DOES IRON DEFICIENCY MAKE YOU SLEEPY?

EVIDENCE FROM INFANTS ON PEMBA ISLAND, ZANZIBAR

A Thesis in

Nutrition

by

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Iron deficiency (ID) and iron deficiency anemia (IDA) affect millions of children throughout the world, with children in the first two years of life most severely affected (1). IDA adversely influences child development, behavior as well as cognitive functioning. But very little evidence exists on whether ID is related to infants’ sleep. It has been shown that iron deficiency is exacerbated by the existence of malarial infection, which may also adversely affect sleep. Both ID and malaria are prevalent in Zanzibar, an island nation off the coast of Tanzania.

We address the following questions: 1) Do IDA and non-IDA infants differ with respect to sleep and daytime activity patterns? 2) Is there a relationship between malaria, and infants’ sleep quality? Activity levels were monitored through the use of accelerometers, which were placed on the infants’ left ankle over a three night period. A nighttime period from 10 PM to 4 AM was isolated for analysis. Sleep quality was defined as the number of wake bouts an infant experienced and the fragmentation of sleep.

Daytime periods between the hours of 8 AM and 11 AM and between 3:30 PM and 6:30 PM were analyzed for total activity levels. Multiple linear regression models were used to analyze cross-sectional data from a community based sample of Zanzibari infants (n=45; 5-18 months of age).

The mean hemoglobin level was 9.4 ± 1.4 g/dL; the mean zinc protoporphyrin level was 161.0 ± 150.4 µmol/mol heme, with 55.6 % of the sample being anemic, 66.7 % (ZPP) iron deficient, and 48.9 % iron-deficient anemic. Malaria parasitemia was determined by blood films, with 42.2% of infants being infected. Infants with Hb <10.0
g/dL spent more time moving than infants with higher Hb levels (145.9 ± 142.4 min vs. 86.6 ± 111.9 min, p<0.022) at night and had more fragmented sleep (60.1 ± 40.2% vs. 35.9 ± 33.5%; p<0.017). Infants with ID had significantly more moving minutes than iron-replete infants (137.0 ± 139.5 min vs. 86.4 ± 107.5 min; p<0.040). Total morning activity levels significantly decreased in infants who were infected with 1000+ malaria parasites and had the following: Hb levels <10 (p<0.028), or ZPP ≥ 90 (p<0.025), or IDA (p<0.040). Total afternoon activity levels were not significantly affected by malaria parasitemia or iron status. Using objective measures of nighttime activity patterns, our study confirms an association between infant iron status and sleep quality.
# TABLE OF CONTENTS

List of Tables ........................................................................................................................................ vi
List of Figures .......................................................................................................................................... vii
Acknowledgements ................................................................................................................................... viii

Chapter 1. REVIEW OF THE LITERATURE ....................................................................................... 1
  Developmental Effects of ID .................................................................................................................. 1
  Effects of Iron Supplementation .......................................................................................................... 2
  Sleep in Children .................................................................................................................................. 3
  Measurement of Sleep Behaviors ......................................................................................................... 4
  Iron and Sleep ....................................................................................................................................... 5
  Malaria, ID and Sleep ........................................................................................................................... 7

Chapter 2. JOURNAL MANUSCRIPT .............................................................................................. 11
  Introduction ........................................................................................................................................ 11
  Subjects and Methods ......................................................................................................................... 14
  Results ................................................................................................................................................. 20
  Discussion ........................................................................................................................................... 23

Chapter 3. CONCLUSION .................................................................................................................. 29

Bibliography .......................................................................................................................................... 32

APPENDIX A: Schema of the Life Cycle of Malaria .......................................................................... 37
LIST OF TABLES

Table 1: Characteristics of Study Sample.........................................................38
Table 2: Sleep outcomes of ID and non-ID infants.............................................39
Table 3: Sleep outcomes of anemic and non-anemic infants...............................40
Table 4: Sleep outcomes of IDA and non-IDA infants.......................................41
Table 5: Morning and afternoon activity levels.................................................42
LIST OF FIGURES

Figure 1. Picture of an Actigraph.................................................................43
Figure 2. Example of an actigraphic record......................................................44
Figure 3. Sleep Fragmentation: malaria, Hb interaction.................................45
Figure 4. Sleep Fragmentation: malaria, IDA interaction..................................45
Figure 5. Moving minutes: malaria, Hb interaction..........................................45
Figure 6. Moving Minutes: malaria, IDA interaction.........................................45
Figure 7. Actual Wake %: malaria, Hb interaction..........................................46
Figure 8. Actual Wake %: malaria, IDA interaction.........................................46
Figure 9. Total activity: malaria, Hb interaction................................................46
Figure 10. Total activity: malaria, IDA interaction............................................46
Figure 11. Wake bouts: malaria, Hb interaction..............................................47
Figure 12. Wake bouts: malaria, IDA interaction............................................47
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Chapter 1

REVIEW OF THE LITERATURE

Iron deficiency (ID) is the most common nutrient deficiency worldwide. Over 2 billion people—approximately 30% of the world’s population—are anemic, many due to ID, and in resource-poor areas, this is frequently exacerbated by infectious diseases (2). Malaria, HIV/AIDS, hookworm infection, schistosomiasis, and other infections such as tuberculosis are particularly important factors contributing to the high prevalence of anemia in some areas (3).

Developmental Effects of ID

IDA is associated with adverse behavioral consequences and cognitive deficiencies. Infants and children aged 2 years and younger are especially susceptible to the effects of iron deficiency and anemia. The brain is at its most vulnerable during critical periods of development, including the last trimester of fetal life and the first 2 years of childhood—a period of rapid brain growth termed the “brain growth spurt” (1). Because of the rapid rate of growth and development, these children have an increased biological need for iron in order for proper neurological and physiological development to occur.

IDA has been associated with adverse behavioral consequences. It has been shown that children with IDA are more likely to maintain closer contact with caregivers, show less pleasure and delight, vocalize less, be more wary, hesitant and easily tired, and generally less playful (4). They also exhibit decreased levels of motor activity (5) and are
less likely to explore their physical environment (4), which can lead to “functional isolation” (6), where the lack of stimulation seeking from the environment on the part of the infant leads to decreased stimulation given from the caretaker. These behaviors and the caretakers’ response are thought to delay the acquisition of new skills (7). In a study of 52 Costa Rican infants ages 12-23 months, it was found that iron-deficient anemic infants were more likely to be asleep, irritable, doing nothing, being carried or in bed, and less likely to be on the patio or playing interactively with objects. When the Bayley Scales of Infant Developmental test was administered, the anemic children made fewer attempts at test tasks, were less playful and had poorer attention than non-anemic children (4).

**Effects of Iron Supplementation**

The effects of supplementation with iron and the timing of that supplementation appears to affect the results/effectiveness of treatment (8). In a study of (n=1657) Costa Rican infants, supplementation in order to prevent ID was shown to significantly increase soothability and decrease tremulousness (9). On the other hand, infants who did not receive supplemental iron processed information more slowly, were less likely to show positive affect, interact socially, or check their caregivers’ reactions, crawled somewhat later and were more likely to be tremulous (9). In a follow-up in adolescence of Costa Rican infants who were born at term and were free of health problems other than ID, there were persisting motor differences, more grade repetition, anxiety/depression, social problems and inattention, and a widening gap in cognitive scores to 19 years, despite iron therapy that corrected IDA in infancy (10). Rodent models have shown that decreased
brain iron content, electrophysiological alterations, neurotransmitter changes, and behavioral alterations persist despite iron treatment when iron-deficiency anemia occurs in infancy (8,11). Although this baseline analysis of our data is only a pilot study, the longitudinal analysis of our data should give further insight about the effects of supplementation.

**Sleep in Children**

The daytime behaviors associated with IDA have been well documented. However, much remains unknown about how IDA affects the nighttime sleep patterns and sleep quality in infants. Sleep is vital to an infant’s health and development and is the primary brain activity of the first years of a child’s life (12). Therefore, if sleep is disrupted or insufficient, factors that influence the sleep-wake cycle should be examined.

Increased sleep is associated with increased approachability, rhythmicity, and adaptability (13). There is evidence that sleep enhances memory and learning, particularly in language development (14,15), and that minor but persistent disruption of sleep may have long-term implications for cognitive performance (12). Sleep deprivation has also been shown to have adverse effects on mood (16). Furthermore, a study of n=35 children of ages 8.2 ± 0.4 months showed that even mild reductions in the opportunity to sleep in young children are associated with substantial difficulties in attention and impulsivity control (17). Sleep deprivation is also associated with altered measures of immune function (18) as well as clear decreases in alertness, vigilance and decision making (19). Higher sleep fragmentation and activity level during the night were
associated with lower scores on the Mental Development Index (MDI) in 10-mo old infants (20).

Other studies have shown that certain forms of learning are enhanced by sleep and impaired by sleep loss. One study showed that sleep deprived adults performed significantly worse on verbal tasks than controls who experienced normal sleep (21). In a study of baby birds, it was shown that after hearing the mother’s song, the birds that slept during a rest period learned the song faster and more accurately than the birds that merely rested during the same time period (22).

In the first months of life human infants show dramatic brain development. One of the main sleep changes is the transition from quiet sleep to four differentiated stages of non-rapid-eye-movement (NREM) sleep by around 4 months (23). NREM sleep is characterized by the appearance of sleep spindles (a burst of brain activity visible on an EEG that occurs during stage 2 sleep. It consists of 12-16 Hz waves that occur for 0.5 to 1.5 seconds) in an EEG test (23). There is some evidence that sleep spindles are markers for the ability to learn certain types of tasks (24). These sleep spindles have been hypothesized to be a marker of normal brain function and development (23). Proper myelination is necessary for the development of sleep spindles (23). Because sleep plays such an integral part in infant brain development, greater understanding of the underlying causes of sleep disruption is warranted.

**Measurement of Sleep Behaviors**

Sleep behaviors can be measured in different ways. The advantage of actigraphy over the more traditional polysomnography is that actigraphy can conveniently record all
movements for 24-hours a day across several days. It is also more cost effective, less invasive, can be worn comfortably, and is a valid measure of sleep (25). Another advantage of actigraphy is that it makes home recording more accessible to patients in their home environment which eliminates the laboratory effects that could alter usual sleep patterns (25). Actigraphy appears useful as an outcome measure and may be useful in monitoring circadian rhythm patterns or disturbances in children (26).

Iron and Sleep

Iron plays an important role in brain development, and is particularly important in myelination, neurotransmission, dendritogenesis, and synaptogenesis (10). Studies in animal models have shown that ID disrupts these processes in a region-specific manner, depending on which brain areas are rapidly developing at the time of the deficiency (10). Since adequate iron is required for normal myelination and dendritogenesis, alterations in these processes due to IDA could impact function in many brain systems, including those that underlie the occurrence of sleep spindles (23).

Sleep is regulated, in part, by dopamine, a neurotransmitter found in the brain (27). In rodent models it has been shown that the distribution of iron and dopamine in the brain are closely related, with particularly high concentrations of both found in the adult striatum (8). The striatum (part of basal ganglia) is richly innervated with dopaminergic fibers that become involved in networks that relate to higher order cognitive functions and emotional processes, procedural or implicit memory, motivated behavior, positive affect, reward-related processing, motor functioning (10), and regulation of sleep (28). Neuromodulation by the dopaminergic system plays an important role in sleep regulation
particularly in the basal ganglia which are high in iron concentration and are more interconnected with REM-regulation than other brain regions (28).

Some of the changes induced by early ID in the basal ganglia are not corrected with iron supplementation (11,28). Two studies have used actigraphy to study effects of iron deficiency anemia on sleep. These two studies of Chilean infants had small sample sizes (n=26-35) and were not randomized controlled trials (5,29). One of the Chilean studies found that IDA was associated with reduced motor activity even after iron treatment (5). The other study of Chilean infants found that at 6 months of age there were no differences between anemic and non-anemic infants in time per sleep state (awake, asleep, etc). However, infants with IDA showed an overall increase in motor activity compared to controls. These differences were no longer observed at 12 and 18 months of age (29). In a study designed to measure the effects of discontinuing coffee intake on toddlers’ cognitive development and sleep, the Guatemalan children did not show that reduction in coffee intake influenced iron status (30), which leaves unanswered the question of whether or not improved iron status would have affected sleep behaviors. These studies leave gaps in our understanding about how ID and IDA affect infants’ sleeping behaviors. Would iron supplementation at an earlier or later stage in the Chilean studies (5,29) result in different outcomes? Are the effects of IDA reversible, and if so, at what time frames is supplementation needed to reverse these effects? Are the number of wakings and the fragmentation of sleep specifically influenced by IDA? The relationship between IDA and sleep should be studied more completely in double-blind, randomized, placebo-controlled studies.
**Malaria, ID and Sleep**

Malaria is a common cause of morbidity and mortality among populations where it is endemic (3,31). More than 40% of the world’s population lives with the risk of malaria, with the overwhelming burden affecting children under the age of 5 years in sub-Saharan Africa (32), with approximately 785,000 African children suffering from cerebral malaria (33).

The presence of malaria is well documented among the Tanzanian population (3). Malaria has been shown to affect the physical health and cognitive development in children. One study found that school age children in Kenya who were treated for malaria had higher scores for sustained attention than the placebo group (34). The treatment group also had lower prevalence of anemia than the control group (34). Another study reported that the academic performance of children experiencing an acute attack of uncomplicated malaria was poorer than that of children experiencing an acute attack of non-malarial fever or that of the other healthy controls (35). In a study that compared children with a history of severe malaria and impaired consciousness to control children on tasks measuring information processing, language and behavior, a significant number of cases showed impaired performance e.g. deficits in measures of language and attention and planning (36). Another study found that children with a history of cerebral malaria in Ghana performed significantly poorer than controls in bimanual tactile discrimination, accuracy of visual screening, visual memory, perceptual learning and rule learning skill, right ear auditory information processing, and dominant-hand motor speed. No significant differences were found in non-verbal reasoning, visual-spatial processing, auditory attention and verbal fluency (37). More studies have also noted deficits in
cognitive functioning in children that have experienced cerebral malaria, particularly
deficits in speech and language, attention, memory, and non-verbal functioning (33,38-
40). Another study found that malarial infection significantly predicted less total motor
activity in crawling infants (41).

Iron and malaria share a complex relationship. Iron has proven to be a necessary
micronutrient for normal growth and development of organisms. Iron also plays a critical
role in the relation to the host’s interactions with pathogens (viruses, bacteria, and
protozoa) (42) and finding the delicate balance of optimal iron status is challenging. The
World Health Organization Guidelines for Treatment of Severely Malnourished Children
advise withholding iron until wide-spectrum antibiotics have been used to control
bacterial infection (43), and another study showed that elevated ferritin levels strongly
predicted earlier mortality in HIV patients (44). For malaria specifically, Prentice (42)
outlines four regularly cited suggestions as to possible means by which iron status might
influence susceptibility to malaria: 1) Alterations in iron availability for parasite growth
and replication. The parasite is dependent on the small pool of iron in the cytoplasm and
hence might be sensitive to nutritional influences on the concentration of iron in this
compartment. 2) Iron supplementation might enhance susceptibility by stimulating
erthropoiesis because there is evidence that parasites have a preference for reticulocytes.
This is only true for *P. vivax* and would not explain effects on *P. falciparum* as has
sometimes been erroneously claimed. 3) Zinc protoporphyrin (a product of iron-deficient
erthropoiesis) may inhibit hemozoin formation and hence generate a toxic environment
in a manner similar to that of an anti-malarial drug action. 4) Iron’s influence on host
immunity. Iron repletion contributes to improved immune function (42). Anemia is a
common complication in chronic and acute malarial infection, with the consequences being more pronounced with *Plasmodium falciparum* malarial infection (45). Approximately 300-500 million people are infected worldwide each year (46), and a substantial proportion of these patients live in parts of the world where malaria is endemic.

Our study aims to examine how IDA affects the sleep-wake cycle in infants who participated in a double-blind, randomized, placebo-controlled study conducted on Pemba Island, Zanzibar, where malaria is endemic. Malarial infection is related to hemoglobin levels in infants (3), may impact outcomes of iron supplementation, (47) and contributes to developmental deficits (32). Symptoms of malaria include fever, chills, sweats, headaches, nausea and vomiting, body aches, general malaise (48). All of these symptoms could possibly contribute to altered sleep patterns in children who become infected with malaria. Stoltzfus and colleagues showed that fever was more common among children less than 30 months of age who had ≥ 5000 parasites/µL blood, and that sex, recent fever and malaria parasite density were strongly related to hemoglobin concentrations (3). They also found that children in this age group who were recovering from fever were more anemic. The malaria parasitemia associated with the lower hemoglobin values increases the risk of severe anemia, which could impact the regular sleeping habits of an infant or exacerbate his existing sleep problems.

Because there is still so much that is unknown about the relationship between iron deficiency, sleep and malaria, and because so many individuals are affected by this relationship, further investigation is necessary. With this study, we have the ability to characterize the effects of IDA on infants’ sleep at night and activity during the day. We
used actigraphic measures of leg movements to characterize night sleep activity and fragmentation, and total activity levels during the day. In short, we have the unique opportunity to begin closing the gap in our understanding of IDA effects on sleep-wake patterns in infants.
Chapter 2

JOURNAL MANUSCRIPT

Introduction

Iron deficiency (ID) and iron deficiency anemia (IDA) affect millions of children worldwide, particularly in the first two years of life (2,49). ID and IDA are prevalent in the countries of sub-Saharan Africa (3), including Zanzibar, off the east coast of Tanzania. Anemia in these communities is prevalent and severe, particularly among women and young children (50), and may be partly explained by the endemic malarial infection.

The developmental consequences of IDA have been well documented, particularly as they relate to daytime behaviors. One study of infant rhesus monkeys found that the iron-deficient infant monkeys had reduced spontaneous motor activity compared to iron sufficient controls (51). Another study found that infants with IDA are more likely to be distracted, fatigued, wary, and hesitant (4). Infants with IDA have deficits in their ability to regulate emotions and are not as easily soothed by caregivers as non-IDA infants (4).

On the other hand, the effects of ID and IDA on sleep and nighttime behaviors are not well understood. Sleep is vital to an infant’s health and development and is the primary brain activity of the first years of a child’s life (12). In a recent review of the literature, a strong case is made for a connection between sleep and ID (52). Results from several studies suggest that infants with IDA do not follow the normal neuromaturational pattern (5,23,28,29) and that IDA could alter the ongoing process of synaptogenesis and apoptosis, which remolds neuronal circuits, thus contributing to neurocognitive and neurobehavioral deficits (5,23,28,29). These processes are dependent
upon the presence of iron (10) for normal development. Peirano et al. found that the sleep spindles of infants with IDA were less dense and had a lower frequency and longer inter-spindle intervals during NREM sleep than in iron-replete infants (23), suggesting that iron is also essential for the normal development of sleep patterns.

In addition, there is evidence that IDA affects the number of nighttime wake bouts in deficient infants. We found that infants with IDA were more likely to have increased night waking and reduced total sleep duration, as reported by mothers (53). In one study of Restless Legs Syndrome (RLS), low body iron stores were associated with periods of insomnia in adolescents (54), and another study found that RLS symptoms were augmented by low levels of ferritin among subjects receiving dopaminergic therapy (55).

Sleep patterns and nighttime behaviors may influence the behaviors and activity of infants during the day. Sufficient sleep has been shown to lessen fatigue, increase soothability, and encourage exploratory behaviors (13,56). Increased sleep is associated with increased approachability, rhythmicity, and adaptability (13). On the other hand, restlessness and sleep disturbances at night could exacerbate daytime problem behaviors (56). With millions of young children affected by ID and IDA, it is important to understand the relationship between iron status and sleep and between sleep and behavior of infants.

An important consideration for the study of IDA and developmental outcomes in children living in malaria-endemic regions such as Zanzibar is the effect of malaria on those outcomes. One study found that malaria parasitemia significantly predicted lower total motor activity scores and less time spent in locomotion in infants who were able to crawl (41). Malaria has also been associated with deficits in cognitive function,
specifically language and attention, in children exposed to acute infections of *P. falciparum* (35,36), with greater degree and duration of impairment being consistently associated with increased severity of disease (32).

In this study we investigated the association between iron status and night and daytime activity patterns in 5-18 month old infants measured during the baseline evaluation of a double-blind randomized placebo controlled trial of iron-folic acid and zinc supplementation conducted on Pemba Island, Zanzibar. Specifically, we examined infant daytime activity levels, sleep fragmentation, time spent awake, mobility, and total activity during the night. We hypothesized that infants with IDA will have lower total activity levels during the day than non-IDA infants, and that infants with IDA will spend less time asleep and more time moving than non-IDA infants. Consequently, infants with IDA will have a higher sleep fragmentation index than iron-replete infants. Furthermore, because malaria is also believed to aggravate anemia and iron deficiency (57), we examined the relationship between malaria, daytime activity and sleep quality in infants, hypothesizing that infants with malaria would have poorer quality of sleep and would be less active during the day compared to infants without malaria. We also explored potential effect modification between malaria and iron status on infants’ daytime activity and sleep patterns.
Subjects and Methods

Study population and sample.

This study was carried out on Pemba Island, which is the smaller and northern of the two islands that comprise Zanzibar, located off the east coast of Tanzania. The two main occupations on the island are fishing and subsistence farming. The staple foods are cassava and rice, which are supplemented with vegetables and small amounts of meat. Infants are typically breastfed after birth, with complementary feeding beginning at a few months of age. By the age of 2 years, children are generally weaned onto the family diet. Malaria (caused by *P. falciparum* and *P. malariae*) is holoendemic, with year-round transmission that is highest in June—September, following the rainy season of April—May (3,47). Helminth infections (parasitic worm infections) are also highly endemic (3).

The study took place between March 2002 and July 2003. The sample was 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 of young children (47). All children on Pemba Island who were between 1 and 35 months of age were invited to participate in the trial. Eight municipalities were selected from the main trial and were invited to participate in a Child Development sub-study. All infants aged 5-18 months were invited and 932 infants were enrolled in the sub-study after parental consent was obtained. The study was approved by human subjects review committees at the Johns Hopkins Bloomberg School of Public Health, the Zanzibar Health Research Council, the University of California at Davis, and Cornell University. The study received exempted status and IRB approval was waived for secondary data analysis of
de-identified data by the Office of Research Protections at The Pennsylvania State University.

At the baseline assessment, the actigraphs were pilot-tested with a small number of children (n=50) who wore the devices continuously for three days. There were only 50 infants chosen because the first round of data collection in order to ascertain the viability of using Actiwatches on a larger group. Infants were randomly selected to participate in this pilot study. Of those infants, 4 had missing data on ZPP and IDA status, 2 of those four were missing data on malaria infection status, and 1 infant was missing age data.

**Assessments**

The study began with household visits to carry out intensive behavioral observations and activity measurements. The day before the observations started, field workers placed an actigraph (Actiwatch, MiniMitter Co., Bend, OR) around the infant’s left ankle to be worn throughout the day and night for three consecutive days. An actigraph is a small, watch-like device (28 x 27 x 10mm, 16 g) that can be worn comfortably by infants without movement restriction (Figure 1). It makes recordings of movement intensity in 15-second epoch lengths, when it detects movement in any plane. The data are displayed graphically as an actigram (Figure 2) and can be analyzed for sleep patterns using specialized software (Actiware, MiniMitter Co., Bend, CO). For this study, the sensitivity of the Actiwatches was set to medium, with the wake threshold set to a value of 40 movement units.
During the data acquisition phase, actigraphic data were collected and averaged over the three days. For the night period, the software calculates several sleep patterns. We focused on the following outcomes: **actual wake (%)** refers to the percent of time infants were awake during the night, **wake bouts** is the number of times an infant has sufficiently high activity to be considered awake (>40 movement units for each 15 second epoch), **moving minutes** is the total number of minutes during the night the infant is moving, **total activity score** is the total of all activity levels for the period of interest, **sleep fragmentation index** is the sum of activity level during non-wake periods and immobile minutes, divided by total sleep time. Higher sleep fragmentation and activity level during the night were associated with lower scores on the MDI in 10-mo old infants (20).

For the nighttime sleep analysis we examined the hours between 10 o’clock PM and 4 o’clock AM. These hours were chosen because they corresponded to the normal bedtime hours of the infants in this population, thus increasing the likelihood that the movements recorded by the actigraphs were the movements of the infants during their sleep and not the movements of the mother carrying the baby. For the morning and afternoon assessments, we analyzed activity between the hours of 8 and 11 AM, and between 3:30 PM and 6:30 PM. These times were chosen to correspond with the times of greatest activity of the largest number of infants (based on the previous visual examination of the graphed data) and to avoid most common nap times. The total activity level for each child was calculated separately for the morning and afternoon periods and these were used as dependent variables in the analysis.
At the time of the home visit, caregivers also completed an interview that included questions on the child’s appetite, motor and language development, temperament, illnesses, and sleep habits, as well as demographic characteristics, as described previously (53). Specifically, parents were asked to rate their infant’s recent appetite as very good, good, so-so, poor, or very poor. They indicated which motor and language milestones the infant had achieved, answered questions about infant’s typical behavior, and illnesses during the previous 5 days (fever, rapid breathing, watery stools, cough, blood in stool). They also indicated whether the infant was breastfed in the past week. Socioeconomic status (SES) was computed from information based on a combination of household variables (possessions, father’s occupation, quality of house, etc). In terms of sleep habits, parents provided information on the number and duration of daytime naps, the duration of nighttime sleep, and the number of night wakings. The relationship between parental sleep reports and iron status has been reported elsewhere (53).

On a separate day, parents brought their infants to village clinics, where children were weighed to the nearest 0.1 kg using a digital scale (Seca, Columbia, MD), and measured to the nearest 0.1 cm using a wooden length board (Shorr Productions, Olney, MD). A 3 mL venous blood sample was collected by a trained phlebotomist into a Vacutainer tube (Becton Dickinson, Franklin Lakes, NJ, USA). A few drops of blood were removed from the tube to measure hemoglobin (Hb) and zinc protoporphyrin (ZPP), using HemoCue hemoglobinometer (HemoCue, AB, Angelholm, Sweden) and a hematofluorometer (AVIV Biomedical, Lakewood, NJ), respectively. ZPP was chosen as a marker because it was conducive to the conditions in the field. Additionally, the
generally accepted gold standard for iron status is diminished stainable iron in a bone marrow aspirate, a costly and invasive test that has subjective results. Therefore, ZPP was chosen because it is a good reflection of marrow iron status for erythropoiesis. Blood films were made to count malaria parasites. Malarial parasite count was done until more than 200 leukocytes were counted; if no parasites were seen, assessment was extended until 500 leukocytes had been counted. This process continued up to 800 leukocytes. Standard quality control procedures were implemented for malarial parasite counting, with every observer reading, blinded, his or her own slides from the previous day (within-observer estimation) and those of other observers (between-observer estimation) (47).

**Statistical Analysis**

Statistical analysis was conducted using STATA 8.0 (STATA Corp., College Park, TX). We examined distributions of activity and sleep measures with scatter plots, and log-transformed any non-normally distributed data. Iron deficiency was defined as ZPP levels ≥ 90 μmol/mol heme, and anemia was categorized as hemoglobin levels <10 g/dL. Infants were categorized as having IDA if they met both of the above criteria. Malaria parasite density was highly skewed and was therefore divided into a categorical variable, with the three categories based on infection intensity (0=0, 1=1-999, 2=1000+ parasites/μL blood) for use in the regression models. These divisions were based on previous work conducted in Zanzibari children 0-5 years old (3).

Multiple linear regression models were used to investigate associations between iron status, sleep patterns, and daytime activity, as well as between malaria, sleep patterns and daytime activity. ID, anemia, and IDA were entered as independent variables in
separate models. All analyses were first carried out with hemoglobin and ZPP as continuous variables, and then again as categorical variables. Sex, age, motor milestone attainment (able to walk alone, or not) and malaria parasitemia were controlled for in all the models where iron status was modeled as an independent variable.

During our exploratory analysis of the interactions between iron status (Hb and ZPP) and malaria infection we created a variable within STATA that included the two variables and entered that new variable into our model. The results were graphed for visual inspection and interpretation.

We selected covariates based on biological plausibility and statistical considerations (p<0.25 in relationships between covariates and outcomes). Variables were selected as covariates if they were associated with at least 3 of the 5 sleep outcomes. Sex, age, socioeconomic status, and infant mobility (i.e. whether or not the infant could walk alone) fit these criteria. Socioeconomic status was considered for inclusion in the model but did not significantly change any of the relationships between blood measures and sleep and activity outcomes, possibly due to the small sample size, and we therefore excluded it from the analyses. We tested adherence to regression assumption with standard regression diagnostics tools.
Results

Sample Characteristics, anemia, iron status and malarial infection.

The mean age of the infants was 12.3 ± 3.7 months at the time of their first clinic visit (Table 1). Of the 45 infants, 31.1% were able to walk without assistance (Table 1). Mean ZPP levels were (161.0 ± 150.4 µmol/mol heme), with 66.7% of infants having ID (Table 1). Anemia was present in over half of the sample, with 55.6% of infants having hemoglobin (Hb) < 10 g/dL and 15.5% of infants having Hb values < 8.0g/dL. Malaria parasitemia (defined as ≥ 1 parasites/µL blood) was present in 42.2% of infants. Parent-reported illness was not related to malaria parasitemia; none of the 8 infants whose mothers reported symptoms of illness showed any evidence of malarial infection (data not shown).

Is there a relationship between malaria, iron status and sleep quality in infants?

Infants with ZPP levels ≥90 moved significantly more than infants with normal ZPP levels (Table 2, p<0.040). When ZPP was included in the model as a continuous variable, total nighttime activity significantly increased in infants as their ZPP levels increased (p<0.008). Minutes spent moving during the night, an indicator of poor sleep quality, was significantly higher among infants with hemoglobin values less than 10.0 g/dL (Table 3, p<0.013), and their sleep was significantly more fragmented (p<0.006). Using logistic regression, we assessed the likelihood of children having high sleep fragmentation (defined as fragmentation index ≥80%) and found that the odds ratio was 12.9 for infants that had Hb values <10.0 g/dL (p=0.007) compared to infants with higher Hb. Finally, infants with IDA spent significantly more time moving during the night (Table 4, p<0.009) and had higher mean fragmentation index (p<0.028) than non-IDA...
Infants. Infants with IDA were more likely to have highly fragmented sleep (OR=6.3, p<0.029) than infants without IDA. Malaria parasitemia was not independently associated with sleep outcomes.

Regression diagnostics conformed to linear regression assumptions, except for the models that included moving minutes as the outcome. For these latter models, we ran a sensitivity analysis removing two points identified as being highly influential in leverage plots. We found that when the two influential points were removed, ZPP remained a significant predictor of moving minutes, both as a continuous and categorical variable, and our conclusions were unchanged. When the two influential points were removed, and models rerun testing the association of hemoglobin with sleep, hemoglobin (entered as a continuous variable) remained independently and significantly (p<0.004) associated with the number of moving minutes, but malaria also became independently associated (p<0.045) with the number of moving minutes. The results were similar when hemoglobin was entered into the model as a categorical variable. Hemoglobin was significant (p<0.001), and malaria became a significant predictor (p<0.03) when there were more than 1000 parasites were present. In the IDA models when the two most influential points were removed, IDA remained a significant predictor (p<0.000) and malaria (when more than 1000 parasites were present) became a significant predictor of the number of moving minutes experienced by the infants (p<0.000).

*Is there a relationship between malaria, iron status and daytime activity levels?*

The mean morning activity level of the infants measured at baseline was 57002 ± 23472 activity units. Neither ZPP, Hb, or IDA was significantly associated with daytime activity levels (Table 5). When adjusted for covariates in the ZPP model, children who
had 1000 malarial parasites had lower morning activity levels by 26155 units (p<0.008) compared to children with no parasites. The difference in activity levels between children with no or high malaria infection was very similar when the models were adjusted for Hb or IDA. Afternoon activity levels averaged 55226 ± 23696 activity units. Morning and afternoon activity levels were highly correlated (Spearman's rho = 0.6232, p<0.001).

During this period of time neither hemoglobin, ZPP, IDA nor malaria were significantly associated with infants’ activity levels.

**Exploratory analyses: iron status and malaria**

We tested whether there were any interactions between iron status indicators and malarial infection on nighttime sleep and daytime activity (Figs. 3-12). Given the fairly small sample size (n=50), firm conclusions cannot be drawn from these analyses; however, the findings were interesting and merit further investigation. We found that infants who were anemic moved significantly less (p<0.015) (Fig. 5) and had significantly less fragmented sleep (p<0.023) (Fig. 3) as malaria parasitemia increased. Infants with IDA also had significantly fewer moving minutes (p<0.000) (Fig. 6) and had significantly less fragmented sleep (p<0.000) (Fig. 4) than iron-replete infants as malaria parasitemia increased. The interactions between malaria and the following variables were not significant: actual wake percentage, total nighttime activity score and number of wake bouts (Figs. 7-12).
Discussion

The most salient finding of this study was the increased movement time and increased sleep fragmentation in ID and anemic infants. Angulo-Kinzler et al. found that IDA infants generally spent more time awake in the night and less time in quiet sleep also during the night (29). Kordas et al. also found that infants with IDA had higher odds of sleeping less than 11 hours in total and woke more during the night than non-IDA infants (53). Both studies’ findings are consistent with our results. However, in another study of infants with IDA, researchers found no differences between anemic and non-anemic infants in total recording time, duration of sleep, or motor activity during sleep (5). Still, infants with IDA showed reduced motor activity during waking at all ages and the magnitude of the differences increased at 12 and 18 months (5). Infants in our study showed decreased activity levels during the morning, but only if they were infected with more than 1000 malarial parasites.

Infants with ID and IDA in our study spent more time moving during the night and had more fragmented sleep (which could result from the increased movement). This increase in movement during the night is similar to what is seen in patients with Restless Leg Syndrome (RLS). One study of adolescents showed that symptoms of RLS and insomnia were associated with low body stores of iron, and these symptoms were diminished by iron supplementation (54). This study examined three teenagers who presented with severe sleep onset insomnia, subjective sleep latency exceeding 60 minutes and excessive daytime sleepiness. The three teenagers were found to have RLS and laboratory evaluation confirmed reduced body stores of iron with a low percent iron saturation (mean value 9.7%) and a low serum ferritin level (mean value 17 mg/l). None
had marked anemia. The three patients were treated with oral iron for 4–5 months. As a
group they had an increase in percent iron saturation (from a mean of 9.7 to 22.7%) and
serum ferritin (from a mean of 17 to 27 mg/l) and a marked reduction of the symptoms of
RLS, with mean subjective sleep latency decreasing from 143 to 23 min, sleep efficiency
increasing from 75.7 to 84.0% and the number of periodic movements per hour of sleep
decreasing from 20.5 to 10.5.

Another study (55) compared patients who experienced augmentation in RLS
symptoms with patients who did not experience augmentation. They found that
augmentation of symptoms causing premature discontinuation from the study or which
were tolerated (n = 36, ferritin: 85 + 59 ng/ml) were associated with lower levels of
serum ferritin compared to patients without augmentation (n = 302, ferritin: 118 + 108
ng/ml, p = 0.0062). Finally, Picchietti et al. (58) reported that low serum ferritin levels
were associated with increased severity of RLS in adults as well as in children, and an
increased prevalence of RLS has been found in iron-deficiency anemia as well as in
blood donors.

The relationship between sleep, IDA and malaria is complex and the mechanisms
behind their interactions are not completely understood. Iron plays an important role in
brain development, and is particularly important in myelination, neurotransmission,
dendritogenesis, and synaptogenesis (10). Studies in animal models have shown that ID
disrupts these processes in a region-specific manner, depending on which brain areas are
rapidly developing at the time of deficiency (10). Since adequate iron is required for
normal myelination and dendritogenesis, alterations in these processes due to ID could
impact function in many brain systems, including those that underlie the occurrence and
organization of sleep spindles (23). These sleep spindles have been hypothesized to be a marker of normal brain function and development (23), and the ability to learn certain types of tasks (24). Proper myelination is necessary for the development of sleep spindles (23). Infants spend more time asleep than awake in the first two years of life (12), suggesting that sleep is an important factor for normal growth and development. Our study revealed that iron deficiency with and without anemia, both prevalent among Zanzibari infants, was associated with altered sleep patterns. Malarial infection was also prevalent but was not independently associated with poor sleep outcomes. Iron deficient infants spent significantly more time moving during the night than iron-replete infants. Anemic infants moved more and had more fragmented sleep than non-anemic infants during the night.

Sleep is regulated, in part, by dopamine, a neurotransmitter found in the brain (27). In rodent models it has been shown that the distribution of iron and dopamine in the brain are closely related, with particularly high concentrations of both found in the adult striatum (8). The striatum (part of basal ganglia) is richly innervated with dopaminergic fibers that become involved in networks that relate to higher order cognitive functions and emotional processes, procedural or implicit memory, motivated behavior, positive affect, reward-related processing, motor functioning (10), and regulation of sleep (28), including the modulation of REM sleep, quality, quantity and timing (28). ID has also been shown to reduce D1 and D2 receptor levels and dopamine transporter levels in rats (59), which could influence sleep patterns.

Sleep deprivation has been shown to have adverse effects on mood (16) and studies show that even mild reductions in the opportunity to sleep in young children are
associated with substantial difficulties in attention and impulsivity control (17). Sleep deprivation, along with iron deficiency, is also associated with altered measures of immune function (18) as well as clear decreases in alertness, vigilance and decision making (19). One study of low-risk infants found that higher motor activity during sleep and more episodes of night waking were negatively correlated with cognitive scores, and high sleep efficiency was correlated with higher cognitive attainment (20). It is possible that cognitive and behavioral deficits and IDA in infants are mediated by fragmented sleep.

Iron and malaria also share a complex relationship. In our sample, anemia was present in 55.6% of infants. Anemia is a common complication in chronic and acute malarial infection, with the consequences being more pronounced with *Plasmodium falciparum* malarial infection (45). In our sample, approximately 42.2% of infants were infected with malarial parasites, mostly of the *P. falciparum* variety (3). Iron is a necessary micronutrient for normal growth and development of humans and other organisms. Iron plays a critical role in the relation to a host’s interactions with pathogens (viruses, bacteria, and protozoa) (42). On the other hand, many studies have shown that iron deficiency is associated with cognitive and developmental delays and other adverse health outcomes (1,9,11,41). Malaria has also been associated with deficits in cognitive function, specifically language and attention, in children exposed to acute infections of *P. falciparum* (35,36), with greater degree and duration of impairment being consistently associated with increased severity of disease (32).

It is unclear how the presence of malaria would affect the relationship between iron status, activity patterns and sleep. We explored these interactions and found that
infants who were anemic moved less and had less fragmented sleep as malaria parasitemia increased. Infants with IDA also had significantly fewer moving minutes and had significantly less fragmented sleep than iron-replete infants as malaria parasitemia increased. Due to our small sample size, we could not confidently compute the statistical interactions between iron status indicators and malaria parasitemia, but these unexpected results provide encouragement to further explore this relationship. One possible explanation of the decrease in activity levels in the IDA infants with high malaria parasitemia levels could be that the fatigue caused by the malaria parasitemia is compounded when combined with low iron levels. Stoltzfus and colleagues have shown that malarial infection is related to hemoglobin levels in infants (3), may impact outcomes of iron supplementation, (47) and contributes to developmental deficits (32). It is also possible that malaria affects sleep due to the symptoms associated with the disease. Symptoms of malaria include fever, chills, sweats, headaches, nausea and vomiting, body aches, general malaise (48,60). All of these symptoms could contribute to altered sleep patterns in children who become infected with malaria. Stoltzfus, et al, showed that fever was more common among children less than 30 months of age who had ≥ 5000 parasites/µL blood, and that sex, recent fever and malaria parasite density were strongly related to hemoglobin concentrations (3). They also found that children in this age group who were recovering from fever were more anemic. The malaria parasitemia associated with the lower hemoglobin values increases the risk of severe anemia, which could impact the regular sleeping habits of an infant or exacerbate their existing sleep problems. Our results indicate that malaria negatively influences infant activity, particularly in the morning, and once malaria parasitemia levels are over 1000. Longitudinal analysis of our
data will provide us with more statistical power to determine the relationship between malaria and iron status.

Our study has some limitations, particularly the small sample size. With only 50 participants, we were unable to determine if there were any interactions of statistical significance between malaria and iron status variables, a relationship which should be investigated more carefully in light of our preliminary findings. Despite the small sample size, the activity data in this study was collected over a three day period and averaged, which gave us a good idea about the movement, activity levels and sleep patterns of the infants. The infants were selected completely at random without prior knowledge of their iron status, greatly reducing selection bias. There were no significant differences between infants who were included in the analysis and those who were excluded (data not shown).

In sum, we found that infants with poorer iron status spent more time moving at night and had more fragmented sleep than iron-replete infants. Morning activity levels were also negatively influenced by malarial infection, particularly if the level of parasitemia was greater than 1000. The relationship between iron status, malaria infection and sleep is complex, yet important to understand. Because there are so many variables that could possibly be interacting, more research is needed. Millions of infants and children worldwide are affected by both IDA and malaria and the eradication of these problems has the potential to improve the overall health of the affected children as well as their sleep, with important implications for cognitive and behavioral development. Future studies should focus on understanding whether iron supplementation could improve sleep patterns of infants with ID and IDA.
CHAPTER 3

CONCLUSION

We found that infants who were iron deficient moved significantly more during the night and had significantly more fragmented sleep than iron-replete infants. These results suggest that iron plays a role in influencing sleep patterns and behaviors. Because sleep is vital to an infant’s health and development, it is important to understand the factors that influence sleep patterns and behaviors, and do what is possible to correct and modify these factors, including iron status, to optimize health and well-being in individuals around the world.

Because of the complex relationship between iron status, malaria, sleep and the environment, there is a need to control for more of the potential confounding variables in a randomized, placebo-controlled study with a larger sample size.

Ideally, future studies should be of sufficient size to consider the interactions between malaria and iron status because our results suggest that iron deficiency and high malaria parasitemia have quite opposite effects on sleep patterns, with ID clearly increasing movement and sleep fragmentation, and malaria reducing nighttime activity. Furthermore, information about inflammation factors should be collected to determine if the iron deficiency anemia is possibly being exacerbated by inflammation caused by chronic infection by the malaria parasite. HIV and AIDS infection information should also be collected, as these diseases can affect iron absorption and metabolism. Lastly, the effects of iron supplementation on sleep patterns and activity levels, both during the day and night time, should be considered for future studies.
Our study, in addition to testing associations between iron status and sleep patterns, was a pilot to test the usefulness of the actigraphic methods for studying infants’ sleep in a field setting. Several lessons can be learned from this experience and used to guide future studies of infants’ sleep. Sleep behaviors can be measured in different ways. The advantage of actigraphy over the more traditional polysomnography is that actigraphy can conveniently record all movements for 24-hours a day across several days. It is also more cost effective, less invasive, can be worn comfortably, and is a valid measure of sleep (25). Another benefit of actigraphy is that it makes activity and sleep recording more accessible to participants in their home environment, which eliminates the laboratory effects that could alter the patients’ usual sleep patterns (25). This is particularly relevant to our study setting as cultural norms would prohibit invasion into private spaces of Muslim homes on Pemba.

Additionally, polysomnography would be expensive and cumbersome to set up, especially because of the problems stemming from shortages of electricity and power outages. Actigraphy appears valuable as an outcome measure and may be useful in monitoring circadian rhythm patterns or disturbances in children (26). Our study pilot-tested the actigraphs with 50 infants over a three day period to ascertain the viability of using actigraphs on more participants. Despite the small sample size, the recorded actigraphic data provide results that encourage continued investigation of sleep patterns.

Some considerations should be made when using actigraphs in infants, such as whether or not they are co-sleeping with their mothers while breastfeeding, as the movement of the mother could possibly register as movement from the infant. Also, the location of the infant should be noted throughout the recording period (e.g. Is he/she at
home playing with siblings? Or, is the mother/sister/grandmother carrying the infant around town or in the field during the day?). Aspects of the environment of the child could be of interest as well. Are there toys or other interesting objects that could catch the attention of the child and motivate him/her to move around in order to reach said object? Additionally, it might be helpful to note the birth order of the child and the number of children in the home during the recording time, as the presence of a playmate could also influence activity levels.

In conclusion, this study showed that actigraphy is useful and showed associations between iron status and movement and activity levels in infants during sleep, and should be considered for use in future studies as an objective measure of activity and sleep.
Bibliography


48. CDC (2008), Atlanta, GA.


60. Prevention, C. f. D. C. a. (2008), Atlanta, GA.
The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host 1. Sporozoites infect liver cells 2 and mature into schizonts 3, which rupture and release merozoites 4. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony A), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony B). Merozoites infect red blood cells 5. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites 6. Some parasites differentiate into sexual erythrocytic stages (gametocytes) 7. **Blood stage parasites are responsible for the clinical manifestations of the disease.**

The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal 8. The parasites’ multiplication in the mosquito is known as the sporogonic cycle C. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes 9. The zygotes in turn become motile and elongated (ookinetes) 10 which invade the midgut wall of the mosquito where they develop into oocysts 11. The oocysts grow, rupture, and release sporozoites 12, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites 1 into a new human host perpetuates the malaria life cycle.

http://www.cdc.gov/malaria/biology/life_cycle.htm
<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>M ± SD, %</th>
<th>Range</th>
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<td>Age (mo)</td>
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<td>12.3 ± 3.7</td>
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</tr>
<tr>
<td>% girls</td>
<td>45</td>
<td>55.6 %</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>45</td>
<td>9.4 ± 1.4</td>
<td>5.9-11.8</td>
</tr>
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<td>% &lt;10 g/dL.</td>
<td>27</td>
<td>55.6 %</td>
<td></td>
</tr>
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<td>ZPP (µmol/mol heme)</td>
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<td>161.0 ± 150.4</td>
<td>11-807</td>
</tr>
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<td>%≥ 90 µmol/mol heme</td>
<td>30</td>
<td>66.7 %</td>
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<td>Malaria parasitemia</td>
<td>45</td>
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<tr>
<td>0 (0 parasites)</td>
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<td>57.8%</td>
<td>0</td>
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<td>1 (1-999)</td>
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<td>22.2 %</td>
<td>1-999</td>
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<td>2 (1000+)</td>
<td>4</td>
<td>20.0 %</td>
<td>1000+</td>
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<td>Able to walk alone</td>
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<td>Wake bouts (#)</td>
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<td>Moving Minutes</td>
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<td>50.2 ± 38.5</td>
<td>11.7-100</td>
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<td>Time awake (%)</td>
<td>45</td>
<td>21.1 ± 12.6</td>
<td>1.8-54.8</td>
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<tr>
<td>Total Activity Units</td>
<td>45</td>
<td>11201 ± 8372</td>
<td>194-39314</td>
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M=Mean  
SD= Standard Deviation  
ID= ZPP≥ 90 µmol/mol heme  
Anemia= Hb<10 g/dL  
IDA= Both ZPP≥ 90 µmol/mol heme and Hb<10 g/dL
Table 2. Sleep outcomes of ID and non-ID infants (Mean ± SD)

<table>
<thead>
<tr>
<th>Sleep Parameter</th>
<th>ZPP&gt;90.0 µmol/mol heme</th>
<th>ZPP&lt;90.0 µmol/mol heme</th>
<th>Adj. *B</th>
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<tbody>
<tr>
<td>Wake bouts (#)</td>
<td>41.6 ± 19.6</td>
<td>42.9 ± 22.8</td>
<td>-3.1</td>
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<tr>
<td>% Time spent awake</td>
<td>22.2 ± 14.0</td>
<td>19.6 ± 9.7</td>
<td>3.0</td>
</tr>
<tr>
<td>Moving minutes</td>
<td>137.0 ± 139.5</td>
<td>86.4 ± 107.5</td>
<td>96.8*</td>
</tr>
<tr>
<td>Fragmentation Index</td>
<td>55.2 ± 39.7</td>
<td>43.7 ± 37.0</td>
<td>18.2</td>
</tr>
<tr>
<td>Total Activity Score</td>
<td>12373± 9162</td>
<td>8856± 6130</td>
<td>4075.0*</td>
</tr>
</tbody>
</table>

*Adjusted for sex, age, malaria parasitemia, ability to walk alone; * p-value <0.05

* ID= ZPP≥90 µmol/mol heme; Anemia= Hb<10 g/dL; IDA= Both ZPP≥90 µmol/mol heme and Hb<10 g/dL.
<table>
<thead>
<tr>
<th>Sleep Parameter</th>
<th>Hb&gt;10.0 g/dL</th>
<th>Hb&lt;10.0 g/dL</th>
<th>Adj. *B</th>
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</thead>
<tbody>
<tr>
<td>Wake bouts (#)</td>
<td>44.2 ± 16.0</td>
<td>44.5 ± 21.8</td>
<td>2.5</td>
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<td>% Time spent awake</td>
<td>20.5 ± 10.3</td>
<td>23.3 ± 14.0</td>
<td>4.9</td>
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<tr>
<td>Moving minutes</td>
<td>86.6 ± 111.9</td>
<td>145.9 ± 142.4</td>
<td>95.6*</td>
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<tr>
<td>Fragmentation Index</td>
<td>35.9 ± 33.5</td>
<td>60.1 ± 40.2</td>
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<td>Total Activity Score</td>
<td>8728 ± 5694</td>
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*Adjusted for sex, age, malaria parasitemia, ability to walk alone; *p-value <0.05

a ID= ZPP≥ 90 μmol/mol heme; Anemia= Hb<10 g/dL; IDA= Both ZPP≥ 90 μmol/mol heme and Hb<10 g/dL.
### Table 4. Sleep outcomes of IDA and non-IDA infants (Mean ±SD)

<table>
<thead>
<tr>
<th>Sleep Parameter</th>
<th>Non-IDA</th>
<th>IDA</th>
<th>Adj. *B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wake bouts (#)</td>
<td>41.5 ± 22.3</td>
<td>42.7 ± 18.9</td>
<td>-1.9</td>
</tr>
<tr>
<td>% Time spent awake</td>
<td>18.9 ± 10.3</td>
<td>24.0 ± 14.5</td>
<td>5.6</td>
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<tr>
<td>Moving Minutes</td>
<td>82.7 ± 103.0</td>
<td>159.4 ± 146.9</td>
<td>116.9*</td>
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<tr>
<td>Fragmentation Index</td>
<td>40.7 ± 34.7</td>
<td>62.7 ± 40.5</td>
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<tr>
<td>Total Activity Score</td>
<td>9468 ± 6797</td>
<td>13608 ± 9640</td>
<td>5593.1</td>
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*Adjust for sex, age, malaria parasitemia, ability to walk alone; * p-value <0.05

* ID= ZPP≥ 90 µmol/mol heme; Anemia= Hb<10 g/dL; IDA= Both ZPP≥ 90 µmol/mol heme and Hb<10 g/dL.
<table>
<thead>
<tr>
<th></th>
<th>Morning Activity Score</th>
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<tbody>
<tr>
<td><strong>Iron Deficiency</strong></td>
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<tr>
<td>No</td>
<td>62439 ± 22588</td>
<td>60275 ± 25331</td>
</tr>
<tr>
<td>Yes</td>
<td>50952 ± 22421</td>
<td>50798 ± 21586</td>
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<tr>
<td><strong>Anemia</strong></td>
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<td></td>
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<tr>
<td>No</td>
<td>62158 ± 26213</td>
<td>58328 ± 25962</td>
</tr>
<tr>
<td>Yes</td>
<td>48880 ± 18288</td>
<td>50460 ± 20310</td>
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<tr>
<td><strong>IDA</strong></td>
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<tr>
<td>No</td>
<td>61226 ± 25014</td>
<td>57468 ± 24351</td>
</tr>
<tr>
<td>Yes</td>
<td>48043 ± 18673</td>
<td>50287 ± 21568</td>
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</table>

*ID = ZPP ≥ 90 µmol/mol heme; Anemia = Hb < 10 g/dL; IDA = Both ZPP ≥ 90 µmol/mol heme and Hb < 10 g/dL.*
Figure 1. Picture of an Actiwatch
Figure 2. Example of an actigraphic record

User identification
Start date 25-Jun-2002
Subject age 01
Day number 1
Actogram Scale 1753

Start time 15:00
Subject gender F
Epoch length 0.25 (Mins)

Sensitivity: MED (40)

Tue 25-Jun-2002

Wed 26-Jun-2002
Figure 3. SleepFragmentation: malaria, Hb interaction

Figure 4. SleepFragmentation: malaria, IDA interaction

Figure 5. Moving minutes: malaria, Hb interaction

Figure 6. Moving Minutes: malaria, IDA interaction
Figure 7. Actual Wake %: malaria, Hb interaction

Figure 8. Actual Wake %: malaria, IDA interaction

Actual wake % among ID and non-ID infants

<table>
<thead>
<tr>
<th>Malaria infection</th>
<th>Time spent awake (%)</th>
</tr>
</thead>
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<tr>
<td>0</td>
<td>Hb&gt;10: 30, Hb&lt;10: 20</td>
</tr>
<tr>
<td>1</td>
<td>Hb&gt;10: 35, Hb&lt;10: 25</td>
</tr>
<tr>
<td>2</td>
<td>Hb&gt;10: 40, Hb&lt;10: 30</td>
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</table>

Figure 9. Total activity: malaria, Hb interaction

Figure 10. Total activity: malaria, IDA interaction

Total activity among ID and non-ID infants

<table>
<thead>
<tr>
<th>Malaria infection</th>
<th>Total Activity Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Hb&gt;10: 10000, Hb&lt;10: 5000</td>
</tr>
<tr>
<td>1</td>
<td>Hb&gt;10: 15000, Hb&lt;10: 7500</td>
</tr>
<tr>
<td>2</td>
<td>Hb&gt;10: 20000, Hb&lt;10: 10000</td>
</tr>
</tbody>
</table>

Figure 7. Actual Wake %: malaria, Hb interaction

Figure 8. Actual Wake %: malaria, IDA interaction

Actual wake % among IDA and non-IDA infants

<table>
<thead>
<tr>
<th>Malaria Infection</th>
<th>Time spent awake (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Non-IDA: 20, IDA: 25</td>
</tr>
<tr>
<td>1</td>
<td>Non-IDA: 25, IDA: 30</td>
</tr>
<tr>
<td>2</td>
<td>Non-IDA: 30, IDA: 35</td>
</tr>
</tbody>
</table>
Figure 11. Wake bouts: malaria, Hb interaction

Wakeouts among ID and Non-ID infants

Number of Wakeouts

Malaria Infection

0 1 2

Hb>=10

Hb<10

Figure 12. Wake bouts: malaria, IDA interaction

Wakeouts in IDA and non-IDA infants

Number of wakeouts

Malaria infection

0 1 2

Non-IDA

IDA