Intestinal SR-BI is upregulated in insulin-resistant states and is associated with overproduction of intestinal apoB48-containing lipoproteins

Amanda A. Hayashi,1 Jennifer Webb,1 Joanna Choi,1 Chris Baker,1 Marsel Lino,1 Bernardo Trigatti,2 Karin E. Trajcevski,3 Thomas J. Hawke,3 and Khosrow Adeli1

1Molecular Structure & Function, Research Institute, The Hospital for Sick Children, University of Toronto, Toronto; and Departments of 2Biochemistry and 3Pathology & Molecular Medicine, McMaster University, Hamilton, Ontario, Canada

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Intestinal SR-BI is upregulated in insulin-resistant states and is associated with overproduction of intestinal apoB48-containing lipoproteins. Am J Physiol Gastrointest Liver Physiol 301: G326–G337, 2011. First published May 5, 2011; doi:10.1152/ajpgi.00425.2010.—Intestinal lipid dysregulation is a common feature of insulin-resistant states. The present study investigated alterations in gene expression of key proteins involved in the active absorption of dietary fat and cholesterol in response to development of insulin resistance. Studies were conducted in two diet-induced animal models of insulin resistance: fructose-fed hamster and high-fat-fed mouse. Changes in the mRNA abundance of lipid transporters, adenosine triphosphate cassette (ABC) G5, ABCG8, FA-CoA ligase fatty acid translocase P4, Niemann-Pick C1-Like1 (NPC1L1), fatty acid transport protein 4 (FATP4), and Scavenger Receptor Class B Type I (SR-BI), were assessed in intestinal fragments (duodenum, jejunum, and ileum) using quantitative real-time PCR. Of all the transporters evaluated, SR-BI showed the most significant changes in both animal models examined. A marked stimulation of SR-BI expression was observed in all intestinal segments examined in both insulin-resistant animal models. The link between SR-BI expression and intestinal lipoprotein production was then examined in the Caco-2 cell model. SR-BI overexpression in Caco-2 cells increased apolipoprotein B (apoB) 100 and apoB48 secretion, whereas RNAi knockdown of SR-BI decreased secretion of both apoB100 and apoB48. We also observed changes in subcellular distribution of SR-BI in response to exogenous lipid and insulin. Confocal microscopy revealed marked changes in SR-BI subcellular distribution in response to both exogenous lipids (oleate) and insulin. In summary, marked stimulation of SR-BI expression was observed in all intestinal segments examined in both insulin-resistant animal models. The link between SR-BI expression and intestinal lipoprotein production was then examined in the Caco-2 cell model. SR-BI overexpression in Caco-2 cells increased apolipoprotein B (apoB) 100 and apoB48 secretion, whereas RNAi knockdown of SR-BI decreased secretion of both apoB100 and apoB48.

Characteristics of enterocytes (14), and it largely depends on the active absorption of dietary fat and cholesterol. In hamsters fed a fructose-enriched diet, intestinal cells increase the rate of apoB48 secretion in the fasting state as a result of increased lipogenesis and facilitated lipoprotein assembly (11). The role of the intestinal contribution to dyslipidemia via chylomicron overproduction has been well documented (1). Absorption of dietary fat and cholesterol is largely dependent on intestinal expression of a number of active transporters. There is growing evidence that lipid transporters, highly expressed on the apical surface of the intestine, facilitate the fatty acid and cholesterol transfer/homeostasis in the enterocyte. These include the adenosine triphosphate cassette (ABC) proteins A1, G1, G5 and G8, which regulate cholesterol homeostasis by promoting efflux of cholesterol from the cell, the Scavenger Receptor Class B Type I (SR-BI), and the Niemann-Pick C1-Like1 (NPC1L1), which are involved in cholesterol absorption, and the FA-CoA ligase fatty acid translocase (CD36/FAT) and fatty acid transport protein 4 (FATP4/ACSL4), which also acts as a FA-CoA ligase. The different transporters required for intestinal uptake of lipids are well characterized; however, the understanding of the molecular regulation of their expression under different pathophysiological conditions, including the insulin-resistant state, is still in its infancy.

In the present study, our first aim was to identify potential changes in gene expression of intestinal transporter proteins in diet-induced animal models of insulin resistance and dyslipidemia. Among the transporters examined, SR-BI consistently showed the greatest change in expression in response to development of insulin resistance in two different animal models used. The second aim of this study was to elucidate the role of SR-BI in the modulation of intestinal lipoprotein production. Our results show evidence that increased expression of SR-BI in the insulin-resistant state may contribute to the intestinal overproduction of apoB48-containing particles in insulin resistance. Finally, we demonstrate that one of the mechanisms by which SR-BI responds to insulin and dietary lipids involves changes in its cellular localization and that insulin-mediated SR-BI membrane translocation is partially phosphatidylinositol 3-kinase (PI3-kinase) dependent.

MATERIALS AND METHODS

Animals and Diets

Fructose-fed hamster model. All procedures involving animals were carried out in compliance with the guidelines of the Hospital for Sick Children Animal Ethics Committee (Toronto, Canada). The studies were reviewed and approved by the animal ethics committee of the Hospital for Sick Children, Toronto, Ontario, Canada. Male
Syrian golden hamsters weighing 100–110 g (Mesocricetus auratus; Charles River, Montreal, Quebec, Canada) were randomly allocated to either chow or fructose-enriched diet (hamster diet with 60% fructose, pelleted; Dyets, Bethlehem, PA). The fructose feeding protocol used in the current study has been used extensively in our laboratory and has been shown to cause insulin resistance (1, 9, 11). The diet was continued for 2 wk, and body weight and food consumption were monitored every 2 days. Before tissue collection, animals were fasted for 5 h; animals were then anesthetized using isoflurane, and tissues were collected and flash-frozen in liquid nitrogen. The intestine was separated into the following three sections: duodenum (2- to 3-cm portion taken at the pylorus), ileum (2- to 3-cm portion taken ending at the cecum), and the jejunum (2- to 3-cm portion taken from the middle of the small intestine). Intestinal segments of duodenum, jejunum, and ileum were stored at −80°C before RNA and protein analyses.

Fasted blood samples (5-h fast) were collected from each animal on days 0 and 14 to determine circulating concentrations of glucose and plasma lipids (cholesterol and triglycerides). Body weight, food intake, and glucose and plasma lipid concentrations were analyzed using either a two-tailed paired Student’s t-test analysis or one-way ANOVA. Baseline measurements (i.e., day 0) were used as covariates in their respective analyses. Data are presented as least-square means and SE of the mean.

High-fat-fed mouse model. Because the response to high-fructose feeding in mice is variable and strain dependent, we used a high-fat diet to induce insulin resistance in the mouse model. Male C57BL/6J mice at 10 wk of age (18.4–23 g) (Jackson Laboratories, Bar Harbor, ME) were randomly allocated to receive either high-fat diet [pelleted, TestDiet, catalog no. 58126: energy (kcal/g) from protein (18.3%), fat (25%), and carbohydrate (55%)] or standard mouse chow [pelleted, H11002, 80°C before RNA and protein analyses. The tissue used in this study was collected from a subset of animals described in a previously published report (25). All experiments were approved by the Animal Care Committee at York University and were conducted in accordance with guidelines set forth by the Canadian Council for Animal Care.

Table 1. Quantitative RT-PCR primer sequences

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<th>Forward Primer</th>
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<td>5′-ATGACGCTTCGACACACTTGG-3′</td>
<td>5′-GAGACGATGGATCTTG-3′</td>
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<td>FATP4</td>
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<td>106</td>
</tr>
<tr>
<td>NPC1L1</td>
<td>5′-TTAATGGTCTAGTGTTAAAGG-3′</td>
<td>5′-GATTCGGAGACATCGATTTG-3′</td>
<td>154</td>
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<td>SR-B1</td>
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</tr>
<tr>
<td>18S</td>
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<tr>
<td>Actin</td>
<td>5′-AAATGCGGCTGCTTAAAGG-3′</td>
<td>5′-ATGCGGCTGCTTAAAGG-3′</td>
<td>135</td>
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</tbody>
</table>

CD36, FA-CoA ligase fatty acid translocase; ABC, adenosine triphosphate cassette; FATP4, FA-CoA ligase fatty acid translocase; NPC1L1, Niemann-Pick C1-Like1; SR-B1, Scavenger Receptor Class B Type I; HPRT, GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
SDS-PAGE and Western Blotting

For Western blotting analysis, 300 mg of frozen tissue from individual animals were lysed in extraction buffer (26). Lysates were centrifuged at 13,000 g for 10 min to remove debris, and protein concentrations in the resulting supernatants were determined as described by Bradford (5). Aliquots of lysate containing equal amounts of protein were used for SDS-PAGE and Western blotting, which were performed as described previously (21). Blots were visualized using the enhanced chemiluminescence (ECL) method. Immobilon P membranes were purchased from Millipore, and ECL reagents were purchased from Perkin Elmer (Sunnyvale, CA). The graphs show the densitometry (mean ± SE for 6 animals/treatment for the hamster model and mean ± SE for 10 animals/treatment for the mouse model). ImageJ software (available at rsb.info.nih.gov/ij/) was used for quantification where indicated. In the case of Western blotting, a representative blot is shown.

Immunohistochemical Staining of Intestinal Tissue

Jejunal segments were removed from hamsters immediately after death, and short lengths of tissue were placed in molds, covered with freezing compound (Histoprep; Fisher Scientific, Pittsburgh, PA), wrapped in foil, and submersed in liquid nitrogen. Blocks were stored at −80°C until use. Tissue sections (8 μm) were cut and placed on positively charged microscope slides and then stored at −80°C with dessicant. All subsequent reactions were performed at room temperature. Sections were fixed and permeabilized by covering the tissue for 1 min each with 4% formaldehyde in PBS. Slides were blocked with 1% FCS in PBS for 1 h. SR-BI specific antibody (Novus Biologicals, Littleton, CO) was diluted 1:100 with 1% BSA and placed for 1 h followed by three 5-min washes with PBS. IgG antibody (Sigma, St. Louis, MO) at similar concentration was used as a negative control.

Cell Culture

Caco-2 cells (American Type Culture Collection, Manassas, VA) were grown in 75-cm² tissue culture flasks at 37°C in air and 5% CO₂ in Eagle’s minimal essential medium (EMEM; GIBCO), supplemented with 20% FBS (GIBCO) and 100 IU/ml penicillin (Wisent, St. Bruno, QC, Canada). The culture medium was changed every other day. For the subculture, the medium was removed and the cells were detached from the culture dish with 0.25% trypsin diluted in PBS containing 0.2 g/l EDTA. Culture medium with FBS was added to stop trypsinization. Cells were seeded on six-well cell culture plates (2 × 10⁵ cells/cm²; Corning, Corning, NY) for all experiments.

Knock Down of SR-BI Expression

Double-stranded small-inhibitory RNA (siRNA) specifically targeting a sequence of human SR-BI was purchased from Ambion predesigned siRNA (Austin, TX). The sequence of the sense RNA strand was 5′-GCCUCUAUGAAUCUGUT and antisense strand was 5′-ACAGAUUUCAUGUAGGCtc. siRNA was transfected into Caco-2 cells using Ambion’s SiPort Lipid reagent. Cells were used 96 h posttransfection.

SR-BI Overexpression

The enhanced green fluorescent protein (EGFP)-SRBI fusion protein-encoding plasmid, which produces a protein consisting of an NH₂-terminal EGFP tag followed by full-length murine SR-BI, was kindly provided by Dr. Dr. R. van der Westhuyzen (24). Plasmids encoding EGFP-SRBI were transfected into cells using Lipofectamine X reagent (Invitrogen, Carlsbad, CA). Cells were used 96 h posttransfection.

Preparation of Bile Acid-Containing Micelles

Solutions of 6.6 mM taurocholate (Sigma) and 1 mM oleic acid (Sigma) were prepared in methanol. Solutions of 0.6 mM egg yolk phosphatidylcholine (Sigma) and 0.5 mM cholesterol were prepared in chloroform. Required amounts of each stock solution were transferred to a Pyrex glass test tube and mixed. Solvents were evaporated under a mild stream of argon. One hour before the start of the experiment, the lipid film was hydrated in serum-free EMEM, sonicated, and incubated at 37°C. Solutions were filtered through a 0.45-μm cellulose acetate filter before addition to cells.

Expression of Green Fluorescence Fusion SR-BI and Confocal Laser Scanning Microscopy

Plasmids encoding EGFP-SRBI were transfected in cells using a Lipofectamine X reagent (Invitrogen). Cells were used 48 h after transfection. For experiments, EGFP-SRBI transfected cells were serum deprived for at least 3 h and then treated with or without a preparation of mixed micelles, insulin, wortmannin, and LY294002 for the indicated time periods. Cells were then washed, fixed with 1% paraformaldehyde in PBS, and incubated for 15 min with DAPI for nucleus visualization. Glass cover slips containing the fixed cells were mounted on glass slides with mounting media (Sigma). Cells were analyzed with a confocal laser scanning microscope.

Data Analysis

Body weight, glucose, and plasma lipid concentrations were analyzed using the two-tailed Student’s t-test, and all other data comparisons were analyzed for statistical significance using ANOVA. Data are presented as least-square means and SE of the mean. Probability values <0.05 were considered statistically significant.

RESULTS

Body Weight, Food Intake, Plasma Glucose, and Lipids in the Fructose-Fed Hamster Model

We have shown in previous studies that 2 wk of fructose feeding in hamsters induces a state of insulin resistance (1, 15). In the hamster model, 2 wk of fructose feeding increased plasma cholesterol (from 2.89 ± 0.2 to 3.80 ± 0.5 mmol/l, P = 0.01, in the fasted state; from 3.16 ± 0.5 to 4.72 ± 0.3 mmol/l, P < 0.001, in the postprandial state) and plasma triglycerides (from 1.45 ± 0.3 to 3.43 ± 0.9 mmol/l, P < 0.01, in the fasted state; from 2.16 ± 0.9 to 6.36 ± 0.7 mmol/l, P < 0.001, in the postprandial state) compared with baseline. No significant changes were observed in circulating blood glucose levels after fructose feeding (data not shown). No significant difference in body weight was observed between diet groups after fructose feeding (data not shown); similarly, no significant changes were observed in blood glucose levels after fructose feeding.

Body Weight, Food Intake, Plasma Lipids, and Glucose Tolerance in the High-Fat-Fed Mouse Model

In the high-fat-fed mouse model, 8 wk of high-fat feeding increased body weight significantly compared with control diet (control: 24.2 ± 0.5 g, n = 9; high fat fed: 31.67 ± 1.38 g, n = 10, P = 0.0001). Plasma insulin levels in this model have been evaluated previously, and it has been shown that mice fed a high-fat diet for 8 wk show increased insulin compared with chow controls (25). An intraperitoneal glucose tolerance test was performed on mice fasted overnight (16 h) after 7 wk of...
diet intervention. Glucose was injected (2 g/kg body wt ip), and blood glucose was measured using a hand-held glucometer (Roche, Mississauga, ON) via tail vein bleeds at 0, 15, 30, 60, 90, 120, and 150 min. Baseline blood glucose levels were not significantly different (control: 9.4 ± 0.9 mmol/l, n = 9 vs. high-fat diet: 11.6 ± 1.1 mmol/l, n = 9, P = 0.149); after glucose challenge, differences in glucose tolerance were seen, since high-fat feeding resulted in slower recovery from glucose injection as assessed by a two-way ANOVA (main effect of diet, P = 0.01). No significant differences were observed in plasma cholesterol (control: 2.27 ± 0.07 mmol/l, n = 9; high fat fed: 2.59 ± 0.18 mmol/l, n = 10, P = 0.14) or triglycerides (control: 0.54 ± 0.03 mmol/l, n = 9 vs. high fat fed: 0.57 ± 0.06 mmol/l, n = 10, P = 0.6).

**mRNA Levels of Intestinal Transporters in Insulin-Resistant Hamster and Mouse Models**

Expressions of several intestinal transporters were assessed in the different models of insulin resistance by measuring mRNA levels in different sections of the small intestine (duodenum, jejunum, and ileum). Table 2 shows the expression levels (mRNA abundance) of intestinal transporters, CD36, ABCG5, ABCG8, FATP4, NPC1L1, SR-BI, ABCA1, and ABCG1, in chow and fructose-fed hamsters, as well as in chow and high-fat-fed mice in the fasting state.

In the fructose-fed hamster model, the mRNA expression of ABCG8, ABCG5, FATP4, and NPC1L1 did not change in response to fructose feeding in the fasted state in any of the intestinal segments examined. CD36 mRNA expression in the hamster intestine was significantly lower in the duodenum of fructose-fed animals in the fasted state compared with control animals (decreased 1.7-fold). In addition, SR-BI mRNA expression in the hamster intestine was significantly higher in the jejunum of the fructose-fed animals in the fasting state (+7.8-fold). Unfortunately, we could not detect the gene expression of ABCA1 and ABCG1 in the intestine of the hamster because of the absence of available sequence information, suggesting a lack of homology to other rodent species.

In the high-fat mouse model, the gene expression of CD36, ABCG8, ABCG5, FATP4, NPC1L1, ABCA1, and ABCG1 did not change in response to high-fat diet in the duodenum and ileum. Similarly, the gene expression of ABCG1 did not change in the jejunum of the animals receiving the high-fat diet compared with the animals receiving the control diet. In the jejunum, the gene expressions of CD36, ABCG8, ABCG5, FATP4, SR-BI, and ABCA1 were all increased in response to high-fat diet (Table 2). Interestingly, SR-BI gene expression was increased consistently (±7-fold) in the jejunum of both fructose-fed hamster and high-fat-fed mouse models. To evaluate if the feeding state had any effect on expression levels of intestinal lipid transporters, we assessed transporter expression in chow and fructose-fed hamsters following an oral fat load. Similar to the results seen in the fasted hamsters, the mRNA levels of ABCG8, ABCG5, FATP4, and NPC1L1 were unaffected by fructose feeding in the postprandial state, with the exception of ABCG5 mRNA, which decreased in the jejunum (−1.7-fold) in response to fructose feeding in the postprandial state. In addition, SR-BI mRNA expression was significantly higher in the duodenum and ileum of fructose-fed hamsters compared with chow (+1.5-and +3.0-fold), and CD36 mRNA expression was decreased in the jejunum of fructose-fed hamsters (−1.4-fold).

Table 2. Effect of diet on CD36, ABCG5, ABCG8, FATP4, NPC1L1, SR-BI, ABCA1, and ABCG1 gene expression (relative mRNA levels) in the duodenum, jejunum, and ileum

<table>
<thead>
<tr>
<th>Gene Name Symbol</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Mice, high fat fed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasting</td>
<td>PP</td>
<td>Fasting</td>
<td>PP</td>
</tr>
<tr>
<td>Fatty acid translocase</td>
<td>CD36</td>
<td>-1.7*</td>
<td>-1.7**</td>
<td>-1.3</td>
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<td>NPC1L1</td>
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<tr>
<td>Scavenger receptor class B type I</td>
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<td>1.5*</td>
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mRNA levels were estimated as described in MATERIALS AND METHODS. PP, postprandial. The values were normalized using two reference genes as an endogenous internal standard. The data represent fold change in gene expression of 6 hamsters on each diet (hamsters, fructose fed) and 10 mice on each diet (mice, high fat fed). ND, gene expression was not possible to be detected in the intestine segments of the hamsters. Values were normalized using two reference genes as endogenous internal standard. *P < 0.05 and **P < 0.001 compared with control diet.
Protein Levels of Intestinal Transporters in the Fructose-Fed Hamster and Fat-Fed Mouse Models

To evaluate the posttranscriptional changes in the intestinal transporters that occurred in response to the insulin-resistant state, Western blot analysis was performed to assess intestinal transporter protein abundance. The protein levels of CD36, FATP4, SR-BI, ABCG5, ABCG8, and NPC1L1 in the duodenum, jejunum, and ileum of chow-fed and fructose-fed hamsters in the fasting condition are shown in Fig. 1.

In the fasting condition (Fig. 1), fructose feeding increased the protein abundance of SR-BI in all intestinal segments. Similarly, NPC1L1 levels were increased in the duodenum of fructose-fed hamsters but did not change in the jejunum or ileum.

**Fig. 1.** Intestinal protein abundance of fatty acid transport protein 4 (FATP4), FA-CoA ligase fatty acid translocase (CD36), adenosine triphosphate cassette (ABC) G5, ABCG8, Niemann-Pick C1-Like1 (NPC1L1), and Scavenger Receptor Class B Type 1 (SR-BI) fructose-fed hamsters. Intestinal segments (duodenum, jejunum, and ileum) from 6 hamsters fed either chow or fructose diets were homogenized, and equal amounts of protein were separated by SDS-PAGE and immunoblotted with antibody against each specific transporter protein (as described in MATERIALS AND METHODS). Blots were visualized with enhanced chemiluminescence. Actin was used as a loading control. A, C, and E: blots shown are results for 3 of the 10 mice examined and are representative. B, D, and F show the average ± SE (n = 6 hamsters/group). *P < 0.05 and **P < 0.01 vs. chow.
ileum. On the other hand, ABCG8 protein levels were decreased in the duodenum and jejunum of fructose-fed hamsters but did not change in the ileum. The protein levels of CD36, FATP4, and ABCG5 were not changed in response to fructose feeding in any of the intestinal segments examined.

Figure 2 shows the protein levels of CD36, FATP4, SR-BI, ABCG5, ABCG8, and NPC1L1 in the duodenum, jejunum, and ileum of chow-fed and high-fat-fed mice. In the duodenum, the high-fat diet increased the protein levels of SR-BI, but it did not change the levels of any of the other transporters studied. In the jejunum, the high-fat diet increased the protein levels of SR-BI and decreased the protein levels of ABCG5 and NPC1L1. No changes were observed in the levels of either FATP4 or ABCG8 in the jejunum. In the ileum, high-fat feeding caused a decrease in the levels of ABCG8, but it did not significantly change the protein levels of CD36, FATP4, SR-BI, ABCG5, and NPC1L1.

Considerable variability was observed in the intestinal level of various transporters examined. This is likely because of animal-to-animal variability and potential differences in response to the test diets and/or differences in transporter concentration, depending on the specific regions within the intes-

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**Fig. 2.** Intestinal protein abundance of FATP4, CD36, ABCG5, ABCG8, NPC1L1, and SR-BI in the high-fat-fed mice. Intestinal segments (duodenum, jejunum, and ileum) from 6 animals fed either chow or high-fat diet were homogenized, and equal amounts of protein were separated by SDS-PAGE and immunoblotted with antibody against each specific transporter protein (as described in MATERIALS AND METHODS). Blots shown are results for 3 of the 6 hamsters examined and are representative. B, D, and F show the average ± SE (n = 10 mice/group). *P < 0.05 and **P < 0.01 vs. chow.
tinal tract. However, despite the variability observed, the effect of diet (high fructose or high fat) on the expression of SR-BI was consistent and statistically significant and based on analysis of multiple intestinal segments from a large number of animals per group (n = 6–10).

Together, these results show evidence of increased intestinal mRNA and protein levels of SR-BI in two different models of insulin resistance. This led to further investigation of the role of SR-BI in intestinal lipoprotein overproduction commonly observed in insulin-resistant states.

Effect of SR-BI Knock Down and Overexpression on Intestinal Lipoprotein Metabolism

The fructose-fed hamster model of insulin resistance has been previously shown to exhibit increased chylomicron production, marked by an increase in apoB48 secretion (11). Here, we tested the hypothesis that the increased SR-BI expression observed in the same model may be a key underlying factor. We examined whether SR-BI overexpression would increase apoB48 secretion in the cultured intestinal model, Caco-2, and if silencing SR-BI would have the opposite effect. We thus overexpressed SR-BI (using lipofectamine and a full-length murine SR-BI construct and enhanced green fluorescent protein (GFP) vector (control) (A), or Caco-2 cells were transfected with scrambled small-inhibitory RNA (siRNA) (control) or siRNA-SR-BI (siRNA) (B). In both experiments, Caco-2 cells were cultured on collagen-coated plates for 96 h. Lipid micelles were added 20 h before the end of the experiment. Cell lysates were analyzed by immunoblot with antibodies against SR-BI, actin (as loading control), and apoB.

Intestinal SR-BI Protein Levels and Distribution in Animal Models of Insulin Resistance

To verify which of the intestinal segments had the highest abundance of SR-BI, we conducted Western blot analysis of SR-BI in the hamster intestinal segments (Fig. 4). The results showed that most of the SR-BI was detected in the duodenum and jejunum, with the least seen in the ileum (P < 0.05). We also studied whether fructose feeding and induction of insulin resistance leads to changes in the localization of SR-BI within the jejunum (Fig. 5). The results show a net increase in apical SR-BI but no change in distribution in the intestine of the fructose-fed hamster (Fig. 5B) compared with that in chow-fed hamsters (Fig. 5A). No staining was seen in the negative control tissue (Fig. 5C). The intensity of SR-BI staining was quantified in these intestinal segments, showing significantly higher intestinal SR-BI staining in fructose-fed hamsters (control/chow fed: %staining intensity = 100 ± 38%, fructose-fed: %staining intensity = 197 ± 45%, t-test P < 0.01) (Fig. 5D). SR-BI expression was observed at both the tip of the villi and...
on the apical side of the hamster intestine in both chow- and fructose-fed models. In the fructose-fed hamster, SR-BI immunostaining appeared to be more concentrated in basolateral compared with the apical compartment, but this difference was not statistically significant.

**Intestinal SR-BI Subcellular Distribution: Rapid Change in Response to Lipid Micelles and Insulin**

To visualize SR-BI and assess its subcellular distribution, we transfected Caco-2 cells with a plasmid containing EGFP-SR-BI. As shown in Fig. 6, SR-BI was mainly localized in the cytoplasm of Caco-2 cells in the basal state (no stimulation; control). After 1 h incubation with lipid micelles, SR-BI moved from the cytoplasm to the cell surface (50%) and formed clusters around the nucleus or in the perinuclear fraction (40%). These results show that only a small percentage of the cells visualized had SR-BI localized in the cytoplasm after 1 h (Fig. 6). This distribution pattern persisted for up to 3 h after lipid micelle treatment.

We also assessed SR-BI subcellular distribution in response to insulin. As shown in Fig. 7, A and B, SR-BI was mainly localized in the cytoplasm of Caco-2 cells in the basal state (no stimulation; control). After 15 min of insulin treatment, SR-BI was predominantly concentrated in clusters around the nucleus or in the perinuclear area (~60%), and this effect was maximal at 30 min and lasted for 2 h after insulin stimulation (Fig. 7A). Similar results were seen with another PI3-kinase inhibitor, LY-204002 (80 μmol) (Fig. 7, E and F).

To examine the involvement of the PI3-kinase pathway in insulin-mediated SR-BI cellular redistribution, we used wortmannin, a PI3-kinase inhibitor. Based on dose-response studies, 250 nM of wortmannin was found to be sufficient to decrease protein kinase B phosphorylation (Ser473) to basal levels (data not shown). As shown in Fig. 7B, wortmannin treatment (250 nM) partially blocked insulin-induced SR-BI cellular redistribution to the perinuclear region, with most of the SR-BI concentrated on the cell surface.

**DISCUSSION**

The present study demonstrates that the overproduction of intestinal apoB48-containing lipoproteins observed in two different animal models of diet-induced insulin resistance and hypertriglyceridemia (fructose-fed hamster and high-fat mouse model) is accompanied by changes in mRNA and protein levels of different transporters in the intestine, including CD36, ABCG5 and ABCG8, FATP4, ABCA1, and SR-BI. With the exception of SR-BI, all of the changes in mRNA levels were less than twofold. These results suggest that the chylomicron overproduction that occurs in conditions of insulin resistance is accompanied by changes in the turnover of dietary lipids and cholesterol in the intestine. We also measured the protein abundance of the same transporters in the three different intestinal segments of hamsters and mice. Small changes were seen in ABCG8, NPC1L1, CD36, and ABCG5 protein levels, but a major increase in the levels of SR-BI in the intestine of fructose-fed hamsters and high-fat-fed mice was observed. Some discrepancy was noted between the mRNA levels and protein levels of some of the transporter proteins examined. This is not surprising, since cellular levels of an expressed protein depend on several factors (other than mRNA abundance), such as translational efficiency of the coding mRNA and protein stability/degradation. We also observed that the expression of SR-BI was highest in the jejunum of all intestinal segments examined, and we postulate that jejunal SR-BI may be more sensitive to fat-induced changes, since this part of the intestine is responsible for the bulk of fat absorption. Fat absorption in the duodenum and ileum may be quantitatively insignificant compared with that by the jejunum. Interestingly, the ileal SR-BI level appeared to respond to fat feeding in the fructose-fed hamster, which may either be a direct response to fat or an indirect hormonal response from the more proximal segments of the intestine. This response was, however, not observed in the fat-fed mouse model. Overall, studies in these rodent models of insulin resistance showed a consistent upregulation of intestinal SR-BI. Similar observations were made by van der
Velde et al. (29) who showed increased SR-BI mRNA and protein levels in the intestine of mice fed a high-fat diet. These observations are, however, in stark contrast to those recently reported by Levy et al. (15), in which decreased SR-BI gene and protein expression was noted in the hyperinsulinemic and hyperglycemic Psammomys obesus. The reasons for the discrepancies between these studies could lie in the inherent differences between the species. In addition, our models utilize nutrient excess (excessive levels of fructose or fat) to induce hyperlipidemia and insulin resistance, whereas, in the model used by Levy et al. (15), animals were fed standard chow; thus, the observed differences could be

Fig. 6. Subcellular distribution of SR-BI in the presence of lipid micelles. Caco-2 cells were transfected with enhanced GFP (EGFP)-SR-BI and used 48 h after transfection. Cells were stimulated with lipid-containing micelles for different times as shown. Paraformaldehyde-fixed cells were analyzed by confocal microscopy. A: confocal image of SR-BI (green channel) and nucleus (blue channel) in Caco-2 cells cultured in the absence (control) or presence of lipid-containing micelles (Micelles). B: the graph shows the percentage of SR-BI intensity in different cellular compartments (as percentage of total intensity). Average ± SE of 3 slides/treatment. *P < 0.05 and **P < 0.01 compared with surface and perinuclear.
Fig. 7. Insulin modulation of SR-BI subcellular localization. Caco-2 cells were transfected with an EGFP-SR-BI plasmid and used 48 h after transfection. Cells were stimulated with wortmannin or insulin for different times as shown. Paraformaldehyde-fixed cells were analyzed by confocal microscopy.

A: confocal images of SR-BI (green channel) in Caco-2 cells cultured in the absence (control) or presence of insulin (Insulin) during different time points.

B: confocal image of SR-BI (green channel) in Caco-2 cells cultured in the absence (control) or presence of wortmannin (Wort) at different time points.

C and D: the graphs show the percentage of SR-BI in different cellular compartments (as percentage of total intensity) in the insulin- and wortmannin + insulin-treated cells. E and F: graphs show the percentage of SR-BI in different cellular compartments (as percentage of total intensity) in insulin- and LY-294002 + insulin-treated cells. Average ± SE of 3 slides/treatment. *P < 0.05 and **P < 0.01 compared with surface and perinuclear. #P < 0.05 compared with surface and cytoplasm. **P < 0.05 compared with perinuclear and cytoplasm.
the result of the nutrient excess we show in our model. Finally, the *P. obesus* model exhibits hyperglycemia, which is not seen in our model and may act as a confounding factor.

SR-BI facilitates cellular uptake of cholesterol from the hydrophobic cores of lipoproteins by first mediating the binding of the lipoprotein to the outer surfaces of the cells (8). Most of our knowledge of SR-BI comes from studies performed in liver cells and macrophages (2, 8, 24, 28). However, recent evidence from human intestinal cells (Caco-2) has shown the importance of SR-BI protein in micelle uptake and transport inside the cells. To test whether SR-BI is a causative factor in intestinal overproduction of apoB-containing lipoproteins (11), we employed the intestinal cell culture model, Caco-2 cells. Caco-2 cells overexpressing SR-BI showed increased secretion of apoB100 and apoB48 (Fig. 4A), whereas inhibition of SR-BI expression (using siRNA) led to decreased secretion. These data appear to suggest that SR-BI expression may be a key determinant of intestinal chylomicron production in insulin-resistant states. It is unclear, however, whether this may relate to a putative role of SR-BI in intestinal cholesterol absorption.

Conflicting results have been reported on the role of intestinal SR-BI in cholesterol absorption. Intestinal cholesterol absorption was initially reported as being unchanged in SR-BI knockout mice (8, 11, 15, 29). However, recent work from Nguyen et al. (19) have shown decreased cholesterol influx in SR-BI knockout mice, although this was found not to be rate limiting. In vitro silencing of SR-BI in Caco-2 cells was found to increase cholesterol uptake (6). In contrast, Bietrix et al. (4) have shown increased cholesterol and triglyceride absorption in transgenic mice overexpressing SR-BI.

These studies appear to suggest a potential role for SR-BI in modulating intestinal lipid transport and support our current observations that altered expression of SR-BI may be a causative factor in the overproduction of intestinal chylomicrons observed in insulin-resistant states. A rabbit study published in 2005 (27) also showed that SR-BI overexpression is associated with increased levels of apoB-containing lipoproteins in the plasma and decreased levels of apoAI. Interestingly, polymorphisms in the SR-BI gene have been linked to insulin resistance in humans (18). These authors also showed that the SR-BI gene lies in a region on chromosome 12q24 that has been linked to type 2 diabetes and that genetic variations in the SR-BI gene are associated with increased risk of coronary artery disease and obesity. Although it is possible that the increases in SR-BI expression and apoB secretion are unrelated effects, this seems unlikely, since alterations in cholesterol level have been linked to changes in apoB production (13, 17, 23).

Intestinal SR-BI localization and its change in response to the development of insulin resistance were also assessed. SR-BI was found to be predominantly concentrated in the proximal intestine (duodenum and jejunum) (Fig. 5), in accordance with results reported in a previous study from Bietrix et al. (4, 7). SR-BI distribution was also observed in both the apical and basolateral surfaces of the hamster intestine (Fig. 6). Examination of jejunal fragments from hamsters fed with chow or fructose diets showed no appreciable differences in SR-BI localization (Fig. 6). Thus, intestinal SR-BI distribution appears not to be grossly altered in the insulin-resistant state.

Subcellular localization of SR-BI and alteration in response to various metabolic stimuli were examined in the Caco-2 cell model using confocal microscopy. We found marked changes in cellular distribution of SR-BI in response to lipid micelles, leading to SR-BI redistribution from the cytoplasm to the cell surface and clusters around the nucleus. Beaslas et al. (3) had previously shown that SR-BI was involved in the apoB translocation process from the apical site to the intracellular pool in response to lipid micelles. These authors also showed that SR-BI forms clusters in the apical membrane of the Caco-2 cells in response to lipid micelles. The clustering of SR-BI in the perinuclear region indicates that there is translocation of SR-BI to the endoplasmic reticulum (ER). This is the site of apoB production (13), and, as such, it seems possible that exposure of lipid promotes movement of SR-BI to the ER to promote chylomicron production. This is supported by our finding that apoB production is increased with increased SR-BI expression.

Intracellular distribution of SR-BI was also highly sensitive to insulin. Insulin treatment induced SR-BI redistribution from the cytoplasm to a predominantly perinuclear localization (~60%). This effect was seen as fast as 30 min posttreatment. Blocking insulin signaling using a PI3-kinase pathway inhibitor (wortmannin) partially blocked the SR-BI cluster formation around the nucleus induced by insulin. In previous studies of SR-BI localization, Shetty et al. (24) have shown that SR-BI concentrates at the cell surface of HepG2 cells after treatment with insulin and that wortmannin blocks this effect. Results from Tondu et al. (28) using adipocytes have also shown that insulin induces SR-BI translocation from intracellular pools to membrane. However, in pig enterocytes, Hansen et al. (12) have demonstrated that, during absorption of dietary fat, SR-BI is endocytosed from the enterocyte brush border and accumulates in cytoplasmic lipid droplets. These results are consistent with our observations. Altogether, the data suggest that perturbations in the PI3-kinase pathway, such as in states of insulin resistance, may disrupt proper intracellular trafficking of SR-BI in the intestine and may impact on dietary lipid uptake. The physiological significance of insulin-stimulated changes in SR-BI localization are currently unclear. We postulate that subcellular localization and traffic between plasma and intracellular membranes are likely critical in SR-BI function and response to exogenous stimuli. Movement of SR-BI from the cell surface to perinuclear membranes (i.e., ER membrane) may signal activation and SR-BI-mediated lipid transfer intracellularly. On the other hand, cell-surface localization may signal inhibited function and reduced lipid transfer. This hypothesis is consistent with the established inhibitory effect of insulin postprandially on intestinal fat absorption and chylomicron production.

In conclusion, the evidence presented in this study suggest that intestinal overproduction of apoB48-containing particles that occurs in response to the insulin-resistant state may result in part from upregulation of SR-BI in the intestine. We postulate that the inhibitory effect of insulin on intestinal chylomicron production may at least partially relate to its effect on the translocation of SR-BI and the cluster formation around the nucleus. Further work is underway to examine the mechanisms by which SR-BI modulates intestinal lipid metabolism and the assembly and secretion of apoB-containing lipoproteins in the enterocyte.
GRANTS
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