Metabolic Messengers: bile acids

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Abstract

Bile acids (BAs) are amphipathic steroids whose production and diversity depend on both host and microbial metabolism. These metabolites have emerged as biologically active signaling molecules informing organs of nutrient availability. Their actions, through activation of the dedicated BA receptors, FXR and TGR5, control the body's integrated physiological metabolic responses. Alterations in BA abundance or signaling are associated with multiple metabolic diseases including obesity, type 2 diabetes, non-alcoholic steatohepatitis, and atherosclerosis. Consequently, modulation of the BA pool could be a valid therapeutic approach, as demonstrated in preclinical and clinical models. Here we provide a historical summary of the discovery of BAs and their receptors, as well as on the role of BA signaling in the control of energy homeostasis.

History of bile and bile acids: from bodily fluids to hormones

Bile — the fluid in which bile acids (BAs) are stored — has been studied for centuries for its unique beneficial properties. While the first written record depicting bile as a therapeutic dates back to the ancient Egyptian period (Ebers Papyrus, 1550 B.C.)[1], it came to prominence with Hippocrates (460-377 B.C.), who postulated bile as one of the four bodily fluids, the "humours". Humoral imbalances were the basis of all diseases and harmonizing the four humors was the main therapeutic approach of the time and one of the cornerstones of traditional Chinese medicine, which proposed the extraction of bile from different animals to treat multiple diseases[2]. However, the biochemical and molecular basis by which BAs govern health and disease was only addressed in the last 150 years (extensively reviewed elsewhere[3–5]) (Figure 1). BAs were isolated and purified in the second half of the 19th century, but the greatest achievements in the BA field were obtained in the 20th century. In the early 1930s, several laboratories began to elucidate the chemical structure of BAs. In the following years, other breakthroughs followed, including the development of the chromatographic separation of BAs, the discovery of cholesterol as substrate for primary BA synthesis in the liver, and the identification of secondary BAs as intestinal derivatives from primary BAs in both rats and humans. These studies were instrumental in the identification of the key intermediates, enzymes and mechanisms controlling BA biosynthesis[3–5]. Moreover, they considerably advanced our knowledge on BA diversity and BA recirculation, two processes that are described in the next sections.

In the last three decades, several discoveries have led to a better understanding of the mechanisms of action of BAs. In 1999, three different research groups cloned and identified farnesoid X receptor (FXR; NR1H4) as the chief nuclear receptor responsive to BAs[6–8]. Shortly after, BAs were described as agonists of the G protein-coupled receptor (GPCR) Takeda G-protein receptor 5 (TGR5; GPBAR1)[9,10]. Another major advance involved the chemical modification of natural BAs into semi-synthetic analogs with enhanced selectivity

for a specific BA receptor[11–14]. These receptor-tailored molecules have moved the field forward, not only because of their utility as research tools to dissect the functions of FXR and TGR5, but also because they laid the foundation for biomedical discoveries and therapeutic opportunities. Among all the semi-synthetic BA derivatives, obeticholic acid was the first to reach the clinic as an FDA-approved FXR agonist for the treatment of primary biliary cholangitis in patients who are unresponsive to the hydrophilic BA specimen, ursodeoxycholic acid[15]. Of note, these semi-synthetic molecules, as well as BAs *per se*, can also trigger non-receptor-mediated effects[16], which due to space constraints will not be described here.

Biology of BAs

BAs are the main constituents of bile, which also contains cholesterol, phospholipids, bilirubin, fatty acids, vitamins and minerals[17]. Originally identified as amphipathic steroid metabolites facilitating intestinal absorption of lipids and fat-soluble vitamins, they are now recognized as true hormones capable of reaching virtually every organ of our body to fine-tune metabolic functions. BAs are initially synthesized from cholesterol in hepatocytes through two different well-characterized metabolic pathways, comprised of multiple enzymes (Figure 3)[18]. BAs can be synthesized through two distinct pathways. Under physiological conditions, the bulk of primary BAs is produced by the classical pathway initiated by cholesterol 7α-hydroxylase (CYP7A1). The alternative branch of BA production, which is dependent on sterol 27-hydroxylase (CYP27A1), contributes to BA synthesis to a minor extent, but becomes important in response to environmental stresses to mediate adaptive responses[19]. Together, these pathways contribute to the hepatic abundance of the primary BAs, chenodeoxycholic acid (CDCA) and cholic acid (CA), with oxysterol 12α-hydroxylase (CYP8B1) being the enzyme that determines the abundance of CA versus CDCA (Figure 3).

In rodents, CDCA is rapidly converted to the more hydrophilic muricholic acids (MCAs). Primary BAs are then conjugated in the liver to either taurine or glycine, mainly in mice and humans respectively, secreted into bile and released into the intestinal lumen after food ingestion. Once in the gut, BAs are bio-transformed into secondary BAs (deoxycholic acid (DCA), lithocholic acid (LCA), ωMCA, and hyodeoxycholic acid (HDCA)) by the intestinal microbiota through different reactions including deconjugation, 7α-dehydroxylation, 6α-hydroxylation and epimerization[20]. When BAs reach the distal part of the small intestine, only a minor fraction transits through the colon and is excreted in the feces. The bulk of BAs is reabsorbed by the gut epithelium and returns to the liver via the enterohepatic circulation. During this process, BAs can spill over into the systemic circulation and signal energy availability to almost all organs through the binding and activation of dedicated BA receptors.

BA receptors

BA receptors trigger genomic and non-genomic adaptive responses in target tissues following changes in BA pool size and/or composition. While several BA receptors have been identified over the last 3 decades[21], FXR and TGR5 have been particularly well studied for their regulatory role in metabolic health and disease[21–28], and their characterization has led to the notion that BA species bind to and activate receptors with distinct potencies *in vitro*. However, it is important to emphasize that the extent of receptor activation *in vivo* is largely determined by the total BA pool composition, composed of weak and strong agonists and antagonists.

FXR is abundant in the liver and intestine but also found in other metabolic tissues. Originally discovered as a farnesol receptor[29], FXR represents the first nuclear receptor that confers BA responsiveness[6–8]. CDCA is a potent natural agonist for FXR and binding to the FXR-retinoic acid receptor α (RXRα) complex activates the transcription of its target

genes[23]. This process is further modulated by the association of coregulators of the FXR-RXRα complex and by post-translational modifications of FXR itself, as described elsewhere[30,31].

While FXR coordinates many of the transcriptional programs elicited by BAs, TGR5 mediates the rapid non-genomic effects. TGR5 is a member of the Rhodopsin-like subfamily of GPCRs and is modestly expressed in almost every tissue, with the exception of the gallbladder epithelium where it is highly abundant[32]. Secondary BAs are the most potent agonists for TGR5 and their conjugation to taurine or glycine further increases their potency[9]. Binding of BAs to TGR5 triggers a Gαs-adenylate cyclase-cAMP signaling cascade resulting in the activation of multiple downstream targets[21].

BAs are thus dual agonists for FXR and TGR5 and their relative abundance, along with their different spatial expression pattern, will ultimately determine receptor activity and biological response. As such, TGR5 and FXR often complement each other in multiple organs and are required to orchestrate whole-body metabolism, as described in the following sections.

Regulation of BA production and flow

Despite the multiple benefits of BAs described below, levels need to be maintained in place to avoid BA accumulation and toxicity. FXR is the master regulator of BA homeostasis and controls BA levels by acting on multiple target genes in enterohepatic organs. For instance, hepatic BA synthesis is controlled by feedback mechanisms involving the FXR-mediated induction of Fibroblast Growth Factor 15/19 (FGF15/19) in the small intestine[33] and the orphan nuclear receptor/transcriptional corepressor small heterodimer partner (SHP; NR0B2)[34,35] in the liver. In addition, FXR regulates the expression of dedicated BA transporters, thereby controlling bile formation and BA recycling[36]. A further level of complexity is given by the TGR5-dependent regulation of bile secretion and flow in

cholangiocytes and gallbladder epithelium[37]. Disruption of these control mechanisms results in altered BA size and composition, and can lead to the development of metabolic diseases[23,24,38] and cancer[38].

BAs and microbiome

BA homeostasis is tightly controlled by the gut microbiome through a complex interaction that leads to modulation of whole-body metabolism, as extensively described elsewhere[39,40]. As a direct consequence of their detergent properties, BAs act as antimicrobial molecules capable of altering the microbial ecology of the gut. Conversely, microbial metabolism of BAs increases the diversity of the BA pool[41], as demonstrated by enrichment of primary conjugated BAs in germ-free (GF) and antibiotic-treated mice. These changes in BA composition impact FXR and TGR5 activation. For example, the absence of microbiota in GF mice has been associated with an increase in BAs acting as FXR antagonists, such as tauro-βMCA (TβMCA)[42]. Diet and pharmacological interventions also regulate FXR activation by modulating the levels of *Lactobacillus*, a bacterial strain expressing bile salt hydrolase, which catalyzes the deconjugation of BAs, including TβMCA[24]. More recently, the gut microbiome has been shown to be required for the production of low abundant phenylalanine- and tyrosine-conjugated CA derivatives, which were proposed to act as FXR agonists[43]. Further studies are warranted to explore the physiological relevance of this subset of conjugated BAs in humans.

The role of the gut microbiome in the generation of TGR5 agonists has been well explored. In particular, 7α-dehydroxylating bacteria transform the primary BAs CA and CDCA into DCA and LCA respectively[44,45]. Diet composition (fat and protein amount) and pharmacological interventions (antibiotics) strongly influence the availability of TGR5 endogenous agonists, in part by modulating the proportion of intestinal 7α-dehydroxylating

bacteria[46–49]. A recent study also reported that a decrease in *Bacteroides vulgatus* and an increase in *Ruminococcus torques* abundance induces DCA[50]. Thus, changes in the activity or quantity of these bacterial species could greatly influence host metabolism via TGR5. While these studies are promising, further research will be needed to fully understand the metabolic benefits of microbiome therapeutics in the context of TGR5 and FXR signaling.

Metabolic actions of BA signaling

Food intake is the main trigger for intestinal BA release, recirculation, and spillover into the systemic circulation. Consequently, BAs have emerged as postprandial messengers that fine-tune whole-body metabolism through the coordinated activation of FXR and TGR5 in multiple organs (Figure 2), as extensively reviewed elsewhere[21,23,28]. In addition to its role in maintaining BA homeostasis, FXR regulates the expression of multiple genes involved in hepatic lipid, glucose and amino acid metabolism, thereby orchestrating the postprandial hepatic response. BAs also modulate whole-body glucose homeostasis, for instance by controlling the secretion of intestinal enteroendocrine hormones, including the insulinotropic and satiety-inducing hormone Glucagon-Like Peptide-1 (GLP-1). Furthermore, intestinal BA signaling is essential for nutrient and cholesterol absorption, as well as for maintaining intestinal integrity, regeneration and motility. Any alterations in these homeostatic control mechanisms may result in profound metabolic dysfunctions. Due to space constraints, this review will hereafter focus on the peripheral and central role of receptor-mediated BA signaling in energy homeostasis.

BA signaling and energy homeostasis

The regulation of energy expenditure and food intake is a tightly controlled biological event, but can be compromised by chronic high-fat diet (HFD) feeding or by excess caloric intake.

Energy homeostasis involves complex inter-cellular and intra-organ communication between peripheral tissues and the central nervous system. BAs are established key regulators of the energy balance during diet-induced obesity (DIO) and orchestrate both peripheral and central responses by modulating TGR5 and FXR activity (Figure 3), as described in the next paragraphs.

BA-TGR5 signaling in peripheral organs. BAs have been identified as metabolites with potent body weight-reducing properties. In 2006, Watanabe *et al*. showed that supplementation of CA to a HFD prevented diet-induced increase in adipose mass, and reversed weight gain after obesity was already established[51]. This phenotype was proposed to result from the activation of the TGR5-deiodinase 2 (DIO2) pathway in murine brown adipose tissue (BAT) and in human skeletal muscle myoblasts[51]. The enzyme DIO2 is involved in the cellular production of bioactive thyroid hormone, and increased DIO2 activity has been associated with enhanced mitochondrial function, adaptive thermogenesis and energy expenditure[52]. Like for CA, feeding mice with CDCA also induced body weight loss[53,54], and enhanced Uncoupling Protein 1 (UCP-1)[54], one of the best studied mitochondrial uncouplers, and DIO2[53] expression in BAT. A similar CDCA-DIO2 mechanism was shown to increase energy expenditure in humans[55]. The use of TGR5-selective agonists and genetically modified mouse models further established that these anti-obesogenic effects are at least partially dependent on TGR5 activation[14,32,51,53,56–61]. While some studies have corroborated these findings and observed increased energy expenditure after BA administration or TGR5 activation[14,51,55,59,61], others, on the contrary, did not[54,62]. Similarly, while some studies report that BAs are not associated with energy metabolism in humans[63,64], others support this association[55,65]. Differences in the experimental interventions, environment and genetics could explain these discrepancies. Indeed, the genetic background

198 and the endogenous BA pool size have been proposed as factors that likely contribute to the
199 efficacy of the response[62]. These findings underscore the complexity of BAs in coordinating
200 the energy balance and urge for additional studies to comprehensively evaluate the
201 contribution of genetics and diet to this phenotype. Finally, other uncharacterized
202 mechanisms may also contribute to the BA-mediated body weight maintenance and further
203 research is needed to clarify this point in both mice and humans.

204 Separately, recent studies have also supported the role of BA-TGR5 signaling in inducing
205 muscle cell differentiation and hypertrophy, thereby improving muscle function[66] and
206 increasing glucose utilization without affecting energy expenditure or physical activity[67]. On
207 the other hand, endurance exercise was recently demonstrated to increase the circulating
208 levels of LCA and DCA, which has been proposed to contribute to the increased energy
209 expenditure that is seen through TGR5 activation[68].

210 In addition to their function in BAT and muscle, three independent studies demonstrated that
211 TGR5 activators are potent triggers of beiging in subcutaneous fat (Figure 3)[56,57,69]. Although
212 the contribution of this mechanism to the regulation of whole-body energy homeostasis is
213 still under debate, activation of TGR5 enhanced lipolysis as well as mitochondrial biogenesis
214 and function in DIO mice[56,69]. Interestingly, this TGR5-mediated adipocyte reprogramming is
215 also triggered by cold exposure and is independent of adrenergic stimulation[69], one of the
216 common signaling pathways for beiging. It will be interesting to investigate whether
217 physiological or pathological fluctuations in endogenous BAs are sufficiently potent to
218 modulate the white-to-brown conversion of adipocytes.

219 Cold exposure, a well-established approach to increase energy homeostasis, not only boosts
220 mitochondrial activity, but also reshapes microbiota composition[70]. Transplantation of cold-
221 remodeled microbiota to GF mice improved both energy and glucose homeostasis[70] and was
222 at least in part dependent on changes in the BA pool size and composition[71,72]. Cold exposure

stimulated the expression of genes related to BA synthesis, mainly of the alternative pathway[71], and increased the production of tauro-conjugated BAs[71,72] including the FXR antagonist TβMCA[42]. On the other hand, deletion of TGR5 in the adipose tissue blunted cold-induced beiging of subcutaneous WAT[69]. Altogether, these changes in BA pool size/composition and their differential impact on TGR5 (upregulation) and FXR (downregulation) activity seem essential for adjusting whole-body energy homeostasis to sustained cold.

BA-TGR5 signaling in the control of central energy homeostasis. Although BAs emerged as satiety-inducing factors in 1968[73], the physiological relevance and mechanisms underlying this action remained unexplored for decades. Several recent studies have provided insight into these aspects (Figure 3). While studying the brain regions that control food intake, Perino *et al*. discovered that BAs spike in the hypothalamus shortly after feeding to prime the onset of satiety by turning off the hypothalamic orexigenic Agouti-Related Peptide/Neuropeptide Y (AgRP/NPY) neurons[74]. BAs acutely prevent the release of hunger-stimulating AgRP and NPY neuropeptides during the first minutes following binding of TGR5 through Rho/ROCK/actin-remodeling, but then further sustain the repression by blunting their expression[74]. In line with previous studies conducted in mice fed a normal chow diet (CD)[14,32,58], these homeostatic mechanisms coordinate the physiological transition between fasting and feeding.

During HFD feeding, compensatory neuronal circuits are recruited to counteract DIO. In this context of obesity, another report revealed that chronic activation of central BA-TGR5 signaling not only led to reduced food intake, but also to increased energy expenditure[75] (Figure 3), adding an additional layer of complexity to the established knowledge of peripheral TGR5 activation[14,51,69,76–79]. Mechanistically, chronic selective central TGR5

activation increased energy expenditure by engaging the sympathetic nervous system[75]. Of note, the enhanced sympathetic tone was not observed in genetic models of obesity[75], suggesting that dietary lipid-induced stress, rather than obesity itself, activates these peripheral neuronal circuits. Several possibilities may account for this diet-specific phenotype. HFD feeding triggers significant alterations in hypothalamic structure and function, including hypothalamic inflammation[80] and endoplasmic reticulum stress[81], and may even lead to partial damage of the neuronal projections[80]. However, HFD feeding also profoundly alters the size and composition of the BA pool[49,82,83], which in turn may modulate hypothalamic BA signaling. More studies are needed to fully address these possibilities.

The central anorexigenic action of BAs can be further enhanced by the regulation of fat preference in lingual taste bud cells[59]. These findings, which were shown to be TGR5-dependent, may point to an additional mechanism to prevent obesity. TGR5 is also expressed in vagal afferent neurons and colocalizes with cholecystokinin A-receptor (CCK-AR)[84]. Both BAs and CCK are released after a meal, and inhibit food intake and body weight through activation of Pro-opiomelanocortin/Cocaine- and Amphetamine-Regulated Transcript (POMC/CART) hypothalamic neurons[84], a well-established satiety-inducing neuronal population[85]. In addition, BA-TGR5 signaling coordinates enteroendocrine cell differentiation[83,86,87] and function, in particular in L cells, for which BAs and TGR5-specific BA derivatives act as secretagogues of GLP-1 and other incretin hormones[14,88,89]. Of note, GLP-1 is a well-established regulator of energy homeostasis[90] and likely acts, together with the above described mechanisms, as an integrated response to amplify BA-induced satiety (Figure 3).

BA-FXR signaling in peripheral organs. Several groups have investigated the role of BA-FXR signaling in obesity and reported that FXR deficiency decreased body weight gain and

fat mass in both genetic and DIO mouse models (Figure 3)[91,92]. Deletion of intestinal FXR was sufficient to prevent weight gain[93–95]. In contrast, liver-specific FXR knockout mice were not protected against DIO[91]. Despite the general consensus that FXR loss-of-function protects against obesity, the effects of its selective modulation by BAs or synthetic molecules are more controversial. Both FXR antagonists and agonists were recently shown to act as anti-obesity molecules. In agreement with the phenotype observed in intestine-specific FXR knockout mice, selective inhibition of FXR signaling by administration of the synthetic Gly-MCA[94] or the natural TβMCA[93,95] protects against DIO by reducing ceramide levels and increasing beiging. However, administration of the gut-restricted agonist fexaramine also attenuates DIO in mice by increasing the thermogenic response in BAT and WAT[96]. In contrast, the function of the synthetic FXR agonist GW4064 in obese mice is less clear and findings diverge, most likely due to different experimental procedures[51,97–99]. The changes in microbiome and BA composition and/or the existence of off-target effects may also explain some of the discrepancies. Of interest, the gut microbiota controls weight gain in an FXR-dependent manner[100] and changes in the level of secondary BAs, as was described with fexaramine administration[96,101], could activate TGR5 and contribute to differential modulation of whole-body energy metabolism. In addition, FXR activation induces *Tgr5* expression in the intestine[102], which could also contribute to the complexity of BA signaling. In line with these findings, dual FXR/TGR5 agonists, such as INT-767, significantly prevent the development of obesity in wildtype mice[79]. However, other unknown factors may also weigh in, and further studies will be needed to clarify the apparent controversial actions of FXR-modulating molecules.

BA-FXR signaling in the control of central energy homeostasis. Although FXR expression has been reported in the brain and hypothalamus[103], its exact function remains ill-

defined. Until recently, the direct role of FXR in the brain was thought to be marginal, as central activation of FXR by intracerebroventricular (icv) administration of GW4064 did not modulate energy metabolism[104]. Instead, the central action of FXR would indirectly depend on the induction of FGF15/19 in the intestine (Figure 3). Consistent with these findings, FGF19 can cross the blood brain barrier[105] and FGF receptors and β-Klotho, the obligate co-receptor mediating FGF15/19 signaling, are expressed in the hypothalamus[106], supporting a central action of FGF15/19. Accordingly, activation of central FGF15/19 signaling reduces body weight[107–109] and food intake[108,109] in animal models of obesity. A recent report, however, showed that icv delivery of FXR agonists in the hypothalamus can decrease sympathetic tone and energy expenditure in chow diet-fed mice[110]. It will be interesting to evaluate the relative importance of each of these pathways in the context of whole-body energy homeostasis.

BA metabolism and genetics in obesity

Decades of preclinical research have revealed how BAs counteract several processes that contribute to the onset of obesity and its associated comorbidities, and have led to the concept that BAs could be used as biomarkers for human disease progression. Lessons from mice should nevertheless be taken with caution, as BA metabolism is not perfectly conserved in evolution. For instance, humans lack enzymes that transform secondary back to primary BAs upon return to the liver, and cannot synthesize the endogenous FXR antagonist TβMCA, two features that fundamentally impact TGR5 and FXR signaling.

Monitoring BA levels in patients with obesity is important to understand how patients with metabolic syndrome could benefit from BA-based therapies. Although findings diverge across studies, fasting circulating BAs tend to augment, while the post-prandial increase of BAs seems to be blunted during obesity[23,111]. BAs are known to show large inter-individual

variations. This is not surprising as they represent several dozens of species, each with unique turn-over and properties. These aspects, together with their multi-compartment physiology and complex regulation by diet, genetics, microbiome and disease, render their investigation highly challenging. For long, human studies have failed to establish solid correlations between changes in BA entities and disease development. A major limitation has been the relatively small sample size of patients, since plasma BA concentrations and composition are notoriously known for their high inter-individual variability. Recent GWAS studies are starting to provide crucial insights into the contribution of genetics and the microbiome to individual BA species in human[112,113] and murine[114] obesity. With the identification of hundreds of quantitative trait loci, these *in silico* studies open up an exciting area of research in which the rigorous validation of these genetic correlations could reveal meaningful causality, and ideally lead to personalized therapeutic perspectives to modulate BA levels and, consequently, whole-body metabolism.

BAs and bariatric surgery

To date, the most promising clinical approach for severe obesity is bariatric surgery (BS), including Roux-en-Y gastric bypass (RYGB), vertical sleeve gastrectomy (VSG) and bile diversion to the ileum (GB-IL). Following these surgical interventions, patients show elevated levels of BAs and incretin hormones in the systemic circulation, as well as gut microbial changes, factors that have been proposed to contribute to the metabolic benefits of BS[115]. The earliest report linking body weight loss to increased BA levels in BS patients dates back to 2009[116], and more recent studies showed that this BA phenotype is sustainable up to at least 5 years after BS[117]. Others confirmed elevation in BA levels after RYGB but found no correlation with weight[118]. Interestingly, the BS-dependent increase in circulating BAs was independent from caloric restriction[119]. Despite the large number of clinical and

preclinical studies, there is still no unequivocal consensus on the molecular mechanisms underlying BS-induced body weight loss, especially in the context of BA signaling. For instance, a functional FXR signaling is required to maintain the VSG-mediated weight loss in obese mice[120], but the beneficial effects of VSG were preserved when FXR was selectively deleted in the intestine or liver[121], suggesting that FXR inactivation and VSG may enhance energy metabolism through yet uncharacterized mechanisms. Recent literature proposed that intestinal FGF15/19 is an important component of the metabolic responses to VSG[122]. In contrast, intestinal FXR appears to be sufficient to support GB-IL-dependent weight loss in obese mice[123], whereas FXR signaling seems to be dispensable in RYGB[124]. Similarly, the role of TGR5 signaling in regulating energy metabolism after BS is debated. While some studies demonstrated that intact TGR5 signaling is required for weight loss after RYGB[125] and VSG[78], others have suggested that RYGB and GB-IL decreased body weight independently of TGR5[123,126]. Altogether, these studies suggest divergent molecular mechanisms following different bariatric interventions.

Conclusion and future perspectives

BAs are peripheral and central regulators of energy homeostasis, and alterations in the BA pool size and composition, or in their biological actions, may shift the balance toward obesity. Despite the extensive characterization of the functions of BAs and their receptors in both preclinical models and patients, multiple aspects of BA signaling remain unknown, and BA analogs or drugs targeting BA signaling are not yet available in the clinic for the treatment of obesity and eating disorders. Studies aimed at increasing the accessibility of BAs to the brain could open up exciting therapeutic perspectives. In addition, modulating the size and composition of the peripheral BA pool by altering the gut microbiota through a change in diet, exercise, cold exposure, antibiotics, probiotics or fecal transplantation could be a

373 promising strategy to modulate energy homeostasis. More studies, especially in humans, will
374 be needed to validate these preclinical findings into effective therapies.
375

Figure legends

Figure 1. Timeline of major discoveries in the BA field. The beneficial effects of bile in the treatment of multiple diseases were already known in ancient times, but the discovery of the chemistry and mechanisms of action of BAs have only been elucidated in the last 150 years, as illustrated in this timeline. B.C., before Christ; BAs, bile acids; UDCA, ursodeoxycholic acid; CDCA, chenodeoxycholic acid; PBC, primary biliary cholangitis; FXR, farnesoid X receptor; TGR5, Takeda G-protein receptor 5; FDA, food and drug administration.

Figure 2. Target tissues and biological activity of BAs. TGR5 and FXR coordinate the BA-mediated whole-body metabolic response by acting in multiple organs. The biological functions listed in this figure are non-exhaustive and the receptor indicated in parentheses represents the most studied one. Most observations on the biological action of the BA-TGR5 signaling have been made in mice, but are supported by *in vitro* studies in human cells and/or by clinical data. TGR5, Takeda G-protein receptor 5; HFD, high-fat diet; FXR, farnesoid X receptor.

Figure 3. BA signaling controls whole-body energy homeostasis through direct and indirect mechanisms. a) Genetic deletion of FXR (*Nr1h4*) or TGR5 (*Gpbar1*) in obese mice is sufficient to alter body weight gain. **b)** BAs activate FXR and TGR5 in enterocytes and enteroendocrine cells to indirectly modulate food intake through increased secretion of FGF15/19 and GLP-1, respectively. **c)** Primary BAs are produced in the liver through the classical or alternative pathways (main enzymes are illustrated in the box) and converted to secondary BAs in the intestine by the gut microbiome. Dashed arrow: not fully defined reaction as deduced from[127]. **d)** BAs reach peripheral organs via the systemic circulation and orchestrate whole-body energy homeostasis by directly activating TGR5 in the brain, BAT

and WAT. Primary (yellow) and secondary (red) BAs can activate both TGR5 and FXR but with different potency (higher potency = more BAs of a color near the receptor). This figure represents an overview and is not exhaustive. *Nr1h4*, nuclear receptor subfamily 1 group H member 4; Gpbar1, G protein-coupled bile acid receptor 1; FXR, farnesoid X receptor; KO, knockout; TGR5, Takeda G-protein receptor 5; FGF15/19, fibroblast Growth Factor-15/19; EEC, enteroendocrine cell; GLP-1, glucagon-like peptide 1; BA, bile acid; CYP7A1, cholesterol 7α-hydroxylase; CYP8B1, sterol 12α-hydroxylase; CYP27A1, sterol 27-hydroxylase; CYP7B1, oxysterol 7α-hydroxylase; CA, cholic acid; CDCA, chenodeoxycholic acid; MCA, muricholic acids; BAT, brown adipose tissue; WAT, white adipose tissue. Created with BioRender.com.

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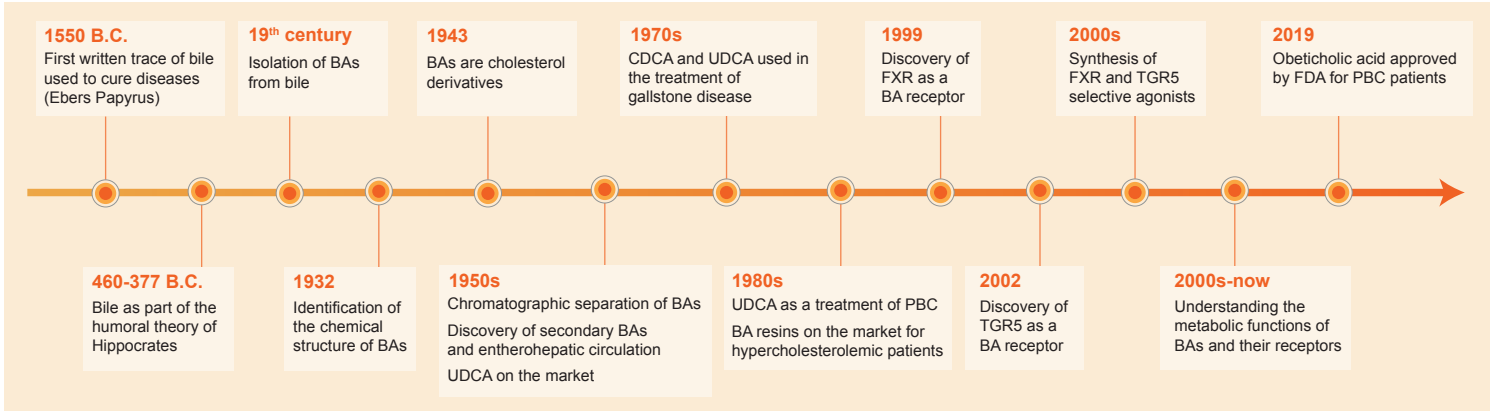
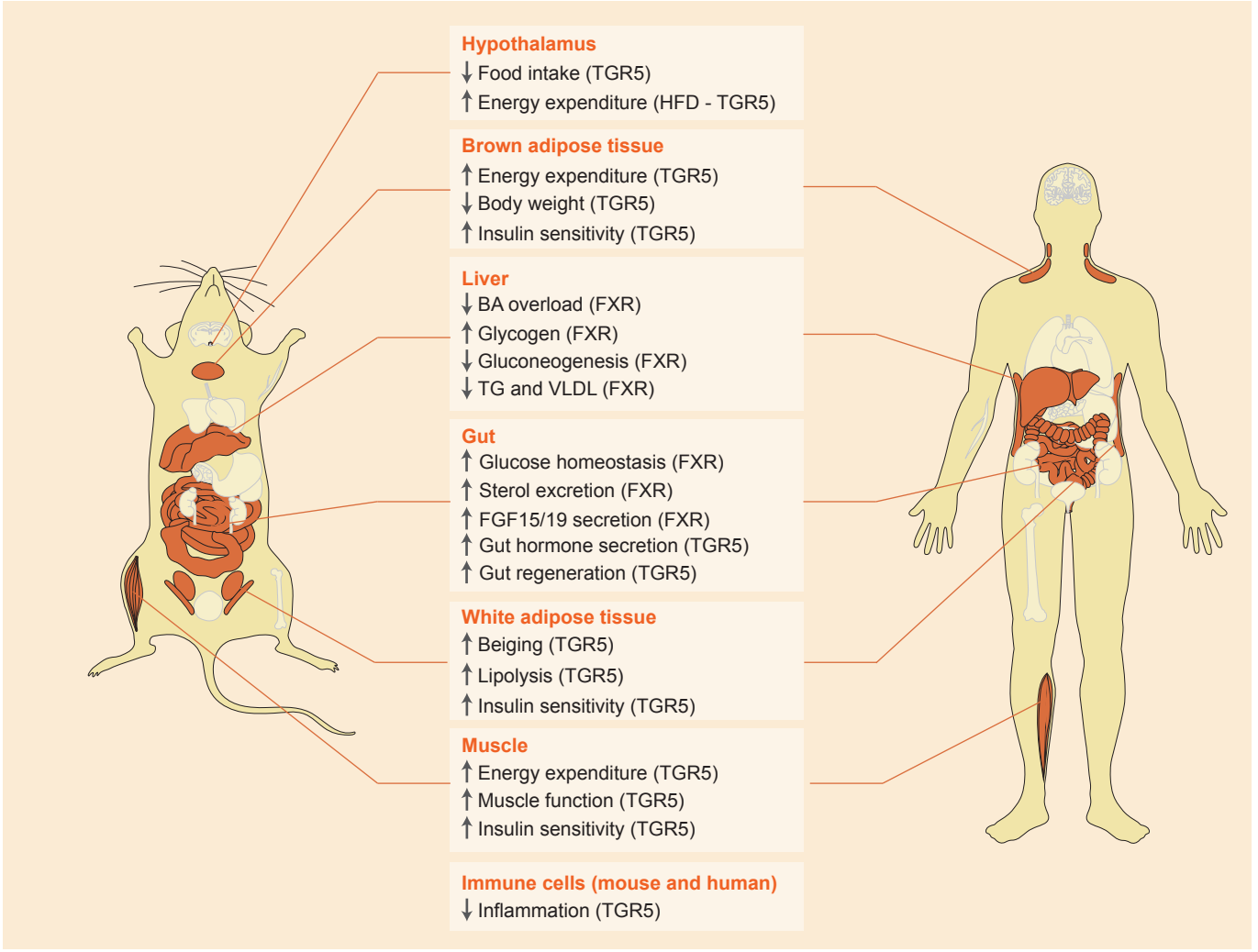
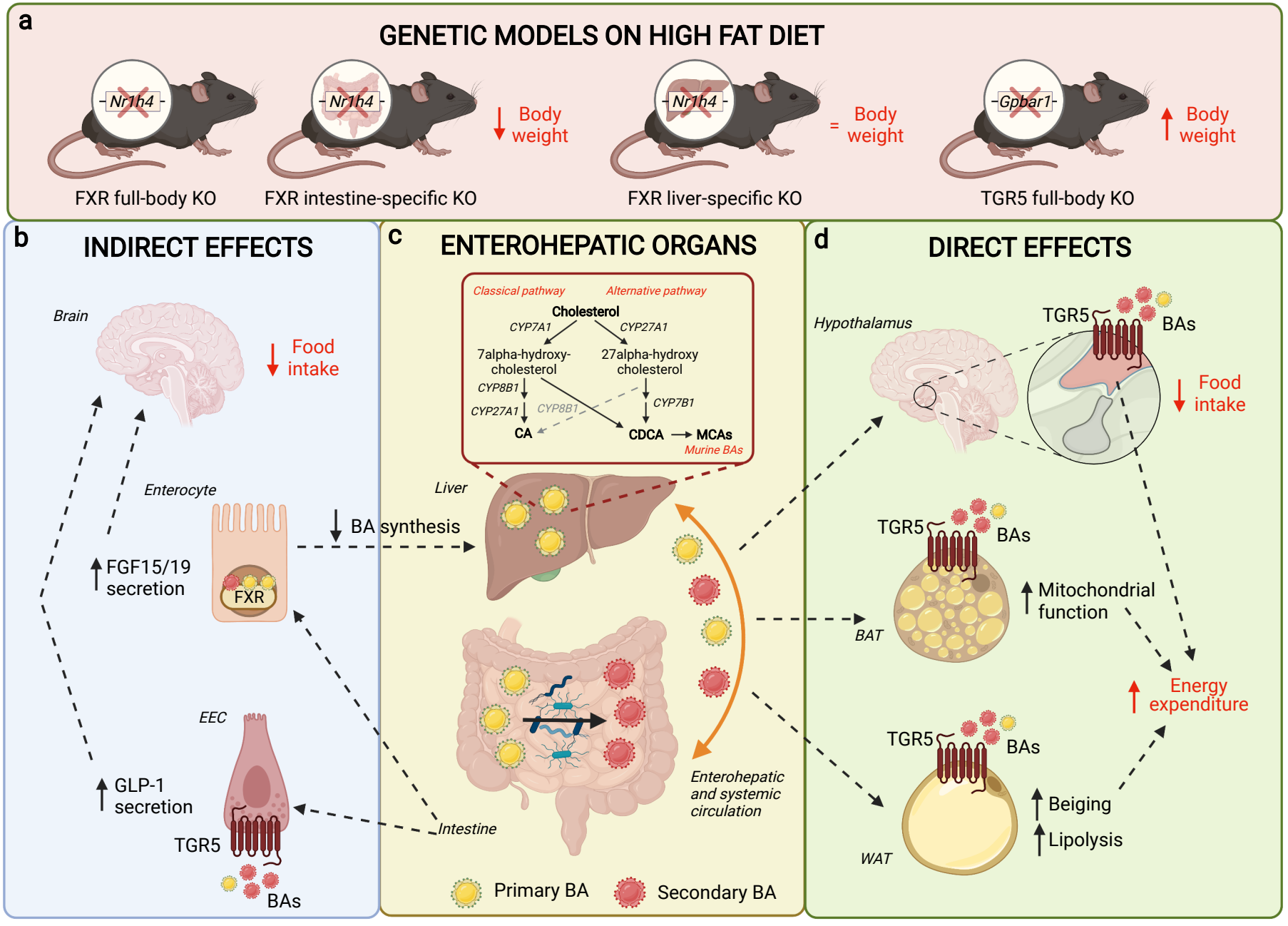

1550 B.C.
First written trace of bile used to cure diseases (Ebers Papyrus)
460-377 B.C.
Bile as part of the humoral theory of Hippocrates
19th century
Isolation of BAs from bile
1932
Identification of the chemical structure of BAs
1943
BAs are cholesterol derivatives
1950s
Chromatographic separation of BAs
Discovery of secondary BAs and entherohepatic circulation
UDCA on the market
1970s
CDCA and UDCA used in the treatment of gallstone disease
1980s
UDCA as a treatment of PBC
BA resins on the market for hypercholesterolemic patients
1999
Discovery of FXR as a BA receptor
2002
Discovery of TGR5 as a BA receptor
2000s
Synthesis of FXR and TGR5 selective agonists
2000s-now
Understanding the metabolic functions of BAs and their receptors
2019
Obeticholic acid approved by FDA for PBC patients


Hypothalamus
↓ Food intake (TGR5)
↑ Energy expenditure (HFD - TGR5)
Brown adipose tissue
↑ Energy expenditure (TGR5)
↓ Body weight (TGR5)
↑ Insulin sensitivity (TGR5)
Liver
↓ BA overload (FXR)
↑ Glycogen (FXR)
↓ Gluconeogenesis (FXR)
↓ TG and VLDL (FXR)
Gut
↑ Glucose homeostasis (FXR)
↑ Sterol excretion (FXR)
↑ FGF15/19 secretion (FXR)
↑ Gut hormone secretion (TGR5)
↑ Gut regeneration (TGR5)
White adipose tissue
↑ Beiging (TGR5)
↑ Lipolysis (TGR5)
↑ Insulin sensitivity (TGR5)
Muscle
↑ Energy expenditure (TGR5)
↑ Muscle function (TGR5)
↑ Insulin sensitivity (TGR5)
Immune cells (mouse and human)
↓ Inflammation (TGR5)


a
GENETIC MODELS ON HIGH FAT DIET
Nr1h4
Body weight
FXR full-body KO
Nr1h4
Body weight
FXR intestine-specific KO
Nr1h4
= Body weight
FXR liver-specific KO
Gpbar1
Body weight
TGR5 full-body KO
b
INDIRECT EFFECTS
Brain
Food intake
Enterocyte
BA synthesis
FGF15/19 secretion
FXR
EEC
GLP-1 secretion
TGR5
BAs
Intestine
c
ENTEROHEPATIC ORGANS
Classical pathway
Alternative pathway
Cholesterol
CYP7A1
CYP27A1
7alpha-hydroxy-cholesterol
27alpha-hydroxy cholesterol
CYP8B1
CYP27A1
CYP8B1
CYP7B1
CA
CDCA
MCAs
Murine BAs
Liver
Enterohepatic and systemic circulation
Primary BA
Secondary BA
d
DIRECT EFFECTS
Hypothalamus
TGR5
BAs
Food intake
TGR5
BAs
BAT
Mitochondrial function
Energy expenditure
TGR5
BAs
Beiging
Lipolysis
WAT