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Myeloid cell-specific ABCA1 deletion does not worsen insulin resistance in HF diet-induced or genetically obese mouse models

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Abstract

Obesity-associated low-grade chronic inflammation plays an important role in the development of insulin resistance. The membrane lipid transporter ATP-binding cassette transporter A1 (ABCA1) promotes formation of nascent HDL particles. ABCA1 also dampens macrophage inflammation by reducing cellular membrane cholesterol and lipid raft content. We tested the hypothesis that myeloid-specific ABCA1 deletion may exacerbate insulin resistance by increasing the obesity-associated chronic low-grade inflammation. Myeloid cell-specific ABCA1 knockout (MSKO) and wild-type (WT) mice developed obesity, insulin resistance, mild hypercholesterolemia, and hepatic steatosis to a similar extent with a 45% high-fat (HF) diet feeding or after crossing into the ob/ob background. Resident peritoneal macrophages and stromal vascular cells from obese MSKO mice accumulated significantly more cholesterol. Relative to chow, HF diet markedly induced macrophage infiltration and inflammatory cytokine

expression to a similar extent in adipose tissue of WT and MSKO mice. Among pro-inflammatory cytokines examined, only IL-6 was highly upregulated in MSKO-ob/ob versus ob/ob mouse peritoneal macrophages, indicating a nonsignificant effect of myeloid ABCA1 deficiency on obesity-associated chronic inflammation. In conclusion, myeloid-specific ABCA1 deficiency does not exacerbate obesity-associated low-grade chronic inflammation and has minimal impact on the pathogenesis of insulin resistance in both HF diet-induced and genetically obese mouse models.

Keywords: cholesterol, macrophage, inflammation, obesity, high-fat

In the past decade, obesity-associated chronic low-grade inflammation has been recognized as a major cause of insulin resistance and type 2 diabetes. For example, overexpansion of adipose tissues increases the production of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) (1), and chemokines, such as monocyte chemoattractant protein-1 (MCP-1) (2). TNF-α-activated c-Jun N-terminal kinase (JNK) impairs insulin signaling, which contributes to insulin resistance (3). MCP-1 recruits immune cells, especially macrophages (40% of infiltrated immune cells), to adipose tissue, exacerbating inflammation and insulin resistance (4). Interestingly, Toll-like receptor 4 (TLR4), a pathogen-associated molecular pattern receptor, has been implicated as a link between inflammation and insulin resistance by functioning as a receptor for saturated fatty acids (5–7). Genetic deletion of key components of the TLR4 signaling pathway, such as TLR4 (8), JNK1 (9), or inhibitor of NF-κB-kinase β (IKKβ) (10) in hematopoietic or myeloid cells further established the mechanistic link between macrophage inflammation and insulin resistance.

ATP-binding cassette transporter A1 (ABCA1), a member of a large family of membrane transporters, effluxes free cholesterol (FC) and phospholipids across the plasma membrane to combine with apolipoprotein A-I, forming nascent high density lipoprotein (HDL) particles (11, 12). Mutations that inactivate ABCA1 lead to Tangier disease, a disorder characterized by near absence of HDL, elevated plasma triglycerides (TGs), reduced low density lipoprotein concentrations, and increased storage of cholesteryl esters (CEs) in macrophages (13–15). Insulin resistance has not been documented in people with Tangier disease, perhaps because the disease is so rare (16). However, ABCA1 single nucleotide polymorphisms are associated with altered glucose metabolism and insulin resistance in humans (17–20). Of interest, leukocyte ABCA1 gene expression is associated with fasting glucose concentrations in normoglycemic men, and reduced leukocyte ABCA1 gene expression is associated with type 2 diabetes (21, 22). To date, only one study has directly linked ABCA1 expression to hyperglycemia, reporting that pancreatic β cell-specific ABCA1 deletion results in defective insulin release from islet cells (23). Whether deletion of ABCA1 expression in other cell types, such as macrophages, alters glucose metabolism is unknown.

Using myeloid cell (macrophage and neutrophil)-specific ABCA1 knockout (MSKO) mice, we demonstrated that macrophages from MSKO mice have a significant increase in FC and are more responsive to pro-inflammatory stimuli [e.g., lipopolysaccharide (LPS)] in vivo and in vitro compared with wild-type (WT) mice (24). This response was mediated through the TLR and myeloid differentiation primary-response protein 88 (MyD88)-dependent pathway and was

independent of alterations in plasma lipid concentrations (24). Hypersensitivity to LPS is most likely due to increased lipid raft content and increased trafficking of TLR4 into plasma membrane lipid rafts in MSKO mouse macrophages (25). Furthermore, MSKO mice challenged with the *Listeria monocytogenes* cleared the bacterium better than WT mice (26). These studies clearly demonstrated a regulatory role of myeloid ABCA1 in macrophage inflammation and innate immunity.

Based on the documented relationship between macrophage inflammation and insulin resistance, we hypothesized that myeloid-specific ABCA1 deficiency may exacerbate obesity-induced chronic inflammation and insulin resistance in mice fed a HF diet or crossed into the leptin-deficient ob/ob background. However, MSKO and WT mice developed obesity, adipose inflammation, and insulin resistance to a similar extent, suggesting that myeloid ABCA1 expression does not significantly worsen obesity-induced chronic inflammation and insulin resistance.

METHODS

Animals

WT ($ABCA1^{+/+}$) and MSKO ($ABCA1^{-M/-M}$) mice were generated as described previously (24, 25). Mice were backcrossed into the C57BL/6 background for six generations before use in these studies and housed in a specific pathogen-free facility with a 12 h light/dark cycle. Experiments were conducted in conformity with the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the experimental protocol was approved by the Wake Forest University Animal Care and Use Committee. At 8 weeks of age, male mice were switched from chow to a HF diet containing 45% of energy as lard and 0.015% cholesterol for 16–24 weeks. The HF diet was made by our institutional diet kitchen (see detailed diet composition in supplementary Table I). Body weight (BW) was measured biweekly. To generate MSKO-ob/ob mice, MSKO mice were first bred with heterozygous ob/ob (ob/+) mice. The resulting double heterozygous MSKO-ob/+ mice were intercrossed to generate ob/+ or MSKO-ob/+ mice, which were then intercrossed to generate ob/ob or MSKO-ob/ob mice, respectively. Ob/ob and MSKO-ob/ob male mice were fed chow for 14–15 weeks for the described studies.

Cell culture

Peritoneal macrophages were harvested from mice by flushing the peritoneal cavity with cold PBS. The peritoneal cells were plated in RPMI media containing 100 U/ml penicillin, 100 μg/ml streptomycin, and 1% Nutridoma SP media (Roche Applied Science). After a 2 h incubation, floating cells were removed by washing with PBS and adherent macrophages were used for experiments.

Plasma lipid, glucose, and insulin analysis

Blood samples were taken from tail veins following a 4 h fast during the light cycle (chow phase) before, and at 2–4 week intervals after initiation of HF diet feeding. Plasma cholesterol (Wako)

and TGs (Roche) were determined by enzymatic analysis according to the manufacturer's instructions. Plasma glucose levels were measured using a glucometer (Ascensia Contour, Bayer). Plasma insulin levels were measured by ELISA (Crystal Chem, Inc., Downers Grove, IL). For fed and overnight fasting plasma samples, blood was collected by tail vein at 9:00 AM in ad libitum fed mice (fed) or in mice fasted during the dark cycle for 15 h (5:00 PM–8:00 AM).

Hepatic lipid analysis

Liver lipids were extracted with chloroform:methanol (2:1) and the extract was used for enzymatic assays (cholesterol, Wako; TG, Roche). Data were normalized to liver protein mass, measured by the Lowry protein assay.

Glucose homeostasis analysis

Glucose tolerance tests (GTTs) and insulin tolerance tests (ITTs) were done after 12–20 weeks of HF diet consumption or with ob/ob mice at 13–14 weeks of age. Briefly, for GTTs, mice were fasted overnight before intraperitoneal (ip) injection of 1 g glucose/kg BW. Blood was collected before and after injection (0, 15, 30, 60, and 120 min) to measure glucose concentrations using a commercial glucose monitor. One week later, the same groups of mice were used for ip injection of 1.5 U of regular human insulin/kg BW (HF diet-fed mice) or 3 U/kg BW (ob/ob mice) after a 5 h fast. Blood glucose concentrations were measured at 0, 15, 30, 60, and 120 min after injection.

Real-time PCR

Total RNA in peritoneal macrophages and white adipose tissue was extracted using Trizol (Invitrogen) and RNeasy lipid tissue kits (Qiagen), respectively, according to the manufacturer's protocols. cDNA preparation and real-time PCR were conducted as described previously (24).

Immunohistochemistry staining

Epididymal fat tissue sections were incubated with the primary antibodies to CD68 (abD Serotec) or cleaved caspase-3 (Cell Signaling), followed by the biotinylated secondary antibody. The staining was visualized using ABC reagent (ABC vector kit; Vector) and DAB substrate chromogen (Dako). The area of fat sections covered by $CD68^+$ or cleaved $caspase\text{-}3^+$ cells was measured using Image-Pro software to quantify the percentage of positive staining cells in adipose tissue.

Stromal vascular cell isolation

Stromal vascular cells were fractioned according to the procedure described by Brown et al. (27). Briefly, epididymal fat was minced and enzymatically digested for 45 min with 5 ml/g tissue of collagenase type I (1 mg/ml, Worthington Biochemical Corporation) media. The digestion mixture was filtered through a 100 μm cell strainer and stromal vascular cells were pelleted by centrifugation (3,000 rpm for 10 min). The stromal vascular fraction (SVF) was then used for flow cytometry.

Flow cytometry

After blocking the Fcγ receptor with purified anti-mouse CD16/CD32 antibody (Fcγ receptor III/II; BD Biosciences), SVF cells were incubated at 4°C for 30 min with isotype controls or the following Abs: PE-anti-F4/80 (BM8; BD Pharmingen), PE-Cy7-anti-CD11c (HL3; BD Pharmingen), and Alexa Fluor 647-anti-CD206 (MR5D3; AbD Serotec). Cell fluorescence was measured using a FACSCanto II flow cytometer and data analyzed with FlowJo software.

Cholesterol content of macrophages and stromal vascular cells

Macrophages or stromal vascular cells isolated from adipose tissue were extracted with isopropanol (including 5α-cholestane as internal standard) at room temperature overnight and analyzed for cholesterol content by gas-liquid chromatography (27).

Statistical analysis

Data are presented as the mean ± SEM unless indicated otherwise. Differences were compared with two-tailed Student's *t*-test or one-way ANOVA using GraphPad Prism software. $P < 0.05$ was considered statistically significant.

RESULTS

Macrophages from MSKO versus WT obese mice had significantly more cholesterol accumulation, but comparable inflammatory cytokine expression

MSKO macrophages have increased plasma membrane FC and lipid rafts, resulting in increased response to TLR4 stimulation (LPS) compared with WT cells (24). Saturated fatty acids also activate TLR4 and induce chronic inflammation in the setting of obesity (5–7). We hypothesized that increased dietary saturated fatty acids (i.e., HF diet), functioning as TLR4 agonists, might increase inflammation during diet-induced obesity, worsening development of insulin resistance in MSKO versus WT mice. To test this possibility, we first measured cholesterol content in the resident peritoneal macrophages from obese mice. Consistent with our previous findings, macrophages from HF diet-fed MSKO mice (**Fig. 1A, C**) or chow-fed MSKO-ob/ob mice (Fig. 1B, D) had significantly more FC and CE accumulation compared with their WT counterparts. However, similar inflammatory cytokine gene expression was observed for macrophages from 24 week HF diet-fed WT and MSKO mice (Fig. 1E). Among the examined cytokines, only IL-6 was upregulated in macrophages from chow-fed MSKO-ob/ob versus ob/ob mice (Fig. 1F). In addition, the expression of TLR4 and its lipid-binding coreceptor CD36 (28) did not differ between genotypes (Fig. 1G, H). These data suggest that despite significant increases in macrophage cholesterol accumulation resulting from ABCA1 deficiency, HF diet feeding was not sufficient to stimulate pro-inflammatory activation of macrophages in vivo.

Myeloid-specific ABCA1 deficiency does not worsen insulin resistance in HF diet-fed mice or chow-fed ob/ob mice

Obesity-associated low-grade chronic inflammation underlies the pathogenesis of type 2 diabetes and insulin resistance (29, 30). Macrophage ABCA1 dampens pro-inflammation via downregulating MyD88-dependent TLR signaling (24, 25). We hypothesized that myeloid-specific ABCA1 deficiency may exacerbate chronic inflammation and insulin resistance associated with diet-induced or genetically obese mice. To test this hypothesis, we first fed the WT and MSKO male mice a HF diet containing 45% of energy as lard and 0.015% cholesterol. When challenged with the HF diet, WT and MSKO mice gained similar BW over the 24 week diet feeding period (**Fig. 2A**). Liver and fat mass were comparable between genotypes after 24 weeks of diet consumption (supplementary Fig. IA, B).

We then assessed several key parameters of glucose homeostasis in WT and MSO mice. Blood glucose concentrations after a 4 h fast were comparable between both groups of mice (Fig. 2B). MSKO mice also exhibited similar plasma glucose clearance (GTT) compared with WT mice (Fig. 2C). Consistent with this finding, the ability of insulin to lower blood glucose concentrations during ITTs was equivalent in both groups of mice (Fig. 2D). We next measured plasma insulin concentrations in overnight-fasted and fed mice; again, no differences were observed between genotypes (Fig. 2E, F). Comparable glucose homeostasis between the two genotypes also occurred with prolonged diet feeding (e.g., 12–24 weeks) using different sets of mice. Adding cholesterol (0.2%) to the HF diet did not significantly alter the phenotype (data not shown). Similar outcomes were also observed for HF diet-fed female mice (data not shown). Together, our data suggest that myeloid cell-specific ABCA1 deletion does not affect the development of systemic insulin resistance in the presence of HF diet-induced obesity.

Next, we tested our hypothesis using an early-onset genetic obesity mouse model by crossing MSKO mice into the ob/ob background. At 8 and 14 weeks of age, ob/ob and MSKO-ob/ob mice had comparable BWs (**Fig. 3A, B**). No differences in liver or fat mass were observed between genotypes (supplementary Fig. IC, D). A GTT was performed when mice were 13 weeks old, followed by an ITT the next week. Due to the low yield of the MSKO-ob/ob mice, we combined the data from three sets of mice by normalizing data to baseline values. Similar to the HF diet study, we saw no differences in the GTTs and ITTs for ob/ob and MSKO-ob/ob mice (Fig. 3C, D), indicating comparable systemic insulin resistance. Similar plasma insulin concentrations (Fig. 3E, F) further confirmed that insulin resistance did not differ between genotypes.

Myeloid-specific ABCA1 deficiency does not worsen adipose tissue inflammation

We next examined the inflammatory status in fat, an important insulin target tissue. We first measured expression of F4/80 and CD68 (macrophage markers) in epididymal fat from HF diet-fed mice (17 weeks). Compared with chow, the HF diet significantly increased F4/80 and CD68 expression in adipose tissue, indicating macrophage infiltration into fat tissue (Fig. 3A). Unexpectedly, MSKO versus WT fat had significantly lower F4/80 expression and a trend toward less CD68 expression. Furthermore, immunohistochemistry staining of macrophages using CD68 antibody revealed that $CD68^+$ cells in MSKO adipose tissues were greatly reduced compared with WT (**Fig. 4B**), indicating significantly less macrophage infiltration into fat. The comparable level of cleaved caspase-3 in adipose tissues (supplementary Fig. IIA, B) rules out a major role for apoptosis in the differential accumulation of macrophages between genotypes. Interestingly, this

difference was not observed with a longer period of HF diet feeding (24 weeks, supplementary Fig. IIIA).

Obesity induces a phenotypic switch from M2-polarized state (alternatively activated) to an M1 (classically activated) pro-inflammatory state in adipose macrophages (31). We examined expression of the M1 type of inflammatory cytokine/chemokine and M2 macrophage markers in fat. Feeding the HF diet for 17 weeks significantly induced pro-inflammatory cytokine (TNF-α) or chemokine (MCP-1) expression in adipose tissues compared with chow (Fig. 4C). However, no genotypic differences were observed between the chow-fed or HF diet-fed mice (Fig. 4C). Not surprisingly, the expression of M2 macrophage markers did not show any difference between genotypes (Fig. 4D). Again, no significant difference was observed in TLR4 or CD36 expression between genotypes (supplementary Fig. IIIB**)**. Increasing the duration of HF diet feeding from 17 to 24 weeks did not significantly increase adipose inflammation in MSKO versus WT mice (supplementary Fig. IIIC).

Next, we examined the macrophage infiltration and inflammatory cytokine expression in ob/ob mouse adipose tissue. Flow cytometry revealed that chow-fed MSKO-ob/ob versus ob/ob mice had significantly less ($F4/80^+$) and M1 ($F4/80^+CD11C^+$), but similar M2 ($F4/80^+CD206^+$) macrophage infiltration into fat (**Fig. 5A**). Furthermore, MSKO-ob/ob versus ob/ob mice had similar levels of IL-1β, TNF-α, and MCP-1 mRNA expression and a trend toward decrease in IL-6 and IL-12p40 mRNA expression in fat tissue (Fig. 5B). Note that similar to peritoneal macrophages, stromal vascular cells from MSKO-ob/ob fat had significantly higher FC (23% increase) and CE (254% increase) accumulation relative to their WT counterparts (Fig. 5C). Thus, our data suggest that myeloid cell-specific ABCA1 deletion enhances cholesterol accumulation in adipose macrophages; however, it does not enhance obesity-induced macrophage infiltration or chronic inflammation in adipose tissue.

Myeloid cell-specific ABCA1 deficiency does not alter plasma and liver lipid profiles in HF diet-induced or genetically-induced obese mice

To determine whether ABCA1 deficiency in myeloid cells affects lipid homeostasis in HF diet-fed or chow-fed ob/ob obese mice, we examined the plasma and liver lipid concentrations. There was no genotypic difference in plasma total cholesterol (TC) and TG concentrations during the 24 weeks of HF diet feeding (**Fig. 6A, C**) or after mice were crossed into the ob/ob background (Fig. 6B, D). We also saw comparable levels of hepatic TC (Fig. 6E, F), FC, and CE (data not shown) accumulation between HF diet-fed WT and MSKO mice. WT and MSKO mice developed hepatic steatosis to a similar extent when fed a HF diet for 24 weeks (Fig. 6G) or crossed into the ob/ob background (Fig. 6H), respectively. Together, these data suggest that myeloid cell-specific ABCA1 deletion does not significantly affect plasma and liver lipid metabolism in the setting of obesity-induced insulin resistance.

DISCUSSION

Obesity and insulin resistance are chronic low-grade inflammatory diseases associated with macrophage accumulation and activation in adipose tissue (29, 30). We previously demonstrated that myeloid cell-specific deletion of ABCA1 results in increased macrophage inflammatory

response to TLR agonists (24), and human studies suggest an association between ABCA1 single nucleotide polymorphisms, leukocyte ABCA1 expression, and insulin resistance (17–22). However, whether there is a link between macrophage ABCA1-regulated innate immunity and insulin resistance is unknown. In the present study, HF diet-fed MSKO mice and chow-fed MSKO-ob/ob mice did not exhibit exacerbated obesity-associated chronic inflammation or insulin resistance compared with their WT counterparts, despite a significant increase in cholesterol content in both resident peritoneal macrophages and adipose stromal vascular cells that accompanies ABCA1 deletion. Our results suggest that increased dietary saturated fatty acid intake (i.e., HF diet feeding) is not sufficient to induce an augmented inflammatory response and insulin resistance in MSKO mice compared with their WT counterparts.

Macrophages play a central role in HF diet-induced obesity and subsequent development of systemic insulin resistance through secretion of pro-inflammatory cytokines that act on local tissues or circulate to distal tissues. The inflammatory cytokines then block insulin receptor signaling by serine phosphorylation of insulin-like substrate, interfering with its ability to engage in insulin receptor signaling (32). During development of diet-induced obesity, saturated fatty acids can activate TLR4, triggering a low-grade inflammatory state in macrophages and other cell types, such as adipose tissue and muscle (7, 8). Deletion of TLR4 in bone marrow-derived cells blunts the macrophage and adipose tissue response to dietary fatty acids and protects mice from HF diet-induced insulin resistance (8). Deletion of hematopoietic cell JNK1 (a key component of the TLR4 signaling pathway) attenuates inflammation in metabolic tissues and improves insulin sensitivity in HF diet-fed mice (9). Moreover, myeloid-specific IKKβ deficiency improves systemic insulin sensitivity in mice on a HF diet (10), suggesting a causative link between increased macrophage inflammation and insulin resistance.

Our previous studies reported that myeloid-specific ABCA1 deficiency exacerbates macrophage inflammatory response to a moderate LPS dose in vivo (3 mg/kg) and in vitro (100 ng/ml), and this response was mediated through a MyD88-dependent TLR4 pathway (24). Unstimulated resting macrophages were indistinguishable between WT and MSKO mice, except for a small (∼10%) but significant increase in cellular FC. Furthermore, MSKO mice cleared infection with *L. monocytogenes* more efficiently than WT mice (26). Collectively, these studies clearly establish a regulatory role for myeloid ABCA1 in acute macrophage inflammation and innate immunity. In addition, leukocyte ABCA1 expression protects against atherosclerosis, a chronic disease with well-characterized inflammatory components (33–35). Based on the causative association between macrophage inflammation and insulin resistance, we hypothesized that myeloid-specific ABCA1 deficiency would exacerbate obesity-associated chronic inflammation, worsening insulin resistance. However, MSKO and WT mice developed obesity, insulin resistance, and mild hepatic steatosis to a similar extent with HF diet feeding. Relative to chow, HF diet feeding markedly induced macrophage infiltration into adipose tissue and inflammatory cytokine expression. However, we did not observe enhanced macrophage infiltration and adipose inflammation in MSKO versus WT mice after a HF diet feeding. Similar data were obtained with chow-fed MSKO mice crossed into the ob/ob background. Consistent with our previous findings, macrophages from HF diet-fed MSKO versus WT mice accumulated significantly more cholesterol, but cytokine and chemokine gene expression was similar to WT macrophages. Therefore, myeloid-specific ABCA1 deficiency impairs cholesterol efflux, leading to intracellular cholesterol accumulation, but does

not exacerbate obesity-associated low-grade chronic inflammation and does not lead to insulin resistance.

Our study shows that absence of myeloid cell ABCA1 expression is not sufficient to mount an exaggerated inflammatory response in the setting of obesity and does not result in insulin resistance. In our previous study, an increased macrophage inflammatory response in ABCA1-deficient versus WT macrophages was triggered by a moderate dose of LPS in vivo or in vitro, and not observed in nonstimulated macrophages (24). There may be several explanations for the lack of an exaggerated inflammatory response with HF diet feeding in our current study. First, the amount of saturated fatty acid necessary to differentially activate an inflammatory response in macrophages with and without ABCA1 expression may have been insufficient. Inflammatory stimulation of macrophages via TLR4 requires high concentrations of saturated fatty acids (~500 μM) in vitro or intravenous infusion of high amounts of TGs (~17 μmol/h) in vivo (7). Second, previous studies demonstrating TLR4-specific activation of inflammation by HF diets used mice lacking TLR4 globally (7) or in bone marrow cells (8), leaving open the possibility that cells other than macrophages could have contributed to the exacerbated inflammation and systemic insulin resistance. In fact, to our knowledge, there are only two examples in which myeloid-specific deletion of genes (IKK-β or JNK) involved in canonical inflammatory signaling pathways resulted in HF-induced systemic insulin resistance (10, 36). However, IKK-β and JNK are directly involved in inflammatory signaling, whereas ABCA1 indirectly affects TLR signaling by increasing membrane lipid raft content, resulting in more TLR4 in lipid rafts (25). In addition, the concept that saturated fatty acids activate TLR4 is not universally accepted. For example, functional TLR4 was necessary to attenuate trans fatty acid-induced obesity, hyperglycemia, and hyperinsulinemia (37). Similarly, mice lacking MyD88, a TLR4 signaling adaptor protein, were more susceptible to diet-induced insulin resistance, suggesting a protective role for MyD88 signaling in obesity-associated insulin resistance (38). Finally, in vitro studies suggest that TLR4 activation by fatty acids can potentially be confounded by LPS and lipopeptide contamination (39).

In summary, myeloid-specific ABCA1 deficiency impairs cholesterol efflux, but does not significantly exacerbate macrophage infiltration and low-grade inflammation in adipose tissue, or insulin resistance in both HF diet-induced and genetically obese mouse models. However, because we examined advanced obesity resulting from 17 weeks of HF diet feeding, we cannot rule out the possibility that myeloid-specific ABCA1 deficiency might worsen adipose inflammation or metabolic abnormalities in the context of less advanced obesity.

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Abbreviations:

BW	body weight
CE	cholesteryl ester
FC	free cholesterol
GTT	glucose tolerance test
HF	high-fat
IKKβ	inhibitor of NF-κB-kinase β
ITT	insulin tolerance test
JNK	c-Jun N-terminal kinase
LPS	lipopolysaccharide
MCP-1	monocyte chemoattractant protein-1
MSKO	myeloid cell-specific ABCA1 knockout
MyD88	myeloid differentiation primary-response protein 88
SVF	stromal vascular fraction
TG	triglyceride
TLR4	Toll-like receptor 4
TNF-α	tumor necrosis factor-α
WT	wild-type

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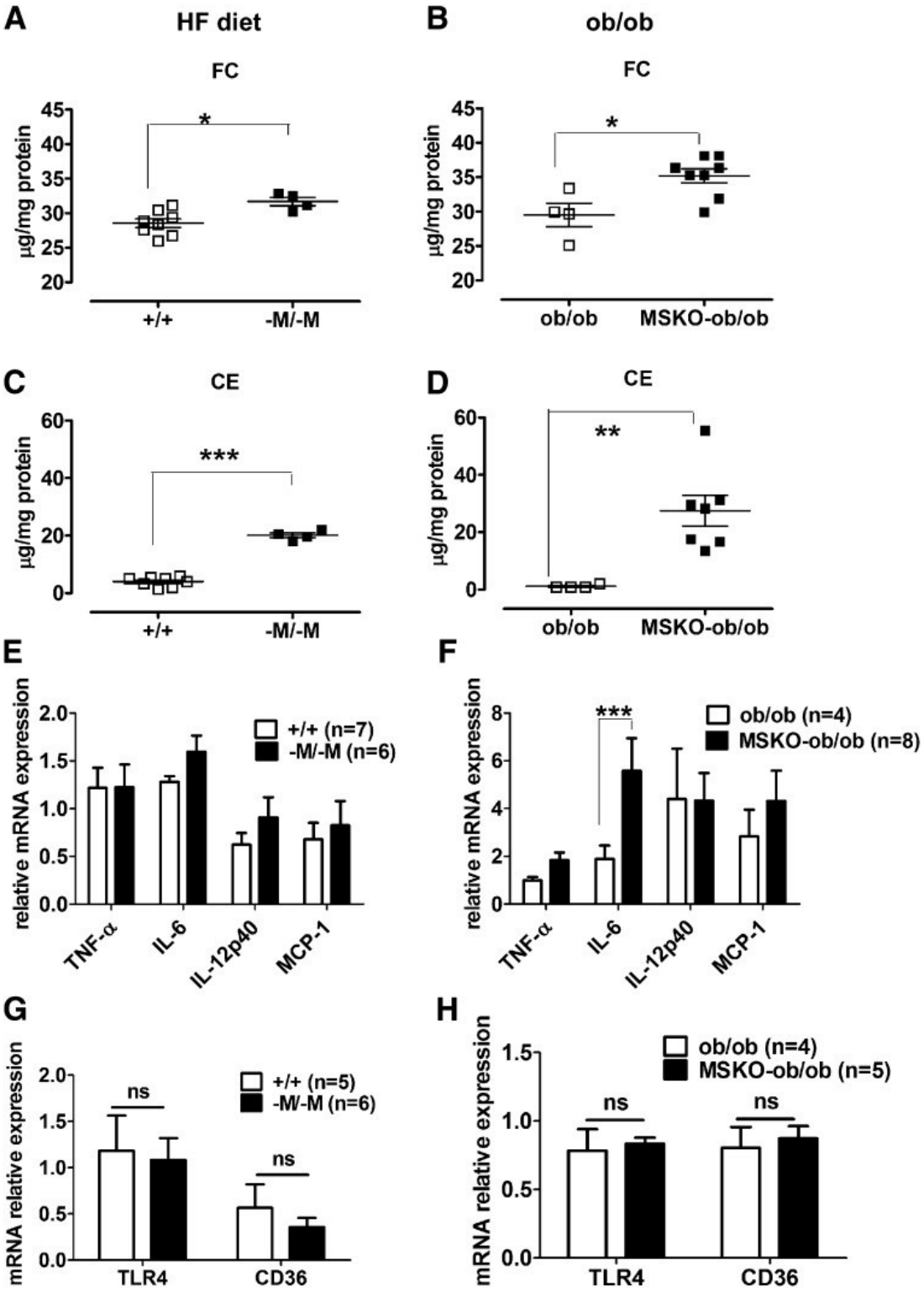
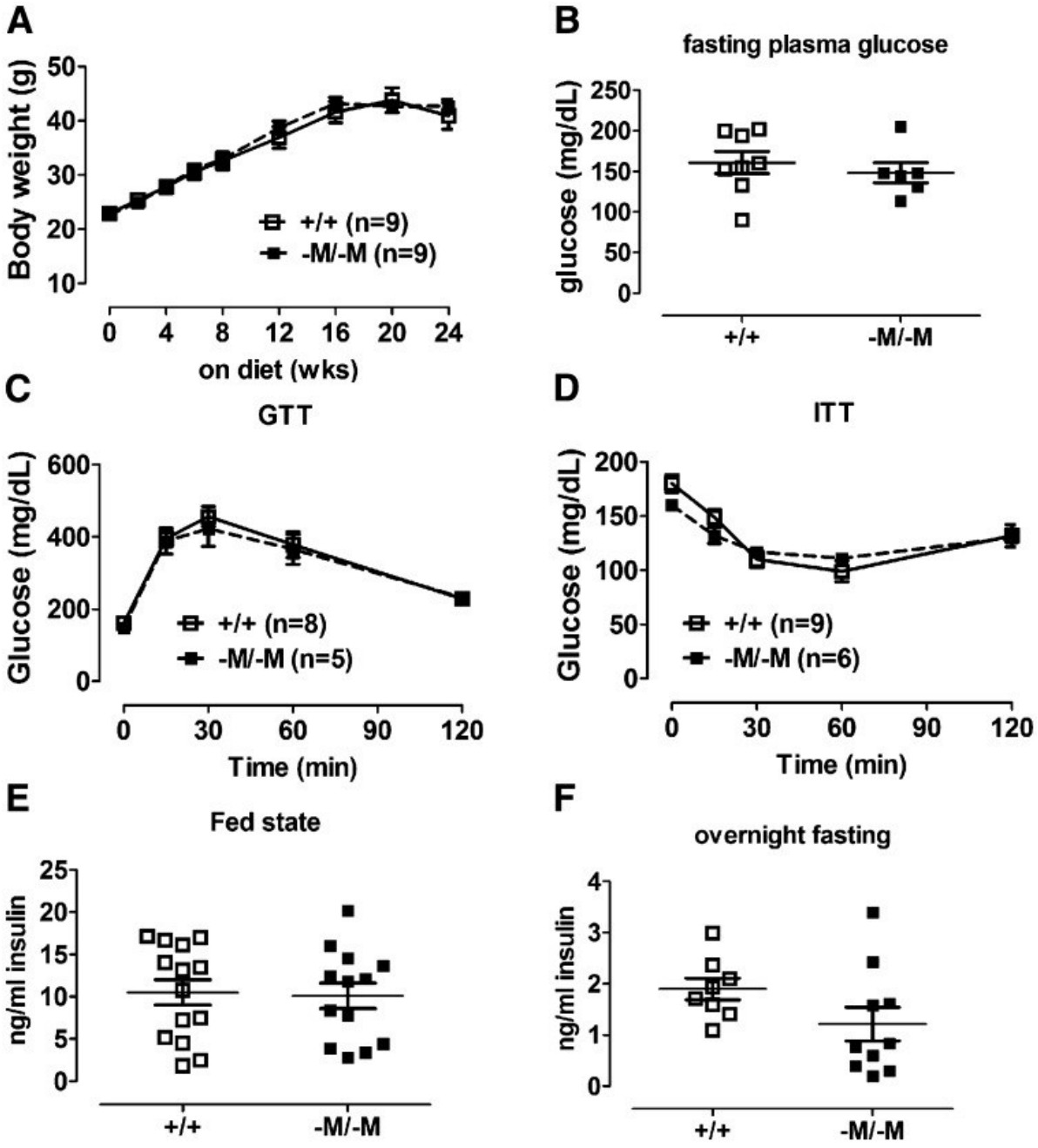
Figures and Tables

Fig. 1.



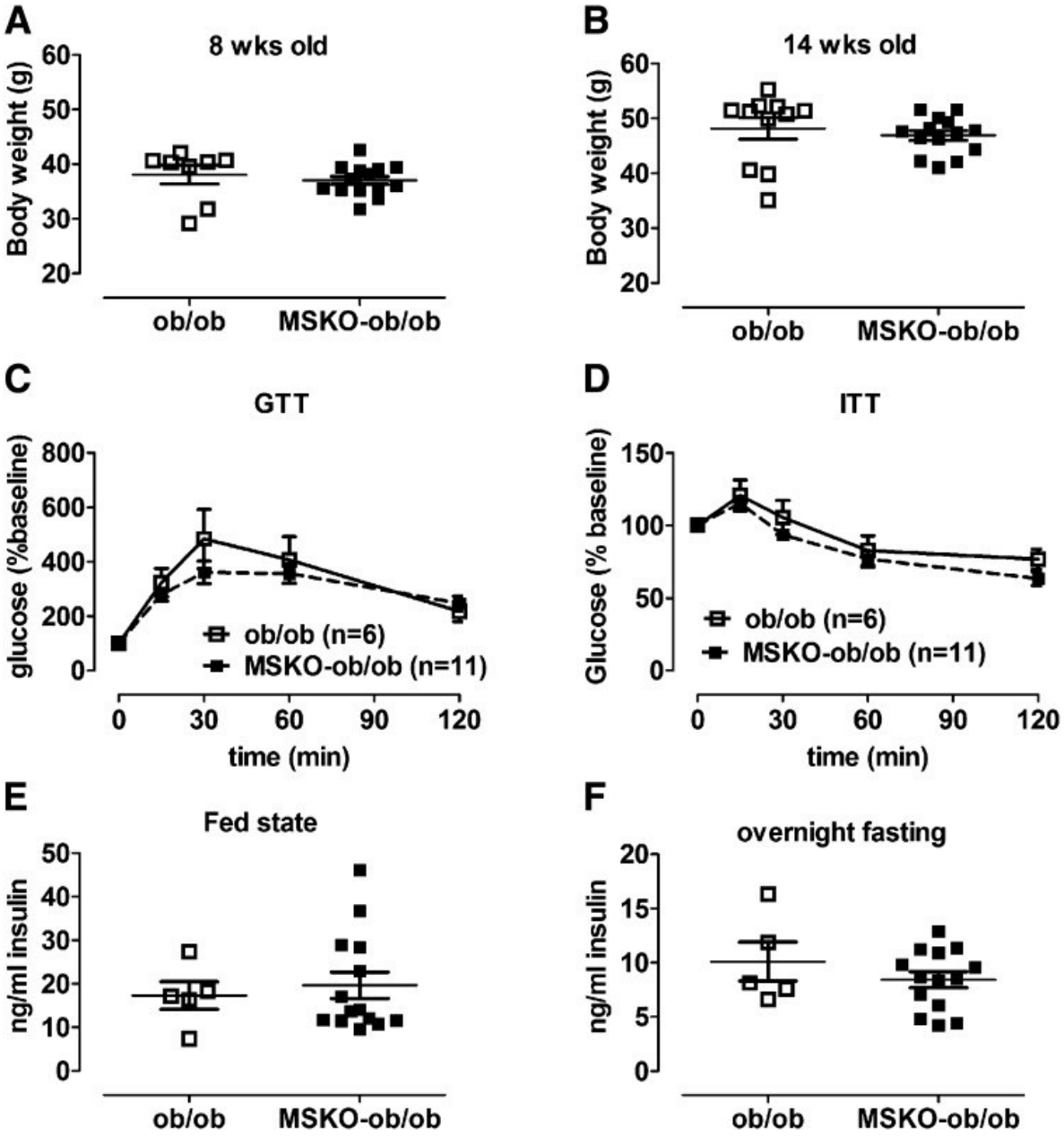
Myeloid-specific ABCA1 deficiency increases cholesterol accumulation but does not enhance obesity-associated inflammation in peritoneal macrophages. Mice were euthanized and resident peritoneal macrophages were isolated for quantification of cholesterol by gas-liquid chromatography or gene expression by quantitative real-time PCR. A, C: FC and CE content of resident peritoneal macrophages from WT and MSKO mice fed the HF diet for 24 weeks. B, D: FC and CE content in resident peritoneal macrophages from 14-week-old chow-fed ob/ob and MSKO-ob/ob mice. E, F: Cytokine and chemokine mRNA expression in resident peritoneal macrophages from HF diet-fed WT and MSKO mice (E) and chow-fed ob/ob and MSKO-o/ob mice (F). G, H: TLR4 and CD36 mRNA expression in resident peritoneal macrophages from HF diet-fed WT and MSKO mice (G) and chow-fed ob/ob and MSKO-o/ob mice (H). $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. ns, no significance. +/+, WT; −M/−M, MSKO.

Fig. 2.



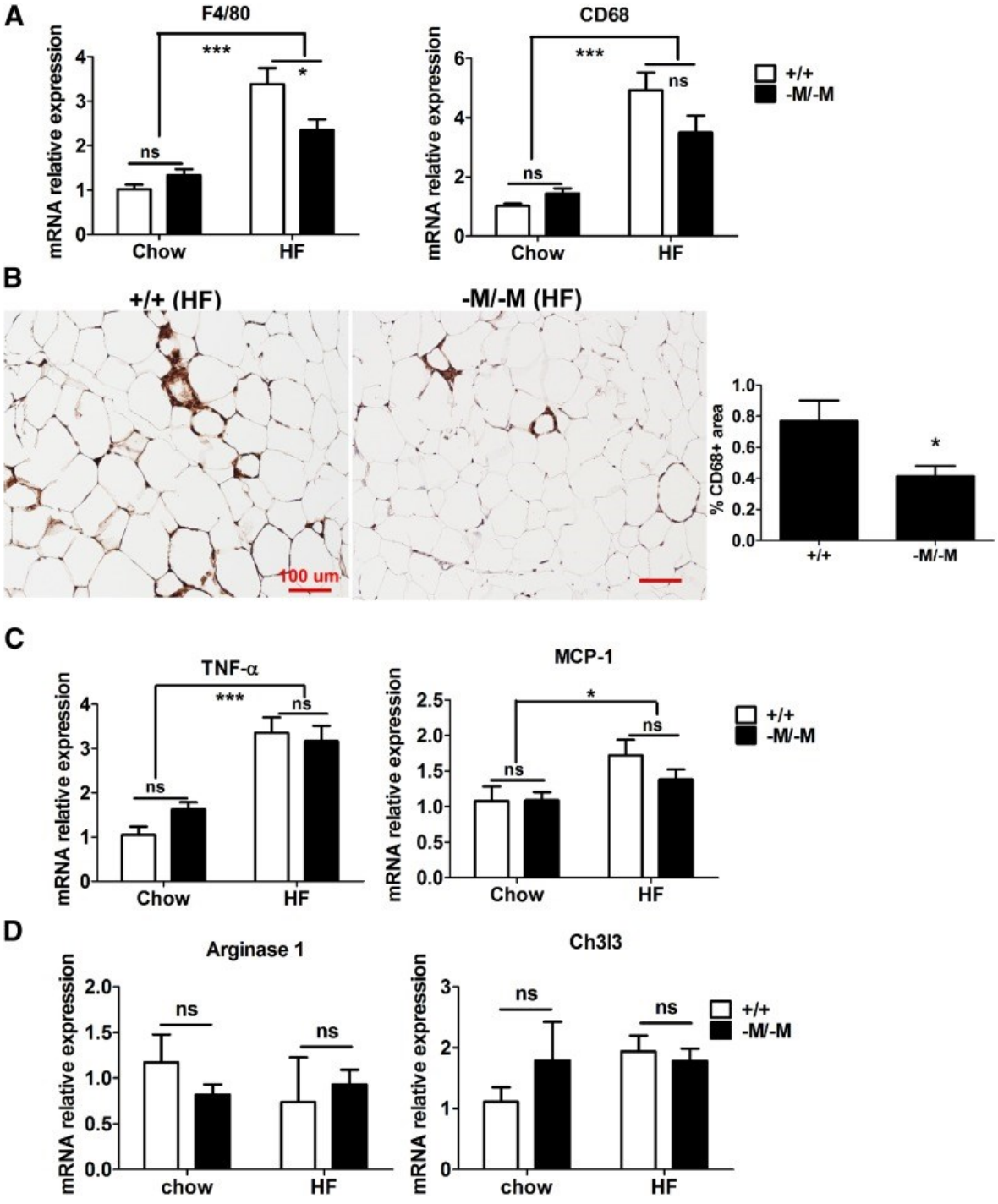
Myeloid-specific ABCA1 deficiency does not worsen insulin resistance in HF diet-fed mice. Male mice, 8–9 weeks old, were fed a HF diet consisting of 45% calories from lard and 0.015% cholesterol for 24 weeks. A: BW over the 24 week diet feeding period. B: Fasting plasma glucose was measured after 20 weeks of HF diet consumption, after a 5 h fast. GTTs (C) and ITTs (D) were performed after 20 weeks of HF diet feeding. Plasma insulin levels of fed (E) or fasted (overnight) (F) mice were measured after 20 weeks of HF diet feeding. +/+, WT; −M/−M, MSKO.

Fig. 3.



Myeloid-specific ABCA1 deficiency does not worsen insulin resistance in male ob/ob mice. MSKO mice were crossed with ob/ob mice to induce obesity. A, B: BW was measured at 8 and 14 weeks of age. GTTs (C) and ITTs (D) were performed at 13 and 14 weeks of age, respectively. Plasma insulin levels in fed (E) or overnight-fasted (F) mice were measured at 13 weeks of age.

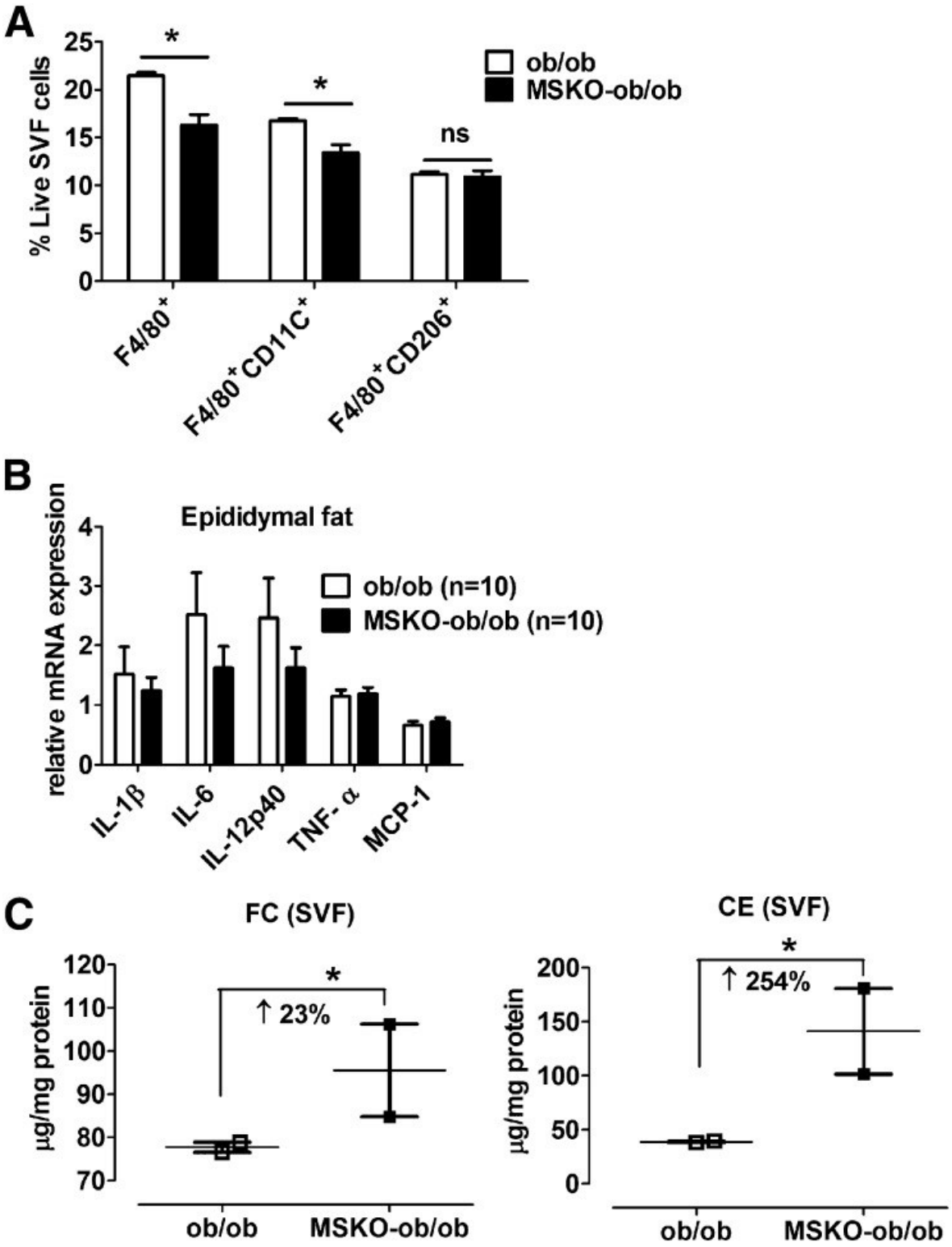
Fig. 4.



Myeloid-specific ABCA1 deficiency does not worsen adipose macrophage infiltration and inflammation in HF diet-fed mice. A: Epididymal fat mRNA expression of macrophage markers (F4/80 and CD68) from mice fed the HF diet for 17 weeks and mice fed the chow diet for 20 weeks. B: Macrophages in adipose tissue were visualized by immunohistochemistry staining using CD68 antibody (objective magnification ×10). CD68+ cells were quantified by Image-Pro

software. Data are presented as percentage of total area that is CD68+. C, D: Epididymal fat mRNA expression of TNF-α, MCP-1, arginase I, and Ch3l3 from mice fed chow for 20 weeks or HF diet for 17 weeks. Data are expressed as mean ± SEM. *$P < 0.05$. ***$P < 0.001$. ns, no significance. +/+, WT; −M/−M, MSKO.

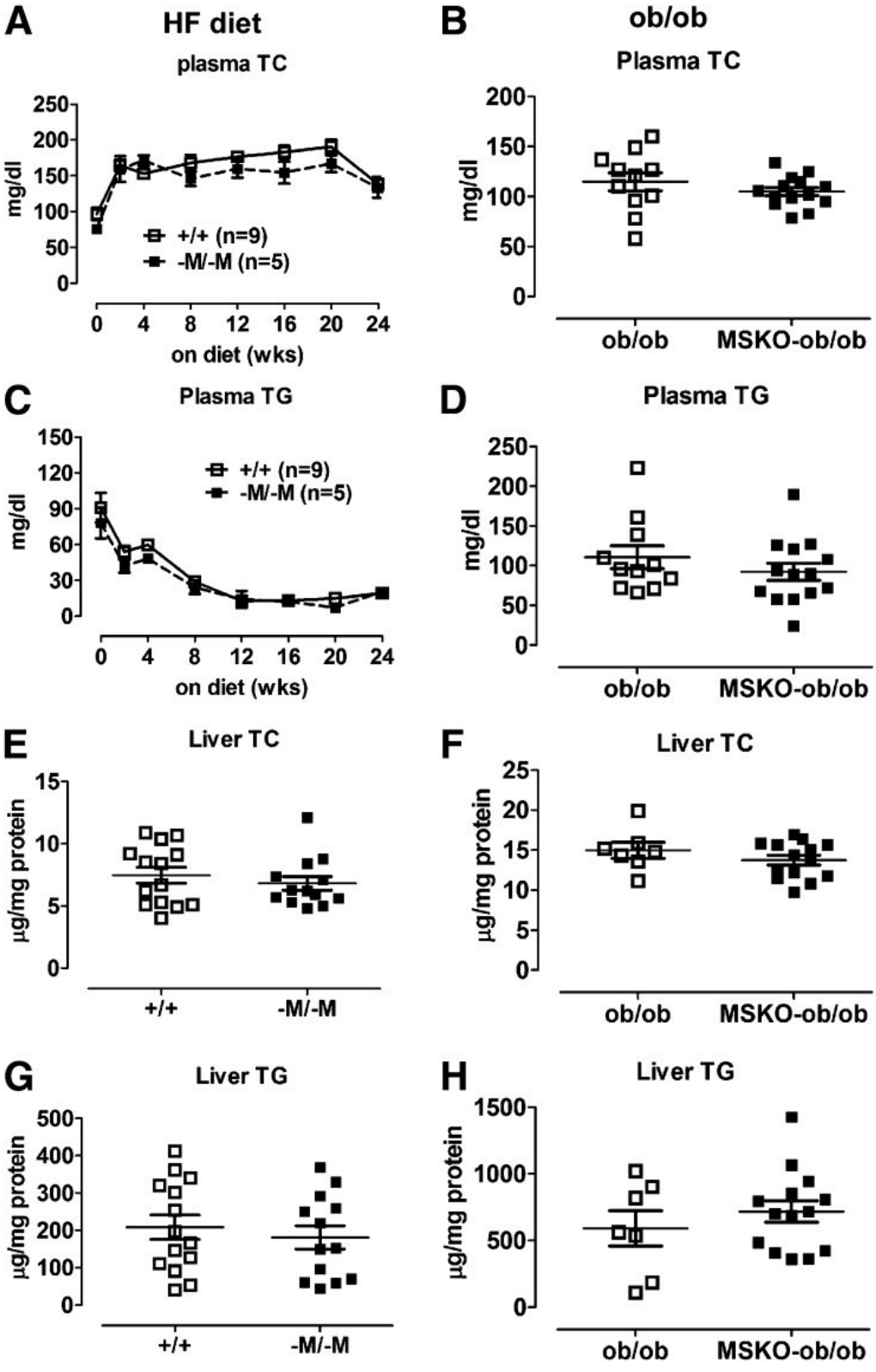
Fig. 5.



Myeloid-specific ABCA1 deficiency does not worsen adipose macrophage infiltration and inflammation in ob/ob mice. A: Percentage of macrophages ($F4/80^{+}$), M1 macrophages

(F4/80^{+}CD11C^{+}), or M2 macrophages (F4/80^{+}CD206^{+}) in stromal vascular cells isolated from ob/ob epididymal fat. Data are expressed as mean ± SEM. B: Epididymal fat mRNA expression of macrophage markers and inflammatory cytokines and chemokines from chow-fed 14-week-old MSKO-ob/ob and ob/ob mice. Data are expressed as mean ± SEM. C: FC and CE content in stromal vascular cells (SVF). Data are expressed as mean ± range. *$P < 0.05$. ns, not significantly different.

Fig. 6.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Plasma TC
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Myeloid-specific ABCA1 deficiency does not alter plasma and liver lipid homeostasis in the setting of obesity. A, C: Blood samples from HF diet-fed mice were collected periodically to assay total cholesterol (TC) and TGs by enzymatic assays. B, D: Blood samples were collected from 14-week-old MSKO-ob/ob and ob/ob mice to assay TC and TGs by enzymatic assays. E–H: After 24 weeks HF diet consumption or at the age of 14 weeks for ob/ob mice, mice were euthanized and liver lipids were quantified by enzymatic assay and normalized to liver protein content. +/+, WT; −M/−M, MSKO.

Supplemental Materials

Myeloid Cell -Specific ABCA1 Deletion Does Not Worsen Insulin Resistance in High Fat Diet-Induced or Genetically Obese Mouse Models

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Supplemental Table I: High-fat diet composition

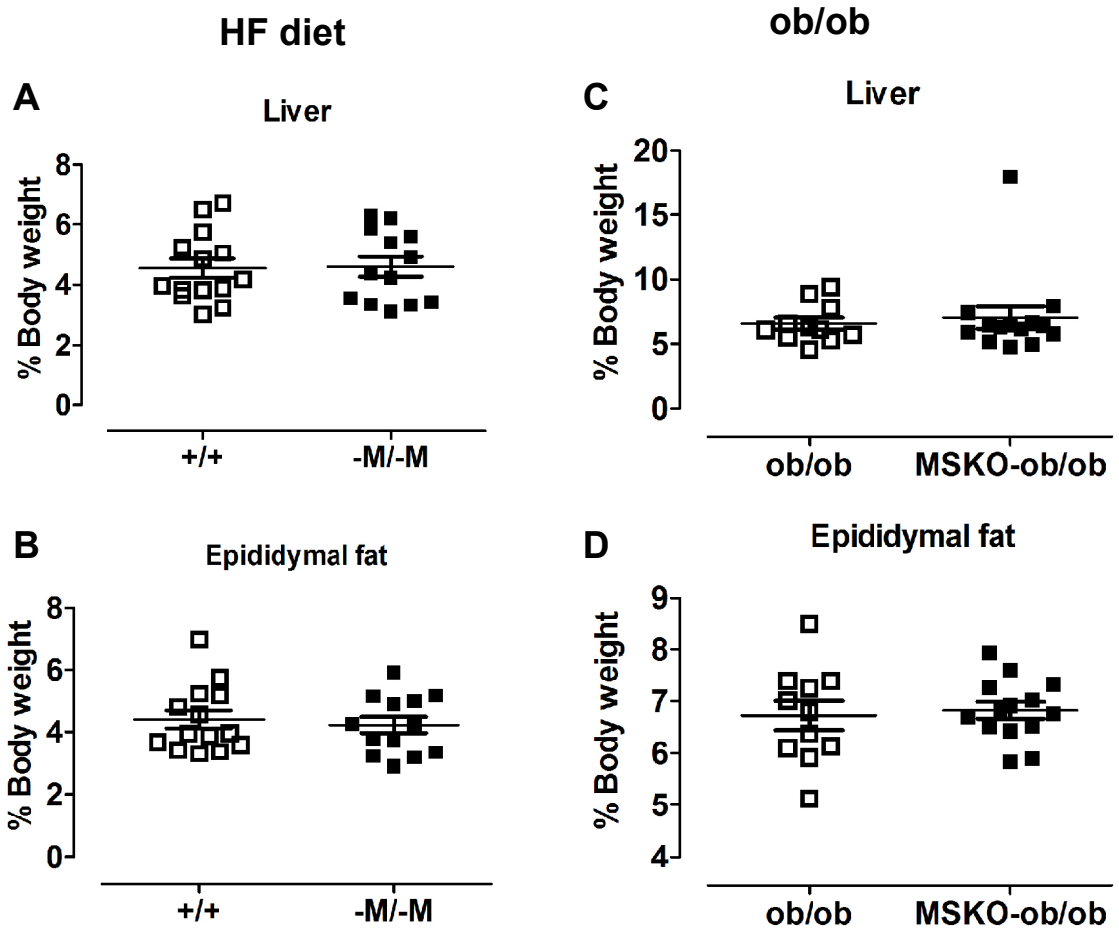
Ingredient	Grams/100 g	Grams of fat	Grams of carbohydrate	Grams of protein
Casein	23.30			23.30
L-cysteine	0.35			0.35
Dextrin	8.00			
Maltodextrin	11.30		8.00	
Sucrose	20.13		11.30	
Cellulose	5.82		20.13	
Soybean oil	2.90	2.90		
Lard	20.50	20.50		
Hegsted Salts	5.0			
Vitamin Mix	2.50		2.50	
MtS-50 (Vitamin E)	0.20			
Total	100	23.40	41.93	23.65
(calories)	472.9	210.6	167.7	94.6
% of Calories		45%	35%	20%

Supplemental Figure 1. Liver (**A** and **C**) and epididymal fat (**B** and **D**) mass from mice consuming the HF diet for 24 wks or chow-fed mice crossed into the ob/ob background (14 wks old). +/+= WT; -M/-M=MSKO.

Supplemental Figure 2. Immunohistochemical staining and quantification of cleaved caspase-3 positive cells (apoptotic) in adipose tissues from mice fed a HF diet for 17 wks (objective magnification ×10). Cleaved caspase-3 positive cells were quantified using Image Pro software. ns= not statistically significant. ns= not significant; $p>0.05$.

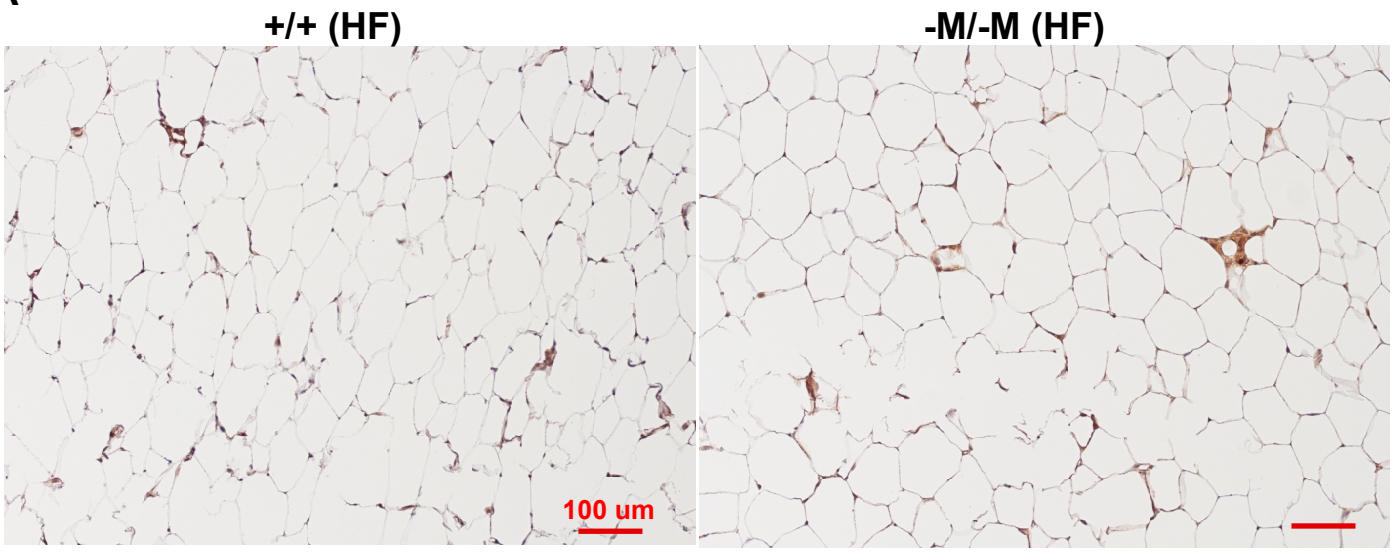
Supplemental Figure 3. (**A**) mRNA expression of F4/80 and CD68 in epididymal fat from mice consuming a HF diet for 24 wks. (**B**) mRNA expression of TLR4 and CD36 in epididymal fat from mice consuming chow (20 wks) or HF diet (17 wks). (**C**) mRNA expression of inflammatory cytokines/chemokines in epididymal fat from mice consuming a HF diet for 24 wks. Gene expression was evaluated by quantitative real-time PCR. +/+= WT; -M/-M=MSKO. ns= not significant; $p>0.05$.

Supplemental Figure 1

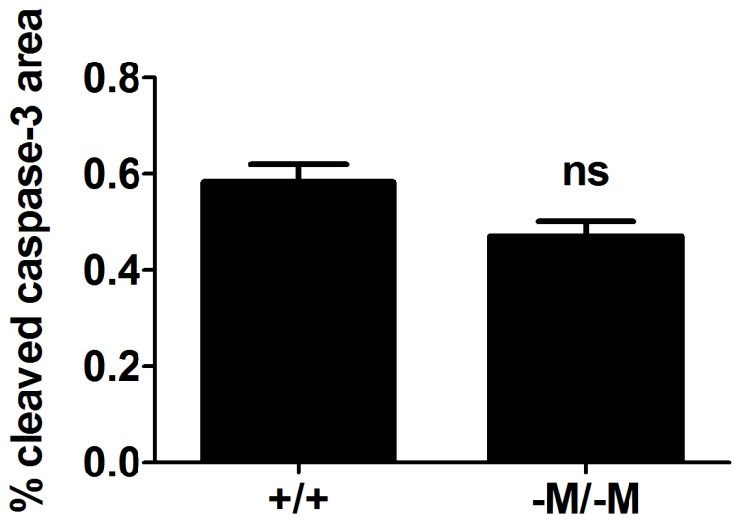


Supplemental Figure 2

A



B



Supplemental Figure 3