Original article

MOLECULAR METABOLISM

Metabolic reprogramming through fatty acid transport protein 1 (FATP1) regulates macrophage inflammatory potential and adipose inflammation

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ABSTRACT

Objective: A novel approach to regulate obesity-associated adipose inflammation may be through metabolic reprogramming of macrophages (MΦs). Broadly speaking, MΦs dependent on glucose are pro-inflammatory, classically activated MΦs (CAM), which contribute to adipose inflammation and insulin resistance. In contrast, MΦs that primarily metabolize fatty acids are alternatively activated MΦs (AAM) and maintain tissue insulin sensitivity. In actuality, there is much flexibility and overlap in the CAM-AAM spectrum *in vivo* dependent upon various stimuli in the microenvironment. We hypothesized that specific lipid trafficking proteins, e.g. fatty acid transport protein 1 (FATP1), would direct MΦ fatty acid transport and metabolism to limit inflammation and contribute to the maintenance of adipose tissue homeostasis.

Methods: Bone marrow derived MΦs (BMDMs) from *Fatp1*$^{-/-}$ and *Fatp1*$^{+/+}$ mice were used to investigate FATP1-dependent substrate metabolism, bioenergetics, metabolomics, and inflammatory responses. We also generated C57BL/6J chimeric mice by bone marrow transplant specifically lacking hematopoetic FATP1 (*Fatp1*$^{B-/-}$) and controls *Fatp1*$^{B+/+}$. Mice were challenged by high fat diet (HFD) or low fat diet (LFD) and analyses including MRI, glucose and insulin tolerance tests, flow cytometric, histologic, and protein quantification assays were conducted. Finally, an FATP1-overexpressing RAW 264.7 MΦ cell line (FATP1-OE) and empty vector control (FATP1-EV) were developed as a gain of function model to test effects on substrate metabolism, bioenergetics, metabolomics, and inflammatory responses.

Results: *Fatp1* is downregulated with pro-inflammatory stimulation of MΦs. *Fatp1*$^{-/-}$ BMDMs and FATP1-OE RAW 264.7 MΦs demonstrated that FATP1 reciprocally controled metabolic flexibility, i.e. lipid and glucose metabolism, which was associated with inflammatory response. Supporting our previous work demonstrating the positive relationship between glucose metabolism and inflammation, loss of FATP1 enhanced glucose metabolism and exaggerated the pro-inflammatory CAM phenotype. *Fatp1*$^{B-/-}$ chimeras fed a HFD gained more epididymal white adipose mass, which was inflamed and oxidatively stressed, compared to HFD-fed *Fatp1*$^{B+/+}$ controls. Adipose tissue macrophages displayed a CAM-like phenotype in the absence of *Fatp1*. Conversely, functional overexpression of FATP1 decreased many aspects of glucose metabolism and diminished CAM-stimulated inflammation *in vitro*. FATP1 displayed acyl-CoA synthetase activity for long chain fatty acids in MΦs and modulated lipid mediator metabolism in MΦs.

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Conclusion: Our findings provide evidence that FATP1 is a novel regulator of MΦ activation through control of substrate metabolism. Absence of FATP1 exacerbated pro-inflammatory activation *in vitro* and increased local and systemic components of the metabolic syndrome in HFD-fed *Fatp1*$^{B-/-}$ mice. In contrast, gain of FATP1 activity in MΦs suggested that *Fatp1*-mediated activation of fatty acids, substrate switch to glucose, oxidative stress, and lipid mediator synthesis are potential mechanisms. We demonstrate for the first time that FATP1 provides a unique mechanism by which the inflammatory tone of adipose and systemic metabolism may be regulated.

Keywords Adipose tissue macrophage; M2 macrophage; Obesity; Glycolysis; Crown-like structures; Mitochondria

Abbreviations

9-HODE	9-hydroxy-10,12-octadecadienoic acid
AAM	Alternatively activated macrophage
ACK	Ammonium-Chloride-Potassium
ACSL	Long chain acyl-CoA synthetase
ATM	Adipose tissue macrophage
BMDM	Bone marrow derived macrophage
BMT	Bone marrow transplant
BSA	Bovine serum albumin
CAM	Classically activated macrophage
CDP-choline	Cytidine diphosphate-choline
CLS	crown-like structures
CoA	coenzyme A
DMEM	Eagle's minimal essential medium
ECAR	Extracellular acidification rate
eQTLs	Expression quantitative trait loci
eWAT	Epididymal white adipose tissue
FATP1	Fatty acid transport protein 1
FATP1-EV	FATP1- empty vector
FATP1-OE	FATP1- over-expresser
GLUT1	Glucose transporter 1
GTT	Glucose tolerance test
HFD	High fat diet
Hmox-1	Heme oxygenase − 1
$HOMA_{IR}$	Homeostasis model assessment of insulin resistance
IFNγ	Interferon gamma
IL-1β	Interleukin 1β
IL-4	Interleukin 4
IL-6	Interleukin 6
iNOS	Inducible nitric oxide synthase
ITT	Insulin tolerance test
Lamp2	Lysosome-associated membrane protein 2
LCFA	Long chain fatty acids
LFD	Low fat diet
Lipa	Lipase A
LPS	Lipopolysaccharide
MΦ	Macrophage
MCP-1	Monocyte chemoattractant protein-1
METSIM	The METabolic Syndromes In Men
MTT	3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide
MuTHER	The Multiple Tissue Human Expression Resource
NADPH	Nicotinamide adenine dinucleotide phosphate
NLRP3	NLR family, pyrin domain containing 3
OCR	Oxygen consumption rate
$PGF_{2\alpha}$	Prostaglandin F2 alpha
PPARγ	Peroxisome proliferator-activated receptor gamma
PPP	Pentose phosphate pathway
PRPP	phosphoribosyl pyrophosphate
Pycard	Apoptosis-associated speck-like protein containing a CARD
SAM	S-adenosylmethionine
SVF	Stroma vascular fraction
TLR4	Toll-like receptor 4
TNF-α	Tumor necrosis factor alpha

1. INTRODUCTION

Chronic over-nutrition results in low-grade inflammation in metabolically sensitive tissues that contributes to systemic metabolic dysregulation. In obese individuals, as much as 40% of total body composition is adipose. Adipose tissue MΦs (ATMs) may account for up to 50% of the cellularity in the obese adipose microenvironment compared to just 10–20% in non-obese [1,2], demonstrating that ATMs play a central role in shaping the adipose inflammatory milieu. Indeed, ATMs are the primary source of inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) in adipose tissue, and, thus, perpetuate obesity-associated inflammation and subsequent comorbidities. Adipose inflammation in obesity has been linked to insulin resistance, type 2 diabetes, cardiovascular disease, and cancer [3,4]. Understanding the etiology of metabolic associated inflammation is critical for combating metabolic diseases. Dramatic changes within the adipose microenvironment occur with the onset and progression of obesity, including an influx of monocytes [5]. Initial studies in the early 2000s described dichotomous ATM phenotypes, either pro- or anti-inflammatory [6]. Several models suggested that monocytes differentiate into pro-inflammatory, or "classically activated" MΦs (CAM), in response to the conditions encountered within the obese adipose microenvironment. Persistent CAM activation is purported to sustain adipose inflammation, eventually leading to impaired function of this tissue [4,7,8]. Resident anti-inflammatory MΦs (AAM), also termed "alternatively activated", are recognized to maintain tissue homeostasis, including insulin sensitivity, by supporting remodeling and secretion of anti-inflammatory cytokines [4]. *In vitro*, CAMs are modeled by activation of the so-called "M1" classical pathway upon exposure to type 1 T-helper cytokines [9]. In contrast, AAMs are activated to the "M2" phenotype by exposure to type 2 T-helper cytokines *in vitro* [6]. Recent evidence also has suggested that in both obesity and weight loss, ATMs can be "metabolically" activated and direct lipid trafficking, thus buffering against the excessive free fatty acid concentrations resulting from enhanced lipolysis in adipose [10–12]. These MΦs are characterized by expression of both CAM- and AAM-associated surface markers, enhanced lysosome biogenesis, expression of PPARγ responsive genes and inhibited autophagy [10,11].

As appreciation of the complexity of the ATM inflammatory phenotype has evolved, so has understanding of the metabolic signature associated with ATMs [13–15]. We and others have demonstrated that CAMs exhibit a significant up-regulation of glucose metabolism, particularly flux through the pentose phosphate pathway (PPP) to generate reactive oxygen species (ROS) [16–19]. We reported that pro-inflammatory activation is achievable by enhancing glucose metabolism via glucose transporter 1 (GLUT1) overexpression using an *in vitro* model, even in the absence of external stimuli [20], in a