HHS Public Access

Author manuscript

Clin Chem Lab Med. Author manuscript; available in PMC 2017 September 25.

Published in final edited form as:

Clin Chem Lab Med. 2013 March 01; 51(3): 591–599. doi:10.1515/cclm-2012-0635.

Neuroprotective actions of perinatal choline nutrition

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Abstract

Choline is an essential nutrient for humans. Studies in rats and mice have shown that high choline intake during gestation or the perinatal period improves cognitive function in adulthood, prevents memory decline of old age, and protects the brain from damage and cognitive and neurological deterioration associated with epilepsy and hereditary conditions such as Down's and Rett syndromes. These behavioral changes are accompanied by modified patterns of expression of hundreds of cortical and hippocampal genes including those encoding proteins central for learning and memory processing. The effects of choline correlate with cerebral cortical changes in DNA and histone methylation, thus suggesting an epigenomic mechanism of action of perinatal choline.

Keywords

brain; choline; DNA methylation; memory; nutrition; pregnancy; epilepsy; Down's syndrome; Rett syndrome

Choline, an essential nutrient for humans

In 1998 the Food and Nutrition Board (FNB) of the Institute of Medicine of the National Academy of Sciences of the United States of America issued a report entitled "Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline" that for the first time included choline as an essential nutrients for humans among other water-soluble vitamins (1). Because 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. Given the high nutritional needs for pregnant and breast feeding women, the AI is increased for them in order to satisfy the requirements of the fetus and baby whose choline is supplied via placenta (2) and milk (3, 4), 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 (5). Subsequent studies have shown that choline deficiency also causes muscle damage (6) and induces apoptotic death of lymphocytes (7). Since the issuance of the FNB report, the establishment of the United States Department of Agriculture (USDA) Database for the Choline Content of Common Foods (8) – has become one of the most valuable resources for

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epidemiological studies on choline nutrition and has helped to investigate the relationship between choline nutrition and disease. A common finding in such studies is the realization that even in an affluent country like the United States the majority of people consume less choline than the AI value (9–12) (Fig. 1). The use of the USDA database also revealed that women in the highest quintile of choline and its metabolite, betaine, consumption as adults had reduced risk of breast cancer (10) and that high betaine intake lowers the risk of colorectal adenoma in women (13) and of esophageal cancer in both men and women (14). Moreover, high choline consumption during pregnancy reduced the risk of neural tube defects in offspring (15, 16). The latter studies were the first to provide evidence for the significance of choline nutrition during pregnancy for normal development of the human central nervous system. In this minireview we summarize the results of studies in animal models on the significance of choline nutrition in early development on brain function later in life. The overall message from these investigations is that high choline intake during the perinatal period is neuroprotective in a variety of animal models of neuronal dysfunction, including that evoked by aging (17–19), seizures (20–23), alcohol consumption (24–29) and genetic variation (30–36).

Choline nutrition and cognitive function: protection against age-related memory decline and advancement of hippocampal development

In rats, high maternal choline consumption during pregnancy has profound and long-term cognitive enhancing effects in offspring (19, 37–47). Interestingly, choline is not effective in all periods of pregnancy (pregnancy in rats lasts 20–22 days) but rather exerts its effects during the second half of gestation. This has been established by the studies of Meck and Williams and their colleagues who provided pregnant rats with approximately 4 times more choline than that present in control rodent diets during embryonic days (E) 6–11 and 12–17 and found that the offspring of dams supplemented with choline during the latter (but not the former) period outperformed the control animals in a radial maze spatial memory task (19). This model of choline supplementation or deprivation in pregnant rats during ~E11–17, has become quite common and many investigators adapted it for studies on choline and brain development and cognitive function. The overall observations from these studies is that choline deficiency causes impairments in certain memory tasks (17), whereas choline supplementation improves memory and attention (17, 37, 39–41, 43, 48, 49) and, prevents age-related memory decline (17, 49), i.e. 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. Interestingly, the cognition enhancing effects of high prenatal intake of choline can be seen already at very early age as the animals acquire developmental cognitive milestones. One such milestone is the ability to navigate using relational cues, considered to signal the onset of hippocampal function (50). This ability was assessed by Mellott et al (43), who studied spatial/relational and cued navigational performance of 18–22 day old choline supplemented and control rats using the Morris water maze. At this age, both control and choline-supplemented rats could learn the location of a platform that was directly cued (Fig. 2A) indicating that they had similar visual and swimming abilities. In contrast, at P18–19, only the prenatally choline-supplemented rats were able to use relational cues to remember the hidden platform location during the first 5 trials, while

control rats showed no spatial memory ability (Fig. 2B). When these rats were re-tested 3 days later on the same spatial task, both prenatally choline supplemented and control rats shortened their escape latencies over the 5 acquisition trials (43). Thus, prenatal choline-supplementation causes an approximately 3-day advancement in hippocampal development.

Neuroprotective actions of choline in rat models of epilepsy

Status epilepticus, a period of prolonged seizures, is a neurological condition that produces multiple degenerative and regenerative changes in the hippocampus, that are thought to contribute to the development of temporal lobe epilepsy in humans and in rodent models. Hippocampal pathophysiology following status epilepticus includes neuronal loss, γ-aminobutyric acid (GABA) system alterations, reactive gliosis, altered growth factor levels, and abnormal dentate gyrus cell proliferation and neurogenesis (51). These changes following status epilepticus are accompanied by cognitive deficits in hippocampal-dependent tasks, which are present both before and after the emergence of spontaneous recurrent motor seizures (51). Therapeutic methods that could prevent or reduce this seizure-related brain dysfunction are needed and several studies have addressed this need by testing the effects of high choline intake in rat models of chemically-evoked epilepsy. In pilocarpine- (20) and kainic acid- (21, 23) induced models of status epilepticus, prenatal choline supplementation attenuated the impairments of visual-spatial memory assessed with the Morris water maze test. Moreover, these protective actions of choline were accompanied by markedly attenuated seizure-induced hippocampal neurodegeneration and dentate gyrus cell proliferation (22). Choline supplementation also prevented hippocampal loss of the GAD65 mRNA encoding the GABA-synthesizing enzyme, glutamic acid decarboxylase (22). As in the other rodent models (22, 31, 34, 52–55), choline supplementation also increased the hippocampal levels of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 1 (IGF1), observed prior to the administration of the seizure-inducing kainic acid (22), indicating that this nutritional treatment may establish a neuroprotective hippocampal microenvironment that dampens the neuropathological response to and/or helps facilitate recovery from status epilepticus to protect cognitive function.

Neuroprotective actions of choline in mouse models of heritable human disease

In addition to studies on the efficacy of dietary choline in models of epilepsy, several investigators tested the hypothesis that high choline intake early in life could be effective in ameliorating the symptoms of genetically-determined neurological disease.

Down’s syndrome is one of the most common forms of mental retardation affecting approximately 1 in 700 births in the United States (56). The disorder is caused by meiotic non-disjunction of chromosome 21 resulting in babies with 3 copies of this chromosome in their cells (trisomy 21). Moon et al (35) used a mouse model of Down’s syndrome (Ts65DN mice) to test the hypothesis that choline supplementation from conception to weaning could prevent some of the neurological and cognitive deficits observed in these mice. The genome of Ts65Dn mice was engineered to carry a third copy of the distal region of mouse

chromosome 16 which contains approximately 94 genes orthologous to the Down's syndrome critical region of the human chromosome 21 (57). The adult offspring of choline-supplemented Ts65Dn dams performed significantly better than control Ts65Dn mice in several visual attention tasks (35). In some of these tasks the choline supplemented Ts65DN mice did not differ from the wild type controls (35). These findings indicate that perinatal choline supplementation significantly ameliorates cognitive dysfunction in Down's syndrome.

Another series of studies (30–34) tested the above hypothesis in mouse models of Rett syndrome (30–34) – a genetic neurological disorder of childhood that also represents a common (approximately 1 in 10,000 births) forms of mental retardation but affecting almost exclusively girls (58). Rett syndrome is typically caused by a mutation in the X-chromosome linked methyl-CpG-binding protein 2 (*MECP2*) gene, and several mouse models with inactivating mutations of *Mecp2* have been developed. As in human disease the mice are born apparently normal but tend to succumb to severe neurological disease within weeks. Unlike the studies described above, the Rett syndrome model mice were supplemented with choline via mothers' milk from birth to weaning. In *Mecp2* null males, choline supplementation improved motor coordination and locomotor activity and enhanced grip strength in females (30). These changes were accompanied by increase in the total brain volume in females, and cerebellar volume in males (32). As in prenatally choline-supplemented rats (see below), postnatal choline supplementation increased striatal NGF expression in both wild-type and *Mecp2* null mice (31), suggesting that neuronal proliferation and survival may contribute to improved motor performance in this model of Rett syndrome. Choline supplementation also increased the brain levels of *N*-acetyl aspartate, a marker of neuronal integrity, as assessed by nuclear magnetic resonance spectroscopy (33). In mice with a different *Mecp2* mutation, early postnatal choline treatment prevented deficits in locomotor activity (34), ameliorated the decline in the activity of the acetylcholine-synthesizing enzyme, choline acetyltransferase in the striatum and increased NGF and BDNF expression in the cerebral cortex and hippocampus (34). Together, these data suggest that postnatal nutritional supplementation with choline may improve neuronal function in Rett syndrome patients and thus should be considered as a potential therapy for this disease.

In order to evaluate the possibility that perinatal choline treatment could be useful as in preventing certain psychiatric disorders, Stevens et al (36) studied the effects of choline supplementation in the DBA/2 mouse strain that is frequently used as a model of schizophrenia (59). The mice were supplemented with choline from conception to weaning by providing high-choline diet to pregnant and lactating dams. DBA/2 mice raised on control diets displayed the characteristic abnormality in sensory processing (that is also present in patients with schizophrenia), whereas prenatally choline-supplemented mice had a normal sensory processing phenotype (36) suggesting that this nutritional treatment may reduce the risk of schizophrenia.

Molecular and cellular correlates and possible mechanisms of the neuroprotective actions of choline

The molecular and cellular mechanisms that govern the neuroprotective actions of perinatal choline nutrition remain to be elucidated. However, a great deal is known about the correlative brain alterations that permit the formulation of plausible hypotheses regarding these mechanisms. Already during brain development, i.e. at the time of altered choline supply, changes in brain structure are observed. Choline deficiency during pregnancy inhibits fetal cell proliferation and stimulates apoptosis in the hippocampus (60, 61), whereas gestational choline supplementation stimulates hippocampal cell division (62). These structural changes in prenatal brain subsequently are followed by neuroanatomical, neurochemical, electrophysiological, and molecular differences in the adult and aged animal. Some aspects of learning and memory require adult neurogenesis that occurs in the dentate gyrus of the hippocampus throughout the lifetime (63, 64). Prenatal choline supplementation enhances this process while prenatal choline deficiency impairs it (52–54). This effect of choline supplementation was also seen in aged rats and correlated with a highly trophic microenvironment within the hippocampus of the prenatally choline supplemented rats that included increased concentrations of NGF, BDNF, IGF1,insulin-like growth factor 2 (IGF2), and vascular endothelial growth factor (VEGF) in these animals as compared to controls (22, 52–55). Prenatal choline supplementation increases the size of the basal forebrain cholinergic neurons (65) that participate in the processes of learning and memory (66, 67) and augments acetylcholine synthesis and release from these neurons (49, 68). Prenatal choline supplementation also increases the activation of key 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 activation of glutamatergic receptors (43). Interestingly hippocampal electrophysiological synaptic plasticity measures termed long-term potentiation (LTP), that are considered as a correlates of certain neuronal aspects of memory, were modulated by prenatal choline in a fashion consistent with these molecular alterations. Prenatal choline supplementation enhanced hippocampal LTP in the CA1 region by decreasing the stimulus intensity required for LTP induction (70, 71), possibly due to an augmented *N*-methyl-D-aspartate 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 participate in signaling pathways involved in memory processes (73) and thus may mediate the observed choline-induced changes in LTP and behavior.

In addition, recent advances in the field of epigenetics have provided the conceptual and experimental framework to explain how such changes in gene expression patterns can be transmitted following cell mitosis. The central molecular mechanism that permits this type of long-term modulation of cellular phenotypes is methylation of DNA at the 5-position of cytosine residues within CpG sequences to form 5-methylcytosine (5mC). 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 (74). This change results in an 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) (75). The process of DNA methylation is dynamic (76) 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. 3). This effect is explained by the direct relationship between dietary intake of choline (and/or other methyl groups) and tissue levels of *S*-adenosylmethionine (the methyl group donor for most enzymatic methylation reactions) that is frequently observed (77). The hypothesis that choline intake by pregnant rats might alter DNA methylation in the fetus was tested in a study by Kovacheva et al (77) who evaluated these parameters in liver and cerebral cortex on E17 in rats following altered dietary supply of choline that had begun on E11. The investigators focused on the differentially methylated region 2 (DMR2) of the *Igf2* gene because the DMR2 methylation changes during development (78). Choline-deficient embryos had higher degree of DMR2 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. DNMT1 is important for maintaining the methylation pattern of the *Igf2* gene and *Dnmt1* knockout mice have abnormal expression of *Igf2* (79). In liver of choline-deficient embryos, *Dnmt1* mRNA was overexpressed by over 50% as compared to control and choline-supplemented fetuses. The data suggested that maternal choline deficiency causes a compensatory induction of *Dnmt1* expression in the fetus thus preventing the loss of DNA methylation when limited amounts of choline are present. As noted above, IGF2 expression in developing and adult brain was governed by prenatal choline intake (55, 73, 77) and recent studies implicate IGF2 as a critical component of memory consolidation mechanisms (80) suggesting that high IGF2 levels observed in brains of prenatally choline-supplemented rats (55, 73) may be part of the mechanism of cognitive enhancement that characterizes these animals. Data showing that maternal choline supply during pregnancy modifies fetal DNA methylation suggest that an epigenomic mechanism contributes to the long-term developmental effects of varied choline intake *in utero* (77, 81, 82). In addition to the central role of DNA 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 (83–87) and are involved in mechanisms of learning and memory (88–92). Thus, it is likely that choline nutrition influences brain development and cognitive function via its effects on DNA methylation.

Conclusions

High choline intake during gestation and early postnatal period has been repeatedly described as a robust cognitive enhancing regimen and is neuroprotective in a variety of animal models of neuronal damage. Data showing that maternal choline supply during pregnancy modifies fetal DNA and histone methylation suggest that a concerted epigenomic mechanism contributes to these long-term effects of varied choline intake *in utero* (77, 81,

82, 93, 94). Recent data indicate that choline nutrition in adulthood may also be critical for normal cognitive function in people as suggested by a study performed on 1391 normal adult and elderly people (average age 61 years) that reported 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 (12).

Acknowledgments

Some of the studies reviewed here were supported by NIH grants AG009525 and CA120488 to JKB

Abbreviations

AI	adequate intake
BDNF	brain-derived neurotrophic factor
CREB	cAMP response element binding protein
DMR	differentially methylated region
DNMT	DNA methyltransferases
FNB	Food and Nutrition Board
GABA	γ-aminobutyric acid
IGF	insulin-like growth factor
LTP	termed long-term potentiation
MAPK	mitogen-activated protein kinase
MECP2	methyl-CpG-binding protein 2
NGF	nerve growth factor
USDA	United States Department of Agriculture
VEGF	vascular endothelial growth factor

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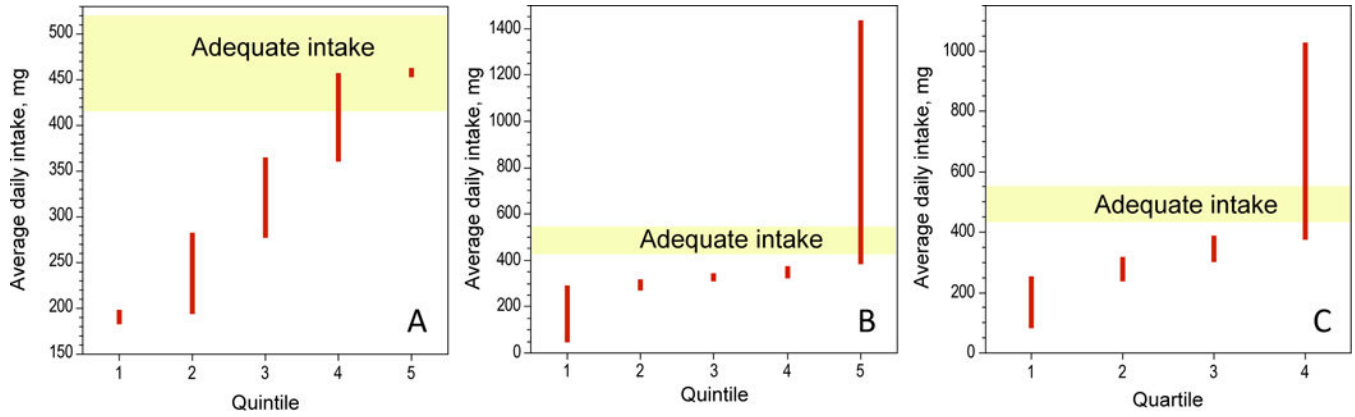

Figure 1. Americans consume less choline than recommended

Average daily choline intake reported in three independent studies. The red bars indicate the intake within the bounding values of the quintiles (**A**, **B**) or quartiles (**C**). The yellow strip indicates the Adequate Intake for adults. Data from refs. 10–12 (panels **A-C**, respectively).

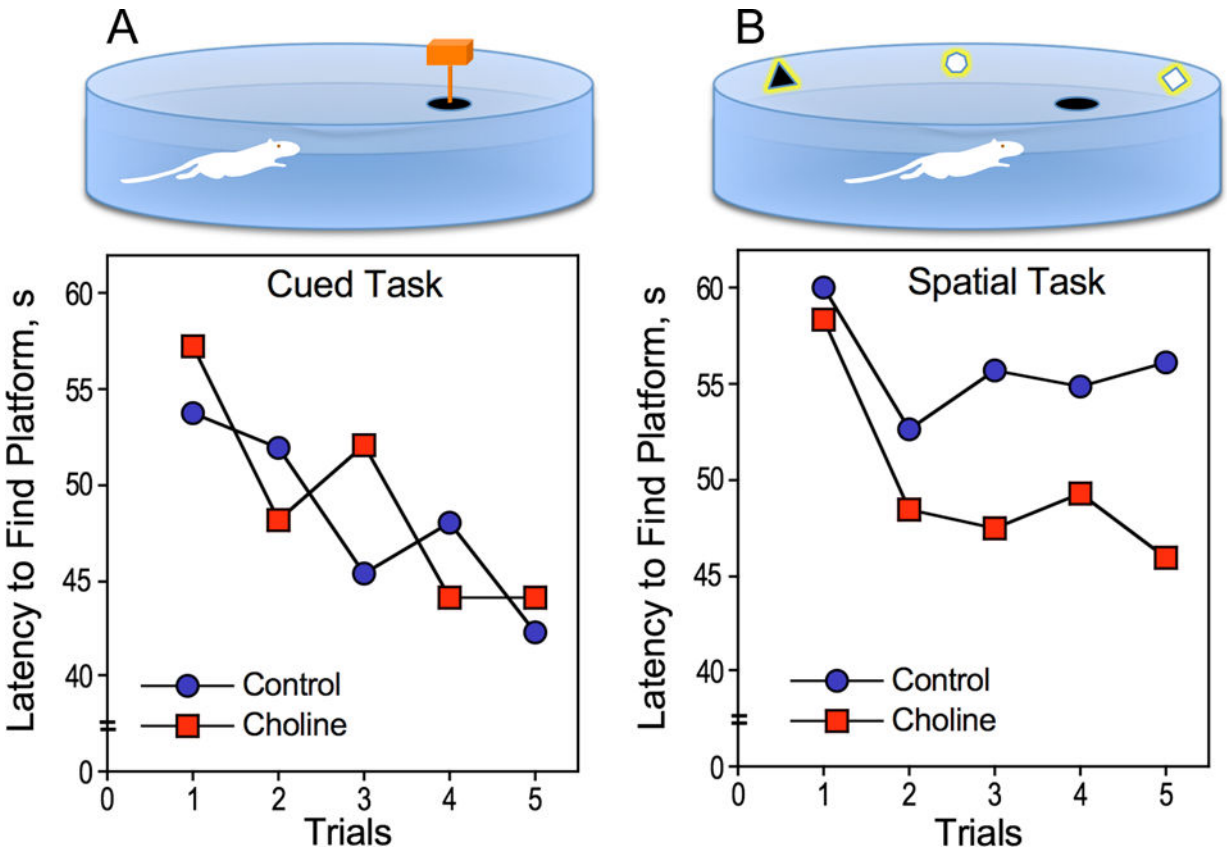

Figure 2. Performance of P18–19 prenatally choline supplemented and control rats during cued- and spatial training in the Morris water maze

A) Cued training, rats learn the marked platform location (top panel): both choline-supplemented and control rats at P18–19 learned the location of the platform (lower panel). **B)** Spatial training, rats use relational cues to learn how to navigate to the platform (top panel): only choline supplemented rats showed improved performance across trials (lower panel). Data from ref. 43.

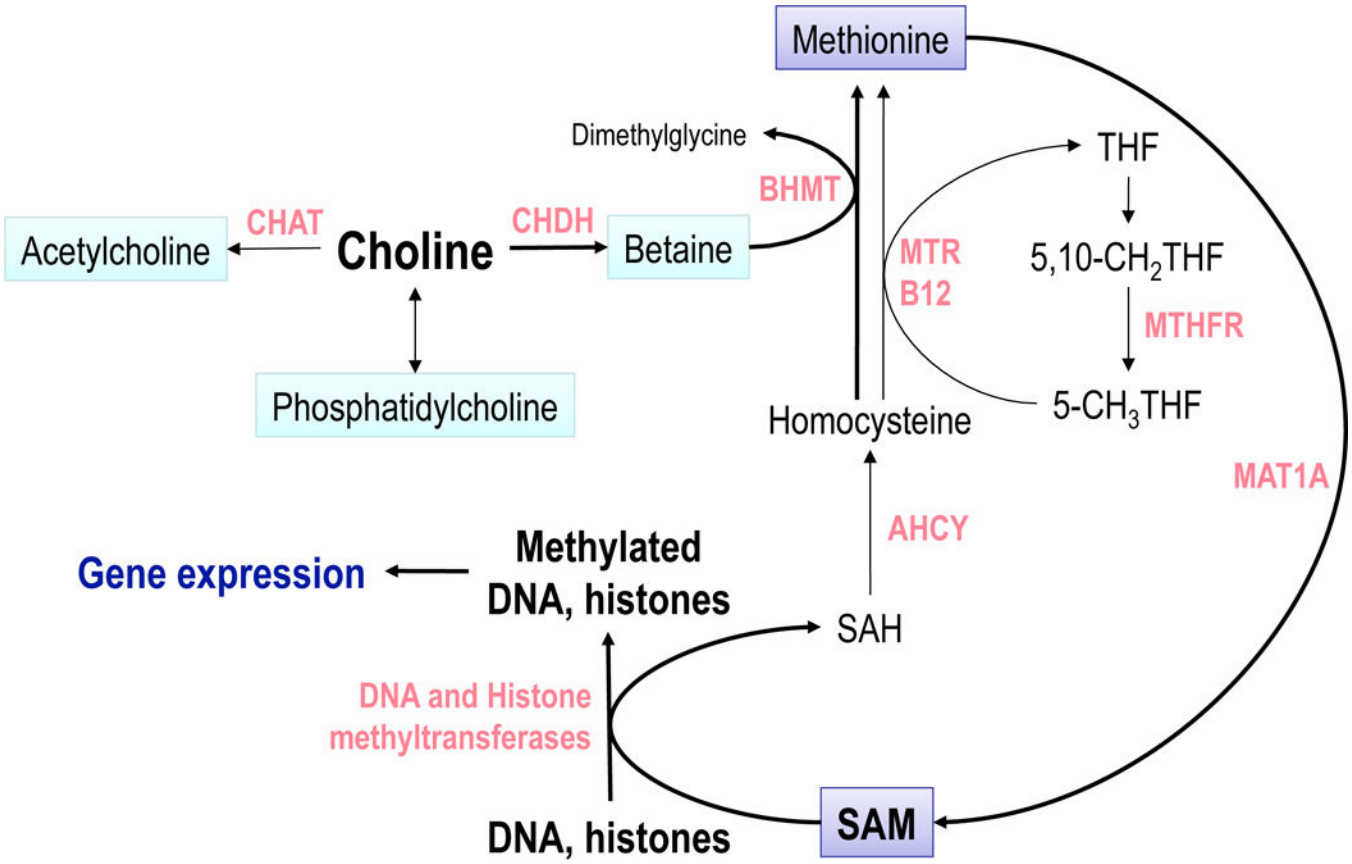

Figure 3. Choline and methyl group metabolism

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 (5-CH_3THF) is used as a methyl donor. Methionine is used as a precursor of S-adenosylmethionine (SAM) in a reaction catalyzed by methionine adenosyltransferase(s) (MAT1A). SAM is used by multiple methylating enzymes including DNA and histone methyltransferases that use SAM as a donor of methyl groups to methylate DNA at the 5-position of cytosine residues within the CpG sequences and histones at specific lysine and arginine residues. The DNA methylation state and pattern exerts a modulatory influence on expression of multiple genes (e.g. *Igf2*). The second product of this, and all other SAM-requiring methylation reactions, S-adenosylhomocysteine (SAH) is hydrolyzed to free homocysteine by SAH hydrolase (AHCY). The metabolic pathway linking choline to DNA and histone methylations is indicated by thick arrows.

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	–