

Choline Nutrition Programs Brain Development Via DNA and Histone Methylation

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Abstract: Choline is an essential nutrient for humans. Metabolically choline is used for the synthesis of membrane phospholipids (e.g. phosphatidylcholine), as a precursor of the neurotransmitter acetylcholine, and, following oxidation to betaine, choline functions as a methyl group donor in a pathway that produces S-adenosylmethionine. As a methyl donor choline influences DNA and histone methylation – two central epigenomic processes that regulate gene expression. Because the fetus and neonate have high demands for choline, its dietary intake during pregnancy and lactation is particularly important for normal development of the offspring. Studies in rodents have shown that high choline intake during gestation improves cognitive function in adulthood and prevents memory decline associated with old age. These behavioral changes are accompanied by electrophysiological, neuroanatomical, and neurochemical changes and by altered patterns of expression of multiple cortical and hippocampal genes including those encoding key proteins that contribute to the biochemical mechanisms of learning and memory. These actions of choline are observed long after the exposure to the nutrient ended (months) and correlate with fetal hepatic and cerebral cortical choline-evoked changes in global- and gene-specific DNA cytosine methylation and with dramatic changes of the methylation pattern of lysine residues 4, 9 and 27 of histone H3. Moreover, gestational choline modulates the expression of DNA (*Dnmt1*, *Dnmt3a*) and histone (*G9a/Ehmt2/Kmt1c*, *Suv39h1/Kmt1a*) methyltransferases. In addition to the central role of DNA and histone methylation in brain development, these processes are highly dynamic in adult brain, modulate the expression of genes critical for synaptic plasticity, and are involved in mechanisms of learning and memory. A recent study documented that in a cohort of normal elderly people, verbal and visual memory function correlated positively with the amount of dietary choline consumption. It will be important to determine if these actions of choline on human cognition are mediated by epigenomic mechanisms or by its influence on acetylcholine or phospholipid synthesis.

Keywords: Brain, choline, DNA, histone, memory, methylation, nutrition, pregnancy.

INTRODUCTION

Choline was first described in 1862 by Strecker [1], who found it in bile, and used the Greek word for bile to name the compound. Determination of the chemical structure of choline and its first chemical synthesis was performed shortly thereafter [2] and in 1868 Strecker found that choline was a part of the molecule of phosphatidylcholine. We now know that phosphatidylcholine is the most abundant structural component of biological membranes. Choline also serves as the precursor of the neurotransmitter, acetylcholine (ACh). The fascinating history of discovery of physiological functions of ACh spans many decades and includes the first demonstration of chemical neurotransmission [see refs. [3, 4] for the 1936 Nobel lectures by Otto Loewi and Sir Henry Dale]. In 1937 Mann and Quastel described the process of enzymatic oxidation of choline to betaine aldehyde and betaine [5] and in 1946 du Vigneaud *et al.*, showed that betaine serves as a donor of metabolic methyl groups, and suggested that the process of methyl group transfer is indirect and occurs via a methionine intermediate [6].

Indeed, in 1953 Cantoni determined the structure of such an intermediate as S-adenosylmethionine (AdoMet) [7]. Most methylating enzymes use AdoMet as the donor of methyl groups [8, 9] generating a methylated product (e.g. 5-methylcytosine in DNA [10] or N-methylated lysine in a histone protein [11]) and S-adenosylhomocysteine (AdoHcy) [8, 9]. This metabolic relationship between choline and DNA methylation is illustrated in Fig. (1). The essential function of dietary choline in preserving normal health in mammals was recognized already in the 1930s initially in the context of studies on pancreas and insulin. It was noted that dogs with surgically removed pancreas, but treated with insulin, developed fatty liver and later died. Feeding with raw pancreas prevented liver disease [12] and the active ingredient of this treatment was subsequently identified as choline [13-15] by, among others, one of the discoverers of insulin, Charles Herbert Best [14, 15].

CHOLINE, AN ESSENTIAL NUTRIENT FOR HUMANS

Remarkably, we had to wait another half a century for the definitive proof that choline was an essential nutrient for humans. In a 1991 rigorous clinical study, Zeisel *et al.*, placed a group of healthy men on a low choline diet and found that they developed liver damage that could be

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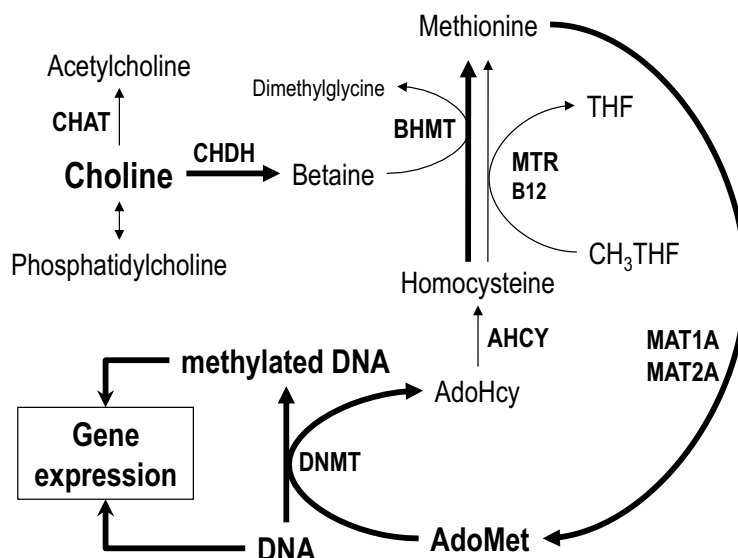


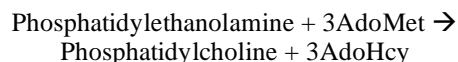
Fig. (1). Choline and methyl group metabolism and its relation to DNA methylation and influence on regulation of gene expression: a simplified diagram. Choline is used as a precursor of phosphatidylcholine, acetylcholine [in a reaction catalyzed by choline acetyltransferase (CHAT)], or betaine [in a reaction catalyzed by choline dehydrogenase (CHDH)]. The methyl groups of betaine are used by betaine: homocysteine S-methyltransferase (BHMT) to regenerate methionine from homocysteine. In an alternative pathway, catalyzed by vit. B12-requiring 5-methyltetrahydrofolate-homocysteine S-methyltransferase (MTR), methyltetrahydrofolate (CH_3THF) is used as a methyl donor. Methionine is used as a precursor of S-adenosylmethionine (AdoMet) in a reaction catalyzed by methionine adenosyltransferase(s) (MAT1A, MAT2A or MAT2B). AdoMet is used by multiple methylating enzymes including DNA methyltransferases (DNMT1, DNMT3A, DNMT3B) that use AdoMet as a donor of methyl groups to methylate DNA at the 5-position of cytosine residues within the CpG sequences. The DNA methylation state and pattern exerts a modulatory influence on expression of multiple genes (see text). The second product of this, and all other AdoMet-requiring methylation reactions, S-adenosylhomocysteine (AdoHcy) is hydrolyzed to free homocysteine by AdoHcy hydrolase (AHCY). The metabolic pathway linking choline to DNA methylation is indicated by thick arrows.

reversed with a standard dose of dietary choline [16]. This landmark discovery served as the initial basis for the classification of choline as an essential nutrient in 1998 when the Food and Nutrition Board (FNB) of the Institute of Medicine of the National Academy of Sciences of the United States of America issued guidelines on its daily intake [17]. Because at that time, and perhaps to this day, there were insufficient data to generate Recommended Daily Allowance values, the FNB issued Adequate Intake (AI) recommendations (Table 1). The AI calls for the average intake of 7.5 mg of choline daily per kg of body weight. Notably, the AI is increased for pregnant and breast feeding women in order to satisfy the needs of the fetus and baby whose choline is supplied via placenta [18] and milk [19, 20], respectively. The AI values were established primarily to ensure that dietary choline is sufficient to prevent liver dysfunction associated with low choline consumption observed in adult men [16] (see above). Subsequent studies have shown that choline deficiency also causes muscle damage [21] and induces apoptotic death of lymphocytes [22].

NUTRIGENETICS AND NUTRIGENOMICS OF CHOLINE IN HUMANS

Since the publication of the FNB report, new research data indicate that even in a developed country like the United States, choline nutrition may be low among surprisingly large segments of population [23] and that additional factors including sex, age, and genotype influence the individual's

requirements for choline. These factors constitute the purview of three relatively new sub disciplines in the science of nutrition and genetics, namely: 1) nutrigenetics – how the genome of an organism governs its response to a nutrient; 2) nutrigenomics – how nutrients interact with the genome to modulate gene expression patterns; and 3) nutrieepigenomics – how a nutrient influences the epigenome and thus modulates gene expression patterns. Recent observations in the area of choline nutrigenetics showed that non-pregnant, non-nursing, women of childbearing age are relatively resistant to choline deficiency as compared to men and older, postmenopausal women [24]. This is explained by the apparently upregulated endogenous biosynthesis of choline (in the form of phosphatidylcholine) in younger women catalyzed by the hepatic enzyme phosphatidylethanolamine N-methyltransferase (PEMT). PEMT activity constitutes the sole mechanism whereby mammals can synthesize choline *de novo*. Note, that the reaction proceeds as follows:



Thus, AdoMet can be produced from choline via betaine and methionine, as described above Fig. (1), but it can also be consumed to generate new choline molecules. Interestingly, it was the presence of PEMT activity in humans that served as the key argument against including choline on the list of essential nutrients. We now know from studies in mice that PEMT is vitally important but its activity is insufficient to create adequate amounts of choline for the maintenance of normal health [25-27]. Remarkably,

expression of the *PEMT* gene is increased by estrogens. In younger women, high levels of circulating estrogen are responsible for inducing and maintaining high *PEMT* activity, and thus rendering the women relatively insensitive to low-choline diets [28, 29]. This, apparently adaptive genomic mechanism, may buffer both the mother and her offspring during gestation and lactation from the detrimental effects of short supply of dietary choline. In contrast, postmenopausal women are sensitive to low-choline diets and deplete their choline pools in a similar fashion to men.

Table 1. Choline Adequate Intake (AI) (mg/day)

Life Stage	Age	Females	Males
Infants	0-6 months	125	125
Infants	7-12 months	150	150
Children	1-3 years	200	200
Children	4-8 years	250	250
Children	9-13 years	375	375
Adolescents	14-18 years	400	550
Adults	19 years and older	425	550
Pregnancy	All ages	450	-
Breast-feeding	All ages	550	-

There are several polymorphic genes that affect humans' requirement for choline, including certain alleles of *PEMT* [29, 30]. One of those alleles (polymorphism rs12325817) apparently renders the *PEMT* gene unresponsive to estrogen conferring high risk of development of choline deficiency-associated liver dysfunction in women carriers on normal diets [31, 32]. Additional genetic polymorphisms that modify choline requirements include alleles of 5,10-methylenetetrahydrofolate dehydrogenase (*MTHFD1*) [33], methyltetrahydrofolate reductase (*MTHFR*) [34-36] and choline dehydrogenase (*CHDH*) [29]. Moreover, it is possible to use metabolomic "signatures" in plasma to identify prospectively individuals at risk of developing liver or muscle dysfunction caused by choline deficiency [37]. Taken together, these findings point to a significant heterogeneity of choline metabolism, and thus dietary needs for choline, among humans.

The establishment of the United States Department of Agriculture (USDA) Database for the Choline Content of Common Foods [38] – one of the most valuable resources for epidemiological studies on choline nutrition – has helped to understand this heterogeneity in the metabolism of choline. Recent studies using this tool found that high intake of choline and betaine during pregnancy was associated with lowered risk of orofacial clefts in infants [39]. Moreover, polymorphisms in genes encoding betaine:homocysteine methyltransferase (*BHMT*) Fig. (1) and CTP:phosphocholine cytidyltransferase (*PCYT1*) (the rate-limiting enzyme in phosphatidylcholine synthesis from choline) were associated with altered risk of orofacial clefts [40], further supporting

the role of choline metabolism and nutrition in this developmental disorder. The use of the USDA database also revealed that women in the highest quintile of choline and betaine consumption as adults had reduced risk of breast cancer [41] and that high betaine intake lowers the risk of colorectal adenoma in women [42] and of esophageal cancer in both men and women [43]. Moreover, high choline consumption during pregnancy reduced the risk of neural tube defects in offspring [44, 45]. These studies are the first to provide evidence for a preventive action of choline in human carcinogenesis and for the significance of choline nutrition during pregnancy for normal development of the human central nervous system. The latter conclusion is supported by the findings that polymorphisms in genes encoding two enzymes of phosphatidylcholine synthesis, *PCYT1A* and choline kinase A (*CHKA*) modify the risk of neural tube defects [46].

CHOLINE NUTRITION AND PROGRAMMING OF BRAIN DEVELOPMENT AND COGNITIVE FUNCTION IN ANIMAL MODELS

In rodent models, maternal choline consumption has profound and long-term effects on brain development and cognition in offspring. Choline deficiency during pregnancy inhibits fetal cell proliferation in the hippocampus and stimulates apoptosis in this brain region [47, 48]. In contrast, gestational choline supplementation stimulates hippocampal cell division [49]. Moreover, in a commonly used model that employs the offspring of pregnant mice or rats consuming diets of varying choline content during only a one week period of the second half of gestation (embryonic days 11-17; pregnancy in mice and rats lasts 19-21 and 20-22 days, respectively), choline deficiency causes impairments in certain memory tasks [50], whereas choline supplementation improves memory and attention [50-57] and, remarkably, prevents age-related memory decline [50, 57]. The two salient conclusions from these studies are: **1.** choline nutrition during gestation programs brain development and cognitive ability, and **2.** cognitive decline is not an inevitable outcome of old age, but rather can be prevented by increased supply of choline during a critical period of prenatal development.

The above-noted structural changes in prenatal brain subsequently translate into neuroanatomical, neurochemical, electrophysiological, and molecular differences in the adult and aged animal. Certain aspects of learning and memory require continual production of new neurons that occurs in the dentate gyrus of the hippocampus throughout the lifetime [58, 59]. Prenatal choline supplementation enhances adult neurogenesis in the dentate gyrus while prenatal choline deficiency impairs this process [60-62]. Moreover, the effect of choline supplementation was seen even in aged rats and correlated with a highly trophic microenvironment within the hippocampus of the prenatally choline supplemented rats that included elevated concentrations of brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), insulin-like growth factor I (IGF1), IGF2, and vascular endothelial growth factor (VEGF) in these animals as compared to controls [60-64]. Prenatal choline supplementation increases the size of the basal forebrain cholinergic neurons [65] that are critical for the processes of

learning and memory [66, 67], and augments ACh synthesis and release from these neurons [57, 68]. In addition, prenatal choline availability alters the activation levels of essential molecular components of memory processing [69], such that phosphorylation of hippocampal mitogen-activated protein kinase (MAPK) and cAMP response element binding protein (CREB) in response to stimulation by glutamate, *N*-methyl-D-aspartate (NMDA), or depolarizing concentrations of potassium is increased by prenatal choline supplementation and reduced by prenatal choline deficiency [51]. Concordant changes were observed in hippocampal electrophysiological synaptic plasticity measures termed long-term potentiation (LTP). Prenatal choline supplementation enhanced hippocampal LTP in the CA1 region by decreasing the stimulus intensity required for LTP induction [70, 71], possibly as a result of an augmented NMDA receptor-mediated neurotransmission [72]. Mellott *et al.*, [73] analyzed gene expression patterns in brains of prenatally choline-deficient, choline-supplemented, and control rats using microarrays and found 530 hippocampal and 815 cerebral cortical mRNA species whose levels were modulated by prenatal choline status. The protein products of several of these genes are known to participate in signaling pathways implicated in memory processes and thus may mediate the observed choline-induced changes in LTP and behavior. The list of these genes includes *Igf2*, protein kinases *Prkcb2*/PKC β 2, *Camk1*, and *Camk2b*/CaMKII β , a receptor for the neurotransmitter γ -aminobutyric acid (*Gabbr1*/GABA $_B$ R1), and a transcription factor *Zif268* (*Egr1*) [73]. These long-term actions on brain structure and function of altered availability of choline during gestation suggest that they may be mediated by epigenetic mechanisms that include DNA- and histone methylation.

4. DNA METHYLATION

Recent advances in the field of epigenetics have provided the conceptual and experimental framework that helps to explain how some phenotypes can be transmitted following

cell mitosis or across generations in complex organisms, including mammals, without a change in primary DNA sequence. The central molecular mechanism that permits this type of inheritance is methylation of DNA at the 5-position of cytosine residues within CpG sequences to form 5-methylcytosine (5mC) Fig. (2). The transcription of genes whose regulatory elements are methylated tends to be different than when the same regions are not methylated due to a concerted change in the interaction of those elements with a complex network of proteins, including transcription factors (see below). This change results in altered phenotype governed by DNA methylation. The pattern of DNA methylation can be propagated through cell divisions because, after DNA replication, the unmethylated daughter strand in hemimethylated DNA becomes symmetrically methylated by the enzyme DNA methyltransferase 1 (DNMT1). The process of DNA methylation is dynamic [74] and responds to the environment, including the availability of nutrients. In particular, DNA methylation is modulated by the availability of nutrients that serve as methyl group donors and cofactors, such as choline, betaine, methionine, folic acid and vitamin B12 Fig. (1). This effect is explained by the direct relationship between dietary intake of choline (and/or other methyl groups) and tissue levels of AdoMet that is frequently observed [75-82]. In proof-of-concept studies it has been shown that very high dietary intake of these compounds in pregnant mice can modify methylation and expression of certain genes and result in modified phenotypes of the offspring [83-88]. Moreover, some of these effects can be transmitted to successive generations [88, 89]. These relatively recent discoveries, however, are based on a long history of investigations of the only nutritional deficiency that causes cancer, namely liver tumors (hepatocarcinoma) in rats fed a choline-deficient diet [90-96]. While many hypotheses for the mechanism of this effect had been advanced, one included the idea that dietary choline (or methyl group) deficiency may result in altered methylation of DNA and thus altered expression of proto oncogenes and tumor suppressor genes because dysregulated

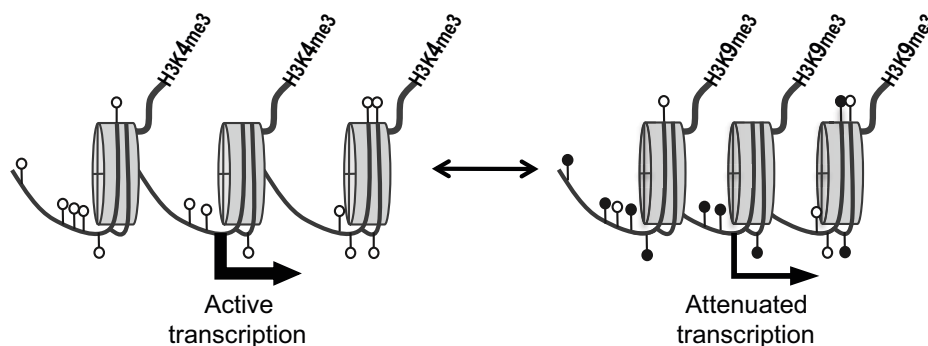


Fig. (2). Methylation of DNA and histone H3 modifies chromatin state and regulates transcription. DNA is wrapped around nucleosomes that are composed of a histone octamer containing two molecules of H3. On the left cytosines within CpGs of DNA are unmethylated (white lollipops) and lysine 4 of the H3 N-terminal tail (thick line above the nucleosomes) is methylated (H3K4me3). Under these conditions transcription tends to be active (thick arrow). On the right, DNA has become more methylated (black lollipops) – initially due to the *de novo* process catalyzed by the DNMT3 enzymes and subsequently maintained in the cell lineage by DNMT1, and H3 is methylated on lysine 9 (H3K9me3). Under these conditions transcription is attenuated (thin arrow). Additional processes, including histone deacetylation, attachment of methylated DNA binding proteins (e.g. MECP2) also contribute to this transcriptional repression and chromatin compaction (illustrated on the right by closer packing of the nucleosomes). The two states are reversible.

DNA methylation is a common pathophysiological feature of carcinogenesis [97-100]. Indeed, abnormalities in DNA methylation have been observed in the preneoplastic lesions and in liver tumors of choline- and methyl-group deficient rats [101-114].

DNA methylation is also important for such regulatory mechanisms of gene expression as X chromosome inactivation, cell differentiation and chromatin structure, the expression changes that occur during aging and genomic imprinting [115-119]. There is some evidence that the latter process may be modified by choline/methyl group availability [83, 120]. In general, CpG methylation causes transcriptional repression [121-129] by recruiting specific proteins (MECP2, MBD1, MBD2, and MBD3) that bind to the methylated DNA via their methylated-CpG binding domains (MBD) [130]. Upon binding to methylated CpG sites, they further recruit transcriptional corepressors and histone deacetylases. Deacetylated nucleosomes are tightly packed which prevents the access of transcriptional activators to their binding sites in the DNA [reviewed in [122, 125, 131]. CpG methylation also contributes to transcriptional repression by specifically preventing transcriptional regulators from binding to their target gene promoters. For example cMyb [132], E2F [133], CREB [134], AP2 [135], NF- κ B [136], and STAT3 [129], cannot bind to methylated forms of their DNA recognition sequences. However, DNA methylation may also upregulate transcription by preventing the binding of a transcriptional repressor to a genomic silencer element, as is the case for *Igf2* gene [137, 138]. DNA methylation is catalyzed by a family of DNA methyltransferase enzymes. The mammalian brain has high levels of expression of DNA methyltransferase 1 (DNMT1) both during development and in adulthood [139-142] and the degree of DNA methylation is higher in the adult brain than in other tissues [143-145]. Moreover, DNA methylation levels in mouse brain undergo dynamic changes perinatally [143], suggesting that DNA methylation is necessary for the differentiation process of the brain. In human brain DNA methylation for most genomic loci tends to increase with age with some loci becoming hypermethylated during childhood, while others after the age of 50, and still others becoming monotonically hypermethylated throughout life until advanced age [146, 147]. Human neurons have a distinct DNA methylation pattern as compared to non-neuronal brain cells and this neuronal DNA methylation pattern is characterized by a large individual variation [148]. Interestingly the expression of genes encoding enzymes of methyl group metabolism and DNA and protein methylation undergoes dramatic changes during brain development as illustrated in Fig. (3), depicting the developmental pattern of their mRNA expression in rat frontal cortex. Note, that the expression of DNA methyltransferase, *Dnmt1*, and of protein methyltransferases, *G9a/Ehmt2/Kmt1c* (that dimethylates histone H3 on lysine 9; H3K9 to H3K9me2 and trimethylates H3K27 to H3K27me3 [149],) and *Prmt1* (that methylates arginine residues in multiple protein substrates [150]) are high in mid-gestation and decline rapidly during the latter part of fetal development. A similar pattern of *Dnmt3a* and *Dnmt3b* expression in mouse brain has been reported [151]. The maturation of mRNA expression of two genes encoding

enzymes that provide metabolic support for the processes of DNA and protein methylation by synthesizing AdoMet (i.e. *Mat2a*) and the hydrolysis of its demethylated metabolite, AdoHcy (i.e. *Ahcy*), follows a similar pattern.

As noted above, DNA methylation patterns acquired during development may be inherited through the cell divisions in a process catalyzed by DNMT1 that methylates hemimethylated CpG sites and thus restores the parental methylation pattern on the daughter DNA strand following DNA replication [152]. In addition, DNMT3A and DNMT3B generate the DNA methylation patterns *de novo* during development and in adulthood [153, 154]. The observed effects of choline- or methyl group deficiency on global- and gene-specific DNA methylation in multiple *in vivo* and *in vitro* models vary: several studies reported hypomethylation [101, 108, 155-163], some no change [164], and some hypermethylation [165-168]. It is noteworthy however, that only some CpG sites respond to the altered availability of methyl groups, suggesting a degree of specificity of this response, that heretofore remains unexplained. Together the results indicate that DNA methylation may respond to the supply of methyl groups in a complex fashion that includes alterations in the activities of DNA methylating and/or demethylating enzymes. Indeed, diet can influence the expression of DNMTs. For example, Fischer male rats fed a methyl-group deficient diet for at least 3 weeks display global DNA hypomethylation and increased expression and activity of cellular DNMTs, (DNMT1 and DNMT3A) possibly as a compensatory mechanism that offsets methyl deficiency [169].

The hypothesis that choline intake by pregnant rats might alter DNA methylation and DNMT expression in the fetus was tested in a study by Kovacheva *et al.*, [82] who measured these parameters in liver and cerebral cortex on embryonic day 17 in rats following altered dietary supply of choline that had commenced on gestational day 11. The investigators focused on the differentially methylated region 2 (DMR2) of the *Igf2* gene because the DMR2 methylation signature changes dramatically during development [170]. Choline-deficient embryos had higher degree of methylation as compared to the control and choline-supplemented rats. One possible mechanism that leads to changes in the global, as well as gene-specific, DNA methylation is via alteration in the activity of DNMTs. It has been shown that DNMT1 is important for maintaining the methylation pattern of the *Igf2* gene and *Dnmt1* knockout mice have abnormal expression of *Igf2* [171]. In liver of choline-deficient embryos, *Dnmt1* mRNA was overexpressed by more than 50% as compared to control and choline-supplemented embryos. The data suggested that maternal choline deficiency causes an apparently compensatory induction of *Dnmt1* expression in the fetus that prevents the loss of DNA methylation when choline is in short supply. While the focus on the *Igf2* gene was driven by the data showing that IGF2 expression in developing and adult brain was governed by prenatal choline intake [64, 73, 82] and by extensive literature on its regulation by DNA methylation [137, 138, 172], IGF2 has recently emerged as a critical component of memory consolidation mechanisms [173] suggesting that high IGF2 levels observed in brains of prenatally choline-supplemented

rats [64, 73] may be part of the mechanism of cognitive enhancement that characterizes these animals.

As noted above, DNA methylation is a dynamic process. Whereas in dividing cells loss of DNA methylation can be accomplished by the failure of DNMT1 to copy the methylation pattern of the mother DNA strand onto the daughter strand, in postmitotic cells, such as neurons, demethylation requires an enzymatic pathway. Recent studies suggest that this process entails initial obligatory conversion of 5mC to 5-hydroxymethylcytosine (5hmC) catalyzed by the Ten-eleven translocation (Tet) family of methyl dioxygenases (TET1, TET2, and TET3) that require 2-oxoglutarate and iron Fe(II) [174-176]. The subsequent enzymatic steps are poorly understood. There is evidence that, in the brain, the activation-induced deaminase (AID)/apolipoprotein B mRNA-editing enzyme complex (APOBEC) family of cytidine deaminases and base excision repair pathway is operational [177]. However, the stepwise oxidation of 5mC to 5-formylcytosine (5fC) and subsequently to 5-carboxylcytosine (5caC) catalyzed by the TET enzymes has also been observed [178, 179] and 5caC could be simply decarboxylated to cytosine in a fashion analogous to the conversion of thymine to uracil [178]. Alternatively 5caC could be processed by thymine-DNA glycosylase in base excision repair pathway [179]. Functionally, the formation of 5hmC may be as significant as demethylation because methylated DNA binding proteins including MECP2 [180] MBD1, MBD2 and MBD4 [181] bind poorly to 5hmC. The dynamic DNA methylation-hydroxymethylation-demethylation pathway is only beginning to be explored. It is important to recognize that the current widely-used general sequencing methods (e.g. sequencing of bisulfite-converted DNA) do not distinguish DNA modified by 5mC from 5hmC [182]. Thus, the body of knowledge on DNA methylation, that has accumulated since the invention of the bisulfite-sequencing technique in 1992 [183], has to be considered as inexact. The development of new methods (e.g. [184]) is important in view of recent studies showing a significant amount of 5hmC in brain DNA [182, 185-187], its increase during postnatal maturation [188], and dynamic changes in DNA 5hmC content regulated by neuronal activity [177].

5. HISTONE METHYLATION

The methylation of histones at specific lysine and arginine residues is essential for the epigenetic regulation of transcription, cell division, and the formation of heterochromatin [189-191] because differentially-modified histones within nucleosomes of a genomic region constitute one of the central modes of regulation of transcriptional activity of the region [192]. The addition of methyl groups to histones exerts different effects depending on what residue is methylated and, for a lysine residue, how many methyl groups are added to the epsilon nitrogen atom. Typically, in the case of histone 3 (H3), di- or trimethylation of lysine 9 (H3K9me2 and H3K9me3) and trimethylation of lysine 27 (H3K27me3) inhibit transcription [193-199]. In contrast, trimethylation of lysines 4, 36 or 79 (H3K4me3, H3K36me3 and H3K79me3, respectively) is associated with transcriptionally active genes as is monomethylation of histone 4 on lysine 20 and histone 2B on lysine 5 (H4K20me

and H2BK5me) [195-202]. There are several enzymes that catalyze these modifications; all use AdoMet as the methyl donor [11]. For example, histone methyltransferase G9a (KMT1C, EHMT2) is responsible for di-methylation of H3K9 to H3K9me2 and for tri-methylation of H3K27 to H3K27me3 [149], and histone methyltransferase Suv39h1 (KMT1A) responsible for tri-methylation at H3K9 to H3K9me3 [203]. Therefore, in addition to modulating DNA methylation, choline supply could affect the methylation of amino acid residues on histone tails [204, 205] leading to alterations in the expression of genes involved in growth and development. [Note dramatic changes in *G9a* and *Prmt1* mRNA expression in the developing rat cortex, Fig. (3)]. Davison *et al.*, [206] examined several components of the histone 3 methylating machinery in rat liver and cerebral cortex on embryonic day 17 of rat fetuses derived from mothers consuming varying amounts of choline. The methylation of H3K9 and H3K27 and expression of *G9a* and *Suv39h1* were directly related to the availability of choline. Similar results were obtained in a mouse model [168]. Consistent with the studies of Kovacheva *et al.*, [82] described above, DNA methylation of the *G9a* and *Suv39h1* genes was dramatically upregulated by choline deficiency [206]. The latter finding points to the possibility that the expression of these histone methyltransferases is under negative control of methylation of their genes.

CONCLUSIONS AND FUTURE DIRECTIONS

Choline nutrition during gestational period in rodents influences brain development and cognitive function throughout lifespan and high choline intake during gestation has emerged as a robust and reliable cognitive enhancing regimen. Moreover, high choline intake during the perinatal period is neuroprotective in a variety of animal models of neuronal damage, including that evoked by seizures [63, 207-209], alcohol consumption [210-215] and genetic mutations [216-222]. Data showing that maternal choline supply during pregnancy modifies fetal histone and DNA methylation suggest that a concerted epigenomic mechanism contributes to the long-term developmental effects of varied choline intake *in utero* [82, 159, 168, 206, 223]. In addition to the central role of DNA and histone methylation in brain development, these processes are highly dynamic in adult brain and there is considerable evidence that they modulate the expression of key genes of synaptic plasticity [224-228] and are involved in mechanisms of learning and memory [229-232]. Thus, it is likely that choline nutrition would have modulatory effects on DNA and histone methylation in adult brain and that it could influence cognitive function in adulthood via an epigenetic mechanism as well. At present this idea remains to be tested experimentally. Importantly however, a recent study performed on 1391 normal adult and elderly people (average age 61 years) showed that verbal and visual memory function correlated positively with the amount of dietary choline consumption, with poorest performance in individuals with lowest choline intake and best performance in those who were consuming the highest amounts of choline [233]. These data further support the notion that adequate choline nutrition is essential for the maintenance of cognitive function in people.

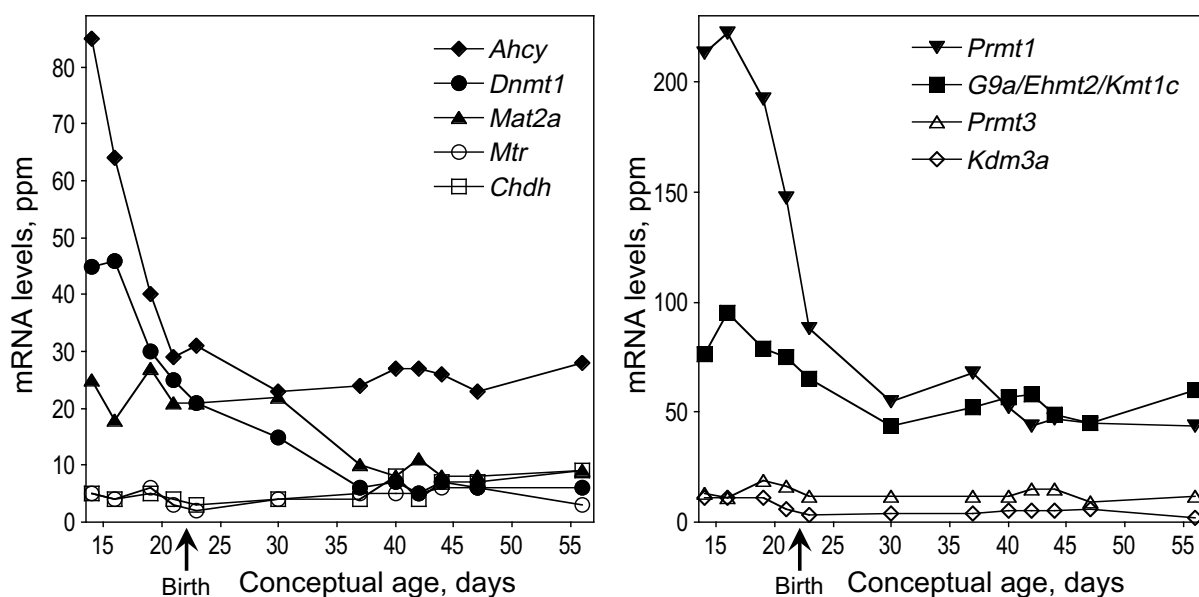


Fig (3). Developmental pattern of expression of selected choline- and methylation-related genes (see Figure 1) in rat frontal cortex. Cortical RNA was analyzed using Affymetrix RG_U34A microarrays as described [73]. Left panel: *Chdh*, choline dehydrogenase; *Mtr*, 5-methyltetrahydrofolate-homocysteine S-methyltransferase; *Mat2a*, methionine adenosyltransferase 2a; *Dnmt1*, DNA methyltransferase 1; *Ahcy*, S-adenosylhomocysteine hydrolase. Right panel: *Kdm3a/Imjd1a*, lysine (K)-specific demethylase 3A; *Prmt3*, protein arginine N-methyltransferase 3; *G9a/Ehmt2/Kmt1c*, euchromatic histone-lysine N-methyltransferase 2; *Prmt1*, protein arginine N-methyltransferase 1. X axis on the graph corresponds to days post-conception. The day of birth is indicated by an arrow. Postnatal days 1, 8, 15, 18, 22, and 34 are conceptual ages 23, 30, 37, 40, 44, and 56, respectively.

CONFLICT OF INTEREST

None declared.

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ABBREVIATIONS

5caC	= 5-carboxylcytosine
5mC	= 5-methylcytosine
5fC	= 5-formylcytosine
5hmC	= 5-hydroxymethylcytosine
ACh	= Acetylcholine
AdoHcy	= S-adenosylhomocysteine
AdoMet	= S-adenosylmethionine
AHCY	= AdoHcy hydrolase
AI	= Adequate intake
BDNF	= Brain-derived neurotrophic factor
BHMT	= Betaine:homocysteine S-methyltransferase
CaMKI	= Calcium/calmodulin-dependent protein kinase I

CaMKII β	= Calcium/calmodulin-dependent protein kinase II beta
CHAT	= Choline acetyltransferase
CHDH	= Choline dehydrogenase
CHKA	= Choline kinase A
CREB	= cAMP response element binding protein
DMR	= Differentially methylated region
DNMT	= DNA methyltransferases
FNB	= Food and Nutrition Board
G9a	= (EHMT2, KMT1C) euchromatic histone lysine N-methyltransferase 2
GABA	= γ -aminobutyric acid
H3	= Histone 3
IGF	= Insulin-like growth factor
LTP	= Termed long-term potentiation
MAPK	= Mitogen-activated protein kinase
MAT	= Methionine adenosyltransferase
MECP2	= Methyl-CpG-binding protein 2
MBD	= Methyl-CpG-binding domain protein
MTHFD1	= 5,10-methylenetetrahydrofolate dehydrogenase 1

MTHFR	= Methyltetrahydrofolate reductase
MTR	= 5-methyltetrahydrofolate-homocysteine S-methyltransferase
NGF	= Nerve growth factor
NMDA	= N-methyl-D-aspartate
PCYT1	= CTP:phosphocholine cytidyltransferase 1
PEMT	= Phosphatidylethanolamine N-methyltransferase
PKC β 2	= Protein kinase C beta-2
PRMT	= Protein arginine N-methyltransferase
Suv39h1	= (KMT1A) suppressor of variegation 3-9 homolog 1
TET	= Ten-eleven translocation
USDA	= United States Department of Agriculture
VEGF	= Vascular endothelial growth factor
Zif268 (EGR1)	= Early growth response

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