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Choline Metabolism Provides Novel Insights into Non-alcoholic Fatty Liver Disease and its Progression

Karen D. Corbin and **Steven H. Zeisel**
University of North Carolina at Chapel Hill, Nutrition Research Institute, Kannapolis, North Carolina, USA

Abstract

Purpose of review—Choline is an essential nutrient and the liver is a central organ responsible for choline metabolism. Hepatosteatosis and liver cell death occur when humans are deprived of choline. In the last few years there have been significant advances in our understanding of the mechanisms that influence choline requirements in humans and in our understanding of choline's effects on liver function. These advances are useful in elucidating why non-alcoholic fatty liver disease (NAFLD) occurs and progresses sometimes to hepatocarcinogenesis.

Recent findings—Humans eating low choline diets develop fatty liver and liver damage,. This dietary requirement for choline is modulated by estrogen and by single nucleotide polymorphisms (SNPs) in specific genes of choline and folate metabolism. The spectrum of choline's effects on liver range from steatosis to development of hepatocarcinomas, and several mechanisms for these effects have been identified. They include abnormal phospholipid synthesis, defects in lipoprotein secretion, oxidative damage caused by mitochondrial dysfunction, and endoplasmic reticulum (ER) stress. Furthermore, the hepatic steatosis phenotype and can be characterized more fully via metabolomic signatures and is influenced by the gut microbiome. Importantly, the intricate connection between liver function, one carbon metabolism, and energy metabolism is just beginning to be elucidated.

Summary—Choline influences liver function, and the dietary requirement for this nutrient varies depending on an individual's genotype and estrogen status. Understanding these individual differences is important for gastroenterologists seeking to understand why some individuals develop NAFLD and others do not, and why some patients tolerate total parenteral nutrition and others develop liver dysfunction.

Keywords

choline; metabolomics; microbiome; non-alcoholic-fatty liver disease; single nucleotide polymorphisms

Introduction

The primary focus of this article will be to relate new understanding about the role of choline metabolism in sustaining normal liver function, development of nonalcoholic fatty liver disease (NAFLD), and hepatocarcinogenesis. The recent discoveries that the dietary requirement for choline varies substantially among individuals due to genetics [1, 2], gender [3], and microbiome composition [4] make it easier to consider the clinical implications of

Correspondence to Steven H. Zeisel, MD, PhD, UNC Nutrition Research Institute, 500 Laureate Way, Room 2218, Kannapolis, NC 28081, USA, Tel: + 1 704 250 5003; fax: +1 704 250 5001, steven_zeisel@unc.edu.

choline in diseases dealt with by gastroenterologists. This review will focus on recent advances on the importance of choline in the liver.

Choline biology

Choline is a constituent of cell and mitochondrial membranes and of the neurotransmitter acetylcholine. Given its essentially ubiquitous incorporation into cellular components and pathways, it is not surprising that this nutrient influences diverse processes such as lipid metabolism [5], signaling through lipid second messengers [6], methylation-dependent biosynthesis of molecules (including epigenetic regulation of gene expression) [7–9], activation of nuclear receptors [10, 11], enterohepatic circulation of bile and cholesterol [12], plasma membrane fluidity [13], and mitochondrial bioenergetics [14].

The two major fates for choline are to be phosphorylated and used to make phospholipids, or to be oxidized and used as a donor of methyl-groups. An especially important choline metabolite in liver is phosphatidylcholine, which is necessary for the packaging and export of triglycerides in very low density lipoprotein (VLDL) [15] and for the solubilization of bile salts for secretion [16]. Aberrant VLDL– mediated secretion of triglycerides is a central mechanism in hepatic steatosis [17]. The role of bile homeostasis in liver physiology is also quite evident, and mostly relates to the causes of gallstones, fibrosis, and hepatocarcinomas [18]. However, new functions attributed to bile salts, including regulation of energy and glucose metabolism [19–21], makes it likely that phosphatidylcholine plays a role in modulating these functions as well.

Choline, folate and methionine metabolism are interrelated as all influence the production of *S*-adenosylmethionine, the universal donor of methyl-groups in biological reactions [5] (Figure 1). Deficiency in one nutrient is associated with an increase in flux of the other nutrients towards methyl donation [5].

Individual choline requirements

Choline is found in a variety of foods, but it is particularly abundant in egg yolks and animal sources of protein (see www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/Choline/Choln02.pdf). Many of these high-choline foods are high in fats or cholesterol (e.g. eggs) and are being avoided by many people who then do not achieve the recommended dietary Adequate Intake for choline [22, 23]. For example, several recent epidemiologic studies reported that 25% of Americans ate diets very low in choline (<203 mg/d in the Framingham Heart Study [24], <217 mg/d in the Atherosclerosis Risk In Communities study [25, 26] and <293 mg/d in the Nurse's Health Study [27]; the Adequate Intake is 450–550 mg/day [22]).

Choline was once believed to be a dispensable nutrient because there is a pathway for endogenous formation of phosphatidylcholine catalyzed by phosphatidylethanolamine N-methyltransferase (PEMT). However, controlled clinical feeding studies demonstrated unequivocally that choline is an essential nutrient; humans deprived of choline developed either fatty liver and liver cell death or developed skeletal muscle damage [1, 2]. These findings were reinforced by clinical evidence that patients fed with total parenteral nutrition solutions low in choline developed fatty liver and liver damage [28].

There are two major sources for variation in human dietary requirements for choline: estrogen status and genetic variation. As mentioned earlier, the dietary requirement for choline can be spared by endogenous biosynthesis of phosphatidylcholine in liver, catalyzed by PEMT [5]. Expression of the gene *PEMT* is induced by estrogen [29], and therefore most premenopausal women [30] and postmenopausal women who are treated with estrogen [30] have a diminished dietary requirement for choline. However, more than 40% of women have

a genetic polymorphism in *PEMT* (rs12325817) that makes this gene unresponsive to estrogen, and these women have the same high choline requirement as men [2, 29, 30]. There are other genetic polymorphisms that modify choline requirements by different mechanisms; choline dehydrogenase (*CHDH*) rs12676 and rs9001 [2], and methylene tetrahydrofolate dehydrogenase 1 (*MTHFD1*) rs2236225 [1].

Choline and non-alcoholic fatty liver disease

Liver is an important organ for metabolism and storage of choline, and liver is dependent on a source of choline [5]. Choline deficient diets, including those that are also deficient in methionine, have long been utilized to study the mechanisms of fatty liver disease and its progression because such diets recapitulate many of the phenotypes seen in humans with NAFLD, including an accumulation of triglycerides in the liver [5, 31]. (Figure 2) Although in many cases NAFLD maintains a benign course, hepatic steatosis is an early manifestation of liver dysfunction that sometimes progresses to steatohepatitis, fibrosis, cirrhosis and liver cancer [32].

Several mouse models with deletion of choline-related genes have given insight into the mechanisms of NAFLD. In several mouse models, deletion of genes needed to use choline as a methyl donor (*Bhmt* [33], *Chdh* [34]), deletion of genes needed to form the choline moiety endogenously (*Pemt* [35]) or deletion of genes needed to make *S*-adenosylmethionine (*Mat1* [36]) result in fatty liver. In humans, polymorphisms in *PEMT* [37, 38] are associated with NAFLD. These observations suggest that the methyl-donation function of choline is important in the mechanism of NAFLD. Earlier, we discussed the hypothesis that phosphatidylcholine was required for normal VLDL secretion from liver. The genetic data suggest that it is phosphatidylcholine that is derived from the PEMT methylation pathway that is important (rather than phosphatidylcholine derived from preformed choline); mouse studies support this conclusion [39].

Although much can be gleaned by studying the genetic mechanisms of disease, several other important levels of control of fatty liver could be concurrently important. Metabolomics, especially when used in combination with other methods to define phenotype, has advanced our understanding of the role of choline in fatty liver. Humans who develop fatty liver on a choline deficient diet exhibit a metabolomic profile at baseline: altered choline metabolites, lipids (including acylcarnitines), and amino acids. This metabolomic profiling (done while people are eating normal diets) accurately predicted which humans would develop fatty liver when fed low choline diets [40]. It is interesting that plasma metabolomic profiling of NAFLD patients (independent of the cause of NAFLD) demonstrated that carnitines, choline metabolites, and bile acids could differentiate healthy controls from NAFLD or non-alcoholic steatohepatitis cases [41], suggesting that similar pathways are involved in NAFLD and choline deficiency.

Choline is an important part of the mitochondrial membrane and mitochondrial dysfunction is a central mechanism in the pathogenesis of NAFLD [32]. Low choline may be important in NAFLD pathophysiology because it perturbs mitochondrial bioenergetics [14] and fatty acid beta oxidation [42]. Choline deficiency alters the composition of mitochondrial membranes; cardiolipin in these membranes is oxidized, and membrane concentrations of phosphatidylethanolamine and phosphatidylcholine are decreased [43, 44]. These membrane changes result in mitochondrial decreased membrane potential [14, 45] and in reduced activity of complex I of the respiratory chain [44, 46]. Decreased ATP production by mitochondria occurs in rats fed a choline deficient diet [47] or a choline-methionine deficient diet [48]. Proteins involved in choline metabolism and transport also influence mitochondrial function. CHDH is a mitochondrial matrix protein that catalyzes the

conversion of choline to betaine. Mice with deleted *Chdh* have abnormal mitochondrial function in multiple tissues [34]. Interestingly, CHDH is upregulated in the mitochondrial proteome of rats with fatty liver induced by alcohol [49]. This could be a compensatory response, since betaine is believed to have a hepato-protective effect [50].

Endoplasmic reticulum (ER) stress is a condition whereby excess unfolded proteins lead to a cascade of stress responses. If stress is chronic, cell death can occur. ER stress is believed to play a role in the pathogenesis of NAFLD [51]. In mice fed methionine-choline deficient diets for up to 21 days, hepatic steatosis was associated with inducing specific ER stress cascades upstream of the unfolded protein response. The integrated ER stress response was unable to cause liver injury in the absence of steatosis, suggesting a coordinated mechanism is necessary for liver disease progression [52]. Another link between choline, NAFLD, and ER stress was found when metabolomic and proteomic studies in obese, leptin deficient mice revealed that the obese phenotype is characterized by ER stress, increased expression of proteins involved in lipogenesis and phospholipid metabolism (including PEMT), and a distinct lipid profile characterized by increased monounsaturated fatty acids and an increased phosphatidylcholine to phosphatidylethanolamine concentration ratio. This altered ratio impairs calcium signaling and ER homeostasis [53].

Choline is a potent modifier of epigenetic marks on genes [7, 8]. It is likely that there are specific epigenetic outcomes that influence NAFLD under choline deprivation. Several genes that are central to the pathophysiology of metabolic disease, such as leptin [54] and PPAR gamma [55], are known to be epigenetically regulated. The specific mechanisms linking choline, epigenetics and NAFLD are areas of active investigation.

The study of the influence of the gut microbiome on human health has advanced tremendously. The gut microbiome integrates many important pathways, including those related to enterohepatic circulation of bile, cholesterol and phospholipids [56]. The gut flora modulates host immunity [57], glucose, lipid, and energy metabolism [58], and choline availability [59], all of which play a role in NAFLD [60]. Gut microbiome composition is influenced by multiple factors such as maternal diet, lifelong diet, environmental exposures, and genetics [61]. Gammaproteobacteria and Erysipelotrichi within the gut microbiome were directly associated with changes in liver fat in humans during choline depletion. Levels of these bacteria, change in amount of liver fat, and a single nucleotide polymorphism (PEMT rs12325817) that affects choline were combined into a model that accurately predicted the degree to which subjects developed fatty liver on a choline-deficient diet [4]. This suggests that understanding the effects of the microbiome can enhance current paradigms defining NAFLD risk and progression.

Choline and progression of fatty liver disease

We need to understand more about the factors that influence the progression of fatty liver disease to more severe liver injury and cancer. Choline and methionine deficiency has been a useful model for identifying potential mechanisms. In rodents, choline deficient diets caused progressive hepatic disease much like what is seen in some humans with fatty liver: steatosis → fibrosis → cirrhosis → hepatocellular carcinoma [6, 62]. This progression from fatty liver to hepatocarcinoma is also seen when a gene in choline metabolism is knocked out in mice. The *Bhmt*$^{-/-}$ mouse, discussed earlier, develops fatty liver and liver injury (elevated ALT and gamma glutamyltransferase 1) at 5 weeks of age and this progresses to hepatocarcinomas by 52 weeks of age [33]. Overall, the hepatic phenotype in the *Bhmt* −/− mouse suggests this model will be a valuable tool to characterize fatty liver progression as related specifically to choline deficiency, altered methylation potential, and perhaps other stress responses that could manifest in mitochondrial dysfunction and ER stress [33].

Multiple mechanisms have been identified that may explain why choline deficiency progresses to hepatocarcinoma. The primary mechanism involves damage to DNA, as assessed by the formation of 8- oxodeoxyguanosine [63, 64], apurinic/apyrimidinic sites [65] and Ogg1-sensitive sites [65] in DNA that accumulate when rats are deprived of choline. Choline deficient hepatocytes overproduce free radicals because their mitochondria become leaky [43, 45, 66–68]. In addition, death of hepatocytes that occurs in choline deprivation [69] causes an inflammatory response with an associated neutrophil/macrophage-mediated generation of reactive oxygen and nitrogen species [70].

Choline, NAFLD and metabolic syndrome

NAFLD is tightly linked to obesity and insulin resistance [32]. There is good reason to believe that choline and 1-carbon metabolism influence obesity and insulin resistance. In mice fed an obesogenic diet, which causes weight gain and hepatic steatosis, a plasma and liver metabolomic approach identified phosphatidylcholine, lysophosphatidylcholine, and betaine as metabolites that differentiated the obese versus lean phenotype [71]. The role of one carbon genes was also prominent in a study that merged genomic and metabolomic data sets to characterize diet-induced obesity in mice [72]. In human studies aiming to characterize insulin sensitivity and diabetes, choline metabolites have also been repeatedly identified as important for distinguishing metabolic states [73–75]. Conversely, altering genes in choline metabolism modifies responses to obesity. *Pemt* knockout mice are protected from obesity due to a high calorie/high fat diet [76]. It is interesting that in obese, leptin receptor deficient mice, genes involved in production of phosphatidylcholine, including *Pemt*, were upregulated [53]. In this mouse, hepatic steatosis was reduced if *Pemt* was silenced [53]. These data suggest that there are phosphatidylcholine-mediated mechanisms that influence responses to obesity.

PPARα, part of the peroxisome-proliferator family of nuclear receptors, is highly expressed in the liver and is involved in fatty acid metabolism, lipoprotein assembly [77] and gluconeogenesis [78]. The endogenous ligand for the PPARα receptor is a specific form of phosphatidylcholine (1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine). Infusion of this phosphatidylcholine recapitulates the protection from fatty liver seen with PPAR agonists [10]. Another nuclear receptor, liver receptor homolog 1 (LRH-1), had no known endogenous ligand until a specific phosphatidylcholine (dilauroyl phosphatidylcholine-DLPC) was identified as the agonist [11]. This receptor is involved in bile acid biosynthesis and activation promotes bile acid synthesis, lowers triglycerides in liver, and decreases serum glucose concentrations [11].

Conclusion

Our understanding of the mechanisms by which choline, and related metabolites, impact liver physiology and of the individual requirements for these nutrients is advancing rapidly. Progress in the utilization of advanced methods, such as metabolomics, and emerging science, such as the area of gut microbiome-host interactions, to broaden our appreciation of the mechanisms by which the multiple functions of choline converge in specific liver phenotypes is particularly exciting. The relatively unexplored non-canonical functions of some genes in the choline metabolism pathway along with the very recent observations linking one carbon and energy metabolism hold much promise for unraveling some of the mysteries of complex metabolic disease while possibly elucidating prevention and treatment targets for NAFLD.

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Key points

- Choline is an essential nutrient with multiple mechanistic roles in NAFLD and its progression including VLDL export, enterohepatic metabolism of bile, mitochondrial function, epigenetics, ER Stress, and VLDL export.
- Choline deficiency in humans is associated with liver dysfunction and susceptibility is dependent on factors, including genetics, gender, and the gut microbiome, which influence choline requirements.
- Recent evidence has identified a prominent role for choline and one carbon metabolism in metabolic syndrome.
- Applying knowledge of individual choline requirements into gastroenterology clinical practice has the potential to improve outcomes.

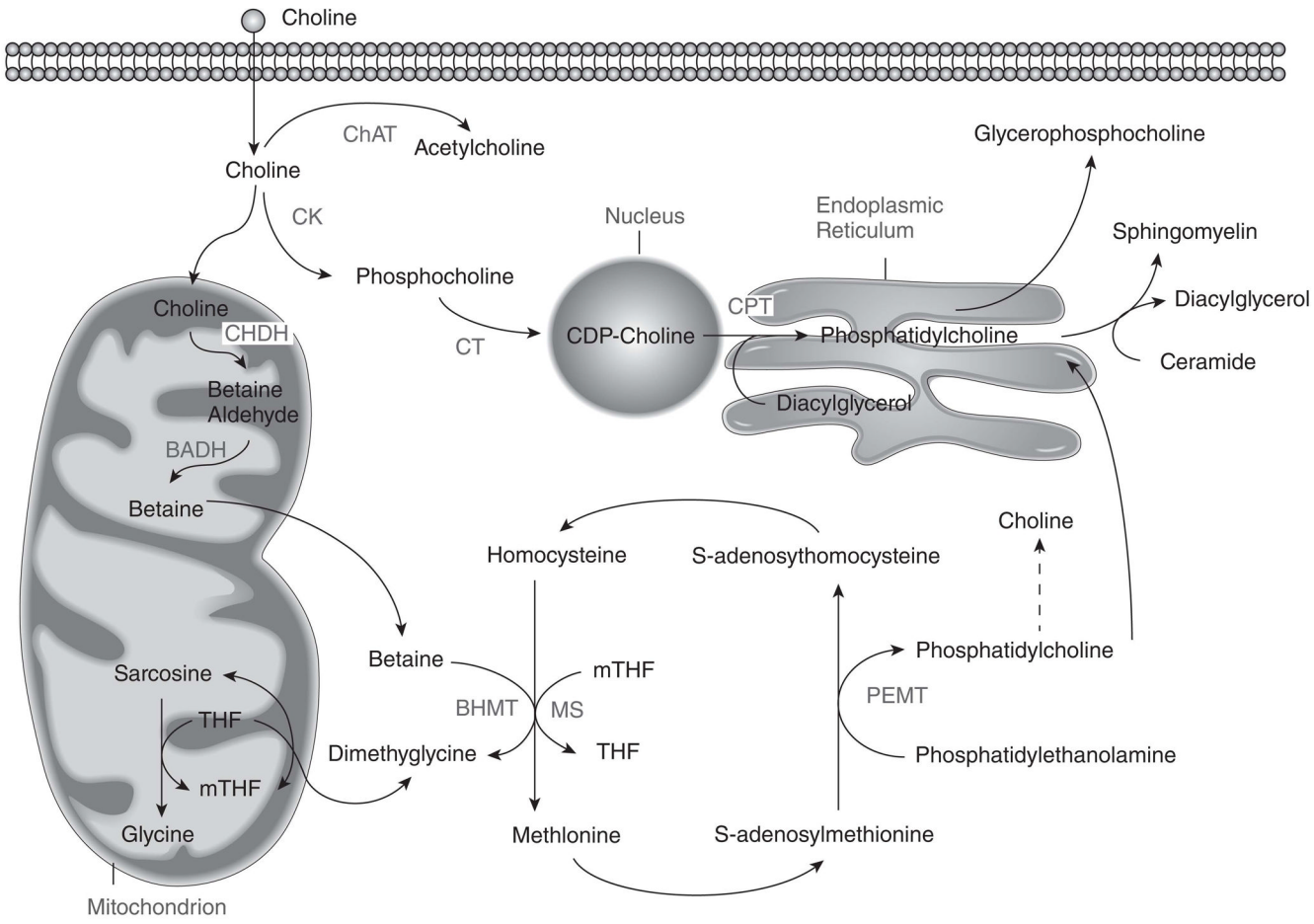

Figure 1. Choline, folate and homocysteine metabolism are closely interrelated

The pathways for the metabolism of these three nutrients intersect at the formation of methionine from homocysteine.

BADH=betaine aldehyde dehydrogenase; BHMT=betaine homocysteine methyltransferase; ChAT=choline acetyltransferase; CHDH=choline dehydrogenase; CK=choline kinase; CPT=choline phosphotransferase; CT=CTP:phosphocholine cytidylytransferase; MS=methionine synthase; mTHF=methyl tetrahydrofolate PEMT=phosphatidylethanolamine-N-methyltransferase; THF=tetrahydrofolate

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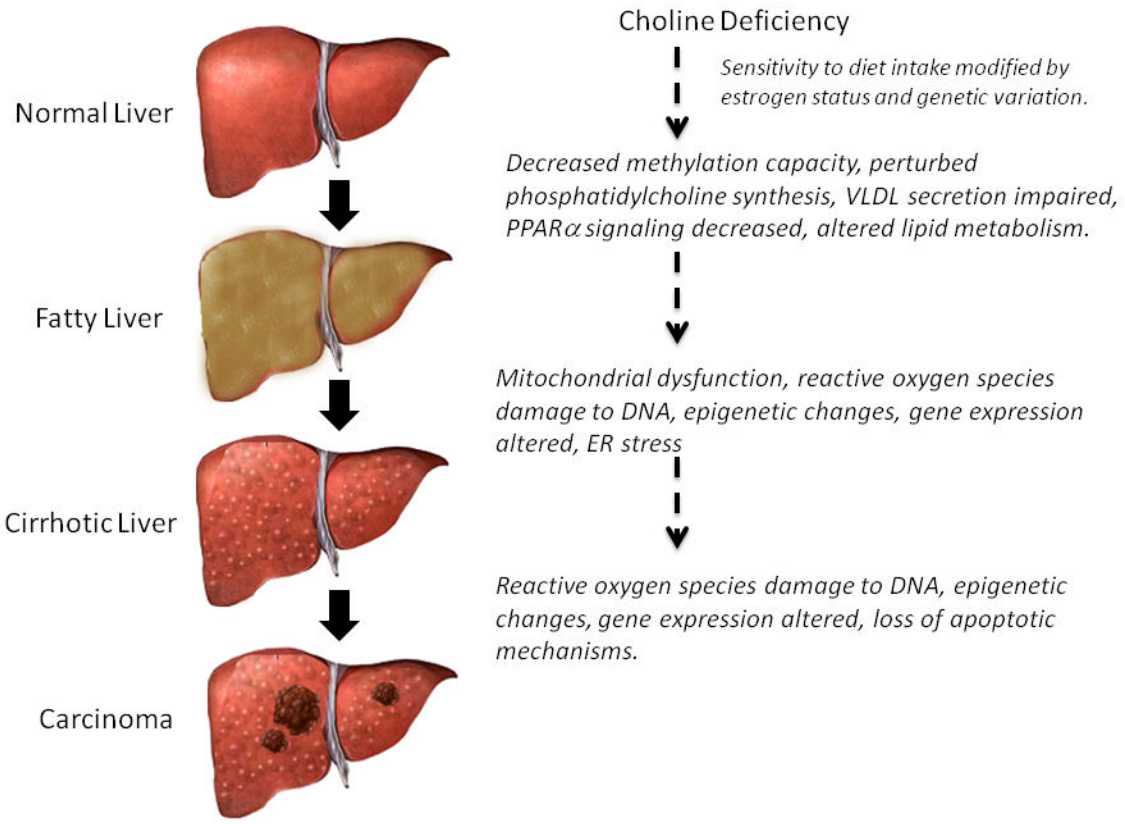

Figure 2. Summary of Choline Deficiency Mediated Mechanisms of Liver Dysfunction

The progression of NAFLD from simple steatosis to hepatocarcinoma is influenced by multiple cholinemediated mechanisms.