



Deficient maternal zinc intake—but not folate—is associated with lower fetal heart rate variability



Marisa N. Spann^{a,b,*}, Jennifer Smerling^{c,1}, Hanna Gustafsson^a, Sophie Foss^a, Margaret Altemus^d, Catherine Monk^{a,b,e}

^a Department of Psychiatry, Columbia University Medical Center, 1051 Riverside Drive, New York, NY 10032, USA

^b New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY 10032, USA

^c Department of Psychology, Columbia University, 1190 Amsterdam Avenue, MC 5501, New York, NY 10027, USA

^d Department of Psychiatry, Weill Cornell Medical College, 525 East 68th Street, New York, NY 10065, USA

^e Department of Obstetrics and Gynecology, Columbia University Medical Center, Herbert Irving Pavilion, 161 Fort Washington Avenue, New York, NY 10032, USA

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ABSTRACT

Objective: Few studies of maternal prenatal diet and child development examine micronutrient status in relation to fetal assessment.

Methods: Twenty-four-hour dietary recall of zinc and folate and 20 min of fetal heart rate were collected from 3rd trimester pregnant adolescents.

Results: Deficient zinc was associated with less fetal heart rate variability. Deficient folate had no associations with HRV. Neither deficient zinc nor deficient folate was related to fetal heart rate.

Conclusions: These findings, from naturalistic observation, are consistent with emerging data on prenatal zinc supplementation using a randomized control design.

Practical Implication: Taken together, the findings suggest that maternal prenatal zinc intake is an important and novel factor for understanding child ANS development.

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1. Introduction

Deficient maternal nutrition during pregnancy is associated with poor child outcomes [1,2]. Few studies on the prenatal origins of these health trajectories have considered maternal micronutrient intake in relation to fetal neurodevelopment [3,4].

The list of nutrients with known prenatal effects on child neurobehavioral trajectories is extensive [1]. We highlight zinc and folate as they have demonstrated effects on nervous system development [1,5], and there is evidence of deficient prenatal intake even in resource-rich countries such as the United States [6,7]. Enzymes in the presence of zinc support metabolism of folate in the body [8,9], suggesting a relationship between these micronutrients.

Prenatal zinc deficiency in animals is associated with abnormal cortical electrophysiology and reduced brain mass in the cerebellum, limbic system, and cerebral cortex [10]; behaviorally, there are

decrements in short-term memory, learning, and reduced novelty-based exploration [11]. A few studies with humans suggest that adequate gestational zinc intake is associated with better infant neurobehavioral development [12]. Specifically, Goldenberg et al. [13] found women who received zinc supplementation during pregnancy had infants with higher birth weights and larger head sizes. Kirksey et al. [14,15] first reported relationships between the prenatal maternal diet and postnatal behavior of infants. Mother–baby pairs were studied in an Egyptian village. Maternal consumption of foods derived from animals that were rich in zinc was positively associated with higher neonatal attention scores on the Brazelton Neonatal Development Assessment Scale. However, maternal zinc intake from plants, dietary phytate and fiber during pregnancy were inversely associated with motor performance scores on the Bayley Scales of Infant Development at 6 months of age.

Folate affects DNA biosynthesis, levels of homocysteine, and methylation processes [16]. Folate supports proper neural tube closure, neurogenesis, cell growth, and myelination of the fetal brain [16,17]. Animal data indicate that deficiency in gestational folate impacts cell mitosis and apoptosis [18] and is associated with anxiety like behavior in the offspring [19]. Some human studies suggest an increased risk for behavioral problems in offspring of folate-deficient women [20,21].

The autonomic nervous system (ANS), the primary self-regulatory and homeostatic system of the body, is a key index for fetal

Abbreviations: ANS, autonomic nervous system; FHR, fetal heart rate; HRV, heart rate variability; ASA24, Automated Self-Administered 24-hour Recall Questionnaire; RDA, recommended dietary allowance; STV, short-term variation; MMR, mean minute range; SD, standard deviation; IQR, interquartile range

* Corresponding author at: New York State Psychiatric Institute, 1051 Riverside Drive, Unit 23, New York, NY 10032, USA. Tel.: +1 11 646 774 5824.

E-mail address: spannma@nyspi.columbia.edu (M.N. Spann).

¹ Joint first authorship, equal contribution.

neurobehavioral assessment [22]. The ANS matures during the fetal period as the brain regions, the medulla oblongata of the brain stem, and the vagus nerve, responsible for fetal cardiac regulation develop [23–25]. The development of the brain stem leads to increases in parasympathetic nervous system activity and a measurable decrease in fetal heart rate (FHR), and an increase in vagal nerve function relates to a measurable increase in heart rate variability (HRV) from the 2nd and 3rd trimesters [22,23,26,27]. FHR and HRV represent primary and stable measures of maturation of the cardiac system and ANS during the fetal period [24]. Higher average levels of fetal HRV predict higher infant HRV [28], as well as better cognitive development at 2 and 2.5 years of age [29]. In pregnant women with zinc deficiency, fetuses of those who received zinc supplementation had lower FHR and higher HRV, as early as 28 weeks [30,31]. In contrast, folate supplementation is standard prenatal care because of its known association with fetal neural tube and heart defects [32,33]; there have not been any studies of it and fetal ANS development [30,31].

We assessed whether pregnant adolescents, of low SES, who are at risk for poor nutrition [34], show differences in fetal ANS function in the 3rd trimester in relation to zinc and folate intake. We hypothesized that deficient intake of zinc or folate would be associated with higher FHR and lower HRV, and the variables would have an additive effect.

2. Methods and participants

Nulliparous pregnant adolescents, ages 14–19 years, were recruited through the Departments of Obstetrics and Gynecology at Columbia University Medical Center (CUMC) and Weill Cornell Medical College, and flyers posted in the CUMC vicinity for a longitudinal pregnancy study. Participants were excluded if they acknowledged smoking or use of recreational drugs, use of medications with an effect on cardiovascular function or lacked fluency in English. Participants gave informed consent, and procedures were in accordance with the Institutional Review Board of the New York State Psychiatric Institute/CUMC.

Two hundred and five participants were enrolled in the 1st or 2nd trimester (see Supplemental Fig. 1 for complete enrollment flow chart). Of the 205, 69 participants had usable data for the 3rd trimester nutrition and fetal assessments, the data points for this study.

2.1. Procedure

Nutrition and fetal EKG data were collected between 34 and 36 gestational weeks; ± 1 week as part of a longitudinal pregnancy study. Medical records were reviewed for delivery data.

Nutrition information was acquired through the Automated Self-Administered 24-hour Dietary Recall (ASA24), which is an internet-based questionnaire provided by the National Cancer Institute [35]. The ASA24 is a detailed questionnaire that asks the participant to recall food intake over the preceding 24 h using detailed probes and portion-size food images. ASA24 estimates relative micronutrient levels using three databases: the USDA's Food and Nutrient Database for Dietary Surveys (FNDDS), the USDA's MyPyramid Equivalents Database (MPED), and the USDA's Center for Nutrition Policy and Promotion's MPED Addendum.

During the fetal session, participants were asked to relax and remain in a 15° right or left lateral position for 20-min periods. The Monica AN24 (Monica Healthcare Ltd, Cardiff, UK) recorded fetal electrocardiographic signals for measurement of FHR and HRV. Four electrodes were placed on the maternal abdomen, one below the umbilicus, one above the pubic hairline, and two laterally, equal distance from the top and bottom electrodes. A fifth electrode for reference was placed lateral to the electrode on the right side.

Gestational age at birth, birth weight, Apgar, and delivery data were determined from the medical record. Gestational age at birth was

determined based on the medical record reporting of dating based on ultrasound examinations and last reported menstrual cycle.

2.2. Data preparation

Women were split into deficient and non-deficient groups for food zinc and folate.

Women in the non-deficient group consumed the recommended dietary allowance (RDA) or more for that particular micronutrient, while women in the deficient group consumed less than the RDA [15, 29]. The recommended dietary allowance for zinc is 11 mg and for folate is 600 mcg [15,29].

The Monica DK 1.4a software (Monica Healthcare Ltd, Cardiff, UK) was used to analyze raw EKG data based on the Dawes and Redman criteria [36]. FHR measures were analyzed in 3.75-second epochs. Fetal heart rate was defined as the average heart rate, in beats per minute (bpm), across the epochs within the 20-min period. HRV was assessed in two different ways: Short-term variation (STV) was defined as the average difference in fetal heart rate between adjacent epochs and was calculated from minutes without decelerations in heart rate or signal loss. Mean minute range (MMR) was the difference between maximum and minimum values for 1-min increments averaged across the 20-min session, and also was calculated from minutes excluding decelerations and signal loss.

2.3. Statistical analyses

The data were examined for normality. One participant, with an outlying folate intake value (>3 standard deviations above the mean) was removed prior to conducting analyses. Pearson and chi-square tests were used to determine associations among variables; *t*-tests were used to compare the deficient (0) and non-deficient [1] groups on fetal measures. Demographic data that were significantly associated with independent and dependent variables were included as covariates. Analyses were performed with IBM SPSS Statistics (IBM, Armonk, NY). Tests were two-tailed with the alpha set at 0.05.

3. Results

3.1. Descriptives

Maternal and fetal demographics, such as maternal age, pre-pregnancy BMI, and annual income, and infant birth weight, Apgar scores, and gender are presented in Table S1. Sample averages for the study variables are provided in Table S2. No significant correlations were found between demographic variables and micronutrient or fetal variables (not shown). There were a significantly greater number of women with a match in their zinc and folate levels, such that women had both deficient zinc and folate or both non-deficient zinc and folate (Table S3). Half the sample was below the RDA threshold for non-deficient zinc ($n = 34$) or folate ($n = 34$).

3.2. Hypotheses testing

Based on independent *t*-tests, non-deficient compared to deficient maternal intake of zinc was associated with greater STV and MMR, but no difference in FHR (Table 1). Non-deficient compared to deficient levels of dietary folate had no associations with fetal variables. Deficient maternal intake of both zinc and folate compared to other levels of maternal intake of the micronutrients showed no associations with fetal variables (Table S4).

4. Discussion

In this sample of pregnant adolescents, half of the women consumed deficient levels of zinc according to the RDA standards and had fetuses

Table 1Results from *t*-tests comparing fetal outcomes of maternal micronutrient non-deficient vs. deficient dietary intake.

	<i>n</i>	Fetal heart rate (bpm)				Short-term variability (ms)				Mean minute range (ms)			
		M	SD	<i>t</i>	<i>p</i>	M	SD	<i>t</i>	<i>p</i>	M	SD	<i>t</i>	<i>p</i>
Zinc													
Deficient	34	141.76	5.74	0.10	0.92	10.02	2.44	−2.23	0.03*	53.18	13.09	−2.29	0.03*
Non-deficient	35	141.61	6.77			11.34	2.38			59.95	12.18		
Folate													
Deficient	34	141.59	7.08	−0.13	0.90	10.69	2.64	0.01	0.99	56.11	14.45	−0.32	0.75
Non-deficient	35	141.78	5.40			10.69	2.37			57.11	11.61		

M = mean; SD = standard deviation; bpm = beats per minute; ms = milliseconds.

with lower levels of HRV indexed via electrocardiograph measures of short-term variability of heart rate and mean minute range collected in the 3rd trimester. However, deficient levels of dietary folate (of which half the sample also was deficient) had no associations with fetal HRV. Dietary deficiency in both micronutrients had no association with fetal HRV. Neither of the maternal micronutrients was related to resting FHR. Since changes in FHR correspond to brain stem development and changes in HRV correspond to vagus nerve development, the findings provide a marker of autonomic nervous system development by fetal cardiac regulation between 34 and 36 weeks of gestation [23–25].

In the two other studies that assess the effects of zinc on fetal ANS development, results were similar to our findings. Zinc-deficient Peruvian women who were supplemented with 15 mg of zinc between 10 and 24 weeks gestation until 4 weeks postpartum had higher fetal HRV at 36 weeks compared to non-supplemented women [30]. In another study, Peruvian women deficient in zinc were given 25 mg supplements between 10 and 16 weeks gestation until 4 weeks postpartum and the fetal HRV findings were replicated [31]. One of the zinc supplementation studies found that higher zinc also was associated with lower fetal FHR [31], ours and the other Peruvian supplementation study did not [30]. Taken together, our results based on self-reported levels of zinc and folate dietary intake and those from controlled trials of zinc and folate supplementation are similar in showing the following: higher levels of zinc, independent of folate, are associated with indices of better fetal ANS development, specifically greater HRV.

In our study, pregnant women with both deficient zinc and folate levels did not differ in fetal HRV measures when compared to pregnant women deficient in only one or adequate in both micronutrients. Zinc and folate may have differential effects on cardiovascular system development. While both zinc and folate are important for heart organ development [33,37], zinc may be involved in the development and function of brain regions, such as the brain stem and hypothalamus, that control heart function [38]. Moreover, given the associations of prenatal levels of maternal zinc and fetal ANS development, and the absence of findings for dietary folate, it is interesting to note that for folate to be metabolized in the body, zinc is required as a cofactor [39]. The body does not readily absorb the natural form of folate found in food because it is conjugated to a polyglutamyl chain that prevents folate from passing through to the intestinal mucosa [39]. Folate conjugase, which requires zinc as a cofactor, removes the polyglutamyl chain allowing natural folate to be absorbed by the body [8,9]. Once the chain is removed, folate monoglutamate then can be absorbed into the intestinal mucosa where it is methylated to form 5-methyl-tetrahydrofolate [40]. This methyl group then will be added, through a complex mechanism, to DNA as an epigenetic modification. Further, while the body readily absorbs folic acid, which is contained in prenatal vitamins though not naturally found in food, it must also be converted to 5-methyl-tetrahydrofolate using the same mechanism to act as a methyl donor to modify DNA [37]. Thus, zinc is required for natural folate absorption and all forms of folate as a methyl donor. Folate acting as a methyl donor for DNA methylation can modify and regulate gene expression through DNA replication and cell division, protein function

and RNA processing, which are critical processes in neurodevelopment [41,42].

This study has several strengths, including prospective data acquisition and a sample with high prevalence of deficient zinc and folate intake. There are weaknesses, including nutrient intake was based on recall, although data suggest this is a reliable approach [43]. The version of the ASA24 available when these data were collected did not include an option to add prenatal vitamins, limiting our data conversion to dietary food equivalents only, without information about zinc and folate consumed from prenatal vitamins.

Prenatal zinc supplementation has been associated with increased HRV in fetuses and infants [30,31]. Here, in urban pregnant adolescents, lower levels of zinc dietary intake were associated with less fetal HRV. The consistency of these findings based on different methodologies—experimentation using a randomized control design and naturalistic observation—suggests that prenatal zinc is important to understanding fetal ANS development. As was evident in this sample, non-deficient intake during pregnancy cannot be assumed. Furthermore, most of the 10 foods richest in zinc—seafood, lamb, wheat germ, spinach, pumpkin seeds, mushrooms—are uncommon for urban teenagers or those in poverty [6,44]. Emerging data show continuity between fetal and infant development [28,45], and fetal ANS features predicting infant neurobehavioral outcomes [28,46]. Thus, these results showing effects of deficient zinc and HRV may have implications for children's long-term outcomes. With greater attention to the prenatal origins of future health, the results highlight the potential of the maternal environment to influence children's developmental trajectory before birth and the public health need for greater knowledge of and access to quality nutrition.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.earlhumdev.2015.01.007>.

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Conflict of interest statement

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.earlhumdev.2015.01.007>.

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