THE ROLE OF MACROPHAGE SUBSTRATE METABOLISM ON OBESITY-INDUCED INFLAMMATION

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ABSTRACT

Megan Huang: The Role of Macrophage Substrate Metabolism on Obesity-Induced Inflammation
(Under the direction of Liza Makowski)

Obesity has become increasingly prevalent worldwide. Studies characterize obesity as a low-grade chronic state of inflammation and identify macrophages as having a role in this response. Macrophage polarization may influence the pro- and anti-inflammatory responses associated with obese and lean phenotypes, respectively. This study exploited the differences in substrate utilization between M1 and M2 polarized macrophages to observe whether there was a change in the inflammatory phenotype with obesity. M1-polarized macrophages are pro-inflammatory and use glucose as fuel whereas M2-polarized macrophages are anti-inflammatory and preferentially use fatty acids as fuel. Specifically in this experiment, mice containing macrophages that were FATP1 knockout or wildtype were fed either a low fat diet (10% kcal from fat, LFD)or a high fat diet (45% kcal from fat, HFD) to test our hypothesis that FATP1 is important in macrophage M2 polarization. Four groups were used in this experiment: low fat-fedwildtype and knockout (LFD $FATP1^{B+/+}$ and LFD $FATP1^{B-/-}$), and high fat-fedwildtype and knockout (HFD $FATP1^{B+/+}$ and HFD $FATP1^{B-/-}$). HFD $FATP1^{B-/-}$ mice gained more weight and had heavier epididymal white adipose tissue (eWAT) than HFD $FATP1^{B+/+}$ mice. HFD $FATP1^{B-/-}$ also had higher percentage body fat and higher plasma leptin levels when compared to HFD $FATP1^{B+/+}$ mice. HFD $FATP1^{B-/-}$ had higher expression of inflammatory markers in eWATcompared to HFD $FATP1^{B+/+}$ mice. The HFD $FATP1^{B-/-}$ group had significantly increased expression of macrophage markers F4/80, CD11C, and CD11B in eWAT. Inflammatory cytokine IL6 was also significantly elevated in eWAT in HFD-fed$FATP1^{B-/-}$ mice when compared to HFD-fed $FATP1^{B+/+}$ mice. Glucose transporter GLUT1 and components of the Nlrp3 inflammasomeexpressionwere also increased in HFD-fed $FATP1^{B-/-}$ in eWAT. Taken together, these data indicate that deletion of FATP1 from macrophages is associated with increased susceptibility to high fat diet-induced weight gain, increased macrophage adipose tissue infiltration, and a shift towards M1-like pro-inflammatory macrophage polarization. ThusM2 macrophages' inability to use fatty acids as fuel drove a more pro-inflammatory phenotype. This study reveals insight into a possible mechanism for the polarization of macrophages that may be relevant to the propagation of obesity.

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I. STUDY AIMS AND HYPOTHESIS

In the current study, we investigate the effects of macrophage substrate availability on obesity-induced inflammation. We hypothesize that manipulating fuel substrate metabolism will alter macrophage polarization and thus affectthe inflammatory response. Past studies have shown that M1 and M2 macrophages preferentially utilize glucose and fatty acid substrates respectively. M1 macrophages are found in obese tissue and are pro-inflammatory while M2 macrophages are found in lean tissue and are anti-inflammatory. However, macrophages are not strictly polarized to M1 or M2. Instead there exists a polarization spectrum between the two phenotypes so that some macrophages may exhibit more M1-like characteristics while others may exhibit more M2-like characteristics. In this study, we propose that blunting fatty acid metabolism will push macrophages to be more M1-like and therefore more pro-inflammatory.

In order to test this hypothesis, this project will block FATP1 in order to blunt fatty acid uptake by macrophages. Mice will either be fed low fat diet (LFD) or high fat diet (HFD). Obese markers will be measured to observe genotype-based changes in inflammation. Gene expression in eWATwill be quantified for several genes using RT-qPCR. GLUT1 expression will be measured to observe any compensatory effects of FATP1 knockout. F4/80, CD11b and CD11c will be used as markers of macrophage infiltration. IL-6 and IL-1β will be used as inflammatory markers. NLRP3, PYCARD AND CASPASE-1 are inflammasome components and measured also as a marker of inflammation.In addition, adipocyte size will be measured to see any differences in tissue structure. Finally, we propose a mechanism through which glucose may activate the inflammatory response via the NLRP3 inflammasome.

SPECIFIC AIMS

AIM 1: To compare obese markers of body weight, body composition, leptin and eWAT weight between genotype groupsand diet groups

HYPOTHESIS: $FATP1^{B-/-}$ groups will have greater body weight, percentage body fat, leptin levels, and heavier eWAT tissue weight compared to $FATP1^{B+/+}$ groups.

RATIONALE: FATP1 knockout in macrophages will reduce the use of fatty acid metabolism and thus reduce anti-inflammatory M2 macrophage polarization and instead increase pro-inflammatory M1 polarizationwith a reliance on glucose as fuel.

EXPERIMENTAL DESIGN:

- Measure body weight weekly throughout the study to track weight gain
- Use MRI to determine body composition
- Measure leptin levels in plasma
- Weigh eWAT at sacrifice
- Compare results of $FATP1^{B-/-}$ groups to $FATP1^{B+/+}$ groups to examine if there is a genotype effect

AIM 2: To determine the metabolic and inflammatory states in eWAT through expression levels of GLUT1, macrophage markers, inflammatory markers, and inflammasome components

HYPOTHESIS 2A: $FATP1^{B-/-}$ groups willhave increased glucose metabolism marked by expression of GLUT1 compared to $FATP1^{B+/+}$ groups.

RATIONALE: In past studies, GLUT1 has been shown to be the primary glucose transporter in all hematopoietic cells and therefore represents an essential part of macrophage glucose metabolism. It has also been shown to be the primary glucose transporter in M1 macrophages (9). Therefore GLUT1 expression is used as a measure of glucose metabolism in macrophages.

HYPOTHESIS 2B: FATP1$^{B-/-}$ groups will have increased inflammation as determined through expression of macrophage markers, inflammatory markers, and inflammasome components compared to FATP1$^{B+/+}$ groups.

RATIONALE: F4/80, CD11b and CD11c are proteins found on macrophage cell surface. Macrophages have been shown to infiltrate adipose tissue in obesity perpetuating the inflammatory response. Measures of the inflammatory response will be confirmed through the expression of inflammatory cytokines and inflammasome components.

EXPERIMENTAL DESIGN:

- Obtain RNA samples from eWAT
- Reverse transcribe RNA into cDNA
- Determine expression levels of GLUT1, macrophage infiltration markers, inflammatory markers, and inflammasome components using RT-qPCR

AIM 3: Measure adipocyte size to observe tissue inflammatory effects

HYPOTHESIS: HFD-fed FATP1$^{B-/-}$ animals will have the largest adipocytes when compared to LFD-fed and FATP1$^{B+/+}$ animals respectively.

RATIONALE: Adipocyte size can be used as a measure of obesity-induced inflammation and thus can be used to determine whether FATP1 knockout contributes to inflammatory response.

EXPERIMENTAL DESIGN:

- Mount and stain eWAT on slides
- Digitally scan slides into ImageScope using Aperio
- Use ImageScope software to measure adipocyte diameters of 100 cells per animal
- Average adipocyte diameters for each group using Microsoft Excel

AIM 4: Explore the NLRP3 inflammasome as a potential mechanism for the glucose-activated inflammatory response

HYPOTHESIS: The NLRP3 inflammasome could be activated in HFD and, to a greater extent, in HFD-fed FATP1$^{B-/-}$, groups producing the inflammatory response observed in the study.

RATIONALE: The NLRP3 inflammasome can be activated by ROS. ROS has been shown to be elevated in obese individuals and has also been shown to be produced as a result glucose metabolism. This increased ROS could be an explanation for the inflammatory response observed in the study.

EXPERIMENTAL DESIGN:

- Determine the expression level of the NLRP3 inflammasome and its components PYCARD and CASPASE-1
- Compare expression levels between the FATP1$^{B-/-}$ group to those of the FATP1$^{B+/+}$ group to examine the relationship between glucose metabolism and inflammasome activation

II. INTRODUCTION

In recent decades, obesity has come to the forefront of global public health issues. It is estimated that worldwide 23.2% of adults were overweight and 9.8% were obese- totaling to about 937 million overweight and 396 million overweight individuals (1). In the United States alone, 33.8% of the population was obese in 2007-2008 (2). These statistics indicate the need to understand the mechanism of obesity propagation. Obesity is associated with a constant state of low-grade inflammation (3,4). Recent studies on macrophages have demonstrated their significant role in obesity.Weisberg et al. demonstrated that obese mice had increased tissue macrophage infiltration leading to activation of adipose inflammatory pathways (4, 5). Macrophages can be polarized towards two general phenotypes: M1 and M2. However, they are not strictly polarized; there exists a spectrum of macrophages showing varying degrees of M1- and M2-like characteristics (6).Broadly speaking, “M1” macrophages are inflammatory and found in obese adipose tissue, whereas “M2” macrophages are anti-inflammatory and present in lean tissue (7). Macrophages are polarized from the M0 undifferentiated form in response to cytokines. Many factors may play a role in the polarization of macrophages and we suggest that the microenvironment and substrates available play a key role in shaping the macrophage phenotype to be discussed below (3).

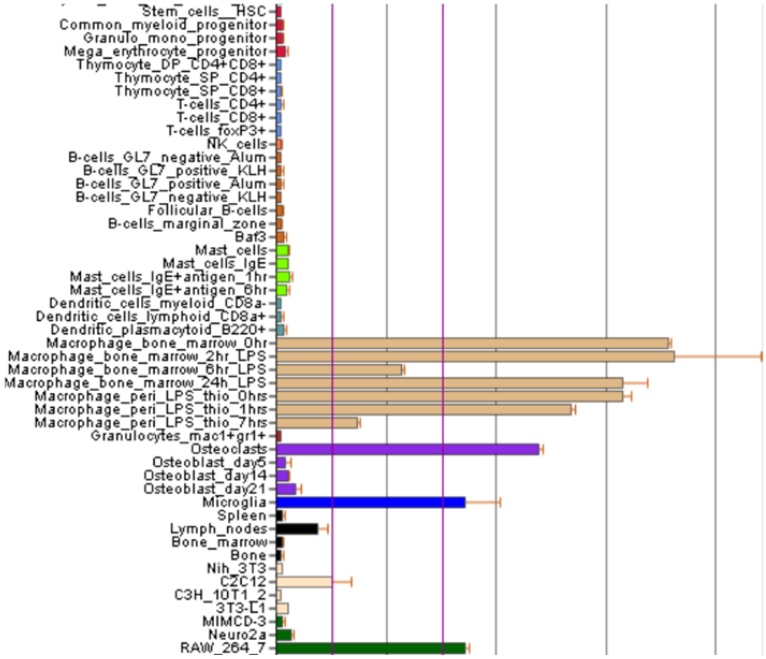
One potential factor that may influence polarization is macrophage substrate availability. Studies have shown that M1 macrophages favor glucose metabolism whereas M2 macrophages rely upon the utilization of fatty acids as fuel. If fat oxidation is blocked, macrophages cannot polarize to the M2 phenotype (8), suggesting the importance of fat metabolism in macrophage biology.

Highly glycolytic macrophages use glucose to produce reactive oxygen species (ROS), which can drive multiple inflammatory pathways.We have previously demonstrated the importance of glucose transporter in driving ROS production and a pro-inflammatory phenotype (9). On the contrary, macrophages that have a preference for fatty acid substrates tend to initiate anti-inflammatory pathways (3).

Fatty acid transport proteins (FATP) contribute to fatty acid uptake by cells. There are six forms of FATP of which FATP1 and FATP4 are the most abundant in macrophages (data not shown). Herein, this study focuses on the role of fatty acid transport protein 1 (FATP1) in macrophage activation. FATP1 is the predominant fatty acid transporter that facilitates very long-chain fatty acid uptake by adipocytes and skeletal muscle (10). In adipocytes, FATP was shown to translocate from inside the cell to the plasma membrane in response to insulin (11).FATP1 is most highly expressed in macrophages when compared to its presence in other hematopoietic stem cells (Fig. 1). Studies have shown blunted insulin-stimulated fatty acid uptake in FATP1 knockout mice, but to date, nothing is known about the role of FATP1 in macrophage biology, polarization, and inflammation (12).

In this study, substrate availability is manipulated via FATP1 knockout to observe if there is an effect on inflammatory outcomes. **We hypothesize that FATP1 knockout from macrophages will block their utilization of fatty acids as fuel pushing M0 macrophages towards the M1 phenotype and perpetuating the diet-induced inflammatory response**.

Figure 1: **FATP1 expression** BioGPS (http://biogps.org/#goto=genereport&id=26457) shows FATP1 expression in mice. Most notably, FATP1 is highly expressed in macrophages when compared to other hematopoietic stem cells (HSCs). The abundance of FATP1 in macrophage makes it an appropriate target for study of FATP1 knockout.



III. METHODS

Animals and diets

3 week old male C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME) and were used as the bone marrow transplant (BMT) recipient mice. Upon receipt, animals were randomized to either a purified diet supplying 10% kcal from fat (Research Diets, LFD) or 45% kcal from fat (Research Diets, HFD). Animals were housed in a climate controlled Department of Laboratory Animals Medicine facility with a 12 hour light:dark cycle. Animals were given *ad libitium* access to food and water. FATP1 total body knockout mice ($FATP1^{-/-}$) and wildtype littermate bone marrow donor mice ($FATP1^{+/+}$) (gifts from Andreas Stahl, U Berkeley) were generated using $FATP1^{-/+}$ breeding pairs. $FATP1^{-/-}$ were backcrossed >12 generations to the C57BL/6J genetic background. All animal procedures were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee.

FATP1 Genotyping

Wildtype and knockout genotypes were confirmed using iProof High-Fidelity DNA Polymerase kit (Bio-Rad Laboratories, Hercules, CA) and the C1000 Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). Mouse tail DNA was purified using the DNeasy Blood & Tissue Kit (QIAGEN Sciences, Valencia, CA). Wildtype and knockout alleles were amplified in separate 50 µl reactions which included 36.5 µl RNase-free water, 10 µl 5x HF buffer, 1 µl dNTP, 1 µl wildtype/knockout primer, 0.25 µl iProof High-Fidelity DNA Polymerase, and 1 µl tail DNA. Reactions were run at 98^{o} C for 10 seconds, 57^{o} C for 30 seconds, and 72^{o} C for 25 seconds for 40 cycles. 4 µl of wildtype and knockout reactions from each DNA sample were combined with 2 µl DNA loading dye. Reactions were loaded and run on a 2% agarose gel with

ethidiumbromide solution (10 mg/ml) in 1x tris-borate EDTA (TBE) buffer at 100V for 30 minutes. Results were visualized using Quantity One software and the VersaDoc Imaging System (Bio-Rad Laboratories, Hercules, CA).

Bone marrow transplant (BMT)

FATP1 macrophage knockout ($FATP1^{B-/-}$) chimeric mice were generated using a bone marrow transplant strategy. BMT was performed when recipient mice reached 6 weeks of age (after three weeks on diets). On the day of transplantation, recipient mice were administered 2 doses of X-ray radiation (500cGy x 2, spaced 4 hours apart; X-RAD-320, Precision X-Ray, North Branford, CT). Bone marrow was harvested from $FATP1^{-/-}$and $FATP1^{+/+}$ donor mice which were matched for age and gender with the C57BL/6J recipient mice. Donor mice were anesthetized with tribromoethanol/amylene hydrate (1.25%, Sigma Aldrich, St. Louis, MO) and euthanized via cervical dislocation. The tibia and femur were isolated from each hind leg. After cutting the ends of the bones, the bone marrow was flushed from the bones using sterile PBS containing 2% fetal bovine serum. Debris was removed from bone marrow using a sterile 70µm cell strainer. The bone marrow was washed in 10mL of sterile Hank's Balanced Sat Solution (HBSS) and centrifuged at 240 x *g* at 4°C for 10 minutes. The supernatant was discarded and the cells again washed and centrifuged in HBSS. Cells were resuspended HBSS and stored on ice until used for injection.

Recipient animals were anesthetized with tribromoethanol/amylene hydrate (1.25%, Sigma Aldrich, St. Louis, MO). Once animals no longer responded to a pain stimulus (a hard tail pinch) $FATP1^{-/-}$ or $FATP1^{+/+}$ bone marrow cells (total volume of 100µL) were transferred to the

recipient mouse via retro orbital injection using a 0.5mL insulin syringe (Becton Dickinson, Durham, NC) with a 28 ½ gauge needle. Following injection, animals recovered on an electric heating mat to maintain body temperature until they woke from anesthesia. Chimeric animals were continued on either the same LFD or HFD for a total of 23 weeks. Control irradiated animals that did not receive a BMT died within 10 days of irradiation.

Metabolic phenotyping

Body composition was measured immediately prior to BMT and again at 23 weeks on diet using magnetic resonance imaging (MRI, EchoMRI, Houston, TX).

Tissue Preparation

Epididymal white adipose tissue (eWAT) was extracted from mice that gained weight at or above mean for the group (n = 7 in LFD FATP1$^{B+/+}$, LFD FATP1$^{B-/-}$ and HFD FATP1$^{B+/+}$; n = 9 in HFD FATP1$^{B-/-}$). The tissue was snap frozen and pulverized with liquid nitrogen. RNA was isolated from each sample using anRNeasy Kit (QIAGEN Sciences, Valencia, CA). RNA was then converted to cDNA usingiScript Reverse Transcriptase kit (Bio-Rad Laboratories, Hercules, CA).

Gene expression

Gene expression was measured using semi-quantitative real-time PCR (qPCR). Expression of 18S was used to standardize gene expression levels. Some genes were quantitated using TaqMan Assays-on-Demand (AOD) and some used Universal Probe Libraries (UPL) (Table 1). All reactions had a volume of 10 µl. AOD reactionscontained 1 µl cDNA (10 ng/µl), 3.5 µl

deionized water, 0.25 µl 18s, 0.25 µl AOD, and 5 µl SsoAdvanced Universal Probes Supermix (Bio-Rad Laboratories, Hercules, CA). UPLreactions contained 1 µl cDNA (10 ng/µl), 3.5 µl deionized water, 0.25 µl 18s, 0.1 µl UPL, 0.2 µl gene primer, and 5 µl Supermix. Reactions were run at 95^{o} C for 10 seconds then 60^{o} C for 20 seconds for 40 cycles in a 7900HT Fast Real-Time PCR System (Life Technologies, Carlsbad, CA). Data were analyzed using SDS 2.4 software and the comparative $\Delta\Delta C_T$ method.

Gene	Probe
GLUT1	UPL 3
F4/80	AOD
CD11b	AOD
CD11c	UPL 69
IL-6	AOD
NLRP3	AOD
PYCARD	AOD
CASPASE-1	AOD
IL-1β	AOD

Table 1. Gene probes
Assays-on-Demand (AOD) or Universal Probe Libraries (UPL) were used to probe for genes.

Tissue Histological Analysis

eWAT was fixed for 24 hours in 10% formalin, embedded in paraffin and sectioned at 5µm at the UNC Histology Research Core Facility. Tissue morphology was assessed on sections stained with hematoxylin and eosin. All histological sections were digitally scanned on an AperioScanScope CS Ultra-Resolution Digital Scanner.Crown like structures were quantified.Adipocyte diameter was measured using ScanScope Image Analysis Toolbox software. The longest diameters(unit of measure: µm) of 50 cells from two sections (N=100 cells) of each tissue were averaged to find the overall adipocyte diameter for each sample.

Statistics

For body weight data analyses, a regression model was run with genotype/diet group and week(0 thru 23) as predictors by the statistician Joe Galanako of the UNC Nutrition Obesity Research Center (NORC).To account for the repeated measures over time within animals, an autoregressive within-subject correlation matrix was fit. F-tests using the resulting parameter and standard error estimates were constructed to make comparisons of interest. P-values less than 0.05 were considered statistically significant. All analyses were performed using SAS Version 9.3 (SAS Institute, Cary NC). For all other *in vivo* data, statistical differences between experimental groups were determined by two-way ANOVA followed by multiple comparisons tests using statistics software within GraphPad Prism (GraphPad Software, Inc., La Jolla, CA).

IV. RESULTS

Genotypes were confirmed using polymerase chain reactions (PCR) (Fig. 2).

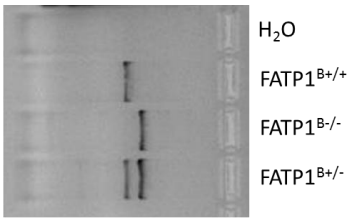


Figure 2. Genotypes
PCR from tail DNA confirms mouse genotypes. Wildtype band is 500 base pairs (bp) and knockout band is 650 bp in size.

Body weight

Starting at 14 weeks into the study, HFD-fed $FATP1^{B-/-}$ mice showed weight gain that was statistically higher than HFD-fed $FATP1^{B+/+}$ mice ($p < 0.0001$ by regression analysis). Both groups started with an average body weight of 13 g. The HFD $FATP1^{B-/-}$ group ended the study with an average body weight of 31.1 g and the HFD $FATP1^{B+/+}$ group averaged a final body weight of 28.2 g (Fig. 3A).

Body composition

HFD-fed mice had a significantly higher percentage of body fat compared to LFD-fed mice ($p < 0.0005$ by two-way ANOVA), but no genotype effect was found (Fig. 3B).

Leptin

Circulating leptin levels were significantly higher in HFD-fed animals compared to LFD-fed animals ($p < 0.0001$ by two-way ANOVA), but no genotype effect was found (Fig. 3C).

eWAT weight

Epididymal white adipose tissue (eWAT) was significantly heavier in HFD-fed mice than inLFD-fed mice ($p < 0.01$).eWAT in the HFD FATP1$^{B-/-}$ group was significantly heavier than the epididymalfat pads in the HFD FATP1$^{B+/+}$ group ($p < 0.01$ by two-way ANOVA) (Fig. 3D).

Crown like structures and Adipocyte diameter in eWAT

There were no significant differences in crown-like structures between diet groups or within genotypes (data not shown). HFD-fed animals had larger adipocyte diameters than LFD-fed mice ($p < 0.0001$). HFD FATP1$^{B+/+}$ and HFD FATP1$^{B-/-}$ mice had an adipocyte diameter average of about 84 µm and 95 µm, respectively, while LFD FATP1$^{B+/+}$ and LFD FATP1$^{B-/-}$ mice had diameters of about 68 µm and 62 µm, respectively. However, no significant genotype effect was found for adipocyte size.

GLUT1 in eWAT

Previous publications suggested that high expression of GLUT1 drives a pro-inflammatory phenotype in macrophages (9). Figure 4A shows that there was increased expression of GLUT1 in eWAT of HFD-fed FATP1$^{B+/+}$ mice, but this increase was not significant.

Macrophage infiltration

The extent of macrophage infiltration was measured based on expression levels of F4/80, CD11b and CD11c. F4/80 is a protein found on macrophage cell surface and can be used to quantify macrophage presence in tissue. CD11b and CD11c are also present on the surface of

macrophages. CD11b regulates leukocyte adhesion and migration during an inflammatory response while CD11c helps trigger the neutrophil respiratory burst. Figure 4b shows a significant increase in expression of these three genes in eWAT in the HFD FATP1$^{B-/-}$ group compared to the HFD FATP1$^{B+/+}$ group.

Inflammatory response

Expression of cytokine IL6 was measured as a marker of inflammation (3). Quantitative PCR showed a significant increase in IL6 expression in eWAT in HFD-fed FATP1$^{B-/-}$ mice compared to HFD-fed FATP1$^{B+/+}$ mice (Fig. 4C).

Inflammasome

Levels of inflammasome components NLRP3, PYCARD, CASPASE-1, and the product IL-1β were measured. All genes but CASPASE-1 were shown to have increased expression in the HFD FATP1$^{B-/-}$ group when compared to the HFD FATP1$^{B+/+}$ group.(Fig. 4D).

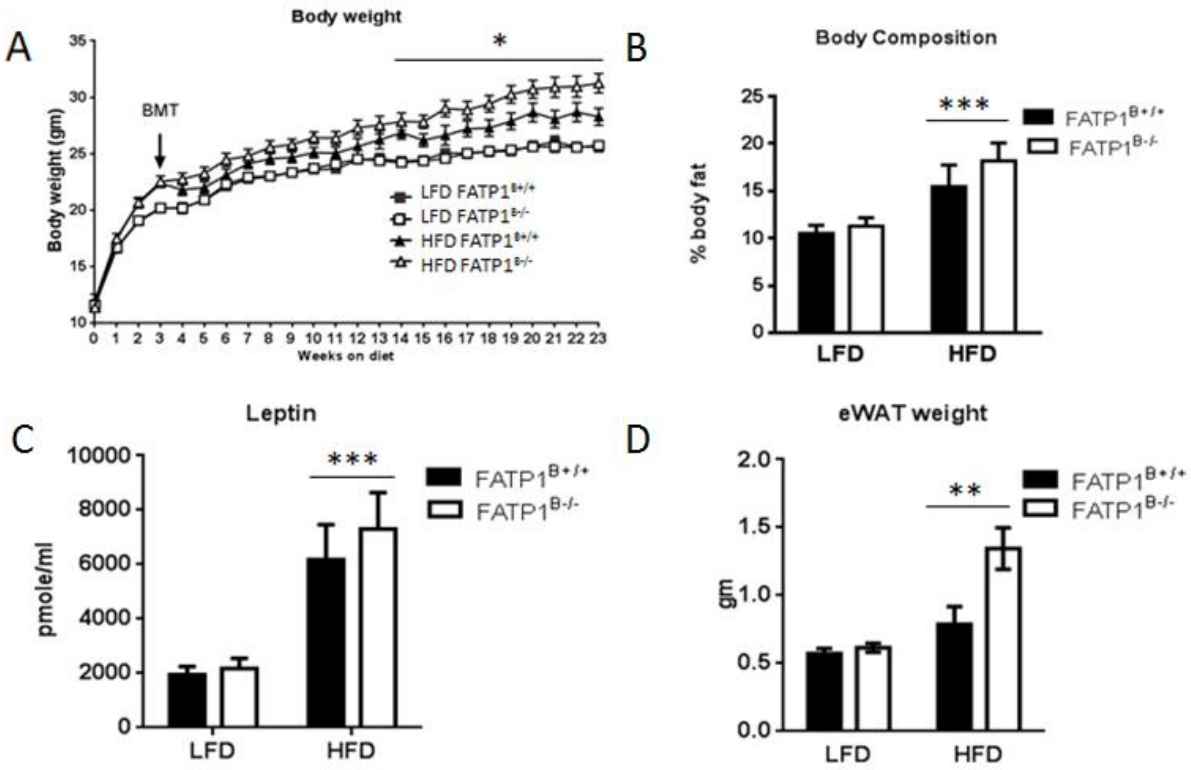


Figure 3: Body weight, body composition, leptin, eWAT weight.

A. Mice in the HFD groups gained significantly more weight compared to mice in the LFD groups, regardless of genotype. Beginning at week 14 and continuing through the end of the study, HFD FATP1$^{B-/-}$ mice were heavier than HFD FATP1$^{B+/+}$ mice ($p < 0.0001$ by regression analysis). B. Adiposity was increased in HFD mice compared to LFD ($p < 0.0005$ by two-way ANOVA); there was no significant genotype effect. C. Circulating leptin concentration was increased in HFD groups, but there was no effect of FATP1 genotype ($p < 0.0001$ by two-way ANOVA). D. Wet weights of eWAT from HFD mice were significantly higher than those from LFD mice ($p < 0.01$); HFD FATP1$^{B-/-}$ mice had higher adipose mass than HFD FATP1$^{B+/+}$ mice (**$p < 0.01$ by two-way ANOVA).

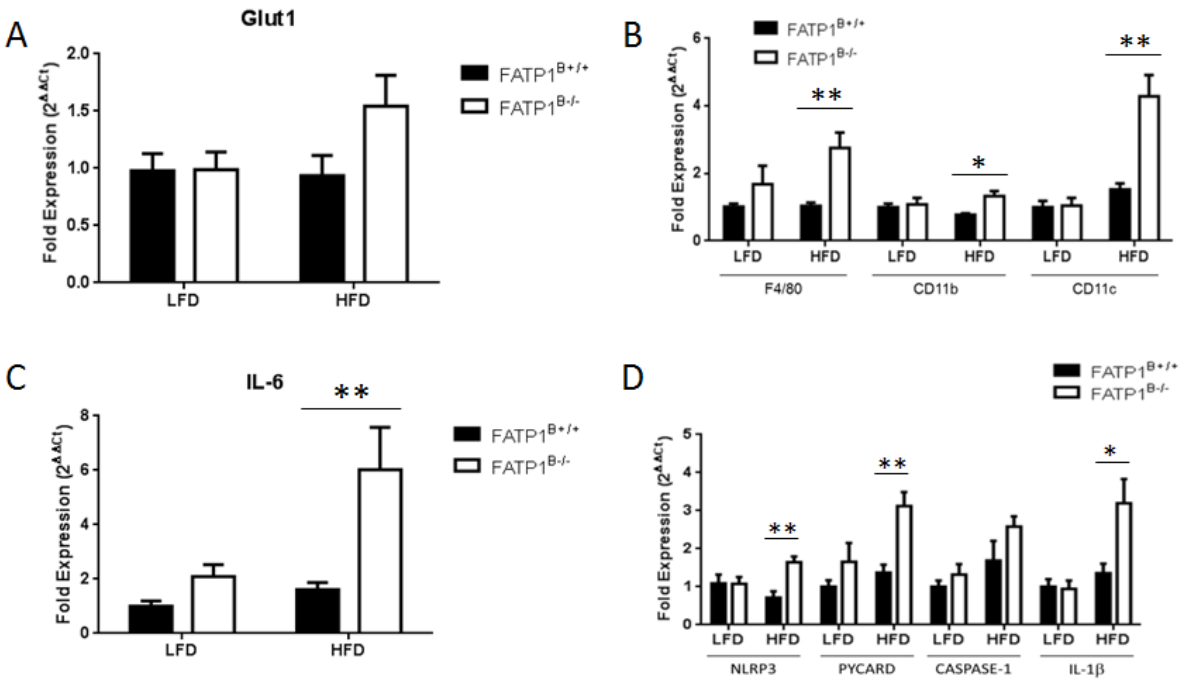
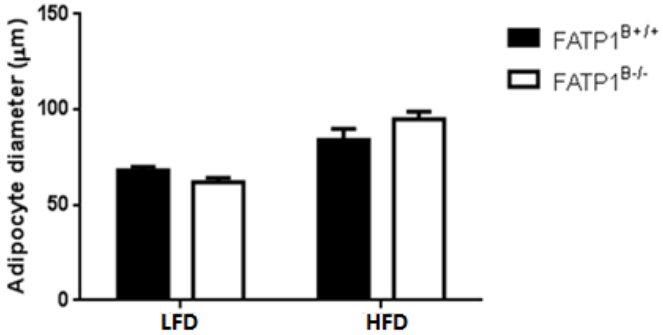


Figure 4: Gene expression

A. There was no statistically significant increase in GLUT1 between wildtype and FATP1 knockout mice. B. There was a significant increase in macrophage infiltration shown by increased expression in $FATP1^{B-/-}$ of F4/80 ($p < 0.01$), CD11b ($p < 0.05$), and CD11c ($p < 0.01$). C. There was significantly increased expression of inflammatory cytokine IL-6 in HFD $FATP1^{B-/-}$ animals in comparison to other groups ($p < 0.01$). D. Inflammasome expression of NLRP3, PYCARD, and IL-1β was elevated in HFD $FATP1^{B-/-}$ mice ($p < 0.01$, $p < 0.01$, and $p < 0.05$ respectively). No difference in expression was found with CASPASE-1. LFD groups showed no significant differences in gene expression for all genes measured.

Figure 5. Adipocyte diameter

The largest diameter of 100 cells from each animal were averaged. The results show a diet effect but not a genotype effect.



V. DISCUSSION

This study tested the effects of FATP1 macrophage knockout on the inflammatory phenotype of mice that remained lean or were fed HFD to induce obesity.Among HFD-fed animals, the $FATP1^{B-/-}$ group gained significantly more weight and had a higher percentage body fat than did the $FATP1^{B+/+}$ group.Increased leptin levels followed the increased weight gain since it has been shown thatmore obese individuals exhibit higher circulating leptin as a result of leptin-resistance, but were not regulated by macrophage FATP1 (13).HFD-fed $FATP1^{B-/-}$ animals also had heavier epididymal fat pads demonstrating that macrophage-restricted deletion of FATP1 contributed to an obesogenic phenotype.

The chronic low-grade state of inflammation associated with obesity is caused by increased macrophage infiltration into stressed tissues as the immune system attempts to restore the body back to its non-inflammatory state. In the present study, F4/80, CD11b and CD11cwere quantified to determine the extent of macrophage infiltration in eWAT. Based on expression levels of classic macrophage markers, there was an increase in macrophage infiltration in HFD-fed $FATP1^{B-/-}$in eWATwhen compared to obese $FATP1^{B+/+}$ controls. We speculate that increased adipose macrophage M1 polarization, due to deletion of FATP1,polarized infiltrating macrophages to be more pro-inflammatory leading to increased inflammation of this tissue.

An increase in expression of inflammation markers was also observed in eWATin theHFD $FATP1^{B-/-}$ group. Interleukin 6 (IL6) and interleukin 1β (IL-1β) weresignificantly elevated in HFD-fed $FATP1^{B-/-}$ animals compared to HFD-fed $FATP1^{B+/+}$ animals. In an obese environment, IL6 release may be stimulated leading to more polarization of M1-like

macrophages.Furthermore, when FATP1 was knocked out, there was increased IL-1β expression in HFD FATP1$^{B-/-}$ animals. IL-1β is a cytokine produced by macrophages that plays a role in cell proliferation, differentiation, and apoptosis.

We next examined the NLRP3 inflammasome as a potential mechanism for the macrophage inflammatory response because it plays an essential role in IL-1β production. Gene expression data showed that there was an increase inexpression of the inflammasomecomponents, specifically NLRP3. This data indicates that activation of the NLRP3 inflammasomemay be a potential mechanism for the macrophage inflammatory response because it plays a role in IL-1β production. NLRP3 inflammasomeactivation of IL-1β may provide a mechanism by which fatty acid metabolism influences macrophage inflammatory response, but future experiments are needed to confirm this hypothesis.

This study was designed to determine whether macrophage substrate availability could be a contributing factor to the obese inflammatory response. FATP1 knockout was proposed to push macrophages to exhibit increased glucose metabolism thus driving M0 macrophages towards the M1 pro-inflammatory phenotype. GLUT1 is the main glucose transporter used by macrophages during glucose metabolism (9).However, herein, expression analysis of GLUT1 in eWAT did not indicate significantly increased expression of this transporter in FATP1 knockout mice. Macrophages' continued reliance on GLUT1 for glucose transport even with FATP1 knockout was assumed, butthere may have been compensatory effects of other GLUT transporters as a result of FATP1 knockout.These effects were not measured suggesting that FATP1$^{B-/-}$ macrophages may still favor glucose, which could be measured using radiolabel glucose uptake

assays. Experiments are currently underway to determine whether a switch in substrate metabolism occurs in the absence of FATP1 or if macrophages' ability to use fatty acids as fuel has been reduced. In sum, findings reported demonstrate that in restriction of fat metabolism in macrophages contributes to release of pro-inflammatory cytokines and increased M1 polarization.

CONCLUSION

This study demonstrated that restricting fatty acid substrate availability via FATP1 knockout from macrophages may increase M1-like macrophage polarization, release of inflammatory cytokines and, thus, a more inflammatory phenotype in obesity.

VI. REFERENCES

1. Kelly T, et al.*Global burden of obesity in 2005 and projections to 2030*. International Journal of Obesity 2008;32:1431-1437.
2. Flegal K, et al. *Prevalence and Trends in Obesity Among US Adults, 1999-2008*. JAMA 2010;303(3):235-241.
3. Johnson A, et al. *The Inflammation highway: metabolism accelerates inflammatory traffic in obesity*. Immunological Reviews 2012;249:218-238.
4. Weisberg S, et al. *Obesity is associated with macrophage accumulation in adipose tissue*.J Clin Invest. 2003;112(12):1796-1808.
5. Xu H, et al. *Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance*. J Clin Invest. 2003;112(12):1821-1830.
6. Gordon S. *The macrophage: Past, present and future*. European Journal of Immunology 2007;37:S9-17.
7. Lumeng C, et al. *Obesity induces a phenotypic switch in adipose tissue macrophage polarization*. J Clin Invest. 2007;117(1):175-184.
8. Vats D, et al. *Oxidative metabolism and PGC-1 beta attenuate macrophage-mediated inflammation*. Cell Metab 2006;4:13-24.
9. Freemerman A, et al. *Glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype*. JBC 2014;289(11):7884-7896.
10. Coe N, et al. *The fatty acid transport protein (FATP1) is a very long chain acyl-CoA synthetase*. JBC 1999;274:36300-36304.
11. Stahl A, et al. Insulin causes fatty acid transport protein translocation and enhanced fatty acid uptake in adipocytes. Developmental Cell 2002; 2:477-488.

12. Wu Q, et al. *FATP1 is an insulin-sensitive fatty acid transporter involved in diet-induced obesity*. Mol Cell Biol 2006;26(9):3455-67.
13. Klok MD, et al. *The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review*. Obes Rev 2007;8(1):21-34.