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Maternal Zinc Supplementation during Pregnancy Affects Autonomic Function of Peruvian Children Assessed at 54 Months of Age[1,2]

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Abstract

Maternal prenatal zinc supplementation improved fetal autonomic regulation in a nutrient-deficient population in Peru. To evaluate whether differences in autonomic regulation existed in early childhood, we studied 165 children from a zinc supplementation trial (80% of original sample) as part of a comprehensive evaluation at age 54 mo. Electrocardiogram (ECG) data were collected from the children at rest and while they underwent a cognitive testing battery following a standardized protocol. Of these, 79 were born to mothers receiving 25 mg/d zinc in addition to 60 mg/d iron and 250 μg/d folic acid during pregnancy, and 86 were born to mothers receiving iron and folic acid only. Derived cardiac measures included heart period (HP), range, HP variability (HPV), mean square of successive differences (MSSD), and a measure of vagal tone (*V*). Children in the zinc supplementation group had greater HP (i.e. slower heart rate), greater range, higher time-independent (HPV) and time-dependent (MSSD) variability in HP, and higher *V* ($P < 0.05$) during baseline. Analyses conducted across the cognitive testing period revealed similar effects of prenatal zinc supplementation on cardiac patterns. Concurrent child zinc plasma concentration was also associated with longer HP, greater variability, and marginally higher range and *V* ($P < 0.10$). Differences in cardiac patterns due to prenatal zinc supplementation were detectable in children at 54 mo of age during conditions of both rest and challenge, indicating that supplementing zinc-deficient pregnant women has beneficial long-term consequences for neural development associated with autonomic regulation. J. Nutr. 141: 327–332, 2011.

Introduction

Zinc is a trace mineral essential to all forms of life because of its fundamental role in gene expression, cell development, and replication. It has direct roles in the central nervous system in enzymes involved in brain growth, in proteins that provide brain structure for neurotransmission, and in neurotransmitters involved in brain memory function (1,2). Zinc also participates in steroid hormone transport, receptor binding and metabolism, and neurotransmitter precursor production, all of which ultimately affect brain function (2). Within the central nervous system, zinc is found predominantly in the hippocampus, cerebellum, and prefrontal cortex, areas of the brain that are responsible, respectively, for learning and memory; posture and balance; and higher intellectual functioning (1). Zinc-containing circuit neuron systems form connections between the cortex and other brain structures, suggesting a role for zinc in the organized response of the organism to its environment.

The autonomic nervous system acts as a control system, with sympathetic and parasympathetic subsystems, which through their opposing neural influences maintain a dynamic balance that provides stability as well as adaptability to environmental events. Therefore, optimal functioning is characterized by organized variability and lability rather than stasis and rigidity. The development of the autonomic system occurs progressively throughout gestation and there is marked continuity in neural development between pre- and postnatal periods (3,4). Measurement of phasic or nonphasic variability in heart rate (HR)[7]

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[7] Abbreviations used: BSA, body surface area; ECG, electrocardiogram; HP, heart period; HPV, heart period variability; HR, heart rate; HRV, heart rate variability; MSSD, mean square of successive difference; *V*, vagal tone.

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patterns provides an accessible indicator of the patency of the autonomic nervous system, particularly related to parasympathetic innervation by the vagus nerve (5,6). Studies in children have focused on detecting markers of physiological regulation and homeostatic control that correspond to infant and child performance and behavior. Higher levels of variability in HR, variously quantified using a variety of dynamic or static metrics, have been linked to better self-regulatory capacity, information processing, and attentional control (7–11). As such, it suggests that variation in these measures reflects the physiological substrate of primarily parasympathetic origin, underlying a child's readiness to engage with the environment (12). This continuity is also noteworthy because of interest in how the prenatal environment contributes to later health (13,14). As reviewed by Thayer and Lane (15) and Thayer et al. (16), decreased vagal function, characterized by higher HR and diminished HR variability (HRV) and related measures, is associated with risk factors for cardiovascular disease, inflammation, and diabetes.

Previously, we reported that prenatal maternal zinc supplementation influenced fetal cardiac measures in 2 different samples. In the first study (n = 55), 15 mg/d supplemental zinc (in addition to iron and folic acid) was associated with greater fetal HRV as well as more large, accelerative, episodic excursions of fetal HR and fewer decelerative ones at 32 and 36 wk of gestation (17). A larger (n = 195) longitudinal investigation with 25 mg/d supplemental zinc, conducted at monthly intervals from 20 to 38 wk of gestation, revealed that zinc supplementation altered developmental trajectories of cardiac patterns in ways consistent with accelerated autonomic maturation and enhanced parasympathetic control (18). Specifically, the HR of fetuses of zinc-supplemented mothers declined more steeply over gestation than those of nonsupplemented fetuses whereas short- and long-term HRV indicators increased more rapidly (19).

We have now conducted a comprehensive evaluation of the growth and development of the offspring at 54 mo of age from the second prenatal supplementation trial. We hypothesized that the alterations to HR patterns observed in the fetus attributable to zinc supplementation would also be expressed as physiologic regulation in early childhood during a baseline undisturbed period as well as during a prolonged period of challenge in which children received a series of behavioral and developmental assessments.

Study Design and Methods

Between 1998 and 2000, a double-blind, randomized, controlled trial of prenatal zinc supplementation was conducted among women receiving prenatal care at Centro Materno Infantil San Jose, in Villa El Salvador, a periurban community in Lima, Peru (19,20). Beginning in 2003, when the children were approaching 54 mo of age, we attempted to locate those children to evaluate their health, nutritional status, developmental outcomes, and autonomic functioning. The protocols for the original study and the follow-up were approved by the institutional review boards of the Instituto de Investigación Nutricional and The Johns Hopkins Bloomberg School of Public Health.

Briefly, 242 women were enrolled in the study at 10–14 wk of gestation and were randomized within strata (based on week of gestation and parity) to receive daily supplements containing 60 mg iron (as ferrous sulfate) and 250 μg folic acid, with or without an additional 25 mg zinc (as zinc sulfate). Compliance with supplementation was high; the mean number of tablets consumed was 157 $\pm$ 27, with no difference by supplement type (19,20). Previously, we described the low dietary zinc intakes and poor zinc status of women during pregnancy in this population, as well as the effect of prenatal zinc supplementation on indicators of zinc and iron status (21–23). Based on the results of our first study (21,22), we increased the dose of zinc from 15 to 25 mg/d for the second pregnancy study. Indicators of zinc status during pregnancy are not very responsive and thus a relatively large sample size is required to detect small changes. We were not able to detect a dose response when comparing the effects of the 2 doses on indicators of zinc status during pregnancy. RBC zinc concentration was the most responsive, with higher mean values at 36 wk of gestation among women consuming supplemental zinc (23).

Of the 242 women enrolled in the supplementation trial, 222 (90.1%) completed the protocol and 195 (80.6%) were included in the formal analysis, 94 in the zinc group and 101 in the control group (19). For those analyses, we excluded the data from 27 mother-infant pairs with obstetrical or medical complications, but for the follow-up study we attempted to locate 10 of these mothers who had completed the protocol and whose babies survived the neonatal period and were free of congenital malformations. From this pool of 205 eligible participants, we located and conducted evaluations of 184 (90%) children, 86 whose mothers had received zinc supplements during pregnancy, and 98 whose mothers had not.

When the children reached 54 mo of age, the study physician conducted a physical exam, which included an assessment of their diet and nutritional status. From this examination, we calculated mean arterial pressure as [diastolic pressure + (systolic pressure − diastolic pressure)/3] and characterized body size in terms of both BMI [weight (kg)/height (m^2)] as well as body surface area [BSA = 0.024265(height in $cm^{0.3964}$) (weight in $kg^{0.5378}$)], the latter being more related to cardiac function measures. Using previously described methods (22,23), we determined each child's hemoglobin concentration and plasma zinc concentration as a measure of zinc status. Hemoglobin concentration is available for almost all children, but due to some parental refusal for a venous blood draw and limited funding for laboratory analyses, we have information on plasma zinc concentration for a subset of 115 children (those with earlier birth dates). We also assessed C-reactive protein concentration, but consideration of inflammatory status did not affect the results presented regarding plasma zinc concentration.

Relevant history on postnatal morbidity, infant feeding, growth, and development were also recorded based on maternal report and abstraction from clinic records. We also interviewed the caregiver about the current social and economic circumstances of the family and compared with 5 y ago at the time of the birth of the study child. A psychologist visited the home and utilized an adapted version of the Home Observation for the Measurement of the Environment Scale (24,25) to measure both the nature and amount of stimulation and support available to the child in his/her home environment.

Over 2 sessions, a broad array of tasks was administered to assess developmental status (26). During these sessions, child HR data were derived telemetrically using 2 pediatric electrodes embedded in a belt secured on the child's chest under a shirt. R-waves were collected, amplified, and timed by a commercially available apparatus (Mini-Logger 2000, Mini Mitter). Five minutes of electrocardiogram (ECG) data were collected with the child sitting quietly to characterize baseline values immediately followed by a series of tasks designed to evaluate child

developmental outcomes (see below). Cardiac data were derived separately for each of the 8 assessments in the test battery. The purpose of this part of the protocol for this analysis was to determine whether zinc supplementation affected physiologic regulation during conditions of rest (i.e. baseline) as well as conditions of challenge (i.e. cognitive and behavioral testing) that elicit an alteration in autonomic regulation.

Data were transferred to a computer, manually edited for artifact, and then processed using MXedit software (Delta-Biometrics). Five cardiac measures were derived to reflect the spectrum of current analysis methods: *1*) heart period (HP), the intervals between R-waves (ms); *2*) range of HP; *3*) HP variability (HPV), SD of HP within epochs (i.e. time-independent variability); *4*) mean square of successive differences (MSSD), a time-dependent method of analyzing variation in successive HP; and *5*) vagal tone (*V*) using methods developed by Porges (27). Briefly, the Porges procedure uses a 21-point polynomial to detrend sequential HP data resampled at 250-ms intervals followed by a band-pass filter to extract the variance within the frequency band consistent with respiration within this age group (i.e. 0.24–1.04 Hz). The estimate of *V*, which corresponds to the respiratory sinus arrhythmia, is calculated as the natural log of the extracted variance. Data were epoched in 30-s intervals and means were computed for each measure.

Missing RCG data for HR was typically the result of ambient signal interference or movement artifact. The main results reported here are from 165 (80%) children, 79 from the zinc group, and 86 from the control group; of these, only 3 in the zinc group and 4 in the control group had not been included in the original analysis (19). The overall number of children providing sufficiently artifact-free data for each cardiac measure across the various tasks varied from 133 to 147.

Following the baseline record, child developmental outcome was assessed using the Spanish adaptation of a standard intelligence test (Wechsler Preschool and Primary Scale of Intelligence) (28) and a comprehensive protocol for studying multiple facets of cognitive and behavioral functioning developed at the Child and Family Research Section of the U.S. Eunice Kennedy Shriver National Institute of Child Health and Human Development and adapted for this age group of Latin American children. Results from this assessment battery by prenatal supplement type are presented elsewhere (26). Children were administered the protocol in consistent order over a 2-d period; to reduce fatigue, we split the administration of the Wechsler Preschool and Primary Scale of Intelligence between the 2 sessions. This splitting resulted in a total of 9 segments yielding HR data in addition to baseline.

All the study personnel, including the physician and psychologists, were unaware of the prenatal supplement type consumed by the mother. The staff editing the ECG data and conducting preliminary analyses were similarly unaware of treatment group.

Data analyses. To assess comparability of the treatment groups, the characteristics of women and children in each group were evaluated by *t*-test or χ^2 analysis. We compared the characteristics of study, maternal, and child variables among participants and nonparticipants in the follow-up study; those with complete or missing ECG data during the follow-up; and those with or without measures of plasma zinc concentration. No statistical differences were found. To examine the consistency of the cardiac measures across cognitive tasks, we computed correlation coefficients. ANOVA was then used to estimate the effects of zinc supplementation on baseline autonomic function. Given the longitudinal nature of the data (baseline, 9 measures during tasks), we estimated comprehensive mixed models to describe the overall mean effect of zinc supplementation on change in autonomic function during tasks. A random effect was specified to allow baseline values to vary across individuals as opposed to making a mean adjustment. We also considered correlated associations between successive tasks using an autoregressive-heteroscedastic structure for the residual covariance matrix, which brought a significant improvement in model fitting as evidenced by the log-likelihood ratio test. Significance was defined as $P < 0.05$. To examine whether the effects of zinc supplementation on the outcomes were diminished or modified by specific study, maternal, or fetal characteristics (e.g. parity, child sex, number of tablets consumed, birth weight, maternal education, and child size at follow-up), we included such variables as well as combined into interaction terms (e.g. zinc × primipara) in the overall regression models. Adjustment did not affect our results and unadjusted treatment differences are reported. No significant interactions were found ($P > 0.15$). All data analyses were performed using SAS version 9.1.3 (SAS Institute).

Results

Characteristics of the sample

There were no differences by supplement type in the maternal or child characteristics at enrollment in the pregnancy study, except that the number of years of schooling was greater among mothers in the group receiving supplemental zinc during pregnancy (**Table 1**). There were no differences in the numbers of tablets consumed by women during pregnancy, with the medians (5th, 95th percentiles) of 155 (99, 194) and 161 (111, 192) being the number of tablets consumed by women in the zinc and control groups, respectively.

At follow-up, most children were characterized by their mother as healthy overall, but almost 30% in each group had a

TABLE 1 Selected characteristics of 165 Peruvian women and children by type of prenatal supplement[1]

	Supplement type	
Characteristic	Iron + folate	Iron + folate + zinc
n	79	86
Maternal age, *y*	28.3 ± 4.8	28.5 ± 5.0
Maternal schooling,[2] *y*	11.1 ± 2.2	10.5 ± 2.6
Child was firstborn, %	58.2	58.1
Child has younger siblings, %	26.9	33.4
Girl, %	46.2	51.8
Birth weight, *g*	3296 ± 441	3268 ± 380
Single, %	21.5	16.3
Family better off now, %		
No	27.9	20.2
Same	21.5	25.0
Yes	50.6	54.8
Economically better off now, %		
No	40.5	33.7
Same	21.5	24.4
Yes	38.0	41.9
Persons per room in household	1.7 ± 0.9	1.9 ± 1.3
HOME inventory[3]	34.5 ± 6.3	32.4 ± 7.1

[1] Values are mean ± SD or percent.
[2] Significantly different by prenatal supplement type, $P < 0.05$.
[3] Home Observation for the Measurement of the Environment.

cold on the first day of testing (**Table 2**). There were no differences in child weight, height, BMI, BSA, or anemia between groups. Using the cutoff point of 9.9 μmol/L for zinc deficiency (29), there were no differences between the zinc (21.8%) and control (13.3%) groups ($P = 0.23$). The only difference between groups noted was a somewhat lower mean diastolic blood pressure among children whose mothers received supplemental zinc during pregnancy ($P < 0.10$).

Cardiac pattern measures

Baseline recording. Significant differences in baseline HR patterns were detected by prenatal supplement type (**Table 3**). Children whose mothers consumed supplemental zinc (along with iron and folic acid) had greater HP (that is, slower HR), greater range, and more variability in HP as assessed by either the time-independent method (HPV) or the time-dependent one (MSSD), and higher *V* compared with children of women in the iron and folic acid only supplement group ($P < 0.05$). Further analyses revealed the following independent influences on cardiac function (not shown). Girls and first-born children had lower HP (faster HR) and first-born children had lower indices of variability: HPV, MSSD, and *V*. BSA was significantly and positively associated with HP and HPV but not with *V* or MSSD. The impact of zinc supplementation on the cardiac measures was not diminished by adjustment for group differences in maternal years of schooling and thus we report the bivariate associations. Further, it was not modified by any characteristic tested including the number of supplement tablets consumed ($P > 0.15$).

Cognitive testing recordings. Correlations across the 10 measurement periods (baseline and 9 cognitive segments) for each of the 4 HR variables ranged from 0.43 to 0.94 ($P < 0.05$), indicating moderate to high degrees of stability for cardiac measures for individual children over time. To reduce the number of analyses, estimated overall mean effects during the entire cognitive testing period were modeled. Results indicate that children in the maternal zinc supplementation group continued to have longer HP (slower HR) and higher time-independent and -dependent indicators of variability (HPV and MSSD) during challenge afforded by testing (**Table 4**). The differences in *V* and range neared significance ($P = 0.08$).

There were no treatment group differences in plasma zinc concentration at follow-up (Table 2). However, plasma zinc concentrations were significantly and positively associated with HP ($r = 0.210$) and range (0.209) and tended to be associated with MSSD (0.167; $P = 0.07$). Adjustment for concurrent plasma zinc concentration did not alter conclusions regarding supplement type differences in the cardiac measures during baseline. Due to limitations in sample size, we could not estimate the effect of plasma zinc concentration on cardiac measures during tasks while adjusting for baseline as a random variable in a comprehensive model. Individual ANCOVA models did not identify significant effects of plasma zinc concentration on change in function to task that were greater than expected due to chance alone.

TABLE 2 Nutritional and cardiovascular status of 165 children at 54 mo of age by type of prenatal supplement consumed by their mothers[1,2]

	Prenatal supplement type	
Characteristic	Iron + folate + zinc	Iron + folate
n	79	86
Health score, %		
Frequently ill	0	2.3
Sometimes ill	5.1	7.0
Fairly healthy	73.4	66.3
Very healthy	21.5	24.4
Cold on day of exam, %	29.1	29.1
Pulse, *bpm*	74.8 ± 5.7	74.6 ± 5.4
Respiratory rate, min^{-1}	24.3 ± 3.4	23.5 ± 3.7
SBP,[2] *mm Hg*	84.7 ± 10.2	84.2 ± 7.8
Diastolic blood pressure,[2] *mm Hg*	57.8 ± 9.6	60.2 ± 7.7
Mean arterial pressure,[2] *mm Hg*	66.8 ± 8.5	68.2 ± 7.1
Weight, *kg*	17.3 ± 2.2	17.4 ± 2.4
Height, *cm*	102.9 ± 3.9	102.5 ± 3.4
BMI, kg/m^2	16.3 ± 1.3	16.5 ± 1.6
BSA, m^2	0.706 ± 0.056	0.708 ± 0.060
Hemoglobin, *g/L*	122.2 ± 10.3	120.0 ± 9.7
Plasma zinc,[3] *μmol/L*	12.2 ± 2.5	11.9 ± 1.8

[1] Values are mean ± SD or percent. Groups did not differ from one another, $P > 0.05$.
[2] *n* were 75 for the iron + folate + zinc group and 85 for the iron + folate group.
[3] *n* were 55 for the iron + folate + zinc group and 60 for the iron + folate group.

TABLE 3 Baseline cardiac function characteristics of Peruvian children at 54 mo by type of prenatal supplement consumed by the mothers[1]

	Prenatal supplement type	
Cardiac measure	Iron + folate + zinc	Iron + folate
n	79	86
HP, *ms*	576.5 ± 53.6*	553.5 ± 53.6
HP range	138 (114–178)*	124 (87–163)
HPV, *ms*	6.91 ± 0.77*	6.59 ± 0.77
MSSD, *ms*	24.3 ± 13.8*	19.8 ± 10.3
V, $ln(ms)^2$	5.63 ± 1.08*	5.29 ± 1.12

[1] Values are mean ± SD or median (IQR). *Different from iron + folate, $P < 0.05$.

Discussion

In this study, we found consistent effects of prenatal zinc supplementation on measures of autonomic functioning indexed by cardiac patterns in early childhood. These findings extend prior demonstration of zinc supplementation effects on cardiac patterns in the fetus (17,19) to the young child. Detected differences in HR and its patterning were evident both during an undisturbed baseline period as well as during an extended period of challenge afforded by a battery of complex developmental

TABLE 4 Estimated overall mean difference in cardiac measures due to prenatal zinc supplementation assessed in Peruvian children across baseline and 9 cognitive tasks

Cardiac measure	Prenatal zinc supplementation, β (SE)
HP	21.6 (9.5)*
HP range	14.3 (0.8)*
HPV	0.31 (0.13)*
MSSD	4.7 (2.0)*
V	0.33 (0.18)

*Different from iron + folate, $P < 0.05$.

tests. Because these findings were based on a randomized controlled trial, they support the interpretation that inadequate maternal intake of zinc during pregnancy alters the development of autonomic regulation in the developing fetus, with reach into childhood.

Unexpectedly, we found that the concurrent plasma zinc concentration in children was positively associated with HP, range, and time-dependent variability (MSSD) during the baseline period. This finding is in contrast to data generated by their mothers during pregnancy, in whom neither supplementation type nor plasma zinc concentration was associated with cardiac patterns during pregnancy (30). It may be that physiologic controls on serum zinc during pregnancy (22) preclude detection of this relation, or it may rather be characteristic of a developing organism, the growing child. To our knowledge, there are no prior reports of this association in the literature and, thus, it awaits confirmation from other studies.

Results of this study suggest that prenatal zinc supplementation is associated with slower HR and multiple measures of variability in children during periods of baseline or rest, regardless of whether the variability measure reflected time-dependent or -independent processes or the effect of respiratory gating via the vagus on variability (i.e. respiratory sinus arrhythmia as estimated by *V*). In addition, prenatal supplementation influenced parasympathetic control during cognitive testing, suggesting that zinc sufficiency during the most intensive period of autonomic nervous system development exerts long-term influences on parasympathetic control during cognitive challenge. Because measures of cardiac variability during attentional effort or cognitive stress reflect the degree to which an organism can adapt to a changing environment (12,31), this suggests an early influence on autonomic regulation and provides support for the critical role of the prenatal environment on later health and well-being (13,14) through either biologic or social/behavioral pathways.

The study has a number of strengths that increase confidence in the results. The supplementation trial included adequate randomization, high compliance with supplementation, and well-measured outcomes during pregnancy. The follow-up participation rate (80%) was relatively high despite the impoverished living conditions of the study sample. Cardiac measures were collected both under conditions of rest and challenge, furthering their generalizability, and the sample size of the followed group was adequate to detect relevant differences in child functioning. One might speculate that differences in motor activity during testing could create differences in the cardiac measures. However, variation in motor activity that accompanies cognitive testing is not sufficiently intense to alter cardiac responsivity (32). We found in 1 study that fetuses of zinc-supplemented mothers had greater motor activity in utero (17), and we have shown that more active fetuses are more active 1-y olds (33). Taken together, these influences would lower the likelihood of finding the differences reported here.

Although few age-specific studies are available to provide comparison data on the cardiac measures used in this study, we identified 3 in U.S. children between the ages of 54 and 60 mo (11,34,35). The U.S. children were also studied at baseline and during tasks designed to be challenging. Those findings suggest somewhat higher baseline HP (~600 ms) and *V* (range 5.5–6.2) than those observed in this study. Differences in size in general, and BSA in particular, might explain some or all of the differences, because American children are likely to be larger, but anthropometric data were not provided in these studies to evaluate this speculation. However, corresponding differences in fetal measures, specifically faster HR and lower HRV, between this Peruvian sample and a North American one have been previously observed (36) and thus may reflect inherent population differences. Gender differences in HP have been previously described (11).

To our knowledge, this is the first randomized trial reporting an impact of maternal zinc deficiency during pregnancy on the autonomic function of offspring in childhood. The clinical importance of persistent effects of zinc on cardiac patterns, and in turn on the development of chronic diseases, awaits future studies as these children age and transition to adolescence and adulthood.

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