Title

The impact of micronutrient supplementation in alcohol-exposed pregnancies on reaction time responses of preschoolers in Ukraine

Authors

Kable, JA
Coles, CD
Keen, CL
et al.

Publication Date

2022-03-01

DOI

10.1016/j.alcohol.2021.12.002

Peer reviewed

Published in final edited form as:

Alcohol. 2022 March ; 99: 49–58. doi:10.1016/j.alcohol.2021.12.002.

The Impact of Micronutrient Supplementation in Alcohol-Exposed Pregnancies on Reaction Time Responses of Preschoolers in Ukraine

JA Kable[1,2], **CD Coles**[1,2], **CL Keen**[3], **JY Uriu-Adams**[3], **KL Jones**[4,5], **L Yevtushok**[6,7,9], **Y Kulikovsky**[6,9], **N. Zymak-Zakutnya**[8,9], **Iryna Dubchak**[8,9], **D Akhmedzhanova**[8,9], **W Wertelecki**[4,9], **CD Chambers**[4,5], **CIFASD**

[1]Departments of Psychiatry and Behavioral Science, Emory University School of Medicine,

[2]Pediatrics, Emory University School of Medicine,

[3]Department of Nutrition, University of California, Davis,

Send correspondence to: Julie A. Kable, Ph.D., 12 Executive Park, Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA 30329, Phone # 404-712-9833, FAX # 404-712-9809, jkabl01@emory.edu, Do not cite without consent of authors.

Authors' Statement

JA Kable: Dr. Kable developed the protocol, analyzed the results, and was the primary author of the manuscript.

CD Coles: Dr. Coles developed other portions of the Ukrainian preschool assessment, provided consultation regarding the analysis, and was involved in reviewing and editing several drafts of the manuscript.

CL Keen: Dr. Keen supervised the methods related to the analysis of choline and its metabolites.
Dr. Keen also reviewed and edited the manuscript.

JY Uriu-Adams: Dr. Uriu -Adams completed the analysis of choline and its metabolites. She also reviewed and edited the manuscript.

KL Jones: Dr Jones is one of the investigators in the Ukrainian project who helped design the assessment protocol. Dr. Jones reviewed and edited the manuscript.

L Yevtushok: Dr. Yevtushok is one of the primary site supervisors in the Ukraine who was responsible for supervising the recruitment and data collection related to the aims of the study that are reflected in the manuscript. Dr. Yevtushok reviewed and edited the manuscript.

Y Kulikovsky: Dr. Kulikovsky was involved in data collection and has reviewed and edited the manuscript.

N. Zymak-Zakutnya: Dr. Zymak-Zakutnya is one of the primary site supervisors in the Ukraine who was responsible for supervising the recruitment and data collection related to the aims of the study that are reflected in the manuscript. Dr. Zymak-Zakutnya reviewed and edited the manuscript.

Iryna Dubchak, Ms. Dubchak was involved in data collection and has reviewed and edited the manuscript.

D Akhmedzhanova: Ms. Akhmedzhanova was involved in supervising and conducting activities related to recruitment, data collection, and transmission. Ms. Akhmedzhanova reviewed and edited the manuscript.

W Wertelecki: Dr. Wertelecki directs the OMNI-Net Ukraine Birth Defects Program that provides administrative oversite of project in the Ukraine. Dr. Wertelecki reviewed and edited the manuscript.

CD Chambers: Dr. Chambers is the principal investigator of the project and is involved in coordinating the activities needed to carry out the aims of the project. She has provided supervision and oversite of data collection, encoding, and sharing among investigators. Dr. Chambers reviewed and edited the manuscript.

the CIFASD : All or part of this work was done in conjunction with the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD), which is funded by grants from the National Institute on Alcohol Abuse and Alcoholism (NIAAA). Additional information about CIFASD can be found at www.cifasd.org.

Ethics approval and consent to participate.

Ethics approval was obtained from the Internal Review Boards at the Lviv National Medical University in Ukraine and the University of California San Diego, La Jolla, California. All participants underwent an informed consent process for the assessments carried in the study. Participant recruitment and the study's procedures were carried out in accordance with the guidelines established by the Declaration of Helsinki.

Competing interests.

The authors have no competing interests to declare.

[4]Departments of Pediatrics, University of California San Diego,

[5]Family Medicine and Public Health, University of California San Diego,

[6]Rivne Regional Medical Diagnostic Center, Rivne, Ukraine,

[7]Lviv National Medical University, Lviv, Ukraine,

[8]Khmelnytsky Perinatal Center, Khmelnytsky, Ukraine,

[9]OMNI-Net Ukraine Birth Defects Program.

Abstract

The potential of micronutrients to ameliorate the impact of prenatal alcohol exposure (PAE) on attentional regulation skills was explored in a randomized clinical trial conducted in Ukraine. Women who differed in prenatal alcohol use were recruited during pregnancy and assigned to one of three groups (No study-provided supplements, Multivitamin/Mineral Supplement (MVM), or MVM plus Choline). Their offspring were seen in the preschool period and a reaction time task was administered. Participants were asked to press a response button as quickly as possible as thirty stimuli from the same category (animals) were presented consecutively and then followed by 6 stimuli from a novel category (vehicles). Number correct, mean latency of the response over trials, and variability in the latency were analyzed separately by sex. During the initial animal trials, boys whose mothers received MVM during pregnancy had more correct responses and reduced response latency compared to boys whose mothers had no MVM treatment. During vehicle trials, maternal choline supplementation was associated with increased response speed in males without a PAE history. Females receiving supplements did not show the same benefits from micronutrient supplementation and were more adversely impacted by prenatal alcohol exposure. Relationships between maternal levels of choline, betaine, and dimethylglycine (DMG) and task performance were also assessed. Although no effects were found for choline after adjusting for multiple comparisons, lower baseline DMG level was associated with greater accuracy and shorter latency of responses in the initial animal trials and shorter latency in the vehicle trials in female preschoolers. Level of betaine in Trimester 3 was associated with reduced variability in the latency of male responses during the animal trials. Maternal micronutrient supplementation in pregnancy appears to improve preschool reaction time performance but the effects varied as a function of sex and PAE exposure status.

Keywords

Prenatal alcohol; micronutrient supplementation; choline; reaction time; attention

---

Prenatal alcohol exposure (PAE) has been associated with a spectrum of neurobehavioral and physical effects that are captured using an umbrella term, Fetal Alcohol Spectrum Disorders (FASDs) (Riley, Infante, & Warren, 2011), with the most extreme variant being Fetal Alcohol Syndrome (FAS)(Stratton, Howe, & Battaglia, 1996). Those diagnosed with FAS have characteristic facial features, growth delays, and neurodevelopmental impairments. The potential of multivitamins and micronutrients to ameliorate the impact of PAE has been the focus of considerable human and animal model research over the

last decade (Helfrich, Saini, Kling, & Smith, 2018; Huebner et al., 2018; Jacobson et al., 2018; Naseer, Y., & M.O., 2010; Nguyen, Risbud, Mattson, Chambers, & Thomas, 2016; Ojeda et al., 2009; Serrano, Han, Brinez, & Linask, 2010; Summers, Rofe, & Coyle, 2009; Thomas, Abou, & Dominguez, 2009; Wozniak et al., 2015) with the effectiveness of choline supplementation receiving considerable attention. Investigations of maternal nutrient status has indicated that mothers of children with FASDs often have poor dietary intake and nutritional status (Carter et al., 2018; Carter, Jacobson, Molteno, & Jacobson, 2007; Carter et al., 2017; May et al., 2016; May et al., 2014).

Although preclinical studies of choline supplementation after a history of PAE have produced promising results, human clinical trials have not been as clear (see review, (Akison, Kuo, Reid, Boyd, & Moritz, 2018)). Choline supplementation provided to older children with PAE has not been found to improve physical status or neurobehavioral outcomes (Nguyen et al., 2016) but has been found to improve memory functioning in preschoolers (Wozniak et al., 2015). In a later assessment of the preschool cohort, enhanced effectiveness of choline supplementation was observed after a four year gap period in the areas of working memory, visual-spatial skills, verbal memory, and behavioral functioning (Wozniak et al., 2020), suggesting effects of the supplementation postnatally may be delayed. Choline supplements taken in pregnancy have resulted in improved physical and developmental outcomes (Jacobson et al., 2018; Kable, Coles, Keen, Uriu-Adams, Jones, Yevtushok, Kulikovsky, Wertelecki, Pedersen, & Chambers, 2015) but to date the results have only been reported on outcomes during the infancy and toddler periods of development due to the age of the cohorts.

In a cohort of women from Ukraine, choline supplementation in pregnancy resulted in improved neurophysiological encoding during visual habituation for both infants with and without PAE. Infants with no PAE who received choline supplementation also showed improvements on the dishabituation trials, suggesting that choline's positive effects were not sufficient to ameliorate the neural damage associated with PAE. Similar results were obtained in information processing speed in a cohort were prenatal choline supplementation was provided but PAE was not assessed (Caudill, Strupp, Muscalu, Nevins, & Canfield, 2018). Relationships between estimates of changes in the nutrient status of the Ukrainian mothers over the course of pregnancy and information processing outcomes suggested that choline's effect may be mediated by the breakdown of choline to betaine and then to dimethylglycine (DMG) (Kable, Coles, Keen, Uriu-Adams, Jones, Yevtushok, Kulikovsky, Wertelecki, Pedersen, & Chambers, 2015).

Although choline was given to women in the Ukrainian cohort, the focus of the study was broader and involved a clinical trial assessing the outcomes of micronutrient supplementation during pregnancy on a sample of infants with PAE. Participants were assigned to either receive the standard of care, which was to recommend multivitamin/mineral supplement use (MVM) but to not provide them to mothers, or to receive MVM supplements from the study. Half of those who received the MVM supplements were also given choline (750 mg) supplements (MVM+choline). During infancy, MVM supplementation was found to reduce the negative impact of alcohol use during pregnancy on developmental outcomes as assessed using a Ukrainian adaptation of the Bayley Scale

of Infant and Toddler Development, 2nd edition© (Coles et al., 2015) but significant effects were not found for choline supplementation on this measure. This cohort was followed again into the preschool period where the impact of PAE was found on nonverbal reasoning and indices of executive functioning and these relationships were dose responsive (Coles et al., 2021).

Choline supplementation was explored in another cohort, consisting of heavy alcohol using women from South Africa who participated in a randomized placebo controlled clinical trial. Growth and neurocognitive impairment was significantly improved in infancy with choline supplementation at higher dosage levels than was used in the Ukrainian cohort (Jacobson et al., 2018). In the South Africa study choline was not given to women who did not have a history of PAE and only 9.7% of the women met minimum requirements for daily choline intake during pregnancy. This combination of factors makes it difficult to discern if the choline was ameliorating the impact of PAE at this higher dosage level or just correcting a choline deficiency frequently present in the cohort. Collectively, these studies suggest that choline intake during pregnancy positively influences neurodevelopmental outcomes but the mechanisms and long-term effects of this intervention have yet to be determined.

One of the most common neurobehavioral impairments of children with an FASD is having attentional regulation problems (Chambers et al., 2019; Coles et al., 2020; Coles et al., 1997; May, Hasken, Baete, et al., 2020; May, Hasken, Bozeman, et al., 2020; May, Hasken, Stegall, et al., 2020; May et al., 2021; Nanson & Hiscock, 1990; Streissguth et al., 1986), leading to a frequent secondary diagnosis of Attention Deficit Hyperactivity Disorder later in childhood (Lange, Rehm, Anagnostou, & Popova, 2018; Nanson & Hiscock, 1990). Early work linking PAE to impaired attentional regulation skills utilized vigilance tasks that measured both the ability to sustain attention and impulsivity in responding (Koelega, 1993). Such tasks require children to maintain alertness in order to respond effectively to target stimuli that are presented at a low frequency relative to distractor stimuli. In school age children, vigilance performance (e.g., errors of commission, reaction time, and an error summary score) on a computerized continuous performance task was negatively impacted by PAE (Streissguth et al., 1986) and significantly related to parent ratings of the children's endurance, persistence, organization, distractibility and impulsivity. This finding has been replicated in some studies of PAE (Brown et al., 1991; Kooistra, Crawford, Gibbard, Ramage, & Kaplan, 2010) but not in others (Boyd, Ernhart, Greene, Sokol, & Martier, 1991; Coles et al., 1997). More recently, performance on the Quotient ADHD System was found to be impacted by prenatal alcohol exposure in a sample of school age children (aged 7–14 years) (Infante et al., 2015).

Assessment of vigilance is complicated by the difficulties associated with getting cooperation over prolonged periods of low arousal in preschool children. In a previous study of a small sample of preschoolers (ages 3–4 years, n =28) exposed to PAE, vigilance behavior was assessed using a computerized task (Herman, Kirchner, Streissguth, & Little, 1980) that increased the frequency of the target stimulus to reduce subject attrition often seen in preschool samples. The number of correct responses on the vigilance task was significantly related to overall IQ (r = .53) and a trend was found for an effect of maternal alcohol use. In a larger study of 4 year-olds (n=245) no significant effects of PAE were

found on a similar computerized vigilance task, suggesting that such measures may be not be effective in identifying early alcohol related neurodevelopmental compromise (Boyd et al., 1991). An alternative to vigilance tasks for preschoolers may be simple reaction time tasks (Weissberg, Ruff, & Lawson, 1990) that require active responding after the presentation of each stimuli but still require sustained attention over the course of multiple trials. Although reaction time tasks are not used to assess sustained attention skills in older children as a result of the simplicity of the response needed for successful performance, responses to these type of tasks in preschoolers have predicted parental report of their child's ADHD symptoms on the Connors' behavior rating scales and have been found to have adequate stability and reliability (Connors, 2008).

To further explore the impact of micronutrient supplementation in pregnancy for heavy alcohol using women on attentional regulation skills in their offspring, a reaction time task was used with preschoolers from the prospective cohort recruited in Ukraine to assess the impact of micronutrient supplementation in pregnancy in individuals with and without a history of PAE. The study's participants were identified during their mothers' pregnancy and followed prospectively until they were between 3 to 5 years old. Performance on a preschool reaction time task was anticipated to be adversely impacted by PAE but MVM and choline supplementation were hypothesized to improve the performance. Specific outcome measures were accuracy of responses, latency associated with the response, and variability in the latency of the response over trials. The latter is often recognized as a key distinguishing variable for detecting attentional deficit symptoms in ADHD with lower variability reflecting improved attentional regulation skills (Elmaghrabi et al., 2018; Gooch, Snowling, & Hulme, 2012; Vasquez, Binns, & Anderson, 2018).

MATERIALS AND METHODS

The study protocol was approved by institutional review boards at the Lviv National Medical University in Ukraine and the University of California San Diego, La Jolla, California. All participants were treated in accordance with the guidelines established by the Declaration of Helsinki and informed written consent was provided by all participants. This study was carried out in two cities (Khmelnytsky and Rivne) in Ukraine in collaboration with the OMNI-Net Birth Defects Program and was part of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD), an international consortium of basic science and clinical investigations funded by the National Institute of Alcohol Abuse and Alcoholism (NIAAA).

Recruitment and Procedures

Participants were children whose mothers enrolled in a clinical trial during their pregnancy and who agreed to follow up during the preschool period. Women were recruited at the first prenatal appointment and were interviewed regarding their alcohol use during pregnancy. All women were provided with information on the risks of alcohol consumption during pregnancy. For the PAE group, those invited to participate in the study were women who reported at least weekly binge drinking episodes (5+ drinks), at least five episodes of 3–4 standard drinks, or at least 10 episodes of 1–2 standard drinks either in the month around

conception or the most recent month of pregnancy. In addition, a sample of women who reported no binge episodes, minimal or no alcohol in the month around conception, and no continued drinking in pregnancy were also invited to participate.

Women were randomly assigned to an intervention group. Those in the standard of care group were encouraged to use MVMs but were not provided the MVMs. MVM supplements had to be purchased by participants in this group as they were not provided by the healthcare system in Ukraine during the interval in which the sample was recruited. MVM use was reported by 28.4% of the No MVM group but was only maintained throughout the pregnancy by one participant in the original cohort (Kable, Coles, Keen, Uriu-Adams, Jones, Yevtushok, Kulikovsky, Wertelecki, Pedersen, Chambers, et al., 2015). Self-initiated single supplement use was more common (No MVM: 49.4 %, MVM: 60.0%, and MVM+choline: 52.8%). Folate and iodine were the most common single supplements reported. An over the counter prenatal MVM supplement (Theravit) was provided throughout the study for those assigned to the MVM group. Half of those assigned to the MVM group were assigned to also receive a daily dose of choline (750 mg).

Interviews were conducted with mothers at enrollment, in the third trimester (~ 32 weeks of gestation), and at postpartum. A structured questionnaire was used to obtain information regarding the mothers' demographic history, lifestyle, and parental substance use in pregnancy. A timeline follow back procedure was used to assess day by day alcohol quantity and type consumed in the periods around conception and in the two weeks prior to enrollment. Absolute ounces of alcohol per day (ozAA/day) and per drinking day (ozAA/drinking day) were computed. Blood samples were obtained from the mother to estimate various micronutrients at enrollment and a third trimester visit. The samples were then shipped to the University of California-Davis for processing and data were sent to the University of California San Diego and Emory University. Estimates of maternal choline, betaine and DMG were obtained using a UPLC-Micro triplequad MS/MS (Waters, Micromass) using modified Holms' procedures (Holm, Ueland, Kvalheim, & Lien, 2003; Innis & Hasman, 2006). Change in choline and its metabolites, betaine and dimethylglycine (DMG), were computed by subtracting baseline concentrations from levels obtained at the second visit.

Preschool Follow Up Assessment Procedures

Mothers and their children were seen again in the preschool period (Mean Age in Years = 3.96 (.34)). During their visit, a medical interview, developmental testing, and the reaction time task were administered.

Preschool Reaction Time Task.—The reaction time task involves the child making a response to a series of chromatic pictures presented on a computer screen. Children were instructed to press a response button as quickly as possible when they saw a target stimulus. An initial fixation slide, consisting of a cross centrally located on the screen, was shown for 1000 msec and was followed by the target stimulus for up to 2500 msec. After the target stimulus, the child was presented with a feedback slide for 1500 msec that was either a large yellow smiley face if they made a correct response or a large yellow frowny face if they

failed to make a response (see Figure 1). A total of 39 stimuli were presented consecutively. The first three were regarded as training trials and were discarded in the analysis. Thirty stimuli from the same category (animals) were presented, followed by 6 stimuli from a new category (vehicles) (see Figure 2 for illustration of the task structure). There were no distractor stimuli presented as part of this task. The child was instructed to respond after each photo presented. Correct responses were defined as those where the child pressed the response button prior to the feedback slide but after the target slide was presented. Failure to make a response (omissions) were treated as incorrect responses as were those that occurred after the feedback slide and before the next stimulus. The computer recorded responses and the time of the response.

Outcomes analyzed relative to task performance were number correct, mean latency of the responses, and variability in the latency of the responses over the trials. Mean latency and variability in the latency of the response was only computed for correct responses as defined by a response within the interval of the presentation of the target stimulus and the presentation of the feedback slide. The results were then analyzed relative to the two different blocks of stimuli. The first phase reflects the capacity to maintain performance on a simple routine task over several trials where a gradual decline in performance is expected as a result of increasing boredom and habituation to the procedures. The second portion reflects the capacity to maintain performance over the remaining trials but incorporates a change in the class of stimuli. Better performance outcomes on the second portion is suggestive of increased attention associated with the change in stimulus class.

Data Analysis

Data was collected and entered on site by team members from the OMNI-Net Birth Defects Program teams in Ukraine and transmitted to the University of California San Diego, La Jolla, California and Emory University, Atlanta, Georgia. Preliminary examination of relationships between the outcome variables and demographic data was done using correlations for continuous data and t-tests for categorical data. The results indicated that there were significant effects for age and sex. Older children were more accurate and responded faster with less variability. Thus, age was included as a covariate. As the preliminary analysis also indicated a significant sex effect in the outcomes, subsequent analyses were then performed separately for each sex. The final analysis was an incomplete factorial design that used univariate analysis of covariance with age as a covariate and between subject's effects of PAE (yes vs. no), MVM treatment (yes vs. no), and choline (yes vs. no). The factorial design was incomplete as choline was never given to participants in the absence of MVM treatment.

Outcomes were examined in relation to estimates of maternal choline and its metabolites, betaine and DMG to determine if preschool attentional regulation skills were related to levels of choline and its metabolites in a linear dose response manner. This adds to the group comparison analysis that was done as the experimental design analysis does not consider variability in compliance and individual dietary preferences for micronutrient enriched foods that may have impacted the neurodevelopmental outcome. In addition to the traditional level of significance ($p < .05$) provided for these relationships, the corrected significance level q*

derived from the Benjamini and Hochberg (1995) adjustment for multiple comparison is also provided. This adjustment corrects for the overall false discovery rate and is used instead of the Bonferroni correction (Nakagawa, 2004) as a result of concerns about making increased Type II errors using the Bonferroni correction. The Bonferroni method adjusts for all p-values but the Benjamini and Hochberg (1995) procedure adjusts for p-values accordingly to their ranking to minimize the false discovery rate while avoiding overcorrecting for the negative results. Both the traditional p-value and adjusted q* value are provided in the results section as the analysis should be considered exploratory relative to the impact of pregnancy levels of choline and its metabolites have on offspring development.

RESULTS

Group Characteristics of Sample

Reaction time data were available on 243 participants. Group characteristics are outlined in Table 1. The table has columns for those that received MVM treatment or not relative to their alcohol status and then breaks out those who received MVM treatment by whether or not they received additional choline supplementation (columns shaded in gray). Main effects for alcohol exposure were obtained for maternal education (PAE: M = 13.2 (SE = .267); No PAE: M = 15.1 (SE = .206), $p < .0001$) and all of the maternal drinking variables (Preconception AA/day: PAE: M = .654 (SE = .054); No PAE: M = .003 (SE = .042), $p < .0001$; Preconception AA/drinking day: PAE: M = 2.040 (SE = .185); No PAE: M = .018 (SE = .143), $p < .0001$); Trimester 1 AA/day: PAE: M = .124 (SE = .029); No PAE: M = .000 (SE = .022, $p < .001$); Trimester 1 AA/drinking day: PAE: M = .562 (SE = .078); No PAE: M = .004 (SE = .061), $p < .0001$)). Maternal education was lower and alcohol consumption was higher among the alcohol using group relative to the contrast group. In addition, an interaction between alcohol exposure and a MVM effect was obtained on the average levels of alcohol use per day (AA/day) in the first trimester. Those who had a history of PAE exposure but were assigned to having no MVM treatment had higher levels of trimester 1 alcohol use than did those assigned to the MVM group (PAE No MVM: M= .229 (SE = .038) vs. PAE MVM: M = .072 (SE = .038), $p < .0045$).

Outcomes by Experimental Design

The averages of the number of correct responses, latency of response, and variability in response time are presented in Table 2A for males and Table 2B for females.

Animal Stimuli Block: For males, a significant MVM treatment effect was found on correct responses ($F(1,119) = 4.084$, $p < .046$) and response latency ($F(1, 119) = 7.859$, $p < .006$) with those receiving MVM having higher correct scores and responding more quickly than did those who did not. There were no significant main effects of PAE for males. For females, there were no significant effects of MVM or choline treatment. A significant effect of PAE was found on number of correct responses ($F(1,110) = 9.609$, $p < .002$) and variability in response time ($F(1, 110) = 5.343$, $p < .023$). Females with a history of PAE had fewer correct responses and more variability than did those without a history of PAE.

Vehicle Stimuli Block: For males, a significant interaction between PAE and choline intervention was found on the mean latency of the response ($F(1,118) = 4.276$, $p < .041$). Male offspring whose mothers were not in the PAE group and received choline had faster mean latency of their responses relative to those who did not have PAE and did not receive choline intervention. The treatment effect was not significant in the PAE group and was not significant for females with or without PAE.

Relationships between Outcomes and Choline and its Metabolites

Biological samples of choline and its metabolites were available on a subset of the sample (Baseline biological samples: Males n=80, Females n=65; Third trimester biological samples: Males n = 71, Females n = 59). These relationships should be viewed as exploratory but add to the group comparisons already conducted as it can contribute to understanding the mechanisms by which levels of choline and its metabolites during pregnancy can impact later development. Table 3 visualizes the correlations between performance measures of the reaction time task and biological samples of maternal choline and its metabolites collected during pregnancy. The information is presented separately for males and females. The strength of the correlation is reflected in the color of each individual cell in the table with dark red indicating strong negative relationships and dark blue indicating strong positive relationships. Significant correlations ($p < .05$) are presented in bold font with dark boxes around the cell.

Baseline choline levels were not related to performance on the initial animal stimuli but lower baseline choline levels were associated with more correct responses on the vehicle trials. This relationship was significant for both sexes and was of similar magnitude (Males: $r = -.281$, $p < .012$, $q^* < .0056$; Females: $r = -.275$, $p < .027$, $q^* < .0111$) but was not significant after adjusting for the multiple comparisons. An increase in choline over time was associated with more correct responses and reductions in variability in the response time on the vehicle trials for females but not males (Correct Responses: Males: $r = .164$, ns; Females: $r = .316$, $p < .024$, $q^* < .0056$: Variability in Latency: Males: $r = -0.047$, ns ; Females: $r = -0.280$, $p < .047$, $q^* < .0111$) but again the findings were not significant when compared to the modified alpha level (q*). Males whose mothers had higher levels of choline in trimester 3 were associated with greater latency or slower responses in males during the changed stimulus class trials but no such relationship was observed in the females (Males: $r = .236$, $p < .$ $p < .049$, $q^* < .0111$; Females: $r = -.041$, ns). The relationship for males, however, did not reach significance using the adjusted alpha level. Lower levels of choline at baseline was associated with improved accuracy for both sexes but greater change in choline over the pregnancy was associated with improved accuracy for females. Trimester 3 levels of choline in mothers of male offspring, however, were associated with longer latencies or slower responses. In each case, the relationships were not significant after adjustments suggesting the effects sizes were not sufficiently strong and larger sample sizes would be needed to clarify whether the obtained relationships were chance false rejections of the null hypothesis or indicative of true relationships.

Betaine effects were sparser and were limited to male responses in the vehicle block of trials where recognition of novelty may have impacted performance. Higher Trimester 3 levels of

betaine were related to lower variability in response times in males ($r = -.355$, $p < .003$, $q^* < .0056$) but not females ($r = -.064$, ns). This effect was significant after adjusting for the multiple comparisons. Males also had a significant relationships between the change in betaine levels and longer latency (-0.258, $p < .043$, $q^* < .0056$) and lower variability in latency in males ($r = -.252$, $p < .048$) but neither relationships was significant after adjusting for multiple comparisons.

Baseline levels of DMG were predictive of preschool responses in females but did not impact males. In females, lower baseline DMG levels were associated with more correct responses, reduced mean latency and reduced response variability on the initial animal trials (Correct ($r = -.526$, $p< .0001$, $q^* < .0056$), Latency ($r = .492$, $p > .0001$, $q^* < .0056$); Variability in Latency ($r = .314$, $p < .011$, $q^* < .0056$). The relationships of baseline DMG were significant for accuracy and latency of the responses after adjustment for multiple comparisons but the variability in latency effect was no longer significant. Baseline DMG levels were also associated with reduced mean latency on the vehicle trials ($r = .362$, $p > .003$, $q^* < .0056$) and this result was significant after adjustment.

After correction for the multiple comparisons performed on the outcome measures, the prenatal estimates of maternal choline were no longer significant but a significant relationship was retained between the impact of maternal betaine levels in the third trimester on variability in response time in preschool males. In addition, the impact of maternal baseline levels of DMG was found to be related to total correct and the mean latency of responses by females in the preschool period on the initial animal trials and reduced latency in the vehicle trials after adjusting the alpha level. Caution should be used when accepting the null hypothesis for those effects that were significant but did not reach the q* threshold. A total of 54 comparisons were made and on average about 3 of these would be significant based on random chance alone. A total of 12 relationships were found using the traditional alpha level of $p < .05$ of which only 4 were below the q* threshold of significance. The 8 remaining significant relationships using traditional thresholds of significance continue to exceed chance levels of false positives, suggesting that some of the findings rejected are likely not due to chance alone and the analysis of these relationships suffers from low statistical power relative to the magnitude of the effect size observed in this study.

DISCUSSION

The impact of the prenatal use of MVM and choline supplementation in pregnancy to ameliorate the negative consequences of PAE on preschool reaction time performance was evaluated in a randomized clinical trial conducted in Ukraine. MVM and choline supplementation during pregnancy was found to improve preschool reaction time performance in males but not females. Male children were more accurate and responded more quickly when their mothers had been provided MVM supplementation during pregnancy regardless of their PAE status. For male preschoolers without PAE, maternal choline supplementation in pregnancy was also associated with faster responses during the block of trials where the class of the stimuli was changed from the original block of trials compared to males whose mothers did not receive choline supplementation. The results for the males are comparable to those found in infancy where both groups of infants

showed positive gains from the supplements provided during pregnancy on infancy encoding but only those who did not have a history of PAE had significant improvements during dishabituation trials (Kable, Coles, Keen, Uriu-Adams, Jones, Yevtushok, Kulikovsky, Wertelecki, Pedersen, & Chambers, 2015). These results suggest that multivitamin/mineral supplementation in pregnancy may have a longer impact or be more advantageous for males.

In contrast, the females in this sample seemed to have a stronger negative impact of PAE on their performance and were less responsive to the intervention than were the males. The existing literature on sex differences of PAE is sparse and conflicting (Otero & Kelly, 2012; Streissguth, 2012). Unique characteristics of the stimuli (animals and vehicles) may have also contributed to the differences found in males and females but examination of the mean levels and ranges in the responses of those without PAE or intervention do not indicate this. An increased severity of outcomes in females with PAE, specifically alcohol-related dysmorphia and neurocognitive functioning, has been reported in another study (May et al., 2017) who attributed to their findings to the lower viability and survival of males. Differential effects of choline by sex have been reported previously in a cohort of preschoolers but the effect was mediated by maternal levels of C-reactive protein (CRP), a marker of inflammation. Improved preschool processing speed and reduced problems with social withdrawal were associated with higher maternal choline in pregnancy but for males this was attenuated by a negative association of maternal CRP on processing speed (Hunter et al., 2021).

An exploratory analysis of the linear relationships between choline and its metabolites to preschool reaction time outcomes suggest that levels of choline and its metabolites in pregnancy may influence offspring's later sustained attention skills. The findings related to choline, however, were not significant after adjusting for multiple comparisons but occurred at higher frequency than chance positive results would have occurred relative to the number of multiple comparisons. Future research with larger sample sizes would be needed to clarify if the relationships persist but were of an insufficient effect size to offset the multiple comparison's carried out in this study. The metabolites of choline were also found to impact preschool reaction time task outcomes and were more robust than the choline effects. Betaine levels, a metabolite that differs from choline in terms of one methyl group, was not related to responses during the initial block of trials but reduced variability in the latency of the response in the second block of trials was associated with higher levels of betaine at trimester 3 in mothers of male children and this relationship persisted after adjusting for multiple comparisons. DMG, a metabolite that differs from choline in terms of two methyl groups, was found to be significant in predicting female performance but not males after adjusting for multiple comparisons. Lower baseline DMG levels were associated with higher accuracy and shorter latency in the original block of trials and shorter latency on the second block of trials. The results support our earlier findings of the importance of metabolites of choline in mediating neurodevelopmental outcome (Kable, Coles, Keen, Uriu-Adams, Jones, Yevtushok, Kulikovsky, Wertelecki, Pedersen, & Chambers, 2015), suggesting a role for methyl group metabolism in understanding the impact of choline on development.

The increased effectiveness seen in the South African clinical trial of choline supplementation during pregnancy (Jacobson et al., 2018) relative to this study, may be

the result of the substantially higher dosage levels of choline supplementation and/or the high frequency of choline insufficiency among South African women (Carter et al., 2018). Levels of choline did not differ by maternal drinking behavior in pregnancy in the Ukrainian women. The South African study did not provide choline to women who did not consume heavy amounts of alcohol during pregnancy so it is unclear from their results if the improvements in growth and neurodevelopmental functioning were the impact of choline offsetting the negative effects of PAE or just resolving the impact of choline deficiency on development (Zeisel, 2013). Choline deficiency is not limited to South Africa as studies have shown a tendency for choline intake to fall below the recommended adequate intake levels in both Europe and the Americas (Derbyshire & Obeid, 2020).

The results of this study are limited by several things. There is some evidence of differential attrition among the mothers of children participating in this study as those who had a history of PAE exposure but were assigned to having no MVM treatment had higher levels of trimester 1 alcohol use than did those who received MVM despite being randomly assigned to intervention group. The increased levels of PAE in the no MVM group may have artificially produced more negative outcomes in this group and biased the results towards a more positive effect of MVM. Choline supplementation was not initiated in the pre-pregnancy period or first trimester of pregnancy so it is possible that a critical window of fetal development was missed. This may be especially true for females as baseline levels of DMG were predictive of preschool outcomes with lower levels associated with better outcomes. It is possible that the higher levels of DMG reflect greater oxidative stress levels of the mother at baseline. The reaction time task was also developed specifically for this cohort so there are no preexisting data on its psychometric characteristic but examination of the range of responses obtained, including those without a history of PAE or intervention, indicated floor effects were unlikely to limit its capacity to pick up significant group differences. Ceiling effects were also unlikely to limit identification of the significance of factors explored in this study in the areas of latency and variability of latency in the response time but may have limited detection of positive effects, particularly in females, on response accuracy where many obtained perfect scores (43.6% on the Animal Block and 70.1% on Vehicle Block).

The findings in this study also may not generalize beyond this developmental window and may be unique to maturational changes in reaction time performance (Mahone & Wodka, 2008) and brain development (Wu et al., 2013). Our results indicated significant sex effects on outcomes of the reaction time task and sex-specific effects of the intervention, the impact of PAE, and relationships between the outcomes and choline or its metabolites. A task that did not have specific sex effects may have been a better outcome to have used to evaluate the study's hypotheses but may not be possible given the sex-specific maturational changes in attentional regulation skills. The fact that ADHD is diagnosed more commonly in boys (Gomez, Harvey, Quick, Scharer, & Harris, 1999) is well known and symptom expression of ADHD symptomatology has been found to differ by sex (Hartung et al., 2002) even among preschoolers (Goggin, 1975; Levy & Hobbes, 1979), suggesting the sex differences are inherent to the construct domain assessed as an outcome in this study.

The sample and the design of the study also limits the study's conclusions and the generalizability of its findings. The sample is unique in that Ukraine does not engage in folic acid enrichment of food products as do many other countries and many of the women in the sample were recruited from areas that may have been impacted by the meltdown of a nuclear power plant in Chernobyl (Dancause et al., 2010). Finally, relative to design of the study, choline supplementation was not provided in the absence of MVM supplementation so the unique contribution of choline in the absence of any potential interactions of choline with the MVM supplementation cannot be identified. By examining the male and female data separately, we also eliminated the potential for assessing potential sex interactions with the other three factors in the study (PAE status, MVM status, and Choline status), including potential three and four way interactions between these variables. Given that some of our sample sizes were as small as 8–10 subjects per cell, there was not sufficient power to reliably assess more complex interactions within the incomplete factorial design used. In addition, estimates of linear relationships that were positive using traditional p-values but failed to reach significance using the adjusted q-values exceeded chance levels of false positives, suggesting the statistical power for these comparisons was low. Future studies are encouraged to use complete factorial designs to eliminate this problem with sufficient power to explore the relationships within the design and between biomarkers and the outcomes..

CONCLUSION

In a cohort of Ukrainian mothers, the impact of MVM and choline supplementation in pregnancy on their children's reaction time performance in the preschool period was assessed. Males demonstrated positive effects of the MVM and choline supplementation on aspects of the attentional regulation skills but females appeared to have stronger negative effects of PAE on their task performance.

Acknowledgements.

The authors would also like to thank Dr. John Newman, Dr. Theresa Pedersen, Dr. Heidrun Gross, Jodi Ensunsa, and Ashley Villalon for technical help with the mass spectrometry analysis. Finally, the authors would like to thank the Ukrainian families who participated in this research and the wonderful staff of the Rivne Regional Medical Diagnostic Center, Khmelnytsky Perinatal Center and the OMNI-Net Ukraine Birth Defects Program.

Funding.

This research was funded by supported by NIH Research Grants #U01AA014835 funded by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) and the NIH Office of Dietary Supplements (ODS)-Christina Chambers, PI. All or part of this work was done in conjunction with the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD), which is funded by grants from the National Institute on Alcohol Abuse and Alcoholism (NIAAA). Additional information about CIFASD can be found at www.cifasd.org.

Availability of data and material.

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

References

Akison LK, Kuo J, Reid N, Boyd RN, & Moritz KM (2018). 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, 42(9), 1591–1611. doi:10.1111/acer.13817 [PubMed: 29928762]

Benjamini Y, & Hochberg Y (1995). Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B-Methodological, 57(1), 289–300. Retrieved from <Go to ISI>://WOS:A1995QE45300017

Boyd TA, Ernhart CB, Greene TH, Sokol RJ, & Martier S (1991). Prenatal alcohol exposure and sustained attention in the preschool years. Neurotoxicol Teratol, 13(1), 49–55. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/2046626 [PubMed: 2046626]

Brown RT, Coles CD, Smith IE, Platzman KA, Silverstein J, Erickson S, & Falek A (1991). Effects of prenatal alcohol exposure at school age. II. Attention and behavior. Neurotoxicol Teratol, 13(4), 369–376. doi:0892–0362(91)90085-B [pii] [PubMed: 1921916]

Carter RC, Jacobson SW, Booley S, Najaar B, Dodge NC, Bechard LJ, … Senekal M (2018). Development and validation of a quantitative choline food frequency questionnaire for use with drinking and non-drinking pregnant women in Cape Town, South Africa. Nutr J, 17(1), 108. doi:10.1186/s12937-018-0411-5 [PubMed: 30466439]

Carter RC, Jacobson SW, Molteno CD, & Jacobson JL (2007). Fetal alcohol exposure, iron-deficiency anemia, and infant growth. Pediatrics, 120(3), 559–567. doi:120/3/559 [pii] 10.1542/peds.2007-0151 [PubMed: 17766529]

Carter RC, Senekal M, Dodge NC, Bechard LJ, Meintjes EM, Molteno CD, … Jacobson SW (2017). Maternal Alcohol Use and Nutrition During Pregnancy: Diet and Anthropometry. Alcohol Clin Exp Res, 41(12), 2114–2127. doi:10.1111/acer.13504 [PubMed: 28940428]

Caudill MA, Strupp BJ, Muscalu L, Nevins JEH, & Canfield RL (2018). Maternal choline supplementation during the third trimester of pregnancy improves infant information processing speed: a randomized, double-blind, controlled feeding study. FASEB J, 32(4), 2172–2180. doi:10.1096/fj.201700692RR [PubMed: 29217669]

Chambers CD, Coles C, Kable J, Akshoomoff N, Xu R, Zellner JA, … Jones KL (2019). Fetal Alcohol Spectrum Disorders in a Pacific Southwest City: Maternal and Child Characteristics. Alcohol Clin Exp Res, 43(12), 2578–2590. doi:10.1111/acer.14213 [PubMed: 31688971]

Coles CD, Kable JA, Granovska IV, Pashtepa AO, Wertelecki W, Chambers CD, & Cifasd. (2021). Measurement of neurodevelopmental effects of prenatal alcohol exposure in Ukrainian preschool children. Child Neuropsychol, 1–16. doi:10.1080/09297049.2021.1919298

Coles CD, Kable JA, Keen CL, Jones KL, Wertelecki W, Granovska IV, … Cifasd. (2015). Dose and Timing of Prenatal Alcohol Exposure and Maternal Nutritional Supplements: Developmental Effects on 6-Month-Old Infants. Matern Child Health J, 19(12), 2605–2614. doi:10.1007/s10995-015-1779-x [PubMed: 26164422]

Coles CD, Kalberg W, Kable JA, Tabachnick B, May PA, & Chambers CD (2020). Characterizing Alcohol-Related Neurodevelopmental Disorder: Prenatal Alcohol Exposure and the Spectrum of Outcomes. Alcohol Clin Exp Res, 44(6), 1245–1260. doi:10.1111/acer.14325 [PubMed: 32173870]

Coles CD, Platzman KA, Raskind-Hood CL, Brown RT, Falek A, & Smith IE (1997). A comparison of children affected by prenatal alcohol exposure and attention deficit, hyperactivity disorder. Alcohol Clin Exp Res, 21(1), 150–161. doi:10.1111/j.1530-0277.1997.tb03743.x [PubMed: 9046388]

Connors C (2008). Conners 3. In. North Tonawanda, NY: MHS, Inc.

Dancause KN, Yevtushok L, Lapchenko S, Shumlyansky I, Shevchenko G, Wertelecki W, & Garruto RM (2010). Chronic radiation exposure in the Rivne-Polissia region of Ukraine: implications for birth defects. Am J Hum Biol, 22(5), 667–674. doi:10.1002/ajhb.21063 [PubMed: 20737614]

Derbyshire E, & Obeid R (2020). Choline, Neurological Development and Brain Function: A Systematic Review Focusing on the First 1000 Days. Nutrients, 12(6). doi:10.3390/nu12061731

Elmaghrabi S, Nahmias MJ, Adamo N, Di Martino A, Somandepalli K, Patel V, … Castellanos FX (2018). Is Increased Response Time Variability Related to Deficient Emotional Self-Regulation in Children With ADHD? J Atten Disord, 1087054718788950. doi:10.1177/1087054718788950

Goggin JE (1975). Sex differences in the activity level of preschool children as a possible precursor of hyperactivity. J Genet Psychol, 127(1st Half), 75–81. doi:10.1080/00221325.1975.10532357 [PubMed: 1194902]

Gomez R, Harvey J, Quick C, Scharer I, & Harris G (1999). DSM-IV AD/HD: confirmatory factor models, prevalence, and gender and age differences based on parent and teacher ratings of Australian primary school children. J Child Psychol Psychiatry, 40(2), 265–274. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10188709 [PubMed: 10188709]

Gooch D, Snowling MJ, & Hulme C (2012). Reaction time variability in children with ADHD symptoms and/or dyslexia. Dev Neuropsychol, 37(5), 453–472. doi:10.1080/87565641.2011.650809 [PubMed: 22799763]

Hartung CM, Willcutt EG, Lahey BB, Pelham WE, Loney J, Stein MA, & Keenan K (2002). Sex differences in young children who meet criteria for attention deficit hyperactivity disorder. J Clin Child Adolesc Psychol, 31(4), 453–464. doi:10.1207/S15374424JCCP3104_5 [PubMed: 12402565]

Helfrich KK, Saini N, Kling PJ, & Smith SM (2018). Maternal iron nutriture as a critical modulator of fetal alcohol spectrum disorder risk in alcohol-exposed pregnancies. Biochem Cell Biol, 96(2), 204–212. doi:10.1139/bcb-2017-0206 [PubMed: 29017023]

Herman CS, Kirchner GL, Streissguth AP, & Little RE (1980). Vigilance paradigm for preschool children used to relate vigilance behavior to IQ and prenatal exposure to alcohol. Percept Mot Skills, 50(3 Pt 1), 863–867. doi:10.2466/pms.1980.50.3.863 [PubMed: 7402868]

Holm PI, Ueland PM, Kvalheim G, & Lien EA (2003). Determination of choline, betaine, and dimethylglycine in plasma by a high-throughput method based on normal-phase chromatography-tandem mass spectrometry. Clin Chem, 49(2), 286–294. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12560353 [PubMed: 12560353]

Huebner SM, Helfrich KK, Saini N, Blohowiak SE, Cheng AA, Kling PJ, & Smith SM (2018). Dietary Iron Fortification Normalizes Fetal Hematology, Hepcidin, and Iron Distribution in a Rat Model of Prenatal Alcohol Exposure. Alcohol Clin Exp Res, 42(6), 1022–1033. doi:10.1111/acer.13754 [PubMed: 29672865]

Hunter SK, Hoffman MC, D'Alessandro A, Walker VK, Balser M, Noonan K, … Freedman R (2021). Maternal prenatal choline and inflammation effects on 4-year-olds' performance on the Wechsler Preschool and Primary Scale of Intelligence-IV. J Psychiatr Res, 141, 50–56. doi:10.1016/j.jpsychires.2021.06.037 [PubMed: 34174557]

Infante MA, Moore EM, Nguyen TT, Fourligas N, Mattson SN, & Riley EP (2015). Objective assessment of ADHD core symptoms in children with heavy prenatal alcohol exposure. Physiol Behav, 148, 45–50. doi:10.1016/j.physbeh.2014.10.014 [PubMed: 25447751]

Innis SM, & Hasman D (2006). Evidence of choline depletion and reduced betaine and dimethylglycine with increased homocysteine in plasma of children with cystic fibrosis. J Nutr, 136(8), 2226–2231. doi:10.1093/jn/136.8.2226 [PubMed: 16857845]

Jacobson SW, Carter RC, Molteno CD, Stanton ME, Herbert JS, Lindinger NM, … Jacobson JL (2018). 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, 42(7), 1327–1341. doi:10.1111/acer.13769 [PubMed: 29750367]

Kable JA, Coles CD, Keen CL, Uriu-Adams JY, Jones KL, Yevtushok L, … Chambers CD (2015). The impact of micronutrient supplementation in alcohol-exposed pregnancies on information processing skills in Ukrainian infants. Alcohol, 49(7), 647–656. doi:10.1016/j.alcohol.2015.08.005 [PubMed: 26493109]

Kable JA, Coles CD, Keen CL, Uriu-Adams JY, Jones KL, Yevtushok L, … Cifasd. (2015). The impact of micronutrient supplementation in alcohol-exposed pregnancies on information processing skills in Ukrainian infants. Alcohol, 49(7), 647–656. doi:10.1016/j.alcohol.2015.08.005 [PubMed: 26493109]

Koelega HS (1993). Stimulant drugs and vigilance performance: a review. Psychopharmacology (Berl), 111(1), 1–16. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7870923 [PubMed: 7870923]

Kooistra L, Crawford S, Gibbard B, Ramage B, & Kaplan BJ (2010). Differentiating attention deficits in children with fetal alcohol spectrum disorder or attention-deficit-hyperactivity disorder. Dev Med Child Neurol, 52(2), 205–211. doi:10.1111/j.1469-8749.2009.03352.x [PubMed: 19549201]

Lange S, Rehm J, Anagnostou E, & Popova S (2018). Prevalence of externalizing disorders and Autism Spectrum Disorders among children with Fetal Alcohol Spectrum Disorder: systematic

review and meta-analysis. Biochem Cell Biol, 96(2), 241–251. doi:10.1139/bcb-2017-0014 [PubMed: 28521112]

Levy F, & Hobbes G (1979). The influences of social class and sex on sustained attention (vigilance) and motor inhibition in children. Aust N Z J Psychiatry, 13(3), 231–234. doi:10.3109/00048677909159141 [PubMed: 293177]

Mahone EM, & Wodka EL (2008). The neurobiological profile of girls with ADHD. Dev Disabil Res Rev, 14(4), 276–284. doi:10.1002/ddrr.41 [PubMed: 19072756]

May PA, Hamrick KJ, Corbin KD, Hasken JM, Marais AS, Blankenship J, … Gossage JP (2016). Maternal nutritional status as a contributing factor for the risk of fetal alcohol spectrum disorders. Reprod Toxicol, 59, 101–108. doi:10.1016/j.reprotox.2015.11.006 [PubMed: 26656914]

May PA, Hamrick KJ, Corbin KD, Hasken JM, Marais AS, Brooke LE, … Gossage JP (2014). Dietary intake, nutrition, and fetal alcohol spectrum disorders in the Western Cape Province of South Africa. Reprod Toxicol, 46, 31–39. doi:10.1016/j.reprotox.2014.02.002 [PubMed: 24568797]

May PA, Hasken JM, Baete A, Russo J, Elliott AJ, Kalberg WO, … Hoyme HE (2020). Fetal Alcohol Spectrum Disorders in a Midwestern City: Child Characteristics, Maternal Risk Traits, and Prevalence. Alcohol Clin Exp Res, 44(4), 919–938. doi:10.1111/acer.14314 [PubMed: 32293735]

May PA, Hasken JM, Bozeman R, Jones JV, Burns MK, Goodover J, … Hoyme HE (2020). Fetal Alcohol Spectrum Disorders in a Rocky Mountain Region City: Child Characteristics, Maternal Risk Traits, and Prevalence. Alcohol Clin Exp Res, 44(4), 900–918. doi:10.1111/acer.14315 [PubMed: 32293732]

May PA, Hasken JM, Stegall JM, Mastro HA, Kalberg WO, Buckley D, … Hoyme HE (2020). Fetal Alcohol Spectrum Disorders in a Southeastern County of the United States: Child Characteristics and Maternal Risk Traits. Alcohol Clin Exp Res, 44(4), 939–959. doi:10.1111/acer.14313 [PubMed: 32293734]

May PA, Marais AS, De Vries MM, Buckley D, Kalberg WO, Hasken JM, … Hoyme HE (2021). The prevalence, child characteristics, and maternal risk factors for the continuum of fetal alcohol spectrum disorders: A sixth population-based study in the same South African community. Drug Alcohol Depend, 218, 108408. doi:10.1016/j.drugalcdep.2020.108408 [PubMed: 33250379]

May PA, Tabachnick B, Hasken JM, Marais AS, de Vries MM, Barnard R, … Hoyme HE (2017). Who is most affected by prenatal alcohol exposure: Boys or girls? Drug Alcohol Depend, 177, 258–267. doi:10.1016/j.drugalcdep.2017.04.010 [PubMed: 28624747]

Nakagawa S (2004). A farewell to Bonferroni: the problems of low statistical power and publication bias. Behavioral Ecology, 15(6), 1044–1045. doi:10.1093/beheco/arh107

Nanson JL, & Hiscock M (1990). Attention deficits in children exposed to alcohol prenatally. Alcohol Clin Exp Res, 14(5), 656–661. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2264593 [PubMed: 2264593]

Naseer MI, Y. LH, & M.O. K (2010). Neuroprotective effect of vitamin C against the ethanol and nicotine modulation of GABA(B) receptor and PKA-alpha expression in prenatal rat brain. Synapse, 64(6), 467–477. [PubMed: 20175221]

Nguyen TT, Risbud RD, Mattson SN, Chambers CD, & Thomas JD (2016). Randomized, double-blind, placebo-controlled clinical trial of choline supplementation in school-aged children with fetal alcohol spectrum disorders. Am J Clin Nutr, 104(6), 1683–1692. doi:10.3945/ajcn.116.142075 [PubMed: 27806977]

Ojeda ML, Nogales F, Vazquez B, Delgado MJ, Murillo ML, & Carreras O (2009). Alcohol, gestation and breastfeeding: selenium as an antioxidant therapy. Alcohol Alcohol, 44(3), 272–277. doi:agp004 [pii] 10.1093/alcalc/agp004 [PubMed: 19213742]

Otero NKH, & Kelly S (2012). Sex differences in the teratogenic effects of alcohol: Findings from animal models. In M. a. K. Lewis L (Ed.), Gender differences in prenatal substance exposure (pp. 139–154). Washington, DC: American Psychological Association.

Riley EP, Infante MA, & Warren KR (2011). Fetal alcohol spectrum disorders: an overview. Neuropsychol Rev, 21(2), 73–80. doi:10.1007/s11065-011-9166-x [PubMed: 21499711]

Serrano M, Han M, Brinez P, & Linask KK (2010). Fetal alcohol syndrome: cardiac birth defects in mice and prevention with folate. Am J Obstet Gynecol, 203(1), 75 e77–75 e15. doi:S0002–9378(10)00315–7 [pii] 10.1016/j.ajog.2010.03.017

Stratton K, Howe C, & Battaglia F (1996). Fetal alcohol syndrome: Diagnosis, epidemiology, prevention and treatment. Washington, D. C.: National Academy Press.

Streissguth AP (2012). Sex differences in prenatal alcohol abuse in humans. In M. a. K. Lewis L (Ed.), Gender differences in prenatal substance exposure (pp. 139–154). Washington, DC: American Psychological Association.

Streissguth AP, Barr HM, Sampson PD, Parrish-Johnson JC, Kirchner GL, & Martin DC (1986). Attention, distraction and reaction time at age 7 years and prenatal alcohol exposure. Neurobehav Toxicol Teratol, 8(6), 717–725. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3808187 [PubMed: 3808187]

Summers BL, Rofe AM, & Coyle P (2009). Dietary zinc supplementation throughout pregnancy protects against fetal dysmorphology and improves postnatal survival after prenatal ethanol exposure in mice. Alcohol Clin Exp Res, 33(4), 591–600. doi:ACER873 [pii] 10.1111/j.1530-0277.2008.00873.x [PubMed: 19183140]

Thomas JD, Abou EJ, & Dominguez HD (2009). Prenatal choline supplementation mitigates the adverse effects of prenatal alcohol exposure on development in rats. Neurotoxicol Teratol, 31(5), 303–311. doi:10.1016/j.ntt.2009.07.002 [PubMed: 19616089]

Vasquez BP, Binns MA, & Anderson ND (2018). Response Time Consistency Is an Indicator of Executive Control Rather than Global Cognitive Ability. J Int Neuropsychol Soc, 24(5), 456–465. doi:10.1017/S1355617717001266 [PubMed: 29208077]

Weissberg R, Ruff HA, & Lawson KR (1990). The usefulness of reaction time tasks in studying attention and organization of behavior in young children. J Dev Behav Pediatr, 11(2), 59–64. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/2324289 [PubMed: 2324289]

Wozniak JR, Fink BA, Fuglestad AJ, Eckerle JK, Boys CJ, Sandness KE, … Georgieff MK (2020). Four-year follow-up of a randomized controlled trial of choline for neurodevelopment in fetal alcohol spectrum disorder. J Neurodev Disord, 12(1), 9. doi:10.1186/s11689-020-09312-7 [PubMed: 32164522]

Wozniak JR, Fuglestad AJ, Eckerle JK, Fink BA, Hoecker HL, Boys CJ, … Georgieff MK (2015). Choline supplementation in children with fetal alcohol spectrum disorders: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr, 102(5), 1113–1125. doi:10.3945/ajcn.114.099168 [PubMed: 26447156]

Wu K, Taki Y, Sato K, Hashizume H, Sassa Y, Takeuchi H, … Fukuda H (2013). Topological organization of functional brain networks in healthy children: differences in relation to age, sex, and intelligence. PLoS One, 8(2), e55347. doi:10.1371/journal.pone.0055347 [PubMed: 23390528]

Zeisel SH (2013). Nutrition in pregnancy: the argument for including a source of choline. Int J Womens Health, 5, 193–199. doi:10.2147/IJWH.S36610 [PubMed: 23637565]

Highlights

1. Maternal alcohol and micronutrient use impacts male and female attention differently
2. Prenatal alcohol exposure resulted in impaired reaction time skills in female preschoolers
3. Maternal micronutrient supplementation improved male reaction time performance

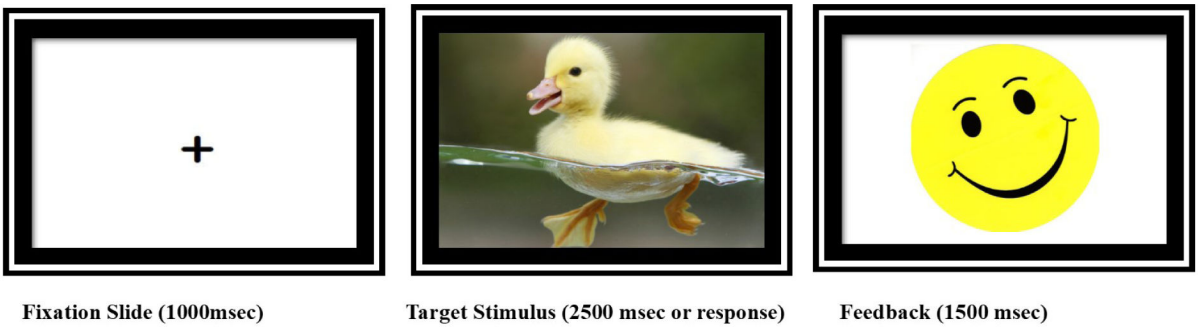

Figure 1.

Outline of the initial fixation slide, a sample target stimulus, and the positive feedback slide associated with the child pressing the response button within 2500 msec.

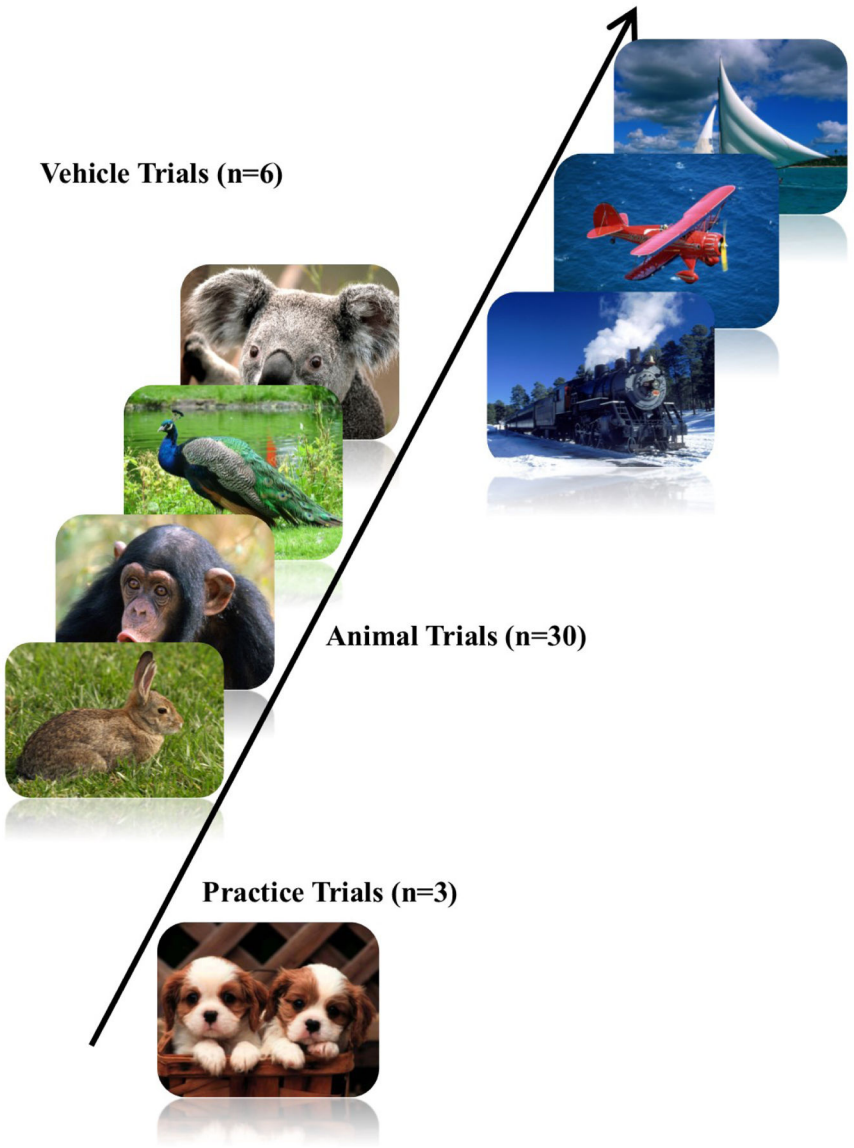

Figure 2.

The timeline of the trials are depicted from top to bottom, including the first 3 practice trials, 30 animal-themed trials, and 6 vehicle-themed trials.

Table 1.

Sample Characteristics by Prenatal Alcohol Use History, Prenatal Multivitamin/Mineral Supplement Treatment (MVM), and Choline Supplement Treatment

Alcohol Exposure Status	Alcohol Use				Contrast			
	(n=45)	(n=51)	(n=18)	(n=33)	(n=69)	(n=78)	(n=34)	(n=44)
Measure	**No MVM Treatment**	**Any MVM Treatment**	**MVM Only**	**MVM & Choline**	**No MVM Treatment**	**Any MVM Treatment**	**MVM Only**	**MVM & Choline**
Child's Age	4.0 (.39)	4.0 (.36)	3.9 (.37)	4.0 (.36)	3.9 (.32)	4.0 (.29)	4.0 (.32)	3.9 (.26)
Maternal Age At Birth (Yrs)	27.6 (6.5)	27.8 (6.0)	28.8 (6.1)	27.3 (6.0)	26.8 (4.7)	26.2 (3.9)	26.0 (3.9)	26.4 (4.0)
Child's Gender (N, %Male)	22 (47.8)	27 (52.9)	10 (55.6)	17 (51.5)	29 (42.0)	49 (62.8)	22 (64.7)	27 (61.4)
Parity	.82 (1.0)	.80 (1.2)	.71 (.92)	.85 (1.3)	.70 (1.0)	.68 (.88)	.82 (1.1)	.57 (.70)
Maternal Education (years) [1]	13.3 (2.4)	13.1 (2.6)	13.4 (2.4)	13.0 (2.7)	14.8 (2.3)	15.2 (2.3)	15.2 (2.7)	15.3 (2.1)
Preconception AA/day-Mean (Oz) [2]	.64 (.73)	.70 (1.0)	.54 (.29)	.78 (1.0)	.01 (.03)	.001 (.01)	.002 (.01)	0 (0)
Preconception AA/drinking day (Oz) [3]	1.97 (1.7)	2.23 (3.3)	1.58 (.76)	2.56 (4.0)	.04 (.19)	.007 (.05)	.017 (.07)	0 (0)
Trimester 1 AA/day-Mean (Oz) [4]	.23 (.57)	.08 (.17)	.04 (.06)	.40 (.20)	.001 (.01)	.000(.00)	0 (0)	.000(.000)
Trimester 1 AA/ drinking day (Oz) [5]	.78 (1.4)	.48 (.80)	.35 (.46)	.55 (.92)	.01 (.07)	.001 (01)	0 (0)	.001 (.01)

[1] Prenatal Alcohol Exposure (PAE) effect (F (1, 236 = 33.567, p < .0001);

[2] PAE effect (F (1, 236 = 108.62, p < .0001);

[3] PAE effect (F (1, 236 = 90.454, p < .0001);

[4] PAE effect (F (1, 236 = 16.595, p < .0001) and PAE*MVM effect (F (1, 236) = 4.236, p < .041);

[5] PAE effect (F (1, 236 = 40.713, p < .0001)

Table 2A (Males) and 2B (Females).

Mean and Standard Deviations of the Indices of the Reaction Time Performance by Group Status

MALES	Alcohol Use - PAE				Contrast			
	(n=21)	(n=27)	(n=10)	(n=17)	(n= 29)	(n=49)	(n=22)	(n=27)
Measure	**No MVM Treatment**	**Any MVM Treatment**	**MVM Only**	**MVM & Choline**	**No MVM Treatment**	**Any MVM Treatment**	**MVM Only**	**MVM & Choline**
Animal Stimuli								
Number Correct	27.9 (2.1)	28.3 (2.9)	29.4 (0.7)	27.6 (3.5)	26.9 (3.6)	28.1 (2.3)	28.0 (1.8)	28.2 (2.7)
Mean Latency of Response	1318.5 (483.7)	1147.7 (443.7)	933.5 (275.1)	1273.7 (481.5)	1380.9 (581.1)	1193.7 (378.1)	1169.8 (366.3)	1213.1 (393.3)
Response Time Variability	782.0 (304.4)	698.8 (282.5)	571.7 (261.3)	773.6 (274.3)	786.9 (325.7)	752.4 (331.1)	793.5 (346.0)	718.9 (320.9)
Vehicle Stimuli								
Number Correct	5.4 (1.3)	5.6 (0.9)	5.7 (0.5)	5.5 (1.1)	5.4 (1.1)	5.5 (1.1)	5.7 (0.6)	5.3 (1.3)
Mean Latency of Response	1568.8 (790.8)	1350.8 (633.8)	1077.5 (469.8)	1511.5 (674.2)	1492.7 (764.1)	1283.6 (541.9)	1390.8 (579.5)	1192.9 (501.2)
Response Time Variability	681.5 (386.6)	595.6 (386.0)	586.7 (424.9)	600.8 (374.7)	654.6 (402.0)	725.1 (433.2)	778.0 (405.2)	680.4 (458.7)
FEMALES	**Alcohol Use - PAE**				**Contrast**			
	(n=24)	**(n=24)**	**(n=8)**	**(n=16)**	**(n=40)**	**(n=29)**	**(n=12)**	**(n=17)**
Measure	**No MVM Treatment**	**Any MVM Treatment**	**MVM Only**	**MVM & Choline**	**No MVM Treatment**	**Any MVM Treatment**	**MVM Only**	**MVM & Choline**
Animal Stimuli								
Number Correct	26.1 (4.9)	27.2 (4.3)	25.4 (6.4)	28.1 (2.6)	28.3 (2.3)	29.0 (1.6)	28.8 (1.9)	29.1 (1.5)
Mean Latency of Response	1458.2 (611.7)	1261.0 (609.5)	1437.4 (776.6)	1172.8 (513.0)	1193.8 (381.5)	1133.8 (511.5)	1094.2 (598.8)	1161.8 (457.6)
Response Time Variability	814.6 (339.5)	748.6 (369.6)	878.8 (339.6)	683.5 (376.9)	736.2 (287.0)	578.6 (312.7)	534.3 (328.0)	609.8 (307.7)
Vehicle Stimuli								
Number Correct	5.3 (1.3)	5.4 (0.8)	5.4 (1.1)	5.4 (0.7)	5.7 (0.5)	5.7 (0.8)	5.6 (0.9)	5.7 (0.7)
Mean Latency of Response	1404.9 (702.4)	1388.2 (616.8)	1546.7 (835.8)	1308.9 (487.0)	1219.8 (546.2)	1244.4 (656.4)	1276.4 (853.5)	1221.9 (501.9)
Response Time Variability	596.9 (399.4)	728.3 (458.3)	672.8 (362.6)	756.0 (508.1)	661.5 (395.4)	530.0 (389.3)	472.3 (336.8)	570.7 (427.8)

Alcohol. Author manuscript; available in PMC 2023 March 01.

Table 3.

Relationship Between Choline, Betaine, and Dimethylglycine and a Preschool Reaction Time Task Outcomes

	Animal Stimuli					
	Correct		Latency		Variability in Latency	
	Males	Females	Males	Females	Males	Females
B[1]-Choline	−0.149	−0.075	0.103	0.013	0.145	−0.059
B-Betaine	−0.003	−0.117	−0.028	0.137	−0.094	−0.108
B-DMG	−0.207	**−0.526** ****	0.125	**0.492** ****	0.158	**0.314** *
T3[2]-Choline	−0.151	0.112	0.140	−0.074	0.186	−0.192
T3-Betaine	−0.047	−0.059	−0.114	0.101	−0.213	−0.048
T3-DMG	−0.198	−0.086	0.083	0.092	0.161	0.047
Δ[3]Choline	0.196	0.227	−0.203	−0.135	−0.099	−0.169
ΔBetaine	−0.011	−0.210	−0.200	0.090	−0.164	0.127
ΔDMG	−0.149	0.191	0.070	−0.237	0.051	−0.207
	Vehicle Stimuli					
	Correct		Latency		Variability in Latency	
	Males	Females	Males	Females	Males	Females
B-Choline	**−0.281** *	**−0.275** *	0.193	0.073	0.113	0.097
B-Betaine	−0.089	−0.045	0.155	0.169	−0.158	−0.178
B-DMG	−0.108	−0.182	0.132	**0.362** **	−0.013	0.123
T3-Choline	−0.169	0.042	**0.236** *	−0.041	0.193	−0.161
T3-Betaine	0.108	−0.051	−0.029	0.002	**−0.355** **	−0.064
T3-DMG	−0.076	−0.041	0.101	0.055	−0.039	−0.111
ΔCholine	0.164	**0.316** *	−0.155	−0.141	−0.047	**−0.280** *
ΔBetaine	0.230	−0.079	**−0.258** *	−0.045	**−0.252** *	0.096
ΔDMG	0.020	0.111	0.020	−0.072	0.043	−0.273

* is $p < .05$,

** is $p < .01$,

*** p < .001,

**** p < .0001;

[1] B refers to baseline value,

[2] T3 refers to trimester three value, and

[3] Δ refers to the change in levels from baseline to trimester 3 values.

The strength of the relationships are reflected in the color hue of the table with the boldest reds reflecting stronger negative relationships and darker blues reflecting stronger positive relationships with white cells reflecting values closer to O or no relationship. Significant relationships are bolded.