Nutrition Research Reviews (2022), 35, 28–38 doi:10.1017/S0954422421000056

Effects of betaine on non-alcoholic liver disease

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Abstract

The increasing prevalence of non-alcoholic fatty liver disease (NAFLD) poses a growing challenge in terms of its prevention and treatment. The 'multiple hits' hypothesis of multiple insults, such as dietary fat intake, *de novo* lipogenesis, insulin resistance, oxidative stress, mitochondrial dysfunction, gut dysbiosis and hepatic inflammation, can provide a more accurate explanation of the pathogenesis of NAFLD. Betaine plays important roles in regulating the genes associated with NAFLD through anti-inflammatory effects, increased free fatty oxidation, anti-lipogenic effects and improved insulin resistance and mitochondrial function; however, the mechanism of betaine remains elusive.

Keywords: **Betaine: NAFLD: AMPK**

(Received 2 November 2020; revised 5 March 2021; accepted 25 March 2021; accepted manuscript published online 5 April 2021)

1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is a very common public health problem, with a recent study reporting that the global prevalence of NAFLD is estimated at 24%[1]. NAFLD is a major liver manifestation of metabolic syndrome and is characterised by excessive accumulation of liver triacylglycerol[2]. Additionally, NAFLD is an important risk factor for many metabolic diseases, and it commonly precedes more serious conditions, including non-alcoholic steatohepatitis (NASH), cirrhosis, hepatocellular carcinoma, type 2 diabetes and cardiovascular disease[2–4]. At present, the pathogenesis of NAFLD is not clear, and the most commonly cited mechanism of NAFLD development is the 'multiple hits' hypothesis[5,6]. Increased levels of serum fatty acids due to diet, environment and obesity lead to adipose tissue fat breakdown and increased dietary fat intake as well as the development of insulin resistance, oxidative stress, mitochondrial dysfunction, gut dysbiosis, impaired hepatic glucose metabolism and inflammation. All of these risk factors may contribute to the pathogenesis of NAFLD (Fig. 1)[6–13]. However, there are still no specific drugs for the treatment of NAFLD.

Betaine (*N*,*N*,*N*-trimethylmethanaminium) is found in many common foods, such as shrimp, shellfish, wheat, beets, whole grains and spinach. Betaine is an obligatory intermediate in the catabolism of choline via oxidation mediated by choline dehydrogenase[14] and choline oxidase[15]. Betaine is formed from glycine and three methyl groups and serves as an effective methyl donor for the methionine homocysteine cycle[13]. Betaine has proved effective in animal models for reducing hepatic lipid accumulation[12,16] and improving insulin resistance, glucose homeostasis and hepatic steatosis[12,17,18]. Male C57BL/6 mice that received betaine (1·5% w/v in drinking water) for 6 weeks exhibited alleviation of oxidative stress, inflammation, apoptosis and autophagy in fatty liver disease[13]. Beneficial effects of betaine supplementation for treating NAFLD have also been demonstrated in NAFLD patients[19]. Another study reported that even though betaine does not improve hepatic steatosis, it may protect against worsening steatosis in NASH patients[11]. However, the mechanism by which betaine improves NAFLD has not been clarified. This review intends to explore the possible mechanisms of exogenous involvement of betaine in NAFLD, including anti-inflammatory effects, alleviation of oxidative stress, increased fatty acid oxidation, improved insulin resistance and anti-lipogenic effects.

2 Regulation of lipid metabolism

A high-fat diet causes metabolic disorders by disturbing lipogenesis and lipolysis, often leading to NAFLD. AMP-activated kinase (AMPK) is a key energy sensor that ameliorates NAFLD by increasing fatty acid utilisation and inhibiting hepatic lipid

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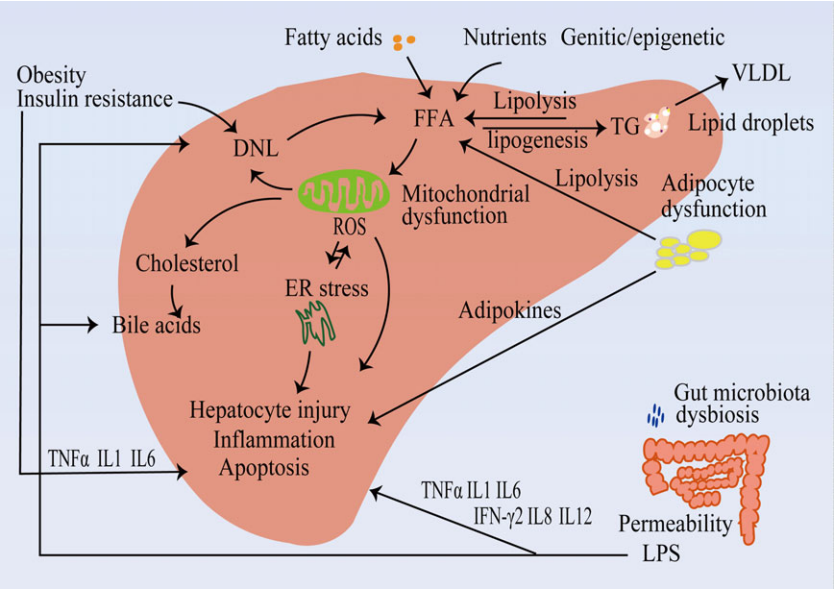

Fig. 1. Schematic of non-alcoholic fatty liver disease. Dietary, genetic and environmental factors increase NEFA uptake, insulin resistance, lipogenesis, adipocyte dysfunction, gut dysbiosis, oxidative stress and endoplasmic reticulum (ER) stress, all of which lead to hepatocyte injury and hepatic inflammation.

synthesis[20–22]. Inhibition of AMPK increases the risk of obesity and diabetes as well as NAFLD[23,24]. High-fat diet-induced obesity-mediated NAFLD results from increased liver NEFA concentrations and triacylglycerol accumulation accompanied by inhibition of betaine homocysteine methyltransferase (BHMT) and decrease in *S*-adenosylmethionine (SAM) levels. Both BHMT and SAM down-regulate *de novo* lipogenesis by enhancing the expression of AMPK; therefore, methyl donor supplementation prevents the progression of hepatic steatosis associated with decreased hepatic triacylglycerol accumulation by increasing the activity of AMPK in obesity-mediated NAFLD mice[24]. One study also reported that a high-fat diet disturbs the homeostasis of hepatic methionine metabolism, leading to fatty liver; betaine, as a methyl donor, prevents fatty liver and hepatic injury by increasing SAM levels and preventing changes in BHMT mRNA expression in rats[25]. Additionally, betaine stimulates energy production and fatty acid synthesis by phosphorylation of AMPK in C2C12 myotubes[26]. These studies indicate that betaine supplementation elevates SAM and BHMT levels via one-carbon metabolites and activates AMPK expression to reduce hepatic NEFAs, thus ameliorating NAFLD.

Although fatty acid and cholesterol synthesis is dependent on acetyl-CoA, the biosynthetic pathways are regulated by distinct sterol regulatory element binding proteins (SREBP). AMPK is the upstream protein of SREBP-1c[27] and carbohydrate response element-binding protein (ChREBP)[17]. Both SREBP-1c and ChREBP are key transcription factors in the process of lipogenesis. Betaine decreases fatty acid synthesis and protects against hepatic steatosis by inhibiting the expression of SREBP-1c[28], mainly by activating AMPK, which also inhibits fatty acid synthase (FASN), stearoyl-CoA desaturase (SCD)[21], ChREBP[17] and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) expression in the liver[29]. Acetyl-CoA carboxylase (ACC) catalyses acetyl-CoA conversion to malonyl-CoA and is the first committed step in *de novo* lipogenesis. Therefore, the inhibitory effects of betaine on fatty acid synthesis are mediated by phosphorylation of AMPK[26], which phosphorylates ACC[30], leading to a decrease in the production of malonyl-CoA. Malonyl-CoA is converted to mevalonate by reduction to mevalonic acid by HMGCR, a key rate-limiting enzyme in cholesterol biosynthesis. Betaine also inhibits HMGCR to decrease the synthesis of cholesterol via AMPK phosphorylation[26].

Hepatic steatosis in high-fructose diet-induced NAFLD rats is related to overexpression of liver X receptor (LXR)[31], which not only significantly increases intracellular triacylglycerol content by increasing SREBP-1c, FAS and SCD1 expression in HepG2 cells[32] but also increases cholesterol levels by up-regulating the expression of CYP7A1[33]. Betaine plays a protective role in decreasing triacylglycerol mainly by depressing the LXRα/nSREBP-1c pathway[28], which is inhibited by AMPK activation[34]. Therefore, the beneficial effect of betaine in hepatic lipogenesis and cholesterol synthesis may be associated with activation of AMPK, which inhibits LXR expression, as well as SREBP-1c and its target genes.

De novo lipogenesis is an integrated process that uses acetyl-CoA to synthesise fatty acids, which are then desaturated and esterified to form triacylglycerol (TG). Liver TG content was associated with VLDL-TG secretion rates, and is exported from hepatocytes in the form of VLDL-TG. Apolipoprotein B100 (apoB100) is lipidated in a process catalyzed by the enzyme microsomal triglyceride transfer protein (MTTP), and is required for VLDL export, suggesting MTTP is important component in maintaining hepatic lipid homeostasis[35]. In a recent study, C57BL/6J mice were fed either a high-fat diet or a control diet and received either drinking water treated with 1% betaine or control untreated drinking water 4–6 weeks before timed mating. Throughout gestation, the livers of fetal mice supplemented with betaine had enhanced mRNA expression of MTTP, which promotes VLDL synthesis and secretion, therefore reducing liver triacylglycerol content[36]. Additionally, previous studies have

https://doi.org/10.1017/S0954422421000056 Published online by Cambridge University Press

reported that a high-fat diet down-regulates MTTP mRNA expression and that betaine increases lipid transport by microsomal MTTP expression by regulating aberrant DNA methylation[37].

3 Regulation of mitochondrial function

Mitochondria play an important role in regulating hepatic lipid metabolism, and mitochondrial β-oxidation maintains the homeostasis of bioactive lipids[38]. The mitochondrial respiratory chain is the main subcellular source of reactive oxygen species (ROS), which can damage mitochondrial proteins, lipids and mitochondrial DNA[39]. Excessive intake of NEFAs increases bioactive lipid accumulation in mitochondria, leading to the formation of ROS. Increasing evidence has reported that mitophagy or autophagy blockade leads to the accumulation of damaged ROS-generating mitochondria. This in turn leads to mitochondrial dysfunction and endoplasmic reticulum (ER) stress[40], increases mitochondrial biogenesis and inhibits ER stress-mediated ROS, leading to the promotion of cell survival during ER stress in eukaryotic cells[41]. Moreover, factors such as oxidative stress, alterations in mitochondrial structure and functional mitochondrial dysfunction are particularly susceptible to ROS attack and play an important role in the physiopathology of NAFLD[39]. These results suggest that the regulation of mitochondrial function is a fundamental mechanism for hepatoprotection. Interestingly, betaine could play a primary role in the hepatoprotective mechanisms of mitochondria through its antioxidative and mitochondria-regulating properties[42,43]; however, the mechanism by which betaine regulates mitochondrial function is still unclear.

Mitochondrial fusion combines two mitochondria into one mitochondrion. Healthy mitochondria eventually fuse to form a healthy mitochondrial pool[44]. The reduced signalling for mitochondrial fusion detected in NAFLD animal models underlines its potential role in the pathology of NAFLD[45]. Mitochondrial fission is the division of one mitochondrion into two mitochondria and is mediated by the interaction of cytoplasmic mitochondrial fission-related protein 1 (Drp1) and other mitochondrial fission proteins, such as mitochondrial fission factor (MFF)[46]. Moreover, mitochondrial fusion and fission-related mechanisms depend on the available energy[47] and the response to metabolic stressors. Ultrastructural mitochondrial lesions, altered mitochondrial dynamics, decreased activity of respiratory chain complexes and impaired ability to synthesise adenosine triphosphate are observed in liver tissues from patients with NAFLD[44]. Cells treated with betaine exhibit enhanced mitochondrial and cellular respiration, mitochondrial potential, and ATP production, thereby increasing energy expenditure[48]. A kinase anchoring protein 1 (AKAP1) is a regulator of mitochondrial fusion that is phosphorylated by AMPK, leading to increased fatty acid oxidation[49]. AKAP1 plays an important role in anchoring PKA to the cytoplasmic face of the mitochondrial outer membrane[50] and maximises ATP production. In mitochondria, PKA phosphorylates DRP1, leading to mitochondrial fission[49]. Moreover, AMPK phosphorylates mitochondrial fission factor (MFF), which is essential for initiating mitochondrial fission,[51] and recruits DRP1 to the outer mitochondrial membrane. Therefore, the mechanism by which betaine indirectly regulates mitochondrial function to control the rates of fatty acid oxidation may involve the phosphorylation of AMPK, which regulates mitochondrial fusion and fission. Future studies investigating the mechanism by which betaine increases fatty acid oxidation may involve enhancing mitochondrial fusion and fission through pathways involving AMPK, AKAP1 activity and DRP1 phosphorylation.

Betaine increases mitochondrial membrane potential and cellular respiration, and increases in mitochondrial biogenesis must be balanced by the removal of damaged mitochondria[52], which is initiated by mitochondrial fission. Mitochondria undergo fusion or fission to maintain mitochondrial energy production and biogenesis to respond to changes in nutrient supplementation[53]. Changes in both the biogenesis and/or mitophagy of mitochondria increase mitochondrial content and cellular energy levels[48], which adapt to the metabolic needs of cells through a balance between fusion and fission events. A key pathway regulating mitochondrial biogenesis is the master transcription factor and transcriptional coactivator peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α) and its target genes nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (TFAM). This pathway is required for mitochondrial DNA (mtDNA) transcription and has been linked to metabolic changes leading to increased mitochondrial biogenesis[54]. The significant up-regulation of the mtDNA copy number by betaine may be due to up-regulation of TFAM[55], an activator of mtDNA replication and transcription[56]. Additionally, mitochondrial biogenesis provides cellular chemical energy in the form of ATP, which stimulates AMPK activation and further increases GLUT-4 expression and regulation of fatty acid oxidation via ACC phosphorylation[57]. Therefore, betaine increases mitochondrial content to alter mitochondrial biogenesis, possibly via the phosphorylation of ACC and AMPK, which up-regulate the network of transcription factors PGC1α, NRF-1, TFAM and Sirt-1[26]. In animal experiments, betaine improves fatty acid oxidation, maintains energy balance and reduces liver fat by up-regulating fibroblast growth factor 21 (FGF21)[18], which also significantly increases the number of mitochondria and the expression of mitochondrial biogenesis-related genes, including PGC1α, NRF1 and TFAM[58].

Autophagy plays an important role in degrading lipids in damaged cells and can not only regulate lipid metabolism and insulin resistance in mice with diet-induced obesity[59] but also protect hepatocytes from injury and cell death[60]. Autophagic flux is impaired in NAFLD patients and NAFLD mouse models, as well as in human hepatocytes with increased ER stress[61]. Betaine indirectly phosphorylates unc-51-like autophagy activating kinase 1 (ULK1)[13], which plays a key role in the initiation stage of autophagy, promotes autophagosome-lysosome fusion[62], promotes mitophagy[63] and responds to mitochondrial fission. Betaine also phosphorylates autophagy activators such as autophagy-related protein 4/5 (ATG4/5), which increases the number of autophagic vesicles and degradation of the autophagic target sequestosome 1/p62 in the livers of NAFLD mice. Betaine also regulates mitophagy through inhibition of mechanistic target of rapamycin complex 1 (mTORC1), which phosphorylates and inhibits ULK1 and its activator

https://doi.org/10.1017/S0954422421000056 Published online by Cambridge University Press

Akt[13]. These latter proteins are regulated by AMPK phosphorylation[59]. Moreover, AMPK phosphorylation regulates mitochondrial transcription by promoting the import of FOXO3 into mitochondria[64]. FOXO3 promotes the translocation of FOXO1 from the nucleus to the cytoplasm, resulting in an increase in FOXO1-induced autophagy via activation of the AKT1 signalling pathway without increasing the expression of FOXO1 at the protein level[65]. Additionally, betaine plays an important role in antiapoptotic pathways and autophagy by increasing the expression of the antiapoptotic protein Bcl-2 and inhibiting the expression of the proapoptotic protein Bax[13], which impairs mitochondrial membrane integrity. Thus, betaine promotes autophagy by activating AMPK, ULK1, and phosphorylated FOXO1 and FOXO3 and by antagonising mTORC1. However, the molecular mechanism by which betaine ameliorates NAFLD by regulating autophagy in hepatocytes requires further study.

4 Enhancement of fatty acid oxidation through epigenetic function of betaine

Mitochondrial dysfunction increases DNA methylation, which can cause the development of metabolic syndrome in obesity[66] and the progression of NAFLD[40,67]. Therefore, the regulation of DNA methylation could prevent NAFLD by attenuating mitochondrial dysfunction[68]. DNA cytosine-5-methyltransferases (DNMTs) mediate DNA methylation by catalysing the transfer of the methyl group from SAM to cytosine during DNA replication[69]. Betaine supplementation significantly elevates DNMT at the mRNA level in animals[70]. The epigenetic effects of SAM via DNA methylation are driven by SAM precursors such as betaine and Met. Betaine acts as a methyl donor for DNA methylation in epigenetic regulation and provides a methyl group to participate in one-carbon metabolism to promote SAM formation[25]. A recent study reported that access to 1% betaine-supplemented water for a total of 8 weeks alters the methylation status of specific gene promoters in mice, leading to persistent changes in gene expression that could be beneficial for the treatment of obesity and type 2 diabetes owing to their valuable metabolic effects on lipolysis, the TCA cycle and mitochondrial oxidative demethylation[15]. However, the mechanism by which betaine methylation regulates mitochondrial oxidative demethylation to ameliorate NAFLD remains unclear.

Fat mass and obesity-associated (FTO) protein is a demethylase that plays a critical role in demethylation. FTO overexpression enhances lipogenesis and ROS production[71] and increases liver damage in NAFLD patients[72]. Interestingly, FTO-dependent demethylation inhibits the methylation of m6A[73], the most prevalent mRNA modification, thereby reducing lipid accumulation and energy metabolism[74]. Moreover, FTO-dependent m6A demethylation is associated with AMPK-related signalling pathways, which reduce lipid accumulation in skeletal muscle cells by regulating FTO expression and FTO-dependent m6A demethylation[73]. Therefore, AMPK plays an important role in regulating lipid accumulation by inhibiting FTO expression and m6A methylation, providing new insights into the molecular regulation of lipid metabolism. In fact, a high-fat diet induces hepatic steatosis by reducing the expression of lipolysis genes such as hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL), and these changes are accompanied by increased hepatic FTO and reduced m6A levels. Betaine plays a hepatoprotective role in preventing these changes by rectifying the m6A mRNA hypomethylation state and down-regulating FTO expression in mouse liver[75]. Additionally, betaine activates AMPKα1, which is beneficial for m6A methylation, and reduces the expression of FTO in high-fat diet-induced wild-type mice[76]. In adipose tissue, the up-regulation of ATGL and HSL expression significantly increases circulating NEFAs, leading to lipid accumulation in the liver; betaine reverses these changes in gene expression by modifying DNA methylation at the promoter in progeny rats[77].

Peroxisome proliferator activated receptor α (PPARα), a transcription factor, regulates the transcription of numerous genes encoding enzymes in fatty acid oxidation and transportation[78] and is involved in the regulation of liver lipid metabolism[79]. Betaine enhances mitochondrial β-oxidation by decreasing hypermethylation of PPARα[78] and modifications of CpG methylation at the gene promoter of the mitochondrial fatty acid oxidation-related gene carnitine palmitoyl transferase 1α (CPT1α)[55], which facilitates fatty acyl-CoA entry into mitochondria. CPT1α is inhibited by ACC production by malonyl-CoA[80-89] and is regulated by PPARα[81]. In the context of DNA methylation, Met is metabolised into SAM by methionine adenosyltransferase in the liver, and the synthesis of SAM serves to enhance DNA methylation, leading to greater expression of PPARα and its target gene FGF21 in lipid metabolism[82]. Betaine plays a role in DNA methylation by increasing the levels of BHMT and SAM, which participate in the functions of mitochondrial oxidative enzymes such as PPARα and ACC. These enzymes are localised in the mitochondrial matrix and have valuable metabolic effects on lipolysis, the TCA cycle and mitochondrial oxidative phosphorylation[15]. Additionally, AMPK increases the expression of PPARα, which in turn enhances the regulatory effect of AMPK on the expression of angiopoietin-like 8 (ANGPTL8), a liver-derived secretory protein that elevates serum triacylglycerol[34].

These studies clarified that the methylation function of betaine plays an essential role in lipid metabolism and links the epigenetic modification of DNA and RNA methylation with lipid accumulation. Future studies should clarify the mechanism by which betaine exerts different regulatory effects on lipid metabolism in different tissues, thereby providing new targets for the regulation of hepatic lipid metabolism and NAFLD.

5 Reduction of insulin resistance

The metabolic processes gluconeogenesis, glycolysis and glycogenolysis coordinately regulate liver glucose, leading to glucose homeostasis. The increase in lipid accumulation induced by a high-fat diet disturbs glucose homeostasis, leading to obesity, which is a major driver of insulin resistance and NAFLD[12]. Interestingly, insulin resistance is closely associated with NAFLD[83]. In fact, betaine supplementation improves insulin sensitivity and reduces insulin resistance to maintain glucose

https://doi.org/10.1017/S0954422421000056 Published online by Cambridge University Press

homeostasis[12,18]; however, the mechanism by which betaine reduces insulin resistance needs to be elucidated. Recent studies have reported that *de novo* lipogenesis and oxidative stress are often related to the lack of insulin sensitivity in the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signalling pathway[84,85], which also plays an important role in maintaining glucose homeostasis and alleviating insulin resistance[86,87]. Interestingly, one study reported that the observed beneficial effects of betaine in methionine-choline-deficient diet-induced NAFLD coincide with increased hepatic phosphorylation of Akt[13]. In the insulin/Akt signalling pathway, Akt-dependent phosphorylation inactivates the expression of FOXO1[87]. This inactivation not only controls hepatic glucose production by stimulating the induction of glucose-6-phosphatase (G6PC) and the repression of glucokinase (Gck)[88] but also notably increases lipogenesis and decreases fatty acid oxidation by up-regulating PPARγ and its target genes FAS and ACC expression[89]. It also positively regulates the transcription of CYP7A1, which regulates cholesterol or bile acid metabolism in the liver, thus linking the carbohydrate and cholesterol metabolic pathways[87]. These studies suggest that FOXO1 may induce obesity, insulin resistance and metabolism disorders[90]. Moreover, AMPK inhibits insulin and AMPK signalling pathways and increases glucose uptake by phosphorylating insulin receptor substrate-1 (IRS), which increases PI3K/AKT phosphorylation[91]. Betaine may phosphorylate IRS-1 to inactivate FOXO1 expression through phosphorylation of PI3K/AKT[83]. Therefore, betaine improves NAFLD by normalising insulin signalling through reduced gluconeogenesis, increased glycogen synthesis and improved hepatic lipid metabolism through the AMPK pathway.

6 Inhibition of liver inflammation by betaine

Lipid metabolism disorders increase the expression of cytochrome P450 2E1 (CYP2E1), which promotes the production of ROS and mitochondrial oxidative stress[92]. Excessive ROS generation leads to oxidative stress in extrahepatic cells[93,94]. Moreover, excess ROS plays an inhibitory role in AMPK activation[23], and scavenging of ROS by the GSH antioxidant system can prevent AMPK inactivation in mice fed a high-fat diet[94]. Betaine significantly reduces excess ROS and increases the levels of GSH and glutathione peroxidase to improve the antioxidative stress ability of the liver[13]. In blunt snout bream fed a high-fat diet, 1·2% betaine supplementation significantly improves antioxidant defences by increasing superoxide dismutase, catalase and GSH levels and reverses the increase in malondialdehyde levels[42]. However, betaine supplementation decreases hepatic triacylglycerol accumulation by increasing CYP2E1 expression in ApoE−/− mice[78]. Therefore, further research is needed to clarify these conflicting effects of betaine treatment.

ROS also induce mitochondrial dysfunction by activating the expression of the nucleotide-binding domain leucine-rich-containing family pyrin domain-containing-3 (NLRP3) inflammasome[95], which is triggered by metabolic dysregulation[45]. Additionally, ROS trigger the inflammatory pathways of nuclear factor kappa B (NF-κB), leading to liver inflammation[96], which is often mediated by chemokines, including IL1 and IL6. Activation of NF-κB leads to the synthesis of inflammatory mediators, stimulates ROS synthesis, deteriorates oxidative stress[97] and accelerates the development of NAFLD[98,99]. Therefore, inhibition of ROS production and NF-κB expression significantly reverses hepatic inflammation and NAFLD. Betaine enhances the removal of ROS by depressing the expression of the NLRP3 inflammasome via inactivation of FOXO1[83]. Betaine also inhibits the hepatic NF-κB/NLRP3 inflammasome activation-mediated inflammation signalling pathway in fructose-fed NAFLD rats[100]. Betaine suppresses NF-κB and IL-1 expression by decreasing the expression of mitogen-activated protein kinases (MAPKs) and IκB/IKK and increases the liver expression of the anti-inflammatory cytokine interleukin-10 (IL10) in methionine-choline-deficient diet-induced NAFLD mice[13]. Additionally, inhibition of the expression of the chemokines IL1, IL6 and IκB through the AMPK/NF-κB signalling pathway is considered a target for inflammatory diseases[101]. Therefore, the anti-inflammatory mechanism of betaine may be mediated by the GSH/AMPK/NF-κB signalling pathways.

7 Regulation of gut dysfunction

The gut microbiome is a functional organ that maintains intestinal homeostasis[102]. There is growing evidence of a close correlation between the gut microbiome and NAFLD[104]. Intestinal injury and increased intestinal permeability accelerate the pathogenesis of NAFLD[105]. Recent studies have shown that a high-fat diet changes bile acid homeostasis, leading to gut microbiota dysbiosis[106-108], which increases hepatic injury, inflammation and NAFLD by influencing lipid metabolism[109,110]. Interestingly, bran-enriched diets, which contain abundant betaine, increase the relative abundances of *Akkermansia*, *Bifidobacterium*, *Coriobacteriaceae*, *Lactobacillus* and *Ruminococcus*, many of which are beneficial for host health[111]. Betaine also significantly improves the microbial community in the gut by reducing the abundances of *Coriobacteriaceae*, *Lachnospiraceae*, *Enterorhabdus* and *Coriobacteriales* and markedly enriching the taxa *Bacteroidaceae*, *Bacteroides*, *Parabacteroides* and *Prevotella* in a model group[112]. Moreover, betaine may have beneficial effects via its osmoprotective role[113] in osmotic regulation and antioxidant activity in human enterocytes. Betaine decreases intestinal injury and intestinal permeability, which limits the entry of bacterial endotoxins into systemic circulation[114]. These results suggest that betaine plays a protective role in reducing intestinal cell damage and inflammation to restore gut microbiota homeostasis. However, the mechanism of betaine in the regulation of gut microbiota homeostasis needs to be further studied.

Gut microbial-derived products, many of which are produced by bacterial fermentation, primarily arrive at the liver through the portal circulation[115]. Lipopolysaccharide (LPS), a component of the Gram-negative bacterial cell membrane and the active component of endotoxin, impairs intestinal permeability[116], and a damaged intestinal mucosal barrier lead to gut dysbiosis.

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https://doi.org/10.1017/S0954422421000056 Published online by Cambridge University Press

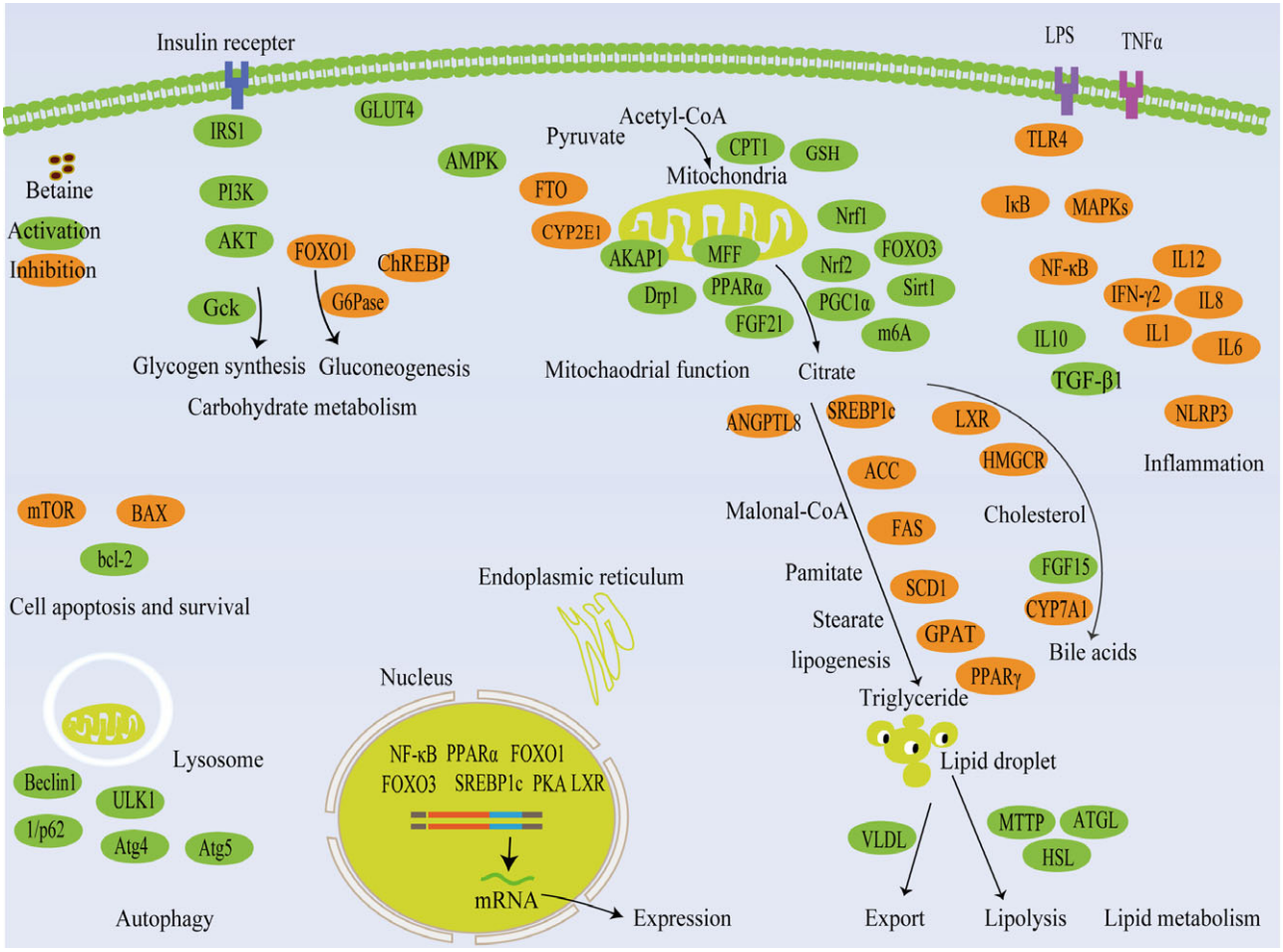

Fig. 2. Schematic of the improvement of non-alcoholic fatty liver disease by betaine. (1) Betaine decreases insulin resistance to reduce *de novo* lipid synthesis and hepatic glucose output. (2) Betaine alters gut dysbiosis to reduce lipid synthesis. (3) Betaine increases fatty acid oxidation and decreases lipid synthesis and accumulation. (4) Betaine reverses intestinal microbiome disorder and inhibits the activation of inflammasomes and pro-inflammatory cytokines. (5) Betaine increases hepatocyte autophagy to decrease lipid accumulation.

Negative microbial metabolites interact with Toll-like receptor 4 (TLR4)[112], a membrane receptor of LPS, triggering an essential inflammatory cascade involving MAPKs and the NF-κB pathway in Kupffer cells[117]. NF-κB induces the transcription of numerous pro-inflammatory cytokines such as pro-inflammatory tumour necrosis factor-α (TNF-α), which regulates lipid metabolism by impairing insulin signalling by inhibiting IRS-1[118]. IL-1β regulates lipid metabolism by suppressing PPARα, resulting in hepatic triacylglycerol accumulation[119]. Additionally, activation of TLR4 triggers the production of pro-inflammatory cytokines, including TNF-α, interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin (IL-12) and interferon gamma (IFN-γ)[105,120,121]. Betaine improves intestinal injury to prevent LPS translocation to systemic circulation[114] and decreases TLR4 and NF-κB expression and histological scores for steatosis, inflammation and necrosis by inhibiting TLR4 signalling pathways in high-fat diet-induced NAFLD rats[122]. Betaine effectively improves intestinal injury in acute liver failure mice by inhibiting the TLR4/MyD88 signalling pathway, improving the intestinal mucosal barrier and maintaining the gut microbiota composition[123]. The appropriate betaine supplementation level for on-growing grass carp (body weight 210–776 g) is estimated to be 4·28 to 4·51 g/kg diet; betaine boosts the growth performance and enhances the immune function of on-growing grass carp by down-regulating intestinal TNF-α, IL-1β, IFN-γ2, IL-6 and IL-8 mRNA expression partly mediated by the [IKKβ, γ/IκBα/NF-κBp65,c-Rel] signalling pathway. In addition, betaine up-regulates the mRNA expression of the intestinal anti-inflammatory cytokines transforming growth factor TGF-β1 and IL-10, partly mediated by the target of rapamycin (TOR) signalling pathway[124]. These studies suggest that the inhibition of TLR4/NF-κB signalling pathways warrants further study to elucidate the effect of betaine on gut microbiota modulation.

8 Conclusion

This paper reviews the recent literature on the regulation of betaine in NAFLD. Betaine improves NAFLD mainly by enhancing the lipid output of the liver, reducing *de novo* lipid synthesis,

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repairing mitochondrial dysfunction to enhance fatty acid oxidation, reducing inflammatory factors to inhibit hepatic inflammation, reducing insulin resistance and regulating gut dysbiosis (Fig. 2). Drug intervention is one of the therapeutic options for NAFLD. Although betaine has been shown to improve NAFLD in various ways, the pathogenesis of NAFLD is complex, and there is no specific drug to treat NAFLD. In a cross-sectional study, a significant inverse association was observed between the plasma betaine concentration and the severity of NAFLD in 1628 community-based participants but not in clinical patients[123]. A randomised placebo-control study of 55 NASH patients who received either oral betaine 20 g daily or placebo for 12 months showed that betaine did not improve hepatic steatosis but may protect against worsening steatosis. These results suggest that although betaine has a beneficial effect in treating hepatic steatosis in animal models, further human studies are needed to explore therapeutic options for NASH[19]. As a novel therapeutic option in drug research and discovery, the potential benefits of betaine in regulating NAFLD require further clarification.

Conflict of Interest

The authors declare that there is no conflict of interest.

Funding

This work was supported in part by the National Natural Science Foundation of China (31860247) and the Natural Science Foundation of Jiangxi Province (20202ACBL206003) to LW, the Science and Technology Project Foundation of Education Department of Jiangxi Provincial (GJJ150961) to MX, The Open Project of Key Laboratory of Prevention and treatment of cardiovascular and cerebrovascular diseases, Ministry of Education (XN201803).

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