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# Asymmetrical Regulation of Scavenger Receptor Class B Type I by Apical and Basolateral Stimuli Using Caco-2 Cells

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**Abstract** Cholesterol uptake and the mechanisms that regulate cholesterol translocation from the intestinal lumen into enterocytes remain for the most part unclear. Since scavenger receptor class B type I (SR-BI) has been suggested to play a role in cholesterol absorption, we investigated cellular SR-BI modulation by various potential effectors administered in both apical and basolateral sides of Caco-2 cells. With differentiation, Caco-2 cells increased SR-BI protein expression. Western blot analysis showed the ability of cholesterol and oxysterols in both cell compartments to reduce SR-BI protein expression. Among the n-3, n-6, and n-9 fatty acid families, only eicosapentaenoic acid was able to lower SR-BI protein expression on both sides, whereas apical α-linolenic acid decreased SR-BI abundance and basolateral arachidonic acid (AA) raised it. Epidermal growth factor and growth hormone, either in the apical or basolateral medium, diminished SR-BI cellular content, while insulin displayed the same effect only on the basolateral side. In the presence of proinflammatory agents (LPS, TNF-α, IFN-γ), Caco-2 cells exhibited differential behavior. SR-BI was downregulated by lipopolysaccharide on both sides. Finally, WY-14643 fibrate diminished SR-BI protein expression when it was added to the apical medium. Biotinylation studies in response to selected stimuli revealed that regulatory modifications in SR-BI protein expression occurred for the most part at the apical cell surface irrespective of the effector location. Our data indicate that various effectors supplied to the apical and basolateral compartments may impact on SR-BI at the apical membrane, thus suggesting potential regulation of intestinal cholesterol absorption and distribution in various intracellular pools. J. Cell. Biochem. 100: 421–433, 2007. 

**Key words:** SR-BI; intestine; cytokines; fatty acids; hormones; fibrates

Cholesterol is an essential constituent of cellular membranes and also serves as a precursor of bile acids and steroid hormones [Soccio and Breslow, 2004]. In fact, cholesterol is needed for normal physiologic processes, but excess cholesterol production or intestinal absorption can influence the progression of atherosclerosis by increasing plasma cholesterol levels or by modifying the composition of lipoproteins [Mancini and Parillo, 1991; Turley and Dietschy, 2003]. Until a few years ago, it was thought that passive diffusion governs intestinal cholesterol uptake. However, several findings have very recently supported the involvement of a protein-mediated specific process [Dawson and Rudel, 1999]. Therefore, new impetus has been given to defining the influence of genetics, environment, hormones, and drugs on dietary cholesterol absorption with the confidence that growing knowledge on the regulation will shed light on the inherent

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mechanisms and the delineation of effective pharmacological treatments for hypercholesterolemia and the prevention of atherosclerosis.

In the small intestine, scavenger receptor class B type I (SR-BI) has been found in the brush border membrane of enterocytes [Hauser et al., 1998; Cai et al., 2001; Lobo et al., 2001], raising the possibility that it plays a role in dietary/biliary cholesterol absorption [Schulthess et al., 2000; Krieger, 2001; Werder et al., 2001]. We recently utilized immunofluorescence staining and demonstrated that the distribution of SR-BI appeared as a gradient, increasing from the developing crypt to the tip of the villus [Levy et al., 2004]. In an effort to better understand the cellular localization of SR-BI, protein A-gold immunocytochemical techniques were applied to thin sections incubated with specific antibodies to disclose SR-BI in the human intestine. These electron microscopic immunocytochemical studies revealed significant immunogold labeling in the luminal region of enterocytes, particularly associated with the apical plasma membrane lining the microvilli. The labeling of SR-BI by gold particles was also present in endosomal invaginations and vesicles. Within the cell, this labeling, although of lower intensity, was present in the rough endoplasmic reticulum (ER), the Golgi apparatus, and the basolateral membrane. In order to define the role of SR-BI in intestinal cholesterol absorption, Caco-2 cells were transfected with a constitutive expression vector (pZeoSV) containing human SR-BI cDNA inserted in the antisense orientation. As noted by immunoblotting and Protein A-gold techniques, stable transformants contained 40%, 60%, and 80% the SR-BI level of control Caco-2 cells and exhibited a proportional drop in free cholesterol uptake without altering the captation of phospholipids (PL) or cholesteryl esters (CE). Confirmation of these data was obtained in intestinal organ culture where SR-BI antibodies significantly lowered cholesterol uptake. These observations suggest that the human intestine possesses a developmental and regional SR-BI pattern of distribution and they extend our knowledge of SR-BI-mediated cholesterol transport.

As mentioned above, SR-BI is expressed more in the apical than the basolateral membrane. It is possible that apical SR-BI may allow the uptake of luminally-derived cholesterol, whereas basolateral SR-BI may interact with lipoproteins originating from the circulation. In support of the latter assumption, early observations documented that intestinal epithelial cells bind apolipoprotein A-I and high-density lipoproteins (HDL) via a receptor-mediated process at the basolateral membrane [Suzuki et al., 1983; Kagami et al., 1984; Sviridov et al., 1986]. In this case, the vectorial distribution of SR-BI would contribute to the cellular polarity of enterocyte lipid metabolism. For example, previous studies reported that plasma free fatty acids (FAs) were primarily oxidized or incorporated into PL in rats and human small gut mucosa [Gangl and Ockner, 1975; Gangl and Renner, 1978], whereas free FA absorbed from the intestinal tract were mainly incorporated into triacylglycerols (TG) [Gangl and Ockner, 1975]. This may be related to several factors, including differences in the plasma membrane composition between the apical and basolateral domains of the enterocytes [Brasitus and Schachter, 1980; Trotter and Storch, 1991]. To our knowledge, neither the modulation of SR-BI in intestinal cells, nor the asymmetrical regulation of protein expression has been addressed.

The purpose of the present investigation was to test the hypothesis that the protein expression of SR-BI in intestinal epithelial cells is highly modulated by various stimuli. This study attempted in particular to detail cellular SR-BI regulation in response to the administration of various effectors in the apical and basolateral sites, including nutrients (sterols and FAs), hormones known as modulators of brush border digestive functions [epidermal growth factor (EGF), insulin, growth hormone (GH)], the microbial glycolipid lipopolysaccharide (LPS), an essential constituent of the outer cell membrane of all Gram-negative bacteria localized in the intestinal lumen, which plays a central role in the transcriptional activation of host defence mechanisms, and inflammatory cytokines and drugs capable of influencing cholesterol metabolism. To this end, we used the Caco-2 cells that differentiate as enterocytes in culture, can be grown as a polarized monolayer when seeded on porous filters on Transwell and allow access to both sides of the bipolar intestinal epithelium. Additionally, experiments were performed to determine whether a number of effectors favor asymmetry with respect to the apico-basal distribution of SR-BI using the biotinylation technique and simultaneously impact on cholesterol uptake.

## MATERIALS AND METHODS

### Cell Culture

Caco-2 cells were grown at 37°C with 5% $CO_2$ in minimum essential medium (MEM; GIBCO-BRL, Grand Island, NY), containing penicillin/streptomycin (100 kU/L) and MEM non-essential amino acids (0.1 mmol/L), and supplemented with 10% decomplemented fetal bovine serum (FBS; Flow, McLean, VA). Caco-2 cells (passages 30–40) were maintained in 17.5 $cm^2$ flasks (Corning, NY). Cultures were split (1:3 to 1:6) when they reached 70–90% confluence, using trypsin-EDTA (50 g/L-0.5 mmol/L; GIBCO-BRL). For individual experiments, cells were plated at a density of $1 \times 10^6$ cells/well on 24.5 mm polycarbonate Transwell filter inserts with 0.4 μm pores (Costar, Cambridge, MA), in MEM supplemented with 5% FBS. The inserts were placed into six-well culture plates, permitting separate access to the upper and lower compartments of the monolayers. Cells were cultured for 21 days, at which time the Caco-2 cells are highly differentiated and suitable for lipid transport, synthesis, and metabolism [Levy et al., 1995; Courtois et al., 2000]. The medium was refreshed every second day. Transepithelial resistance, an index of cell confluence and tight junction formation, was evaluated using a Millicel-ERS apparatus (Millipore, Bedford, MA).

### Cell-Surface Biotinylation of Caco-2 Cells

All stages of this procedure were performed at 4°C. Following incubation with various stimuli in the apical and basolateral compartments, Caco-2 cells on filters were washed with ice-cold phosphate-buffered saline (PBS; containing 1 mmol/L $CaCl_2$ and 1 mmol/L $MgCl_2$) and biotinylated for 1 h through exposure to 1 mg/ml biotinamidocaproate *N*-hydroxysuccinimide ester (Sulfo-NHS-biotin, Pierce Biotechnology, Rockford, IL) in dimethylformamide (40 mmol/L) present in the medium bathing the apical or basolateral surfaces. At the end of the incubation period, cells were washed twice in ice-cold PB and twice more with iodoacetamide buffer (50 mmol/L iodoacetamide in PBS) to remove excess biotin from the cell surface and quench its activity, respectively. Cells were subsequently lysed with 0.5 ml of precipitation assay buffer (pH 8.0) consisting of 50 mmol/L Tris, 150 mmol/L NaCl, 1% (v/v) Nonidet P-40, 0.5% (v/v) deoxycholic acid, 0.1% (v/v) SDS, and EDTA-free complete protease inhibitor mixture, which included 0.2 mmol/L PMSF, 0.002 mmol/L pepstatin A, and 0.01 mmol/L leupeptin. The collected lysate (1 mg/ml) was centrifuged at 10,000*g* for 30 min to pellet debris. Biotinylated proteins were recovered by rotating the clear lysate for 2 h with 200 μl of streptavidin-agarose beads (ImmunoPure ® Immobilized Streptavidin; Pierce) suspended in 0.01 mol/L $NaPO_4$, 0.15 mol/L NaCl (pH 7.2). The beads were recovered by centrifugation for 1 min at 10,000*g* and washed twice in PBS using rotation and centrifugation. Bound proteins were eluted from the beads in Laemmli sample buffer by repeated rounds of agitation and boiling, separated by SDS–polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes as described later in this section. Transfers were probed with antibody SR-BI. Specific staining was visualized by the Enhanced Chemiluminescence technique (Amersham Corp.). For quantitation of the biotinylation experiments, bands were scanned with a laser densitometer.

### Western Blot Analysis

Cells were harvested after incubation with cholesterol (50 μmol/L), 7-ketocholesterol (50 μmol/L), methyl β-cyclodextrin (5 mmol/L), oleic acid (0.5 mmol/L), linoleic acid (0.5 mmol/L), AA (0.5 mmol/L), α-linolenic acid (0.5 mmol/L), eicosapentanoic acid (0.5 mmol/L), docosahexaenoic acid (0.5 mmol/L), insulin (30 mU/ml), EGF (50 ng/ml), GH (5 ng/ml), LPS (5 ng/ml), Interferon-γ (IFN-γ) (5 IU/ml), TNF-α (10 ng/ml), and Fibrate WY-14643 (100 μmol/L) applied either to the apical or the basolateral side. Cells were lysed and rinsed in PBS. Lysis buffer consists of TBS, 5 mmol/L EDTA, 0.2% SDS, 1% Triton, 0.5% sodium desoxycholate, and a cocktail of protease inhibitors (PMSF, BHT, and pepstatin). The buffer was added to the cells and placed on ice for 10 min. All lysates were sonificated for 10 sec and then centrifuged for 10 min at 4°C. The supernatant was collected and stored at −80°C. The protein concentration was measured spectrophotometrically using protein standard (Biorad). To assess the presence of SR-BI and evaluate its mass, Caco-2 cells were homogenized and adequately prepared for Western blotting as described previously [Levy et al., 2001, 2002]. Proteins were denatured in sample buffer containing SDS and β-mercaptoethanol, separated on a 4–20%

gradient SDS–PAGE and electroblotted onto nitrocellulose membranes. Non-specific binding sites of the membranes were blocked using defeated milk proteins followed by the addition of primary antibodies directed against SR-BI. The relative amount of primary antibody was detected with species-specific horseradish peroxidase-conjugated secondary antibody. One of the most commonly used reference proteins is β-actin for endogenous controls. In the present study, β-actin has been scrutinized under the influence of the various stimuli employed for the regulation of SR-BI and was found constant irrespective of the stimuli tested, which allowed us to assume that no significant, quantitative changes occurred at the different experimental conditions. Blots were developed and the mass of SR-BI was quantitated using an HP ScanJet scanner equipped with a transparency adapter and software. Control SR-BI values were set at about 4000 arbitrary units after the background was substrated from control and test measurements.

### [$^{14}$C]-Cholesterol Uptake

To study cholesterol uptake, [$^{14}$C]-cholesterol (10 μCi), was added as a mixed bile salt micelle (6.6 mmol/L sodium taurocholate, 1 mmol/L oleic acid, 0.5 mmol/L monoolein, 0.1 mmol/L cholesterol, and 0.6 mmol/L phosphatidylcholine) [Levy et al., 2004]. Caco-2 cells were incubated at 37°C for 4 h.

### Statistical Analysis

Results are reported as mean ± SEM of at least triplicate samples representative of not less than three separate experiments. Statistical analyses were performed using a Student's *t*-test.

## RESULTS

The first issue addressed by our studies was to explore whether a difference could be noted in the expression of SR-BI amount depending on the maturity of cell differentiation. Indeed, a higher level of SR-BI was found in confluent, more differentiated cells than that in the dividing 50% confluent cells (Fig. 1). Thus, increased intestinal epithelial cell differentiation could upregulate the protein expression of SR-BI. Therefore, we decided to carry out the next experiments, aimed at evaluating SR-BI regulation, using fully differentiated Caco-2 cells. Interestingly, the membrane distribution of SR-BI in differentiated Caco-2 cells showed that 58% was located in the apical site versus 42% in the basolateral membrane.

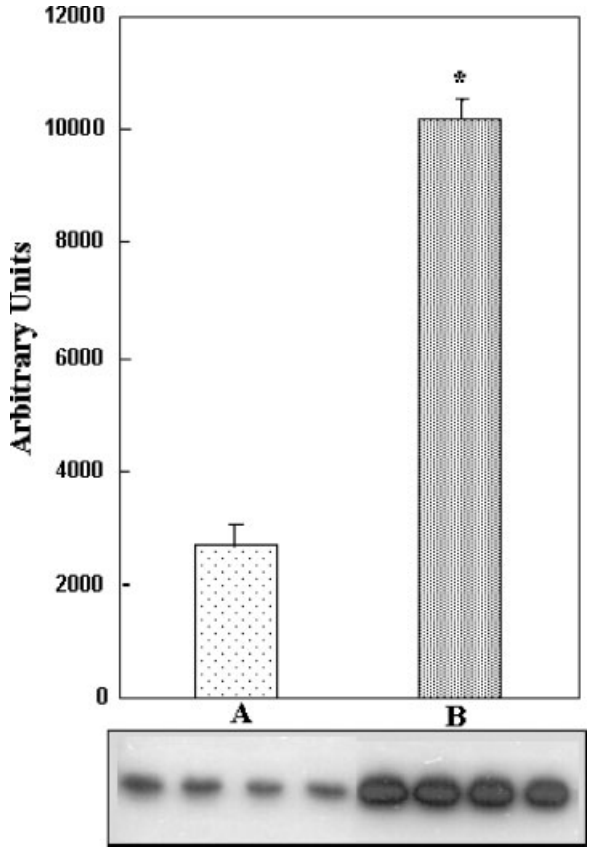


**Fig. 1.** Modulation of SR-BI protein expression in Caco-2 cells under proliferation and differentiation conditions. The effect of the maturation process on SR-BI protein was assessed in Caco-2 cells that were grown until 70–90% confluence and transferred to polycarbonate Transwell filter inserts. SR-BI expression was determined in (**A**) undifferentiated (5-day pre-confluence) and (**B**) differentiated (21-day post-confluence) Caco-2 cells by fractionation on SDS–PAGE, Western blot analysis and chemiluminescence's detection. Results in the histogram represent the mean ± SEM of four separate experiments. *$P < 0.0001$ undifferentiated versus differentiated.

We also established the experimental conditions to examine SR-BI modulation in differentiated Caco-2 cells. Preliminary data with different periods of incubation and concentrations of effectors revealed that the time length of 4-h culture and the quantities of stimuli indicated in the present work were suitable for the study of SR-BI regulation (results not shown). SR-BI values for most stimuli were situated at the initial linear portions of the activity curves when various concentrations were tested.

To determine the effects of sterols on SR-BI protein expression, Caco-2 cells were separately cultured with cholesterol (50 μmol/L) and 7-ketocholesterol (50 μmol/L) for 4 h at 37°C. Sterol-containing micelles were added to the apical compartment, whereas identical concentrations of sterols in albumin solution were administered to the basolateral chamber. At the end of the incubation period, cell lysates were generated, resolved by SDS–PAGE and immunoblotted with SR-BI-specific antibodies. Regardless of the site of supplementation, both cholesterol and 7-ketocholesterol downregulated SR-BI protein expression compared with control cells (Fig. 2). A similar trend in SR-BI expression was observed following exposure of Caco-2 cells to methyl β-cyclodextrin, a cholesterol-sequestering agent from membranes (Fig. 3).

We next tested the influence of FAs on SR-BI protein expression. In particular, it was of interest to ascertain whether different polyunsaturated FA families display distinct effects on SR-BI. The supplementation of intestinal cells with AA (20:4n-6) in the basolateral compartment raised SR-BI expression (Fig. 4), while oleic acid (18:1n-9) and linoleic acid (18:2n-6) did not alter the amount of SR-BI protein in comparison with untreated Caco-2 cells (data not shown). Similarly, a variation in SR-BI protein expression was also observed in response to n-3 FAs, depending on the route of supplementation. α-linolenic acid (ALA. 18:3n-3) decreased SR-BI abundance only when it was added to the apical compartment, whereas eicosapentaenoic acid (EPA, 20:5n-3) was able to reduce SR-BI protein expression on both sides (Fig. 4). Moreover, docosahexaenoic acid (DHA, 22:5n-3) could not change SR-BI protein quantity. These data point out the divergence in SR-BI protein expression by n-3 FAs.

SR-BI protein expression was then evaluated as a function of fibrates, agonists of the orphan nuclear receptors, that is, peroxisome proliferator-activated receptors (PPAR) that control the expression of genes involved in lipid metabolism. WY-14643, a known potent ligand of $PPAR_{\alpha}$, at the concentration of 100 μmol/L decreased SR-BI protein expression only when it was added to the apical compartment of Caco-2 cells (Fig. 5). Therefore, these data suggest that $PPAR_{\alpha}$ is an important regulator of SR-BI protein content in the intestine.

Since LPS produced by gram-negative bacteria is a profuse product of the normal flora that can induce innate immune responses and may be involved in normal physiological processes, we assessed its action on SR-BI expression.

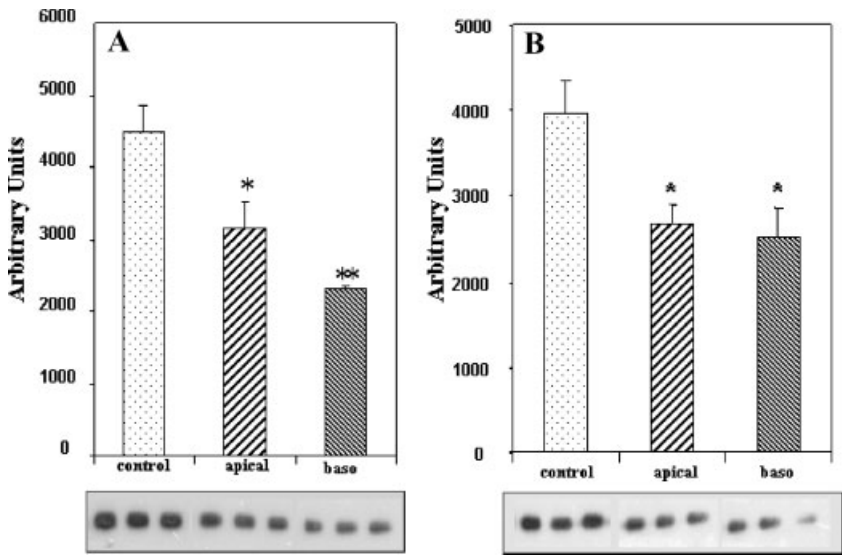


**Fig. 2.** Effects of cholesterol and oxysterols on SR-BI protein expression. Differentiated Caco-2 cells were cultured for 4 h at 37°C in the presence or absence of (**A**) cholesterol (50 μmol/L) and (**B**) 7-ketocholesterol (50 μmol/L) added to the apical and basolateral sites. At the end of the incubation, cell lysates were generated and 50 μg of protein was resolved by SDS–PAGE, transferred to a nitrocellulose membrane, immunoblotted with anti-SR-BI antibody and developed by chemiluminescence procedure. The mass of SR-BI was quantitated using an HP ScanJet scanner equipped with a transparency adapter and software. A representative Western blot is shown. Data are mean ± SEM for three separate experiments. $^{*}P < 0.008$, $^{**}P < 0.001$ versus control.

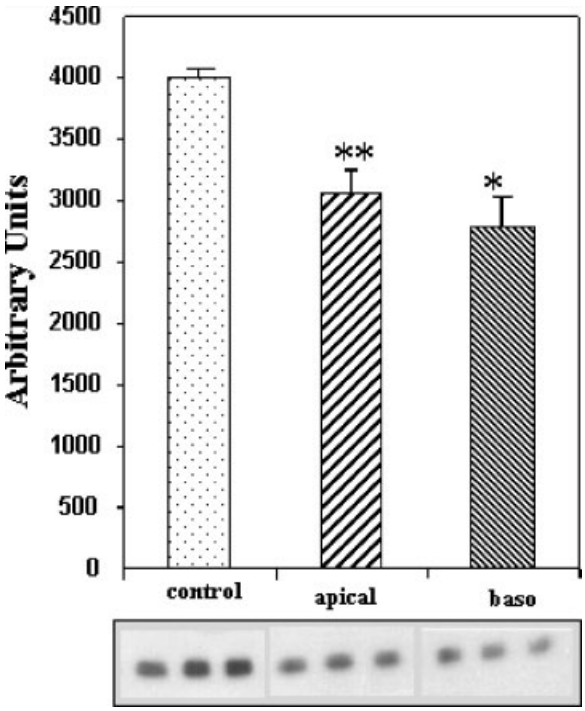


**Fig. 3.** Regulation of SR-BI protein expression by methyl β-cyclodextrin. Differentiated Caco-2 cells were treated for 4 h at 37°C with 5 mmol/L methyl β-cyclodextrin added to the apical and basolateral sites. SR-BI expression was examined as described in Materials and Methods and in Figure 1 legend. Data represented are mean ± SEM for three independents experiments. $*P < 0.002$, $**P < 0.001$ versus control.

Figure 6 displays representative Western blots of SR-BI lysates from Caco-2 cells incubated with LPS. The addition of LPS to the apical and basolateral culture medium for 4 h at 37°C diminished SR-BI levels.

Similarly, given the potency of the proinflammatory cytokines TNF-α and IFN-γ in inducing inflammatory and physiological responses in intestinal epithelial cells, we investigated their effects on SR-BI protein expression. Caco-2 cells cultured with 10 ng/ml of TNF-α exhibited opposite SR-BI outcomes depending on the location of TNF-α supply (Fig. 6). A marked decrease in SR-BI cell content was noted only when IFN-γ (5 IU/ml) was supplied to the apical side.

To assess the role of hormones in SR-BI regulation, Caco-2 cells were incubated with EGF, GH and insulin. The presence of EGF and GH in the apical and basolateral media resulted in a significant reduction in SR-BI protein quantity (Fig. 7). In contrast, insulin displayed a negative effect on SR-BI content in human Caco-2 cells only when it was administered to the basolateral side.

As the above findings show, many stimuli were able to alter the total amount of the SR-BI protein in Caco-2 cells. In order to determine whether alterations in SR-BI protein levels were membrane domain specific, we repeated the experiments with the effectors making a substantial impact on SR-BI expression. The apical and basolateral cell surface proteins were then biotinylated separately and processed for immunoprecipitation, gel electrophoresis and Western blotting. The incubation of Caco-2 cells with apical EPA, EGF, and IFN-γ markedly reduced SR-BI protein mass at the apical membrane (Fig. 8). Similarly, the treatment of the intestinal cells with basolateral EPA, EGF, and IFN-γ lowered SR-BI levels at the apical membrane. Limited changes were noted at the basolateral membrane, suggesting that the regulatory modifications in SR-BI protein expression mainly occur at the apical cell surface.

To further study the functional outcome of SR-BI modulation, [$^{14}$C]-free cholesterol uptake was appraised following the treatment of Caco-2 cells with apical EPA, EGF, and IFN-γ. The apical compartment was chosen, since most stimuli regulated SR-BI at this site. A decreasing trend was observed in cellular cholesterol content in the presence of EPA, EGF, and IFN-γ (Fig. 9).

## DISCUSSION

The major transport functions expressed by gut epithelial cells are the absorption of nutrients by apical membrane transporters and the intimate exchange with the blood circulation via the basolateral membrane. Human intestinal epithelium is a polarized tissue in which enterocytes have access to both an apical and a basolateral nutrient supply from the intestinal lumen and the serosal circulation, respectively. Since SR-BI protein is mainly associated with the apical membrane of the enterocytes and is less pronounced in the basolateral membrane, we hypothesized that its regulation may be different depending on the stimulus location. We, therefore, used an experimental model that makes it possible to mimic the prevailing in vivo conditions and to determine whether the effects of different stimuli on SR-BI protein expression are dependent on their route (apical versus basolateral) of delivery. With Caco-2 cells

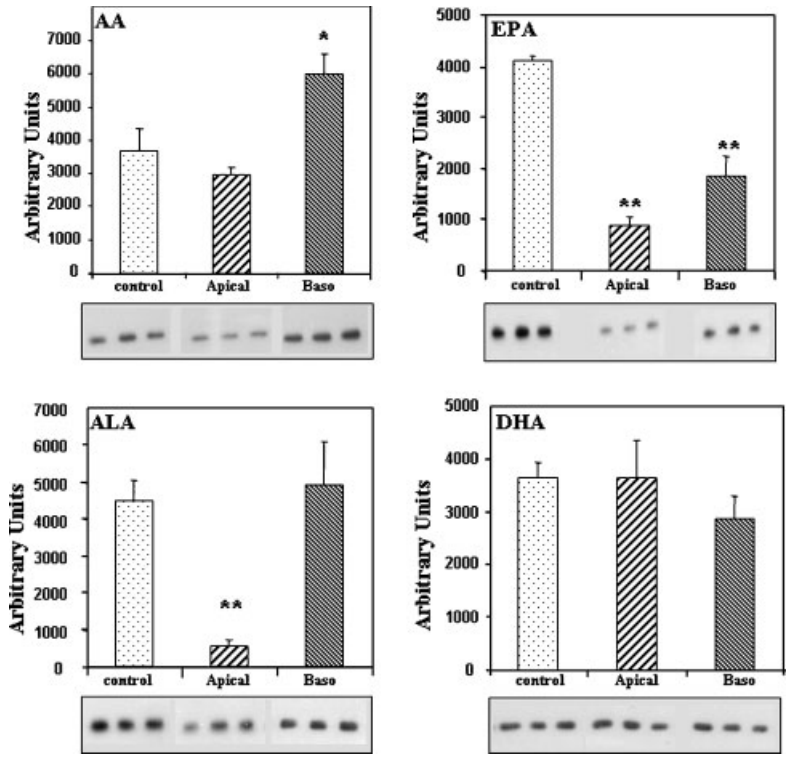


**Fig. 4.** Fatty acid regulation of SR-BI in Caco-2 cells. Differentiated Caco-2 cells were incubated for 4 h at 37°C with 0.5 mmol/L AA (20:4n-6), 0.5 mmol/L α-linolenic acid (ALA, 18:3n-3), 0.5 mmol/L eicosapentaenoic acid (EPA, 20:5n-3) or 0.5 mmol/L docosahexaenoic acid (DHA, 22:5n-3) added to the apical and basolateral sites. After separation in polyacrylamide gel, proteins were transferred electrophoretically onto nitrocellulose membrane for probing with anti-SR-BI antibody as described in Materials and Methods. Data represented are mean ± SEM for three separate experiments. $^{*}P < 0.005$, $^{**}P < 0.001$ versus control.

granting access to both sides of the bipolar intestinal epithelium, not only could we demonstrate the modulation of SR-BI protein content by lipids, hormones, chemical agents, and cytokines, but we were also able to underline the importance of the site of the stimulus in the regulation of SR-BI.

In view of the small intestine's high capacity to absorb lipids and elaborate most of the major lipoprotein classes and considering the well-known actions of a number of nutrients and hormones on lipid metabolism and transport at the intestinal level, the lack of knowledge about the modulation of SR-BI in the gut is perplexing. For the first time, the present work attempted to detail the modulation of SR-BI in intestinal cells. According to our data, there is a discrete regulation of SR-BI from stimuli, originating from apical and basolateral media, such as n-3 and n-6 FAs, fibrate, cholesterol, 7-ketocholesterol, methyl β-cyclodextrin, LPS, TNF-α, IFN-γ, insulin, GH, and EGF. These hormonal, pharmacological and nutritional effectors have been selected in our experiments, since they have been demonstrated: (i) to be present in the intestinal lumen and blood circulation with the ability to play a central role in enterocyte cholesterol homeostasis; (ii) to influence lipid transport and metabolism; and (iii) to regulate SR-BI and its preferential HDL ligand in various cells and organs. The rationalization for the utilization of each modulator is notified underneath. They also provide further insight into the parallel modulation of SR-BI protein expression in the apical membrane of Caco-2 cells and cholesterol absorption.

There has been speculation that SR-BI expression could be sensitive to cellular cholesterol content, with some of the most persuasive evidence coming from cholesterol feeding studies [Fluiter et al., 1999]. A high cholesterol diet was found to simultaneously suppress SR-BI expression in rat liver parenchymal cells and to induce it in Kupffer cells [Fluiter et al., 1998]. The observations that in some cases dietary treatments can induce SR-BI expression in some

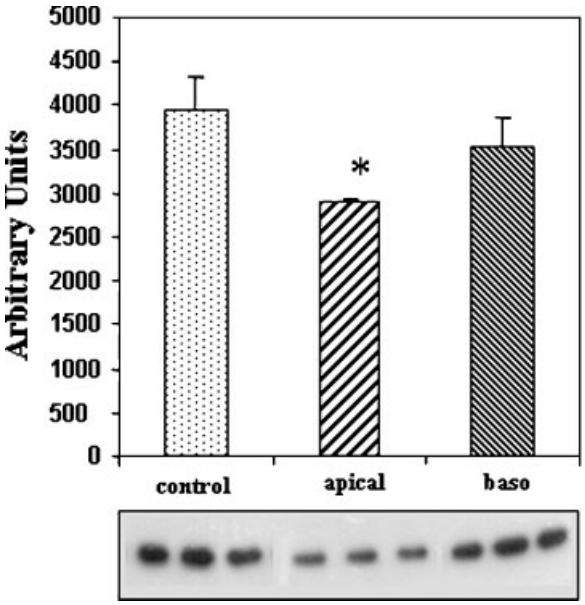


**Fig. 5.** Effect of WY-14643, a specific agonist of PPAR$_\alpha$, on SR-BI protein content. Differentiated Caco-2 cells were exposed to 100 μmol/L WY-14643 for 18 h at 37°C. The fibrate was separately added to the apical and basolateral compartments. Following the incubation period, cells were harvested and SR-BI expression was evaluated as described in Materials and Methods and in Figure 1 legend. Data represented are mean ± SEM for three separate experiments. *$P < 0.008$ versus control.

tissues or cell types and suppress it in others clearly indicate that there are cell type-dependent differences in the systems that regulate SR-BI expression [Fluiter et al., 1998], which prompted us to explore the effects of sterols in intestinal epithelial cells. The results of the present study showed that SR-BI protein expression was responsive to cholesterol and oxysterols in Caco-2 cells. The downregulation of SR-BI by these molecules suggests that dietary sterol intake or their subsequent metabolism affects intestinal SR-BI expression. Further analysis of the molecular and cellular bases of SR-BI regulation and function should provide new insight into the physiology and pathophysiology of intestinal cholesterol metabolism.

Diets rich in dietary polyunsaturated FAs may lower plasma HDL-C concentrations [Shepherd et al., 1978; Chong et al., 1987], whereas oleic acid-enriched diets were shown either to have no significant consequence [Mensink and Katan, 1989] or to elevate the levels of HDL-C [De La Cruz et al., 2000; Judd et al., 2002]. The mechanisms responsible for this effect and the implications they have for reverse cholesterol transport and atherogenesis are not fully understood. Although the role of dietary fat in

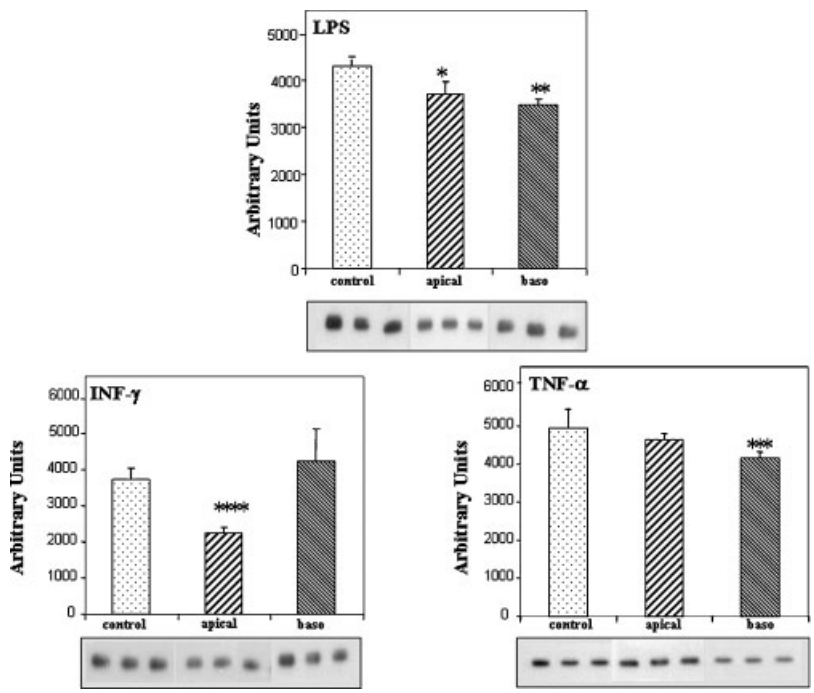


**Fig. 6.** Effects of LPS and cytokines on SR-BI protein expression. Differentiated Caco-2 cells were treated for 4 h at 37°C with 5 ng/ml LPS, 10 ng/ml TNF-α and 5 IU/ml IFN-γ added to the apical and basolateral sites. After incubation, cell lysates were electrophoresed on SDS–PAGE and analyzed by Western blot as described in Materials and Methods and Figure 1 legend. Data represented are mean ± SEM for three separate experiments. *$P < 0.03$, **$P < 0.02$, ***$P < 0.01$, ****$P < 0.002$ versus control.

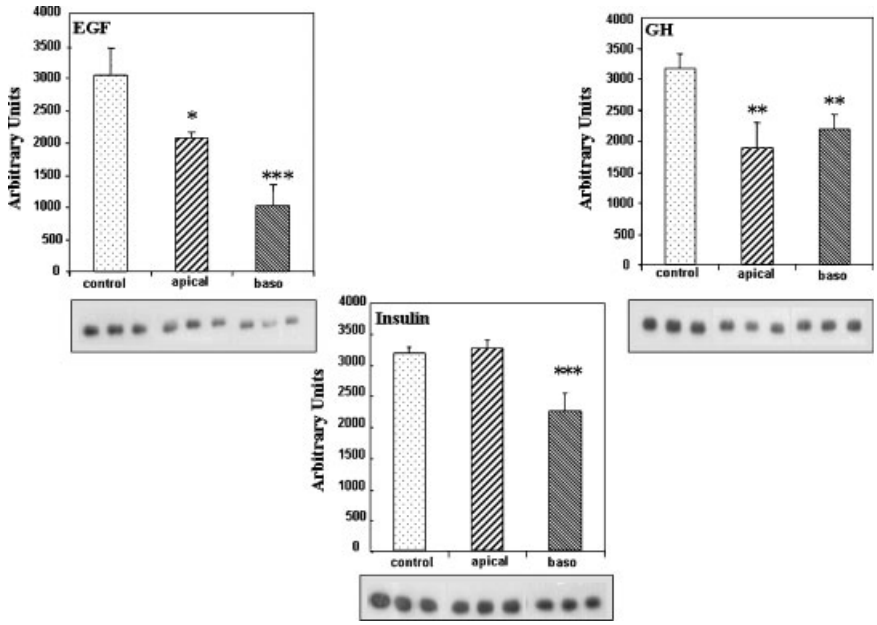


**Fig. 7.** Modulation of SR-BI protein expression by hormonal stimuli. Differentiated Caco-2 cells were treated for 18 h at 37°C with 5 ng/ml GH, 30 mU/ml insulin and 50 ng/ml EGF added to the apical and basolateral sites. Cells were harvested after incubation and their SR-BI content was measured by Western blot as described in Materials and Methods and Figure 1 legend. Data represented are mean ± SEM for three separate experiments. $^{*}P < 0.01$, $^{**}P < 0.003$, $^{***}P < 0.002$ versus control.

modulating the expression of SR-B1 is not completely clear, Spady et al. [1999] suggested that polyunsaturated FAs might increase the hepatic expression of SR-BI in hamsters, thereby augmenting HDL-CE delivery to the liver and lowering plasma HDL-C. However, seemingly inconsistent results were reported by other investigators [Hatahet et al., 2003] and urge us to define SR-BI protein expression in response to different FAs in intestinal epithelial cells. Under our experimental conditions, oleic acid, linoleic acid, and DHA caused little change in SR-BI protein expression in Caco-2 cells. Conversely, basolateral AA increased and apical ALA decreased SR-BI protein expression, while EPA lowered it when administered either in the apical or basolateral compartments. It is not the first time that independent effects of distinct FA families or individual n-3 FA have been recorded. In fact, dietary fish oil was found to markedly reduce plasma TG levels in normal and hypertriglyceridemic individuals, whereas vegetable oils containing predominantly n-6 polyunsaturated FAs had no impact [Harris et al., 1983; Phillipson et al., 1985; Connor, 1988; Harris, 1989]. Furthermore, data from another study in rats also suggested that dietary EPA and DHA may have differential effects on plasma cholesterol and TG levels [Kobatake et al., 1984]. An examination of the mechanisms underlying the SR-BI protein expression differences in response to dietary FAs may provide insights into how these FAs influence intestinal cholesterol transport as well as plasma cholesterol metabolism.

A role for $PPAR_{\alpha}$ in modulating intestinal cholesterol metabolism was suggested by the observation that Gemfibrozil, a pharmacological ligand of $PPAR_{\alpha}$, inhibited cholesterol absorption from the gut into mesenteric lymph in rats [Umeda et al., 2001]. More recently, firm evidence was obtained in WY-14643-fed animals in which the activation of intestinal $PPAR_{\alpha}$ decreased dietary cholesterol absorption through a mechanism that involved an increase in intestinal ATP binding cassette transporter A1 (ABCA1) levels [Knight et al., 2003]. Accordingly, our data revealed the downregulation of SR-BI protein expression by WY-14643 that was added to the apical side of Caco-2 cells. Our understanding of SR-BI and ABCA1 is still in its early stages and additional information is required in order to propose a model of intestinal cholesterol trafficking.

Most studies have focused only on the hormonal regulation of SR-BI in steroidogenic tissues. The expression of SR-BI appears to be

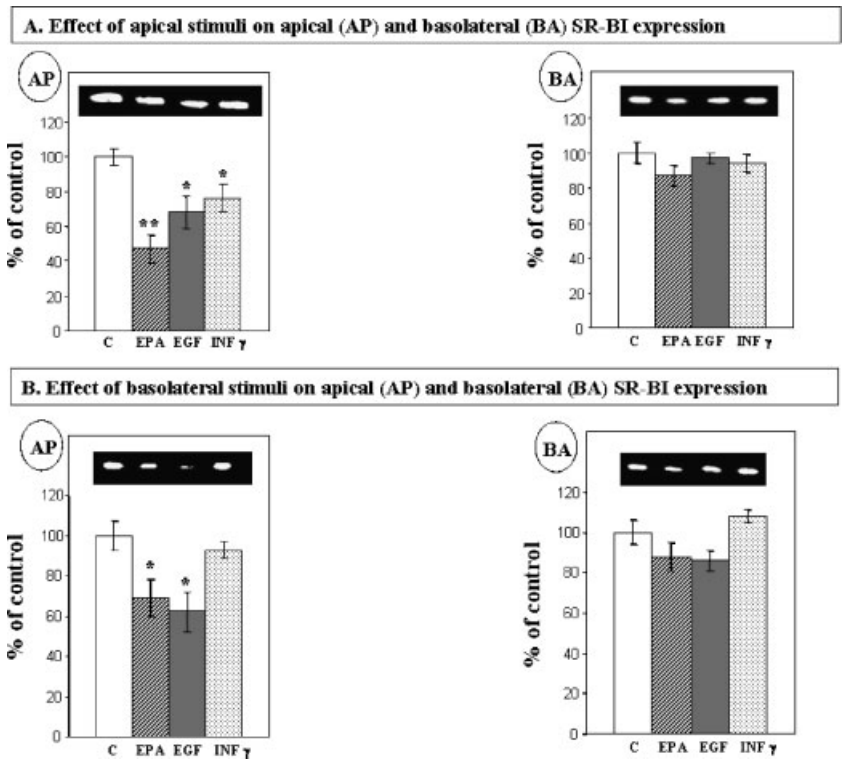


**Fig. 8.** Differential distribution of SR-BI in the apical and basolateral membranes. Caco 2 cells were grown on Transwell filters and submitted to treatments with EPA, EGF, and IFN-γ stimuli added to the apical (AP) and basolateral (BA) compartments. Thereafter, sulfo-NHS-biotin was employed to selectively label the apical or the basolateral surface. The cells were extracted with lysis buffer and the biotinylated proteins were recovered with streptavidin-agarose beads. Proteins were analyzed by SDS–PAGE and Western blot. SR-BI mass was quantitated using an HP ScanJet scanner equipped with a transparency adapter and software. Data are means ± SEM for three separate experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ versus controls (100%) without stimuli.

coordinately regulated with steroidogenesis in the adrenal gland, ovary, and testis [Reaven et al., 1998]. However, little is known about the hormonal modulation of SR-BI in the human intestine. In order to unravel whether SR-BI is hormonally regulated in the intestine, we incubated Caco-2 cells with three powerful effectors that are known as modulators of brush border membrane digestive functions and the synthesis and secretion of lipoproteins in humans [Levy et al., 1992, 1996a,b]. The addition of EGF and GH to both sides of Caco-2 cells, as well as basolateral insulin produced a significant decrease in SR-BI protein expression in the present investigation. Identification of the endocrine, paracrine and autocrine factors involved in the modulation of SR-BI and intestinal cholesterol transport remains to be fully elucidated in future experiments.

Pro-inflammatory mediators such as LPS have been shown to downregulate the mRNA and protein levels of SR-BI in the monocyte and macrophage [Buechler et al., 1999] as well as in hamster liver [Khovidhunkit et al., 2001]. In addition, treatment of hamsters with TNF or IL-1 produced a reduction in hepatic mRNA levels of SR-BI. Previous studies have documented the participation of TNF-α and LPS in the disturbance of lipid metabolism in the gastrointestinal tract and blood circulation [Mehran et al., 1995; Murthy et al., 1996, 2000; Dube et al., 2001; Bernotti et al., 2003; Courtois et al., 2003; Levy et al., 2003]. The results of our study confirm these in vitro observations and demonstrate for the first time the downregulation of SR-BI protein expression by basolateral TNF-α, apical IFN-γ and LPS on both sides. Therefore, intestinal mucosa that is constantly exposed to commensal bacteria and their inflammatory components or the invasion of pathogenic bacteria or mechanical breaks in the continuous epithelial monolayer barrier may undergo alterations in SR-BI levels and cholesterol transport.

Recent studies on luminal cholesterol transport have shown that SR-BI represented a facet

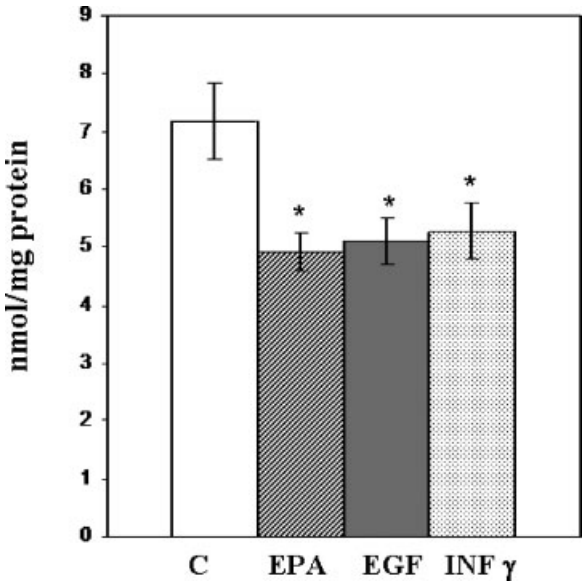


**Fig. 9.** Influence of apical effectors on cholesterol uptake. Caco 2 cells were grown on Transwell filters and submitted to treatments with EPA, EGF, and IFN-γ stimuli added to the apical compartment. Thereafter, cholesterol incorporation was evaluated as described in the Materials and Methods section. $^*P < 0.05$.

of this lipid trafficking [Levy et al., 2004]. In fact, the reduction of endogenous SR-BI protein expression by antisense cDNA affected the ability of Caco-2 cells to capture exogenous cholesterol [Levy et al., 2004]. In the present investigation, the regulation of SR-BI content in Caco-2 by various stimuli essentially reflected changes in apical SR-BI protein amounts. Additionally, cholesterol uptake followed the pattern of apical protein expression modulated by the specified effectors EPA, EGF, and IFN-γ. These results support the hypothesis that SR-BI at the microvillous membrane of intestinal polarized epithelial cells may undergo a thorough regulation, which may impact on cholesterol absorption.

In summary, our data suggest that specific nutrients, hormones and cytokines may exert a significant impact on SR-BI protein expression depending on the route of supply. The alterations of SR-BI protein expression following the treatment of Caco-2 cells with the numerous effectors, examined in our study, may have several consequences on cholesterol absorption. Future exploration is undoubtedly necessary to decipher the role of SR-BI on the apical and basolateral sides of the enterocyte and the intracellular mechanisms triggered by regulatory effectors.

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