Mechanisms of triglyceride metabolism in patients with bile acid diarrhea

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Abstract

Bile acids (BAs) are essential for the absorption of lipids. BA synthesis is inhibited through intestinal farnesoid X receptor (FXR) activity. BA sequestration is known to influence BA metabolism and control serum lipid concentrations. Animal data has demonstrated a regulatory role for the FXR in triglyceride metabolism. FXR inhibits hepatic lipogenesis by inhibiting the expression of sterol regulatory element binding protein 1c via small heterodimer primer activity. Conversely, FXR promotes free fatty acids oxidation by inducing the expression of peroxisome proliferator-activated receptor α. FXR can reduce the expression of microsomal triglyceride transfer protein, which regulates the assembly of very low-density lipoproteins (VLDL). FXR activation in turn promotes the clearance of circulating triglycerides by inducing apolipoprotein C-Ⅱ, very low-density lipoproteins receptor (VLDL-R) and the expression of Syndecan-1 together with the repression of apolipoprotein C-Ⅲ, which increases lipoprotein lipase activity. There is currently minimal clinical data on triglyceride metabolism in patients with bile acid diarrhea (BAD). Emerging data suggests that a third of patients with BAD have hypertriglyceridemia. Further research is required to establish the risk of hypertriglyceridaemia in patients with BAD and elicit the mechanisms behind this, allowing for targeted treatment.

Key words: Bile acids; Bile acid diarrhea; Triglycerides; Farnesoid X receptor
Core tip: Bile acids are essential for the absorption of dietary lipids. The farnesoid X receptor (FXR) has a crucial role in triglyceride metabolism through regulating hepatic de novo lipogenesis and modulating free fatty acids oxidation and triglyceride clearance. There is a reported interruption of the metabolism of triglycerides in patients with bile acid diarrhea (BAD) with a third of patients suffering with hypertriglyceridemia. Emerging treatments for BAD such as therapeutic imitation of the FXR may aid in alleviating symptoms and improving triglyceride levels.

INTRODUCTION

Bile acids (BAs) are essential for the absorption of lipids - hallmark of the modern diet. Bile acid diarrhea (BAD) is commonly overlooked in the differential diagnosis of chronic diarrhea despite it being potentially extremely debilitating for the patient with impact on daily activities due to urgency, increased bowel frequency and the fear of incontinence. Primary bile acid diarrhea (PBAD; formerly known as type 11) is most common with secondary BAD (due to ileal resection, post cholecystectomy etc. formerly known as type 1 and 111 respectively). Since the 1960s, bile acid sequestration has been recognised to influence bile acid metabolism and control serum lipid concentrations however the underlying mechanisms connecting lipid metabolism and bile acids have only started to be recognized more recently[1].

BILE ACID FORMATION

The formation of primary BAs, cholic acid and chenodeoxycholic acid (CDCA), involves hydroxylation of cholesterol catalyzed by the cytochrome P450 enzyme cholesterol 7α-hydroxylase (CYP7A1) resulting in a relative deficiency of hepatic microsomal cholesterol. This is ensued by upregulation of low-density lipoproteins (LDL) receptor expression and activity to harvest cholesterol from the systemic circulation resulting in reduced plasma LDL-cholesterol levels[2]. This mechanism has been reproduced by bile acid sequestrants (BAS) and ileal resection causing an interruption of the enterohepatic circulation of BAs resulting in a hypocholesterolaemic effect and hypertriglyceridemia through increased secretion of triglyceride rich very low-density lipoproteins (VLDL) particles from the liver[3-6]. Resin-bound primary BAs (which have been mixed with BAS resins such as Cholestyramine) become inaccessible for microbial biotransformation into secondary BAs in the intestine which in turn results in a relative reduced proportion of secondary BAs, promoting BA synthesis from cholesterol to restore the BA pool.

Bile acid mediated triglyceride metabolism

BAs emulsify dietary lipids, which are then packaged into chylomicrons by the enterocytes and released into the lymphatic vessels. Chylomicrons are composed of phospholipids, cholesterol esters, triglycerides and apolipoprotein B-48 but only achieve maturation after acquiring Apolipoproteins C-II and E from circulating high-density lipoproteins (HDLs)[7]. The triglycerides are then hydrolysed in the capillaries of adipose and muscle tissue by lipoprotein lipase (LPL) into fatty acids. The residual chylomicrons (which still have a small amount of triglycerides) are finally taken up by the liver and used for the synthesis of VLDLs. Once released into the bloodstream, VLDLs also obtain Apolipoproteins C-II and E from circulating HDLs and release free fatty acids (FFAs) to muscle and adipose tissues. The liver then removes the VLDLs (mainly the larger, triglyceride-rich ones) from the bloodstream. VLDLs may also become LDLs[8].

Farnesoid X-activated receptor mediated metabolism

Changes in expression of the LDL receptor cannot be used to account for the increase in triglyceride levels observed with interruption of the enterohepatic circulation of BAs. Animal data has demonstrated a regulatory role for the farnesoid X-activated receptor (FXR) in triglyceride metabolism. The FXR, a member of the nuclear hormone receptor family, is expressed in hepatocytes and ileal enterocytes. BAs are agonists of the FXR therefore once the ileal FXR is activated, there is release of fibroblast growth factor 19 (FGF-19 in humans, FGF-15 in mice) into the portal circulation which then binds to fibroblast growth factor 4 (FGF-4) in hepatocytes. Through hepatic FXR, this results in downregulation of CYP7A1, which consequently inhibits the classical BA synthetic pathway by induction of small heterodimer primer (SHP) activity, thus inhibiting the conversion of cholesterol to BAs[9]. SHP mediates a signaling cascade to impede the action of liver X receptor (LXR) resulting in an inhibition of the expression of sterol regulatory element binding protein 1c (SREBP-1c) whose expression is otherwise induced by LXR interacting with liver receptor homologue 1. SREBP-1c is an essential transcription factor that regulates hepatic triglyceride synthesis by inducing enzymes such as fatty acid synthase, which are involved in lipogenesis[10].

Other postulated mechanisms may account for the triglyceride-lowering effect of FXR activation. Activation
of FXR modulates FFA oxidation and triglyceride clearance\[11\]. Incubation of human hepatoma HepG2 cells with FXR ligands (CDCA) and agonists demonstrated the induction of expression of peroxisome proliferator-activated receptor α (PPARα) and its target genes to promote FFA oxidation\[12\]. PPARα is thought to play a critical role in mediating triglyceride metabolism. Fibrates activate PPARα, which lowers hepatic apoC-III production and increases LPL mediated lipolysis. This results in increased catabolism of triglyceride-rich particles and reduced secretion of VLDLs causing hypotriglyceridaemia\[13\]. Micronomal triglyceride transfer protein (MTP) is critical for lipoprotein synthesis and secretion in the liver and small intestine by participating in the transfer of triglycerides to newly synthesised apolipoprotein B (apo-B). Hepatocyte nuclear factor-4 (HNF-4) has been demonstrated to regulate MTP gene expression through elevated HNF-1 levels. Hep G2 cells cultured with CDCA, a ligand for FXR, exhibited increased expression of SHP, which suppresses HNF-4 activity and reduces mRNA levels for MTP and apo-B thus reducing the secretion of VLDL\[14\]. Syndecan-1 (SDC1), a heparin sulphate, is involved in the binding, internalisation and degradation of low-density lipoprotein. It is induced by FXR ligands therefore the administration of CDCA results in increased expression of SDC1 causing a hypotriglyceridaemic effect\[15\]. Clearance of triglyceride-rich lipoproteins may be induced by CDCA causing an increased expression of the VLDL receptor\[16\].

**Animal models and clinical data**

A mouse study demonstrated that the administration of synthetic FXR agonists or FGF-15 in mice lacking the apical ileal sodium dependant bile acid transporter (ASBT) gene, a sodium-dependant BA transporter responsible for reabsorbing > 95% of BAs in the ileum, resulted in a decrease in serum triglyceride and cholesterol levels. There was also a 50% reduction in the bile pool size, which was thought to account for the changes seen in lipid metabolism through eliciting a compensatory increase in BA synthesis, which augments the hepatic requirement for cholesterol\[17\]. Another murine study revealed that pharmacological inhibition of this ileal sodium-dependant BA transporter resulted in reduced plasma triglyceride levels\[18\].

It has been demonstrated that activation of the FXR by ligands such as cholic acid results in lower plasma triglyceride levels by influencing LPL activity through induction of apolipoprotein C-II expression\[19\]. BA synthetic activators of the FXR have also exhibited a decrease in triglyceride levels through the repression of apolipoprotein C-III, a cofactor for LPL, which mediates triglyceride hydrolysis\[20\]. Elevated triglyceride concentrations have been demonstrated in patients with monogenic familial hypertriglyceridaemia who were found to display a defect in ileal BA absorption\[21\]. Individuals with decreased BA synthesis secondary to a CYP7A1 deficiency have also shown raised triglyceride levels\[22\]. These clinical observations exhibit an important direct relationship between triglyceride metabolism and BAs.

Despite an increased knowledge of the possible physiological mechanisms underlying triglyceride metabolism in patients with BAD, there is minimal clinical data available to support this occurrence. A study of twenty-four patients with an ileoanal anastomosis (IAA) and ileal pouch, which may disrupt the absorption of bile acids, demonstrated reduced serum total and LDL cholesterol, and LDL and HDL triglyceride levels but similar VLDL triglyceride levels to the control group. Their triglyceride:cholesterol ratio was higher. It was thought that reduced hepatic cholesterol content was secondary to cholesterol malabsorption in these patients with an inadequate increase in cholesterol synthesis, so that biliary cholesterol secretion deteriorated with reduced cholesterol remaining for VLDL production\[23\].

Another study in 12 patients with an IAA and ileal pouch revealed that the malabsorption of BAs resulted in a significant decrease in plasma concentrations of total cholesterol, LDL cholesterol and apolipoprotein B. Plasma concentrations of total triglycerides and VLDL triglycerides increased significantly\[24\].

A more recent study in 47 patients with primary BAD who had a Selenium-75-homophoric acid taurine (SeHCAT) scan retention value of less than 15% demonstrated raised triglycerides in 13 (27.7%) of these patients. Raised FGF-19 levels were associated with higher serum triglycerides. There was a significant negative correlation seen between triglycerides and SeHCAT retention with patients exhibiting high triglycerides having significant lower SeHCAT retention values. Furthermore, triglycerides were positively correlated with age and body mass index (BMI)\[25\].

The few studies that are available are of very small numbers and are detailed in Table 1.

In our series, 25 of the 67 patients studied were found to have raised triglyceride levels (> 1.9 mmol/L). 19/25 (76%) had PBAD, with 6/25 (24%) having SBAD. Of the 25 patients with raised triglyceride levels, 17 (68.0%) patients had their BMI recorded. All 17 (100%) patients had a BMI above the normal range with 11/17 (64.7%) being classified as overweight and 6/17 (35.3%) as obese. Co-morbidity was present in 14/25 (56.0%) patients with raised triglyceride levels.

Over a third of patients (37%) in our series with BAD have hypertriglyceridaemia, with similar ratios among the PBAD and SBAD groups. Of these patients, most had severe BAD (48%) and were all found to be either overweight or obese (BMI > 30). Other high-risk groups identified in patients with hypertriglyceridaemia were those diagnosed with PBAD (76%), BMIs indicative of > 30 (100%) and the presence of co-morbidity (56%). The relationship between triglyceride levels (cut off 1.9 mmol/L) and SeHCAT 7 d retention
FGF-19 or mutations in the ASBT gene. Expression of FGF-19 in ileal biopsies has been associated with transcription of ASBT in patients with primary BAD but this correlation was found to be stronger in the control group[25].

Pattni et al[26] demonstrated the median value of serum FGF-19 in the group of patients with primary BAD was 65% lower of the value in the control group who also had chronic diarrhoea but normal SeHCAT retention value. Lower FGF-19 levels were also found in patients with severe BAD (SeHCAT retention value 0-5%) and increased levels of BAs as well as in the obese patients with primary BAD.

Decreased serum FGF-19 levels have been demonstrated in patients with obesity[27]. In addition to this, microRNA (miR-34a) has been found to be elevated in obesity. miRs function as negative gene regulators and inhibit and/or destabilize target mRNAs. After a meal, FXR induces the expression of FGF-19, which then binds to FGF-4 and its coreceptor β-Klotho (βKL), triggering activation of cellular kinases to mediate postprandial responses. miR-34a has a role in regulating β-Klotho expression therefore raised levels in obesity attenuated hepatic FGF-19 signaling by directly targeting βKL[28]. Taken together, these findings would suggest hypertriglyceridemia is more prevalent in patients diagnosed with primary BAD, severe BAD (SeHCAT retention < 5%) and being obese.

It is unclear whether dietary factors influence bile salt metabolism. In rats, a fat-free diet induced a reduction in BA synthesis likely secondary to a slower recirculation of BAs via the enterohepatic circulation. Further supplementation of cholesterol and saturated fatty acids increased BA synthesis causing an increase in the production of LDL-cholesterol[29]. In humans, ingestion of low and high-fat diets were associated with a 23% and 28% lower synthesis rate of total BAs respectively demonstrating that conversion of cholesterol to bile salts is affected by dietary fat intake[30]. These findings suggest that dietary fat intake may affect BA metabolism and therefore account for the higher levels of hypertriglyceridemia demonstrated in patients with obesity in although the mechanisms behind this remain undefined. An overview of FXR

**DISCUSSION**

**Mechanistic overview**

The mechanisms underlying the high incidence of hypertriglyceridemia demonstrated in clinical studies detailed in Table 1 remains uncertain with most literature pointing to a variety of mechanisms favouring reduced triglyceride levels in these patients. The mixed triglyceride results exhibited in patients with BAD may be a reflection of the differing genetic variation (e.g., polymorphisms of SREBP-1) in components of the signaling pathway seen in this disease group. One possible theory is that the vast majority of our patients were diagnosed with primary BAD, which may indicate that this is secondary to interruption of the enterohepatic circulation of BAs via a physiological mechanism - as noted in our series. This may be due to impaired responsiveness/activation of the FXR to BAs, defective feedback inhibition of BA synthesis by FGF-19 or mutations in the ASBT gene. Expression of FGF-19 in ileal biopsies has been associated with transcription of ASBT in patients with primary BAD but this correlation was found to be stronger in the control group[25].

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**Table 1  Studies measuring lipid levels in patients with bile acid diarrhoea**

| Study         | Type of BAD | Patients | Results                                      |
|---------------|-------------|----------|----------------------------------------------|
| Hakala et al[23] | Secondary   | 24 patients with IAA + ileal pouch | ↓ LDL chol ↓ LDL + HDL triglycerides |
| 1997          |             | 20 controls                          |                                |
| Akerlund et al[24] | Secondary   | 12 patients with IAA + ileal pouch | ↓ LDL chol ↑ VLDL triglycerides |
| 1994          |             | 12 controls                           |                                |
| Walters et al[25] | Primary     | 47 patients with BAD                 | ↑ Triglycerides in 13/47 (27.7%) |
| 2015          |             | 59 controls                           |                                |
| This study    | Total       | 67 patients                           | ↑ Triglycerides in 25/67 (37.3%) |
| 2015          | Primary     | 48 patients                           | 18/48 with PBAD (37.5%)         |
|               | Secondary   | 19 patients                           | 7/19 with SBAD (36.8%)          |

BAD: Bile acid diarrhoea; VLDL: Very low-density lipoproteins; LDL: Low-density lipoproteins.

**Figure 1  Relationship of triglycerides to selenium-75-homophoric acid taurine retention values in patients with primary and secondary bile acid diarrhoea.** The linear regression (R² value 0.0129) and normal values for TGs (1.9 mmol/L) and selenium-75-homophoric acid taurine (SeHCAT) retention (15%) are shown.

Values in the 67 patients are shown in Figure 1.
FXR is expressed by hepatocytes and ileal enterocytes. BAs, agonists of the FXR, are re-absorbed via the ASBT in the ileum, releasing FGF-19 (hormone) into the portal circulation. FGF-19 binds to FGF-4 in hepatocytes and down-regulates CYP7A1 via klotho β and inhibition of SHP. This impedes BA synthesis.

FXR inhibits hepatic lipogenesis by inhibiting the expression of SREBP-1c via SHP. Conversely, FXR promotes FFA oxidation by inducing the expression of PPARα. FXR can reduce the expression of microsomal MTP, which regulates the assembly of VLDL. FXR activation promotes the clearance of circulating triglycerides by inducing Apo-CⅡ, VLDL-R and the expression of SDC-1 together with the repression of Apo-CⅢ, which increases LPL activity.

Chylomicrons (CRs) containing Apo-CⅡ, Apo-E and Apo-B48, lose Apo-CⅡ through hydrolysis by LPL and are then taken up by the liver and used for the synthesis of VLDLs. VLDLs follow the same process of CRs once released into the bloodstream and release FFA to adipose and muscle tissues after LPL activation.

**CONCLUSION**

FXR plays a crucial role in triglyceride metabolism by regulating hepatic de novo lipogenesis and modulating FFA oxidation and triglyceride clearance. Clinical data supports the interrupted metabolism of triglycerides in patients with BAD. We have also identified risk groups with specific phenotypic patterns of BAD who are particularly vulnerable to this effect. Recognising the specific high-risk groups demonstrated in this study of patients with raised triglyceride levels would permit earlier diagnosis and management of hypertriglyceridaemia, which is a strong predictor for coronary heart disease[31].

Further studies linking triglyceride levels with FGF-19 measurement would help in determining the role of FGF signaling in those with BAD. Therapeutic imitation of the FXR signaling pathway in inhibiting hepatic lipogenesis has already been demonstrated in a recent proof-of-concept study investigating the effects of obeticholic acid in patients with primary BAD. This FXR agonist showed an increase in median fasting FGF-19 levels with an improvement in symptoms of diarrhoea and triglyceride levels[32]. Thus emerging treatments for BAD may not only alleviate symptoms but also tackle underlying pathological disturbances in this case hypertriglyceridaemia.

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