THE ROLES OF ATP-BINDING CASSETTE TRANSPORTERS G5 AND G8 IN LIVER X RECEPTOR-MEDIATED STEROL TRAFFICKING

APPROVED BY SUPERVISORY COMMITTEE

Committee Chairperson _____
Helen H. Hobbs

Committee Member _____
Jonathan C. Cohen

Committee Member _____
Jay C. Horton

Committee Member _____
David W. Russell

THE ROLES OF ATP-BINDING CASSETTE TRANSPORTERS G5 AND G8 IN LIVER X RECEPTOR-MEDIATED STEROL TRAFFICKING

by

JENNIFER LYNN YORK

DISSERTATION

Presented to the Faculty of the Medical School

The University of Texas Southwestern Medical Center at Dallas

In Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF MEDICINE WITH DISTINCTION IN RESEARCH

Portions of this work have been previously published in Journal of Biological Chemistry

The University of Texas Southwestern Medical Center at Dallas

April 2004

ABSTRACT

THE ROLES OF ATP-BINDING CASSETTE TRANSPORTERS G5 AND G8 IN LIVER X RECEPTOR-MEDIATED STEROL TRAFFICKING

Publication No. _____

Jennifer Lynn York

The University of Texas Southwestern Medical Center at Dallas, 2004

Supervising Professor: Helen H. Hobbs, M. D.

The liver X receptor (LXR) is a nuclear receptor that plays a critical role in orchestrating the trafficking of sterols between tissues. Treatment of wild type mice with a potent and specific nonsteroidal LXR agonist, T0901317, is associated with increased biliary cholesterol secretion, decreased fractional cholesterol absorption, and increased fecal neutral sterol excretion. The following studies show that expression of two target genes of LXRα, the ATP-binding cassette (ABC) transporters Abcg5 and Abcg8, is required for the increase in sterol excretion, the decrease in fractional cholesterol absorption, and the increase in fecal neutral sterol excretion associated with LXR agonist treatment. Mice lacking ABCG5 and ABCG8 (G5G8$^{-/-}$ mice) and wild type littermate controls were treated for 7 days with T0901317. In control animals, the LXR agonist produced a 3-fold increase in biliary cholesterol concentration, a 25% reduction in fractional cholesterol absorption, and a 4-fold elevation in fecal neutral sterol excretion. In contrast, treatment of G5G8$^{-/-}$ mice with the LXR agonist did not significantly affect any of these parameters. These results demonstrate that ABCG5 and ABCG8 are required for LXR agonist-associated changes in dietary and biliary sterol trafficking and that increased expression of these proteins promotes cholesterol excretion in vivo.

TABLE OF CONTENTS

PRIOR PUBLICATIONS AND PRESENTATIONS

Publications:

2003 "Stimulation of cholesterol excretion by the liver X receptor agonist requires ATP-binding cassette transporters G5 and G8." Yu L, York J, von Bergmann K, Lutjohann D, Cohen JC, Hobbs HH. J Biol Chem. 2003 May 2;278(18):15565-70.

Presentations:

2004 "The Roles of ATP-binding Cassette Transporters G5 and G8 in Liver X Receptor-Mediated Sterol Trafficking" Seminar as partial fulfillment of the requirements for the degree of Doctorate of Medicine with Distinction in Research.

2003 "LXR-agonist Associated Increase in Biliary Cholesterol Secretion Requires ATP Binding Cassette Transporters ABCG5 and ABCG8" Poster and seminar at the 40th Annual UTSW Medical Student Research Forum, Dallas, Texas

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABC – ATP-binding cassette

FPLC – fast protein liquid chromatography

$G5G8^{+/+}$ – homozygous for wild type Abcg5 and Abcg8 allele

$G5G8^{-/-}$ – homozygous for an inactivated Abcg5 and Abcg8 allele

GC – gas chromatography

HDL – high density lipoprotein

LDL – low density lipoprotein

LXR – liver X receptor

SREBP – sterol regulatory element-binding protein

CHAPTER I Introduction

Cholesterol is an important structural component of animal cell membranes. The cholesterol required to maintain membrane integrity can be synthesized de novo from acetyl-CoA or can be obtained from cholesterol-containing foods in the diet. The typical Western diet includes ~400 mg of cholesterol per day, of which between 20-90% is absorbed in the proximal small intestine (1). The major pathway by which cholesterol is eliminated from the body is by excretion into bile, either as free cholesterol or after conversion to bile acids.

A variety of noncholesterol sterols are also present in the diet. The most plentiful of these are two plant sterols, sitosterol and campesterol. The levels of these sterols in tissues are normally low, because plant sterols are poorly absorbed from the intestine and are preferentially secreted into the bile by hepatocytes (2-4).

One mechanism by which excess cholesterol and other sterols are eliminated from the body involves the action of two ATP-binding cassette (ABC) half-transporters, ABCG5 and ABCG8 (5, 6). Mutations in either of these genes in humans cause sitosterolemia, a rare autosomal recessive disorder of sterol trafficking (5, 6). Subjects with sitosterolemia have increased fractional absorption of dietary noncholesterol sterols and decreased biliary secretion of plant- and animal-derived sterols (1, 7). Consequently, these patients accumulate sitosterol, as well as other plant- and shellfish-derived neutral sterols, in the blood and tissues (8, 9). Subjects with sitosterolemia also are frequently hypercholesterolemic and develop tendon xanthomas as well as premature coronary artery disease (8, 10).

The pivotal role of ABCG5 and ABCG8 in enterohepatic sterol transport was demonstrated by manipulating the expression of the encoding genes in mice (11, 12). Transgenic mice containing ~14 copies of a human genomic DNA fragment that includes the ABCG5 and

ABCG8 genes have a ~50% reduction in the fractional absorption of dietary cholesterol, dramatically elevated levels of biliary cholesterol, and a 4.5-fold increase in fecal neutral sterol excretion (11). Plant sterol levels are more than 50% lower in these mice than in wild type littermates. Disruption of the mouse Abcg5 and Abcg8 genes has the opposite effect on dietary sterol trafficking; G5G8$^{-/-}$ mice have 30-fold higher plasma levels of sitosterol than do wild type littermates due to increased fractional absorption of dietary plant sterols and impaired biliary sterol excretion (12).

Abcg5 and Abcg8 are expressed predominantly in the liver and small intestine (4) and are coordinately up regulated at the transcriptional level by dietary cholesterol. The response of Abcg5 and Abcg8 to cholesterol requires the liver X receptor α (LXRα) (13), a nuclear receptor. LXR target genes are known to be involved in lipid, cholesterol and carbohydrate metabolism. LXRα regulates the expression of ABCA1 (14, 15), the gene mutated in Tangier disease (16–19), the apolipoprotein ApoE, C-I, C-IV, and C-II genes, and mouse (but not human) cholesterol 7α-hydroxylase (Cyp7A1) (21–23), the rate limiting enzyme in bile acid synthesis. Additionally, LXRα activation increases expression of sterol regulatory element-binding protein 1c (SREBP-1c) (24) an important transcription factor in the regulation of fatty acid biosynthesis (25), and represses expression of the potent activator of cholesterol biosynthesis, sterol regulatory element-binding protein 2 (SREBP-2) (26). LXRα regulates the fatty acid synthase gene directly and indirectly through SREBP-1c (27), cholesterol ester transfer protein (CETP) gene, which facilitates clearance of HDL cholesterol esters by the liver (28), and possibly itself, as a means of autoregulation (20). By regulating the expression of these and other genes, LXRα coordinates the synthesis and trafficking of cholesterol and fatty acids between tissues. Mice lacking LXRα accumulate large amounts of cholesterol in the liver when fed a high cholesterol diet (22),

whereas wild type mice treated with an LXR agonist have decreased absorption of dietary cholesterol (14) and increased biliary cholesterol excretion (29). The mechanism by which LXRα prevents the accumulation of dietary cholesterol has not been fully defined. The decreased fractional absorption of dietary cholesterol associated with LXR agonist treatment was attributed initially to the action of ABCA1, as levels of ABCA1 mRNA increased in the small intestine of animals given the nonsteroidal synthetic LXR agonist: N-(2,2,2-trifluoro-ethyl)-N-[4-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethyl-ethyl)phenyl]benzenesulfonamide (T0901317) (14). Subsequent characterization of mice expressing no ABCA1 (Abca1$^{-/-}$ mice) revealed no impairment of biliary cholesterol secretion or fecal neutral sterol excretion (29, 30). In the current study, we tested the hypothesis that ABCG5 and ABCG8 mediate the LXR agonist-associated increase in biliary and fecal excretion of cholesterol and reduction in cholesterol absorption.

CHAPTER II Experimental Procedures

Materials—The synthetic LXR agonist T0901317 was purchased from Cayman Chemical Company (Ann Arbor, MI). Sterols were obtained either from Steraloids Inc. (Newport, RI) or from Sigma-Aldrich (St. Louis, MO).

Animals and Diets—Mice homozygous for a disrupted Abcg5 and Abcg8 allele ($G5G8^{-/-}$) were generated as described previously (12). The mice used in these studies were offspring of $G5G8^{-/-}$ mice of mixed genetic background (129S6SvEv × C57BL/6J), and were housed in plastic cages in a temperature-controlled room (22 °C) with a daylight cycle from 6 am to 6 pm. They were fed ad libitum a cereal-based rodent chow diet (Diet 7001, Harlan Teklad, Madison, WI) containing 0.02% cholesterol and 4% fat. All animal procedures were performed with approval of the Institutional Animal Care and Research Advisory Committee at the University of Texas Southwestern Medical Center.

T0901317 Treatment—Diets containing 0.025% of T0901317 (T-diet) were made by mixing powdered chow diet (Diet 7001, Harlan Teklad) with pure T0901317 (Cayman Chemical Company). This "T-diet" was stored in aluminum foil-covered containers at 4 °C for no more than 3 days. Female mice were housed individually 1 week before initiation and throughout the experiment. Mice were fed for 1 week with either the T-diet or regular powdered chow diet dispensed from a feeder jar. The dose and duration of T0901317 treatment were based on prior studies (14, 31).

Lipid Chemistries—Sterol levels in plasma and liver were measured by gas chromatography (GC) as described (32, 33) with some modifications. Briefly, samples were saponified in 3% potassium hydroxide/ethanol at 66 °C for 3 h after addition of 5α-cholestane as a recovery standard. Lipids were extracted using petroleum ether and dried under nitrogen. The residual lipids were dissolved in Tri-Sil reagent (product 48999, Pierce, Rockford, IL) for analysis by GC. Hepatic triglyceride levels were measured using the Infinity triglyceride reagent (Sigma-Aldrich, St. Louis, MO). Lipoproteins were size-fractionated using fast protein liquid chromatography (FPLC), and the total sterol concentration in each fraction was measured using cholesterol/HP kits (catalog number 1127771, Roche Diagnostics Corp., Indianapolis, IN).

Biliary Lipid Composition—Bile was collected from the gallbladder of anesthetized mice using a 30.5-gauge needle. The concentrations of cholesterol, phospholipids, and bile acids were measured as described previously (34).

Fecal Sterol Excretion—Mice were fed either the control or T-diet for 4 days prior to being moved to new cages containing fresh wood shavings. The diets were fed for an additional 3 days and the feces collected thereafter. The feces were dried, weighed, and ground to a fine powder. An aliquot of 0.5 g of feces was used to determine fecal neutral and acidic sterol excretion (11, 32).

Fractional Absorption of Dietary Cholesterol—Mice were fed either the control or T-diet for 4 days prior to administering an oil mixture containing deuterated cholesterol and sitostanol by gavage (12). Mice were then housed individually in new cages containing fresh wood shavings.

The diets were continued, and the feces were collected after 3 days and processed for sterol absorption as described (12). These studies were performed by Liqing Yu.

Quantitative Real-time PCR—Total RNA was extracted from tissues using the RNA Stat-60 kit (Tel-Test Inc., Friendswood, TX), and real-time PCR was performed to assay the relative amounts of selected mRNAs as described (26, 27).

Statistical Analysis—All data are reported as the mean ± S.E.M. The differences between the mean values were tested for statistical significance by the two-tailed Student's t test.

CHAPTER III Results

LXR Activation Increases Plasma Levels of Sitosterol and Campesterol in G5G8$^{-/-}$ Mice— Plasma levels of sitosterol and campesterol were 30-fold higher in G5G8$^{-/-}$ than in G5G8$^{+/+}$ female mice fed a chow diet. Addition of T0901317 (0.025%) to the diet for 7 days resulted in a fall of plasma plant sterol levels to barely detectable levels in wild type mice. Mean plasma sitosterol levels increased from 20.6 to 39.6 mg/dl in the LXR agonist-treated knockout animals (Fig. 1); an increase of similar magnitude occurred in the plasma level of campesterol, the other major dietary plant sterol. Mean plasma levels of cholesterol were lower in chow-fed G5G8$^{-/-}$ mice than in wild type mice, as previously described (12) (Fig. 1). With T0901317 treatment, the levels of plasma cholesterol increased by 50% and by 90% in the wild type and G5G8$^{-/-}$ mice, respectively.

Fig.1

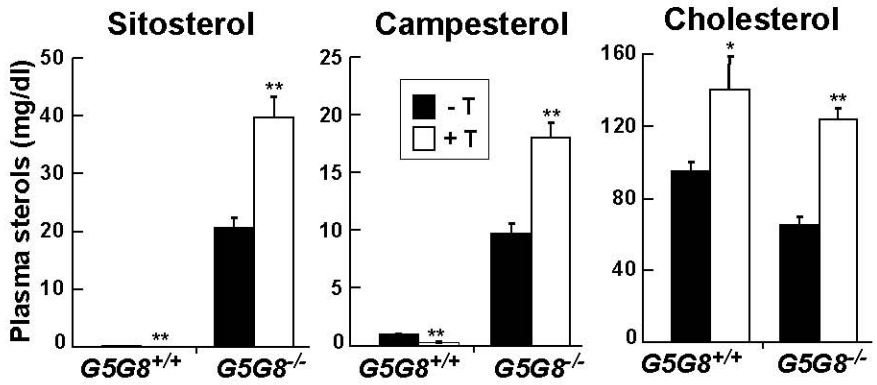

Fig. 1. Plasma sterol levels in chow-fed mice with or without T0901371. 17-week-old female G5G8$^{+/+}$ and G5G8$^{-/-}$ mice (n = 5 in each group) were fed a powdered chow diet with or without 0.025% (w/w) of the synthetic LXR agonist T0901317 for 7 days. Mice were sacrificed during

the daylight cycle after a 4-hr fast, and blood was collected from the inferior vena cava. Plasma was separated by centrifugation, and the levels of plasma sterols were determined by GC as described under "Experimental Procedures." –T, chow diet; +T, chow diet plus 0.025% T0901317, *, $p<0.05$ between chow-fed and T0901317-fed mice of each genotype; **, $p<0.01$ between chow-fed and T0901317-fed mice of each genotype.

FPLC analysis was performed to determine the distribution of sterols in the plasma of these mice. No significant difference was seen in the distribution of sterols in the knockout and wild type mice. The LXR agonist-mediated increase in plasma sterol levels in both experimental groups was due to increases of HDL sterols (Fig. 2). Treatment with T0901317 was associated with widening of the HDL sterol peaks and a shoulder extending into the larger size fractions, as has been reported previously (30, 31).

Fig.2

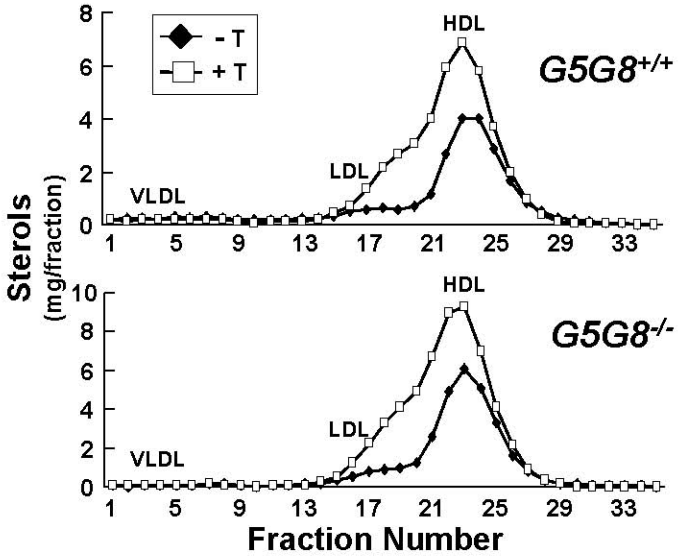

Fig. 2. Sterol profiles of FPLC fractions from plasma of G5G$^{+/+}$ and G5G8$^{-/-}$ mice fed a chow diet with or without T0901317. Plasma isolated from the mice described in the legend to Fig. 1 was subjected to FPLC analysis. The sterol content of each fraction was assayed using a cholesterol/HP kit, as described under "Experimental Procedures." -T, chow diet only; +T, chow diet plus 0.025% T0901317.

T0901317 Treatment Is Not Associated with Increased Biliary Cholesterol Levels in G5G8$^{-/-}$ Mice—T0901317 treatment increased biliary cholesterol levels of wild type mice by almost 3-fold (from 7.13 to 20.12 μmol/ml), as observed previously (30) (Fig. 3). In contrast, no significant increase in mean biliary cholesterol level was seen in the G5G8$^{-/-}$ mice after treatment with T0901317 (0.73 versus 0.97 μmol/ml). These data are consistent with Abcg5 and Abcg8 being required for the increase in biliary cholesterol levels associated with LXR activation. Biliary phospholipid levels were significantly lower in the knockout than in the wild type mice.

A similar difference in biliary phospholipid levels was observed previously in female, but not male, G5G8$^{-/-}$ mice (12). Biliary phospholipid and bile acid levels fell after T0901317 treatment in the G5G8$^{+/+}$ mice but not in the G5G8$^{-/-}$ mice (Fig. 3). Similar reductions in biliary lipid levels were seen previously in wild type mice treated with an LXR agonist (30).

Fig. 3

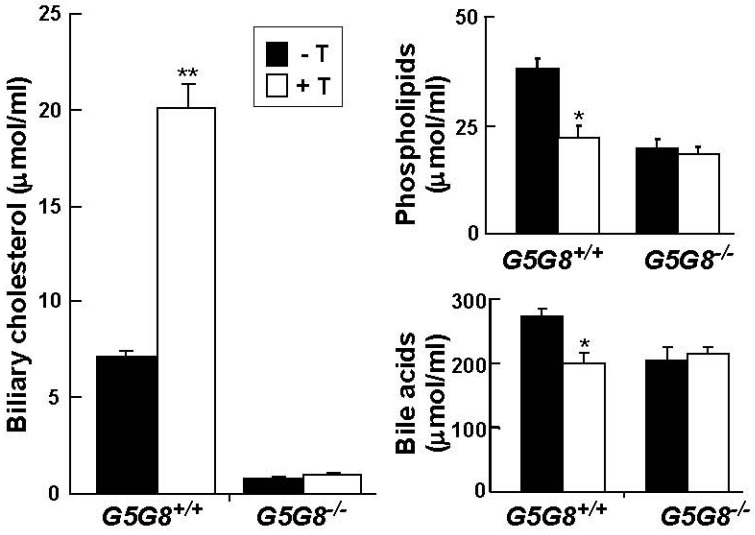

Fig. 3. Biliary lipid levels in G5G8$^{+/+}$ and G5G8$^{-/-}$ mice fed a chow diet with or without T0901317. Gallbladder bile was collected from the mice described in the legend to Fig. 1. The concentrations of biliary cholesterol, phospholipids, and bile acids were measured as described under "Experimental Procedures." -T, chow diet; +T, chow diet plus 0.025% T0901317; *, $p < 0.05$ between chow-fed and T0901317-fed mice of each genotype; **, $p < 0.01$ between chow-fed and T0901317-fed wild type mice.

Hepatic Cholesterol Levels in the G5G8$^{-/-}$ Mice Do Not Fall with T0901317 Treatment—Treatment of G5G8$^{-/-}$ mice with T0901317 resulted in a modest, but significant reduction in hepatic sitosterol and campesterol levels (Fig. 4). The hepatic cholesterol level fell by 30% in the wild type mice after treatment with T0901317, presumably in part due to the increase in biliary cholesterol secretion (Fig. 4). In contrast, no change in hepatic cholesterol levels was seen in the knockout mice after LXR agonist treatment, likely reflecting the lack of increase in biliary cholesterol secretion in these mice (Fig. 4).

Hepatic triglyceride levels were similar in chow-fed G5G8$^{+/+}$ and G5G8$^{-/-}$ mice. Treatment with T090137 increased hepatic triglycerides in both groups of mice (Fig. 4), as previously observed (31). These results are attributed to the activation of LXR target genes SREBP-1c and FAS, which stimulates fatty acid biosynthesis (24, 33).

Fig. 4

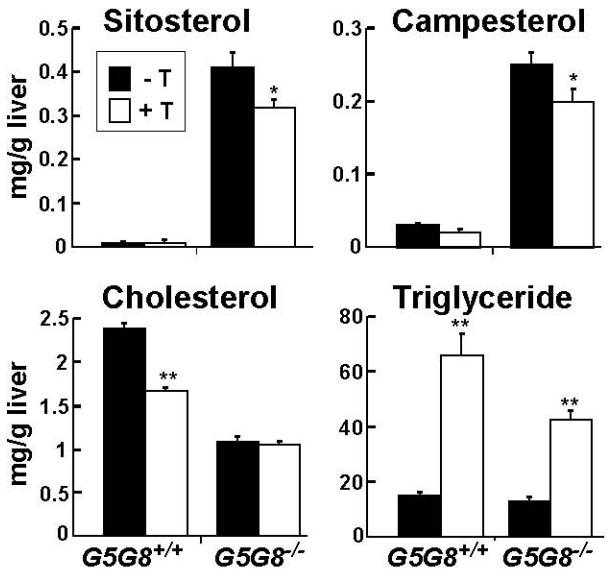

Fig. 4. Hepatic lipid levels in G5G8$^{+/+}$ and G5G8$^{-/-}$ mice fed a chow diet with or without T0901317. Livers were obtained from the mice described in the legend to Fig. 1. An aliquot of tissue was weighed, saponified, and processed for the determination of sterol contents by GC as described under "Experimental Procedures." Hepatic triglyceride levels were measured enzymatically as described under "Experimental Procedures." -T, chow diet only; +T, chow diet plus 0.025% T0901317; *, $p < 0.05$ between chow-fed and T0901317-fed mice of each genotype; **, $p < 0.01$ between chow-fed and T0901317-fed mice of each genotype.

LXR Agonist T0901317 Does Not Reduce Fractional Absorption of Dietary Cholesterol in G5G8$^{-/-}$ Mice—The fractional absorption of dietary cholesterol was reduced from 72% to 52% in the wild type mice treated with T0901317, which is similar to that observed by Repa et al. (14). In contrast, the fractional absorption of cholesterol increased slightly (from 76% to 81%) in T0901317-treated G5G8$^{-/-}$ mice (Fig. 5).

Fig.5

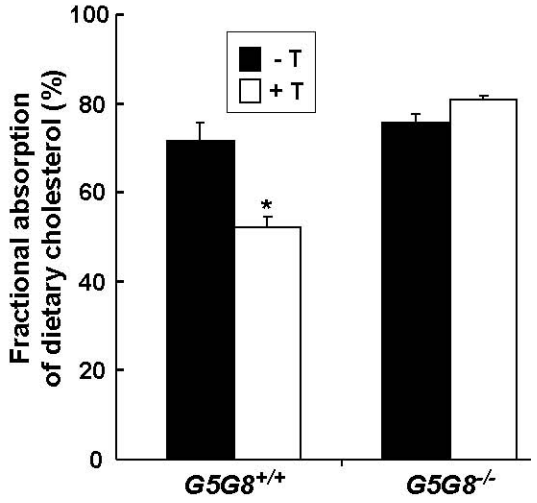

Fig. 5. Fractional absorption of dietary cholesterol in G5G8$^{+/+}$ and G5G8$^{-/-}$ mice fed a chow diet with or without T0901317. Five-month-old female mice of the indicated genotype (n = 7–8 in each group) were housed individually. After treatment with either chow diet or chow plus 0.025% T0901317 diet for 4 days, each mouse was gavaged with 50 μl of an oil mixture containing deuterated cholesterol and sitostanol. Feces were collected after 3 days and sterols were extracted. The fecal sterols were subjected to GC-MS as described previously (12). Deuterated sitostanol was used as a nonabsorbable marker by which the fractional absorption of dietary cholesterol was calculated. *, $p < 0.01$ between chow-fed and T0901317-treated wild type mice.

T0901317 Treatment Fails to Increase Fecal Neutral Sterol Excretion in G5G8$^{-/-}$ Mice— Decreased fractional cholesterol absorption and increased biliary cholesterol secretion both may contribute to the dramatic increase in fecal neutral sterol excretion that occurs with LXR agonist treatment (14, 30). The level of neutral sterols excreted into the feces over the 3-day collection period was ~30% lower in the chow-fed G5G8$^{-/-}$ mice than in the G5G8$^{+/+}$ mice, and this level failed to increase with T0901317 treatment (Fig. 6). Thus, ABCG5 and ABCG8 are required for the stimulation of fecal neutral sterol excretion by the LXR agonist T0901317. Fecal bile acid excretion was similar in the G5G8$^{+/+}$ and G5G8$^{-/-}$ mice and did not change significantly with T0901317 treatment (Fig. 6).

Fig.6

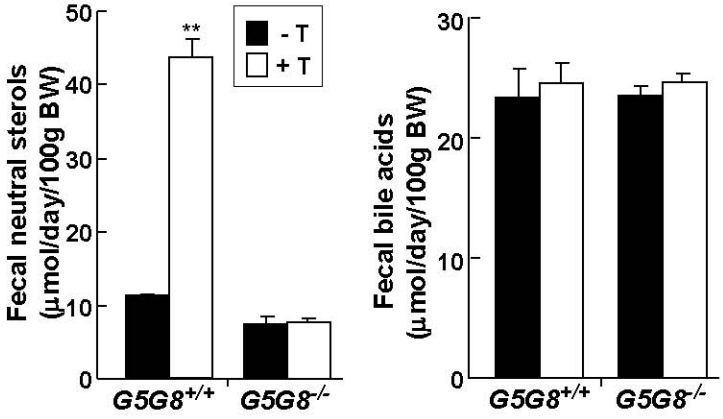

Fig. 6. Fecal neutral sterol excretion in G5G8$^{+/+}$ and G5G8$^{-/-}$ mice fed a chow diet with or without T0901317. Feces were collected for the last 3 days of the experiment described in the legend to Fig. 1. The fecal neutral sterols and bile acids were measured as described under "Experimental Procedures." -T, chow diet only; +T, chow diet plus 0.025% T0901317; *, $p < 0.01$ between chow-fed and T0901317-fed wild type mice.

LXR Agonist (T0901317) Treatment Increases mRNA Levels of Select Target Genes in Liver and Small Intestine of G5G8$^{+/+}$ and G5G8$^{-/-}$ Mice—The mRNA levels of known LXR target genes, including Srebp-1c, Abca1, Abcg1, Abcg5, Abcg8, and Cyp7A1 were determined to confirm that T0901317 had the expected biological effects in the tissues of the treated mice. The levels of all selected LXR target gene mRNAs were increased with T0901317 treatment in both strains of mice with the exceptions of Abcg5 and Abcg8 in G5G8$^{-/-}$ mice. As expected, the levels of Srebp-2 mRNA, which is not a target of LXR, did not increase significantly with T0901317 treatment in either wild type or G5G8$^{-/-}$ mice (Fig. 7).

Fig.7

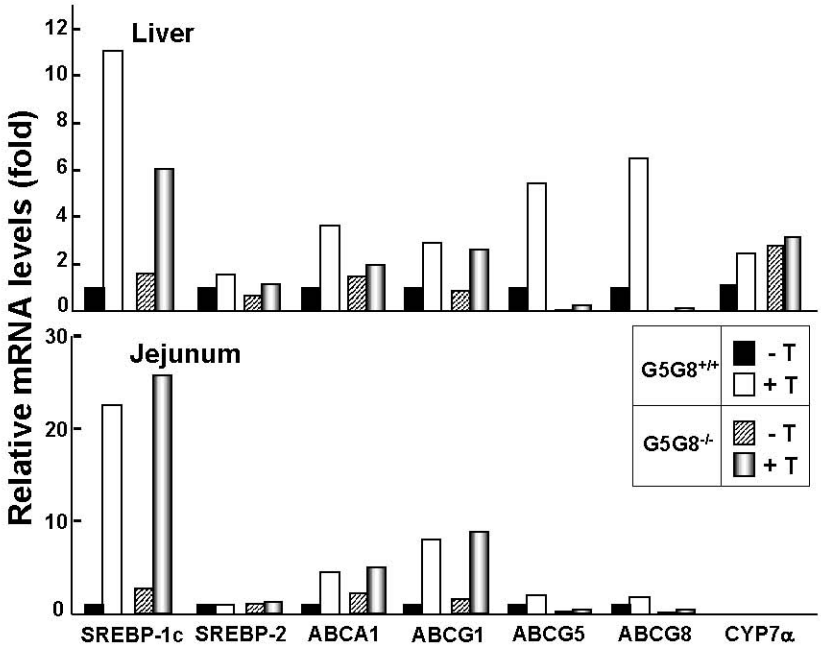

Fig. 7. Relative mRNA levels of LXR target genes, in livers and intestines of chow fed mice with or without the addition of the LXR agonist T0901317. The livers and jejuna were harvested from the mice described in the legend to Fig. 1 and maintained at -80 °C after freezing in liquid nitrogen. Total RNA was extracted from the tissues using the RNA Stat-60 kit (Tel-Test Inc., Friendswood, TX). An equal amount of RNA from each sample was pooled per group, and real-time PCR was performed using gene-specific primers as described previously (31, 32). Cyclophilin was used as an internal control for these studies. Values represent the mRNA levels relative to the mRNA level in the chow-fed wild type mice. -T, chow diet only; +T, chow diet plus 0.025% T0901317.

CHAPTER IV Conclusions and Recommendations

ABCG5 AND ABCG8 ARE REQUIRED FOR LXR-REGULATED CHOLESTEROL EXCRETION

Conclusions

A major finding of this study is that in mice, ABCG5 and ABCG8 are required for the stimulation of biliary and fecal cholesterol excretion by the synthetic LXR agonist, T0901317. Disruption of Abcg5 and Abcg8 abolished the increase in biliary cholesterol levels, the reduction in fractional cholesterol absorption, and the increase in fecal neutral sterol excretion associated with LXR activation in wild type mice (14, 26). These data are consistent with LXR activation promoting the excretion of sterols by increasing Abcg5 and Abcg8 expression.

Recommendations

Future studies are needed to understand the mechanism of action of ABCG5 and ABCG8. This includes further investigation into the formation of heterodimers, their intracellular path to the apical hepatocyte plasma membrane, and the biochemical mechanism by which they transport cholesterol into the biliary canniculi.

ABCG5 AND ABCG8 ARE NECESSARY FOR THE LXR-REGULATED PLANT STEROL TRAFFICKING

Conclusions

In wild type mice, treatment with the LXR agonist was associated with significantly lower plasma levels of both sitosterol and campesterol (Fig. 1). Similar reductions in plasma plant sterol levels were seen in transgenic mice containing 14 copies of the human ABCG5 and

ABCG8 transgenes (11). Conversely, in the G5G8$^{-/-}$ mice, plasma levels of sitosterol and campesterol increased ~2-fold with T0901317 treatment (Fig. 1). These data indicate that increased expression of Abcg5 and Abcg8 is both necessary and sufficient for the LXR agonist-associated reduction in plasma plant sterol levels observed in the wild type animals. In the absence of ABCG5 and ABCG8, plant sterols accumulate in the liver due to increased absorption of dietary plant sterols and reduced ability to efficiently secrete sterols into the bile (12).

Recommendations

In the G5G8$^{-/-}$ mice, increased absorption of dietary plant sterols and decreased secretion of sterols into the bile leads to increased hepatic levels of plant sterols (Fig. 4) as well as increased incorporation of these sterols into lipoproteins and secretion into plasma. At this time, it is not clear why treatment of G5G8$^{-/-}$ with an LXR agonist causes a significant decrease in hepatic plant sterol levels, but a different LXR target gene is a possibility. Further studies are needed to determine if LXR agonist treatment in G5G8$^{-/-}$ mice results in either an increase in the proportion of plant sterols that are incorporated into lipoproteins, an increase in secretion of total lipoproteins into the circulation or both. These possibilities could be investigated by size fractionating and quantifying plant sterols in the plasma of wild type and G5G8$^{-/-}$ mice. Treatment with the LXR agonist may also promote mobilization of plant sterols from the liver or peripheral tissues into the circulation.

TREATMENT WITH A LXR AGONIST INCREASES PLASMA CHOLESTEROL

Conclusions

Plasma cholesterol levels are significantly increased with T0901317 treatment in G5G8$^{+/+}$ and G5G8$^{-/-}$ mice (Fig. 1). The increase in plasma cholesterol was limited to the HDL fraction (Fig. 2) and was associated with an increase in the size of HDL particles, as reported previously (31, 35).

Recommendations

Future studies are needed to determine the relationship between the LXR agonist-induced increase in HDL quantity and size and the known ability of LXR to increase expression of Abca1 in the intestine, liver (Fig. 7), and macrophages (14). ABCA1 has been shown to participate in the efflux of excess cholesterol from macrophages to HDL (16–18, 36) and to promote formation of pre-β-HDL particles by hepatocytes (37) and possibly enterocytes (38). Recent studies have shown that in spite of the absence of HDL, in ABCA1$^{-/-}$ mice, all theoretical downstream aspects of reverse cholesterol transport, including hepatic lipid content, biliary secretion rates, and fecal neutral sterol excretion rates, are intact and functioning normally (29). Therefore the role of macrophage-derived cholesterol transport, including ABCA1 and HDL, in the total magnitude of reverse cholesterol transport is questionable.

Additionally, future studies are required to clarify the role of ABCA1 in whole body cholesterol trafficking and studies with a both G5G8$^{-/-}$ and ABCA1$^{-/-}$ knock out strain of mice may assist in answering these questions. The above observed increase in plasma HDL can be explained by LXR agonist-mediated induction of Abca1 and increased mobilization of cholesterol from peripheral macrophages into HDL. With the role of hepatic ABCA1 still in question, more work is needed to determine if the fall in hepatic cholesterol levels (Fig. 4) in the

G5G8$^{+/+}$ mice with LXR activation is, at least in part due to an ABCA1-mediated increase in the efflux of cholesterol from the liver into the circulation (14, 30) in addition to the increase in biliary cholesterol secretion.

ABCG5 AND ABCG8 ARE RESPONSIBLE FOR LXR AGONIST EFFECTS ON BILIARY LIPID LEVELS

Conclusions

In response to T0901317 treatment mean biliary cholesterol levels to increased 3-fold in wild type mice but did not change significantly in knockout mice. Biliary phospholipid and bile acid levels were lower in the G5G8$^{-/-}$ mice than in their wild type littermates, which is comparable to the reductions observed previously in female mice (12). Both the biliary phospholipid and bile acid levels fell in wild type mice treated with T0901317 (Fig. 3), as was seen previously in mice treated with LXR agonists (30). No reduction in the levels of bile acids or phospholipids in the bile occurred in the T0901317-treated G5G8$^{-/-}$ mice. No change in fecal bile acid excretion was seen in either the knockout or the wild type animals treated with the LXR agonist (Fig. 6). Therefore, the increased excretion of biliary cholesterol associated with LXR agonist treatment in G5G8$^{+/+}$ mice was not quantitatively coupled to bile acid or phospholipid excretion. These results indicate that the effect of LXR agonists of increasing fecal loss of cholesterol is mediated by ABCG5 and ABCG8.

Recommendations

Future studies are needed to distinguish the relative contributions of the liver and the intestine to the increased fecal neutral sterol excretion. The stimulation of fecal cholesterol loss by the LXR agonist may result from an increase in biliary cholesterol secretion by hepatocytes and/or the decreased fractional absorption of dietary cholesterol by enterocytes. Tissue-specific disruptions of Abcg5 and Abcg8 will be required to assess the function of these transporters in the liver and small intestine.

ACKNOWLEDGEMENTS

Helen H. Hobbs for believing in me, fantastic guidance, and inspiring mentorship

Jonathan C. Cohen for intellectual stimulation, always encouraging me to think ahead to consider the next level, and for serving on my Research Distinction committee

Liqing Yu for training me with daily patience, and for his major role in the publication of this work

Jay D. Horton and David W. Russell for serving on my Research Distinction committee and helpful discussion in the publication of this work

Klaus von Bergmann and Dieter Lutjohann from the Department of Clinical Pharmacology, University of Bonn, Bonn 53105, Germany for their assistance with publishing this work

Robert Guzman, Yinyan Ma, Anja Kerksiek, and Silvia Winnen for excellent technical assistance

Scott Clark, Anh Nguyen, Scott M. Grundy, and Gloria Vega for measuring lipids in bile, plasma, and tissues

Joachim Herz for kindly providing the targeting vector for generation of knock out mice

Michael McPhaul, without whose guidance, patience and dedication to student research none of this would have been possible

BIBLIOGRAPHY

1. Miettinen, T. A. (1980) Eur. J. Clin. Invest. **10,** 27–35

2. Schoenheimer, R. (1931) Science **74,** 579–584

3. Gould, R. G., Jones, R. J., LeRoy, G. V., Wissler, R. W., and Taylor, C. B. (1969) Metabolism **18,** 652–662

4. Salen, G., Ahrens, E. H., Jr., and Grundy, S. M. (1970) J. Clin. Invest. **49,** 952–967

5. Berge, K. E., Tian, H., Graf, G. A., Yu, L., Grishin, N. V., Schultz, J., Kwiterovich, P., Shan, B., Barnes, R., and Hobbs, H. H. (2000) Science **290,** 1771–1775

6. Lee, M. H., Lu, K., Hazard, S., Yu, H., Shulenin, S., Hidaka, H., Kojima, H., Allikmets, R., Sakuma, N., Pegoraro, R., Srivastava, A. K., Salen, G., Dean, M., and Patel, S. B. (2001) Nat. Genet. **27,** 79–83

7. Salen, G., Shore, V., Tint, G. S., Forte, T., Shefer, S., Horak, I., Horak, E., Dayal, B., Nguyen, L., and Batta, A. K. (1989) J. Lipid Res. **30,** 1319–1330

8. Salen, G., Horak, I., Rothkopf, M., Cohen, J. L., Speck, J., Tint, G. S., Shore, V., Dayal, B., Chen, T., and Shefer, S. (1985) J. Lipid Res. **26,** 1126–1133

9. Gregg, R. E., Connor, W. E., Lin, D. S., and Brewer, H. B., Jr. (1986) J. Clin. Invest. **77,** 1864–1872

10. Bhattacharyya, A. K., and Connor, W. E. (1974) J. Clin. Invest. **53,** 1033–1043

11. Yu, L., Li-Hawkins, J., Hammer, R. E., Berge, K. E., Horton, J. D., Cohen, J. C., and Hobbs, H. H. (2002) J. Clin. Invest. **110,** 671–680

12. Yu, L., Hammer, R. E., Li-Hawkins, J., Von Bergmann, K., Lutjohann, D., Cohen, J. C., and Hobbs, H. H. (2002) Proc. Natl. Acad. Sci. U. S. A. **99,** 16237–16242

13. Repa, J. J., Berge, K. E., Pomajzl, C., Richardson, J. A., Hobbs, H., and Mangelsdorf, D. J. (2002) J. Biol. Chem. **277,** 18793–18800

14. Repa, J. J., Turley, S. D., Lobaccaro, J. A., Medina, J., Li, L., Lustig, K., Shan, B., Heyman, R. A., Dietschy, J. M., and Mangelsdorf, D. J. (2000) Science **289,** 1524–1529

15. Costet, P., Luo, Y., Wang, N., and Tall, A. R. (2000) J. Biol. Chem. **275,** 28240–28245

16. Brooks-Wilson, A., Marcil, M., Clee, S. M., Zhang, L. H., Roomp, K., van Dam, M., Yu, L., Brewer, C., Collins, J. A., Molhuizen, H. O., Loubser, O., Ouelette, B. F., Fichter, K., Ashbourne-Excoffon, K. J., Sensen, C. W., Scherer, S., Mott, S., Denis, M., Martindale, D., Frohlich, J., Morgan, K., Koop, B., Pimstone, S., Kastelein, J. J., and Hayden, M. R. (1999) Nat. Genet. **22,** 336–345

17. Bodzioch, M., Orso, E., Klucken, J., Langmann, T., Bottcher, A., Diederich, W., Drobnik, W., Barlage, S., Buchler, C., Porsch-Ozcurumez, M., Kaminski, W. E., Hahmann, H. W., Oette, K., Rothe, G., Aslanidis, C., Lackner, K. J., and Schmitz, G. (1999) Nat. Genet. **22,** 347–351

18. Rust, S., Rosier, M., Funke, H., Real, J., Amoura, Z., Piette, J. C., Deleuze, J. F., Brewer, H. B., Duverger, N., Denefle, P., and Assmann, G. (1999) Nat. Genet. **22,** 352–355

19. Marcil, M., Brooks-Wilson, A., Clee, S. M., Roomp, K., Zhang, L. H., Yu, L., Collins, J. A., van Dam, M., Molhuizen, H. O., Loubster, O., Ouellette, B. F., Sensen, C. W., Fichter, K., Mott, S., Denis, M., Boucher, B., Pimstone, S., Genest, J., Jr., Kastelein, J. J., and Hayden, M. R. (1999) Lancet **354,** 1341–1346

20. Milatt, L.J., Bocher V., Fruchart J.C., and Staels, B. (2003) Biochemica et Biophysica Acta **1631,** 107-118

21. Lehmann, J. M., Kliewer, S., Moore, L., Smith-Oliver, T., Oliver, B., Su, J.-L., Sundseth, S., Winegar, D., Blanchard, D., Spencer, T., and Willson, T. M. (1997) J. Biol. Chem. **272,** 3137–3140

22. Peet, D. J., Turley, S. D., Ma, W., Janowski, B. A., Lobaccaro, J. M., Hammer, R. E., and Mangelsdorf, D. J. (1998) Cell **93,** 693–704

23. Chen, J. Y., Levy-Wilson, B., Goodart, S., and Cooper, A. D. (2002) J. Biol. Chem. **277,** 42588–42595

24. Repa, J. J., Liang, G., Ou, J., Bashmakov, Y., Lobaccaro, J. M., Shimomura, I., Shan, B., Brown, M. S., Goldstein, J. L., and Mangelsdorf, D. J. (2000) Genes Dev. **14,** 2819–2830

25. Horton, J. D., Goldstein, J. L., and Brown, M. (2002) J. Clin. Invest. **109,** 1125–1131

26. Yang, J., Goldstein, J. L., Hammer, R. E., Moon, Y. A., Brown, M. S., and Horton, J. D. (2001) Proc. Natl. Acad. Sci. U. S. A. **98,** 13607–13612

27. Liang, G., Yang, J., Horton, J. D., Hammer, R. E., Goldstein, J. L., and Brown, M. S. (2002) J.

Biol. Chem. **277,** 9520–9528

28. Luo, Y., and Tall, A.R. (2000) J. Clin. Invest. **105,** 513-520

29. Groen, A. K., Bloks, V. W., Bandsma, R. H., Ottenhoff, R., Chimini, G., and Kuipers, F. (2001) J. Clin. Invest. **108,** 843–850

30. Plosch, T., Kok, T., Bloks, V. W., Smit, M. J., Havinga, R., Chimini, G., Groen, A. K., and Kuipers, F. (2002) J. Biol. Chem. **277,** 33870–33877

31. Schultz, J. R., Tu, H., Luk, A., Repa, J. J., Medina, J. C., Li, L., Schwendner, S., Wang, S., Thoolen, M., Mangelsdorf, D. J., Lustig, K. D., and Shan, B. (2000) Genes Dev. **14,** 2831–2838

32. Schwarz, M., Russell, D. W., Dietschy, J. M., and Turley, S. D. (1998) J. Lipid Res. **39,** 1833–1843

33. Turley, S. D., Herndon, M. W., and Dietschy, J. M. (1994) J. Lipid Res. **35,** 328–339

34. Turley, S. D., Daggy, B. P., and Dietschy, J. M. (1991) Metabolism **40,** 1063–1073

35. Joseph, S. B., Laffitte, B. A., Patel, P. H., Watson, M. A., Matsukuma, K. E., Walczak, R., Collins, J. L., Osborne, T. F., and Tontonoz, P. (2002) J. Biol. Chem. **277,** 11019–11025

36. Bortnick, A. E., Rothblat, G. H., Stoudt, G., Hoppe, K. L., Royer, L. J., McNeish, J., and Francone, O. L. (2000) J. Biol. Chem. **275,** 28634–28640

37. Vaisman, B. L., Lambert, G., Amar, M., Joyce, C., Ito, T., Shamburek, R. D., Cain, W. J., Fruchart-Najib, J., Neufeld, E. D., Remaley, A. T., Brewer, H. B., Jr., and Santamarina-Fojo, S. (2001) J. Clin. Invest. **108,** 303–309

38. Mulligan, J. D., Flowers, M. T., Tebon, A., Bitgood, J. J., Wellington, C., Hayden, M. R., and Attie, A. D. (2003) J. Biol. Chem. **278,** 13356–13366

VITAE

Jennifer Lynn York was born in Wichita Falls, Texas on April 7, 1977 as the second daughter to Vicky Jean and Larry Brian York. After graduating from Winston Churchill High School in San Antonio, Texas in 1995, she entered The University of Texas at Austin where, in 1999, she was accepted into the Howard Hughes Medical Institute Summer Undergraduate Research Program and her interest in basic science research was ignited. Through that summer and the following two years she worked in the pharmacology laboratory of Rueben A. Gonzales, Ph.D. studying changes in dopamine concentrations in the nucleus accumbens in alcohol self-administering rats, as well as the role of D2 receptors on the alcohol-induced increase in dopamine in rats. Jennifer graduated Cum Laude with a Bachelor of Arts degree in Biology and Collective Collaboration in Psychology in 2000. She entered medical school at The University of Texas Southwestern Medical Center, and continued to pursue her interest in neuroscience by joining Cole Giller M.D., Ph.D., on a Parkinson's research project to transcribe anatomical brain atlas data into computer format for processing with 3ds max software, constructing libraries of wire mesh models of anatomical structures within the brain. The first year in medical school she became interested in the genetics of human disease, which led her to the lab of Helen H. Hobbs, M.D., where she spent one summer and an additional elective month studying the ATP binding cassette transporters G5 and G8 and their role in liver X receptor-mediated sterol trafficking in mice. Jennifer will complete her Doctorate of Medicine, with a Distinction in Research in June of 2005 and plans to pursue a career in pediatric hematology and oncology.

Prior Publications:

"Stimulation of cholesterol excretion by the liver X receptor agonist requires ATP-binding cassette transporters G5 and G8." Yu L, York J, von Bergmann K, Lutjohann D, Cohen JC, Hobbs HH. J Biol

Chem. 2003 May 2;278(18):15565-70.

"Dopamine Activity in the Nucleus Accumbens During Consummatory Phases of Oral Ethanol Self-Administration." Doyon W, York J, Diaz L, Samson H, Czachowshi C, Gonzales R. Alcohol Clin Exp Res. 2003 Oct; 27(10):1573-1582.

Permanent address:

16517 Lone Wolf Dr.

Leander, TX 78641