Gut microbiota and nuclear receptors in bile acid and lipid metabolism

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CHAPTER 2

Bile acid sequestrants: more than simple resins

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ABSTRACT

Purpose of review: Bile acid sequestrants (BAS) have been used for more than 50 years in the treatment of hypercholesterolemia. The last decade, bile acids are emerging as integrated regulators of metabolism via induction of various signal transduction pathways. Consequently, BAS treatment may exert unexpected side-effects. We discuss a selection of recently published studies that evaluated BAS in several metabolic diseases.

Recent findings: Recently, an increasing body of evidence has shown that BAS in addition to ameliorating hypercholesterolemia are also effective in improving glycemic control in patients with type 2 diabetes, although the mechanism is not completely understood. Furthermore, some reports suggested using these compounds to modulate energy expenditure. Many of these effects have been related to the local effects of BAS in the intestine by directly binding bile acids in the intestine or indirectly by interfering with signaling processes.

Summary: A substantial effort is being made by researchers to fully define the mechanism by which BAS improve glycemic control in type 2 diabetic patients. A new challenge will be to confirm in clinical trials the recent discoveries coming from animal experiments suggesting a role for bile acids in energy metabolism.

INTRODUCTION

Bile acids are amphipathic molecules that are synthesized in the liver from cholesterol. Until recently, they were considered to be simple detergents facilitating absorption of dietary fat and lipid-soluble vitamins. During the last decade, it has become clear that bile acids play an important role in the regulation of energy metabolism by acting as key signaling molecules, activating nuclear receptors and cell signaling pathways (1). Because bile acids are synthesized from cholesterol, their removal via sequestration in the intestine lowers LDL cholesterol (LDL-C) levels. Therefore, bile acid sequestrants (BAS) have been developed as a strategy to treat hypercholesterolemia. Interestingly, intestinal sequestration of bile acids also improves glycemic status in type 2 diabetes patients. Moreover, bile acid signaling influences energy expenditure. Modulating bile acid signaling via sequestration could, therefore, have multiple beneficial effects as therapy for the metabolic syndrome. Novel aspects of bile acid metabolism and the effects of intestinal sequestration in basal and clinical research will be covered in this review.

Regulation of bile acid metabolism

Bile acids are formed from cholesterol via a multistep process in two parallel metabolic pathways. The neutral (classic) pathway starts with 7-[alpha]-hydroxylation of cholesterol by cholesterol [alpha]-hydroxylase (CYP7A1) and the acidic pathway is initiated by sterol 27-hydroxylase (CYP27A1). At the step catalyzed by hydroxy delta 5-steroid dehydrogenase, both pathways converge leading to the main end-product cholic acid for the neutral pathway and chenodeoxycholic acid (CDCA) for the acidic pathway (see for review (1)). Particularly, expression and activity of CYP7A1 is regulated via a complex mechanism. In contrast, little is known about the regulation of CYP27A1, despite its role in both pathways and severe phenotype in humans lacking this enzyme (2). In rodents, CDCA is rapidly converted into the hydrophilic [alpha]-muricholic and [beta]-muricholic acids.

Conjugation of bile acids prior to their secretion increases their solubility. Human bile acids are mainly conjugated to glycine (3), whereas bile acids in rodents are almost exclusively taurine conjugated. Note that conjugated and most unconjugated bile acids are fully ionized at neutral pH and formally should be called bile salts. Bile salts

are secreted via the bile salt export pump from the liver into bile and induce secretion of cholesterol and phospholipids from the canalicular space (see for recent review (1)). In the intestine, bacteria deconjugate bile salts and convert primary bile salts into secondary bile salts. In humans, portions of cholic acid and CDCA are converted into the secondary bile salts deoxycholic acid (DCA) and lithocholic acid (LCA), respectively. In mice, DCA is formed from cholic acid and [beta]-muricholic acid is converted into [omega]-muricholic acid. Vice versa, bile salts are known to have antimicrobial activity. Conditions with decreased bile salt secretion, such as liver cirrhosis, are associated with bacterial overgrowth (4). In the ileum and colon, about 95% of bile salts are reabsorbed (except for LCA) by both active and passive mechanisms (for review see (1)). The reabsorbed bile salts are transported back to the liver via the portal venous circulation for resecretion into bile. This constant recycling of the bile salt pool is called the enterohepatic circulation. The remaining bile salts are lost in feces and are replenished by de novo synthesis from cholesterol in the liver. In humans, approximately 500 mg of bile salts are synthesized per day, being an important route for elimination of excess cholesterol.

Bile salts as signaling molecules

It has become clear that bile salts, in addition to solubilizing fat, act as important metabolic signaling molecules. Bile salts can activate nuclear receptors such as the farnesoid X receptor (FXR/NR1H4) and thereby modulate the transcription of genes involved in bile salt, cholesterol and glucose metabolism (5–7). Furthermore, bile salts activate the G protein-coupled bile acid receptor 1 (TGR5/GPBAR1) and (secondary) bile salts have been shown to activate the constitutive androstane receptor (8), pregnane X receptor (PXR/NR1I2)(9) and vitamin D receptor (10). PXR and the vitamin D receptor are involved in detoxifying bile salts as well as inhibiting bile salt synthesis (10–13).

Bile salts regulate their own synthesis via signaling through the nuclear receptor FXR in the liver and intestine. Bile salt activation of hepatic Fxr induces the expression of small heterodimer partner (Shp/Nr0b2)(14). SHP functions as a potent repressor of the nuclear receptor liver homolog receptor-1 (Lrh-1/Nr5a2)(15). Initially, in-vitro studies identified Lrh-1 as a critical transcription factor for Cyp7a1 (16–21). However, as liver-specific Lrh-1 gene deletion did not alter Cyp7a1 expression, the regulatory role of Lrh-1 on Cyp7a1 transcription remained controversial (19, 20). Recently, it was shown

that Lrh-1 is critical in vivo for the activation of Cyp7a1 as Lrh-1 knockdown mice could not increase bile salt synthesis during intestinal bile salt sequestration (18). Additionally, Lrh-1 controls bile salt synthesis by inducing Cyp8b1 transcription (19–21)), hereby changing the pool composition. Thus, bile salt activation of hepatic Fxr via Shp prevents Lrh-1 from activating Cyp7a1 and Cyp8b1 and therefore inhibits bile salt biosynthesis. However, several studies suggest that hepatic Fxr is only activated when bile salt levels are pathologically elevated and under normal physiological conditions, intestinal Fxr mediates feedback regulation of bile salt synthesis (22, 23).

When bile salts are taken up in the ileum, bile salt activation of intestinal Fxr induces the expression of FGF19 (fibroblast growth hormone 19) or Fgf15 (mouse ortholog of the human FGF19), a secreted protein that binds to the hepatic receptor complex Fgfr4/[beta]-Klotho. Via subsequent signal transduction, Cyp7a1 expression is repressed (24–28). Concurrently, Fgf15/FGF19 decreases bile salt absorption by inhibiting the ileal apical sodium-dependent bile salt transporter (Asbt/Slc10a2)(29). Thus, bile salts regulate their own synthesis from two distinct sites in the body. Recently, it was shown that the intestinal Fxr-mediated Fgf15 production contributes to the regulation of hepatic bile salt synthesis in mice mainly during the dark phase (30). However, Fxr-independent mechanisms are likely to play a role in regulating Fgf15 production, as intestinal $Fxr^{-/-}$ mice are still able to upregulate Fgf15 and downregulate Cyp7A1 expression upon TCA feeding (30). There might be a role for Lrh-1 herein, as Fgf15 expression is decreased in Lrh-1 knockdown and intestinal-specific Lrh-1 knockout mice (18, 20). Furthermore, it remains to be investigated whether Lrh-1 might be involved in the downstream signaling cascade of Cyp7A1 repression by Fgf15/FGF19.

There is a tight relation between bile salt and cholesterol metabolism. When cholesterol is converted into bile salts, hepatic microsomal cholesterol content decreases. This causes upregulation of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase (HMGCR) and increases LDL receptor (LDLR), as their expression is controlled by the sterol-sensing sterol regulatory element-binding proteins (SREBPs) (31). Consequently, more cholesterol is synthesized de novo and recruited from plasma LDL particles to deliver sufficient substrate for bile salt synthesis. Thus, modulation of hepatic cholesterol conversion into bile salts serves as a key mechanism by which bile salts can impact on plasma cholesterol levels. In addition, bile salts can bind to TGR5/GPBAR1. The most potent natural agonist is LCA, but several other bile salts such as DCA, CDCA

and cholic acid are able to activate TGR5. TGR5 is expressed in multiple organs lining the enterohepatic axis, such as gallbladder, cholangiocytes and intestine. $Tgr5^{-/-}$ mice showed a decreased total bile salt pool size, for which the mechanism is still unknown. Furthermore, TGR5 is expressed in several organs important for energy homeostasis such as brown adipose tissue (BAT) and skeletal muscle (reviewed in (32)). Thus, as bile salts are important signaling molecules modulating bile salt homeostasis, they could serve as an attractive target to treat several conditions associated with the metabolic syndrome.

BILE ACID SEQUESTRANTS

BAS are large polymers that bind negatively charged bile salts in the small intestine. Binding of bile salts in the intestine disrupts their enterohepatic circulation by preventing reabsorption from the gut, hence increasing their fecal excretion up to more than three times the normal (33). Consequently, bile salt synthesis is increased at the expense of plasma LDL-C concentrations. The cholesterol-lowering action of these drugs, thus, appears to be mainly mediated through increased bile salt excretion. Therefore, these compounds have been used as cholesterol-lowering agents since the early 1960s.

Three compounds are available on the market: cholestyramine, colestipol (first-generation BAS) and colesevelam-HCl. Cholestyramine and colestipol have greater affinity for dihydroxy than trihydroxy bile salts, which in time creates an imbalance in the bile salt pool by increasing the trihydoxy bile salt fraction. In contrast, colesevelam-HCl has been specifically engineered to bind bile salts via both hydrophobic and ionic sites, which enhances the affinity and specificity to bind bile salts compared to the traditional BAS and allows it to be used at lower doses (34, 35).

BAS are considered safe although they are associated with gastrointestinal complaints (*e.g.*, constipation, abdominal pain, nausea, etc.) which often results in treatment discontinuation. Furthermore, BAS can decrease the absorption of fat, fat-soluble vitamins and other nutrients, which should be considered during long-term treatment (36–38). In addition, cholestyramine and colestipol may affect the absorption of several drugs, which may become dangerous in case of drugs with a narrow therapeutic window, such as warfarin. In contrast, studies using colesevelam-HCl treatment reported less side-effects and drug interactions than the traditional BAS (35, 39). Finally, BAS

treatment often results in increased triglyceride levels, which limits the use of these compounds in patients with high plasma triglyceride levels (34).

Cholesterol-lowering properties

BAS have been used for more than four decades as cholesterol-lowering agents in the treatment of dyslipidemias. As monotherapy, these compounds have proven their efficacy in reducing LDL-C levels by 9–28% without changing or slightly increasing HDL-cholesterol (HDL-C) by 0–9% in a dose-dependent manner (for review see (40)). In addition, BAS have also been used in combination with other lowering drugs (such as statins, niacin, fibrates and ezetimibe) in order to achieve stronger LDL-C-lowering effects (Table 1).

Table 1. Effects of bile acid sequestrant therapy on improving plasma lipid profile.

Study	Compound	LDL-c baseline (mmol/l)	% change from baseline			Ref
			LDL-c	HDL-c	TG	
As monotherapy						
Patients with TC > 6.8 mM; 7.4 years; n=3806	Placebo	5.3	-3	2	13	[41]
	Cholestyramine 24 g/d	5.3	-15*	5	17	
Patients with LDL-c > 6.0 mM; 5 years; n=143	Placebo	5.9	-5*	2	26*	[42]
	Cholestyramine 24 g/d	6.3	-26*	8*	28*	
Patients with LDL-c > 4.5 mM; 4 weeks; n=264	Cholestyramine 12 g/d	6.7	-23	8	11	[43]
	Lovastatin 20 mg/d	7.3	-32	9	-21	
	Lovastatin 40 mg/d	7.0	-42	8	-27	
Patients with LDL-c > 4.1 mM; and < 6.5 mM; 8 weeks; n=196	Placebo	4.9	0.3	0.4	11.4*	[44]
	Colestipol 4 g/d	4.8	-5.2*	-0.9	14.8*	
	Colestipol 4 g/d	4.9	-10.9*	0.4	10.2*	
	Colestipol 8 g/d	4.7	-19.8*	-0.5	11.6*	
	Colestipol 16 g/d	4.9	-25.8*	-0.8	15.0*	
Patients with LDL-c > 4.14 mM; 6 weeks; n=137	Placebo	5.0	-0.3	-0.7	3.2	[35]
	Colesevelam-HCl 1.5 g/d	5.0	-2.1	0.7	1.5	
	Colesevelam-HCl 2.25 g/d	5.2	-5.4	0.7	-1.1	
	Colesevelam-HCl 3.0 g/d	5.2	-9.3*	8.9*	1.3	
	Colesevelam-HCl 3.75 g/d	5.2	-19.3*	8.4*	9.2	

Table 1. Continued

Study	Compound	LDL-c baseline (mmol/l)	% change from baseline			Ref
			LDL-c	HDL-c	TG	
Patients with LDL-c 3.4-5.7 mM; 24 weeks; n=494	Placebo	4.0	0	0	2	[45]
	Colesevelam-HCl 2.3 g/d	4.2	-9*	4*	7*	
	Colesevelam-HCl 3.0 g/d	4.1	-12*	4*	3	
	Colesevelam-HCl 3.8 g/d	4.1	-15*	4*	9*	
	Colesevelam-HCl 4.5 g/d	4.0	-18*	4*	7*	
In combination with other cholesterol-lowering drugs						
Patients with LDL-c 4.14-5.69 mM; 3 months; n=26	Cholestyramine 8 g/d	4.5	-13*	2.8	15.4	[46]
	Cholestyramine 8 g/d + Lovastatin 5 mg/d	4.5	-24.7*	4.7	12.3*	
	Lovastatin 5 mg/d	4.5	-20.7*	7.5	-4.6	
Patients with previous coronary bypass surgery; TC 4.79-9.07 mM; 2 years; n=162	Placebo	4.4	-5*	2	-5*	[47]
	Colestipol 30 g/d + Niacin (range 3-12 g/d)	4.4	-43*	37*	-43*	
Patients with previous coronary bypass surgery; TC 4.79-9.07 mM; 4 years; n=103	Placebo	4.4	-6	2	-5	[48]
	Colestipol 30 g/d + Niacin (range 3-12 g/d)	4.4	-40*	37*	-18	
Patients with apoB > 3.2 mM; 2.5 years; n=146	Placebo	4.5	-7*	5*	15.5	[49]
	Colestipol 30 g/d + Lovastatin 40 mg/d	5.1	-46*	15*	-8.8	
	Colestipol 30 g/d + Niacin 4 g/d	4.9	-32*	43*	-22.9*	
Patients with LDL-c < 4.1 mM; 4 weeks; n=135	Placebo	4.4	1	1	2	[50]
	Colesevelam-HCl 2.3 g/d	4.4	-7*	4*	14*	
	Lovastatin 10 mg/d	4.3	-22*	3	5	
	Colesevelam-HCl 2.3 g/d + Lovastatin 10 mg/d					
	Dosed together	4.5	-34*	3	9	
	Dosed separately	4.4	-32*	3	-3	
Patients with LDL-c < 4.1 mM; 6 weeks; n=258	Placebo	4.8	-4*	3	6	[51]
	Colesevelam-HCl 3.75 g/d	5.1	-16*	2	11*	
	Simvastatin 10 mg/d	4.7	-26*	3*	-17*	
	Colesevelam-HCl 3.75 g/d + Simvastatin 10 mg/d	5.1	-42*	10*	-12	
	Colesevelam-HCl 2.3 g/d	4.8	-8*	3*	11	
	Simvastatin 20 mg/d	4.7	-34*	7*	-12*	
	Colesevelam-HCl 2.3 g/d + Simvastatin 20 mg/d	4.9	-42**	4*	-12*	

Table 1. Continued

Study	Compound	LDL-c baseline (mmol/l)	% change from baseline			Ref
			LDL-c	HDL-c	TG	
Patients with LDL-c < 4.1 mM; 4 weeks; n=94	Placebo	4.8	3	4*	9	[52]
	Colesevelam-HCl 3.75 g/d	4.8	-12*	3*	10	
	Atorvastatin 10 mg/d	4.7	-38*	8*	-24*	
	Colesevelam-HCl 3.75 g/d + Atorvastatin 10 mg/d	4.8	-48*	11*	-1	
	Atorvastatin 80 mg/d	4.7	-53*	5*	-33*	
Patients with LDL-c 3.4-4.9 mM; 1 year; n=123	Atorvastatin 30 mg/d	3.8	-47*	12*	-25*	[53]
	Atorvastatin 20 mg/d+ niacin 2 g/d	4.1	-47*	25*	-33*	
	Colesevelam-HCl 3.8 g/d + Atorvastatin 20 mg/d + ER niacin 2 g/d	4.1	-57*	29*	-42*	
Patients with LDL-c > 3.4 mM and TG < 4.5 mM; 6 weeks; n=86	Placebo + ezetimibe 10 mg/d	4.5	-22*	3	4	[54]
	Colesevelam-HCl 3.8 g/d + Ezetimibe 10 mg/d	4.6	-54*	3	3	
Patients with LDL-c > 3.4 mM; 12 weeks; n=20	Ezetimibe 10 mg/d	4.3	-24*	0.9	-19*	[55]
	Colesevelam-HCl 1.875 g/d + Ezetimibe 10 mg/d	4.1	-30-	5.0	36*	
Patients with LDL-c 4.9 mM; 6 weeks; n=12	Ezetimibe 10 mg/d	5.2	-26*	8	4	[56]
	Colesevelam-HCl 3.75 g/d	4.5	-23*	-2	23	
	Colesevelam-HCl 3.75 g/d + Ezetimibe 10 mg/d	4.9	-39*	2	11	
Patients with LDL-c > 3.0 mM; 6 weeks; n=129	Fenofibrate 160 mg/d	4.1	-6*	10	-37*	[57]
	Colesevelam 3.75 g/d + Fenofibrate 160 mg/d	4.1	-17*	12	-32*	
In combination with diet						
Patients with LDL-c < 6.9 mM; 5 years; n=143	Diet	6.0	0	40*	2.5	[58]
	Cholestyramine 24 g/d + Diet	6.3	-25*	35*	9	
Patients with TC > 6.0 mM; 3.25 years; n=90	Placebo	4.8	-3	0	1	[59]
	Lipid-lowering diet	5.0	-16*	0	-20*	
	Cholestyramine 16 g/d + lipid-lowering diet	5.3	-36*	-4	0	

ApoB; apolipoprotein B; TC: total cholesterol concentration; LDL-c: cholesterol concentration in low-density lipoprotein; HDL-c: cholesterol concentration in high-density lipoprotein; TG: triglyceride concentration. *p<0.05 compared to baseline

Bile salts influence plasma triglyceride levels by inhibiting the production of triglyceride-rich very low density lipoprotein (VLDL) particles and accelerating their clearance from the circulation (reviewed in (60)). The impact of BAS on triglyceride metabolism is partly mediated by Fxr. BAS treatment changes the bile salt pool composition towards a pool composed of less potent Fxr agonists (61). Therefore, reduced Fxr activity influences the transcription of several genes involved in triglyceride metabolism, such as ApoC-II [a component of VLDL which activates lipoprotein lipase (Lpl) in capillaries] (62). Indirectly, Fxr also decreases triglyceride synthesis via Shp by inhibiting Srebp1c expression and the Fxr target gene Fgf15/FGF19 has been reported to induce fatty acid oxidation (63, 64). Accordingly, $Fxr^{-/-}$ mice develop severe fatty liver and elevated circulating free fatty acid, plasma cholesterol and triglyceride levels (65,66), whereas Fxr activation by feeding a cholic acid diet (62, 67) or administration of a synthetic Fxr agonist GW4064 (67) prevents hypertriglyceridemia. Thus, by attenuating FXR activation, bile salt sequestration impairs triglyceride metabolism.

One could speculate that BAS treatment in addition to reducing bile salt (re)absorption could also reduce cholesterol absorption, as cholesterol needs to be incorporated into mixed micelles in order to be absorbed. However, the fact that combined treatment of BAS with ezetimibe has additive effects in reducing plasma LDL-C levels, discards this hypothesis. Furthermore, it is likely that binding between BAS and bile salts takes place in the lower small intestine when the acidic pH of the stomach content has been neutralized. Therefore, micelle formation and cholesterol absorption can still take place in the upper small intestine. Taken together, the use of BAS as monotherapy or in combination with other cholesterol-lowering agents will depend on the sensitivity and tolerability of the patients to these drugs.

Cholesterol-lowering treatment is often initiated to reduce the risk of atherosclerosis and cardiovascular diseases, which can lead to ischemic heart disease and stroke. Cholestyramine and colestipol (either as monotherapy or in combination with other cholesterol-lowering agents) have proven their efficacy in reducing the risk of coronary heart disease which is related to the degree of LDL-C reduction and HDL-C increase (Table 2). Although the effects of colesevelam-HCl on reducing the risk of coronary heart disease have not yet been addressed, colesevelam-HCl treatment has been successful in decreasing the total amount of LDL particles, increasing LDL particle size

Table 2. Effects of bile acid sequestrant therapy on reducing the coronary heart disease risk.

Study	Compound	% change from baseline		CHD risk (%)	Ref
		LDL-c	HDL-c		
Patients without CHD; 7.4 years; n=3806	Placebo Cholestyramine 24 g/d	-3 -15*	2 5	Definitive CHD death: 2 Definitive CHD death: 1.6	[41]
Patients with Type II hyperlipoproteinemia and coronary artery disease; 5 years; n=143	Placebo Cholestyramine 24 g/d	-5* -26*	2 8*	Progression: 33; Regression: 10 Progression: 12; Regression: 12	[42]
Patients with previous coronary bypass surgery; 2 years; n=162	Placebo Colestipol 30g/d + Niacin (range 3-12 g/d)	-4* -26*	2 37*	Progression: 35.6 Progression: 25.0	[47]
Patients with previous coronary bypass surgery; 4 years; n=103	Placebo Colestipol 30g/d + Niacin (range 3-12 g/d)	-6 -40*	2 37*	Progression: 21.3 Progression: 17.9	[48]
Patients with previous CHD; 2.5 years; n=146	Placebo Colestipol 30 g/d + Lovastatin 40 mg/d Colestipol 30 g/d + Niacin 4 g/d	-7 -46* -32*	5 15 43	Progression: 46; Regression: 11 Progression: 21; Regression: 32 Progression: 25; Regression: 39	[49]
Patients with previous CHD; 3.25 years; n=90	Placebo Lipid-lowering diet Lipid-lowering diet + Cholestyramine 16 g/d	-3 -16* -36*	0 0 -4	Progression: 46 Progression: 15 Progression: 12	[59]

CHD: coronary heart disease; LDL-c: cholesterol concentration in low-density lipoprotein; HDL-c: cholesterol concentration in high-density lipoprotein. *p<0.05 compared to baseline

(68) and decreasing the levels of high-sensitive C-reactive protein (69,70), markers of cardiovascular risk and inflammation, respectively.

Bile acid sequestrants in diabetes

Type 2 diabetes mellitus (T2DM) is currently an epidemic disease worldwide. The initial treatment of T2DM consists of weight loss, control of hypertension, management of dyslipidemia as well as lifestyle modifications. However, if this is insufficient, a pharmacological approach must be established. Currently, there are 12 antidiabetic drugs available (including insulin) that target different pathological defects. Among them, colesevelam-HCl has been approved as an adjuvant to diet and exercise interventions

in order to improve glycemic control in patients with T2DM. One of the first studies that reported the beneficial effects of cholestyramine in type 2 diabetes appeared in the mid-1990s. Cholestyramine treatment (8 g/day for 6 weeks) achieved reductions in fasting plasma glucose of around 13% (71). Since then, the efficacy of BAS alone or in combination with other antidiabetic drugs (*e.g.*, insulin, sulfonylurea or metformin) on improving glucose control has been tested in several clinical trials (Table 3). The glucose-lowering effects of colesevelam-HCl treatment used as monotherapy was tested in a 16-week, randomized, double-blind study in adults with untreated prediabetes (72). As expected, colesevelam-HCl treatment reduced LDL-C, total cholesterol and apolipoprotein B levels. Additionally, fasting plasma glucose and HbA_{1c} were reduced (-4% and -2%, respectively). The combination of colesevelam-HCl with other antidiabetic drugs achieved additive reductions in fasting plasma glucose and HbA_{1c} of around 10% (61, 75–78). The efficacy of other BAS (colestimide and cholestyramine) in improving glycemic control has been addressed in two different studies. Treatment resulted in both reductions in fasting plasma glucose and HbA_{1c} of around 10% (71, 73).

Table 3. Effects bile acid sequestrant therapy on glycemic control.

Study	Compound	% change from baseline				Ref
		LDL-c	HDL-c	HbA_{1c}	FPG	
Without concomitant anti-diabetic drugs						
Untreated prediabetic subjects; 16 weeks; n=216	Placebo	2	4	0	-2	[72]
	Colesevelam HCl 3.75 g/d	-14*	4	-2*	-4*	
With concomitant antidiabetic drugs						
T2DM subjects with glyburide and/or insulin treatment; 6 weeks; n=21	Placebo	-2	0	-4	4	[71]
	Cholestyramine 16 g/d	-29*	3	-10	-11*	
T2DM subjects with oral antidiabetic or insulin treatment; 12 weeks; n=70	Pravastatin 10 mg/d	-16*	-6	0	0	[73]
	Colestimide 3 g/d	-23*	-6	-12*	-9*	
T2DM subjects with sulfonylurea and/or metformin treatment; 12 weeks; n=65	Placebo	2	-4	2	1	[74]
	Colesevelam-HCl 3.75 g/d	-8*	-7	-4*	-3*	

Table 3. Continued

Study	Compound	% change from baseline				Ref
		LDL-c	HDL-c	HbA_{1c}	FPG	
Controls and T2DM subjects with glipizide or dietary treatment; 8 weeks; n=28	Colesevelam-HCl 3.75 g/d Controls T2DM	 -11.1* -9	 0 0	 0 -8*	 0 -13*	[61*]
T2DM subjects with insulin or in combination with oral anti-diabetic; 16 weeks; n=287	Placebo Colesevelam-HCl 3.75 g/d	0.5 -12.3*	0.4 -0.5	1 -5*	13* -2	[75]
T2DM subjects with metformin, sulphonylurea or insulin treatment; 16-26 weeks; n=509	Placebo Colesevelam-HCl 3.75 g/d	0 -19*	1 0	0 -7*	6 -7*	[76]
T2DM subjects with metformin or in combination with oral anti-diabetic; 26 weeks; n=316	Placebo Colesevelam-HCl 3.75 g/d	3 -13*	0 0	2 -5*	7* -3	[77]
T2DM subjects with sulfonylurea and/or oral anti-diabetic treatment; 26 weeks; n=461	Placebo Colesevelam-HCl 3.75 g/d	0.6 -16*	0 0	2 -5*	4 -3	[78]

FPG: fasting plasma glucose; HbA1c: glycosylated hemoglobin; LDL-c: cholesterol concentration in low-density lipoprotein; HDL-c: cholesterol concentration in high-density lipoprotein; T2DM: type 2 diabetes mellitus. *p<0.05 compared to baseline

The exact mechanism of how BAS improve glycemic control has not been fully defined yet; however, multiple hypotheses have been proposed (79–81). One of the proposed mechanisms is based on the ability of BAS to alter the composition of the bile salt pool (1, 79, 82). In a recent study, bile salt pool sizes and synthesis rates were determined before and after 8 weeks of colesevelam-HCl treatment in patients with T2DM and in healthy controls (61). Although colesevelam-HCl treatment resulted in a more hydrophilic bile salt pool, no associations were found between these bile salts and glucose levels. It has been speculated that changes in bile salt pool composition may result in changes in FXR activity, which may result in improving insulin resistance (83). However, this could not be confirmed in two different studies in diabetic rats receiving BAS (84, 85).

A second proposed mechanism involves increased release of incretins after BAS treatment (84, 85, 86, 87). The decrease in bile salt reabsorption caused by BAS treatment

might increase the luminal concentration of bile salts which in turn would be able to activate TGR-5 (88, 89). This activation would ultimately stimulate the release of incretins such as glucagon-like protein 1 (GLP-1)(88), a potent glucose-lowering hormone produced in enteroendocrine L cells. GLP-1 is secreted into the blood and increases glucose-stimulated insulin release, likely by increasing the sensitivity of pancreatic [beta]-cells to glucose (84, 85, 90). Evidence supporting this hypothesis comes from studies in diabetic rats and T2DM patients receiving either control or BAS treatment (84, 85, 87, 91). BAS treatment resulted in increased glucose-stimulated Glp-1, peptide YY and insulin release (84). T2DM patients treated with colestimide had increased 2-h postprandial GLP-1 levels (87), whereas plasma glucose levels decreased. Similarly, treatment of type 1 diabetic patients with colesevelam-HCl resulted in reductions in HbA_{1c}, which were inversely related to increased GLP-1 levels (91). Thus, the increase in incretin release could be responsible for the observed decrease in fed serum glucose and increase insulin after BAS treatment, which is associated with improved insulin sensitivity.

In addition, it has been proposed that BAS treatment is able to modulate hepatic glucose metabolism (83), although the available data are contradictory. On the one hand, several studies indicate that bile salts suppress expression of genes involved in gluconeogenesis and decrease blood glucose levels (92–94). Bile salts modulate hepatic glucose metabolism via signaling pathways mediated by the nuclear receptor Fxr (92, 95). Fxr-null mice exhibit elevated serum glucose levels and impaired glucose tolerance and insulin sensitivity (96). Moreover, activation of Fxr by GW4064 or hepatic Fxr overexpression lowered blood glucose levels in both diabetic db/db and wild-type mice (97). This effect was the result of repression of hepatic gluconeogenic genes and increased hepatic glycogen synthesis and glycogen content, while increasing insulin sensitivity (97). On the other hand, recently it has been shown that long-term Fxr activation by GW4064 reduced basal energy expenditure and resulted in glucose intolerance and insulin resistance, whereas cholic acid administration had opposite effects. The authors hypothesized these effects to be due to differences in bile salt pool size and decreased Tgr5 activation, although plasma bile salts were unchanged (98). The glucose-lowering effects of bile salts may be mediated by Fgf15/FGF19. Kir et al. (99) recently showed that Fgf15-null mice exhibit enhanced blood glucose levels after an oral glucose bolus and decreased postprandial liver glycogen storage. Like insulin,

Fgf15/FGF19 inhibits hepatic gluconeogenesis (100) and FGF19 was shown to increase glucose uptake in 3T3-L1 adipocytes (26). Moreover, treatment with FGF19 prevented or even reversed the development of diabetes in two different mouse models (64). However, unlike FGF19, treatment of ob/ob mice with an FGF19 variant with selective activation of Fgfr4 failed to improve glucose levels and insulin sensitivity (101). Taken together, it is conceivable that changes in FXR activity resulting from BAS treatment may affect hepatic glucose fluxes. The exact mechanism, however, still needs to be elucidated.

Finally, it has been speculated that BAS may decrease glucose absorption in the intestine (102). However, this could not be confirmed in in-vivo studies (61, 103). In a recent pilot study, the effects of colesevelam-HCl on insulin sensitivity and potential binding to glucose were assessed in T2DM patients (103). Colesevelam-HCl treatment improved whole-body insulin resistance, although not by altering glucose absorption. Therefore, it seems unlikely that the glucose-lowering effects of BAS therapy are because of changes in glucose absorption in the intestine.

Bile acid sequestrants in energy metabolism

Recent data from mouse studies suggest a role for bile salts on energy metabolism. A diet enriched in cholic acid has been shown to increase energy expenditure and prevented diet-induced obesity (104). This effect was mediated by activation of BAT. Bile salt signaling through Tgr5 activated the enzyme deiodinase iodothyronine type 2 (D2) that converts the inactive thyroid hormone thyroxine into its active form, hereby increasing thyroid hormone receptor saturation. Furthermore, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (Pgc-1[alpha]) was activated, which is a master regulator of mitochondrial biogenesis (104). During high-fat feeding, female $Tgr5^{-/-}$ mice gained more weight than controls. Moreover, administration of the Tgr5 agonist INT-777 resulted in attenuation of weight gain as a consequence of enhanced energy expenditure (32, 105).

On the basis of mouse data, the role of bile salts in modulating (postprandial) energy metabolism would suggest that long-term BAS treatment would result in decreased energy expenditure and fat accumulation. To date, only one study investigated the relationship between plasma bile salt levels and energy expenditure in patients with T2DM and healthy controls before and after BAS treatment. Total plasma bile salts tended to

be lower in T2DM; however, no differences were found in resting energy expenditure (REE) between T2DM and controls at baseline or after BAS treatment, neither did plasma bile salts correlate with REE (106). More research needs to be performed in order to define the role of bile salts and their sequestration in regulating energy expenditure in human populations.

Other signaling by bile salts (possible effects of bile acid sequestrants)

BAS treatment results in a more hydrophillic bile salt pool, which is associated with decreased susceptibility for gallstone disease (107). Hydrophobic bile salts activate Tgr5 and decrease gallbladder smooth muscle function, which is a hallmark of gallbladder disease (108). It has been shown that Tgr5$^{-/-}$ mice fed a lithogenic diet are protected against cholesterol gallstone formation (109). A shift towards a more hydrophillic bile salt pool during BAS treatment might therefore lead to decreased TGR5 activation in the gallbladder which could protect against gallstone disease. In addition, the intestinal Fxr target gene Fgf15/FGF19 stimulates gallbladder filling, possibly involving the fibroblast growth factor receptor 3 (110). The fact that Fgf15/FGF19 is decreased during BAS treatment may contribute to diminished gallbladder relaxation. Therefore, BAS treatment might be beneficial for preventing gallstone formation, which has an increased prevalence in type 2 diabetic patients (111).

Furthermore, binding bile salts might interfere with their antimicrobial actions which might lead to bacterial overgrowth. Bacteria are known to have great impact on bile salt, cholesterol and glucose metabolism (reviewed in (112)). The composition of the gut microbiota is linked to energy extraction from the diet, synthesis of gut hormones involved in energy homeostasis, production of butyrate and the regulation of fat storage (112). Therefore, it is increasingly acknowledged that gut microbiota might contribute to the pathophysiology of obesity and T2DM, as these patients exhibit a different bacterial flora (reviewed in (113)). Thus, studies are warranted to investigate the effects of BAS treatment on gut microbiota composition.

CONCLUSION

Bile salt sequestration has major beneficial effects on disturbed human metabolism, it lowers LDL-C and ameliorates hyperglycemia in T2DM patients, thus seems a treatment

of choice in disorders such as the metabolic syndrome. Yet, some future challenges remain in deciphering the mechanisms responsible for the glucose-lowering effects of BAS and exploring possible consequences on energy metabolism, gallstone disease and gut microbiota composition.

REFERENCES

1. Lefebvre P, Cariou B, Lien F et al. Role of bile acids and bile Acid receptors in metabolic regulation. Physiol Rev 2009; 89:147-191.
2. Verrips A, Hoefsloot LH, Steenbergen GC et al. Clinical and molecular genetic characteristics of patients with cerebrotendinous xanthomatosis. Brain 2000; 123 (Pt 5):908-919.
3. Wiggins HS, Wootton ID. Studies in the bile acids. 3. The conjugated bile salts of certain primates. Biochem J 1958; 70:349-352.
4. Hofmann AF, Eckmann L. How bile acids confer gut mucosal protection against bacteria. Proc Natl Acad Sci U S A 2006; 103:4333-4334.
5. Makishima M, Okamoto AY, Repa JJ et al. Identification of a nuclear receptor for bile acids. Science 1999; 284:1362-1365.
6. Parks DJ, Blanchard SG, Bledsoe RK et al. Bile acids: natural ligands for an orphan nuclear receptor. Science 1999; 284:1365-1368.
7. Wang H, Chen J, Hollister K et al. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. Mol Cell 1999; 3:543-553.
8. Zhang J, Huang W, Qatanani M et al. The constitutive androstane receptor and pregnane X receptor function coordinately to prevent bile acid-induced hepatotoxicity. J Biol Chem 2004; 279:49517-49522.
9. Staudinger JL, Goodwin B, Jones SA et al. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. Proc Natl Acad Sci U S A 2001; 98:3369-3374.
10. Makishima M, Lu TT, Xie W et al. Vitamin D receptor as an intestinal bile acid sensor. Science 2002; 296:1313-1316.
11. Xie W, Radominska-Pandya A, Shi Y et al. An essential role for nuclear receptors SXR/PXR in detoxification of cholestatic bile acids. Proc Natl Acad Sci U S A 2001; 98:3375-3380.
12. Schmidt DR, Holmstrom SR, Fon Tacer K et al. Regulation of bile acid synthesis by fat-soluble vitamins A and D. J Biol Chem 2010; 285:14486-14494.
13. Li T, Chiang JY. Mechanism of rifampicin and pregnane X receptor inhibition of human cholesterol 7 alpha-hydroxylase gene transcription. Am J Physiol Gastrointest Liver Physiol 2005; 288:G74-84.
14. Lu TT, Makishima M, Repa JJ et al. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. Mol Cell 2000; 6:507-515.
15. Lee YK, Moore DD. Dual mechanisms for repression of the monomeric orphan receptor liver receptor homologous protein-1 by the orphan small heterodimer partner. J Biol Chem 2002; 277:2463-2467.
16. Goodwin B, Jones SA, Price RR et al. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. Mol Cell 2000; 6:517-526.
17. Nitta M, Ku S, Brown C et al. CPF: an orphan nuclear receptor that regulates liver-specific expression of the human cholesterol 7alpha-hydroxylase gene. Proc Natl Acad Sci U S A 1999; 96:6660-6665.
18. Out C, Hageman J, Bloks VW et al. LRH-1 is critical for adequate upregulation of Cyp7a1 gene transcription and bile salt synthesis during bile salt sequestration. Hepatology 2011; 53:2075-2085.
19. Mataki C, Magnier BC, Houten SM et al. Compromised intestinal lipid absorption in mice with a liver-specific deficiency of liver receptor homolog 1. Mol Cell Biol 2007; 27:8330-8339.
20. Lee YK, Schmidt DR, Cummins CL et al. Liver receptor homolog-1 regulates bile acid homeostasis but is not essential for feedback regulation of bile acid synthesis. Mol Endocrinol 2008; 22:1345-1356.

21. del Castillo-Olivares A, Gil G. Alpha 1-fetoprotein transcription factor is required for the expression of sterol 12alpha -hydroxylase, the specific enzyme for cholic acid synthesis. Potential role in the bile acid-mediated regulation of gene transcription. J Biol Chem 2000; 275:17793-17799.
22. Kim I, Ahn SH, Inagaki T et al. Differential regulation of bile acid homeostasis by the farnesoid X receptor in liver and intestine. J Lipid Res 2007; 48:2664-2672.
23. Houten SM, Volle DH, Cummins CL et al. In vivo imaging of farnesoid X receptor activity reveals the ileum as the primary bile acid signaling tissue. Mol Endocrinol 2007; 21:1312-1323.
24. Holt JA, Luo G, Billin AN et al. Definition of a novel growth factor-dependent signal cascade for the suppression of bile acid biosynthesis. Genes Dev 2003; 17:1581-1591.
25. Inagaki T, Choi M, Moschetta A et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. Cell Metab 2005; 2:217-225.
26. Kurosu H, Choi M, Ogawa Y et al. Tissue-specific expression of betaKlotho and fibroblast growth factor (FGF) receptor isoforms determines metabolic activity of FGF19 and FGF21. J Biol Chem 2007; 282:26687-26695.
27. Lin BC, Wang M, Blackmore C, Desnoyers LR. Liver-specific activities of FGF19 require Klotho beta. J Biol Chem 2007; 282:27277-27284.
28. Yu C, Wang F, Kan M et al. Elevated cholesterol metabolism and bile acid synthesis in mice lacking membrane tyrosine kinase receptor FGFR4. J Biol Chem 2000; 275:15482-15489.
29. Shin DJ, Osborne TF. FGF15/FGFR4 integrates growth factor signaling with hepatic bile acid metabolism and insulin action. J Biol Chem 2009; 284:11110-11120.
30. Stroeve JH, Brufau G, Stellaard F et al. Intestinal FXR-mediated FGF15 production contributes to diurnal control of hepatic bile acid synthesis in mice. Lab Invest 2010.
31. Horton JD, Goldstein JL, Brown MS. SREBPs: transcriptional mediators of lipid homeostasis. Cold Spring Harb Symp Quant Biol 2002; 67:491-498.
32. Pols TW, Noriega LG, Nomura M et al. The bile acid membrane receptor TGR5: a valuable metabolic target. Dig Dis 2011; 29:37-44.
33. Herrema H, Meissner M, van Dijk TH et al. Bile salt sequestration induces hepatic de novo lipogenesis through farnesoid X receptor- and liver X receptor alpha-controlled metabolic pathways in mice. Hepatology 2010; 51:806-816.
34. Insull W,Jr. Clinical utility of bile acid sequestrants in the treatment of dyslipidemia: a scientific review. South Med J 2006; 99:257-273.
35. Davidson MH, Dillon MA, Gordon B et al. Colesevelam hydrochloride (cholestagel): a new, potent bile acid sequestrant associated with a low incidence of gastrointestinal side effects. Arch Intern Med 1999; 159:1893-1900.
36. Watkins DW, Khalafi R, Cassidy MM, Vahouny GV. Alterations in calcium, magnesium, iron, and zinc metabolism by dietary cholestyramine. Dig Dis Sci 1985; 30:477-482.
37. Coronato A, Glass GB. Depression of the intestinal uptake of radio-vitamin B 12 by cholestyramine. Proc Soc Exp Biol Med 1973; 142:1341-1344.
38. West RJ, Lloyd JK. The effect of cholestyramine on intestinal absorption. Gut 1975; 16:93-98.
39. Donovan JM, Stypinski D, Stiles MR et al. Drug interactions with colesevelam hydrochloride, a novel, potent lipid-lowering agent. Cardiovascular Drugs and Therapy 2000; 14:681-690.
40. Hou R, Goldberg AC. Lowering low-density lipoprotein cholesterol: statins, ezetimibe, bile acid sequestrants, and combinations: comparative efficacy and safety. Endocrinol Metab Clin North Am 2009; 38:79-97.

41. Kuipers F, Stroeve JH, Caron S, Staels B. Bile acids, farnesoid X receptor, atherosclerosis and metabolic control. Curr Opin Lipidol 2007; 18:289-297.
42. Brufau G, Stellaard F, Prado K et al. Improved glycemic control with colesevelam treatment in patients with type 2 diabetes is not directly associated with changes in bile acid metabolism. Hepatology 2010; 52:1455-1464.
43. Kast HR, Nguyen CM, Sinal CJ et al. Farnesoid X-activated receptor induces apolipoprotein C-II transcription: a molecular mechanism linking plasma triglyceride levels to bile acids. Mol Endocrinol 2001; 15:1720-1728.
44. Tomlinson E, Fu L, John L et al. Transgenic mice expressing human fibroblast growth factor-19 display increased metabolic rate and decreased adiposity. Endocrinology 2002; 143:1741-1747.
45. Fu L, John LM, Adams SH et al. Fibroblast Growth Factor 19 Increases Metabolic Rate and Reverses Dietary and Leptin-Deficient Diabetes. Endocrinology 2004; 145:2594-2603.
46. Lambert G, Amar MJ, Guo G et al. The farnesoid X-receptor is an essential regulator of cholesterol homeostasis. J Biol Chem 2003; 278:2563-2570.
47. Sinal CJ, Tohkin M, Miyata M et al. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. Cell 2000; 102:731-744.
48. Watanabe M, Houten SM, Wang L et al. Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. J Clin Invest 2004; 113:1408-1418.
49. Rosenson RS. Colesevelam HCl reduces LDL particle number and increases LDL size in hypercholesterolemia. Atherosclerosis 2006; 185:327-330.
50. Bays HE, Davidson M, Jones MR, Abby SL. Effects of Colesevelam Hydrochloride on Low-Density Lipoprotein Cholesterol and High-Sensitivity C-Reactive Protein When Added to Statins in Patients With Hypercholesterolemia. The American Journal of Cardiology 2006; 97:1198-1205.
51. Devaraj S, Autret B, Jialal I. Effects of colesevelam hydrochloride (WelChol) on biomarkers of inflammation in patients with mild hypercholesterolemia. Am J Cardiol 2006; 98:641-643.
52. Garg A, Grundy SM. Cholestyramine Therapy for Dyslipidemia in Non-Insulin-dependent Diabetes Mellitus: A Short-Term, Double-Blind, Crossover Trial. Ann Intern Med 1994; 121:416-422.
53. Handelsman Y, Goldberg RB, Garvey WT et al. Colesevelam hydrochloride to treat hypercholesterolemia and improve glycemia in prediabetes: a randomized, prospective study. Endocr Pract 2010; 16:617-628.
54. Goldberg RB, Fonseca VA, Truitt KE, Jones MR. Efficacy and safety of colesevelam in patients with type 2 diabetes mellitus and inadequate glycemic control receiving insulin-based therapy. Arch Intern Med 2008; 168:1531-1540.
55. Goldfine AB, Fonseca VA, Jones MR et al. Long-term Safety and Tolerability of Colesevelam HCl in Subjects with Type 2 Diabetes. Horm Metab Res 2010; 42:23-30.
56. Bays HE, Goldberg RB, Truitt KE, Jones MR. Colesevelam hydrochloride therapy in patients with type 2 diabetes mellitus treated with metformin: glucose and lipid effects. Arch Intern Med 2008; 168:1975-1983.
57. Fonseca VA, Rosenstock J, Wang AC et al. Colesevelam HCl improves glycemic control and reduces LDL cholesterol in patients with inadequately controlled type 2 diabetes on sulfonylurea-based therapy. Diabetes Care 2008; 31:1479-1484.
58. Yamakawa T, Takano T, Utsunomiya H et al. Effect of colestimide therapy forglycemic control in type 2 diabetes mellitus with hypercholesterolemia. Endocr J 2007; 54:53-58.
59. Staels B, Kuipers F. Bile Acid Sequestrants and the Treatment of Type 2 Diabetes Mellitus. Drugs 2007; 67:1383-1392.
60. Brinton EA. Novel pathways for glycaemic control in type 2 diabetes: focus on bile acid modulation. Diabetes Obes Metab 2008.

61. Goldfine AB. Modulating LDL cholesterol and glucose in patients with type 2 diabetes mellitus: targeting the bile acid pathway. Curr Opin Cardiol 2008; 23:502-511.
62. Bays HE, Cohen DE. Rationale and design of a prospective clinical trial program to evaluate the glucose-lowering effects of colesevelam HCl in patients with type 2 diabetes mellitus. Curr Med Res Opin 2007; 23:1673-1684.
63. Prawitt J, Staels B. Bile acid sequestrants: glucose-lowering mechanisms. Metab Syndr Relat Disord 2010; 8 Suppl 1:S3-S8.
64. Chen L, McNulty J, Anderson D et al. Cholestyramine reverses hyperglycemia and enhances glucose-stimulated glucagon-like Peptide 1 release in zucker diabetic Fatty rats. J Pharmacol Exp Ther 2010; 334:164-170.
65. Shang Q, Saumoy M, Holst JJ et al. Colesevelam improves insulin resistance in a diet-induced obesity (F-DIO) rat model by increasing the release of GLP-1. Am J Physiol Gastrointest Liver Physiol 2010; 298:G419-G424.
66. Beysen C, Deines KC, Tsang EL et al. Colesevelam HCl improves glucose metabolism and increases plasma glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide concentrations in subjects with type 2 diabetes. Diabetologia 2009; 52:171.
67. Suzuki T, Oba K, Igari Y et al. Colestimide lowers plasma glucose levels and increases plasma glucagon-like PEPTIDE-1 (7-36) levels in patients with type 2 diabetes mellitus complicated by hypercholesterolemia. J Nippon Med Sch 2007; 74:338-343.
68. Kawamata Y, Fujii R, Hosoya M et al. A G protein-coupled receptor responsive to bile acids. J Biol Chem 2003; 278:9435-9440.
69. Maruyama T, Miyamoto Y, Nakamura T et al. Identification of membrane-type receptor for bile acids (M-BAR). Biochem Biophys Res Commun 2002; 298:714-719.
70. Kogire M, Gomez G, Uchida T et al. Chronic effect of oral cholestyramine, a bile salt sequestrant, and exogenous cholecystokinin on insulin release in rats. Pancreas 1992; 7:15-20.
71. Garg SK, Ritchie PJ, Moser EG et al. Effects of colesevelam on LDL-C, A1c and GLP-1 levels in patients with type 1 diabetes: a pilot randomized double-blind trial. Diabetes Obes Metab 2011; 13:137-143.
72. Stayrook KR, Bramlett KS, Savkur RS et al. Regulation of carbohydrate metabolism by the farnesoid X receptor. Endocrinology 2005; 146:984-991.
73. Yamagata K, Daitoku H, Shimamoto Y et al. Bile acids regulate gluconeogenic gene expression via small heterodimer partner-mediated repression of hepatocyte nuclear factor 4 and Foxo1. J Biol Chem 2004; 279:23158-23165.
74. De FE, Mitro N, Gilardi F et al. Coordinated control of cholesterol catabolism to bile acids and of gluconeogenesis via a novel mechanism of transcription regulation linked to the fasted-to-fed cycle. J Biol Chem 2003; 278:39124-39132.
75. Cariou B, Duran-Sandoval D, Kuipers F, Staels B. Farnesoid X receptor: A new player in glucose metabolism? Endocrinology 2005; 146:981-983.
76. Ma K, Saha P, Chan L, Moore D. Farnesoid X receptor is essential for normal glucose homeostasis. J Clin Invest 2006; 116:1102-1109.
77. Zhang Y, Lee FY, Barrera G et al. Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. Proc Natl Acad Sci U S A 2006; 103:1006-1011.
78. Watanabe M, Horai Y, Houten SM et al. Lowering Bile Acid Pool Size with a Synthetic Farnesoid X Receptor (FXR) Agonist Induces Obesity and Diabetes through Reduced Energy Expenditure. J Biol Chem 2011; 286:26913-26920.
79. Kir S, Beddow SA, Samuel VT et al. FGF19 as a postprandial, insulin-independent activator of hepatic protein and glycogen synthesis. Science 2011; 331:1621-1624.

80. Potthoff MJ, Boney-Montoya J, Choi M et al. FGF15/19 regulates hepatic glucose metabolism by inhibiting the CREB-PGC-1alpha pathway. Cell Metab 2011; 13:729-738.
81. Wu X, Ge H, Lemon B et al. Selective activation of FGFR4 by an FGF19 variant does not improve glucose metabolism in ob/ob mice. Proc Natl Acad Sci U S A 2009; 106:14379-14384.
82. Thomson AB, Keelan M. Feeding rats diets containing cheno- or ursodeoxycholic acid or cholestyramine modifies intestinal uptake of glucose and lipids. Digestion 1987; 38:160-170.
83. Schwartz SL, Lai YL, Xu J et al. The effect of colesevelam hydrochloride on insulin sensitivity and secretion in patients with type 2 diabetes: a pilot study. Metab Syndr Relat Disord 2010; 8:179-188.
84. Watanabe M, Houten S, Mataki C et al. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. Nature 2006; 439:484-489.
85. Thomas C, Gioiello A, Noriega L et al. TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab 2009; 10:167-177.
86. Brufau G, Bahr MJ, Staels B et al. Plasma bile acids are not associated with energy metabolism in humans. Nutr Metab (Lond) 2010; 7:73.
87. Hay DW, Carey MC. Pathophysiology and pathogenesis of cholesterol gallstone formation. Semin Liver Dis 1990; 10:159-170.
88. Lavoie B, Balemba OB, Godfrey C et al. Hydrophobic bile salts inhibit gallbladder smooth muscle function via stimulation of GPBAR1 receptors and activation of KATP channels. J Physiol 2010; 588:3295-3305.
89. Moore JG. Circadian dynamics of gastric acid secretion and pharmacodynamics of H2 receptor blockade. Ann N Y Acad Sci 1991; 618:150-158.
90. Choi M, Moschetta A, Bookout AL et al. Identification of a hormonal basis for gallbladder filling. Nat Med 2006; 12:1253-1255.
91. Ruhl CE, Everhart JE. Association of diabetes, serum insulin, and C-peptide with gallbladder disease. Hepatology 2000; 31:299-303.
92. Vrieze A, Holleman F, Zoetendal EG et al. The environment within: how gut microbiota may influence metabolism and body composition. Diabetologia 2010; 53:606-
93. Kootte RS, Vrieze A, Holleman F et al. The therapeutic potential of manipulating gut microbiota in obesity and type 2 diabetes mellitus. Diabetes Obes Metab 2011.
94. The Lipid Research Clinics Coronary Primary Prevention Trial results. I. Reduction in incidence of coronary heart disease. JAMA 1984; 251:351-364.
95. Brensike JF, Levy RI, Kelsey SF et al. Effects of therapy with cholestyramine on progression of coronary arteriosclerosis: results of the NHLBI Type II Coronary Intervention Study. Circulation 1984; 69:313-324.
96. A multicenter comparison of lovastatin and cholestyramine therapy for severe primary hypercholesterolemia. The Lovastatin Study Group III. JAMA 1988; 260:359-
97. Hunninghake DB, Stein EA, Bremner WF et al. Dose--Response Study of Colestipol Tablets in Patients with Moderate Hypercholesterolemia. Am J Ther 1995; 2:180-189.
98. Insull W, Toth P, Mullican W et al. Effectiveness of colesevelam hydrochloride in decreasing LDL cholesterol in patients with primary hypercholesterolemia: A 24-week randomized controlled trial. Mayo Clin Proc 2001; 76:971-982.
99. Denke MA, Grundy SM. Efficacy of low-dose cholesterol-lowering drug therapy in men with moderate hypercholesterolemia. Arch Intern Med 1995; 155:393-399.
100. Blankenhorn DH, Nessim SA, Johnson RL et al. Beneficial effects of combined colestipol-niacin therapy on coronary atherosclerosis and coronary venous bypass grafts. JAMA 1987; 257:3233-3240.

101. Cashin-Hemphill L, Mack WJ, Pogoda JM et al. Beneficial effects of colestipol-niacin on coronary atherosclerosis. A 4-year follow-up. JAMA 1990; 264:3013-3017.
102. Brown G, Albers JJ, Fisher LD et al. Regression of coronary artery disease as a result of intensive lipid-lowering therapy in men with high levels of apolipoprotein B. N Engl J Med 1990; 323:1289-1298.
103. Davidson MH, Toth P, Weiss S et al. Low-dose combination therapy with colesevelam hydrochloride and lovastatin effectively decreases low-density lipoprotein cholesterol in patients with primary hypercholesterolemia. Clin Cardiol 2001; 24:467-474.
104. Knapp HH, Schrott H, Ma P et al. Efficacy and safety of combination simvastatin and colesevelam in patients with primary hypercholesterolemia. Am J Med 2001; 110:352-360.
105. Hunninghake D, Insull W,Jr., Toth P et al. Coadministration of colesevelam hydrochloride with atorvastatin lowers LDL cholesterol additively. Atherosclerosis 2001; 158:407-416.
106. Moore A, Phan BA, Challender C et al. Effects of adding extended-release niacin and colesevelam to statin therapy on lipid levels in subjects with atherosclerotic disease. J Clin Lipidol 2007; 1:620-625.
107. Bays H, Rhyne J, Abby S et al. Lipid-lowering effects of colesevelam HCl in combination with ezetimibe. Curr Med Res Opin 2006; 22:2191-2200.
108. Knopp R, Tsunehara C, Retzlaff B et al. Lipoprotein effects of combined ezetimibe and colesevelam hydrochloride versus ezetimibe alone in hypercholesterolemic subjects: a pilot study. Metabolism 2006; 55:1697-1703.
109. Zema MJ. Colesevelam HCl and ezetimibe combination therapy provides effective lipid-lowering in difficult-to-treat patients with hypercholesterolemia. Am J Ther 2005; 12:306-310.
110. McKenney J, Jones M, Abby S. Safety and efficacy of colesevelam hydrochloride in combination with fenofibrate for the treatment of mixed hyperlipidemia. Curr Med Res Opin 2005; 21:1403-1412.
111. Levy RI, Brensike JF, Epstein SE et al. The influence of changes in lipid values induced by cholestyramine and diet on progression of coronary artery disease: results of NHLBI Type II Coronary Intervention Study. Circulation 1984; 69:325-337.
112. Watts GF, Lewis B, Brunt JN et al. Effects on coronary artery disease of lipid-lowering diet, or diet plus cholestyramine, in the St Thomas' Atherosclerosis Regression Study (STARS). Lancet 1992; 339:563-569.
113. Zieve FJ, Kalin MF, Schwartz SL et al. Results of the glucose-lowering effect of WelChol study (GLOWS): A randomized, double-blind, placebo-controlled pilot study evaluating the effect of colesevelam hydrochloride on glycemic control in subjects with type 2 diabetes. Clin Ther 2007; 29:74-83.