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# Hyperglycemia promotes overexpression of *SR-BII* isoform of the scavenger receptor class B type I in type 2 diabetes mellitus: A study in Juana Koslay City, San Luis, Argentina

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## ABSTRACT

**The scavenger receptor class B type I (*SR-BI*) is a high-density lipoprotein (HDL) receptor involved in reverse cholesterol transport. Some studies reported the association to be stronger in the presence of diabetes. The full length gene encoding *SR-BI* is comprised in 13 exons that are alternatively spliced to produce two major transcripts: the full length *SR-BI* and the splice variant *SR-BII*, in which exon 12 is skipped. Considering that type 2 diabetes status is characterized by changes in the concentration of plasma lipids, modifications in lipoprotein size and composition, which may be important modulators of the *SR-BI* expression; the aims of the study were to examine the influence of *SR-BI* polymorphism (rs838895) on lipid profile and *SR-BI* mRNA expression in a population of diabetic patients living in Juana Koslay City. Blood samples were drawn from controls (n = 40) and Type 2 diabetic patients (n = 66) and DNA and total RNA were obtained. *SR-BI* mRNA expression was measured by RT-PCR and *SR-BI* polymorphism was detected by Tetra Primer ARMS-PCR. Compared to controls, diabetic patients had higher fasting serum glucose, glycated hemoglobin, triglycerides, total cholesterol, low-density lipoprotein cholesterol, and lower high-density lipoprotein cholesterol. *SR-BI* mRNA expression was lower in T2DM when compared to controls, suggesting that the hyperglycemia presents in T2DM patients down-regulates *SR-BI* mRNA expression. Interestingly, we found that decreased *SR-BI* expression resulted in markedly increased plasma LDL concentrations in T2DM subjects, and the overexpression of *SR-BII* isoform is responsible for the markedly increased plasma LDL-c concentrations. The polymorphism (rs838895) did not modify the mRNA level of *SR-BI* in leucocytes from control and diabetic patients. This study provides novel evidence suggesting that hyperglycemia may affect reverse cholesterol transport by controlling SR-BI expression in diabetic patients. LDL cholesterol levels are associated with low *SR-BI* mRNA expression in T2DM.**

**Keywords:** Type 2 Diabetes Mellitus; Hyperglycemia; Scavenger Receptor Class B Type I and Polymorphism

## 1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) has emerged as one of the most prevalent chronic diseases worldwide. Decreased plasma high-density lipoprotein cholesterol (HDL-c) is one of the most common lipid disorders in diabetic subjects [1-3]. Moreover, low HDL-c concentrations have been defined as a key sign of the insulin resistance syndrome, a common metabolic condition linked to a higher cardiovascular risk, which has been estimated to be present in one fourth of all Americans [4,5]. HDL-c is believed to exert its antiatherogenic role mainly through the process of delivering cholesterol from peripheral tissues back to the liver for removal from the body, which is called reverse cholesterol transport [6,7].

Scavenger receptor class B, type I (*SR-BI*), a major

 

HDL receptor [8,9], plays an important role in reverse cholesterol transport, a major pathway for the clearance of excess cholesterol from the body. In this process, peripheral cholesterol is packaged into HDL from which it is subsequently removed in the liver and excreted into bile. *SR-BI* mediates the uptake and biliary secretion of HDL-c by the liver [10,11].

Besides its role as a functional HDL receptor involved in reverse cholesterol transport, *SR-BI* also participates in the metabolism of Apolipoprotein B-containing lipoproteins, including low density lipoprotein (LDL) [12,13] and very low density lipoprotein (VLDL) [14,15].

The full length gene encoding *SR-BI* (gene symbol *SCARB*1) is comprised of 13 exons that are alternatively spliced to produce two major transcripts: the full length *SR-BI* and the splice variant *SR-BII*, in which exon 12 is skipped. *SR-BI* and *SR-BII* splice forms, conserved in both mouse and rat genomes, have different tissue distributions and may influence cellular cholesterol trafficking and homeostasis in different ways [16]. *SR-BII* is reported to be a minor splice variant in human liver and has shown to be less efficient at reverse cholesterol transport [17].

Studies using animal models have demonstrated that *SR-BI* has an atheroprotective role. *SR-BI* overexpression in the liver is associated with reduction of atherosclerosis in LDL receptor-deficient mice under a high fat and cholesterol diet [18]. On the other hand, *SR-BI* deficiency results in lipid deposition in the aorta and atherosclerosis in mice [19]. In contrast, much less is known about the role of *SR-BI* in humans.

Genome-wide association studies have uncovered a number of loci associated with type 2 diabetes, but together they account for little of the overall variance and provide little predictive power over traditional risk factors [20]. It is likely that many loci still remain to be discovered. Genes located in chromosomal regions showing linkage to type-2 diabetes in family-based studies are rational candidates for more detailed investigation. *SCARB*1 lies in a region on chromosome 12q24 that has been linked to type-2 diabetes [21] and more recently to abdominal obesity, a risk factor for type-2 diabetes, in three studies [22-24]. Acton *et al*. [25] were the first to identify single nucleotide polymorphisms (SNPs) of the ***SCARB*1** in a white European population and associated some of these common variants with plasma lipid levels and body mass index. Genetic variation in the *SCARB*1 has also been associated with increased risk of coronary artery disease [26], obesity [25], triglycerides [27,28], and HDL-c [29-33], all facets of the metabolic syndrome. Furthermore, there is evidence that diabetes status may modify the *SCARB*1 association with HDL-c [34].

A poorly studied polymorphism is intron 11 (IVS11) *SCARB*1 where G is changed by C (rs838896). Chiba-Falek *et al*. [35] showed that in liver tissue of young adult females (but not men) genotypes that carried the mutant allele (C) were associated with generally lower level of *SR-BI*, a non-significant lower level of *SR-BII* and a lower level of overall *SCARB*1, compared to young women not carrying this allele.

Considering that type 2 diabetes status is characterized by changes in the concentration of plasma lipids, modifications in lipoprotein size, and composition [2,36,37] that may be important modulators of the *SCARB*1 expression; the aims of the current work were: 1) to assess the phenotypic effect on lipid and lipoprotein concentrations of the genetic variant (rs838896) at *SCARB*1 in T2DM patients who reside in Juana Koslay, San Luis, Argentina, 2) to examine the association of the genetic variation at the *SCARB*1 locus with the type 2 diabetes status in these patients, 3) to estimate whether diabetes status modifies the association between the *SCARB*1 variation and the examined phenotypic traits, and 4) to examine the influence of this genetic variant (rs838896) on mRNA expression in peripheral leucocytes.

## 2. RESEARCH DESIGN AND METHODS

### 2.1. Subjects

The present study was carried out in accordance to the guidelines of the Helsinki Declaration. A total of 106 volunteers (66 patients with type 2 diabetes and 40 healthy age-matched controls) participated in this investigation. Criteria published by the American Diabetes Association were used to diagnose T2DM [38]. These patients reside in Juana Koslay, San Luis, Argentina. The protocol for this study was approved by the local Institutional Review Board, and a written informed consent was obtained from each patient to be enrolled. During an initial interview with each patient, they were asked for diseases, medication and smoking histories. Exclusion criteria included liver, kidney and thyroid diseases, as well as the use of anti-lipemic drugs.

### 2.2. Anthropometric and Clinical Data

For each subject enrolled, height (meters) and weight (Kg) measurements were acquired. Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. The body mass index (BMI) was calculated as weight divided by height squared ($Kg/m^2$).

### 2.3. Blood Sampling

Venous blood samples for glucose, lipid, and lipoprotein analysis were collected into EDTA-containing (1 g/liter) tubes from all subjects after a 12-h overnight fast at the beginning of the study. Blood was also collected to extract DNA and RNA (see below).

 

## 2.4. Biochemical Measurement

Fasting plasma glucose (FPG) was measured by using a glucose oxidase method with a commercial enzymatic kit (Wiener Lab, Rosario, Argentina) and Glycated hemoglobin (HbA1c) concentration was measured with a coupled ionic-exchange chromatography/spectrophotometric assay (BioSystems, Barcelona, Spain) in a Bayer Express Plus Chemistry Analyzer (Bayer Diagnostics, Siemens, Germany). Total cholesterol (TC), triglycerides (TG) and HDL-c concentrations were measured using commercial kits by following manufacturer's instructions ((Wiener Lab, Rosario, Argentina) in a Bayer Express Plus Chemistry Analyzer (Bayer Diagnostics, Siemens, Germany). Low density lipoprotein-cholesterol (LDL-c) was calculated with the Friedewald formula: LDL-c= total cholesterol (mg/dL) – HDL-c (mg/dL) – triglycerides (mg/dL)/5 [39].

## 2.5. Genotyping and SNP Analysis

Genomic DNA was isolated from diabetic patients and healthy volunteers using conventional protocol by Qiagen kits (Qiagen, Inc., Valencia, CA). DNA concentration was detected by UV-VIS spectroscopy and diluted to a final concentration of 20 ng/μL. The *SCARB*1 polymorphism (rs838896) was analyzed by Tetra Primer AMRS-PCR [40]. Two pairs of primers were used, one which amplifies a fragment of 431 bp, common to both alleles (outer primers: forward 5' GTAGATAGTGAGCTTTG-ACATGGAGGCCAG 3' and reverse 5' ACTTCCGTT-CCCTCCTGATAGCAGCT 3') and another pair specific for the SNP (inner primers): forward 5' TTGGAACTGA-TTCTGGGAGTGACAGTGG 3' for the G allele and reverse 5' GATTCCCCTCTTTAAAAGCCTCCAAAGG 3' for the C allele. Nucleotide sequence and SNP details were obtained from NCBI website (*http://www.ncbi.nlm.nih.gob*). Primers were designed ''in silico'' in a free access web (*http://cedar.genetics.soton.ac.uk*) and then checked for specificity (*http://blast.ncbi.nlm.nih.gov/Blast.cgi*). Each PCR reaction was performed containing 200 ng of genomic DNA, 1 pmol of each outer primer and 5 pmol of each inner primer (1:5 ratio of outer to inner primer concentration) , 200 μM dNTPs, 2.5 mM $MgCl_2$, 1X buffer, and 1 unit of AmpliTaq Gold DNA polymerase (Perkin-Elmer). PCRs were carried out in 35 μL reactions. The template DNA was denatured for 3 minutes at 95˚C before undergoing 35 cycles of denaturation for 1 minute at 95˚C, primer annealing for 1 minute at 68˚C, and extension for 1 minute at 72˚C, and final extension at 72˚C for 3 minutes. The resultant products obtained after PCR were separated by electrophoresis on 2.5% agarose gel containing GelRed. This resulted in 3 DNA fragments of: 431 bp common to both alleles (outer primers amplification), 292 bp for the C allele and 195 bp for the G allele. The image was visualized and photographed under UV transillumination. Allele frequencies for this SNP were calculated by allele counting. Randomly selected 20% of samples were re-genotyped for cross validating initial genotypes. In case of unclear genotyping results, the samples were repeated again in duplicates until clear genotype was available. Unclear genotyping results, even after repetition was excluded from the study. No genotyping error was observed during cross validation.

## 2.6. Measurement of Gene Expression

Total RNA was isolated from leucocytes using the TRIzol reagent following manufacturer's instructions (Life Technologies, Carlsbad, CA). Agarose gel electrophoresis and GelRed staining confirmed the purity and integrity of isolated RNA. RNA concentration was assessed by spectrophotometric measurements at 260/280 nm. Then, ten micrograms of total RNA were reverse-transcribed with 200 units of Moloney-murine leukemia virus (MMLV) reverse transcriptase (Promega) using random hexamers as primers in 20-μL reaction mixture following the instructions provided by the manufacturer. The reverse transcription reactions were performed using a GeneAmp PCR system 2400 (PerkinElmer, Wellesley, MA) with the following conditions: 65˚C for 10 min, 37˚C for 60 min, and 90˚C for 5 min.

Given the existence of alternative splice forms of *SCARB*1 involving inclusion/exclusion of exon 12, primers able to detect both isoforms were used. Primers were constructed from the published cDNA (*http://www.ncbi.nlm.nih.gob*) sequence of *SCARB*1 (*Forward* 5' CTGCGTCCTGCTGCTGGTCC 3' and *Reverse* 5' GGC-TCACGGTGTCCTCAGGA 3') and the house-keeping gene *beta-Globin* (*Forward* 5' CAACTTCATCCAC-GTTCACC 3' and *Reverse* 5' GAAGAGCCAAGGAC-AGGTAC 3'), used as internal control. *SCARB*1 primers were designed to generate a 197 bp fragment for the *SR-BI* transcript (exon 12 including) and a 68 bp fragment for the *SR-BII* transcript (exclusion of exon 12) (**Figure 1**).

PCR was used to quantify human overall *SCARB*1 products (*SR-BI* and *SR-BII* isoforms) and beta-Globin expression from the cDNA samples. PCRs were carried out in 35 μL reactions. Each amplification reaction included 20 ng of cDNA, 20 pmol of each PCR primer and 1 unit of AmpliTaq Gold DNA polymerase (Perkin-Elmer,

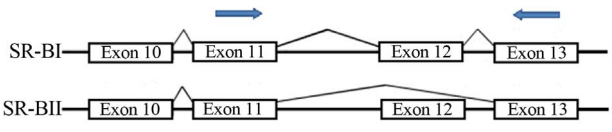


**Figure 1.** Schematic illustration of the location of primers (arrows) used for RT-PCR.

 

Waltham, MA). These reactions were performed in a buffer 1X containing 1.5 mmol/L $MgCl_2$, 50 mmol/L KCl, 20 mmol/L Tris-HCl (pH 8.4), and 200 pmol/L of each deoxynucleotide triphosphate. cDNA and control preparations were amplified using the following conditions: 95˚C for 3 minutes followed by 35 cycles of: denaturation for 1 minute at 95˚C, primer annealing for 1 minute (at 62˚C for *SCARB*1 and 55˚C for beta-Globin), and extension for 1 minute at 72˚C; followed by a final extension at 72˚C for 3 minutes. We controlled for DNA contamination by running three randomly selected, RNA control samples that were not converted to cDNA and no-cDNA/RNA sample in each plate. No observable amplification was detected. The amplification is predicted to generate a 197 bp fragment for the *SR-BI* transcript and a 68 bp fragment for the *SR-BII* transcript. PCR products were separated on a 2.5% agarose gel containing GelRed. The image was visualized and photographed under UV transillumination. The intensity of each band was measured using NIH *Scion Image* 1.6.3 software and reported as the values of band intensity units.

### 2.7. Statistical Analysis

Chi square test was used to check adjustment of the data to the Hardy-Weinberg equilibrium and to compare the allelic frequencies between controls and diabetic subjects. Comparison of allele frequencies and genotype distributions between case and control samples were done by Pearson's chi-square test. To analyze the association between *SCARB*1 genotypes, clinical and biochemical parameters a Student *t*-test was used when variables were continuous, whereas a Fisher's exact test was used for the categorical variables. A $p < 0.05$ was considered to be statically significant.

## 3. RESULTS

### 3.1. Subjects Characteristics

One hundred and six individuals were included in this study, 66 of them were diabetic and 40 were healthy controls. **Table 1** shows the demographic characteristics of both groups. 51.4% were women and 48.6% were men, age distribution was not different between the groups with a media age of 56.3 years old. The average value of BMI was 25.3 kg/m$^2$ for the control group and 31.2 kg/m$^2$ for the diabetic one. There was a significant difference in weight and BMI between both groups. Diabetic female subjects were more obese than diabetic male subjects.

As shown in **Table 2** FPG and $HbA_{1c}$ concentrations were higher in the diabetic group when compared with age-matched control subjects. Total cholesterol, TG and LDL-c were increased in the diabetic group, while HDL-c was significantly lower in these patients, when compared to controls. LDL-c was lower in diabetic female than in diabetic male subjects. The atherogenic index (AI) was higher in the diabetic patients, what suggests a risk for cardiovascular diseases in these patients.

**Table 1.** Anthropometric characteristics in control and T2DM patients.

| | Control (n = 40) | T2DM (n = 66) | *p* |
|---|---|---|---|
| Age (years) | 54.63 ± 10.63 | 58.13 ± 10.96 | 0.186 |
| Weight (kg) | 68.9 ± 12.4 | 85.9 ± 14.5 | <0.0001 |
| Height (m) | 1.65 ± 1.1 | 1.7 ± 0.9 | 0.04 |
| BMI (kg/m$^2$) | 25.3 ± 4.3 | 31.2 ± 3.7 | <0.0001 |

Data are shown as mean ± SD. Abbreviations used: BMI, body mass index.

**Table 2.** Biochemical characteristics in control and T2DM patients.

| | Control (n = 40) | T2DM (n = 66) | *p* |
|---|---|---|---|
| FPG (mg/dL) | 83.0 ± 11.3 | 165.8 ± 76.1 | <0.0001 |
| HbA1c (%) | 5.5 ± 0.7 | 9.2 ± 2.4 | <0.0001 |
| TC (mg/dL) | 169.6 ± 26.2 | 190.69 ± 47.3 | 0.04 |
| HDL-c (mg/dL) | 41.8 ± 5.2 | 36.4 ± 7.8 | 0.002 |
| LDL-c (mg/dL) | 116.2 ± 28.0 | 141.0 ± 32.0 | 0.001 |
| TG (mg/dL) | 122.0 ± 31.3 | 250.0 ± 110.2 | <0.0001 |
| AI | 4.2 ± 0.8 | 5.4 ± 1.9 | 0.003 |

Data are shown as mean ± SD. Abbreviations used: TG, triglycerides; TC, total cholesterol; LDL-c, low density lipoprotein; HDL-c, high density lipoprotein; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; AI: TC/HDL-c.

### 3.2. Genotype Frequencies

During the amplification reaction, a 431 bp region of *SCARB*1 was amplified with Outer forward primer and Outer reverse primer that served as internal control for the quality of the PCR amplification. The allele-specific amplification (Forward inner/Reverse outer and Forward outer/Reverse inner) resulted in the 195 bp PCR product specific for the G allele and the 292 bp PCR products specific for the C allele (**Figure 2**).

**Table 3** shows the absolute number and frequency (%) of controls and T2DM patients having each genotype. The distribution of genotypes was as expected from the Hardy-Weinberg equilibrium because no statistically significant differences ($p > 0.05$) were detected between the observed and the expected frequencies in any case. We did find statistically significant differences between diabetes status and controls in the genotype and allele frequencies of the *SCARB*1 variants. The frequency of the C allele was significantly higher in diabetic subjects

 

than that in controls (0.5 vs. 0.30), and this SNP showed strong association with T2DM [OR = 2.429 with 95% CI (1.359 - 4.339), $p = 0.004$].

### 3.3. Genotype Associations with Lipid Traits

**Table 4** shows plasma concentrations of lipids and lipoproteins by the *SCARB*1 polymorphism in controls and T2DM. Because of the small number of subjects, homozygotes for the minor C allele and heterozygotes were grouped as carriers of the C allele (GC and CC). In controls, no differences in lipids concentrations were detected in carriers of the C allele of intron 11 polymorphism (rs838896), whereas in diabetics the presence of the C allele was associated to an increase in LDL-c and slight decrease in HDL-c when compared to the GG homocygotes.

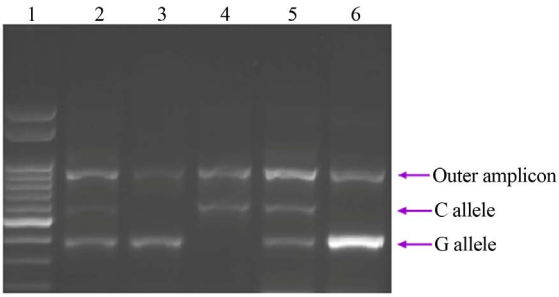


**Figure 2.** Representative agarose gel of Tetra Primer ARMS-PCR amplification of DNA. Lane 1 shows the molecular weight marker; Lanes 2 and 5 show a typical G/C genotype; Lanes 3 and 6 show a typical G/G genotype; and Lane 4 shows a typical C/C genotype.

**Table 3.** Distribution of *SCARB*1 genotypes and allele frequencies in controls and T2DM patients.

| | Control (n = 40) | T2DM (n = 66) | OR (95% CI) | *p* |
|---|---|---|---|---|
| ***Genotypes*** | | | | |
| G/G | 20 (50.0%)[a] | 16 (24.2%)[b] | 5.313 (1.48 - 18.96) | 0.015 |
| G/C | 15 (37.5%) | 33 (50.0%) | 1.93 (0.554 - 6.735) | 0.452 |
| C/C | 4 (12.5%) | 17 (25.8%) | 1.0 | |
| ***Allele*** | | | | |
| G | 0.70 | 0.49 | 2.429 (1.359 - 4.339) | |
| C | 0.30 | 0.51 | 1.0 | 0.004 |

[a]Percentages, odds ratio (OR) and *p* with respect to C/C genotype (shown as 1.0); [b]Percentages, OR and *p* with respect to C allele (shown as 1.0).

### 3.4. mRNA Expression Levels and Presence of Alternative Splice Variants

Given the existence of alternative splice forms of *SCARB*1 involving inclusion/exclusion of exon 12, we hypothesized that a SNP located within the associated haplotype block could modulate splicing efficiency. As different isoforms of *SCARB*1 probably might modulate receptor function, the expression of *SCARB*1 isoforms was investigated in human leucocytes by RT-PCR using primers designed to detect both *SR-BI* and *SR-BII* transcripts. We quantified the amounts of the full-length *SR-BI* isoform (including exon 12), the alternative *SR-BII* isoform (skipping exon 12) and overall *SCARB*1 mRNA (all splice forms) in human leucocytes from 66 T2DM and 40 controls.

As shown in **Figure 3**, overall *SCARB*1 expression was significantly higher in leucocytes from controls when compared to type 2 diabetic patients ($p = 0.0002$).

**Figure 4** shows a comparative graph of the percentage expression based on the total expression of *SR-BI* and *SR-BII* in controls and diabetics. In normal subjects the highest percentage of expression corresponds to *SR-BI* (77% ± 2%) compared to *SR-BII* (21% ± 3%) showing an extremely significant difference, while in diabetics although there is a trend to increased expression of *SR-BI* (55% ± 11%) it showed no significant differences in the expression of *SR-BII* (45% ± 11%). Diabetic subjects showed overexpression of *SR-BII* isoform and a decresed expression of *SR-BI* when compared to controls.

In order to determine the functional consequences of SNP (rs838896), we carried out genetic association analysis of the associated SNP and expression of *SCARB*1. We then compared relative levels of each splice form (ratio to overall *SCARB*1 expression) in T2DM and controls, and found no differences by genotype. We then compared relative levels of each splice form in men ver-

**Table 4.** Plasma levels of lipids and lipoproteins by the *SCARB*1 polymorphism in controls and T2DM.

| | Control (n = 40) | | *p* | T2DM (n = 66) | | *p* |
|---|---|---|---|---|---|---|
| | GG | GC/CC | | GG | GC/CC | |
| **TC (mg/dL)** | 173.55 ± 28.63 | 172.84 ± 25.87 | 0.977 | 163.38 ± 38.93 | 188.95 ± 83.82 | 0.634 |
| **HDL-c (mg/dL)** | 42.95 ± 6.86 | 40.84 ± 3.52 | 0.630 | 39.94 ± 6.29 | 36.23 ± 6.45 | 0.075 |
| **LDL-c (mg/dL)** | 123.65 ± 28.80 | 110.84 ± 21.27 | 0.132 | 93.09 ± 40.23 | 195.95 ± 50.25 | <0.0001 |
| **TG (mg/dL)** | 127.85 ± 40.68 | 121.32 ± 24.98 | 0.423 | 174.23 ± 92.42 | 135.03 ± 37.54 | 0.341 |

Data are shown as mean ± SD. Abbreviations used: TG, triglycerides; TC, total cholesterol; LDL-c, low density lipoprotein; HDL-c, high density lipoprotein.

 

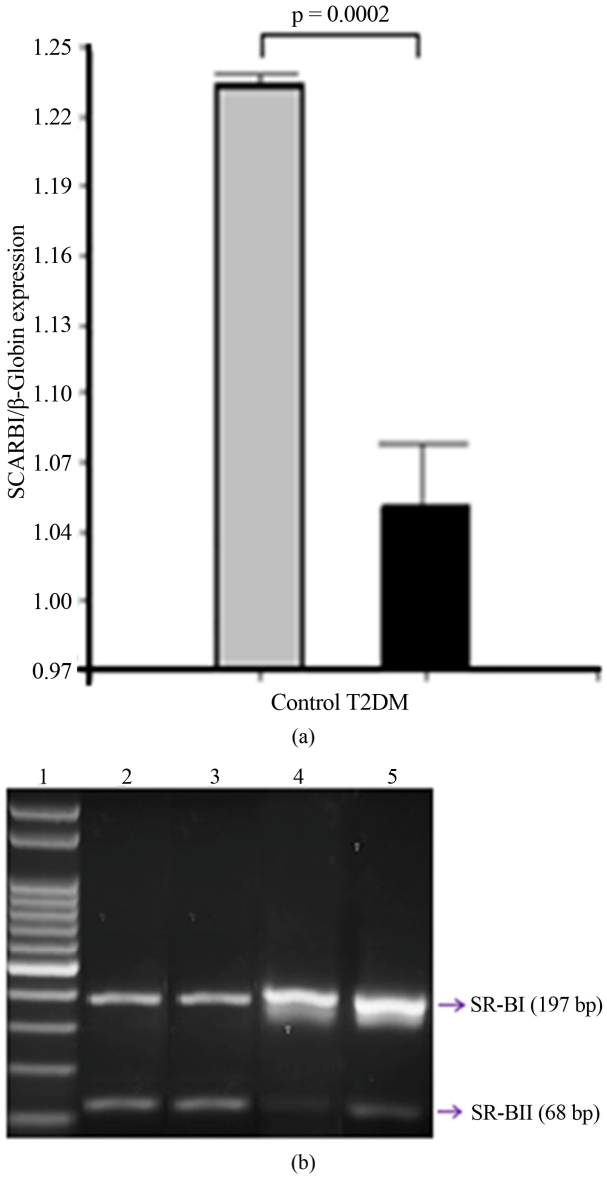


**Figure 3.** Expression of overall *SCARB*1. (a) The bar graphs represent the densitometric analysis of *SCARB*1 expression in leucocytes of controls and type 2 diabetic patients; (b) Representative agarose gel: Lane 1 shows the molecular weight marker; Lanes 2 and 3: T2DM; and Lanes 4 and 5: Control. The expression of *SCARB*1 isoforms was assessed by RT PCR, using specific primers, and it was normalized against the expression of the housekeeping gene beta-Globin, as reported in *Material and Methods*. Data are expressed as mean ± SEM.

sus women, but no significant associations were found (data not shown).

### 3.5. Correlation and Lineal Regression Studies

Because *SCARB*1 mRNA was reduced in the diabetic group, we performed correlations to determine whether this reduction was associated to metabolic factors that

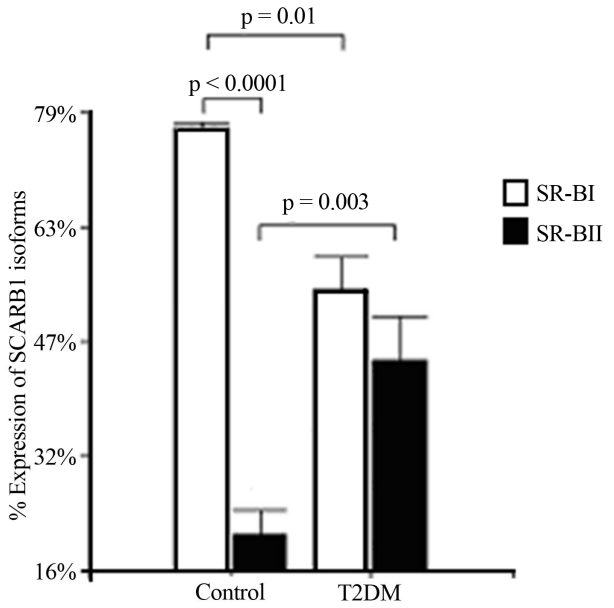


**Figure 4.** Relative levels of each splice form (ratio to overall *SCARB*1 expression) in T2DM and controls.

are important for insulin resistance. When the data from all diabetic patients were pooled together, interestingly *SCARB*1 was negatively correlated to LDL-c levels ($r = -0.89$, $p < 0.0001$), FPG ($r = -0.944$, $p = 0.001$) and there was a trend to correlate with HDL-c levels ($r = -0.29$, $p = 0.08$).

A subsequent linear regression analysis revealed that there was a significant and negative correlation between overall *SCARB*1 expression and LDL-c levels and the changes of the *SR-BI* and *SR-BII* isoforms expression levels in T2DM. Furthermore there was a strong positive correlation among the expression of *SR-BII* and LDL-c levels and a negative relationship between *SR-BI* and LDL-c (**Figure 5**).

## 4. DISCUSSION

No large population studies examining the association between genetic polymorphisms at the *SCARB*1 gene and diabetic status have been reported to date. The studies of the *SCARB*1 gene have been limited to a few candidate SNPs in exon 1, intron 5, and exon 8 [34].

In this study we analyzed the G/C change at intron 11 (rs838896) and confirmed the association of the minor C allele at *SCARB*1 with type 2 diabetes in an Argentinean small city [OR = 2.429 with 95% CI (1.359 - 4.339), $p = 0.004$]. Further studies will be needed to evaluate the impact of *SCARB*1 polymorphism on genetic predisposetion to T2DM in the Argentinean population.

A series of population studies examining the association of polymorphisms in the *SR-BI* gene with different lipoprotein parameters has suggested that *SCARB*1 genetic variability plays a significant role in lipoprotein

 

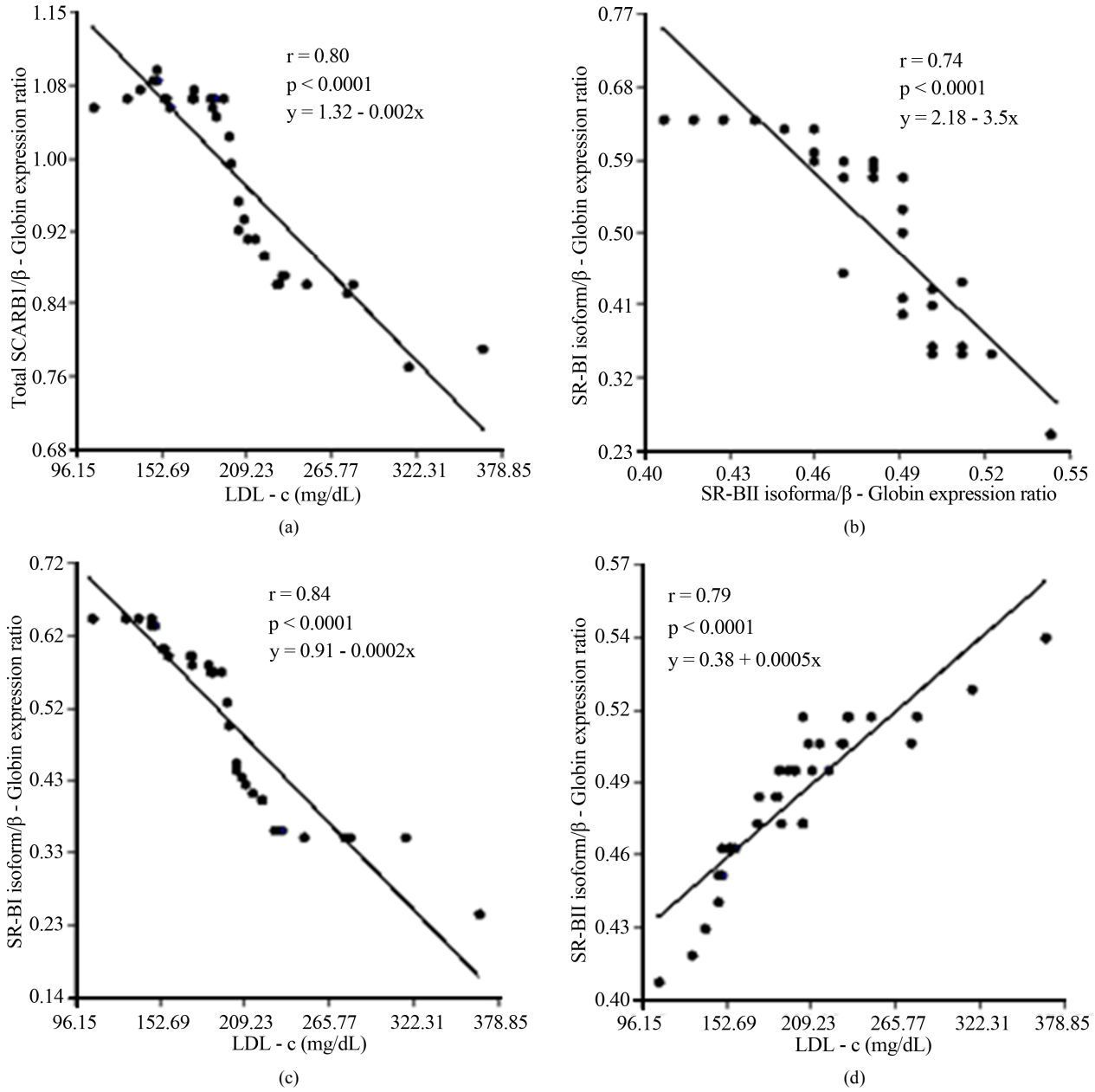


**Figure 5.** (a) Lineal regression analysis between the expression of total *SCARB*1 and LDL-c levels; (b) Lineal regression analysis between the expression of *SR-BI* and *SR-BII* isoforms; (c) Lineal regression analysis between the expression of *SR-BII* isoform and LDL-c levels; (d) Lineal regression analysis between the expression of *SR-BI* isoform and LDL-c levels.

metabolism in humans [23,25,36,41]. Furthermore, considering that type 2 diabetes is characterized by low plasma HDL-c concentrations, high triglycerides, a preponderance of small, dense LDL, an increase in oxidized lipoproteins, as well as by other insulin-resistance related parameters that may modulate the expression of the *SR-BI* gene [20,36]; we have examined the interaction effects between diabetes status and *SR-BI* gene variants determining plasma lipids and lipoproteins.

The specific contribution of the *SCARB*1 locus to the homeostasis of lipid metabolism in humans remains mostly undefined. In our study, we have found significant associations between carriers of the C allele at the intron 11 polymorphism (rs838896) and elevated LDL-c concentrations as compared with GG homozygous diabetic subjects. Earlier experimental studies demonstrated that the *SR-BI* receptor could bind both HDL-c and LDL-c lipoproteins in a nonreciprocal cross competition. Analyses in cultured cells have demonstrated that *SR-BI* binds other classes of lipoproteins in addition to HDL-c. Hamster *SR-BI* was originally identified through its ability to bind modified human LDL-c [42] and the human

*SR-BI*, binds VLDL in addition to LDL-c and HDL-c [43]. These studies suggested a role for *SR-BI* in LDL-c metabolism in humans.

In studies *in vivo*, alterations in hepatic *SR-BI* expression have been associated to changes in plasma concentrations of apoB-containing lipoproteins. Sustained, high-level expression of *SR-BI* in livers of transgenic mice results in reduced plasma concentrations of LDL-c and apoB [44,45], as well as decreased VLDL and IDL/LDL particle size [45].

The increase in LDL-c concentrations that we observed in diabetic carriers of the C allele is more compatible with a reduced expression of the *SR-BI* than with an overexpression of this gene variant. These observations, and the fact that the G/C change at intron 11 (rs838896) do not result in a change in the amino acid sequence, suggest that the intron 11 SNP could be in linkage disequilibrium with critical regulatory regions in intron 11 of *SCARB*1, or alternatively with another functional variant at a neighboring locus [46].

In the present study, we examined the expression of *SCARB*1 from Type 2 diabetes patients. Hepatocytes and macrophages are appropriate samples for the evaluation of cholesterol transport; however collecting these specimens is not practical in human subjects. Thus, we measured mRNA levels in circulating leucocytes, where mononuclear leucocytes would become macrophages in peripheral tissues. Powell *et al*. reported that the basic regulatory mechanism in liver and mononuclear leucocytes is similar [47].

In this report, we demonstrated that diabetic patients had significantly reduced expression of overall *SCARB*1 mRNA in circulating leucocytes cells compared to controls. The hyperglycemia present in T2DM patients downregulated *SCARB*1 mRNA expression. This suppressive effect has also been reported in HepG2 cells [48] and Caco-2/15 cell line [49] following exposure to high glucose concentrations.

Decreased *SCARB*1 expression resulted in markedly increased plasma LDL-c concentrations in T2DM subjects. A possible explanation for the increase of LDL-c is that LDL-c lipoproteins may be a poor substrate for *SR-BI* receptor, because LDL-c is not taken up as efficiently as HDL-c via *SR-BI*. Studies *in vitro* have demonstrated that LDL-c can serve as a substrate for selective uptake by *SR-BI*. However, lipid transfer mediated by *SR-BI* from LDL-c particles appeared to be less efficient when compared with HDL-c [50,51].

Our results suggest that the marked downregulation of overall *SCARB*1 and its impact on LDL-c lipoproteins may lessen the anti-atherogenic properties normally associated to elevated reverse cholesterol transport. Although high LDL-c values were associated to low expression of *SCARB*1 in T2DM leucocytes, there are some characteristics of *SR-BI* receptor that might cause difficulty in the interpretation and discussion of this result. First, *SR-BI* has multiple functions and has a dual role in cholesterol metabolism, participating in the intake and efflux of cholesterol traffic among cells and HDL-c particles [52], as well as contributing to the capture of apoB-containing lipoproteins, LDL and VLDL [14,53]; second, *SR-BI* displays specific expression profiles among several tissues that may result from different mechanisms of transcription regulation [54]; and third, there is limited information explaining the factors that modulate the *SCARB*1 mRNA expression in humans tissues *in vivo*.

Diabetes also induces some changes in lipid and lipoproteins that may be important modulators. In this regard, it has been described that elevated triglyceride content that frequently occurs in type 2 diabetes may impair the beneficial effects of the *SR-BI* overexpression [55]. On the other hand, increasing evidence indicates that *SR-BI* may play additional roles that might be of particular importance in type 2 diabetes. Thus, the *SR-BI*, as a scavenger receptor, can also bind oxidized LDL that adds support to its antiatherogenic properties [56,57].

Although there are no studies in humans examining the association between the *SR-BI* expression and atherosclerosis, several results from animal models are consistent in their results showing a decreased atherosclerosis associated to a higher *SCARB*1 expression [9]. We have shown that the *SCARB*1 locus is significantly associated to LDL-c concentrations. Our data suggest that, through its association with lipid risk factors and more specifically, with the kinetics of reverse cholesterol transport, the *SR-BI* may modulate the risk of atherosclerosis in the general population and especially in diabetic subjects.

Given the existence of alternative splice forms of SR-BI involving inclusion/exclusion of exon 12, we hypothesized that a SNP (rs838896) located within the associated haplotype block may modulate splicing efficiency. We quantified the amounts of the full-length SR-BI isoform (including exon 12), the alternative *SR-BII* isoform (skipping exon 12) and overall *SCARB*1 mRNA (all splice forms) in leucocytes from controls and T2DM individuals. In normal subjects the highest percentage of expression corresponds to *SR-BI* compared to *SR-BII*, consistent with other studies [35]; while in diabetics the expression of *SR-BI* showed no differences when compared to *SR-BII*. Although there are no studies in humans examining the expression of both isoforms in T2DM, we conclude that the sustained hyperglycemia promotes an overexpression of *SR-BII* isoform.

In order to determine the functional consequences of *SCARB*1 polymorphism (rs838896), we carried out genetic association analysis of the associated SNP and expression of *SCARB*1 isoforms. Our data do not support

 

an effect of this SNP on relative amounts of *SR-BI* or *SR-BII* in leucocytes. In order to determine whether the rs838896 variant is the cause of the different expression levels of *SCARB*1 isoforms, follow up functional studies using cell culture and other systems need to be done.

In the other hand, estrogen is known to have a profound impact on serum lipid levels, resulting in a decrease in LDL-c and triglycerides, affording younger women relative protection from coronary artery disease. Estrogen is also a potent regulator of *SR-BI* receptor, influencing the relative expression of *SR-BI* and *SR-BII* isoforms. Zhang *et al*. [58] proposed that estrogen regulation of alternative splicing of *SR-BI* in the rat occurs via regulatory splicing factors that interact with regulatory sequences in intron 11 of *SCARB*1 [46,59]. Similarly, treatment of HepG2 liver cell lines with estradiol has been shown to result in a downregulation of *SR-BI* and up-regulation of the *SR-BII* splice form [59,60].

Chiba-Falek *et al*. [35] have detected sex-specific expression of *SCARB*1 isoforms *in vivo*, in human liver tissue, and identified polymorphisms in intron 11 of the gene that modify this effect. They also found these same genetic variants to influence triglyceride and HDL-c levels in an endogenous estrogen-dependent manner in a human population study. Our findings do not agree with those of Chiba-Falek due to the patients included in the current study. It should be noted that this was an elderly population of men and women, with relatively low levels of estradiol.

Furthermore, our data also revealed that there is a strong and negative correlation among the expression of *SR-BII isoform* and LDL-c levels in T2DM. *SR-BII* is reported to be a minor splice variant in human liver and has been shown to be less efficient at reverse cholesterol transport [61]. We conclude that *SR-BII* have reduced capacity to de-lipidate LDL-c particles due to its less efficient activity on LDL-c particle catabolism.

## 5. CONCLUSION

To our knowledge, the results from this study provide novel evidence that hyperglycemia may affect reverse cholesterol transport by controlling *SCARB*1 expression in diabetic patients. The linear regression analysis revealed that there is a strong and negative correlation between the changes of *SCARB*1 expression and LDL-c levels. We conclude that the sustained hyperglycemia promotes overexpression of *SR-BII* isoform, which is less efficient in reverse cholesterol transport and leads to elevate LDL-c concentrations in T2DM patients.

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