REVIEW

Association between Maternal Choline, Fetal Brain Development, and Child Neurocognition: Systematic Review and Meta-Analysis of Human Studies

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ABSTRACT

We studied associations between prenatal and early postnatal choline intake, brain development, and neurocognitive function of children. We conducted a systematic review followed by a meta-analysis and critical appraisal of human studies published from 1997 to 2021. Thirty publications were identified. The meta-analysis included 5 of 7 case-control studies studying neural tube defects (NTDs) in relation to maternal choline intakes/circulating concentrations. Low maternal choline intake/circulating concentrations were associated with a higher OR for NTDs among 1131 mothers of newborns with NTDs and 4439 control mothers (pooled estimate = 1.36; 95% CI: 1.11, 1.67). The 95% prediction intervals were 0.78, 2.36. Findings and critical evaluation of 10 publications with interventional designs showed that higher maternal choline intakes during the second half of pregnancy and early postnatal period (550 mg up to 1 g/d on top of the diet) or a child intake of 513 to 625 mg/d from supplements were safe and likely to demonstrate favorable effects on several domains of child neurocognition, such as memory, attention, and visuospatial learning versus the comparators. Findings from observational studies (n = 13) partly supported the association between maternal choline intake/serum concentrations and child neurocognition, but there was low confidence in the use of plasma choline concentrations as a choline intake marker. In conclusion, low maternal choline intakes were associated with a higher OR for NTDs. The risk could be up to 2.36-fold in some populations. Despite limitations of available trials and observational studies, higher maternal choline intake was likely to be associated with better child neurocognition/neurodevelopment. The results should be used to guide choline intake recommendations in pregnancy and lactation, especially because most young women are not achieving the reference intake of choline. This meta-analysis is registered at PROSPERO as CRD42021233790. *Adv Nutr* 2022;13:2445–2457.

Statement of Significance: This systematic review and meta-analysis is novel. Low maternal choline intake/circulating choline concentrations were associated with a higher OR for neural tube defects in the offspring. The study summarizes present evidence and gaps in knowledge in the field and the need to conduct well designed randomized controlled trials.

Keywords: brain, choline, essential nutrient, first trimester, infant, lactation, neurocognition, neural tube defects, pregnancy, prenatal

Introduction

Choline is regarded as being an essential nutrient, needed for human health (1, 2). Dietary requirements for choline are higher during pregnancy and lactation compared with those for nonpregnant women (1, 2). However, the average dietary choline intake in women is presently lower than the Adequate Intakes (3, 4). The fetus and infant accumulate choline at the expense of maternal stores (5, 6).

Limited choline intake during pregnancy and lactation increases the susceptibility of the mother to develop choline deficiency (7). The prenatal and early postnatal periods are both critical developmental periods, regarded as being "sensitive windows of development," including to choline insufficiency [reviewed in (8)]. Liver, fish, eggs, and milk are examples of some dietary sources that are abundant in choline (9). Foods can contain and provide both water- and

lipid-soluble choline derivatives (10). In dietary supplements, choline bitartrate, choline chloride, and phosphatidylcholine (PtdCholine) are commonly used.

Choline is oxidized in the cell's mitochondria to betaine. Betaine and folate are methyl donors in one-carbon metabolism. Folate deficiency depletes choline stores in the liver (11) and increases the requirements for betaine as a source of methyl groups. In addition, choline is a source of PtdCholine that participates in lipid transport, lipid signaling, and membrane structure (1). Choline is converted to acetylcholine, which, in turn, as a neurotransmitter plays a role in behavior and neurocognition, including memory and learning (12). A choline-deficient diet in animals has been found to deplete brain choline and acetylcholine, and influence nicotinic acetylcholine receptors in the brain (13, 14). Mouse embryos exposed to inhibitors of PtdCholine synthesis or choline uptake also showed brain malformations and a dose-dependent craniofacial hypoplasia (15, 16). In contrast, in a spina bifida mouse model, supplementation of the maternal diet with 2% choline from the initiation of pregnancy substantially decreased the prevalence and severity of spina bifida in the offspring (17). Thus, insufficient maternal choline intake during early embryogenesis could be associated with brain malformations such as neural tube defects (NTDs).

Brain development begins in utero and continues throughout childhood (18). Studies in rats have shown that limited choline intake during the second half of pregnancy and early postnatal periods caused life-long abnormalities in spatial and temporal memory and attention in the offspring, which were possibly attributed to mechanisms involving reduced brain acetylcholine (19, 20). Insufficient choline intake during late pregnancy and the early postnatal period could, in theory, impact infants' neurocognitive development. Exposure to alcohol during pregnancy causes damage to the hippocampus (21). In contrast, studies in animals have shown that choline supplementation ameliorated alcohol-induced hyperactivity and visual spatial learning deficits in the offspring (22–24), suggesting that the same effects could be expected in human fetal alcohol spectrum disorders (FASDs).

A growing body of evidence from animal studies [reviewed in (25)] strongly suggests that maternal choline insufficiency could influence brain development in the offspring. However, studies in humans have shown mixed results and data have not presently been collated on the topic from clinical studies. Therefore, we aimed to evaluate the evidence from human studies investigating associations between maternal (and child) choline intake, fetal brain development, and child neurocognition. For this purpose, we conducted a systematic review followed by meta-analysis and critical appraisal of human studies published after the recognition of choline as an essential nutrient in 1998.

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The authors declare that they will share data for research purposes upon request to the corresponding author.
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Supplemental Figures 1–10, Supplemental Material, and Supplemental Tables 1–10 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/advances/.
Abbreviations used: FASD, fetal alcohol spectrum disorder; IOM, Institute of Medicine; NTD, neural tube defect; PtdCholine, phosphatidylcholine; RCT, randomized controlled trial; ROB2, version 2 of the Cochrane risk-of-bias tool for randomized trials; SB-5, Stanford–Binet Intelligence Scale.

Methods

This study was conducted according to an a priori protocol that was registered at the International Prospective Register of Systematic Reviews (PROSPERO; ID: CRD42021233790).

Study population, exposures, and outcomes

The target population consisted of pregnant/lactating women, infants, and/or children. We included prospective and retrospective studies investigating maternal or child choline intake. The exposure variable was maternal or infant choline intake from the regular diet or from supplements (any form and dose) and maternal serum/plasma and/or breast-milk concentrations of free choline, PtdCholine, or total choline.

The brain-development outcomes needed to be assessed in the fetus, newborns, infants, or children. The primary outcome was structural abnormalities of the brain such as spina bifida and anencephaly. The secondary outcomes were as follows—*1*) neurocognitive impairment including 1 or more of the following: global intellectual impairment, executive functioning deficit, learning impairment, visual-spatial reasoning deficit, or memory deficit; *2*) self-regulation deficit including 1 or more of the following: impairment of mood or behavioral regulation, attention deficit, or impulse control deficit; and *3*) adaptive functioning deficit such as communication impairment, social communication and interaction deficit, daily living skills deficit, or motor skills deficit. The neurocognitive outcomes were adopted according to widely applied criteria used to diagnose brain function abnormalities in children (26).

Search strategy

The study protocol was finalized after a pilot phase. The search was conducted in the PubMed platform on 11 February 2021 using the terms shown in **Supplemental Table 1**. The titles and abstracts of the initial search results were screened. Publications identified as potentially relevant then became candidates for full-text screening. Additionally, reference lists of previous systematic reviews and studies retained after full-text screening were reviewed and relevant publications extrapolated. A further hand-search for relevant studies was conducted in ClinicalTrials.gov and the Cochrane Library. Two authors (RO and CS) independently conducted the search, screening, data extraction, and evaluation of quality and risk of bias. Discrepancies between the study team members were resolved after discussion.

The inclusion criteria were as follows: English and German publications of human studies published from

1 January 1997 until 11 February 2021. This date was allocated due to choline being first recognized as an essential nutrient by the US Institute of Medicine (IOM) in 1998 (1). We included case-control, cohort, cross-sectional, or interventional study designs. Interventional studies included randomized, or quasi-randomized, controlled (placebo or any appropriate comparator), blinded, or open-label studies. Intervention studies conducted with pregnant and lactating women, infants, or children were also included. We planned to include studies that recruited vulnerable groups, such as preterm births, exposure to drugs, or infections during pregnancy, due to these being established stressors affecting brain development. Studies addressing FASDs were also included as alcohol is known to damage the hippocampal cholinergic system that has been shown to be a molecular target of choline (23).

We excluded animal studies, case reports, case series, narrative reviews, studies not reporting the exposure (choline intake, choline supplements, or plasma/serum/milk levels), studies supplementing choline without an appropriate comparator, and studies not reporting clinical outcomes related to brain development or the outcomes specified. In addition, studies using advanced neuroimaging techniques (i.e., magnetic resonance spectroscopy) to assess brain metabolites including choline were excluded because the link between brain choline and choline insufficiency is not established in humans.

Data extraction and quality assessment

We used a standardized form to extract the following information: first author, year of publication, PubMed ID (PMID), country of residence of the participants, folic acid fortification in the country during recruitment (yes or no), exposure definition (i.e., choline intake, blood concentrations, supplementation), how was the exposure assessed (i.e., FFQs, assay methods), when was the exposure assessed (i.e., pregnancy week or postnatal age), the exact definition of exposed versus nonexposed subjects [i.e., intake cutoff, or the comparator in randomized controlled trials (RCTs)], the form and dose of choline in studies administering supplemental choline, the start and duration of the intervention, study setting, study inclusion and exclusion criteria, primary and secondary outcomes, age of the child at outcome assessment, methods of outcome assessment (i.e., tests of neurocognition), description of the target population (total number, mean age of the mothers and the children, gestational age, or lactation stage), the study-specific effect size of the association, and the covariates that were accounted for in the studies.

For the publications that were retained in the final stage, the study quality and risk of bias were evaluated using the Newcastle-Ottawa Scales for case-control, cohort, and cross-sectional studies. The Newcastle-Ottawa Scales included specific questions on the selection of the participants, comparability of subjects, and assessment of the outcome in cohort studies (or assessment of the exposure in case-control studies). Version 2 of the Cochrane risk-of-bias tool for randomized trials (RoB2; version of 22 August 2019) and the Excel Macro Form (Microsoft Corporation) freely available online were used to evaluate the RCTs (**Supplemental Material**). The RoB2 tool evaluates the risk of bias in 5 domains: *1*) bias arising from the randomization process, *2*) bias due to deviations from the intended interventions, *3*) bias due to missing outcome data, *4*) bias in measurement of the outcome, and *5*) bias in selection of the reported results. The evaluation of the RCTs was conducted on the outcome level by the reviewers (RO and CS) who agreed on the evaluation in all studies.

Data analyses

We undertook a meta-analysis of studies when the same outcome was reported in publications and the number of studies was $\geq$3 for the main analysis. For example, this was undertaken for maternal choline intake/serum choline concentration and NTDs in the offspring. The effect size analyzed was the OR and 95% CIs. These were calculated from the contingency tables showing the presence and the absence of NTDs in the child among women with high choline intake or circulating blood concentrations compared with women with low intake or concentrations (**Table 1**).

Main data analyses

The meta-analysis was undertaken at the study level. Thus, for studies presenting contingency tables in subgroups that shared the same reference group, we assumed that the correlation coefficient between the subgroups of a study was equal to 1.0. The Mantel-Haenszel method was used to estimate the pooled OR for all strata, assuming a random-effect model. Each study was weighted by the inverse variance of its effect size. Pooled estimates and 95% CIs were calculated and presented in forest plots. The Cochran's Q statistic (calculated as the weighted sum of squared differences between individual study effects and the pooled effect across the studies) and I^2 statistics were used to study the heterogeneity between the studies. The pooled estimate and the SE were used to calculate the 95% prediction intervals that estimate the range where the true effects are to be expected for 95% of similar (exchangeable) studies that might be conducted in the future (27).

Additional data analyses

Meta-analyses were additionally performed at the sub-study level, assuming that the sub-studies were independent (although the denominator was the same control group). The aim of the analyses at the sub-study level was to evaluate the consistency in the direction and strength of the association within and across the studies and to identify trends and factors that could explain different patterns of association among the subgroups.

The sensitivity analysis included using alternative cutoff values to define low maternal choline intakes (when applicable). A leave-one-out meta-analysis was run to investigate whether removing any specific study from the analysis would

TABLE 1 Quantitative data extracted from 5 case-control studies on the association between maternal choline intake or serum total choline and NTDs in the newborns[1]

Study	Total numbers, *n* without NTD [*n*, all NTDs]	Maternal choline intake (in mg/d) or serum total choline concentration (in mM)		Offspring with NTD		Offspring without NTD		OR (95%CI)[3]
		Low intake or concentration[2]	Reference group[2]	Low choline (a)	High choline (c)	Low choline (b)	High choline (d)	
Carmichael et al., 2010 (34)	534 [279]	<293 (Q1)	≥506	72	65	133	132	1.10 (0.73, 1.66)
	534 [279]	293 to 506 (Q2 + Q3)	≥506	142	65	269	132	1.07 (0.75, 1.54)
	534 [279]	<506 (Q1 + Q2 + Q3)	≥506	214	65	402	132	1.08 (0.77, 1.52)
Petersen et al., 2019 (35)	2831 [164]	<200	>200	21	143	269	2562	1.40 (0.87, 2.25)
	2831 [164]	<200 (no suppl. use)	>200	21	131	269	2345	1.40 (0.87, 2.25)
	2831 [164]	<231 (Q1)	≥306 (Q4)	38	42	653	653	0.90 (0.58, 1.42)
	2831 [164]	230 to 306 (Q2 + Q3)	≥306 (Q4)	84	42	1525	653	0.86 (0.58, 1.25)
	2831 [164]	<306 (Q1 + Q2 + Q3)	≥306 (Q4)	122	42	2178	653	0.87 (0.61, 1.25)
Lavery et al., 2014 (32) combined and by fortification[4]	225 [184]	<545	>1008	47	41	56	56	1.15 (0.66, 2.01)
	225 [184]	545 to 760	>1008	58	41	56	56	1.41 (0.82, 2.44)
	225 [184]	760 to 1008	>1008	38	41	57	56	0.91 (0.51, 1.62)
	225 [184]	<760	>760	105	79	112	113	1.34 (0.91, 1.98)
	225 [184]	<545	>545	47	137	56	169	1.04 (0.66, 1.62)
(−)	146 [111]	<545	>1008	34	18	42	31	1.39 (0.67, 2.91)
(−)	146 [111]	545 to 760	>1008	34	18	36	31	1.63 (0.77, 3.43)
(−)	146 [111]	760 to 1008	>1008	25	18	37	31	1.16 (0.54, 2.52)
(−)	146 [111]	<760	>760	68	43	78	68	1.38 (0.83, 2.28)
(−)	146 [111]	<545	>545	34	77	42	104	1.09 (0.64, 1.88)
(+)	79 [56]	<545	>1008	11	17	14	25	1.16 (0.42, 3.15)
(+)	79 [56]	545 to 760	>1008	19	17	20	25	1.40 (0.58, 3.37)
(+)	79 [56]	760 to 1008	>1008	9	17	20	25	0.66 (0.24, 1.80)
(+)	79 [56]	<760	>760	30	26	34	45	1.53 (0.77, 3.04)
(+)	79 [56]	<545	>545	11	45	14	65	1.13 (0.47, 2.73)
Shaw et al., 2004 (33) combined and according to folate intake	440 [424]	≤290	>498	147	80	110	110	1.84 (1.26, 2.69)
	440 [424]	290 to 372	>498	99	80	110	110	1.24 (0.83, 1.84)
	440 [424]	372 to 498	>498	98	80	110	110	1.23 (0.82, 1.82)
	440 [424]	<498 (lowest 3 Qs combined)	>498	344	80	330	110	1.43 (1.04, 1.98)
	440 [424]	<372	>372	246	178	220	220	1.38 (1.06, 1.81)
Folate <350.55 μg/d	220 [211]	<372	>372	166	45	149	71	1.76 (1.14, 2.71)
Folate ≥350.56 μg	220 [213]	<372	>372	80	133	71	149	1.26 (0.85, 1.88)
Shaw et al., 2009 (36)[5]	409 [80]	Serum choline <2.7	≥2.7	33	46	100	308	2.21 (1.34, 3.65)

[1] NTD, neural tube defect; Q, quartile of choline intake.
[2] All possible subgroups of choline intake (mg/d) are shown. The subgroups may overlap depending on the cutoff shown. Cutoff values were rounded for easiness.
[3] OR for NTDs in mothers with low (vs. high) choline intake/or concentration = (a × d)/(b × c).
[4] "(+)" Refers to mandatory folic acid fortification in place during recruitment; "(−)" Refers to no mandatory folic acid fortification in the country during recruitment.
[5] Serum total choline consists of free choline and choline esters obtained after enzymatic release of choline from phospholipids.

have a major effect on the pooled estimate and thus invalidate the conclusions.

The association between maternal choline intake and NTDs could be theoretically attenuated at higher maternal folate intakes. Therefore, we conducted subgroup analysis according to maternal folate intake, assuming that women would have a higher intake if they originated from countries with mandatory folic acid fortification compared with women not exposed to folic acid fortification. Moreover, subgroup analyses were conducted according to NTD types.

P values less than 0.05 were considered to be statistically significant, whereas *P* values between 0.05 and 0.10 were considered to show a trend. The data analyses were conducted using version 3 of the Comprehensive-Meta-Analysis Software program (Biostat Inc.®).

The studies on outcomes related to child neurocognition were heterogeneous and not directly suitable for a meta-analysis. Thus, we conducted a critical appraisal of the evidence from these individual studies.

Results

Search results

The initial search identified 346 publications from the PubMed platform using the search terminologies shown in Supplemental Table 1. After screening the titles and the abstracts, 33 articles qualified for a full-text screening. Four additional articles and 3 conference abstracts were identified from the hand-search using the keywords "choline AND pregnancy" (e.g., in ClinicalTrials.gov and Cochrane Library) and screening of references used in previous systematic reviews (25, 28–31). Full texts of 40 publications were screened (**Figure 1**). A further 10 publications were excluded due to fulfilling at least 1 of the exclusion criteria (**Supplemental Table 2**). Of the remaining 30, 7 publications addressed NTDs as a main outcome and 23 publications addressed various outcomes related to neurocognition in children.

Maternal choline and NTDs in the offspring

Seven independent case-control studies investigated the association between maternal choline intake/serum total choline and NTDs in the offspring (Table 1 and **Supplemental Table 3**) (32–38). Two of the 7 studies were not suitable for quantitative data analyses: a Chinese study (37) reporting nonquantitative metabolomics data using human placentas from cases and controls and an Irish study (38) that did not present the data in a form that could be combined with the remaining studies. The 5 remaining case-control studies originated from the United States or Canada, recruited women of mixed ethnic origins, and were included in a novel meta-analysis (32–36).

The main exposure variable in 4 studies was "maternal choline intake during preconception and early pregnancy" (32–35). In the fifth study, the exposure was "maternal serum total choline concentrations" that were measured at 16–18 weeks of gestation (36). Different definitions of low choline intake were reported (Table 1). The results were presented in subgroups of spina bifida and anencephaly in 4 of the 5 studies (**Supplemental Table 4**) (32–35). In 1 study, women were recruited between 1989 and 1991 (before applying mandatory folic acid fortification in the United States) (33); in 3 studies, women were recruited after the start of mandatory folic acid fortification in the United States or Canada (34–36); and in 1 study, women were recruited both before and after folic acid fortification (32).

The 5 case-control studies included 1131 women who gave birth to offspring with NTDs and 4439 mothers of healthy children. The contingency tables and the OR (95% CI) for NTDs in women with low choline intake are shown for all possible categories of intake compared with the study-specific upper intake group (reference group) (Table 1). **Supplemental Figure 1** shows the ORs (95% CIs) of NTDs among the intake subgroups ordered from the highest to the lowest. The figure also shows the corresponding definition of low maternal intakes, types of NTD, and the presence of fortification in the country or folate intake in the mother (high and low).

The pooled OR (95% CI) for having a child with an NTD in women with low maternal choline intakes or low circulating maternal choline concentrations was 1.36 (1.11, 1.67) ($n = 5$ studies, random-effects model) compared with women with a high intake or serum concentration (**Figure 2**A). The sub-studies were consistent in the direction of the association, except for a subgroup of Mexican-American women with choline intake between 760 mg/d and 1008 mg/d compared with women with intakes >1008 mg/d (OR: 0.91; 95% CI: 0.51, 1.62) (32) (Figure 2B). The heterogeneity statistics were as follows: the Q statistics were not significant (Q value = 6.374, $df = 4$, $P = 0.173$), suggesting that the true effect size was not likely to differ between the studies. The $I^2 = 37.24$ suggests that 37% of the dispersion in the observed association reflects variance in the true effect and 63% is related to sampling/random errors (low heterogeneity). The 95% prediction intervals were 0.78 to 2.36, suggesting that the ORs to be expected for 95% of similar future studies would fall between 0.78 and 2.36 (**Supplemental Figure 2**).

The studies showed no significant publication bias (Egger's regression intercept = 3.04; 95% CI: −2.60, 8.69; $P = 0.185$) (funnel plot in **Supplemental Figure 3**A). Removing any of the 5 studies from the analysis did not substantially affect the results (Supplemental Figure 3B). For instance, the estimate was 1.26 (1.07, 1.48) when we excluded the study of Shaw et al. (36), which reported maternal serum total choline concentrations (i.e., the sum of free choline and choline esters) as an exposure variable.

The sensitivity analysis using a higher cutoff to define low choline intake (< vs. >1008 mg/d instead of < vs. >760 mg/d) in the study of Lavery et al. (32) yielded a similar pooled estimate of 1.34 (95% CI: 1.09, 1.64) (**Supplemental Figure 4**). The corresponding heterogeneity statistics were as follows: Q value = 7.431, $df = 4$, $P = 0.115$, and $I^2 = 46.17$. There was no significant publication bias and the leave-one-out meta-analysis showed consistent results (**Supplemental Figure 5**A, B). When the definition of low maternal choline

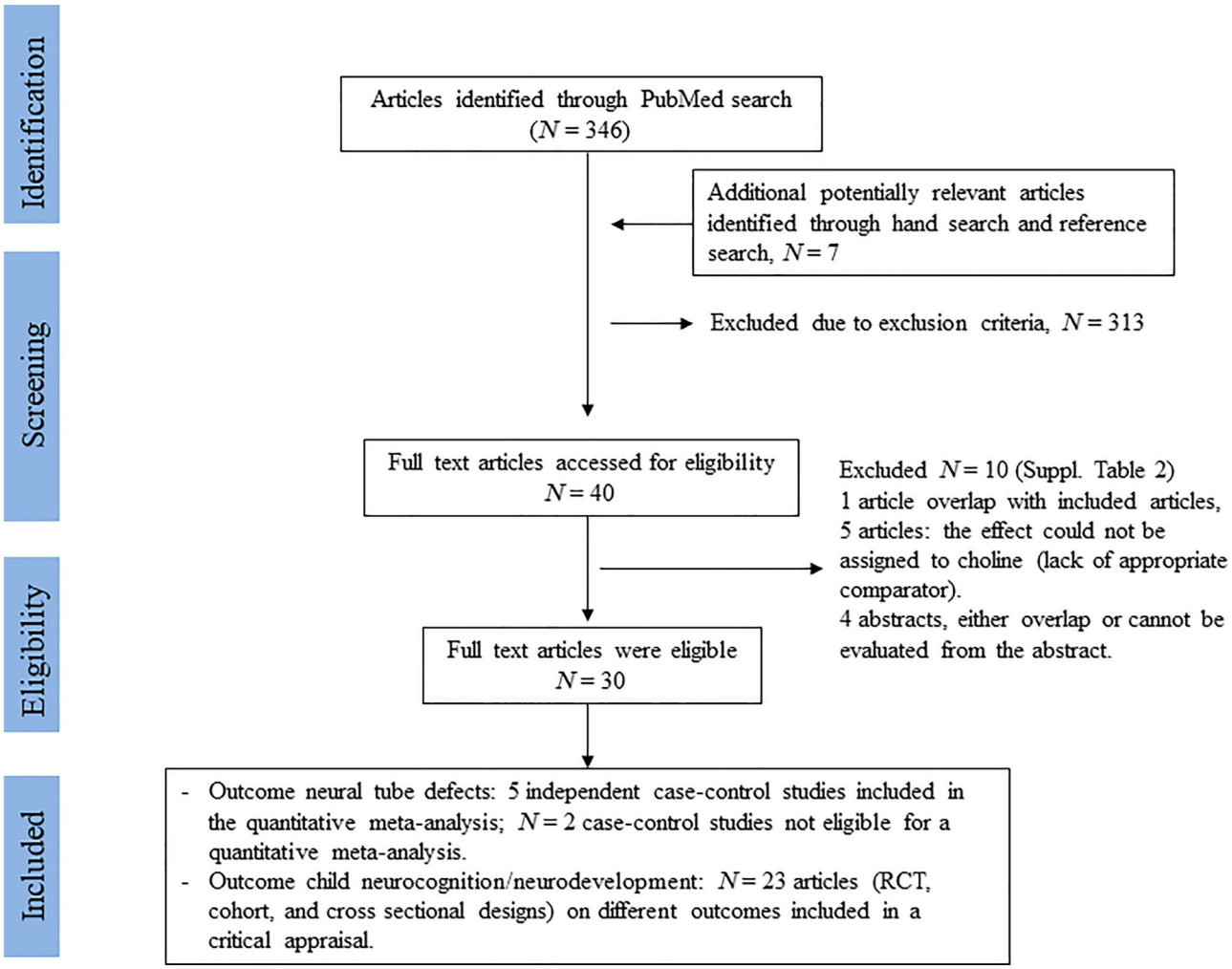

FIGURE 1 Study flow chart. RCT, randomized controlled trial; Suppl., Supplemental.

intake of <200 mg/d in the study of Petersen et al. (35) was replaced by <306 mg/d (lowest 3 quartiles) compared with >306 mg/d (the upper quartile), the pooled estimate of the association with NTDs was 1.27 (95% CI: 0.98, 1.65) (data not shown).

Generally higher pooled estimates were observed in the subgroup analysis of studies that included women who were likely to have low folate intake (before applying mandatory folic acid fortification in the country or women with a low folate intake of <350.56 μg/d) (pooled estimate = 1.56; 95% CI: 1.15, 2.12; $n = 2$ studies) (32, 33) (**Supplemental Figure 6**). Moreover, studies including women who were likely to have high folate intake (i.e., folate intake ≥350.56 μg/d or studies conducted after the fortification with folic acid) showed significant pooled estimates, but the association between maternal choline intake and NTDs was weakened (pooled estimate = 1.23; 95% CI: 1.00, 1.53; $n = 4$ studies) (**Supplemental Figure 7**A). The associations were generally consistent in the subgroups of the studies, except for 1 subgroup with maternal choline intake between 760 mg/d and 1008 mg/d (32) (Supplemental Figure 7B and **Supplemental Figure 8**).

Figure 3 and Supplemental Table 4 show the associations between maternal choline and anencephaly (pooled estimate = 1.20; 95% CI: 0.98, 1.48 ; $P = 0.079$; 3 studies) and spina bifida in the newborns (pooled estimate = 1.33; 95% CI: 1.11, 1.58; 4 studies). The ORs were generally consistent for the NTD subtypes, except for 2 subgroups with nonsignificant ORs <1.0 (**Supplemental Figure 9**).

An additional Chinese study (115 cases and 144 controls) that was not included in the meta-analysis showed lower choline in the placentas of women pregnant with an anencephalic child compared with control women (37). In contrast, an Irish study (71 cases and 214 controls) reported no significant differences in nonfasting plasma choline concentrations between mothers of the cases and mothers of the controls [mean (SD) plasma total choline = 2.8 (1.0) vs. 2.9 (0.9) mmol/L, respectively] (38).

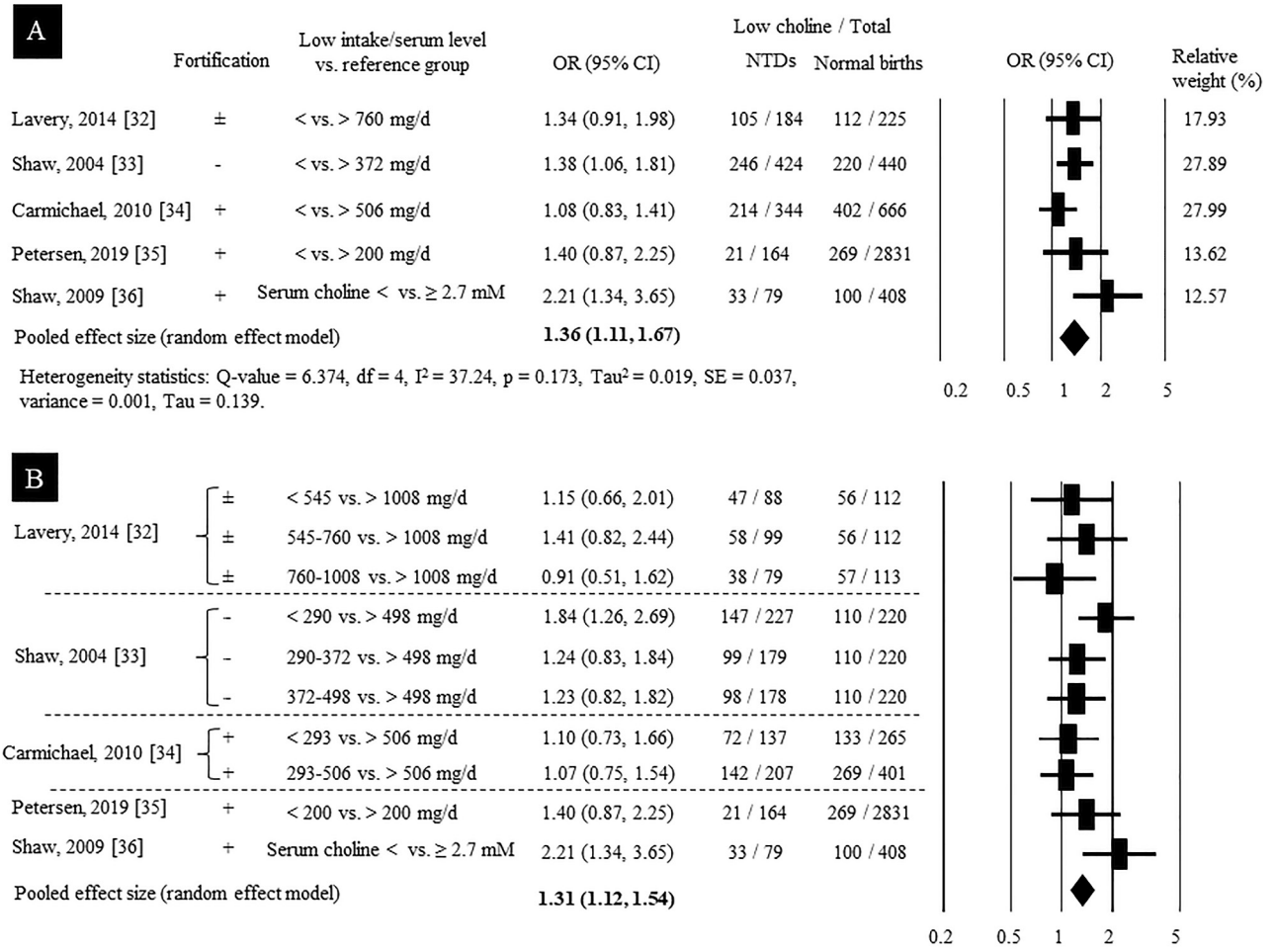

FIGURE 2 (A) Forest plot of the association between low maternal choline intake or serum total choline concentration (vs. high intake/concentration) and the OR and 95% CIs of NTDs. The analysis was run at the study level and the studies were weighted by the inverse variance. (B) The analysis was performed at the sub-study level to evaluate the consistency of the association across the subgroups. Serum total choline in the study of Shaw et al. (36) consisted of the sum of all choline-containing compounds. NTD, neural tube defect.

Choline and neurocognition and neurodevelopment of the child: evidence from interventional trials

Ten publications with RCT designs addressed the effect of supplementing the mother or the child with choline and its effects on neurocognition or neurodevelopment of the child. The studies were heterogeneous in terms of population characteristics, exposures, and outcomes (**Supplemental Tables 5** and **6**). The results are discussed below on a study-by-study basis according to 3 categories: healthy pregnant women, women exposed to alcohol during pregnancy, and infants/children exposed to alcohol during the prenatal life.

Choline supplementation in healthy pregnant women.

We identified 4 publications with RCT designs from 3 independent groups of healthy pregnant women (39–42). Caudill et al. (39) conducted a 12-wk double-blind RCT with 930 mg choline/d (380 mg dietary choline plus 550 mg choline from choline chloride) or 480 mg choline/d (380 mg from the diet plus 100 mg choline chloride) in third-trimester US pregnant women (n = 24 women were evaluated). The primary outcome, mean saccade reaction time for the stimulus-guided fixation shifts, was measured in the child at age 4, 7, 10, and 13 mo. The personnel who conducted the cognitive testing and statistical analysis were blinded to the randomization. The saccade reaction time is a visual attention task that predicts information-processing speed and childhood intelligent quotient scores (Supplemental Table 6). This task distinguishes between visually guided reactive saccades and memory-guided anticipatory saccades (39). The adjusted mean (95% CI) saccade reaction time was 33.8 (2.7, 54.8) ms faster in the high-choline-intake group compared with the low-intake group (39). Infants from mothers with a high choline intake (vs. low-intake group) were faster to react to pictures at age 4, 7, 10, and 13 mo. The number of predictive saccades (the secondary outcome) did not differ according to maternal choline intake (39). Therefore, infants whose mothers achieved 930 mg choline/d

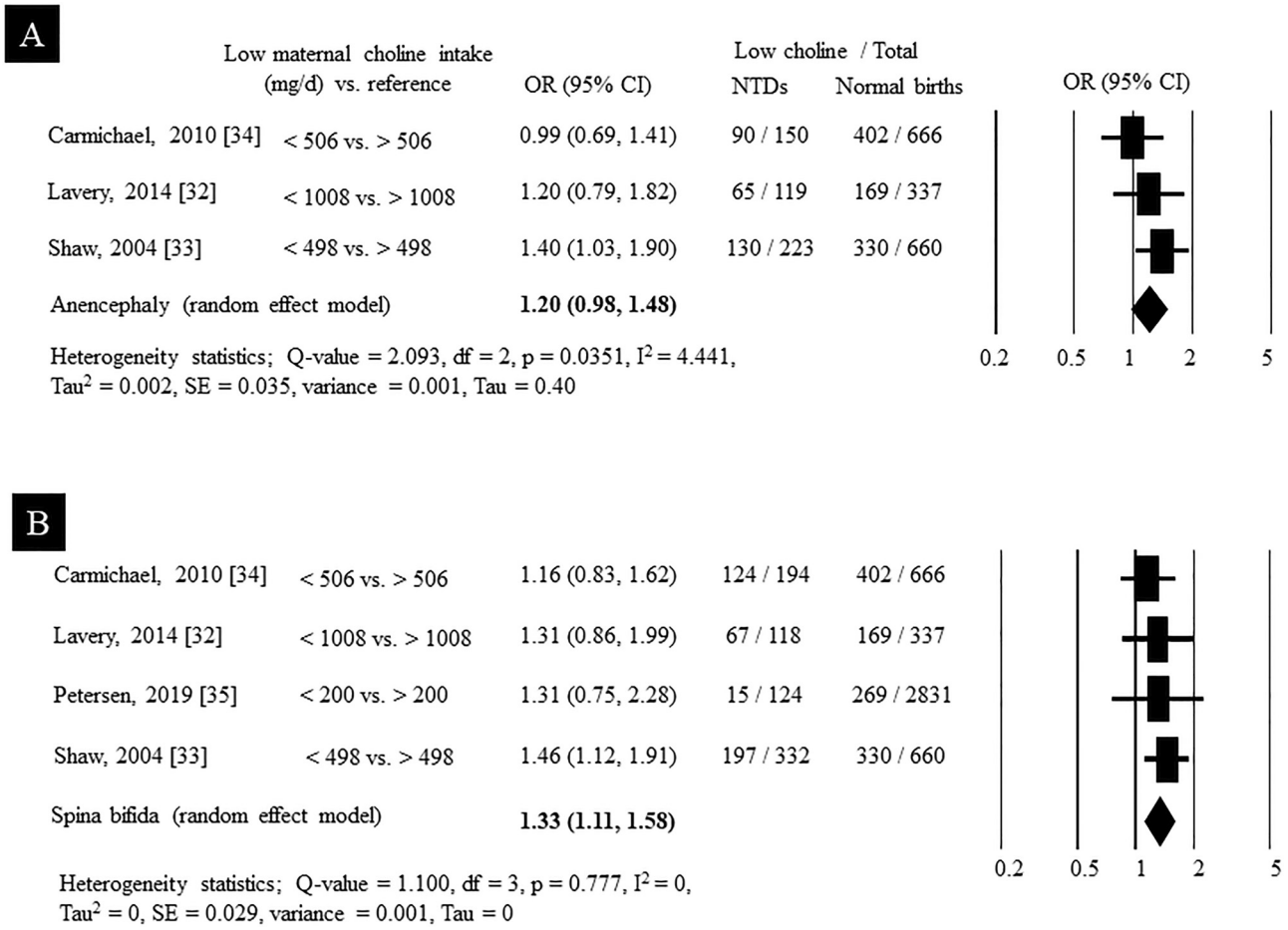

FIGURE 3 Forest plot showing subgroup analysis of the association between low maternal choline intake (vs. high intake) and the OR and 95% CIs of anencephaly (A) and spina bifida (B). NTD, neural tube defect.

(vs. 480 mg/d) showed consistently faster average saccade reaction time in the first year of life.

In a further randomized double-blind trial, Ross et al. (40, 41) used a PtdCholine equivalent to 900 mg/d choline or placebo in 100 healthy US pregnant women (76 women completed the study) from the second trimester until birth. After birth, the infants received 100 mg choline/d until the age of 3 mo (40, 41). Sensory gating deficiency in the infants was studied at the age of 1 mo and 3 mo by using electroencephalographic recordings of inhibition of the P50 component of the cerebral evoked response to paired sounds. The inhibition of the auditory P50 cerebral evoked response was defined as the amplitude of the P50 response to the second of paired auditory stimuli divided by the amplitude of the response to the first stimulus (40). An intact cerebral inhibition was defined a priori as a P50 ratio <0.5. In infants aged 1 mo, more infants in the choline group had a P50 ratio <0.5 compared with the placebo group (76% vs. 43%; $P = 0.009$). At the age of 3 mo, the percentage of infants with a normal P50 ratio did not differ between infants in the choline and placebo groups (76% vs. 72%, respectively) (40). At the age of 6 mo, the choline and placebo groups did not differ in other domains of general development such as visual perception and discrimination and receptive or expressive language (40). Therefore, 1-mo-old infants of mothers who received 900 mg choline/d (vs. placebo) were more likely to have intact cerebral inhibition as a response to stimuli, suggesting a reduced future risk of attentional dysfunction and schizophrenia (40). However, the high percentage of loss to follow-up (24%) and applying unplanned neurocognitive tests (i.e., the Mullen Scales of Early Learning) limit the generalizability of the results.

Ross et al. (41) compared parent ratings on the Child Behavior Checklist between the choline and the placebo arms in a subgroup of 49 children at the age of 40 mo (50% of the original cohort). Parents were blinded for the intervention. The sex-adjusted parent ratings of the attention subscale ($P = 0.038$) and the withdrawn subscale ($P = 0.003$) were lower in children from the choline group compared with those in the placebo group (41). Scores of primary behavior problems did not differ between the interventions. The results suggest that choline supplementation in the mother could have a favorable effect on child attention and withdrawal at the age of 40 mo (41). Limitations of this study

were the unplanned analyses and the fact that the outcome was available from only 50% of those randomized, which could cause selection bias for the 40-mo outcome.

Cheatham et al. (42) administered 750 mg choline/d (from PtdCholine) or placebo to pregnant women from week 18 of pregnancy until 90 d postpartum in a randomized double-blind design. The average dietary choline intake in the women was 360 mg/d (42). The short-term visuospatial memory delayed response task, long-term episodic memory, and language development were investigated in the child at age 10 and 12 mo (42). The compliance rate was taken into account in the data analyses. The intervention had no significant effect on neurocognition of the child at the age of 10 mo. At 12 mo, the long-term episodic memory task tended to be lower in the choline compared with the placebo group [mean (SD) = 0.45 (0.22) vs. 0.53 (0.20); $P = 0.056$]. The intervention had no significant effects on the composite index of global development, number of words spoken, short-term visuospatial memory, or long-term episodic memory (42). The results implied that 750 mg choline/d from PtdCholine had no effect on infant brain development in middle-class American mothers consuming a Western diet (42). However, low compliance and a high drop-out rate (41 of 140 participants dropped out) may limit the generalizability of the results.

Choline supplementation in pregnant women exposed to alcohol.

Three publications with RCT designs were conducted in 2 independent cohorts of women who consumed alcohol during pregnancy (43–45). Jacobson et al. (43) provided placebo or 1.25 × 2 g choline bitartrate/d (equivalent to ∼1 g bioavailable choline with molecular weight = 104.17 g/mol) on top of the diet starting from the 20th week of gestation until delivery. Eyeblink conditioning was the primary outcome that was analyzed in 62 infants (31 choline and 31 placebo) at the age of 6.5 mo (43). Eyeblink conditioning detects abnormalities in cerebellar-dependent learning and memory. A trend towards a favorable effect of choline supplementation (vs. placebo) on eyeblink conditioning was observed in the whole group ($P = 0.090$). The effect of choline (vs. placebo) on eyeblink conditioning was significant ($P = 0.036$) after excluding 4 subjects with <20% adherence. The Fagan Test of Infant Intelligence test showed improved visual recognition memory at 12 mo in infants from the choline group compared with those from the placebo group. This study was well designed, although there was some concern regarding whether outcomes were planned.

Kable et al. (44) and Coles et al. (45) provided 750 mg choline/d (the supplemental form was unreported) plus multivitamin nutrients (vs. multivitamin nutrients) from week 19 of gestation until delivery to pregnant women who were exposed to alcohol. In 1 publication, visual and auditory stimuli were measured in the child at the age of 4 to 11 mo using a fixed-trial habituation/dishabituation paradigm and cardiac responses to stimuli (Supplemental Table 6) (44). The psychologists were blinded to the intervention, while the participants were not blinded. Children in the choline group showed a significant change in heart rate after visual stimuli compared with those in the group without choline (adjusted $P < 0.001$) (44). Choline supplementation showed a significant effect on latency in the visual habituation trials [Wald chi-square $F(1, 150) = 9.0$; adjusted $P < 0.003$] (44), but no effect on the results of the Bayley Scales of Infant Development test at the age of 6 mo (45). Limitations of the study design included the fact that it was unblinded and there was a selective loss to follow-up in high-risk drinkers.

Choline supplementation in children with FASDs.

Three publications with RCT designs were conducted in infants or children born to mothers who ingested alcohol during pregnancy (46–48). Two of the publications were based on the same group of infants (46, 47).

In a pilot study, Wozniak et al. (46, 47) administered 1.25 g choline bitartrate (513 mg choline/d) or placebo to 60 children (aged 2.5–5 y) for a duration of 9 mo (46, 47). Global cognitive development of the child was the primary outcome that was measured by the Mullen Scales of Early Learning. The test provides total score and subscores of components of hippocampal-dependent memory (early learning composite, visual reception, fine motor, receptive language, and expressive language) (46). The study groups did not differ in global cognitive development or in elicited imitation memory paradigm (a hippocampus-dependent memory task). The largest improvement in the elicited imitation delayed performance occurred in children aged between 2.5 and 4.0 y from the choline group (46), suggesting an effect moderation by age.

A subgroup of 31 children was then retested 4 y after the end of the original trial (46). The long-term cognitive and behavioral tests and domain-specific outcomes were studied focusing on memory, attention, and executive functioning using the Stanford–Binet Intelligence Scale (SB-5) test (47). The elicited imitation memory paradigm was repeated with more difficult event sequences and a longer memory delay to account for the age of the children at follow-up (47). Children in the choline group showed 8.2% higher SB-5 nonverbal IQ subscores [$F(1, 28) = 5.17$, $P = 0.03$] and 11.7% higher subscores of working memory [$F(1, 28) = 7.74$, $P = 0.01$] compared with the placebo group (47). A post hoc test showed significant effects of choline versus placebo on the subscores of nonverbal visual-spatial reasoning component ($P = 0.004$) and the nonverbal working memory component ($P = 0.018$) (47). Therefore, choline supplementation showed no effects on memory in the short term (46), but the nonverbal aspects of working memory and visual spatial processing improved in the long term in children with FASDs (47). These long-term outcomes after 4 y were tested in a subgroup of the children and were not planned a priori (47).

Nguyen et al. (48) administered glycerolphosphocholine liquid corresponding to 625 mg choline/d or placebo for 6 wk to 55 children with FASDs (mean age: 8.3 y; range: 5–10 y) and investigated a standardized neuropsychological test battery at the end of the intervention. The intervention had

no significant effects on paired associates learning, design fluency, spatial working memory, spatial working memory strategy, quotient attention-deficit/hyperactivity disorder (ADHD), and grooved pegboard (48). However, the lack of effect might be due to the short duration of the study and possible irreversible symptoms in older children.

Choline safety in RCTs

None of the intervention studies exceeded the Tolerable Upper Intake Level for choline (3.5 g/d) (1). No serious adverse outcomes in the mothers or the children were observed in any of the included studies all providing different forms and dosages of choline in the aforementioned trials. A fishy body odor was the only characteristic side effect reported after choline intake (1) (**Supplemental Table 7**).

Evidence from noninterventional studies

Associations between maternal or child choline intake/serum choline concentration and child neurocognition and neurodevelopment were addressed in 13 publications with longitudinal or cross-sectional designs (**Supplemental Table 8**) (49–62). A critical evaluation of the publications is provided in the Supplemental Material. The overall evaluation of this section in the context of the present study is addressed in the discussion.

Quality of the studies and risk of bias assessment

Case-control, cohort, and cross-sectional studies identified were of low risk of bias (i.e., scored ≥8 of 9) (**Supplemental Tables 9** and **10**). However, these forms of studies have inherent limitations such as retrospective measurements of choline intake in case-control studies and the use of plasma choline as a biomarker of choline intake or status in cohort studies. The risk of bias in publications with RCT designs was evaluated for each reported outcome (**Supplemental Figure 10**). Two RCTs with a low risk of bias reported results in favor of choline's role in brain function and were conducted either in pregnant women (39) or in children with FASDs (46). One study in women with alcohol consumption during pregnancy (43) showed some concerns regarding the risk of bias due to likely unplanned outcomes, but it reported favorable effects of choline in women who adhered to the supplements. The 6-wk RCT with a low risk of bias was conducted in children with FASDs aged between 5 and 10 y (48), which could explain the lack of effect in the intervention group. The remaining publications were of a high risk of bias mostly due to missing results and unplanned results.

Discussion

Brief interpretation of the results of the meta-analysis

We found that low maternal choline intake/circulating maternal choline concentrations were associated with a 36% higher risk of NTDs (OR: 1.36; 95% CI: 1.11, 1.67). The predictive intervals (0.78, 2.36) suggest that low choline intake could be associated with an OR for NTDs of up to 2.36 in future studies. At present, the results should be interpreted with caution due to the limited number of available studies and possible recall bias, including underreporting in studies interviewing women several months after delivery.

Subgroup analyses according to maternal folate intake suggest that the association between low maternal choline intake and NTDs is independent of folate but could be stronger in women with low folate intake (Supplemental Figures 6 and 7). The interaction between choline and folate could be explained by the mutual functions of these nutrients as methyl donors and the possibility that choline could be spared for other developmental pathways when folate intake is sufficient. Studies in mice have shown that, compared with folic acid, choline added to maternal diets prevented more cases of spina bifida (17). Collectively, these results suggest that the role of choline in early brain development is unique and not fully exchangeable with the role of folate. A pooled analysis of individual data from the case-control studies is timely and would enable adjusting for potential confounding variables.

Coherent evidence from RCTs

Despite limitations of available RCTs, the results generally support favorable effects of higher maternal choline intake on some domains of neurodevelopment and neurocognition in the child (i.e., self-regulation, learning, and memory) (39–41).

Choline supplementation was initiated in the second or third trimester and lasted until birth or up to 3 mo postpartum. Different forms of choline (PtdCholine, choline chloride, choline bitartrate, and glycerophosphocholine) and doses (≤1 g/d) have been used on top of the diet in RCTs. Choline intake in the available RCTs was higher than the average intake that women could achieve through the usual diet (3) and exceeded the adequate intake of 450 mg/d or 480 mg/d for pregnant women as set by the IOM or the European Food Safety Authority. Thus, maternal choline intake above the present adequate intake appears to be necessary to influence neurocognition of the child.

Results on choline supplementation in pregnancies exposed to alcohol provided supportive evidence that choline could impact brain functions. Choline supplementation (vs. placebo) to pregnant women exposed to alcohol from the second trimester to birth had positive effects on learning and memory of the infants (43). The study among 2.5–5-y-old children with FASDs supports favorable effects of choline on nonverbal aspects of working memory and on visual spatial processing (46). Furthermore, the null results in the 6-wk trial among older children (48) could reflect a critical time window for improving brain outcome, at least in FASDs, a model of disturbed cholinergic system in the brain.

Further high-quality interventional studies with well-planned outcomes, longer follow-up time, and sufficient power are needed. Choline supplements could start before pregnancy or in the first trimester and last until birth or at least 3–6 mo of lactation. The repeated neurocognitive tests should account for the age of the child at follow-up and minimize potential measurement errors due to ceiling effects

(47). Showing a dose–response relation between maternal choline intake and child neurocognetive test scores can strengthen the evidence that choline is causally involved in brain development. Moreover, future multilevel meta-analysis combining correlated neurocognitive outcomes in the child could address the overall effects of RCTs across the studies.

Observational studies

Results of observational studies are mixed. Some studies have shown associations supporting that higher choline intake or circulating concentrations in the mother are associated with better neurocognitive outcomes in the child (51, 58), especially in women with prenatal stressors (52–57). Results of observational studies generally provide coherence to the hypothesis that maternal choline intake can impact brain development and functions.

In contrast to interventional studies, observational studies are subject to more confounding by external and contextual factors (including cultural, socioeconomic, and dietary) that may influence maternal choline intake and child cognition at the same time. There is currently no optimal blood marker for choline intake. Normal fasting plasma free choline concentrations in humans are approximately 10 μmol/L (63, 64), with concentrations reported in early pregnancy (58). Plasma choline concentrations are low in deficient people (65); they increase in response to high choline intake in adults (66, 67) and in late pregnancy (58, 68, 69). Plasma choline concentrations do not accurately reflect moderate changes in choline intake (70) and are thus not a reliable marker for choline status. Circulating choline concentrations in plasma reflect endogenous synthesis, intake, uptake, losses, and likely a degree of homeostatic and metabolic regulations (71). During pregnancy, plasma choline concentrations are subject to additional dynamic changes due to transport to the fetus and maternal liver phosphatidylethanolamine methyl transferase (PEMT) induction by estrogen (72). Moreover, the association between maternal choline intake and child neurocognitive outcomes could be subject to effect modification by folate intake (50), DHA (59), and betaine (58, 61, 62). Understanding these interactions could further offer an opportunity to identify additive or synergistic effects of multiple nutrients, but this issue requires in-depth investigations.

Epidemiological studies reported average dietary intakes of choline in pregnant women of approximately 350 mg/d (42, 50, 58). This corresponds to 100–180 mg/d less than the Adequate Intake for pregnant women (1, 2). Adequate choline intakes are difficult to attain through the usual diet. Thus, antenatal multivitamin supplements should contain choline in addition to folate. Like folate, choline supplementation should be part of standard health care for women planning a pregnancy and pregnant and lactating women.

The present study has some limitations. First, our search was limited to English and German articles published in PubMed. Although we cannot exclude that we may have missed relevant studies, a systematic search in Cochrane-library.com during preparation of this article (30 August 2021) identified 83 potential publications but did not yield any further publications compared with the search in PubMed. Second, due to the limited number of available studies, the present meta-analysis included 1 study reporting maternal serum total choline in millimoles per liter (36). Serum total choline was measured as free choline and choline esters obtained after enzymatic release of choline from phospholipids (73). This marker is not well established in the literature. However, excluding this single study did not substantially change the association between maternal choline intake and NTDs, suggesting that the conclusion is not likely to be biased. Finally, case-control studies are commonly used to investigate rare diseases, but this design has inherent limitations.

Conclusions

Low maternal choline intake/circulating serum total choline in early pregnancy was associated with a 36% higher OR for NTDs. In general, RCTs providing up to 1 g choline/d to women during the second part of the pregnancy showed favorable effects on certain neurocognitive domains of the child. Despite limitations of longitudinal and cross-sectional studies, the results were generally supportive of potential associations between higher choline intake/circulating plasma choline concentrations and favorable neurocognition/neurodevelopment in children. The role of choline in brain development is biologically plausible and strongly supported by animal studies [reviewed in (25, 74)]. Thus, associations reported in the present review are likely to be reflective of a cause-and-effect relation. Choline intake exceeding current recommendations appears to be necessary to influence child neurocognition. Updated, robust recommendations from obstetric societies about the need to fulfill choline intake during pregnancy and lactation to support brain development are warranted. In order to achieve recommended choline intake thresholds, the need to add choline to antenatal supplements should be urgently considered.

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References

1. Institute of Medicine. Dietary Reference Intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington (DC): National Academy Press; 1998.
2. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Dietary reference values for choline. EFSA J 2016;14:4484.
3. Vennemann FB, Ioannidou S, Valsta LM, Dumas C, Ocke MC, Mensink GB, et al. Dietary intake and food sources of choline in European populations. Br J Nutr 2015;114(12):2046–55.

4. Wiedeman AM, Barr SI, Green TJ, Xu Z, Innis SM, Kitts DD. Dietary choline intake: current state of knowledge across the life cycle. Nutrients 2018;10(10):1513.
5. Molloy AM, Mills JL, Cox C, Daly SF, Conley M, Brody LC, et al. Choline and homocysteine interrelations in umbilical cord and maternal plasma at delivery. Am J Clin Nutr 2005;82(4): 836–42.
6. Ilcol YO, Ozbek R, Hamurtekin E, Ulus IH. Choline status in newborns, infants, children, breast-feeding women, breast-fed infants and human breast milk. J Nutr Biochem 2005;16(8):489–99.
7. Zeisel SH, Mar MH, Zhou Z, da Costa KA. Pregnancy and lactation are associated with diminished concentrations of choline and its metabolites in rat liver. J Nutr 1995;125:3049–54.
8. Blusztajn JK. Choline, a vital amine. Science 1998;281(5378):794–5.
9. Patterson K, Bhagwat S, Williams JR, Howe JC, Holden JM, Zeisel SH, et al. USDA Database for the Choline Content of Common Foods, release 2. Nutrient Data Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service USDA. 2008. Available from: https://data.nal.usda.gov/dataset/usda-database-choline-content-common-foods-release-2-2008 (accessed 24 April 2021).
10. Zeisel SH, Mar MH, Howe JC, Holden JM. Concentrations of choline-containing compounds and betaine in common foods. J Nutr 2003;133(5):1302–7.
11. Kim YI, Miller JW, da Costa KA, Nadeau M, Smith D, Selhub J, et al. Severe folate deficiency causes secondary depletion of choline and phosphocholine in rat liver. J Nutr 1994;124(11):2197–203.
12. Hasselmo ME. The role of acetylcholine in learning and memory. Curr Opin Neurobiol 2006;16(6):710–15.
13. Cohen EL, Wurtman RJ. Brain acetylcholine: increase after systemic choline administration. Life Sci 1975;16(7):1095–102.
14. Morley BJ, Robinson GR, Brown GB, Kemp GE, Bradley RJ. Effects of dietary choline on nicotinic acetylcholine receptors in brain. Nature 1977;266(5605):848–50.
15. Fisher MC, Zeisel SH, Mar MH, Sadler TW. Perturbations in choline metabolism cause neural tube defects in mouse embryos in vitro. FASEB J 2002;16(6):619–21.
16. Fisher MC, Zeisel SH, Mar MH, Sadler TW. Inhibitors of choline uptake and metabolism cause developmental abnormalities in neurulating mouse embryos. Teratology 2001;64(2):114–22.
17. Rogers SW, Tvrdik P, Capecchi MR, Gahring LC. Prenatal ablation of nicotinic receptor alpha7 cell lineages produces lumbosacral spina bifida the severity of which is modified by choline and nicotine exposure. Am J Med Genet A 2012;158A(5):1135–44.
18. Thomason ME. Development of brain networks in utero: relevance for common neural disorders. Biol Psychiatry 2020;88(1):40–50.
19. Meck WH, Williams CL. Characterization of the facilitative effects of perinatal choline supplementation on timing and temporal memory. Neuroreport 1997;8(13):2831–5.
20. Meck WH, Smith RA, Williams CL. Organizational changes in cholinergic activity and enhanced visuospatial memory as a function of choline administered prenatally or postnatally or both. Behav Neurosci 1989;103(6):1234–41.
21. Berman RF, Hannigan JH. Effects of prenatal alcohol exposure on the hippocampus: spatial behavior, electrophysiology, and neuroanatomy. Hippocampus 2000;10(1):94–110.
22. Thomas JD, Idrus NM, Monk BR, Dominguez HD. Prenatal choline supplementation mitigates behavioral alterations associated with prenatal alcohol exposure in rats. Birth Defects Res A Clin Mol Teratol 2010;88(10):827–37.
23. Monk BR, Leslie FM, Thomas JD. The effects of perinatal choline supplementation on hippocampal cholinergic development in rats exposed to alcohol during the brain growth spurt. Hippocampus 2012;22(8):1750–7.
24. Thomas JD, Tran TD. Choline supplementation mitigates trace, but not delay, eyeblink conditioning deficits in rats exposed to alcohol during development. Hippocampus 2012;22(3):619–30.
25. Derbyshire E, Obeid R. Choline, neurological development and brain function: a systematic review focusing on the first 1000 days. Nutrients 2020;12(6):1731.
26. Mattson SN, Bernes GA, Doyle LR. Fetal alcohol spectrum disorders: a review of the neurobehavioral deficits associated with prenatal alcohol exposure. Alcohol Clin Exp Res 2019;43:1046–62.
27. IntHout J, Ioannidis JP, Rovers MM, Goeman JJ. Plea for routinely presenting prediction intervals in meta-analysis. BMJ Open 2016;6(7):e010247.
28. Freedman R, Hunter SK, Hoffman MC. Prenatal primary prevention of mental illness by micronutrient supplements in pregnancy. Am J Psychiatry 2018;175(7):607–19.
29. Akison LK, Kuo J, Reid N, Boyd RN, Moritz KM. Effect of choline supplementation on neurological, cognitive, and behavioral outcomes in offspring arising from alcohol exposure during development: a quantitative systematic review of clinical and preclinical studies. Alcohol Clin Exp Res 2018;42(9):1591–611.
30. Leermakers ET, Moreira EM, Kiefte-de Jong JC, Darweesh SK, Visser T, Voortman T, et al. Effects of choline on health across the life course: a systematic review. Nutr Rev 2015;73(8):500–22.
31. Wallace TC, Blusztajn JK, Caudill MA, Klatt KC, Zeisel SH. Choline: the neurocognitive essential nutrient of interest to obstetricians and gynecologists. J Diet Suppl 2020;17(6):733–52.
32. Lavery AM, Brender JD, Zhao H, Sweeney A, Felkner M, Suarez L, et al. Dietary intake of choline and neural tube defects in Mexican Americans. Birth Defects Res A Clin Mol Teratol 2014;100(6):463–71.
33. Shaw GM, Carmichael SL, Yang W, Selvin S, Schaffer DM. Periconceptional dietary intake of choline and betaine and neural tube defects in offspring. Am J Epidemiol 2004;160(2):102–9.
34. Carmichael SL, Yang W, Shaw GM. Periconceptional nutrient intakes and risks of neural tube defects in California. Birth Defects Res A Clin Mol Teratol 2010;88(8):670–8.
35. Petersen JM, Parker SE, Crider KS, Tinker SC, Mitchell AA, Werler MM. One-carbon cofactor intake and risk of neural tube defects among women who meet folic acid recommendations: a multicenter case-control study. Am J Epidemiol 2019;188(6):1136–43.
36. Shaw GM, Finnell RH, Blom HJ, Carmichael SL, Vollset SE, Yang W, et al. Choline and risk of neural tube defects in a folate-fortified population. Epidemiology 2009;20(5):714–19.
37. Chi Y, Pei L, Chen G, Song X, Zhao A, Chen T, et al. Metabonomic profiling of human placentas reveals different metabolic patterns among subtypes of neural tube defects. J Proteome Res 2014;13(2):934–45.
38. Mills JL, Fan R, Brody LC, Liu A, Ueland PM, Wang Y, et al. Maternal choline concentrations during pregnancy and choline-related genetic variants as risk factors for neural tube defects. Am J Clin Nutr 2014;100(4):1069–74.
39. Caudill MA, Strupp BJ, Muscalu L, Nevins JEH, Canfield RL. Maternal choline supplementation during the third trimester of pregnancy improves infant information processing speed: a randomized, double-blind, controlled feeding study. FASEB J 2018;32(4): 2172–80.
40. Ross RG, Hunter SK, McCarthy L, Beuler J, Hutchison AK, Wagner BD, et al. Perinatal choline effects on neonatal pathophysiology related to later schizophrenia risk. Am J Psychiatry 2013;170(3):290–8.
41. Ross RG, Hunter SK, Hoffman MC, McCarthy L, Chambers BM, Law AJ, et al. Perinatal phosphatidylcholine supplementation and early childhood behavior problems: evidence for CHRNA7 moderation. Am J Psychiatry 2016;173(5):509–16.
42. Cheatham CL, Goldman BD, Fischer LM, da Costa KA, Reznick JS, Zeisel SH. Phosphatidylcholine supplementation in pregnant women consuming moderate-choline diets does not enhance infant cognitive function: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr 2012;96(6):1465–72.
43. Jacobson SW, Carter RC, Molteno CD, Stanton ME, Herbert JS, Lindinger NM, et al. Efficacy of maternal choline supplementation during pregnancy in mitigating adverse effects of prenatal alcohol

exposure on growth and cognitive function: a randomized, double-blind, placebo-controlled clinical trial. Alcohol Clin Exp Res 2018;42(7):1327–41.
44. Kable JA, Coles CD, Keen CL, Uriu-Adams JY, Jones KL, Yevtushok L, et al. The impact of micronutrient supplementation in alcohol-exposed pregnancies on information processing skills in Ukrainian infants. Alcohol 2015;49(7):647–56.
45. Coles CD, Kable JA, Keen CL, Jones KL, Wertelecki W, Granovska IV, et al. Dose and timing of prenatal alcohol exposure and maternal nutritional supplements: developmental effects on 6-month-old infants. Matern Child Health J 2015;19(12):2605–14.
46. Wozniak JR, Fuglestad AJ, Eckerle JK, Fink BA, Hoecker HL, Boys CJ, et al. Choline supplementation in children with fetal alcohol spectrum disorders: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr 2015;102(5):1113–25.
47. Wozniak JR, Fink BA, Fuglestad AJ, Eckerle JK, Boys CJ, Sandness KE, et al. Four-year follow-up of a randomized controlled trial of choline for neurodevelopment in fetal alcohol spectrum disorder. J Neurodev Disord 2020;12(1):9.
48. Nguyen TT, Risbud RD, Mattson SN, Chambers CD, Thomas JD. Randomized, double-blind, placebo-controlled clinical trial of choline supplementation in school-aged children with fetal alcohol spectrum disorders. Am J Clin Nutr 2016;104(6):1683–92.
49. Signore C, Ueland PM, Troendle J, Mills JL. Choline concentrations in human maternal and cord blood and intelligence at 5 y of age. Am J Clin Nutr 2008;87(4):896–902.
50. Villamor E, Rifas-Shiman SL, Gillman MW, Oken E. Maternal intake of methyl-donor nutrients and child cognition at 3 years of age. Paediatr Perinat Epidemiol 2012;26(4):328–35.
51. Boeke CE, Gillman MW, Hughes MD, Rifas-Shiman SL, Villamor E, Oken E. Choline intake during pregnancy and child cognition at age 7 years. Am J Epidemiol 2013;177(12):1338–47.
52. Freedman R, Hunter SK, Law AJ, D'Alessandro A, Noonan K, Wyrwa A, et al. Maternal choline and respiratory coronavirus effects on fetal brain development. J Psychiatr Res 2020;128:1–4.
53. Freedman R, Hunter SK, Law AJ, Wagner BD, D'Alessandro A, Christians U, et al. Higher gestational choline levels in maternal infection are protective for infant brain development. J Pediatr 2019;208:198–206.
54. Hoffman MC, Hunter SK, D'Alessandro A, Noonan K, Wyrwa A, Freedman R. Interaction of maternal choline levels and prenatal marijuana's effects on the offspring. Psychol Med 2020;50(10):1716–26.
55. Hunter SK, Hoffman MC, D'Alessandro A, Wyrwa A, Noonan K, Zeisel SH, et al. Prenatal choline, cannabis, and infection, and their association with offspring development of attention and social problems through 4 years of age. Psychol Med 2021;1–10. doi: 10.1017/S0033291720005061. Online ahead of print.
56. Hunter SK, Hoffman MC, D'Alessandro A, Noonan K, Wyrwa A, Freedman R, et al. Male fetus susceptibility to maternal inflammation: C-reactive protein and brain development. Psychol Med 2021;51(3):450–9.
57. Hunter SK, Hoffman MC, McCarthy L, D'Alessandro A, Wyrwa A, Noonan K, et al. Black American maternal prenatal choline, offspring gestational age at birth, and developmental predisposition to mental illness. Schizophr Bull 2021;47(4):896–905.
58. Wu BT, Dyer RA, King DJ, Richardson KJ, Innis SM. Early second trimester maternal plasma choline and betaine are related to measures of early cognitive development in term infants. PLoS One 2012;7(8):e43448.
59. Cheatham CL, Sheppard KW. Synergistic effects of human milk nutrients in the support of infant recognition memory: an observational study. Nutrients 2015;7(11):9079–95.
60. Wiedeman AM, Whitfield KC, March KM, Chen NN, Kroeun H, Sokhoing L, et al. Concentrations of water-soluble forms of choline in human milk from lactating women in Canada and Cambodia. Nutrients 2018;10(3):381.
61. Wiedeman AM, Chau CMY, Grunau RE, McCarthy D, Yurko-Mauro K, Dyer RA, et al. Plasma betaine is positively associated with developmental outcomes in healthy toddlers at age 2 years who are not meeting the recommended Adequate Intake for dietary choline. J Nutr 2018;148(8):1309–14.
62. Strain JJ, McSorley EM, van Wijngaarden E, Kobrosly RW, Bonham MP, Mulhern MS, et al. Choline status and neurodevelopmental outcomes at 5 years of age in the Seychelles Child Development Nutrition Study. Br J Nutr 2013;110(2):330–6.
63. Smolders L, de Wit NJW, Balvers MGJ, Obeid R, Vissers MMM, Esser D. Natural choline from egg yolk phospholipids is more efficiently absorbed compared with choline bitartrate; outcomes of a randomized trial in healthy adults. Nutrients 2019;11(11): 2758.
64. Savendahl L, Mar MH, Underwood LE, Zeisel SH. Prolonged fasting in humans results in diminished plasma choline concentrations but does not cause liver dysfunction. Am J Clin Nutr 1997;66(3): 622–5.
65. Zeisel SH, da Costa KA, Franklin PD, Alexander EA, Lamont JT, Sheard NF, et al. Choline, an essential nutrient for humans. FASEB J 1991;5(7):2093–8.
66. Wallace JM, McCormack JM, McNulty H, Walsh PM, Robson PJ, Bonham MP, et al. Choline supplementation and measures of choline and betaine status: a randomised, controlled trial in postmenopausal women. Br J Nutr 2012;108(7):1264–71.
67. Lemos BS, Medina-Vera I, Malysheva OV, Caudill MA, Fernandez ML. Effects of egg consumption and choline supplementation on plasma choline and trimethylamine-N-oxide in a young population. J Am Coll Nutr 2018;37(8):716–23.
68. Yan J, Jiang X, West AA, Perry CA, Malysheva OV, Devapatla S, et al. Maternal choline intake modulates maternal and fetal biomarkers of choline metabolism in humans. Am J Clin Nutr 2012;95(5): 1060–71.
69. Jacobson SW, Carter RC, Molteno CD, Meintjes EM, Senekal MS, Lindinger NM, et al. Feasibility and acceptability of maternal choline supplementation in heavy drinking pregnant women: a randomized, double-blind, placebo-controlled clinical trial. Alcohol Clin Exp Res 2018;42(7):1315–26.
70. Abratte CM, Wang W, Li R, Axume J, Moriarty DJ, Caudill MA. Choline status is not a reliable indicator of moderate changes in dietary choline consumption in premenopausal women. J Nutr Biochem 2009;20(1):62–9.
71. Wurtman RJ, Cansev M, Ulus IH. Choline and its products acetylcholine and phosphatidylcholine. In: Lajtha A, Tettamanti G, Goracci G (eds), Handbook of Neurochemistry and Molecular Neurobiology. Boston, MA: Springer; 2009. p. 443–501.
72. Resseguie M, Song J, Niculescu MD, da Costa KA, Randall TA, Zeisel SH. Phosphatidylethanolamine N-methyltransferase (PEMT) gene expression is induced by estrogen in human and mouse primary hepatocytes. FASEB J 2007;21(10):2622–32.
73. Midttun O, Kvalheim G, Ueland PM. High-throughput, low-volume, multianalyte quantification of plasma metabolites related to one-carbon metabolism using HPLC-MS/MS. Anal Bioanal Chem 2013;405(6):2009–17.
74. McCann JC, Hudes M, Ames BN. An overview of evidence for a causal relationship between dietary availability of choline during development and cognitive function in offspring. Neurosci Biobehav Rev 2006;30(5):696–712.