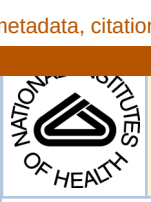

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Dietary Choline Deficiency causes DNA Strand Breaks and Alters Epigenetic Marks on DNA and Histones

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Abstract

Dietary choline is an important modulator of gene expression (via epigenetic marks) and of DNA integrity. Choline was discovered to be an essential nutrient for some humans approximately one decade ago. This requirement is diminished in young women because estrogen drives endogenous synthesis of phosphatidylcholine, from which choline can be derived. Almost half of women have a single nucleotide polymorphism that abrogates estrogen-induction of endogenous synthesis, and these women require dietary choline just as do men. In the US, dietary intake of choline is marginal. Choline deficiency in people is associated with liver and muscle dysfunction and damage, with apoptosis, and with increased DNA strand breaks. Several mechanisms explain these modifications to DNA. Choline deficiency increases leakage of reactive oxygen species from mitochondria consequent to altered mitochondrial membrane composition and enhanced fatty acid oxidation. Choline deficiency impairs folate metabolism, resulting in decreased thymidylate synthesis and increased uracil misincorporation into DNA, with strand breaks resulting during error-prone repair attempts. Choline deficiency alters DNA methylation, which alters gene expression for critical genes involved in DNA mismatch repair, resulting in increased mutation rates. Any dietary deficiency which increases mutation rates should be associated with increased risk of cancers, and this is the case for choline deficiency. In rodent models, diets low in choline and methyl-groups result in spontaneous hepatocarcinomas. In human epidemiological studies, there are interesting data that suggest that this also may be the case for humans, especially those with SNPs that increase the dietary requirement for choline.

Keywords

Choline; DNA; SNP; mitochondria; reactive oxygen species; epigenetics; methylation

Choline is an essential nutrient for most people [1], and in some areas of the world (including the USA) dietary intake is marginal [2]. Dietary choline deficiency is associated with important modifications to the structure of DNA and chromosomes. These effects of choline deficiency result in changes in gene expression, genomic instability and carcinogenesis.

Choline Requirements

Choline is needed to form membranes (phosphatidylcholine and sphingomyelin are choline-containing phospholipids), it is an important methyl-group donor (choline metabolism intersects with folate metabolism at the methylation of homocysteine to form methionine) and it is needed to form the neurotransmitter acetylcholine [3]. Choline can be made available from endogenous synthesis of phosphatidylcholine in the liver [4], and is a part of the diet; the food sources of choline have been comprehensively catalogued by the United States Department of Agriculture (see www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/Choline/Choln02.pdf) [5, 6]. Many of the foods that have high choline content also are high in fats or cholesterol (e.g. eggs) and people are decreasing their intake of these foods such that today only a minority of people achieve the recommended dietary Adequate Intake [1] for choline [2]. A number of studies report that 20–25% of Americans eat only a portion of the recommended adequate intake for choline (<203 mg/d in the Framingham Heart Study [7], <217 mg/d in the Atherosclerosis Risk In Communities study [8, 9] and <293 mg/d in the Nurse's Health Study [10]; Adequate Intake is 450–550 mg/day [1]).

Almost all men and postmenopausal women, when fed a diet low in choline, develop fatty liver, liver damage and/or muscle damage, as well as increased apoptosis in tissues [11, 12], but less than half of premenopausal women develop such organ dysfunction when fed a low choline diet [11]. Premenopausal women have a lower requirement for choline because estrogen induces *PEMT* (phosphatidylethanolamine-*N*-methyltransferase) [13], the gene in liver enabling endogenous biosynthesis of choline moiety (as part of phosphatidylcholine). Postmenopausal women treated with estrogen had a dietary choline requirement similar to that of premenopausal women [14]. However, almost half of women in the USA have one or more single nucleotide polymorphisms (SNPs) in the *PEMT* gene, and this makes them unable to induce this gene with estrogen [15], thereby abrogating much of endogenous production of choline. These women develop organ dysfunction on a low choline diet [16]. There are several other common SNPs in genes of choline and folate metabolism (*PEMT, CHDH, BHMT* and *MTHFD1)* that alter dietary choline requirements in men and women [16, 17].

Our understanding of dietary intake and the factors that influence dietary requirements for choline, suggest that many people have low or marginal intakes of this nutrient. This is important for scientists interested in diet and DNA mutations, as one consequence of this choline insufficiency is alteration of the structure of DNA and histones resulting in DNA strand breaks in humans [12], and in rats [18, 19]. The mechanisms for these strand breaks are not fully elucidated but are probably related to oxidative damage, folate deficiency-related uracil misincorporation, and to altered DNA methylation and subsequent effects on DNA repair processes.

Choline and Oxidative DNA damage

Oxidative damage to DNA, as assessed by the formation of 8-oxodeoxyguanosine [20, 21], apurinic/apyrimidinic sites [22] and Ogg1-sensitive sites [22] in DNA accumulate when rats are deprived of choline. The oxidative stress to DNA is reflected in changes in genes for DNA repair enzymes with significant increases in expression of apurinic/apyrimidinic endonuclease 1 (*Ape*), poly(ADP-ribose) polymerase 1 (*Parp*), and DNA polymerase beta (*Polβ*), 8-oxyguanine DNA glycolase 1 (Ogg1) and O6-methylguanine-DNA methyl transferase [21, 22]. Choline deficiency and resulting liver cell death [23] causes an inflammatory response, and elicited neutrophils and macrophages generate reactive oxygen and nitrogen species [24] that can directly induce DNA base oxidation and deamination.

However, In addition, hepatocytes grown in culture medium low in choline overproduce free radicals in the absence of an inflammatory response [25, 26]. Thus, overproduction of reactive oxygen species (ROS) in choline deficiency is likely due to abnormal mitochondrial function; in rats, choline deficient mitochondria leak large amounts of ROS [25, 27–30]. Excess ROS triggers apoptosis in many tissues in both rodent and human studies [12, 23, 25, 31, 32]. There are a number of mechanisms whereby choline deficiency induces mitochondrial dysfunction and over generation of ROS. Choline deficiency alters the composition of mitochondrial membranes; rats fed low choline diets oxidize cardiolipin in mitochondrial membranes, and have lower phosphatidylethanolamine and phosphatidylcholine concentrations in these membranes [25, 33]. In addition, the phosphatidylcholine in mitochondria consists of species with longer chain fatty acids that are present in controls [34]. These membrane changes are associated with mitochondrial membrane potential decreases [30, 35] and reduced activity of complex I of the respiratory chain (NADH-ubiquinone oxidoreductase) [33, 34] (this can be restored by adding cardiolipin to the choline deficient mitochondria [33]; cardiolipin is required for electron transfer by complex I [36, 37]). Decreased ATP production by mitochondria was also seen in rats fed a choline deficient diet [38], or choline-methionine deficient diet [39]. This could be caused by a proton leak secondary to abnormal membrane function [40] or by abnormally high expression of *UCP2* (uncoupling protein-2) in choline deficient hepatocytes [39]. It is interesting that, in mice, deletion of a gene in choline metabolism (*Chdh*; choline dehydrogenase is an inner mitochondrial leaflet protein that catalyzes conversion of choline to betaine) causes gross distortion of mitochondrial morphology and decreased mitochondrial function including decreased ATP production, uncoupling and abnormal membrane polarization [41].

These changes in mitochondrial function are exacerbated by the changes that choline deficiency causes in metabolism. During choline deficiency, hepatocytes are supplied with large amounts of fatty acids which increase mitochondrial respiratory activity [42] and oxygen utilization [29]. The combination of excess activity in pathways of lipid metabolism with increased generation of ROS forms lipid peroxides in nuclear and mitochondrial membranes [43–45] which are sources of free radicals in the nucleus that modify DNA [46].

Choline and Folate-related DNA damage

Because folate metabolism and choline metabolism are intermingled, perturbing metabolism of choline results in compensatory changes in folate-related metabolic pathways [47–49]. Thus, diets that are low in choline also result in decreased tissue folate [47, 48]. Folate is a cofactor for metabolic pathways that methylate homocysteine, forming methionine, but also folate is a cofactor for the formation of thymidylate needed for DNA synthesis [19, 50, 51]. When thymine is not available, uracil is misincorporated into DNA [19], and subsequent efforts to repair the mistake can result in errors that cause DNA strand breaks to occur at folate-sensitive fragile sites [19, 52–55]. Though this mechanism for strand breaks that occur in choline deficiency remains a possibility, humans who were deprived of choline but supplemented with folic acid still developed strand breaks in DNA [12].

Choline and Epigenetic Marks

Choline is an important source of methyl-groups for synthesis of *S*-adenosylmethionine which is needed for epigenetic marking of DNA and histones. [56, 57]. A choline deficient-low methionine diet in rats results in global hypomethylation of hepatic DNA [58]. Choline deficiency also results in altered methylation status of DNA cytosines, usually at repetitive elements such as cytosine-guanine repeats (CpG islands) in the promoters of specific genes [57, 59]. Choline and methyl deficiency can cause hypomethylation of some genes while

paradoxically hypermethylating certain other genes (including tumor suppressor genes) by increasing the recruitment of methyl-binding proteins to the CpG islands [12, 58, 60, 61]. This occurs in part because of limitation in the availability of *S*-adenosylmethionine, but also because several DNA methyltransferases (*Dnmt1* and *3a*) are regulated via methylation of their promoter regions [62]. Depending on the balance between these two mechanisms near a specific gene, choline deficiency can be associated with reproducible hypo- or hypermethylation of a specific codon in that gene. Choline deficiency also changes the expression and activity of G9a histone methylase with direct consequences for global and gene specific levels of histone methylation [56]. These changes in gene methylation can permanently alter phenotype; in the Agouti mouse, maternal diet (choline, folate and methionine) during pregnancy directly influences the coat color of pups [63]. Similarly, maternal diet during pregnancy determines whther pups have straihght or kinky tails in the *Axin-fused* mouse [64],

Choline influences DNA methylation (see above) which in turn influences genomic stability [65]. As noted above, choline availability modulates expression of *Dnmt1* and *Dnmt3a* [62]. Mice (or cultured cells) with partial loss of function of *Dnmt1* have increased mutation rates (by as much as 10-fold) at specific regions of DNA (microsatellite repeats) [65, 66]. Similarly, genomic stability was decreased in *Dnmt1* single or *Dnmt3a*/*Dnmt3b* double knockout cells [67]. The likely mechanism for these *Dnmt*-associated effects on mutation rates are defects in mismatch repair (MMR) due to decreased levels of protein complexes (MutLα and MutSα) that are needed to complete DNA repair [65]. Choline also influences histone methylation (see above), which in turn is important for the activation of DNA damage response pathways that consist of complex signaling networks that detect and repair DNA damage before the cell divides [68]. Histone methylation also is important in DNA double strand break repair because *53BP1*, which is required for proper homologous recombination, is recruited to sites of DNA damage by methylated histones [69].

Choline and Cancer

Increased mutation rates are usually associated with increased risk for cancer formation. In addition, as discussed earlier, choline modulates epigenetic marking of genes, and choline deficiency is correlated with the silencing of several tumor suppressor genes responsible for DNA repair (*BRCA1, hMLH1*) [21], cell cycle regulation (*p15, p16*) [70] and carcinogen metabolism (*GSTP1*) [71]. Indeed, choline deficiency is one of the few single nutrient deficiencies that causes increased spontaneous carcinogenesis. Rats and mice fed a choline (and methyl) deficient diet first develop fatty liver, progress to liver fibrosis, followed by development of foci of abnormal enzyme-altered hepatocytes that are similar to those induced during initiation of cancer with one of many different chemical carcinogens [72–76]. In choline deficiency these altered foci of hepatocytes, which express γ-glutamyltranspeptidase [77] and the placental form of glutathione S-transferase [78], precede the formation of adenomas and hepatocellular carcinomas [79]. A diet containing 0.8% added choline completely prevents the development of cancer in experimental animals [80]. Choline deficiency also sensitized rodents to hepatic carcinogens such as aflatoxin B1 [81] and breast carcinogens such as dimethylbenz[a]anthracene (DMBA) [81], medroxyprogesterone acetate (MPA) [82] and procarbazine [83]. For example, rats fed a choline-deficient/methionine-low diet were sensitized to aflatoxin B1 with greatly increased hepatocarcinogenesis and reduced time to first tumors [84].

Given the body of research linking dietary choline (methyl) deficiency to increased mutation rates, strand breaks and liver cancer, it is plausible that SNPs that increase human dietary requirements for choline (in *PEMT, CHDH, BHMT* or *MTHFD1*) will alter the risk of cancer. An epidemiological study (The Long Island Breast Cancer Study) suggests that high

dietary choline intake decreases the risk for developing breast cancer and that the *PEMT* rs12325817 SNP was associated with a 30% increased risk of breast cancer mortality (OR: 1.30; 95% CI: 1.01–1.67) while the *BHMT* (betaine homocysteine methyltransferase) rs3733890 polymorphism was associated with reduced breast cancer-specific mortality (hazard ratio, 0.64; 95% confidence interval, 0.42–0.97) [85, 86]. It is interesting that diminished expression of *Pemt2*, one of two PEMT isoforms in rodent liver, has been reported to occur during hepatocarcinogenesis in rats and in cell culture models [87]. Cell proliferation of the hepatoma-derived cell line, McArdle RH777, which normally has low PEMT activity, was suppressed when transfected with *Pemt2* [88]. These hepatoma cells, when transfected with *Pemt2*, failed to form anchorage-independent colonies in soft agar (a property of cancer transformed cells), while the vector-transfected control cells grew efficiently [89]. In addition, hepatocellular carcinomas induced by the chemical carcinogens aflatoxin B1, diethylnitrosamine or methylnitrosourea had diminished *Pemt2* expression and PEMT activity compared with non-tumor liver tissue [87, 89]. This change in *PEMT* expression and activity was also observed in human hepatocellular carcinomas [90]. Collectively, these studies demonstrate an important role for choline metabolism, and specifically PEMT in cancer. While the mechanism for this cancer-promoting effect of choline deficiency is not fully elucidated, an important component is likely to be the modification of DNA and chromosome structure, mistakes in DNA repair and resulting increased mutation rate.

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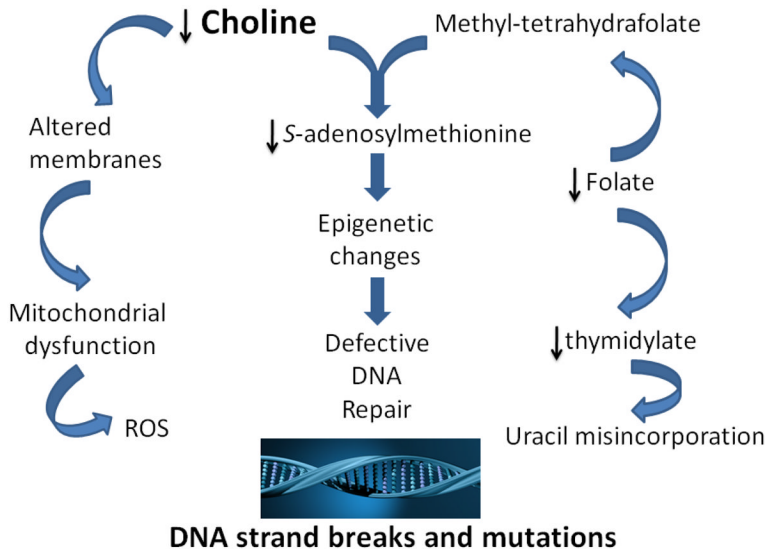

Figure 1. Mechanisms for effects of choline deficiency on DNA strand breaks and mutation

Choline deficiency increases leakage of reactive oxygen species from mitochondria consequent to altered mitochondrial membrane composition and enhanced fatty acid oxidation. Choline deficiency impairs folate metabolism, resulting in decreased thymidylate synthesis and increased uracil misincorporation into DNA, with strand breaks resulting during error-prone repair attempts. Choline deficiency alters DNA methylation, which alters gene expression for critical genes involved in DNA mismatch repair, resulting in increased mutation rates.