Building better babies: should choline supplementation be recommended for pregnant and lactating mothers? Literature overview and expert panel consensus

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ABSTRACT

Accumulating evidence confirms choline as a critical, essential perinatal nutrient. Choline contributes to fetal and infant brain development with possible lifelong effects on cognitive function. Maternal choline supply supports placental health and reduces the risk of birth defects. Further, choline is involved in gene regulation during growth and development. Despite these apparent health benefits, choline intake in many pregnant and lactating women is insufficient. While US obstetricians and gynecologists are encouraged to recommend choline supplementation, their peers in Europe are less familiar with its importance. We provide an overview of the evidence and seek interpretation from an expert panel.

KEYWORDS

Choline; prenatal supplementation; pregnancy outcomes; fetal brain development.

Introduction

There is current interest in the potential benefits of maternal choline supplementation. First, choline is ubiquitously found in the body as a constituent of cell and organelle membranes [1-4]. In tissues, choline concentrations are tightly regulated [1-4]. It is also a component of lipoproteins, which have an important role in lipid metabolism, and is an element in bio-active molecules such as the neurotransmitter acetylcholine [1-4]. Second, choline is needed for fetal growth, brain development and placental function [5,6]. Various studies report that an inadequate maternal supply of choline to the developing fetus may result in birth defects and impaired postnatal cognitive ability [5,6]. Third, the production of choline by the maternal liver is insufficient to meet requirements, which progressively increase during pregnancy [5-9]. In a significant proportion of the population, ordinary dietary intake is generally insufficient to meet their needs [5,6]. This is particularly so during pregnancy and lactation [5,6].

In this article, we examine the role of choline in the perinatal stage, present dietary recommendations, summarize relevant animal studies, and provide a review of the literature on the potential benefits of choline in humans. Our objective is to raise awareness as to the importance of adequate choline during pregnancy and lactation. We address the question of whether obstetricians and gynecologists, midwives and health advisors should now recommend supplementation.

Article history

Received 31 Jan 2020 - Accepted 16 Apr 2020

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What is the function of choline?

Until recently, the nutritional and health benefits of choline were eclipsed by those of folic acid (folate). It is increasingly recognized that choline has many structural, biochemical, metabolic and neurological roles. Apart from cell membrane biogenesis, it is required for nerve myelination, synthesis of the neurotransmitter acetylcholine, bile secretion, alveolar surfactant formation, hepatic lipid export, and one-carbon metabolism (see also Box 1) [1-4,10-14]. Moreover, there is a strong association between choline supply and regulation of gene expression (epigenetics, see Box 1) [15]. All these physiological processes are particularly important for fetal development.

Choline demand and intake in pregnancy and lactation

Choline is considered to be an essential nutrient, because its endogenous synthesis via the PEMT pathway (Box 1) does not meet the amount required for tissue growth, homeostasis

and repair [5,6]. Pregnant and breastfeeding women exhibit exceptionally high demand for choline, yet consumed dietary choline is generally suboptimal [5,6]. Surveys show that the vast majority of men and women consume dietary choline at levels below recommendations (Table 1) [5,16]. Data from the US and Canada demonstrate that this applies in particular to pregnant and breastfeeding women [17-21]. Fewer nutritional surveys have been performed in Europe; however, two studies similarly confirm that pregnant women consume less choline than is recommended (Table 1) [16,22]. Data also strongly suggest that pregnant women in developing countries suffer from inadequate choline intake [23]. Recognizing the necessity of nutritional choline, the Food and Nutrition Board of the American Institute of Medicine (IOM, known today as the National Academy of Medicine) published the first official recommendations on adequate intake levels in 1998 [24]. These recommendations state that pre-menopausal women should ingest 425 mg/day, pregnant women should ingest 450 mg/day, whereas breastfeeding women should ideally ingest 550 mg/day (Table 1). To avoid hypotension, an upper level of 3500 mg/day has been suggested [24]. In 2016, the European Food Safety Authority (EFSA) made similar recommendations for pre-menopausal (400 mg/day), pregnant (480 mg/day) and breastfeeding (520 mg/day) women (Table 1) [25]. To fill the gaps in maternal choline intake, the American Medical Association and the American Academy of Pediatrics advocate adding choline to prenatal pills and infant formulas [26,27]. Unlike the American agencies, the equivalent European bodies have provided no advice regarding maternal supplementation of choline. Although health claims have been submitted to the EFSA, the agency has not so far reached a similar conclusion [25,28]. Recent studies may reinforce the importance of advocating maternal choline supplementation.

Choline metabolism during pregnancy and lactation

Human liver cells are capable of endogenously producing choline in limited amounts via the PEMT pathway [1-4]. Of note, choline itself is a major source of SAM (Box 1), which, in turn, participates in endogenous choline formation [29,30]. Yet, we predominantly obtain choline through our diet [3]. Choline-rich food sources include animal products, such as eggs, fish, meat and milk, and to a lower extent mushrooms, cruciferous vegetables, grains, nuts and beans [3]. There are several commercially available types of choline in the form of salts or phospholipids (i.e., lecithin) and other organic esters (i.e., glycerophosphocholine) to be found in common nutritional supplements or formulas.

The fetus has little capability of producing its own choline, and therefore it relies on transport of choline from the mother [8,31-37]. To support fetal development, elevated estrogen levels during gestation stimulate maternal choline (i.e., in the form of phosphatidylcholine, PC) production in the liver via the PEMT pathway [38,39]. Thus, choline delivered to the fetus comes from two maternal sources – diet-derived choline and hepatic choline production [8] (Figure 1). The increased supply of choline to the fetus is reflected in rising levels of circulating choline, as observed in the maternal blood, the amniotic fluid and the cord blood [40-42]. Choline concentration in cord blood is 3-4-fold higher than in maternal blood, thereby helping to support

Figure 1 The developing fetus is influenced by the maternal supply of choline. There are two primary maternal sources – food and estrogen-induced synthesis via the hepatic PEMT pathway. Dietary sources of folate, betaine and SAM (see Box 1) fulfil some overlapping functions. The placenta mediates the transport of these compounds to the fetus, but also stores and transports acetylcholine. PC – phosphatidylcholine, PE - phosphatidylethanolamine.

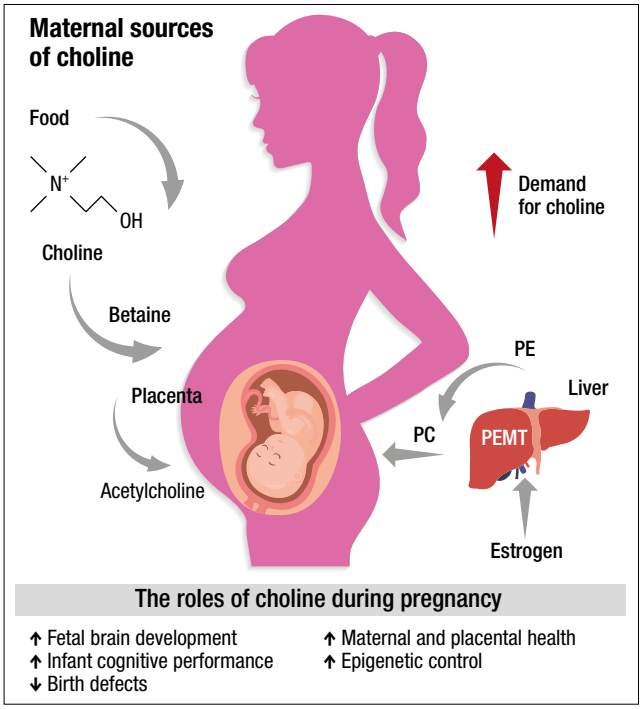

Table 1 X.

	Premenopausal women	Pregnant women	Lactating women	Infants
IOM (1998)	425	450	550	125 (0-6 months) 150 (6-12 months)
EFSA (2016)	400	480	520	N/A
Mean intake (US & Canada)	271-280*	304-347*	346	N/A
Mean intake (Europe)	291-374*	268-356*	N/A	N/A

*Recommended adequate intake levels and mean intake levels in premenopausal, pregnant and lactating women as well as recommended intake levels in infants [5,16-21,24,25,172]. * These numbers do not represent ranges, such as an interquartile range, but rather means from the different referenced studies. EFSA - European Food Safety Authority; IOM - American Institute of Medicine; N/A – data not available.*

fetal needs [9,40,42]. In this, the placenta has a unique role in facilitating active transport of free choline to the developing fetus [9,43,44]. Also, the placenta is the only non-neuronal organ capable of synthesizing, storing and transporting choline in the form of acetylcholine [43,44]. In order to meet the demands of the fetus, maternal stores of choline and choline-related compounds (see Box 1) are depleted despite the endogenous estrogen-enhanced choline synthesis [8,9]. Of note, the endogenous PEMT pathway (Box 1) also depends, in part, on exogenous choline [45]. Because some women are particularly susceptible to choline insufficiency due to their genetic makeup (e.g., PEMT variants), ensuring an adequate dietary intake of choline is even more important [46].

Human breastmilk is rich in choline and choline derivatives to fulfil the developmental requirements of the breastfed infant during the lactation period [47-49], which equate to ~18mg/kg of choline per day [24]. To satisfy this need, the mammary glands facilitate the synthesis of choline-containing molecules (e.g., phosphocholine and PC) [50]. In turn, these molecules are actively incorporated into breastmilk [36,51]. To further optimize choline supply, lactating women excrete lower levels of choline in their urine, indicating active choline retention in the body [52]. It is noteworthy that choline concentration is higher in the serum of lactating women and breastfed infants as compared with non-lactating women and formula-fed infants, respectively [48]. According to this study, infants fed on formula products did not obtain sufficient choline in their diet [48]. Becoming aware of this insufficiency, the FDA published a requirement for all commercial infant formulas, namely, the inclusion of choline in a concentration equivalent to 7 mg/kg/day [53]. This concentration is still substantially lower than the adequate intake values for infants, as recommended by the IOM [24]. Choline intake positively correlates with concentrations of choline and choline-containing compounds in maternal plasma and breastmilk [52,54]. The total concentration of choline in breastmilk is highly variable and depends on dietary choline intake and the PEMT genotype of the mother. Choline concentration in breastmilk also decreases gradually after birth [48]; the infant's choline requirement therefore correlates with its decreasing growth rate.

Collectively, these data imply that choline is an essential nutrient for the fetus and infant, and that maternal choline intake is generally sub-optimal.

Health benefits of choline: findings in animal studies

Animal studies support the notion that nutritional choline confers health benefits. Numerous experiments, using choline deficiency and supplementation models, have shown that an adequate maternal choline supply is required for normal fetal brain development [4,42,55,56]. Choline deficiency may lead to impaired neurogenesis and neural tube defects (NTDs) [33,57-69]. By comparison, maternal choline supplementation in rodents accelerates neurogenesis and memory processing in the hippocampus at a time equivalent to a human gestational age of 24 weeks [58,70-73]. Given the fact that the hippocampus processes memory, insufficient prenatal choline intake affects long-term cognitive functions in animal offspring, and may do so in humans as well. This was shown by enhanced visuospatial, auditory and temporal memory capacities in the offspring of choline-supplemented rodent dams [74-83]. Notably, prenatal choline supplementation attenuates the development of cognitive deficits in brain-afflicted animal disease models. These include in-utero alcohol exposure [84,85], Rett syndrome [86,87], Down syndrome [88-92], Alzheimer's disease [92,93], autism [94,95], epilepsy [96,97] and schizophrenia [98,99]. These findings on brain development and function in animals appear clinically relevant to a broad range of human cerebral pathologies.

Another area from animal studies where gestational choline supply appears to be necessary is the optimization of placental function [43,100-102]. Here, choline insufficiency probably results in preterm delivery, birth defects or stillbirths [103]. Of note, choline supplementation normalizes fetal weight and adiposity in a mouse model of maternal obesity [104-107]. Taken together, results in animals studies suggest that adequate choline intake could be of clinical importance in women with risk factors for poor pregnancy outcomes.

Health benefits of choline in humans: overview of clinical studies

Promising results in animals, particularly with regard to neurogenesis and brain development, have prompted researchers to carry out clinical studies in pregnant women, newborns and infants. Most of the data on the consequences of maternal choline supply has so far been generated from observational studies [5,6]. These typically involve estimating choline intake in populations exposed to known dietary patterns or measuring choline concentrations in the maternal, cord or infant blood (Table 2 - categories 1 & 2). Following data collection, the respective pregnancy outcomes are monitored. With the advent of choline supplementation, predominantly in the US, interventional studies have become more common. These studies use a defined intake of choline (e.g., supplements, dietary modifications or a combination of both) and then investigate the respective pregnancy outcomes (Table 2 - category 3). In most clinical studies, pregnant and lactating women were provided with different doses of supplemental choline or placebo in addition to their regular diet.

Health benefits of choline in humans: neural tube defects (NTDs), brain development and cognitive function

NTDs (incidence of 5 to 10 cases per 10,000 live births) are multifactorial birth defects that can, in part, be prevented by supplementing folate [108]. Because both choline and folate are necessary for fetal brain development and show a metabolic crossover, it has been hypothesized that choline insufficiency could be associated with NTDs. Sufficient maternal choline intake has been shown to be associated with a lower risk of NTDs in observational studies [109-112]. For instance, Shaw et al. have shown that maternal choline intake of ≥ 498 mg/day is

Table 2 Maternal choline supply and pregnancy outcomes: review of the literature in the last decade.

Carry-out	Study readouts; Regimen	Pregnancy outcomes and main findings	Reference
(1) Surveys on gestational diet* (also considering the intake of supplements during pregnancy)	Effect of gestational diet on the risk of hypospadias.	Choline-rich diet at pregnancy is associated with reduced risk of hypospadias in newborns.	*Carmichael et al., J Urol. 2009* [149]
	Effect of gestational diet on the risk of transverse and longitudinal limb deficiencies.	No association between maternal choline intake and limb deficiencies in newborns.	*Robitaille et al., Birth Defects Res A Clin Mol Teratol. 2009* [159]
	Effect of gestational diet on the risk of NTDs.	No association between maternal choline intake and NTD risk.	*Carmichael et al., Birth Defects Res A Clin Mol Teratol. 2010* [113]
		High maternal choline intake shows a trend in reducing NTD risk, whereas betaine intake shows a stronger association.	*Lavery et al., Birth Defects Res A Clin Mol Teratol. 2014* [111]
		High maternal choline intake is associated with lower NTD risk.	*Petersen et al., Am J Epidemiol. 2019* [112]
	Effect of gestational diet on the risk of craniosynostosis.	A choline-rich diet during pregnancy is associated with an increased risk of metopic synostosis, but not with other types of craniosynostosis.	*Carmichael et al., Birth Defects Res A Clin Mol Teratol. 2010* [160]
	Effects of methylmercury pollution and maternal nutrition on the cognitive performance of infants. Scores among 9 and 30-month-old infants on the Bayley Scales Infant Development-II (BSID-II) test.	Choline-rich diet in pregnancy does not affect the cognitive performance of infants from highly-exposed mothers.	*Lynch et al., Environ Res. 2011* [144]
	Global epigenetic state in cord blood.	Choline-rich diet during pregnancy is inversely associated with cord blood methylation levels in male newborns.	*Boeke et al., Epigenetics 2012* [169]
	Scores among 3-year-old children on the Peabody Picture Vocabulary Test III and the Wide Range Assessment of Visual Motor Abilities (WRAML) test.	No association between maternal choline intake and cognitive performance.	*Villamor et al., Paediatr Perinat Epidemiol. 2012* [141]
	Scores among 7-year-olds on the Kaufman Brief Intelligence Test and the WRAML2 test.	Choline-rich diet in pregnancy is associated with better visual memory.	*Boeke et al., Am J Epidemiol. 2013* [131]
	Choline intake in preterm infants. Effect of gestational diet on the risk of preterm deliveries.	A low maternal choline intake is associated with a very low birth weight among preterm infants.	*Bernhard et al., Eur J Nutr. 2013* [156]
		Low maternal choline intake is associated with preterm deliveries.	*Carmichael et al., Am J Perinatol. 2013* [152]
	Polymorphism of genes associated with neuroblastoma risk.	Maternal choline intake shows interactions with altered gene polymorphism. The effect on neuroblastoma risk is unknown.	*Mazul et al., Cancer Causes Control. 2016* [182]
	Associations between spontaneous preterm deliveries, gestational diet and global epigenetic state.	Low maternal choline intake is associated with an increased rate of preterm delivery (excluding newborns with very low birth weight).	*Chen et al., J Pregnancy Child Health. 2016* [153]
	Effects of nitrogen dioxide (NO2) pollution and prenatal intake of supplements on congenital heart defects (CHDs).	The risk of peri-membranous ventricular septal defect is synergistically reduced (high NO2 exposure with low choline intake compared to low NO2 exposure with high choline intake). No effect of maternal choline intake on other CHD types.	*Stingone et al., Am J Epidemiol. 2017* [158]
	Epigenetic state of specific genes in buccal samples from 6-month-old infants.	Choline-rich diet in pregnancy alters the DNA methylation patterns of genes involved in growth and metabolism.	*Pauwels et al., Clin Epigenetics 2017* [172]

Carry-out	Study readouts; Regimen	Pregnancy outcomes and main findings	Reference
(2) Gestational and newborn choline concentrations*	Effect of gestational diet on the risk of NTDs.	Gestational serum choline concentrations show an inverse correlation with NTDs.	*Shaw et al., Epidemiology 2009* [110]
		No association between gestational plasma choline concentrations and NTD risk.	*Mills et al., Am J Clin Nutr. 2014* [114]
	Scores of 18-month-old infants on the BSID-III test.	Gestational plasma choline concentrations show a positive correlation with cognitive scores.	*Wu et al., PLoS One 2012* [132]
	Determination of choline concentrations in the cord blood and plasma of preterm infants. Determination of choline concentrations in the breastmilk of the respective mothers.	Cord blood choline concentrations show an inverse correlation with postpartum age. Choline plasma concentrations drop rapidly after preterm birth.	*Bernhard et al., Eur J Nutr. 2015* [155]

Carry-out	Study readouts; Regimen	Pregnancy outcomes and main findings	Reference
		Lower choline concentrations are detected in the breastmilk of mothers of preterm infants as compared to the breastmilk of mothers of in-term infants.	*Maas et al., Eur J Nutr. 2017* [154]
	Anthropometric values at birth, at 14 days and 5 years after birth.	Cord blood betaine/choline concentrations show an inverse correlation with birth weight. Gestational plasma betaine/choline concentrations do not show a correlation.	*Hogeveen et al., Pediat Res. 2013* [164]
		Gestational plasma betaine/choline concentrations show an inverse correlation with adiposity in infants.	*van Lee et al., Am J Clin Nutr. 2016* [162]
		Gestational plasma choline concentrations show a positive correlation with adiposity in newborns. This association is lost later in life.	*van Lee et al., Int J Epidemiol. 2019* [163]
	The occurrence of pathogenic infections at 16-34 weeks of gestation. Neonatal cerebral inhibition via sensory P50 wave recordings; Scores of 1-year-old infants on the Infant Behavior Questionnaire-Revised Short Form.	Maternal infection decreases cerebral inhibition of auditory response at 1 month of age and behavioral self-regulation at 1 year of age. Infants born to infected mothers with high gestational plasma choline concentrations show normalized cerebral inhibition and behavioral self-regulation (i.e., similarly to infants of non-infected mothers).	*Freedman et al., J Pediatr. 2019* [133]
	Marijuana use during pregnancy. Neonatal cerebral inhibition via sensory P50 wave recordings; Scores of 3-month-old infants on the Infant Behavior Questionnaire-Revised Short Form.	Maternal substance usage decreases cerebral inhibition of auditory response at 1 month of age and behavioral self-regulation at 3 months of age. High gestational plasma choline concentrations show partial mitigating effects.	*Hoffman et al., Psychol Med. 2019* [134]

Carry-out	Study readouts; Regimen		Pregnancy outcomes and main findings	Reference
(3) Maternal choline supplement* (interventional studies)	Scores of 10-12-month-old infants on Visuospatial Memory Delayed Response Task, long-term episodic memory by Deferred Imitation, MacArthur-Bates Short Form Vocabulary Checklist: Level I, and the Mullen Scales of Early Learning, AGS edition. Regimens of 750 mg PC/d vs. placebo (perinatal 18 w through 90 d postpartum).		No association between maternal choline intake and cognitive performance.	*Cheatham et al., Am J Clin Nutr. 2012* [143]
	DNA damage in maternal blood leukocytes and the epigenetic state of specific genes.	Regimens of 930 vs. 480 mg choline/d (perinatal 12 w until delivery).	In comparison with nonpregnant women, pregnant women exhibit higher levels of DNA damage in blood leukocytes. However, high maternal choline intake has minimal effects on DNA damage.	*Jiang et al., PLoS One 2012* [183]
	Epigenetic and expression state of cortisol-regulating genes in the placenta and cord blood.		High maternal choline intake is associated with altered methylation of cortisol-regulating genes in the placenta and cord blood.	*Jiang et al., FASEB J 2012* [170]
	Gene expression profiles in the placenta and maternal blood.		High maternal choline intake is associated with reduced sFLT1 expression in the placenta and maternal blood.	*Jiang et al., FASEB J 2013* [171]
	Partitioning of choline into different metabolic pathways in pregnant and lactating women.		Pregnant women show a different choline partitioning pattern compared with nonpregnant women.	*Yan et al., FASEB J 2013* [8]
			High maternal choline intake restores choline partitioning in women with variants in genes that regulate folate metabolism.	*Ganz et al., FASEB J 2017* [174]
	Scores of 4-13-month-old infants on visual attention tasks, which measure the latency of saccadic eye movements.		High maternal choline intake is associated with faster mean reaction time.	*Caudill et al., FASEB J 2018* [135]

Carry-out	Study readouts; Regimen		Pregnancy outcomes and main findings	Reference
	Scores of 7-year-old children on color-location memory tasks.		High maternal choline intake is associated with better visual performance (e.g., passing more levels at low retention intervals).	*Bahnfleth et al., Curr Dev Nutr. 2019* [136]
	Vitamin B12 concentrations in the maternal serum.		High maternal choline intake shows a positive correlation with vitamin B12 levels.	*King et al., J Nutr Biochem 2019* [178]
	Neonatal cerebral inhibition via sensory P50 wave recordings; The scores of 6-month-old infants on Mullen Scales of Early Learning.	Regimens of 6.3 gr PC/d vs. placebo (perinatal 2nd trimester until delivery), followed by 100 mg PC/d vs. placebo (infants until 3 months of age, orally).	P50 response, lack of which is associated with schizophrenia risk, improves in 5-week-old infants who receive choline supplementation compared with placebo.	*Ross et al., Am J Psychiatry 2013* [137]
	Scores of 40-month-old children on the Child Behavior Checklist, version 1.5-5.		Fewer attention problems and less social withdrawal in children who receive choline supplementation compared with the placebo group.	*Ross et al., Am J Psychiatry 2016* [138]
	Scores of 6-month-old infants in the BSID-II test.	Three groups of heavy-drinking pregnant women – no treatment, prenatal multivitamin supplements, and multivitamin supplement + 750 mg choline/d. A fourth control group of no drinkers.	Prenatal alcohol exposure is associated with lower scores. High maternal choline intake improves cognitive performance only in infants of mothers in the alcohol groups.	*Coles et al., Matern Child Health J. 2015* [139]
	Cardiac orienting responses of 6-12-month-old infants in visual and auditory tests.		High maternal choline intake is associated with better and faster visual performance. Also, gestational choline concentrations show positive correlation with visual performance. No effect of choline intake on auditory performance.	*Kable et al., Alcohol 2015* [140]
	Adverse effects of high choline intake.	Heavy-drinking pregnant women – regimens of 2 g choline/d vs. placebo (perinatal 2nd trimester until delivery).	A small increase in nausea/dyspepsia in the choline group. No other observed adverse effects.	*Jacobson et al., Alcohol Clin Exp Res. 2018* [184]
	Scores of 6.5-12-month-old infants on the eyeblink conditioning (ECB) test and the Fagan Test of Infant Intelligence.		High maternal choline intake is associated with better visual performance and higher novelty preference (e.g., almost twice as many pass the adherence test of ECB). Infants of choline-treated mothers also show catch-up growth in weight and head circumference.	*Jacobson et al., Alcohol Clin Exp Res. 2018* [142]

** Including nutritional choline derivatives, such as betaine and dimethylglycine and phosphatidylcholine (PC). CHDs - congenital heart defects; EBC - eyeblink conditioning; NTDs- Neural tube defects; sFLT1- fms-like tyrosine kinase-1. Significant results indicated by $p < 0.05$.*

associated with lower NTD risk when compared with an intake of ≤ 290 mg/day [109]. However, other studies failed to confirm an association [113,114]. This could be attributed to differences in methodology. For example, conclusions from reported nutritional choline intake cannot be directly compared with conclusions from measured plasma choline concentrations [113,114]. Furthermore, increased folate intake over the years could have a masking effect on NTD rates, considering the implementation of folate fortification recommendations in the US [115]. Of interest, it has been suggested that single nucleotide polymorphisms in genes that regulate choline metabolism could be possible risk factors for NTDs [22].

There appears to be a positive association between prenatal maternal choline supply and the offspring's cognitive function, as measured using a range of specifically developed and appropriately applied tests [116-130]. Infants who were exposed to high maternal choline supply before birth showed better scores in tasks that assessed visual memory, attention, behavioral and language abilities [131-142]. By comparison, such infants did not necessarily show an advantage in auditory and motor skills [139,140]. While not all tests indicate associations between choline supply and cognitive performance, a functional link does seem to exist [141,143,144]. Additionally, children who directly consumed larger amounts of nutritional choline scored better on cognitive tests [145,146]. Although association studies do not prove causality, it is assumed that optimized choline supply is also essential beyond birth – in infancy and early childhood. This assumption requires further investigation by means of appropriate interventional studies.

An important outcome demonstrated by cognitive studies is the positive effect of maternal choline supply on children whose development is delayed due to environmental toxins. Women who suffer from severe infections, substance abuse or alcohol exposure during pregnancy tend to give birth to chil-

dren with increased risks of disability and impaired cognitive performance. In observational and interventional studies, high maternal choline supply lowered these risks in 'compromised' women and their infants [133,134,139,140,142,145]. Improvements in cognitive functions or growth parameters could be demonstrated [133,134,139,140,142,145]. Hence, these studies suggest that nutritional choline has the ability to "restore" fetal brain development and protect against neural insults. Gestational alcohol exposure leads to neurodevelopmental deficits and disturbances in DNA methylation [147,148], in which choline plays a major role (Box 1). Thus, we suggest that maternal alcohol exposure could be an excellent model to show that incorporating choline into these metabolic pathways improves brain function; this awaits further investigation. However, not all serious brain-related conditions are positively affected by choline. For example, infants and children born to mothers who were exposed to mercury contamination did not benefit from higher maternal choline supply [144]. Altogether, these findings indicate that maternal choline supply during pregnancy can improve brain function in "compromised" infants; this could be of lifelong benefit.

Health benefits of choline in humans: other pregnancy outcomes

In addition to cognitive function, are there any other physical properties that may improve in association with a high maternal choline supply? The risk of non-neuronal birth defects, such as orofacial clefts, urethral and diaphragmatic malformations, has been found to show a decrease following high choline intake [149-151]. Likewise, the risk of pre-term delivery is lower [152,153]. It has been suggested that preterm infants suffer from choline undernutrition, reflected by low choline concentrations in cord blood and the mother's breastmilk [37,154-156]. In support, preterm infants are more dependent on the endogenous PEMT pathway to synthesize PC [37]. Further prospective randomized trials with choline supplementation are required to validate this hypothesis. Of interest, air pollution is known to increase the risk of certain congenital heart defects (CHDs) [157]. A recent study points to maternal choline as a risk-lowering factor in air pollution-associated CHDs [158]. In contrast, high choline intake does not lower the risk of some other birth defects, such as limb deficiencies and cranial malformations [159,160]. Because of the rarity of most birth defects, conducting large-scale clinical studies in these areas will likely be a challenge.

It is well-established that malnutrition affects fetal development and subsequently infant health. In the western world, maternal obesity and gestational diabetes may promote long-term complications in both the mother and the infant [161]. Despite promising findings of choline in mouse models of gestational diabetes [104-107], results of studies in humans are so far inconclusive. One study shows inverse associations between gestational choline/betaine concentrations and neonatal weight and adiposity [162], whereas another study shows a positive correlation with neonatal adiposity [163]. Previous studies have not demonstrated an association between gestational betaine/choline concentrations and birth weight; however, neonatal adiposity was not directly determined [164,165]. In one of these studies, an inverse correlation between cord blood betaine/choline concentrations and birth weight was noted [164]. While sufficient choline supply is associated with a lower risk of pre-term deliveries, some studies suggest it is associated with lower birth weight. This is not necessarily a discrepancy, as choline delivery to the fetus could be matched to its growth and development. Further investigation is required to draw conclusions regarding the health benefits of choline in gestational diabetes and its contribution to fetal growth.

Maternal choline supply: epigenetic control and placental health

With the advent of genomic sequencing and analytics, and their application in epidemiological and nutritional research [166], attention has turned to choline-dependent gene regulation. It has been suggested that choline directly affects epigenetic control of gene expression via DNA and histone methylation (Box 1) [167,168]. Indeed, high maternal choline intake alters global DNA methylation and gene expression in the placenta and the cord blood of newborns [169-171]; the implications of this are currently unknown. Despite evidence in adults [6,167], the effect of choline insufficiency on epigenetic control has not been addressed in the context of pregnancy.

Periconceptional choline intake shows an inverse association with methylation of genes involved in growth and metabolism in samples from infants [172]. This observation is of particular interest for the assessment of preterm delivery risk due to its association with aberrant gene methylation and low maternal choline intake alike [153]. Furthermore, altered methylation and expression of cortisol-regulating and vascular-function-regulating genes have been detected in the placenta and blood of pregnant women receiving choline supplementation [170,171]. This includes reduced expression of the preeclampsia risk marker fms-like tyrosine kinase-1 [171,173]. Of note, high plasma choline concentrations are detected in women with preeclampsia; it is not yet known whether this is a feedback response or a bystander effect [165]. These results highlight the importance of choline for placental function and macronutrient supply, which are crucial for the health of both the mother and the developing fetus.

This choline-gene-regulation axis is controlled in both directions; specific genetic variants can make individuals more susceptible to choline deficiency. For example, pregnant and lactating women with genetic dysregulation of folate metabolism become more dependent on choline metabolism [8,54,168,174]. This dysfunctional metabolism can be corrected by choline supplementation [174]. The implications of these gene polymorphisms on pregnancy outcomes are yet to be investigated.

Consensus and recommendations from the expert panel

Pregnancy depletes maternal choline supply, which suggests that the endogenous PEMT pathway is generally insufficient to meet the choline demands of pregnant and breastfeed-

ing women [5-9]. Thus, choline-rich foods are recommended. However, most pregnant women fail to achieve an adequate intake of choline. Insufficient choline intake may influence fetal development, notably affecting the brain, and may increase the risk of birth defects (see above). An expert panel, meeting in Frankfurt, Germany in Q2 2019, concluded that on balance there is enough evidence to recommend choline supplementation during pregnancy and lactation, especially for healthy brain development.

Choline supplementation is well-tolerated and unlikely to cause harm. Mild adverse effects, such as hypotension, body odor and nausea are only encountered at concentrations more than a magnitude higher than recommended intake levels (>7.5 g/day) [24]. While the practice of recommending choline supplementation in the US is now established, European obstetricians and gynecologists remain largely unaware of its importance. The EFSA has yet to publish an official statement recommending choline as a prenatal supplement [25]; the panel concluded that the body of evidence supports such a recommendation.

Unanswered questions and future directions

In the near future, what can we expect from clinical research investigating the effects of prenatal choline supplementation? It is only in recent years that scientists have been engaged in interventional studies involving choline supplementation [5,6]. It seems likely that the effects of maternal choline on fetal/infant development and health will not be limited to studies on cognitive performance [135-140,142,143]. Improvements in pre-term delivery rates and the frequency of birth defects, notably NTDs, have been well-demonstrated by observational studies on maternal choline intake [37,109-112,149-156]. Thus, choline supplementation could potentially reduce the rates of these unfortunate pregnancy outcomes. Also, we find it interesting that folate, which is nowadays a widespread fortified nutrient, reduces the risk of birth defects [108]. In support of these overlapping and complementary functions, both folate and choline participate in one-carbon metabolism (Box 1) [4,13,175]. Moreover, one nutrient has been shown to compensate, in part, for deficiency of the other nutrient, as observed in pregnant female rodents and women [168,174,176,177]. A correlation between maternal choline intake and vitamin B12 levels has also recently been noted [178]. The interplay between these essential nutrients warrants further research that will allow a better understanding of how maternal nutrients control fetal development.

Warranting further research, a prominent finding emerges from clinical trials (Table 2). Adequate maternal choline intake is likely to have a profound neuroprotective effect on "compromised" infants, normalizing their cognitive performance [133,134,139,140,142,145]. It is therefore possible that adequate choline supply can counteract other environmental exposures that have long-term harmful effects on the health of newborns, such as maternal smoking, drug abuse and depression. Can adequate choline supply during pregnancy reduce the detrimental impact of congenital illnesses that affect brain development and function? Choline supplementation can delay age-related neurodegeneration and memory impairment in demented patients [179]. Thus, choline supplementation might display corrective features of brain malfunction at much earlier stages of life.

There has been little focus on the benefits of choline on maternal health during pregnancy and lactation. Preliminary observations suggest that choline may modify the concentrations of neonatal stress hormones and preeclampsia-associated risk factors [170,171]. These observations may prompt initiation of clinical research on the direct effects of choline on stress-related diseases and maternal health. The role of choline in cell growth may indicate a relationship between maternal choline intake during pregnancy and birth weight and preterm delivery. Hence, researchers should consider addressing the contribution of choline supplementation to the prevention of preterm deliveries.

Evidence suggests that choline content in breastmilk is crucial for infant growth [52,54,154,155]; so far, however, this has not been demonstrated in choline supplementation research. The studies, in which the infants show improved cognition, examined the consequences of prenatal choline supplementation without controlling for the postnatal period [135-140,142]. In other words, maternal choline supply via breastfeeding may have been a confounding factor that eventually contributed to the improved cognition. Choline supplementation during the lactation period could therefore be investigated from the perspective of its possible effects on infant growth and cognitive function.

Ethical considerations prohibit interventional studies that investigate the health impacts of choline deficiency on pregnant and lactating women. Nonetheless, placebo/untreated subjects with limited choline intake do exist, particularly among vulnerable subpopulations, which could usefully serve as "insufficiency" controls. Taken together, appropriate future studies may reveal that the hallmark of choline supplementation lies in its abundant preventive health benefits, as opposed to a sole status as an 'enhancement' nutrient.

Conclusion

This article seeks to raise awareness of the health benefits of nutritional choline intake and prenatal choline supplementation for the community of pregnant and lactating women. Newborns may show a lower prevalence of birth defects and improved long-lasting cognitive performance, especially in pregnancies at risk of complications. Hence, prenatal choline supplementation may reduce the risk of undesirable pregnancy outcomes. We consider choline to be an underappreciated, essential nutrient that should be supplemented during pregnancy and lactation.

Box 1 - Scientific terms.

Acetylcholine – A major neurotransmitter. Its functions include cognitive neurotransmission and peripheral muscle activation. It accounts for ~1% of the total choline pool.

Betaine, dimethylglycine and S-adenosylmethionine (SAM) – Betaine is an oxidized product of choline and a precursor of SAM (see Epigenetics). Dimethylglycine is a demethylation product of betaine. Betaine is a methyl donor that participates in the re-methylation of homocysteine to methionine (see one-carbon metabolism). Plasma betaine and choline are directly influenced by choline intake [180], but because of rapid tissue uptake and engagement of homeostatic mechanisms, fasting concentrations show little association with dietary intake [181].

Choline – A small positively-charged molecule (molecular weight: 104.17) that is classified as an essential nutrient by the EFSA and the National Academy of Medicine. It is a constituent of phospholipids in cell membranes and the myelin sheaths surrounding nerve fibers. It is also a precursor for the neurotransmitter acetylcholine and for a group of methyl-donor metabolites (see one-carbon metabolism). Choline is mainly obtained via the diet (e.g., choline-rich foods). Humans have a limited capacity for choline biosynthesis in the liver (see PEMT pathway). Adequate trans-placental and mammary choline supply routes are necessary for overall fetal and neonatal brain development.

Epigenetics – Refers to gene regulation that is not encoded in the DNA sequence. This occurs via biochemical reactions, such as DNA methylation and histone modification (e.g., methylation and acetylation), thereby regulating gene expression. Choline is a precursor for SAM, a universal methyl donor used by DNA and histone methyltransferases (see also one-carbon metabolism).

One-carbon metabolism – A series of life-essential cellular biochemical reactions in which a one-carbon unit (e.g., methyl [CH3] or methylene [CH2]) is transferred to an accepting molecule. Choline, together with folate and vitamin B12, are nutrients that drive one-carbon metabolism. These processes are involved in the synthesis of nucleic acids and amino acids, lipoprotein assembly and DNA methylation (see epigenetics).

Phosphatidylcholine (PC) – A phospholipid. Phospholipids are amphipathic biomolecules that constitute cell and organelle membranes; lecithin is a mixture of phospholipids. The basic form consists of a polar head group, such as phosphocholine in PC, a glycerol backbone and two fatty acids. PC is involved in the emulsification of fat by bile, alveolar surfactant function and triglyceride export to the blood circulation. The latter is done via secretion of very-low-density-lipoproteins from the liver, chylomicrons from the intestine, and high-density lipoproteins from the periphery. Sphingomyelin, another common phospholipid, also contains choline.

Phosphatidylethanolamine N-methyltransferase (PEMT) pathway – De novo synthesis of PC from phosphatidylethanolamine (PE) in the liver, using SAM as a methyl donor. It is estimated that approximately 30% of PC is produced through the hepatic PEMT pathway, whereas the remaining 70% is produced from dietary choline through the CDP-choline pathway. Elevated estrogen levels during pregnancy induce the endogenous PEMT pathway. This enables some women to meet the increased demand for choline during pregnancy. Common single nucleotide polymorphisms of the PEMT pathway blunt endogenous choline synthesis.

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Acknowledgements: *Funding to develop this manuscript was provided by Procter & Gamble. The company had no involvement in the contents.*
Conflict of Interest Statement*: SHZ is the founder of SNP Therapeutics, he is on advisory boards for Ingenuity Brands, ByHeart, and receives grant funding from Balchem; all these companies have an interest in choline. MAC has provided scientific advisory services regarding choline to Balchem Corporation, P&G Health, Natals, Inc. (Ritual), Bayer and Nu Skin Enterprises. RO participated in advisory boards on choline for P&G Health Germany. The remaining authors have no disclosures.*
Contribution to Authorship: *MAC, RO, ED, WB and SHZ conceptualized and carried out this project. KL and SJW assisted in writing and editing the review.*