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Lack of myeloid *Fatp1* increases atherosclerotic lesion size in *Ldlr*$^{-/-}$ mice

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ABSTRACT

Background and aims: Altered metabolism is an important regulator of macrophage (MΦ) phenotype, which contributes to inflammatory diseases such as atherosclerosis. Broadly, pro-inflammatory, classically-activated MΦs (CAM) are glycolytic while alternatively-activated MΦs (AAM) oxidize fatty acids, although overlap exists. We previously demonstrated that MΦ fatty acid transport protein 1 (FATP1, *Slc27a1*) was necessary to maintain the oxidative and anti-inflammatory AAM phenotype *in vivo* in a model of diet-induced obesity. The aim of this study was to examine how MΦ metabolic reprogramming through FATP1 ablation affects the process of atherogenesis. We hypothesized that FATP1 limits MΦ-mediated inflammation during atherogenesis. Thus, mice lacking MΦ *Fatp1* would display elevated formation of atherosclerotic lesions in a mouse model lacking the low-density lipoprotein (LDL) receptor (*Ldlr*$^{-/-}$).

Methods: We transplanted bone marrow collected from *Fatp1*$^{+/+}$ or *Fatp1*$^{-/-}$ mice into *Ldlr*$^{-/-}$ mice and fed chimeric mice a Western diet for 12 weeks. Body weight, blood glucose, and plasma lipids were measured. Aortic sinus and aorta lesions were quantified. Atherosclerotic plaque composition, oxidative stress, and inflammation were analyzed histologically.

Results: Compared to *Fatp1*$^{+/+}$*Ldlr*$^{-/-}$ mice, *Fatp1*$^{-/-}$*Ldlr*$^{-/-}$ mice exhibited significantly larger lesion area and elevated oxidative stress and inflammation in the atherosclerotic plaque. Macrophage and smooth muscle cell content did not differ by *Fatp1* genotype. There were no significant systemic alterations in LDL, high-density lipoprotein (HDL), total cholesterol, or triacylglyceride, suggesting that the effect was local to the cells of the vessel microenvironment in a *Fatp1*-dependent manner.

Conclusions: MΦ *Fatp1* limits atherogenesis and may be a viable target to metabolically reprogram MΦs.

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1. Introduction

Macrophages (MΦs) play a central role in the pathogenesis of atherosclerosis through clearance of modified LDL particles, efferocytosis, and control of the immune milieu [1–3]. MΦs are a diverse population of cells based on site of origin, location, surface markers, functional analysis, and metabolic phenotype [4,5]. While most work has focused on the former characteristics, little work has been conducted on understanding the metabolic phenotype of MΦs, or how MΦ metabolic phenotype influences disease progression, until recent years [6,7]. In general, *in vitro* studies have shown that pro-inflammatory classically activated MΦs (LPS and IFNγ-stimulated, CAM or M1-like) are highly glycolytic while immunoregulatory alternatively activated MΦs (IL4-stimulated, AAM or M2-like) primarily oxidize fatty acids [8,9]. However, *in vivo* MΦ phenotype is highly dynamic, with mixed inflammatory and metabolic phenotypes [7,10–13].

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Previous reports from our lab and others have provided evidence that when substrate metabolism is modulated, a macrophage's response to specific stimuli is dramatically altered. Fatty acid transport protein 1 (FATP1, *Slc27a1*) is an acyl-CoA synthetase wherein expression within hematopoietic populations is limited to MΦs and plasmacytoid dendritic cells [14], but not other cells that may contribute to inflammation in atherosclerosis, including monocytes, microglia, B cells, T cells, neutrophils and eosinophils [9]. We have recently reported through loss and gain of function models that FATP1 is necessary to maintain the immunoregulatory AAM phenotype *in vivo* and *ex vivo*. *Fatp1* is downregulated with pro-inflammatory stimulation of bone marrow derived MΦs [9]. Chimeric mice lacking *Fatp1* through bone marrow transplantation and fed high fat diet gained more weight and had impaired glucose tolerance on high fat diet, along with greater white adipose mass, MΦ infiltration, and crown-like structures formation. Consequently, in white adipose tissue of $Fatp1^{-/-}$ transplanted mice, inflammation was elevated with evidence of oxidative stress. $Fatp1^{-/-}$ adipose tissue MΦs (ATMs) isolated from white adipose were more CAM-like compared to ATMs isolated from $Fatp1^{+/+}$ transplanted mice fed a high fat diet [9]. *In vitro* studies additionally demonstrated that MΦs lacking *Fatp1* displayed a hyper-inflammatory response to CAM stimuli, with elevations in iNOS (*Nos2*), among others, and reductions in immunoregulatory AAM marker arginase 1 (Arg*1*). Furthermore, absence of *Fatp1* led to a metabolic shift from fatty acid uptake and oxidative metabolism to increased glucose transporter GLUT1 expression, glycolysis, and metabolic intermediates associated with oxidative stress and pentose phosphate pathway [9]. Conversely, an *in vitro* gain of function model was utilized by over-expressing *Fatp1* in the RAW264.7 CAM-like MΦ cell line. RAW MΦs over-expressing *Fatp1* failed to be CAM activated, displayed blunted GLUT1 expression and reduced glucose metabolism, and exhibited increased fatty acid uptake and metabolism [9]. Thus, *Fatp1* cannot only direct the metabolic flexibility of MΦs, but the inflammatory phenotype *in vitro* and in high fat diet-exposed mice, which begs the question of the role of MΦ *Fatp1* in other metabolic diseases.

Cardiovascular diseases such as atherosclerosis are the cause of approximately 1 in 3 deaths within the United States and are the leading cause of death worldwide [15]. MΦ-mediated inflammation is a critical component of atherosclerotic plaque formation with MΦ glycolysis associated with poorer outcomes [16]. Atherosclerosis is an ongoing inflammatory process, during which MΦs mediate all stages of the disease, from initiation through progression and, ultimately, thrombotic complications, as well as resolution [1]. Our previous work indicated that there is a critical link between fatty acid transport/metabolism and inflammation in atherosclerosis, demonstrating the need to further understand novel regulators of MΦ substrate metabolism [17–20]. Because MΦ lipid metabolism plays a central role in the pathogenesis of atherosclerosis, we hypothesized that MΦs, with demonstrated blunted fatty acid metabolism and elevated glycolysis due to lack of *Fatp1,* would display increased atherogenesis. Herein, we report that while lack of hematopoietic *Fatp1* led to no systemic alterations in lipids, $Fatp1^{-/-}Ldlr^{-/-}$ mice displayed greater plaque formation, with evidence of oxidative stress and inflammation compared to $Fatp1^{+/+}Ldlr^{-/-}$ mice.

2. Materials and methods

2.1. Animals and maintenance

Animal studies were performed with approval and in accordance with the guidelines of the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill. Mice were housed in a climate controlled animal facility and had *ad libitium* access to food and water. $Fatp1^{-/-}$ mice [21] were provided by Dr. Andreas Stahl (University of California, Berkeley). *Fatp1* total body knockout ($Fatp1^{-/-}$) and *Fatp1* wild type ($Fatp1^{+/+}$) bone marrow donor mice were generated using $Fatp1^{-/+}$ breeding pairs to generate littermate controls. $Ldlr^{-/-}$ mice (stock number. 002207, Jackson Labs) were obtained at 4 weeks of age. At 6 weeks of age, bone marrow transplant was performed, (BMT, below). Chimeric $Ldlr^{-/-}$ mice were then housed in a sterilized environment. Mice were maintained on chow diet for four weeks before challenge with Western Diet (Harlan Teklad, TD88137) for 12 weeks.

2.2. Bone marrow transplantation

Upon arrival, N = 34 4-week-old male $Ldlr^{-/-}$ mice were randomized to two experimental groups. At 6 weeks of age, recipient $Ldlr^{-/-}$ mice were administered two doses of X-ray irradiation (500 cGy × 2, spaced 4 h apart; X-RAD, North Branford, CT) as in previous work [9]. Simultaneously, bone marrow was harvested from 6 to 8 week old male $Fatp1^{-/-}$ and $Fatp1^{+/+}$ donor mice. The femur and tibia were collected and bone marrow cells were flushed by ice-cold PBS, then centrifuged at 1200 RPM at 4 °C for 5 min. Cell pellets were resuspended in HBSS buffer. Each recipient mouse received approximately 5×10^6 bone marrow cells through retro-orbital injection under anesthesia in 100 μl. Control animals were transplanted with the HBSS buffer only, and died within 10 days of lethal irradiation.

2.3. Body weight and composition

Body weight was measured prior to starting mice on diet, and weekly until sacrifice. Body composition was measured at 6, 10, 13, 16, 19 and 22 weeks of age using the EchoMRI-100 quantitative magnetic resonance whole body composition analyzer (Echo Medical Systems, Houston, TX). Fat mass is presented as percent fat mass over total body weight [22].

2.4. Lipids and glucose measurement

After 6 h of fasting, blood was collected. Total cholesterol, LDL-cholesterol, HDL-cholesterol, and triacylglyceride were measured in the UNC Animal Clinical Chemistry and Gene Expression Core Facility by Vt350 Automated Chemical Analyzer from Ortho-Clinical Diagnostics Company (Rochester, NY). Mice were fasted for 6 h before blood glucose was measured and GTT was conducted. Blood glucose was measured using a FreeStyle Freedom Lite glucometer (Abbot Diabetes Care, Inc., Alameda, CA). Intraperitoneal GTT was performed after 7 weeks on diet at 16 weeks of age. Briefly, 2.0 gm/kg body weight of glucose was injected intraperitoneally and blood glucose was measured over 120 min [9].

2.5. Histology and quantification

Hearts were either transferred from 10% formalin to 70% ethanol stored at 4 °C for creation of formalin fixed paraffin embedded (FFPE) sections or transferred to 30% sterile sucrose for 72 h for cryosectioning. Serial interrupted sections from FFPE hearts and frozen hearts were either cut at 5 μm or 10 μm thickness. FFPE sections were stained with Masson's trichrome for quantification of collagen content, necrotic core areas, and subendothelium cell numbers, MOMA2 (macrophage/monocyte monoclonal antibody) for MΦs, anti α-SMA (alpha-smooth muscle actin) for smooth muscle cells (SMC), anti 4HNE (4-hydroxynonenal) for oxidative stress, and anti-IL6 (interleukin 6) for inflammation. The antibodies used for immunostaining were: MOMA2 (BioRad, MCA519G), anti

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