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*The Role of SR-B1 in Lipid Metabolism and
Inflammation in 3T3-L1 Adipocytes*

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Abstract

Obesity is associated with a number of complications that may increase the risk for chronic disease, including inflammation and dysfunctional high-density lipoprotein (HDL) particles. Scavenger receptor class B member 1 (SR-B1) is an HDL receptor found in the cell plasma membrane involved in cholesterol exchange and the initiation of intracellular signaling cascades. During the process of adipocyte (fat cell) formation (adipogenesis), there is a delicate balance of transcriptional programs that affect cholesterol transport and facilitate lipid accumulation. Mice fed a high fat diet have increased SR-B1 mRNA expression in adipose tissue depots. Furthermore, SR-B1 mRNA expression was significantly increased in mature adipocytes. In this study, 3T3-L1 pre-adipocytes were differentiated into mature adipocytes and transfected with SR-B1 siRNA. There was a 70% suppression of SR-B1, indicating a successful knockdown. Mature adipocytes were stimulated through lipopolysaccharides (LPS) and macrophage-conditioned media (MCM) to determine SR-B1 function in inflammatory conditions. Inflammatory stimulations demonstrated that there is increased mRNA expression of the inflammatory marker monocyte chemoattractant protein 1 (MCP-1) when cells are treated with MCM or LPS, however no significant effects of SR-B1 silencing were observed. Future experiments will include performing additional trials for inflammatory experiments, conducting Western blot analysis to determine protein suppression, and using small molecules to inhibit SR-B1 lipid transfer activity, such as block-lipid transport 1.

Literature Review

Introduction

Adipose tissue has the capacity to store large amounts of energy in the form of triglycerides. Adipose tissue is also the largest reservoir for cholesterol in the body (~25% of whole-body cholesterol in humans), and adipocytes depend upon pre-formed sterol uptake due to their limited cholesterol biosynthetic capacity.¹ The majority of cholesterol in adipocytes comes from circulating lipoproteins, such as high-density lipoproteins (HDL), and adipocytes maintain cholesterol homeostasis primarily through regulating efflux.² Cholesterol accumulates in proportion to triglycerides in adipose tissue,³ and cholesterol-loading enhances fatty acid uptake in adipocytes.⁴ Evidence suggests that cholesterol imbalance in adipocytes may contribute to adipocyte dysfunction.^{2,5} Cholesterol overload is an indicator of enlarged adipocytes. Cholesterol uptake from HDL by adipocytes is directed towards lipid droplets and insulin stimulates this process.^{6,7}

Obesity is characterized by excess adipose tissue, which may lead to chronic diseases such as type 2 diabetes and cardiovascular disease. Dysmetabolic conditions are attributed to the low-grade systemic inflammation that occurs with obesity,⁸ which involves macrophage infiltration of adipose tissue. When adipocytes grow to the point where they can no longer accommodate any more triglycerides, they acquire a dysfunctional metabolic profile, leading to the secretion of inflammatory cytokines that will attract macrophages to the tissue. Adipocytes will become resistant to insulin, leading to ectopic lipid deposition and systemic inflammation.⁸ Altered cholesterol homeostasis is a characteristic of hypertrophied adipocytes in the obese state.² Enlarged adipocytes from obese animals do not respond to hormones like adipocytes from lean controls and exhibit an altered metabolic state. The adipocytes in obese individuals contain more cholesterol

than lean individuals because excess cholesterol is detrimental in the circulation and thus, needs to be taken up by adipocytes. Therefore, recognizing how cholesterol homeostasis and inflammatory pathways impact adipocytes is key to understanding many metabolic disorders.

Scavenger Receptor Class B, Type 1 as an HDL Receptor and Cholesterol Sensor

Scavenger Receptor Class B, Type 1 (SR-B1) plays an important role as a cholesterol sensor and in facilitating cellular cholesterol bidirectional flux.⁹ It has high affinity for apolipoprotein A-I (apoA-I) residing on high-density lipoprotein (HDL) particles, and is responsible for the uptake of cholesteryl esters from HDL by steroidogenic tissues and the liver,⁷ where it is highly expressed. Furthermore, SR-B1 facilitates the bidirectional flux of free cholesterol and phospholipids between lipoproteins and the cell plasma membrane.⁹ SR-B1 was shown to be a plasma membrane cholesterol sensor through its C-terminal transmembrane domain.⁹ Because of its role in the efflux of cholesterol from cells to lipoproteins, SR-B1 plays a role in reverse cholesterol transport (RCT)⁹ and thus, eliminates excess cholesterol from the body.

The function of SR-B1 as an HDL receptor in atherosclerosis and reverse cholesterol transport (RCT) has been extensively studied.¹⁰ Mice deficient in SR-B1 (SR-B1 knockout) display reduced RCT¹¹ and increased atherosclerosis.¹² On the contrary, hepatic overexpression of SR-B1 in mice reduced plasma HDL-C levels and lowered atherosclerosis,¹³ suggesting that SR-B1 overexpression may promote cholesterol flux through RCT pathways.

Role of Adipose SR-B1 in Lipid Homeostasis and Regulation by Metabolic Signals

Adipose SR-B1 expression and intracellular location are regulated by insulin and refeeding in mice.⁷ There is in vivo evidence that insulin and angiotensin II can mediate HDL homeostasis

through their regulation of SR-B1 translocation in adipose tissue.⁷ Insulin also induces the transcription of several genes involved in lipogenesis by activating the lipogenic transcription factors, sterol regulatory element binding-protein-1c (SREBP1c) and liver X receptor (LXR).¹⁴ Intracellular sterol levels regulate SR-B1 gene expression via SREBP-1a, a transcription factor that binds to two sterol-responsive elements (SREs) on the SR-B1 promoter in rats.¹⁵ LXR α and β and peroxisome proliferator-activated receptors (PPAR) α and γ bind to distal LXRE and PPARE, respectively, on the human and rat SR-B1 promoters.¹⁵ The nuclear receptor LXR is activated by oxidized cholesterol and induces the expression of target genes to help maintain cellular homeostasis in response to increased cellular cholesterol.¹⁶ LXR activation, via oxysterol-loading, increased lipid accumulation in adipocytes.¹⁷ In vitro studies using 3T3-L1 adipocytes showed that HDL induced lipogenesis by increasing LXR binding activity in a process independent of insulin,⁷ demonstrating a link between adipose lipid storage and SR-B1-HDL interactions. Potentially, as adipocytes increase in size to store more triglycerides, more adipocyte SR-B1 is needed to provide cholesterol to the cells beyond their biosynthetic capabilities.

In addition to LXR, PPARs are important ligand-activated nuclear receptors regulating lipid metabolism in cells. PPAR γ , a PPAR subtype highly expressed in mature adipocytes, has anti-inflammatory properties and is critical for adipogenesis.¹⁸ PPAR γ ligands, including fatty acid derivatives such as 15-deoxy- Δ^{12-14} prostaglandin J₂, increased SR-B1 expression in human and mouse monocytes and macrophages.¹³ Furthermore, PPAR γ activation using a PPAR γ agonist drug increased SR-B1 mRNA and protein expression in mouse adipose tissue, which in turn increased cholesterol uptake from circulating HDL particles.¹⁹ In contrast to PPAR γ , Pref-1, a growth factor highly expressed in 3T3-L1 pre-adipocytes, inhibits adipogenesis and its expression is suppressed during adipocyte maturation.²⁰ Adiponectin (adipoQ) is an adipose-derived hormone

down-regulated in obesity and regulated by PPAR γ .²¹ Low plasma adiponectin concentrations are associated with co-morbidities of obesity, such as type 2 diabetes, coronary heart disease, and hypertension.²¹ Furthermore, adiponectin decreased mRNA levels of inflammatory markers such as TNF- α and NF- κ B, and reduced anti-inflammatory marker IL-10 in mouse macrophages.^{8,21}

When adipocytes are under metabolic stress, such as under excess cholesterol accumulation, they become dysfunctional and increase the production of cytokines. Macrophage accumulation initiates metabolic dysfunction of adipose tissue through pro-inflammatory pathways.²² Macrophage-conditioned media (MCM) can stimulate a pro-inflammatory response in mature adipocytes.²² Lipopolysaccharide (LPS) is a pro-inflammatory molecule derived from the cell wall of gram-negative bacteria. Circulating LPS has been shown to be increased with obesity and is associated with adipose tissue inflammation and insulin resistance, and will inhibit adipocyte differentiation.²³ In macrophages, SR-B1 can bind and internalize LPS,²⁴ which can reduce their inflammatory response to LPS.²⁵ A co-culture of adipocytes and macrophages in the presence of LPS enhanced the secretion of interleukin-6 and monocyte chemoattractant protein-1 (MCP-1).²² In mice, hepatic SR-B1 was shown to be promote LPS clearance,²⁵ furthering the evidence that SR-B1 has anti-inflammatory properties.

SR-B1 helps regulate cholesterol transport across the plasma membrane, which can help traffic cholesterol away from extrahepatic tissues to prevent atherosclerosis. The role of SR-B1 in RCT has been heavily studied in hepatocytes and macrophages, but its function in adipocytes has yet to be fully understood. Adipocyte SR-B1 expression and intracellular location are responsive to metabolic status. Evidence suggests that cholesterol imbalance in adipocytes may contribute to adipocyte dysfunction. However, information regarding SR-B1 function in adipocytes and role in obesity development is lacking. Experiments in mice have demonstrated that overall SR-B1

suppression results in obesity, but tissue-specific SR-B1 expression, specifically in the adipocytes has not been determined. In addition, the role of SR-B1 during inflammatory states has not been investigated either.

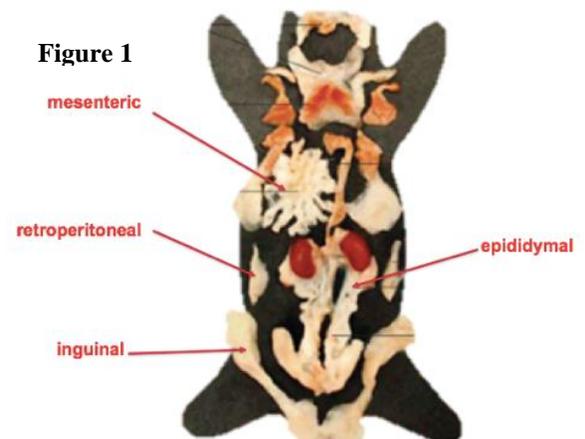
Methods

Differentiation of 3T3-L1 pre-adipocytes

3T3-L1 pre-adipocytes were thawed, seeded into T-75 flasks and incubated at 37°C and 5% CO₂. The cells were maintained in 10% calf serum/Dulbecco's Modified Eagle's medium (DMEM) containing penicillin-streptomycin antibiotic until they reached 100% confluency. After 2 days at 100% confluency, the cells were differentiated using an induction media containing 10% fetal bovine serum (FBS)/DMEM media, 500 mM 3-isobutyl-1-methylxanthine (IBMX), 167 nM insulin (in 100 μM HCl), and 1 μM dexamethasone. After 2 days in the induction media, the media was changed to 10% FBS + insulin 167 nM insulin (in 100 μM HCl) and maintained in this media for 12 days until adipocytes became mature.

Animals and Diets

C57BL/6J mice (8 weeks of age, n = 56) were obtained from The Jackson Laboratory and allowed to acclimate for 2 weeks on chow diet. Mice were then fed either a purified high fat lard-based diet comprised of 60% energy from fat (HFD, n = 16) or a purified low fat diet containing 10% of energy from fat (LFD, n = 8).²⁶ After 16 weeks on this diet, mice were fasted for 6-8 hours before being euthanized. Adipose tissues were perfused with saline



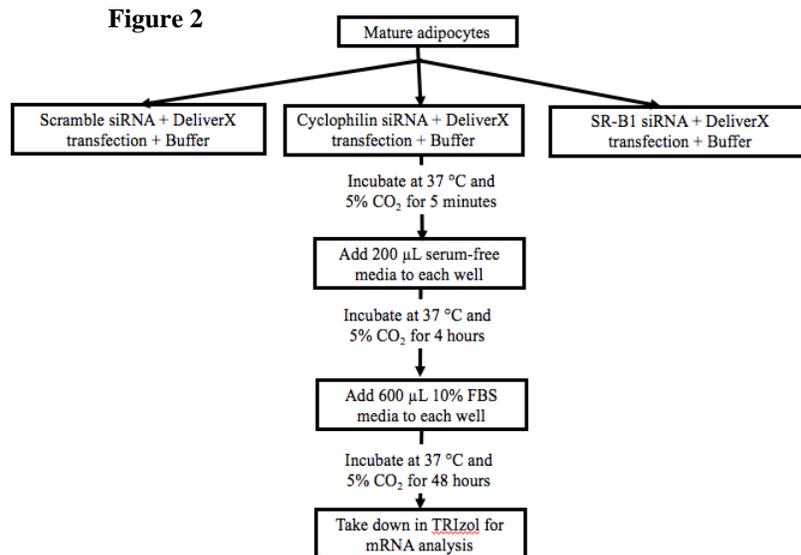
before being harvested, snap-frozen in liquid N₂ and stored at -80°C.

In a separate animal study²⁶, C57BL/6J mice (6 weeks of age, n = 52) were fed a diet comprised of 60% kcal from fat for 10 weeks (HFD, n=14). Adipose tissue was obtained from the epididymal, mesenteric, inguinal, and retroperitoneal regions of the mice (Figure 1)²⁵ for gene expression.

RAW 264.7 Macrophage Culture

RAW 264.7 murine macrophages were obtained from ATCC. The cells were seeded into T-75 flasks and maintained in complete media containing 10% FBS/DMEM and penicillin-streptomycin antibiotic and incubated at 37°C and 5% CO₂. After 3 days, the macrophage-conditioned media was aspirated and frozen at -20°C for future experiments.

siRNA Knockdown



When the adipocytes were fully mature (12 days post-differentiation), the cells were prepared for transfection by transferring them into 24-well plates. The adipocytes were dislodged from the flasks using 0.5 mg/mL collagenase and pelleted by centrifugation at 400 x g for 5

minutes. The collagenase solution was aspirated and complete culture media (10% FBS/DMEM) was added to the tubes. The cells were counted and plated at a concentration of 2.0×10^5 cells/well on a 12-well plate and allowed to incubate overnight at 37°C and 5% CO₂.

Pooled siRNAs were obtained from Dharmacon (siGENOME SMARTpool) and used for knockdown of selective gene expression. Transfection was conducted using a complex containing siRNA and DeliverX Plus Reagent (Affymetrix) following a validated transfection protocol for 3T3-L1 adipocytes (Panomics).²⁷ The three siRNA (5 μM each) used were scramble (negative control), cyclophilin (positive control), and SR-B1 (target gene) (**Figure 2**). To confirm knockdown of SR-B1, the cells were treated under 5 conditions 1) cells only 2) cells + transfection reagent 3) scramble 4) cyclophilin and 5) SR-B1. After transfection with siRNA + DeliverX, the cells were incubated for 4 hours at 37° C and 5% CO₂ before 10% FBS/DMEM media was added. The cells were incubated for an additional 48 hours and were removed in TRIzol reagent for mRNA analysis. siRNA and transfection reagent were confirmed to work based on gene expression levels after treatment using real-time qRT-PCR. The SR-B1 expression was measured for the scramble siRNA and SR-B1 siRNA.

Inflammation was simulated through either 10 ng/mL LPS or macrophage-conditioned media (MCM). After a 48-hour incubation in siRNA and transfection media, cells in each siRNA knockdown condition (scramble, cyclophilin, SR-B1) were treated with 10 ng/mL LPS for 4 hours before being removed in TRIzol reagent for mRNA analysis. For macrophage experiments, 1 mL of MCM was added to each experimental well and incubated for 4 hours before being collected in TRIzol reagent.

RNA Isolation, cDNA synthesis, and real-time qPCR

Total RNA was isolated using TRIzol reagent. Total RNA was treated with DNase I and reverse transcribed using iScript cDNA synthesis kit (BioRad). Gene expression was assessed by real-time quantitative polymerase chain reaction (qPCR) using iTaq Universal SYBR Green Supermix (BioRad). Gene expression was normalized to the expression of the reference gene, 36-B4, using the $2^{-\Delta\Delta C_t}$ method. Genes primers used are listed in **Table 1**.

<i>Gene</i>	Protein	5'-Forward Primer-3'	5'-Reverse Primer-3'	Amplicon Size
<i>36-B4</i>	Acidic ribosomal phosphoprotein PO	GCAGACAACGTGGGCTCCAAGCAGAT	GGTCCTCCTTGGTGAACACGAAGCCC	190
<i>Adipoq</i>	Adiponectin	CAACCAACAGAATCATTATG	GGTAAGAGAAGTAGTAGAGT	77
<i>MCP-1</i>	Monocyte chemoattractant protein-1	TTCCTCCACCACCATGCAG	CCAGCCGGCAACTGTGA	64
<i>Pparg</i>	PPAR γ	CACAATGCCATCAGGTTTGG	GCTGGTCGATATCACTGGAGATC	82
<i>Scarb1</i>	SB-B1	CTCATCAAGCAGCAGGTGCTCA	GAGGATTCGGGTGTCATGAA	710

Statistical Analysis

Data was analyzed using Graph Pad Prism 6. Means were compared using a one-way ANOVA test with Tukey's *post hoc* comparisons, when appropriate, or Student's *t*-test. Data are reported as mean \pm SEM. Significance is reported as $p < 0.05$.

Results

Epididymal Adipose SR-B1 mRNA increased in mice with high fat diet (HFD)-induced obesity

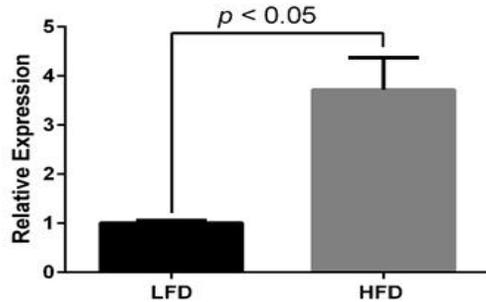


Figure 3:

Mice were fed a LFD or HFD for 16 weeks. Epididymal adipose tissue was analyzed for SR-B1 expression. Relative expression was standardized to the LFD control. Values are mean \pm SEM. n = 4

A four-fold increase in SR-B1 expression was seen in the HFD mice, compared to the LFD control mice. The difference between the LFD and HFD SR-B1 expression was significant at a p value less than 0.05.

SR-B1 expression in four fat depots in mice

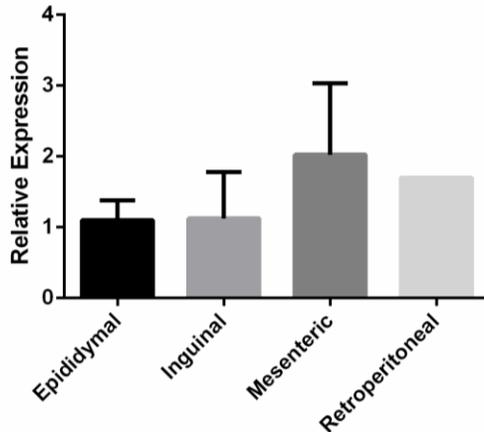


Figure 4:

Adipose tissue from four different fat depots were analyzed for SR-B1 expression. Values are standardized to the SR-B1 expression of the epididymal tissue. Values are mean \pm SEM. n = 5, n=3 for retroperitoneal

Mice were fed a high fat diet (HFD) for 10 weeks and the mRNA expression of SR-B1 in four different white adipose tissue depots showed no significant difference (**Figure 4**).

3T3-L1 Differentiation

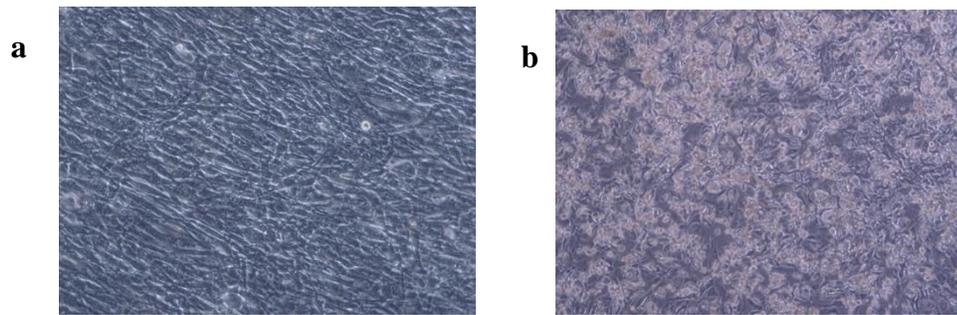


Figure 5a-b:
3T3-L1 pre-adipocytes at 100% confluence (A), mature 3T3-L1 adipocytes 12 days after differentiation (B)

Cells were maintained in 10% calf-serum until 100% confluent (**Figure 5a**) before being treated with a differentiation cocktail comprised of isobutyl-methylxanthine, insulin and dexamethasone. There was lipid accumulation 12 days after differentiation (**Figure 5b**).

SR-B1 mRNA is increased in mature adipocytes

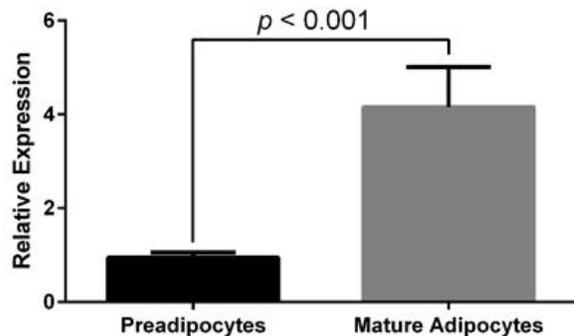


Figure 6:
The SR-B1 mRNA expression levels were measured pre-differentiation (pre-adipocytes) and post-differentiation (mature adipocytes). Values are mean ± SEM. n = 3

Cells at 70% confluence, 100% confluence, and Day 0 of differentiation were combined into “pre-adipocytes” for mRNA analysis of SR-B1. Post-differentiation days 3 and 7 were combined into “mature adipocytes.” There is about a four-fold increase in SR-B1 expression between the pre-adipocytes and mature adipocytes and the difference is significant at $p < 0.001$ (**Figure 6**).

SR-B1 knockdown after siRNA transfection protocol

An approximate 70% knockdown in SR-B1 was observed in the cells treated with SR-B1 siRNA (**Figure 7**). The change was significant at $p < 0.05$. The scramble siRNA is a negative control sequence. Because the sequence does not complement any gene sequence, there should not be any change in gene expression. After the cells were transfected with the appropriate siRNA sequence, gene expression was analyzed using Rplp0 (36-B4) as a reference gene.

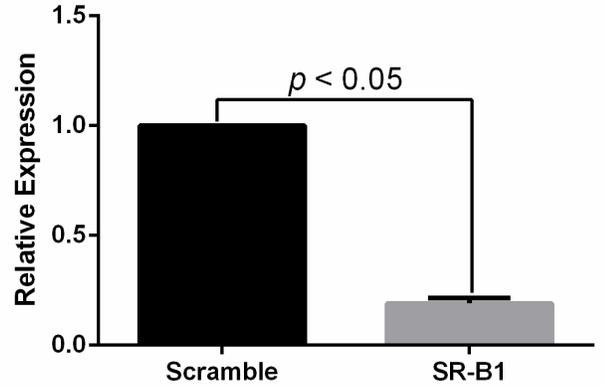


Figure 7: Mature 3T3-L1 adipocytes were treated with scramble siRNA or SR-B1 siRNA to determine the efficiency of the transfection protocol. Relative expression of SR-B1 was standardized to the scramble siRNA. Values are mean \pm SEM. $n = 3$

Transfected 3T3-L1 cells treated with lipopolysaccharides (LPS)

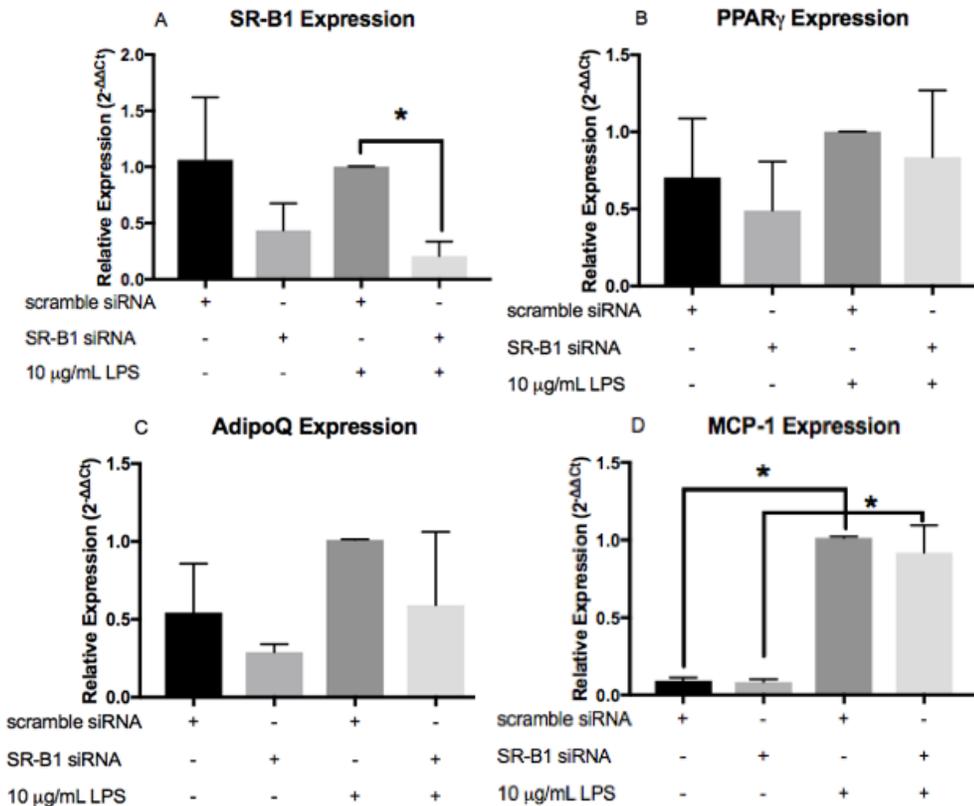


Figure 8 (a-d): Mature 3T3-L1 cells were transfected with scramble siRNA or SR-B1 siRNA for 48 hours and exposed to 10 $\mu\text{g/mL}$ LPS for 4 hours. Gene expression was standardized to scramble siRNA+MCM. Values are mean \pm SEM.

* $p < 0.05$ with t -test

SR-B1 mRNA expression decreased significantly with SR-B1 siRNA in the presence of LPS (**Figure 8a**), demonstrating that SR-B1 was suppressed with the transfection in the stimulated condition. There is no significant change in PPAR γ mRNA after SR-B1 suppression or in the presence of MCM (**Figure 8b**). When SR-B1 siRNA was added, AdipoQ mRNA expression tended to decrease in the presence of MCM, with a p-value of 0.0596 (**Figure 8c**). MCP-1 expression increased with MCM; however, its expression in cells transfected with SR-B1 siRNA is not different than the scramble siRNA (**Figure 8d**).

Transfected cells treated with macrophage-conditioned media (MCM)

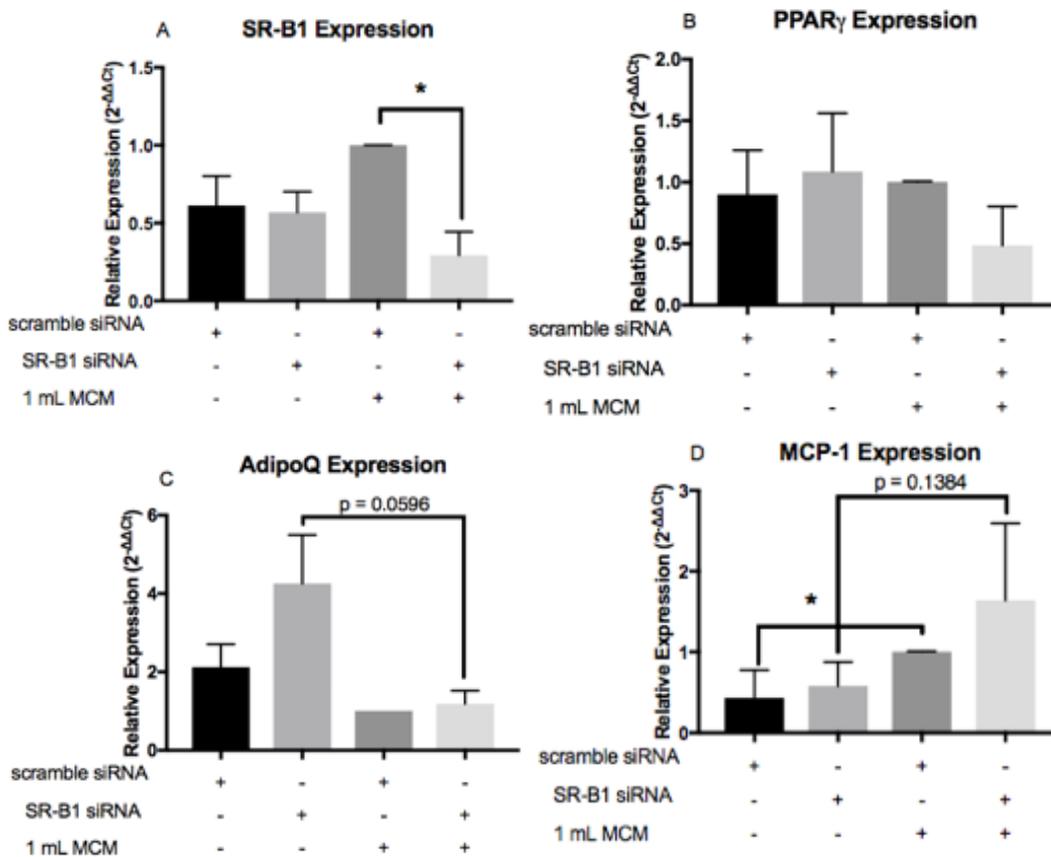


Figure 9 (a-d): Mature 3T3-L1 cells were transfected with scramble siRNA or SR-B1 siRNA for 48 hours and exposed to 1 mL MCM for 4 hours. Gene expression was standardized to scramble siRNA+LPS. Values are mean \pm SEM. n = 3

*p < 0.05 with *t*-test

SR-B1 mRNA was decreased in the presence of MCM with SR-B1 siRNA (**Figure 9a**), but there were no significant changes in PPAR γ . AdipoQ mRNA expression between siRNA conditions (**Figure 9b-c**). MCP-1 increased significantly in the presence of LPS in cells treated with either scramble siRNA or SR-B1 siRNA (**Figure 9d**). There was no significant difference in MCP-1 expression between either siRNA conditions treated with LPS (**Figure 9d**).

Discussion

SR-B1 plays a role in cholesterol metabolism by binding HDL and facilitating the selective transportation of cholesteryl esters across the plasma membrane.⁹ Additionally, it is involved in facilitating the bidirectional flux of free cholesterol across the membrane.⁹ Its significance in the liver has been extensively studied; however, its role in adipocytes has yet to be determined. One study has shown that SR-B1 deficiency in mice leads to altered adiposity, leading to the hypothesis that SR-B1 plays a role in lipid metabolism in adipocytes.²⁸ There is, however, research that suggests that SR-B1 suppression is protective in obesity by being more resistant to hepatic lipid deposition.²⁹ Adipose tissue was obtained from mice fed either a low-fat diet (LFD) with 10% kcal as fat or a high-fat diet (HFD) with 60% kcal as fat for 10 weeks and SR-B1 mRNA expression in the epididymal adipose tissue was found to be much higher in the mice fed a HFD. The significant difference suggests that SR-B1 may play a crucial role in adipose tissue expansion and energy storage.

Mice have multiple white adipose tissue depots in their body, including epididymal/perigonadal, mesenteric, inguinal and retroperitoneal tissues. After SR-B1 expression was found to be higher in HFD mice, it was hypothesized that SR-B1 expression might vary between the different adipose depots. Adipose tissue samples from the four depots were analyzed

for SR-B1 expression to determine if there was a specific depot that SR-B1 expression was greatest. Analyzing the depots from five mice did not show any significant differences between the four depots, suggesting that SR-B1 might have a similar function across all white adipose tissue in mice.

3T3-L1 pre-adipocytes need to be differentiated to mature adipocytes through a process that lasts about 14 days. It was hypothesized that SR-B1 may play a role at separate intervals during the differentiation process. Cells were obtained in TRIzol reagent at different time points in the differentiation process, including before maturation (70% and 100% confluency, day of differentiation), and during maturation (3 days after differentiation, and 7 days after differentiation). SR-B1 mRNA expression was significantly higher in the mature adipocytes than the pre-adipocytes; furthering the hypothesis that SR-B1 plays a significant role in adipogenic conditions.

To determine the impact that SR-B1 has on the metabolism of adipocytes, knockdown experiments using siRNA were conducted to silence the SR-B1 gene. Through three trials of the transfection protocol, SR-B1 expression was reduced by ~70% in the SR-B1 transfected cells, demonstrating that the SR-B1 siRNA and the transfection protocol were valid for use in future experiments.

LPS was used to initiate an inflammatory response in the mature 3T3-L1 adipocytes. SR-B1 has been shown to bind and internalize LPS²⁴ and be anti-inflammatory in macrophages,²⁵ so it was hypothesized that SR-B1 would be a similar role in adipocytes in the presence of LPS. In the LPS experiments, SR-B1 mRNA expression was significantly decreased in cells transfected with SR-B1 siRNA in the presence of LPS. The expressions of PPAR γ , MCP-1 and AdipoQ were also analyzed. PPAR γ and AdipoQ did not change significantly with the transfection or LPS

stimulation. MCP-1, an inflammatory chemokine, was induced with LPS treatment of mature adipocytes. The significant increase in MCP-1 observed in the transfected cells in the presence of LPS shows it is an effective inflammatory stimulant in adipocytes. However, no differences in MCP-1 expression were observed between scramble siRNA and SR-B1 siRNA conditions.

Macrophage-conditioned media (MCM) contains inflammatory cytokines released from macrophages, such as TNF α , which can initiate an immune response in the presence of adipocytes. The SR-B1 mRNA was significantly reduced in cells treated with SR-B1 siRNA in the presence of MCM, demonstrating that the transfection was effective. MCP-1 expression was significantly increased in the cells treated with scramble siRNA in the presence of LPS; however, the SR-B1 suppressed cells had a trending increase in MCP-1 expression with a p value of 0.1384. These data provides preliminary evidence that MCM can be used initiate an inflammatory response in adipocytes. Consistent with this, AdipoQ mRNA expression tended to decrease when cells were treated with MCM. However, there were no significant changes in PPAR γ . In healthy adipocytes, PPAR γ has a positive association with adipocyte maturation; however, there was no difference between the conditions.

SR-B1 plays a role in adipose tissue, as shown by its increased expression in mice fed a HFD as well as mature adipocytes. While its role has been determined in hepatocytes, this study demonstrated its role in lipid metabolism. Its function in inflammation is inconclusive from this study due to the complications involved in transfecting mature adipocytes. These experiments demonstrated that LPS and MCM are both effective inflammatory stimulants in mature adipocytes, which can be used in future experiments regarding the role of SR-B1 in inflammation.

Limitations

In vitro studies are limited by the fact that cells are in an isolated environment that does not provide a complete understanding of lipid metabolism in vivo. Because the differentiation of adipocytes requires a longer time than other cell types, it was time-consuming to culture them until they were fully mature cells ready to be used for experiments. In the fall of 2016, there was contamination in the shared cell culture incubator, so cells could not be grown during the time of de-contamination and cleaning. Another limitation for this study was the lack of protein expression data to confirm SR-B1 knockdown. Transfection to suppress protein may require a longer transfection than the 48 hours conducted. Lastly, there are variations in MCM media based on the length of time that the macrophages were exposed to their media. Although both siRNA conditions received the same batches of MCM, it would be more effective in future experiment to collect MCM frequently and pool together to reduce variation across experiments.

Future Directions

In order to increase statistical power, more trials should be done to reduce experimental variation and error. With only three trials in both the LPS and MCM experiments and large variation in gene expression, no definitive conclusions can be made from this data. Due to time constraints, more trials were not able to be done for the inflammation experiments. Furthermore, the dose- and time-dependent responses were not studied. A 4-hour incubation period might not have been enough time to observe an effect on mRNA for some genes. Future studies will use a longer incubation period.

Additionally, the expression of more genes could be tested to determine the effects of SR-B1 knockdown on inflammatory pathways. The TNF α and IL-1beta primers were used; however,

there was no PCR signal, indicating that these genes are expressed at very low levels, if at all, in the mature adipocytes. Currently, there are some trends that can be pursued further in the future, such as increased MCP-1 when SR-B1 is knocked down in the presence of MCM. Western blot analysis can also be used to determine protein expression after knockdown.

Finally, the function of SR-B1 in lipid homeostasis can be studied using BLT compounds, a class of small molecule chemical inhibitors that block lipid transport through selective uptake and efflux pathways.³⁰ The effects of BLT compounds are highly specific to the SR-B1 pathway and do not affect several clathrin-dependent and -independent endocytic pathways, the secretory pathway, or the actin or tubulin cytoskeletal networks.³⁰ Although under the influence of these compounds SR-B1 had a higher binding affinity to HDL, they prevented SR-B1 from efficiently transferring lipid.³⁰ Out of the different BLT compounds identified, BLT-1 was the most potent inhibitor and can be used to modify SR-B1 activity and HDL metabolism at low micromolar concentrations.³⁰ Therefore, using BLT-1 can help better understand the effect SR-B1 has on lipid metabolism.

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