# Effects of Alfalfa Saponins on mRNA Expression of Reverse Cholesterol Transport Genes ATP-binding Cassette Transporter A1 and Scavenger Receptor Class B Type I: Postprint

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## Abstract

This study investigated the effects of alfalfa saponins on the mRNA expression of cholesterol reverse transport genes ATP-binding cassette transporter A1 (ABCA1) and scavenger receptor class B type I (SR-BI) in rat liver and liver cells (BRL cells) as well as mouse macrophages (ANA-1 cells) at both animal and cellular levels. Thirty-two male healthy SD rats were randomly divided into four groups (n=8 per group): normal control group, alfalfa saponin group, high-fat model group, and high-fat alfalfa saponin group. The normal control and alfalfa saponin groups were fed a basal diet, while the other two groups were fed a high-fat diet. After 4 weeks of feeding, the alfalfa saponin and high-fat alfalfa saponin groups received daily gavage of 240 mg/kg alfalfa saponins for 4 weeks. A lipid accumulation model was established by treating BRL cells with fetal bovine serum for 48 h. BRL cells were divided into normal control group (normal cells), alfalfa saponin group (normal cells), lipid accumulation model group (lipid-accumulated cells), and lipid accumulation saponin group (lipid-accumulated cells). Alfalfa saponins were added to the culture medium of the alfalfa saponin and lipid accumulation saponin groups at a final concentration of 300  g/mL, and cells were cultured for 24 h. A lipid-loaded model was established by treating ANA-1 cells with oxidized low-density lipoprotein (ox-LDL) for 48 h. ANA-1 cells were divided into normal control group (normal cells), alfalfa saponin group (normal cells), lipid-loaded model group (lipid-loaded cells), and lipid-loaded saponin group (lipid-loaded cells). Alfalfa saponins were added to the culture medium of the alfalfa saponin and lipid-loaded saponin groups at a final concentration of 300  g/mL, and cells were cultured for 24 h. Fluorescence quantitative PCR was used to determine the mRNA expression levels of ABCA1 and SR-BI in rat liver, BRL cells, and ANA-1 cells. The results

showed that: 1) Alfalfa saponins significantly increased the mRNA expression of ABCA1 and SR-BI in normal rat liver ($P<0.05$), significantly increased ABCA1 mRNA expression in high-fat rat liver ($P<0.05$), but had no significant effect on SR-BI mRNA expression in high-fat rat liver ($P>0.05$); 2) Alfalfa saponins significantly increased the mRNA expression of ABCA1 and SR-BI in normal BRL cells ($P<0.05$), but had little effect on ABCA1 and SR-BI mRNA expression in lipid-accumulated BRL cells ($P>0.05$); 3) Alfalfa saponins significantly decreased ABCA1 mRNA expression in normal ANA-1 cells ($P<0.05$). These findings indicate that alfalfa saponins can promote cholesterol reverse transport and enhance hepatic cholesterol excretion by upregulating the mRNA expression of ABCA1 and SR-BI in rat liver and normal liver cells, thereby exerting preventive and therapeutic effects on hyperlipidemia.

## Full Text

# Effects of Alfalfa Saponins on mRNA Expression of Reverse Cholesterol Transport Genes ATP-Binding Cassette Transporter A1 and Scavenger Receptor Class B Type I

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### Abstract

This study investigated the effects of alfalfa saponins (AS) on mRNA expression of reverse cholesterol transport genes—ATP-binding cassette transporter A1 (ABCA1) and scavenger receptor class B type I (SR-BI)—using rat liver, rat liver cells (BRL cells), and mouse macrophages (ANA-1 cells) as experimental models. Thirty-two healthy male SD rats were randomly divided into four groups (n=8): normal control group, AS group, hyperlipidemic model group, and hyperlipidemic saponins group. Rats in the normal control and AS groups were fed a basal diet, while the other two groups received a high-fat diet. After four weeks, rats in the AS and hyperlipidemic saponins groups were administered AS at 240 mg/kg body weight daily by gavage for four weeks (weeks 5–8). BRL cells were incubated with fetal bovine serum for 48 h to establish a lipid accumulation model, then divided into normal control (normal cells), AS (normal cells), hyperlipidemic model (lipid-laden cells), and hyperlipidemic saponins (lipid-laden cells) groups. AS was added to the culture medium of the AS and hyperlipidemic saponins groups at a final concentration of 300  g/mL for 24 h. ANA-1 cells were incubated with oxidized low-density lipoprotein (ox-LDL) for 48 h to establish a lipid-loaded model, then divided into normal control (normal cells), AS (normal cells), lipid-loaded model (lipid-loaded cells), and lipid-loaded saponins (lipid-loaded cells) groups. AS was added to the culture medium of

the AS and lipid-loaded saponins groups at a final concentration of 300 g/mL for 24 h. mRNA expression levels of ABCA1 and SR-BI in rat liver, BRL cells, and ANA-1 cells were measured by quantitative real-time PCR. The results showed that: (1) AS significantly increased ABCA1 and SR-BI mRNA expression in livers of normal rats ($P<0.05$) and ABCA1 mRNA expression in livers of hyperlipidemic rats ($P<0.05$), but had no significant effect on SR-BI mRNA expression in hyperlipidemic rat livers ($P>0.05$); (2) AS significantly increased ABCA1 and SR-BI mRNA expression in normal BRL cells ($P<0.05$) but showed no significant effects in lipid-laden BRL cells ($P>0.05$); and (3) AS significantly decreased ABCA1 mRNA expression in normal ANA-1 cells ($P<0.05$). These findings demonstrate that AS can promote reverse cholesterol transport and enhance hepatic cholesterol excretion by upregulating ABCA1 and SR-BI mRNA expression in rat liver and normal liver cells, thereby exerting preventive and therapeutic effects against hyperlipidemia.

**Keywords:** alfalfa saponins; BRL cells; ANA-1 cells; rats; reverse cholesterol transport; mRNA expression

## Introduction

With modern societal development and improving living standards, dyslipidemia —particularly elevated blood cholesterol—has emerged as a major risk factor for atherosclerosis and coronary heart disease. Under normal physiological conditions, most cholesterol serves as a structural component of cell membranes, while the remainder is transported via blood to the liver, adrenal glands, ovaries, testes, skin, and other tissues for synthesis into bile acids, hormones, and vitamin D. Cholesterol combines with other lipids and various apolipoproteins to form several types of lipoproteins [1]. Approximately 80% of cholesterol is synthesized in extrahepatic tissues, with only a small portion derived from plasma low-density lipoprotein cholesterol (LDL-C). To prevent cholesterol accumulation in extrahepatic tissues, cholesterol synthesized peripherally is transported back to the liver by high-density lipoprotein (HDL) for conversion to bile acids [2]. HDL plays a crucial role in this reverse transport process, and large-scale clinical trials have confirmed an inverse correlation between HDL levels and cardiovascular disease. HDL metabolism is complex, involving multiple pathways. As a cholesterol receptor, HDL continuously removes cholesterol from cell membranes, leading to efflux of excess intracellular cholesterol. This process is mediated by apolipoprotein A-1 (apoA-1) and scavenger receptor class B type I (SR-BI). Recently, Lu et al. [3] reported a non-receptor-mediated pathway involving ATP-binding cassette transporter A1 (ABCA1) for clearing cholesterol from peripheral tissues. ABCA1 belongs to the ATP-binding cassette (ABC) transporter family, and its encoded protein—called cholesterol efflux regulatory protein (CERP)—participates in cholesterol efflux, facilitating cholesterol transfer to apoA-1 and HDL.

Plant-derived compounds with cholesterol-lowering effects primarily include saponins, ketones, and terpenoids. Saponins possess multiple biological func-

tions and represent important active components for reducing cholesterol levels in plants, making alfalfa saponins a key focus for research and development of lipid-lowering phytopharmaceuticals. Alfalfa saponins are biologically active compounds extracted from alfalfa, consisting of cyclic acetal structures formed by dehydration condensation between hydroxyl groups of sugars or non-sugar compounds. Their structure comprises pentacyclic triterpenoid compounds [4]. Research on alfalfa saponins began abroad in the 1950s, with extensive work on extraction, isolation, and structural identification. Chinese scholars have also made significant progress in recent years. Wang et al. [5] demonstrated that alfalfa saponins enhance hepatic cholesterol excretion by promoting expression of cholesterol 7$\alpha$-hydroxylase (CYP7A1) and low-density lipoprotein receptor (LDL-R), thereby exerting preventive and therapeutic effects on hyperlipidemia. Liang et al. [6] investigated the effects of alfalfa saponins on cholesterol metabolism-related gene expression in rat liver cells (BRL cells) to explore regulatory mechanisms at the cellular level. This study examined the effects of alfalfa saponins on mRNA expression of ABCA1 and SR-BI—key genes involved in reverse cholesterol transport (RCT)—in rat liver, BRL cells, and mouse macrophages (ANA-1 cells) to elucidate the impact and mechanism of alfalfa saponins on RCT and provide a scientific basis for their application in animal production.

## Materials and Methods

### 1.1 Experimental Materials

Alfalfa saponins (Hebei Baoen Company; extracted from alfalfa meal by alcohol extraction and purified by macroporous resin column chromatography; main component identified as alfalfa saponins by thin-layer chromatography; total saponin content 51% by UV spectrophotometry), 32 healthy male SPF-grade SD rats (Henan Experimental Animal Center), automatic biochemical analyzer (HITACHI 7170A, HITACHI), high-speed refrigerated centrifuge (Eppendorf), total cholesterol (TC) assay kit (Beijing Beihua Kangtai Clinical Reagent Co., Ltd.), total bile acid assay kit (Nanjing Jiancheng Bioengineering Institute), microplate reader (Multiskan GO 1.00.40, Thermo), HDL and LDL assay kits (Guangzhou Darske Biotechnology Co., Ltd.), triglyceride (TG) detection kit (Ningbo Meikang Biotechnology Co., Ltd.), rat BRL cells and ANA-1 cells (Cell Resource Center, Shanghai Institute of Life Sciences, Chinese Academy of Sciences), MTT (Solarbio), oxidized low-density lipoprotein (ox-LDL) (Beijing Xiesheng Biotechnology Co., Ltd.), high-glucose DMEM medium (Solarbio), fetal bovine serum (Hangzhou Sijiqing Bioengineering Materials Co., Ltd.), RPMI-1640 medium (Solarbio), trypsin (Solarbio), dimethyl sulfoxide (DMSO) (Sigma), 25 $cm^2$ culture flasks (Corning), 6-well and 96-well plates (Corning), hemocytometer (Shanghai Qiujing Biochemical Reagent and Instrument Co., Ltd.), total RNA extraction reagent (Invitrogen), fluorescent quantitative PCR kit [Toyobo (Shanghai) Biotechnology Co., Ltd.], reverse transcription kit and DNA gel recovery kit [Takara Biotechnology (Dalian) Co., Ltd.], 2×Taq PCR

Master Mix (Beijing Kangwei Century Biotechnology Co., Ltd.), and agarose (Invitrogen).

### 1.2 Animal Experiments

After a one-week acclimation period, 32 healthy male SD rats [initial body weight (191.41$±$16.01) g] were randomly divided into two groups: normal group and hyperlipidemic group, with no statistically significant differences in serum TC content or body weight between groups (P>0.05). A high-fat diet was used to establish the hyperlipidemic model. The normal group received a basal diet, while the hyperlipidemic group received a high-fat diet (composition: 1.0% cholesterol, 0.1% pig bile salt, 10.0% lard, 5.0% egg yolk powder, 5.0% whole milk powder, and 78.9% basal diet) for four weeks.

After model establishment, both the normal and hyperlipidemic groups were each randomly subdivided into two groups based on serum TC content and body weight, yielding four experimental groups: (1) Normal control: basal diet with daily gavage of 2 mL distilled water at 09:00; (2) Alfalfa saponins: basal diet with daily gavage of 240 mg/kg AS in 2 mL at 09:00; (3) Hyperlipidemic model: high-fat diet with daily gavage of 2 mL distilled water at 09:00; and (4) Hyperlipidemic saponins: high-fat diet with daily gavage of 2 mL distilled water for weeks 1–4, then 240 mg/kg AS in 2 mL daily at 09:00 for weeks 5–8. All rats had free access to water and feed.

Sample collection: At the end of the experiment, rats were fasted overnight, anesthetized, and the liver was excised. Liver tissue from the same anatomical region was blotted dry, wrapped in aluminum foil, snap-frozen in liquid nitrogen, and stored at -80°C.

### 1.3 BRL Cell Experiments

**1.3.1 Establishment of Lipid-Laden BRL Cell Model [7]** BRL cells were seeded in 50 mL culture flasks and cultured in high-glucose DMEM medium containing 10% fetal bovine serum at 37°C in a 5% $CO_2$ incubator. Upon reaching confluence, cells were trypsinized, counted, and seeded in 6-well plates at 1.5$×10^{5}$ cells/well. After reaching 80% confluence, the medium was replaced with fresh medium containing 50% fetal bovine serum, and cells were cultured for 24–48 h until intracellular lipid droplets or foam-like deposits appeared, indicating successful establishment of the lipid-laden cell model. Cells were then maintained in high-glucose DMEM medium containing 0.1% fetal bovine serum for 24 h before subsequent treatments.

**1.3.2 Experimental Groups** Cells were cultured in 6-well plates and divided into four groups (n=6 replicates/group): (1) Normal control: 2.9 mL high-glucose DMEM with 10% fetal bovine serum for 24 h, medium change, then 48 h additional culture, followed by 24 h in 0.1% serum medium, then 100 L fresh medium for 24 h; (2) Alfalfa saponins: same culture protocol but with addition

of 100 L AS solution (300 g/mL, final concentration 100 g/mL) for the final 24 h; (3) Lipid-laden model: culture with 10% serum medium for 24 h, then 50% serum medium for 48 h, followed by 24 h in 0.1% serum medium, then 100 L fresh medium for 24 h; and (4) Lipid-laden saponins: same as lipid-laden model but with addition of 100 L AS solution (300 g/mL, final concentration 100 g/mL) for the final 24 h.

### 1.4 ANA-1 Cell Experiments

ANA-1 cells were seeded in 25 $cm^2$ flasks with RPMI-1640 medium containing 10% fetal bovine serum and cultured at 37°C in a 5% $CO_2$ incubator. Fresh medium was changed regularly, and cell morphology was monitored microscopically. When cell density reached 2$×10^{6}$ cells/mL, the medium was replaced.

**1.4.1 Establishment of Lipid-Loaded ANA-1 Cell Model** Prior to the experiment, cells were cultured in serum-free RPMI-1640 medium for 12 h to induce quiescence. Lipid-loaded ANA-1 cells were established by treatment with 50 mg/L ox-LDL for 48 h.

**1.4.2 Experimental Groups** Cells were cultured in 6-well plates and divided into four groups (n=6 replicates/group): (1) Normal control: 2.9 mL RPMI-1640 with 10% fetal bovine serum for 48 h, then 24 h in 0.1% serum medium, followed by 100 L fresh medium for 24 h; (2) Alfalfa saponins: same protocol but with addition of 100 L AS solution (300 g/mL, final concentration 100 g/mL) for the final 24 h; (3) Lipid-loaded model: 2.8 mL RPMI-1640 with 10% fetal bovine serum plus 100 L ox-LDL (1.45 g/ L, final concentration 50 mg/L) for 48 h, then 24 h in 0.1% serum medium, followed by 100 L fresh medium for 24 h; and (4) Lipid-loaded saponins: same as lipid-loaded model but with addition of 100 L AS solution (300 g/mL, final concentration 100 g/mL) for the final 24 h.

### 1.5 MTT Assay and Gene Expression Analysis

**1.5.1 MTT Assay for Cell Viability** Cell suspensions (2$×10^{4}$ cells/mL) were seeded in 96-well plates (100 L/well) and cultured for 24 h. Various AS concentrations (0, 50, 100, 200, and 250 g/mL) were added (n=6 replicates/group). After 24 h incubation at 37°C in 5% $CO_2$, supernatants were removed, 90 L fresh medium and 10 L MTT were added, and cells were incubated for an additional 4 h. Then 150 L DMSO was added per well, plates were shaken for 10 min, and absorbance (OD) was measured at 490 nm using a microplate reader, with cell-free wells as blanks.

**1.5.2 Determination of Relative mRNA Expression 1.5.2.1 Total RNA Extraction**: Total RNA was extracted from liver tissue and cells using the Trizol method and stored at -80°C.

**1.5.2.2 RNA Concentration Measurement**: RNA concentration was determined using a Thermo micro-UV spectrophotometer at 260 nm, with $OD_{260}/OD_{280}$ ratios of 1.8–2.0 considered acceptable.

**1.5.2.3 RNA Quality Assessment**: RNA integrity was evaluated by 1% agarose gel electrophoresis.

**1.5.2.4 Reverse Transcription**: cDNA was synthesized using the Reverse Transcriptase M-MLV (RNase H-) kit (Takara) according to the manufacturer' s instructions.

**1.5.2.5 Primer Design**: Primers were designed using Primer 5.0 software based on GenBank sequences for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ABCA1, SR-BI, and mouse $\beta$-actin, ABCA1, SR-BI. Primer sequences and parameters are shown in .

**1.5.2.6 Quantitative Real-Time PCR**: SYBR qPCR Mix was used as the fluorescent dye. The optimized reaction system (10  L total) contained: SYBR-qPCR Mix 5  L, DEPC-treated water 3.8  L, template cDNA 1  L, and 0.1  L each of forward and reverse primers. Cycling conditions were: 95°C for 2 min; 40 cycles of 95°C for 15 s, 60°C for 20 s, and 72°C for 30 s; and final hold at 20°C for 10 min. Data were analyzed using the 2^(-ΔΔCt) method.

### 1.6 Statistical Analysis

Data were analyzed using SPSS 13.0 software by one-way ANOVA, with Duncan' s multiple range test for intergroup comparisons. Significance was set at $P<0.05$. Results are expressed as means ± standard deviation.

## Results

### 2.1 Effects of Alfalfa Saponins on BRL and ANA-1 Cell Viability

As shown in , BRL cell viability did not differ significantly from controls at AS concentrations of 50 and 100  g/mL, but increased significantly at 200 and 250  g/mL ($P<0.05$). ANA-1 cell viability showed no significant differences from controls at any AS concentration ($P>0.05$), although a decreasing trend was observed at concentrations above 100  g/mL. Therefore, 100  g/mL was selected as the non-toxic dose for subsequent experiments.

### 2.2 Effects of Alfalfa Saponins on Hepatic ABCA1 and SR-BI mRNA Expression in Rats

shows that compared with the normal control group, ABCA1 mRNA expression was significantly increased in the AS, hyperlipidemic model, and hyperlipidemic saponins groups (8.34-fold, 3.86-fold, and 9.57-fold increases, respectively; $P<0.05$). SR-BI mRNA expression was also significantly elevated in the AS group (12.07-fold; $P<0.05$) but showed smaller increases in the hyperlipidemic model and hyperlipidemic saponins groups (2.77-fold and 2.94-fold,

respectively). Compared with the hyperlipidemic model group, the hyperlipidemic saponins group showed significantly higher ABCA1 mRNA expression ($P<0.05$) but no significant change in SR-BI mRNA expression ($P>0.05$).

### 2.3 Effects of Alfalfa Saponins on ABCA1 and SR-BI mRNA Expression in BRL Cells

As shown in , ABCA1 mRNA expression was significantly increased in the AS, lipid-laden model, and lipid-laden saponins groups compared with normal controls (5.97-fold, 1.46-fold, and 1.47-fold increases, respectively; $P<0.05$), with no significant difference between the lipid-laden model and lipid-laden saponins groups ($P>0.05$). SR-BI mRNA expression was significantly increased in the AS group (1.69-fold; $P<0.05$) but significantly decreased in the lipid-laden model and lipid-laden saponins groups (0.17-fold and 0.35-fold of control, respectively; $P<0.05$). The lipid-laden saponins group showed a slight but non-significant increase in SR-BI mRNA compared with the lipid-laden model group ($P>0.05$).

### 2.4 Effects of Alfalfa Saponins on ABCA1 and SR-BI mRNA Expression in ANA-1 Cells

demonstrates that ABCA1 mRNA expression was significantly decreased in the AS, lipid-loaded model, and lipid-loaded saponins groups compared with normal controls (0.59-fold, 0.24-fold, and 0.26-fold of control, respectively; $P<0.05$). SR-BI mRNA expression showed a non-significant decrease in the AS group (0.60-fold; $P>0.05$) but was significantly increased in the lipid-loaded model and lipid-loaded saponins groups (4.56-fold and 4.07-fold, respectively; $P<0.05$). No significant differences were observed between the lipid-loaded model and lipid-loaded saponins groups for either gene ($P>0.05$).

## Discussion

Blood lipids comprise various lipid components including cholesterol, triglycerides (TG), phospholipids (PL), and free fatty acids (FFA). Our previous research demonstrated that alfalfa saponins can reduce serum TC, TG, and LDL-C levels in hyperlipidemic rats, indicating significant lipid-lowering effects [8].

The anti-atherosclerotic action of HDL is primarily based on its participation in reverse cholesterol transport (RCT). Alfalfa saponins exhibit hemolytic activity, causing red blood cell lysis even at low concentrations when injected intravenously. This hemolytic effect is thought to involve interactions between saponins and cholesterol in red blood cell membranes. We used the MTT assay to assess AS effects on BRL and ANA-1 cell viability to determine appropriate non-toxic concentrations for subsequent experiments. The results showed that AS concentrations below 100  g/mL had no significant effect on BRL cell viability, while ANA-1 cell viability was not significantly affected at any tested concentration, although a decreasing trend was observed above 100  g/mL. Therefore, 100  g/mL was selected as the experimental dose.

Cholesterol homeostasis—encompassing uptake, synthesis, and efflux—is critical for maintaining membrane integrity and fundamental cellular activities. RCT describes the process by which nascent discoidal HDL extracts excess cholesterol from peripheral cells (including arterial wall cells). This cholesterol is esterified by lecithin cholesterol acyltransferase (LCAT) in plasma, converting free cholesterol to cholesteryl esters that migrate to the HDL core, ultimately forming spherical mature HDL particles that transport cholesterol to the liver for excretion primarily as bile acids [9-10]. This process prevents foam cell formation and represents a key anti-atherosclerotic mechanism of HDL. Three cholesterol efflux pathways have been identified: the apoA-1/ABCA1 pathway, the SR-BI pathway, and aqueous diffusion. This study examined two key RCT genes—ABCA1 and SR-BI—to investigate the regulatory mechanisms of alfalfa saponins at both organismal and cellular levels.

ABCA1, a member of the ABC transporter superfamily, promotes intracellular cholesterol efflux and participates in RCT to remove excess tissue cholesterol [11]. As a newly identified key factor initiating cholesterol efflux and HDL formation, ABCA1 mediates the first and rate-limiting step of RCT, critically influencing lipid metabolism and atherosclerosis development. Studies in ABCA1-overexpressing transgenic mice have shown increased serum HDL, decreased LDL, and accelerated cholesterol efflux [12-14]. ABCA1 also plays an important role in clearing excess cholesterol from macrophages to prevent atherosclerotic progression [15]. Our results showed that AS significantly increased ABCA1 mRNA expression in both normal and hyperlipidemic rat livers and in normal BRL cells, suggesting that AS enhances RCT by upregulating ABCA1 expression. However, AS had minimal effects on ABCA1 mRNA in lipid-laden BRL cells, suggesting that AS may exert compensatory rather than direct effects on hepatic ABCA1, warranting further mechanistic investigation. The significant decrease in ABCA1 mRNA in normal ANA-1 cells and minimal changes in lipid-loaded ANA-1 cells indicate that AS does not promote RCT in macrophages. Collectively, these findings suggest that AS primarily enhances RCT in hepatocytes rather than macrophages.

SR-BI, a member of the CD36 superfamily, is predominantly expressed in the liver and steroidogenic tissues (adrenal glands, ovaries, testes) where it facilitates selective HDL-cholesterol uptake. SR-BI content is high in tissues that selectively take up HDL-C, with lower expression in other tissues. As an HDL receptor with multiple ligand-binding sites, SR-BI mediates selective cholesterol ester uptake upon HDL binding. HDL efflux rate correlates positively with cellular SR-BI mRNA expression. Studies have shown that hepatic SR-BI overexpression reduces plasma HDL-C while increasing biliary cholesterol, whereas SR-BI knockout increases circulating TC, particularly HDL-C [16]. Our results indicate that AS primarily affects SR-BI mRNA expression in normal rat liver and normal BRL cells, with minimal effects in hyperlipidemic rats and lipid-laden BRL cells. This suggests that AS regulates SR-BI expression in normal hepatocytes but has limited effects on lipid-laden cells, indicating that blood lipid levels influence cholesterol metabolism and should be considered when

evaluating AS effects. The minimal impact of AS on SR-BI mRNA in both normal and lipid-loaded ANA-1 cells suggests no significant role in regulating SR-BI-mediated RCT in macrophages, requiring further investigation [17].

In conclusion, alfalfa saponins promote reverse cholesterol transport and enhance hepatic cholesterol excretion by upregulating ABCA1 and SR-BI mRNA expression in rat liver and normal liver cells, thereby exerting preventive and therapeutic effects against hyperlipidemia.

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*Note: Figure translations are in progress. See original paper for figures.*