Hyperglycemia suppresses hepatic scavenger receptor class B type I expression

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Materials and Methods

Materials. Wortmannin, LY-294002, bisindolylmaleimide I, and SB-203580 were purchased from Calbiochem. α-Amanitin was purchased from Sigma (St. Louis, MO) and used at 2 μg/ml.

Cell culture. Human hepatoma HepG2 cells (obtained from Riken Cell Bank, Ibaragi, Japan) were grown in DMEM (Life Technologies, Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine serum (Dainippon Pharmaceutical, Osaka, Japan).

Western blot analysis. An antibody directed against the extracellular domain of hSR-BI between amino acid residues 185 and 300 of the reported sequence of the isomer containing 509 amino acid residues (10) was generated. The corresponding cDNA fragment was amplified from THP-1 cDNA by PCR. The amplified fragment was inserted into a pGEX-2T vector (Pharmacia) and sequenced, and the protein was expressed in Escherichia coli. The fusion protein was isolated with glutathione-Sepharose 4B beads (Pharmacia) and used to generate an antisemur in guinea pigs as described previously (40). The membranes were blocked with 0.1% Tween-20 in PBS containing anti-hSR-BI antibody (diluted 1/3,000 from whole antisemur) (40) or anti-GAPDH antibody (diluted 1/1,000; Biomol Research, Plymouth Meeting, PA).

Northern blot analysis. A full-length cDNA of hSR-BI was synthesized by PCR using reverse-transcribed RNA from HepG2 cells and labeled with [32P]dCTP (3,000 Ci/mmol) by the random priming method. The amplified fragment was inserted into a pGEM-2 vector (Pharmacia) and sequenced, and the protein was expressed in Escherichia coli. The fusion protein was isolated with glutathione-Sepharose 4B beads (Pharmacia) and used to generate an antisemur in guinea pigs as described previously (40). The membranes were blocked with 0.1% Tween-20 in PBS containing anti-hSR-BI antibody (diluted 1/3,000 from whole antisemur) (40) or anti-GAPDH antibody (diluted 1/1,000; Biomol Research, Plymouth Meeting, PA).

Real-time reverse transcriptase-polymerase chain reaction. Polymerase chain reactions (PCR) were performed in LightCycler (Roche, Mannheim, Germany) glass capillaries. The reaction mixture consisting of 2 μl of LightCycler-FastStart DNA Master SYBR Green I (Roche), 2 μl of the cDNA template for each gene of interest, and 1 μl of 10 μM each primer. The sequences of the forward and reverse hSR-BI primers were 5'-TGAACTTCTGGGCAAATG-3' and 5'-TGGGGATGCCCTTCAACAC-3', respectively. The cycling program consisted of initial denaturation for 60 s at 95°C followed by 55 cycles of 95°C for 5 s, 62°C for 5 s, and 72°C for 15 s, with 20°C/s cycles. Known amounts of DNA were then diluted to provide standards and a regression curve of crossing points vs. concentration generated using a LightCycler.

HDL-selective CE uptake. Human HDL (d = 1.070–1.20 g/ml) was isolated by preparative ultracentrifugation from fresh plasma collected in EDTA (1 mg/ml) as described (22). HDL was passed through a heparin-Sepharose affinity column to remove particles collected in EDTA (1 mg/ml) as described (22). HDL was passed through a heparin-Sepharose affinity column to remove particles containing apolipoprotein E (39). The isolated lipoproteins were iodinated by the Pierce iodogen method to a specific activity of 400–600 cpm/ng protein for HDL. For specific uptake studies, the lipid moiety of human HDL was labeled with [3H]cholesterol ester ([3H]CE) and the apoA-I with 125I. The former ([3H]CE) was prepared from [3H]cholesterol and oleic anhydride, as described previously (42). Human apolipoprotein A-I was purified from HDL, labeled with 125I, and then exchanged into the [3H]HDL by a 24-h incubation at 37°C (42). The values for the selective uptake of HDL CE were obtained as previously described (40).

Transfection of siRNA. The siRNAs were designed to target the following cDNA sequences: scrambled, 5'-CCGTTCACTGACGAGGAGTACT-3'; and Sp1 siRNA, 5'-AATGGGCTGCATTTCTCGCAG-3' (Santa Cruz Biotechnology, Santa Cruz, CA). Transfection of Sp1 siRNA was performed using siPORT Amine (Ambion, CA).

Protocol for diabetic animals. Four-week-old male Long-Evans Tokushima Otsuka (LETO) rats (n = 4) and Otsuka Long-Evans Tokushima Fatty (OLETF) rats (n = 4) were supplied by Tokushima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan). All rats were kept in specific pathogen-free conditions at Kagawa University Animal Center in rooms controlled for both temperature and light (on from 8:00 AM to 8:00 PM). The experiments were approved by the local animal ethics committee. Some rats (LETO n = 4, OLETF n = 4) were provided with sucrose (20% sucrose solution as drinking water) for 4–20 wk of age. In OLETF rats (n = 4), food intake was restricted to 70% of average daily consumption for 8–16 wk of age. Otherwise, the rats had free access to tap water and a diet of standard chow (Clea Japan, Tokyo, Japan).

Statistical analysis. Statistical comparisons were made by one-way analysis of variance and Student’s t-test, with P < 0.05 considered significant.

Results

Effects of high-glucose conditions on hSR-BI expression in HepG2 cells. We measured the endogenous expression of SR-BI in HepG2 cells by Western blot analysis to determine whether glucose affected hSR-BI expression. There was an inverse correlation between hSR-BI expression and the concentration of glucose to which the cells were exposed. hSR-BI in the cells treated for 24 h with 22.4 mM glucose decreased to approximately one-half of that in the cells treated with the control medium containing 5.6 mM glucose (Fig. 1A); however, no such effect was observed in the cells treated with the osmotic control, i.e., mannitol.

To further confirm this observation, we used Northern blot analysis to assess hSR-BI expression in HepG2 cells. Results (Fig. 1B) showed a marked decrease in the abundance of hSR-BI mRNA following treatment with 22.4 mM glucose (Fig. 1B); however, GAPDH expression remained unaltered. To understand how hSR-BI expression was downregulated by high glucose concentrations, we examined whether mRNA stability was altered (Fig. 1C). hSR-BI mRNA stability was
examined in the absence or presence of high glucose concentrations after mRNA synthesis had been blocked by α-amanitin, an RNA polymerase II inhibitor. High glucose concentrations did not affect hSR-BI mRNA stability, indicating that downregulation of hSR-BI mRNA is not due to accelerated mRNA degradation. Previous reports, including reports from our laboratory (40, 42), showed that cells take up CE from HDL by a selective nonendocytotic pathway. To test whether high glucose concentration-mediated inhibition of hSR-BI expression affected this process, we measured the kinetics of CE uptake using doubly labeled HDL. Results showed that cellular HDL-CE uptake (Fig. 2) following high-glucose treatment was decreased compared with the control cells. These findings support the idea that high-glucose treatment inhibits the surface expression and function of hSR-BI in hepatocytes.

Since glucose lowered the abundance of both hSR-BI protein and mRNA in HepG2 cells, we speculated whether glucose regulated transcriptional activity of the hSR-BI promoter in HepG2 cells. For these studies, we measured luciferase activity in the HepG2 cells transfected with pCLA-LUC and exposed to varying concentrations of glucose (Fig. 3A). In agreement with the protein and mRNA levels, high-glucose...
treatment also inhibited the activity of the promoter. Together these results clearly show that glucose suppresses the activity of the hSR-BI/CLA-1 gene in HepG2 cells.

p38 MAPK signaling mediated high glucose-induced hSR-BI suppression. To further examine how glucose suppressed the activity of the hSR-BI gene, we postulated the potential involvement of signaling pathways. Therefore, we tested the effects of known signaling cascade inhibitors on glucose suppression of hSR-BI promoter activity. In this study, HepG2 cells were exposed to 22.4 mM glucose plus either a phosphatidylinositol 3-kinase (PI3K) inhibitor (10 μM wortmannin, WM; LY-294002), a PKC inhibitor (10 μM bisindolylmaleimide I), or a p38 MAPK inhibitor (1 μM SB203580). Results showed that the inhibitory effect of glucose on hSR-BI promoter activity persisted in the presence of PKC and PI3K inhibitors (Fig. 3B). Unexpectedly, glucose failed to suppress hSR-BI promoter activity in the presence of SB203580, a p38 MAPK inhibitor. This finding suggests that p38 MAPK may mediate high glucose-induced suppression of hSR-BI promoter activity.

Kinetics of p38 MAPK phosphorylation by high-glucose treatment. The preceding studies have shown that p38 MAPK may be required for the inhibitory effects of glucose on hSR-BI. Assuming this is true, glucose should induce p38 MAPK activity that is measurable by analyzing the kinetics of Thr180 phosphorylation by Western blot analysis. This phosphorylation is a prerequisite for induction of p38 MAPK catalytic activity. Results showed that p38 MAPK phosphorylation was evident within 15 min and peaked at 30 min following exposure of the HepG2 cells to 22.4 mM glucose (Fig. 4). These findings provide additional support that glucose induces p38 MAPK catalytic activity.

p38 MAPK regulates hSR-BI promoter activity. Assuming that glucose activates p38 MAPK, which, in turn, suppresses hSR-BI promoter activity, the use of constitutively active forms of p38 MAPK should mimic the effects of glucose. To
test this hypothesis, we expressed constitutively active forms of p38 MAPK (p38α, -β, and -γ) in HepG2 cells carrying pCLA-LUC. Results showed that the constitutively active form of p38 MAPK (p38-DN) blocks hyperglycemia-induced inhibition of hSR-BI transcription. HepG2 cells were transfected with pCLA-LUC and either the empty vector or p38-DN and then treated with 22.4 mM glucose for 24 h prior to cell harvesting. The results are expressed as relative luciferase activity compared with the control cells arbitrarily set at 100. Each data point shows the mean ± SE of 4 separate transfections that were performed on separate days. *Significant difference (P < 0.01). N.S., no significant difference.

hSR-BI promoter activity (Fig. 5B). Consistent with previous results, high-glucose treatment inhibited hSR-BI promoter activity; however, the expression of p38-DN masked the ability of high glucose to suppress hSR-BI promoter activity. Together, these findings support the idea that the p38 MAPK pathway is required for glucose suppression of hSR-BI promoter activity in HepG2 cells.

A DNA motif mediated glucose suppression of hSR-BI promoter activity. Since the preceding data point to the hSR-BI promoter as the site of action of the p38 MAPK cascade, we searched for a cis-acting site(s) within the DNA that may mediate the effects of high glucose. Therefore, templates were created by serial deletions of the hSR-BI promoter contained within pCLA-LUC. Constructs arising from the removal of 250, 725, and 992 base pairs from pCLA-LUC yielded the templates pCLA2-LUC, pCLA4-LUC, and pCLA6-LUC, respectively. Glucose suppression of the activity of pCLA-LUC, and of the pCLA2-LUC, and pCLA4-LUC deletion constructs was indistinguishable at 40 ± 4, 50 ± 7, and 55 ± 5%, respectively (Fig. 6). However, glucose could not suppress the activity of the pCLA6-LUC deletion construct (−209 to +2). Together, these observations show that glucose suppression of hSR-BI promoter activity requires the −476 to −210 fragment of the promoter.

Sp1 mediated high glucose-induced suppression of hSR-BI gene expression. Previous studies (47) have shown that the transcription factor Sp1 may mediate the actions of glucose on hSR-BI promoter activity. Therefore, we conducted a DNA homology search that revealed the presence of six putative Sp1 binding sites within the hSR-BI promoter. To determine whether Sp1 mediates the actions of glucose on hSR-BI pro-

Fig. 5. Role of p38 MAPK signal transduction pathway on hSR-BI promoter activity under conditions of hyperglycemia. A: effects of p38 MAPK isoforms on hSR-BI promoter activity. HepG2 cells were transfected with pCLA-LUC and either an empty vector (control) or expression vectors of constitutive active forms of p38 MAPK (p38α, -β, -γ) for 24 h prior to cell harvesting. All assays were corrected for β-galactosidase activity, and the total amount of protein in every reaction was identical. The results are expressed as relative luciferase activity compared with the control cells arbitrarily set at 100. Each data point shows the mean ± SE of 4 separate transfections that were performed on separate days. *Significant difference (**P < 0.01, **P < 0.005). B: dominant-negative p38 MAPK (p38-DN) blocks hyperglycemia-induced inhibition of hSR-BI transcription. HepG2 cells were transfected with pCLA-LUC and either the empty vector or p38-DN and then treated with 22.4 mM glucose for 24 h prior to cell harvesting. The results are expressed as relative luciferase activity compared with the control cells arbitrarily set at 100. Each data point shows the mean ± SE of 4 separate transfections that were performed on separate days. *Significant difference (P < 0.01). N.S., no significant difference.

Fig. 6. Deletion of the −476 to −210 fragment of the hSR-BI promoter abolished the response to hyperglycemia. HepG2 cells were transfected with 1 μg of several constructs [empty vector (vector), pCLA-LUC (CLA), pCLA2-LUC (CLA2), pCLA4-LUC (CLA4), or pCLA6-LUC (CLA6)] and treated with 22.4 mM glucose for 24 h prior to cell harvesting. All assays were corrected for β-galactosidase activity, and the total amount of protein in every reaction was identical. The results are expressed as relative luciferase activity compared with the control cells arbitrarily set at 100. Each data point shows the mean ± SE of 4 separate transfections that were performed on separate days. *Significant difference (P < 0.01).
Fig. 7. Transcriptional factor specificity protein-1 (Sp1) is involved in high-glucose treatment-induced suppression of hSR-BI transcriptional activity.  

A: dose-dependent induction of Sp1 protein in nuclear protein by glucose. HepG2 cells were exposed to the indicated amounts of glucose for 24 h. Sp1 in nuclear protein was detected by Western blot analysis probed with an anti-Sp1 antibody. Abundance of TFIIID served as the control and is shown at the bottom of each lane. The Sp1/TFIID ratio is shown as %control in the figure. Each data point shows the mean ± SE of 3 separate experiments. *Significant difference (P < 0.01).

B: effects of glucose on Sp1 binding to the Sp1 probe. Cells were exposed to varying concentrations of glucose as indicated (lane 1, only the probe; lane 2, 5.6 mM glucose; lane 3, 11.2 mM glucose; lane 4, 22.4 mM glucose) for 24 h before the nuclear extracts were prepared. The binding activity of Sp1 was examined by EMSA, as described in MATERIALS AND METHODS. Abundance of OCT1 served as the control and is shown at the bottom of each lane. An identical experiment that was independently performed yielded similar results.

C: deletion of the −476 to −210 fragment of the hSR-BI promoter abolished the response to Sp1. HepG2 cells were transfected with 1 μg of several constructs (vector, CLA, CLA2, CLA4, or CLA6) and cotransfected with the Sp1 expression vector. All assays were corrected for β-galactosidase activity, and the total amount of protein in every reaction was identical. The results were expressed as relative luciferase activity compared with the control cells arbitrarily set at 100. Each data point shows the mean ± SE of 4 separate transfections that were performed on separate days. *Significant difference (P < 0.01).

D: effects of Sp1 knockdown on hSR-BI promoter activity in HepG2 cells. siRNA for Sp1 or scramble siRNA (sc) was transfected into HepG2 cells with the pCLA-LUC plasmid for 24 h prior to cell harvesting. Top: efficiency of siRNA on Sp1 expression by Western blot analysis. All assays were corrected for β-galactosidase activity, and the total amount of protein in every reaction was identical. The results are expressed as relative luciferase activity compared with the control cells arbitrarily set at 100. Each data point shows the mean ± SE of 4 separate transfections that were performed on separate days. *Significant difference (P < 0.01).
pressive effects of glucose on hSR-BI promoter activity in the basic transcriptional factor TFIID was affected to a very small extent by glucose treatment with varying concentrations of glucose. Results showed that glucose induced the abundance of Sp1 in a dose-dependent manner (Fig. 7). In contrast, basal transcriptional factor TFIID was affected to a very small extent by glucose (Fig. 7A).

To confirm this observation, electrophoretic mobility shift analysis (EMSA) with a specific Sp1 binding motif was performed. The Sp1 binding activities were assayed in the nuclear extracts prepared from the HepG2 cells exposed to 5.6, 11.2, or 22.4 mM glucose for 12 h. Results showed that glucose stimulated Sp1 DNA binding activity (Fig. 7B). In contrast, the DNA binding activity of the transcription factor OCT1 to its motif was not affected by glucose.

To determine whether Sp1 affects the transcriptional activity of the hSR-BI promoter and to locate the site that mediates the actions of Sp1, we cotransfected the above deletion constructs into the HepG2 cells along with the Sp1 expression vector. Results (Fig. 7C) showed that Sp1 expression inhibited the activity of pCIA-LUC, pCIA2-LUC, and pCIA4-LUC but not of pCIA6-LUC. To further explore the role of Sp1 in glucose suppression of hSR-BI promoter activity, we used siRNA to block Sp1 expression. Results showed that Sp1 expression was attenuated by a specific Sp1 siRNA but not by a scrambled siRNA (Fig. 7D). Next, the HepG2 cells were exposed to Sp1-specific or scrambled siRNA and then treated with 22.4 mM glucose. Results (Fig. 7D) showed that hSR-BI promoter activity was inhibited in the cells exposed to the control siRNA following treatment with 22.4 mM glucose. However, the inhibitory activity of 22.4 mM glucose was attenuated in the cells carrying Sp1 siRNA. These findings are in accordance with the idea that the glucose suppression of hSR-BI gene expression requires Sp1.

**Effect of high glucose on hSR-BI expression in vivo.** Although in vitro experiments on cultured cells, as mentioned above, have helped to elucidate the action of glucose, it remains unknown whether glucose has the same effect on an in vivo model. To answer this question, we used OLETF rats, an animal model of DM, and their lean nondiabetic counter parts, LETO rats. Sucrose feeding markedly increased blood glucose levels in OLETF rats (18.2 ± 1.2 mmol/l) compared with that in LETO rats (6.8 ± 0.4 mmol/l), as previously reported (32). We speculated whether hyperglycemia in the sucrose-fed animals would decrease hSR-BI mRNA and protein, as observed in the in vitro model. First, we measured the levels of endogenous SR-BI mRNA expression in the liver of these animals by a real-time PCR method. As expected, hepatic SR-BI mRNA in OLETF rats was decreased compared with that in the euglycemic controls (Fig. 8A). Furthermore, the abundance of the SR-BI protein also decreased in OLETF rats (Fig. 8B). These findings show that, in rats, hyperglycemia decreases hepatic SR-BI expression, and this finding is similar to the observations of the in vitro study on cultured HepG2 cells.

**DISCUSSION**

In this report, we summarized the results of the studies on glucose suppression of hSR-BI gene transcription. The suppressive effects of glucose on hSR-BI promoter activity in the HepG2 cells require the participation of the p38 MAPK cascade and the transcription factor Sp1. More importantly, the observations in vitro were extended to an in vivo diabetic rat model to demonstrate that hyperglycemia suppresses hSR-BI mRNA expression. Controversially, Milliat et al. (38) had reported that early and strong enhancement of hepatic SR-BI expression was observed in streptozotocin-induced diabetes in hypercholesterolemic rats. The discrepancy between our findings and those of that previous report may be due to the differences in the experimental methods and the rat species used. Further investigations will be needed to clarify the regulation of SR-BI by glucose in vivo.

Recent reports show that the p38 MAPK pathway is activated in response to hyperglycemia and in models of DM. In vascular smooth muscle cells, exposure to high glucose concentrations induces the activation of p38 MAPK (6). Another report (30) indicated that high glucose causes a fourfold increase in p38 MAPK in rat aortic smooth muscle cells. p38 MAPK activity was increased in the glomeruli of diabetic rats compared with the controls. In the same cells, the investigator observed increased phosphorylation of heat shock protein 25, a downstream substrate of p38 MAPK (15). Although the published findings pointed toward the involvement of the p38 MAPK cascade in mediating the actions of glucose, more
direct and detailed evidence was needed to further define its role. In the current study, we demonstrate clearly the intermediary role of p38 MAPK in glucose suppression of hSR-BI promoter activity by using both constitutively active and dominant-negative forms of p38 MAPK on hSR-BI promoter activity (Fig. 5). Our data support the model that hyperglycemia activates p38 MAPK, which, in turn, triggers a series of steps to suppress hSR-BI promoter activity. In agreement with this hypothesis, the constitutively active form of p38 MAPK mimics the inhibitory action of hyperglycemia on hSR-BI promoter activity, whereas its dominant-negative mutant blocks the effect of glucose. Although we have pointed out the role of p38 MAPK on hSR-BI expression, a recent report (49) indicated that PI3K activation posttranslationally stimulates hepatic SR-BI function by regulating subcellular localization of SR-BI in a PI3K-dependent manner. Further studies will be needed to determine the detailed regulatory mechanisms of hepatic SR-BI expression.

Sp1 plays a vital role in the regulation of transcription from TATA-less promoters that commonly encode housekeeping genes (12). Cellular Sp1 activity and concentration are regulated during development, cellular proliferation, apoptosis, and other cellular processes (8, 17). Sp1 is involved in mediating responses to various stimuli, including induction of the transforming growth factor-β receptor gene (7), epidermal growth factor-mediated expression of the gastrin gene (13), and cAMP-dependent induction of the CYP11A gene (2). A recent report (37) showed that p38 MAPK participates in the LPS-induced activation of Sp1, which, in turn, regulates the transcription of the hIL-10 gene. Another report detailed the relationship between p38 MAPK and Sp1 in that the activation of the p38 MAPK cascade enhances Sp1 phosphorylation and mediates the interaction of Sp1 with p38 MAPK. These processes are essential for force-induced filamin expression in fibroblasts (14).

On the basis of several reports that have detailed the connection between p38 MAPK and Sp1, we speculated whether p38 MAPK and Sp1 were components of the pathway leading to glucose suppression of hSR-BI promoter activity. Deletional studies on the hSR-BI promoter led to the identification of a 266-bp DNA fragment (−476 to −210) that is required for the effects of p38 MAPK, and embedded within this sequence were six putative Sp1 binding sites. This finding stands in contrast with the feature of other cellular genes, i.e., the fact that they are regulated by multiple transcription factors. Our studies do not exclude the participation of other factors that may cooperate with Sp1 to regulate hSR-BI transcription. Such factors may remain masked in transient transfections because this approach is known to generate high plasmid copy numbers and bring about the accumulation of aberrant chromatin structure in these cells.

Eflux of cellular free cholesterol from peripheral cells to the acceptor HDL particles is the first step in reverse cholesterol transport and requires the binding of HDL to the hepatocyte membrane. Several reports (1, 35) have suggested that rodent SR-BI is functionally related to hSR-BI, and this protein is likely to be the receptor that selectively takes up the HDL cholesterol ester. SR-BI is believed to act as a docking receptor for HDL (52).

Hepatic overexpression of SR-BI causes increased biliary secretion of cholesterol without a concomitant increase in the secretion of phospholipids or bile salts (48). SR-BI enhances the exchange of cholesterol between the surface of HDL and the cell. SR-BI deficiency did not affect the expression of the key regulators of hepatic cholesterol homeostasis, including HMG-CoA reductase, the low-density lipoprotein (LDL) receptor, and cholesterol 7α-hydroxylase (18). However, SR-BI deficiency reduced the cholesterol content in bile by ~40%. Rigotti et al. (44) generated mice with a targeted null mutation in the SR-BI gene and showed that plasma cholesterol concentrations were increased because of the formation of large apolipoprotein A-I containing particles in heterozygous and homozygous mutants relative to the wild-type controls. The plasma concentration of apolipoprotein A-I, the major protein in HDL, remained unchanged in the mutants. These data, in conjunction with the increased lipoprotein size, suggested that the higher plasma cholesterol concentration in the mutants was due to decreased selective cholesterol uptake. These results suggest that SR-BI may stimulate hepatic uptake of HDL free cholesterol and its transport into bile.

Several potential links between diabetes and atherosclerosis have been identified, and many clinical observations point toward the correlation between the risk of vascular complications in diabetes and poor glycemic control. A primarily important step in the development of atherosclerosis is the uptake of modified plasma LDL by monocyte-derived macrophages in the vascular wall (55). This process is largely mediated by the cell surface scavenger receptor CD36. Griffin et al. (21) recently reported a fivefold increase in macrophage CD36 expression after prolonged exposure to high glucose concentrations (11 and 33 mmol/l) in vitro, leading to increased translational efficiency of CD36 mRNA. It is possible that the increased monocyte CD36 expression observed in DM could be due to this mechanism. A previous report (46) indicated a significant increase in the baseline expression of the monocyte-macrophage LDL scavenger receptor CD36 on peripheral venous monocytes of patients with type 2 DM. Both good “HDL” and bad “modified LDL” metabolism may be affected by a hyperglycemic state, such as in DM.

In summary, the results of this study show that hyperglycemia inhibits hepatic endogenous hSR-BI expression. This inhibitory effect of hyperglycemia on hSR-BI promoter activity is mediated by the p38 MAPK/Sp1 signaling cascade. These findings raise the possibility that hyperglycemia may affect reverse cholesterol transport by controlling hSR-BI expression in diabetic patients.

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REFERENCES


