# Iron-Deficiency Anemia is Associated with Altered Characteristics of Sleep Spindles in NREM Sleep in Infancy

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## Abstract

**Objective—**To determine the effects of iron-deficiency anemia on the development of non-rapid-eye-movement (NREM) sleep stages, as indexed by sleep spindles.

**Study design—**Patterns of sleep spindles during NREM sleep stages 2 and 3–4 (slow-wave-sleep, SWS) were compared in 26 otherwise healthy 6-month-old Chilean infants with iron-deficiency anemia and 18 non-anemic control infants. From polygraphic recordings, EEG activity was analyzed for sleep spindles to assess their number (density), duration, frequency, and inter-spindle interval.

**Results—**Iron-deficient anemic infants differed from the control group by having sleep spindles with reduced density, lower frequency, and longer inter-spindle intervals in NREM sleep stage 2 and SWS.

**Conclusions—**These results provide evidence of delayed sleep spindle patterns in iron-deficient anemic infants, suggesting that iron is an essential micronutrient for the normal progression of NREM sleep pattern development in the human.

### Keywords

Infants; Iron-deficiency anemia; Sleep spindles; NREM sleep; NREM sleep stage 2; SWS

## Introduction

Pioneering work by Youdim and colleagues and others in the 1970s and 1980s suggested that iron-deficiency anemia produced sleep-wake cycle alterations in animal models [1,2], perhaps due to effects of iron deficiency on dopamine systems [3,4]. However, there has been little sleep-related research in human iron deficiency, despite the fact that iron-deficiency anemia continues to be the most common single nutrient deficiency in the world [5,6]. In developing countries, over 50% of pregnant women are anemic [7,8], as are 46–66% of children <4 years, with half attributed to iron deficiency [9]. Poor, minority and/or immigrant infants and toddlers in the US and other developed countries remain at increased risk for iron deficiency[10]. Infancy is a period of peak prevalence due to rapid growth and limited dietary sources of iron. Since the organization of sleep-wake states undergoes dramatic changes in early infancy, the sleep-wake cycle might be particularly vulnerable to the effects of iron deficiency in this age period.

Iron is required for proper function of many enzymes and proteins. Thus, there are several mechanisms by which iron deficiency could produce diffuse and subtle changes in the central nervous system. For instance, rodent studies have shown effects of iron deficiency on neurotransmitter systems, neurometabolism, dendritogenesis, and myelination (see reviews [11–13]). Rodent models have also shown decreased brain iron content, electrophysiological alterations, neurotransmitter changes, and behavioral alterations that persist despite iron treatment when iron-deficiency anemia occurs in infancy [11–13].

Finding direct evidence of central nervous system effects in human infants is challenging. We have tried to advance the field by using assessments that reflect patterns of functional development and integrity of the central nervous system. For instance, using auditory brainstem evoked potentials, we showed slower neural transmission throughout the auditory pathway [14] and using actigraphic recordings, altered spontaneous motor activity modulation as a function of sleep-wake states [15,16] in otherwise healthy 6-month-old iron-deficient anemic infants. Here, we pursue the issue of sleep state maturation in more depth.

The first months of postnatal age show dramatic brain development in the human. 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 [17–19]. The sleep spindle is one of the most characteristic EEG patterns during sleep and a hallmark of NREM sleep stage 2, with a known anatomic generator (the nucleus reticularis thalami) [20]. Sleep spindles are defined by discrete bursts of relatively sinusoidal 12–14 Hz waves, which become clearly distinguishable during quiet sleep between 4 and 9 weeks post-term age [19,21–23]. Sleep spindles reach adult-like mature patterns at about 3 months, and their activity is maximal between 3 and 6 months [19,22–25]. Spindles have been postulated to be a marker of normal brain functional development and integrity, and their absence or abnormality strongly suggests cerebral dysfunction or pathology. Indeed, deviations from normal maturational patterns have been observed among infant groups with conditions that put them at high risk for poorer health and development [26–28]. Thus, maturational patterns of sleep spindles provide a useful noninvasive tool for investigating central nervous system functioning and integrity during early development in the human.

The purpose of the present study was to compare sleep spindle patterns as a function of NREM sleep stages in otherwise healthy 6-month-old iron-deficient anemic and non-anemic control infants. We predicted that iron-deficiency anemia would be associated with altered spindle patterns, reflecting irregular progression or delay in the normal maturation of NREM sleep development.

## Procedures

### Sample

The study was conducted in conjunction with a clinical trial of the developmental effects of preventing iron-deficiency anemia in infancy[29]. The sample was drawn from healthy infants receiving routine pediatric care in community clinics in 4 working-class communities on the southeastern outskirts of Santiago, Chile. The infants who received sleep studies at 6 months were identified during screening for the preventive trial. At the 4-month routine pediatric visit, infants were evaluated to make sure that those invited to participate were healthy. Entrance criteria included birth weight ≥3.0 kg, singleton birth, routine vaginal delivery, no major congenital anomalies, no major birth or neonatal complications, no jaundice requiring phototherapy, no hospitalization for other than an uncomplicated problem, no chronic illness, no iron therapy, and no evidence of failure to thrive or other nutrient deficiency. Other exclusion criteria were specific to successful completion of the study: residence outside the identified neighborhoods; no stable, literate caregiver available to accompany the infant for project

appointments; another infant less than 12 months of age in the household; infant in day care. Mixed breast- and bottle-feeding was the norm, and infants were growing normally by US standards.

Between 5 and 6 months a finger stick hemoglobin determination was performed (HemaCue, Leo Diagnostics, Helsingborg, Sweden). If the HemaCue value was ≤ 103 g/l (10.3 g/dl), a venipuncture was performed for determination of iron status: hemoglobin, hematocrit, mean cell volume (using Coulter Model ZBI, Hialeah FL); serum ferritin (International Nutritional Anemia Consultative Group, Washington, D.C.); and erythrocyte protoporphyrin (Hematofluorometer, Helena Laboratories, Beaumont TX). Anemia at 6 months was defined as a venous hemoglobin ≤ 100 g/l (10.0 g/dl). Iron deficiency was defined as two or more of the following: mean cell volume <70 fl, erythrocyte protoporphyrin >1.77 μmol/l (100 μg/dl) packed red blood cells, serum ferritin <12 μg/l. As expected, iron-deficiency anemia was uncommon at this age, detected in only about 3% of over 2000 infants with HemaCue determinations. For each iron-deficient anemic 6-month-old, an infant with a normal HemaCue level was randomly selected to receive a venipuncture specimen. Those who were clearly non-anemic (hemoglobin ≥120 g/l [12.0 g/dl]) constituted the control group. These infants did not enter the preventive trial, participating instead in the neuromaturation component of the project.

The infants were studied during a spontaneous afternoon nap, with about half receiving recordings of brainstem auditory evoked potentials [14] and half, polygraphic recordings of sleep-wake parameters, with actigraphy for some [15,16]. A variety of technical difficulties made data unusable for 12 other infants who received polygraphic recordings. All infants were given appropriate iron therapy and close pediatric supervision. The study was explained to parents of qualifying infants, and signed informed consent was obtained. The research protocol was approved by the Institutional Review Boards of the University of Michigan Medical Center, Ann Arbor, and INTA, University of Chile, Santiago. The present report is based on the polygraphic recordings and compares sleep spindle characteristics in both NREM sleep stage and SWS in 26 iron-deficient anemic infants and 18 infants without iron-deficiency anemia (non-anemic control) at 6 months of age.

## Sleep Data Collection

All sleep data were acquired and processed without knowledge of whether a given infant was iron-deficient anemic or non-anemic control. The wake-up time and the length of the waking episode that preceded the nap recording were estimated from information provided by the mother. Polygraphic recordings were conducted in the sleep laboratory of INTA, University of Chile. The procedures were standardized to limit potential influences of ambient environment, circadian rhythms, and/or food intake on sleep-waking patterns and related physiological activities. Studies took place in a special quiet and comfortable room, during the infant's spontaneous afternoon nap. Infants and their mothers were transported from home to the laboratory so that they arrived at least one hour before their habitual noon meal based on parental report of the infant's daily routine. During this one-hour period, electrodes were attached according to routine procedures. Mothers then fed their infant and engaged in their own routines before putting the infant down for a nap in his or her own clothing. Infants were placed in the supine position. The ambient temperature was maintained constant at 22–23°C. Mothers stayed in the laboratory throughout.

Recordings were made continuously using a TECA lA97 18-channel polygraph as follows: the electroencephalographic (EEG) activity with electrodes placed according to the international 10–20 system, with bipolar montages Fp1-C3, C3-O1, Fp2-C4, C4-O2, and Cz-Pz; rapid eye movements, monitored by electro-oculogram; tonic chin and diaphragmatic electromyograms, using surface electrodes; motor activity of both upper and lower limbs recorded independently by piezo-electric crystal transducers; abdominal respiratory movements, using a mercury strain

gauge; nostril airflow, by a thermistor; ECG using surface electrodes. For some infants, rectal and axillary temperatures and oximetry were also recorded. All data were simultaneously recorded on paper and computer, converted on-line from analog-to-digital signals, collected on hard disk, and then transferred to laser media for off-line analyses. Infant behavior was also observed directly and noted on the polygraphic paper.

## Data Processing and Analysis

**Coding waking and sleep states—**Waking and sleep (NREM sleep with its four stages, REM sleep, and indeterminate sleep) states were visually coded by the temporal concordance of EEG, EOG and EMG criteria according to Rechtschaffen and Kales' criteria [30], adapted for infants by Guilleminault and Souquet [31]. The minimum length of a state was 1 min. Interruption of the concordance between the given parameters for 1 min or more was considered as an interruption of the state; shorter changes were included in the preceding state. Recordings were terminated after a spontaneous waking episode lasting 15 min or more. Two independent scorers analyzed all recordings visually without knowledge of the infants' iron status. Overall inter-scorer agreement was 94.6% for sleep-waking states. Discrepancies were discussed and codes thus agreed upon were used in the data analysis.

**Sleep spindle measures from EEG signal processing—**Sleep spindles were defined as follows: duration >0.5 s, amplitude >10 μV, frequency 11–15 s. The beginning and end of each individual spindle were identified in one of the Fp-C derivations (Fp1-C3 or Fp2-C4) by two observers, who followed the same rules to resolve scoring discrepancies. The following spindle parameters were then determined for NREM sleep stages 2 and SWS:

– duration: time between the beginning and end of each spindle (in seconds),

– frequency: number of waves (or cycles) within each spindle expressed as cycles/s (or Hz),

– spindle index (density): number of spindles expressed as spindles/min,

– inter-spindle interval: time spent between the end of each spindle and the beginning of the next one (in seconds).

**Statistical methods—**For each individual infant, the duration of waking and NREM sleep stages was calculated. The following spindle variables were computed for NREM sleep stage 2 and SWS: frequency, duration, index (spindles/min), and inter-spindle interval. A series of independent t tests and $\chi^2$ tests were conducted to identify significant differences between iron-deficient anemic and control groups in background characteristics. Any background characteristic that was even weakly associated ($p < 0.10$) with more than one sleep variable was considered for covariate control. Due to colinearity among measures of growth and family characteristics, the set of covariates was simplified to age, gender, birth weight, weight-for-age z-score, and mother's IQ. Because some of the sleep spindle variables were significantly skewed, general linear regressions with a log normal distribution model were performed utilizing generalized estimated equation (GEE) methodology [32]. The regressions tested for differences in sleep spindle variables between iron-deficient anemic and non-anemic infants controlling for the above covariates. All tests of statistical significance were two-sided, with alpha set at 0.05. Statistics analyses were conducted using SAS v9.1.

## Results

Table 1 shows the infant and family background characteristics of the iron-deficient anemic and non-anemic control groups. Iron-deficient anemic infants were somewhat shorter and lighter at birth, as has been observed in some other studies [33–35]. They were also slightly

older at the time of assessment. There was some indication that they came from less advantaged family backgrounds, as has been considered previously [36–37].

Table 2 shows the comparisons between iron-deficient anemic and non-anemic infants in the duration of relevant sleep-wake states and the sleep spindle parameters, controlling for covariates. Statistically significant differences were the same with and without covariate control. On average, as noted before [15], the iron-deficient anemic group had been awake for a shorter time before napping than non-anemic controls, but the groups did not differ in the duration of NREM sleep stage 2 or SWS sleep during the nap. When spindle patterns were compared as a function of NREM sleep stages, both groups showed the same pattern: spindle index, spindle duration, spindle frequency, and the inter-spindle interval were similar in NREM sleep stage 2 and SWS. However, iron-deficient anemic infants showed a reduced spindle index during NREM sleep stage 2 and SWS relative to non-anemic control infants. The effect sizes were large (0.6–1.1 SD). Since spindle duration was similar across groups, the differences in the spindle index appears to be due to the significantly longer inter-spindle intervals in the iron-deficient anemic group during both NREM sleep stage 2 and SWS (effect sizes 0.6–0.8 SD). In addition, sleep spindle frequency was lower in iron-deficient anemic infants than in non-anemic control infants in both NREM sleep stage 2 and SWS. The effect sizes were very large in both stages (1.7 SD).

## Discussion

This study shows altered sleep spindle patterns in 6-month-old infants with iron-deficiency anemia. During a spontaneous afternoon nap, their NREM stage 2 and SWS were characterized by reduced spindle index, longer inter-spindle interval, and lower spindle frequency, compared to non-anemic controls. The duration of sleep stages and sleep spindles did not differ between the groups.

To the best of our knowledge, no data on sleep spindle patterns in iron deficiency have been reported previously. Although the results must be replicated, our findings are reminiscent of the developmental changes in sleep spindles that have been observed in several other biological risk conditions. NREM sleep appears to be a vulnerable state to a variety of insults occurring during the first months of postnatal life [38–42]. Infant groups considered at higher than average epidemiological risk for poorer developmental or neurological outcome also present different patterns of sleep spindle organization. For example, infants with mental retardation, prematurity, congenital hyperthyroidism, hyperbilirubinemia, phenylketonuria, or autism are characterized by different patterns of sleep spindles [26–28,42–46].

The differences in sleep spindle patterns observed in the iron-deficient anemic group correspond to less mature characteristics. Since spindles have been shown to arise from synchronized activities in functionally important neuronal networks linking the thalamus and the cortex [20,47,48], our results suggest that iron is required to assure the normal progression of the oscillating thalamocortical network that regulates the spindle patterning within NREM sleep stages.

Promising structural explanations for the observed sleep spindle alterations relate to impaired myelination and dendritic growth. Early iron deficiency alters myelin content and compaction [49–53] by directly affecting oligo-dendrocytes, which are responsible for myelin formation (see reviews [12,13]). Myelination is at least partly postnatal in the human [54], coinciding with the period of quiet sleep-NREM sleep restructuring [17–19]. Disrupted growth and organization of dendrites has also been reported in early iron deficiency in rodent models [55]. Although sleep spindles represent one of the few EEG patterns with a known anatomic generator [20], rapidly developing changes in early infancy appear to reflect myelination and

dendritic growth [56] within the neuronal networks that link the thalamus and the cortex. Since adequate iron is required for normal myelination and dendritogenesis, alterations in these processes due to iron-deficiency anemia could impact function in many brain systems, including those that underlie the occurrence of sleep spindles at the macroscopic EEG level. Future research in animal models may be particularly useful in evaluating the developmental profile of spindles in iron-deficiency anemia and understanding the NREM sleep stage differences observed in iron-deficient anemic infants.

Other mechanisms should be considered as well, particularly those related to a potential imbalance of brain neurotransmitter systems with early iron deficiency [12,13]. Beginning with the work of Youdim and colleagues in rodent models [4], iron deficiency has been implicated in later functioning of the dopamine (DA) system, especially the D2 receptor [12,13]. Recent studies of developmental iron deficiency suggest that transporters for DA, serotonin, and norepinephrine, DA levels, and D1 receptors are affected as well [57–59]. These neurotransmitter systems are involved in sleep spindle patterns. For example, there is a progressive disappearance of EEG sleep spindles in the rat under dietary-induced reduction of endogenous brain serotonin levels [60]. In experimental models of hyper-phenylalaninemia, serotonin (5-HT2) receptors decrease together with D2 receptors [61]. Therefore, if brain neurotransmitter imbalance results from iron-deficiency anemia, there might be effects on the biochemical substrate of spindle generation.

Sleep spindles appear to do more than reflect network properties (i.e., promoting the formation of thalamocortical networks by providing endogenous signals with repetitive and synchronized activity [62]). Some investigators have suggested that sleep spindles provide necessary conditions for the plastic modifications underlying memory formation [48,63]. Although the functions of sleep remain largely unknown, one of the most exciting hypotheses is that sleep contributes importantly to processes of memory and brain plasticity (for review see ref. [64]). As the multifaceted relationships between sleep and memory were recognized, initial attention concentrated on rapid-eye-movement (REM) sleep [64]. More recently, NREM sleep stages 2 and SWS have become a major focus of attention, with particular emphasis on sleep spindles. There is evidence that the spindles are markers for ability to learn certain kinds of tasks [65–67] even during a daytime nap [68]. The role of sleep in learning and memory has been shown by studies at the behavioral, systems, cellular, and molecular levels, including the modulation during sleep of cerebral protein synthesis and expression of genes involved in neuronal plasticity [64]. However, research to date continues to be fragmentary and has been conducted almost exclusively in adults (human or animal). Large amounts of sleep in infancy suggest that sleep may play a role in brain maturation [69], and sleep state organization and especially quiet sleep-NREM sleep in early infancy correlate with measures of cognitive functioning and attention in later childhood and early adolescence [70]. Yet the relationships between sleep spindles and learning have not been characterized in infants and young children. If the connections between sleep spindles and learning also apply in infancy, it is possible that the altered patterns of sleep spindles in iron-deficient anemic infants restrict their cognitive and memory-related abilities and contribute to the poorer developmental outcome that is consistently observed [13,71].

Finally, another aspect that deserves attention is the potential relationship between sleep spindles and motor control issues during sleep. Sleep spindles have been associated with a suppression of muscle tone. In the study of Chase and Harper [72], cats were trained to produce 12–14 Hz EEG activity during waking and, with acquisition of the spindle task; they showed concurrent loss of nuchal EMG together with entrainment of both respiratory and cardiac rhythms. This motoric relationship may be particularly relevant to our observations, since restless legs syndrome (RLS) and periodic limb movements during sleep (PLMS) have been associated with iron deficiency and conditions characterized by compromised iron status

[73]. Moreover, dietary iron supplementation has been shown to alleviate RLS symptoms and to reduce PLMS in adult patients [74] and to be associated with clinical improvement in some children with PLMS [75,76].

There are several limitations to our study. With a bipolar montage, we could not evaluate the precise location of spindle waves. Frontal and parietal spindles, for instance, appear to follow somewhat different developmental paths, suggesting the existence of different generators or a topographical difference during maturation of the thalamocortical network [77]. Because our recordings were performed during naturally-occurring early afternoon naps in healthy infants, we could not evaluate the influence of known modifiers of spindle characteristics, such as sleep restriction or deprivation, circadian phase, or pharmacologic effects [78]. Also, we could not evaluate the modulation of spindle characteristics as a function of the ongoing temporal structure within a long sleep episode [24,78].

Despite these drawbacks, our results provide further evidence that iron deficiency adversely affects the functional development of the central nervous system in the human infant. The observation of delayed maturation of sleep spindles – a key characteristic of NREM sleep stages – suggests that iron is an essential micronutrient for the normal progression of sleep development in infancy.

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**Table 1**

Background characteristics[*]

| Group | Iron-deficient anemic | Non-anemic | *P*-value |
|---|---|---|---|
| *N* | 26 | 18 | |
| Infant | | | |
|   Age at recording (months) | 7.1 ± 0.4 | 6.8 ± 0.4 | 0.05 |
|   Gender (% male, *n*) | 69% (18) | 50% (9) | 0.20 |
|   Gestational age (weeks) | 39.1 ± 1.1 | 39.6 ± 1.3 | 0.19 |
|   Birth weight (kg) | 3.4 ± 0.4 | 3.7 ± 0.4 | 0.01 |
|   Birth length (cm) | 50.6 ± 2.1 | 51.9 ± 1.6 | 0.04 |
|   Milk intake (ml/day) | 572.7 ± 251.3 | 544.5 ± 288.7 | 0.73 |
|   Growth in infancy (6 months) | | | |
|     Weight-for-age *z*-score | 0.8 ± 0.7 | 0.4 ± 1.0 | 0.19 |
|     Height-for-age *z*-score | 0.1 ± 0.8 | 0.2 ± 0.9 | 0.80 |
|   Iron status at study entry in infancy | | | |
|     Hemoglobin (g/L) | 94.2 ± 6.9 | 123.3 ± 4.0 | 0.0001 |
|     Mean corpuscular volume (fl) | 65.3 ± 4.2 | 73.8 ± 1.9 | 0.0001 |
|     Erythrocyte protoporphyrin (µg/dl rbcs) | 194.2 ± 76.9 | 83.1 ± 17.4 | 0.0001 |
|     Ferritin (µg/l) | 8.7 ± 9.03 | 20.7 ± 13.9 | 0.0015 |
| Family | | | |
|   Socioeconomic status index | 40.1 ± 9.0 | 39.0 ± 5.4 | 0.66 |
|   HOME score in infancy | 28.8 ± 5.1 | 31.2 ± 4.0 | 0.11 |
|   Father's education (years) | 9.6 ± 3.2 | 11.7 ± 1.8 | 0.07 |
|   Mother's education (years) | 9.4 ± 3.1 | 10.4 ± 1.9 | 0.23 |
|   Mother's IQ | 82.7 ± 8.8 | 88.2 ± 11.1 | 0.08 |

[*] Values are mean ± SD

**Table 2**

Duration of sleep stages and characteristics of sleep spindle patterns depending on infant iron status[*]

|  | Iron-deficient anemic | Non-anemic | *P*-value | Significant covariates |
|---|---|---|---|---|
| Waking prior to nap |  |  |  |  |
| Duration of stage (min) | 200.4 ± 13.9 | 251.1 ± 11.3 | 0.01 | Birth weight, WAZ |
| NREM stage 2 |  |  |  |  |
| Duration of stage (min) | 25.8 ± 5.9 | 30.5 ± 6.1 | 0.88 | Mother's IQ |
| Spindle index (spindles/min) | 2.4 ± 0.2 | 2.9 ± 0.2 | 0.05 | Age |
| Spindle duration (s) | 1.9 ± 0.1 | 2.0 ± 0.1 | 0.39 |  |
| Inter-spindle interval (s) | 27.9 ± 3.3 | 19.6 ± 1.7 | 0.02 | Age |
| Spindle frequency (Hz) | 12.3 ± 0.1 | 13.4 ± 0.1 | <0.0001 |  |
| SWS |  |  |  |  |
| Duration of stage (min) | 37.7 ± 4.6 | 30.5 ± 5.0 | 0.23 | Mother's IQ |
| Spindle index (spindles/min) | 2.0 ± 0.2 | 2.8 ± 0.2 | 0.001 | Gender |
| Spindle duration (s) | 1.9 ± 0.1 | 2.0 ± 0.1 | 0.72 | WAZ |
| Inter-spindle interval (s) | 31.3 ± 2.7 | 21.0 ± 2.2 | 0.009 |  |
| Spindle frequency (Hz) | 12.3 ± 0.1 | 13.2 ± 0.1 | <0.0001 |  |

[*] Values are mean ± SE