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Targeted deletion of adipocyte Abca1 impairs diet-induced obesity

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Abstract

Objective—Adipose tissue cholesterol increases with adipocyte triglyceride (TG) content and size during development of obesity. However, how adipocyte cholesterol affects adipocyte function is poorly understood. The aim of this study was to evaluate the role of the cellular cholesterol exporter, ATP binding cassette transporter A1 (Abca1), on adipose tissue function during diet-induced obesity.

Approach and Results—Adiponectin *Cre* recombinase transgenic mice were crossed with *Abca1* $^{flox/flox}$ mice to generate adipocyte-specific *Abca1* knockout (ASKO) mice. Control (Cntl) and ASKO mice were then fed a high fat, high cholesterol (45% calories as fat, 0.2% cholesterol) diet for 16 weeks. Compared to Cntl mice, ASKO mice had a 2-fold increase in adipocyte plasma membrane cholesterol content and significantly lower body weight, epididymal fat pad weight, and adipocyte size. ASKO vs. Cntl adipose tissue had decreased peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer binding protein expression, nuclear sterol regulatory element binding protein 1 (SREBP1) protein, lipogenesis, and TG accretion, but similar Akt activation after acute insulin stimulation. Acute siRNA-mediated *Abca1* silencing during 3T3L1 adipocyte differentiation reduced adipocyte Abca1 and PPARγ protein expression and triglyceride content. Systemic stimulated TG lipolysis and glucose homeostasis was similar between Cntl and ASKO mice.

Conclusions—Adipocyte Abca1 is a key regulator of adipocyte lipogenesis and lipid accretion, likely due to increased adipose tissue membrane cholesterol, resulting in decreased activation of lipogenic transcription factors PPARγ and SREBP1.

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Keywords

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Subject codes

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Introduction

Obesity continues to increase at an alarming rate in the US population, with 67% of adults classified as overweight or obese based on body mass index [1, 2]. Obesity is a risk factor for other chronic diseases and is associated with increased prevalence of type 2 diabetes, hypertension, dyslipidemia, and cardiovascular disease [2, 3]. With the onset of obesity, adipocytes increase in size due to increased triglyceride (TG) accumulation, which protects against lipotoxicity in other organs because excess circulating fatty acids (FAs) are esterified into TG and stored in adipocytes. However, if FA flux into adipose tissue exceeds the ability of adipocytes to store the excess energy as TG, adipocyte dysfunction and ultimately cell death occurs. Large dysfunctional adipocytes are charactized by decreased responsiveness to insulin [4, 5], decreased glucose uptake, increased endoplasmic reticulum and mitochondrial stress [6], reactive oxygen species [7, 8], and proinflammatory cytokine secretion [9–11]. However, obesity and adipocyte hypertrophy does not lead to adipose tissue dysfunction in all cases [12–14], suggesting that additional factors contributing to adipose tissue dysfunction remain to be identified.

Cholesterol and TG accumulate proportionately in adipocytes during weight gain [15]. While much research has focused on adipocyte TG accumulation, little is known about how cholesterol accumulation affects adipocyte function. Unlike other tissues, adipocytes store nearly all cholesterol as free cholesterol (FC; >93% of total cholesterol). In obesity, adipocytes can contain up to 50% of the body's FC, making it the largest FC pool [16]. Adipocytes also exhibit very low levels of cholesterol synthesis compared to other tissues, suggesting that most adipocyte cholesterol comes from dietary sources [17, 18]. Several studies suggest a link between cholesterol content and adipose tissue inflammation [19, 20] and insulin resistance [21]; however, how adipocyte cholesterol content influences adipose tissue inflammation and insulin resistance is poorly understood.

To determine how cholesterol accumulation affects adipocyte function, our lab [22] and others [23] have used *in vivo* mouse models to delete the ATP binding cassette transporter A1 (Abca1) from adipocytes. Abca1 is a membrane protein that exports cellular cholesterol and phospholipids to apolipoproteins, generating nascent high-density lipoprotein (HDL) particles [24]. Abca1 is highly expressed in adipose tissue [25] and its expression is important in mobilizing adipose tissue cholesterol for transport to the liver for excretion [22, 26]. Previous studies suggest that adipocyte Abca1 contributes significantly to plasma cholesterol concentrations and is the major cholesterol efflux mechanism in adipocytes [22, 23]. However, the model used (i.e., aP2 *Cre recombinase* mouse) also results in partial and variable

myeloid cell (i.e., macrophage, monocyte, neutrophil) Abca1 deletion [22, 27]. Myeloid-specific *Abca1* gene deletion results in proinflammatory macrophages that are functionally distinct from macrophages expressing Abca1 [28–30]. This is an important point because macrophages are the primary source of inflammatory cytokine expression in adipose tissue [31], and cytokines contribute to development of insulin resistance.

To address this issue, we generated an adipocyte-specific *Abca1* knockout (ASKO) mouse model using an adiponectin *Cre* recombinase mouse, so that macrophage Abca1 expression remained intact [27]. This model allowed us to study the impact of ABCA1 expression and adipocyte cholesterol accumulation on adipocyte function. Our results demonstrate that adipocyte Abca1 is critical for regulating adipose tissue cholesterol content and that genetic deletion of adipocyte Abca1 expression results in mice that are resistant to diet-induced obesity.

Methods and Methods

A detailed description of the materials and methods is available in the online-only data supplement.

Results

Abca1 is selectively deleted from adipose tissue

Previous reports on *in vivo* adipocyte Abca1 deletion used an $Abca1^{flox/flox}$ mouse crossed with an aP2 *Cre* recombinase mouse [22, 23]; however, the resulting knockout mice also demonstrated partial and variable myeloid *Abca1* deletion, complicating interpretation of results. In this study, we created an adipocyte-specific *Abca1* knockout mouse model by crossing $Abca1^{flox/flox}$ mice with adiponectin *Cre* recombinase expressing mice obtained from Dr. Evan Rosen [32, 33]. Abca1 protein expression was reduced in white adipose tissue (WAT; 17% (HT) and 58% (ASKO) of Cntl) and brown adipose tissue (BAT; 28% (HT) and 93% (ASKO) of Cntl) and the reduction exhibited the expected gene dosage effect (i.e., homozygous< heterozygous<Cntl) (Figure 1A). In contrast, no apparent decrease in Abca1 protein expression was observed in macrophages (peritoneal or bone marrow-derived) or liver, demonstrating selectivity for elimination of adipocyte Abca1. Abca1 gene expression was significantly reduced in adipose tissue, but not in liver (Figure 1B). Although other Abca1-expressing tissues were not examined, previous work has shown adipocyte specificity with the adiponectin *Cre* recombinase mouse [27, 32].

Adipocyte Abca1 deletion significantly reduces plasma HDL concentration

The previous adipocyte Abca1 knockout mouse model (made with an aP2 *Cre* recombinase mouse) had a slight, but significant decrease in total plasma cholesterol and HDL cholesterol in two separate studies [22, 23]. In our study, plasma total, LDL, and HDL cholesterol concentrations were measured in mice before (chow) and after 16 weeks of HFHC diet. ASKO mice had significantly reduced (~30%) total plasma cholesterol (Figure 1C; genotype, $p<0.015$) and HDL cholesterol (Figure 1D; genotype, $p<0.028$) concentrations compared to control mice. However, because few age-matched chow-fed mice were analyzed, post-hoc analyses did not show a significant difference for total plasma cholesterol

and HDL cholesterol between chow-fed control and ASKO mice. No differences were observed in plasma concentrations of LDL, VLDL, or TG between the two genotypes (Figure 1E and F).

ASKO mice are resistant to diet-induced weight gain

To determine the systemic effects of adipocyte-specific Abca1 gene deletion, mice were fed a HFHC diet for 16 weeks starting at 8 weeks of age. A smaller cohort was maintained on chow from weaning until 24 weeks of age. All three possible genotypes of Cntl mice (*adiponectin Cre*$^{+}$ *Abca1*$^{+/+}$, *Abca1*$^{+/+}$, *Abca1*$^{flox/flox}$) were used, since their phenotypes (body and fat pad weight, plasma cholesterol, adipocyte size, and adipose cholesterol content) were indistinguishable from *Abca1*$^{+/+}$ mice. Both genotypes gained weight at a similar rate while consuming a chow diet. Strikingly, ASKO mice failed to gain weight on the HFHC diet compared to their Cntl counterparts (Figure 2A). In fact, weight gain for HFHC diet-fed ASKO mice was similar to chow-fed mice, resulting in terminal body weights significantly lower in HFHC-fed ASKO than in HFHC-fed Cntl mice (32.0 ± 1.4 g versus 39.9 ± 1.1 g; $p<0.0017$) (Figure 2B). These results demonstrate that adipocyte-specific Abca1 deletion confers resistance to HFHC diet-induced obesity.

Similar patterns were observed in white adipose tissue weight. On a HFHC diet, Cntl mice had two-fold higher epididymal white adipose tissue (EAT) mass compared to ASKO mice (1.99 ± 0.14 vs. 1.12 ± 0.12 g, respectively, $p < 0.0001$), whereas EAT mass was similar for chow-fed Cntl and ASKO (0.48 ± 0.10 vs. 0.55 ± 0.09 g, respectively; $p = 0.6$) (Figure 2C). HFHC diet-fed ASKO mice had larger EAT mass than their chow-fed counterparts ($p = 0.02$) despite having no difference in body weight; however, the difference in fat pad weight between chow and HFHC diet-fed ASKO mice (0.57 g) was much smaller than that for Cntl mice (1.51 g). These differences were unaltered when EAT weight was normalized to body weight (data not shown). Liver:body weight ratio was similar for all mice (data not shown). To measure body fat composition, the total amount of fat in EAT, subcutaneous (SAT), brown (BAT), and retrorenal depots was measured and normalized to body weight. HFHC diet-fed ASKO mice had a lower percentage of body weight as fat compared to Cntl mice (11.4 ± 0.9% vs. 8.8 ± 0.8%, $p = 0.04$) (Figure 2D). Snout-to-tail length, measured at necropsy, was similar for both genotypes, supporting no growth retardation in ASKO mice (Figure 2E). Together, these data show that ASKO mice are resistant to HFHC diet-induced weight gain.

Abca1 deletion results in smaller adipocytes

Adipocyte hypertrophy is believed to be the major mechanism for adipose tissue expansion during weight gain and hypertrophied adipocytes are associated with metabolic dysfunction [34]. We determined whether adipocyte Abca1 deletion alters adipocyte size. Adipose tissue sections from ASKO and Cntl mice fed HFHC and chow diets were stained with hematoxylin and eosin and examined microscopically. ASKO EAT adipocytes from HFHC diet-fed mice appeared smaller than those from Cntl mice (Figure 2F), which was confirmed by quantitative size analysis (Figure 2G). SAT adipocytes from HFHC diet-fed ASKO mice were also significantly smaller than their Cntl counterparts (Supplemental Figure I A and B).

EAT adipocyte size distribution was similar for chow-fed mice (Supplemental Figure I C and D).

Adipocyte-specific *Abca1* deletion has minimal impact on metabolic phenotype

Since ASKO mice gained less weight on the HFHC diet compared with Cntl mice, we subjected both genotypes to metabolic phenotyping. Mice fed the HFHC diet for 16 weeks were placed in metabolic cages (CLAMS) for four days, two days for acclimation and two days for measurements. Although ASKO mice had significantly ($p<0.05$) increased oxygen consumption, carbon dioxide production, and heat production during the dark cycle when the data were normalized for body weight (data not shown), this was not the case when the data were expressed on a per mouse basis (Supplemental Figure II A–C), as suggested in recent literature [35, 36]. Analysis of covariance of body weight vs. heat production [35] showed no significant genotype difference between ASKO and Cntl mice (data not shown). Physical activity (Supplemental Figure II D), respiratory exchange ratio (Supplemental Figure II E), and food intake (Supplemental Figure II F) were also similar between genotypes. Despite no difference in food intake, plasma leptin was significantly decreased in ASKO compared to Cntl mice (8.0 ± 3.6 vs. 20.9 ± 2.4 ng/ml, $p = 0.01$) (Supplemental Figure II G). Leptin mRNA abundance was also significantly decreased in EAT from ASKO mice compared to Cntl mice (fold change: 0.47 ± 0.20 vs. 1.03 ± 0.10, respectively; $p< 0.03$) (Supplemental Figure II H). EAT adiponectin mRNA abundance trended lower in ASKO mice compared to Cntl mice ($p=0.086$; data not shown).

ASKO mice have higher adipose tissue cholesterol content

We next examined the impact of adipocyte Abca1 deletion on adipose tissue cholesterol content. EAT from ASKO mice had ~3-fold higher levels of cholesterol compared to Cntl mice, regardless of diet (Figure 3A). However, EAT stromal vascular cells from both genotypes had similar cholesterol content (7.2 ± 1.1 and 6.4 ± 1.1 μg/mg protein, respectively, n=4/genotype), demonstrating that adipocyte Abca1 deletion had minimal effect on stromal vascular cell cholesterol content. ABCG1 gene expression increased and cholesterol synthesis (HMGCoA synthase) and lipoprotein receptor gene (LDLr and VLDLr) expression decreased significantly in response to deletion of adipocyte Abca1, in ASKO vs. Cntl mice fed the HFHC diet (Figure 3B). Gene expression results were confirmed for ABCG1 and LDLr protein expression by Western blot analysis (Figure 3C, 3D). Previously, we demonstrated that increased plasma membrane FC and lipid raft content resulted in agumented macrophage inflammatory signaling in macrophage-specific Abca1 knockout mice [28, 29]. We isolated adipocyte plasma membranes and observed a two-fold increase in cholesterol content for ASKO vs. Cntl mice (Figure 3E). Lipid rafts were also visualized using fluorescent-labeled beta-cholera toxin on adipose tissue sections; ASKO adipose tissue demonstrated increased lipid raft staining (Figure 3F). These data demonstrate a remarkable increase in adipocyte cholesterol in the absence of Abca1 that cannot be overcome by the usual compensatory cellular responses to increased cellular cholesterol.

ASKO mice have reduced adipose tissue TG

Previous studies have shown that the adipocyte cholesterol to TG ratio remains relatively constant regardless of adipocyte size [17]. Given the smaller adipocyte phenotype along with

increased adipose cholesterol in ASKO mice, we next examined whether adipose TG metabolism was affected. As anticipated, EAT from HFHC diet-fed ASKO mice contained less TG than EAT from Cntl mice (Figure 4A). There are several reasons why TG may be lower in ASKO adipocytes. We first explored whether ASKO adipocytes were more lipolytic compared to Cntl adipocytes. After 8 weeks of HFHC diet feeding, Cntl and ASKO mice were stimulated with a β3-specific adrenergic receptor agonist to induce lipolysis. There was no significant difference in plasma non-esterified free fatty acid levels before and after β3-agonist stimulation between ASKO and Cntl mice, indicating that deletion of adipocyte Abca1 does not alter *in vivo* lipolytic potential (Figure 4B). We next examined [^{3}H]-oleic acid incorporation into EAT TG. Less [^{3}H]-TG was found in ASKO than Cntl EAT, suggesting either decreased [^{3}H]-oleic acid upake or diminished esterification into TG (Figure 4C). Finally, we examined *de novo* lipogenesis by incubating EAT with [^{14}C]-acetic acid. ASKO EAT incorporated less [^{14}C]-acetate into TG, suggesting decreased *de novo* lipogenesis (Figure 4D). Taken together, these results demonstrate that ASKO adipocytes are smaller and contain less TG due to decreased lipogenesis.

Adipocyte Abca1 deletion does not affect adipose tissue insulin sensitivity

Since HFHC diet-fed ASKO mice had smaller adipocytes that contained less TG and synthesized less TG relative to Cntl adipocytes, we hypothesized that insulin signaling might also be attenuated in ASKO adipose tissue. Fasting blood glucose concentrations were similar in ASKO and Cntl mice, regardless of diet (Figure 5A). Plasma insulin concentrations were similar between genotypes in chow-fed mice, but significantly higher in HFHC diet-fed ASKO vs. Cntl mice (Figure 5B). Insulin signaling was examined in EAT, liver and skeletal muscle collected 5 minutes after portal vein insulin injection. Contrary to our hypothesis, insulin signaling, measured as Akt phosphorylation, was similar between genotypes in all three tissues (Figure 5C, 5D). To examine systemic glucose homeostasis, glucose and insulin tolerance tests were performed on mice fed chow until 24 weeks of age or the HFHC diet for 16 weeks, starting at 8 weeks of age. Although HFHC diet-fed mice were defective in plasma glucose disposal compared to chow-fed counterparts, glucose tolerance (Figure 5E) and insulin tolerance (Figure 5F) curves were indistinguishable between genotypes, suggesting that deletion adipocyte *Abca1* did not affect systemic insulin resistance.

EAT lipid accretion pathways are down-regulated in ASKO mice

Since insulin signaling could not explain reduced adipocyte TG content and lipogenesis in ASKO mice, we further examined lipid accretion pathways. PPARγ and CCAAT/enhancer binding protein (C/EBP1)α and β gene expression were significantly decreased in EAT of HFHC-fed ASKO mice (Figure 6A). These genes are the major transcription factors that govern TG accumulation during adipocyte differentiation [37]. Other genes involved in adipocyte TG synthesis, such as DGAT 1 and 2, or lipid uptake (i.e., CD36) were also decreased in ASKO EAT. These data were confirmed by Western blot analysis of PPARγ, Stearoyl-CoA Desaturase 1 (SCD-1), and CD36 (Figure 6B and C).

SREBP1c is a major regulatory pathway responsible for the promotion of *de novo* lipogenesis and TG storage [38]. Accordingly, we examined nuclear (i.e., activated) SREBP1c

levels in adipocytes isolated from EAT of mice fed HFHC diet for 16 weeks; nuclear SREBP1 protein was decreased in ASKO vs. Cntl adipocytes (Figure 6D). To determine whether transient loss of adipocyte Abca1 protein expression impaired adipocyte differentiation by decreasing PPARγ expression, we silenced Abca1 in 3T3L1 cells during differentiation into adipocytes and examined PPARγ and aP2 protein expression. Abca1 protein expression was strikingly reduced in 3T3L1 adipocytes by day 2 of Abca1 siRNA treatment compared to control-treated cells (Figure 6E), and began to increase during days 6–8. Reduction of Abca1 protein was associated with decreasing expression of PPARγ and aP2, a target of PPARγ, (Figure 6E) and reduced adipocyte TG content (Figure 6F). Based on these results, we proposed that deletion of Abca1 resulted in decreased expression and activation of Pparγ, which in turn, leads to decreased lipid accretion and smaller adipocytes.

Deletion of Abca1 in BAT has minimal effect on BAT function

BAT weight did not differ between Cntl and ASKO animals (Supplemental Figure III A), although cholesterol content was elevated >2-fold in ASKO mice (Supplemental Figure III B). Expression of prototypical BAT genes was similar for both genotypes of mice (Supplemental Figure III C). However, ASKO mice were better able to maintain body temperature during acute cold exposure compared with Cntl mice (Supplemental Figure IIID), suggesting a modest increase in thermogenesis in ASKO mice.

Deletion of Abca1 in adipose tissue does not increase adipose tissue inflammation

In previous studies, increased adipose tissue cholesterol content driven by dietary cholesterol intake was associated with increased adipose tissue inflammation [19, 39]. Despite a 3-fold increase in EAT cholesterol (Figure 3A), pro-inflammatory and ER stress-related gene expression did not increase in EAT of ASKO relative to Cntl mice, although inflammatory gene expression did increase in mice fed the HFHC diet versus the chow diet (Supplemental Figure IV A). CD68 immunofluorescence was similar for ASKO and Cntl adipose tissue (Supplemental Figure IV B), in agreement with the gene expression data. Previous studies have shown a direct correlation between dietary cholesterol, adipose tissue cholesterol, and inflammation [39, 40]; however, this is not the case in our ASKO model, likely due to selective increases in cholesterol in adipocytes, but not stromal vascular cells.

Discussion

This study was designed to elucidate the role of adipocyte Abca1 expression on adipocyte lipid homeostasis. Previous studies have shown that Abca1 is abundantly expressed in adipose tissue [22]; however, its role in adipocyte lipid homeostasis and function *in vivo* is poorly understood. Previous studies using aP2 *Cre* recombinase mediated-deletion of *Abca1* $^{flox/flox}$ alleles were complicated by concomitant and variable *Abca1* gene deletion in macrophages in addition to adipocytes [22, 23]. In the present study, we generated a mouse model with adipocyte-specific deletion of the *Abca1* gene using adiponectin *Cre* recombinase-mediated deletion, allowing us to more clearly detect the effects of Abca1 expression in adipocytes.

ASKO mice had significantly lower total plasma and HDL cholesterol concentrations compared to Cntl mice (Figures 1C, 1D). This agrees with our previous findings [22] that adipose Abca1 contributes minimally (<15%) to total plasma cholesterol levels *in vivo*, which are primarily determined by Abca1 expression in the liver [41] and small intestine [42].

Despite marginal impact on plasma cholesterol concentrations, adipocyte Abca1 deletion resulted in significantly higher levels of adipose tissue cholesterol content. We observed a 3-fold increase in total cholesterol in both WAT and BAT (Figure 3A and Supplemental Figure IIIB), despite the fact that ABCG1 was upregulated and cholesterol synthesis and uptake genes were downregulated in ASKO adipose tissue (Figure 3B). These results strongly suggest that Abca1 is the major cholesterol efflux system in adipocytes – and that in its absence, other pathways of cellular cholesterol homeostasis cannot compensate. These results are in contrast to those of early *in vitro* experiments, which reported that Abca1 only effluxes cholesterol under conditions of prolonged lipolysis [43]. On the other hand, our findings agree with those in other *in vivo* models that examined adipose Abca1 expression *in vivo* [22, 23].

Adipocyte cholesterol balance may play a significant role in determining adipocyte size and TG content, particularly during progression of obesity [21, 44, 45]. For example, adipocyte cholesterol accumulation is associated with TG accretion during adipocyte hypertrophy [15]. However, in our study, ASKO mice had a 3-fold increase in adipose tissue cholesterol, but reduced adipose tissue TG content and adipocyte size (Figure 2G and 4A). The reason for the difference in outcomes between our study and previous ones may be attributed to the high levels of plasma membrane cholesterol in ASKO adipocytes (Figure 3E). Adipocyte cholesterol moves from the plasma membrane to the lipid droplet during hypertrophy, leading to enlarged adipocytes and relative depletion of plasma membrane cholesterol [21]. Adipocytes appear to sense cholesterol redistribution from the plasma membrane to lipid droplets as a form of cellular cholesterol depletion [21, 43, 44, 46]. Supporting this notion, hypertrophied adipocytes activate SREBP2 and upregulate cholesterol synthesis genes, despite higher levels of cellular cholesterol [21]. In the absence of adipocyte Abca1, cholesterol redistribution from the plasma membrane to lipid droplets may be compromised.

To determine why ASKO mice had lower fat pad weight and smaller adipocytes, we examined adipose tissue TG metabolism. We determined that β3 adrenergic-stimulated TG lipolysis *in vivo* was similar between genotypes (Figure 4B). In contrast, ASKO EAT was less able to incorporate radiolabeled oleic acid and acetate into TG (Figure 4C and D) and expression of major lipogenic genes and transcription factors (i.e., PPARγ, C/EBPs, SREBP1c) were downregulated in the adipose tissues of ASKO mice compared to their Cntl counterparts (Figure 6A and B). The most likely explanation for our findings is that accumulation of cholesterol without adipocyte Abca1 expression inhibited SREBP1c processing by increasing endoplasmic reticulum cholesterol content [47], diminishing *de novo* lipogenesis and PPARγ expression, which is a direct transcriptional target of SREBP1c [48]. Reduced lipogenesis and fatty acid uptake would decrease potential ligands for PPARγ activation as well as substrate for TG synthesis [49].

Interestingly, despite a 3-fold increase in EAT cholesterol content in ASKO mice, we observed only minor effects on adipose tissue inflammation (Supplemental Figure IVA and B). Previous work has shown that addition of dietary cholesterol alone can lead to adipose inflammation [19, 39]. Several studies, including our own, have demonstrated that dietary cholesterol leads to increased adipose tissue cholesterol content, macrophage infiltration, and inflammation [39, 40, 50]. These studies show ~ 50% increase in adipose cholesterol content as a result of increased dietary cholesterol intake. In contrast, ASKO mice had a ~300% increase in EAT cholesterol, suggesting that adipocyte cholesterol content alone does not determine adipose tissue inflammation and that macrophages, not adipocytes, are primarily responsible for dietary cholesterol-induced inflammation. The absence of increased EAT inflammation in HFHC diet-fed ASKO vs. Cntl mice may also explain why systemic insulin resistance was similar between genotypes.

Macrophage infiltration into adipose tissue is likely the main contributor to adipose tissue inflammation that accompanies weight gain and obesity [19, 51–55]. We also showed that a small increase in macrophage membrane FC and lipid raft content induced by macrophage-specific Abca1 deletion results in an exaggerated proinflammatory response to Toll-like 4 receptor (TLR4) agonists (i.e. lipopolysaccharide, LPS) [29], supporting the idea that increased adipose tissue macrophage cholesterol may result in increased adipose tissue inflammation. However, increased macrophage inflammation *per se* does not result in the systemic insulin resistance that accompanies obesity [56], suggesting that adipose tissue macrophage inflammation and adipocyte dysfunction are both necessary to display the full metabolic phenotype in obese states.

ASKO mice consuming a HFHC diet had significantly less weight gain compared to Cntl mice. One explanation for this outcome may be related to higher energy expenditure by ASKO mice during the dark cycle, (Supplemental Figure II). However, when energy expenditure data were not normalized to body weight, there was no difference between genotypes. Although the appropriate way to report metabolic data has been debated [36], most studies normalize energy expenditure to body weight as we did. Different energy expenditures could not be explained by differences in BAT mass or UCP1 expression. We also found no consistent evidence for beiging or browning of WAT (subcutaneous and visceral) based on UCP1 gene and protein expression (data not shown). However, in an acute cold tolerance experiment, ASKO mice had a slight, but significant, elevation of body temperature compared with Cntl mice, in agreement with the increased energy expenditure data.

We also observed similar food intake and activity between Cntl and ASKO mice. However, a small decrease in food intake in ASKO mice may not have been detected over the relatively short food intake experiments. For example, an 8 g difference in body weight between Cntl and ASKO mice over a 16 week feeding period is an average body weight gain of 0.071 g/day (8g/112 days); detecting a difference in food consumption needed to produce this small change in body weight over a two day (CLAMS experiment) or one week (manual food intake experiment) observational period may be very difficult (Supplemental Figure II F). More detailed studies will be necessary to understand the mechanisms for lower body weights in HFHC diet-fed ASKO mice.

The results of our study may have clinical implications. During development of obesity, as adipocyte size and TG content increases, adipocyte cholesterol also increases, reaching 50% of the total body cholesterol pool in extreme cases of obesity [15]. Cellular cholesterol accumulation normally would increase expression of ABCA1 and other cholesterol export proteins through activation of the transcription factor liver X receptor (LXR). We have shown increased visceral fat expression of ABCA1 and apolipoprotein E, two LXR target genes, in non-human primates fed increased dietary cholesterol, which may provide protection from adipocyte cholesterol overload [39]. Obesity is also accompanied by increased adipose tissue and systemic inflammation and we and others have shown that inflammatory stimuli downregulate ABCA1 expression [30, 57]. Based on results from the present study, this may afford adipocytes a protective mechanism to avoid excessive TG accumulation, which may lead to adipocyte dead, by downregulating TG synthesis as adipocyte FC accumulates.

In summary, the results from our mouse model provide additional mechanistic insights into cholesterol metabolism in adipose tissue. Surprisingly, deletion of Abca1 in adipocytes was mostly beneficial, resulting in lower weight gain, reduced body fat, and increased energy expenditure, with no alteration in systemic glucose and insulin sensitivity or adipose tissue inflammation. We posit that most of these results are due to the high level of membrane cholesterol in ASKO adipocytes. By preventing cholesterol efflux through Abca1, we artificially raised membrane cholesterol levels and prevented the adipocyte hypertrophy normally observed in HFHC diet-fed mice. Our results demonstrate an important role for adipocyte Abca1 in the regulation of adipocyte function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

TG	Triglyceride
Abca1	ATP Binding Cassette Transporter A1
ASKO	Adipose-specific *Abca1* knockout
HT	Heterozygous adipocyte-specific *Abca1* knockout
C/EBP	CCAAT/enhancer binding protein
HFHC	High Fat High Cholesterol

HDL	High Density Lipoprotein
EAT	Epididymal Adipose Tissue
BAT	Brown Adipose Tissue
SAT	Subcutaneous Adipose Tisue
WAT	White Adipose Tissue
SREBP1/2	Sterol Regulatory Element Binding Protein 1/2
PPARγ	Peroxisome Proliferator-activated Receptor γ

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Highlights

- Adipocyte-specific deletion of the cellular cholesterol exporter Abca1 prevents body weight gain induced by high fat, high cholesterol diet feeding without affecting glucose or insulin sensitivity.
- Mouse adipocytes lacking Abca1 had a 3-fold increase in cholesterol content, but were smaller in size and contained less triglyceride due to defective triglyceride synthesis, which likely occurred due to decreased activation of transcription factors SREBP1c and PPARγ.
- This study provides compelling evidence that Abca1 is a major regulator of adipocyte cholesterol content and triglyceride storage.

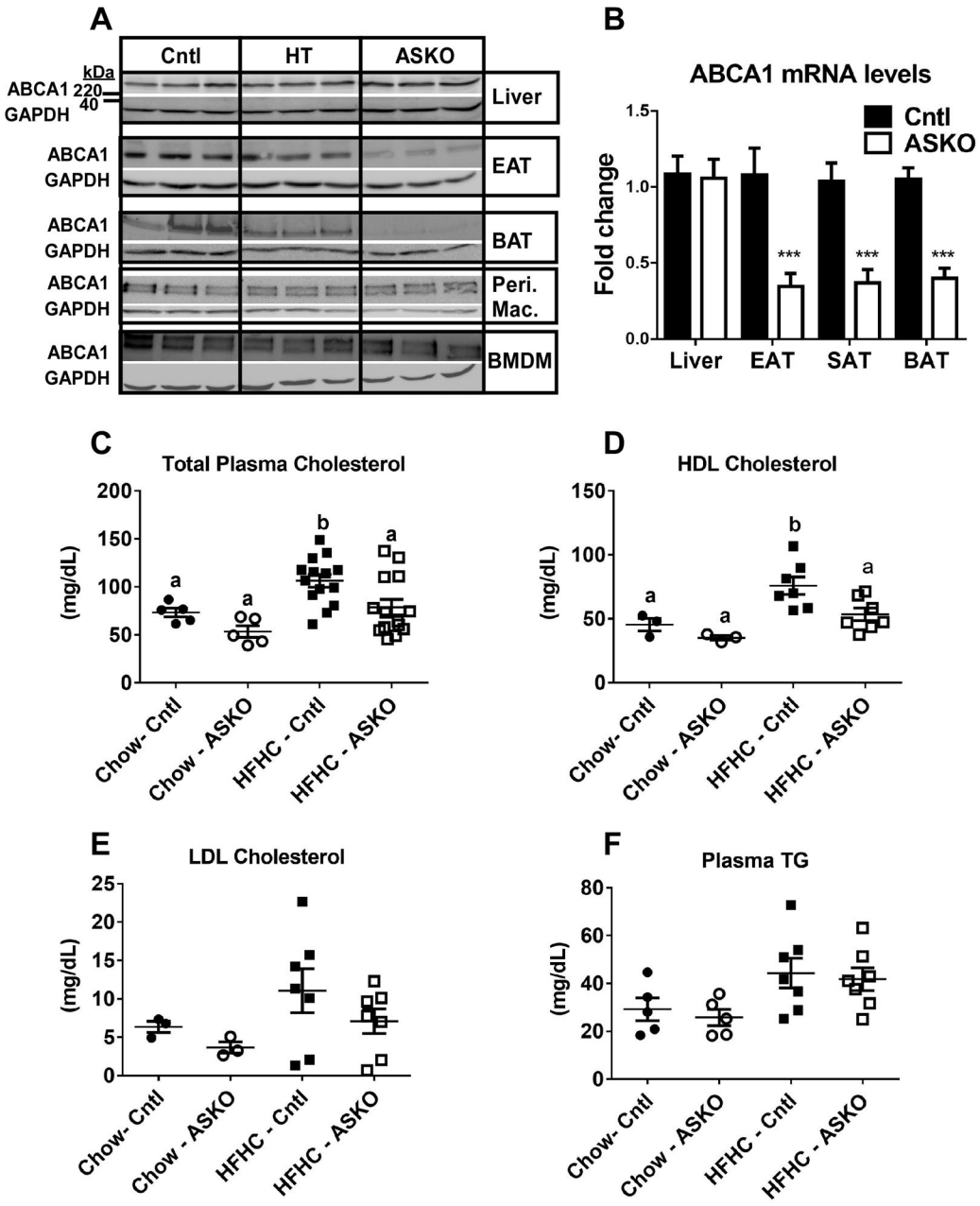
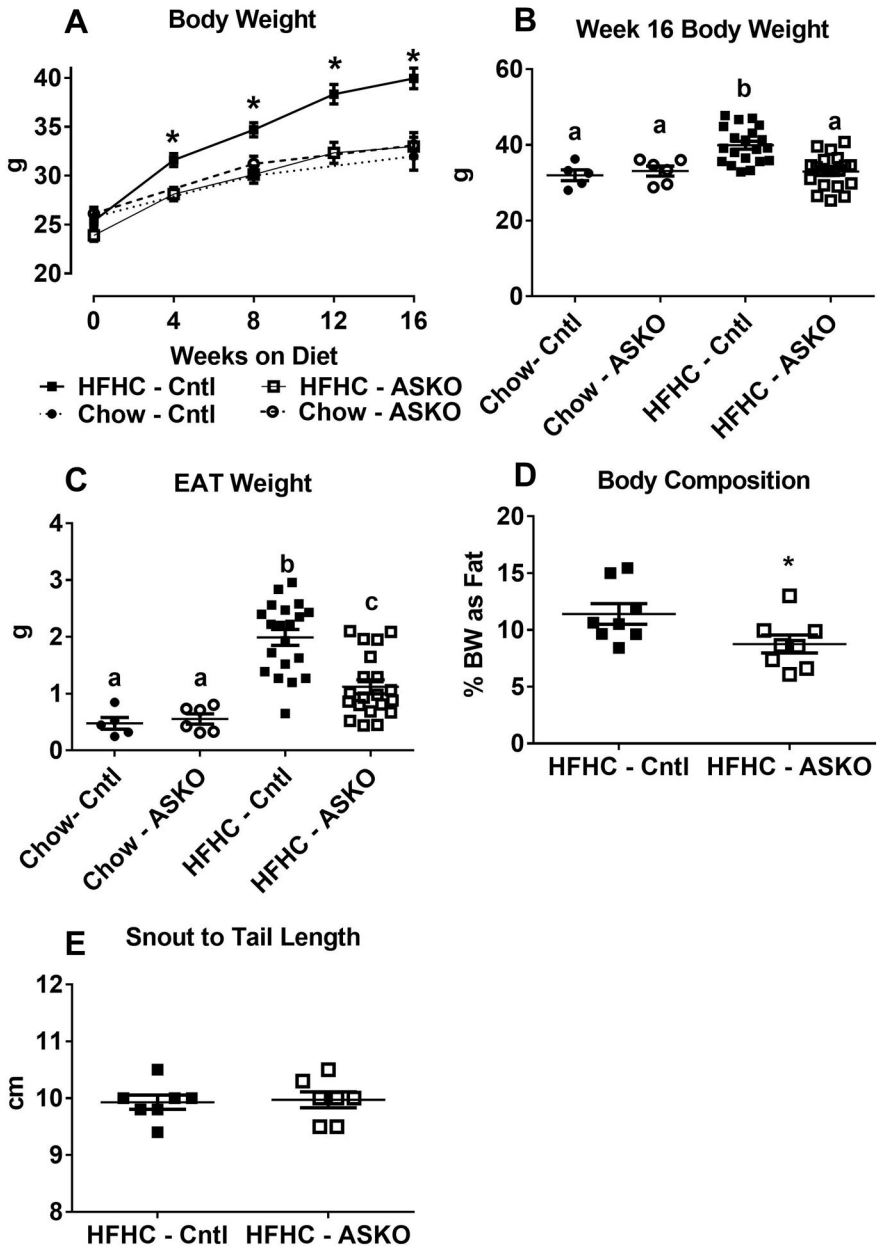


Figure 1.

A) Abca1 and GAPDH Western blots of tissues from Cntl, heterozygous ASKO (HT), and ASKO mice. EAT=epididymal adipose tissue, BAT=brown adipose tissue, Peri. Mac.=resident peritoneal macrophages, BMDM=bone marrow-derived macrophages. B) Abca1 gene expression in liver, EAT, SAT and BAT (n=6 per group). *** denotes statistical difference by Student's t-test, $p<0.0001$. Plasma concentrations of: C) total cholesterol, D) HDL cholesterol, E) LDL cholesterol, and F) triglyceride (TG) in male Cntl and ASKO mice fed chow for 24 weeks or a HFHC diet for 16 weeks starting at 8 weeks of age. Total plasma cholesterol and TG concentrations were determined from individual plasma samples. LDL and HDL cholesterol concentrations were determined after FPLC fractionation of plasma

and enzymatic assay of cholesterol as described in the Methods. Each data point represents an equal volume pooled from 2 mice. Panels C and D, different letters denote statistical difference by ANOVA and Tukey's multiple comparisons test, $p<0.05$.



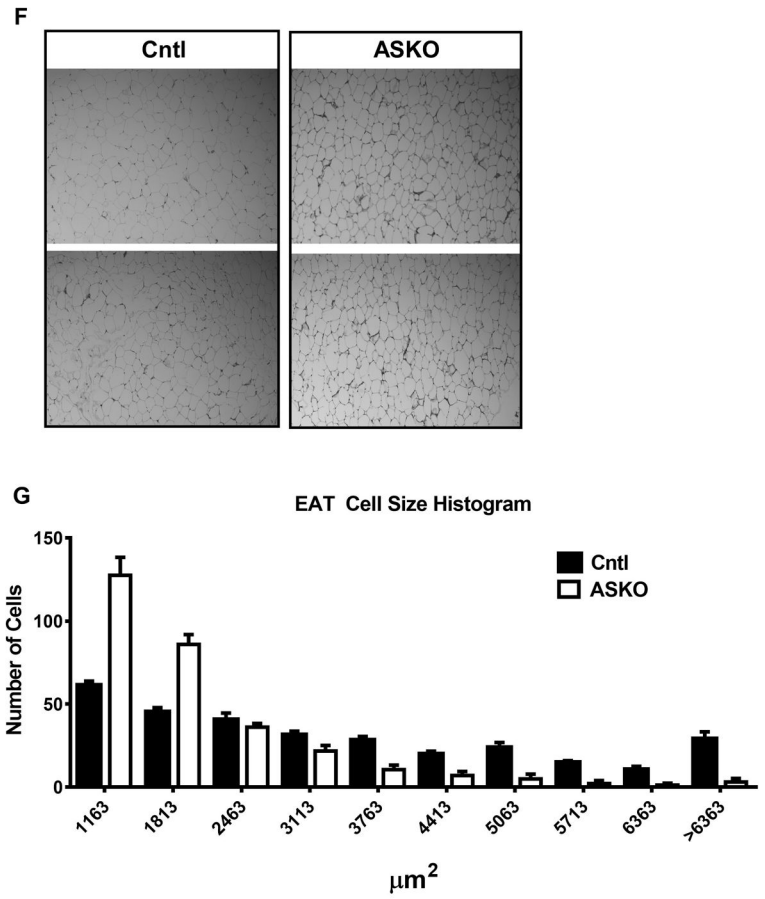
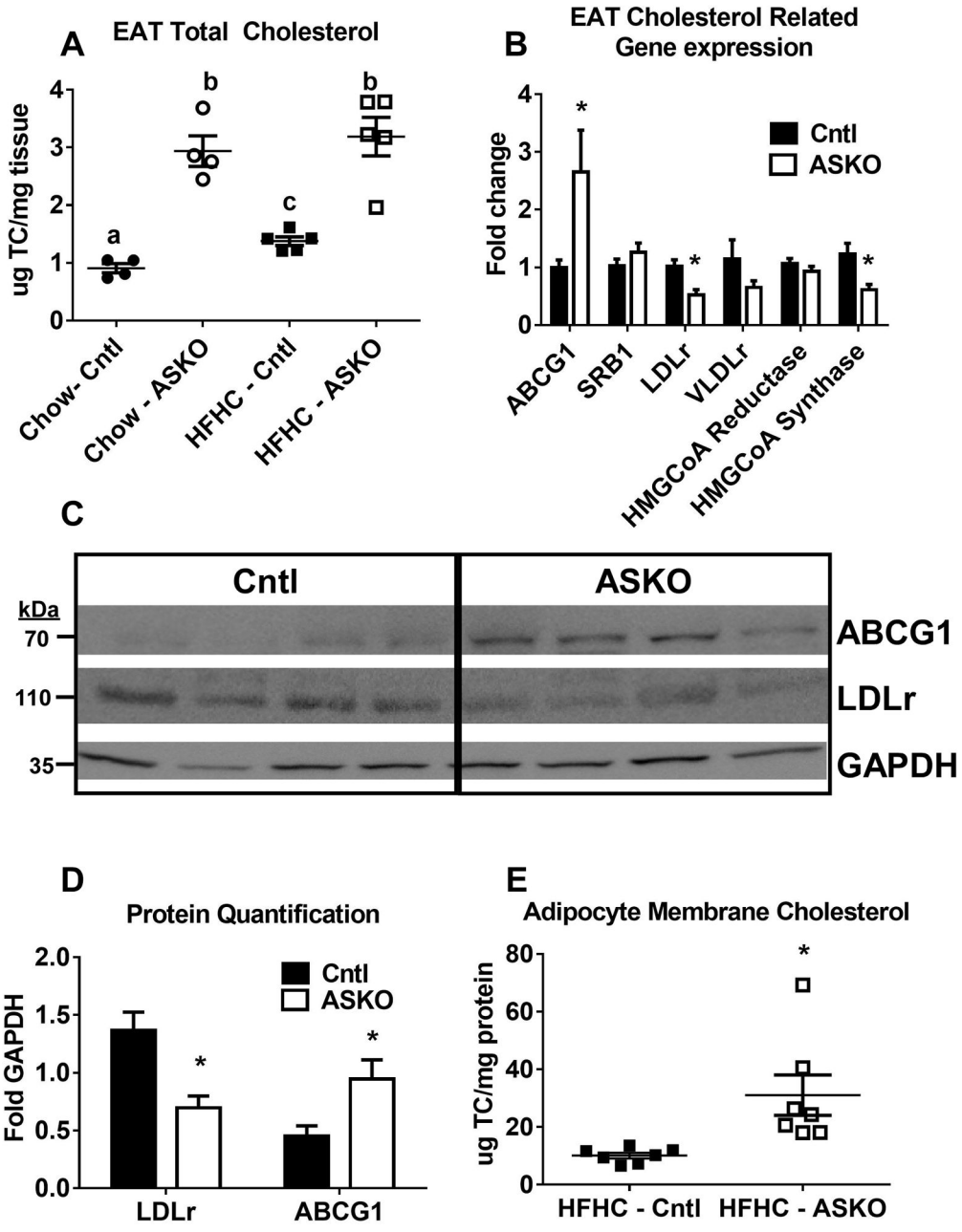


Figure 2.

Male Cntl and ASKO mice were fed chow for 24 weeks (n=5–6) or a HFHC diet for 16 weeks starting at 8 weeks of age (n=20). Mice were sacrificed at 24 weeks of age to harvest adipose tissue. A) Body weight gain during dietary feeding. B) Terminal body weights. C) Epididymal adipose tissue (EAT) weight harvested at 24 weeks of age. D) Percentage of body weight as fat was calculated by summing the weight of epididymal, retrorenal, subcutaneous, and brown fat depots and dividing the sum by total body weight X 100% for each mouse. E) Snout to tail length of mice at 24 weeks of age. F) Representative photomicrographs of EAT harvested at 24 weeks of age. G) Histogram of EAT adipocyte cross-sectional area; 300 cell measurements per mouse were taken from n=6 mice per genotype. Adipocyte size was significantly smaller in ASKO vs. Cntl mice by Chi square analysis ($p<0.001$). Asterisks (panels A and D) and different letters (panels B and C) denote statistical difference by ANOVA and Tukey's multiple comparisons test or unpaired Student's t-test, $p<0.05$.



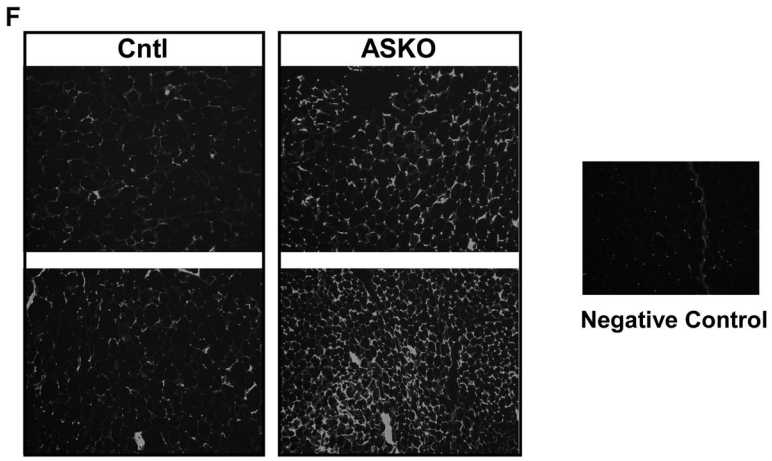


Figure 3.

A) Male Cntl and ASKO mice were fed chow for 24 weeks or a HFHC diet for 16 weeks starting at 8 weeks of age before EAT was harvested to measure total cholesterol content. B) Expression of genes involved in cholesterol metabolism in EAT from HFHC-fed mice diet for 16 weeks (n= 6 per group). C) Western blot of EAT ABCG1, LDLr and GAPDH from mice fed the HFHC diet diet for 16 weeks. D) Quantification of Western blot data in panel C. E) EAT plasma membrane cholesterol content from mice fed the HFHC diet for 16 weeks. F) Lipid raft staining of EAT from mice fed the HFHC diet for 16 weeks. Lipid rafts detected by fluorescent-labeled beta-cholera toxin, which binds to GM1 gangliosides. Negative control contained no fluorescent-labeled beta-cholera toxin. Different letters (panel A) denote statistical difference by ANOVA and Tukey's multiple comparisons test. Asterisks (panels B, D, and E) denote statistical differences between genotypes by unpaired Student's t-test, $p<0.05$.

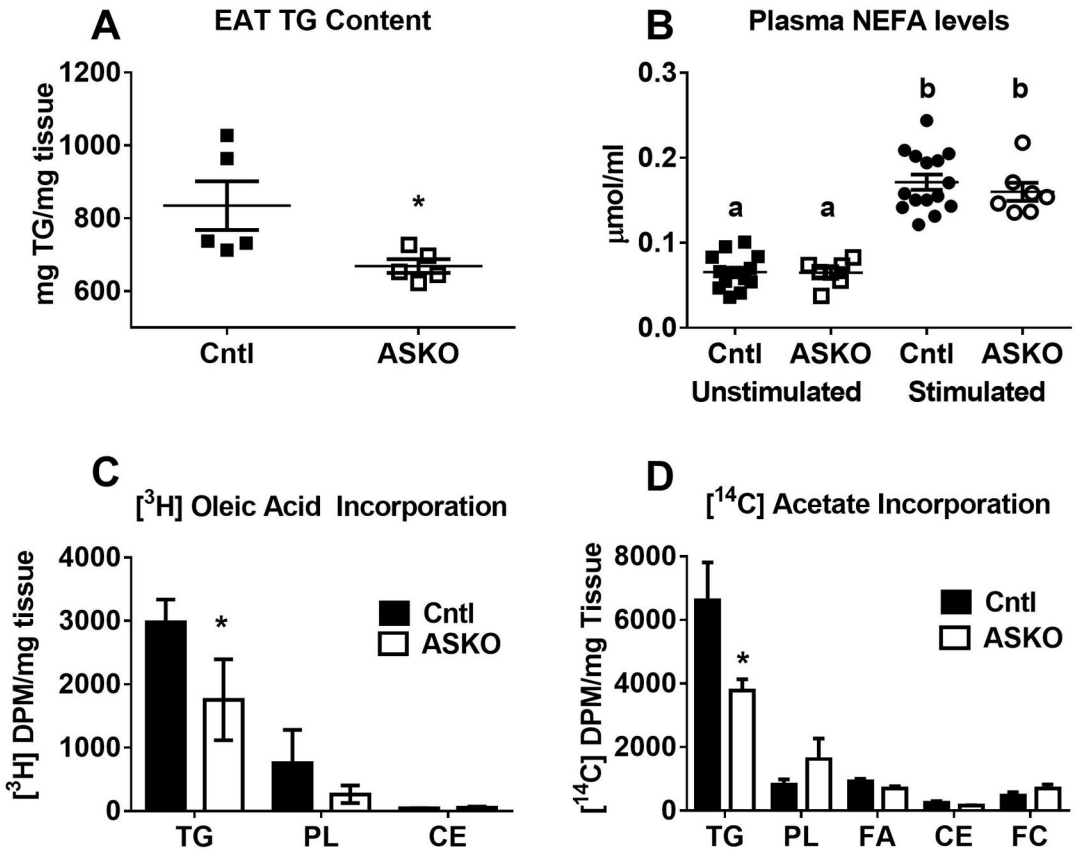


Figure 4.

A) EAT TG content of male Cntl and ASKO mice fed HFHC diet for 16 weeks starting at 8 weeks of age. B) Plasma NEFA concentrations before and after stimulation with a β-3 specific adrenergic agonist in male mice fed HFHC diet for 8 weeks starting at 8 weeks of age. C) Incorporation of [^{3}H]-oleic acid into EAT explant lipid fractions (n=6 per group). D) Incorporation of [^{14}C]-acetate into EAT explant lipid fractions (n=4 per group). Different letters (panel B) denote statistical difference by ANOVA and Tukey's multiple comparisons test. Asterisks (panels A, C, and D) denote statistical difference between genotypes by Student's t-test, $p<0.05$.

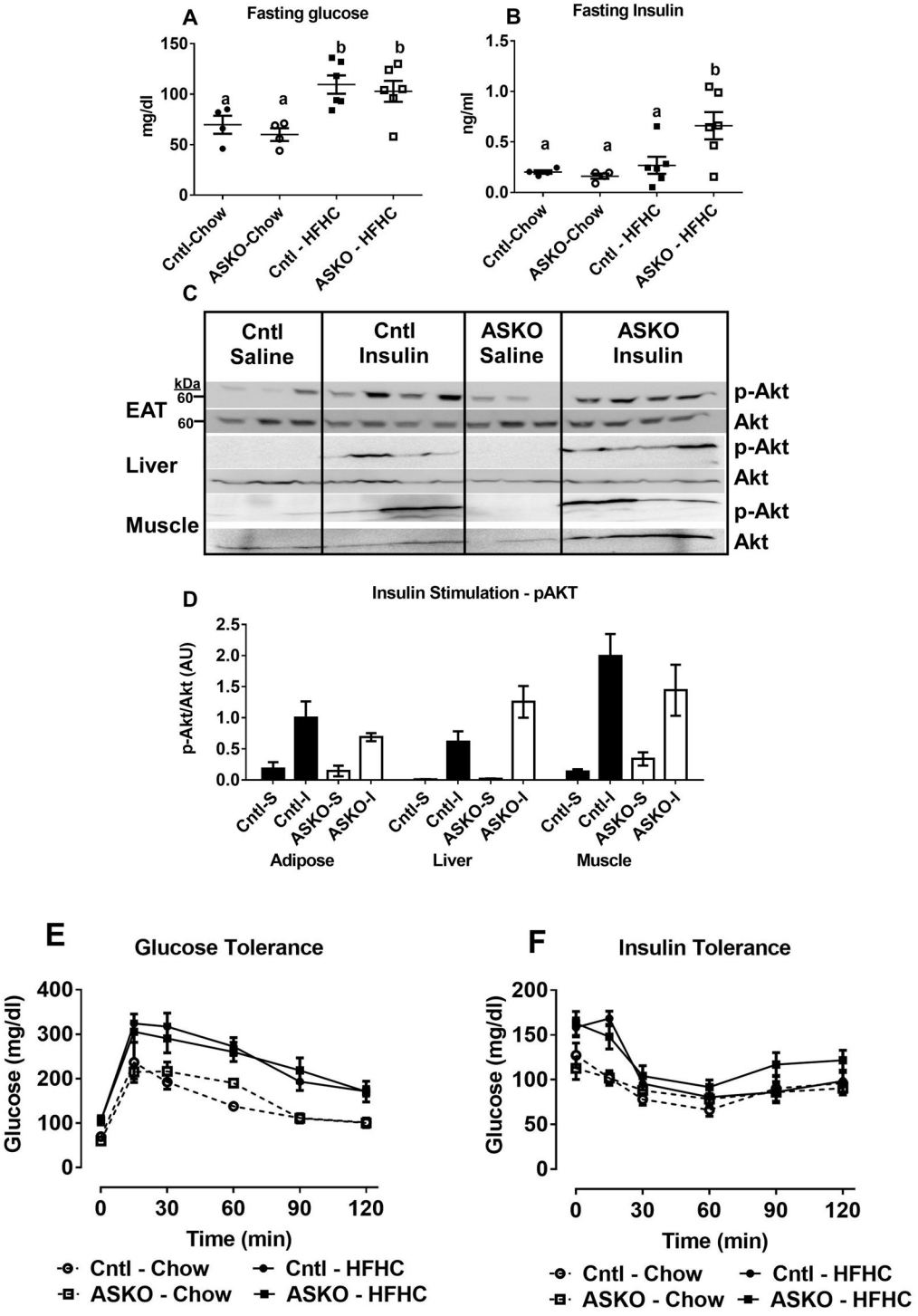


Figure 5.

Male Cntl and ASKO mice were fed chow for 24 weeks or a HFHC diet for 16 weeks starting at 8 weeks of age and then fasted for 12 hours before blood and plasma were collected for glucose (A) and insulin (B) measurements, respectively. C) Male mice fed a HFHC diet for 16 weeks were fasted overnight, anesthetized, and injected with insulin

(1U/Kg) in the portal vein. Five minutes later, mice were euthanized and EAT, liver, and skeletal muscle were harvested for Western blot analysis of p-Akt and total Akt expression. D) Western blot quantification of p-Akt/total Akt from blots in C. E) Glucose tolerance tests were performed on mice fed the HFHC diet for 16 weeks after a 12 hour fast. F) Insulin tolerance tests were performed on mice fed the HFHC diet for 16 weeks after a 12 hour fast. Different letters (panel A, B) denote statistical difference by ANOVA and Tukey's multiple comparisons test, $p<0.05$.

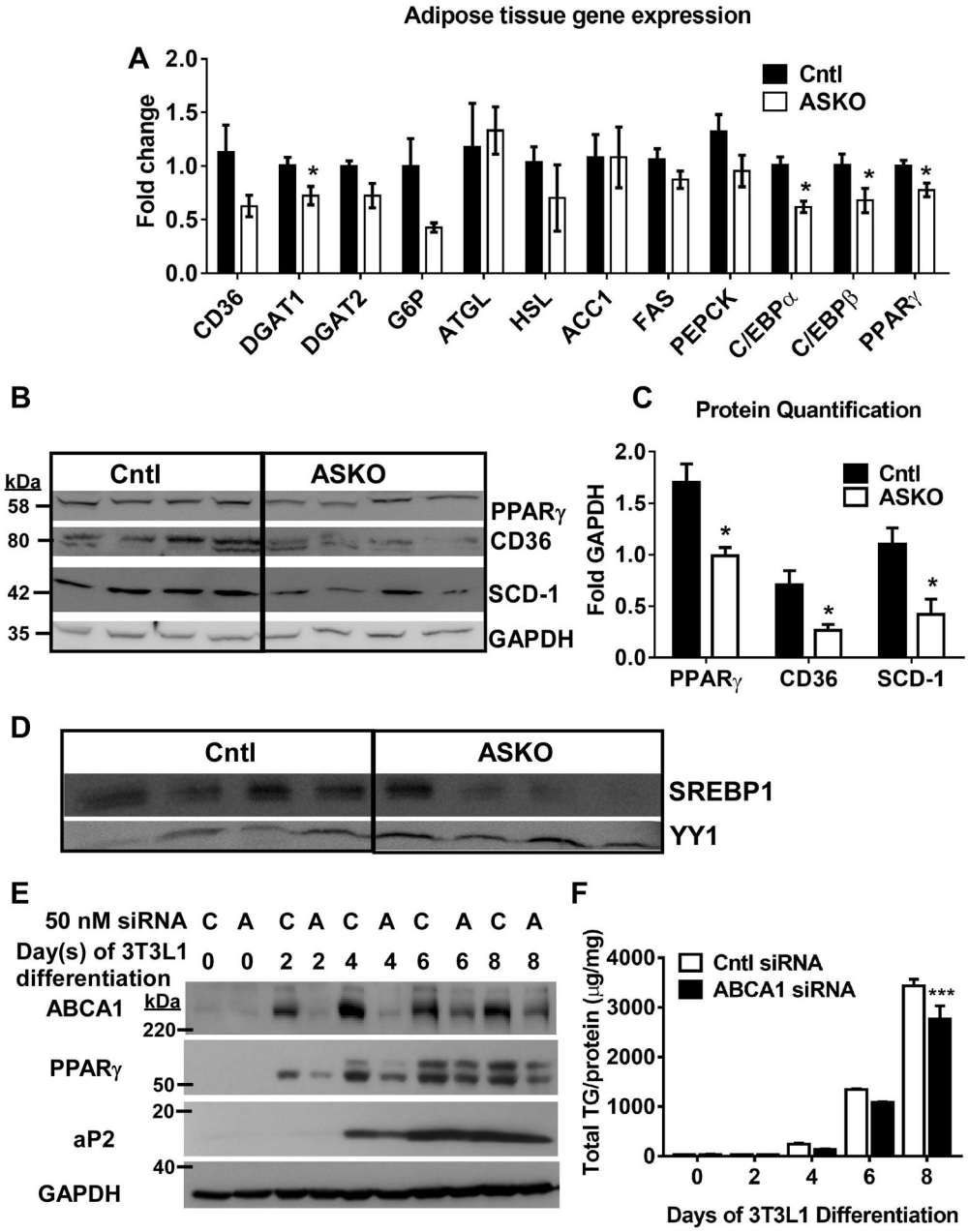


Figure 6.

Male Cntl and ASKO mice were fed a HFHC diet for 16 weeks, starting at 8 weeks of age, before harvesting EAT for gene and protein expression. A) EAT gene expression of lipogenic genes and transcription factors (n=6 per group). B) Western blot for PPARγ, CD36, SCD-1, and GAPDH in EAT tissue. C) Quantification of Western blot results in panel B; asterisk denotes statistical difference by upaired Student's t-test, $p<0.05$. D) Western blot of SREBP1 and YY1 in nuclear fraction of EAT isolated adipocytes. E) and F) 3T3-L1 cells were transfected with scrambled control siRNA (C) or with Abca1 siRNA (A) for 6 h and then differentiated for 8 d. Abca1, PPARγ, aP2, and GAPDH expression was examined by Western blot and cellular triglyceride (TG) content was measured by enzymatic TG assay.

Two-way ANOVA results showed significant differences for time (0.0001), siRNA treatment (0.03), and interaction (0.01); asterisks denote Bonferroni's multiple comparisons test results; *** $p<0.001$.