SERUM MICRONUTRIENT STATUS, SLEEP AND NEUROBEHAVIORAL FUNCTION IN EARLY ADOLESCENTS: A COHORT STUDY

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DEDICATION

To my husband, for his love, inspiration, encouragement and unwavering support.

To my grandfather, for always believing in me and giving me the strength to finish this journey.

To my mom and dad, for fostering my love of learning.

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ABSTRACT

SERUM MICRONUTRIENT STATUS, SLEEP AND NEUROBEHAVIORAL FUNCTION IN EARLY ADOLESCENTS: A COHORT STUDY

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Adolescence represents a critical period of neurobehavioral development. Prior research has proposed suboptimal micronutrient and sleep status as individual risk factors for neurobehavioral impairment in adolescents. Additionally, there is a small but growing body of literature that has documented an association between micronutrient status and sleep patterns, suggesting a complex relationship among micronutrients, sleep and neurobehavioral function. It was thus the aim of the present study to provide a systematic review on micronutrients and sleep, empirically examine the associations between micronutrient status and sleep with a focus on early adolescents, and to characterize the contribution of sleep to the relationship between micronutrient status and neurobehavioral function.

Study One (Chapter 2) systematically reviewed the existing studies (n=26) on the relationship between micronutrients and sleep across populations, thereby providing the background and generating hypotheses for the empirical sub-studies. Data sets from the China Jintan Child Cohort were used to conduct cross-sectional and longitudinal analyses (n=777) of serum zinc/iron concentrations and adolescent sleep assessed by the Pittsburgh Sleep Quality Index (Chapter 3), as well as a mediation analysis (n=226) of sleep quality between serum iron/zinc and neurobehavioral function in early adolescents

aged 11-14 years (Chapter 4). Cross-sectional analyses found significant associations of higher serum zinc concentrations with better global sleep quality, as well as decreased odds of insufficient sleep duration and sleep disturbances in early adolescents. Longitudinal analyses reported a trend towards better sleep efficiency at early adolescence with increasing serum zinc concentrations at preschool age. Serum iron concentrations were significantly associated with concurrent sleep latency but not global sleep quality and other sleep domains in early adolescents. Similarly, the interaction effects between serum iron and zinc on sleep quality did not reach statistical significance. Results of the mediation analyses indicate that early adolescents with low levels of serum iron and zinc were significantly associated with fast but error-prone performance on nonverbal reasoning task. Sleep quality partially mediated the relationship between low serum zinc and non-verbal reasoning but not low iron.

Findings from this dissertation study provide preliminary evidence for understanding the multifaceted and interrelated role of micronutrient status, sleep, and neurobehavioral function during early adolescence, which may be useful for developing interventions to optimize sleep-related health in adolescents. Future research is needed to validate and examine the clinical relevance of these findings.

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CHAPTER 1: INTRODUCTION

Introduction to the Problem

Insufficient sleep duration and impaired sleep quality are significant public health concerns that affect about 14 - 68.9% of adolescents worldwide (National Sleep Foundation, 2007; Chung & Cheung, 2008; Eaton et al., 2010; Gradisar, Gardner, & Dohnt, 2011). Suboptimal sleep elicits variable negative health outcomes in adolescents. Of them, impaired neurobehavioral functions, such as executive control, working memory, and attention, are of particular concern for adolescents. Neurobehavioral function supports school performance, academic achievement, as well as skills essential to behavioral control and stress coping in adolescents (Curcio, Ferrara, & De Gennaro, 2006; Liang, Matheson, Kaye, & Boutelle, 2014; Slattery, Grieve, Ames, Armstrong, & Essex, 2013). Given the critical development of the brain during adolescence, sleep problems may substantially affect neurogenesis and alter the developmental trajectory of the brain (Dewald, Meijer, Oort, Kerkhof, & Bögels, 2010). Therefore, identifying the potential targets to improve sleep health has significant implications for adolescent development, especially neurobehavioral function.

Micronutrient status has received increasing attention as a modifiable factor for both neurobehavioral dysfunction (Benton, 2008; Halterman, Kaczorowski, Aligne, Auinger, & Szilagyi, 2001) and sleep impairment (Peuhkuri, Sihvola, & Korpela, 2012; Takeda, Minami, Seki, & Oku, 2004). Micronutrients are known to support neurotransmitter synthesis and brain function (Singh, 2004). Despite their importance,

micronutrient deficiencies, particularly iron and zinc deficiencies, are prevalent in children and adolescents globally (Black, 2003).

Considerable studies in children have suggested that suboptimal micronutrient status, such as iron deficiency, is associated with lower scores on testing of attention (Falkingham et al., 2010), working memory (Lambert, Knaggs, Scragg, & Schaaf, 2002), intelligence (Lomagno et al., 2014), and course performance (Halterman et al., 2001). Concerning micronutrients and sleep, several lines of evidence have reported the correlations between micronutrient status and sleep-related neurotransmitters in animal models (Peuhkuri et al., 2012; Takeda et al., 2004), highlighting the biological plausibility of the impact of micronutrients on sleep regulation. Human studies have also documented the associations between micronutrient status and sleep patterns among infants (Kordas et al., 2009), young children (Kordas et al., 2007), and adults (Grandner, Jackson, Gerstner, & Knutson, 2013). However, no study has critically reviewed the current literature on the relationship between micronutrients and sleep.

Additionally, the evidence is sparse regarding the association between micronutrient status and sleep in the adolescent population. Given the rapid brain growth (Bryan et al., 2004) and developmental changes in sleep patterns throughout adolescence (Hagenauer & Lee, 2013), sleep patterns during this developmental period may be particularly vulnerable to suboptimal micronutrient status. Importantly, while the current literature has reported the main effect of each micronutrient on sleep duration and sleep quality, such sleep effect may change depending on the status of another micronutrient. For example, a clinical trial documented that iron and zinc supplements alone increased

the duration of night sleep in infants, yet the group receiving iron together with zinc supplements did not exhibit such benefits on sleep (Kordas et al., 2009). However, the potential interaction effect between micronutrients on adolescent sleep has received insufficient attention. Furthermore, prior research has documented an association between iron deficient anemia (IDA) in infancy and altered sleep architecture later in 4-year-olds as relative to the non-IDA controls (Peirano, Algarín, Garrido, & Lozoff, 2007). The predictive effect of suboptimal micronutrient status in early childhood on later sleep during adolescence, however, has yet to be characterized.

Another limitation in the current literature is that few studies have explored the interrelationships between micronutrient status, sleep and neurobehavioral function. The majority of studies have considered suboptimal micronutrient status (Benton, 2008; Halterman et al., 2001) and sleep impairment (Buckhalt, El-Sheikh, & Keller, 2007) as individual risk factors of neurobehavioral dysfunction in adolescents. However, suboptimal micronutrient status has also been proposed as a predisposing factor of impaired sleep (Kordas et al., 2009) which, in turn, may affect neurobehavioral function. According to this conceptualization, sleep may meet the criteria for functioning as a mediator of the relationship between micronutrient status and neurobehavioral function (Baron & Kenny, 1986). In other words, sleep, to some extent, may account for decreased neurobehavioral function resulting from suboptimal micronutrient status.

The aims of this dissertation study were: 1) to provide a systematic review of the existing literature regarding the relationship between micronutrients and sleep and explore their biological underpinnings (Chapter 2); 2) to test the associations between

serum micronutrient status (iron and zinc) and sleep quality in early adolescents (Chapter 3); and 3) to test the mediating effect of sleep quality on the relationship between serum micronutrient status (iron and zinc) and neurobehavioral function in early adolescents (Chapter 4). This dissertation study is an important first step towards understanding the complex interplay among micronutrients, sleep, and neurobehavioral function in adolescents. Findings from this study may inform future interventions to optimize sleep health and neurobehavioral development in adolescents, and may ultimately shed light on the full extent of their consequences on pubertal development.

Background and Significance

This section provides operationalized definitions of key concepts and a comprehensive discussion of developmental characteristics of sleep, micronutrient status and neurobehavioral functions during adolescence. In addition, the relationships among sleep, micronutrient status, and neurobehavioral functions are reviewed. This information provides the basic knowledge upon which the subsequent studies of the dissertation were built.

Definitions of Key Concepts

For the purpose of clarity and consistency, the following definitions will be used throughout this proposal. Table 1.1 summarizes the operational definition of key concepts.

Adolescence. Adolescence is a period of rapid and significant human growth and development situated between childhood and adulthood (Hanson & Chen, 2007). Based

on the definition by the World Health Organization, this study defines adolescence as a period between 10 to 19 years old. Adolescence includes two developmental ages, early (10–14 years) and late adolescence (15–19 years) (United Nations Children's Fund, 2011).This dissertation will focus on the population at early adolescence when physical, cognitive and psychosocial changes commence and develop.

Sleep. Sleep is a multidimensional construct that is characterized by physiological and neurobehavioral markers of nocturnal sleep as well as perceived sleep quality (Buysse et al., 2010). In this study, self-report sleep health is operationalized as the additive function of perceived sleep quality, sleep onset latency, sleep duration, sleep efficiency, sleep disturbances, sleep medication usage, and daytime dysfunction (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989; Buysse et al., 2010).

Micronutrient status. Micronutrients refer to minerals and vitamins consumed in milligram or microgram amounts that are essential to human health (Katz, 2014). In the systematic review (Chapter 2), micronutrient status is operationalized as two sets of indicators widely used in the literature: (1) biochemical indicators, such as serum/plasma zinc concentration, serum ferritin and iron (De Benoist, Darnton-Hill, Davidsson, Fontaine, & Hotz, 2007; World Health Organization, 2001); (2) the amount of habitual dietary intake. Two empirical studies (Chapter 3 and 4) further specify micronutrient status as the amount of micronutrient in serum with a focus on iron and zinc for two reasons. First, iron and zinc deficiencies are two of the most prevalent types of suboptimal micronutrient status in adolescents (Allen, De Benoist, Dary, & Hurrell, 2006; Yang, Chen, & Feng, 2007). Second, unlike the biochemical indicators, dietary

assessment may not define micronutrient status in the human body per se but reflect the risk of micronutrient deficiency (De Benoist et al., 2007).

Neurobehavioral function. The term neurobehavioral function refers to a wide spectrum of cognitive behaviors underpinned by the specific central nervous system and brain function (Lezak, 2004). Subtle neurobehavioral changes may not be clinically observable but can be detected by a thorough measurement of functional indices (Green et al., 2004), thus providing an opportunity for early identification and intervention. Functional domains of interest include attention, abstraction/mental flexibility, memory, spatial processing ability and non-verbal reasoning, which can be classified into three categories as below (Gur et al., 2012).

(1) Executive control comprises a range of cognitive processes with the intention to control one's behavior, such as the inhibitory control of attention (focusing on what we choose and suppressing attention to other stimuli), working memory, and cognitive flexibility (i.e. creatively thinking "outside the box," seeing anything from different perspectives, and quickly and flexibly adapting to changed circumstances) (Diamond, 2013).

(2) Episodic memory refers to consciously accessible memory for unique events that allow us to represent past experiences and to flexibly employ these representations in service of current and future goals (Rugg & Vilberg, 2013).

(3) Complex cognition involves all cognitive processes used by individuals for deriving new information out of given information in the service of solving problems,

decision making, and planning actions (Knauff & Wolf, 2010). Spatial processing ability and non-verbal reasoning reflect such higher order mental abilities (Gur et al., 2012).

Table 1.1.

Operationalized definition of key concepts

Concept	Operationalized definition
Adolescent	The group of people aged 10-19 years old. Early adolescents refer to those in the early developmental stage (10-14 years of old) at adolescence.
Sleep	A multidimensional construct characterized by habitual sleep duration and sleep quality (additive function of perceived sleep quality, sleep onset latency, sleep duration, sleep efficiency, sleep disturbances, sleep medication usage, and daytime sleepiness and dysfunction)
Micronutrient status	Biochemical indicators of micronutrient and habitual dietary intake.
Neurobehavioral function	A wide spectrum of cognitive behaviors underpinned by the specific central nervous system and brain function. Major domains in this study include executive control, episodic memory, and complex cognition.

Sleep in Adolescents

Adolescents are particularly vulnerable to insufficient sleep duration and reduced sleep quality due to a multitude of intrinsic and environmental factors. Pubertal changes in sleep–wake regulation include a decrease in sleep electroencephalographic (EEG) slow-wave activity (SWA), delayed melatonin onset phase, reduced sensitivity to light and lengthened circadian rhythms (Carskadon, Acebo, & Jenni, 2004; Wolfson & Carskadon, 1998). When coupled with psychosocial pressure and behavioral factors, such as early school start times, increased academic burden, stressful events and electronic media use, adolescents often experience sleep deprivation, large night-to-night variability

in sleep timing and reduced sleep quality (Cain & Gradisar, 2010; Dahl & Lewin, 2002; Knutson & Lauderdale, 2009). For example, whereas laboratory research suggests that 9.2 hours is optimal for adolescents (Wolfson & Carskadon, 1998), the mean sleep duration on school nights ranges from 7.46 to 8.44 hours worldwide (Gradisar et al., 2011).

Insufficient sleep, sleep disruptions, and reduced sleep quality can adversely affect mental health (Ivanenko, Crabtree, & Gozal, 2005), neurobehavioral function and academic performance (Dewald et al., 2010). For example, a number of recent studies have underscored the potential impact of sleep debts and other sleep issues on depression, mood disturbances, and suicidal ideation (Owens & Group, 2014). Sleep deprivation can also selectively affect daily functioning, such as vigilance, motivation and high-order cognitive abilities (i.e. decision-making skills and executive control function) (Owens & Group, 2014). In addition, impaired sleep has been linked to risk-taking behaviors (O'Brien & Mindell, 2005) and metabolic syndrome in adolescents (Redline et al., 2007). Given the significant burden of sleep impairment in adolescents, identification of markers that best predict impaired sleep quantity and quality has important public health implications for this vulnerable population.

Micronutrient Deficiency in Adolescents

Although severe micronutrient deficiencies are considered rare, mild-to-moderate micronutrient deficiencies, mainly from insufficient dietary intake (Yang et al., 2007), contribute considerably to the global burden of health problems. The World Health Organization (WHO) estimated that more than two billion people worldwide suffer from

deficiencies in a certain type of micronutrients (Allen et al., 2006). Micronutrient deficiencies affect all populations; children and adolescents from developing countries are among the most vulnerable groups (Allen et al., 2006). Among micronutrient deficiencies, iron and zinc deficiencies are of greatest concern due to their high prevalence. A systematic review has reported that approximately 8-40% of children (<18 years) suffered from iron deficiency, and 30-50% of adolescents aged 11-16 years reported zinc deficiency in China (Wong, Chan, Chui, Sutcliffe & Wong, 2014).

Micronutrient deficiencies, such as zinc and iron deficiency, can manifest as clinical diseases (Wong et al., 2014). Micronutrient deficiencies are also associated with subclinical impairments that can substantially reduce resistance to infection, increase susceptibility to metabolic disorders, and delay or impair physical and psychomotor development (Allen et al., 2006). Moreover, prior research has indicated that deficiencies in iron and zinc decrease cognitive ability and increase behavioral problems in children and adolescents (Liu et al., 2014; Liu, Raine, Venables, Dalais, & Mednick, 2003). The high prevalence and pervasive health consequences underscore an urgent need for early detection and management of suboptimal micronutrient status, especially iron and zinc deficiencies.

Neurobehavioral Function in Adolescents

Adolescence represents a critical period in the maturation of the brain systems and in the associated cognitive and behavioral development (Brenhouse & Andersen, 2011; Spear, 2011). Recent research using a neural system approach has provided insight into the process of neurodevelopment during adolescence, characterized by synaptic

refinement, a developmental increase in myelination, functional connectivity between the brain regions, and neurotransmitter innervation (Brenhouse et al., 2011; Jung & Haier, 2007). Developmental changes in the brain systems parallel cognitive and behavioral improvement during adolescence and into adulthood. Behavioral studies have observed substantial improvement with age in neurobehavioral performance, and the magnitude of these age-related changes varies across cognitive domains (Gur et al., 2012). Based on the performance on a computerized neuropsychological battery, a study in a sample aged 8-21 years found that executive-control functions, specifically attention and motor speed, presented the most profound age-related improvement, whereas the effect size of age was least on memory (Gur et al., 2012).

Adolescence has been viewed as a period of vulnerability for neurobehavioral dysfunction. The introduction of exogenous risk factors, such as insufficient sleep and micronutrient status, may interfere with brain development and, in turn, affect neurobehavioral function (Falkingham et al., 2010; Owens & Group, 2014). Suboptimal neurobehavioral function is known to have an impact on daily functioning in adolescents, such as academic performance, behavioral control and stress coping (Curcio, Ferrara, & De Gennaro, 2006; Liang, Matheson, Kaye, & Boutelle, 2014; Slattery, Grieve, Ames, Armstrong, & Essex, 2013). Of note, neuronal malleability during adolescence may also exert a lasting detrimental effect into adulthood (de Bruin, van Run, Staaks, & Meijer, 2016), suggested by the associations between childhood neurobehavioral deficits and internalizing and externalizing psychopathology during adulthood (Owens & Hinshaw,

2016). It is therefore of particular interest to examine the domain-specific responses of neurobehavioral function to health risk factors during adolescence.

The Relationship between Micronutrient Status, Sleep and Neurobehavioral Function

Neurobehavioral performance in adolescents is multifactorial. Micronutrient status and sleep are a part of the complex influences on neurobehavioral development. While prior research has considered suboptimal micronutrient status and sleep as two individual risk factors, several lines of evidence have suggested a possible association between micronutrients and sleep, which adds to the complexity of the relationships among micronutrient status, sleep and neurobehavioral function. The following sections provide a brief literature review on the interplays.

Micronutrient Status and Neurobehavioral Function. The profile of micronutrient status has been known as a significant predictor of neurobehavioral function (Benton, 2008; Halterman et al., 2001). For example, iron and zinc deficiencies can place children and adolescents at risk for depressed motor development, cognitive delays, and academic problems, probably mediated by alterations in neuronal metabolism in the hippocampus and prefrontal projections (Beard, 2003). By comparison, micronutrient treatment, such as zinc supplementation, has shown benefits on reasoning and psychomotor capacity in Chinese and Mexican-American children (Sandstead, 2012). However, such effects may be moderated by the baseline micronutrient status. A meta-analysis reveals that micronutrient supplementation only improves cognitive performance

in children with poor micronutrient status, but not those with sufficient nutrition (Benton, 2001). Hence, neurobehavioral research should give more attention to children and adolescents who are at a higher risk of micronutrient deficiencies.

Prior research has also suggested a possible long-term effect of micronutrient deficiencies. Suboptimal micronutrient status early in life, such as iron and zinc deficiencies, have been found to predict later cognitive deficits (Liu et al., 2003) and externalizing behavioral problems (Liu, Raine, Venables, & Mednick, 2004). Specifically, three longitudinal studies have shown reduced visual-spatial performance (Lozoff, Jimenez, Hagen, Mollen, & Wolf, 2000), executive function (Lukowski et al., 2013) and inhibitory control (Algarín et al., 2013) in children and adolescents who had iron deficient anemia in infancy. Therefore, identifying and modifying suboptimal micronutrient status is critical for both immediate neurobehavioral function and long-term neurobehavioral development throughout childhood and adolescence.

Sleep and Neurobehavioral Function. Research concerning the relationship between sleep and neurobehavioral function is highly relevant due to the interaction of developmental changes in intrinsic sleep regulation systems and the brain maturation process during adolescence. Several reviews and meta-analyses have highlighted relationships of sleep deprivation and poor sleep quality with decreased neurobehavioral function, learning capacity and school performance in children and adolescents (Astill, Van der Heijden, Van IJzendoorn, & Van Someren, 2012; Curcio et al., 2006; de Bruin et al., 2016). One meta-analytic review also compares the effect size of sleep indicators on school performance, suggesting that daytime sleepiness shows the strongest relationship,

followed by sleep duration and sleep quality (Dewald et al., 2010). The potential neurobiological mechanisms hypothesized in prior research involve an interference with neuronal reactivation and reorganization of memory traces, hemostatic downscaling of synaptic strength during sleep, as well as the functional integrity of the frontoparietal networks that support sustained attention (Astill et al., 2012).

Neurobehavioral domains appear to have differential sensitivity to inadequate sleep time and reduced sleep quality. de Bruin et al. (2016) aggregated prior studies that tested the cognitive effect of sleep manipulation in adolescents, showing decreased vigilance after sleep deprivation, improved working memory obtained from sleep extension, and enhanced memory consolidation following a post-learning sleep. However, the cognitive effects of experimentally induced sleep deprivation or poor sleep quality may not reflect the nature of habitual sleep in a non-laboratory setting. A recent meta-analysis in healthy schoolchildren aged 5-12 years revealed a trend toward better executive functioning, multiple-domain cognitive functioning, and school performance with increased habitual sleep duration, but not intelligence, sustained attention and memory (Astill et al., 2012). A further complexity is that neurobehavioral domains and performance tasks used in prior studies vary, thus limiting the comparability of the findings across studies. Future research using an established performance assessment tool that covers multiple neurobehavioral domains is warranted to provide insight into naturally occurring sleep behavior.

Micronutrient Status and Sleep. Whereas current findings consistently support the impact of sleep on dietary intake and metabolic outcomes (Spiegel, Tasali, Penev, &

Van Cauter, 2004; Spaeth, Dinges, &Goel, 2014), there is a small but growing literature focused on a reversed relationship between sleep quality and nutritional indicators (Grandner et al., 2013; Peuhkuri et al., 2012). Researchers found that macronutrients, such as carbohydrates and amino acids (specifically, tryptophan), can influence the levels of neurotransmitters in the intrinsic sleep processes and affect sleep patterns and sleep quality (Peuhkuri et al., 2012). Micronutrients have not received as much attention as macronutrients in the sleep literature. However, current evidence, primarily stemming from studies in infants and adults, supports the presence of an association of dietary micronutrient intake or biochemical indicators of micronutrient status with sleep parameters.

Sleep parameters associated with iron and zinc are of particular interest due to their high prevalence of deficiency. Iron deficiency anemia may be associated with more night waking and shorter total sleep than better-nourished infants (Kordas et al., 2008), whereas iron supplement can increase the total sleep duration in infants regardless of the anemia status at baseline (Kordas et al., 2009). In light of zinc status, randomized controlled trials in infants found a longer maternal-reported night and total sleep in those receiving supplemental zinc than the placebo group (Kordas et al., 2009). Other micronutrients associated with human sleep in the current literature include copper (Song, Kim, & Jung, 2012), magnesium (Dralle & Bodeker, 1980), as well as Vitamins D (Massa et al., 2015) and B12 (Beydoun et al., 2014). However, the results of the associations have been insufficient and inconsistent.

Gaps in the Literature

Although increasing evidence has suggested the interrelationship between micronutrients, sleep and neurobehavioral function, significant and important questions remain. First, several systematic review and meta-analyses have been conducted on the relationship between micronutrient status and neurobehavioral function (Eilander et al., 2009), and between sleep and neurobehavioral function (Astill et al., 2012). In contrast, no systematic review has examined the current studies on the relationship between micronutrient status and sleep patterns. Second, research on micronutrients and sleep has primarily focused on infants (Kordas et al., 2008), young children (Kordas et al., 2007), adults (Grandner et al., 2013) and older adults (Held et al., 2002). There is a dearth of evidence regarding the association between micronutrient status and sleep in adolescents. Given that the metabolic characteristics of micronutrients (Iglesia et al., 2010) and the biological regulation of sleep (Ohayon, Carskadon, Guilleminault, & Vitiello, 2004) during adolescence differ from other life stages, existing findings may not reflect the nature of the relationship in an adolescent population. Third, considerable evidence supports the direct neurobehavioral benefits from optimal micronutrient status and sleep. Together with the possible association between micronutrients and sleep in adolescents, micronutrient status may affect sleep, thereby influencing neurobehavioral function. However, this mediating role of sleep has yet to be investigated.

There are also several methodological limitations in the current literature. First, the majority of studies examined the short-term effect of micronutrient status on sleep or the cross-sectional correlations. Given the potential long-term influences of micronutrient

deficiency (Peirano et al., 2007), a longitudinal design should be employed to find the nature of the associations, especially, how suboptimal micronutrient status in early childhood predicts later sleep during adolescence. Second, while most of the studies tested the main effect of individual micronutrients; little attention has been given to the interaction effect between micronutrients on sleep. Third, prior studies focus only on selected aspects of sleep and neurobehavioral function. The role of micronutrients in multiple dimensions of sleep, as well as their relationships with various neurobehavioral domains, have barely been explored within one study.

To fill the gaps in the literature, this dissertation study addressed the following research questions:

(1) Is serum micronutrient status (iron and zinc) associated with multiple sleep domains at early adolescence?

(2) Can serum micronutrient status (iron and zinc) in early childhood predict later sleep at early adolescence?

(3) What are the contributions of sleep in the pathway between micronutrient status and neurobehavioral domains at early adolescence?

Conceptual Framework

The conceptual framework underpinning this study is adapted from the "Conceptual model of impaired sleep" developed by Lee and colleagues (Lee et al., 2004). The parent model conceptualizes impaired sleep as a potential health problem, with bio-psycho-social health outcomes related to it. It encompasses the influential

factors and adverse health outcomes of sleep deprivation, sleep disruption, and reduced sleep quality. According to this model, health conditions such as inadequate nutrition, along with other bio-socio-environmental factors, can cause sleep disruption, and in turn predict significant individual differences in health outcomes, such as impaired neurobehavioral and physiological function, as well as emotional outcomes.

The adapted model focuses on the relationship between micronutrients, night-time sleep and neurobehavioral functions (see Figure 1.1). The association of reduced sleep quality and neurobehavioral function frames an important consideration for adolescents for whom our social norms raise the expectation of intense learning. Also, as sleep plays a role in the brain development, concerns must be raised for suboptimal micronutrient status that predicts reduced sleep quality during adolescence.

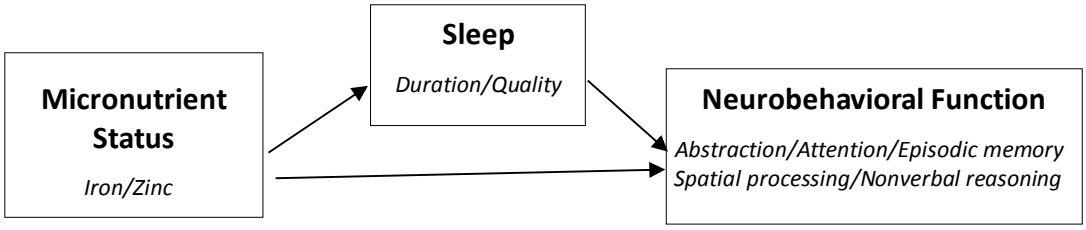

Figure 1.1. The conceptual framework of micronutrient status, sleep and neurobehavioral function

Purpose and Specific Aims

The purpose of this dissertation was to examine the relationships among micronutrient status, sleep and neurobehavioral functions in adolescents, thereby

providing preliminary evidence for identifying prevention and intervention factors of adolescent health.

The specific aims of this dissertation were to:

Aim 1 (Chapter 2): Systematically review empirical research on the relationship between micronutrient status and sleep patterns, outline neurobiological underpinnings, and provide recommendations for future research and public health practice.

Aim 2 (Chapter 3): Test the associations between serum micronutrient status (iron and zinc) and sleep quality in early adolescents using both cross-sectional and longitudinal designs.

Hypothesis 2a: Low serum micronutrient status (iron and zinc) at **preschool age** is significantly predictive of reduced sleep quality at **early adolescence**.

Hypothesis 2b: Low serum micronutrient status (iron and zinc) at **early adolescence** is significantly associated with reduced sleep quality at **early adolescence**.

Aim3 (Chapter 4): Test the partial mediating effect of sleep quality on serum micronutrient status (iron and zinc) and neurobehavioral function.

Hypothesis 3a: Sleep quality significantly mediates the relationship between serum micronutrient status and each neurobehavioral domain in early adolescents.

To our knowledge, this dissertation represents the only research using an adolescent sample to date. This systematic review extended to the relevant studies across the lifespan, thereby generating hypotheses for the subsequent quantitative studies on adolescent sleep. The two data-based components of this dissertation study used

subsamples from the China Jintan Child Cohort Study (Liu et al., 2010; Liu et al., 2015). The Jintan cohort (Liu et al., 2010; Liu et al., 2015) contains datasets on serum micronutrient concentrations (iron and zinc), subjective sleep measurements at both preschool age and early adolescence, as well as neurobehavioral performance (i.e. attention, episodic memory et. al) tested on Penn Computerized Neuropsychological Battery (CNB) (Gur et al., 2010) during early adolescence. Thus, this cohort provides a unique research opportunity to address the research aims.

Specifically, the second component of this dissertation tested the cross-sectional and longitudinal relationships between serum micronutrient status (iron and zinc) and sleep quality in adolescents (Chapter 3), using data from 777 subjects who had complete sleep and micronutrient data at both preschool age (3-5 years old) and early adolescence (11-14 years old). The empirical findings of the second component helped elucidate the relationships that were underdeveloped in the literature but also provided a basis for the third component of this dissertation (Chapter 4). The third component secondarily analyzed the existing data from 226 subjects, in order to identify the contribution of sleep quality to the relationship between micronutrient status and neurobehavioral function.

Significance

Micronutrient deficiency, sleep impairment and neurobehavioral dysfunction in adolescents are all significant public health issues worldwide. Despite the emerging evidence that suggests the interrelationships among them, the predictive effect of micronutrient status on sleep quality, as well as the mediating effect of sleep underlying relationship between micronutrient status and neurobehavioral dysfunction are presently

underexplored and pose a critical barrier to promote adolescent health. This dissertation study provides insight into the multifaceted and interrelated public health issues of nutrition, sleep and neurobehavioral function. In particular, the findings regarding the association between micronutrient status and sleep quality proposes a novel target for optimizing adolescent sleep health. The contribution of sleep to the association between micronutrients and neurobehavioral function further frames an important consideration for adolescents. Overall, this dissertation study represents an initial step to develop potential targets for optimizing sleep and neurobehavioral function, in order to shed light on the full extent of their consequences on pubertal development.

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CHAPTER 2: THE RELATIONSHIP BETWEEN MICRONUTRIENT STATUS AND SLEEP PATTERNS: A SYSTEMATIC REVIEW

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Authorship: X.J. developed the idea for the study, performed the systematic searches and the article retrieval, analyzed the data, and wrote and revised the article. J.L. mentored the review process, including conceptualizing the research question, the literature search and the interpretation of results, and critically revised the article. M.A.G. contributed to the conceptualization of the research question, the initial outline of the manuscript and article revision.
Ethics of human subject participation: Not applicable.

Abstract

Objective This study aims to review articles on the relationship of dietary and circulating micronutrients with sleep patterns, and identify issues surrounding implications for future research and public health practice.

Design A systematic review was conducted. PubMed, Embase and Scopus were searched through January 2016.

Setting Both experimental and observational studies were included in the review. However, studies that focused on secondary sleep impairment due to comorbidities were excluded.

Subjects This review included individuals in different age groups, from infants to older adults.

Results A total of 26 articles were selected. In the articles reviewed, researchers generally supported a potential role of micronutrients, iron, and magnesium in particular, in the development of sleep stages among infants and reversing age-related alterations in sleep architecture in older adults. Micronutrient status has also been linked to sleep duration, with sleep duration positively associated with the levels of iron, zinc, and magnesium, and negatively associated with copper, potassium, and Vitamin B_{12} levels. The mechanism underlying these relationships include the impact of micronutrients on excitatory/inhibitory neurotransmitters and the expression of circadian genes.

Conclusions Although the number of studies on the relationship between micronutrient status and sleep remains low, evidence has emerged that suggests a link between dietary/circulating micronutrients and sleep. Future research is needed to investigate the dose-dependent as well as the longitudinal relationships between micronutrient levels and human sleep across populations, test the interactions between micronutrients on sleep outcomes, and ultimately examine the clinical relevance of micronutrients on sleep health.

Key words: micronutrient, mineral, vitamin, trace element, sleep

Introduction

An optimal sleep pattern has important implications for health maintenance and health promotion [1]. However, cross-sectional studies have suggested that between 14% and 40% of the general population have impaired sleep, including insufficient sleep duration, long sleep-onset latency, frequent and long nocturnal awakenings, and other sleep disturbances [2-6]. Sleep deprivation and sleep impairment can affect cognitive performance in children [7] and adults [8]. Over time, impaired sleep patterns have been linked to depression [9], obesity [10], metabolic [11] and cardiovascular diseases [12], cancer [13] and increased risk of mortality [14]. The high prevalence and consequent negative impact of sleep impairment highlight the importance of understanding potentially modifiable factors.

As the relationships between insufficient sleep, weight gain and obesity have been observed in both children [10] and adults [15], increasing attention has been given to the potential links between sleep, dietary intake, and nutrition. Whereas current findings consistently support the impact of sleep deprivation and sleep problems on dietary intake and metabolic outcomes [15-17], recent studies have suggested a reversed relationship between dietary or serum nutrient levels and sleep problems [18-20]. Researchers found that macronutrients, such as carbohydrates and amino acids (specifically, tryptophan), can involve and influence the levels of neurotransmitters in the intrinsic sleep processes and affect sleep patterns [21,22]. For example, low proportion of carbohydrate intake was found to increase the percentage of slow wave sleep (deep sleep) and reduce the percentage of REM sleep among healthy good sleepers [18].

Micronutrient intake has not received as much attention as macronutrients as a modifiable factor for sleep deprivation and sleep problems. However, experimental studies indicated that micronutrients may impact important nerve-signaling chemicals or neurotransmitters of sleep regulation, including serotonin [22], N-methyl-D-aspartate glutamate (NMDA) [23] and melatonin secretion [24]. Several epidemiological studies and clinical trials have also provided evidence to support the relationship between micronutrient intake and sleep patterns. For example, randomized controlled trials in infants found a longer nighttime and total sleep duration in those receiving supplemental zinc or iron than the placebo group[25]. To date, however, no study has critically reviewed the current literature on the association between micronutrients and sleep in a developmental perspective for sleep patterns.

The aim of this review is twofold: to examine empirical research on the relationship of dietary or biological micronutrient levels with sleep patterns, and to identify issues surrounding implications for future research and public health practice. As micronutrient deficiency and poor sleep are of particular concern in both developed and developing countries [26-29], understanding the possible roles of micronutrients in sleep will inform future prevention and intervention programs for the multifaceted and interrelated public health issues of nutrition and sleep; and shed light on the full extent of their consequences on health.

Methods

This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement checklist [30]. We searched

articles in PubMed, Embase, and Scopus through January 2016. PubMed was searched using MeSH to identify articles with the medical subject headings *micronutrients, minerals, iron compounds, zinc, copper, cobalt, potassium, magnesium, calcium, sodium, phosphorous, manganese,* and *sleep,* and the key words *vitamin**, which yielded 153 articles. Embase was searched using EMTREE with *trace element, vitamin, mineral intake, mineral deficiency, mineral blood level, trace metal blood level, zinc deficiency, vitamin deficiency, sodium deficiency, selenium deficiency, potassium deficiency, phosphate deficiency, calcium deficiency, copper deficiency, iron deficiency, magnesium deficiency, cobalt,* and *sleep,* yielding 100 articles. Additionally, Scopus was searched using the key words *micronutrient*, vitamin*, trace element, sleep, sleep pattern,* and *sleep quality*, identifying 538 results. As shown in Figure 2.1 (The PRISMA flow diagram of article selection process), duplicates were identified and deleted in Refworks, yielding 749 potentially relevant articles. Twenty-three articles were identified for additional scrutiny after reviewing titles and abstracts. The reference lists of the 23 articles were then manually searched. Only articles published in English and Chinese and with full-text available were considered. No year restriction was set in the literature search.

Inclusion and exclusion criteria

Studies relevant to the research question were expected to focus on micronutrient intake or biological micronutrient levels and sleep patterns in humans. More specifically, the term "micronutrients" refers to the three types of nutrients: (a) vitamins; (b) trace elements, such as iron, zinc, copper, cobalt, selenium, manganese; (c) minerals, such as

calcium, magnesium, and potassium [31]. The term "sleep pattern" is used to denote frequently examined parameters in subjective and objective sleep studies, including sleep duration (the time one spends sleeping), sleep-onset latency (the amount of time from lights out, or bedtime, to the commencement of sleep), night awakenings (number of times waking up in the middle of the night), sleep stages (rapid eye movement (REM) and four stages of non-NREM sleep scored according to standard polysomnographic criteria), as well as sleep phases in circadian sleep rhythm, indicated by habitual bedtime and wake time [32]. A total of 70 articles met the inclusion criteria. To examine the direct correlation between micronutrients and sleep patterns, articles were excluded if they met any of the following criteria: (a) the study focused on secondary sleep impairment due to comorbidities such as mood disorders, pain, and treatment effects (n=3); (b) the study investigated the relationship of micronutrient status with physiologic sleep disorders, such as restless leg syndrome or obstructive sleep apnea (n=25); (c) the study primarily examined the molecular or genetic pathways linking micronutrients and sleep patterns using animal models (n=9); (d) the study was a narrative literature review, expert opinion or case study (n=9); (e) the study examined the effect of multiple treatments with micronutrient supplementation as one of the therapeutic elements (n=1).

Data extraction and analysis

Extracted data included research design, subjects (age, sex, and sample size), the covariates that were adjusted for in observational studies, study site, micronutrient measurement, sleep measurement and key findings (See Table 2.1 and 2.2). The methodological strengths and limitations of each study were also summarized in Table

2.1 and 2.2. The research quality of each study was then further evaluated using the validity questions of the American Dietetic Association Quality Criteria Checklist (QCC) for primary research [33]. Each study was classified as positive, neutral or negative according to the rating criteria of the QCC.

Results

A final sample of 26 articles assessing the association between micronutrients and sleep was identified for review, including 19 observational studies (Table 2.1) and 7 clinical trials (Table 2.2). The reviewed articles covered a range of non-institutionalized samples from infants to older adults across several different countries. However, adolescents who may experience significant developmental sleep alteration and metabolic changes were rarely reported in the articles reviewed. Micronutrients studied in the existing literature included iron, zinc, copper, magnesium, as well as Vitamins D and B_{12}. Studies with an observational design measured micronutrient status by either 24-hour dietary recall (one day only) (n=1), generic food frequency questionnaires (n=3), micronutrient-intake recall (n=2), or laboratory indices of serum/ hair concentrations (n=13). The seven clinical trials specifically examined the sleep effect of dietary supplements with various treatment periods, from 1 week to 12 months. The body of literature on sleep-pattern variables associated with micronutrients is broadly categorized into sleep stage, sleep duration, sleep latency, waking after sleep onset, and circadian rhythm of sleep. Along with retrospective questionnaires and prospective sleep logs, objective measurements of actigraphy and polysomnography (PSG) have been used to collect sleep data. Table 2.3 shows the scientific validity of each study.

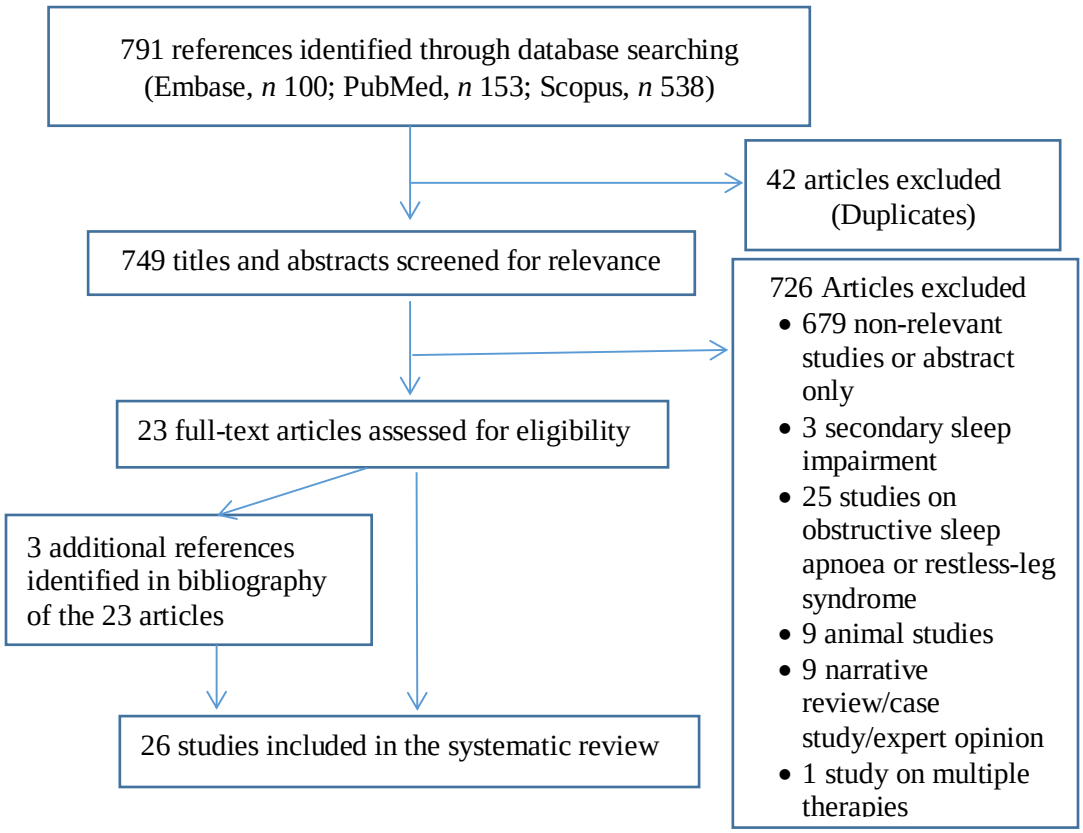

Figure 2.1. PRISMA flow diagram of article selection process

Trace Elements and Sleep Patterns

Iron. The association between iron and sleep duration has been consistently reported in infants and the general adult population. Based on results from a cross-sectional study and a clinical trial, iron deficiency anemia (IDA) may be associated with more night waking and shorter total sleep duration than better-nourished infants [(34)], whereas iron supplement can decrease the length of daytime naps among IDA infants, and increase the night- and total sleep duration in infants regardless of the IDA status at

baseline [25]. A recent observational study extended such associations to adults, showing an association between decreased iron intake and very short sleep (< 5 h) after controlling for overall diet [35]. Iron deficiency is also associated with altered characteristics of sleep stages. As compared with non-IDA infants, IDA infants showed more awake times, shorter quiet-sleep duration, and delayed sleep-spindle patterns in non-rapid-eye-movement (NREM) at night [36]. Such alterations in the temporal organization of sleep architecture may be long lasting. A longitudinal study followed up children with and without IDA in infancy, indicating that former IDA infants exhibited an altered distribution of NREM and REM sleep at 4-year old relative to the non-IDA controls [37].

Zinc and copper. Although the association between zinc and sleep phase in circadian rhythm has been suggested in several studies [38, 39], no consensus has been reached on this effect. In the sample of children aged 6–8 years, researchers found no significant association between low serum zinc and bedtime or wake-up time [39, 40]. In contrast, the study by Sato-Mito et al., which classified participants into quintiles (Q) by the midpoint of sleep (Q1/earliest=2:32am, Q2=3:10am, Q3=3:37am, Q4=4:11am, Q5/latest=5:31am), reported a significant reduction of energy-adjusted zinc intake in women who had the latest midpoint of sleep [40].

With regard to sleep duration, five studies provided evidence to support the association of zinc and copper with sleep duration among different populations. The randomized controlled trials, using maternal reports of sleep patterns, found a longer night and total sleep duration in infants who received supplemental zinc than the placebo group [25]. This finding agrees with an observational study that found an association

between decreased zinc and very short sleep in a general adult population [35]. In terms of nutritional biomarkers, researchers found that shorter sleep duration or increased odds of sleep insufficiency were associated with lower serum zinc levels in women [41] and children in early adolescence [42]. Copper levels showed mixed effects in prior research. Whereas researchers reported a negative relationship between hair copper levels and sleep duration in 126 women aged 21-72 years [41], a study of 2570 men aged 42-60 years old showed highest average levels of serum copper in the group with the longest sleep duration [43].

Minerals and Sleep Patterns

Magnesium. The involvement of magnesium in sleep patterns has been investigated in infants and older adults. Researchers found that increased serum magnesium was associated with increased quiet sleep and decreased active sleep in full-term infants [44]. In a longitudinal study, Black, Holditch-Davis, Schwartz, and Scher [45] reported that preterm infants whose mothers received prenatal MgSO4 treatment (whether or not they also received steroids) had more active sleep without REM, whereas the MgSO4-only group showed higher quiet-sleep regularity and fewer state changes. In older adults, a clinical trial also suggested that oral magnesium supplement may increase SWS delta power and sigma power measured by PSG [46].

Potassium. Using sleep log and actigraph data, the randomized controlled trial by Drennan, Kripke, Klemfuss, and Moore [47] reported reduced sleep duration and wakefulness after sleep onset in young males following potassium supplements compared to controls. These researchers also identified a later bedtime after potassium

supplementation, which is contrary to an observational study on female students aged 18-20 years old that found a negative association between the midpoint of sleep and dietary potassium intake [48].

Vitamins and Sleep Patterns

Vitamin B12. The results showed mixed effects of Vitamin B_{12} on sleep patterns. No significant or definitive effect of Vitamin B_{12} on sleep phase and sleep duration at night was reported in early human studies [24,49,50], except the clinical trial by Mayer, Kroger, and Meier-Ewert [51] that reported an alerting effect of Vitamin B_{12} supplement with a decrease in sleep duration. A recent study consistently found an independent inverse relationship between serum Vitamin B_{12} concentrations and sleep duration in adults [52]. Additionally, one study investigated the relationship between Vitamin B_{12} and sleep timing, measured as the midpoint of sleep. This study showed that young women with lower intakes of Vitamin B_{12} were more likely to have a later sleep period [48].

Vitamin D. Several studies have indicated a potential protective effect of Vitamin D on sleep. Analyses on data from the National Health and Nutrition Examination Surveys (NHANES) 2005–2006 and MESA (2000-2013) found inverse correlations of serum Vitamin D with sleep latency (minutes to fall asleep) [53] and daytime sleepiness [52], but not sleep duration [53] in adults. While these relationships were found from heterogeneous populations including both young adults and older adults, studies specific to community-dwelling older adults reported a positive association between Vitamin D levels in serum and sleep duration [20, 54-55]. Current studies also examined the link between Vitamin D and sleep phase but showed inconsistent findings. In a study on

postmenopausal women, participants with higher intake of dietary Vitamin D showed a later sleep acrophase (which refers to the peak of a fitted 24-h cosine wave that was an indicator of sleep timing measured by actigraphy; this is an indicator of mathematically-modeled curve peak) [56]. In contrast, an inverse relationship was reported in another study conducted with female students aged 18–20 years in Japan [40].

Possible Interactions of Micronutrients on Sleep Patterns

While the main effect of each micronutrient on sleep patterns has been reported in current research, such sleep effect may change depending on the level of another micronutrient, suggesting an interaction among micronutrients. For example, although iron and zinc supplements alone reduced the length of naps as well as increased the duration of night sleep and total sleep in infants, infants receiving iron together with zinc supplements did not exhibit such sleep effect [25]. Zinc/copper ratios in serum and hair also significantly predicted sleep duration in women, with longer sleep duration associated with a medium tertile of zinc/copper ratio in serum or a high tertile of hair ratio [41]. In combining effects of vitamins, researchers of a cross-sectional study reported negative effects from a multivitamin or multiple single-vitamin use on sleep patterns in the number and duration of nighttime awakenings compared to non-vitamin users [57]. Considering the significant effects of vitamins mentioned, this finding suggested possible antagonistic effects between vitamins on sleep outcomes.

Discussion

Sleep Stages in Relation to Micronutrient status

The reviewed studies consistently supported the hypothesis that micronutrient levels can predict the organization of sleep stages, iron, and magnesium in particular. However, researchers should interpret these findings from a developmental perspective for sleep patterns across the life span. Two distinct sleep states are defined on the basis of polysomnography: REM and NREM sleep, which are called active sleep and quiet sleep in infants respectively [1]. Given one of the main alterations in infant sleep is the transition from predominantly active sleep to increased quiet sleep by around 4 months [36], decreased quiet sleep and delayed spindle patterns in NREM sleep from iron deficiency anemia [36] indicate that iron may be essential for the normal development of quiet sleep/NREM sleep in infants.

Studies on prenatal exposure to magnesium in preterm infants [45] and postnatal serum magnesium concentrations in full-term infants [44] have been consistently associated with an increase in the duration of quiet sleep, suggesting a potentiating role of magnesium in the maturity of quiet sleep during infancy. In contrast, preterm with prenatal exposure to MgSO4 was also associated with more active sleep without REM [45]. REM sleep in infants has been linked to the development of the neuromuscular and sensory system, as well as brain function [58]. As the amount of active sleep without REM often decreases over the preterm period [59], increased active sleep without REM suggests delayed development of active sleep and possible influence on the brain development, thus raising a concern of magnesium treatment for pregnant women and infants. The effects of magnesium on infant sleep need to be interpreted cautiously due to the

coexistence of acceleration in the development of quiet sleep and a delay in the maturity of active sleep.

The benefit of magnesium supplements on increased slow wave sleep (SWS) in the elderly is of particular interest. Whereas the most prominent alterations in elderly sleep are known to be reductions of SWS, REM sleep, and sleep efficiency [(1)], magnesium may have beneficial effects on sleep patterns due to its action on reversing age-related sleep changes. Additionally, given that the delta power of SWS in EEG reflects the restorative property of sleep [(60)], magnesium may improve the physiological function of sleep in older adults, which warrants the future research on the clinical application of magnesium supplement for older adults.

Sleep duration in relation to micronutrient status.

Most trace elements and minerals in this review correlated significantly with sleep duration in different populations, despite the discrepancy in the direction of these relationships across micronutrients. Specifically, iron, zinc, and magnesium may positively associate with sleep duration, whereas the relationship between sleep duration is inverse to the levels of hair copper, as well as potassium and Vitamin B_{12} supplements. The relationship between Vitamin D and sleep duration is controversial. Considering the negative correlation between the number of waking episodes and sleep duration at night [(34)], together with associated sleep onset latency [(39)], the effect of iron, zinc, and magnesium on sleep duration might be moderated or mediated by the number of awakenings after sleep onset and sleep latency at night. This hypothesis may not be supported by the study on potassium due to the coexistence of reduced sleep duration and

wakefulness after sleep onset [47]. However, delayed sleep time reported by sleep logs in that study could explain such paradoxical findings.

Although micronutrient status could be a modifiable factor to improve sleep duration, micronutrient levels may not show a linear association with sleep, with the longest sleep duration found in the middle tertile level of zinc and copper in women [41]. This finding, together with evidence that both short- and long-sleep duration have been documented to increase all-cause mortality (cardiovascular-related, cancer-related, and all) [14], indicate that optimal rather than high or low micronutrient levels are related to normal sleep patterns.

Sleep phase in relation to micronutrient status.

The published literature supported the presence of an association between sleep phase with micronutrient variables of zinc, potassium, and Vitamin D. However, the limited evidence and lack of consensus on such associations preclude the conclusion of causal links or concrete clinical recommendations. The different results could be attributed to the large night-to-night variation of the indicators for sleep phase: sleeping time and wake-up time. Sleep habits in humans vary greatly between weekdays and weekends [61], and human chronotype is known to correlate better with the midpoint of sleep on free days than on work days in a non-experimental environment [62]. However, studies in this review did not differentiate weekdays from weekends for bedtime and wake-up time, thus leading to substantial measurement error and biased findings.

The inconsistent results of micronutrients and sleep phase may also result from the variability of micronutrient and sleep measurements. Dietary intake may be positively

associated with biological micronutrient levels in red blood cells and serum [25, 51, 63]. However, metabolic characteristics enable some micronutrients, such as Vitamin B12, to maintain a normal range for a short period of dietary deficiency [31], suggesting a possible discrepancy between dietary intake and biological levels of micronutrients. Similarly, sleep data were collected by different questionnaires; and thus, it is possible that inconsistent patterns of statistical significance reflect different psychometrical properties rather than true differences in the associations. Besides, subjective sleep measurements do not highly correlate with estimates based on physiologic measures [64], and may lead to different results as compared with objective measurements. Therefore, standardized objective measures of micronutrients and sleep should be incorporated into future studies.

Neurobiological mechanism of current findings.

Although the possible neurobiological mechanisms underlying the main effects of micronutrients on sleep patterns are not fully understood, researchers have suggested that a number of causal pathways linking micronutrients with sleep in experimental studies. For example, micronutrients may be essential in the synthesis and transportation of neurotransmitters that are related to sleep homeostasis. Whereas iron, zinc, copper, and magnesium may be associated with antagonists of excitatory transmissions, including the N-methyl-D-aspartate receptor [65, 66], and dopaminergic neurons [67, 68], micronutrients can also potentiate inhibitory transmissions, such as gamma-Aminobutyric acid ($GABA_A$) receptors [69, 70]. In addition to the neurobiological pathways, researchers have documented that retinoic acid, a metabolite of Vitamin A, significantly up-regulates the expression of *Circadian Locomotor Output Cycles Kaput (CLOCK)*/*brain and muscle*

arylhydrocarbon receptor nuclear translator (BMAL)-dependent circadian genes, thus modulating the circadian sleep regulatory process and affecting sleep phase, sleep duration, as well as the organization of sleep stages [71].The mechanisms underlying the association between micronutrients and sleep regulation warrant future examination of the effect of micronutrient status on sleep patterns.

The long-term effect of suboptimal levels of micronutrients, such as iron deficiency, may be due to an irreversible impact on the brain. Several studies on rats have shown that iron deficiency causes disturbances and damage to brain iron distribution and sleep-related neurotransmitter systems, and such disturbances could not be completely normalized by iron replenishment [72]. Current literature has also supported the interactions between micronutrients on sleep patterns, which possibly result from the antagonism between micronutrients in either metabolic absorption or the binding to receptors of neurotransmitters [73, 74]. Specifically, a high intake of zinc may interfere with absorption of iron and copper [73]. Additionally, under physiological conditions, zinc ion would liberate copper ion from the $GABA_A$ receptor and inhibit the effect of copper on sleep regulation [74]. These interactions have made it hard to disentangle the direct effect of a single micronutrient on sleep outcomes from current findings.

Implications for Future Nutrition Research

The results from this review have important implications for future research. Due to the possibility of concentration-dependent effect and intertwined actions of micronutrients, further studies demonstrating the nature of the effects of micronutrient levels on sleep are warranted. In terms of studied populations, whereas articles involving

human subjects have primarily focused on infants, young children, general adults, postmenopausal women, and older adults, very few studies have specifically investigated children in adolescence when substantial developmental changes in sleep pattern occur. Additionally, many of the current studies were heterogeneous in age, including all young, middle-age and older adults [35, 52]. Given that the metabolic characteristics of micronutrients and the biological regulation of sleep may vary across the life span, researchers should take into account the developmental effect when interpreting results. More than half of human studies are observational studies, and the degree of control for confounders varies across studies. Confounders that were typically adjusted for included age, sex, education, family income, body mass index, energy intake, smoking status, and race/ethnicity. There are only four studies involved covariates of depressive symptoms or antidepressant use [20, 43, 52-53]. Future research should take into account important covariates, such as a medical history of psychiatric disorder or medication usage known to affect micronutrient absorption or sleep, to uncover the nature of the associations. Furthermore, a majority of reviewed studies were cross-sectional or focused on the short-term effects of micronutrient supplements. Research with a longitudinal design is needed to investigate how micronutrient status early in life predictors later sleep patterns, and how the trajectory of micronutrient profile over the parts of the lifespan are associated with age-related changes in sleep patterns.

Implications for Public Health Practice

Both micronutrient deficiency and poor sleep are significant public health issues worldwide. Although the clinical relevance of micronutrients on sleep patterns needs

further examination, health care providers, particularly those practicing in primary care settings, should take into account several findings from current literature into health care practice for individuals and communities. First, health care providers should be attentive about micronutrient levels in infants due to the irreversible influence on brain function and sleep organization, iron status in particular. Second, when using magnesium in prenatal settings, particular attention should be given to mixed effects on the development of active sleep and quiet sleep in infants. However, magnesium may be beneficial for elderly sleep and brain function. Third, despite the indefinite recommendation of optimal doses at this moment, it is important to note that intake of optimal rather than high or low micronutrient is needed to maintain normal sleep patterns.

Limitation of This Review

This review has several potential limitations. First, only articles focusing on a direct relationship between micronutrient variables and sleep-pattern variables are included. Studies on physiologic sleep disorders such as restless leg syndrome, obstructive sleep apnea, and health complaints such as pain, which were not reviewed here, may uncover other missed relationships between micronutrients and sleep patterns. Second, this review did not stratify findings by gender, race and cultural context due to the unavailability of relevant data, and thus, it cannot disentangle the interplays between social factors and micronutrients on sleep patterns.

Conclusion

Although no definite clinical recommendations can be made at this point due to the limited evidence, current studies have linked the trace elements, minerals, and

vitamins to sleep patterns in humans. In the articles reviewed, researchers have observed a beneficial effect of adequate serum iron on the development of sleep stages in infants, and magnesium supplements on inhibiting age-related sleep changes in older adults. Published literature also supported an association between sleep duration and micronutrients, with sleep duration positively associated with iron, zinc, and magnesium, and negatively associated with copper, potassium and Vitamin B_{12}. However, the results of the associations between micronutrients and sleep phase were insufficient and inconsistent. Future research is needed to investigate the concentration-dependent as well as the longitudinal relationships between micronutrient levels and human sleep across populations, test the interactions among micronutrients on sleep outcomes, and ultimately examine the clinical relevance of micronutrients on sleep health.

Table 2.1 Observational studies on micronutrients and sleep patterns in humans.

Study*	Methods	Samples/settings	Micronutrient Measures	Sleep Measures	Major Findings	Strengths	Limitations
Luojus (2015) [43]	Cross-sectional study V: age, cumulative smoking history, alcohol consumption, Human Population Laboratory depression scale scores, physical activity, cardio metabolic syndrome, cardio-metabolic syndrome, cardiovascular disease history.	Men aged 42-60 years (n=2570)/Eastern Finland	Serum zinc and copper concentrations	Self-reported sleep duration	Sleep duration was significantly associated with levels of both serum copper and high-sensitivity C-reactive protein in adjusted models. Zinc no longer significantly associated with sleep duration after adjustment.	A large sample size Biomarkers of zinc and copper Controlled for important confounders	Self-reported sleep duration may lead to recall bias. Sleep duration was categorized into 9 levels, which decreased statistic power; Only included men in the study and may reduce its generalizability.
Ji (2015) [42]	Longitudinal study V: grade, sex, education level in mother and in father.	Followed children from 3-5 years old to 11-15 years (n=1295)/ China	Serum zinc concentrations	Self-reported sleep patterns, Pittsburgh Sleep Quality Index	Cross-sectional analyses showed negative correlations of blood zinc concentrations with insufficient sleep duration, sleep disturbances and poor sleep quality in adolescence, but no association at preschool age. Longitudinal analyses indicated that blood zinc concentrations at preschool age predict poor sleep efficiency and poor sleep quality in adolescence.	Include two-wave longitudinal data from a preschool cohort Large sample size, Used blood zinc status	Used only subjective sleep measures; Did not adjust for dietary nutrients, environmental influential factors
Massa (2015) [55]	Cross-sectional study V: age, clinic, season, comorbidities, body mass index, and physical and cognitive	Men aged 68 years or older (n=3,048)/	Total 25(OH) vitamin D combining 25(OH) vitamin	Nightly total sleep time, sleep efficiency, and wake time after sleep onset	Low levels of total serum 25(OH)D are associated with poorer sleep including short sleep duration and lower sleep	Objective sleep measures for an average of 5 consecutive 24-h periods	Findings may not be generalizable to young men or women.

	function		D2 and 25(OH) vitamin D3	(WASO) obtained using wrist actigraphy worn for an average of 5 consecutive 24-h periods	efficiency	Used biomarkers of nutrient Data was from a large cohort study	Lack of additional hand scoring of actigraphic records.
Bertisch (2015) [20]	Cross-sectional study V: Model a: adjusted for age, sex, race/ethnicity, examination site, and waist circumference. Model b: additionally adjusted for education, family income, physical activity, smoking status, and alcohol intake. Model c: additionally adjusted for antidepressant use, depression score, history of osteoarthritis, history of asthma, and glomerular filtration rate.	Adults with mean age of 67.4 years (n=1,721)/ US	Serum 25(OH)D concentration	Sleep duration, efficiency, and symptoms were measured by polysomnography, actigraphy, and questionnaires	Vitamin D deficient individuals slept shorter than sufficient individuals, with strongest associations shown in African Americans. Chinese Americans with Vitamin D deficiency had a higher apnea-hypopnea index (AHI) versus sufficient individuals.	Investigated the ethnic disparity in sleep effect of Vitamin D. Used both objective and subjective sleep measures; Used biomarker of nutrient. Rigorously controlled for multiple potential confounders.	Vitamin D measurement preceded the collection of sleep outcomes by an average of 10.3 y. Did not adjust for season variation of Vitamin D levels.
Kim (2014) [54]	Cross-sectional study V: sex, age, body mass index (BMI), cigarette smoking, alcohol consumption, and self-reported daily sun exposure	Adults aged 60 to 80 (n = 1,614)/South Korea	Serum 25(OH)D concentration	Self-reported sleep duration	Serum vitamin D level is positively associated with self-reported daily sleep duration in elderly Korean individuals.	Used a nationally representative sample of older adults; Considered sun exposure as a vitamin D resource	Did not adjusted for dietary intake, supplementation Of vitamin D, and season influence. Self-reported sleep duration and sun exposure may lead to recall bias.
Beydoun (2014) [52]	Cross-sectional study V: age, sex, race/ethnicity, education, marital status and family income, BMI, smoking, physical activity, self-reported chronic conditions, anti-depressant medication use; dietary Intakes	US NHANES, 2005–2006 (aged 20-85, n=2459) / US	Serum concentrations of key nutrients	sleep questionnaire included items on sleep habits and disorders; and a subscale of the Functional	Independent inverse associations were found between serum vitamin B-12 and sleep duration, 25(OH)D and sleepiness (as well as insomnia), and between folate and sleep disturbance.	Used a nationally representative sample; Biomarkers of nutrients; Adjusted important confounders	Did not stratify age groups Self-reported sleep may lead to recall bias.

				Outcomes of Sleep Questionnaire			
Shiue (2013) [53]	Cross-sectional study V: sex, ethnicity, body mass index, high blood pressure, active smoking, depressive symptom	NHANES, 2005–2006 (aged 16 and above, n = 6139) / US	Serum 25(OH)D concentrations	Self-reported sleeping hours, minutes to fall asleep and sleep complaints; sleep disorders diagnosed by doctors	No association between serum 25(OH) D concentrations and sleeping hours was observed while a significant inverse association was found between serum 25(OH) D concentrations and minutes to fall asleep. Moreover, people with higher vitamin D levels could be more likely to complain sleep problems, although the reason is unclear.	Used a nationally representative sample	Did not stratify age groups Did not adjust the status of other nutrients which may affect sleep patterns Self-reported sleep duration and latency may lead to recall bias.
Grandner (2013) [35]	Cross-sectional study V: total energy intake, total number of foods consumed, age, gender, income, education, BMI and exercise	Adults aged 18+ in the NHANES 2007-2008 (n = 5587) / US	24-h food recall	Self-reported sleep duration	After adjustment for overall diet, only decreased phosphorus, magnesium, iron, zinc, and selenium in the context of very short (<5 h) sleep and decreased phosphorus in the context of long sleep remained significant. Some of the effects of the vitamin for very short (<5 h) sleep remained including decreased thiamin, total folate, folic acid, and folate DFE in fully adjusted analyses.	Sampling was performed to ensure generalizability to the US population. Effects of covariates were examined separately and totally.	Self-reported sleep duration and dietary intake may lead to recall bias. Did not differentiate sleep duration in weekdays and weekends. Direction of effect cannot be determined
Song (2012) [41]	Cross-sectional study V: age, smoking status, and occupation	Women aged 21 to 72 years (n=126)/Korea	Zn, Cu, and Zn/Cu ratio in	Self-reported questionnaire (7-day recall):	The participants in the middle tertile of Zn and Zn/Cu ratio in the serum	Compared results from different	Results of the present study may not generalize to men and other people.

			the serum and hair	average sleep hours separately on weekdays and weekends.	had significantly longer sleep duration compared to those in the lowest tertile. An increasing Zn/Cu ratio in the hair was associated with longer sleep hours, whereas sleep duration decreased significantly from the lowest to the highest tertile of hair Cu level.	micronutrient measures	Self-reported sleep duration and dietary intake may lead to recall bias. Did not mention the method used to calculate mean hours of sleep based on weekday and weekend sleep hours. Direction of effect cannot be determined
Sato-Mito (2011) [48]	Cross-sectional study V: current smoking status, supplement use, energy intake	Women aged 19–36 (n=112)/ Japan	Self-administered diet history questionnaire (One month recall)	Morningness–Eveningness Questionnaire (MEQ), preferred bedtime and rise time.	A lower MEQ score showed a significant association with a lower energy-adjusted intake of protein, calcium, magnesium, zinc, vitamins (D, riboflavin, and B6), and vegetables, and with a higher intake of noodles. Furthermore, a later midpoint of sleep showed a significant association with a lower energy-adjusted intake of protein, cholesterol, potassium, calcium, magnesium, zinc, vitamins (D, riboflavin, B6, and B12), soy, fish and shellfish, and eggs, and with a higher intake of noodles, bread, and confections.	Supplement use was addressed in data analyses	The sample used may be limited in its generalizability. Self-reported dietary intake may lead to recall bias. Investigated preferred rather than actual sleep schedule. Did not consider known confounders such as SES, course load Direction of effect cannot be determined
Sato-Mito. (2011) [40]	Cross-sectional study V: The means of dietary intake , dietary behaviors (time at which meals began, eating	Female dietetics students aged 18–20 years (n=3304)/Japan.	Self-administered diet history questionnaire	Self-reported bedtimes and rise times on weekdays.	Late midpoint of sleep was significantly negatively associated with the percentage of	Used large sample size.	The sample used may be limited in its generalizability.

	duration, the number of skipped meals, and the number of occasions when TV was watched during weekday meals), lifestyle variables, residential block, the size of the residential area, and current smoking status.		(One month recall)		energy from protein and carbohydrates, and the energy-adjusted intake of cholesterol, potassium, calcium, magnesium, iron, zinc, vitamin A, vitamin D, thiamin, riboflavin, vitamin B6, folate, rice, vegetables, pulses, eggs, and milk and milk products.	Important confounders were controlled.	Self-reported dietary intake may lead to recall bias. Did not consider sleep-latency adjusted midpoint of sleep Did not consider known confounders such as SES, course load Investigated sleep schedule on weekdays only. Direction of effect cannot be determined
Grandner (2010) [56]	Cross-sectional study V: adjusted for age, income, education, total dietary amount, BMI, and minutes of moderate-strenuous physical activity	Post-menopausal women (n=459)/USA	Food frequency questionnaire (Three- month recall).	Objective sleep: one week of actigraphy. Subjective sleep: sleep diary.	Later sleep acrophase, an indicator of sleep timing, was associated with more dietary Vitamin D.	Used large sample size. Incorporated subjective and objective sleep measurement.	The sample used may be limited in its generalizability. Self-reported dietary intake may lead to recall bias. Did not differentiate weekdays from weekends for sleep schedule. Direction of effect cannot be determined.
Kordas (2008) [34]	Cross-sectional study V: age, sex, SES, breastfeeding, caste, Malarial parasite count, Trichuris egg densities, illness in the previous week, and walking unassisted	Infants aged 6-18 months: study 1(n=174)/ Pemban and Zanzibar study 2(n =770)/ Pemban and Zanzibar study3(n=326)/ Nepal	Hb, serum zinc protoporphyrin	Sleep questionnaire (parental report)	IDA infants were associated with shorter night sleep duration and higher frequency of night waking.	Used large sample size. Used biomarkers of micronutrient status. Compared results across 3 places.	Parental reports/recall may lead to measurement bias. Did not mention if infants with medication were excluded. Adjusted different covariates in the studies and inhibited the precision of the comparison across studies.

							Direction of effect cannot be determined
Kordas (2007)[39]	Cross-sectional study V: anxiety, bedroom sharing, who decides when to get up and go to bed, age, sex, crowding in the home.	Children aged 6–8 years (n=550)/ Mexico	Serum zinc, serum ferritin (SF), hemoglobin (Hb)	Sleep questionnaire (parental report)	Children with anemia tended to have an earlier bedtime, and be less likely to have long sleep onset latency. Low SF was related to longer sleep onset latency. Zinc deficiency was not related to sleep, behavior, or activity.	Used large sample size. Used biomarkers of micronutrient status.	Parental recall may lead to measurement bias. Did not differentiate weekdays from weekends for sleep schedule. Did not classify the type of anemia. The direction of effect cannot be determined.
Peirano (2007)[36]	Cross-sectional study V: age, gender, birth weight, weight-for-age z-score, and mother's IQ.	Otherwise healthy 6-month-old infants with IDA(n=26) and non-anemic control infants(n=18)/ Chile	Hb, SF	Sleep PSG(EEG activity)	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	Used objective sleep measurement	Did not evaluate the precise location of spindle waves which reflect different developmental paths; Did not tease apart the pharmacologic effects Direction of effect cannot be determined
Peirano (2007)[37]	Longitudinal cohort study Exposure: IDA in infancy V: age and sex	55 healthy 4-y-old children (former IDA = 27, nonanemic controls = 28)/ Chile	IDA in infancy	Sleep PSG(EEG activity)	Relative to controls, former IDA children showed: a) longer duration of REM sleep episodes in the first third and shorter in the last third; b) more REM sleep episodes in the first third and fewer in the second third; and c) shorter latency to the first REM sleep episode and shorter NREM stage 2 and SWS episodes within the first sleep cycle.	Time-order relationship was generally clear. Used objective sleep measurement	Only recorded a single night-sleep in the laboratory; Did not assess daytime naps and could not determine whether disrupted nighttime sleep was caused by a long daytime nap. Did not consider current iron status

Lichstein (2007) [57]	Cross-sectional study V: age, ethnicity and sex	72 people, ranging in age from 20 to 9/ USA	Self-reported vitamin use (one-month recall)	Sleep diaries and sleep questionnaires	For those individuals taking a multivitamin or multiple single vitamins, sleep diaries revealed poorer sleep compared to non-vitamin users in the number and duration of awakenings during the night. After controlling for age, ethnicity, and sex the difference in the number of awakenings was still marginally significant.	Used large sample size.	Self-reported vitamin intake may lead to recall bias. Sleep diary may be not reliable. Did not stratify the type of vitamin use. Did not collect data on herbal supplements Direction of effect cannot be determined
Black (2006) [45]	Longitudinal cohort study: Exposure: antenatal MgSO4 supplement V: N/A	Preterm infants (n=134) MgSO4 only (n=5) Steroids only (n=46) MgSO4+ Steroids (n=45) Non-treatment (n=38)/ USA	N/A	Sleep EEG	Infants exposed to MgSO4 had more active sleep without rapid eye movement. The MgSO4-only group had higher quiet sleep regularity scores and fewer state changes.	Time-order relationship was generally clear. Used objective sleep measurement	Did not mention if confounders were controlled in regression models. Dose or duration of MgSO4 treatment was not adjusted. Sampling issues may limit the generalizability of these findings. Small size of the MgSO4 only group is small.
Dralle (1980) [44]	Cross-sectional study V: N/A	Full-term newborn infants between the 5th and 15th days of life (n=14)/ Germany	Serum magnesium	Sleep EEG, EMG from chin muscles, EOG and ECG	With increasing serum Mg, quiet sleep increased, whereas active sleep decreased.	Used objective sleep and micronutrient measures	Used small sample size. Magnesium treatment was not controlled in regression models.

Note: ***** For each study, the first author and the publication year were presented in the table. PSG = polysomnograhy; EEG = electroencephalography; EOG = electro-oculogram; ECG = electrocardiography; V= variables adjusted for; N/A = not applicable.

Table 2.2. Clinical trials on micronutrients and sleep patterns in human subjects

Study	Methods	Samples/settings	Sleep Measurement	Findings	Strengths	Limitations
Kordas (2009) [25]	Two RCTs with 2 × 2 factorial design: G1: 12.5 mg elemental iron + 50 ug folic acid G2:10 mg zinc G3:iron–folic acid and zinc G4:placebo	Study1: Pemban and Zanzibar infant (n=877) Study 2: Nepali infants (n=567)	Maternal reports of sleep patterns (napping frequency and duration, nighttime sleep duration, the frequency of night waking) were collected.	Serum zinc levels and Zn/Cu ratio were lowest in ⩽6 h sleep. Serum copper levels were highest in ⩾10 h sleep. Supplemental iron was consistently associated with longer night and total sleep duration. There was also a positive main effect of zinc on total sleep duration	Used large sample size and multiple study sites	Maternal recall may lead to bias. It is also possible that micronutrient treatment increased daytime activity (reduced lethargy), in which case infants would be tired and require longer sleep. Side effects were not assessed.
Held (2002) [46]	RCT with cross-over design: two Mg2+ treatment intervals of 20 days duration separated by 2 weeks washout	Elderly subjects (n=12)	Sleep EEG	Mg2+ led to a significant increase in slow wave sleep delta power and sigma power.	Used subjective sleep measures	Used small sample size The long-term effected was not assessed.
Honma (1992) [24]	RCT with a crossover design Vitamin B12 supplement was taken for 4 weeks	Healthy subjects (n=9)	Sleep-log Serum melatonin	No significant differences were observed between groups in the timing and duration of sleep.	The RCT design allows for the examination of causal-effect. Explored the mediators of Vitamin B12 and sleep	The sample size is small. Side effects were not assessed. Sleep log may not reveal the real sleep-wake cycle.
Okawa (1997) [49]	RCT Methylcobalamin (3 mg/day) or placebo was administered for 4 weeks.	50 patients with delayed sleep phase syndrome aged 13–55 years: methylcobalamin	Sleep-log	No significant differences were observed between the 2 groups in drowsiness during the daytime or in night sleep duration.	The RCT design allows for the examination of causal-effect.	Sleep duration from sleep log may be biased due to the lack of information on sleep latency.

		group: n=27; control group: n=23				Side effects were not assessed.
Takahashi (1999) [50]	RCT Methylcobalamin 6 mg/day, for 8 weeks Control: 0.03 mg/day	Patient with DSPS (age: N/A) Methylcobalamin group: n=21; control group: n=27	Sleep diary and questionnaire	Significant improvement of the parameters of the sleep–wake cycle, was observed in the test group compared to the control group at the end of the 4th week but not the 8th week of administration.	Examined the does effect of Vitamin B12	Sleep diary may not reveal the real sleep-wake cycle. Did not design placebo group. Did not compare the baselines
Mayer (1996) [51]	Pretest-posttest design G1: 3 mg cyanocobalamin (CB12) treatment G2: methylcobalamin (MB12) treatment	Healthy adults (mean age 35.17 in women, 37.2 in men) CB12: n=9 MB12: n=10/ Germany	Wrist actigraph	Only MB12 has a positive psychotropic alerting effect with a distribution of the sleep-wake cycle toward sleep reduction.	Compared the effects of different forms of Vitamin B12	Did not design a placebo group. The ceiling effect may exist due to the optimal pre-treatment scores in many participants. The comparison between groups was questioned due to the unequal baselines.
Drennan (1991) [47]	RCT with a crossover design: 1 week of oral potassium chloride supplements (96 meq/day) to I week of identical placebo capsules	Healthy male volunteers aged 18-33 years (n=9) /US	Sleep log and wrist actigraphy	Potassium supplementation significantly delayed sleep-log-identified Bedtime. Potassium reduced Sleeping Interval for both sleep-log and wrist-actigraph data. Potassium significantly increased actigraphic Sleep Efficiency due to a reduction in actigraphic Wake after Sleep Onset (WASO).No effect of potassium on actigraphic sleep phase was observed.	Compared findings from subjective and objective sleep measures Side effects were assessed.	The sample size is small. The experimental period is short (2 weeks), and the long-term effects were not assessed.

Note: PSG = polysomnograhy; EEG = electroencephalography; G=group, DSPS= delayed sleep phase syndrome.

Table 2.3 Research validity assessment using the Quality Criteria Checklist (QCC)

Study	1.Clear research question	2.Participant selection free of bias	3.Comparable study groups#	4.Withdrawals or response rate described	5.Use of blinding	6.Intervention protocol and/or data collection procedure described in detail	7. Outcomes clearly defined and the measurements valid and reliable	8.Appropriate statistical analysis	9.Conclusion supported by results	10.Unlikely funding bias	Overall quality rating*
Observational studies											
Luojus et al. (2015) [43]	Y	Y	Y	N	Unclear	Y	Y	Y	Y	Y	+
Ji et al. (2015) [42]	Y	Y	Y	Y	Unclear	Y	Y	Y	Y	Y	+
Massa et al. (2015)[55]	Y	Y	Y	Y	Unclear	Y	Y	Y	Y	Y	+
Bertisch et al. (2015)[20]	Y	Y	Y	Y	Unclear	Y	Y	Y	Y	Y	+
Kim et al. (2014) [54]	Y	Y	Y	N	Unclear	Y	Y	Y	Y	Y	+
Beydoun et al. (2014) [52]	Y	Y	Y	Y	Unclear	Y	Y	Y	Y	Y	+
Shiue et al.(2013)[53]	Y	Y	Y	N	Unclear	N	Unclear	Y	Y	Unclear	Ø
Grandner et al.(2013)[35]	Y	Y	Y	N	Unclear	Y	Y	Y	Y	Y	+
Song et al.(2012) [41]	Y	Unclear	Y	N	Unclear	Y	Y	Y	Y	Y	Ø
Sato-Mito et al. (2011) [48]	Y	Unclear	Unclear	N	Unclear	Y	Y	Y	Y	Y	Ø
Sato-Mito et al.(2011)[40]	Y	Y	Y	Y	Unclear	Y	Y	Y	Y	Y	+
Grandner et al.(2010)[56]	Y	Y	Y	N	Unclear	Y	Y	Y	Y	Unclear	+
Kordas et al.(2008) [34]	Y	Y	Y	Y	Unclear	Y	Y	Y	Y	Unclear	+
Kordas et al.(2007)[39]	Y	Y	Y	Y	Unclear	Y	Y	Y	Y	Unclear	+
Peirano et al.(2007) [36]	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	+
Peirano et al.(2007) [37]	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	+
Lichstein et al. (2007)[57]	Y	Y	Y	Y	Unclear	Y	Y	Y	Y	Y	+

Black et al. (2006) [45]	Y	Y	Y	N	Unclear	Y	Y	Y	Y	Unclear	+
Dralle et al.(1980) [44]	Y	Unclear	Unclear	N	Unclear	Y	Y	Y	Y	Unclear	Ø
Clinical Trials											
Kordas et al.(2009) [25]	Y	Y	Y	N	Unclear	Y	Y	Y	Y	Y	+
Held et al.(2002)[46]	Y	Unclear	Y	Y	Unclear	Y	Y	Y	Y	Unclear	
Honma et al. (1992)[24]	Y	N	Y	Y	Unclear	Y	Y	Y	Y	Unclear	Ø
Okawa et al. (1997)[49]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Unclear	+
Takahashi et al. (1999)[50]	Y	Y	N	N	Y	Y	Y	Y	Y	Unclear	Ø
Mayer et al. (1996)[51]	Y	Unclear	N	N	Unclear	Y	Y	Y	Y	Unclear	Ø
Drennan et al. (1991) [47]	Y	N	Y	N	Unclear	Y	Y	Y	Y	Unclear	Ø

Y=yes, N=no, unclear=not clearly reported.
#: RCTs: baseline comparison between groups; observational studies: were preexisting differences accounted for by using appropriate adjustments?
**: positive (+):If most (six or more) of the answers to the above validity questions are "Yes" (including criteria 2, 3, 6, 7), the report should be designated with a plus symbol (+) on the Evidence Worksheet. Neutral(Ø): If the answers to validity criteria questions 2, 3, 6, and 7 are "Yes" but several other criteria indicate study weaknesses, the report should be designated with a neutral (Ø) symbol on the Evidence Worksheet; Negative (-):If most (six or more) of the answers to the above validity questions are "No," the report should be designated with a minus (-) symbol on the Evidence Worksheet.*

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CHAPTER 3: SERUM MICRONUTRIENT STATUS AND SLEEP IN EARLY ADOLESCENTS[1]

Abstract

Introduction. Little evidence is available regarding the relationship between micronutrient status and sleep in adolescents. The present study aimed to examine the cross-sectional and longitudinal associations of serum iron and zinc concentrations with self-reported sleep in adolescents, and explore the interactions between iron and zinc status.

Methods. A total of 777 adolescents from the Jintan China Child Cohort were included in this study. Blood samples were analyzed for iron and zinc concentrations when the children were at preschool age (3–5 years old) and early adolescence (11–14 years old). Global sleep quality and sleep subdomains were assessed by the Pittsburgh Sleep Quality Index (PSQI) during early adolescence. Statistical methods included the generalized linear regression and ordered logistic regression models.

Results: the cross-sectional analyses found that increased serum zinc concentrations were associated with better global sleep quality (β=-0.20, p=0.03), as well as decreased odds of insufficient sleep duration (OR=0.28, p <0.001) and sleep disturbances (OR=0.45, p=0.04) in early adolescents. The longitudinal analyses revealed that higher serum zinc concentrations at 3–5 years old were associated with better sleep efficiency (OR=0.17, p=0.03) at 11–14 years old. Regarding iron, higher serum iron concentrations showed significant cross-sectional association with better sleep latency (OR=0.68, p=0.03) at 11–14 years old. Yet, the longitudinal associations of serum iron and sleep, as well as the interaction effects between iron and zinc on sleep quality did not reach statistical significance (ps>0.05).

Conclusions. Our findings suggest that increased serum zinc and iron concentrations are associated with decreased risk for sleep impairment. Future research is warranted to examine the clinical relevance of micronutrient status on sleep in early adolescents.

Keywords: serum iron; serum zinc; sleep quality; adolescents; micronutrients

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Introduction

Inadequate sleep or reduced sleep quality represent significant public health concerns in adolescents. It is estimated that between 14% and 68.9% of adolescents worldwide report any kind of sleep problems, such as sleep deprivation, poor sleep quality and sleep disturbances (National Sleep Foundation, 2007; Chung & Cheung, 2008; Eaton et al., 2010; Gradisar et al., 2011). Prior research has shown that suboptimal sleep contributes to impaired mental health (Ivanenko et al., 2005), decreased neurocognitive function and academic performance (Dewald et al., 2010), as well as an increased risk for obesity (Liu, Zhang, et al., 2012). Epidemiological evidence linking psychosocial and environmental factors to sleep problems in adolescents is rapidly growing, including early school start times, homework burden, co-sleep status, stressful events and electronic media use (Cain & Gradisar, 2010; Knutson & Lauderdale, 2009; Li et al., 2010; Usami et al., 2013). In contrast, the associations between the nutritional factors, especially suboptimal micronutrient status, and impaired sleep have yet to be investigated.

The relationship between micronutrients and sleep is biologically plausible. Animal studies have implicated the involvement of micronutrients in intrinsic sleep regulation, showing their associations with sleep-related neurotransmitters (Campbell, Gustafson, & Feinberg, 2002; Carskadon, 2002; Frederickson, Suh, Silva, Frederickson, & Thompson, 2000) and melatonin secretion (Honma, Kohsaka, Fukuda, Morita, & Honma, 1992). Several cross-sectional studies and clinical trials have also provided evidence to support the relationship between micronutrient status and sleep, with a focus on infants (Kordas et al., 2009), young children (Kordas et al., 2007), and adults (Grandner, Jackson, Gerstner, & Knutson, 2013). For example, infants with iron deficiency anemia (IDA) tended to have

more night waking and short sleep duration than better-nourished infants (Kordas et al., 2008). The cross-sectional study by Grandner et al. (2013) also showed that lower zinc intake was in alignment with short sleep in adults.

Despite the emerging evidence suggesting the relationship between micronutrient status and sleep, the number of studies in this field remains low, especially in the adolescent population. Given the rapid brain maturation and developmental changes in sleep regulations throughout adolescence (Bryan et al., 2004), adolescent sleep may be particularly vulnerable to suboptimal micronutrient status. Additionally, the extant literature primarily focused on the short-term effect of micronutrient status on sleep parameters or the cross-sectional correlations, yet the long-lasting effect remains uncertain. Furthermore, prior evidence also suggested possible antagonisms between micronutrients in binding to receptors of sleep neurotransmitters in rats (Sharonova, Vorobjev, & Haas, 2000). The interaction effect between micronutrients on sleep, however, has yet to be explored in human beings.

The primary aims of the present study were to examine the cross-sectional association between serum micronutrient status and sleep at early adolescence, and the longitudinal association between micronutrient status at preschool age and sleep at early adolescence. This study also explored the interactions between iron and zinc. The findings from this study may provide foundations to understanding the biological risk factors of sleep impairment and inform future interventions for optimizing sleep health in adolescents.

Methods

Participants and Procedures

The present study is an extension of the China Jintan child cohort that aims to

investigate early health factors for the later neurobehavioral development in childhood and adolescence (Liu et al., 2015; Liu et al., 2010). Using a multi-stage sampling method, this cohort study enrolled 1656 children aged 3–5 years who represented preschoolers from different school districts in Jintan city in 2004. According to their grade (1st, 2nd, and 3rd) in preschools, children were classified into lower, middle and upper cohort, respectively. In 2005–2007, we collected Wave I data, including serum micronutrient concentrations, when children graduated from preschools with a response rate of 83.6% (n=1385) (Liu et al., 2010). About 1110 (54% males) children participated in the Wave II study when they were in their last month of sixth grade in 2011–2013 (11–14 years old). Both micronutrient concentrations and self-reported sleep were collected in the Wave II. Detailed sampling and research procedures of the cohort study were described elsewhere (Liu et al., 2015; Liu et al., 2010).

The present study used a subsample who met the following criteria: 1) had complete sleep data at 11–14 years old (n=839); 2) had complete data on serum iron and zinc concentrations at both 3-5 years old and 11–14 years old (n=1110). A total of 777 early adolescents were eligible for the present study. Compared with those who were not included (n=333), there were no significant differences in key variables but sex and parental education (p=0.005–0.018). Thus, child sex and parental education were adjusted for in data analyses to reduce the bias of sample selection. Written informed consent was obtained from parents and adolescents. Institutional Review Board (IRB) approval was obtained from the University of Pennsylvania and the Ethical Committee for Research at Jintan Hospital in China.

Measures

Serum micronutrient concentration

Blood specimens were collected by trained pediatric nurses using a strict research protocol at two time points: the first was in Fall 2004–Spring 2005 when children were in preschools (3–5 years old), and the second was in Summer 2011 to Summer 2013 (early adolescence) when they were in 6th grade (11–14 years old). Approximately 0.5 mL of venous blood samples were collected in a lead-free Ethylenediaminetetraacetic acid (*EDTA*) tube (Liu et al., 2011).

At the preschool point, blood samples were frozen at −20℃ and shipped to the Child Development Center at Nanjing Medical University, China. Iron and zinc concentrations were determined by atomic absorption spectrophotometry (BH model 5.100 manufactured by Beijing Bohu Innovative Electronic Technology Corporation), with duplicate readings taken within an integration time of 2 seconds (Liu et al., 2011). At early adolescence, specimens were restored at −40℃ until being analyzed using inductively coupled plasma mass spectrometry in Xin Hua Hospital, Shanghai, China. The detailed analytical procedure was reported elsewhere (Su et al., 2012).

Sleep

Adolescents filled out the Chinese version of the Pittsburgh Sleep Quality Index (PSQI) in June-July 2013. The PSQI measures seven self-described sleep domains over the prior month, including subjective sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, use of sleeping medication, as well as daytime sleepiness and dysfunction. The PSQI total score was generated by summing up the subdomain scores with

a range from 0 to 21 (sub-domains range = 0–3). Higher PSQI total scores reflect greater sleep impairment. As the responses designed for adult sleep duration may not fit the recommended optimal sleep duration for adolescents, John et al. (2016) modified the scoring of the subdomain of sleep duration as "> 9 hours=0, 8.5–9 hours = 1, 8–8.5 hours = 2, and <8 hours = 3". There are no established cut-offs for adolescents' PSQI total score, despite the wide use of the adult cut-offs (PSQI global score >5) (John et al., 2016). This study, therefore, used PSQI total score as a key dependent variable. The PSQI has an overall Cronbach's alpha coefficient of 0.82–0.83 for community-dwelling adults in China, indicating high internal consistency (Tsai et al., 2005). In a Chinese adolescent population, the PSQI has an alpha coefficient of 0.87 for overall reliability, and scores ranged from 0.46 to 0.85 for subscales. The cumulative variance of principal components was 70.72% (Zhou et al., 2012).

Covariates

Covariates included age, sex, mother and father education levels, home district (rural, suburb or urban) and environmental noise. Sleep quality at preschool age, which was reported by parents using the seven sleep items in the Chinese version of Child Behavior Check List (CBCL) (Achenbach & Rescorla, 2001; J. Liu, Zhou, et al., 2012), was also adjusted for in the analysis.

Statistical analysis

Descriptive statistics were calculated, including frequencies and percentages used to characterize categorical demographic variables, as well as means and standard deviations for continuous variables. Student t test and ANOVA were applied to examine the association of

PSQI total score with sample characteristics. If different between groups in ANOVA, the post hoc Tukey's HSD test was performed for multiple comparisons.

Serum iron/zinc concentrations were log-transformed before entering the adjusted regression models. Generalized linear regression models were used to test the relationship between serum micronutrient concentrations (at preschool and adolescence) and the PSQI total score during adolescence. For specific sleep domains, ordered logistic regression models were used to examine the associations between micronutrient status and sleep subdomains. The odds ratios from the ordered regression models reflect the odds of being at a greater impairment level of sleep subdomain associated with 1 unit increase in log-transformed micronutrient concentration. Both micronutrient concentrations and log-transformed concentrations between waves showed weak and insignificant correlations, multicollinearity was not a concern when adding them into the same model. Thus, the log-transformed zinc or iron concentrations at two-time points were simultaneously added to the same models. Iron and zinc entered the models separately to test their main effects. Regarding the interactions between zinc and iron, the interaction effects at preschool age and early adolescence were tested separately. For each time point, iron concentrations were median-split into two levels to facilitate interpretation. The interaction term between zinc concentrations and binary iron variable were then added into the regression models. Interaction analyses were repeated with iron concentrations and median-split of zinc levels.

Among covariates, education level in mother and father were collinear, resulting in high variance inflation factor values. Thus, only father’s education was added into models as a covariate. The estimated standard errors were adjusted at the school level. All the analyses were performed using STATA version 14.2, with statistical significance taken at the two-

sided level of $p < 0.05$.

Results

Sample Characteristics

Among the 777 early adolescents, about 51.09 % (n=397) were males. Participants were aged 11-14 years old (12.14 ± 0.55) when sleep data was collected. Table 3.1 presents descriptive characteristics of socio-demographic variables and associated PSQI total scores at early adolescence. The average PSQI score is 5.04 ± 2.54, with 306 (39.38%) early adolescents classified as poor sleepers (PSQI>5). PSQI total score was significantly associated with father education level (F=0.35, p=0.004) and age (r=0.17, $p < 0.001$), yet there were no significant trends with other sample characteristics (ps > 0.05). Specifically, early adolescents whose father received at least college education had better sleep quality than their peers whose father received middle school or less education, suggested by lower PSQI total scores. A positive linear trend was observed between age and PSQI total score, indicating that older adolescents were associated with worse global sleep quality.

Table 3.1. Socio-demographic characteristics and associated PSQI total score.

	N(%)/M ± SD	**PSQI Total**	t/F/r	*p* value
Sex				
Boys	397(51.09)	4.99±2.43	0.48	0.49
Girls	380(48.91)	5.12±2.63		
Age	12.14±0.55	5.05±2.53	0.17	<0.001**
Father's education [a]				
Middle school or less	222(29.68)	5.50±2.64	5.35[b]	0.004**
High school	215(28.74)	4.89±2.31		
College or higher	311(41.58)	4.82±2.58		
Home district				
Rural	138(17.76)	5.48±2.46	2.76	0.06
Suburb	312(40.15)	4.87±2.54		

Urban	327(42.08)	5.04±2.55		
Noise [b]				
No	442(54.70)	5.05±2.49	0.15	0.70
Yes	166(27.30)	4.96±2.45		
CBCL sleep (preschool)	2.85±2.17	5.05±2.53	-0.04	0.31

Note.[a] Turkey's HSD test: Father's education: College or higher < Middle school or less, p=0.01

[b] Variables have missing data; **p<0.001

Table 3.2 describes the domain scores of the PSQI. There were only 25.23% of our sample (n=196) slept the recommend 9 hours (score=0) at night, and 27.80% self-rated good sleep quality (score=0). About 83.27% (n=647) of adolescents reported some form of perceived sleep disturbances. Table 3.3 described the mean zinc and iron concentrations at preschool age and early adolescence. There were no significant associations of iron or zinc between waves; yet, iron and zinc concentrations at the same wave showed positive and significant correlations (ps<0.01).

Table 3.2. Descriptive analysis of sleep domains

Domain Score	0	1	2	3
Sleep duration	196(25.23)	317(40.80)	213(27.41)	51(6.56)
Sleep disturbances	130(16.73)	596(76.71)	50(6.44)	1(0.13)
Sleep latency	272(35.01)	341(43.89)	123(15.83)	41(5.28)
Daytime sleepiness &dysfunction	264(33.98)	340(43.76)	143(18.40)	30(3.86)
Sleep efficiency	678(87.26)	71(9.14)	19 (2.45)	9 (1.16)
Subjective sleep quality	216(27.80)	421(54.18)	114(14.67)	26(3.35)
Sleep medication use	747(96.14)	23(2.96)	3(0.39)	4(0.51)

Note. Data were presented as N (%). 0 stands for optimal status. Higher domain scores mean worse sleep problems.

Table 3.3. Micronutrient concentrations and their pairwise correlations [a]

	Mean ± SD	Zn1	Zn2	Fe1	Fe2
Zn1	82.26±13.24		0.05	0.29**	0.02

Zn2	87.92±15.82	-0.01	0.15**
Fe1	81.33±8.41		0.01
Fe2	115.45±39.68		

Note. [a] coefficients of Pearson correlations; 1=measured at preschool age, 2=measured at early adolescence; unit of zinc/iron concentration: μg/dL; ***p*<0.001

Table 3.4 shows the concurrent association of serum zinc or iron concentrations with sleep domains in early adolescents, after adjusting for micronutrient status at preschool age and covariates. Higher log-transformed serum zinc concentrations were significantly associated with decreased PSQI total score (β=-0.62, p=0.03), which indicated better global sleep quality. For specific domains, increased serum zinc concentrations at adolescence showed decreased likelihood of insufficient sleep duration (OR=0.28, p<0.001) and sleep disturbances (OR=0.45, p=0.04). Regarding iron status, adolescents with higher serum iron concentrations tended to have decreased PSQI total scores (β=-0.20), yet this association did not reach statistical significance (p=0.40). Among sleep subdomains, whereas higher serum iron concentrations were significantly associated with better sleep latency (OR=0.68, p=0.03), there were no significant relationships with other sleep subdomains.

Table 3.4. Adjusted regression models for serum zinc/iron concentrations and sleep

Adolescent Sleep	Zn 1	Zn 2	Fe 1	Fe 2
Global sleep quality, B (Robust SE)				
PSQI total score	-1.18(0.66)	-0.62(0.21)*	-1.33(1.24)	-0.20(0.23)
Sleep domains, OR (Robust SE)				
Sleep duration	0.28 (0.04)	0.28 (0.04)**	1.28(1.08)	0.99(0.17)
Sleep disturbances	1.26 (0.33)	0.45 (0.17)*	1.87(1.74)	1.52(0.59)
Sleep latency	0.86 (0.45)	0.94 (0.15)	1.09(0.83)	0.69(0.12)*
Daytime sleepiness &dysfunction	0.67(0.47)	0.99 (0.26)	0.27(0.24)	0.76(0.19)
Sleep efficiency	0.17(0.07)**	1.21 (0.76)	0.78(1.01)	1.31(0.43)

Subjective sleep quality	0.59 (0.23)	0.97 (0.19)	0.54(0.51)	0.77(0.27)
Sleep medication use	1.10 (1.04)	1.91 (1.82)	0.73(0.94)	0.98(0.44)

Note: 1. Micronutrient concentrations were log-transformed. 1=measured at preschool age, 2=measured at early adolescence; 2. Due to unbalanced sample sizes across categories, the categories with small sizes in sleep disturbances, efficiency, latency and medication use were merged into the closet category.

*$p<0.05$, **$p<0.001$.

Regarding the longitudinal relationships (Table 3.4), serum zinc status at preschool was not significantly predictive of the PSQI total score ($p>0.05$). However, higher serum zinc concentrations at preschool age were associated with decreased odds of poor sleep efficiency (OR=0.17, p=0.03) during early adolescence. For iron status, there were no significant associations between serum iron concentrations at preschool age and sleep at early adolescence (ps>0.05). All these longitudinal associations were independent of zinc or iron levels measured at early adolescence.

In terms of the exploratory aim, the interaction effects between serum iron categories and zinc concentrations during early adolescence were not statistically significant on their concurrent PSQI global score and sleep subdomains. Similarly, serum iron and zinc at preschool age showed no significant interactions on sleep (ps>0.05). Repeated analyses using serum iron concentrations and zinc categories produced similar results (ps>0.05).

Discussion

The present study is one of the first to characterize the associations between serum micronutrient status and sleep quality in a healthy adolescent sample. For the zinc–sleep relationship, adolescents with higher zinc concentrations at preschool age (3–5 years old) tended to have better sleep efficiency (ratio of sleep length/ time in bed) at early adolescence (11–14 years old). Serum zinc concentrations at early adolescence showed a domain-

specific relationship with adolescent sleep independent of early zinc status at preschool age, with significant associations with sleep duration, sleep disturbances and global sleep quality. Regarding the iron–sleep relationship, our sample exhibited a significant cross-sectional association between higher iron concentrations and decreased odds of long sleep latency. However, the longitudinal associations between iron and sleep were not statistically significant. Additionally, there were no interactions between iron and zinc at both time points on adolescents sleep.

Although prior evidence in adolescents is limited, our findings regarding the cross-sectional associations between serum zinc and sleep duration align with previous research in infants (Kordas et al., 2009) and adults (Grandner, Jackson, Gerstner, & Knutson, 2014; Song et al., 2012). For example, the randomized controlled trials by Kordas et al. (2009) suggested longer nighttime sleep and total sleep duration in infants receiving supplemental zinc compared with the placebo group. Researchers also found that shorter sleep duration was associated with lower serum zinc levels in women (Song et al., 2012). We also found an association between increased serum zinc concentrations and decreased sleep disturbances. Similarly, one clinical trial showed the efficacy of a combined supplementation including zinc, magnesium, and melatonin on primary insomnia among older adults (Rondanelli et al., 2011). However, in the absence of the main effect of zinc, the findings from this clinical trial are not comparable to our results of sleep disturbances. The neurobiological underpinnings of the relationship between zinc and sleep remain unclear. Possible explanations may be the role of zinc as a coenzyme required for neurogenesis, neuronal migration, and synaptogenesis (Bhatnagar & Taneja, 2001). In addition, zinc has been documented as an antagonist of excitatory transmissions, such as the N-methyl-d-aspartate receptor (Takeda,

Minami, Seki, & Oku, 2004), as well as an agonist of inhibitory transmissions of gamma-Aminobutyric acid (GABA) receptors (Turgeon & Albin, 1992).

The present study also provided preliminary evidence to clarify temporal associations between childhood serum zinc and adolescent sleep quality. Specifically, increased serum zinc in early childhood showed a significant relationship with better sleep efficiency at early adolescence, which reflected the ratio of sleep duration to total time in bed (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). This longitudinal finding further supports the potential relationship between zinc status and sleep characteristics. Of note, this longitudinal relationship was independent of zinc status at early adolescence, suggesting a potentially long-lasting sleep effect of low zinc status in early childhood. However, only one out of seven domains achieved statistical significance for the longitudinal analyses. Additional research is needed to validate the longitudinal conceptualization.

In terms of iron status, there was a significant relationship between serum iron concentrations and sleep latency in early adolescents. Sleep latency, which was assessed by the minutes to fall asleep, is a proxy measure for sleep propensity. According to the PSQI instrument, sleep latency over 30 minutes is one of the indicators for potential insomnia (Buysse et al., 1989). The finding of the present study suggested that decreased serum iron concentrations were associated with increased risk of sleep-onset difficulty, probably due to the correlation between iron and excitatory transmission, such as dopaminergic neurons (Beard, Erikson, & Jones, 2002). There were no significant relationships between iron and other sleep subdomains, which is inconsistent with prior research that reported shorter total sleep duration was associated with iron deficiency anemia (IDA) infants (Kordas et al., 2008) and low iron intake in adults (Grandner et al., 2013). The discrepancy may be due to

the differences in assessing iron status and sleep. Additionally, whereas no longitudinal association was found in this study, prior research on IDA infants reported long-lasting impact on sleep architecture in the follow-ups during childhood (Peirano, Algarin, Garrido, & Lozoff, 2007). Future research is warranted to examine the long-term biological action of suboptimal iron status on sleep.

The interaction effects of iron and zinc on sleep were not significant in our sample. It has been documented that a high intake of zinc may interfere with iron absorption (Singh, 2004), thus influencing the effect of iron supplements. One experimental study provided evidence to support the possible interactions, showing that iron and zinc supplements alone increased sleep duration in infants, whereas infants receiving iron together with zinc supplements did not exhibit such sleep effect (Kordas et al., 2009). Iron and zinc status were measured by serum concentrations in the present study, thereby the interaction at the metabolic absorption level may not apply to our sample. However, prior research has also suggested potential antagonism between micronutrients in binding to neurotransmitter receptors (Sharonova et al., 2000), highlighting the need for more research on the interactions between iron and zinc status on sleep outcomes.

Limitations

The strengths of this paper include two-wave longitudinal data from a preschool cohort, the large sample size, and the use of biological micronutrient measurement. However, there are several potential limitations. First, sleep quality in adolescents was based on a self-report questionnaire. Although the PSQI has good psychometric properties, future studies with polysomnography and actigraphy combined with a sleep diary, are warranted to replicate this study. Second, because of the data availability, it is unknown whether

micronutrient and sleep data were collected in the absence of medication or chronic or acute diseases. This uncertainty may affect the sensitivity of measures of both serum micronutrient levels and sleep quality. Third, the usage of micronutrient concentrations, instead of clinical classifications, may limit the clinical implication of our findings. However, most of the previous findings from epidemiological studies were based on micronutrient concentrations. Using similar micronutrient indicator may facilitate direct comparisons with prior findings. Fourth, the data of habitual dietary intake is not available in the present study. Therefore, this study did not adjust for dietary nutrients, such as vitamin D, dodecanoic acid and total carbohydrate, which may relate to sleep outcomes (Grandner et al., 2014; Sato-Mito et al., 2011). Future studies should incorporate possible confounders of dietary intake and dietary habits into analyses. Finally, we cannot make causal inferences of the results due to the observational design.

Implications for research and practice

Despite the limitations, the findings of the present study have significant implications for future research and practice. Micronutrient deficiency displays a declining trend over the past few decades, however, children and adolescents from developing countries remain one of the most vulnerable groups (Allen et al., 2006), especially for iron and zinc deficiencies (Yang et al., 2007). Although the causal relationship is not conclusive, this study provided preliminary evidence to suggest the importance to involve micronutrient status, such as zinc and iron, into risk assessment and risk management to enhance sleep health. Additionally, suboptimal micronutrient status and impaired sleep have been considered as predisposing factors for multiple physical and psychological outcomes in adolescents. A better understanding of these complex relationships may help identify the

most promising factors around which to design prevention and intervention efforts to enhance adolescent health.

Conclusion

The findings from this population-based sample extended the current knowledge about the micronutrient–sleep associations to the adolescent population. Higher serum zinc concentrations at preschool age are predictive of better sleep efficiency at early adolescence. After controlling for serum status at preschool age, the cross-sectional associations of serum zinc concentrations with adolescent sleep quality are present across domains of sleep duration and sleep disturbances. Serum iron concentrations at early adolescence are also associated with concurrent sleep latency. There are no significant interaction effects between iron and zinc on adolescent sleep in early adolescents. Future research is needed to examine the clinical relevance of the relationship between micronutrient status and sleep parameters in early adolescents.

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CHAPTER 4: MICRONUTRIENT STATUS, SLEEP QUALITY AND NEUROBEHAVIORAL FUNCTION IN EARLY ADOLESCENTS: A MEDIATION ANALYSIS

Abstract

Introduction. The relationship among micronutrient status, habitual sleep and neurobehavioral function remains underexplored in adolescents. This study aimed to investigate the association between habitual sleep quality and neurobehavioral function, and the mediating effect of sleep quality on the relationship between micronutrient status and neurobehavioral function in early adolescents.

Methods. The sample was comprised of 226 early adolescents (12.14 ± 0.55 years) from Jintan, China. Habitual sleep quality was measured by the Pittsburgh Sleep Quality Index (PSQI), with poor sleeper defined as a total score ≥ 5. Iron and zinc status (normal/low) were determined by serum concentrations according to their clinical reference ranges. The neurobehavioral function was measured by the Penn Computerized Neuropsychological Battery (CNB). The mediation analyses were performed based on Baron & Kenny's procedures.

Results. Compared with normal sleepers, poor sleepers showed decreased accuracy (β=-1.21, p=0.001) on task for episodic memory, slower reaction speed (β=123.38, p=0.004) on abstraction/mental flexibility, and fast (β=-669.06, p=0.03) but error-prone (β=-0.81, p=0.03) pattern on nonverbal reasoning task. Both zinc and iron deficiencies were significantly associated with performance on nonverbal reasoning ($p<0.05$) relative to the normal controls. Low serum zinc was also significantly associated with worse performance on spatial processing ability and attention. Sleep quality partially mediated the relationship between low serum zinc and nonverbal reasoning, yet other mediation models did not show statistical significance.

Conclusion. Habitual sleep quality may contribute to neurobehavioral function in a domain-specific manner among early adolescents. Poor sleep quality may also partially mediate the relationship between low micronutrient status and decreased neurobehavioral performance. A

further investigation of the complex relationship can provide a basis for developing interventions to optimize sleep health and neurobehavioral function in adolescents.
Keywords: serum iron; serum zinc; sleep quality; adolescents; neurobehavioral function

Introduction

Adolescence represents a critical period of brain maturation and neurodevelopment processes (Castellanos-Ryan et al., 2016). Thus, this transitional stage, may be particularly vulnerable to neurobehavioral dysfunction. Emerging evidence has indicated that decreased neurobehavioral function, such as executive control and working memory, have a negative impact on learning, academic achievement, behavioral control and stress coping in adolescents (Curcio, Ferrara, & De Gennaro, 2006; Liang, Matheson, Kaye, & Boutelle, 2014; Slattery, Grieve, Ames, Armstrong, & Essex, 2013). Childhood neurobehavioral deficits also showed associations with internalizing and externalizing psychopathology during adulthood (Niendam et al., 2003; Owens & Hinshaw, 2016), suggesting that neuronal malleability during adolescence may interfere with developmental brain maturation and exert a lasting detrimental effect into adulthood (de Bruin, van Run, Staaks, & Meijer, 2016). Therefore, identifying risk factors for neurobehavioral impairment and intervening have significant implications for daily functioning in adolescence and beyond.

Sleep and micronutrient status have been considered as two modifiable lifestyle factors of neurobehavioral function in adolescents. Recent work on sleep highlights its importance to specific neurobehavioral domains, with a focus on experimental sleep manipulation (de Bruin et al., 2016). A recent review of sixteen experimental studies reported a reduction in vigilance after sleep deprivation, improvements in working memory after sleep extension, and memory consolidation following post-learning sleep (de Bruin et al., 2016). However, these findings may not apply to habitual sleep in natural

settings, highlighting the need to delineate the relationship between habitual sleep and neurobehavioral domains. Regarding micronutrient status, prior research has reported that iron deficiency was associated with lower scores on attention tests (Falkingham et al., 2010) and working memory (Lambert, Knaggs, Scragg, & Schaaf, 2002) in children, as well as abstraction/mental flexibility and spatial processing ability in early adolescents (Ji, Cui, & Liu, 2017). Similar to iron, zinc deficiency has also shown detrimental effects on cognitive development in animal models (Bhatnagar & Taneja, 2001). However, evidence regarding zinc and neurobehavioral function in humans, especially the adolescent population, is limited and controversial (Bhatnagar & Taneja, 2001; Gogia & Sachdev, 2012).

Although micronutrient status and sleep have been proposed as individual risk factors for neurobehavioral development, prior evidence also suggests a potential mediating effect of sleep (Baron& Kenny, 1986). First, suboptimal micronutrient status correlated with decreased neurobehavioral function (Falkingham et al., 2010; Ji et al., 2017). Second, sleep patterns were associated with neurobehavioral function in adolescents (de Bruin et al., 2016). Third, the literature suggests that micronutrient status may predict sleep patterns. A recent systematic review has shown that suboptimal micronutrient status, such as low levels of iron and zinc, were associated with shorter sleep duration, more sleep disruptions and decreased global sleep quality in infants, children and adults (Ji, Grandner, & Liu, 2016). Specific to adolescent sleep, a longitudinal study reported that serum zinc concentrations in 3-5 years old and 11-14 years old were associated with multiple sleep domains measured by the Pittsburgh Sleep Quality Index (PSQI) in adolescents (Ji & Liu, 2015). These conceptual pathways

indicate the possibilities that micronutrient status may affect sleep and, in turn, influence neurobehavioral domains. However, the contribution of sleep to the neurobehavioral effects of micronutrient status has yet to be examined.

The aim of this study was two-fold: 1) to investigate the associations between habitual sleep quality and multiple neurobehavioral domains, and 2) to examine the mediating effect of habitual sleep quality on the relationship between serum micronutrient status and neurobehavioral domains among an early-adolescent sample from a healthy school population in Jintan, China. We hypothesized that: a) early adolescents with poor sleep quality (as measured by the global PSQI score) will exhibit worse performance on tasks for neurobehavioral function, and b) sleep quality significantly mediates the relationship between micronutrient status and neurobehavioral domains. The findings from this study extend the current knowledge on micronutrients, habitual sleep and neurobehavioral function, and provide a foundation for developing and testing interventions to optimize neurobehavioral development in adolescents.

Methods

Participants and Procedures

The present study is an extension of the China Jintan child cohort that aims to investigate early health factors for the later neurobehavioral development in childhood and adolescence (Liu et al., 2015; Liu et al., 2010). Using a multi-stage sampling method, the Jintan cohort study enrolled 1656 children aged 3–5 years who represented preschoolers from different school districts in Jintan city in 2004. According to their grade (1st, 2^{nd,} and 3rd) in preschool, children were classified into lower, middle and upper cohort. A total of 1110 (54% male) children participated in the wave II study when they were in their last

month of sixth grade in 2011–2013 (11-14 years old). Detailed sampling and research procedures of this larger cohort study have been described elsewhere (Liu et al., 2015; Liu et al., 2010).

This cross-sectional study used a subsample of early adolescents in Wave II who met the following criteria: 1) had complete data on micronutrient concentrations (both iron and zinc), sleep information and neurobehavioral test scores, and 2) blood test of zinc/iron was drawn prior to sleep measurement and neurobehavioral tasks. A total of 226 early adolescents were eligible for this study. The Jintan research team obtained written informed consent from parents and adolescents, and Institutional Review Board (IRB) approval from both the University of Pennsylvania and the ethical committee for research at Jintan Hospital in China.

Measures

Micronutrient Status

Blood specimens were collected by trained pediatric nurses using a strict research protocol from summer 2011 to summer 2013 when participants were in the last few months of 6th grade (11-14 years old). Approximately 0.5 mL of venous blood samples were collected in a lead-free Ethylenediaminetetraacetic acid (*EDTA*) tube, stored at −40°C and shipped to Xin Hua Hospital, Shanghai, China. Micronutrient concentrations were analyzed using inductively coupled plasma mass spectrometry. The detailed analytical procedure was reported elsewhere (Liu et al., 2011; Su et al., 2012). We classified iron concentrations lower than 75 ug/dl as low iron level (Liu et al., 2014), and zinc concentrations lower than 70 ug/dl as low zinc level (Food & Administration, 2001).

Sleep

Adolescents were asked to fill out the Chinese version of the Pittsburgh Sleep Quality Index (PSQI) with sleep schedules in June–July 2013. The PSQI measures self-described sleep quality and quality over the prior month. It is composed of 19 items that are scored to determine seven component scores: subjective sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, use of sleeping medication, as well as daytime sleepiness and dysfunction (sub-domains range=0–3). Sleep duration is calculated by bedtime and wake-time and classified into 0-3 levels according to the adolescent cutoff used in the National Sleep Foundation (John, Bellipady, & Bhat, 2016). The component scores summed up produce a global score which ranges from 0 to 21, with poor sleeper defined as total scores>5. The PSQI has been shown to demonstrate acceptable reliability and validity in adults (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989; Tsai et al., 2005). The PSQI has an overall Cronbach's alpha coefficient of 0.82–0.83 for community-dwelling adults in China, indicating high internal consistency (Tsai et al., 2005). In a Chinese adolescent population, the PSQI has an alpha coefficient of 0.87 for overall reliability, and scores ranged from 0.46 to 0.85 for subscales. The cumulative variance of principal components was 70.72% (Zhou et al., 2012).

Neurobehavioral Function.

Trained research assistants administrated the Penn Computerized Neuropsychological

Battery (CNB) in a quiet testing room in the Children's Health Laboratory located in the Jintan Hospital. The present study focused on five tests, including abstraction and mental

flexibility, attention, episodic memory, spatial processing ability and nonverbal reasoning. The Penn CNB has been validated with functional neuroimaging to define the recruitment of specific brain systems (Gur et al., 2012; Gur et al., 2010; Roalf et al., 2014) and has shown good psychometric properties in children aged 8-21 years (Gur et al., 2012). The instruction for each test was translated into Chinese and then back translated and revised by our research team. In a pilot study using 122 subsamples in our cohort, the Cronbach's alpha coefficients for the full scale and each test of the Penn CNB were greater than 0.8, suggesting adequate internal consistency (not published yet).

Abstraction and mental flexibility: the short Penn Conditional Exclusion Test (sPCET) asked participants to decide which of four objects did not belong with the other three, based on one of three sorting principles (e.g., size, shape, line thickness).

Attention: the Penn Continuous Performance Test (PCPT) asked participants to press the space bar whenever the display formed a digit (for the first half of the PCPT test) or a letter (for the second half of the PCPT test).

Episodic memory**:** the Short Visual Object Learning Test (sVOLT) asked participants to memorize Euclidean shapes that were presented at a rate of 1 shape(s). They were then asked to choose whether a shape was presented in the original list on a scale of 1- 4 (1=*definitely yes to 4=definitely not*).

Spatial processing ability**:** the short Penn Line Orientation Test (sPLOT) presented two lines at an angle and asked respondents to click on a button to rotate one line until it showed the same angle as the other.

Non-verbal reasoning: the Penn Matrix Reasoning Test (PMRT) included the matrix reasoning problem as used in the Raven Progressive Matrices Test (RPMT). PMR requires reasoning by geometric analogy and contrast principles.

Each test yields measures of accuracy (number of the correct response) and speed (median response time for correct responses with a unit of a millisecond), thereby allowing an evaluation of individual differences in cognitive strategy for speed-accuracy tradeoff (Gur et al., 2010). Problem-solving strategies are usually classified into four groups: fast-accurate, slow-inaccurate, slow-accurate (reflective), and fast-inaccurate (impulsive) (Ault, 1973). Poor function in the present study was defined as: 1) slow and/or inaccurate performance; or 2) fast but inaccurate performance (Ault, 1973).

Covariates

Covariates included socio-demographic variables, such as age, sex, home district and parental education. Neurobehavioral domains may be influenced by heritable cognitive traits, such as general intelligence quotient (IQ) (Kuula et al., 2015). Thus, IQ assessed by the Chinese version of the Wechsler Intelligence Scale for Children-Revised (WISC-R) was also controlled for in this study. Additionally, the time of day when neurobehavioral tests were performed has been linked to cognitive performance in children and adolescents (Blatter & Cajochen, 2007); we, therefore, treated it as a covariate in this study.

Statistical analysis

Descriptive statistics, including means ± standard deviations and frequencies/percentages, were used to characterize continuous and categorical factors,

respectively. Logistic regression models were used to address the predictive effect of sleep quality on neurobehavioral domains, while controlling for micronutrient deficiencies (iron and zinc) and covariates, including age, sex, parental education, home district, WISC IQ score and time of neurobehavioral tests. Each neurobehavioral domain entered the model separately as a dependent variable.

For the mediation models, the Baron & Kenny's procedures were taken to estimate the mediation effect of sleep quality (Baron & Kenny, 1986; MacKinnon & Dwyer, 1993): (1) testing the direct effect (c) of low zinc and iron levels on neurobehavioral performance; (2) testing the effect (a) of low zinc and iron levels on sleep quality; (3) testing the effect (c′) of low zinc and iron levels on neurobehavioral performance controlling for sleep quality. Mediating effect was determined by the following criteria: first, sleep quality (mediator) was a significant predictor of the task performance for the neurobehavioral domain (outcome); second, low iron/zinc (predictor) was a significant predictor of sleep quality (mediator) and this neurobehavioral domain (outcome); third, the coefficient (c′) of micronutrient variable was reduced in absolute size compared to it is in the first model (c). For the relationships that met the criteria, the post estimation function in the generalized structural equation modeling (GSEM) was used to calculate the effect size, standard error, p values, and 95% CI of the direct effect and indirect effect. The covariates adjusted in the mediation modeling included age, sex, parental education, home district, WISC IQ score and time of CNB tests. The cohort was clustered in the regression models. The significance level was set at $\alpha = 0.05$. All analyses were performed using STATA 14.2.

Results

Sample Characteristics

The final dataset was comprised of 226 early adolescents, aged 11-14 years old (12.14 ± 0.55). There were 53.10% boys (n=120) and 46.90% girls (n=106). As shown in Table 4.1, about 27.88% (n= 63) of the early adolescents were classified as poor sleepers with a PSQI total score greater than five. According to the clinical reference range, about 18.14% (n = 41) of adolescents had low serum iron and 5.75% (n=13) exhibited low serum zinc. Because of the collinearity between mother and father education, only father education was further included in the regression models.

Table 4.1. Sample characteristics (n=226)

	M±SD/ N (%)
Sex	
Male	120 (53.10)
Female	106 (46.90)
Age	12.14±0.55
Mother's education	
Middle school or less	79(36.41)
High school	41(18.89)
College or higher	97(44.70)
Father's education	
Middle school or less	55(25.23)
High school	48(22.02)
College or higher	115(52.75)
Home district	
Rural	32 (14.16)
Suburb	94 (41.59)
Urban	100 (44.25)
Time of neurobehavioral test	
AM (8:00-11:59)	18 (8.11)
PM (12:00-17:00)	204 (91.89)
WISC-R IQ	104.92±12.19
Sleep	

Normal	163 (72.12)
Poor	63 (27.88)
Zinc status	
Normal	213 (94.25)
Low	13 (5.75)
Iron Status	
Normal	185(81.86)
Low	41(18.14)

Sleep Quality and Neurobehavioral Domains

Table 4.2 shows the distribution of neurobehavioral performance by sleep groups. After adjusting for zinc and iron status, as well as covariates, early adolescents with poor global sleep quality (PSQI>5) tended to have declined performance on abstraction/mental flexibility (sPECT), episodic memory (sVOLT), and nonverbal reasoning (PMRT) compared with normal sleepers. Specifically, while there was no statistical difference in reaction speed (β=-51.97, p=0.53) on episodic memory, poor sleepers showed significant decrements in the performance accuracy on this test (β=-1.21, p<0.01), relative to their peers with normal sleep. Poor sleepers also tended to exhibit longer reaction times on abstraction/mental flexibility (β=123.38, p<0.01) to achieve statistically similar accuracy (β=2.30, p>0.05). Regarding nonverbal reasoning, poor sleep quality predicted a fast (β=-669.06, p=0.03) but error-prone (β=-0.81, p=0.04) performance. There were no significant associations of poor sleep quality with sustained attention and spatial processing ability.

Table 4.2. Mean Scores of CNB Tasks and Coefficients for Poor Sleeper (Adjusted)

	Sleep Quality			
Model Outcome	Normal (M ± SD)	Poor(M ± SD)	*B* (robust SE) [a]	*p*
sPCET				
Speed, ms	1646.11±411.08	1762.98±500.59	123.38 (0.68) **	0.004**

Accuracy	25.47±6.11	27.32±7.50	2.30 (0.32)	0.19
PCPT				
Speed, ms	514.24±135.79	503.79±118.20	-13.83(10.24)	0.31
Accuracy	79.75±35.77	73.38±41.34	-5.75 (2.40)	0.32
sVOLT				
Speed, ms	1618.83±664.19	1579.42±633.09	-57.97 (64.39)	0.53
Accuracy	15.11±2.99	13.87±3.19	-1.21 (0.02) **	0.001**
PMRT				
Speed, ms	7795.92±5491.38	7225.94±5250.4	-669.06 (120.95)*	0.03*
Accuracy	11.80±4.41	11.13±4.59	-0.81 (0.14)*	0.03*
sPLOT				
Speed, ms	7682.66±2258.645	8225.36±3019.74	655.32 (91.09)	0.09
Accuracy	9.42±3.84	9.48±4.32	0. 16 (0.16)	0.42

Note. a. The reference level was normal sleep. PCPT = Penn Continuous Performance Test; PMRT = Penn Matrix Reasoning Test; sCTAP = short Computerized Finger-Tapping task; sPCET = short Penn Conditional Exclusion Test; sPLOT = short Penn Line Orientation Test; sVOLT = short Visual Object Learning Test;
*$p < 0.05$, **$p < 0.01$

Micronutrient Status and Neurobehavioral Domains

In Table 4.3, the model set 1 represents the predictive effects of micronutrient status on neurobehavioral domains, after adjusting for covariates. Compared with normal zinc, low zinc level was significantly associated with a fast but less accurate performance on tasks for non-verbal reasoning (PMRT) and spatial processing (sPLOT) (ps<0.05). Low zinc level was also significantly associated with longer reaction time (β=15.63, p=0.03) on PCPT that reflected sustained attention. Similar to low zinc, adolescents with low serum iron tended to exhibit faster but less accurate performance (ps<0.05) on non-verbal reasoning (PMRT) as relative to their peers with a normal iron. However, there were no significant associations between low iron and other neurobehavioral domains.

Table 4.3. The Associations of Zinc/Iron Status with Sleep and CNB Performance

Model Outcome	Low Zinc		Low Iron	
	Model Set 1	Model Set 2	Model Set 1	Model Set 2
PSQI Sleep, OR(Robust SE)				
Sleep quality	1.28(0.03)**	n/a	1.25(0.16)	n/a

CNB Test, B (Robust SE)				
sPCET				
Speed, ms	-133.77 (23.79)	-148.93 (22.92)	54.33 (8.41)	49.47 (11.99)
Accuracy	-0.60 (0.12)	-0.88 (0.07)*	1.15(0.24)	1.06 (0.32)
PCPT				
Speed, ms	15.63 (2.55)*	16.68 (3.24)*	15.53 (6.13)	16.21 (6.33)
Accuracy	13.24 (3.47)	13.68 (3.67)	-9.24 (6.53)	-8.95 (6.78)
sVOLT				
Speed, ms	-440.33 (41.60)	-433.21 (50.01)	12.03 (6.67)	14.31 (10.90)
Accuracy	0.72 (0.20)	0.87 (0.19)	0.64 (0.28)	0.69 (0.31)
PMRT				
Speed, ms	-2422.72 (280.69)*	-2368.94 (286.58)*	-1390.02 (62.84)**	-1353.65 (44.17)**
Accuracy	-1.87 (0.30)*	-1.80 (0.30)*	-1.10 (0.14)*	- 0.96 (0.16)*
sPLOT				
Speed, ms	-1816.04 (116.55)*	-1893.97 (122.04)*	263.73 (21.69)	227.76 (34.10)
Accuracy	-1.75 (0.09)*	-1.79 (0.11)*	0.79 (0.56)	0.78 (0.57)

Note. The model set 1 adjusted for age, sex, parental education, home district, full WISC IQ score and time of CNB tests; the model set 2 adjusted for covariates in the model set 1 and global sleep quality. Reference level: normal iron or zinc level.

Mediation Effect of Sleep Quality

According to the mediation criteria proposed by Baron & Kenny (1986), sleep quality significantly mediated the relationship between low zinc and task performance (speed and accuracy) for nonverbal reasoning (PMRT). First, as shown in Table 4.3, adolescents with low zinc (OR=1.28, $p<0.001$) were significantly associated with increased likelihood of poor global sleep quality. Low iron showed a similar trend for poor sleep quality but the association showed a lack of statistical significance (OR=1.26, $p=0.09$). Second, as described above, both poor sleep quality and low zinc significantly predicted fast but error-prone performance on nonverbal reasoning. Third, as shown in the model set 2, the absolute magnitudes of low zinc on nonverbal reasoning performance declined after controlling for sleep quality. However, low zinc still exerted a significant

direct effect on accuracy and speed of nonverbal reasoning performance, suggesting a partial mediating effect of sleep quality. Furthermore, the GSEM output (Table 4.4) further showed that both direct and indirect effect were significant. Sleep quality mediated 16.38% of the total effect of low zinc on performance accuracy, and 10.93% of the total effect of zinc deficiency on test speed. The relationships between low zinc/iron levels, sleep quality, and other neurobehavioral domains did not meet the criteria of a significant mediation model.

Table 4.4. The Statistic Effect of Low Zinc on PMRT Performance (Direct & Indirect)

Low zinc	PMRT accuracy		PMRT speed	
	B (Robust SE)	95%CI	B (Robust SE)	95%CI
Direct effect	-1.80 (0.30)	(-2.38,-1.22)	-2368.94 (278.97)	(-2915.71, -1822.16)
Indirect effect*	-0.35 (0.06)	(-0.47, -0.24)	-290.62 (51.57)	(-391.69, -189.55)
Total effect	-2.16 (0.33)	(-2.80, -1.51)	-2659.55 (286.86)	(-3221.79,-2097.31)

Note. *Indirect effect was through sleep quality; PMRT reflects nonverbal reasoning performance.

Discussion

The present study is among the first to examine the relationship between micronutrient status, habitual sleep and neurobehavioral function in early adolescents. Three main findings emerged from this study. First, neurobehavioral patterns associated with poor global sleep quality suggested worse function across abstraction/mental flexibility, episodic memory, and nonverbal reasoning. Second, zinc and iron deficiencies, relative to normal micronutrient status, significantly predicted faster but less accurate performance on the task for nonverbal reasoning. Low zinc also significantly predicted decreased function across spatial processing ability and sustained attention. Third, whereas sleep quality partially mediated the relationship between low zinc and nonverbal reasoning, there was no significant mediating effect of sleep quality on other relationships.

Sleep Quality and Neurobehavioral Domains

The present study extended the relationship between sleep manipulations and neurobehavioral function to habitual sleep quality in naturalist settings, with significant associations across abstraction/mental flexibility, nonverbal reasoning, and episodic memory. A slower reaction speed on the task for abstraction/mental flexibility reflects decreased executive function that is essential for effective problem solving (Moore, Reise, Gur, Hakonarson, & Gur, 2015). Our findings are congruent with a recent observational study that reported an association between reduced actigraph-measured sleep quality and longer reaction times on executive function task among girls (Kuula et al., 2015). The present study also found a fast but error-prone pattern on task for nonverbal reasoning among poor sleepers. This speed-accuracy trade-off indicates an impulsive problem-solving strategy, suggesting poor sleepers are cognitively immature (Ault, 1973; Rozencwajg & Corroyer, 2005). The study by Kuula et al. (2015) also indicated an association between reduced sleep quality and fast but less accurate cognitive performance among boys; however, these results were not comparable because nonverbal reasoning was not specifically assessed in that study. As the nonverbal reasoning measurement in CNB is commensurate with Raven's Progressive Matrices test (RPMT) for fluid intelligence quotient (IQ), our findings are in line with the positive relationship between sleep spindle and the fluid IQ reported in a sleep EEG study (Bódizs, Gombos, Ujma& Kovács, 2014). A possible explanation is that sleep may play a potential role in maintaining the functional integrity of the frontoparietal networks that support executive control and reasoning (Astill, Van der Heijden, Van IJzendoorn, & Van Someren, 2012). Regarding episodic memory, the results extended the previous findings on

post-learning sleep (de Bruin et al., 2016) to habitual sleep quality, which further strengthens the possible contribution of sleep to memory (Astill et al., 2012).

Micronutrient Status and Neurobehavioral Domains

The results on zinc and neurobehavioral domains suggested a potential neurobehavioral impact of low zinc in early adolescents, with more profound associations with nonverbal reasoning ability, sustained attention and spatial processing. In line with these findings, a cross-sectional study showed that plasma zinc levels were positively associated with nonverbal reasoning ability and negatively associated with reaction time among adolescent girls in India (Chiplonkar & Kawade, 2014). Our findings also agree with previous experimental studies that indicated neurobehavioral improvement after zinc supplements, specifically on domains of attention and reasoning, among Chinese children and Mexican-American children (Sandstead, 2012).

Regarding low iron, we have conflicting findings when compared with previous research. Whereas prior research suggested the associations of iron deficiency with multiple neurobehavioral domains in children and adolescents, such as attention (Falkingham et al., 2010), executive function (Lukowski et al., 2013) and working memory (Lambert et al., 2002), low serum iron was only associated with non-verbal reasoning in our sample. The heterogeneity of measures and scales used to assess neurobehavioral function may be one of the reasons for the inconsistent findings, thus highlighting the need for additional research in this area.

Potential Mechanism: Mediation Effect of Sleep Quality

The mechanism underlying the relationship between micronutrient status and neurobehavioral function lacks a clear consensus. The findings of the mediating effect of sleep proposed a novel pathway linking micronutrient status and neurobehavioral function, particularly in nonverbal reasoning. In the present study, low zinc was associated with increased odds of having poor sleep which, in turn, predicted worse nonverbal reasoning. These conceptual links are biologically plausible. First, zinc has been documented as an antagonist of excitatory neurotransmitters, such as the N-methyl-d-aspartate receptor (Takeda, Minami, Seki, & Oku, 2004), and an agonist of inhibitory neurotransmitters, such as gamma-Aminobutyric acid (GABA) receptors (Turgeon & Albin, 1992), thus influencing intrinsic sleep regulation process. Second, reduced sleep quality may further affect nonnonverbal reasoning through its detrimental effect on frontoparietal networks (Astill et al., 2012).

However, sleep quality only accounted for part of the neurobehavioral effect of low zinc, indicating that there are unmeasured factors mediating the effects. For example, zinc has been considered a coenzyme for proteins that support brain function and a nutrient essential for neurogenesis, neuronal migration and synaptogenesis (Bhatnagar & Taneja, 2001). Low zinc status may also alter DNA methylation of brain-derived neurotrophic factor (BDNF) gene in the hippocampus, thereby exerting a direct effect on neurobehavioral function. In the present study, low iron also predicted worse performance on nonverbal reasoning, likely by altering the prefrontal−subcortical dopaminergic network and the frontostriatal networks (Carpenter et al., 2016; Muñoz & Humeres, 2012). However, the mediating effect of sleep quality on the relationship between iron and neurobehavioral function did not show statistical significance. Future

research is needed to examine the complex relationship between micronutrient, sleep and neurobehavioral function.

Study Strengths and Limitations

The main strength of this study was that we were able to illustrate domain-specific relationships between micronutrient status, sleep and neurobehavioral function in an early adolescent sample. However, several potential limitations should be taken into account in the interpretation of results. First, this study did not specifically test the relationship between habitual sleep duration and neurobehavioral domains. Instead, habitual sleep duration was considered as one element that contributed to global sleep quality. Second, habitual daytime napping and weekend compensatory sleep may obscure brain responses to global sleep quality measured by the PSQI. Because of the missing data and insufficient statistical power, napping behaviors and weekend catch-up sleep were not included in data analyses. Additionally, self-reported sleep measures may pose a potential risk for recall bias. Thus, future research should employ an objective measure of sleep such as actigraphy and take into account daytime sleep and day-to-day variability in sleep duration. Third, moderating and mediating effects may not be mutually exclusive. However, the regression models were not stratified by sleep groups to further examine the moderating effect of sleep quality due to the lack of statistical power. Future research is warranted to test more complex models, such as moderator mediation and mediator moderation. Finally, the prevalence of low zinc in our sample may not represent the micronutrient status in Chinese children and adolescents (Yang, Chen, & Feng, 2007). Whereas prior epidemiological findings primarily relied on dietary zinc intake, the

discrepancy may result from different micronutrient indicators. In addition, the small sample size of low zinc may lead to type II error. Nevertheless, the associations between low zinc and neurobehavioral domains achieved statistical significance in this small sample, indicating a substantial difference in sleep quality and neurobehavioral function between adolescents with and without low zinc status. A further investigation of the relationships with a larger sample size is needed to illustrate the nature of these relationships.

Despite the limitations, our findings on the relationship between zinc/iron status, sleep quality and neurobehavioral function have important implications for future research and practice. During adolescence, the theoretical links between interrelated health issues are complicated by developmental processes. Neurobehavioral function, especially nonverbal reasoning, may be particularly vulnerable to low micronutrient status and reduced sleep quality in early adolescents. The partial mediating effect of sleep found in the present study suggests a novel conceptual pathway between micronutrient status, sleep and neurobehavioral function. Although the clinical relevance of the complex relationships is not yet fully understood, this study represents an initial step to recommend potential prevention and intervention programs for the interrelated public health issues of nutrition, sleep and cognition. Routine nutrition monitoring among early adolescents, especially assessment of adequate micronutrient concentrations/levels, is necessary due to its suggested importance to sleep health from the present study. More importantly, as the optimal neurobehavioral function may require the consideration of multiple interconnected factors, health providers and parents may include both

micronutrient status and suboptimal sleep into risk assessment and risk management to optimize neurobehavioral function in early adolescents.

Conclusion

The findings from a healthy early adolescent sample suggest that both poor sleep quality and low levels of zinc/iron are associated with decreased neurobehavioral function in a domain-specific fashion. In addition, sleep quality may partially explain the relationship between micronutrient status and neurobehavioral performance. Specifically, low zinc in the serum is associated with increased likelihood of poor sleep quality in early adolescents, which may further predict worse neurobehavioral performance, nonverbal reasoning in particular. A further investigation of the relationship could provide the foundation for issuing guidelines to optimize micronutrient status, sleep health and neurobehavioral function in adolescents.

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CHAPTER 5: CONCLUSION

Overview of Background and Specific Aims

Adolescence is a time of marked changes in cognitive ability and in the behaviors underpinning these cognitive transitions. The vulnerability to suboptimal sleep during adolescence may give rise to neurobehavioral concerns among adolescents who undergo the brain maturation process. However, neurobehavioral function, a sensitive indicator of central nervous system functioning, requires the consideration of multiple factors to proceed optimally (Lezak, 2004). Micronutrient status has been considered as a predisposing factor for both sleep patterns and neurobehavioral dysfunction, thereby providing an opportunity for the prevention and intervention of pubertal sleep and neurobehavioral problems. This dissertation study contributes to the current body of knowledge regarding adolescent sleep health and neurobehavioral development by identifying the cross-sectional and longitudinal relationships between serum micronutrient status and habitual sleep quality, and by exploring the contributions of habitual sleep quality to the relationship between micronutrient status and neurobehavioral function.

The three sub-studies presented in this dissertation study collectively accomplished the following aims: 1) systematically reviewing the existing literature on micronutrient status and sleep patterns to identify known and hypothesized associations as well as neurobiological underpinnings (Chapter 2). Since this dissertation was one of the first studies on this phenomenon among adolescents, the systematic review extended to the

relevant studies across all age groups.; 2) empirically testing the cross-sectional and longitudinal associations between serum micronutrient concentrations (iron and zinc) and adolescent sleep measured by the PSQI, as well the interaction effect between iron and zinc status on sleep (Chapter 3); and 3) constructing a mediating model of global sleep quality (assessed by the PSQI) on the relationship between serum micronutrient status (iron and zinc) and multiple neurobehavioral domains (Chapter 4).

This chapter summarizes the study findings, outlines methodological constraints, and highlights theoretical and clinical contributions and implications for sleep health and neurobehavioral development in adolescents.

Summary of Findings

The science state of micronutrient status and sleep patterns. The findings from the systematic review provided the foundation for understanding the relationship between micronutrient status and sleep characteristics across the lifespan. The number of studies in this area remains low, with 19 observational studies and 7 clinical trials. Although a definitive recommendation cannot be made, prior research has suggested that micronutrients, such as iron and magnesium, were associated with the development of sleep stages among infants as well as a delay of age-related alterations in sleep architecture among older adults. Sleep duration was found to be positively correlated with the levels of iron, zinc, and magnesium, and negatively correlated with copper, potassium, and Vitamin B_{12} levels. The mechanism underlying these relationships remains unclear. Possible explanations include the impact of micronutrients on excitatory/inhibitory neurotransmitters and the expression of circadian genes. The

unknown questions identified in the systematic review were further addressed in the remaining analyses in Chapter 3, with a focus on an early adolescent sample from Jintan, China.

Iron and sleep. The systematic review (Chapter 2) indicated associations of suboptimal iron levels with reduced sleep duration in infants (Kordas et al., 2008) and adults (Grandner, Jackson, Gerstner, & Knutson, 2013). Our early adolescent sample showed a trend towards decreased odds of long sleep latency with increasing iron concentrations (Chapter 3), although there were no statistically significant relationships of serum iron concentrations with global sleep quality and other sleep subdomains. The systematic review (Chapter 2) also identified a potentially long-lasting effect of iron deficiency on the development of quiet sleep/NREM sleep in infants. However, findings from the present study (Chapter 3) revealed no longitudinal associations between childhood serum status and adolescent sleep quality. The discrepancy may be a result of the subjective and objective sleep measures employed, as well as different developmental stages of the participants in each study.

Zinc and sleep. The findings of the systematic review suggest a positive association between zinc levels (zinc intake or biochemical levels) and sleep duration in adults. The results in Chapter 2 extended the relationship to an early adolescent sample, showing both cross-sectional and longitudinal relationships between serum zinc and sleep quality. Specifically, higher serum zinc concentrations measured at early adolescence were significantly associated with better concurrent global sleep quality, which might be attributed to decreased odds of insufficient sleep duration and sleep disturbances. In

terms of the longitudinal relationship between zinc status and sleep, increased serum zinc concentrations at a preschool age significantly predicted better sleep efficiency at early adolescence. There were no significant interaction effects between iron and zinc on sleep outcomes, including global sleep quality and sleep subdomains.

Sleep and neurobehavioral function. Poor global sleep quality showed domain-specific associations with neurobehavioral function in this early adolescent sample, with statistically significant correlations with abstraction/mental flexibility, episodic memory, and nonverbal reasoning ability. Specifically, when compared to normal sleepers, poor sleepers displayed similar reaction times, but experienced decreased performance accuracy on the test for episodic memory. Similarly, poor sleepers were also more apt to using longer reaction times to achieve similar accuracy on test for abstraction/mental flexibility. Furthermore, with respect to nonverbal reasoning, adolescents with poor sleep quality showed a trend towards a fast but error-prone performance, suggesting an impulsive cognitive style that marks brain immaturity in children and adolescents (Ault, 1973).

The mediation effect of sleep. In Chapter 4, the mediation analyses found an insignificant pathway linking low iron and global sleep quality measured by the PSQI, which substantiates the findings on serum iron concentrations in Chapter 3. The conceptualization of sleep quality as a potential mediator of iron status and its association with neurobehavioral function were not supported based on Baron & Kenny's (1986) criteria. Instead, low iron was associated with a fast but error-prone performance for nonverbal reasoning independent of global sleep quality.

In terms of zinc status, the findings of the mediation analyses were also congruent with the results presented in Chapter 3, suggesting low zinc status in the serum to be predictive of global sleep quality. This finding provides evidence to support the pathway between the conceptualized predictor (zinc) and mediator (sleep). The mediation analyses also indicated that there were significant associations between low zinc and decreased neurobehavioral performance, especially nonverbal reasoning, spatial processing ability, and attention. Whereas global sleep quality partially mediated the pathway linking low zinc and nonverbal reasoning, no statistical significance was found for other mediation models.

Limitations

There were a number of strengths to the present study; however, several limitations must be acknowledged. The study limitations include the observational study design, difficulty in measuring certain variables of interest and potential biases in the available study sample. Specifically, the findings of this dissertation study cannot offer evidence for causation in the absence of a randomized control design. Although the temporal aspects of the variables of interest were incorporated, the questions of whether suboptimal micronutrient status is a contributory cause of reduced sleep quality, and whether decreased neurobehavioral function is the outcome of suboptimal micronutrients and sleep remain unanswered.

Second, serum iron and zinc concentrations were measured with different laboratory methods at baseline and follow-up during adolescence. The methodological differences make the interpretation of results more challenging, especially the findings of

weak correlations between two waves as well as the substantial decrement in the prevalence of low zinc at early adolescence. As a secondary analysis, the health and medication data when blood samples were collected were not available, which adds to the uncertainty of assessment reliability. In addition, sleep data based on adolescent self-report raises the concerns of recall bias. Although the PSQI has good psychometric properties, future studies with objective sleep measures, such as polysomnography and actigraphy, combined with a sleep diary, are warranted to confirm the findings in this study.

Third, there was variation over the course of the parent study (Jintan Cohort study) in terms of what data were available and the temporal order of the variables of interest. Thus, the subsamples included in each sub-study may not be representative of the Jintan cohort. Additionally, sleep patterns and eating behaviors are socially and culturally constructed (Eertmans, Baeyens, & Van Den Bergh, 2001; Jenni & O'Connor, 2005). The findings from a Chinese context may not be generalizable to other countries or racial/ethnic groups.

Implications for Future Research

This work represents the first step to tackling the multifaceted public health issues of nutrition, sleep and cognition. Additional studies are warranted to validate the associations identified in this body of work.

This study provides evidence of the link between micronutrient status and sleep health in adolescents, especially serum zinc status. Although low zinc was not prevalent in our sample, the World Health Organization estimated that more than two billion people

worldwide suffer from deficiencies in a certain type of micronutrients (Allen et al., 2006). Furthermore, this dissertation work found consistent associations with adolescent sleep based on both serum zinc concentrations and zinc levels defined by clinical reference range in China. Future studies should consider exploring the dose-response relationship between micronutrient status and sleep duration/quality using different clinical cutoffs in the literature, so as to enhance the comparability and generalizability of the research findings. Clinical trials are also needed to examine the impact of dietary intake of micronutrient on sleep quality, which may provide a basis for the development of public health recommendations.

This dissertation work also contains a novel conceptualization of the mediating effect of sleep quality on the relationship between micronutrient status and neurobehavioral function. However, it also raises several new questions. First, only one out of five neurobehavioral measures achieved statistical significance for the mediation model. Additional research with a large sample size is needed to validate the partial mediating conceptualization and explore the biological underpinnings of the variations across neurobehavioral domains. Second, sleep quality only accounted for 10.93-16.38% of the total effect of low serum zinc on neurobehavioral function. Together with the significant but low odds ratio of micronutrient status on sleep quality, it is reasonable to hypothesize alternative models to depict the nature of the complex relationship between micronutrient status, sleep and neurobehavioral function, such as additive or multiplicative effect of micronutrient and sleep on neurobehavioral function, as well as a moderator mediation model with sex as an additional moderator.

Future research should also address several challenges specific to adolescent assessment. The self-reported instrument used in this dissertation can capture perceived experience in habitual sleep among adolescents. However, adolescents tended to have problematic responding behaviors in self-report surveys, including providing "extreme, and potentially untruthful, responses to multiple questions" (Fan et al., 2006; Robinson-Cimpian, 2014). These uncertainties reflect the need for improved methods to assess adolescents sleep. For example, studies using actigraphy together with sleep diary is necessary to validate the findings from this dissertation. In addition, time-of-day effects on performance for cognitive tasks remain a challenge, especially for large-scale epidemiological studies which are unrealistic to conduct cognitive tasks at the same time for each participant. Future research on sleep-cognition relationships should incorporate well-defined theoretical approaches and standard procedures to control for time-dependent changes in task performance for neurobehavioral function.

Implications for Future Practice

This dissertation work presents a first step towards better understanding neurobehavioral function by acknowledging the importance of interwoven lifestyle factors, micronutrient status and sleep, among adolescents. Although the causal relationships are not yet fully understood, the findings have significant implications for health practice.

Adolescents are vulnerable to insufficient sleep time and reduced sleep quality. This dissertation extends the existing literature on neurobehavioral effects of sleep manipulations to habitual sleep quality. This finding will be useful for designing

programs aimed at optimizing neurobehavioral development and daily functioning in adolescents. The common sleep promotion programs, such as sleep hygiene education program (Tan, Healey, Gray, & Galland, 2012), institutional provision (i.e. delayed school start time) (Owens, Belon, & Moss, 2010), cognitive-behavior therapy (Bei et al., 2013), may enhance sleep health and, thereby, contribute to neurobehavioral development during adolescence. Importantly, this dissertation work proposed a novel modifiable factor, serum micronutrient status, which is associated with both sleep health and neurobehavioral function in adolescents.

This study identifies suboptimal micronutrient status as a potential contributor to sleep health. Health providers may consider to include micronutrient levels into risk assessment for sleep problems, zinc status in particular. Serum zinc concentration does not reflect an immediate response to dietary zinc intake due to the homeostatic mechanism; however, lower zinc concentrations signal many weeks of even severe dietary zinc restriction (Table & Table, 2001). Thus, sufficient dietary zinc intake is essential to maintain biological functions of zinc and promote sleep health. Relative to serum status, serum iron only showed significant associations with sleep latency in adolescents. In contrast, the systematic review identified several associations between iron and sleep in other population, warranting additional efforts to maintain normal iron status to reduce the risk for suboptimal sleep.

Of note, monitoring micronutrient levels may have significant implications for sleep health in adolescents, which may further benefit their neurobehavioral function. Additionally, interventions aimed at micronutrient status, therefore, may also carry a

direct benefit for optimizing neurobehavioral function in adolescents. It has been documented that micronutrients, such as zinc, function as a component of various enzymes essential for the structural integrity of proteins and regulation of gene expression (Table & Table, 2001). Most importantly, preventions and interventions with considerations of the intertwined risk factors of micronutrient status and suboptimal sleep may substantially promote neurobehavioral function and shed light on pubertal development.

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