Stanol esters decrease plasma cholesterol independently of intestinal ABC sterol transporters and Niemann-Pick C1-like 1 protein gene expression

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Abstract **Possible mechanisms for the cholesterol-lowering effects of plant stanol esters were addressed by feeding hamsters diets containing stanol esters, cholesterol, or cholestyramine/lovastatin. ABCA1, ATP binding cassette G1 (ABCG1), ABCG5, ABCG8, and Niemann-Pick C1-like 1 (NPC1L1) mRNA levels were then estimated in duodenum, jejunum, and ileum. Plasma cholesterol was decreased by 36% and 94% in animals fed stanol esters and cholestyramine/lovastatin, respectively. Cholesterol feeding increased plasma cholesterol by 2.5-fold. Plasma plant sterols were unchanged by stanol ester feeding but became undetectable by feeding cholestyramine/lovastatin. Cholesterol and stanols accumulated in enterocytes of animals fed cholesterol and stanol esters, respectively. ABCG5 and ABCG8 mRNA levels were decreased by stanol esters and cholestyramine/lovastatin. Cholesterol feeding markedly increased ABCA1 and ABCG1 expression and modestly increased ABCG5/ABCG8. NPC1L1 mRNA was not significantly altered by any of the diets. ABCG1, ABCG5, ABCG8, and NPC1L1 mRNAs were highest in cells of the upper villus, whereas ABCA1 mRNA was highest in cells of the lower villus. The results suggest that cholesterol lowering effect of stanol esters is unrelated to changes in mRNA levels of intestinal ABC sterol transporters or NPC1L1. Cholesterol flux regulates ABC expression but not NPC1L1. The different localization of ABCA1 suggests a different function for this protein than for ABCG1, ABCG5, ABCG8, and NPC1L1.**—Field, F. J., E. Born, and S. N. Mathur. **Stanol esters decrease plasma cholesterol independently of intestinal ABC sterol transporters and Niemann-Pick C1-like 1 protein gene expression.** ***J. Lipid Res.*** **2004.** 45: **2252–2259.**

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Dietary sterols derived from plants decrease plasma cholesterol levels (1, 2). Mechanisms for why plant sterols should do this have been debated (for review, see Ref. 3). Most evidence, however, points to an effect at the level of the intestine leading to a decrease in cholesterol absorption (4–7). In the lumen of the intestine, plant sterols compete with cholesterol for space within bile salt micelles. Without micellar solubilization, cholesterol is poorly absorbed, if at all. By displacing cholesterol from the micelle, plant sterols impede the absorption of cholesterol (8–10). Because dietary cholesterol contributes to LDL cholesterol and decreasing LDL cholesterol is proven to be "cardioprotective," the food industry is taking advantage of this property by packaging plant sterols into a variety of different foodstuffs.

Recommending the ingestion of plant sterols would not be a sensible way to decrease plasma cholesterol and thus, the risk of atherosclerosis, if plant sterols themselves were readily absorbed. Fortunately, this is not the case. Despite structurally differing from cholesterol by only a methyl (campesterol) or an ethyl (β-sitosterol) group attached to the C-24 position of the side chain, these major plant sterols are absorbed to a much lesser extent than cholesterol (for reviews, see Refs. 11, 12). The reasons for this are not clear. Recently, mutations in the tandem genes ATP binding cassette G5 (Abcg5) and Abcg8 were shown to cause the rare autosomal recessive disorder β-sitosterolemia (13, 14). Individuals with this disorder have impaired biliary secretion of sterols, "hyperabsorb" sterols, have increased plasma and tissue levels of plant sterols, and have an increased risk of atherosclerosis. It is speculated that in the intestine, the proteins encoded by these genes function as sterol transporters, facilitating the efflux of absorbed plant and animal sterols into the lumen of the gut (13). Although an attractive hypothesis, this has not been demonstrated at the cellular level.

There has been a suggestion that plant sterols enhance the gene expression of intestinal ABCA1, another sterol

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transporter that has been implicated in facilitating the efflux of cholesterol at the basolateral membrane of intestinal cells (15). If dietary plant sterols also enhance gene expression of intestinal ABCG5 and ABCG8 or ABCA1, this could theoretically explain why plant sterols interfere with the absorption of cholesterol and why plant sterol absorption is inefficient.

Another cholesterol transporter, Niemann-Pick C1-like 1 (NPC1L1) protein, was recently described and found to be critical for the normal absorption of cholesterol and phytosterols (16, 17). The expression of its gene was highest in jejunum, and the protein was enriched in jejunal brush border membranes. In NPC1L1-null mice, cholesterol and sitosterol absorption were markedly attenuated, leading to reduced levels of plasma phytosterols. It has yet to be described whether NPC1L1 gene expression is regulated by plant sterols.

In the present study, we asked if a diet enriched in stanol esters would enhance the gene expression of intestinal ABC transporters as a potential mechanism to limit cholesterol and plant sterol absorption. Because the newly described NPC1L1 protein may be the putative intestinal sterol transporter, we also investigated whether stanol esters might attenuate its expression. Stanol esters, saturated derivatives of plant sterols esterified to rapeseed fatty acids, were studied because they are being packaged in margarines and sold as capsules (Benecol®) for their cholesterol-lowering effects. In addition to the effects of stanol esters, we studied whether changes in cholesterol flux across the intestine would alter the gene expression of ABC transporters or NPC1L1 in hamster intestine. The results suggest that the cholesterol-lowering effect of stanol esters is not mediated by enhanced gene expression of intestinal ABCG5, ABCG8, ABCA1, or ABCG1, nor by the decreased expression of NPC1L1. Whereas changes in cholesterol flux did not alter NPC1L1 mRNA levels, gene expression of intestinal ABC transporters was increased by cholesterol influx and decreased by "cholesterol depletion."

MATERIALS AND METHODS

TriReagent was purchased from Sigma (St. Louis, MO). RNase-free DNase was purchased from Promega Corp. (Madison, WI). Superscript III RNase H$^-$ reverse transcriptase was obtained from Invitrogen Life Technologies (Carlsbad, CA). SYBR Green PCR mix was purchased from Applied Biosystems (Foster City, CA). Benecol® soft gels containing stanol esters were from McNeil Nutritionals (Fort Washington, PA). Lovastatin was a gift from Merck (Rahway, NJ). Cholestyramine was obtained from Bristol-Myers Squibb Co. (Princeton, NJ).

Animals and diet

Male Golden Syrian hamsters weighing 90–120 g were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). They were maintained for 1 week on NIH-31 modified mouse/rat diet 7013 (Harlan/Teklad, Madison, WI) before starting the test diets. The animals were fed the test diets ad libitum, and the light cycle was from 6:00 AM to 6:00 PM. The control chow diet was prepared by adding 1% olive oil to Teklad 4% mouse/rat meal 7001. The 2% stanol esters or cholesterol diets were prepared by dissolving the sterols in the olive oil and thoroughly mixing with the control chow diet. To prepare the "cholesterol-depletion" diet, control chow was supplemented with 4% (w/w) cholestyramine and 0.15% (w/w) lovastatin (dissolved in olive oil). The start of the dietary period was staggered so that all hamsters were fed the respective diets for exactly 14 days.

Sterol analysis of plasma and intestinal cells

Intestinal cells were homogenized in phosphate-buffered saline, pH 7.0. Protein content in the homogenate was determined using the BCA method (Pierce Endogen, Rockford, IL). A 0.4 ml sample of intestinal cells or 0.05 ml of plasma was taken and mixed with 0.1 ml of 10 N NaOH, 1.5 ml of methanol, and 25 μg of cholestane as an internal standard. This mixture was kept in boiling water for 1 h to hydrolyze sterol esters and other lipids. After the addition of 1 ml of water, the sterols were extracted twice with 3 ml of hexane. Cholesterol and noncholesterol sterols were then separated by gas-liquid chromatography on a Supelco 3% SP-2100 on a 100/120 Supelcoport column using a Hewlett-Packard 5790A gas chromatograph. The oven was set at 275°C, and the run time was 20 min.

Isolation of enterocytes

Cells along the villus axis were isolated from duodenum, jejunum, and ileum of hamster intestine exactly as described by us previously (18).

Estimation of mRNA levels by real-time RT-PCR

RNA was extracted from intestinal cells using TriReagent. Two micrograms of RNA was treated with RNase-free DNase to remove contaminating genomic DNA. After inactivating the DNase by the addition of EGTA and heating at 65°C for 10 min, the RNA was transcribed to cDNA by SuperScript III RNase H$^-$ reverse transcriptase using random hexamers at 24°C for 10 min and 50°C for 4 h. After stopping the reaction by incubation at 90°C for 15 min, 4% of the RT mixture was mixed with 2× SYBR green PCR master mix and gene-specific primers in a final volume of 20 μl. The primers were designed with Primer Express software. The primer pairs were selected to yield a single amplicon based on dissociation curves (**Fig. 1**) and analysis by acrylamide gel electrophoresis. **Table 1** provides the sequences of the primers used for each gene. Because published mouse mRNA sequences were used to design primers for hamster ABCA1, ABCG1, ABCG5, ABCG8, and NPC1L1, the hamster PCR products were cloned in a plasmid, pCR4-TOPO (Invitrogen), and sequenced. The percentage homology observed between hamster and mouse sequences is listed in Table 1 as well. The sequences were analyzed by BLAST (National Center for Biotechnology Information, National Institutes of Health) to verify that the primers used were specific for the given gene. Real-time quantitative PCR was performed in a model ABI Prism 7000 sequence detection system. The thermal cycler parameters were as follows: hold for 2 min at 50°C and 10 min at 95°C for one cycle followed by amplification of cDNA for 40 cycles with melting for 15 s at 95°C and annealing and extension for 1 min at 60°C. The values were normalized using 18S rRNA as an endogenous internal standard. A serial dilution of a standard was run on each plate for each mRNA and used to calculate the relative levels of mRNA.

Statistical analysis

To determine whether significant differences existed among dietary groups, the data were analyzed at $P < 0.05$ by one-way ANOVA and the Tukey's procedure, using SigmaStat software (SPSS, Chicago, IL).

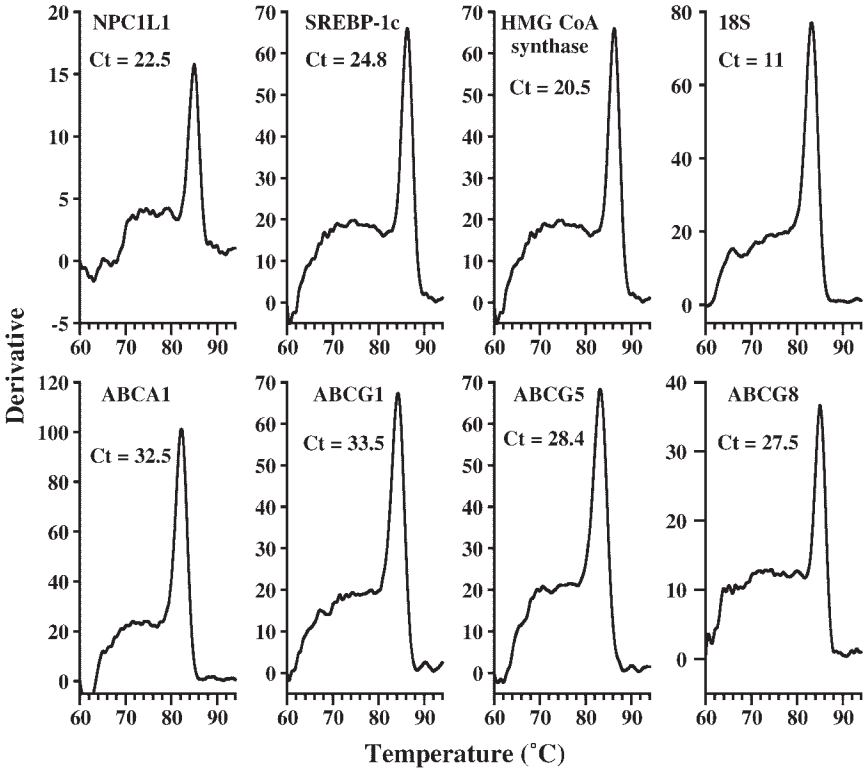

Fig. 1. In hamster enterocytes, quantitative RT-PCR for a given mRNA yields a single amplicon. The Ct value represents the threshold cycle number for the gene. ABCG1, ATP binding cassette G1; NPC1L1, Niemann-Pick C1-like 1; SREBP-1c, sterol-regulatory element binding protein-1c.

RESULTS

Effect of diets on tissue and plasma sterol levels

To address the effect of stanol esters and cholesterol flux on plasma and intestinal sterol levels, hamsters were fed the following diets for 2 weeks: control rat chow; chow containing 2% stanol esters (Benecol®); chow containing 2% cholesterol; or chow containing 4% cholestyramine and 0.15% lovastatin. After the dietary period, plasma was collected and analyzed by gas-liquid chromatography for cholesterol and noncholesterol sterols (collectively called plant sterols). Intestines were removed and divided into three equal segments representing duodenum, jejunum, and ileum. Sterol content was estimated by gas-liquid chromatography in isolated cells prepared from the individual intestinal segments.

TABLE 1. Real time RT-PCR primers

Gene Name	Sequence	Homology to Mouse	PCR Product	Accession Number or Reference
		%	*bp*	
ABCA1	5′-ATAGCAGGCTCCAACCCTGAC-3′ 5′-GGTACTGAAGCATGTTTCGATGTT-3′	97	103	Mouse NM_013454
ABCG1	5′-GGGATCAGAACAGTCGCCTG-3′ 5′-CGAGGTCTCTCTTATAGTCAGCGTC-3′	95	72	Mouse AF323659
ABCG5	5′-TGATTGGCAGCTATAATTTTGGG-3′ 5′-GTTGGGCTGCGATGGAAA-3′	100	69	Mouse AF312713
ABCG8	5′-TGCTGGCCATCATAGGGAG-3′ 5′-TCCTGATTTCATCTTGCCACC-3′	94	92	Mouse AF324495
SREBP-1c	5′-GCGGACGCAGTCTGGG-3′ 5′-ATGAGCTGGAGCATGTCTTCAAA-3′	NA	95	Hamster (18)
HMG-CoA synthase	5′-CCTATGACTGCATTGGGCG-3′ 5′-CCCAGACTCCTCAAACAGCTG-3′	NA	101	Hamster L00326
NPC1L1	5′-CCTGACCTTTATAGAACTCACCACAGA-3′ 5′-GGGCCAAAATGCTCGTCAT-3′	95	102	Mouse AY437866
18S	5′-TAAGTCCCTGCCCTTTGTACACA-3′ 5′-GATCCGAGGGCCTCACTAAAC-3′	NA	71	Hamster M33069

ABCG1, ATP binding cassette G1; NA, not applicable as primers were designed from hamster sequence; NPC1L1, Niemann-Pick C1-like 1; SREBP-1c, sterol-regulatory element binding protein-1c.

In hamsters fed diets enriched in stanol esters, plasma cholesterol levels were significantly decreased by 36%, 70 versus 109 mg/dl (**Table 2**). Adding cholesterol to the chow diet increased plasma cholesterol levels by 2.5-fold to 276 mg/dl. In animals ingesting cholestyramine and lovastatin, plasma cholesterol levels were markedly reduced to 6 mg/dl. Plasma plant sterols in hamsters fed control chow were ~1% of the levels of plasma cholesterol, 1.2 mg/dl. Compared with controls, similar plasma plant sterol levels were observed in hamsters fed either stanol esters or cholesterol. In contrast, plant sterols were essentially undetectable in plasma of animals ingesting cholestyramine and lovastatin. Thus, similar to observations in humans, plant sterols in hamsters constitute a small fraction of total plasma sterols and adding stanol esters to the diet decreases plasma cholesterol levels. Unlike the marked increase observed in plasma cholesterol by adding cholesterol to the diet, adding similar amounts of stanol esters did not increase plasma levels of the plant sterol. This finding suggests that, like humans, hamsters do not absorb stanols. Depriving the gut of bile acids and suppressing cholesterol synthesis markedly diminishes the absorption of both plant and animal sterols.

The total cholesterol content of intestinal cells isolated from duodenum, jejunum, and ileum of animals ingesting the diet enriched in stanol esters was not different from that in controls. In contrast, there was a significant accumulation of cholesterol in intestinal cells of hamsters ingesting the cholesterol-enriched diet, particularly in the jejunum and ileum. A decrease in cholesterol was found in intestinal cells of animals ingesting cholestyramine and lovastatin. Small amounts of plant sterols were detected in intestines of animals fed control chow. The amount of plant sterols in intestinal cells increased down the length of the gut, being highest in ileum. Adding stanol esters to the diet caused a modest but significant accumulation of plant sterols along the entire length of the intestine. In contrast, plant sterols in intestinal cells of hamsters fed the cholesterol or cholestyramine and lovastatin diets were essentially undetectable. These findings suggest that absorptive cells of the small intestine take up small amounts of plant stanols from the diet. In animals ingesting a cholesterol-enriched diet, it is likely that excess cholesterol in the lumen competes with plant sterols for uptake, thus decreasing the amount found in intestinal cells. Lack of bile acids in the lumen diminishes the uptake of plant sterols by the intestine.

TABLE 2. Effect of diet on tissue and plasma sterol levels

Diet	Duodenum	Jejunum	Ileum	Plasma
	μg/mg protein			*mg/dl*
Cholesterol				
Control	28 ± 1	28 ± 1	22 ± 1	109 ± 3
Stanol esters	25 ± 3	27 ± 3	24 ± 2	70 ± 7[a]
Cholesterol	42 ± 2[a,b]	77 ± 6[a,b]	127 ± 3[a,b]	276 ± 6[a,b]
Cholestyramine plus lovastatin	19 ± 1[a,c]	19 ± 1[a,c]	19 ± 1[c]	6 ± 1[a,b,c]
Plant sterols				
Control	0.9 ± 0.2	1.5 ± 0.1	2.1 ± 0.1	1.2 ± 0.2
Stanol esters	1.5 ± 0.1[a]	4.4 ± 1.0[a]	3.8 ± 0.7[a]	0.7 ± 0.3
Cholesterol	0.0 ± 0.0[a,b]	0.1 ± 0.0[a,b]	0.2 ± 0.1[a,b]	1.2 ± 0.6
Cholestyramine plus lovastatin	0.1 ± 0.0[a,b]	0.0 ± 0.0[a,b]	0.0 ± 0.0[a,b]	0.1 ± 0.1[a,c]

Cholesterol and noncholesterol (plant) sterols in intestinal cells and plasma were estimated by gas-liquid chromatography as described in Materials and Methods. Each value represents the mean ± SEM of six animals.

[a] $P < 0.05$ compared with the control diet.

[b] $P < 0.05$ compared with the stanol esters diet.

[c] $P < 0.05$ compared with the cholesterol diet.

Effect of diets on ABC gene expression

After the dietary period, RNA was extracted from intestinal cells isolated from duodenum, jejunum, and ileum. mRNA levels for the ABC transporters ABCA1, ABCG1, ABCG5, and ABCG8 were estimated by quantitative RT-PCR. To document changes in intestinal cholesterol flux (cellular sterol levels do not necessarily represent flux), HMG-CoA synthase and sterol-regulatory element binding protein-1c (SREBP-1c) mRNA levels were also estimated. In response to an increase in cellular cholesterol flux, HMG-CoA synthase and SREBP-1c mRNA levels decreased and increased, respectively. The reverse happened in response to cholesterol depletion.

In hamsters fed a control chow diet, ABCA1 and ABCG1 mRNA levels were significantly higher in ileum compared with duodenum (**Fig. 2**). In contrast, mRNA levels of ABCG5 and ABCG8 did not significantly differ down the length of the intestine. Compared with intestines of control animals, mRNA levels of ABCA1 or ABCG1 were not significantly altered by the ingestion of diets enriched in stanol esters. Both ABCG5 and ABCG8 gene expression, however, was modestly decreased in intestines of these animals.

Enriching the diet in cholesterol significantly increased ABCA1 and ABCG1 expression in all three segments. In addition, mRNA levels of ABCG5 in jejunum and ileum and ABCG8 in duodenum were also increased in hamsters ingesting cholesterol. Neither ABCG5 nor ABCG8 mRNA levels, however, were increased by cholesterol influx to the extent observed with ABCA1 or ABCG1. Compared with controls, expression of ABCG1, ABCG5, and ABCG8 was markedly decreased in animals ingesting cholestyramine and lovastatin. In contrast, ABCA1 expression was similar to that in control animals. NPC1L1 mRNA levels were highest in jejunum and lowest in duodenum. NPC1L1 gene expression was not altered by feeding stanol esters, cholesterol, or cholestyramine and lovastatin. Gene expression of SREBP-1c was increased in all three intestinal segments of animals ingesting cholesterol and was markedly decreased in animals ingesting cholestyramine and lovastatin. In hamsters fed stanol esters, SREBP-1c mRNA levels were decreased in jejunum and ileum. mRNA levels of HMG-CoA synthase were dramatically increased in all three segments by feeding cholestyramine and lovastatin. In hamsters fed cholesterol, HMG-CoA synthase expression was modestly decreased in jejunum and ileum. The addition of stanol esters to the diet, however, did not significantly alter HMG-CoA synthase mRNA levels. These results suggest that supplemental dietary stanol esters do not enhance the gene expression of intestinal ABC transporters, nor do they suppress the expression of NPC1L1.

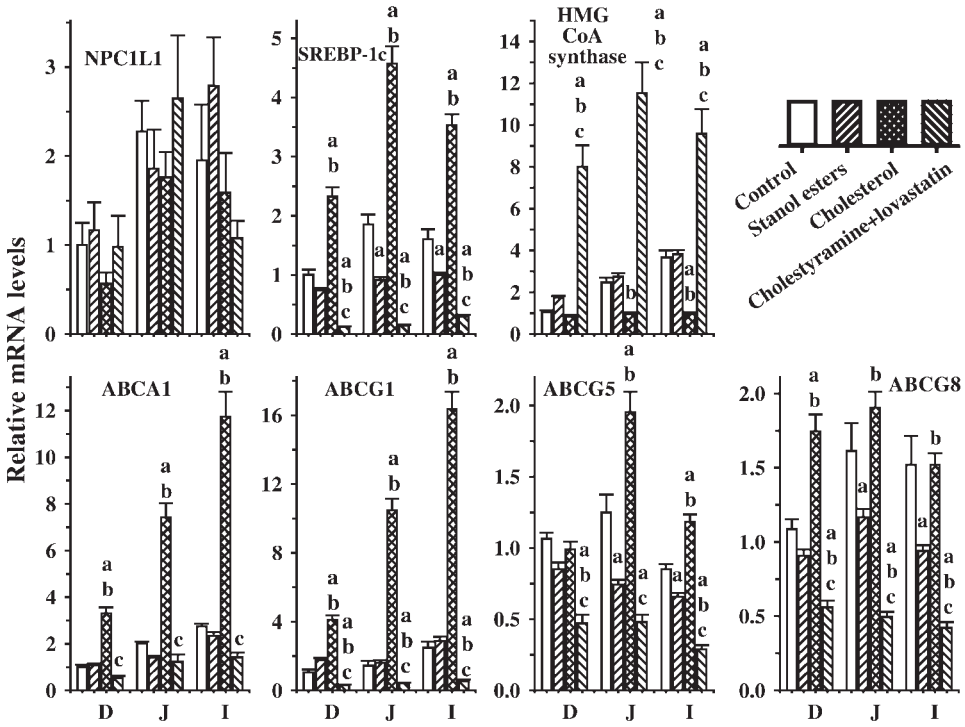

Fig. 2. Effect of diet on ABCA1, ABCG1, ABCG5, ABCG8, NPC1L1, SREBP-1c, or HMG-CoA synthase gene expression in duodenum (D), jejunum (J), and ileum (I). The total enterocyte population from the three segments was isolated, and mRNA levels were estimated as described in Materials and Methods. The values were normalized using 18S rRNA as an endogenous internal standard. The data represent means ± SEM of six hamsters on each diet. [a] $P < 0.05$ compared with the control diet. [b] $P < 0.05$ compared with the stanol esters diet. [c] $P < 0.05$ compared with the cholesterol diet.

Increased cholesterol influx, as indicated by an increase in SREBP-1c and a decrease in HMG-CoA synthase expression, enhanced intestinal ABC mRNA levels, particularly ABCA1 and ABCG1. Decreasing cholesterol flux suppressed gene expression of ABCG transporters without altering ABCA1 expression. In contrast, NPC1L1 expression was unaltered by changes in cholesterol flux.

To address the localization of the ABC transporters in cells along the villus axis, we sequentially isolated villus cells of duodenum, jejunum, and ileum from hamsters fed the cholesterol or cholestyramine and lovastatin diets. These dietary groups were chosen for analysis because they demonstrated the largest changes in the expression of these genes. Cell fractions 1 and 2, 3 and 4, and 5 represent cells from the upper, mid, and lower villus, respectively. In cholesterol-fed animals, ABCG1, ABCG5, ABCG8, and NPC1L1 mRNA levels were highest in cells of the upper villus, and their expression decreased progressively as cells of the lower villus were reached (**Fig. 3**). In contrast, ABCA1 mRNA levels had the opposite pattern. In cholesterol-fed hamsters, ABCA1 mRNA abundance was highest in cells of the lower villus and least in cells of the upper villus. Because of lower levels of expression in animals ingesting cholestyramine and lovastatin, the trends were not as evident. In each intestinal cell fraction of animals ingesting cholestyramine/lovastatin, ABCG1, ABCG5, and ABCG8 mRNA levels were lower than in animals ingesting cholesterol.

DISCUSSION

It is clear from the results of this study that dietary stanol esters decrease plasma cholesterol by a mechanism that is independent of enhancing gene expression of intestinal ABC transporters. Ingestion of stanol esters did not alter ABCA1 or ABCG1 mRNA levels and caused a modest decrease in ABCG5/ABCG8 gene expression. These results, therefore, do not support a role for transcriptional regulation of intestinal ABC transporters in the cholesterol-lowering effect of plant stanols. Similar results were observed in mice fed a potent inhibitor of cholesterol absorption, an ezetimibe analog. In that study, both intestinal ABCG5/ABCG8 mRNA levels were consistently lower in mice fed the inhibitor (19). From those results and the present study, instead of implicating a direct inhibitory effect of stanols on ABCG5/ABCG8 gene expression, we would argue that stanols (and ezetimibe) decrease cholesterol flux through the intestine, causing the suppression of ABCG5/ABCG8 mRNA levels (discussed further below). Because we have no data that address the protein expression or activity of these intestinal sterol transporters, we cannot exclude the possibility of posttranscriptional regulation of these proteins by stanols.

The recent seminal discovery suggesting that NPC1L1 may be the putative apical intestinal sterol transporter has opened a new area for investigation (16). Before the description of NPC1L1, it was postulated that this proposed

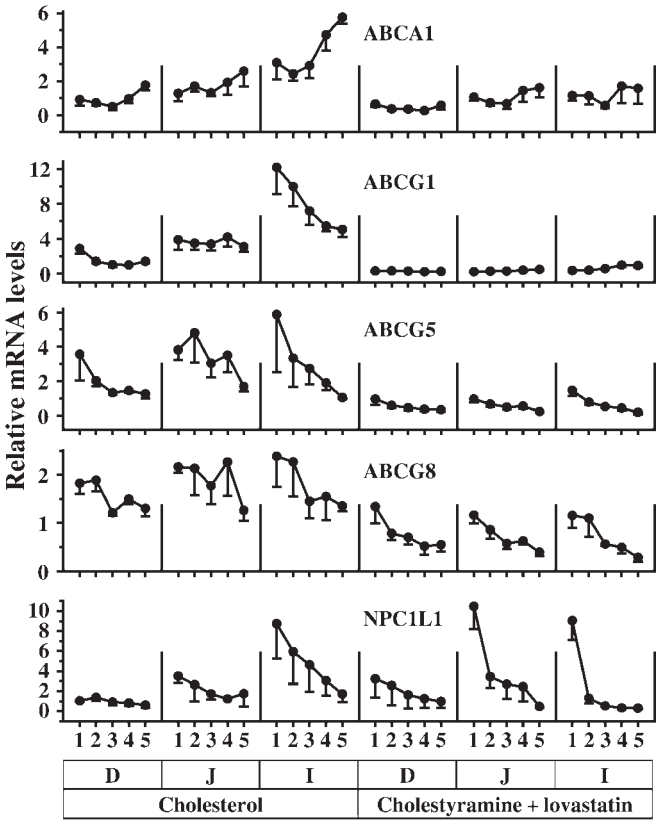

Fig. 3. ABCA1, ABCG1, ABCG5, ABCG8, and NPC1L1 mRNA levels in cells isolated from villus tips to crypts in duodenum (D), jejunum (J), and ileum (I) from hamsters fed cholesterol or cholestyramine and lovastatin. Cells along the villus axis were isolated from duodenum, jejunum, and ileum of hamster intestine, and mRNA levels were determined as described in Materials and Methods. The values were normalized using 18S rRNA as an endogenous internal standard. Data represent means ± SEM of three hamsters on each diet.

apical sterol transporter likely was responsible for the discrimination between plant and animal sterol absorption by the intestine (20). Understanding how this protein is regulated would be important in advancing our understanding of how the intestine regulates the absorption of different sterols. It is clear from our results, however, that plant stanols do not interfere with cholesterol absorption by attenuating the expression of NPC1L1. Further studies will be necessary to examine the possible role of this transporter in plant sterol absorption.

The present results demonstrate that flux of cholesterol through the intestine regulates the gene expression of ABC transporters. Although this is not a new finding, we have extended previous observations by investigating ABC expression in animals in which cholesterol flux through the intestine has been modestly (stanol diet) or markedly (cholestyramine and lovastatin diet) suppressed. In earlier experiments in mice, intestinal ABCA1, ABCG5, and ABCG8 mRNA levels were increased by ~2-fold by feeding either 1% or 2% cholesterol (19, 21). Our results in hamsters, in general, agree with those observed in mice. We were impressed, however, with the marked enhancement of ABCA1 and ABCG1 expression compared with ABCG5 or ABCG8 by cholesterol feeding. In all three intestinal segments from duodenum to ileum, ABCA1 expression was increased by 3- to 4-fold, and ABCG1 expression was increased by 4- to 7-fold, by cholesterol feeding. In contrast, the effects of cholesterol feeding on ABCG5/ABCG8 expression were, at best, modest, and in some segments, nonexistent. From previous data, it is clear that cholesterol per se is likely not the true regulator of ABC gene expression. It is postulated that cholesterol is oxidized to a ligand molecule that is recognized by liver X receptors (LXRs), transcription factors that when activated by ligand binding enhance the transcription of their target genes (22, 23). ABCA1, ABCG1, ABCG5, and ABCG8 are such target genes (21, 24, 25). In intestinal cell cultures, we also observed that gene expression of ABCG5/ABCG8 was not very responsive to LXR activation compared with ABCA1 and ABCG1 (25). Although it seems clear that upregulation of intestinal ABCG5/ABCG8 expression by dietary cholesterol is dependent upon LXR (21), we argue that compared with intestinal ABCA1 and ABCG1, the LXR-mediated enhancement of ABCG5/ABCG8 expression in intestine is a weak regulator.

Interestingly, a decrease in cholesterol flux across the intestine affected ABCG5/ABCG8 expression more that ABCA1 expression, the reverse of what was seen with cholesterol influx. In intestines of animals ingesting stanols, neither ABCA1 nor ABCG1 mRNA levels were altered. In contrast, gene expression for both ABCG5/ABCG8 was consistently decreased. This became even more apparent in animals ingesting cholestyramine and lovastatin. In each intestinal segment, ABCG5/ABCG8 mRNA levels were significantly depressed. Such was not the case for ABCA1 expression, which remained similar to that in controls. The effect of changes in cholesterol flux on intestinal ABCG5/ABCG8 gene expression is reminiscent of what we previously observed for intestinal HMG-CoA reductase or HMG-CoA synthase expression. Despite marked increases in cholesterol flux through the intestine, gene expression of either reductase or synthase decreases only modestly, if at all. However, if cholesterol is removed from the intestinal lumen and the flux of cholesterol is decreased, transcription of these genes is enhanced dramatically (18). It would seem that similar to intestinal HMG-CoA reductase and synthase, the regulation of ABCG5/ABCG8 expression by cholesterol flux is much more sensitive to cholesterol removal than to cholesterol excess. Because the true regulators of these genes are oxysterols, this suggests that in the intestine these genes are normally fully repressed (HMG-CoA reductase/synthase) or activated (ABCG5/ABCG8) by oxysterols. This may be because the intestine is continually bathed by biliary and dietary cholesterol. Further influx of sterols, therefore, would cause only modest regulation of these genes. By preventing cholesterol absorption and removing the oxysterol ligands, however, the expression of these genes is unmasked and they respond more robustly.

It is noteworthy that NPC1L1 mRNA levels were not altered by changes in cholesterol flux. Obviously, this indi-

cates that this gene is regulated differently from ABC transporters, SREBP-1c, or HMG-CoA synthase in intestine. This lack of regulation by dietary cholesterol was somewhat unexpected, as this protein does contain a sterol-sensing domain that is found in key proteins that regulate several different aspects of cellular cholesterol homeostasis (16). This observation does not imply, however, that this protein is not important in cholesterol absorption. Clearly, that is not the case (16, 17). It may be that this gene is constitutively expressed and that regulation occurs posttranscriptionally. To prevent excessive amounts of cholesterol from entering the body, it would be advantageous for the organism to suppress NPC1L1 in response to an increased influx of cholesterol. In hamsters fed cholesterol, the observed 2.5-fold increase in plasma cholesterol and the rather massive accumulation of cholesterol within intestinal cells suggest that little regulation of cholesterol uptake, either at the gene or protein level of NPC1L1, occurs. During the review of this article, Davis et al. (17) demonstrated a modest downregulation of intestinal NPC1L1 mRNA expression in mice fed cholesterol and cholate. Obviously, further studies will be required to determine whether the regulation of intestinal NPC1L1 gene expression by dietary cholesterol is species-specific or is dependent upon the combination of a bile salt together with dietary cholesterol.

ABCA1 mRNA levels were highest in cells of the lower villus. In contrast, gene expression of the other ABC transporters, ABCG1, ABCG5, and ABCG8, and the putative intestinal sterol transporter, NPC1L1, were highest in cells of the upper villus. This difference in cellular location along the villus axis between ABCA1 and the other sterol transporters suggests different functions of the proteins. At present, we do not know the function of ABCG1 in the intestine. In a previous study with CaCo-2 cells, we could not separate the functions of ABCA1 and ABCG1 (25). Both genes were regulated in parallel, and enhancement of their expression caused increased efflux of cholesterol at the basolateral membrane. The observation that ABCG1 localizes to the upper villus along with the other ABCG transporters suggests that the function of intestinal ABCG1 differs from that of ABCA1. Experiments are ongoing to investigate basolateral cholesterol efflux in cells that have ABCG1 expression inactivated.

The observation that ABCA1 is predominantly localized to cells of the lower villus in the ileum also suggests that ABCA1 is likely not involved in facilitating the uptake of cholesterol from the lumen, a function that is attributed to cells of the upper villus in the distal duodenum and jejunum. Indeed, it is now clear that intestinal ABCA1 does not function at the apical membrane and is not responsible for the entry of cholesterol into the cell (25–27). These results further suggest that in the ileum, cells of the lower villus are responsible for most of the production of intestinal HDL. It also makes sense that ABCG5/ABCG8 and NPC1L1 are located in cells of the upper villus if they are important in neutral sterol uptake/efflux. From in situ hybridization experiments, Repa et al. (21) showed that transcripts for ABCG5/ABCG8 mRNA were confined exclusively to cells that line the intestinal villus. Although it was not possible to discern a gradient of ABCG5/ABCG8 transcripts from tip to crypt, it appeared from that study that cells within the crypt were almost devoid of label. Our results are in agreement with that observation. Similarly, NPC1L1 protein and gene expression were found in proximal jejunal enterocytes bordering the luminal space, with the protein enriched in the brush border membrane (16). Our results in hamster intestine agree with those results in rat and mouse.

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