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Choline's role in maintaining liver function: new evidence for epigenetic mechanisms

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Abstract

Purpose of review—Humans eating diets low in choline develop fatty liver and liver damage. Rodents fed choline–methionine-deficient diets not only develop fatty liver, but also progress to develop fibrosis and hepatocarcinoma. This review focuses on the role of choline in liver function, with special emphasis on the epigenetic mechanisms of action.

Recent findings—Dietary intake of methyl donors like choline influences the methylation of DNA and histones, thereby altering the epigenetic regulation of gene expression. The liver is the major organ within which methylation reactions occur, and many of the hepatic genes involved in pathways for the development of fatty liver, hepatic fibrosis, and hepatocarcinomas are epigenetically regulated.

Summary—Dietary intake of choline varies over a three-fold range and many humans have genetic polymorphisms that increase their demand for choline. Choline is an important methyl donor needed for the generation of *S*-adenosylmethionine. Dietary choline intake is an important modifier of epigenetic marks on DNA and histones, and thereby modulates the gene expression in many of the pathways involved in liver function and dysfunction.

Keywords

choline; DNA methylation; epigenetics; fatty liver; liver carcinoma

INTRODUCTION

Humans must eat diets containing choline [1] because its metabolite phosphatidylcholine constitutes 40–50% of cellular membranes and 70–95% of phospholipids in lipoproteins, bile and surfactants [2]; it is needed to form acetylcholine, an important neurotransmitter [2]; its metabolite betaine is needed for normal kidney glomerular function, and perhaps for mitochondrial function [2]; and it provides one-carbon units, via oxidation to betaine, to the methionine cycle for methylation reactions [2].

There is a recommended adequate intake for choline (about 550 mg/day) [3], but choline intake in the diet has been estimated to vary by as much as three-fold – the lowest quartile

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Conflicts of interest
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and the highest quartile of intake were approximately 150mg and 500 mg/day choline equivalents, respectively, in the Framingham Offspring Study [4], the Atherosclerosis Risk In Communities study [5,6], and the Nurse's Health Study [7]. Intake of choline is likely to be lower in low-income countries. Patients fed with total parenteral nutrition (TPN) solutions receive only small amounts of choline (from the lipid emulsions) and many become choline deficient [8].

CHOLINE AND LIVER FUNCTION

Much of choline metabolism occurs in the liver, and this is among the first organs to accumulate choline absorbed from the intestine [2]. When humans eat diets low in choline, fatty liver is one of the earliest adverse events, and in some people significant hepatic damage occurs (as assessed by release of hepatic enzymes into blood) [9]. People with one of several very common genetic polymorphisms (SNPs) in the genes of choline metabolism are more likely to develop hepatic dysfunction when deprived of choline [10–12], and these people have abnormal plasma metabolomic profiles even when fed a normal diet containing choline [13]. Patients fed with TPN solutions often develop liver dysfunction, and in some, this resolves when they are fed a source of choline [8,14]. We do not know whether the patients who are susceptible to TPN-associated liver damage are those who have SNPs in the genes of choline metabolism.

Rodents also develop fatty liver when fed diets low in choline and methionine [2,15], and this animal model is commonly used for the study of nonalcoholic fatty liver disease (NAFLD) which affects 20% of the global population, 50% of diabetic patients, and 90% of morbidly obese people [16]. The likely mechanism responsible for the development of fatty liver in choline deficiency is related to the synthesis of very-low-density lipoprotein (VLDL), which is the primary package within which triglycerides are secreted from the liver [17]. Phosphatidylcholine is a required component of the VLDL envelope, and when it is not available, triglycerides cannot be exported from liver and hence accumulate in the cytosol [17]. Phosphatidylcholine is formed in the liver by methylation of phosphatidylethanolamine or from incorporation of preformed choline (usually from the diet) [17]. Premenopausal women are less likely to develop fatty liver on a low-choline diet because estrogen induces the hepatic gene (*PEMT*) that is responsible for *de novo* formation of phosphatidylcholine [10]. In more than 20% of premenopausal women, a SNP in *PEMT* leaves them less responsive to estrogen induction of this gene, and they must eat choline to prevent development of fatty liver [10,11,18].

In people, NAFLD sometimes progresses to liver injury and hepatocarcinoma [19], and the choline–methionine-deficient rodent model may help us to understand the underlying reasons for this progression. Rats and mice fed a diet low in choline–methionine content first develop fatty liver, then the liver becomes fibrotic, followed by the development of foci of enzyme-altered hepatocytes which express γ-glutamyltranspeptidase [19] and the placental form of glutathione *S*-transferase [20] similar to those precancerous cells induced by chemical carcinogens [21,22▪▪]. Eventually, these animals develop adenomas and hepatocellular carcinomas [21]. Adding choline to this deficient diet completely prevents the development of cancer in experimental animals, suggesting that choline itself has an important role [21]. It is interesting that hepatocytes in cell culture, which are slowly shifted to growth media low in choline concentration, also transform into hepatocarcinoma cells [23]. This suggests that the underlying mechanisms for this response to low choline are intrinsic to the hepatocytes. Choline–methionine deficiency also sensitizes rodents to liver carcinogens such as aflatoxin B1 [24]. For example, the dose of aflatoxin B1 needed to induce hepatocarcinomas was greatly reduced in rats fed low-choline–methionine diet [24].

Thus, choline–methionine deficiency acts as an initiator and as a promoter of carcinogenesis.

Several potential mechanisms whereby diets low in choline and methionine result in hepatocarcinogenesis have been explored. These include [22▪▪] liver necrosis with consequent regeneration; induction of oxidative DNA damage and lipid peroxidation because of free radical leaks from mitochondria, with subsequent oxidation of DNA bases resulting in nicks and deletions during base repair; altered protein kinase C signaling because of accumulation of diacylglycerol; and loss of liver apoptotic responses. This review will focus on yet another proposed mechanism – the alteration in the status of labile epiloci induced by methyl devoid diets [25].

ROLE OF METHYL-DONOR NUTRIENTS (METHIONINE, CHOLINE, AND FOLATE) IN SUSTAINING METHYLATION CAPACITY

After *S*-adenosylmethionine (SAM) is used tomethylate a substrate, *S*-adenosylhomocysteine and then homocysteine is formed. To regenerate methionine, homocysteine (which is toxic for cells) must be methylated, and the resulting methionine can then be converted to SAM [26]. This methionine cycle in liver utilizes methyl groups from methyl-tetrahydrofolate or from betaine to convert homocysteine to methionine. Choline is the precursor for betaine formation and, therefore, for many of the methyl groups donated to homocysteine via the enzyme betaine homocysteine methyltransferase (BHMT). BHMT processes a significant portion of cellular homocysteine as *Bhmt* knockout mice become hyperhomocysteinemic even under adequate supply of dietary methyl-tetrahydrofolate [22▪▪].

As discussed later, methylation of DNA and histones constitutes an important mechanism for modulating gene expression called epigenetic regulation. It is not surprising that alterations in dietary choline supply or utilization shape the epigenome.

EPIGENETICS

Epigenetic regulation of gene expression involves the chemical modification of nucleotides in DNA at specific locations. Usually, DNA is not present in cells in the linear form that we so commonly picture it as being, but rather DNA is tightly wound around proteins (histones) [27] (Fig. 1). The positively charged DNA is attracted to negatively charged histones, forming a compact spherical complex. One mechanism for modifying this tightly wound structure occurs when specific cytosine residues are methylated [approximately 70% of the cytosine residues adjacent to guanines (CpG) in genes are methylated as are the intergenic CpG islands and the CpGs in transposable elements that are so common in the human genome [27]]. This CpG methylation is achieved by the enzymatic transfer of methyl groups from SAM to cytosine. When CpGs on DNA are methylated, they attract methyl-binding proteins, which then attract histone deacetylases [27]. These enzymes remove acetyl groups on specific lysine residues in histones and thereby increase the negative charge on the protein. The tight chromatin complex formed by positively charged DNA and negatively charged histones prevents the transcription factors from reaching the DNA to activate gene expression. When DNA CpGs are not methylated, the DNA histone interaction is weaker, opening up the chromatin and creating a permissive environment for gene transcription [28,29]. Simply stated, DNA methylation usually shuts genes off.

As discussed above, acetylation of histones is important for maintaining the structure of chromatin, but methylation of histones is also an important epigenetic signal (mono-methyl and di-methyl lysine 9 on histone H3) that represses gene transcription [30], while di-methyl

and tri-methyl lysine 4 on histone H3 are enriched in areas with transcriptional active chromatin [31]. Using SAM as the methyl donor, mono-methyl and di-methyl lysine 9 on histone H3 are formed by *G9a* histone methylase [32], whereas tri-methyl lysine 9 on histone H3 is formed by *SUV39* methylase [33]. DNA methylation and histone methylation mechanisms exhibit crosstalk, creating a reinforcing signaling system controlling gene expression.

Alterations in the epigenome are more frequent during sensitive periods of development when progenitor cells are dividing and not yet differentiated [34]. During development, profound epigenomic transformations take place, including DNA methylation catalyzed by DNMT3 [35]. Once established, the epigenome maintains a relatively stable state of transcription in mature somatic cells. During mitosis, this pattern of methylation is faithfully copied to the sister DNA strands by the maintenance and chromatin maturation genes *DNMT1*, *HDAC1*, and *SMARCAD1* [36]. However, selected loci on genes exhibit a degree of epigenetic plasticity and remain responsive to nutrient levels later in life.

Why is epigenetic flexibility important? As in any other living cell, the genetic information encoded in the hepatocyte genome is fixed, but an epigenetic regulatory mechanism is superim-posed to achieve flexibility in processing the genetic information. Epigenetic marks determine why cells with the same genetic code can have different differentiated phenotypes (somatic individuality): hepatocytes express different genes than do Kupffer cells, stellate cells, endothelial cells or fibroblasts in the liver [37]. Epigenetic marks can permit metabolic flexibility (adaptation of metabolic pathways in response to the environmental signals) [37]. Several genes central to hepatic metabolism are epigenetically regulated, including peroxisome proliferator- activated receptor γ (PPARγ [38]), nuclear sterol response element-binding protein 1-c (SREBP-1c) [39], alcohol dehydrogenase [39,40], glutathione S-transferase [39,40], serine dehydrase [40], CYP450 2c11 [40], glucokinase [41], pyruvate kinase [41,42], phosphoenolpyruvate carboxykinase [42], and enzymes of cholesterol metabolism via epigenetic regulation of the liver X receptor [43]. Thus, liver function is dependent, in part, on how well epigenetic regulatory mechanisms are established. At the same time, liver is probably the most important organ that controls the availability of the SAM needed to establish epigenetic marks.

THE LIVER IS AN IMPORTANT ORGAN CONTROLLING METHYLATION

Half of the methionine coming from diets is utilized by the liver for forming SAM that is needed for methylation reactions and more than 85% of methylation reactions take place in liver. Interestingly, the critical genes for controlling methyl metabolism and DNA methylation capacity are themselves regulated by methylation. For example, *MAT1A* (forms SAM) is underexpressed when it is hypermethylated [44], and the expression of the DNA methyltransferases *DNMT1* AND *DNMT3A* are controlled by methylation of specific CpG sites [21,45▪,46]. The expression of *G9a* histone methylase is also decreased when CpGs at specific sites in the gene are methylated [47,48]. Thus, rodents fed diets low in choline and methionine undermethylate these methyltransferase genes and therefore overexpress these methyltransferases [21,45▪,46–48]. This explains why some genes are paradoxically overmethylated despite methyl-donor deficiency [46]. Interspersed elements containing repetitive DNA sequences represent 30% of the mammalian genome [49], and the methylation status of these elements is modified by the availability of dietary choline in rodents [50]. An additional potential mechanism for methyl-deficiency modulation of DNA methyltransferase activity in liver is focused on mitochondria. Abnormal membrane composition causes the release of free radicals and oxidizes the nucleotides, forming 8-hydroxydeoxyguanosine which inhibits cytosine methylation [51]. Mechanistically, the accumulation of intracellular fat, inflammation, fibrosis and eventually carcinogenesis are

multi-factorial, and epigenetic mechanisms occupy central roles in this scenario (Fig. 2). We revisit here the effects of choline deficiency on the liver epigenome and on signal transduction involved in the inflammation pathways.

EPIGENETIC MECHANISMS INVOLVED IN NONALCOHOLIC FATTY LIVER DISEASE, NONALCOHOLIC STEATOHEPATITIS, AND PROGRESSION TOWARD LIVER TUMORIGENESIS

Some of the major signals and mechanisms involved in NAFLD, nonalcoholic steatohepatitis (NASH), and progression toward liver carcinogenesis are signaling by cytokines/chemokines (TNFα [52,53], TGFβ [54,55], IL-6 [56], and IL-10 [57]), CCL2/MCP1 targeting PPARα [58–60] (there is an increase in promoter methylation of antifibrotic PPARα receptor protein in choline-deficient livers [61]), CCL5 increased by hepatocellular lipid accumulation [62–64] and CXCL8/IL-8 [65]; increased *de novo* synthesis of triglycerides [66]; decreased VLDL synthesis and export [67]; and decreased long-chain fatty acid oxidation.

As discussed earlier, many of the hepatic genes involved in pathways for the development of fatty liver are epigenetically regulated, including PPARγ [38], SREBP-1c [39], glucokinase [41], pyruvate kinase [41,42], phosphoenolpyruvate carboxykinase [42], and enzymes of cholesterol metabolism [43]. Fatty liver can progress to liver damage that is accompanied by fibrosis [scar tissue synthesized by activated hepatic stellate cells (aHSCs, i.e., myofibroblasts)]. Recent studies explain how liver fibrosis is increased in low methyl-donor environments [68–70]. Low levels of inflammatory signals combined with epigenetic mechanisms normally keep hepatic stellate cells (HSCs) quiescent [69]. TGF-β1 signaling mediates the activation of HSCs [71] by decreasing the expression of Phosphatase and Tensin homolog (PTEN), a repressor of phosphatidylinositol 3,4,5 triphosphate kinase/serine-threonine kinase Akt (PI3K/AKT) and extracellular signal-regulated kinase (ERK) signaling pathways [72]. A new set of epigenetic marks is acquired by aHSCs and these act to control gene expression so as to maintain the aHSC cellular phenotype [69]. Once achieved, the epigenome of aHSCs results in increased *DNMT1* expression and in increased MECP2 levels with recruitment of histone modifiers [69,73,74] (Fig. 1). These changes stabilize the aHSCs' chromatin and maintain low PTEN expression, thereby ensuring the progression of fibrosis. In summary, the fibrosis process is initiated upon HSC activation via cytokines and growth factors and a new set of epigenetic marks is acquired that maintains their new cellular phenotype [69]. The decreased availability of methyl donors thereby can initiate and sustain hepatic fibrosis (Fig. 2). Interestingly, these modified epigenetic mechanisms driving liver repair are heritable [75▪▪]. Newer generations are less responsive to fibrosis because they generate decreased numbers of HSCs, increased expression of antifibrogenic PPAR-γ, and decreased TGF-β. These adaptations were epigenetically transmitted through the male germline, via histone modifications. This may explain the presence of hypomethylated PPAR-γ in humans harboring mild forms of fibrosis in severe methyl-deficient environments.

Mechanisms responsible for tumor-initiating events or tumor progression are also, in part, epigenetic [76,77]. Many tumor suppressor genes are epigenetically regulated, including genes for cell-cycle regulation (p15 and p16), apoptosis (DAPK and APAF-1), cell adherence (CDH1 and CDH3), and DNA repair (BRCA1 and hMLH) [22▪▪]. Hepatocellular carcinomas have an epigenome that is profoundly different from normal hepatocytes, with gene-specific DNA overmethylation or undermethylation, altered histone epigenetic marks, and abnormal expression of genes for DNA methyltransferases and histone-modifying enzymes [77]. Methyl-deficient diets which caused hepatic cancers were associated with

global and gene-specific epigenetic changes [25,78,79], including hypomethylation of *c-myc* [80], *c-fos*, and *c-Ha-ras* [81]. These changes in cytosine methylation patterns occur after short-term feeding of choline-deficient diets and before hepatocarcinomas develop, suggesting a causal rather than a consequential role. Interestingly, mouse models in which methylmetabolism has been perturbed by genetic manipulation, such as *Mat1a*$^{-/-}$ mice [in which methionine adenosyltransferase (forms SAM) is deleted [82–84]] and *Bhmt*$^{-/-}$ mice (in which BHMT, needed to transfer methyl moiety from betaine to homocysteine, is deleted [22■■]), develop hepatic steatosis and hepatocarcinomas.

Other products of choline metabolism influence carcinogenesis and involve epigenetic mechanisms. Lysophosphatidic acid (LPA), via G-protein-coupled transmembrane receptors, regulates cellular proliferation, differentiation, morphogenesis, and protection from apoptosis [85,86]. Phosphatidylcholine is a precursor for LPA formation [87]. The genes encoding the receptors for LPA signal are regulated by the epigenetic mechanisms [88]. Rodents fed choline–methionine-deficient diets had aberrant methylation of the gene for the LPA1 receptor in a pattern similar to the methylation abnormalities described in hepatocellular carcinomas [89].

CONCLUSION

Liver is the organ where choline, methyl folate, methionine, and SAM metabolic pathways are most active, and it is the organ where most methylation reactions occur. The liver is very sensitive to the availability of methyl donors in the diet, including choline. When deprived of these nutrients, the liver becomes fatty, hepatocytes die, fibrosis develops, and eventually foci of carcinomas appear. This progression occurs not only because these nutrients are needed to produce important structural components (membranes) and signaling molecules (e.g., LPA and acetylcholine), but also because these nutrients influence the epigenetic regulation of gene expression.

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■ of special interest

■■ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 364).

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KEY POINTS

- Choline and other dietary methyl donors are important for liver function.
- These nutrients are important modulators of epigenetic regulation of gene expression.
- Pathways important for the development of fatty liver, hepatic fibrosis, and hepatocarcinoma are regulated via epigenetic mechanisms.

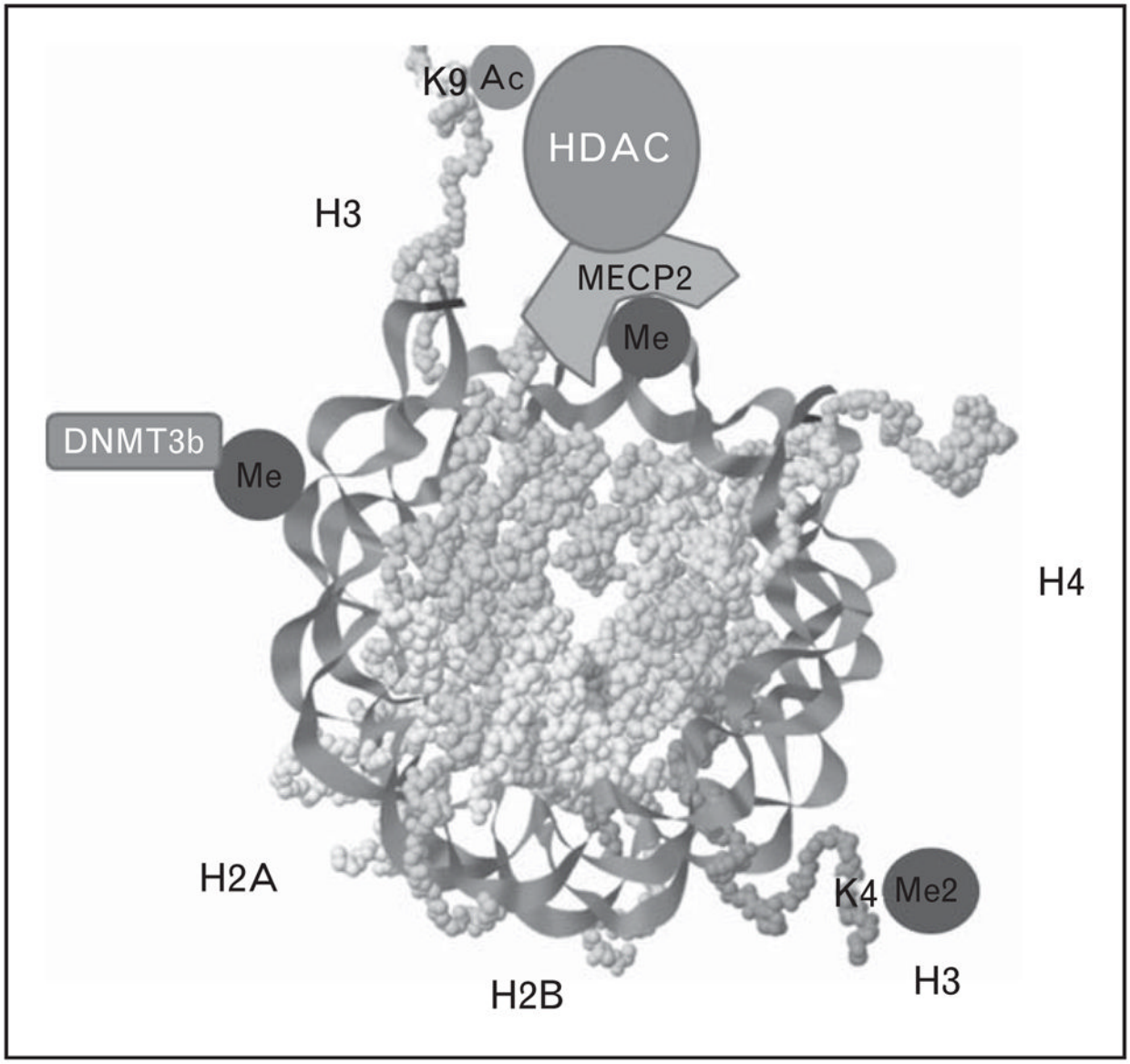

FIGURE 1.

The nucleosome structure. DNA is tightly coiled in nucleosome repeating units (rendering captured with open-source java applet Jmol) composed of 147 base pairs of DNA wrapped around a histone octamer made of two copies of four core histones H2a, H2b, H3, and H4. The transcription of genes is modulated by how tightly the chromatin is packed, and epigenetic marks on histones (at specific lysines such as K9 and K4) and on DNA can modify this chromatin structure (see text). *De novo* methylation of the core nucleosome unit by DNA methyltransferase 3b (DNMT3b) and the mechanism of histone deacetylation by the methyl CpG-binding protein 2–histone deacetylase complex (MECP2–HDAC) are also depicted.

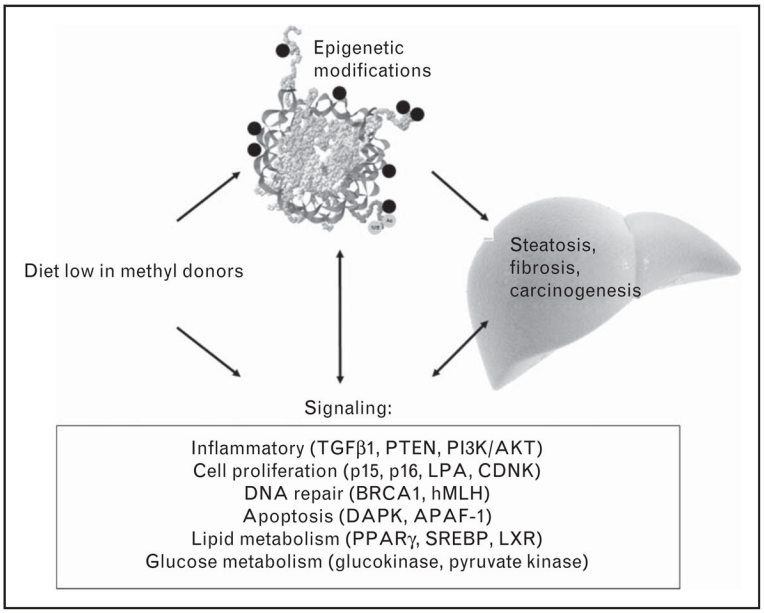

FIGURE 2.

Epigenetic mechanisms modify liver function. Dietary intake of methyl donors such as methionine and choline modifies hepatic inflammatory signaling pathways as well as the epigenetic marks regulating the expression of genes relevant to signaling pathways involved in hepatic steatosis, fibrosis, and carcinogenesis.