Published in final edited form as:

Biochim Biophys Acta Mol Cell Biol Lipids. 2020 February ; 1865(2): 158554. doi:10.1016/j.bbalip.2019.158554.

Scavenger Receptor Class B, Type 1 Facilitates Cellular Fatty Acid Uptake

Wei Wang^{1,*†§}, Zhe Yan^{1,*†**}, Jie Hu^{*†‡2}, Wen-Jun Shen^{*†‡3}, Salman Azhar^{*†}, Fredric B. Kraemer^{*†‡3}

* Division of Endocrinology, Gerontology and Metabolism, Stanford University, Stanford, CA 94305

†Veterans Affairs Palo Alto Health Care System, Palo Alto, CA 94304

§Department of Endocrinology, Peking University First Hospital, Beijing, China

**Department of Endocrinology and Metabolism, West China Hospital of Sichuan University, Chengdu, China

Abstract

SR-B1 belongs to the class B scavenger receptor, or CD36 super family. SR-B1 and CD36 share an affinity for a wide array of ligands. Although they exhibit similar ligand binding specificity, SR-B1 and CD36 have some very specific lipid transport functions. Whereas SR-B1 primarily facilitates the selective delivery of cholesteryl esters (CEs) and cholesterol from HDL particles to the liver and non-placental steroidogenic tissues, as well as participating in cholesterol efflux from cells, CD36 primarily mediates the uptake of long-chain fatty acids in high fatty acid-requiring organs such as the heart, skeletal muscle and adipose tissue. However, CD36 also mediates cholesterol efflux and facilitates selective lipoprotein-CE delivery, although less efficiently than SR-B1. Interestingly, the ability or efficiency of SR-B1 to mediate fatty acid uptake has not been reported. In this paper, using overexpression and siRNA-mediated knockdown of SR-B1, we show that SR-B1 possesses the ability to facilitate fatty acid uptake. Moreover, this function is not blocked by BLT-1, a specific chemical inhibitor of HDL-CE uptake activity of SR-B1, nor by sulfo-N-succinimidyl oleate, which inhibits fatty acid uptake by CD36. Attenuated fatty acid uptake was also observed in primary adipocytes isolated from SR-B1 knockout mice. In conclusion, facilitation of fatty acid uptake is an additional function that is mediated by SR-B1.

Keywords

CD36; SR-B1; fatty acids; knockdown; knockout mice

Address correspondence to: Fredric B. Kraemer, M.D., Division of Endocrinology, Gerontology and Metabolism, S025,300 Pasteur Drive, Stanford, CA 94305-5103, Phone: 650-723-6054, fbk@stanford.edu. ³ To whom correspondence should be addressed at wenjun@stanford.edu or fbk@stanford.edu.

¹These authors contributed equally to the work.

²Current address: PCET Division, Aragen Bioscience, 260 Cochrane Circle, Morgan Hill, CA 94307.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Disclosure Summary: Authors declare no conflict of interests.

Introduction

The class B scavenger receptor family is also known as the CD36 super family and consists of three principal family members and their splice variants. The principal members are 1) SR-B1 (scavenger receptor class B, type 1, also known as SCARB1 or its human homologue CLA-1 [CD36 and LIMP2 Analogous-1]), 2) cluster determinant 36 (CD36), also known as fatty acid translocase (FAT), and 3) lysosomal integral membrane protein type 2 (LIMP-2), also known as SCARB2 (1–6). In addition, there are splice variants of SR-B1 termed SR-BII and CLA-2 in lower species and humans, respectively. Proteins in this family are located in plasma or lysosomal membranes and function to transport lipids, enzymes and viral particles through the membrane of the cells or organelles (lysosomes) and, therefore, play pivotal roles in maintaining lipid homeostasis.

SR-B1 (82-kDa) and CD36 (88-kDa) share 30% sequence identity and both proteins are structurally composed of a large heavily glycosylated extracellular domain, two transmembrane domains and two cytoplasmic N- and C-terminal tails (7, 8). Organs with the highest expression of CD36 include white and brown adipose tissues, lung, heart, mammary gland, and macrophages, whereas a relatively high expression has been demonstrated in the ovary, adrenal cortex, testicular Leydig cells, kidney and skeletal muscle (9). Likewise, SR-B1 is expressed in a variety of tissues and cells including adipose tissue, vascular endothelial cells, smooth muscle cells, macrophages, phagocytes, and intestinal cells, but is predominantly expressed in hepatocytes, adrenocortical cells, and ovarian granulosa/luteal cells, as well as hormonal stimulated testicular Leydig cells (10).

While LIMP-2 is located within lysosomal membranes and mediates lysosomal delivery of β -glucocerebrosidase and serves as a receptor for enterovirus 71 and coxsackieviruses, both SR-B1 and CD36 are located within the plasma membrane. SR-B1 and CD36 share an affinity for a wide array of ligands, including native and modified lipoproteins, advanced glycation end products, and anionic phospholipids (11–13). Although they exhibit similar ligand binding specificity, SR-B1 and CD36 have some very specific lipid transport functions. Whereas SR-B1 primarily facilitates the selective delivery of cholesteryl esters (CEs) and cholesterol from HDL particles to the liver and non-placental steroidogenic tissues (13–16), as well as participating in cholesterol efflux from cells (17, 18), CD36 primarily mediates the uptake of long-chain fatty acids by high fatty acid-requiring organs such as the heart, skeletal muscle and adipose tissue (7). However, CD36 also mediates cholesterol efflux and facilitates selective lipoprotein-CE delivery (7, 19). Based on the use of various CD36/SR-B1 chimeras and *in vitro* transient expression experiments, it has been suggested that CD36 is less efficient than SR-B1 in mediating selective HDL-CE uptake (12, 20–22). Interestingly, the ability or efficiency of SR-B1 to mediate fatty acid uptake has not been reported. We addressed this issue in the current paper and show that SR-B1 possesses the ability to facilitate fatty acid uptake. Moreover, this function is not blocked by BLT-1 (block lipid transport-1), a specific chemical inhibitor of HDL-CE uptake activity of SR-B1, nor by SSO (sulfo-N-succinimidyl oleate), an inhibitor of fatty acid uptake by CD36.

Materials and Methods

Chemicals and reagents-

Reagents were obtained from the following sources: Cholesteryl BODIPY FLC12 (cholesteryl 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoate) was obtained from Molecular Probes (Life Technologies, Grand Island, NY). Bicinchoninic acid assay protein kit was from Pierce Biotechnology, Inc. (Rockford, IL); organic solvents were from J. T. Baker (Phillipsburg, NJ); TRIzol reagent and SuperScript II were from Invitrogen (Carlsbad, CA); RNeasy kit was from QIAGEN (Valencia, CA); SyBr green Taqman PCR kit was from Applied Biosystems (Foster City, CA); Odyssey blocking buffer, goat antimouse IgG-IRDye 800, donkey antigoat IgG-IRDye 680, and goat antirabbit IgG-IRDye 680 were from Li-Cor Biosciences (Lincoln, NE); block lipid transport-1 (BLT-1), sulfo-N-succinimidyl oleate (SSO), isobutyl-methylxanthine, dexamethasone, and insulin were from Sigma-Aldrich (St. Louis, MO). QBT Fatty Acid Uptake Assay Kit was from Molecular Devices Corporation, Sunnyvale, CA, USA; siRNAs for mouse SR-B1 and CD36 were purchased from Thermo Fisher Scientific (Waltham, MA).

Animals-

Heterozygote mating pairs for SR-B1 and homozygote CD36 knockout mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Heterozygote mating pairs of SR-B1 mice were used to generate homozygote knockout mice of SR-B1. All animal experiments were performed according to procedures approved by the VA Palo Alto Health Care System Animal Care and Use Committee. Animals were fed standard normal chow and housed in laboratory cages at 23°C under a 12-hour light-dark cycle. Four-month old wild type, homozygote knockout mice of SR-B1 and CD36 were used for studies.

Cell Culture-

HEK293 cells and COS7 cells (obtained from ATCC) were grown to confluence in Dulbecco's modified Eagle's medium, containing 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. OP9 cells (obtained from ATCC) were grown to confluence in Dulbecco's modified Eagle's medium, containing 20% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. For differentiation, OP9 cells were treated with 0.5 mM isobutyl-methylxanthine and 1 µM dexamethasone on day 0 and with 1 µg/ml insulin on day 2. Cells were harvested on day 5 for RNA isolation, immune-blot analysis, HDL uptake and fatty acid uptake analysis.

Primary adipocytes were isolated by collection of white adipose tissue from SR-B1 and CD36 knockout and wild-type control mice, followed by collagenase I digestion. Primary adipocytes were used for fatty acid uptake experiments.

RNA isolation and quantitative real time PCR analysis-

For RNA isolation, subcutaneous and visceral white adipose tissues were collected from wild type, SR-B1 knockout, and CD36 knockout mice. RNA were isolated using TRIzol® reagent (ThermoFisher Scientific, Waltham, MA), and followed the procedure from the manufacturer. After the ethanol precipitation step, total RNA was dissolved in 30 µl RNase

free water, re-amplified to aRNA, then converted to cDNA using Superscript II reverse transcriptase (ThermoFisher Scientific, Waltham, MA). Real-time PCR was performed with the cDNA prepared as above using an ABI Prism 8500 System using SYBR green master mix reagent (Applied Biosystems Inc., Foster City, CA). The relative mass of specific RNA was calculated by the comparative cycle of threshold detection method (23) using acidic ribosomal phosphoprotein, large, P0 (36B4 or Rplp0) as the reference gene. Genes examined included: *Fabp4*, *Cd36*, *Abca1*, *Acs11*, *Fatp1*, *Fatp4*, *Sr-b1*. Supplemental Table 1 shows the primer sets used for each gene.

Transfections-

SR-B1 wild type and SR-B1 C384S mutant cDNA were cloned in pcDNA6/V5-HisB vector (ThermoFisher Scientific, Waltham, MA) and sequenced. HEK293 and COS7 cells were plated in 96-well culture plates at a density of 2×10^4 cells per well, and transfection was performed with PolyJet™ reagent (SignaGen Laboratories, Gaithersburg, MD, USA) following a reverse transfection protocol. Cells were cultured at 37°C, 5% CO₂ for 5 hours, then the media were replaced with fresh growth media containing 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. Forty-eight hours after transfection, cells were used for fatty acid uptake assays and western blot analyses. For silencing of SR-B1 and CD36 in OP9 cells, Silencer Pre-designed siRNAs for SR-B1 and CD36 were purchased from Thermo Fisher Scientific (Waltham, MA). Scrambled siRNA was purchased from Santa Cruz Biotechnology and used as a negative control. OP9 cells were used for transfection with SR-B1, CD36 or scrambled siRNA. Cells were plated in 96-well culture plates at a density of 2×10^4 cells per well, and transfection was performed with PolyJet™ reagent (SignaGen Laboratories, Gaithersburg, MD, USA) following a reverse transfection protocol. Cells were cultured at 37°C, 5% CO₂ for 6 hours, then the media were replaced with fresh medium containing 0.5mM isobutyl-methylxanthine and 1µM dexamethasone and, subsequently, cultured according to the differentiation protocol. After 120 hours (5 days), the cells were used for fatty acid uptake assays and western blot analyses.

HDL-CE uptake assay-

For these studies, human apoE-free high-density lipoprotein-3 (hHDL₃) was used to prepare reconstituted (*rec*) HDL-BODIPY-cholesteryl ester (C12) particles (*rec*-HDL-BODIPY-CE) as described previously (24). HEK293 cells and COS7 cells (5×10^4 cells/well) were transfected with SR-B1, SR-B1 C384S, CD36 construct or control DNA (0.25 µg per well) in 24 well plates. Forty-eight hours after transfection, cells were incubated with *rec*-HDL-BODIPY-CE (50 µg/mL protein) with dibutyryl cAMP (2.5 mM) for 60–180 min at 37 °C. For BLT-1 treatment, the cells were pre-incubated with 10 µM BLT-1 for 60 min before incubation with *rec*-HDL-BODIPY-CE (50 µg/mL protein) with dibutyryl cAMP (2.5 mM) for 60–180 min at 37 °C. Lipids were extracted from washed cells with a hexane/isopropyl alcohol mixture [3:2 (v/v)] as described previously (25). In each case, a portion of the hexane/isopropyl alcohol extract was transferred to a microplate well, and the fluorescence was measured at an excitation wavelength of 503 nm and emission wavelength of 512 nm using a fluorescence plate reader (Molecular Devices Corp., Sunnyvale, CA). Results are reported as arbitrary units and expressed as relative % HDL-BODIPY-CE uptake. For OP9

cells transfected with control, SR-B1 or CD36 siRNA, HDL-CE uptake experiments were performed after 5 days of adipogenic differentiation.

Fatty acid uptake assay-

Isolated primary adipocytes were seeded into a black-wall/clear-bottom 96-well plate (Corning Costar) at a density of 2×10^4 cells per well. Forty-eight hours after transfection in HEK293 cells or COS7 cells and on day 5 of differentiation of OP9 cells (following transfection), all cultured to confluence in black-wall/clear-bottom 96-well plates, fatty acid uptake assays were performed using a QBT fatty acid uptake kit from Molecular Devices (San Jose, CA) according to the manufacturer's protocol. Briefly, the media were removed from the wells and replaced with 90 μ l/well serum free media and 10 μ l/well HBSS (1x) containing 0.2 % fatty acid free BSA. For BLT-1 treatment, the cells were pre-incubated with 10 μ M BLT-1, which was added in serum free DMEM+0.1%DMSO. For SSO treatment, the cells were pre-incubated with 25 μ M SSO. The assay plate was then incubated for one hour at 37°C, 5% CO₂. Before the fluorescent labeled fatty acid was added, a time zero read was performed. 100 μ l of the test compound Bodipy-dodecanoic acid (CH₃(CH₂)₁₀COOH), diluted in HBSS (1x) and 0.2% BSA, was added to each well and immediately read in a fluorescence microplate reader for a kinetic reading at 485nm (every 30 seconds for 30 min) using bottom-read mode (SpectraMax 5E, Molecular Devices, San Jose, CA).

Immunoblotting analysis:

After transfection, cells were washed twice with 1x PBS and extracted in RIPA lysis buffer with protease and phosphatase inhibitor cocktail (Santa Cruz Biotechnology, Santa Cruz, CA), and protein concentrations determined with the BCA Protein Reagent (Pierce Biotechnology, Rockford, IL). Samples were reduced for SDS-PAGE application, and 10–20 μ g protein per lane was electrophoresed on 12% bis-tris polyacrylamide gels (Bio-Rad, Hercules, CA) and subsequently transferred to nitrocellulose membranes (GE Healthcare, Little Chalfont, UK). Membranes were blocked with 5% BSA in PBS with 0.1% tween 20 and incubated with each of the following antibodies: rabbit anti-SR-B1, rabbit anti-CD36 (Abcam, Cambridge, UK), mouse monoclonal anti-V5 (Thermo Fisher Scientific, Waltham, MA), and mouse monoclonal anti- β -actin (Cell Signaling, Danvers, MA). Subsequently, membranes were incubated with IRDye 680LT goat anti-rabbit or goat anti-mouse secondary antibody (LI-COR Biosciences) and protein bands were visualized and quantified with the Odyssey Infrared Imaging System (LI-COR Biosciences).

Statistics-

Data are expressed as means $\pm$ SEM. Statistical analyses were performed by one-way ANOVA using Prism 6.02 for Mac OS X (GraphPad Software, Inc., La Jolla, CA, USA). Differences between groups were considered statistically significant when $P < 0.05$.

Results

Increased fatty acid uptake in HEK293 cells with overexpression of SR-B1:

To examine whether SR-B1 can facilitate fatty acid uptake, plasmid vectors with rat SR-B1 cDNA, CD36 cDNA as a positive control, or empty vector were transfected into HEK293 cells. Subsequently cells were assayed for fatty acid uptake. As shown in Figure 1, cells with overexpression of SR-B1 displayed increased fatty acid uptake compared to empty vector and to a degree that was comparable to cells overexpressing CD36. Due to the rapid fatty acid uptake into the cells, the accumulative uptake over the first 5 minutes was calculated and presented as AUC in Figure 1B. Similar results were also observed when experiments were performed using COS7 cells (data not shown).

BLT-1, an inhibitor of HDL-CE uptake by SR-B1, does not block fatty acid uptake by SR-B1

BLT-1 is a specific inhibitor that binds to SR-B1 at Cys384 and blocks the ability of SR-B1 to mediate HDL-CE uptake (26–29). When HEK293 cells with overexpression of wild type SR-B1 were treated with BLT-1, there was a ~ 60% decrease in HDL-CE uptake by the cells ($p < 0.05$) (Figure 2A), consistent with the ability of BLT-1 to inhibit this function. Mutation of SR-B1 Cys384 to serine (C384S) also resulted in ~60% decrease of HDL-CE uptake by the cells ($p < 0.01$). BLT-1 treatment did not further decrease HDL-CE uptake by the cells overexpressing SR-B1 C384S. In contrast to HDL-CE uptake, there was no difference in fatty acid uptake in cells overexpressing wild type SR-B1 or the C384S mutant of SR-B1 (Figure 2B). Although BLT-1 treatment showed a trend of decreasing fatty acid uptake in both the wild type and SR-B1C384S mutant, the same trend was observed with cells transfected with control pcDN6 plasmid (Figure 2B). Thus, there were no significant differences in fatty acid uptake for the wild type and SR-B1 C384S mutant with BLT-1 treatment. Mutation of SR-B1 at cysteine 384 to serine does not affect the fatty acid uptake activity of SR-B1.

SSO does not block FA uptake mediated by SR-B1

SSO was shown to inhibit CD36 mediated FA uptake (36, 37). To investigate whether SSO can also inhibit the FA uptake mediated by SR-B1, HEK 293 cells were transfected with plasmid carrying SR-B1, CD36 or control vector. Forty-eight hours after transfection, cells were treated with SSO (25 μ M) for one hour before assaying for fatty acid uptake activity. As shown in Figure 3, transfection of CD36 into HEK 293 cells resulted in greater than two fold increase in fatty acid uptake ($P < 0.001$) compared with control and corrected for relative CD36 expression. Treatment with SSO reduced fatty acid uptake by ~30 %. Overexpression of SR-B1 resulted in ~ 25% increase in fatty acid uptake when compared with control ($p < 0.01$) and corrected for relative SR-B1 expression, again demonstrating that SR-B1 can facilitate fatty acid uptake, but less efficiently than CD36 when normalized for their level of expression. Treatment with SSO, however, did not affect fatty acid uptake mediated by SR-B1. SSO had no effect on FFA uptake in control cells.

Attenuated fatty acid uptake with silencing of SR-B1 with siRNA

To evaluate the contributions of SR-B1 and CD36 to fatty acid uptake, OP9 cells were transfected with siRNA against SR-B1, CD36 or scrambled siRNA and subsequently differentiated into adipocytes. Fatty acid uptake activity was assayed at day 5 after transfection and differentiation. As shown in Figure 4, SR-B1 siRNA treatment resulted in a 60% decrease in SR-B1 protein, which was paralleled by a 65% decrease in HDL-CE uptake. CD36 siRNA treatment resulted in ~90% decrease in CD36 protein, and HDL-CE uptake was reduced to less than 50% of that in control cells. When fatty acid uptake was assayed using the QBT assay system, a decrease in fatty acid uptake was observed throughout the time course of fatty acid uptake due to the reduced levels of SR-B1 ($P < 0.01$) and CD36 ($p < 0.01$). The cumulative fatty acid uptake at 5 min was ~27% lower in cells with knockdown of SR-B1 ($p < 0.01$) and ~34% lower in cells transfected with siCD36 ($p < 0.01$) compared with control scrambled siRNA, consistent with both SR-B1 and CD36 facilitating fatty acid uptake.

Decreased fatty acid uptake in primary adipocytes isolated from SR-B1 and CD36 knockout mice

To compare the physiological significance of fatty acid uptake facilitated by SR-B1 and CD36, primary adipocytes were isolated from SR-B1 and CD36 knockout mice and analyzed for relative rates of fatty acid uptake. As shown in Figure 5, primary adipocytes isolated from both SR-B1 knockout mice and CD36 knockout mice display decreased fatty acid uptake, with the cumulative uptake at 5 min significantly less than that of WT control ($p < 0.05$, and $p < 0.01$ for SR-B1 and CD36, respectively). We measured mRNA levels of genes involved in fatty acid metabolism in both subcutaneous and retroperitoneal fat of SR-B1 and CD36 knockout mice to determine whether there were any compensatory changes due to gene deletion. As shown in Figure 6, the expression ABCA1, ACSL1, and FATP4 were not changed in the white adipose tissue of either the SR-B1 knockout or CD36 knockout mice. There was decreased expression of FATP1 in the subcutaneous white adipose and increased FABP4 expression in the retroperitoneal white adipose tissue of CD36 knockout mice. There were no changes in CD36 mRNA expression in the SR-B1 knockout adipose tissue; however, there was a small reduction in CD36 mRNA expression in subcutaneous fat in SR-B1 knockout mice.

Discussion:

Fatty acids are essential building blocks of biological membranes and also serve as an energy source of almost all living cells. In addition, fatty acids play important roles in signal-transduction pathways, cellular fuel sources, composition of hormones and lipids, the modification of proteins, and energy storage within adipose tissue in the form of triglycerides. Apart from adipose tissue, most other cell types have limited storage of fatty acids in the form of triglycerides in lipid droplets; therefore, the majority of fatty acids are supplied through uptake of circulating fatty acids from the plasma (30–32). The mechanisms responsible for fatty acid uptake into cells have been controversial (33, 34) and have been attributed either to a bidirectional flip-flop model for fatty acid transport or to specific long

chain fatty acid transport systems, including specific transporter proteins, for instance belonging to the fatty acid transporter family (35).

One member of the scavenger receptor super family, CD36, has been implicated in facilitating long chain fatty acid transport across the plasma membrane (7, 8). Indeed, sulfo-N-succinimidyl oleate (SSO), which binds to CD36 (36–38), inhibits CD36-mediated fatty acid uptake (36, 37). SR-B1 shares ~30% sequence identity with CD36 and both proteins have very similar functional structural domains (8). Based on the crystal structure of LIMP-2, the ectodomains of both SR-B1 and CD36 contain a predominantly hydrophobic tunnel that can enable facilitated lipid transfer, particularly cholesterol and cholesterol esters (26). Whether fatty acids traverse the ectodomain tunnel is not known. However, SSO, which interacts with lysine 164 in CD36 (37), not only inhibits CD36 facilitated fatty acid uptake, but also the ability of CD36 to transport CE from oxidized LDL (36, 37). It is noteworthy that SR-B1 does not contain a lysine at or near the analogous position, and is not affected by SSO. In contrast, BLT-1, a thiosemicarbazone reagent that interacts with cysteine 384 located within the SR-B1 tunnel (26, 29), inhibits cholesterol and CE transport by SR-B1 (27).

Although they exhibit similar ligand binding specificity (1, 12, 13), SR-B1 and CD36 have some very specific lipid transport functions. Whereas CD36 has been shown to be able to facilitate fatty acid uptake in adipose tissue as well as heart, and skeletal muscle (7), SR-B1 is known primarily to facilitate the selective delivery of cholesteryl esters and cholesterol from HDL particles to the liver and non-placental steroidogenic tissues (13–15). However, the structural similarity of SR-B1 to CD36 and the facts that SR-B1 is expressed at relatively high levels in adipose tissue (1) and its expression increases during adipose differentiation (1) provided a rationale to explore whether SR-B1 can also function to facilitate fatty acid transport. In the current study, we have provided several lines of evidence showing that SR-B1 has fatty acid uptake activity. First, heterologous expression of SR-B1 in either HEK293 or COS7 cells increased cellular fatty acid uptake to a degree that approached that observed with heterologous expression of CD36; however, the ability of SR-B1 to facilitate fatty acid uptake is less efficient than CD36. Second, knockdown of SR-B1 in OP9 adipocytes using siRNA reduced fatty acid uptake to a similar degree as siRNA-mediated knockdown of CD36. Third, fatty acid uptake in primary adipocytes isolated from both SR-B1 and CD36 global knockout mice was similarly reduced compared with wild-type adipocytes without significant compensatory changes in other fatty acid transporters. Thus, a substantial body of experimental evidence supports the conclusion that SR-B1 facilitates cellular fatty acid uptake. Intriguingly, BLT-1, which effectively interferes with SR-B1-mediated CE transport by obstructing the hydrophobic tunnel within the ectodomain (26–29) displayed no effect on the ability of SR-B1 to facilitate fatty acid transport. Likewise, SSO, which effectively interferes with CD36-mediated fatty acid uptake by binding to lysine 164, displayed no effect on the ability of SR-B1 to facilitate fatty acid transport, which is not surprising in view of the fact that SR-B1 does not contain a site analogous to lysine 164 found in CD36. These observations have implications for possible mechanisms underlying SR-B1- and, by analogy, possibly CD36-facilitated fatty acid uptake. Thus, either the attachment of the bulky BLT-1 to cysteine 384 within the SR-B1 tunnel is not sufficient to prevent the transit of fatty acids through the tunnel or, alternatively, SR-B1-facilitated fatty acid uptake might not

involve fatty acids traversing the tunnel, but the presence of SR-B1 within the plasma membrane might alter the physicochemical properties of the membrane, in particular lipid rafts where SR-B1 is preferentially localized (39), to favor fatty acid flux through the plasma membrane. Additional studies will be needed to address these possibilities. Another point to be considered is that although CD36 and SR-B1 show roughly 30% sequence homology, the C-terminal domains of these two transporters contain different amino acid lengths; SR-B1 has ~45 amino acids, whereas CD36 contains only 9 amino acids (2, 8). Given this, we speculate that the C-terminal domains of these two transporters do not participate in fatty acid uptake. Moreover, since the C-terminal domain of CD36 does not contain any putative PDZ protein binding sites as compared to SR-B1, which contains multiple potential binding sites (8, 40), it is also unlikely that PDZ proteins contribute to the regulation of CD36/SR-B1-mediated fatty acid uptake.

The functional activity of SR-B1 to facilitate cellular fatty acid uptake might provide an explanation to the observation that ablation of SR-B1 in mice results in decreased adiposity and reduced body weight gain when animals are fed a western diet (41). Moreover, SR-B1 has been linked to cholesterol content and HDL mediated triglyceride accumulation in 3T3-L1 cells (42) and in adipose tissue *in vivo* (43). Another potentially interesting association is the observation that fasting non-esterified free fatty acid concentrations and plasma glucose levels are lower in SR-B1 GA genotype carriers as compared to subjects carrying the SR-B1 GG genotype when fed a diet rich in monounsaturated fatty acids (44); however, it is not known whether these SR-B1 genotypes result in alterations in SR-B1 expression.

In conclusion, using overexpression and siRNA-mediated knockdown of SR-B1, as well as primary adipocytes isolated from SR-B1 knockout mice, we show that SR-B1 possesses the ability to facilitate fatty acid uptake. Moreover, this function is not blocked by BLT-1, a specific chemical inhibitor of HDL-CE uptake activity of SR-B1, nor by SSO, which inhibits fatty acid uptake by CD36. Thus, facilitation of fatty acid uptake is an additional function that is mediated by SR-B1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by Merit Review Award # I01BX001923 (SA), and # I01BX000398 (FBK) and Senior Research Career Scientist Award # IK6B004200 (SA) from the United States Department of Veterans Affairs, Biomedical Laboratory Research Development Program and NIH grant P30 DK116074 (FBK).

Abbreviations

BLT-1	block lipid transport 1
CD36	cluster of differentiation 36
CE	cholesteryl ester
CLA-1	CD36 and LIMP2 Analogous-1

LIMP-2	lysosomal integral membrane protein type 2
rec	reconstituted
SR-B1	scavenger receptor class B, type 1
SSO	sulfo-N-succinimidyl oleate

References

1. Acton SL, Scherer PE, Lodish HF, and Krieger M. 1994 Expression cloning of SR-BI, a CD36-related class B scavenger receptor. *J Biol Chem* 269: 21003–21009. [PubMed: 7520436]
2. Calvo D, Dopazo J, and Vega MA. 1995 The CD36, CLA-1 (CD36L1), and LIMPII (CD36L2) gene family: cellular distribution, chromosomal location, and genetic evolution. *Genomics* 25: 100–106. [PubMed: 7539776]
3. Calvo D, and Vega MA. 1993 Identification, primary structure, and distribution of CLA-1, a novel member of the CD36/LIMPII gene family. *J Biol Chem* 268: 18929–18935. [PubMed: 7689561]
4. Oquendo P, Hundt E, Lawler J, and Seed B. 1989 CD36 directly mediates cytoadherence of *Plasmodium falciparum* parasitized erythrocytes. *Cell* 58: 95–101. [PubMed: 2473841]
5. Rigotti A, Miettinen HE, and Krieger M. 2003 The role of the high-density lipoprotein receptor SR-BI in the lipid metabolism of endocrine and other tissues. *Endocr. Rev.* 24: 357–387. [PubMed: 12788804]
6. Webb NR, de Villiers WJ, Connell PM, de Beer FC, and van der Westhuyzen DR. 1997 Alternative forms of the scavenger receptor BI (SR-BI). *J Lipid Res* 38: 1490–1495. [PubMed: 9254074]
7. Pepino MY, Kuda O, Samovski D, and Abumrad NA. 2014 Structure-function of CD36 and importance of fatty acid signal transduction in fat metabolism. *Annu Rev Nutr* 34: 281–303. [PubMed: 24850384]
8. Shen WJ, Hu J, Hu Z, Kraemer FB, and Azhar S. 2014 Scavenger receptor class B type I (SR-BI): a versatile receptor with multiple functions and actions. *Metabolism* 63: 875–886. [PubMed: 24854385]
9. Febbraio M, Hajjar DP, and Silverstein RL. 2001 CD36: a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism. *J Clin Invest* 108: 785–791. [PubMed: 11560944]
10. Rigotti A, Miettinen HE, and Krieger M. 2003 The role of the high-density lipoprotein receptor SR-BI in the lipid metabolism of endocrine and other tissues. *Endocr Rev* 24: 357–387. [PubMed: 12788804]
11. Acton SL, Scherer PE, Lodish HF, and Krieger M. 1994 Expression cloning of SR-BI, a CD36-related class B scavenger receptor. *J Biol Chem* 269: 21003–21009. [PubMed: 7520436]
12. Calvo D, Gomez-Coronado D, Suarez Y, Lasuncion MA, and Vega MA. 1998 Human CD36 is a high affinity receptor for the native lipoproteins HDL, LDL, and VLDL. *J Lipid Res* 39: 777–788. [PubMed: 9555943]
13. Rigotti A, Acton SL, and Krieger M. 1995 The class B scavenger receptors SR-BI and CD36 are receptors for anionic phospholipids. *J Biol Chem* 270: 16221–16224. [PubMed: 7541795]
14. Azhar S, and Reaven E. 2002 Scavenger receptor class BI and selective cholesteryl ester uptake: partners in the regulation of steroidogenesis. *Mol Cell Endocrinol* 195: 1–26. [PubMed: 12354669]
15. Hu J, Zhang Z, Shen WJ, and Azhar S. 2010 Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones. *Nutr Metab* 7: 47.
16. Shen WJ, Asthana S, Kraemer FB, and Azhar S. 2018 Scavenger receptor B type 1: expression, molecular regulation, and cholesterol transport function. *J Lipid Res* 59: 1114–1131. [PubMed: 29720388]
17. Phillips MC 2014 Molecular mechanisms of cellular cholesterol efflux. *J Biol Chem* 289: 24020–24029. [PubMed: 25074931]
18. Shen WJ, Azhar S, and Kraemer FB. 2018 SR-B1: a unique multifunctional receptor for cholesterol influx and efflux. *Annu Rev Physiol* 80: 95–116. [PubMed: 29125794]

19. Febbraio M, and Silverstein RL. 2007 CD36: implications in cardiovascular disease. *Int J Biochem Cell Biol* 39: 2012–2030. [PubMed: 17466567]
20. Connelly MA, Klein SM, Azhar S, Abumrad NA, and Williams DL. 1999 Comparison of class B scavenger receptors, CD36 and scavenger receptor BI (SR-BI), shows that both receptors mediate high density lipoprotein-cholesteryl ester selective uptake but SR-BI exhibits a unique enhancement of cholesteryl ester uptake. *J Biol Chem* 274: 41–47. [PubMed: 9867808]
21. de Villiers WJ, Cai L, Webb NR, de Beer MC, van der Westhuyzen DR, and de Beer FC. 2001 CD36 does not play a direct role in HDL or LDL metabolism. *J Lipid Res* 42: 1231–1238. [PubMed: 11483624]
22. Gu X, Trigatti B, Xu S, Acton S, Babitt J, and Krieger M. 1998 The efficient cellular uptake of high density lipoprotein lipids via scavenger receptor class B type I requires not only receptor-mediated surface binding but also receptor-specific lipid transfer mediated by its extracellular domain. *J Biol Chem* 273: 26338–26348. [PubMed: 9756864]
23. Livak KJ, and Schmittgen TD. 2001 Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻($\Delta\Delta C_T$) Method. *Methods* 25: 402–408. [PubMed: 11846609]
24. Reaven E, Tsai L, and Azhar S. 1996 Intracellular events in the “selective” transport of lipoprotein-derived cholesteryl esters. *J. Biol. Chem.* 271: 16208–16217. [PubMed: 8663101]
25. Hu Z, Shen WJ, Kraemer FB, and Azhar S. 2012 MicroRNAs 125a and 455 repress lipoprotein-supported steroidogenesis by targeting scavenger receptor class B type I in steroidogenic cells. *Mol Cell Biol* 32: 5035–5045. [PubMed: 23045399]
26. Neculai D, Schwake M, Ravichandran M, Zunke F, Collins RF, Peters J, Neculai M, Plumb J, Loppnau P, Pizarro JC, Seitova A, Trimble WS, Saftig P, Grinstein S, and Dhe-Paganon S. 2013 Structure of LIMP-2 provides functional insights with implications for SR-BI and CD36. *Nature* 504: 172–176. [PubMed: 24162852]
27. Nieland TJ, Penman M, Dori L, Krieger M, and Kirchhausen T. 2002 Discovery of chemical inhibitors of the selective transfer of lipids mediated by the HDL receptor SR-BI. *Proc Natl Acad Sci USA* 99: 15422–15427. [PubMed: 12438696]
28. Nieland TJ, Shaw JT, Jaipuri FA, Duffner JL, Koehler AN, Banakos S, Zannis VI, Kirchhausen T, and Krieger M. 2008 Identification of the molecular target of small molecule inhibitors of HDL receptor SR-BI activity. *Biochemistry* 47: 460–472. [PubMed: 18067275]
29. Yu M, Romer KA, Nieland TJ, Xu S, Saenz-Vash V, Penman M, Yesilaltay A, Carr SA, and Krieger M. 2011 Exoplasmic cysteine Cys384 of the HDL receptor SR-BI is critical for its sensitivity to a small-molecule inhibitor and normal lipid transport activity. *Proc Natl Acad Sci USA* 108: 12243–12248. [PubMed: 21746906]
30. Berg JM TJ, Stryer L 2002 *Biochemistry*. 5th ed. W H Freeman, New York.
31. Glatz JF 2011 Challenges in fatty acid and lipid physiology. *Front. Physiol.* 2: 45. [PubMed: 21852975]
32. Das UN 2006 Essential fatty acids: biochemistry, physiology and pathology. *Biotechnol J* 1: 420–439. [PubMed: 16892270]
33. Hamilton JA, Guo W, and Kamp F. 2002 Mechanism of cellular uptake of long-chain fatty acids: Do we need cellular proteins? *Mol Cell Biochem* 239: 17–23. [PubMed: 12479564]
34. Schaffer JE 2002 Fatty acid transport: the roads taken. *Am J Physiol Endocrinol Metab* 282: E239–246.
35. Stahl A 2004 A current review of fatty acid transport proteins (SLC27). *Pflugers Arch* 447: 722–727. [PubMed: 12856180]
36. Coort SL, Willems J, Coumans WA, van der Vusse GJ, Bonen A, Glatz JF, and Luiken JJ. 2002 Sulfo-N-succinimidyl esters of long chain fatty acids specifically inhibit fatty acid translocase (FAT/CD36)-mediated cellular fatty acid uptake. *Mol Cell Biochem* 239: 213–219. [PubMed: 12479588]
37. Kuda O, Pietka TA, Demianova Z, Kudova E, Cvacka J, Kopecky J, and Abumrad NA. 2013 Sulfo-N-succinimidyl oleate (SSO) inhibits fatty acid uptake and signaling for intracellular calcium via binding CD36 lysine 164: SSO also inhibits oxidized low density lipoprotein uptake by macrophages. *J Biol Chem* 288: 15547–15555. [PubMed: 23603908]

38. Harmon CM, and Abumrad NA. 1993 Binding of sulfosuccinimidyl fatty acids to adipocyte membrane proteins: isolation and amino-terminal sequence of an 88-kD protein implicated in transport of long-chain fatty acids. *J Membr Biol* 133: 43–49. [PubMed: 8320718]
39. Babitt J, Trigatti B, Rigotti A, Smart EJ, Anderson RGW, Xu S, and Krieger M. 1997 Murine SR-BI, a high density lipoprotein receptor that mediates selective lipid uptake, is N-glycosylated and fatty acylated and colocalizes with plasma membrane caveolae. *J Biol Chem* 272: 13242–13249. [PubMed: 9148942]
40. Shen W-J, Asthana S, Kraemer FB, and Azhar S. 2018 Scavenger receptor B type 1: expression, molecular regulation, and cholesterol transport function. *J Lipid Res* 59: 1114–1131. [PubMed: 29720388]
41. Karavia EA, Papachristou NI, Sakellaropoulos GC, Xepapadaki E, Papamichail E, Petropoulou PI, Papakosta EP, Constantinou C, Habeos I, Papachristou DJ, and Kypreos KE. 2015 Scavenger Receptor Class B Type I Regulates Plasma Apolipoprotein E Levels and Dietary Lipid Deposition to the Liver. *Biochemistry* 54: 5605–5616. [PubMed: 26313465]
42. Yvan-Charvet L, Bobard A, Bossard P, Massiéra F, Rousset X, Ailhaud G, Teboul M, Ferré P, Dagher G, and Quignard-Boulangé A. 2007 In vivo evidence for a role of adipose tissue SR-BI in the nutritional and hormonal regulation of adiposity and cholesterol homeostasis. *Arterioscler Thromb Vasc Biol* 27: 1340–1345. [PubMed: 17363694]
43. Toh SA, Millar JS, Billheimer J, Fuki I, Naik SU, Macphee C, Walker M, and Rader DJ. 2011 PPARgamma activation redirects macrophage cholesterol from fecal excretion to adipose tissue uptake in mice via SR-BI. *Biochem Pharmacol* 81: 934–941. [PubMed: 21291868]
44. Perez-Martinez P, Perez-Jimenez F, Bellido C, Ordovas JM, Moreno JA, Marin C, Gomez P, Delgado-Lista J, Fuentes F, and Lopez-Miranda J. 2005 A polymorphism exon 1 variant at the locus of the scavenger receptor class B type I (SCARB1) gene is associated with differences in insulin sensitivity in healthy people during the consumption of an olive oil-rich diet. *J Clin Endocrinol Metab* 90: 2297–2300. [PubMed: 15671101]

Highlights:

- SR-B1 can facilitate fatty acid uptake
- BLT-1, a specific inhibitor of SR-B1-mediated HDL-CE uptake, does not block SR-B1-mediated FA uptake
- Primary adipocytes from SR-B1 knockout mice have attenuated FA uptake
- SSO, an inhibitor of CD36-mediated FA uptake, does not block SR-B1-mediated FA uptake.

Figure 1. Overexpression of SR-B1 increases cellular fatty acid uptake.

Forty-eight hours after transfection with pcDNA6, pcDNA6-SR-B1 and pcDNA-CD36, HEK293 cells were serum deprived for 1 hour at 37°C, 5% CO₂. At the end of the incubation, 100 µl of fatty acid mixture was added into the wells, and kinetic readings at 485nm (every 30 seconds for 30 minutes) were started immediately with a SpectraMax M5E. A. Relative fluorescent reading over time for HEK293 cells transfected with pcDNA6, pcDNA6-SR-B1 and pcDNA-CD36. B. Cumulative relative fluorescent reading calculated at 300 seconds after the start of the uptake assay. Data presented are representative of three

independent experiments (n=8–10). * $p < 0.05$ vs. pcDNA6 control. C. Immunoblotting with anti-V5 antibody and anti- β -actin antibody for control.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2. BLT-1 does not block fatty acid uptake by SR-B1.

Forty-eight hours after transfection with pcDNA6, pcDNA6-SR-B1 and pcDNA-CD36, HEK293 cells were pre-treated with or without 10 μ M BLT-1 for 1 hour at 37°C, 5% CO₂. At the end of the incubation, cells were assayed for Bodipy-CE-HDL uptake (A) or fatty acid uptake (B). For fatty acid uptake, the accumulated uptakes at 5 minutes after the initiation of the assay were calculated. Data presented are representative of three independent experiments with six replicates for each experiment (n=6). * p < 0.05 vs. pcDNA6 control, ** p < 0.01 vs. pcDNA-SR-B1, # p < 0.05 vs. no BLT-1. C.

Immunoblotting analysis for expression of SR-B1 and mutant SR-B1 C384 using anti-V5 antibody and anti- β -actin antibody for control.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 3: SSO does not block FA uptake by SR-B1.

HEK293 cells were transfected with plasmid carrying SR-B1, CD36 or control vector. Forty-eight hours after transfection, cells were treated with SSO (25 μ M) for one hour before assaying for fatty acid uptake activity. Data presented are representative of two independent experiments with (n=8) for each treatment. * $p < 0.05$, ** $p < 0.01$, vs WT.

Figure 4. Attenuated fatty acid uptake with silencing of SR-B1 with siRNA.

OP9 cells were transfected with siRNA for control (scrambled), SR-B1 or CD36 for six hours, and then treated with adipogenic cocktail for differentiation. Five days later, cells were assayed for Bodipy-CE-HDL uptake (A) and fatty acid uptake (B). Panel (C) shows the immunoblotting for expression of SR-B1 and CD36. Data presented are representative of three independent experiments with eight replicates for each experiment (n=8). * $p < 0.05$, ** $p < 0.01$, vs control.

Figure 5. Decreased fatty acid uptake in primary adipocytes isolated from SR-B1 and CD36 knockout mice.

Primary adipocytes were isolated from WT, SR-B1 knockout and CD36 knockout mice. 2×10^4 cells were plated in each well of 96 well plates and fatty acid uptake was assayed. Data presented are representative of three independent experiments with six replicates for each experiment (n=6). * $p < 0.05$, ** $p < 0.01$, vs WT.

Figure 6: Expression of fatty acid transporters in adipose tissue from WT, SR-B1 and CD36 knockout mice.

Subcutaneous (A) and retroperitoneal (B) white adipose tissues were collected from four month old wild type, SR-B1 knockout, and CD36 knockout mice. RNA was prepared and expression of genes involved in fatty acid metabolism were analyzed using RT-PCR. Data presented are representative of three independent experiments with at least three replicates for each experiment (n=3–5). * $p < 0.05$, ** $p < 0.01$, vs WT.