Received: 30 July 2021 | Revised: 22 October 2021 | Accepted: 8 November 2021

DOI: 10.1096/fj.202101217R

THE FASEB JOURNAL

RESEARCH ARTICLE

Prenatal choline supplementation improves child sustained attention: A 7-year follow-up of a randomized controlled feeding trial

Charlotte L. Bahnfleth[1] | **Barbara J. Strupp**[1,2] | **Marie A. Caudill**[1] | **Richard L. Canfield**[1]

[1]Division of Nutritional Sciences, Cornell University, Ithaca, New York, USA

[2]Department of Psychology, Cornell University, Ithaca, New York, USA

Correspondence
Barbara J. Strupp, Division of Nutritional Sciences, Cornell University, 102 Savage Hall, Cornell University, Ithaca, NY 14853, USA.
Email: bjs13@cornell.edu

Richard L. Canfield, Division of Nutritional Sciences, Cornell University, 115 Savage Hall, Cornell University, Ithaca, NY 14853, USA.
Email: rlc5@cornell.edu

Funding information
NIFA/USDA, Grant/Award Number: 1007195; Balchem Corp.; NICHD Traineeship, Grant/Award Number: 1T32HD087137-01; Egg Nutrition Center

Abstract

Numerous rodent studies demonstrate developmental programming of offspring cognition by maternal choline intake, with prenatal choline deprivation causing lasting adverse effects and supplemental choline producing lasting benefits. Few human studies have evaluated the effect of maternal choline supplementation on offspring cognition, with none following children to school age. Here, we report results from a controlled feeding study in which pregnant women were randomized to consume 480 mg choline/d (approximately the Adequate Intake [AI]) or 930 mg choline/d during the 3rd trimester. Sustained attention was assessed in the offspring at age 7 years ($n = 20$) using a signal detection task that showed benefits of maternal choline supplementation in a murine model. Children in the 930 mg/d group showed superior performance (vs. 480 mg/d group) on the primary endpoint (SAT score, $p = .02$) and a superior ability to maintain correct signal detections (hits) across the 12-min session ($p = .02$), indicative of improved sustained attention. This group difference in vigilance decrement varied by signal duration ($p = .04$). For the briefest (17 ms) signals, the 480 mg/d group showed a 22.9% decline in hits across the session compared to a 1.5% increase in hits for the 930 mg/d group ($p = .04$). The groups did not differ in vigilance decrement for 29 or 50 ms signals. This pattern suggests an enhanced ability to sustain perceptual amplification of a brief low-contrast visual signal by children in the 930 mg/d group. This inference of improved sustained attention by the 930 mg/d group is strengthened by the absence of group differences for false alarms, omissions, and off-task behaviors. This pattern of results indicates that maternal 3rd trimester consumption of the choline AI for pregnancy (vs. double the AI) produces offspring with a poorer ability to sustain attention—reinforcing

Abbreviations: ACh, acetylcholine; AI, Adequate Intake; IQR, interquartile range; RCT, randomized controlled trial; SAT, sustained attention task.

Barbara J. Strupp and Richard L. Canfield contributed equally to this study.

Clinical Trial Registry: NCT01127022 (www.clinicaltrials.gov)

concerns that, on average, choline consumption by pregnant women is approximately 70% of the AI.

KEYWORDS
attention, child development, choline, cognition, dietary supplements

1 | INTRODUCTION

Physiological demands for choline increase markedly during pregnancy,[1,2] due to choline's numerous roles in fetal development. Specifically, choline is a precursor for several biomolecules with key ontogenetic roles: (1) acetylcholine (ACh), a neurotransmitter which regulates multiple aspects of early brain development[3,4] and also plays a pivotal role in attentional function[5]; (2) phosphatidylcholine, a major component of biological membranes[6]; (3) sphingomyelin, a primary constituent of myelin[7]; and (4) betaine, a methyl donor that (through DNA methylation) can exert life-long effects on gene expression.[8–10] Consistent with these roles, over 40 years of research in rodents has demonstrated the importance of maternal choline intake for the developmental programming of offspring brain development and cognitive function. Specifically, maternal choline deprivation produces lasting offspring cognitive impairment,[11,12] whereas prenatal choline supplementation improves offspring attention[13–16] and spatial memory.[12,17,18] In addition, maternal choline supplementation is broadly neuroprotective for the offspring in conditions as diverse as fetal or early postnatal alcohol exposure,[19–22] prenatal stress exposure,[23] autism,[24] down syndrome,[14,15,25–28] epilepsy,[29–31] Rett syndrome,[32–34] cognitive aging,[16,17] and Alzheimer's disease.[28,35,36]

Despite a large body of rodent research on this topic, little is known about the functional effects of maternal choline intake on offspring cognition in humans or the maternal intake level needed to fully support fetal neurodevelopment. An Adequate Intake (AI) level of 450 mg choline/d for pregnant women was established in 1998; however, this value was extrapolated from evidence pertaining to the amount of choline needed to prevent liver dysfunction in men and not on endpoints related to offspring cognitive function.[37] The few studies that have evaluated the enduring effects of maternal choline intake on offspring cognition in humans are inconclusive.[38–44] Two observational studies found that greater concentrations of choline metabolites in maternal plasma[38] or greater estimated prenatal dietary choline intake[39] was positively associated with child performance on cognitive tests, but two other observational studies found no association.[40,41] Only three randomized controlled trials (RCTs) have explored this topic in typically-developing children. Two trials reported beneficial effects of maternal choline supplementation on aspects of infant attention—one on attentional orienting speed[43] and the other on an electrophysiological index of auditory attentional gating[44]—and one trial detected no benefits to infant memory.[42] Notably, no RCTs of maternal choline supplementation have followed children into school age, a time when tests of complex cognitive functioning can be used to more adequately evaluate the hypothesized enduring effects.

To address this need, the present study leveraged a controlled choline feeding trial in which women had been randomized to one of two levels of choline intake during the 3rd trimester of pregnancy. Beneficial effects of the higher level of maternal choline intake on infant attention had been demonstrated previously in this cohort[43] (one of the two RCTs detecting beneficial effects noted above). We followed these children to age 7 years to assess multiple aspects of attentional control by employing a signal detection task previously used in a rodent study which manipulated maternal choline intake and reported that prenatal choline availability affected the ability of offspring to detect visual cues and sustain attention throughout the testing session.[13] Importantly, this type of signal detection task has also been widely used for basic science investigations into the role of cholinergic pathways in stimulus detection and sustained attention in rodents.[5,45–49] Thus, the use of this task served a dual function: (1) it provided the opportunity to directly test the translational hypothesis that the enduring attentional benefits of maternal choline supplementation observed in rodents are also seen in humans; and (2) by comparing the effects of maternal choline supplementation in this task to the effects of cholinergic manipulations, it provided an opportunity to inform the hypothesis, advanced by Mohler and colleagues,[13] that the enduring attentional benefits of maternal choline supplementation are at least partly mediated by lasting changes in cholinergic neuronal pathways projecting from the basal forebrain to the frontal cortex.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

The present study is a 7-year follow-up of the children born to women who participated in a randomized,

double-blind, parallel-group controlled choline feeding study during their 3rd trimester of pregnancy (NCT01127022). The sample size for the original feeding study was set to achieve 80% power to detect group differences for the primary endpoints—biomarkers of choline status—at an α of .05.[1,50] Secondary outcomes included genomic expression, metabolomic profiling of plasma and placental tissue,[8,51] and offspring cognition during infancy.[43] The present study is an ancillary follow-up of the children at age 7 years to test for effects on child cognition, using pre-specified primary and secondary endpoints.

Details of the controlled feeding study, including the study diet, have been published elsewhere.[1] Briefly, 3rd trimester pregnant women (27 weeks gestation) aged ≥21 years were recruited from the Ithaca, NY region in 2008–2009, with eligibility contingent on a variety of factors including general good health and willingness to comply with the study protocol. Exclusion criteria included: (1) use of alcohol or tobacco products during pregnancy, (2) non-singleton pregnancy, and (3) pregnancy-related complications such as preeclampsia, gestational diabetes, or intrauterine growth restriction.

Women were randomized to consume either 480 mg choline/d (approximately the AI) or 930 mg choline/d from enrollment until delivery (i.e., approximately 12 weeks). To achieve these total choline intake levels, all women consumed the same study diet, which provided 380 mg choline/d, and an additional choline supplement of either 100 or 550 mg choline/d. The choline supplement (choline chloride, Balchem Corp., New Hampton, NY, USA) was mixed with cran-grape juice by study personnel and served in color-coded tubes so that participants and investigators remained blinded to the dose. No adverse effects of either choline dose were reported for the controlled feeding study.[1] On weekdays, women consumed one meal/d and the choline supplement while supervised by study personnel in the Human Metabolic Research Unit at Cornell University; all other meals were prepared for carry-out to be consumed off site. On weekend days, participants were instructed to consume the choline supplement with a meal of their choice. Adherence to the study diet and choline supplement was high based on in-lab monitoring of supplement and food consumption, return of supplement and food containers for weekend days, and greater fasting plasma concentrations of choline and its metabolites in the 930 mg/d (vs. 480 mg/d) group.[1] In addition to the study diet and choline supplement, all women consumed a daily prenatal multivitamin (Pregnancy Plus, Fairhaven Health LLC, Bellingham, WA, USA), a daily 200 mg docosahexaenoic acid supplement (Nature's Way Neuromins, Schwabe NorthAmerica, Green Bay, WI, USA), and thrice weekly 250 mg potassium and 250 mg magnesium supplements (General Nutrition Corp., Pittsburgh, PA, USA).

Between August 2016 and March 2017, children born to the enrolled women were invited to participate in a 7-year follow-up to investigate the effects of 3rd trimester choline intake on child cognitive functioning. Children were tested between ages 7.0 and 7.7 years at Cornell University ($n = 16$), or at an alternate location if travel to Ithaca, NY was not possible ($n = 4$). Characteristics of the participants and their mothers were obtained via parent report at the time of follow-up and included sex, child age, use of English in the home, grade in school, computer keyboard experience, visual acuity, race, ethnicity, maternal age at child conception, current maternal level of education, and family income. Maternal and infant characteristics assessed at the time of the feeding study were evaluated to assess possible bias from loss to follow-up and for sensitivity analyses. These included maternal race, ethnicity, education, and age at child conception, infant gestational age, birthweight, and breastfeeding duration.

2.2 | Behavioral assessment

Children were administered the Sustained Attention Task (SAT)[52] by one of two trained examiners blinded to group assignment as part of a two-day cognitive testing protocol. The SAT is a signal detection task designed to make demands on several aspects of cognitive control of voluntary attention, with a specific emphasis on attentional processes responsible for amplifying the perceptual salience of low-quality signals, filtering of distractions, suppressing competing and prepotent responses, and for sustaining these effortful processes to prevent a deterioration in signal detection performance over an uninterrupted 12-min testing period.[53] This task has been used with rodents and humans to characterize attentional control abilities, and is sensitive to experimental manipulations and phenotypic variations in basal forebrain cholinergic system function.[5,45–49] The SAT has excellent test-retest reliability in children.[52]

A detailed description of the SAT is presented in Figure 1. Briefly, the computer-administered SAT consisted of 216 trials on which the child indicated whether they saw or did not see a brief, low-contrast, gray square of variable duration (17, 29, or 50 ms) presented on a light gray background (the signal). On a randomly selected 108 trials, the signal appeared in the center of the screen. On the remaining 108 trials, no signal appeared. After every trial, a computer-generated voice command ("Go") prompted the child to indicate whether a signal had or had not appeared by pressing one of two response keys with distinct tactile and visual markings. Automated feedback (a 500 ms reward tone) was provided only after correct responses. Preceding the 216 test trials, each child

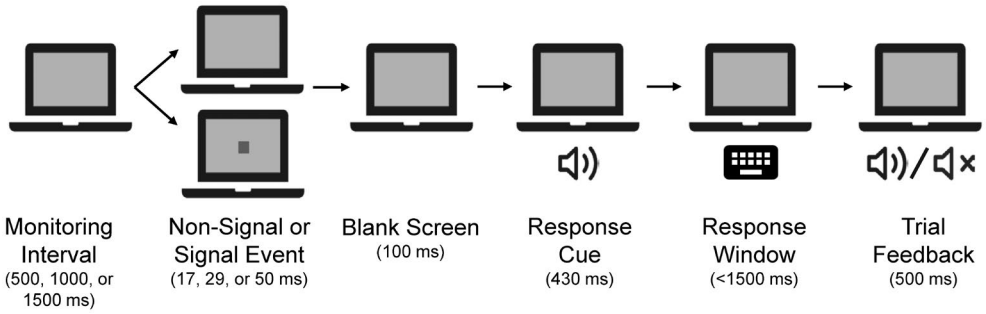

FIGURE 1 Time course of events within each trial of the Sustained Attention Task (SAT). Each trial began with a variable monitoring interval (500, 1000, or 1500 ms) to prevent anticipatory responding. Following the monitoring interval, a non-signal or signal event occurred. The child was instructed to indicate whether they saw or did not see a brief, low-contrast signal (a 5 mm × 5 mm gray square) of variable duration (17, 29, or 50 ms) on a light gray screen. A signal was presented randomly on 50% of the trials, with an equal number of signal and non-signal trials occurring every 18 trials. One hundred ms after the signal or non-signal event, a 430 ms auditory response cue ("Go") indicated the opening of the response window; i.e., that it was time to respond. Children had 1500 ms to indicate whether they saw or did not see a signal by pressing the appropriate, pre-specified key on the laptop keyboard (key assignments were determined by child handedness). Correct responses were followed by a 500 ms positively-valenced tone. No feedback was given after incorrect responses or omissions (Icons sourced from Microsoft Powerpoint).

TABLE 1 SAT trial outcomes defined by trial type and child response

	Child response to trial		
	Saw signal	**Did not see signal**	**No response**
Trial type			
Signal	Hit	Miss	Omission
Non-signal	False alarm	Correct rejection	Omission

performed a minimum of 12 practice trials to verify a full understanding of task rules and procedures. Four children (480 group: $n = 3$, 930 group: $n = 1$) did not demonstrate mastery of task rules following completion of the 12 practice trials and were administered an additional 12 trials. The mean number of practice trials for the 480 and 930 groups was 16.0 and 14.4, respectively.

For each of the 216 trials, there were five possible outcomes: hit, miss, correct rejection, false alarm, or omission. As defined in Table 1, a hit is correctly identifying a displayed signal, a miss is incorrectly reporting that a signal had not been presented (when a signal had in fact been displayed), a correct rejection is correctly noting the absence of a signal, and a false alarm is incorrectly reporting a signal when no signal had been presented. If no response is made during the 1500 ms window following the "Go" command, an omission was recorded.

The key endpoints for the SAT are defined by Equations 1–3 in Table 2. The primary endpoint is the SAT score (Table 2, Equation 3), a nonparametric measure of perceptual sensitivity developed within a signal detection theory framework that can be compared across individuals or groups that differ in response bias (i.e., different dominant tendencies to respond either by affirming or by denying that a stimulus was presented).[54] A key advantage of the SAT score is that it provides a single bias-free measure of overall performance across signal and non-signal trials. A key disadvantage is that performance on signal and non-signal trials involve somewhat distinct attentional and cognitive functions and neural pathways.[48] This type of combined index is difficult to interpret and, to the extent that effects are specific to one of the two types of trials, it will be a less sensitive index of treatment effects. In the present study, there was reason to believe that this would be the case, based on the hypothesis that an important mechanism underlying the lasting benefits of maternal choline supplementation is altered cholinergic activity in the frontal cortex. This hypothesis suggests that performance on signal trials (hits) is expected to provide the most sensitive and valid indicator of intervention effects, based on multiple studies showing that manipulations affecting the cholinergic modulation of attention impact signal trial performance while leaving non-signal trial performance unaffected.[45,46,55–57] For these reasons, we also examined performance on signal and non-signal trials separately.

An omission could arise for several reasons. For example, the child could be engaged in prolonged decision-making regarding whether or not a stimulus had appeared and/or which of the two keys should be pressed. Such difficulties would tend to increase omissions but likely would also manifest as a reduced hit percentage and an increase in false alarms for trials on which a keypress was made

TABLE 2 Equations for SAT task endpoints

(1)	$\text{Percentage Hits} = \frac{\text{Number of Hits}}{\text{Number of Hits} + \text{Number of Misses}}$
(2)	$\text{Percentage False Alarms} = \frac{\text{Number of False Alarms}}{\text{Number of False Alarms} + \text{Number of Correct Rejections}}$
(3)	$\text{SAT score}^{a} = \frac{\text{Percentage Hits} - \text{Percentage False Alarms}}{2(\text{Percentage Hits} + \text{Percentage False Alarms}) - (\text{Percentage Hits} + \text{Percentage False Alarms})^2}$

Note: There is no equation for omissions because it was analyzed as a binary outcome and percentages were not computed.

[a]The SAT score (the primary endpoint) ranges from −1 to +1. Scores ≤ 0 represent an inability to distinguish signal trials from non-signal trials. A score of +1 indicates perfect responding.

within the response window. However, omissions, especially in young children, could indirectly reflect off-task behavior related to deficits in self-control, or an inability to resist distraction. More direct information about children's off-task behaviors was obtained from videos recorded while the children performed the task. These videos were independently coded by two trained observers blinded to group assignment, using Behavioral Observation Research Interactive Software (BORIS Version 4.1.4[58]). Scorers viewed a split-screen display showing the child's upper torso, face, and gaze direction on one panel alongside a synchronized view from behind the child that included the testing computer's display screen. Scorers coded each incidence and duration of off-screen looking, with excellent interrater reliability (kappa = 0.92). All coding discrepancies were jointly resolved. Two variables were constructed as measures of off-task behavior: trials missed due to off-screen looking and total time looking off-screen.

In addition to the SAT, children were administered other behavioral tests tapping areas of cognition hypothesized to benefit from maternal choline supplementation. Unlike the SAT, these other tests are not direct analogs of tests which have previously shown the benefits of maternal choline supplementation in rodents and, therefore, do not have the same translational implications; the results for these tests are described elsewhere.[59–61]

2.3 | Statistical analysis

Maternal and child characteristics for the two treatment groups in the final analytic sample were compared using Student's *t* tests for continuous variables and Fisher's exact tests for categorical variables. The same approach was used to compare participants included in the final analysis to the six children who did not provide cognitive endpoint data (lost to follow-up, $n = 5$; data collection failure, $n = 1$).

Recognizing the limitations of estimating multiple statistical models in a small sample with multiple endpoints, the analysis plan (completed prior to unblinding) prespecified one basic linear mixed-effects model for estimating the effect of 3rd trimester choline intake for the SAT score and for the percentages of hits and false alarms. This *a priori* model included fixed effects for choline group status (480 or 930 mg/d), task block (three blocks of 72 trials) and, for endpoints related to signal trials, signal duration (17, 29, and 50 ms), in addition to all interactions. A prespecified fixed main effect for child sex was included because it is prognostic of child attention performance but was not controlled by the experimental design[53] (women were randomized to treatment without knowledge of fetal sex). The small number of females in both treatment groups precluded testing for interactions involving sex. Random effects were specified for the individual child and for the task block within the child. Treatment effects on omissions used the same mixed model described above, except that the fixed effect for signal duration had four levels because it also included non-signal trials. Because omissions were very rare (more than half the children made no omissions), treatment effects were estimated using a generalized linear mixed model with a binomial distribution. Fisher's exact test was used to test for group differences in the proportion of children who looked off-screen and a Wilcoxon rank-sum test was used to test for group differences in time spent looking off-screen.

Sustained attention was defined in terms of a vigilance decrement,[52] i.e., a decline in signal-trial performance across the session. The vigilance decrement, assessed for SAT score and hit percentage, was operationalized for analysis as the linear change in performance across trial blocks (block 3–block 1) and tested using a planned single degree of freedom linear interaction contrasts.[62] The same analysis was conducted to assess performance change over blocks for non-signal trials, but is not described in terms of a vigilance decrement because this concept only pertains to measures involving signal trials. Tests for quadratic trends were also conducted, but in every case found to be nonsignificant ($.17 \leq p \leq .95$) and thus are not reported.

The robustness of the results from the primary analyses was evaluated in a series of sensitivity analyses. First, to assess the influence of possible imbalance in potentially prognostic child and maternal demographic characteristics, we entered each as an individual covariate into the *a priori* models and estimated the change in treatment effect. In addition, we evaluated the possible influence of the number of trials missed due to off-screen looking

on the estimation of the choline effect by excluding those trials in a separate analysis. Finally, to assess the possible influence of response bias on the results of the hits analysis, we conducted an additional analysis which included the percentage of false alarms and the false alarm by block interaction as covariates.

Statistical tests were two-sided with $p < .05$ indicating statistical significance for main effects, interactions, and planned single degree of freedom contrasts. *p*-Values for *post hoc* pairwise comparisons were Bonferroni-corrected.[63] All data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

3 | RESULTS

3.1 | Participant characteristics

Of the 26 children whose mothers completed the randomized choline feeding trial, 21 were re-recruited for the 7-year cognitive follow-up (77% retention; see Figure 2). One of these children did not comply with the cognitive testing protocol and, prior to unblinding the investigators to group assignment, it was decided to exclude all cognitive data from this child. Children included in the final analytic sample ($n = 20$) did not differ from those who were not included ($n = 6$) on available demographic measures including child sex, maternal race/ethnicity, and maternal age and education level at conception (all $p \geq .27$; data not shown).

Characteristics of participant children and their mothers are reported in Table 3. Children were predominantly non-Hispanic white and male, and most had completed 1st grade at the time of testing. Mothers of the children were mostly highly educated, the majority having earned a bachelor's degree or higher. A non-significant trend indicated slightly higher educational attainment for mothers in the 480 mg choline/d group versus the 930 mg choline/d group (Fisher's Exact test $p = .09$); treatment groups were otherwise similar in demographic and birth characteristics (all other $p \geq .59$).

3.2 | Overall description of task performance

Of the 4320 trials administered, a valid response was recorded on 4315 trials; five trials were excluded due to

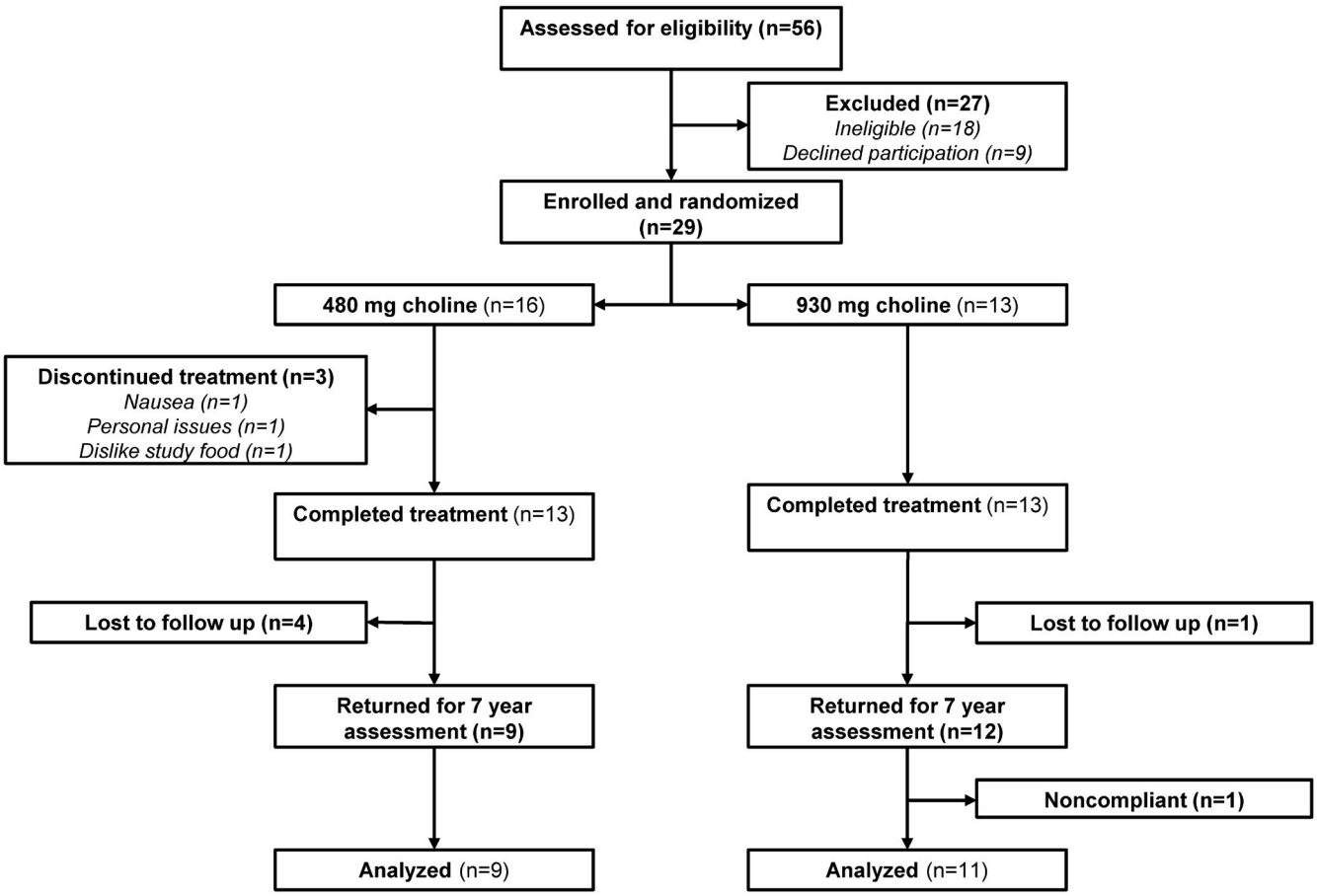

FIGURE 2 Participant flowchart for the assessment of the effect of 3rd trimester choline supplementation on child sustained attention task (SAT) performance at age 7 years

TABLE 3 Select characteristics of children included in final analytic sample[a]

	3rd trimester maternal choline intake		
	480 mg/d ($n = 9$)	930 mg/d ($n = 11$)	p[b]
Child characteristics			
Sex (male)	6 (67)	8 (73)	1.00
Mean birthweight in grams (SD)	3487 (540)	3467 (420)	.93
Mean gestational age in weeks (SD)	39.2 (1.5)	38.9 (1.2)	.61
Mean breastfeeding duration in weeks (SD)	17 (8)	15 (13)	.81
Mean age at testing in years (SD)	7.2 (0.2)	7.3 (0.2)	.68
English not primary language	1 (11)	3 (27)	.59
Normal or corrected to normal vision	9 (100)	11 (100)	1.00
Highest grade completed			.62
Kindergarten	3 (33)	2 (18)	
1st Grade	6 (67)	9 (82)	
Keyboard experience			.81
None	1 (11)	0 (0)	
Minimal	5 (56)	7 (64)	
Frequent	3 (33)	4 (36)	
Race			1.00
Asian	1 (11)	2 (18)	
Black	0 (0)	1 (9)	
Native American	0 (0)	1 (9)	
White	8 (89)	7 (64)	
Hispanic ethnicity	2 (22)	2 (18)	1.00
Maternal characteristics			
Mean age at conception in years (SD)	28.4 (3.0)	27.6 (3.7)	.61
Education			.09
High School/Associate degree	0 (0)	4 (36)	
Bachelor's degree	3 (33)	4 (36)	
Masters/Doctoral degree	6 (67)	3 (28)	
Family income (per year)			.67
<$50 000	0 (0)	2 (18)	
$50 000–<$100 000	4 (44)	4 (36)	
≥$100 000	5 (56)	5 (45)	

Abbreviation: SD, standard deviation.

[a]All values n (%) unless otherwise noted.

[b]Reported p-values from Student's t test (continuous variables) and Fisher's exact test (categorical variables).

technical problems. On average, children correctly identified the presence of the signal on 78% (Median: 83%, interquartile range [IQR]: 67%–92%) of all signal trials and correctly noted the absence of a signal on 78% (Median: 82%, IQR: 71%–91%) of non-signal trials. Across all children, the 17 ms signal was more difficult to detect than either the 29 or 50 ms signals. Specifically, children averaged 70% hits on 17 ms trials, compared to 86% hits for the 29 ms and 85% for 50 ms trials (17 ms vs. average of 29 and 50 ms: $t(114) = 7.69$, 95% CI [−0.20, −0.12], $p < .001$). A vigilance decrement for the hit percentage was also seen in the group as a whole, as evidenced by a lower hit rate during the third trial block (76%) compared to the first trial block (84%; $t(38) = 2.51$, 95% CI [0.02, 0.15], $p = .02$). The overall omission rate was very low (Median: 3.7%, IQR: 2.1%–13.4%) and did not change from block 1 to block 3

($t(86.94) = 0.43$, 95% CI [−0.67, 2.07], $p = .67$), indicating that children responded consistently throughout the task. Results are next presented for each endpoint in models that include choline treatment group and planned tests of hypotheses concerning interactions between choline group, signal duration, and trial block.

3.3 | SAT score (signal and non-signal trials)

The mean (SE) SAT score for the 930 and 480 mg/d groups, respectively, was 0.71 (0.04) and 0.56 (0.04). Analyses from the mixed model revealed a significant main effect of choline treatment group. Children whose mothers consumed 930 mg choline/d during the 3rd trimester of pregnancy more accurately identified signals, while also correctly rejecting non-signals, as compared to children whose mothers consumed 480 mg choline/d ($F_{(1,17)} = 7.19$, 95% CI [0.03, 0.27], $p = .02$; see Figure 3). This effect of maternal choline intake on SAT score did not interact with signal duration ($F_{(2,108)} = 0.30$, $p = .74$) and the three-way interaction of group, signal duration, and trial block was also not significant ($F_{(3,94.16)} = 0.35$, $p = .79$).

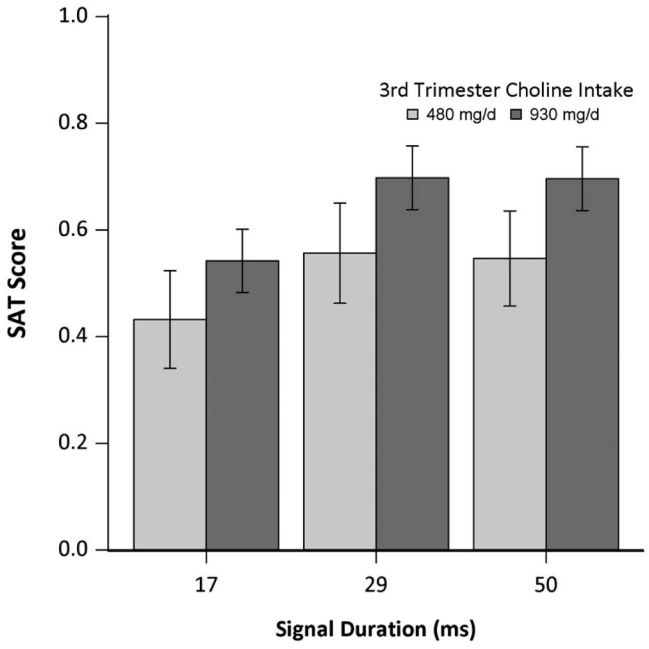

FIGURE 3 Effect of 3rd trimester choline intake and signal duration on SAT score. Children in the 930 mg/d group had a higher average SAT score than children in the 480 mg/d group (main effect of treatment group: $p = .02$). SAT score differed significantly by signal duration ($p < .0001$), but the 3rd trimester choline intake by signal duration interaction was non-significant ($p = .74$). Values represent least square means ± SEM. 480 mg/d: $n = 9$; 930 mg/d: $n = 11$

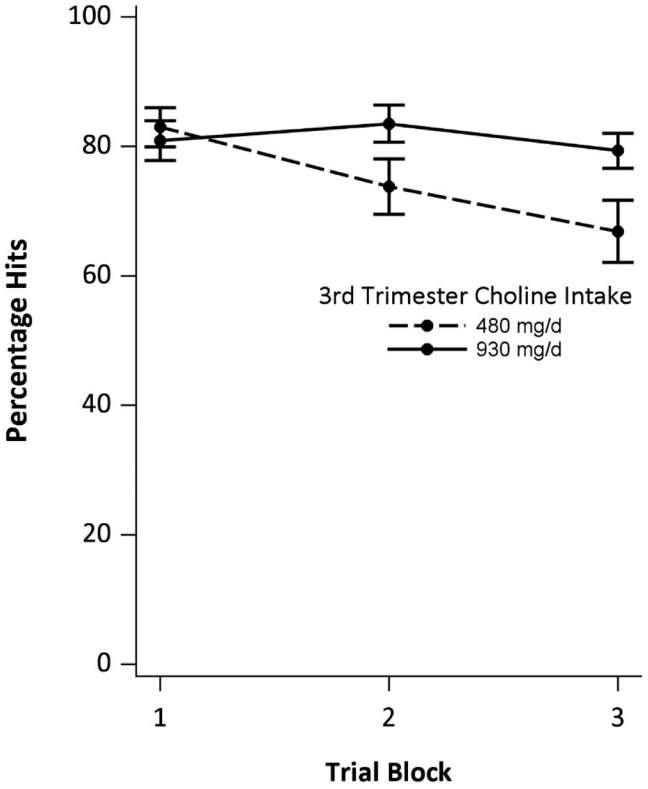

FIGURE 4 Effect of 3rd trimester choline intake on vigilance decrement for percentage hits. Linear change in percentage hits (correct signal detections) across the task blocks varied by 3rd trimester choline intake ($p = .02$): the 480 mg/d group exhibited significant decline across blocks ($p = .001$) whereas the 930 mg/d group exhibited no change in performance across blocks ($p = .71$). Values represent least square means ± SEM. 480 mg/d: $n = 9$; 930 mg/d: $n = 11$

Finally, there was no evidence of a vigilance decrement for SAT score (block 3–block 1) in either group. The mean (SE) vigilance decrement for the 930 and 480 mg/d groups, respectively, was 0.05 (0.05) and 0.10 (0.08). Neither value differed from zero (930 mg/d: $t(36) = 0.77$, [−0.09, 0.19], $p = .45$; 480 mg/d: $t(36) = 1.29$, [−0.06, 0.26], $p = .21$). In addition, the two groups did not differ in the magnitude of the vigilance decrement for SAT score ($t(36) = 0.44$, [−0.17, 0.26], $p = .66$). As noted above, SAT score was expected to be a less sensitive index of vigilance decrement than percentage hits because it combines performance on both signal and non-signal trials.[53]

3.4 | Percentage hits (signal trials)

Performance on signal trials revealed superior stimulus detection by children in the higher maternal choline intake group. The mean (SE) for percentage hits in the 930 and 480 mg/d groups, respectively, was 84% (3.2) and 76% (3.5). Although the main effect of choline group was

not statistically significant ($F_{(1,17)}$ = 2.62, 95% CI [−2.2, 17.0], p = .12), this result was qualified by higher-order interactions. As depicted in Figure 4, there was a significant interaction of choline group and block, revealing a group difference in vigilance decrement across blocks (t(36) = 2.37, 95% CI [2.1, 27.0], p = .02). Simple slopes analysis revealed a significant vigilance decrement of 16% for the 480 mg/d group (t(36) = 3.54, 95% CI [0.07, 0.25], p = .001), but a nonsignificant 1.6% decrement for the 930 mg/d group (t(36) = 0.38, 95% CI [−6.8, 9.9], p = .71).

Importantly, the choline group difference in the vigilance decrement also varied by signal duration ($F_{(3,108)}$ = 2.78, p = .04; Figure 5). The vigilance decrement for 17 ms signals in the 480 mg choline/d group was 22.9%, significantly greater than the 1.5% increase in hits across blocks for the 930 mg choline/d group (t(108) = −2.54, 95% CI [−37.7, −4.7], p = .04, Bonferroni corrected). The groups did not differ significantly in vigilance decrement for 29 and 50 ms trials (t(108) = 1.30, 95% CI [−5.7, 27.3], p = .59 and t(108) = −0.45, 95% CI [−20.2, 12.8], p = .99, respectively, Bonferroni corrected). Additionally, the results show a non-significant trend for the 480 mg/d group in which the magnitude of the vigilance decrement appears to increase with each reduction in signal duration, whereas the 930 mg/d group shows no such pattern. Because vigilance declines most rapidly for stimuli that are the most difficult to detect, this result suggests that children in the 480 mg/d group found the 17 ms signal increasingly difficult to detect across the trial blocks, whereas the 17 ms signals did not become more challenging than the longer signals for children in the 930 mg/d group.

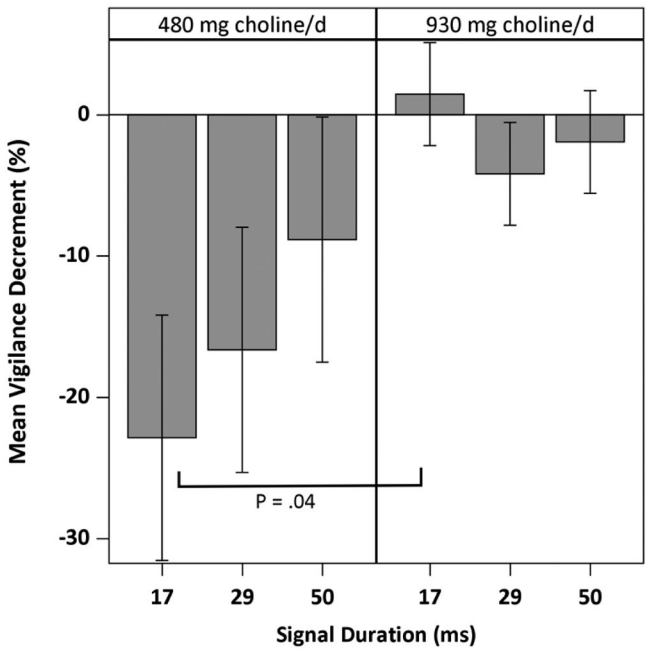

FIGURE 5 Effect of 3rd trimester choline intake on vigilance decrement for percentage hits as a function of signal duration. The choline group difference in the vigilance decrement varied by signal duration (p = .04). The vigilance decrement for 17 ms signals was significantly greater for the 480 mg choline/d group than for the 930 mg choline/d group (p = .04, Bonferroni corrected). The groups did not differ significantly in vigilance decrement for 29 and 50 ms trials. Notably, for the 480 mg/d group there is an apparent increase in the magnitude of the vigilance decrement for each decrease in signal duration, whereas the 930 mg/d group shows no vigilance decrement for any signal duration. Values represent least square means ± SEM. 480 mg/d: n = 9; 930 mg/d: n = 11

3.5 | Percentage false alarms (non-signal trials)

The mean (SE) false alarm rate for the 930 and 480 mg/d groups was, respectively, 15.3% (5.0) and 22.9% (5.3). These rates did not differ by choline group ($F_{(1,17)}$ = 1.21, 95% CI [−22.4%, 7.0%], p = .29) and the change in false alarms from trial block 1 to block 3 did not differ from 0 for either group (both p > .15, not shown).

3.6 | Omissions and off-screen looking (signal and non-signal trials)

The mean proportion (SD) of omissions for the 930 and 480 mg/d groups, respectively, was 0.05 (0.10) and 0.10 (0.17). The omission rate did not differ by choline group status ($F_{(1,17.06)}$ = 0.54, 95% CI [−0.23, 0.11], p = .47) and the change in omission rates from block 1 to block 3 also did not differ by choline group (t(86.94) = 1.14, 95% CI [−0.46, 0.70], p = .26). The omission rate varied by trial type; i.e., the 4 level trial-type variable denoting non-signal, 17, 29, and 50 ms trials ($F_{(3,215)}$ = 53.01, p < .001). Specifically, omissions were more likely to occur on non-signal trials than on signal trials of any duration (17 ms: t(215) = 6.57, 29 ms: t(215) = 8.42, 50 ms: t(215) = 8.42, all Bonferroni corrected p < .001). There were no statistically significant interactions between trial type and choline group ($F_{(3,215)}$ = 0.91, p = .44) and the change in omissions across trial blocks did not differ by choline group for any trial type (Joint $F_{(4,154.2)}$ = 0.74, p = .56).

Video recordings were available for behavioral coding of off-screen looking for 19 of the 20 participants (930 mg/d: n = 10, 480 mg/d: n = 9). On only 0.4% of trials (17 out of 4099 video recorded trials) did a child miss a trial due to off-screen looking, and ten of the 19 children did not look away from the screen during the task. The proportion of children who did not look away from the screen during the task did not differ by choline group (Fisher's exact test: p = .66). Furthermore, the median amount of time spent looking off-screen during the entire

11.5-min session was 0 s (IQR: 0–4.7 s), and this also did not differ between choline groups ($Z = 0.66$, $p = .51$).

3.7 | Sensitivity analyses

Sensitivity analyses were conducted to test the robustness of the main findings: (1) The choline group effect on SAT score, (2) the choline by block interaction for percentage hits, and (3) the choline by block by signal duration interaction for percentage hits. Accordingly, each variable in Table 3 was entered as a single added covariate to the respective *a priori* regression models (child sex was included in all models). For SAT score, three covariates altered the estimate of the choline effect by >10%. Including either maternal education or the child's highest grade completed increased the effect estimate by 13% whereas including the covariate for the child's race decreased the effect estimate by 14%. Only the inclusion of child race altered the statistical significance of the effect (from $p = .02$ to $p = .05$). For hits, no covariates altered the effect estimate of the interaction of choline and block by more than 5%. For the interaction of choline, block, and signal duration, only the inclusion of infant birth weight altered the estimated effect by 10%—this increased the effect size favoring the 930 mg/day group.

In sensitivity analyses designed to assess the possible impact of biased responding on the percentage hits results, the percentage of false alarms and the false alarm by block interaction were included in the *a priori* model for percentage hits. After adding these variables, the choline by block effect estimate increased by 23% and the choline by block by signal duration estimate increased by 2%.

Finally, sensitivity analyses designed to assess the possible impact of (1) trials missed due to off-screen looking and (2) differential numbers of practice trials did not reveal any instance in which estimates of choline group differences were changed by more than 5%.

4 | DISCUSSION

The findings of this study revealed that 7-year-old children born to women randomly assigned to 930 mg choline/d during the 3rd trimester of pregnancy performed better on a challenging sustained attention task than children born to women assigned to 480 mg choline/d. Children from the 930 mg/d group achieved higher SAT scores, indicating a superior ability to detect visual signals while also correctly identifying non-signal events. This superiority in SAT score implicates one or more aspects of attentional control that affect signal detection performance.[5,47,48]

Insight into the specific nature of the prenatal choline-induced attentional control benefit was provided by the pattern of results across trial blocks for hits (signal trial performance), coupled with the lack of treatment effects for the other endpoints (false alarms, omissions, off-task behavior). For hits, the significant choline group by trial block interaction revealed a steeper vigilance decrement for children in the 480 mg/d group than for children in the 930 mg/d group. Notably, hits significantly declined from block 1 to block 3 by 16% for children in the 480 mg/d group, in contrast to the negligible (1.5%) and non-significant decline for children in the 930 mg/d group. This differential vigilance decrement indicates a role for prenatal choline availability in shaping offspring sustained attention into childhood.

A more refined understanding of the nature of this prenatal choline effect on sustained attention was revealed by the significant 3-way interaction of choline group, block, and signal duration observed for hits. This interaction showed that the group difference in vigilance decrement was greatest for 17 ms trials and appeared to become progressively smaller as the signal duration increased. Whereas children from the 480 mg/d group showed their largest vigilance decrement on the 17 ms trials (nearly 23%), children from the 930 mg/d group showed no decrement for 17 ms trials. The groups did not differ in vigilance decrement for the more easily detected[53,64] 29 and 50 ms signals. These results suggest that greater maternal choline intake improved children's ability to sustain their application of attentional mechanisms that amplify the perceptual salience of a degraded visual signal.[5,45,65,66] Because we did not observe a two-way interaction of choline group and signal duration, there was no evidence that the groups differed in their overall ability to detect the briefest signals, only in their ability to sustain that detection performance across the session.

Multiple lines of evidence make it clear that the group difference in sustaining detection performance for the briefest signals cannot be attributed to group differences in motivation, arousal, off-task behaviors, or shifts in response bias across the session. Most pertinent is the fact that the groups did not differ in vigilance decrement for the 29 and 50 ms signals; reduced motivation or arousal in the 480 mg/d group would have affected performance for all signal durations, not only for the 17 ms signals. Similarly, group differences across the session in children's ability to suppress off-task behaviors or thoughts (e.g., mind wandering) would have affected all signal durations and would likely have manifested as a group difference in omissions and/or in looks away from the testing screen; no such effects were observed. Finally, the group difference in vigilance decrement was not due to differential changes in response bias across the session because the groups did not differ in false alarms overall, neither group showed a change in false alarms across the session,

and sensitivity analyses showed that controlling for false alarms and the false alarms by block interaction in the percentage hits model slightly strengthened the original results. Taken together, this pattern of effects supports the inference that greater 3rd trimester maternal choline intake causes lasting improvements in offspring sustained attention in humans.

The present findings are consistent with those from a mouse model which varied maternal choline intake during pregnancy and measured offspring performance on a rodent analog of the SAT.[13] This rodent study reported that: (1) Maternal choline supplementation improved performance on signal trials but not non-signal trials; (2) maternal choline deficiency impaired offspring sustained attention. These similar findings across species support the general argument that higher maternal choline intake during pregnancy improves offspring attentional control.

Several lines of evidence suggest that the lasting attentional effects of prenatal choline availability may be mediated by fetal programming that affects cholinergic system activity throughout life.[12] Perhaps most importantly, the specific constellation of effects which differentiate the two choline groups in our study parallels effects of manipulations which selectively alter the activity of basal forebrain cholinergic neurons projecting to the cortex.[5,47,65] Notably, in signal detection tasks, selective cholinergic manipulations specifically affect performance on signal trials but leave non-signal trial performance unaffected.[5,45] In addition, these cholinergic manipulations produce their largest effects on signal detection when demands on effortful attention are high (e.g., diminished cue salience, presence of distractors, greater time on task).[5] Similarities in the patterns of effects seen in this task following maternal choline supplementation to those seen in animals following selective cholinergic manipulations implicate increased cortical cholinergic activity as a likely mechanism for the observed effects.

Further support for this hypothesis is provided by studies showing that maternal choline supplementation in rodents produces lasting changes to the morphology and/or activity of cholinergic neurons in the basal forebrain.[26,67,68] This research has primarily focused on cholinergic basal forebrain neurons projecting to the hippocampus, not those which project to the cortex, but one exception is highly pertinent to the present findings. Using a frontal cortex slice preparation, Napoli and colleagues[69] reported that prenatal choline supplementation dramatically potentiated ACh release induced by depolarization and/or administration of insulin-like growth factor 2, an endogenous modulator of sustained ACh release. Importantly, these effects were seen at postnatal day 80, demonstrating lasting effects of the prenatal dietary manipulation. When coupled with an earlier demonstration that maternal choline supplementation reduces the activity of acetylcholinesterase (the enzyme which degrades ACh) in the offspring,[70] such results suggest a plausible mechanism for the findings in the present study. Specifically, these findings are consistent with the view that the superior sustained attention seen in the 930 mg/d group children may be due to a greater ability to sustain cholinergic activity in the prefrontal cortex, an area which modulates attentional control.

Importantly, the present findings are not likely to be due to altered coeruleocortical noradrenergic activity, despite the fact that this system has also been linked to sustained attention.[71] Manipulations altering this system typically affect attentional lapses[72] and distractibility,[73,74] with performance changes typically seen most prominently in the false alarm rate rather than the hit rate.[72,75] The finding that maternal choline supplementation produced differences only in the hit rate enhances the plausibility that a cholinergic mechanism is involved.

4.1 | Strengths and limitations

The present study has several key strengths. First, the design of the study allows for strong causal inferences. All food and choline supplements were provided by the study, and participants consumed more than 70% of the choline supplements under study personnel supervision, making it the only maternal choline supplementation study to ensure that choline intake for the two treatment groups truly differed by a substantial amount. A second strength of the study was the use of a task previously shown to reveal beneficial attentional effects of maternal choline supplementation in rodents, which allows our findings to speak to the cross-species translation of these effects. Third, we followed an *a priori* statistical analysis plan designed to preserve power in a small sample and avoid capitalizing on chance findings, and the interpretation of results was further strengthened by the results of sensitivity analyses. Finally, careful analysis of videos of the children's behavior while they performed the task enabled verification of data quality and exclusion of off-task behavior as a possible sources of group differences.

This study also has limitations. Most importantly, the small sample size raises a concern that the findings, albeit statistically significant, might not reflect a true effect of the different levels of maternal choline intake; i.e., that the present results may have low positive predictive value.[76] The positive predictive value of a result depends on the statistical power of the study, the actual level of statistical significance observed, and on the prior probability that the effect is true. The statistical power was adequate to detect effects of the prenatal

intervention with modest statistical significance and the prior probability that the reported effects are likely to be true effects of choline is quite strong. Maternal choline supplementation produced a similar pattern of effects in rodents performing a very similar task[13] and significant effects of higher maternal choline intake were found in this same cohort of children when they performed a visual attentional orienting task as infants.[43] The present results may be seen as a test of the positive predictive value of these earlier small studies because the findings largely replicate those reported previously. Moreover, the sensitivity analyses help to rule out the possibility that the reported effect sizes were influenced by a minor imbalance between the treatment groups. Nevertheless, a larger sample size would increase confidence in the representativeness of our participant sample. More definitive conclusions about the effects of maternal choline supplementation in humans await larger intervention studies with diverse participants. One additional limitation is that the inference of improved sustained attention in the higher choline group is based on only one test of this aspect of attention. It is well-documented that performance on various sustained attention tasks depends not only on sustained attention but on other cognitive processes as well (e.g., working memory, perceptual abilities, inhibitory control).[77] The pattern of group differences in the current task—where the groups did not differ in hit performance early in the session, but only as the session progressed, and even then, only for the briefest cues—helps to exclude differences relating to other cognitive processes. Future studies should ideally include additional tests of sustained attention to determine the generality of the effects reported here.

4.2 | Summary and conclusions

In summary, maternal intake of the recommended amount of choline during the 3rd trimester resulted in poorer offspring sustained attention than was demonstrated by the offspring of mothers who consumed twice that amount, when children were assessed at age 7 years. Sustained attention (and attentional control more broadly) contributes to a wide variety of higher cognitive functions such as problem-solving and working memory and is positively associated with school performance.[78–83] Therefore, if subsequent research confirms the adverse effects of low choline intake on offspring sustained attention, it is likely that such effects would extend beyond performance on a laboratory task.[84] When interpreting the present findings one must note that both choline intake levels in this study are greater than the average consumption of pregnant women in North America, which is approximately 350 mg/d.[39,85–88] It would have been unethical to feed pregnant women a total dietary choline intake less than the AI, but as a result, the data do not directly address the effect of increasing maternal choline intake from current average maternal intake levels to either the 480 mg/d or the 930 mg/d levels administered in this study.

Another important aspect of these findings is the correspondence seen between the effects of varied maternal choline intake in rodents and humans in homologous tasks. The similarity in findings is striking and suggests that the many other benefits of maternal choline supplementation documented for rodents may also translate to humans. In addition to improving attention and memory in young adult animals, these benefits include a lessening of impairment in diverse conditions including prenatal stress exposure,[23] autism,[24] Down syndrome,[14,15,25–28] epilepsy,[29–31] Rett syndrome,[32–34] cognitive aging,[16,17] Alzheimer's disease,[28,35,36] and fetal or early postnatal alcohol exposure.[19–22] Indeed, three recent human studies have reported that either maternal[89,90] or early postnatal[91] choline supplementation improves cognitive outcomes in children exposed to alcohol prenatally.

The findings from this cohort at age 7 years extend the results from infancy[43] and provide new evidence that the beneficial effects of maternal choline supplementation during pregnancy for offspring attentional function endure into early childhood. Moreover, emerging evidence from other tests administered to these children at age 7 indicates that the benefits of higher maternal choline intake during the third trimester are not limited to sustained attention, but also include improved working memory[59,61] and problem-solving.[60] Although replication in a larger clinical trial is needed, these findings suggest that the choline AI for pregnant women may not be sufficient for optimal child cognition because consumption of 930 mg choline/d produced superior child cognition relative to consumption of approximately the AI. These findings raise concerns about the evidence that approximately 90% of pregnant women in North America consume choline at levels below the AI and that prenatal vitamins commonly contain little or no choline.[92]

ACKNOWLEDGMENTS

The 7-year follow-up was funded by NIFA/USDA (Hatch Accession Number: 1007195) and Balchem Corp. CLB was supported by an NICHD Traineeship (1T32HD087137-01) and the Egg Nutrition Center Young Investigator Research Award for Early Exploration. We thank Bailey Drewes for her contribution to data collection and Faith Park for her contribution to video coding. Additionally, we thank Dr.

Martin Sarter for his important insights into attentional control and signal detection task outcome measures, and for feedback on an earlier version of this manuscript. Finally, we thank Dr. Lynn Johnson and the Cornell Statistical Consulting Unit for the assistance with statistical analyses.

AUTHOR CONTRIBUTIONS

The authors' responsibilities were as follows: Barbara J. Strupp, Richard L. Canfield, and Marie A. Caudill designed the research; Charlotte L. Bahnfleth conducted the research; Charlotte L. Bahnfleth and Richard L. Canfield analyzed the data; Richard L. Canfield, Barbara J. Strupp, and Charlotte L. Bahnfleth wrote the paper; Richard L. Canfield and Barbara J. Strupp had primary responsibility for final content and contributed equally to this work. All authors read and approved the final manuscript.

DISCLOSURES

The authors report no conflict of interests. Funders had no role in the study design, data collection, or the analysis and interpretation of the data.

ETHICAL APPROVAL

Ethical approval for the present study was obtained from the Institutional Review Board for Human Participants at Cornell University in Ithaca, NY (USA). Written parental consent and child assent were obtained from all study participants.

DATA AVAILABILITY STATEMENT

Data described in the manuscript, code book, and analytic code will be available upon request pending review of application and approval by the authors.

REFERENCES

1. Yan J, Jiang X, West AA, et al. Maternal choline intake modulates maternal and fetal biomarkers of choline metabolism in humans. *Am J Clin Nutr*. 2012;95:1060-1071. doi:10.3945/ajcn.111.022772
2. 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(12):3049-3054.
3. Lauder JM, Schambra UB. Morphogenetic roles of acetylcholine. *Environ Health Perspect*. 1999;107(suppl 1):65-69.
4. Abreu-Villaça Y, Filgueiras CC, Manhães AC. Developmental aspects of the cholinergic system. *Behav Brain Res*. 2011;221(2):367-378. doi:10.1016/J.BBR.2009.12.049
5. Sarter M, Givens B, Bruno JP. The cognitive neuroscience of sustained attention: where top-down meets bottom-up. *Brain Res Rev*. 2001;35:146-160. doi:10.1016/S0165-0173(01)00044-3
6. Kent C. Regulation of phosphatidylcholine biosynthesis. *Prog Lipid Res*. 1991;29(4):87-105. doi:10.1016/S0163-7827(05)80001-3
7. Hanada K, Horii M, Akamatsu Y. Functional reconstitution of sphingomyelin synthase in Chinese hamster ovary cell membranes. *Biochim Biophys Acta*. 1991;1086(2):151-156. doi:10.1016/0005-2760(91)90002-Y
8. Jiang X, Yan J, West AA, et al. Maternal choline intake alters the epigenetic state of fetal cortisol-regulating genes in humans. *FASEB J*. 2012;26:3563-3574. doi:10.1096/fj.12-207894
9. Mehedint MG, Niculescu MD, Craciunescu CN, Zeisel SH. Choline deficiency alters global histone methylation and epigenetic marking at the Re1 site of the calbindin 1 gene. *FASEB J*. 2010;24:184-195. doi:10.1096/fj.09-140145
10. Niculescu MD, Craciunescu CN, Zeisel SH. Dietary choline deficiency alters global and gene-specific DNA methylation in the developing hippocampus of mouse fetal brains. *FASEB J*. 2006;20:43-49. doi:10.1096/fj.05-4707com
11. Blusztajn JK. Choline, a vital amine. *Science*. 1998;281(5378):794-795. doi:10.1126/science.281.5378.794
12. Meck WH, Williams CL. Metabolic imprinting of choline by its availability during gestation: implications for memory and attentional processing across the lifespan. *Neurosci Biobehav Rev*. 2003;27(4):385-399. doi:10.1016/S0149-7634(03)00069-1
13. Mohler EG, Meck WH, Williams CL. Sustained attention in adult mice is modulated by prenatal choline availability. *Int J Comp Psychol*. 2001;14:136-150.
14. Moon J, Chen M, Gandhy SU, et al. Perinatal choline supplementation improves cognitive functioning and emotion regulation in the Ts65Dn mouse model of down syndrome. *Behav Neurosci*. 2010;124:346-361. doi:10.1037/a0019590
15. Powers BE, Kelley CM, Velazquez R, et al. Maternal choline supplementation in a mouse model of Down syndrome: effects on attention and nucleus basalis/substantia innominata neuron morphology in adult offspring. *Neuroscience*. 2017;340:501-514. doi:10.1016/J.NEUROSCIENCE.2016.11.001
16. Meck WH, Williams CL. Simultaneous temporal processing is sensitive to prenatal choline availability in mature and aged rats. *NeuroReport*. 1997;8(14):3045-3051.
17. Meck WH, Williams CL, Cermak JM, Blusztajn JK. Developmental periods of choline sensitivity provide an ontogenetic mechanism for regulating memory capacity and age-related dementia. *Front Integr Neurosci*. 2008;1:1-11. doi:10.3389/neuro.07.007.2007
18. Meck WH, Smith RA, Williams CL. Pre- and postnatal choline supplementation produces long-term facilitation of spatial memory. *Dev Psychobiol*. 1988;21(4):339-353. doi:10.1002/dev.420210405
19. Thomas JD, La Fiette MH, Quinn VRE, Riley EP. Neonatal choline supplementation ameliorates the effects of prenatal alcohol exposure on a discrimination learning task in rats. *Neurotoxicol Teratol*. 2000;22(5):703-711. doi:10.1016/S0892-0362(00)00097-0
20. Thomas JD, Biane JS, O'Bryan KA, O'Neill TM, Dominguez HD. Choline supplementation following third-trimester-equivalent alcohol exposure attenuates behavioral alterations in rats. *Behav Neurosci*. 2007;121(1):120-130. doi:10.1037/0735-7044.121.1.120
21. Ryan SH, Williams JK, Thomas JD. Choline supplementation attenuates learning deficits associated with neonatal alcohol exposure in the rat: effects of varying the timing of choline administration. *Brain Res*. 2008;1237:91-100. doi:10.1016/j.brainres.2008.08.048

22. Schneider RD, Thomas JD. Adolescent choline supplementation attenuates working memory deficits in rats exposed to alcohol during the third trimester equivalent. *Alcohol Clin Exp Res*. 2016;40(4):897-905. doi:10.1111/acer.13021
23. Schulz KM, Pearson JN, Gasparrini ME, et al. Dietary choline supplementation to dams during pregnancy and lactation mitigates the effects of in utero stress exposure on adult anxiety-related behaviors. *Behav Brain Res*. 2014;268:104-110. doi:10.1016/j.bbr.2014.03.031
24. Langley EA, Krykbaeva M, Blusztajn JK, Mellott TJ. High maternal choline consumption during pregnancy and nursing alleviates deficits in social interaction and improves anxiety-like behaviors in the BTBR T+Itpr3tf/J mouse model of autism. *Behav Brain Res*. 2015;278:210-220. doi:10.1016/j.bbr.2014.09.043
25. Velazquez R, Ash JA, Powers BE, et al. Maternal choline supplementation improves spatial learning and adult hippocampal neurogenesis in the Ts65Dn mouse model of Down syndrome. *Neurobiol Dis*. 2013;58:92-101. doi:10.1016/j.nbd.2013.04.016
26. Ash JA, Velazquez R, Kelley CM, et al. Maternal choline supplementation improves spatial mapping and increases basal forebrain cholinergic neuron number and size in aged Ts65Dn mice. *Neurobiol Dis*. 2014;70:32-42. doi:10.1016/j.nbd.2014.06.001
27. Kelley CM, Powers BE, Velazquez R, et al. Maternal choline supplementation differentially alters the basal forebrain cholinergic system of young-adult Ts65Dn and disomic mice. *J Comp Neurol*. 2014;522(6):1390-1410. doi:10.1002/cne.23492
28. Strupp BJ, Powers BE, Velazquez R, et al. Maternal choline supplementation: a potential prenatal treatment for Down syndrome and Alzheimer's disease. *Curr Alzheimer Res*. 2016;13:97-106.
29. Yang Y, Liu Z, Cermak JM, et al. Protective effects of prenatal choline supplementation on seizure-induced memory impairment. *J Neurosci*. 2000;20(22):RC109. doi:10.1523/JNEUROSCI.20-22-j0006.2000
30. Holmes GL, Yang Y, Liu Z, et al. Seizure-induced memory impairment is reduced by choline supplementation before or after status epilepticus. *Epilepsy Res*. 2002;48(1):3-13. doi:10.1016/S0920-1211(01)00321-7
31. Wong-Goodrich SJE, Glenn MJ, Mellott TJ, Liu YB, Blusztajn JK, Williams CL. Water maze experience and prenatal choline supplementation differentially promote long-term hippocampal recovery from seizures in adulthood. *Hippocampus*. 2011;21(6):584-608. doi:10.1002/hipo.20783
32. Nag N, Berger-Sweeney JE. Postnatal dietary choline supplementation alters behavior in a mouse model of Rett syndrome. *Neurobiol Dis*. 2007;26(2):473-480. doi:10.1016/j.nbd.2007.02.003
33. Nag N, Mellott TJ, Berger-Sweeney JE. Effects of postnatal dietary choline supplementation on motor regional brain volume and growth factor expression in a mouse model of Rett syndrome. *Brain Res*. 2008;1237:101-109. doi:10.1016/j.brainres.2008.08.042
34. Ricceri L, De Filippis B, Fuso A, Laviola G. Cholinergic hypofunction in MeCP2-308 mice: beneficial neurobehavioural effects of neonatal choline supplementation. *Behav Brain Res*. 2011;221(2):623-629. doi:10.1016/j.bbr.2011.03.051
35. Velazquez R, Ferreira E, Knowles S, et al. Lifelong choline supplementation ameliorates Alzheimer's disease pathology and associated cognitive deficits by attenuating microglia activation. *Aging Cell*. 2019;18(6):e13037. doi:10.1111/acel.13037
36. Mellott TJ, Huleatt OM, Shade BN, et al. Perinatal choline supplementation reduces amyloidosis and increases choline acetyltransferase expression in the hippocampus of the APPswePS1dE9 Alzheimer's disease model mice. *PLoS One*. 2017;12(1):e0170450. doi:10.1371/journal.pone.0170450
37. Institute of Medicine. *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. National Academies Press; 1998. doi:10.17226/6015
38. Wu BTF, 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:e43448. doi:10.1371/journal.pone.0043448
39. 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;177:1338-1347. doi:10.1093/aje/kws395
40. 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;87:896-902. doi:10.1093/ajcn/87.4.896
41. 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;26:328-335. doi:10.1111/j.1365-3016.2012.01264.x
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;96:1465-1472. doi:10.3945/ajcn.112.037184
43. 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;32:2172-2180. doi:10.1096/fj.201700692RR
44. Ross RG, Hunter SK, McCarthy L, et al. Perinatal choline effects on neonatal pathophysiology related to later Schizophrenia risk. *Am J Psychiatry*. 2013;170:290-298. doi:10.1176/appi.ajp.2012.12070940
45. McGaughy J, Kaiser T, Sarter M. Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: selectivity of the behavioral impairment and relation to cortical AChE-positive fiber density. *Behav Neurosci*. 1996;110(2):247-265. doi:10.1037/0735-7044.110.2.247
46. McGaughy J, Sarter M. Sustained attention performance in rats with intracortical infusions of 192 IgG-saporin-induced cortical cholinergic deafferentation: effects of Physostigmine and FG 7142. *Behav Neurosci*. 1998;112(6):1519-1525. doi:10.1037/0735-7044.112.6.1519
47. Hasselmo ME, Sarter M. Modes and models of forebrain cholinergic neuromodulation of cognition. *Neuropsychopharmacology*. 2011;36(1):52-73. doi:10.1038/npp.2010.104
48. Sarter M, Hasselmo ME, Bruno JP, Givens B. Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. *Brain Res Rev*. 2005;48(1):98-111. doi:10.1016/j.brainresrev.2004.08.006

49. Parikh V, Kozak R, Martinez V, Sarter M. Prefrontal acetylcholine release controls cue detection on multiple timescales. *Neuron*. 2007;56(1):141-154. doi:10.1016/j.neuron.2007.08.025
50. Yan J, Jiang X, West AA, et al. Pregnancy alters choline dynamics: results of a randomized trial using stable isotope methodology in pregnant and nonpregnant women. *Am J Clin Nutr*. 2013;98(6):1459-1467. doi:10.3945/ajcn.113.066092
51. Jiang X, Bar HY, Yan J, et al. A higher maternal choline intake among third-trimester pregnant women lowers placental and circulating concentrations of the antiangiogenic factor fms-like tyrosine kinase-1 (sFLT1). *FASEB J*. 2013;27(3):1245-1253. doi:10.1096/fj.12-221648
52. Demeter E, Guthrie SK, Taylor SF, Sarter M, Lustig C. Increased distractor vulnerability but preserved vigilance in patients with schizophrenia: evidence from a translational Sustained Attention Task. *Schizophr Res*. 2013;144:136-141. doi:10.1016/j.schres.2013.01.003
53. Parasuraman R, Warm JS, Dember WN. Vigilance: taxonomy and utility. In: Mark LS, Warm JS, Huston RL, eds. *Ergonomics and Human Factors*. Recent Research in Psychology. Springer; 1987:11-32. doi:10.1007/978-1-4612-4756-2_2
54. Demeter E, Sarter M, Lustig C. Rats and humans paying attention: cross-species task development for translational research. *Neuropsychology*. 2008;22(6):787-799. doi:10.1037/a0013712
55. Burk JA, Sarter M. Dissociation between the attentional functions mediated via basal forebrain cholinergic and GABAergic neurons. *Neuroscience*. 2001;105(4):899-909. doi:10.1016/S0306-4522(01)00233-0
56. Burk JA, Herzog CD, Porter MC, Sarter M. Interactions between aging and cortical cholinergic deafferentation on attention. *Neurobiol Aging*. 2002;23(3):467-477. doi:10.1016/S0197-4580(01)00315-3
57. McGaughy J, Sarter M. Effects of ovariectomy, 192 IgG-saporin-induced cortical cholinergic deafferentation, and administration of estradiol on sustained attention performance in rats. *Behav Neurosci*. 1999;113(6):1216-1232. doi:10.1037/0735-7044.113.6.1216
58. Friard O, Gamba M. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol Evol*. Published online 2016. doi:10.1111/2041-210X.12584
59. Bahnfleth C, Canfield R, Nevins J, Caudill M, Strupp B. Prenatal choline supplementation improves child color-location memory task performance at 7 y of age (FS05-01-19). *Curr Dev Nutr*. 2019;3(Supplement_1):1260-1261. doi:10.1093/cdn/nzz048.FS05-01-19
60. Nevins J, Beckman K, Bahnfleth C, et al. Maternal choline supplementation during pregnancy improves executive functioning in children at age 7 y (E10–06). *Curr Dev Nutr*. 2018;2(nzy043):3-4. doi:10.1093/cdn/nzy043
61. Bahnfleth CL. *The Effect of Maternal Choline Intake on Child Attention and Memory: A Seven-year Follow-up*. Doctoral dissertation. Cornell University; 2019.
62. Abelson RP, Prentice DA. Contrast tests of interaction hypotheses. *Psychol Methods*. 1997;2(4):315-328. https://psycnet.apa.org/record/1997-43554-001
63. VanderWeele TJ, Mathur MB. Some desirable properties of the Bonferroni correction: is the Bonferroni correction really so bad? *Am J Epidemiol*. 2019;188(3):617-618. doi:10.1093/aje/kwy250
64. Nuechterlein KH, Parasuraman R, Jiang Q. Visual sustained attention: image degradation produces rapid sensitivity decrement over time. *Science*. 1983;220(4594):327-329. doi:10.1126/science.6836276
65. Sarter M. Behavioral-cognitive targets for cholinergic enhancement. *Curr Opin Behav Sci*. 2015;4:22-26. doi:10.1016/j.cobeha.2015.01.004
66. Kang JI, Huppé-Gourgues F, Vaucher E. Boosting visual cortex function and plasticity with acetylcholine to enhance visual perception. *Front Syst Neurosci*. 2014;8:172. doi:10.3389/fnsys.2014.00172
67. Williams CL, Meck WH, Heyer DD, Loy R. Hypertrophy of basal forebrain neurons and enhanced visuospatial memory in perinatally choline-supplemented rats. *Brain Res*. 1998;794:225-238. doi:10.1016/S0006-8993(98)00229-7
68. Loy R, Heyer D, Williams CL, Meck WH. Choline-induced spatial memory facilitation correlates with altered distribution and morphology of septal neurons. *Adv Exp Med Biol*. 1991;295:373-382.
69. Napoli I, Blusztajn JK, Mellott TJ. Prenatal choline supplementation in rats increases the expression of IGF2 and its receptor IGF2R and enhances IGF2-induced acetylcholine release in hippocampus and frontal cortex. *Brain Res*. 2008;1237:124-135. doi:10.1016/j.brainres.2008.08.046
70. Cermak JM, Holler T, Jackson DA, Blusztajn JK. Prenatal availability of choline modifies development of the hippocampal cholinergic system. *FASEB J*. 1998;12(3):349-357. doi:10.1096/fasebj.12.3.349
71. Unsworth N, Robison MK. A locus coeruleus-norepinephrine account of individual differences in working memory capacity and attention control. *Psychon Bull Rev*. 2017;24(4):1282-1311. doi:10.3758/s13423-016-1220-5
72. Chamberlain SR, del Campo N, Dowson J, et al. Atomoxetine improved response inhibition in adults with attention deficit/hyperactivity disorder. *Biol Psychiat*. 2007;62(9):977-984. doi:10.1016/j.biopsych.2007.03.003
73. Bunsey MD, Strupp BJ. Specific effects of idazoxan in a distraction task: evidence that endogenous norepinephrine plays a role in selective attention in rats. *Behav Neurosci*. 1995;109(5):903-911. doi:10.1037/0735-7044.109.5.903
74. Greene CM, Bellgrove MA, Gill M, Robertson IH. Noradrenergic genotype predicts lapses in sustained attention. *Neuropsychologia*. 2009;47(2):591-594. doi:10.1016/j.neuropsychologia.2008.10.003
75. van den Brink RL, Murphy PR, Nieuwenhuis S. Pupil diameter tracks lapses of attention. *PLoS One*. 2016;11(10):e0165274. doi:10.1371/journal.pone.0165274
76. Button KS, Ioannidis JPA, Mokrysz C, et al. Power failure: why small sample size undermines the reliability of neuroscience. *Nat Rev Neurosci*. 2013;14:365-376. doi:10.1038/nrn3475
77. Shalev L, Ben-Simon A, Mevorach C, Cohen Y, Tsal Y. Conjunctive Continuous Performance Task (CCPT)—a pure measure of sustained attention. *Neuropsychologia*. 2011;49(9):2584-2591. doi:10.1016/j.neuropsychologia.2011.05.006
78. Barkley RA. Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. *Psychol Bull*. 1997;121(1):65-94.
79. Metallidou P, Malegiannaki AC, Konstantinopoulou E, Kiosseoglou G. Effects of different functions of attention on

school grades in primary school children. *J Educ Hum Dev*. 2016;5(3):68-79. doi:10.15640/jehd.v5n3a8

80. Muris P. Relation of attention control and school performance in normal children. *Percept Mot Skills*. 2006;102(1):78-80. doi:10.2466/pms.102.1.78-80
81. McClelland MM, Cameron CE, Connor CM, Farris CL, Jewkes AM, Morrison FJ. Links between behavioral regulation and preschoolers' literacy, vocabulary, and math skills. *Dev Psychol*. 2007;43(4):947-959. doi:10.1037/0012-1649.43.4.947
82. Bixler R, D'Mello S. Automatic gaze-based user-independent detection of mind wandering during computerized reading. *User Model User-Adap Inter*. 2016;26:33-68. doi:10.1007/s11257-015-9167-1
83. Kane MJ, Conway ARA, Hambrick DZ, Engle RW. Variation in working memory capacity as variation in executive attention and control. In: ARA Conway, C Jarrold, MJ Kane, A Miyake, JN Towse, eds. *Variation in Working Memory*. Oxford University Press; 2007:21-48.
84. Smilek D, Carriere JSA, Cheyne JA. Failures of sustained attention in life, lab, and brain: ecological validity of the SART. *Neuropsychologia*. 2010;48(9):2564-2570. doi:10.1016/j.neuropsychologia.2010.05.002
85. Visentin CE, Masih S, Plumptre L, et al. Maternal choline status, but not fetal genotype, influences cord plasma choline metabolite concentrations. *J Nutr*. 2015;145(7):1491-1497. doi:10.3945/jn.115.211136
86. Lewis ED, Subhan FB, Bell RC, et al. Estimation of choline intake from 24 h dietary intake recalls and contribution of egg and milk consumption to intake among pregnant and lactating women in Alberta. *Br J Nutr*. 2014;112:112-121. doi:10.1017/S0007114514000555
87. Fischer LM, da Costa KA, Galanko J, et al. Choline intake and genetic polymorphisms influence choline metabolite concentrations in human breast milk and plasma. *Am J Clin Nutr*. 2010;92:336-346. doi:10.3945/ajcn.2010.29459
88. Wallace TC, Fulgoni VL. Usual choline intakes are associated with egg and protein food consumption in the United States. *Nutrients*. 2017;9(8):839. doi:10.3390/nu9080839
89. Jacobson SW, Carter RC, Molteno CD, 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):1327-1341. doi:10.1111/acer.13769
90. Kable JA, Coles CD, Keen CL, et al. The impact of micronutrient supplementation in alcohol-exposed pregnancies on information processing skills in Ukrainian infants. *Alcohol*. 2015;49(7):647-656. doi:10.1016/j.alcohol.2015.08.005
91. Wozniak JR, Fink BA, Fuglestad AJ, 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. doi:10.1186/s11689-020-09312-7
92. Bell CC, Aujla J. Prenatal vitamins deficient in recommended choline intake for pregnant women. *J Fam Med Dis Prev*. 2016;2:048.

How to cite this article: Bahnfleth CL, Strupp BJ, Caudill MA, Canfield RL. Prenatal choline supplementation improves child sustained attention: A 7-year follow-up of a randomized controlled feeding trial. *FASEB J*. 2022;36:e22054. doi:10.1096/fj.202101217R