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BRIEF CUTTING EDGE REPORT

Obesity Biology and Integrated Physiology

Reversal of obesity development in *Ceacam1*$^{-/-}$ male mice by bone marrow transplantation or introduction of the human *CEACAM1* gene

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Abstract

Objective: Although *Ceacam1*$^{-/-}$ male mice become obese on normal chow, the effect of bone marrow transplantation or introduction of the carcinoembryonic antigen-related cell adhesion molecule 1 (*CEACAM1*) gene has not been studied, to the knowledge of the authors.

Methods: This study analyzed *Ceacam1*$^{-/-}$ mice on normal diet or high-fat diet (HFD), including effects of bone marrow transplantation or introduction of the *CEACAM1* gene.

Results: Male *Ceacam1*$^{-/-}$ mice on normal diet versus HFD for 24 weeks gained significantly more weight than controls, and *Ceacam1*$^{-/-}$ mice aged up to 2 years had a high frequency of liver cancer. Transplantation of wild-type bone marrow into *Ceacam1*$^{-/-}$ mice or introduction of the human *CEACAM1* gene fully or partially reversed the obesity phenotype. Liver lipidomics on *Ceacam1*$^{-/-}$ versus wild-type controls on an HFD revealed a significant increase in diacyl glycerides. An increase in fatty acid transporter CD36 levels further suggests that loss of *Ceacam1* leads to a major dysregulation of free fatty acid uptake.

Conclusions: CEACAM1 expression in both the liver and immune cells regulates obesity and lipid storage pathways in the liver. Bone marrow reconstitution of the immune system or introduction of the human *CEACAM1* gene can fully or partially reverse the phenotype.

INTRODUCTION

Obesity and associated nonalcoholic fatty liver disease (NAFLD) are major worldwide health threats, in that NAFLD often proceeds to nonalcoholic steatohepatitis (NASH) and hepatocellular cancer (1). NAFLD prevalence is as high as 45%, with up to 50% of patients developing steatohepatitis, fibrosis, cirrhosis, and end-stage liver failure or hepatocellular carcinoma (2). NAFLD is associated with obesity (3) and metabolic syndrome (4), including insulin resistance in the liver (5). Despite these connections, the mechanism behind obesity and NAFLD and hepatic insulin resistance is unknown. A striking prevalence of NAFLD in male individuals (6) and the sexual dimorphism associated with inflammation (7) are important clues. *Ceacam1*$^{-/-}$ (cardioembryonic antigen-related cell adhesion molecule 1) mice that have impaired insulin clearance, develop steatosis, and exhibit a proinflammatory phenotype even on a normal diet (ND) (8) are an important model of obesity and fatty liver disease. Liver-specific deletion of

Ceacam1 induces hepatosteatosis (9), whereas reconstitution of liver-specific expression of *Ceacam1* in *Ceacam1*$^{-/-}$ mice reverses the phenotype (10).

CEACAM1 signals via two immunotyrosine inhibitory motifs in its cytoplasmic domain (11) that were recently shown to vary in their regulation between mouse and man (12). Lack of CEACAM1 inhibitory signaling in *Ceacam1*$^{-/-}$ mice may explain the lipid accumulation in the liver that contributes to the development of obesity and NAFLD. To further develop this model, we performed comparative bone marrow transplantation (BMT) studies in wild-type (WT) versus *Ceacam1*$^{-/-}$ mice on an ND versus a high-fat diet (HFD) and generated a *CEACAM1* transgenic mouse to determine whether the human gene could substitute for the murine gene.

Study Importance

What is already known?

- *Ceacam1*$^{-/-}$ male mice become obese with visceral adiposity and fatty liver on a normal diet and, with age, develop progressive nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, and liver cancer. This model is of interest because the sex dependence mimics human studies and does not require a prolonged high-fat diet. However, evolutionary changes in CEACAM1 signaling complicate interpretation of mechanistic studies.

What does this study add?

- The obesity and fatty liver phenotype of *Ceacam1*$^{-/-}$ male mice can be reversed by bone marrow transplantation or introduction of the human *CEACAM1* transgene.

How might these results change the direction of research?

- A novel murine model of male obesity and nonalcoholic fatty liver disease that expresses the human *CEACAM1* transgene is relevant to the study of human obesity, in that the human gene can mimic the effects of the murine gene. Given the evolutionary changes in signaling between the murine and human genes, we expect this model to provide new information.

METHODS

Animal studies

This study was conducted under protocol number 08017 and was approved by the Institutional Animal Care and Use Committee of the City of Hope (Association for Assessment and Accreditation of Laboratory Animal Care [AAALAC] assurance number A3001-01). The HFD was from TestDiet (catalog #1810740). Mice were housed in a specific pathogen-free facility, four per cage, and were fed ad libitum. Human *CEACAM1* transgenic mice (huTg) were generated on the FVB/NJ mice background (13) and backcrossed over seven generations to the *Ceacam1*$^{-/-}$ background C57/BL6 mice in our lab. Because the homozygous huTg mice were sterile, only heterozygous huTg mice were used in this study. The number of animals per group (total of 92), parameters measured, and outcomes for each study are shown in the figures and figure legends.

BMT and flow cytometry

BMT on 3.5-month-old mice was performed as previously described (14). Peripheral blood leukocytes analysis, with anti-mouse CEACAM1-PE (catalog #134506, BioLegend) on a Canton II Flow cytometry machine (BD Biosciences) and Flowjo software, was used to confirm BMT efficiency.

Lipidomics

Lipodomics was performed as previously described (12). A complete analysis of all lipids in the cell lines is available on request. Serum triglycerides (TG) were quantitated using the Triglyceride Assay Kit-Quantification (catalog #ab65336, Abcam).

Immunoblot analysis

Hepatocytes lysed in 1% NP-40 lysis buffer were immunoblotted as previously described (15), using antibodies shown in Supporting Information Table S1 and analyzed on an Odyssey Infrared Imaging System (LI-COR Biosciences).

Statistical analysis

Results (mean [SEM]) were analyzed using unpaired Student *t* tests or ANOVA (GraphPad Prism software [version 5.0]).

RESULTS

Phenotypes of male WT versus *Ceacam1*$^{-/-}$ mice on an ND versus an HFD

Whereas previous studies (16) indicated that male *Ceacam1*$^{-/-}$ (KO) mice develop visceral adiposity starting at 24 weeks on an ND, we found a weight gain for the KO versus WT mice on an ND that was 20% higher starting at 14 weeks (Figure 1A). KO versus WT mice on an HFD diverged as early as 12 weeks, with a maximum increase of 28% to 30% over the remaining time (Figure 1A). Serum TG were

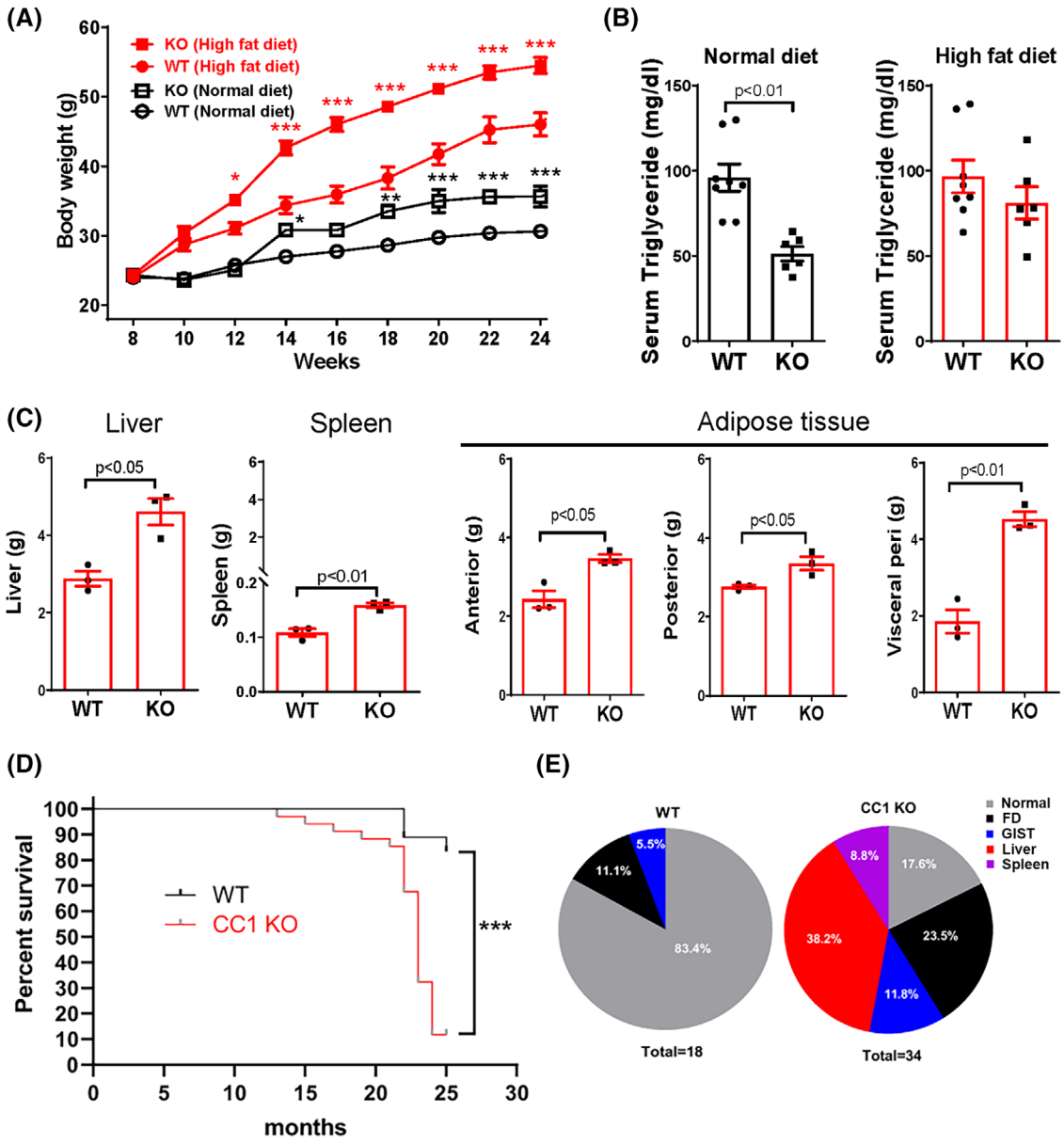

FIGURE 1 Analysis of body, tissue weights, survival, and serum TG of male KO vs. normal mice. (A) Weight gain (mean [SEM], $n = 8$) for male WT and KO mice on an ND (black symbols) and an HFD (red symbols). (B) Serum TG levels (mean [SEM]) for WT ($n = 8$/group) vs. KO ($n = 6$/group) mice on an ND and HFD. (C) Organ weights (mean [SEM], $n = 3$) in liver, spleen, and adipose tissues for KO vs. WT mice on an HFD. (D) Kaplan-Meier plot of survival of male WT ($n = 18$) vs. KO ($n = 34$) mice on an ND. (E) Along with mice FD, all mice at 25 months were euthanized and necropsied for presence of liver cancer, GIST, or splenomegaly. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. FD, found dead; GIST, gastrointestinal stroma tumors; HFD, high-fat diet; KO, *Ceacam1* $^{-/-}$; ND, normal diet; TG, triglycerides; WT, wild type [Color figure can be viewed at wileyonlinelibrary.com]

significantly lower in KO versus WT mice for the ND but not the HFD (Figure 1B). Serum TG for WT mice were in accordance with the literature (17), but they differed from previously reported plasma levels (18). The weight changes in extrahepatic tissues (Figure 1C) were most dramatic in the visceral fat pad. When the long-term effects of KO mice on life-span were followed for 25 months, only 17.6% of KO mice survived, in comparison with 83.3% for WT mice (Figure 1D). The major cause of death in KO mice on an ND was liver cancer (38%) in comparison with none in WT mice (Figure 1E; Supporting Information Figure S1). Therefore, male KO mice developed increasing liver cancer with age, even on an ND.

Effect of BMT on weight gain and NAFLD phenotype

Because fatty liver disease is associated with an inflammatory phenotype (16), we performed BMT of WT bone marrow into *Ceacam1* $^{-/-}$ mice. When KO mice transplanted with WT bone marrow (Figure 2A)

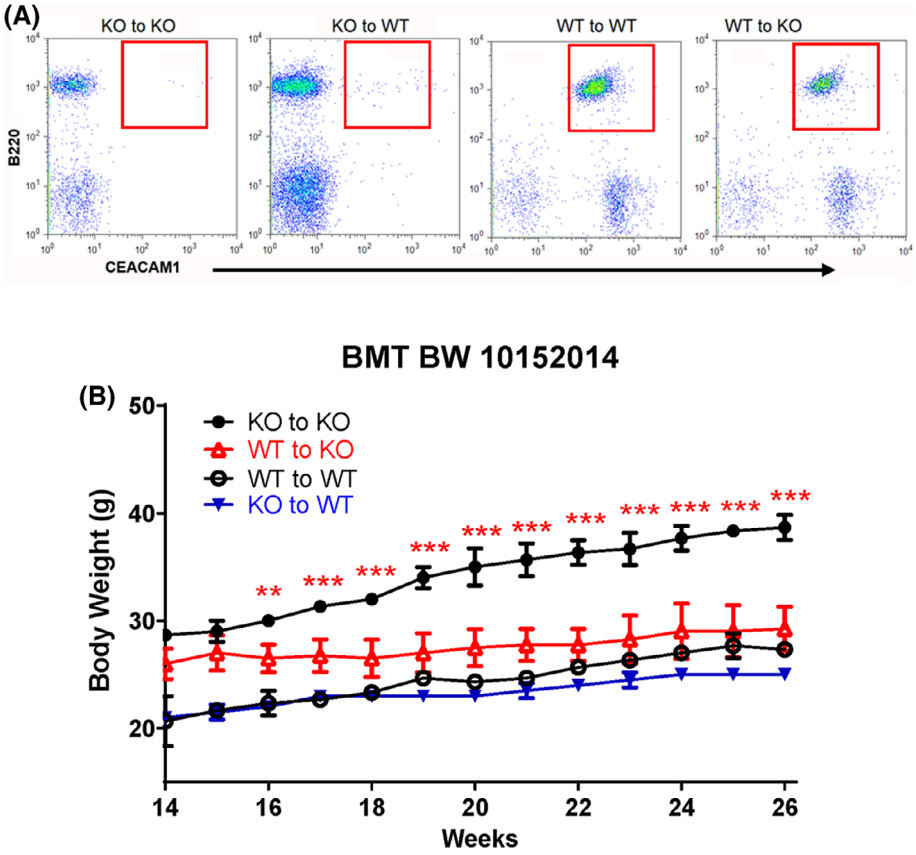

FIGURE 2 Reconstitution of WT bone marrow cells in KO mice decreases *Ceacam1* $^{-/-}$-induced obesity. (A) Reconstitution efficiency of WT → WT, WT → KO, KO → KO, and KO → WT. (B) The body weight of chimeric mice fed a normal diet was measured at the indicated times (mean [SEM], $n = 4$/group). $^{**}p < 0.01$, $^{***}p < 0.001$, in comparison with WT to KO. BMT, bone marrow transplantation; KO, *Ceacam1* $^{-/-}$; WT, wild type [Color figure can be viewed at wileyonlinelibrary.com]

were fed an ND starting at 14 weeks of age, the KO → KO controls started at a 40% higher weight compared with WT → WT controls and exhibited a further 25% weight gain over 26 weeks (Figure 2B). Although the KO → WT recipients experienced little or no effect on weight gain, the WT → KO recipients that started at a weight of 26 g at 14 weeks experienced about a 10% weight loss (Figure 2B). Therefore, the transplantation of WT bone marrow into KO mice consistently reversed the weight gain phenotype.

Effect of *CEACAM1* gene on obesity

Because murine and human CEACAM1 may signal differently in the liver (12), we asked whether the human *CEACAM1* gene could substitute for the murine *Ceacam1* gene. Previously, we generated a transgenic FVB/NJ mouse expressing both murine and human CEACAM1 in which the human CEACAM1 mimicked the expression of murine CEACAM1 in the liver (13). To remove the murine *Ceacam1* gene, these mice were backcrossed to *Ceacam1* $^{-/-}$ C57/BL6 mice to generate mice expressing only the human *CEACAM1* gene (Figure 3A–C). Because the hu/hu males were sterile and few males were produced per litter, the study was conducted with *CEACAM1* heterozygous transgenic mice, referred to as huTg mice. As shown in Figure 3D, huTg mice showed similar weight gains on an ND from 8 to 24 weeks as WT mice, demonstrating that expression of the human gene was able to correct the weight gain phenotype of KO mice on an ND. Although the huTg mice had a higher weight gain than WT mice on an HFD, they exhibited less of a weight gain than the KO mice. In addition, these mice had less liver cancer ($n = 16$, 12.5%) at 23 months than KO mice ($n = 34$, 38.2%) (Supporting Information Figure S2 vs. Figure 1E). These results suggest that the heterozygous genotype was capable of partly correcting the HFD phenotypes, consistent with a gene dosage effect. CEACAM1 staining for the liver of a heterozygous huTg mouse is shown in Supporting Information Figure S3.

When WT mice were compared with huTg mice, the serum TG levels were similar, indicating that the *CEACAM1* gene was able to normalize serum TG levels (Figure 3E). However, for male mice on an HFD, serum TG were unchanged for WT mice versus a slight nonsignificant drop for KO mice and huTg mice. Therefore, all three strains of mice maintained their serum TG levels on an HFD, suggesting a strong homeostatic mechanism.

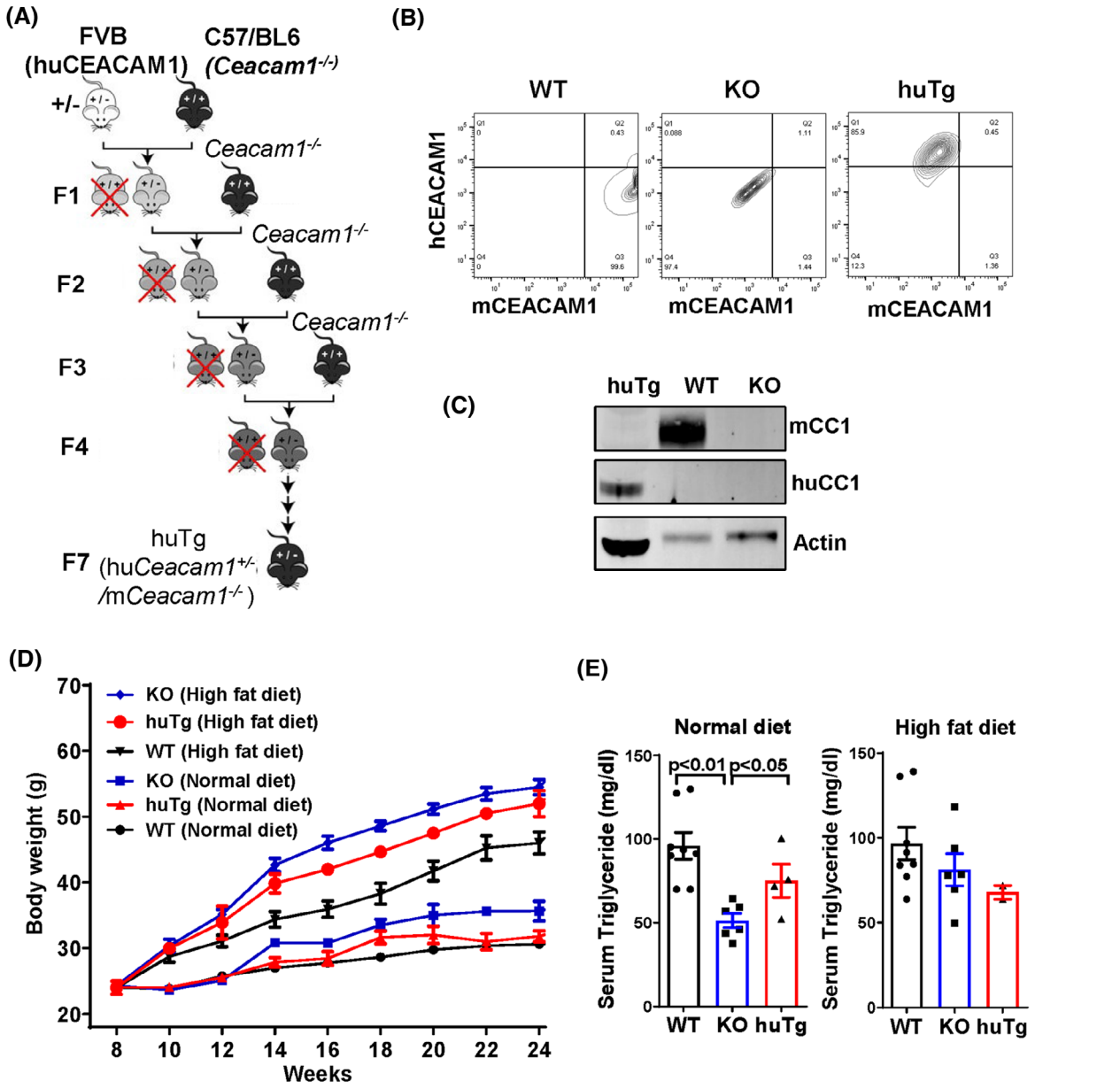

FIGURE 3 huTg rescues body weight and restores serum triglycerides in KO mice on a normal diet and high-fat diet. (A) Breeding human *CEACAM1* in *CEACAM1* $^{-/-}$ C57/BL6 mice. (B) Flow cytometric verification of human CEACAM1 expression on hepatocytes. (C) Western blot verification of human CEACAM1 expression. (D) Body weight changes ($n = 6$/group). (E) Serum TG levels. huTg, human CEACAM1 transgene; KO, *Ceacam1* $^{-/-}$; WT, wild type [Color figure can be viewed at wileyonlinelibrary.com]

Lipidomics of WT versus KO mice

When lipidomic analysis was performed on WT versus KO male mice livers on an ND, the most abundant lipid for WT and *Ceacam1* $^{-/-}$ mice was TG (18:2/18:2/18:2), with a KO/WT ratio of 1.9 (Supporting Information Table S2). The most dramatic increase in KO/WT was for TG (16:0/16:0/18:2), with a 23.6-fold increase in KO mice, suggesting a change in the synthesis or storage of this lipid. Both WT and KO mice had diacylglycerol (DG) as their most abundant lipids, with KO/WT ratios of 6.3 to 9.0 for the top four DGs (Supporting Information Table S2), suggesting that both strains of mice partly reduced their storage of TG by lipolysis.

The most abundant lipid in the adipose tissue of WT male mice on an ND was TG (16:0/18:2/18:2) versus TG (18:2/18:2/18:2) in KO mice, with KO/WT ratios of 1.2 (Supporting Information Table S3), whereas for male mice on an HFD, the most abundant lipid was TG (16:0/18:1/18:2), with a KO/WT ratio of 1.3, and for KO mice, DG (18:1/18:2), with a KO/WT ratio of 2.7 (Supporting Information Table S3). The high level of DG in KO mice on an HFD suggests that these animals reduced their lipid storage burden by lipolysis of TG to

DG. We conclude that lipid storage and composition are altered in both the liver and adipose tissue of KO mice.

CD36 levels in livers of WT and *Ceacam1*$^{-/-}$ mice

Lipid accumulation in NALFD is associated with altered import of nonesterified free fatty acids transported by CD36 (19) that, in turn, is associated with CEACAM1 signaling (12). Immunoblot analysis of CD36 in WT versus KO mice on an ND versus an HFD revealed lower levels of CD36 in KO mice on an ND but elevated levels in KO versus WT mice on an HFD (Supporting Information Figure S4). Therefore, the predicted role of CD36 in fatty liver development was found in this model.

DISCUSSION

CEACAM1 expression in both the liver and immune cells regulates obesity and lipid storage pathways in the liver. Bone marrow reconstitution of the immune system or introduction of the human CEACAM1 gene can fully or partially reverse the phenotype.O

CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHOR CONTRIBUTIONS

ZZ, DLP, GG, AT, and KL performed the experiments. ZZ wrote the first draft, and ZZ and JES wrote the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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