



# Zinc supplementation, mental development and behaviour in low birth weight term infants in northeast Brazil

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**Objective:** To test whether zinc supplementation reduces the deficits in mental development and behaviour that are found in term infants of low birth weight in the study population.

**Design:** A prospective double-blind, part-randomised efficacy trial.

**Setting:** A low-income population in Pernambuco, northeast Brazil, where the economy is largely dependent on sugar-cane production, and where over 90% of deliveries occur in health facilities.

**Subjects:** During a 20-month period, all singleton, term infants weighing 1500–2499 g born to families of low income (< US \$280/month) were enrolled at birth ( $n = 205$ ). At 6 and 12-months, the numbers tested were 163 and 138 respectively.

**Intervention:** Infants born from January 1993–January 1994 were randomly assigned to receive daily, except Sundays, a placebo ( $n = 66$ ) or 1 mg zinc ( $n = 68$ ). Those born February–August 1994 were given 5 mg zinc ( $n = 71$ ). Supplementation was for eight weeks, starting at birth. Field workers visited each infant at home to administer the supplement.

**Results:** At 6 and 12-months, mental and psychomotor development was assessed with the Bayley Scales of Infant Development and no significant differences in the scores of the three groups were found. At 12-months, behaviour was also assessed on 5 ratings. Ratings were highest in infants given 5 mg zinc ( $P = 0.042$ ).

**Conclusions:** Zinc supplementation (5 mg/d) for eight weeks may reverse some of the poor behaviours, particularly responsiveness, exhibited by low birth weight infants. No amelioration of their mental and psychomotor deficits was found.

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**Descriptors:** zinc supplementation; infant development; behaviour; low birth weight

## Introduction

Mild zinc deficiency is thought to be widespread in poor populations with limited access to a varied diet (Sandstead, 1991). Among such populations, infants of low birth weight (LBW) may be particularly vulnerable as intrauterine growth retardation is associated with reduced liver size at birth (Usher & McLean, 1974) and hepatic zinc-metallotheonein is thought to act as a zinc reserve in infants (Aggett, 1994). Consequently term LBW infants can be expected to have a smaller zinc reserve than other infants. In northeast Brazil, the prevalence of LBW is about 9%. We surmised that term LBW infants in the northeast of Brazil are likely to be deficient in zinc because of reduced zinc stores at birth, limited access to dietary sources because of poverty, and poor bioavailability since breastfeeding durations are short.

Animal studies show an adverse effect of zinc deficiency in utero and early life on brain weight, composition and function (Sandstead, 1985; Frederickson, 1989; Wallwork & Sandstead, 1993). When behaviour is tested concurrently with zinc deprivation in immature animals, the prominent characteristics are reduced activity and responsiveness, but

learning, attention and memory are also affected (Golub *et al*, 1995, 1996). We have previously reported that term LBW infants in northeast Brazil performed less well in the Bayley tests of motor and mental development and were more inhibited than infants of appropriate birth weight (ABW), and less active, cooperative, vocal and happy during their developmental assessments (Grantham-McGregor *et al*, 1998). At 6-months of age, the mean difference in their mental development index (MDI) was 4 points and this widened to 7 points at 12-months. For the psychomotor development index (PDI), the mean difference was 7 points at 6-months and 10 points at 12-months. We hypothesised that zinc deficiency may be impairing their mental and motor development, and that zinc supplementation might be beneficial. In this paper we therefore compare the development and behaviour of term LBW infants who received 5 mg zinc daily during their first eight weeks of life with infants who received a placebo or 1 mg zinc. Testing was performed at 6 and 12-months of age.

## Subjects and methods

Details of the location, subject eligibility, and study procedures have been given in previous publications (Lira *et al*, 1996; Grantham-McGregor *et al*, 1998). Briefly, the

study took place in the interior of the state of Pernambuco where most of the population are poor and where the economy is heavily dependent on sugar-cane production. Five maternity centres serve this area, and over 90% of deliveries occur there. During a 13-month period beginning in January 1993, all singleton LBW term infants weighing 1500–2499 g born to families of low income (equivalent to < US\$280 per month) were recruited for the study. Gestational age was assessed by one of two paediatricians by the method of Capurro *et al* (1978). Infants with congenital anomalies, abnormal neurological signs, or signs of asphyxia were excluded. All were well enough to go home within two days of birth and no infant had respiratory infections in the first week. The intention was to give 5 mg zinc or a placebo daily except Sundays, for eight weeks from birth, in a randomized, double-blind trial. A mistake in the manufacture of the zinc solution resulted in the initial cohort being given 1 mg instead of 5 mg. When this was discovered, the design was modified and the enrolment period was extended to include a second cohort of infants who would all receive 5 mg zinc. Inclusion and exclusion criteria remained the same and all field workers and participating families were unaware of the change and remained blind to treatment allocation. Thus, during a 13-month period from January 1993, 66 LBW term infants were randomly allocated to receive the placebo and 68 received 1 mg zinc. During February–August 1994, 71 LBW term infants received 5 mg zinc.

#### Zinc supplement/placebo

The supplement was zinc sulphate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) masked in sorbitol and flavour. The placebo was similar in appearance and taste (sorbitol and flavour). A field worker, using a disposable syringe, administered 0.5 ml of the supplement/placebo at home in the presence of the mother or caretaker daily except Sundays, from birth for eight weeks. If an infant was hospitalised, the field worker went to the hospital to give the dose. Bottles were kept by the field worker to prevent tampering, and a photograph of the infant's mother was affixed to the bottle to prevent misallocation.

#### Other zinc sources

At 4, 8, 17 and 26 weeks, any zinc-containing ointments, creams or tonics given by parents were recorded. Consumption of specific zinc-rich foods was also ascertained on these occasions.

#### Data collection

**Developmental tests.** Infants were scheduled to have their development assessed at 6 and 12-months using the two subscales of the Bayley Scales of Infant Development. They were tested in the presence of their mothers and any infant who was sick at the scheduled test time was treated and the test postponed. Two paediatricians shared the testing at 6-months, and one of them undertook all the 12-month tests. Both were blind to treatment allocation.

**Behaviour.** At 12-months the infants' behaviour was rated on 5 scales, each with a 9-point range. These were: *responsiveness* to the tester in the first 10 min of the test (avoiding = 1, friendly and inviting = 9), *emotional tone* (unhappy throughout = 1, happy throughout = 9), *activity level* (very still = 1, very active = 9), *cooperation* with the test procedure (resists all suggestions = 1, always

complies = 9) and *amount of vocalisation* (very quiet = 1, constant vocalisation = 9). The scales were adapted from the rating scales of Bayley (1993) and Wolke *et al* (1990). We first selected behaviours that we hypothesised were likely to be affected by nutritional factors (Grantham-McGregor *et al*, 1990) and retained those that proved reliable when tested during the pilot phase.

**Home Environment.** The Caldwell Home Inventory (Caldwell 1967) was used to provide an index of stimulation. It was modified for local cultural and socioeconomic conditions and rescaled. The index comprised 43 questions or observation items, and the total score was used for the analysis. The infants' socioeconomic environment was assessed on the basis of four indicators: (i) family income for the month preceding birth (expressed as minimum salaries), (ii) household resources (scaled 0–5), (iii) housing quality (scaled 0–16) and (iv) water and sanitation (scaled 0–6). Reported parental literacy was also recorded. The mothers were interviewed at home by one of two female interviewers.

**Morbidity and feeding.** Infant morbidity and feeding status were recorded daily during home interviews (except Sundays) from birth to eight weeks of age, and then twice weekly until 6-months. The morbidity questionnaire focused on maternally perceived symptoms of diarrhoea, cough, vomiting, fever and *cansaço* (a local term for rapid breathing). The feeding questionnaire focused on the number of breastfeeds and other feeds (excluding water, juice and tea).

**Anthropometry.** Birth weight and length were measured for 67% of infants within 12 h of birth, and only two were measured after 24 h. Procedures and equipment have been described previously (Lira *et al*, 1996). Weight and length were remeasured at 4, 8, 17, 26 and 52 weeks.

#### Quality control

The zinc content of the supplement and placebo solutions was verified by the Pernambuco State Institute for Technology, Brazil and the Middlesex Hospital, UK. A 10% sample of individual bottles was also checked against the randomization code. No errors were detected. Gestational age was assessed independently by the two paediatricians for 20% of the infants with good interobserver reliability ( $\kappa = 0.86$ ). Approximately 8% of the Bayley tests were witnessed by a second observer and scored independently. Interobserver correlations were high for both MDI and PDI and were above  $r = 0.94$  at 6 and 12-months. For the behaviour ratings, interobserver agreement was assessed between the tester and trainer in 10 consecutive tests before the study began. During the study, 18 further tests were witnessed independently by a second observer. Interobserver correlations were high, ranging from 0.84 for cooperation to 0.96 for activity level. Approximately 10% of interviews to assess home stimulation were scored independently by a second observer. Interobserver agreement was high, with complete agreement for at least 41 of the 43 variables.

#### Statistical methods

Questionnaires were pre-coded and checked daily for completeness, accuracy and consistency. Double data entry was performed by two data clerks using dBase-

III + (Ashton Tate, Torrance CA) and verified. Differences among the groups were assessed using a standard chi-squared test for proportions (Kirkwood, 1988), analysis of variance (ANOVA) for normally-distributed continuous variables (Kirkwood, 1988), and the non-parametric Kruskal-Wallis test for non-normally distributed continuous variables (Altman, 1991). The Kruskal-Wallis test is the multi-sample generalisation of the more familiar two-sample Wilcoxon (Mann-Whitney) rank sum test. In addition, because the five behaviour ratings were correlated one with another, we tested the hypothesis that the population means for all five behaviour scores were simultaneously no different across the three treatment groups using Multivariate Analysis of Variance. The test statistic selected to test this hypothesis was Hotelling's Trace (Olsen, 1976), but Pillai's Trace and Wilk's Lambda both gave almost identical *P*-values. Three of the five behaviour scores were first transformed (raised to the power three) to remove the pronounced skew in the distributions, which would otherwise have affected the validity of the test result. Analyses were conducted using the Statistical Package for the Social Sciences (SPSS) for Windows, version 6.1 (SPSS Inc., Chicago IL).

### Ethics

Ethical permission was obtained from the ethics committees of the London School of Hygiene and Tropical Medicine, the Universidade Federal de Pernambuco, and the Fundação Nacional de Saúde. Mothers knew they could withdraw their child from the study at any time. Sick infants were referred to a local health service according to a standard protocol.

## Results

### Losses from the study

By the 6-months' test, 29 infants (14%) had moved out of the area and 13 (6%) died. Thus Bayley tests were carried

out at 6-months on 53 (80%) placebo infants, 56 (82%) 1 mg and 54 (76%) 5 mg infants. By the 12-months' test, there were 25 further losses (9 placebo infants and 8 in each of the zinc groups).

### Comparability of the groups

Since infants were randomly allocated to placebo and 1 mg zinc treatments, between-group differences in characteristics that might influence development could only have arisen by chance. The 5 mg zinc group, however was not randomly allocated. Formal statistical tests were therefore used to compare the three groups with respect to characteristics of the infants, their mothers and their family environments. Table 1 shows selected variables from among those examined. At birth, the groups were comparable except the 5 mg zinc group had smaller head size and were longer, and their mothers were taller (data not shown). During the trial, infants who received 5 mg zinc had significantly less diarrhoea than other infants. Very few infants in any group regularly received zinc-containing ointments, tonics, or zinc-fortified formulas. Few consumed zinc-rich foods. Examination of the characteristics of infants lost to follow-up showed that these were comparable across the three treatment groups.

### Supplementation and Bayley scores

Table 2 shows for each treatment group mental and psychomotor scores at 6 and 12-months. Zinc supplementation for eight weeks from birth did not improve the scores. Adjusting for the smaller head size/length ratio in the 5 mg zinc group did not affect the outcome.

### Supplementation and behaviour

The rating for responsiveness to tester was significantly higher in infants given 5 mg zinc (Kruskal-Wallis test, *P* = 0.023). The other behaviour ratings did not differ significantly when analyzed individually (Table 2), but a

**Table 1** Birth anthropometry, socioeconomic characteristics, and home stimulation index according to treatment group. Values are means (s.d.) except where indicated otherwise

	Placebo <i>n</i> = 53	1 mg zinc <i>n</i> = 56	5 mg zinc <i>n</i> = 54	<i>P</i> -value
Measurements at birth				
Length (cm)	45.9 (1.47)	46.2 (1.22)	46.5 (1.24)	0.048 <sup>a</sup>
Weight (g)	2329 (164)	2353 (137)	2351 (153)	0.65 <sup>a</sup>
Head Circumference (cm)	32.6 (0.79)	32.6 (0.91)	32.2 (0.95)	0.042 <sup>a</sup>
Head/length ratio	0.71 (0.023)	0.71 (0.026)	0.69 (0.021)	0.0004 <sup>a</sup>
Gestation (weeks)	39.0 (1.34)	38.8 (1.39)	39.0 (1.42)	0.56 <sup>a</sup>
Sex (% female)	62%	55%	56%	0.71 <sup>b</sup>
Socioeconomic status				
Income (minimum salaries: 1 = US\$70)	1.51 (0.70)	1.38 (0.70)	1.46 (0.61)	0.57 <sup>a</sup>
Resource index (0–5)	3.0 (1.54)	3.2 (1.56)	3.5 (1.33)	0.30 <sup>a</sup>
Housing index (0–16)	5.3 (1.21)	5.5 (1.50)	5.2 (1.13)	0.32 <sup>a</sup>
Water index (0–6)	3.2 (1.26)	3.1 (1.21)	3.0 (1.27)	0.86 <sup>a</sup>
Maternal literacy (%)	76% (40/53)	73% (41/56)	82% (41/54)	0.57 <sup>b</sup>
Paternal literacy (%)	86% (44/51)	84% (46/55)	79% (41/52)	0.60 <sup>b</sup>
Diarrhoea (% days 0–6 months)	median 1.1 IQR <sup>d</sup> 0.0–5.0	median 2.5 IQR 0.0–7.1	median 0.0 IQR 0.0–2.5	0.007 <sup>c</sup>
Stimulation index at 12 months	22.8 (6.94) <i>n</i> = 44	22.6 (6.18) <i>n</i> = 47	22.0 (6.17) <i>n</i> = 45	0.83 <sup>a</sup>
Breast feeding duration (months)	median 2.9 IQR 1.7–5.4	median 2.9 IQR 1.5–4.4	median 2.4 IQR 1.5–4.3	0.74 <sup>c</sup>

<sup>a</sup> ANOVA.

<sup>b</sup>  $\chi$ -squared.

<sup>c</sup> Kruskal-Wallis.

<sup>d</sup> IQR = interquartile range.

**Table 2** Bayley scores at 6 months and 12 months (mental development index MDI and psychomotor development index PDI) and behaviour ratings according to treatment group. Values are means for MDI and PDI (s.d)

	Placebo	1 mg zinc	5 mg zinc	P-value
Bayley scales (6 months)	<i>n</i> = 53	<i>n</i> = 56	<i>n</i> = 54	
MDI	91.5 (5.4)	89.6 (6.9)	90.1 (7.4)	0.32 <sup>a</sup>
PDI	93.6 (6.6)	91.3 (8.7)	93.7 (8.4)	0.19 <sup>a</sup>
12 months	<i>n</i> = 44	<i>n</i> = 48	<i>n</i> = 46	
MDI	109.1 (12.2)	106.7 (11.1)	106.9 (12.1)	0.57 <sup>a</sup>
PDI	100.4 (11.3)	101.1 (11.0)	100.0 (11.6)	0.87 <sup>a</sup>
Behaviour ratings (12 months)	<i>n</i> = 44	<i>n</i> = 48	<i>n</i> = 46	
Responsiveness	median 4 IQR 3.25–6	median 5 IQR 4–5.75	median 5.5 IQR 5–6	0.023 <sup>b</sup>
Emotional tone	median 6 IQR 5–7	median 7 IQR 5.25–7	median 7 IQR 6–7	0.67 <sup>b</sup>
Activity level	median 7 IQR 6–7	median 7 IQR 6–7	median 7 IQR 7–7	0.68 <sup>b</sup>
Cooperation	median 6 IQR 4–7	median 6 IQR 4–7	median 6.5 IQR 5–7	0.60 <sup>b</sup>
Vocalisation	median 5 IQR 3–7	median 4 IQR 3–6	median 5 IQR 4–6	0.51 <sup>b</sup>

Joint test of between-group difference in all five ratings by Hotelling's Trace:  $F(10,260) = 1.929$ ;  $P = 0.042$ .

<sup>a</sup> ANOVA.

<sup>b</sup> Kruskal-Wallis.

joint test of between-group differences in all five ratings taken together did indicate a significant difference (Hotelling's Trace,  $F[10,260] = 1.929$ ;  $P = 0.042$ ). The 5 mg zinc group had the highest scores on all five ratings.

## Discussion

Since zinc deficiency is believed to be common in developing countries, any associated impairment in cognitive or psychomotor development would be of profound public health significance. Animal experiments, including studies in non-human primates indicate that long-term impairment does occur in animals exposed to severe zinc deficiency during the period of rapid brain growth (Golub *et al*, 1995). Characteristic of rhesus monkeys marginally deprived of zinc from conception onwards is reduced responsiveness associated with hypoactivity. Human studies are very limited as yet and, because many factors affect child development, a causal association between zinc deficiency and development can only be inferred from supplementation trials. We have located six randomised trials. Three focused on specific cognitive functions. The first was in southern Ontario, Canada, among 60 boys aged 5–7 y who were of short stature (Gibson *et al*, 1989). Supplementation was for 12-months and attention span and working memory were assessed on four subtests of the Detroit Tests of Learning Aptitude. No treatment effect was found. Similarly in Guatemala with the same Test but utilising three subtests, no benefit of zinc supplementation was detected in 162 children aged 5–7 y from poor families. The mean duration of supplementation was 90 d (Cavan *et al*, 1993). In contrast, in China, neurocognitive function was better in children aged 6–9 y supplemented with zinc for 10 weeks, or zinc with other micronutrients, compared with children with micronutrients alone (Penland *et al*, 1997). In one study of very low birth weight infants in Newfoundland, Canada, psychomotor development in the first year of life was examined and was found to improve with 3 months' zinc supplementation (Friel *et al*, 1993). Mental development, however, did not improve. In two other trials, investigators observed children's behaviour and found changes in activity patterns. One was in India with 93

poor children aged 12–23 months and those randomised to receive zinc for 6-months were markedly more active than placebo children (Sazawal *et al*, 1996). The other trial was in Guatemala in 85 infants who were supplemented for 7 months from the age of 6–9 months (Bentley *et al*, 1997). At the end of the trial, infants who had received zinc were more frequently observed to be sitting up and playing, and less likely to be lying down, than placebo infants. These effects were not apparent after 3 months' supplementation.

Our study is thus the first to focus on term LBW infants. Although we have previously shown that these infants have substantial deficits in MDI and PDI compared to ABW infants, zinc supplementation was not associated with any improvement in their scores. The study had 85% power to detect a difference of 4 DQ points at 6-months. Diarrhoea detrimentally affects the development of LBW infants in this population and since 5 mg zinc was associated with a significant reduction in diarrhoea (Lira *et al*, 1997), the lack of a significant impact on development may seem surprising. The prevalence of diarrhoea was, however, quite low in the first 6-months of life, and the difference in PDI score attributable to diarrhoea was modest (<1 point at 6-months).

Infants receiving 5 mg zinc were more responsive to the tester than other infants in that they were more friendly and less inhibited or fearful ( $P = 0.023$ ). That responsiveness should be associated with zinc supplementation is interesting, given the suggestion of Golub and her colleagues that changes in activity and responsiveness provide a sensitive index of dietary zinc adequacy (Golub *et al*, 1995). Although this could be a chance finding we think this unlikely as the 5 mg zinc group had the highest scores on all five behaviour ratings. Undernourished children in general have been described as shy and inhibited, and it is possible that zinc deficiency contributes to this behaviour. Since infants receiving 5 mg zinc were not randomly allocated, unlike the 1 mg and placebo groups, we cannot be entirely certain that zinc was the causal factor in their improved behaviour. Nevertheless, the 5 mg zinc group had comparable socioeconomic and demographic characteristics to the other groups and the macroenvironment was similar in 1993 and 1994. Infants were tested by the same

testers and under the same conditions throughout. During the study, losses tended to be among infants with the lowest Bayley scores. Since losses were similar in all three groups, this bias should not affect interpretation of the results.

The supplementation period was just eight weeks because of perceived health service implications as we considered that if, in the future, zinc supplements were to be provided through existing health service channels, compliance may be of limited duration. Although eight weeks' supplementation appears sufficient for a beneficial effect on diarrhoea prevalence, it may be too short to benefit mental and motor function. If the duration of zinc supplementation were to be restricted for logistical or other reasons, it may be preferable to start supplementation when breastfeeding ends, ie when intakes of bioavailable zinc decrease.

The mechanisms whereby zinc affects brain function and behaviour are not yet clearly defined, but zinc appears to induce the release of the neurotransmitter  $\gamma$  aminobutyric acid (GABA) and thus influence neuronal excitability and modulate synaptic transmission (Frederickson, 1989; Smart, 1990; Xie & Smart, 1991). Zinc is concentrated in specific neuronal structures, notably in the nerve terminals of the hippocampus (mossy fibre system), cortex and pineal body. GABA may also have a trophic role in neuronal cell growth and differentiation (Ben-Ari & Cherubini, 1991).

## Conclusions

This study perhaps raises more questions than it answers. The data indicate that supplementation with 5 mg zinc may reverse some of the poor responsiveness exhibited by LBW infants. No amelioration of the MDI and PDI deficits was evident. Further supplementation studies are warranted in which zinc is given to LBW infants later and for longer.

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