed a HFD. At the end of the study, total protein was extracted and resolved by SDS-PAGE. Protein identification was determined by western analysis using anti-FXR and anti-SHP polyclonal antibodies. Ponceau S staining and the Bradford method were used in order to load equivalent amounts of protein in each well (92,93). Protein levels were determined using densitometry. Panels B & D, WT, black bars; Arv1/-, white bars. *p<0.01. (n=8).

**Fig. 18.** Model showing the effects due to loss of ARV1.
Figure 3

Survival, %

NC

Age, weeks

Survival, %

HFD

Age, weeks

Downloaded from http://www.jbc.org/ on July 24, 2018
Figure 6

| Age (wks) | Experimental Week |
|-----------|-------------------|
| 8         | 1                 |
| 9         | 2                 |
| 10        | 3                 |
| 11        | 4                 |
| 12        | 5                 |
| 13        | 6                 |
| 14        | 7                 |
| 15        | 8                 |
| 16        | 9                 |
| 17        | 10                |
| 18        | 11                |
| 19        | 12                |
| 20        | 13                |
| 21        | 14                |
| 22        | 15                |

- Nonfasted bleed
- Fasted bleed
- OGGT
- Glu + Ins
- Metabolic activity
- Food intake
- ITT
- DEXA
- Fasted bleed
- OGGT + Ins

ACE
Adiponectin
Leptin
Ghrelin
Tissue collection
Blood collection
Chol
TAG
VLDL
LDL
HDL
NC/HFD

ACE
Adiponectin
Leptin
Chol
TAG
VLDL
LDL
HDL
Tissue collection
Bile acids
Blood collection
Figure 15

A

PPARα

75 kDa
50 kDa
37 kDa

B

Relative PPARα expression

WT KO

***

C

FGF21

25 kDa
15 kDa

D

Relative FGF21 expression

WT KO

***
Mice lacking ARV1 have reduced signs of metabolic syndrome and non-alcoholic fatty liver disease
Christina Gallo-Ebert, Jamie Francisco, Hsing-Yin Liu, Riley Draper, Kinnari Modi, Michael D. Hayward, Beverly K. Jones, Olesia Buiakova, Virginia McDonough and Joseph T. Nickels

J. Biol. Chem. published online February 28, 2018

Access the most updated version of this article at doi: 10.1074/jbc.RA117.000800

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts