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Choline and betaine in health and disease

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Abstract Choline is an essential nutrient, but is also formed by de novo synthesis. Choline and its derivatives serve as components of structural lipoproteins, blood and membrane lipids, and as a precursor of the neurotransmitter acetylcholine. Pre-and postnatal choline availability is important for neurodevelopment in rodents. Choline is oxidized to betaine that serves as an osmoregulator and is a substrate in the betaine–homocysteine methyltransferase reaction, which links choline and betaine to the folate-dependent one-carbon metabolism. Choline and betaine are important sources of one-carbon units, in particular, during folate deficiency. Choline or betaine supplementation in humans reduces concentration of total homocysteine (tHcy), and plasma betaine is a strong predictor of plasma tHcy in individuals with low plasma concentration of folate and other B vitamins (B_2, B_6, and B_{12}) in combination TT genotype of the methylenetetrahydrofolate reductase 677 C->T polymorphism. The link to one-carbon metabolism and the recent availability of food composition data have motivated studies on choline and betaine as risk factors of chronic diseases previously studied in relation to folate and homocysteine status. High intake and plasma level of choline in the mother seems to afford reduced risk of neural tube defects. Intake of choline and betaine shows no consistent relation to cancer or cardiovascular risk or risk factors, whereas an unfavorable cardiovascular risk factor profile was associated with high choline and low betaine concentrations in plasma. Thus, choline and betaine showed opposite relations with key components of metabolic syndrome, suggesting a disruption of mitochondrial choline oxidation to betaine as part of the mitochondrial dysfunction in metabolic syndrome.

Abbreviations

PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PEMT	Phosphatidylethanolamine N-methyltransferase
BHMT	Betaine-homocysteine methyltransferase
tHcy	Total homocysteine
PML tHcy	Post-methionine-load tHcy
NAFLD	Nonalcoholic fatty liver disease

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Introduction

Choline and betaine are metabolically related quaternary ammonium compounds (Fig. 1). They are metabolically linked to both lipid and folate-dependent one-carbon metabolism, and studies in animals and humans have provided results suggesting their involvement in neurodevelopment and the pathogenesis of various chronic diseases and points to a role in risk assessment and disease prevention. This review covers key aspects of this growing research field, from biochemistry and experimental inves-

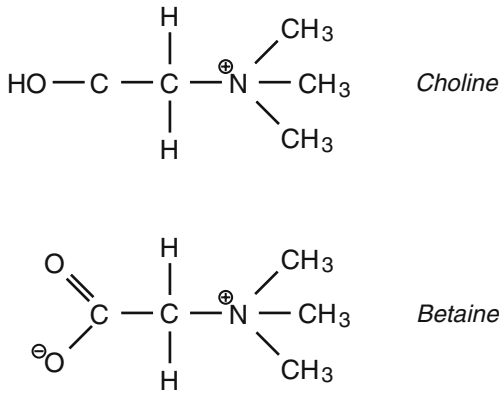

Fig. 1 Chemical structures of choline and betaine

tigations to epidemiological studies, with emphasis on recent data relevant to human disease.

Biochemistry

Choline and betaine are obtained from diet or by synthesis de novo in tissues. Phosphatidylcholine (PC) is a phospholipid and the most abundant choline species, which accounts for 95% of the total choline pool in mammalian tissue. It is synthesized de novo from phosphatidylethanolamine (PE), a reaction catalyzed by the S-adenosylmethionine-dependent enzyme phosphatidylethanolamine N-methyltransferase (PEMT). The remaining 5% includes choline, phosphocholine, glycerophosphocholine, cytidine 5-diphosphocholine, and acetylcholine (Ueland et al. 2005; Zeisel 2000). Their metabolic relationships are depicted in Fig. 2.

Synthesis of PC catalyzed by PEMT consumes 3 molecules of S-adenosylmethionine and generates 3 molecules of S-adenosylhomocysteine per molecule PC formed. Recent animal studies on PEMT knockout mouse and estimates of methyl balance in humans suggest that PC synthesis (and not creatine synthesis) is quantitatively the most important S-adenosylmethionine-dependent transmethylation reaction and therefore the most important source of homocysteine in mammals (Stead et al. 2006). In the liver and kidney, choline is oxidized to betaine. This is a two-step enzymic reaction in which choline is first converted to betaine aldehyde, a reaction catalyzed by the mitochondrial choline oxidase (choline dehydrogenase, EC 1.1.99.1), and betaine aldehyde is further oxidized in the mitochondria or cytoplasm to betaine by betaine aldehyde dehydrogenase (EC 1.1.1.8) (Lin and Wu 1986). Formation of betaine links choline to folate-mediated one-carbon metabolism, because betaine serves as a methyl donor in the betaine-homocysteine methyltransferase (BHMT) reaction (Fig. 2). In the liver and kidney, BHMT catalyzes the conversion of homocysteine to methionine. Homocysteine remethylation is also catalyzed by the ubiquitous methionine synthase, which requires 5-methyltetrahydrofolate as methyl donor and cobalamin as cofactor (Ueland et al. 2005). During choline deprivation leading to low betaine content, more 5-methyltetrahydrofolate is used for homocysteine remethylation, thereby increasing folate requirements. Conversely, during folate deficiency, methyl groups from choline and betaine are used, thereby increasing choline requirements. Thus, 5-methyltetrahydrofolate and choline/betaine have been regarded as fungible sources of methyl groups (Kim et al. 1994; VarelaMoreiras et al. 1992).

Metabolic ramifications

Choline and betaine have ramifications to processes vital to cellular structure and function. In cholinergic neurons, choline is acetylated to form the neurotransmitter acetylcholine. Choline is a precursor for the synthesis of membrane phospholipids, including PC, which accounts for about 50% of phospholipids in mammalian membranes and thereby affect signalling and transport across membranes (Zeisel 2006b). PC, derived from both phosphatidylcholine biosynthetic pathways (the cytidine 5′-diphosphocholine and the PE methylation pathways), is involved in very low density lipoprotein (VLDL) assembly and secretion from the liver (Vance 2008). Choline and betaine promote homocysteine remethylation to methionine and thereby affect the concentration of the universal methyl donor S-adenosylmethionine. Altered concentration of S-adenosylmethionine may influence DNA methylation at cytosine bases that are followed by a guanosine (5-CpG-3 sites) via change in methyl group availability and may thereby influence gene transcription, genomic imprinting, and genomic stability. Increased DNA methylation usually leads to gene silencing and reduced gene expression (Robertson 2005). Animal experiments have demonstrated changes in global and gene-specific DNA methylation following altered choline intake (Christman et al. 1993; Niculescu et al. 2006), and in mouse models during gestation, consumption of diets abundant in methyl group donors and cofactors (choline, betaine, methionine, folic acid, and vitamin B_{12}) affects the phenotype of offspring in a way that relates to hypermethylation of the relevant genes (Niculescu et al. 2006; Waterland et al. 2006; Waterland and Jirtle 2003).

Betaine and osmoregulation

The concentrations of betaine in tissue are in the millimolar range and orders of magnitude higher than in plasma (Slow et al. 2009). Intracellular betaine serves as an osmolyte that

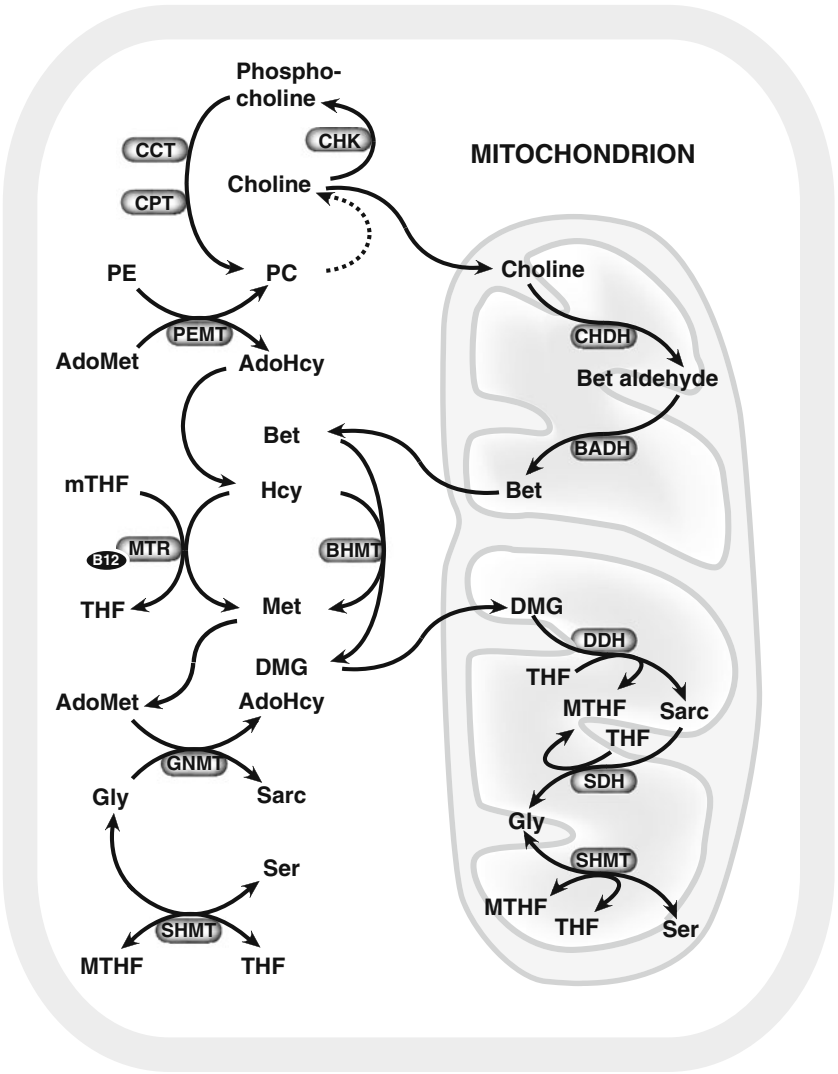

Fig. 2 Metabolism of choline and betaine and its relationship to one-carbon metabolism. *AdoHcy* S-adenosylhomocysteine, *AdoMet* S-adenosylmethionine, *BADH* betaine aldehyde dehydrogenase, *Bet* betaine, *BHMT* betaine-homocysteine S-methyltransferase, *CCT* CTP-phosphocholine cytidylyltransferase, *CHK* choline kinase, *CHDH* choline dehydrogenase, *CPT* cytidine 5-diphosphate (CDP) choline: diacylglycerol cholinephosphotransferase, *DDH* dimethylglycine dehydrogenase, *DMG* dimethylglycine, *Gly* glycine, *GNMT* glycine *N*-methyltransferase, *Hcy* homocysteine, *Met* methionine, *mTHF* 5-methyltetrahydrofolate, *MTHF* methylenetetrahydrofolate, *MTR* methionine synthase, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PEMT* phosphatidylethanolamine *N*-methyltransferase, *Sarc* sarcosine (monomethylglycine), *SDH* sarcosine dehydrogenase, *Ser* serine, *SHMT* serine hydroxymethyltransferase, *THF* tetrahydrofolate. Modified from Ueland et al. (2005)

regulates cell volume and thereby tissue integrity (Lang 2007; Schliess and Haussinger 2002). It also serves as a "compensatory" or "counteracting" solute that stabilizes proteins and is particularly effective at countering the denaturing effect of urea (Venkatesu et al. 2009). These functions of betaine have been most thoroughly studied in renal medulla, where cells are normally exposed to high extracellular osmolarity during normal operation of the urinary concentrating mechanism (Neuhofer and Beck 2005). Cells in other tissues (Lang 2007), such as liver (Haussinger 2004; Weik et al. 1998; Zhang et al. 1996), brain (Olsen et al. 2005), intestine (Kettunen et al. 2001; Lim et al. 2007), and skin (Warskulat et al. 2004), may also be exposed to hyperosmolality, albeit to a lesser extent than renal medulla, and they also accumulate methylamines serving as organic osmolytes, including betaine. Betaine has been shown to protect preimplantation mouse embryos against increased osmolarity in vitro (Anas et al. 2008). Osmolyte-mediated volume regulation is under tight control (Burg and Ferraris 2008; Haussinger 2004). Cellular accumulation of betaine is mediated by the osmoregulated betaine/γ-aminobutyric acid (GABA) transporter, designated BGT-1 (Yamauchi et al. 1992), which is expressed in kidneys (Kempson and Montrose 2004) and other tissues (Olsen et al. 2005; Petronini et al. 2000; Warskulat et al. 2008). Other osmoregulated mammalian betaine transporters exist that are not specific to betaine (Anas et al. 2008; Burg and Ferraris 2008). BHMT expression in kidney and liver is decreased during high sodium chloride intake (Delgado-Reyes and Garrow 2005), and osmoregulation of BHMT (Schafer et al. 2007) may control the partitioning of betaine between its use as a methyl donor and its accumulation as an osmoprotectant. Betaine synthesis from choline is not affected by hypertonicity (Burg and Ferraris 2008) but seems to be controlled by the choline transport into the mitochondria (O'Donoghue et al. 2009).

Homocysteine status in humans

The function of several B vitamins related to one-carbon metabolism converges on homocysteine, and plasma total homocysteine (tHcy) serves as a useful probe of changes in overall one-carbon metabolism in clinical and epidemiological studies (Hustad et al. 2007). High doses (6 g/day and higher) of betaine, alone or in combination with B vitamins, have been used for years to treat patients with homocystinuria (Ogier de Baulny et al. 1998; Yap 2003). Such treatment reduces plasma tHcy and partly corrects other biochemical abnormalities but also improves the clinical condition. Betaine supplementation reduces the increase in tHcy after methionine loading [post-methionine-load (PML) tHcy] but not fasting tHcy in renal patients who are folate and vitamin B_6 replete (McGregor et al. 2002). In healthy individuals, supplementation with betaine (Alfthan et al. 2004; Olthof et al. 2003; Olthof and Verhoef 2005; Schwab et al. 2002) or phosphatidylcholine (Olthof et al. 2005a) reduces fasting tHcy (by 20%) and PML tHcy (by 29–40%). Folic acid exerts a similar effect on fasting tHcy but does not affect PML tHcy. Betaine seems be more efficient and acts faster than phosphatidylcholine, probably because phosphatidylcholine needs to be metabolized to betaine to enhance homocysteine remethylation (Olthof et al. 2005a). Thus, oral betaine or choline, at doses similar to the amounts found in some diets, have a homocysteine-lowering effect.

PML tHcy is inversely associated with plasma betaine in cardiovascular patients. This effect is attenuated after the patients have been supplemented with B vitamins (folate, vitamin B_6 and cobalamin) (Holm et al. 2004). In a large study on 500 healthy individuals (Holm et al. 2005), plasma betaine was a stronger predictor of the PML tHcy (mean change in tHcy of 7.2 μmol/L across the extreme betaine quartiles) than folate, cobalamin, and vitamin B_6. The inverse association between the PML tHcy and plasma betaine was strongest at low folate. Smaller studies on the relationship between fasting tHcy and betaine provided inconsistent results, demonstrating weak or no associations (Allen et al. 1993; Lever et al. 2005; McGregor et al. 2001; Schwahn et al. 2004). A large study of 10,700 healthy individuals allowed the investigation of betaine as a predictor of fasting tHcy in strata according to folate and vitamins B_2, B_6, and B_{12} status and methylenetetrahydrofolate reductase (MTHFR) genotype. Betaine was a strong determinant of fasting plasma tHcy in individuals with low serum folate and the MTHFR TT genotype. The association was further strengthened at low levels of the other B vitamins. Thus, in individuals with the combination of serum folate in the lowest quartile, low vitamin B_2, B_6, and B_{12} status, and the MTHFR TT genotype, the difference in tHcy across extreme plasma betaine quartiles was, amazingly, 8.8 μmol/L. Thus, betaine takes over as a methyl donor and sustains methionine synthesis under conditions of impaired B-vitamin status (Holm et al. 2007).

Dietary requirements and intake

Dietary sources of choline are eggs, beef, pork, liver, soybean, and wheat germ (Zeisel et al. 2003), whereas betaine is obtained from wheat bran, wheat germ, and spinach (Sakamoto et al. 2002; Slow et al. 2005). Recently, a comprehensive database on the content of choline and betaine in common foods was compiled (http://www.nal.usda.gov/fnic/foodcomp/Data/Choline/Choline.html). Choline intake by humans on ad libitum diets averages 8.4 mg/kg per day and 6.7 mg/kg per day for men and women, respectively (Fischer et al. 2005), which equals the recommended daily intake of 7 mg/kg per day (550 mg/d for men and 425 mg/d for women) set in 1998 by the Institute of Medicine (Yates et al. 1998). The intake by some women is below this value (Fischer et al. 2005). A recommended daily intake has not been established for betaine, but the recently estimated dietary intake ranges from 100–300 mg/d (Bidulescu et al. 2007; Chiuve et al. 2007; Cho et al. 2006; Detopoulou et al. 2008; Fischer et al. 2005).

De novo biosynthesis of phosphatidylcholine catalyzed by PEMT in the liver is a significant source of choline relative to dietary intake. The importance of the PEMT pathway is demonstrated by animal experiments demonstrating low choline pool in the liver of *Pemt -/-* mice fed adequate amounts of choline (Zhu et al. 2003). The *PEMT* gene has multiple estrogen-responsive elements, and increased *PEMT* transcription has been demonstrated in human hepatocytes exposed to 17-β-estradiol (Resseguie et al. 2007). The estrogen-dependent *PEMT* increases the capacity for the endogenous synthesis of PC in premenopausal women, which may become paramount under conditions of increased requirements for choline, such as pregnancy and lactation, and explain why premenopausal women are relatively resistant to choline deficiency (Zeisel 2009b).

The choline and betaine intake estimates based on the Food-Frequency Questionnaire (FFQ) have recently been validated by investigating the relationship between intake and plasma tHcy in 1,960 participants from the Framingham Offspring Study (Cho et al. 2006). High intakes of choline and betaine were related to low tHcy. Notably, the inverse associations were most pronounced in individuals with low folate intake and in individuals consuming alcohol, which is in line with observation based on measurement of plasma concentrations of betaine and tHcy (Holm et al. 2007), demonstrating that choline, betaine, and folate are interchangeable sources of one-carbon units.

In a population of middle-aged and elderly men and women recruited from an area without folic-acid fortification and low folate intake (Norway), plasma free choline showed a positive relationship to intake of eggs and cholesterol but not to consumption of other food items rich in choline. Plasma betaine was positively related to intake of high-fiber bread and nutrients such as complex carbohydrates, fiber, folate, and thiamine. Thus, only a few food items are major determinants of plasma choline and betaine (Konstantinova et al. 2008b). Notably, betaine was negatively associated with a Western dietary pattern with a high loading for meat, pizza, sugar, and fat and was positively associated with total energy intake, whereas choline was not significantly associated with any identified dietary patterns or total energy intake (Konstantinova et al. 2008b)

Choline deficiency

Dietary deficiency of choline in humans causes fatty liver (Buchman et al. 1995) and liver (Zeisel 1991) and muscle (Fischer et al. 2007) damage. Fatty liver may reflect impaired export of triacylglycerol from the liver, whereas release of liver and muscle proteins into blood suggesting tissue damage is attributable to induction of apoptosis and muscle membrane fragility by choline deficiency (daCosta et al. 2004; daCosta et al. 2006; Fischer et al. 2007). As expected, choline deficiency caused an increase in plasma tHcy (da Costa et al. 2005), which, however, was uniform (20%) and unrelated to signs of organ damage (Fischer et al. 2007). The amount of choline required to maintain normal organ function showed large interindividual variability. Some individuals required more than the recommended adequate intake (AI) (550 mg/day), whereas others required <50 mg/day. Signs of organ dysfunction developed less commonly in premenopausal women than in men and postmenopausal women, probably because of upregulation of endogenous PC synthesis by estrogen (Fischer et al. 2007). Common genetic polymorphisms in genes encoding for the phosphatidylethanolamine *N*-methyltransferase (*PEMT*; rs12325817), choline dehydrogenase (*CHDH*; rs9001 and rs12676), and 5,10-methylenetetrahydrofolate dehydrogenase (*MTHFD1*; rs2236225) were strongly related to the occurrence of organ damage (da Costa et al. 2006; Kohlmeier et al. 2005).

Liver steatosis

Deletion of the *PEMT* gene in *Pemt* -/- mice caused low level of PC in the liver, but these animals had otherwise a normal phenotype with no dyslipidemia and liver pathology. When fed a choline-deficient diet, they rapidly developed steatohepatitis and drastic reduction in liver PC combined with extremely low concentrations of triacylglycerol and cholesterol in plasma (Vance et al. 2007). These observations demonstrate the importance of the PEMT pathway to provide PC under conditions of insufficient dietary choline and could be explained by the requirement of PC for normal VLDL assembly and secretion. Chronic alcohol consumption in rat causes liver steatosis. The concurrent biochemical changes include impaired PC formation via the PEMT pathway and reduced VLDL secretion (Kharbanda et al. 2007). Betaine is known to ameliorate the adverse effects of alcohol on the liver, in particular, fatty liver (Barak et al. 1997; Barak et al. 1996; Barak and Tuma 1983). Notably, dietary betaine supplementation of rats given alcohol enhanced the synthesis of PC catalyzed by PEMT, normalized VLDL secretion, and prevented the development of steatosis (Kharbanda et al. 2007; Kharbanda et al. 2009). The mechanism may involve lowering homocysteine and stimulating methionine synthesis via the BHMT pathway, thereby increasing the S-adenosylmethionine/S-adenosylhomocysteine ratio, which leads to PEMT activation (Barak et al. 2003; Purohit et al. 2007). These experimental results point to a possible role of betaine administration in treatments of hepatic steatosis.

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease and is associated with all components of the metabolic syndrome (Kotronen and Yki-Jarvinen 2008). Treatment of NAFLD with betaine has been evaluated in four human studies (Purohit et al. 2007), including a short-term, randomized, double-blind trial including 191 patients (Miglio et al. 2000). Improved liver function, including reduction of aminotransferases and liver steatosis, were observed in a significant portion of the patients in most, but not all (Abdelmalek et al. 2009), studies. A common genetic variant of PEMT characterized by an amino acid substitution (V175M) and reduced enzyme activity was more frequent (67.9%) in patients with NAFLD (n=28) than in healthy control (40.7%; n=59) (Song et al. 2005). Although based on a small number of patients, this observation emphasizes the importance of impaired PEMT pathway in the pathogenesis of fatty liver in humans. Notably, the incidence of NAFLD is lower in premenopausal women than in men or postmenopausal women, which could be related to a protective effect from induced expression of PEMT by estrogens (Zeisel 2007).

Pregnancy

During pregnancy, there is a high need for choline, which is transported across a concentration gradient from the mother to the fetus (Sweiry and Yudilevich 1985). The circulating concentrations of free choline in the fetus and newborn are

six- to sevenfold higher than in the mother (Ozarda Ilcol et al. 2002; Zeisel et al. 1980), who actually may become choline depleted (Zeisel et al. 1995). The increased need concurs with stimulation of de novo choline synthesis by estradiol, which increases progressively during pregnancy (Adeyemo and Jeyakumar 1993). During pregnancy, choline and betaine in plasma show some unique features compared with concentrations of other metabolites and vitamins related to one-carbon metabolism (tHcy, folate, and cobalamin). Plasma choline shows no relationship to tHcy and increases throughout pregnancy (whereas other metabolites decline) (VelzingAarts et al. 2005), which may reflect maintenance of adequate blood choline concentrations to facilitate active transport to the fetus. Betaine became a gradually stronger and folate a weaker predictor of tHcy during the course of pregnancy (VelzingAarts et al. 2005); in pregnant women (from Seychelles) with high choline intake (from eggs) and no folic acid supplementation, betaine was a tHcy predictor only in women low in methionine (Wallace et al. 2008). Thus, betaine may serve as a source of one-carbon units when other sources (folate, methionine) become limited. At delivery, maternal (Molloy et al. 2005) and cord (Braekke et al. 2007) plasma choline shows a positive relationship to tHcy (which contrasts to the negative relationship with folate and vitamin B_{12}), possibly because of enhanced homocysteine formation during upregulation of PC synthesis (Stead et al. 2006). Cord plasma concentration of free choline was threefold higher than maternal plasma choline, and there was no relationship between concentrations in the fetal and maternal compartments. For other one-carbon metabolites, concentration gradients were less dramatic, and there was a positive relationship between concentrations in cord and maternal plasma (Molloy et al. 2005). These observations are in agreement with an active transport mechanism delivering substantial amounts of choline to the fetus; after birth, the choline decreases and approaches adult levels within days (McMahon and Farrell 1985).

Choline and animal brain development and function

Choline is transported across the blood–brain barrier by a specific carrier (Cornford et al. 1978; Lockman and Allen 2002), and choline supplementation increases the brain content of choline and choline esters (Garner et al. 1995). The neonatal brain has a high-capacity choline transporter (Cornford et al. 1982) and very active form of PEMT (Blusztajn et al. 1985), factors that favor delivery of high amounts of choline to the developing brain. There is a large body of experimental data suggesting that choline deficiency or supplementation during the second half of gestation (E 11–17) and later in the newborn affects neurodevelopment in rodents. The offsprings of choline-supplemented pregnant rats or mice have improved visuospatial and auditory memory or performance in behavioral test, whereas choline deficiency seems to have the opposite effect. Prenatal and early postnatal choline supplementation also prevents age-related memory decline and protects against the adverse effects of some neurotoxic agents (McCann et al. 2006; Zeisel 2006c), including alcohol (Thomas et al. 2009), in offsprings. Notably, inhibition of choline uptake and metabolism causes neural tube defects (NTD) in mouse embryo in vitro (Fisher et al. 2002).

Increased levels of brain phosphatides and synaptic proteins, improved cognition, and enhanced neurotransmitter release was observed in several animal models after administration of choline in combination with uridine and docosahexaenoic acid, i.e., precursors of phosphatide biosynthesis. These effects were markedly enhanced when animals received all three compounds together and have been explained by increasing the substrate saturation of low-affinity enzymes involved (Wurtman et al. 2009). Experimental studies suggest that cognitive dysfunction in folate-deficient rats was not associated with plasma homocysteine or brain content of S-adenosylmethionine or S-adenosylhomocysteine but related to depletion of PC in the brain; both cognitive impairment and low PC were prevented by methionine supplementation (Troen et al. 2008).

Electrophysiological, biochemical, and morphological studies have been carried out to elucidate the mechanisms behind the role of prenatal choline in neurodevelopment, but the plethora of effects does not point to a unifying hypothesis. Prenatal choline availability affects fetal hippocampal cell proliferation, apoptosis, and differentiation. Choline supplementation during pregnancy enhances hippocampal neurogenesis, increases the size of cholinergic neurons, and enhances acetylcholine storage and release in the basal forebrain in adulthood, elevates brain concentrations of neurotrophins and growth factors, enhances long-term potentiation and depolarization-induced mitogen-activated signal transmission in postnatal hippocampus, and changes hippocampal and cerebral cortical gene expression during postnatal development and in adulthood (McCann et al. 2006; Zeisel 2006c). The view prevails that the change in DNA methylation, gene expression, and genomic imprinting rather than a change in acetylcholine neurotransmission explains the effects of choline on neurodevelopment and function (McCann et al. 2006; Zeisel 2009a).

Choline and the human central nervous system development and function

Data on choline and the function of the central nervous system (CNS) in humans are sparse. The elderly (Cohen et

al. 1995) or patients with Alzheimer's disease (Nitsch et al. 1992) have reduced levels of free choline and PC in the brain. Some (Alvarez et al. 1997; Ladd et al. 1993; Levy 1982; Little et al. 1985; Sitaram et al. 1978; Spiers et al. 1996) but no all (Brinkman et al. 1982; Drachman et al. 1982; Fitten et al. 1990; Harris et al. 1983; Mohs and Davis 1980; Weinstein et al. 1991) studies including healthy or demented individuals reported improved performance on memory and learning tasks following supplementation with choline, CDP-choline, or PC. A limited number of individuals (n=9-95) was included in these studies. A placebo-controlled study on the combination of choline, uridine, and docosahexaenoic in 212 patients with Alzheimer's disease demonstrated improved memory in patients with mild disease (Wurtman et al. 2009). These observations should be confirmed in larger trials.

Administration of citicoline, CDP-choline, increases phosphatidylcholine concentration in the brain and inhibits neuronal phospholipid membrane breakdown and enhances repair of the neuronal membrane after neuronal injury and ischemia (Saver 2008). A recent meta-analysis of ten trials enrolling 2,279 patients with stroke suggests that patients receiving citicoline had substantially reduced frequencies of death and disability (Saver 2008).

In a recent large population-based study involving 5,918 men and women, low plasma concentrations of free choline were significantly associated with high anxiety levels. No relationship was observed between choline and depression (Bjelland et al. 2009). Whether these results reflect an effect of anxiety on choline level and intake or a predisposition to anxiety in subjects with low choline status cannot be ascertained from a study with a cross-sectional design.

The relationship between periconceptional choline intake and NTD in pregnant women recruited from California between 1989 and 1991 was investigated by Shaw and colleagues (2004). Women in the highest quartile of choline intake had a risk reduction of about 50% compared with those in the lowest quartile. High betaine intake also seemed to be association with risk reduction, which, however, was attenuated after adjustment for covariates. This is not surprising given that the BHMT pathway does not appear to be active in the postimplantation embryo and is only found in the early fetus, a time when neural tube closure is nearly complete (Fisher et al. 2002). Shaw and colleagues (2009) recently published an investigation of the association between total choline (mainly PC) in midpregnancy serum specimens and NTD risk. The specimens were collected in California from 2003 to 2005, i.e., after implementation of mandatory folic acid fortification (Shaw et al. 2009). Compared with the 25–75 percentiles, women with low choline <25 percentile had increased risk [odds ratio (OR) = 1.8] and those with high choline >75 percentile had decreased risk (OR=0.4). No other B vitamins were associated with NTD risk, which may reflect widespread vitamins supplement use and vitamin fortification of food.

The first study on maternal choline status and intelligence in their children was recently published by Signore et al. (2008). Free and total choline (mainly PC) in maternal serum at gestational weeks 16–18, 24–26, 30–32, and 36–38 and in cord blood were determined in 404 maternal–child pairs. Intelligence (IQ) scores at the age of 5 years were investigated in relationship to free and total choline in maternal and cord blood by linear regression analyses, and no associations were found.

Cardiovascular disease and metabolic syndrome

Studies based on mice models of atherosclerosis point to several mechanisms connecting choline and betaine to atherogenesis. In apolipoprotein E (apoE)-deficient mice, betaine administration had a dose-dependent antiatherogenic effect and reduced aortic inflammatory response. These beneficial effects were explained by a choline-spearing effect, increasing PC available for VLDL assembly and section, and seemed to outweigh a marked exacerbation of hyperlipidemia in mice given betaine (Lv et al. 2009). In another study on LDL-receptor (*Ldlr*-/-) knockout mice fed high-fat/high-cholesterol diet, lack of PEMT (in *Pemt* -/- /*Ldlr* -/- mice) markedly attenuated atherosclerosis by 80%. The effect was attributable to decreased plasma concentration of apoB-containing lipoproteins, and the atheroprotective lipid profile was explained by decreased synthesis and increased clearance of VLDL particles with low content of PC (Zhao et al. 2009).

The homocysteine-lowering effect of choline and betaine has motivated investigations in humans of the possible relationship of cardiovascular disease and risk factors with intake or plasma concentrations of choline and betaine. High doses of betaine have been reported to increase plasma LDL cholesterol (Olthof et al. 2005b), which might offset the health benefit from homocysteine reduction, but the significance of the lipid effects has been questioned (Zeisel 2006a). Short- or long-term betaine supplementation does not improve flow-mediated vasodilation, a marker of endothelial function, despite reduction of tHcy (Olthof et al. 2006; Olthof and Verhoef 2005). One study demonstrated impaired vasodilation in individuals given betaine and no effect from low doses of folic acid and enhanced vasodilation in individuals given folic acid at doses exceeding those required to obtain maximal homocysteine reduction, suggesting improved endothelial function by mechanisms independent of homocysteine (Moat et al. 2006). A recent study on 3,000 healthy Greek men and women demonstrat-

ed low plasma levels of inflammatory markers, such as C-reactive protein, interleukin-6, and tumor necrosis factor-alfa, in individuals with high intake of choline and betaine (Detopoulou et al. 2008). Because inflammation plays a role in atherogenesis, high intake of choline and betaine may protect against cardiovascular disease. However, two recent large prospective studies, based on the participants in the Dutch PROSPECT–EPIC cohort (Dalmeijer et al. 2007) and in the Atherosclerosis Risk in Communities (ARIC) study (Bidulescu et al. 2007), respectively, demonstrated no association between intake of choline and betaine and cardiovascular disease. Whether these null findings are related to the large measurement error of intake estimates for micronutrients such choline and betaine (Bidulescu et al. 2009) should be addressed in future studies.

Plasma choline and betaine were investigated in relation to life style and cardiovascular risk factors in 7,074 healthy men and women (Konstantinova et al. 2008a). Choline showed a positive relationship to serum triglycerides, glucose, body mass index (BMI), body fat, and waist circumference, whereas plasma betaine was inversely related to these factors in addition to non-high-density-lipoprotein (non-HDL) cholesterol, and systolic and diastolic blood pressure, and positively related to HDL cholesterol. Thus, an unfavorable cardiovascular risk factor profile is associated with high choline and low betaine concentrations, and choline and betaine show opposite relationships with key components of metabolic syndrome. In the same cohort, neither plasma choline nor betaine was positively associated with consumption of animal products, fruit, or vegetables, but each was positively associated with the intake of specific food items such as eggs (choline) and bread (betaine) (Konstantinova et al. 2008b). These observations do not support the contention that the link between plasma choline and betaine and the metabolic syndrome reflects dietary intake. We hypothesized that the divergent associations of the substrate (choline) and product (betaine) of mitochondrial choline oxidation reflect disruption of this pathway as part of the mitochondrial dysfunction that prevails in metabolic syndrome (Konstantinova et al. 2008a).

Free choline in blood has recently been recognized as a potentially useful marker for diagnosis and risk stratification of ischemic heart disease, especially if cardiac troponins are negative on admission (Apple et al. 2005). Choline in plasma (Adamczyk et al. 2006) or serum (LeLeiko et al. 2009) has been associated with early events related to tissue ischemia, whereas whole-blood choline predicts events related to coronary plaque instability (such as myocardial infarction) during follow-up (Danne et al. 2007), particularly in patients with low to moderate risk (Mockel et al. 2008). The reported associations between ischemic heart diseases and choline in plasma versus whole blood are complex and somewhat inconsistent (Body et al. 2009) and have been explained by activation of phospholipases A2 and D in ischemic heart tissue and activated blood cells, leading to release of choline into plasma and secondary uptake into blood cells (Danne et al. 2007). Diagnostic performance of choline in blood is an important subject for future research, which must address issues such as possible interference from smoking (which reduces plasma choline (Konstantinova et al. 2008a)), from postprandial increase in choline (Konstantinova et al. 2008a), changes in choline metabolism in metabolic syndrome (Konstantinova et al. 2008a), specificity and sensitivity in various patient categories, and reliability coefficient as obtained by longitudinal measurements.

Cancer

Aberrations in choline phospholipid metabolism have been demonstrated in a variety of cancers, including breast (Morse et al. 2009), prostate, and brain (Glunde and Serkova 2006). These changes are characterized by elevated phosphocholine and total choline-containing compounds and are explained by elevated choline uptake and increased choline kinase and phospholipase C and D activities in cancer cells. Altered choline phospholipid metabolism detected by noninvasive magnetic resonance spectroscopy is used as an endogenous biomarker of cancer (Glunde and Serkova 2006), and enzymes involved in choline metabolism, such as choline kinase (Janardhan et al. 2006) and phospholipase D (Huang and Frohman 2007), have been suggested as new therapeutic targets. Dietary choline deficiency is associated with an increased incidence of spontaneous liver cancer and increased sensitivity to carcinogenic chemicals in rats. Suggested mechanisms include liver damage and regeneration, decreased DNA methylation and impaired DNA repair, increased oxidative stress, and activation of protein kinase C (Zeisel and Blusztajn 1994). A recent study shows that increasing maternal dietary choline from a deficient to an adequate intake during the second half of pregnancy in rats slows down the growth of mammary tumors induced later in life by a carcinogen in female offspring. This beneficial effect was associated with overexpression of genes that confer favorable prognosis in human cancers and underexpression of those associated with aggressive disease. Choline may modulate DNA methylation of developing mammary cells, thereby creating an epigenetic setting affecting tumor growth (Kovacheva et al. 2009).

There are only a few studies on intake of choline and betaine and cancer risk in humans, because food composition data have not been available until recently. In a large population-based study (Xu et al. 2008b), breast cancer risk

was reduced by 24% among women with choline intake in the upper quintile and increased in women homozygous for the variant allele of *PEMT* rs12325817, a single nucleotide polymorphism (SNP) assumed to alter estrogen responsiveness of the promoter of the choline-synthesizing enzyme, PEMT (Xu et al. 2008b). Investigations among women in the Nurses' Health Studies demonstrated no relationship between breast cancer risk and intake of choline or betaine (Cho et al. 2007b), whereas risk of distal colorectal adenomas was inversely and weakly associated with betaine intake and positively associated with choline intake (Cho et al. 2007a). The positive association with choline was strongest among women with low folate intake or high alcohol consumption, an observation that supports the involvement of one-carbon metabolism. The association may reflect stimulation of growth of established adenomas by choline or other dietary factors present in food rich in choline (Cho et al. 2007a). Likewise, results of experimental studies and recent intervention trials with folic acid suggest that folate deficiency promotes carcinogenesis, whereas folate enhances the growth of established neoplasias (Kim 2008). Secondary analyses of the Aspirin/Folate Polyp Prevention Study demonstrated that individuals treated with folic acid had an increased risk of prostate cancer (Figueiredo et al. 2009). Notably, in the Northern Sweden Health and Disease Cohort, doubling of plasma choline was associated with 46% increase of prostate cancer risk (Johansson et al. 2009). These findings point to a possible role of one-carbon metabolism in growth enhancement of prostate cancer.

Common polymorphism in the BHMT gene and disease risk

A common single polymorphism (c.716G>A, also know as 742G>A) in the *BHMT* gene was first reported by Park and Garrow (1999). BHMT c.716G>A was found not to be related to plasma tHcy concentration (Fredriksen et al. 2007; Heil et al. 2000; Morin et al. 2003; Weisberg et al. 2003), but a recent large epidemiological study demonstrated decrease in dimethylglycine (the product of the BHMT reaction) according to the number of c.716A alleles (Fredriksen et al. 2007), suggesting that this polymorphism may have metabolic effects. The variant c.716A allele has been associated with increased risk (Morin et al. 2003), decreased risk (Boyles et al. 2006), or no change in risk (Zhu et al. 2005) of spina bifida, and decreased risk of coronary artery disease (Weisberg et al. 2003) and no association with risk of cardiovascular disease (Heil et al. 2000) or aortic aneurysm (Giusti et al. 2008). Furthermore, carriers of the variant allele have been reported to have increased risk of colorectal cancer (Koushik et al. 2006) and possibly decreased risk of colorectal adenoma when combined with high methyl status (Hazra et al. 2007). The BHMT c.716G>A polymorphism was not associated with breast cancer risk (Xu et al. 2008b), but breast cancer patients with the variant allele had increased overall mortality (Xu et al. 2008a). Thus, studies on BHMT c.716G>A and disease risk have provided somewhat inconsistent results, which provide no clue to a role of betaine in the pathogenesis of birth defects, cardiovascular disease, and cancer.

Summary and conclusion

Choline is an essential nutrient in humans that serves as a precursor of phospholipids and acetylcholine and has been shown to effect neurodevelopment in rodents. Its oxidation to betaine provides a link to folate-dependent, one-carbon metabolism. The metabolic ramifications and results from experimental studies demonstrate an important role of choline and betaine in normal physiology and suggest the involvement in pathogenesis of common diseases. Recent establishment of analytical methods and food composition data for choline and betaine have motivated clinical and epidemiological studies on choline–betaine status and disease risk, mainly for conditions previously investigated in relation to folate status. Human data are sparse, the number of studies is limited, and no large placebo-controlled intervention trial on choline/betaine supplementation has been published. Thus, choline and betaine in humans is a research area in its infancy but with the potential to generate data leading to strategies for disease prevention.

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