8 The Influence of Gut Microbial Metabolism on the Development and Progression of Non-alcoholic Fatty Liver Disease

Wei Jia and Cynthia Rajani

Abstract

Non-alcoholic fatty liver disease (NAFLD) is defined as the presence of excess fat in the liver parenchyma in the absence of excess alcohol consumption and overt inflammation. It has also been described as the hepatic manifestation of metabolic syndrome (Than NN, Newsome PN, Atherosclerosis. 239:192–202, 2015). The incidence of NAFLD has been reported to be 43–60% in diabetics, ~90% in patients with hyperlipidemia and 91% in morbidly obese patients (Than NN, Newsome PN, Atherosclerosis. 239:192–202, 2015, Machado M, Marques-Vidal P, Cortez-Pinto H, J Hepatol, 45:600–606, 2006, Vernon G, Baranova A, Younossi ZM, Aliment Pharmacol Ther, 34:274–285, 2011). The risk factors that have been associated with the development of NAFLD include male gender, increasing age, obesity, insulin resistance, diabetes and hyperlipidemia (Attar BM, Van Thiel DH, Sci World J, 2013:481893, 2013, Gaggini M, Morelli M, Buzzigoli E, DeFronzo RA, Bugianesi E, Gastaldelli A, Forum Nutr, 5:1544–1460, 2013). All of these risk factors have been linked to alterations of the gut microbiota, ie., gut dysbiosis (He X, Ji G, Jia W, Li H, Int J Mol Sci, 17:300, 2016). However, it must be pointed out that the prevalence of NAFLD in normal weight individuals without metabolic risk factors is ~16% (Than NN, Newsome PN, Atherosclerosis. 239:192–202, 2015). This fact has led some investigators to hypothesize that the gut microbiota can impact lipid metabolism in the liver independently of obesity-related metabolic factors (Marchesi JR, Adams DH, Fava F, Hermes GD, Hirschfield GM, Hold g, et al., Gut, 65:330 339, 2016) (Le Roy T, Llopis M, Lepage P, Bruneau A, Rabot S, Bevilacqua C, et al., Gut, 62:1787–1794, 2013). In this chapter, we will explore the effect of the gut microbiota on hepatic lipid metabolism and how this affects the development of NAFLD.

Keywords

Non-alcoholic fatty liver disease · Gut microbiota · Diabetes · Steatosis · Metabolic syndrome · Bile acids

W. Jia (✉)
University of Hawaii Cancer Center, Honolulu, HI, USA

Shanghai Jiao Tong University School of Medicine, Shanghai, China
e-mail: wjia@cc.hawaii.edu

C. Rajani
University of Hawaii Cancer Center, Honolulu, HI, USA
e-mail: crajani@cc.hawaii.edu

J. Yu (ed.), *Obesity, Fatty Liver and Liver Cancer*, Advances in Experimental Medicine and Biology 1061, https://doi.org/10.1007/978-981-10-8684-7_8

8.1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined as the presence of excess fat in the liver parenchyma in the absence of excess alcohol consumption and overt inflammation. It has also been described as the hepatic manifestation of metabolic syndrome. A much broader definition of NAFLD that has come into common use is that it can be considered as the entire spectrum of liver disease which progresses from simple steatosis → steatohepatitis → fibrosis → cirrhosis and finally leading to either liver transplantation or hepatocarcinoma (HCC) [1]. The incidence of NAFLD has been reported to be 43–60% in diabetics, ~90% in patients with hyperlipidemia and 91% in morbidly obese patients [1–3]. The risk factors that have been associated with the development of NAFLD include male gender, increasing age, obesity, insulin resistance, diabetes and hyperlipidemia [4, 5]. All of these risk factors have been linked to alterations of the gut microbiota, ie., gut dysbiosis [6]. The gut microbiota are considered to be an additional organ in the body which, as a collection of many different cells, works together with the host to promote health but can also malfunction and initiate disease [7]. Although gut microbiota have been implicated as part of the etiology of the risk factors leading to NAFLD, it must be pointed out that the prevalence of NAFLD in normal weight individuals without metabolic risk factors is ~16% [1]. The fact that not all persons with NAFLD are obese or have other associated metabolic risk factors has led some investigators to hypothesize that the gut microbiota can impact lipid metabolism in the liver independently of obesity-related metabolic factors [8]. In this chapter, we will explore the effect of the gut microbiota on hepatic lipid metabolism and how this affects the development of NAFLD.

8.2 The Gut Microbiota and Development of NAFLD

NAFLD is prevalent among obese persons, however, not all obese people develop NAFLD. In this section, we will discuss the evidence from pre-clinical and clinical studies that provide evidence for gut microbiota involvement in the etiology of NAFLD. High fat diet (HFD) is a standard method for inducing obesity, steatosis and insulin resistance in mice [9]. Early studies showed that germ-free (GF) mice treated with HFD gained less weight and exhibited less glycaemia, insulinemia, and better glucose tolerance and insulin sensitivity relative to conventional mice [10]. These differences in metabolism may be partially explained by the increased fatty acid (FA) oxidation and decreased lipogenesis observed in germ-free (GF) mice [11]. It has also been shown that diabetes-susceptible and resistant mice of the same genetic background are associated with different gut microbiota [12]. A recent study, which will be discussed below, was undertaken to examine NAFLD with the hypothesis that NAFLD could be dissociated from the degree of obesity and diabetes via the gut microbiota in mice [8].

In order to understand the role of the gut microbiota in NAFLD development, a conventional strain of C57BL/6J mice were fed a common high fat diet (HFD) for 16 weeks [8]. Within the same mouse strain, HFD treatment produced mice that responded to the diet by developing high levels of glycaemia, systemic inflammation and steatosis (responders) and also several mice that did not develop metabolic disorders (non-responders). From these two groups of mice, one responder and one non-responder was chosen that had similar body weight, fat pad mass and food intake to become a fecal donor mouse. Two groups of germ-free (GF) C57BL/6J mice were then submitted to fecal transplantation from either the responder mouse or the non-responder to generate RR mice and NRR mice, respectively. The RR and NRR groups were fed the same HFD for 16 weeks. Both NRR and RR groups exhibited similar food intake, weight gain and size of epididymal fat pads, but the RR group had enhanced levels of fasting glycaemia and insulinemia. The HOMO-IR index was 2.4-fold greater in the RR group indicating development of much more insulin resistance. Total caecal concentrations of short-chain fatty acids (SCFAs) were similar between NRR and RR but isobutyrate and isovalerate, bacterial fermentation prod-

ucts of valine and leucine were significantly higher in the caecum of RR mice. The NRR group developed slight to mild steatosis while the RR group developed marked steatosis with a 30% higher triglyceride (TG) level. The transcription factors, sterol regulatory binding protein (SREBP) 1c and carbohydrate response element binding protein (ChREBP) were found to be increased ~2-fold in RR *vs.* NRR mice. Both of these factors affect hepatic *de novo* lipogenesis (DNL) [13].

The microbiota of the mice on HFD showed a clustering pattern with two bacterial species, *Lachnospiraceae bacterium 609* and *Barnesiella intestinihominis*, higher in RR mice at both week 3 and 16, and *Bacteroides vulgates* was higher in NRR mice [8]. *Barnesiella intestinihominis* belongs to the family Porphyromonadaceae which was shown previously to be increased in inflammasome deficient mice that developed marked steatosis and inflammation and also in a clinical study of obese NAFLD patients relative to healthy lean [14]. On the other hand, *Bacteroides vulgates* was previously found to be decreased in patients with type-2 diabetes (T2D) suggesting this bacterium may exert protective effects against T2D [15]. More generally, *Barnesiella* and *Roseburia* genera were found to be more represented in RR mice while *Allobaculum* was increased in the NRR group. RR mice had significantly increased Firmicutes species than NRR mice even though the degree of adiposity was the same for both groups.

Other findings that were remarkable in this study were that there was no significant difference in systemic and hepatic inflammation or in body and liver weights between RR and NRR indicating that the gut microbiota can impact hepatic lipid metabolism independently of a systemic pro-inflammatory state and that insulin resistance does not depend on a greater degree of obesity [8]. Based on this study, the impact of microbiota on steatosis and NAFLD may be explained by their function in regulating glucose homeostasis via the transcription factors ChREBP and SREBP, which control transcription of lipogenic genes. Both ChREBP and SREBP transcription factor activities are under the control of another important hepatic transcription factor, the bile acid (BA) sensitive farnesoid X receptor (FXR). In the next section, we shall briefly review hepatic lipid metabolism and its connectivity with glucose metabolism and how BA activation of FXR influences lipid and glucose metabolism in the liver.

8.3 Hepatic Lipid Metabolism and Its Interface with Glucose Metabolism in NAFLD

Lipid metabolism begins in the intestine where lipids are emulsified by bile acids (BAs). Lipid emulsification allows them to become hydrolyzed and subsequently absorbed by the enterocytes where they become converted to lipoprotein particles called nascent chylomicrons. Nascent chylomicrons then travel through the lymphatic system into the circulation where they are processed further via replacement of apoproteins A-I and IV (apoI,IV) with apoE and apoC-II which allows them to be broken down into free fatty acids (FFAs), glycerol and chylomicron fragments. FFAs are then partially removed from the blood by adipose tissue while the cholesteryl-ester enriched and TG depleted chylomicron fragments are endocytosed by the liver and broken down in the lysosomes into recyclable hepatic glycerol, FA, cholesterol, amino acid and phosphate residues [16]. Therefore, hepatic FAs come from four sources, (1) lipolysis of adipose tissue, (2) dietary ingestion, (3) endogenous production via *de novo* lipogenesis (DNL) and, (4) released from hepatic lysosomes by autophagy. In a clinical study of NAFLD patients, it was determined that ~50–60% of TGs in the liver were derived from nonesterified FFAs (from lipolysis of adipose tissue and chylomicron fragments), ~19–33% from DNL and 8–22% from dietary sources [16, 17]. The increase in DNL in NAFLD was thought to be due to dysregulation of SREBP1c and FoxO-modulation of insulin signaling, thereby providing a link between hepatic lipid and glucose metabolism [18]. Hepatic FA synthesis, on the other hand, is initi-

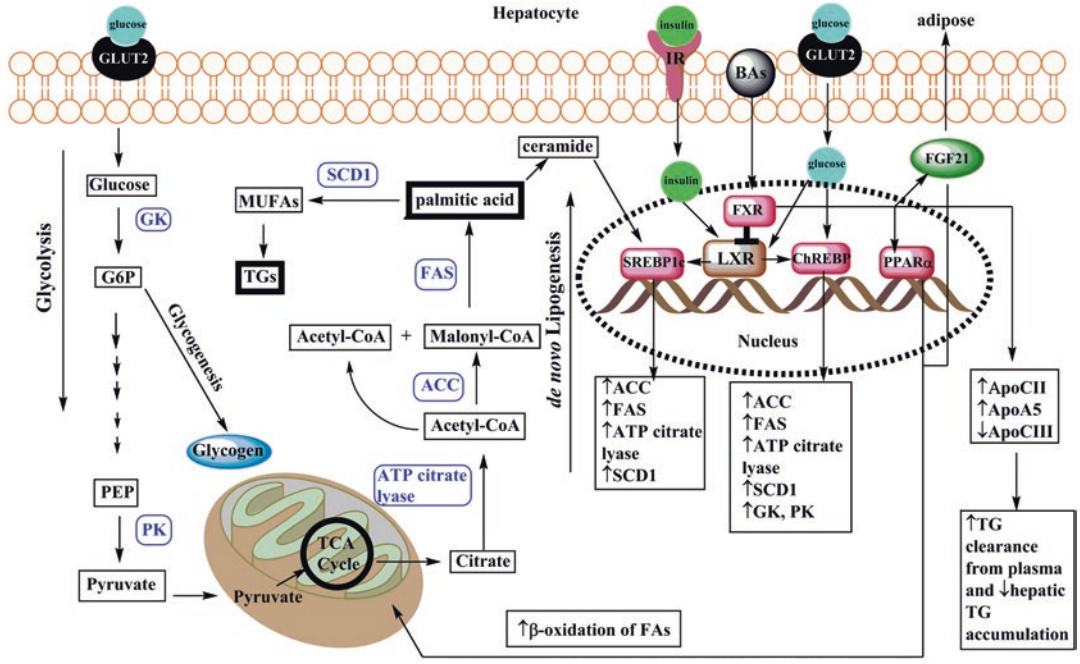

Fig. 8.1 The interface between hepatic lipid and glucose metabolis. Both glucose and insulin activate LXR and this causes increased expression and activation of SREBP1c and ChREBP which, in turn, increases *de novo* lipogenesis. In addition to LXR, glucose can also directly activate ChREBP and ceramide, a product of lipogenesis can directly activate SRBEP1c. ChREBP transcribes key enzymes for the glycolysis/glycogenesis cycles (GK, PK) and both ChREBP and SREBP1c synergistically transcribe important enzymes for *de novo* lipogenesis (ATP citrate lyase, ACC, FAS, SCD1). BAs activate the nuclear receptor FXR which modulates both glycolysis/glycogenesis and *de novo* lipogenesis cycles via it inhibitory effect on LXR. FXR activation also leads to increased expression of PPARα which in turn, transcribes genes for the increase of mitochondrial β-oxidation of FAs. Therefore, hepatic FXR activation leads to a decrease in the glycolysis → *de novo* lipogenesis → TG axis and reduces hepatic lipid accumulation and also increases the use of FAs for energy expenditure in the liver via upregulation of PPARα. Both of these FXR mediated effects reduce hepatic lipid accumulation to forestall NAFLD [16, 19, 20, 22, 23, 25] *Abbreviations: GLUT2* glucose receptor-2, *GK* glucokinase, *G6P* glucose-6-phospate, *PEP* phosphenolpyruvate, *PK* pyruvate kinase, *ACC* acetyl-CoA carboxylase, *FAS* fatty acid synthase, *FAs* fatty acid, *SCD1* steroyl-CoA-desaturase-1, *IR* insulin receptor. *BAs* bile acids, *FXR* farnesoid X receptor, *LXR* liver X receptor, *PPARα* peroxisome proliferator-activated receptor-α, *ChREBP1c* carbohydrate response element binding protein-1c, *SREBP* sterol response element binding protein, *FGF21* fibroblast growth factor-21

ated via two enzymes, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). The lipid sensitive SREBP1c and the glucose sensitive ChREBP transcription factors together induce expression of FAS and ACC in a synergistic way thus giving increased support to the idea of an interface between glucose and lipid metabolism in the liver [19, 20].

Figure 8.1 is a diagram depicting a brief overview of the interface between hepatic glucose and lipid metabolism in a normal liver. Glucose uptake via GLUT2 transporters can be shunted into either glycolysis or glycogenolysis. Activation of ChREBP results in the increased transcription of genes for glucokinase (GK) which phosphorylates glucose to become glucose-6-phophate which, in turn, can be used as a substrate for either glycolysis or glycogenesis. ChREBP also acts to upregulate pyruvate kinase (PK) which is a key enzyme in glycolysis that converts phosphoenolpyruvate into pyruvate. Pyruvate is then taken into the mitochondria where it enters TCA cycle. The result of this is the production of citrate which is converted into acetyl-CoA via the enzyme ATP citrate lyase, an enzyme that is controlled by both ChREBP and SREBP-1c. ChREBP and SRBEP-1c are regulated

by glucose and insulin, respectively. Together, ChREBP and SRBEP-1c transcribe genes for enzymes involved in *de novo* lipogenesis (Fig. 8.1). Acetyl-CoA formed previously from citrate is then catalyzed by acetyl-CoA carboxylase (ACC) to form malonyl Co-A. Malonyl CoA and acetyl-CoA together can then be reacted with fatty acid synthase (FAS) to form palmitic acid, an important FA that is the substrate for production of monounsaturated FAs (MUFAs) via steroyl-CoA-desaturase-1 (SCD1). MUFAs are then eventually packaged into TGs or else undergo β-oxidation in the mitochondria [16, 19, 21].

Both SREBP1c and ChREBP expression are regulated by the BA sensitive nuclear receptor, farnesoid X receptor (FXR) via inhibition of liver X receptor (LXR) [22]. The primary evidence for FXR involvement in hepatic lipid metabolism came from studies of FXR KO mice which clearly showed that FXR deletion resulted in hepatic lipid accumulation and elevated plasma TGs. On the contrary, activation of FXR by either BAs or an agonist such as GW4064 or INT-747 reduced both glycolysis and de novo lipogenesis, leading to a reduction in hepatic TGs in mice [23, 24]. FXR activation also leads to the increased expression of peroxisome proliferator-activated receptor-α (PPARα) resulting in increased β-oxidation of FAs for energy expenditure and decreased hepatic TGs in mice [21]. This was shown in PPARα$^{-/-}$ mice which are incapable of upregulating FA oxidation in the liver and develop severe steatosis [25, 26]. When placed on a methionine/choline deficient diet, PPARα$^{-/-}$ mice develop NASH [26]. Furthermore, administration of PPARα agonists prevented the development of methionine- and choline-deficient diet-induced NASH in mice [27]. Clinical data is inconclusive in humans in the use of PPARα agonists for prevention of steatosis in NAFLD which has been attributed to small sample size and the use of combined treatments [28]. Lastly, hepatic FXR activation leads to the increased expression of fibroblast growth factor -21 (FGF21) which is secreted from the liver and acts mainly in adipose tissue via binding to fibroblast growth receptor-4 (FGFR4) (Fig. 8.2) to increase expression of adiponectin, a beneficial adipokine that has been shown to reduce the level of ceramide [29]. FGF21 has also been shown to activate an extracellular signal-related kinase ½ (ERK1/2) signaling pathway in adipose tissue (Fig. 8.2) that leads to increased expression of GLUT1 glucose transporters resulting in increased glucose uptake by adipose tissue and a lowering of blood glucose levels thus protecting against hyperglycemia, hyperinsulinemia and insulin resistance [30].

FXR activation also plays a critical role in VLDL clearance from the plasma. VLDL TGs are cleared from the plasma via their hydrolysis by lipoprotein lipase (LPL), an enzyme which lines the endothelial cells of extrahepatic tissues. FXR induces apoCII and apoA5 which are activators of LPL and suppresses apoCIII which is an LPL inhibitor [22, 31, 32]. FGF21 produced by FXR activation also acts in an endocrine way in the liver mitochondria to increase β-oxidation of FAs into acetyl-CoA for use in the ketogenesis pathway [22, 33] (Figs. 8.1 and 8.2).

FXR activation in the intestine has consequences for hepatic lipid metabolism and progression to NAFLD as shown in Fig. 8.2. In the intestine, FXR is known to target the expression of genes that lead to the synthesis of ceramide. This was shown in mice using an intestine specific FXR inhibitor, glyco-muricholic acid (G-MCA), which cannot be hydrolyzed by the gut microbiota. The G-MCA treatment protected the mice that were exposed to HFD from adiposity, hyperglycemia, insulin resistance and hepatic steatosis by decreasing the expression of ceramide and the ceramide synthetic enzymes, sphingomyelin phosphodiesterase 3 (Smpd3) and serine palmitoyltransferase long-chain base subunit 2 (Sptlc2) [34]. Increased ceramide activates three different signaling pathways in the liver, inhibitor of nuclear factor κB kinase subunit β (IKK2), c-Jun N-terminal kinase (JNK) and protein kinase C-ζ (PKCζ) that all result in insulin resistance (Fig. 8.2) [35]. However, FXR activation in the ileum exerts a hepatoprotective effect by increasing the production of FGF19/(15 in mice), a hormone that when secreted into the circulation binds to the hepatic FGFR4 receptor. Hepatic FGF19/15-FGFR4 binding decreases

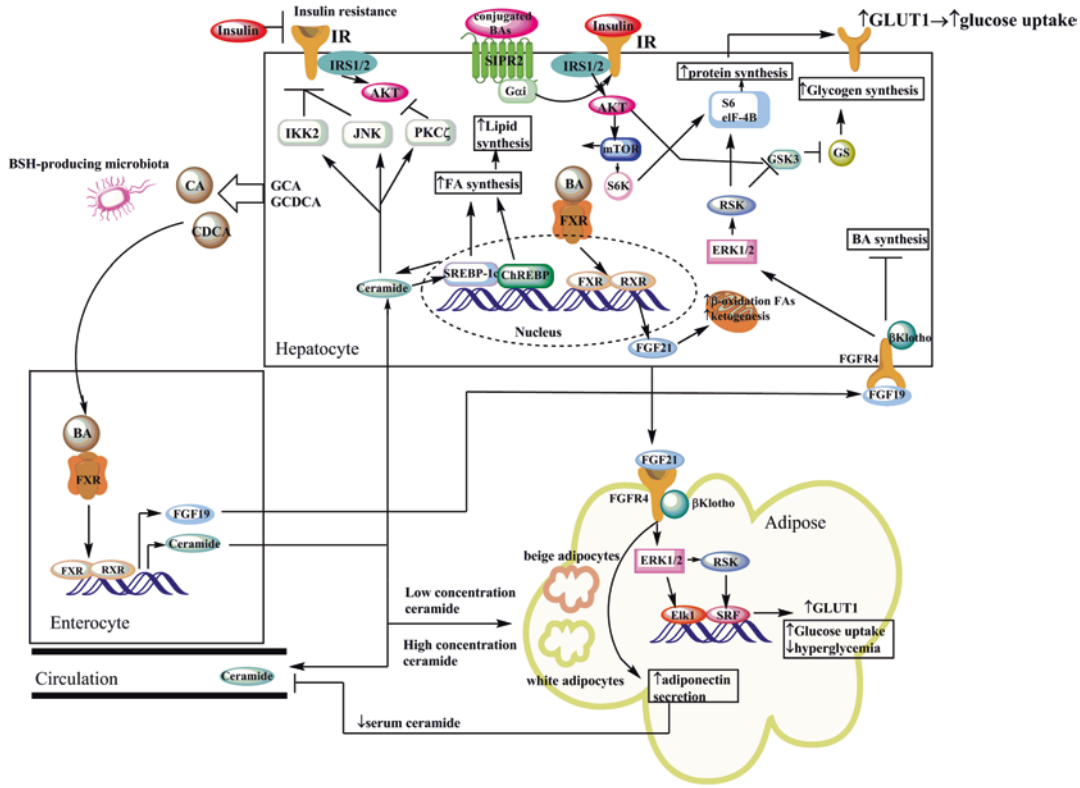

Fig. 8.2 The gut microbiota-BA-FXR- FGF21/FGF19-adiponectin-ceramide pathway role in metabolic diseases, including NAFLD. BSH producing microbiota deconjugate BAs secreted from the liver. Unconjugated, primary BAs (CA, CDCA) then activate intestinal FXR which leads to the production of FGF19. FXR activation also targets two genes for enzymes important for the synthesis of ceramide, *Smpd3* and *Sptlc2* and thus causes an increase in ceramide. FGF19 subsequently binds to FGFR4/β-Klotho which causes, (1) inhibition of BA synthesis, (2) activation of ERK1/2 → ↑protein (ie., GLUT1 glucose transporters) and glycogen synthesis. Ceramide, on the other hand, (1) activates SREBP-1c to ↑FA synthesis, (2) activates IKK2, JNK and PKCζ which effectively block the effects of insulin on its receptor, ie., insulin resistance. Insulin, also shown in this diagram, can be activated by the BA sensitive G-protein-coupled receptor SIPR2 and shows some parallel activity to the effects of FGF19 in that it augments the effect of insulin via the pathway leading to increased S6/elF-4B which causes increased protein (GLUT1) and glycogen synthesis. In addition, insulin targets mTOR to cause ↑lipid synthesis. FXR activation in the liver causes production of FGF21 which, after secretion, targets FGFR4/β-Klotho in WAT where it, (1) activates the ERK 1/2 → RSK → Elk1/SRF pathway that leads to increased expression of GLUT1 transporters which in turn cause enhanced uptake of glucose into WAT and a decrease in hyperglycemia, (2) causes an increase in adiponectin secretion that in turn, lowers serum ceramide. Lower serum ceramide means more beige adipocytes and increased energy utilization to fight obesity while high serum ceramide means more WAT and less energy expenditure [29, 30, 35–37]

Abbreviations: BA bile acid, *CA* cholic acid, *CDCA* chenodeoxycholic acid , *FXR* farnesoid X receptor, *RXR* retinoid X receptor, *FGF19/21* fibroblast growth factor-19/21, *IR* insulin receptor, *IRS1/2* insulin receptor substrate ½, *IKK2* inhibitor of nuclear factor κB kinase subunit beta, *JNK* c-Jun N-terminal kinase, *PKC* protein kinase C, *FA* fatty acid, *AKT* protein kinase B, *SREBP-1c* sterol response element binding protein-1c, *ChREBP* carbohydrate responsive element binding protein, *mTOR* mammalian target of rapamycin, *S6K* S6 ribosomal protein kinase-beta-1, *S6* S6 ribosomal protein, *elF-4B* eukaryotic translation initiator factor -4B, *GSK3* glycogen synthase kinase 3, *GS* glycogen synthase, *RSK* ribosomal S6 kinase, *ERK1/2* extracellular signal-related kinase ½, *FGFR4* fibroblast growth factor receptor-4, *Elk1* ETS domain containing protein-1, *SRF* serum response factor, *BSH* bile salt hydrolase, *WAT* white adipose tissue, *SIPR2* sphingosine-1-phosphate receptor-2

BA synthesis but activates ERK1/2 signaling pathways, increasing protein (GLUT1) and glycogen synthesis. These activities increase glucose uptake and storage of excess glucose as glycogen, conferring protection against hyperglycemia and hepatic insulin resistance [36]. Conjugated BAs also bind to another hepatic BA sensitive G-protein coupled receptor, sphingosine-1-phosphate receptor-2 (SIPR2), which has been shown to transactivate the insulin receptor (IR) to augment insulin signaling, the result is protein kinase B activation (AKT) which stimulates mammalian target of rapamycin (mTOR) to increase glycogenesis and protein synthesis (ie. GLUT1) [37].

In summary of this section, we have reviewed hepatic lipid metabolism and the signaling pathways that mediate it. Further we have discussed how BAs impact these signaling pathways and hepatic lipid metabolism via the nuclear receptor, FXR and the G-protein coupled receptor SIPR2 which not only directly impact transcription factors the govern lipogenesis, glycolysis and glycogenesis, but also cause transcription of important FGF hormones that positively affect metabolism. The gut microbiota is responsible for the composition of the BA pool which are the endogenous agonists for FXR and SIPR2. In the next section, we will discuss the gut microbiota-BA axis and its effect on NAFLD development.

8.4 The Gut Microbiota-BA Axis and Development of NAFLD

The gut microbiota shapes the composition of the BA pool producing the endogenous ligands for the BA sensitive receptors discussed so far in this chapter, FXR and SIPR2. Early evidence for the existence of a gut microbiota-BA axis came from examination of the BA pool in GF mice/rats. GF rodents have only primary conjugated BAs, an expanded intestinal BA pool, increased BA synthesis and decreased BA reabsorption [25]. Gut microbiota are essential for modifying the structure of the primary BAs produced in the liver and these modifications include deconjugation of the primary BAs, GCDCA (or TCDCA) and GCA (or TCA) into CDCA and CA, which must precede subsequent, multiple 7α-dehydroxylation steps to produce the secondary BAs, deoxycholic acid (DCA) and lithocholic acid (LCA). These gut microbiota transformed BA have been shown to be high affinity ligands for FXR and their affinities have been ranked as CDCA> LCA = DCA > CA [24, 38, 39]. Reconjugation in the liver of the secondary BAs LCA and DCA to TLCA (or GLCA)and TDCA (or GDCA) gives rise to the most potent ligands for the intestinal BA sensitive G-protein coupled receptor, Takeda G-protein coupled receptor 5 (TGR5) (TLCA > GLCA>LCA >TDCA> GDCA> DCA > TCDCA> GCDCA >CDCS > TCA > GCA > CA) [24, 37, 40, 41]. The hepatic BA sensitive G-protein coupled receptor, SIPR2, is only activated by conjugated Bas [37]. Notably, the genes for the two conjugating enzymes for BAs, BA-CoA synthase (BACS) and BA-CoA:amino acid N-acyltransferase (BAT) are FXR targets [22, 42]. Thus, the gut microbiota, by modifying the BA pool control FXR and SIPR2 signaling and the accumulation of TGs in the liver that lead to NAFLD.

A recent study nicely demonstrated the alteration of the BA pool that occurs with metabolic changes in mice [43]. A group of obesity-prone (129S6/SvEvTac=129T) and obesity resistant mice (129S6/SvlmJ=129J) from the same strain were treated with HFD along with another group of obesity-prone mice from a different strain (C571BL/6J=B6J). Both B6J and 129T mice gained a significant and similar amount of weight while the 129J mice remained lean. However, both 129T and 129J groups maintained normal blood glucose and insulin levels and remained insulin sensitive despite their significantly different BMIs. The B6J mice developed hyperinsulinemia, hyperglycemia and insulin resistance. Insulin resistance is strongly associated with the development of NAFLD [44]. The investigators then used a metabolomic technique to analyze the BAs in all of the mice with the following results. The BA profiles indicated a unique baseline (no HFD) gut microbiota for each group based on the differences in the BA abundances found which was altered by HFD in a unique way for each

Table 8.1 BA profiles reflect changes in microbiota

Mouse strain	Treatment	BA profile + dominant bacterial phlya
B6J	Chow + placebo	HDCA/UDCA > MCA = CDCA > DCA > CA > LCA
	HFD + placebo	CA > > MCA > DCA > HDCA/UDCA > CDCA >LCA
		Firmicutes >> Bacteroidetes >>>>> Actinobacteria
	HFD + V	CA > MCA > HDCA/UDCA > CDCA (no LCA, DCA)
		Proteobacteria >> Firmicutes >>> Tenericules
	HFD + M	MCA >> CA > HDCA/UDCA > CDCA > DCA (no LCA)
		Firmicutes > Proteobacteria >>>> unclassified
129T	Chow + placebo	CA > MCA >> HDCA/UDCA = CDCA >> CA > LCA
	HFD + placebo	CA > MCA > DCA > HDCA/UDCA > CDCA > LCA
		Firmicutes >> Bacteroidetes~ Verrucomicrobia >> Deferribacteres
	HFD + V	CA > MCA >> HDCA/UDCA > CDCA > DCA (no LCA)
		Firmicutes > Proteobacteria >> Deferribacteres>>> Tenericutes
	HFD + M	MCA > CA >>> HDCA/UDCA > CDCA (no DCA, LCA)
		Firmicuttes > > Proteobacteria >>>>> Actinobacteria
129J	Chow + placebo	MCA >> HDCA/UDCA > DCA = CDCA >> CA > LCA
	HFD + placebo	MCA > CA >> HDCA/UDCA > CDCA (no DCA, LCA)
		Verrucomicrobia >>> Firmicutes >> Bacteroidetes>>>> Proteobacteria
	HFD + V	CA > MCA >> HDCA/UDCA > CDCA (no DCA, LCA)
		Proteobacteria (2/3) >> Firmicutes (1/3)
	HFD + M	MCA >>> HDCA/UDCA > CDCA > CA (no DCA, LCA)
		Firmicutes >> Proteobacteria = Verrucomicrobia

group (Table 8.1). Both mouse [45] and human [46] obesity phenotypes have been associated with an decrease in the ratio of the two dominant phyla in the microbiota, Bacteroidetes/ Firmicutes relative to lean controls and thus the next strategy was to administer two antibiotics to two groups of HFD mice from each strain, metronidazole (M), a broad spectrum antibiotic that is absorbable by anaerobes and vancomycin (V) that is absorbable only by gram positive bacteria which would include *Firmicutes* and the third most common phylum in the gut, Actinobacteria [47]. Using the 129J strain (lean control) as a point of reference, the HFD treatment transformed the BA profile of 129T mice to be similar to the B6J in terms of rank ordering of BA abundance. The BA composition for both 129T and B6J mice on antibiotic treatment changed to become more similar to the 129J lean control. The gut microbiota differences among the different treatment groups showed an increase in Firmicutes with HFD only for the obesity-prone strains.

The V and M treated B6J mice showed improved glucose, glucose tolerance and insulin sensitivity with no changes in insulin levels. Finally, transplantation of fecal matter to HFD treated GF-B6J from V and M-treated B6J resulted in improved glucose, glucose tolerance and insulin sensitivity relative to the original HFD treated B6J mice, indicating that these differences were due to the transplanted microbiota. The major conclusions from this study are; (1) that development of metabolic syndrome does not depend on obesity but is strongly affected by the gut microbiota, (2) although 129T and 129J have the same genetic background, they can have different microbiota and therefore, different obesity tendencies, (3) changes in the gut microbiota may be visualized by changes in the BA pool composition. (Table 8.1).

8.5 Angiopoietin-Like Protein-4 and Development of NAFLD

BAs are not the only regulators of hepatic lipid accumulation under the control of the gut microbiota. In this section, we will examine the effect of the gut microbiota metabolites on the pathogenesis of NAFLD via their ability to impact LPL activity and alter the availability of choline. These pathways are summarized in Fig. 8.3.

The following pivotal study clearly revealed the involvement of the gut microbiota as a regulator of both hepatic and adipose lipid storage [48]. This experiment involved the comparison of GF C57BL/6J (B6J) mice with conventionalized mice (CONV-D) from a WT donor, as well as, conventionally raised WT mice (CONV-R). CONV-R mice contained 42% more total body fat than the GF mice. When the GF mice were conventionalized using a fecal transplant from the CONV-R mice (CONV-D), they increased their total body fat by 57% with a 61% increase in epididymal fat. The predominant caecal bacteria genera in both CONV-R and CONV-D were found to be Bacteroides and Clostridium. Relative to GF mice, CONV-D showed a 2.3-fold increase in hepatic TGs with no appreciable changes in liver FFAs or cholesterol. An increase in the mRNA for ChREBP and to a lesser extent, SREBP-1c, was observed along with mRNA increases for the enzymes ACC and FAS suggesting that these mice were displaying an increase in *de novo* lipogenesis. A doubling of capillary density in the small intestine was observed for CONV mice compared to GF and a single gavage of a mixture of glucose and 2-deoxyglucose and measurement 15 min. Later revealed 2-fold higher levels of 2-deoxy 6-phosphate in CONV-D mice relative to GF. Lipoprotein lipase (LPL) activity was increased and this was found to be due to less transcription of the gene for angiopoietin-like protein-4 (ANGPTL4), in the small intestine but not in the adipose tissue or liver confirmed by qRT-PCR of ANGTPL4 mRNA levels. The conclusions from these findings were proposed to be: (1) an increase in the processing of dietary polysaccharides by gut microbiota and increased delivery of monosaccharides to the liver resulted in increased TG synthesis and (2) a decrease in intestinal ANGTPL4 upon CONV resulted in increased LPL activity and thus increased FFA transport and subsequent storage as TGs in adipose. Both of these conclusions thus explained the observed increase in hepatic TGs and total body fat in CONV *vs.* GF mice [48].

The ability of the gut microbiota, independently of PPARs, to affect ANGTPL4 gene transcription in the intestine was confirmed in an experiment using specific pathogen-free (SPF) C57B/6J (B6J) treated with HFD and a probiotic bacterial strain thought to have anti-obesity effects, *Lactobacillus paracasei ssp paracasei F19* (F19) [49]. Relative to controls, F19 treated mice had elevated levels of ANGTPL4 and a significantly lower body fat. HCT116, LoVo, HT29 and SW480 colonocytes were then treated with F19 and all cell lines were stimulated by F19 to produce elevated levels of ANGTPL4. Heat-killed F19 could not produce a ANGTPL4 response while conditioned media from F19/ cells, even if heat-killed, could produce a response. Supernatants of F19 cultured alone could also mount a ANGTPL4 response when added to colonocytes. When PPARα and PPARγ specific ligands were applied to colonocytes, an increase in ANGTPL4 was observed indicating that there is also regulation of ANGTPL4 by PPAR nuclear factors. The PPAR that is highly expressed in the intestine is PPARγ [22, 49].

Pursuing the idea of a gut microbiota secretion factor as a control of ANGTPL4 expression, another group used imaging to determine that ANGTPL4 was most highly expressed in enteroendocrine cells (EEC) and thus did experiments on the intestinal EEC cell line HuTu-80, a line known to express high levels of ANGTPL4 [50]. They then treated the cells with various nutrients and found that the short chain fatty acids (SCFAs) butyrate and propionate but not acetate, significantly induced AGTPL4 secretion into the medium and that this was accompanied by an increase in AGPTL4 mRNA. Some BAs were also tested and CDCA and DCA were found to inhibit AGTPL4 secretion. Therefore, from the three experiments discussed above, it would

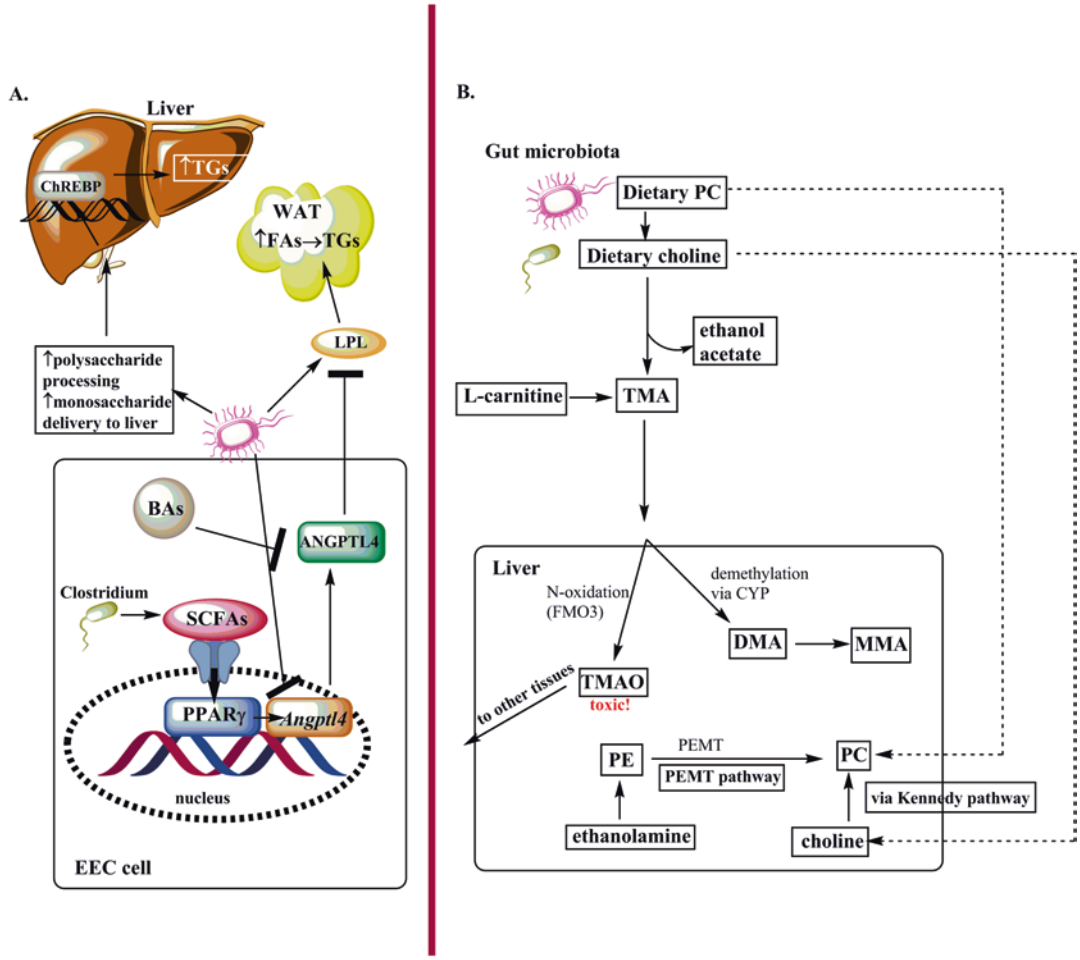

Fig. 8.3 (**a**) **The effect of gut microbiota on fat storage in NAFLD.** Gut microbiota in the colon that are capable of fermenting polysaccharides to provide an increased energy harvest are abundant in obesity and NAFLD. NAFLD is associated with increased capillary density which allows rapid transit of monosaccharides to be transported to the liver where they activate ChREBP which in turn, initiates *de novo* lipogenesis to produce more TGs to accumulate in the liver. The gut microbiota have also been shown to block transcription of the *Angptyl4* gene and thus increase activity of LPL to cause more FFAs to enter adipose for storage as TGs. Other types of gut microbiota such as *Clostridium sp.* produce SCFAs as a metabolite and these were found to increase secretion of ANGPTL4 presumably via a PPAR nuclear factor. Increased ANGPTL4 would cause a decrease in LPL activity and a decrease in fat storage. BAs, on the other hand were found to inhibit ANGPTL4 secretion from the EECs. This mechanism was proposed to explain the observed transmission of an NAFLD phenotype via gut microbiota [6, 27, 48–51]. (**b**) **The metabolism of dietary choline and PC by gut microbiota prevents PC synthesis in the liver resulting in NAFLD.** Dietary PC can be metabolized to choline in the gut. All choline in the gut can then be metabolized to TMA by certain species of gut microbiota. Diversion of choline into this metabolic pathway results in diminished synthesis of PC in the liver via the mammalian Kennedy pathway and PEMT pathways. In the liver, TMA can be demethylated by CYP enzymes to DMA and MMA or it can be N-oxidized by FMO3 enzymes to produce TMAO, a toxic substance that can be secreted to other tissues such as macrophages and arterial epithelium where it causes inflammation and atherosclerosis, respectively. If the microbiota cause choline deficiency in the liver via excess TMAO synthesis, then not enough PC can be produced to export VLDL and TGs accumulate in the liver and NAFLD results. A polymorphism in the PEMT gene causes the PEMT pathway to shut down and mammalian synthesis of PC decrease by ~30%. The combination of a PEMT polymorphism and high abundance of gut microbiota that produce TMAO is a risk factor for the development of NAFLD [55, 56, 60]
Abbreviations: TGs triglycerides, *ChREBP* carbohydrate responsive element binding protein, *WAT* white adipose tissue, *BAs* bile acids, *FAs* fatty acids, *ANGPTL4* angiopoietin-like protein-4, *PPARγ* peroxisome proliferator-activated receptor-γ, *SCFAs* short chain fatty acids, *PC* phophatidylcholine, *TMA* trimethylamine, *DMA* dimethylamine, *MMA* monomethylamine, *TMAO* trimethylamine-N-oxide, *PE* phosphoethanolamine, *FMO3* flavin mono-oxygenase enzyme-3, *PEMT* phosphatidylethanolamine-N-methyltransferase

seem that the gut microbiota mediates LPL activity via AGTPL4 induction or suppression with their metabolites SCFAs or BAs, respectively and this, in turn, impacts hepatic lipid and adipose TG accumulation. SCFAs are known to activate PPARγ in the intestine which may account for the effect of SCFAs on increased AGPTL4 secretion [51]. Gut microbiota that are known to be producers of SCFAs include the Clostridial clusters IV and XIVa of *Firmicutes*, including species of the genera *Eubacterium, Roseburia, Faecalibacterium* and *Coprococcus* [52]. Figure 8.3a summarizes the above discussion.

8.6 Gut Microbiota Choline Metabolism and Development of NAFLD

Choline deficiency has been associated with NALFD in both animal models and humans [53]. It is an essential nutrient as it is a major methyl donor for the biosynthesis of the important cell membrane lipids, phosphatidylcholine, lysophosphatidylcholine and sphingomyelin [54, 55]. (Fig. 8.3b) It is also necessary for the synthesis of the neurotransmitter, acetylcholine [55]. Phosphatidycholine (PC) deficiency increases *de novo* lipogenesis which causes an increase in TGs. Lack of PC in hepatic lipid droplets reduces their surfactant properties and larger lipid droplets that are less likely to undergo lipolysis are formed. PC is required for both VLDL synthesis and secretion from the liver [55, 56]. PC has also been identified as a cell wall component of ~10–15% of all bacteria [57].

Several experiments have been done to examine the role of the microbiota on the bioavailability of choline for the host. Metabolomic profiling of urine samples from the inbred mouse strain 129S6, a strain that is susceptible to HFD-induced NAFLD, revealed increased amounts of microbiota-derived methylamines including trimethylamine (TMA) and trimethylamine-N-oxide (TMAO) which are breakdown products of choline that are not derived from mammalian metabolism. Serum PC levels were also low in spite of the fact that the diet was supplemented with choline. This metabolic profile was not observed in another NAFLD-resistant strain, BALB/c and may be a distinct metabotype for NAFLD [54]. Figure 8.3 diagrams the three pathways for choline catabolism, two are pure mammalian and one is a bacterial pathway [54]. In a subsequent metabolomic study, human gut isolates were used to identify eight bacterial species from two different phyla, *Firmicutes* and *Proteobacteria*, and six genera that exhibited significant choline consumption and TMA accumulation: *Anaerococcus hydrogenalis, Clostridium, asparagiforme, Clostridium hathewayi, Clostridium sporogenes, Escherichia gergusonii, Proteus penneri, Providencia rettgeri,* and *Edwarsiella tarda*. These strains could be cultured *in vitro* in media containing deuterated choline where they consumed 60% of the provided choline. They also encoded component genes for the metabolism of choline. When these bacteria were gavaged into GF mice containing a core community of non-TMA producers, there was a significant decrease in the abundance of fecal choline and decreased levels of serum choline. Therefore, bioavailabilty of choline for the host was shown to be affected by the presence of TMA producing gut microbiota [58]. A rigorously controlled longitudinal study of the effect of choline deficiency on human gut microbiota was performed on 15 healthy women who were cooked in-house meals to assure dietary compliance and to control choline supplementation for 2 months [59]. Each subject was tested with three diets, (1) a standard research diet containing a recommended amount of choline (for 10 d), (2) a choline deficient diet (for 42 d) and (3) a choline recovery diet (for 10 d) that contained significant amounts of choline added to the standard research diet. Their liver fats were measured by MRI at the beginning and end of the baseline diet, at 21 and 42 d during the choline deficient diet and at the end of the diet recovery period. Patient urine and blood samples were taken for baseline values at day 1, at the end of every dietary phase and every 3–4 days in between to monitor the health status of the subjects. Stool samples were collected at the beginning and end of each dietary phase and at the middle of the choline deficient phase and recovery phase for pyrosequencing of 16S

rRNA. Even though the gut microbiota remained distinct for each subject throughout the study, variations in the amounts of two classes of bacteria, *Gammaproteobacteria* and *Erysipelotrichi* showed significant increase in abundance in subjects with low level of choline and were negatively correlated with liver fat. The elevated abundances were reversed when choline was restored to the diet indicating that these two bacteria classes respond to choline levels and may potentially be used as a potential biomarker for the detection of choline deficiency which may lead to the development of NAFLD [59].

8.7 Therapeutic Intervention for NAFLD

Gut dysbiosis has been implicated in NAFLD pathogenesis and previous studies have highlighted several benefits of using probiotic strains and or prebiotic compounds to adjust the gut microbiota, which include reduction in liver TGs, as well as improvement in glucose/insulin homeostasis and inflammation. Table 8.2 is a summary of some of the pre-clinical studies in mice and clinical studies in humans that have provided evidence that probiotics and synbiotics may help to alleviate NAFLD.

8.8 The Metabolomic Approach to NAFLD

The research discussed in this chapter all made use of a technique called metabolomics. Metabolomics is quite literally, "the measurement of metabolites" and it is considered one of the system biological approaches capable of capturing the changes of an entire spectrum of metabolites (untargeted approach) or a set of specific metabolites (targeted approach). The most

Table 8.2 Summary of pre-clinical and clinical intervention studies of probiotics and synbiotics in NAFLD

Subjects	Strain/prebiotic	Time weeks	Outcome	References
20 obese children	*Lactobaculus rhamnous GG*	8 week	↓ALT	[61]
28 adults	*Lactobacillus bulgaris*	12 week	↓ALT and γ-GTP	[62]
	Streptococcus thermophilus			
72 adults	*Lactobacillus acidophilus*	8 week	↓ALT, ASP, TC, LDL-C	[63]
	Bifidobacterium breve			
44 obese children	*Bifidobacteria, lacrobacilli*	16 week	↓fatty liver index, BMI, ↑GLP1	[64]
	Streptococcus thermophila			
40 rats HFD induced NAFLD	*Bifidobacterium longum*	10 week	↓liver TGs	[65]
	Lactobacillus acidophilus		*B.longum* > *L.acidophilus*	
40 mice HFD induced NAFLD (C57BL/6 J)	*Lactobacillus rhamnous*	12 week	↓BMI, liver TGs, adipose macrophage infiltration	[66]
	Bifidobacterium animalis ssp. lactis		Improved glucose/insulin homeostasis	
	Lactobacillus paracasei			
22 adults	*VSL#3*	3 month	↑MDA, 4-HNE,S-NO	[67]
66 adults	*Bifidobacterium longum*	24 week	↓liver TGs, AST	[68]
	FOS			
52 adults	*L.casei, L. rhamnous, S. thermophilus, B. breve, L. acidophilus, B. longum, L. bulgaricus* and FOS	30 week	↓NF-κB, TNFα	[69]
50 adults	Synbiotic Protexin	28 week	↓FBS, TGs, ALT, AST, GGT,LDL, cholesterol	[70]

Abbreviations: *ALT* alanine aminotransferase, *LDL* low density lipoprotein, *AST* aspartate aminotransferase, *GGT* γ-glutamyltranspeptidase, *TNFα* tumor necrosis factor α, *NF-κB* nuclear factor –κB, *MDA* malondialdehyde, VSL#3 combination of *B. breve, B. infantis, L. casei, L. plantarum, L. acidophilus, L. delbrueckii ssp. bulgaricus, S. thermophiles, FBS* fasting blood sugar, *4-HNE* 4-hydroxynonenal, *S-NO* S-nitrothiols

common platforms employed are gas chromatography (GC) or high-performance liquid chromatography (HPLC) interfaced to a mass spectrometer, commonly referred to as GC-MS or HPLC-MS [6]. By examining the end-products of metabolism between two treatment groups of mice, for example, one can distinguish between the two groups based on their metabotype rather than on phenotype. This was highlighted in this chapter when it was discovered that two obese strains of mice with comparable BMI were metabolically very different from one another in that one had insulin resistance and the other did not. The measurements of BAs, lipids, cytokines and bacterial metabolites such as TMA and butyrate all make use of the metabolomic techniques.

Metabolomis is also a way to condense a large amount of data into a more workable format. For example, there are many functional redundancies among the microbiota in that more than one species is capable of producing butyrate. Therefore, instead of putting the focus on which of more than one thousand bacteria are present in any given patient, it may be more cogent to think in terms of whether the patient has a healthy gut based on the amount of beneficial bacterial metabolite he or she has. Analysis of metabolic endpoints allows one to look at a patient's situation in terms of a functional metabolome rather than the actual physical microbiome when assessing the health of his/her gut microbiota.

The metabolomic approach also has the capability of being able to handle large numbers of samples and to generate data from multiple biochemical pathways occurring simultaneously either at one time point or a series of time points. The clinician can then visualize a more complete picture of the functional status of his patient's health. A practical application was highlighted in this chapter with respect to choline deficiency. If the amount of bacterial choline metabolites increases over time in a patient, this may signal additional choline supplementation as a treatment to forestall development of NAFLD. FDA recommendations for daily choline intake may not be effective for everyone. Metabolomic techniques thus open up the possibility of a more "personalized" medical approach to someone's health. Ultimately the goals of this approach are: (1) to realize a distinct metabotype for the progression of any human pathological condition, (2) to discover which metabolites signify a risk for development of any future health problems. In the case of NAFLD, early, effective intervention and subsequent monitoring of both host and microbiota metabolites may prevent progression to more serious chronic liver disease or metabolic syndrome such as diabetes.

References

1. Than NN, Newsome PN. A concise review of non-alcoholic fatty liver disease. Atherosclerosis. 2015;239:192–202.
2. Machado M, Marques-Vidal P, Cortez-Pinto H. Hepatic histology in obese patients undergoing bariatric surgery. J Hepatol. 2006;45:600–6.
3. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 2011;34:274–85.
4. Attar BM, Van Thiel DH. Current concepts and management approaches in nonalcoholic fatty liver disease. Sci World J. 2013;2013:481893.
5. Gaggini M, Morelli M, Buzzigoli E, DeFronzo RA, Bugianesi E, Gastaldelli A. Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. Forum Nutr. 2013;5:1544–60.
6. He X, Ji G, Jia W, Li H. Gut microbiota and nonalcoholic fatty liver disease: insights on mechanism and application of metabolomics. Int J Mol Sci. 2016;17:300.
7. Marchesi JR, Adams DH, Fava F, Hermes GD, Hirschfield GM, Hold G, et al. The gut microbiota and host health: a new clinical frontier. Gut. 2016;65:330–9.
8. Le Roy T, Llopis M, Lepage P, Bruneau A, Rabot S, Bevilacqua C, et al. Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. Gut. 2013;62:1787–94.
9. Fearnside JF, Dumas ME, Rothwell AR, Wilder SP, Cloarec O, Toye A, et al. Phylometabonomic patterns of adaptation to high fat diet feeding in inbred mice. PLoS One. 2008;3:e1668.
10. Rabot S, Membrez M, Bruneau A, Gerard P, Harach T, Moser M, et al. Germ-free C57BL/6J mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism. FASEB J. 2010;24:4948–59.
11. Backhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-

induced obesity in germ-free mice. Proc Natl Acad Sci U S A. 2007;104:979–84.
12. Serino M, Luche E, Gres S, Baylac A, Berge M, Cenac C, et al. Metabolic adaptation to a high-fat diet is associated with a change in the gut microbiota. Gut. 2012;61:543–53.
13. Musso G, Gambino R, Cassader M. Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD). Prog Lipid Res. 2009;48:1–26.
14. Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature. 2012;482:179–85.
15. Wu X, Ma C, Han L, Nawaz M, Gao F, Zhang X, et al. Molecular characterisation of the faecal microbiota in patients with type II diabetes. Curr Microbiol. 2010;61:69–78.
16. Bechmann LP, Hannivoort RA, Gerken G, Hotamisligil GS, Trauner M, Canbay A. The interaction of hepatic lipid and glucose metabolism in liver diseases. J Hepatol. 2012;56:952–64.
17. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest. 2005;115:1343–51.
18. Liu HY, Han J, Cao SY, Hong T, Zhuo D, Shi J, et al. Hepatic autophagy is suppressed in the presence of insulin resistance and hyperinsulinemia: inhibition of FoxO1-dependent expression of key autophagy genes by insulin. J Biol Chem. 2009;284:31484–92.
19. Dentin R, Girard J, Postic C. Carbohydrate responsive element binding protein (ChREBP) and sterol regulatory element binding protein-1c (SREBP-1c): two key regulators of glucose metabolism and lipid synthesis in liver. Biochimie. 2005;87:81–6.
20. Denechaud PD, Dentin R, Girard J, Postic C. Role of ChREBP in hepatic steatosis and insulin resistance. FEBS Lett. 2008;582:68–73.
21. Berlanga A, Guiu-Jurado E, Porras JA, Auguet T. Molecular pathways in non-alcoholic fatty liver disease. Clin Exp Gastroenterol. 2014;7:221–39.
22. Li T, Chiang JY. Bile acid signaling in metabolic disease and drug therapy. Pharmacol Rev. 2014;66:948–83.
23. Zhang Y, Lee FY, Barrera G, Lee H, Vales C, Gonzalez FJ, et al. Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. Proc Natl Acad Sci U S A. 2006;103:1006–11.
24. Li Y, Jadhav K, Zhang Y. Bile acid receptors in non-alcoholic fatty liver disease. Biochem Pharmacol. 2013;86:1517–24.
25. Ip E, Farrell GC, Robertson G, Hall P, Kirsch R, Leclercq I. Central role of PPARalpha-dependent hepatic lipid turnover in dietary steatohepatitis in mice. Hepatology. 2003;38:123–32.
26. Kashireddy PV, Rao MS. Lack of peroxisome proliferator-activated receptor alpha in mice enhances methionine and choline deficient diet-induced steatohepatitis. Hepatol Res. 2004;30:104–10.
27. Ip E, Farrell G, Hall P, Robertson G, Leclercq I. Administration of the potent PPARalpha agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice. Hepatology. 2004;39:1286–96.
28. Tailleux A, Wouters K, Staels B. Roles of PPARs in NAFLD: potential therapeutic targets. Biochim Biophys Acta. 2012;1821:809–18.
29. Holland WL, Adams AC, Brozinick JT, Bui HH, Miyauchi Y, Kusminski CM, et al. An FGF21-adiponectin-ceramide axis controls energy expenditure and insulin action in mice. Cell Metab. 2013;17:790–7.
30. Ge X, Chen C, Hui X, Wang Y, Lam KS, Xu A. Fibroblast growth factor 21 induces glucose transporter-1 expression through activation of the serum response factor/Ets-like protein-1 in adipocytes. J Biol Chem. 2011;286:34533–41.
31. Claudel T, Inoue Y, Barbier O, Duran-Sandoval D, Kosykh V, Fruchart J, et al. Farnesoid X receptor agonists suppress hepatic apolipoprotein CIII expression. Gastroenterology. 2003;125:544–55.
32. Claudel T, Sturm E, Duez H, Torra IP, Sirvent A, Kosykh V, et al. Bile acid-activated nuclear receptor FXR suppresses apolipoprotein A-I transcription via a negative FXR response element. J Clin Invest. 2002;109:961–71.
33. Inagaki T, Dutchak P, Zhao G, Ding X, Gautron L, Parameswara V, et al. Endocrine regulation of the fasting response by PPARalpha-mediated induction of fibroblast growth factor 21. Cell Metab. 2007;5:415–25.
34. Jiang C, Xie C, Lv Y, Li J, Krausz KW, Shi J, et al. Intestine-selective farnesoid X receptor inhibition improves obesity-related metabolic dysfunction. Nat Commun. 2015;6:10166.
35. Gonzalez FJ, Jiang C, Patterson AD. An intestinal microbiota-Farnesoid X receptor Axis modulates metabolic disease. Gastroenterology. 2016;151:845–59.
36. Kir S, Beddow SA, Samuel VT, Miller P, Previs SF, Suino-Powell K, et al. FGF19 as a postprandial, insulin-independent activator of hepatic protein and glycogen synthesis. Science. 2011;331:1621–4.
37. Copple BL, Li T. Pharmacology of bile acid receptors: evolution of bile acids from simple detergents to complex signaling molecules. Pharmacol Res. 2016;104:9–21.
38. Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, et al. Bile acids: natural ligands for an orphan nuclear receptor. Science. 1999;284:1365–8.
39. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, et al. Identification of a nuclear receptor for bile acids. Science. 1999;284:1362–5.
40. Kawamata Y, Fujii R, Hosoya M, Harada M, Yoshida H, Miwa M, et al. A G protein-coupled receptor responsive to bile acids. J Biol Chem. 2003;278:9435–40.

41. Maruyama T, Miyamoto Y, Nakamura T, Tamai Y, Okada H, Sugiyama E, et al. Identification of membrane-type receptor for bile acids (M-BAR). Biochem Biophys Res Commun. 2002;298:714–9.
42. Thomas C, Pellicciari R, Pruzanski M, Auwerx J, Schoonjans K. Targeting bile-acid signalling for metabolic diseases. Nat Rev Drug Discov. 2008;7:678–93.
43. Fujisaka S, Ussar S, Clish C, Devkota S, Dreyfuss JM, Sakaguchi M, et al. Antibiotic effects on gut microbiota and metabolism are host dependent. J Clin Invest. 2016;126:4430–43.
44. Moschen AR, Kaser S, Tilg H. Non-alcoholic steatohepatitis: a microbiota-driven disease. Trends Endocrinol Metab. 2013;24:537–45.
45. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A. 2005;102:11070–5.
46. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006;444:1022–3.
47. Musso G, Gambino R, Cassader M. Obesity, diabetes, and gut microbiota: the hygiene hypothesis expanded? Diabetes Care. 2010;33:2277–84.
48. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A. 2004;101:15718–23.
49. Aronsson L, Huang Y, Parini P, Korach-Andre M, Hakansson J, Gustafsson JA, et al. Decreased fat storage by lactobacillus paracasei is associated with increased levels of angiopoietin-like 4 protein (ANGPTL4). PLoS One. 2010;5:e13087.
50. Alex S, Lichtenstein L, Dijk W, Mensink RP, Tan NS, Kersten S. ANGPTL4 is produced by enteroendocrine cells in the human intestinal tract. Histochem Cell Biol. 2014;141:383–91.
51. Alex S, Lange K, Amolo T, Grinstead JS, Haakonsson AK, Szalowska E, et al. Short-chain fatty acids stimulate angiopoietin-like 4 synthesis in human colon adenocarcinoma cells by activating peroxisome proliferator-activated receptor. gamma Mol Cell Biol. 2013;33:1303–16.
52. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, et al. Host-gut microbiota metabolic interactions. Science. 2012;336:1262–7.
53. Buchman AL, Dubin MD, Moukarzel AA, Jenden DJ, Roch M, Rice KM, et al. Choline deficiency: a cause of hepatic steatosis during parenteral nutrition that can be reversed with intravenous choline supplementation. Hepatology. 1995;22:1399–403.
54. Dumas ME, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, et al. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. Proc Natl Acad Sci U S A. 2006;103:12511–6.
55. Sherriff JL, O'Sullivan TA, Properzi C, Oddo JL, Adams LA. Choline, its potential role in nonalcoholic fatty liver disease, and the case for human and bacterial genes. Adv Nutr. 2016;7:5–13.
56. Krahmer N, Guo Y, Wilfling F, Hilger M, Lingrell S, Heger K, et al. Phosphatidylcholine synthesis for lipid droplet expansion is mediated by localized activation of CTP: phosphocholine cytidylyltransferase. Cell Metab. 2011;14:504–15.
57. Geiger O, Lopez-Lara IM, Sohlenkamp C. Phosphatidylcholine biosynthesis and function in bacteria. Biochim Biophys Acta. 2013;1831:503–13.
58. Romano KA, Vivas EI, Amador-Noguez D, Rey FE. Intestinal microbiota composition modulates choline bioavailability from diet and accumulation of the proatherogenic metabolite trimethylamine-N-oxide. MBio. 2015;6:e02481.
59. Spencer MD, Hamp TJ, Reid RW, Fischer LM, Zeisel SH, Fodor AA. Association between composition of the human gastrointestinal microbiome and development of fatty liver with choline deficiency. Gastroenterology. 2011;140:976–86.
60. Russell WR, Hoyles L, Flint HJ, Dumas ME. Colonic bacterial metabolites and human health. Curr Opin Microbiol. 2013;16:246–54.
61. Vajro P, Mandato C, Licenziati MR, Franzese A, Vitale DF, Lenta S, et al. Effects of lactobacillus rhamnosus strain GG in pediatric obesity-related liver disease. J Pediatr Gastroenterol Nutr. 2011;52:740–3.
62. Aller R, De Luis DA, Izaola O, Conde R, Gonzalez Sagrado M, Primo D, et al. Effect of a probiotic on liver aminotransferases in nonalcoholic fatty liver disease patients: a double blind randomized clinical trial. Eur Rev Med Pharmacol Sci. 2011;15:1090–5.
63. Nabavi S, Rafraf M, Somi MH, Homayouni-Rad A, Asghari-Jafarabadi M. Effects of probiotic yogurt consumption on metabolic factors in individuals with nonalcoholic fatty liver disease. J Dairy Sci. 2014;97:7386–93.
64. Alisi A, Bedogni G, Baviera G, Giorgio V, Porro E, Paris C, et al. Randomised clinical trial: the beneficial effects of VSL#3 in obese children with non-alcoholic steatohepatitis. Aliment Pharmacol Ther. 2014;39:1276–85.
65. Xu RY, Wan YP, Fang QY, Lu W, Cai W. Supplementation with probiotics modifies gut flora and attenuates liver fat accumulation in rat non-alcoholic fatty liver disease model. J Clin Biochem Nutr. 2012;50:72–7.
66. Wang J, Tang H, Zhang C, Zhao Y, Derrien M, Rocher E, et al. Modulation of gut microbiota during probiotic-mediated attenuation of metabolic syndrome in high fat diet-fed mice. ISME J. 2015;9:1–15.
67. Loguercio C, Federico A, Tuccillo C, Terracciano F, D'Auria MV, De Simone C, et al. Beneficial effects of a probiotic VSL#3 on parameters of liver dysfunction in chronic liver diseases. J Clin Gastroenterol. 2005;39:540–3.
68. Malaguarnera M, Vacante M, Antic T, Giordano M, Chisari G, Acquaviva R, et al. Bifidobacterium longum with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. Dig Dis Sci. 2012;57:545–53.

69. Eslamparast T, Poustchi H, Zamani F, Sharafkhah M, Malekzadeh R, Hekmatdoost A. Synbiotic supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study. Am J Clin Nutr. 2014;99:535–42.

70. Mofidi F, Poustchi H, Yari Z, Nourinayyer B, Merat S, Sharafkhah M, et al. Synbiotic supplementation in lean patients with non-alcoholic fatty liver disease: a pilot, randomised, double-blind, placebo-controlled, clinical trial. Br J Nutr. 2017;117:662–8.