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Review Article

# BILE ACIDS AND SAPONINS IN INCRETIN MODULATION: MANY SIMILARITIES, FEW DIFFERENCES

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**Abstract**

**Objective**: Bile acids (BA) are a large complex family of amphipathic molecules with a steroid backbone. They exhibit considerable structural diversity within different cells, body compartments and pathophysiological states, and there is considerable interspecies variability. In addition to their role in the solubilization of dietary lipids and the fat-soluble vitamins A, K, D and E, bile acids are signaling molecules that regulate many cell types by activating specific receptors in the nucleus and at the plasma membrane. Bile acids are natural ligands for the farnesoid X receptor (FXR). Bile acids may play an additional role in modulating incretin release through binding to a recently identified G-protein–coupled cell surface receptor known as Takeda G-Protein Receptor 5 (TGR5). **Methods**: TGR5 activation in enteroendocrine cells stimulate the release of glucagon-like peptide-1 (GLP-1) which maintains blood glucose homeostasis by promoting glucose-induced insulin secretion, suppressing glucagon release, delaying gastric emptying, promoting satiety, and increasing glucose disposal in the peripheral tissues. In brown adipose tissue and skeletal muscle, TGR5 mediates energy expenditure through a BA–TGR5–cAMP–D2 (type 2 iodothyronine deiodinase) signaling pathway. **Results**: However, endogenous bile acids have a lower affinity and selectivity for TGR5, and they have specific biological properties about localization and cycling. Several research evidence has identified saponins as the antidiabetic principle in plants and its incretin modulating effect have shown several similarities to that of BA by stimulating TGR5/GLP1 pathway, enhancement of insulin secretion, promoting glycolysis, decreasing gluconeogenesis as well as inhibition of αglucosidase. **Conclusion**: Therefore, this work is said to review the similarities and differences between bile acid and saponin in incretin modulations

**Key words:** Saponin, Bile acids, TGR5, Incretin, Signaling.

## INTRODUCTION

### Bile acids and its traditional role

Bile acids are effective digestive surfactants that stimulate absorption of lipids including fat-soluble vitamins (such as vitamin A, D, E and K), acting as emulsifiers [1, 2]. Synthesis of bile acid is the major pathway for cholesterol catabolism in most species other than human and account for ~50% of the daily turnover of cholesterol [2].Its synthesis occurs solely in the liver by a series of enzymatic reactions in the hepatocyte that convert hydrophobic cholesterol into more water-soluble amphipathic compounds [3].The synthesis of bile acids is localized primarily in the perivenous hepatocytes; the cells surrounding the central hepatic vein [4]. The direct products of the bile acid synthetic pathways are referred to as primary bile acids. Cholic acid and chenodeoxycholic acid are the primary bile acids formed in humans. The action of intestinal bacterial flora on primary bile acids results in the formation of secondary bile acid species: deoxycholic and lithocholic acids, derived from a cholic acid and chenodeoxycholic acid, respectively [3].

The steps leading to the formation of primary bile acids involve hydroxylation of cholesterol, catalyzed by the cytochrome P450 enzyme cholesterol 7α-hydroxylase (CYP7A1), the first and rate-limiting step of the so-called classic or neutral pathway of bile acid biosynthesis [5,3, 2]. The activity of CYP7A1 is subject to complex modes of regulation. The conversion of cholesterol to bile acids is primarily determined by this pathway[1]. Bile acid synthesis can also occur by an “alternative” or “acidic” pathway, which is control by the enzyme CYP27A1 and converts oxysterols to bile acids [3]. It is estimated that only 6% of bile acid synthesis occurs through this pathway [6].But data also suggest that under definite conditions, such as fetal development and chronic liver disease [7] this pathway may contribute more significantly to bile acid synthesis. The consecutive conversion of bile acid intermediates from either the classical or alternative pathways to cholic acid or chenodeoxycholic acid is regulated by CYP8B1; interaction of these intermediates with this enzyme determines the amount of cholic acid versus chenodeoxycholic acid formed. Hydroxylation via CYP8B1 results in the formation of the more hydrophilic cholic acid molecule. Thus, the cholic acid/chenodeoxycholic acid ratio determines the overall hydrophobicity (and biological properties) of the bile acids pool, before their secretion into the bile canalicular lumen for storage in the gallbladder as mixed micelles with phospholipids and cholesterol, further enhancing their hydrophilicity [3]. Upon absorption of a meal, gallbladder contraction releases micellar bile acids into the intestinal lumen to aid digestion. Enterohepatic circulation enabled 95% of bile acids to be reabsorbed from the distal ileum and transported back to the liver via the portal circulation. Interestingly, the perivenous hepatocytes, which account for the synthesis of bile acids, are not involved in the reuptake of bile acids; bile acids are taken up and transported mainly by pericentral hepatocytes that surround the portal triads, where portal blood enters the liver acinus[8]. The zonation differences accounting for where bile acids are produced and re-enter the liver are relatively unexplored; thus, the pathophysiological importance of these observations is unknown at this time [3]. Only ~5% of bile acids are not reabsorbed and is eliminated in the feces. This small amount of loss is replenished through *novo* synthesis of bile acids in the liver[1,7]. The size of the bile acid pool is tightly regulated within the liver and intestine to prevent cytotoxic accumulation of bile acids [2]. As the bile acid pool size increases, a feedback mechanism, regulated by the interplay of several nuclear receptors, is activated to inhibit *de novo* bile acid synthesis. In the liver, a nuclear receptor called living receptor homolog (LRH)-1 activates gene transcription of the CYP7A1 gene [3]. In 1999, bile acids were identified as the natural ligands for the farnesoid X receptor (FXR). By binding to the nuclear receptor FXR, bile acids mediate control of their synthesis [11]. (Makishima*et al.,* 1999). FXR is thus a bile acid sensor. FXR can be activated by both primary and secondary conjugated bile acids, but chenodeoxycholic acid appears to be the most potent natural bile acid ligand [9]. FXR functions as a biological regulator of bile acid synthesis via its transcriptional induction of the inhibitory nuclear receptor SHP [3]. In the liver, small heterodimer partner (SHP) exerts its inhibitory effect by interacting with LRH-1 and subsequently repressing CYP7A1 transcription activation by LRH-1 [3].Bile acids can also inhibit transcription of CYP7A1 by repressing another nuclear receptor, hepatocyte nuclear factor (HNF)-4α, intestinal FXR activation due to trans intestinal bile acid flux after a meal also induces the expression of fibroblast growth factor (FGF)-19, which is released by small intestine epithelial cells and circulates to bind to

hepatocyte FGF receptor 4 (FGFR4), receptor that signals a reduction in bile acid synthesis via c-Jun NH2-terminal kinase (JNK) pathway activation [3]. Repression of CYP7A1 results in the reduction of bile acid synthesis from intra-hepatic cholesterol in response to the daily feeding-fasting cycle. Finally, emerging evidence suggests that expression of intestinal bile acid–binding protein (IBABP), which may be involved in the shuttling of bile acids from the apical to basolateral side of enterocytes on reabsorption, as well as $Na^+$taurocholateco-transporting polypeptide (NTCP), which uptakes bile acids returning to the liver, may also be under partial FXR control, thus, FXR activation serves as a critical modulator of the enterohepatic circulation and *de novo* synthesis of bile acids to provide tight regulation of the bile acid pool[3,10]

**Bile acid and lipid metabolism**

Manipulation of bile acid metabolism by bile acid sequestration has been recognized as a means of regulating systemic lipid concentrations since the 1960s, the underlying molecular mechanisms linking bile acids and lipid metabolism have been unraveled over the last decade. Bile acids together with ileal resection interrupt enterohepatic circulation of bile acids resulting in the reduction of total plasma and LDL cholesterol as well as the elevation of HDL cholesterol, apolipoprotein (apo)-AI, and triglycerides[9, 11]

As a direct consequence of interjecting the return of bile acids to the liver, CYP7A1 expression becomes de-repressed, and conversion of cholesterol into bile acids is stimulated. The depletion of hepatic cholesterol due to increased diversion to bile acid synthesis leads to increased hepatic LDL receptor expression to harvest cholesterol from the systemic circulation[9]. It is this indirect effect on LDL receptor expression that accounts for the decline in total proteinand LDL cholesterol produced by bile acids or ilealresection. However, the elevation in HDL cholesterol and triglyceride levels observed with interruption of the enterohepatic circulation of bile acids cannot be explained by changes in LDL receptor expression. Animal data have revealed an independent regulatory role for FXR in both HDL cholesterol and triglyceride metabolism. With regard to HDL cholesterol, FXR represses apoAI expression and plays a role in HDL particle remodeling through induction of phospholipid transfer protein [12, 13].FXR activation increases clearance of triglycerides by influencing lipoprotein lipase (LPL) activity through introduction of apoC-II and repression of apoC-III and by inducing peroxisome proliferator–activated receptor-α expression [9].

**Bile acid and Glucose metabolism**

The first clinical hypothesis that manipulation of the bile acid pool plays a role in glucose homeostasis resulted from observations made in a small study conducted by Garg and Grundy [14]. In this study, the effectiveness of 8 g of cholestyramine or placebo was evaluated in a crossover fashion over 12 weeks in 21 patients with type 2 diabetes stabilized on insulin or glyburide, with a baseline LDL cholesterol >3 mmol/l (>130 mg/dl) and triglycerides <8 mmol/l (<300 mg/dl). Unexpectedly, cholestyramine was associated with a modest improvement in glycemic control, with mean plasma glucose values lowered by 13% and a median reduction in urinary glucose excretion of 0.22 g/day (*P*< 0.001) and a trend toward lower glycated hemoglobin concentrations. These changes occurred without a dosage adjustment for insulin or glyburide. These results were later corroborated with colesevelam and colestimide [14, 15].Similar data on the glucose-lowering effects of bile acids and ileal biliary diversion have been observed in animal studies as well [16].Systematic data on the influence of bile acids on glucose metabolism have been mounting. Effects on bile acid pool composition, TGR5-mediated alterations in hepatic glucose production and intestinal glucose absorption, influences on peripheral insulin sensitivity, incretin effects, and energy use may all contribute to glucose regulation. There is evidence that the bile acid pool size and composition are altered in animal models of either type 1 or type 2 diabetes [16]. (Cao, 2016).as well as in humans with type 1 or 2 diabetes [17].The mechanisms behind these observations are unclear, but preliminary evidence suggests a role for insulin and glucose in modulation of bile acid synthesis [18].

**Bile acid and Incretin modulation**

Bile acids might play an supplementary role in modulating incretin release through binding to a recently identified G-protein–coupled cell surface receptor known as TGR5 [19] This receptor is expressed in various tissues, including the gallbladder, liver, intestine, brown adipose tissue, central nervous system, and monocytes/macrophages [9] Cholic acid appears to be the most effective bile acid agonist for the receptor, TGR5 biology is incompletely understood, but it may play a role in immune modulation and hepatocyte protection from the cytotoxic effects of bile acids [19]. Bile acid activation of TGR5 was also recently shown to induce intestinal glucagon-like peptide (GLP)-1 secretion [17]. In type 2 diabetic patients, bile acid sequestration with colestimide may also increase GLP-1 release. This observation has lent support to the involvement of bile acids in mediating the entero-insular response to feeding. Bile acids may also play a role in metabolic regulation through modulation of energy expenditure. This effect appears to be mediated through modulation of thermogenesis. For example, bile acids given to high fat–fed mice increase energy expenditure in brown adipose tissue, preventing obesity and insulin resistance. This effect appears to be mediated by induction of the cAMP-dependent thyroid hormone–activating enzyme type 2 iodothyronine deiodinase (D2); bile acids increase D2 activity and oxygen consumption in brown adipose tissue, an effect believed to be mediated by TGR5, not FXR [20].

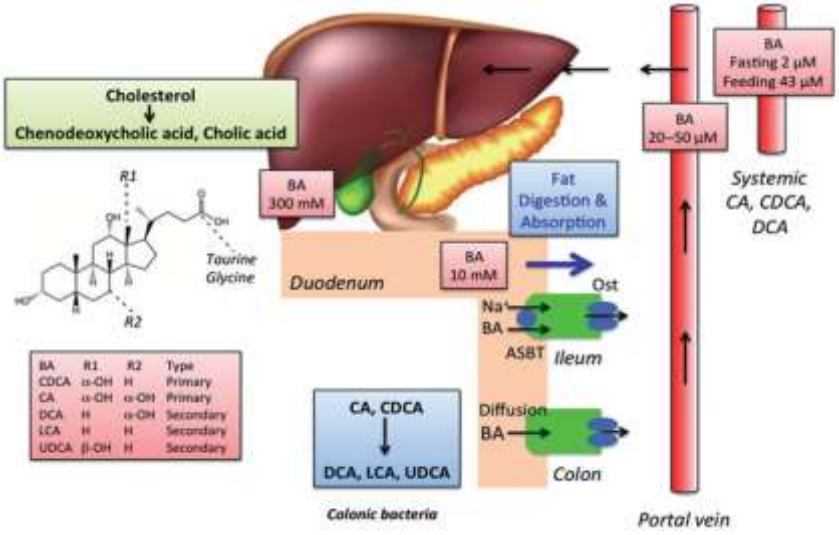



**Fig.1: The synthesis, metabolism, and enterohepatic circulation of bile acids. [9]**

**Bile acids are signaling molecules with hormonal-like actions**

Bile acids are a large complex family of amphipathic molecules with a steroid backbone. They exhibit substantial structural diversity within different cells, body compartments and pathophysiological states, and there is considerable interspecies variability [21]. Bile acids are synthesized and metabolized in mammalian cells and colonic bacteria by multiple and mostly well-characterized enzymatic pathways [3]. Bile acids are synthesized in hepatocytes from cholesterol by an enzymatic pathway that includes hydroxylation and side-chain shortening, which confers bile acids with detergent-like properties that are required for their principal digestive function, namely the emulsification and absorption of dietary fats and lipid-soluble vitamins [7]. Primary bile acids, the direct products of cholesterol metabolism in the liver, include chenodeoxycholic acid (CDCA) and cholic acid (CA) in humans. Before secretion into the biliary system, primary bile acids are conjugated to taurine or glycine [21] However, Bile acids can be further modified in the liver by sulphation and glucuronidation. Bile acids are stored within the gall bladder and are secreted into the intestinal lumen after feeding in response to cholecystokinin, an intestinal hormone that is released by luminal fats and which stimulates contraction of the gall bladder [7]. Primary bile acids are actively and efficiently absorbed in the terminal ileum by the apical sodium-dependent bile acid transporter (ASBT) and the bile acids collateral heterodimeric organic solute transporter. Despite the efficiency of these transport processes, a small proportion of bile acids escape ileal absorption and pass to the colon, where they are de-conjugated, oxidized and dehydroxylated by bacterial enzymes to form secondary bile acids, which include lithocholic acid (LCA) and deoxycholic acid (DCA) in humans (Figure 1). These reactions increase the hydrophobicity of bile acids, which facilitates their passive flux across colonocytes. The absorbed primary and secondary bile acids then enter the hepatic portal vein and recycle to the liver for re-use (Figure 1). This pathway of bile acid secretion, reabsorption and recycling comprises the enterohepatic circulation of the bile acid pool, which mostly comprises CA, CDCA and DCA. The synthesis, transport and secretion of bile acids are tightly regulated by multiple physiological mechanisms that can regulate the concentration of bile acids in different compartments and tissues, and the overall composition of the bile acid pool. For example, given the episodic nature of bile secretion, the concentrations of bile acids in the intestinal lumen and portal and systemic circulations wax and wane during feeding and fasting, reminiscent of the circulating concentrations of gut hormones. Moreover, disruption to the enterohepatic circulation of bile acids, which can occur during disease or therapy, leads to marked alterations in the concentrations and composition of bile acids in the intestinal lumen and the circulation. These physiological and pathological alterations in the concentrations and composition of bile acids are likely to be of direct relevance to the hormonal-like actions of bile acids. In addition to their role in the solubilization of dietary lipids and the fat-soluble vitamins A, K, D and E, bile acids are signalling molecules that regulate many cell types by activating specific receptors in the nucleus and at the plasma membrane [7]

**Takeda G-Protein Receptor 5 (TGR5)**

Takeda G-protein-coupled receptor 5 (TGR5), also known as GPBAR1, M-BAR, or GPCR19, was identified first as a G protein-coupled receptor responsive to bile acids in 2002 [22] It shows: high expression in the gallbladder; moderate expression in the intestine, spleen and placenta; and low expression in the lung, brown adipose tissue (BAT), skeletal muscle and brain [23, 24]. TGR5 activation in enteroendocrine cells increases the release of GLP-1 which maintains homeostasis of blood glucose by promoting glucose-induced insulin secretion, suppressing glucagon release, delaying gastric emptying, promoting satiety, and increasing glucose disposal in the peripheral tissues [25]. In brown adipose tissue and skeletal muscle, TGR5 mediates energy expenditure through a BA–TGR5–cAMP–D2 signaling pathway [20] Therefore, TGR5 activation provides a promising strategy for treatment of type 2 diabetes mellitus and associated metabolic disorders [17]. Thus TGR5 has drawn considerable attention from both academia and industry [26-30] However, TGR5 activation in other tissues can cause some side effects, of which those in the gallbladder and heart are the main concerns. Assays in mice have revealed that TGR5 activation in the epithelium of the gallbladder by administration of either bile acids derivatives (e. g. INT-777), or synthetic small molecule TGR5 agonist causes smooth-muscle relaxation, prevents bile secretion, and greatly increases gallbladder volume [29] Several absorbed TGR5 agonists have been shown to change heart rate and blood pressure in dogs [31, 32]. Therefore, it was suggested that localized activation of TGR5 within the intestinal tract while avoiding systemic exposure (i.e. intestinally-targeted) could be a promising anti-diabetes mellitus strategy with minimal side effects [33, 34]. While no intestinally-targeted TGR5 agonists with robust activity were reported, especially in a diabetic model, there was still doubt about the validity of this strategy. The concern exist that whether healthy hypoglycemic efficacy could be achieved by TGR5 activation in the intestine alone without additional effects in the brown adipose tissue or skeletal muscle and whether the possible side effects in gallbladder and heart could be eliminated by low systemic drug concentration. A compound from *Tithonia diversifolia* was isolated with low systemic exposure, and its gallbladder filling effect was thus reduced. It displayed a great hypoglycemic efficacy in normal and diabetic adult male wistar rats [35]. TGR5 is one of ~800 human G protein-coupled receptors (GPCRs) that impact all levels of biology by transducing signals from a diverse spectra of events. TGR5 belongs to the family A group of GPCRs, also known as the rhodopsin-like subfamily of GPCRs. The receptor is conserved among mammals, and is also found in lower vertebrates, such as fish [19]. TGR5 was discovered as a membrane receptor reactive to bile acids [22, 36] since then, TGR5 has been investigated in several cell types, such as muscle cells, adipocytes, endothelial cells (ECs), and macrophages [24]. The functions of TGR5, which are dependent on cell type, range from glucagon-like peptide-1 (GLP-1) release and mitochondrial energy homeostasis to the control of inflammatory responses [37]

**TGR5 is a GPCR for Bile acids**

Bile acids can regulate the activity of several GPCRs. Conjugated forms of LCA and DCA can modulate the activity of muscarinic receptors expressed in Chinese Hamster Ovary cell (CHO) and chief cells and DCA and CDCA are antagonists of the formyl-peptide receptor. However, multiple bile acids can activate TGR5 (also known as Gpbar1, M-BAR and GPR131), which is recognized as a *bona fide* bile acid receptor [22]. TGR5 cDNA is encoded by a single exon gene located on mouse chromosome 1c3 and human chromosome 2q35. The open reading frame of the receptor encodes 330 amino acids and is predictive of the seven transmembranes spanning TGR5 with 28% identity to human sphingosine-1-phosphate receptor (EDG-1), a member of the class A (rhodopsin-like) GPCR family [22] Several endogenous bile acids activate TGR5, albeit with graded potencies. The rank order of potency with which the bile acids stimulate cAMP generation in TGR5 expressing CHO cells is taurolithocholic acid (TLCA, 0.33 μM) >LCA (0.53 μM) >>DCA (1 μM) >CDCA (4.4 μM) >CA (7.7 μM), whereas ursodeoxycholic acid (UDCA) and cholesterol have little activity [36]. Oleanolic acid (OA), an active component from the leaves of the European olive tree *Oleaeuropaea*, activates TGR5 with a similar potency to lithocholic acid, but does not activate the farnesoid X receptor [29]. Multiple steroidal and non-steroidal TGR5 ligands have been developed as potential treatments for metabolic and inflammatory disorders [30].

**TGR5 and glucose homeostasis**

A sum up study of metabolites in the circulation of human subjects after glucose challenge shown marked increases in the circulating levels of bile acids after glucose that simultaneous with measures of insulin sensitivity that are beneficial to glucose metabolism [36]. Oleanoleic acid extracted from olive leaves is a TGR5-selective agonist that does not activate the farnesoid X receptor [38] Oleanoleic acid partially corrects the diet-induced insulin resistance in mice fed a high-fat diet as demonstrated by its ability to lower plasma glucose and insulin levels [39]. At least two mechanisms may account for the anti-diabetic effect of TGR5

agonists. Firstly, TGR5 activation may activate type 2 iodothyronine deiodinase (D2) and thyroxine, thereby increasing mitochondrial energy expenditure in brown adipose tissue and skeletal muscle and improving glucose utilization [38]. Secondly, TGR5 agonists can promote secretion of glucagon-like peptide 1 (GLP-1), a hormone derived from intestinal L-cells that stimulates insulin secretion and suppresses appetite and gastrointestinal transit [40]Collectively, these effects reduce circulating blood glucose. Bile acids and TGR5 selective agonists stimulate the release of GLP-1 from the murine enteroendocrine cell line STC-1 [41], and TGR5-stimulated GLP-1 release improves liver and pancreatic function and glucose tolerance in obese mice [19] The effects of a TGR5 selective agonist on GLP-1 release from STC-1 cells is linked to an increased ATP/ADP ratio, which causes the closure of ATP-dependent potassium channels (KATP) and a subsequent calcium influx, leading to GLP-1 secretion [19] Interestingly, the use of bile acid sequestrants, which complex with bile acids in the intestine and prevent reabsorption into the enterohepatic circulation, has been associated with improvements in diabetes mellitus type II [42]. The use of bile acid sequestrants correlates with the increased GLP-1 release, which is known to improve glycaemic response. Additionally, the ability of bile acid sequestrants to induce GLP-1 release and lower glycaemia was decreased in mice lacking TGR5 [42]. These anti-diabetic consequences of TGR5 activation provide automatic awareness into improved glycaemic control and have sparked interest into a possible avenue for the development of alternative drug therapy in type II diabetes mellitus.

**Incretin modulators and their development**

Endogenous bile acids are physiological ligands that activate TGR5, even though with different affinities.[24] There has been a rush to design and validate new compounds that are able to modulate the activity of the TGR5 receptor and, until now, this research has focused on TGR5 ligands that improve glucose homeostasis and limit body weight gain. The design and synthesis of TGR5 agonists has especially been deemed necessary because endogenous bile acids generally have a lower affinity and selectivity for TGR5, and they have specific biological properties with regard to localization and cycling. The grading of bile acid affinity for TGR5 is as follows: lithocholic acid (LCA) > DCA > CDCA > CA [38].In agreement with computational methods that have been used to investigate bile acid binding to TGR5, a recent TGR5 mutation study confirmed key residues within the TGR5 binding pocket that are involved in bile acid binding to TGR5 [43] Different research groups and companies have used step-by-step strategies to design selective agonists via the extensive screening of bile acid derivatives and the subsequent modification of their individual structures. Medicinal chemistry plans yielded potent bile acid (steroidal) derivatives through modification of the bile acid scaffold and screening of chemical libraries. Subsequent hit-to-lead optimization produced potent nonsteroidal heterocyclic compounds. [44] Evidently, the steroidal agonists contain a steroidal core that includes bile acids, steroid hormones, and semisynthetic derivatives, whereas this is not the case for the nonsteroidal agonists. Besides the selective TGR5 agonists, compounds have been developed with dual affinity for both TGR5 and the nuclear bile acid receptor, farnesoid X receptor (FXR). Although many compounds have been identified and tested for attraction, efficiency, and selectivity, only a limited number have made it to preclinical testing in the fields of atherosclerosis, colitis, and liver diseases. Most compounds were investigated with respect to specific aspects of obesity and type 2 diabetes mellitus [43]

**Semi-synthetic bile acids as TGR5 agonists**

One of the best identified semisynthetic bile acid TGR5 agonists may be 6α-ethyl-23(S)-methylcholic acid (S-EMCA, INT-777), which was developed through the incorporation of vital 6α-ethyl and 23(S)-methyl moieties in the scaffold of CA. Likewise, other semisynthetic bile acid receptor ligands have been developed solely with FXR (INT-747) or with combined FXR/TGR5 (INT-767) activity [45] The TGR5 agonist, INT-777 (Figure 4) stimulates receptor activation and GLP-1 release from enteroendocrine L-cells, increases energy expenditure, reduces hepatic steatosis, improves insulin sensitivity, and stimulates insulin release in cultured pancreatic MIN6 cells and human pancreatic islets. Furthermore, INT-777 stimulates bile flow, gallbladder filling, and gallbladder relaxation [46]. In 2011, it became clear that INT-777 was also able to inhibit macrophage inflammation and atherosclerosis [46]

**Triterpenoid as Incretin modulators**

The screening of a library of plant extracts for TGR5 activity led to the finding of oleanolic acid as a triterpenoid TGR5 agonist. Oleanolic acid was known as an olive leaf-derived oleanane-type triterpenoid and has weak anti-inflammatory and anti-carcinogenic effects [47].Multiple studies, primarily on glucose metabolism, have shown the biological action of oleanolic acid as a TGR5 agonist. Oleanolic acid improved glucose tolerance in mice fed a high-fat diet [38].Furthermore, oleanolic acid increases pancreatic beta-cell insulin release via TGR5 and improves prokinetic action (motility) of the colon [48]

Betulinic acid was identified as a triterpenoid able to activate TGR5 by a biological screening of a collection of naturally occurring triterpenoids. These triterpenoid derivatives were generated, and found to be potent TGR5 agonists. Even the most potent Betulinic acid derivative, however, showed marginal effects on glucose metabolism, despite its beneficial toxicity profile; these effects may have been due to its low bioavailability [49] Other findings indicate that Betulinic acid may play a role in intraluminal duodenal $HCO3-$ secretion after luminal administration and inhibits high glucose-induced vascular smooth muscle cell proliferation and migration [50]. Betulinic acid decreased body weight, as well as blood glucose and lipid levels, while increasing insulin and leptin in mice fed a high-fat diet. However, none of these studies definitely proved a TGR5-mediated mechanism, leaving the relevance of TGR5 in these effects of betulinic acid uncertain. The same holds true for the triterpenoid, ursolic acid. Before the discovery of ursolic acid as a TGR5 agonist, ursolic acid was shown to improve glucose metabolism without affecting body weight [51].Later on, these findings were more or less confirmed in other studies that showed ameliorated glucose metabolism, decreased abdominal obesity, and improved hepatic steatosis [52]

**Saponin**

The saponins are naturally occurring surface-active glycosides. They are mainly made by plants, but also by lower marine animals and some bacteria. They got their name from their ability to form stable, soap-like foams in aqueous solutions. This easily visible character has attracted human interest from ancient times. Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be tri-terpenoid (figure 2a) or steroid (Figure 2b) in nature [53, 35]. The aglycone may contain one or more unsaturated C–C bonds. The oligosaccharide chain is normally attached at the C3 position (monodesmosidic), but many saponins have an additional sugar moiety at the C26 or C28 position (bidesmosidic). The great complexity of saponin structure arises from the variability of the aglycone structure, the nature of the side chains and the position of attachment of these moieties on the aglycone [54]. Experiments demonstrating the physiological, immunological and pharmacological properties of saponins have provoked considerable clinical interest in this class of substances.

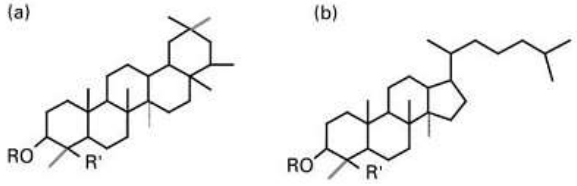



**Fig. 2a and 2b: Basic structures of sapogenins: a triterpenoid (a) and a steroid (b).**

**Triterpenoid saponins**

Triterpenoid saponins are triterpenes, which belong to the group of saponin compounds. Triterpenes are a type of terpene containing 30 carbon atoms. Triterpenes are assembled from a five-carbon isoprene unit through the cytosolic mevalonate pathway to make a thirty-carbon compound [54]. Some triterpenes are steroidal in nature. Cholesterol, phytosterols, and phytoecdysteroids are triterpenes. The triterpenes are subgrouped into some 20 groups, depending on their particular structures. Some triterpenoid compounds are found as saponin glycosides, which refers to the attachment of various hexose molecules to the triterpene unit. These sugars can be cleaved off in the gut by bacteria, sometimes allowing the aglycone (triterpene) to be absorbed into the blood stream or to insert into cell membranes [55]. Saponin glycosides decrease surface tension of water with foaming and will break down lipids. Usually, triterpene saponins are designated as such by the suffix ending –side, such as ginsenoside or astragaloside, named for the plant genera they were first discovered in. Some, such as the ginsenosides and eleutherosides are designated Rx where the suffix x = a, a1, b2, is indicative of the relative position of the saponin spots from top to bottom of a thin layer chromatogram

**Biological effects of Saponins**

Triterpenes makeup a large structurally diverse group of natural compounds biogenetically derived from active isoprene. Two C15 units build squalene or related acyclic 30-carbon precursors. As the result of their cyclization and oxidation, various structures are formed. Alterations occur in two ways, one producing tetra- and pentacyclictriterpenes and the other one leading through cycloartenole to cucurbitacines or to cholesterol and further to phytosterols, cardiac glycosides and steroid saponins. The most common structures of triterpenes include pentacyclic—oleanane, ursane, taraxerane, taraxastane, lupane, and tetracyclic—dammarane and cucurbitane (Figure 7) [54].Another group consists of nortriterpenoids formed from tetracyclic triterpene precursors through oxidation and degradation, resulting in fewer than thirty carbon atoms in the basic skeleton. These are divided into two groups: limonoids (C26) and quassinoids (C20 and C19) [56].

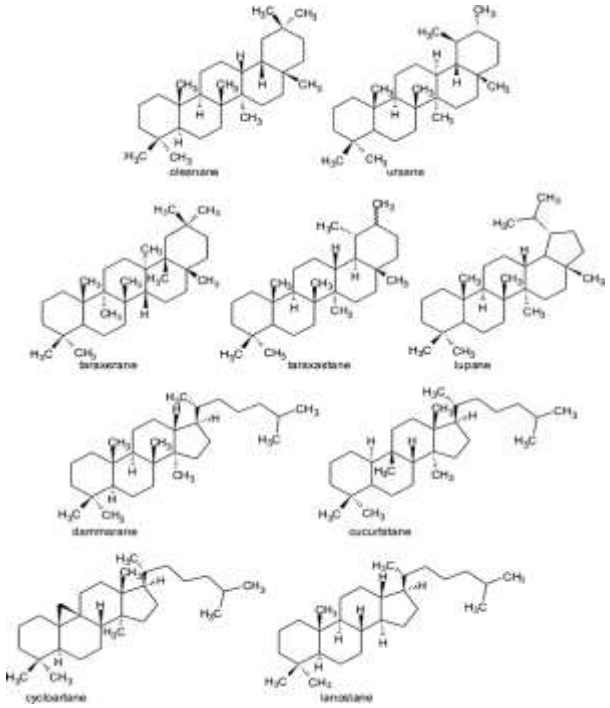



**Fig. 3: Chemical structures of the main subclasses of triterpenes [54].**

The influence of saponins on the TGR5 receptor and its implication in diabetes

TGR5, a G protein-coupled receptor, was identified as a membrane receptor for bile acids. The expression of TGR5 and its function are discrete from the previously identified nuclear bile acid receptor, the farnesoid X receptor (FXR). These two bile acid receptors supplement each other in maintaining bile acid homeostasis and mediating bile acid signaling. Both receptors also play roles in regulating inflammation and glucose metabolism [57]. An interesting finding for TGR5 is its role in energy metabolism. The discovery of TGR5 manifestation in brown adipocyte tissues (BATs) and the recent discovery of BAT in the adult human body suggest a potential approach to combat obesity by targeting TGR5 to increase thermogenesis. The agonists of this receptor can also be used for the inhibition of the development of insulin resistance in early stages of diabetes mellitus[57] Oleanolic acid, a selective TGR5 agonist, which does not influence FXR is isolated from the leaves of *Oleaeuropaea* (Oleaceae) [38] . Other compounds with agonistic properties include betulinic acid and ursolic acid [49] Betulinic acid is the most active (83 % efficacy with respect to litocholic acid used as positive control) of these three triterpenes mentioned above. The values of EC50 for oleanolic, betulinic and ursolic acids were 2.25; 1.04; 1.43 μM, respectively [49]A comparison with other compounds having similar structure has proven that the hydroxyl group at C-3 plays a role in this type of activity. Unfortunately oleanolic acid has weak metabolic stability when administrated orally to rats and very low bioavailability [49]. Nomilin, a highly oxygenated limonoid-type triterpene specific for *Citrus* sp., has also been recognized as an activator of TGR5. Its influence on TGR5 was higher than the natural agonist, chenodeoxycholic acid. Similar to the compounds mentioned above it does not induce FXR activity. Experimental animals treated with nomilin (0.2 %) had a lower body weight, decreased serum glucose and serum insulin, and an enhanced glucose tolerance [58].

**Effects of saponins on cell membranes**

A large number of the biological effects of saponins have been ascribed to their action on membranes. In fact, their specific ability to form pores in membranes has contributed to their common use in physiological research [58] . Saponins have long been known to have a lytic action on erythrocyte membranes and this property has been used for their detection. The hemolytic action of saponins is thought to be the result of the affinity of the aglycone moiety for membrane sterols, (particularly cholesterol), with which they form insoluble complexes [59]Isolated cell membranes from human erythrocytes when treated with saponin developed pores of 40 – 50 A ˚ diameter as against the 80 A ˚ pores produced in artificial membranes compared with the reversible perforations caused by substances such as vitamin A, the membrane pores or defects produced by saponins were long lasting and such membranes were then permanently permeable to large molecules like ferritin[60] The lesions that are caused by saponins are thought to be a micelle-like buildup of saponins and cholesterol in the plane of the membrane, possibly with saponin molecules arranged in a ring with their hydrophobic moieties combined with cholesterol around the outer perimeter [54]Other reports depict the interactions between saponins and biological membranes to be more complex. [6]showed that insertion of the aglycone into the lipid bilayer is independent of the presence of cholesterol. Saponins could induce a permeability change on liposomal membrane without cholesterol when they are glycosylated at both C3 and C28 (bidesmosidic) of the oleanolicaglycone [62]

**Hypoglycemic activity of saponins**

Saponins isolated from plants such as fenugreek [63],*Puerariathunbergiana* [57] and *Calendula officinalis*[64]have been shown to have hypoglycaemic effects. [42] found regularly higher plasma insulin levels, probably caused by stimulation of the b-cells in male Wistar rats given 10 and 100 mg fenugreek extract/300 g bodyweight mixed with food while [65] did not find insulin-releasing activity in rats given oleanolic acid glycosides orally. The hypoglycaemic action here was due to suppression of the transfer of glucose from the stomach to the small intestine and the inhibition of glucose transport across the brush border of the small intestine. The saponinmomordin was also found to significantly and dose-dependently inhibit gastric emptying [66].

The inhibitory activity here was dependent on the level of serum glucose and mediated at least in part by the capsaicin-sensitive sensory nerves and the central nervous system. [64]showed that the oleanolic acid 3-monodesmosideswith hypoglycaemic activity also inhibited gastric emptying showing a correlation between the two effects, while other saponins showing no hypoglycaemic activity in the mixture also did not affect gastric emptying.

**Effects of saponins on cholesterol metabolism**

A number of studies have shown that saponins from different sources lower serum cholesterol levels in a variety of animals including human subjects [54, 35]. Large mixed micelles formed by the relations of saponins with bile acids account for their increased excretion when saponin-rich foods such as soyabean, lucerne and chickpea are taken [67]. The resulting enhanced metabolism of cholesterol in the liver causes its serum levels to go down. Decreased intestinal cholesterol absorption induced by some saponins, however, was seen to be without interfering with the enterohepatic bile acid recirculation [68] Saponins also reduced the more harmful LDL-cholesterol selectively in the serum of rats, gerbils and human subjects [69]. Morehouse *et al.,*[70] found that the mechanism of action of saponins was luminal but did not involve stoichiometric complexation with cholesterol. They also found that the synthetic saponinstiqueside and pamaqueside were much more potent than naturally occurring saponins such as those from lucerne in preventing hypercholesterolaemia and that the *in vivo*influence of pamaquecide was 10-fold that of tiqueside even though it differs from tiqueside only by an additional keto group. Other suggested mechanisms of action of saponins include delaying the intestinal absorption of dietary fat by inhibiting pancreatic lipase activity.[71] Not all reports, however, agree on the anticholesterolaemic activity of saponins. Soyabean and lucerne saponins were found to be not singularly responsible for the hypo-cholesterolaemic effect of diets containing them [72] The level of muscle cholesterol in tilapia fed small amounts of Quillaja saponins (up to 300 mg/kg) was higher than that of controls [72]while the serum levels were not different from control. It has also been noted that a saponin-induced reduction of serum cholesterol occurred only when a hypercholesterolaemic diet had been fed, and there is also evidence of increased cholesterol synthesis to compensate for saponin-induced excretion [73]It can be concluded that several dietary saponins do have a hypocholesterolaemic action. Since cholesterol binding takes place in the intestinal lumen, factors such as quantity of saponins and cholesterol, and the presence of other ligands of both these compounds may play a role, and these may have caused the observed discrepancies among the various results. Knowledge of the nature of the inter-action between the particular saponin and cholesterol and the nature of the cholesterol moieties and other ligands in the diet are essential to arrive at an effective dietary dose of that particular saponin that could have a significant hypocholesterolemic effect.

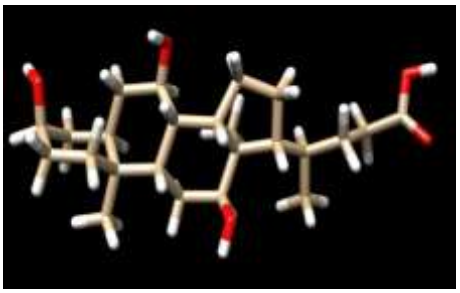
**Bile acid and Saponin as antidiabetic agent**

Most of the synthetic drugs employed in the treatment of diabetes mellitus involve either administration of exogenous insulin or oral hypoglycemic drugs and generally have side effects like weight gain that aggravate other risk factors to promote diabetic cardiovascular complications [60]Recent evidence has identified saponin as a potent antidiabetic agent. Ginsenoside Re, a class of saponin, has been reported to remarkably reduce diabetes-associated cerebral decline and also confirmed the involvement of oxidative stress and inflammation in the development of cognitive impairment caused by diabetes [21]. Saponin act as the antidiabetic agent through its hypoglycemic activity may be by stimulating TGR5/GLP1 pathway, enhancement of insulin secretion, promoting glycolysis, decreasing gluconeogenesis as well as inhibition of α-glucosidase.

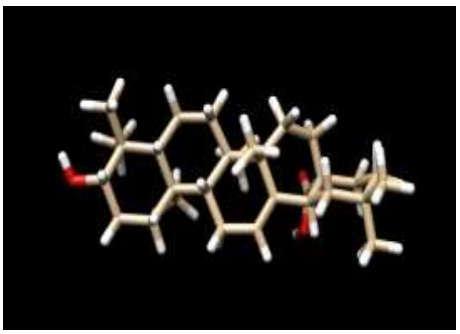
**CONCLUSION**

Knowledge of bile acid physiology has dramatically evolved from the concept of digestive detergents to an elegant story of bile acid functioning as hormones involved in the modulation of a variety of metabolic processes and saponins from various plants has being found to perform all this hormonal functions also as shown in this review. Manipulation of bile acids composition and pool size through bile acid sequestration takes advantage of this physiology and has found clinical application for dyslipidemia and, more recently, type 2 diabetes. This review has been able to relate the differences and similarities between bile acid functions and saponin to help researchers understanding the importance of this phytocompound in drug design.

This will contribute to potential additional therapeutic applications for these complex molecules.



(A)



(B)

**Fig. 4: (A) 3D structure of Cholic acid (a primary bile acid)and (B) 3D structure of triterpenoid (a type of saponin).**

A B

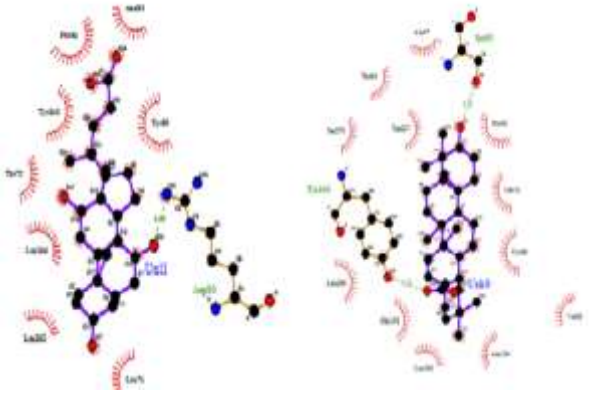


**Fig. 5: 2D representation of the interaction between (A) cholic acid and TGR5 compared with (B) saponin and TGR5.**

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