# The effect of silymarin on expression of selected ABC transporters in the rat

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**ABSTRACT**: Silymarin (standardized extract from the seeds of the *Silybum marianum*) has been used in the supportive therapy of liver diseases and its cytoprotective activity is believed to be based on antioxidant properties. Our previous works showed hypolipidemic effects of silymarin. The ATP-binding cassette (ABC) transporters G5 and G8 play a major role in biliary cholesterol secretion. The ABCA1 transporter plays a significant role in movement of cellular cholesterol to high density lipoproteins. We investigated the possibility that silymarin affects the regulation of lipid metabolism via selected ABC transporters using rats fed a high-cholesterol diet with silymarin. The major finding in this study is that silymarin up-regulated mRNA of ABC transporters (G5, G8 and A1). This result suggests that silymarin positively affects the plasma lipoprotein profile via up-regulation of ABC transporters involved in lipid metabolism. Furthermore, this study shows for the first time that silymarin up-regulates the expression of these ABC transporters at the mRNA level.

**Keywords**: ABC transporter; lipid metabolism; mRNA; rat; silymarin

**List of abbreviations**

**ABC** = ATP-binding cassette; **EDTA** = ethylenediaminetetraacetic acid; **HDL** = high-density lipoprotein; **HPRT** = hypoxanthine-guanine phosphoribosyltransferase; **TRIS** = tris(hydroxymethyl) aminomethane; **VLDL** = very low-density lipoprotein

Silymarin is a standardized extract from the seeds of the medical plant *Silybum marianum* (milk thistle). For centuries, silymarin has been used in the supportive therapy of liver diseases and its cytoprotective activity is believed to be based on antioxidant properties (Valenzuela and Garrido, 1994). This extract has been promoted as a nutritional supplement for healthy liver function (Wellington and Jarvis, 2001) and because of its beneficial influence on certain risk factors of atherosclerosis (Simanek et al., 2001). Our previous studies have shown a hypolipidemic influence of silymarin, which was demonstrated by a decrease in total plasma cholesterol and cholesterol content in the liver, and an increase in the HDL/VLDL cholesterol ratio in rats fed a high-cholesterol diet (Skottova et al., 2003, 2004). The ATP-binding cassette (ABC) transporters are transmembrane proteins that utilize the energy from ATP (adenosine triphosphate) hydrolysis to transport substrates across the cell membrane (Dean et al., 2001). The ABCG5 and ABCG8 transporters play a major role in the intestinal absorption of sterols and in biliary cholesterol secretion (Zhang and Mangelsdorf, 2002). The ABCA1 transporter plays a significant role in the movement of cellular cholesterol into high-density lipoproteins (HDL) (Oram and Vaugham, 2000).

A possible explanation for the hypolipidemic effect of silymarin is a lowering of cholesterol absorption in the intestine (Sobolova at al., 2006). In the current study, the possibility that silymarin influences the regulation of lipid metabolism by affecting selected ABC transporters (G5, G8 and A1) was studied.

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## MATERIAL AND METHODS

### Animals

As a study model we used SPF (specific pathogen free) male Wistar rats (180–220g of body weight). The animals were maintained under standard laboratory conditions with free access to water. The rats were fed *ad libitum* on a standard laboratory diet (STD; KrmiMo Mohelsky, Czech Republic) or on an experimental high-cholesterol diet (HCD) prepared by adding 1% (w/w) of cholesterol to the standard laboratory diet. Silymarin (1% or 3%, w/w; SM 1% and SM 3%; Sigma Aldrich) was administered as a dietary supplement to the high-cholesterol diet (HCD). The doses of silymarin (1% or 3%) were selected according to previously published improvements in the lipoprotein profile (Skottova et al., 2003). The animals were fed their respective diets for 20 days and the amount of food consumed was checked daily per cage of two rats (not shown). After 20 days of feeding the rats were deprived of food overnight and were then anesthetized by intramuscular (*i.m.*) administration of fentanyl (40 µg/kg of body weight) in combination with medetomidin (200 µg/kg of body weight), followed by an *i.m.* administration of diazepam (5 mg/kg of body weight). The liver was removed and rinsed in an ice-cold sucrose solution (pH 7.4; 0.25M sucrose, 1mM $Na_2EDTA$, 0.025M TRIS), weighed, divided into several portions, and frozen in liquid nitrogen. All experiments with animals were approved by the Ethics Committee of the Ministry of Education, Czech Republic.

### RNA isolation and RT-PCR procedures

A 40mg piece of liver tissue was homogenized (FastPrep, Qbiogene, Illkirch, France) and RNA was isolated using TRIzol reagent according to the manufacturer's instructions (Invitrogen, Basel, Switzerland). Contaminating genomic DNA was removed by DNase I treatment (Roche, Rotkreuz, Switzerland). One microgram of RNA was reverse-transcribed with SuperScript II (Invitrogen, Carlsbad, CA, USA) using random primers. The generated cDNA was used for real-time PCR using SYBR Green PCR Master Mix (Applied Biosystems, Weiterstadt, Germany) in an ABI PRISM 7700 Sequence Detection system with the following thermal cycling conditions: 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min for denaturation, annealing and elongation. Cycle-to-cycle fluorescence emission was monitored and quantified using SDS 1.1.9 software (Applied Biosystems, Weiterstadt, Germany). All samples were analyzed in duplicate. Rat primers were synthesized by Operon (Cologne, Germany).

The following primer sequences were used:

HPRT Fw 50-TCGACCCTCAGTCCCAGC-30
HPRT Rev 50-CAGCATAATGATTAGGTATGCAAAATAAA-30
ABCA1 Fw 50-TACACCTGACACACCAGCTACAAG
ABCA1 Rev 50-GGAACAAAGCCAGCTCCTGA
ABCG5 Fw 50-GGCCAGACCATGTGCATCTT
ABCG5 Rev 50-CCAGAGATGGCGTCCAGC
ABCG8 Fw 50-GATGCTGGCTATCATAGGGAGC
ABCG8 Rev 50-CTCTGCCTGTGATAACGTCGAG

### Statistical Analysis

Data were normalized to hypoxanthine-guanine phosphoribosyltransferase (HPRT) and quantified using the comparative $C_t$ method. Results are given as means ± S.E., $n = 7$. Intergroup differences were evaluated using ANOVA and Student's *t*-test (Excel, Microsoft Office 2003).

## RESULTS AND DISCUSSION

The experimental diet supplemented with silymarin caused significant up-regulation of ABCG8 transporter mRNA (2.1 ± 0.5 and 4.1 ± 0.6 fold for the low (1%) and high (3%) dose of silymarin, respectively, relative to the HCD value); the results are shown in Figure 1. The HCD (Figure 1) itself elicited significant down-regulation of the mRNA of this transporter (4.6 ± 0.8 fold), relative to the STD value. ABCG5 mRNA (Figure 2.) was significantly induced by silymarin (1.6 ± 0.3 and 1.9 ± 0.2 fold for the low (1%) and high (3%) dose of this extract). The high-cholesterol diet itself also showed significant down-regulation of mRNA (2.6 ± 0.2 fold) of this transporter, as well as of ABCG8. Surprisingly, the mRNA levels of ABCA1 (Figure 3) were significantly up-regulated only after a low dose (1%) of silymarin in the experimental diet (1.3 ± 0.1 fold). Neither a high dose of silymarin

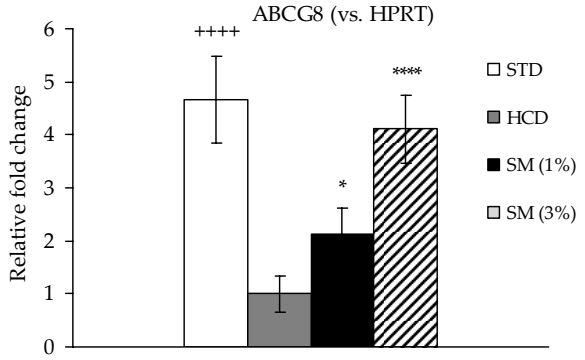


Figure 1. Liver expression of ABCG8 mRNA in rats fed experimental diets

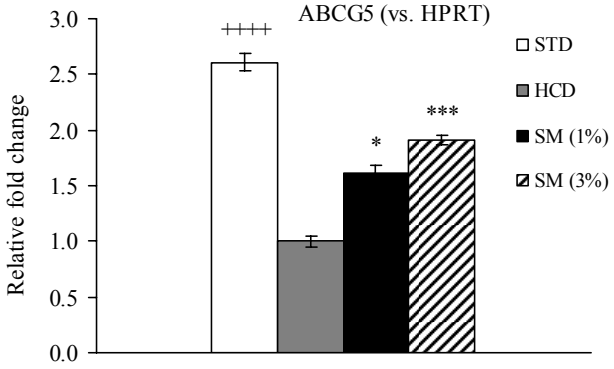


Figure 2. Liver expression of ABCG5 mRNA in rats fed experimental diets

STD = standard diet; HCD = high-cholesterol diet; SM (1% or 3%) = HCD supplemented with silymarin
Data are expressed as the mean ± S.E. derived from seven animals; $^{++++}P < 0.001$ vs. HCD; $^{*}P < 0.05$; $^{****}P < 0.001$ vs. HCD

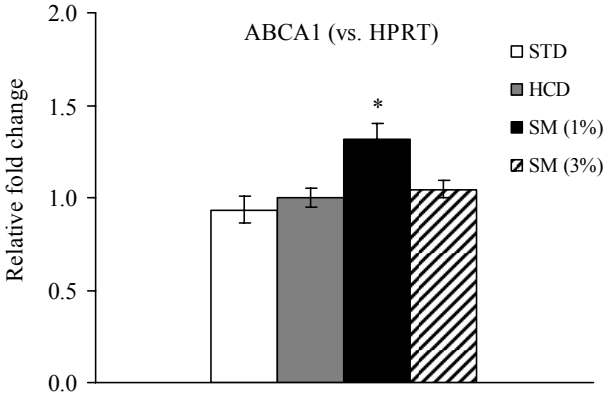


Figure 3. Liver expression of ABCA1 mRNA in rats fed experimental diets

STD = standard diet; HCD = high-cholesterol diet; SM (1% or 3%) = HCD supplemented with silymarin
Data are expressed as the mean ± S.E. derived from seven animals; $^{*}P < 0.05$ vs. HCD

nor HCD itself significantly affected ABCA1 transporter mRNA levels.

The hypolipidemic effects of silymarin are well documented in the literature (Nassuato et al., 1991; Simanek et al., 2001; Lin et al., 2009). In our previous study we demonstrated that a possible explanation for the positive effects of silymarin on lipoprotein profile lies in its lowering of cholesterol absorption in the intestine (Sobolova et al., 2006). The major finding in this study is that silymarin causes up-regulation of the mRNA of the ABC transporters (G5, G8 and A1) which plays an important role in the regulation of lipid metabolism, e.g., intestinal absorption of sterols, biliary cholesterol secretion, or movement of cellular cholesterol into HDL (Oram and Vaugham, 2000; Zhang and Mangelsdorf, 2002). These results suggest that silymarin positively affects the plasma lipoprotein profile not only via reduction of cholesterol absorption from the intestine (Sobolova et al., 2006), but probably via up-regulation of the ABC transporters connected with lipid metabolism.

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