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The Effects of Iron and/or Zinc Supplementation on Maternal Reports of Sleep in Infants from Nepal and Zanzibar

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Abstract

Background—There is some evidence that sleep patterns may be affected by iron deficiency anemia but the role of iron in sleep has not been tested in a randomized iron supplementation trial.

Objective—We investigated the effect of iron supplementation on maternal reports of sleep in infants in 2 randomized, placebo-controlled trials from Pemba Island, Zanzibar, and Nepal.

Design—In both studies, which had parallel designs and were carried out in years 2002 to 2003, infants received iron–folic acid with or without zinc daily for 12 months, and assessments of development were made every 3 months for the duration of the study. Eight hundred seventy-seven Pemban (12.5 ± 4.0 months old) and 567 Nepali (10.8 ± 4.0 months) infants participated. Maternal reports of sleep patterns (napping frequency and duration, nighttime sleep duration, frequency of night waking) were collected.

Results—Mean Hb concentration was 9.2 ± 1.1 for Pemban and 10.1 ± 1.2 g/dL for Nepali infants. Approximately, one-third of the children were stunted. Supplemental iron was consistently associated with longer night and total sleep duration. The effects of zinc supplementation also included longer sleep duration.

Conclusions—Micronutrient supplementation in infants at high risk for iron deficiency and iron deficiency anemia was related to increased night sleep duration and less night waking.

Index terms

infants; sleep; iron deficiency anemia; iron supplementation; developing countries

Iron deficiency anemia (IDA) in children is associated with developmental delays and deficits in cognitive function,[1] particularly when IDA develops before the second year of life.[2] Studies of iron supplementation among infants and toddlers have shown limited benefits for cognitive

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function[2] but significant improvements in motor development.[3,4] Iron treatment and prevention of IDA have also been related to more positive effect, greater soothability, and greater exploration.[4,5] With few exceptions,[6,7] investigations of IDA and iron supplementation effects on behavior have been limited to daytime hours. Nighttime behavior and sleep have not received much attention, and there are no randomized, placebo-controlled supplementation trials of iron supplementation on infant sleep.

Infants with IDA are more likely to be carried, asleep or irritable, and more easily fatigued during testing.[5,8] Two actigraph studies investigated activity of iron-deficient Chilean infants at 6, 12, and 18 months, one during a midday nap,[7] and second over a 24-hour period.[6] Infants with IDA fell asleep faster during naps than non-IDA infants.[7] At 6 and 12 months infants with IDA spent more time awake at night and less time in quiet sleep than controls.[6] In both studies infants received supplemental iron for 12 months. After 6 and 12 months of treatment, differences in nighttime activity between IDA and control infants disappeared, suggesting a benefit of iron. However, this was not a randomized, placebo-controlled trial so changes in activity cannot be attributed to iron specifically. In addition, there were fewer than 20 infants per group to evaluate treatment effects. Another study of Guatemalan toddlers did not find any relationship between anemia and sleep (daytime, nighttime, and total sleep hours, nighttime waking, and napping frequency) when measured before an intervention to reduce coffee intake.[9] The intervention also did not impact sleep, but it had no effect on iron status,[10] possibly explaining lack of sleep effects.

We recently reported on baseline relationships between IDA and infant sleep in 3 supplementation trials.[11] We found that 6- to 18-month-old infants with IDA slept longer and awoke at night more than infants without IDA.[11] In this report, we investigated the effect of iron supplementation on infants' sleep. The evaluation of sleep in iron-deficient children is needed because inadequate sleep is adversely associated with cognitive and behavioral development.[12] We examined these relationships in 2 randomized, placebo-controlled, 2 × 2 factorial trials from Pemba Island, Zanzibar, and Nepal, in which iron–folic acid was given with or without zinc. The studies were designed as "sister" trials and purposely carried out in 2 different cultural settings with varying degrees of malnutrition and malarial infection to understand if these factors would impact the efficacy of the supplements in improving infant nutritional status, growth, and development.

METHODS

Setting and Participants

Pemba Island, Zanzibar—The study took place between March 2002 and July 2003 on Pemba Island, Zanzibar, with the sample drawn from a large, community-based trial investigating the effects of daily zinc and/or iron–folic acid supplementation on mortality, morbidity, growth, and anemia.[13] Eight municipalities, both urban and rural, were selected from the large trial for inclusion in a child development substudy. All infants aged 5 to 18 months were invited; 932 were enrolled with parental consent and 877 were randomized. The study was approved by human subjects review committees at Johns Hopkins Bloomberg School of Public Health, Zanzibar Health Research Council, University of California at Davis, and Cornell University.

The population on Pemba Island, approximately 350,000 inhabitants, is mostly Muslim. The primary economic activities on Pemba are fishing, farming, and cultivation of cloves. Malaria is endemic and transmitted year-round and parasitic infections like hookworm or schistosomiasis are also common.[14] We found a high prevalence of stunting and anemia among infants and toddlers,[15] likely due to the introduction of complementary foods that are low in

micronutrients. Young children eat foods available to the rest of the household, including maize porridge, potatoes, bread, vegetables, fruit, and some meat.

Cosleeping is common, especially when infants are breastfeeding. Infants may sleep in the same bed as their parents or in a separate, smaller bed right next to the parents. Pemban households have a range of bed styles, depending on socioeconomic status. Most typical is a wooden frame with a foam mattress. Adults from poorest families may sleep on the floor but all infants sleep on beds raised off the floor, usually uncovered for fear of suffocation. In families with few resources beds may consist of 4 posts with rope stretched among them and a simple mat on top.

Typical bedtime is around 9 p.m. Infants often do not fall asleep in bed but in the mother's arms or lap, and mothers may pat their infants' back to help them fall asleep. Young children are allowed to sleep as long as they need to and when they continue to sleep after their mothers have gotten up, they are left in the care of another person or alone in bed propped up with pillows. If awake, an infant is taken to the kitchen area where the mother can watch him while doing house chores. During the day, infants also nap close to the mother. Infants are often swaddled and carried on the mother's back if they need to be taken to the field or on errands. Grandmothers and maternal sisters share in childcare, but young children are also carried and cared for by their older sisters, sometimes as young as 7 years old.

Sarlahi District, Nepal—The study was carried out in south central Nepal. The sample was drawn from a large, community-based trial of zinc and/or iron–folic acid supplementation. Infants aged 4 to 17 months from one Village Development Committee were invited to participate in a child development substudy. After verbal consent from caregivers, 567 infants were randomized to treatment and assessed. The study population and sample were described elsewhere.[16,17] The study was approved by Johns Hopkins Bloomberg School of Public Health and Nepal Health Research Council. Baseline data collection took place between January and March 2002.

The study population, mostly Hindu, rural, and composed of farmers and laborers, is considered poor by Nepali standards.[17] Under-nutrition, especially wasting is a problem among Nepali infants and toddlers. In our study population, over 65% of infants were fed exclusively with breast milk at 4 to 5 months of age, with limited complementary feeding.[18] After 6 months of age, nonexclusive breastfeeding continued for over 90% of the infants, but infants were also fed diets consisting largely of rice, lentils, greens, and little meat (whereas rice consumption in the previous 7 days was reported for 80% of 9- to 11-month-old infants, less than 20% of these infants had consumed any meat[18]). In another study, 1- to 6-year-old children from this region were observed to eat an average of 3.9 meals per day, and consume predominantly grains, pulses, and dairy products, with lower amounts of fruits, vegetables, and meat.[19] Children in this age range either eat alone or share a plate of food with adults or siblings,[19] with the father usually eating first. Similar to Pemba Island, typical diets are characterized by low micronutrient bioavailability.

In Nepali households, young children usually go to sleep at around 9 p.m., which is dictated by availability of light, and are allowed to sleep as long as they need.[20] Like on Pemba, Nepali children cosleep with adults, especially when breastfeeding. When older, boys sleep with their father and girls sleep with their mother. Young children often do not fall asleep in their bed and are moved for the night. Beds are usually raised off the ground for protection against snakes, and children are often covered for protection against mosquitoes and flies. Infants nap during the day in small hammocks outside the house so that mothers may hear them when they stir or wake up.[20]

Interventions

The 2 studies had nearly identical research designs. The randomization and supplementation procedures have been described previously for both studies.[13,17] Infants were randomized to receive a single tablet daily for 12 months (infants <1 year of age received half the dose). The vanilla-flavored tablets (Nutriset, Malaunay, France) were dissolvable in water or breast milk, and contained (1) 12.5 mg elemental iron plus 50 μg folic acid, (2) 10 mg elemental zinc, (3) iron–folic acid and zinc, or (4) placebo. The tablets were formulated as ferrous sulfate and zinc sulfate.

In the Pemba Island study, individual infants were randomized to one of the supplementation groups. In the Nepal study, infants were randomized by sectors, stratified by geographic area. This was accounted for in the analysis by adjusting for cluster randomization. On Pemba, a community worker made a weekly delivery of tablets packaged in blister packs, and recorded tablet consumption in the previous week as well as signs of illness. In Nepal, home visits occurred twice a week to record compliance and morbidity. In neither setting did community workers dispense nutritional advice.

Assessments

Parents answered questions regarding their infants' illness, sleep, and appetite during home visits, which were conducted approximately every 3 months, for a total of 5 visits. In both studies, weight was measured to the nearest 0.1 kg and length/height to the nearest 0.1 cm. In both studies, blood was drawn (venous on Pemba, capillary in Nepal) and used to measure hemoglobin (HemoCue, Angelholm, Sweden) and zinc protoporphyrin (AVIV Biomedical, Lakewood, NJ) concentrations. Trained field workers also asked questions about infant sleep, including: Does the child usually nap? If so, then how long and how often? How many hours does the child sleep during the night? How often does the child wake up during the night? The use of clocks or watches is not common in Nepal or on Pemba Island. However, both cultures have standard ways of telling time that are tied to an agricultural calendar and daily activities. People in both settings are proficient in telling time. In Nepal, the standard is to use current agricultural cycle, sunrise, and sunset to determine the hour. On Pemba, sunrise and sunset are widely used, but people also rely on the 5 daily calls to prayer at the mosques, which occur at fixed times. Also relevant is the fact that in either culture, the mothers did not have a problem with the concept of "hour" and had no difficulty understanding or answering the questions. In addition, in both cultures, unless working, mothers are never far away from their infants and are attentive to signs of hunger, fussing, or waking either during the night or daytime naps.

Parents provided information about their infants' health and family's socioeconomic status. In the Pemba study, malarial parasite counts in blood films[21,22] were made. Both studies asked about fever, rapid breathing, cough, and watery stools in the previous 5 days. The Pemba study also asked about blood in stools. Breastfeeding in the previous week was queried, as was infant's appetite (coded as good/very good vs. so-so/poor/very poor). Parents were asked about their infant's highest achieved motor milestone. Household possessions and house conditions were used to construct indices of socioeconomic status.[15,18] In Nepal, family's caste/religion was recorded as high- or lower-caste Hindu, and Muslim.

Data Analysis

Data Reduction—Anemia was defined as Hb <10 g/dL; iron deficiency (ID) as zinc protoporphyrin ≥90 μmol/mol heme; iron deficiency anemia combined the 2 cutoffs. Both cutoffs were used previously to describe the Pemba Island population[15] and adapted here for consistency. Height-for-age, weight-for-age, and weight-for-height z-scores, respectively were calculated using Epi Info based on the CDC/WHO 1978 international growth reference (Centers for Disease Control, Atlanta, GA). Stunting was defined as height-for-age< −2 SD,

and wasting as weight-for-height z-score < −2 SD. In each study, nap and nighttime sleep duration were summed into "total sleep." Zero values for malaria parasite counts were set to 1; variables were log transformed.

Statistical Analysis—Analysis was performed using STATA 10 (STATA, College Park, TX). Sleep patterns at visit 4 were modeled as a function of sleep at visit 1 and appropriate covariates. Visit 4 instead of 5 was used because of concern about data validity in the Pemba study. When we examined changes in sleep patterns over time, night sleep duration of Pemban infants declined gradually, until visit 5 (mean age 25.7 ± 4.1 months), when it dropped substantially. There was no concurrent increase in illness to explain this sudden decline and we could find no other plausible explanation. More detailed analysis of visit 5 showed that 43% of mothers reported sleep length of 3 hours, possibly due to reporting bias or data entry errors; we did not see unusual data patterns at visit 4. For consistency, we also used visit 4 to examine supplementation effects on sleep in Nepali infants.

Infants with complete data on initial and final sleep patterns, and predictors were included in regression models. In the Pemba study, complete data were available for the following sleep patterns: 696 (does nap), 551 (number of naps), 516 (nap duration), 630 (night sleep duration), 600 (number of wakings), and 457 (total sleep). However, 50 infants had missing data on breast-feeding, further reducing the analyzable sample size. For the Nepal study, 514 (does nap), 405 (number of naps), 403 (nap duration), 479 (night sleep duration), 515 (number of wakings), and 374 (total sleep) infants had complete sleep data. However, there was missing data on zinc protoporphyrin (60), and stunting (1), further reducing the analyzable sample size. There is a sizable difference in response rate between "does nap" and nap frequency and duration because answers on frequency/duration are conditioned on whether infants napped.

All analyses were performed as intention-to-treat in multivariate linear and logistic regressions. Adjustments were made for variables that were unbalanced among treatment groups at baseline. Main effects of treatments, plus their interaction, were tested, first for the full sample, and then stratified by baseline iron deficiency anemia status. If interaction terms were nonsignificant ($p > .10$), they were removed, and models were rerun testing for main effects only.

RESULTS

Background Characteristics of Study Participants

The Nepal sample was slightly younger than Pemban infants and had a higher prevalence of acute rather than chronic malnutrition (Table 1). There were some baseline differences among treatment groups. In the Pemba Island study, groups differed on breastfeeding rates ($p = .006$), with the zinc group having the lowest rate (82.3% compared with 90.4 and 92.8% in placebo and iron groups, respectively). The zinc group also had lower prevalence of anemia, iron deficiency, and iron deficiency anemia (IDA) (Table 1). In the Nepal study, differences were found on sex, hemoglobin, zinc protoporphyrin, stunting, Muslim caste (Table 1), socioeconomic status (higher in the zinc than the iron or combined groups, $p < .01$), and breastfeeding rates (lower in the zinc group, 93.6%, than either placebo 98.7%, $p = .04$ or the combined group, 98.1%, $p = .06$). Table 2 provides a summary of participant flow through the studies, specifying numbers of infants randomized to treatment, and infants with data on sleep at baseline and follow-up.

Treatment Effects on Sleep—Overall

There were no main effects of iron or zinc on sleep patterns among Pemban infants (Table 3), but we found 2 iron–folic acid by zinc interactions: on nighttime and total sleep duration.

Pemban infants receiving iron–folic acid slept a mean 1.1 hours longer at night ($p < .05$) and 1 hour longer overall ($p = .06$) than infants receiving placebo. Infants who received zinc slept a mean 0.7 hour longer at night and 1.0 hour overall ($p = .08$) compared with infants receiving placebo. However, infants receiving both iron–folic acid and zinc did not differ on sleep duration from the placebo group, suggesting a possible antagonism between iron–folic acid and zinc.

Nepali infants who received any iron–folic acid slept 0.3 hours more at night (Table 3; $p < .01$) and 0.4 hours more overall ($p < .01$) than infants not receiving any iron. There was also a positive main effect of zinc on total sleep duration (Table 3).

Supplementation Effects by Iron Deficiency Anemia at Baseline

Among Pemban infants who were non-IDA at baseline, supplementation with iron–folic acid alone or zinc alone resulted in 1.6 hours and 1.3 hours longer night duration, respectively than in infants receiving placebo ($p < .05$, Table 4). Also, infants who received supplemental zinc slept a total 1.7 hours longer ($p < .05$) than the placebo group. Infants who received iron–folic acid together with zinc did not differ from placebo on nighttime or total sleep duration. Among infants who were IDA at baseline, iron–folic acid supplementation resulted in a mean 0.6 hour increase in nighttime sleep duration ($p < .05$). Zinc, either alone or with iron–folic acid reduced the number of nap compared with placebo ($\beta = -0.1$, $p < .05$).

In the Nepal study, infants who were non-IDA at baseline and received any iron–folic acid had slept longer by 0.3 hours (Table 4, $p < .05$) than infants not receiving iron–folic acid. Among infants who were IDA at baseline, iron–folic acid or zinc supplementation reduced the length of naps ($p < .1$ and $p < .05$, respectively; Table 4) compared with placebo. Infants receiving both iron–folic acid and zinc did not differ from the placebo group on mean length of naps. Infants receiving any iron–folic acid experienced longer night sleep duration than infants not receiving any iron–folic acid, whereas infants receiving any zinc had longer total sleep duration than infants not receiving zinc.

DISCUSSION

It is well recognized that iron deficiency anemia affects infant behavior. But most studies describing these findings have been conducted during the daytime. Sleep and nighttime activity of infants with iron deficiency anemia (IDA) have not received equal attention despite the fact that reduced sleep clearly influences the daytime mood, behaviors, and learning of infants, and despite evidence that IDA may affect sleep. We investigated the efficacy of iron–folic acid supplementation (alone or combined with zinc) to improve sleep among infants on Pemba Island and in south-central Nepal. These studies were chosen for the analysis of sleep in infants because they were conducted in settings characterized by moderate-to-high prevalence of IDA and general under-nutrition and were designed as parallel trials.

Our analysis revealed that micronutrient supplementation does affect infant sleep, as assessed by maternal reports. Iron–folic acid supplementation was associated with longer nighttime and total sleep in both studies. The effects of zinc were manifested as longer sleep duration among infants from Nepal, and less napping among infants with IDA in both settings. The increase in nighttime sleep duration was a particularly consistent finding. These results suggest that iron (and/or folate) status is related to infants' sleep. The effects of zinc supplementation on sleep were less consistent between the 2 studies. These inconsistencies and the limitations of the 2 trials indicate the need for additional studies on the effects of IDA on infant sleep.

We chose to present the 2 studies together, despite different cultural, nutritional, and morbidity contexts, because the studies were designed with a comparison in mind. Iron supplementation

trials are often criticized for not being comparable—infants are given different levels of treatment, on a different schedule, for a differing period of time, assessed in differed ways. To address these issues, the Pemba Island and Nepal trials were essentially identical in design: each study had a placebo-control group, infants received the same amount of supplement, made by the same manufacturer, for the same period of time, questions about sleep were identical and conducted on a similar schedule. Setting the 2 trials in different cultures also had a purpose, namely to answer the question of whether iron–folic acid and/or zinc supplementation would be equally efficacious in populations that differed on the prevalence of IDA (moderate in Nepal, high on Pemba), wasting (high in Nepal, low on Pemba), and malaria/parasitic infections (very low in Nepal, high on Pemba). It could be argued that a comparison on functional outcomes (development, sleep) between such different settings should not be attempted but we argue that it is essential to understand under what circumstances iron supplementation will or will not improve infant outcomes.

The use of a randomized design with placebo control is a major strength of both studies. With well-randomized treatments, factors that influence sleep, including differences in recall or time-telling ability, would be distributed equally among treatment groups, essentially rendering those factors noninfluential. The use of a placebo control answers the question of whether changes in sleep are truly due to the active supplement. Due to the internal consistency and validity of each study, we do not need to expect the same effect to consider them together. Any differences in outcomes between the 2 studies would then be discussed in the context of underlying differences in nutritional status, culture of sleep, or time-telling practices. Despite differences in the prevalence of IDA (higher on Pemba than Nepal), however, we found consistent benefits of iron–folic acid on sleep duration, suggesting that iron deficiency is an important predictor in sleep pattern development.

Another difference between the 2 settings is the high prevalence of malaria and parasitic infections on Pemba. These infections have been associated with the presence of anemia[14] and total motor activity[23] in this population. Other studies have also found cognitive and behavioral consequences.[24] In the large supplementation trial, we found that infants who received iron–folic acid had higher likelihood of being hospitalized or dying.[13] Increased rates of adverse outcomes were attributed to high prevalence of malaria. In the present study, 32.6% of infants had malaria parasites in blood. However, the relationship between malaria morbidity and iron deficiency/supplementation is complex and it is unclear how iron–folic acid supplementation would affect sleep in a population where malaria is holoendemic but a clear reduction in anemia rate is expected. Presumably, if iron had a uniformly detrimental effect in a malaria-endemic population, we would expect to see an increase in sleep duration and night waking due to illness. We did observe increased sleep duration due to iron–folic acid supplementation on Pemba, but we also found increased sleep duration in Nepal, where malaria is uncommon. We examined the effects of iron supplementation on sleep among infants who did and did not have malaria at baseline, and found no effects of iron among infants with malaria and reduced sleep duration among infants without malaria (data not shown).

Our findings need to be interpreted with caution because of certain limitations. First, the infants were supplemented for 12 months but because of possible problems with the final study visit on Pemba, we analyzed sleep patterns in both studies after 9 months of treatment. The effects of supplementation after another 3 months might have differed for a variety of reasons, including increased age. Whereas micronutrient supplementation may improve sleep in young infants, it may have no effect as children age. Second, we used maternal recall to determine sleep duration. In addition to typical concerns about recall data, people in these cultures do not use watches. Although both Nepali and Zanzibari populations are quite proficient at telling time and anchor time telling to sunrise, sunset, and other fixed events, the possibility remains that their estimates of sleep duration may not be accurate, or that some people are more accurate

at telling time than others. But because of randomized designs, errors or differences in time telling should have been evenly distributed among treatment groups. In addition, even in Western populations recall of sleep duration may be approximate, and we would expect time keeping to vary among individuals because of differences in number and type of watches used, and the rigor in or reliance on time keeping in daily activities.

People on Pemba and in Nepal use slightly different methods for time estimation, which could lead to differences in findings. However, as mentioned previously, the mothers did not need explanation for the concept of "hour" and had no problem understanding or answering questions about sleep duration. The 2 studies were also remarkably similar in terms of sleeping arrangements and practices—with cosleeping being common and bedtime dictated by the availability of light. Similar also was the practice of letting infants sleep as long as needed and allowing daytime naps, in close proximity to the mother. If sleep duration in our study is interpreted in terms of general trends and not as measures of effect sizes, then our results serve both to provide evidence on the link between IDA and sleep, and to stimulate further research. Additional studies, using more objective measures of sleep, are needed to confirm our findings.

In our baseline report, IDA was associated with longer sleep duration.[11] Here, we found iron-supplemented infants to sleep longer than nonsupplemented infants. At first glance these findings seem contradictory. However, at baseline infants with IDA also woke up at night more frequently than non-IDA infants.[11] Increased night waking may result from increased emotional reactivity—infants with IDA tend to be fearful, clingy, and difficult to soothe.[5,8] It is possible that infants with IDA wake up more easily and remain awake longer than non-IDA infants. Supplementation may reduce emotional reactivity that is related to micronutrient deficiencies, and that may affect sleep through increased endocrine reactivity. Since we did not ask about nighttime activity, the duration of waking episodes, or difficulty in falling back to sleep, further studies need to investigate the involvement of micronutrient deficiencies in these aspects of sleep.

It is also possible that micronutrient treatment increased daytime activity (reduced lethargy), in which case infants would be tired and require longer sleep. We found that iron–folic acid improved attainment of independent walking in Pemban infants.[3] Bangladeshi infants receiving iron and zinc had a smaller decrease in psychomotor development scores and exploratory behavior than infants receiving riboflavin.[4] Zinc-supplemented Guatemalan infants were observed laying down less, sitting up and playing more than nonsupplemented infants.[25] Thus, increased mobility, and presumably, greater exploration, in infants supplemented with iron and/or zinc may be partly responsible for longer sleep duration in our study. To test this, day and night activity/behavior should be examined together.

The primary aim of this study was to examine the effects of iron–folic acid supplementation on infant sleep patterns because there is growing evidence that IDA may be adversely associated with sleep. However, the original design of the studies gave us the opportunity to examine the effects of iron–folic acid combined with zinc. Although the relationship between zinc deficiency and infant sleep is not well studied, the association among zinc deficiency and daytime behaviors and emotions, as well as locomotion[4,25] warrants this examination. Furthermore, potential antagonisms between iron and zinc on functional outcomes have been of interest for programmatic reasons. There is evidence of antagonisms between iron and zinc on iron status and less so on infant development.[26] We found some evidence of iron–zinc antagonisms. For example, among Pemban infants without IDA, iron–folic acid alone and zinc alone increased sleep duration, whereas iron–folic acid with zinc did not differ from placebo. These findings suggest that combined iron–folic acid–zinc supplementation may not yield optimal results, and that antagonism may depend on the underlying prevalence of micronutrient deficiencies.

In sum, we examined changes in infant sleep after iron or iron–folic acid supplementation, combined with zinc. We found increased sleep duration, and conclude that micronutrient supplementation improves sleep in infants. One caveat to our interpretation is that any findings on sleep are best examined in light of concurrent changes in daytime activity, a recommendation we make for future studies. In addition, we recommend that future studies use more objective measures of sleep. Sleep patterns of infants and toddlers with IDA deserve further attention because sleep fragmentation and inadequate sleep have been associated with poorer cognitive and behavioral development.[12,27]

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Katarzyna Kordas analyzed the data and drafted the manuscript. Emily H. Siegel, Deanna K. Olney, Sabra S. Khalfan, Patricia K. Karriger, Steven C. LeClerq, and Subarna K. Khatry participated in study design, data collection, and manuscript revisions. Joanne Katz, James Tielsch, and Rebecca J. Stoltzfus conceived the study designs, secured funding, oversaw data analysis, and participated in manuscript revisions.

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Table 1

Baseline Characteristics of Infants Randomized to Treatment Groups

	Placebo N = 215	Iron/FA N = 223	Zinc N = 219	Combined Tx N = 220
Pemba Island, Zanzibar				
Age (mo)	12.1 ± 3.8[a]	12.4 ± 4.0	12.9 ± 3.9	12.7 ± 4.2
Percent girls	49.8	48.0	53.0	45.0
Hb[b]	9.5 ± 1.6	9.2 ± 1.5	9.6 ± 1.5	9.4 ± 1.5
Percent w/Hb <10[b]	57.3	63.3	53.9[c]	59.5
Percent w/ZPP ≥90[d]	80.7	79.1	72.2[e]	73.0
Percent IDA	53.3	58.2	44.8[c]	52.5
Percent stunted	36.7	36.6	31.6	32.7
Percent wasted	4.2	4.5	4.6	7.7
Percent underweight	34.1	32.7	29.4	31.8
	N = 152	**N = 129**	**N = 125**	**N = 161**
Sarlahi District, Nepal				
Age (mo)	11.2 ± 4.1	10.6 ± 3.7	10.4 ± 4.2	10.9 ± 4.1
Percent girls	53.9	45.0	58.4	44.1[f]
Hb[b]	9.9 ± 1.2	10.0 ± 1.1	10.7 ± 1.2[c,e,g]	10.1 ± 1.3
Percent w/Hb <10[b]	48.6	40.6	20.5[c,e,g]	43.4
Percent w/ZPP ≥90[c]	60.3	68.3	39.0[c,e,g]	63.2
Percent IDA	36.8	35.8	12.3[c,e,g]	35.5
Percent stunted	43.1	29.3[e]	25.6[e]	34.4
Percent wasted	21.2	20.3	26.9	25.6
Percent underweight	58.2	49.6	44.9	52.9
Low-caste Hindu	64.4	85.9	80.7[c,e]	77.4
Muslim	32.2	9.3[e]	7.2[e,g]	16.1[e]

IDA, iron deficiency anemia; ZPP, zinc protoporphyrin; FA, folic acid.

[a] Values shown as mean ±SD.

[b] Units given in g/dL.

[c] Different from iron–folic acid.

[d] Units given in μmol ZPP/mol heme.

[e] Different from placebo.

[f] Different from zinc.

[g] Different from the combined treatment, all $p < .05$.

Table 2

Participant Flow[a]

Randomized	**Placebo N = 215**	**Iron-FA N = 223**	**Zinc N = 219**	**Both N = 220**
Pemba Island, Zanzibar				
Have baseline sleep data	190	205	202	197
Have follow-up sleep data	184	182	184	189
	N = 152	**N = 129**	**N = 125**	**N = 161**
Sarlahi District, Nepal				
Have baseline sleep data	150	127	125	159
Have follow-up sleep data	133	122	119	145

FA, folic acid.

[a]For information on Study 2, refer to Olney et al, [3] and on Study 3 to Tielsch et al.[17]

Table 3

Supplementation Effects on Reports of Sleep in Young Children

	Iron-FA	Zinc	Interaction Term
Pemba Island, Zanzibar[a]			
Does nap[b]	1.0 (0.7 to 1.6)	1.0 (0.6 to 1.5)	—
Number of naps[c]	0.03 (−0.04 to 0.1)	−0.03 (−0.1 to 0.03)	—
Nap duration[c]	−0.05 (−0.2 to 0.1)	0.01 (−0.1 to 0.1)	—
Nighttime sleep[c]	1.1 (0.2 to 2.1)**	0.7 (−0.2 to 1.6)	−1.5 (−2.8 to −0.3)*
Night waking[c]	−0.1 (−0.5 to 0.2)	−0.3 (−0.6 to 0.1)	—
Total sleep[c]	1.0 (0.04 to 2.1)	1.0 (−0.1 to 2.1)*	−1.9 (−3.5 to −0.3)*
Sarlahi District–Nepal[d]			
Does nap[b]	0.9 (0.4 to 2.0)	0.6 (0.3 to 1.2)	—
Number of naps[c]	0.06 (−0.02 to 0.1)	−0.01 (−0.1 to 0.1)	—
Nap duration[c]	0.1 (−0.1 to 0.3)	0.1 (−0.1 to 0.3)	—
Nighttime sleep[c]	0.3 (0.1 to 0.5)**	0.1 (−0.1 to 0.3)	—
Night walking[c]	0.1 (−0.1 to 0.3)	−0.01 (−0.2 to 0.2)	—
Total sleep[c]	0.4 (0.1 to 0.7)**	0.3 (0.03 to 0.5)*	—

FA, folic acid, IDA, iron deficiency anemia; ZPP, zinc protoporphyrin.

[a] Adjusted for baseline sleep, IDA, and breastfeeding; Sample sizes for regression analysis were: n = 646 for does nap, n = 508 for number of naps, n = 476 for nap duration, n = 584 for night sleep duration, n = 600 for night waking, n = 457 for total sleep duration.

[b] Values given as odds ratio (95% CI).

[c] Values given as β(95% CI).

[d] Adjusted for sex, hemoglobin, ZPP, stunting, and caste/religion; adjusted for cluster randomization; robust estimates of variance; Sample sizes for regression analysis were: n = 455 for does nap, n = 354 for number of naps, n = 352 for nap duration, n = 415 for night sleep duration, n = 446 for night waking, n = 327 for total sleep duration.

* $p < .05$,

** $p < .01$.

Table 4

Supplementation Effects Stratified by Baseline IDA Status

	Non-IDA				IDA			
	N	**Iron-FA**	**Zinc**	**Interaction**	**N**	**Iron-FA**	**Zinc**	**Interaction**
Pemba Island[a]								
Number of naps	209	0.1 (0.0 to 0.2)[c]	−0.01 (−0.1 to 0.1)	—	248	−0.03 (−0.1 to 0.1)	−0.1 (−0.2 to 0.01)*	—
Nap duration	190	−0.3 (−0.2 to 0.1)	0.01 (−0.2 to 0.2)	—	236	−0.01 (−0.2 to 0.2)	0.02 (−0.2 to 0.2)	—
Nighttime sleep	251	1.6 (−0.1 to 3.0)*	1.3 (0.01 to 2.6)*	−2.5 (−4.4 to −0.5)*	270	0.6 (0.3 to 1.6)*	−0.2 (−1.2 to 0.8)	—
Number wakings	257	0.1 (−0.4 to 0.6)	−0.4 (−0.9 to 0.1)		278	−0.3 (−0.8 to 0.2)	−0.1 (−0.7 to 0.3)	—
Total sleep	183	1.2 (−0.6 to 2.9)	1.7 (0.1 to 3.3)*	−3.2 (−5.5 to −0.7)*	227	0.7 (−0.4 to 1.7)	−0.1 (−1.2 to 0.9)	—
Nepal[b]								
Number of naps	241	0.1 (−0.02 to 0.2)	0.005 (−0.1 to 0.1)	—	113	0.02 (−0.1 to 0.1)	0.02 (−0.1 to 0.1)	—
Nap duration	239	0.2 (−0.1 to 0.4)	0.1 (−0.1 to 0.3)	—	113	−0.2 (−0.4 to 0.03)	−0.3 (−0.6 to −0.04)*	0.6 (0.2 to 1.0)**
Nighttime sleep	280	0.3 (0.01 to 0.5)*	0.1 (−0.2 to 0.3)	—	135	0.5 (0.1 to 0.9)*	0.3 (−0.1 to 0.6)	—
Number wakings	304	0.002 (−0.2 to 0.3)	−0.1 (−0.3 to 0.2)	—	142	0.2 (−0.1 to −0.6)	0.01 (−0.3 to 0.3)	—
Total sleep	219	0.4 (0.01 to 0.7)	0.2 (−0.1 to 0.6)	—	108	0.4 (−0.02 to 0.8)	0.5 (0.1 to 0.9)*	—

FA, folic acid, IDA, iron deficiency anemia; ZPP, zinc protoporphyrin.

[a] Adjusted for baseline sleep, and breastfeeding.

[b] Adjusted for sex, hemoglobin, ZPP, stunting, and caste/religion; adjusted for cluster randomization; robust estimates of variance.

[c] Values given as β (95% CI).

* $p < .05$,

** $p < .01$.