Public Health Nutrition: 24(17), 5815–5825 doi:10.1017/S1368980021002329

Serum micronutrient status, sleep quality and neurobehavioural function among early adolescents

Xiaopeng Ji[1], Charlene W Compher[2], Sharon Y Irving[2], Jinyoung Kim[3], David F Dinges[4] and Jianghong Liu[2,*]

[1]College of Health Sciences, School of Nursing, University of Delaware, Newark, DE 19713, USA: [2]School of Nursing, University of Pennsylvania, Philadelphia, PA, USA: [3]School of Nursing, University of Nevada, Las Vegas, USA: [4]Department of Psychiatry, University of Pennsylvania, School of Medicine, Philadelphia, PA, USA

Submitted 22 September 2020: Final revision received 5 May 2021: Accepted 18 May 2021: First published online 26 May 2021

Abstract

Objective: To examine associations between serum micronutrients and neurobehavioural function and the mediating role of sleep quality in early adolescents.
Design: In this cross-sectional study, peripheral blood samples were analysed for Fe and Zn levels. The Pittsburgh Sleep Quality Index and Penn Computerized Neurocognitive Battery were used to assess sleep quality and neurobehavioural function, respectively. The logistic/linear regressions and generalised structural equation modelling were performed to estimate the associations.
Setting: Jintan, China
Participants: In total, 226 adolescents (106 females) from the Jintan Child Cohort study.
Results: Adolescents with low Fe (<75 μg/dl) (OR = 1·29, $P = 0·04$) and low Zn (<70 μg/dl) (OR = 1·58, $P < 0·001$) were associated with increased odds for poor sleep quality. Adolescents with low Fe and Zn were associated with fast (Fe: $\beta = -1353·71$, $P = 0·002$, Zn: $\beta = -2262·01$, $P = 0·02$) but less-accurate (Fe: $\beta = -0·97$, $P = 0·04$; Zn: $\beta = -1·76$, $P = 0·04$) performance on non-verbal reasoning task and poor sleep quality partially mediated the associations between low Fe/Zn and non-verbal reasoning ($P < 0·05$). Additionally, low Fe was associated with a slower reaction on spatial processing task ($\beta = 276·94$, $P = 0·04$), and low Zn was associated with fast ($\beta = -1781·83$, $P = 0·03$), but error-prone performance ($\beta = -1·79$, $P = 0·04$) on spatial processing ability and slower reaction speed ($\beta = 12·82$, $P = 0·03$) on the attention task. We observed similar trends using a cut-off point of 75 μg/dl for low serum Zn, except for the association with attention task speed ($P > 0·05$).
Conclusion: Fe and Zn deficiencies may possibly be associated with poor sleep and neurobehavioural function among early adolescents. Poor sleep may partially mediate the relationship between micronutrients and neurobehavioural function.

Keywords
Serum Fe
Serum Zn
Sleep quality
Adolescents
Neurobehavioural function

Although severe micronutrient deficiencies are considered rare, children and adolescents from developing countries are among the most vulnerable groups for mild-to-moderate micronutrient deficiencies, particularly Fe and Zn deficiencies[1]. Approximately 8–40 % of children and adolescents (<18 years) suffer from Fe deficiency, and 10–50 % of adolescents aged 11–16 years report Zn deficiency in China[1,2]. Micronutrients are known as cofactors essential for neurotransmitter synthesis and brain function[3]. Prior research has linked Fe deficiency with diminished verbal memory, attention and executive function in children[4] and adolescents[5,6]. However, a systematic review suggests that Fe supplementation improves attention and concentration but no other cognitive domains[7]. Regarding the role of Zn, a consensus on the relationship between Zn and neurobehavioural function in adolescents remains elusive. Children and adolescents who took Zn supplementation have shown improvement in psychomotor development, reasoning and executive function in some[8,9], but not all[10] studies. Neurobehavioural function is essential for learning, academic achievement, behavioural control and stress coping in adolescents[11,12]. Early neurobehavioural deficits in childhood are associated with

*Corresponding author: Email jhliu@nursing.upenn.edu

internalising and externalising psychopathology during adulthood[13], suggesting a long-lasting detrimental effect of neurobehavioural dysfunction. Thus, elucidating the relationship between micronutrient deficiencies and neurobehavioural domains has significant public implications for health promotion during adolescence and beyond.

In addition to micronutrient deficiencies, sleep deficiencies and sleep disturbances have been public health concerns for adolescents. More than half of adolescents worldwide report insufficient sleep duration, poor sleep quality and excessive daytime sleepiness[14]. Impaired sleep may exponentially increase the risk for depression[15], neurobehavioural dysfunction[16] and cardiovascular risk factors[17] in adolescents. Micronutrient deficiency may be an understudied risk factor for impaired sleep[18]. Experimental studies show the impact of micronutrients on nerve-signalling chemicals or neurotransmitters essential to intrinsic sleep regulation[19,20], indicating that the link between micronutrients and sleep is biologically plausible. Most clinical trials and epidemiological studies in this area are based on samples of infants, children and adults[18,21–23]. Fe deficiency anaemia is associated with more night waking and shorter sleep duration than better-nourished infants[21], and Fe supplement increases nocturnal and total sleep duration among Fe deficiency anaemia infants[22]. In the general adult population, there is an association between low Zn intake and short sleep duration[23]. Very few studies have examined the relationship between micronutrient status and sleep quality among adolescents who are at high risk of sleep impairment. Our previous work suggests that serum Zn concentrations in 3–5-year-olds and 11–14-year-olds predict multiple sleep domains measured by the Pittsburgh Sleep Quality Index (PSQI) during adolescence[24]. However, the role of Fe deficiency and Zn deficiency in sleep quality remains less clear in this vulnerable population.

The primary aim of this study was to investigate the associations of serum micronutrient status (Fe and Zn) with sleep quality and multiple neurobehavioural domains in a healthy adolescent sample. We hypothesised that adolescents with low Fe/Zn would exhibit an increased risk for poor sleep quality and poor performance on neurobehavioural tasks. Additionally, although experimental studies suggest the role of micronutrients in brain function on a molecular level[3], the mechanism through which micronutrient deficiencies alters neurobehavioural performance in adolescents remains unclear. There is extensive evidence demonstrating the neurocognitive and behavioural consequences of experimental sleep restriction, including declined vigilance, executive function and working memory, as well as longer reaction time[16]. Given the cognitive effect of sleep, micronutrient deficiencies may affect sleep quality[18], thereby leading to poor neurobehavioural function. Thus, we also tested whether sleep quality mediated the relationship between low Fe/Zn and neurobehavioural function. The findings from this study will help understand the role of intertwined health issues of nutrition and sleep in predicting neurobehavioural function, and ultimately inform tailored nutritional interventions for health care professionals to improve sleep and neurobehavioural development in adolescents.

Methods

Study design and participants

The present study is part of the China Jintan child cohort study that aims to investigate early health risk factors and neurobehavioural development throughout childhood and adolescence[25,26]. The Jintan research team used a multi-stage sampling method and enrolled 1656 children (3–5 years old) who represented preschoolers from each school district (urban, suburban and rural) in Jintan in 2004 (Wave I). According to their year (1st, 2nd and 3rd) in preschool, children were classified into the lower, middle and upper cohort. When participants were in their last month of sixth grade in 2011–2013 (approximately 12 years old), they were invited to Wave II data collection. A total of 1110 participants participated in the Wave II study. Of them, 343 participants had blood drawn for Zn/Fe testing in the same period for sleep measurement and neurobehavioural tasks. Sleep-related questionnaires were completed under the supervision of a research assistant in student classrooms. Also, paediatric nurses collected fasting blood samples and conducted physical examinations (e.g. height/weight measurement) for participants in each school. Regarding neurocognitive assessment, trained research assistants instructed participants to perform the Penn Computerized Neurocognitive Battery in the Children's Health Laboratory, Jintan Hospital. This cross-sectional study used a subsample (n 226) who had complete data on serum micronutrient concentrations (Fe and Zn), sleep and neurobehavioural test scores. There were no differences in social and demographic variables (i.e. age, sex, home location, parent education, BMI z-scores) between the subsample (n 226) and the whole sample in Wave II (n 1110) ($P > 0{\cdot}05$). Detailed sampling and research procedures of this cohort study have been described in Liu et al.[25,26].

Measures

Micronutrient Status

Peripheral blood specimens (fasting) were drawn at 07.00–08.00 in each school by paediatric nurses from the Jintan Hospital. Research assistants provided fasting instructions to participants the day before the blood draw. Approximately 0·5 ml of blood samples were collected in a lead-free ethylenediaminetetraacetic acid tube, stored at −40°C and shipped to Xin Hua Hospital, Shanghai, China. Micronutrient concentrations were analysed using inductively coupled plasma MS. The detailed analytical

https://doi.org/10.1017/S1368980021002329 Published online by Cambridge University Press

procedure was reported elsewhere[25,26]. The lower cut-off point of <75 μg/dl was used to classify individuals as on low Fe status[27]. Low Zn status was identified by fasting serum concentrations below 70 μg/dl, as proposed by the International Zinc Nutrition Consultative Group[28]. This cut-off has been used in a nationally representative sample in China[2], thus enhancing the comparability of our results. Due to the small number of participants who had Zn concentrations below 70 μg/dl, we conducted sensitivity analyses using 75 μg/dl[29] and 76·5 μg/dl[27] as cut-off points for low Zn to examine the stability of estimations. Of them, the threshold serum Zn concentration of <75 μg/dl was selected because it was reported to be more responsive to nutritional treatment than other thresholds in undernourished children[29].

Sleep quality

Adolescents completed the Chinese version of the Pittsburgh Sleep Quality Index (PSQI) and questions about sleep schedules in their classrooms in June–July 2013. The PSQI comprises nineteen items categorised into seven sleep domains: subjective sleep quality, sleep efficiency, sleep latency, sleep duration, sleep disturbances, use of sleeping medication and daytime sleepiness and dysfunction[30]. Sleep duration was calculated by bedtime and wake-time and classified into 0–3 levels according to the adolescent cut-off used in the National Sleep Foundation[31]. The PSQI global score ranged from 0 to 21 (sub-domains range = 0–3), with poor sleeper defined as total scores>5[30]. The PSQI is a widely used instrument in measuring sleep quality in diverse groups, including adolescents[30,32]. In the adolescent population, the internal consistency for the Chinese version of PSQI was reported to be 0·87, with Cronbach's alpha ranged from 0·46 to 0·85 for sub-domains[32]. The internal consistency in the current study was 0·78.

Neurobehavioural Function

Adolescents performed the Penn Computerized Neurocognitive Battery, including tests on attention, abstraction and mental flexibility, episodic memory, spatial processing ability, and non-verbal reasoning. The Penn Computerized Neurocognitive Battery has been validated with functional neuroimaging to define the recruitment of specific brain systems and has shown good psychometric properties among children and adolescents aged 8–21 years[33]. In a subsample (*n* 122) of the Jintan child cohort, the Cronbach's alpha coefficients for the overall test and each domain of the Penn Computerized Neurocognitive Battery were reported to be greater than 0·8, suggesting adequate internal consistency.

Executive control: (1) In the short Penn Conditional Exclusion Test (sPCET), participants needed to decide which object did not belong with the other three based on sorting principles, such as shape, size, and line thickness. The sPCET assessed abstraction ability and mental flexibility. (2) The Penn Continuous Performance Test measured the ability to focus and sustain attention. Participants pressed the space bar on a computer keyboard whenever the display formed a digit (for the first half of the test) or a letter (for the second half of the test). Episodic memory: The Short Visual Object Learning Test (sVOLT) measured spatial memory for shapes. Participants were asked to memorise a series of three-dimensional shapes (two-dimensional shapes and their locations), at a rate of 1 shape/second, to correctly answer test trials. During the immediate recognition phase, participants were shown twenty shapes, one at a time, and asked to choose whether a shape was included in the original list on a scale of 1–4 (1 = 'Definitely yes' to 4 = 'Definitely not'). While ten shapes were those participants were asked to memorise, the other ten were distractors. The accuracy score indicates the number of correctly recognised shapes and distractors. Complex cognition: (1) The short Penn Line Orientation Test (sPLOT), which measured spatial processing ability, showed two lines at an angle and asked participants to click on the button to rotate one of the lines until it matched the same angle as the other. (2) The Penn Matrix Reasoning Test (PMRT) showed matrices that required geometric analogy and contrast principles reasoning skills, thus assessing the ability of non-verbal reasoning.

Tests were approximately 30 min in length depending on individual performance. 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 evaluation of individual differences in cognitive strategy for the speed-accuracy tradeoff. Poor function in the present study was defined as: (1) slow and/or inaccurate performance, or (2) fast but inaccurate performance.

Covariates

Social and demographic characteristics such as age, sex, parental education (years of formal education from elementary school to the present day) and home location (urban, suburban, and rural areas) were assessed and treated as covariates in this study. These covariates were selected since they have been linked to micronutrient intake[1,2], sleep patterns[14,34], and/or cognition[35] in the literature. Pediatric nurses performed physical examinations for participants in each school, including height and weight assessment. BMI was then calculated using the formula weight (kg) divided by height in meters squared (m^2). Gender-specific BMI-for-age *z*-scores were calculated using the WHO AnthroPlus software[36]. Neurobehavioural testing performance may be confounded by heritable cognitive traits such as general intelligence quotient (IQ)[37]. Thus, the Chinese version of the Wechsler Intelligence Scale for Children-Revised (WISC-R) was used to assess IQ in adolescents. Additionally, given the potential diurnal variation in cognitive performance, we recorded the time of day when neurobehavioural tests were performed.

https://doi.org/10.1017/S1368980021002329 Published online by Cambridge University Press

Statistical methods

Sample characteristics were summarised using descriptive statistics. We performed logistic regression models to examine the relationship between micronutrient status (normal Fe/Zn as the reference category) and sleep quality, controlling for age, sex, parental education, home location, BMI *z*–scores. The distributions of continuous variables were tested for normality using the Shapiro-Wilk test as well as the standardised normal probability (pnorm) and quintile-normal (qnorm) plots. Gender-specific BMI-for-age *z*-scores ($P = 0{\cdot}39$), mother education years ($P = 0{\cdot}88$), father education years ($P = 0{\cdot}92$), but not age, WISC-R IQ scores and neurobehavioural testing scores ($P < 0{\cdot}05$), were normally distributed. A series of linear regressions were then conducted to estimate the associations between micronutrient status and neurobehavioural performance. Accuracy and speed scores of each neurobehavioural domain entered the model separately as a dependent variable. For each neurobehavioural domain, the predictive effect of micronutrient status on neurobehavioural testing scores (accuracy and speed) was first modeled, while controlling for age, sex, parental education, home location, BMI *z*-scores, WISC IQ score, and time of neurobehavioural tests (Model 1). Sleep quality was then additionally adjusted for in Model 2. Because of the multicollinearity between mother and father education, only father education was included in models. The nesting of assessment within each cohort (low, middle, and upper) was clustered in each model. The normality in the residuals of linear regression models (Table 4) were tested using pnorm and qnorm plots.

We used the generalised structural equation modelling with bootstrap approaches to test the direct and indirect pathways among micronutrient status, sleep quality and neurobehavioural scores. A generalised structural equation modelling is a preferred method for mediation models featuring categorical variables[38], as is the case with micronutrient status and sleep quality in this study. Age, sex, parental education, home location, BMI *z*-scores, WISC IQ score, and time of neurobehavioural tests were entered into models as covariates. The 95 % bias-corrected and accelerated (BCa) CI for direct, indirect and total effects were estimated from 5000 random bootstrap samples. The 95 % CI (BCa) that does not cross zero indicates a significant mediating (indirect) effect. Sensitivity analyses were conducted to assess whether the threshold for low Zn (70 *v.* 75 *v.* 76·5 μg/dl) affected results. Statistical tests were two-tailed with a significance level of 0·05. All analyses were performed in Stata 16.

The final sample composed of 226 early adolescents aged 11–14 years, 53·10 % (*n* 120) were boys. Demographic characteristics, BMI, IQ and time of neurobehavioural tests are summarised in Table 1. A total of sixty-three participants (27·88 %) were classified as poor sleepers (PSQI > 5) (Table 2). The mean serum Fe concentration was 100·32 (SD = 30·21) μg/dl, with forty-one participants (18·14 %) having low serum Fe. In terms of serum Zn, thirteen (5·86 %) had serum Zn concentrations below 70 μg/dl, thirty five (15·49 %) had serum Zn concentrations <75 μg/dl and forty one (17·70 %) had serum Zn concentrations <76·5 μg/dl.

Table 1 Sample characteristics (*n* 226)

	Mean	SD	*n*	%
Sex				
Male			120	53·10
Female			106	46·90
Age	12·14	0·55		
Mother's education years	13·35	2·99		
Father's education years	12·70	3·16		
Home location				
Rural			32	14·16
Suburb			94	41·59
Urban			100	44·25
Time of neurobehavioural test*				
AM (08:00–11:59)			18	8·11
PM (12:00–17:00)			204	91·89
WISC-R IQ	104·92	12·19		
BMI	19·44	3·23		
BMI *z*-score	0·38	1·11		
Iron concentrations, μg/dl	100·32	30·21		
Zinc concentrations, μg/dl	87·95	12·49		

BMI, body mass index; WISC-R IQ, Wechsler Intelligence Scale for Children-Revised, intelligence quotient.
*There were missing data in testing time (*n* 2).

Table 2 summarises serum Fe and Zn status of participants with normal and poor sleep quality. The proportion of poor sleepers was higher in individuals with low Fe (32 % *v.* 27 %) and low serum Zn (38 % *v.* 27 %) compared with those who had normal micronutrient levels. After controlling for influences of age, gender, parental education, home location and BMI *z*-scores, low Fe status (OR = 1·29, $P = 0{\cdot}04$) predicted a 29 % increase in odds for poor sleep compared with normal Fe status. Adolescents with low Zn (<70 μg/dl) were estimated to have a 58 % increase in odds for poor sleep (OR = 1·58, $P < 0{\cdot}001$). We repeated analyses using a cut-off value of 75 μg/dl for low serum Zn and consistently found a significant association between low serum Zn and increased odds of poor sleep quality (OR = 1·29, $P = 0{\cdot}04$). However, there is no association between low Zn defined as serum concentrations below 76·5 μg/dl and poor sleep ($P > 0{\cdot}05$), and we did not repeat mediation analyses using this cut-off.

The descriptive statistics of neurobehavioural scores by micronutrient status are summarised in Table 3. In adjusted models, individuals with low Zn status (<70 μg/dl) were significantly associated with fast (t = 2·17, $P = 0{\cdot}03$) but less accurate (t = 2·34, $P = 0{\cdot}03$) performance on spatial processing (sPLOT) task compared with those who had normal serum Zn. The associations of low Zn with sPLOT accuracy ($\beta = -1{\cdot}79$, $P = 0{\cdot}04$) and speed ($\beta = -1781{\cdot}83$, $P = 0{\cdot}03$) remained significant in linear regression models after adjusting for covariates (Table 4). Adolescents with low serum Zn tended to have similar performance patterns on task for non-verbal reasoning (PMRT): shorter reaction time ($\beta = -2262{\cdot}01$, $P = 0{\cdot}02$) but lower accurate scores ($\beta = -1{\cdot}76$,

https://doi.org/10.1017/S1368980021002329 Published online by Cambridge University Press

Table 2 Association between serum iron/zinc and sleep quality (logistic regression)†

	Total (*n* 226)		Normal sleep (*n* 163)		Poor sleep (*n* 63)		Regression*	
Serum micronutrients	*n*	%	*n*	%	*n*	%	OR	SE
Iron (cut-off:75 μg/dl)								
Normal	185	81·85	135	72·97	50	27·03		
Low	41	18·14	28	68·29	13	31·71	1·29**	0·14
Zinc (cut-off:70 μg/dl)								
Normal	213	94·24	155	72·77	58	27·23		
Low	13	5·86	8	61·54	5	38·46	1·58***	0·10
Zinc (cut-off:75 μg/dl)								
Normal	191	84·51	140	73·30	51	26·70	1·29**	0·15
Low	35	15·49	23	65·71	12	34·29		
Zinc (cut-off:76·5 μg/dl)								
Normal	185	82·38	135	72·97	50	27·03		
Low	41	17·70	28	68·29	13	31·71	1·12	0·35

**$P < 0·05$,
***$P < 0·01$.
†Logistic regression models adjusted for age, sex, parental education, home district, full WISC IQ score, time of CNB tests and BMI *z*-score.

Table 3 Description of neurobehavioural scores by serum iron/zinc status

	Zinc				Iron			
	Normal		Low (<70 μg/dl)		Normal		Low (<75 μg/dl)	
CNB Test	Mean	SD	Mean	SD	Mean	SD	Mean	SD
sPCET								
Speed, ms	1687·25	441·35	1538·42	404·85	1668·51	433·36	1724·65	470·97
Accuracy	26·04	6·64	25·15	5·38	25·76	6·66	27·00	6·10
PCPT								
Speed, ms	518·23	139·94	527·95	162·43	508·46	127·03	523·95	148·03
Accuracy	77·71	36·87	83·94	41·38	79·38	36·33	71·63	41·94
sVOLT								
Speed, ms	1570·23	572·98	1492·24	440·74	1609·95	671·88	1598·33	577·08
Accuracy	14·72	3·07	14·88	3·14	14·70	3·03	15·36	3·03
PMRT								
Speed, ms	7567·37	5274·61	5971·46	3813·03	7846·59	5470·10	6707·83	5152·43
Accuracy	11·63	4·35	10·82	5·71	11·74	4·38	11·05	4·82
sPLOT								
Speed, ms	7925·45	2536·93	6386·35	1087·37	7799·99	2461·89	7997·87	2700·58
Accuracy	9·85	3·76	7·25	3·05	9·23	3·97	10·41	3·89

PCPT, Penn Continuous Performance Test; PMRT, Penn Matrix Reasoning Test; sCTAP, short Computerised Finger-Tapping Task; sPCET, short Penn Conditional Exclusion Test; sPLOT, short Penn Line Orientation Test; sVOLT, short Visual Object Learning Test; ms, milliseconds.

$P = 0·04$) (Table 4, Model 1). The associations between low serum Zn and PMRT scores were attenuated but remained significant with the addition of sleep quality into analyses (Table 4, Model 2). Additionally, compared with those with normal Zn, the low Zn group was significantly associated with longer reaction time ($\beta = 12·82$, $P = 0·03$) on Penn Continuous Performance Test that reflected sustained attention. We found similar results using a cut-off point of 75 μg/dl for low serum Zn, except for the association with Penn Continuous Performance Test speed ($P > 0·05$) (Table 4). In terms of Fe status, low serum Fe was significantly associated with faster ($\beta = -1318·60$ and $-1353·71$, Ps $= 0·002$) but error-prone performance ($\beta = -0·92$, $P = 0·05$; and $P = -0·97$, $P = 0·04$) on non-verbal reasoning (PMRT) in models with and without adjusting for sleep quality. Furthermore, adolescents with low Fe were estimated to be 270-ms slower in reaction speed on spatial processing (sPLOT) task ($\beta = 269·65$, $P = 0·04$), while accuracy scores did not statistically differ between the normal and low Fe groups ($\beta = 0·79$, $P = 0·39$). Neither low Fe nor low Zn was predictive of task performance on abstraction and mental flexibility (sPCET) and spatial memory (sVOLT) (ps $> 0·05$) in the adjusted regression models.

As shown in Table 5, among neurobehavioural domains, the indirect effects of low Fe and low Zn on non-verbal reasoning (PMRT) function were statistically mediated by sleep quality. Poor sleep quality accounted for 17·16% of the total effect of low Zn (<70 μg/dl) on non-verbal reasoning accuracy and 11·46% of the total effect on performance speed. The mediation pathway remained significant when low Zn was defined as concentrations <75 μg/dl, with 22·14 % of the total effect on PMRT accuracy and 13·05% of the total

https://doi.org/10.1017/S1368980021002329 Published online by Cambridge University Press

https://doi.org/10.1017/S1368980021002329 Published online by Cambridge University Press

Table 4 The associations between zinc/iron status and neurobehavioural performance (linear regression)†

	Low Zinc (<70 μg/dl)				Low Zinc (<75 μg/dl)				Low Iron (<75 μg/dl)			
	Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
CNB Test	*B*	SE	*B*	SE	*B*	SE	*B*	SE	*B*	SE	*B*	SE
sPCET												
Speed, ms	−142·72	23·27	−157·86	22·35	−17·87	90·12	−17·01	80·59	53·78	23·27	48·86	19·38
Accuracy	−0·60	1·61	−0·88	1·63	0·13	0·74	0·15	0·54	1·19	1·10	1·09	1·15
PCPT												
Speed, ms	12·82*	2·19	13·27*	2·51	12·04	13·24	12·94	14·19	15·70	6·88	16·39	7·10
Accuracy	10·96	2·93	11·28	3·16	6·50	4·90	6·44	4·39	−4·93	5·42	−4·69	5·65
sVOLT												
Speed, ms	−232·27	40·46	−232·03	49·63	−224·45	118·64	−224·82	118·91	11·74	11·45	14·05	15·59
Accuracy	0·64	0·13	0·80	0·12	−0·11	0·32	−0·13	0·21	0·54	0·41	0·58	0·45
PMRT												
Speed, ms	−2262·01*	328·73	−2209·48*	333·09	−1916·01*	277·88	−1904·72*	294·64	−1353·71**	58·87	−1318·60**	73·63
Accuracy	−1·76*	0·37	−1·69*	0·38	−1·02*	0·25	−1·01*	0·26	−0·97*	0·20	−0·92*	0·22
sPLOT												
Speed, ms	−1781·83*	92·97	−1858·08*	98·88	−634·85*	17·78	−666·75*	25·58	269·65*	10·25	234·20†	21·73
Accuracy	−1·79*	0·14	−1·83	0·15	−1·13*	0·21	−1·13*	0·22	0·79	0·58	0·77	0·58

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; ms, milliseconds.
Reference level: normal iron or zinc level.*$P \leq 0{\cdot}05$, **$P < 0{\cdot}01$.
†Model 1 adjusted for age, sex, parental education, home district, full WISC IQ score, time of CNB tests and BMI *z*-score; the model set 2 adjusted for covariates in the model 1 and sleep quality.

Table 5 Mediating role of sleep in the relationship between micronutrients and Penn Matrix Reasoning Test (PMRT) scores (GSEM)*

	PMRT accuracy		PMRT speed	
	B	95 % CI (BCa)†	*B*	95 % CI (BCa)†
Low zinc (<70 μg/dl)				
Direct effect	−1·69	−1·99, −1·45	−2209·48	−2431·22, −2032·80
Indirect effect‡	−0·35	−0·42, −0·29	−286·11	−343·08, −238·12
Total effect	−2·04	−2·41, −1·74	−2495·58	−2495·58, −2270·91
Low zinc (<75 μg/dl)				
Direct effect	−1·01	−1·02, −0·81	−1904·72	−1950·92, −1709·95
Indirect effect	−0·29	−0·32, −0·27	−285·75	−316·74, −253·98
Total effect	−1·31	−1·56, −1·13	−2190·47	−2435·12, −2075·83
Low Iron (<75 μg/dl)				
Direct effect	−0·92	−1·11, −0·72	−1318·60	−1364·55, −1252·05
Indirect effect	−0·22	−0·27, −0·18	−180·59	−220·62, −140·56
Total effect	−1·14	−1·35, −0·94	−1499·19	−1522·65, −1478·97

GSEM, generalised structural equation modelling.
*Models adjusted for age, sex, parental education, home district, full WISC IQ score, time of neurobehavioural tests and BMI *z*-score.
†BCa: bias-corrected accelerated CI from 5000 bootstrap samples.
‡Indirect effect was through sleep quality.

effect on PMRT speed mediated by sleep quality. In terms of serum Fe, sleep quality was also a significant mediator between the association between low Fe and fast but error-prone performance on the PMRT. Although poor sleep quality was also associated with longer reaction time on task for abstraction/mental flexibility (sPECT) ($\beta = 102{\cdot}46$, $P = 0{\cdot}02$) and declined accuracy on episodic memory task (sVOLT) ($\beta = -1{\cdot}16$, $P = 0{\cdot}001$) (Supplement Table 1), sleep quality did not significantly mediate the association between serum micronutrient status and these neurobehavioural domains. Supplemental Figure 1 shows the direct and indirect pathways between micronutrient deficiencies and PMRT scores.

Discussion

Eighteen percent of participants had low serum Fe and up to 17 % had low serum Zn depending on the lower cut-off points of Zn. Adolescents with low serum Fe and low Zn tended to have a fast but error-prone performance on non-verbal reasoning task. Low serum Zn and Fe status were associated with increased odds for poor sleep quality, which in turn partially mediated the relationship between micronutrient deficiencies and non-verbal reasoning. Additionally, adolescents with low serum Zn were estimated to have faster but less accurate performance on spatial processing task and slower reaction speed on sustained attention task than the group with normal Zn. We also observed an association between low serum Fe and slower reaction speed on spatial processing task. However, the indirect effect through sleep quality on spatial processing and attention was not statistically significant. To our knowledge, this is one of the first studies that examine the complex relationship between micronutrient deficiencies, sleep and neurobehavioural function in an adolescent sample.

Despite improvements in nutritional status over the past decades, mild-to-moderate micronutrient deficiencies, mainly from insufficient dietary intake and poor bioavailability, remain a public health concern in China[2,39]. There were 28 % of school-age children reporting insufficient Fe intake and 38 % reporting insufficient Zn intake based on the estimated average requirements in China[2]. Compared with rates not meeting the estimated average requirements, we observed lower rates of Fe and Zn deficiencies defined by fasting serum concentrations in our sample. Following the decreasing trend of micronutrient deficiencies in China, the prevalence of low serum Fe in our sample (18 %) decreased as relative to the rate at baseline (24 %) when participants were at 3–5 years old[27]. The prevalence rates of serum concentrations below 76·5 μg/dl (17 %) and low Zn <75 μg/dl (15 %) were half of the rate at baseline (3–5 years old)[27]. In data analysis, we used a cut-off value of <70 μg/dl for Zn deficiency to ensure comparability with other studies[2]. The prevalence of low Zn <70 μg/dl (6 %) in our sample was similar to that in Iranian adolescents aged 12–18 years (5·4 %) but lower than the prevalence (10 %) reported in a large rural sample of children in China[2]. Given that this study included participants from urban, suburban and rural areas, the discrepancy may be attributed to the geographic disparity in nutritional status.

The relationship between micronutrient deficiencies and neurobehavioural function was most pronounced in the domain of non-verbal reasoning. We observed a fast but error-prone pattern on task for non-verbal reasoning among individuals with low serum Zn and low Fe. Specific areas of the brain involved in higher-order cognitive function, such as logic and reasoning, continue to develop and mature until mid-teenage years[40]. The speed-accuracy trade-off reflects an impulsive problem-solving pattern, thus indicating less cognitive maturity in adolescents with micronutrient deficiencies than their counterparts[41]. Our findings are congruent with a cross-sectional study showing a trend towards increasing abstract reasoning intelligence with increasing plasma Zn

https://doi.org/10.1017/S1368980021002329 Published online by Cambridge University Press

concentrations among adolescent girls in India[42]. A systematic review consistently shows a selective response in non-verbal fluid intelligence following micronutrient supplementation among children and adolescents with micronutrient deficiencies, particularly Fe deficiency[40]. Non-verbal cognitive ability, which comprises reasoning, logical thinking and problem-solving, may be more directly related to biological function, thus sensitive to micronutrient-related brain biochemistry[40,43]. We also observed worse performances on spatial processing task among those with low Zn or low Fe. These findings, to some extent, converge with the neuroimaging research showing a positive association between Fe concentrations in the basal ganglia and visuospatial intelligence in children aged 7–11 years[44]. The associations of low Fe and Zn with sustained attention did not follow a consistent pattern. Our finding that adolescents with low serum Zn were estimated to have worse sustained attention parallels with prior research suggesting enhanced attention after Zn supplementation among Chinese and Mexican-American low-income children[9]. However, we did not find variation in attention scores between Fe groups, which is contradictory to previous work that suggests the Fe-attention relationship[7,40]. The disparity may result from different neurobehavioural measures or the time for attention test. Future studies are needed to examine the generalisability of our findings.

More than one-fourth of our samples were classified as poor sleepers. Our findings that the odds of poor sleep were higher in adolescents with low serum and Fe both support and extend previous work. Several lines of evidence have suggested the importance of optimal micronutrient status to sleep health in other age groups[23,45,46]. As compared with the healthy control, infants with Fe deficiency anaemia showed more awake times, shorter sleep duration and delayed sleep-spindle patterns in non-rapid-eye-movementPFA at night[45]. Research also shows that short sleep duration was associated with insufficient dietary Zn intake[23] and low serum Zn concentrations[46] in adults. Not previously shown is our finding that poor sleep may act as a mediator in the relationship between micronutrient deficiencies and neurobehavioural deficits, particularly the fast but error-prone performance on non-verbal reasoning task. This pathway is partially supported by an actigraphy study showing an association between reduced sleep quality and fast but less accurate cognitive performance among adolescent boys[37]. However, very few studies have examined micronutrient deficiencies, poor sleep and neurobehavioural deficits together in one study. Given the pubertal changes in intrinsic sleep regulation (e.g. delayed melatonin onset phase)[47] and brain functioning[40], understanding the neurobehavioural function in response to intertwined risk factors of micronutrient deficiencies and poor sleep has substantial clinical implications to this vulnerable population.

Sleep quality did not mediate the association between micronutrient deficiencies and worse spatial processing ability and sustained attention, although the direct effects of micronutrient status were significant. This may be partially explained by the absence of association between poor sleep quality and these neurobehavioural domains. Our findings converge with a prior observational study suggesting no associations between PSQI total score and attention task performance[48] but contradict the findings from experimental studies, which showed declined vigilance following experimental sleep restrictions[16]. The immediate effect of experimentally imposed sleep restriction may not be comparable to our findings, which probably reflect a long-term association between chronic poor sleep quality and neurobehavioural function in naturalist settings.

The mechanisms underlying the complex relationships among micronutrients, sleep modulation and neurobehavioural function remain unclear. Fe and Zn have been documented as an antagonist of excitatory neurotransmitters, such as the N-methyl-d-aspartate receptor[19] and dopaminergic neurons[20] and an agonist of inhibitory neurotransmitters, such as gamma-aminobutyric acid receptors[19], thereby potentially influencing intrinsic sleep regulation process. We also conducted an exploratory analysis to examine the threshold concentrations of low serum Zn for predicting poor sleep quality. While we did not find the association between serum Zn below 76·5 μg/dl and sleep quality, low serum Zn defined by cut-off points of 70 and 75 μg/dl was associated with increased odds of poor sleep quality. Poor sleep quality may further predict non-verbal reasoning ability through its detrimental effect on frontoparietal networks. Of note, both direct and indirect effects of low Fe and Zn on non-verbal reasoning were significant in mediation models, suggesting a partial mediating role of sleep quality. Animal models suggest that impaired neuronal growth, myelination and synaptogenesis are potential mechanisms that explain the direct effect of Fe and Zn deficiencies on cognitive impairment[49,50].

Collectively, our data show that while micronutrient deficiencies and poor sleep are individual risk factors for impaired neurobehavioural performance, low serum Fe and Zn may be novel risk factors for habitually poor sleep, which in turn predicts poor non-verbal reasoning ability. Several potential limitations should be considered in the interpretation of results. First, habitual daytime napping and weekend compensatory sleep may obscure brain responses to sleep quality. Because of missing data and insufficient statistical power, napping behaviours and weekend catch-up sleep were not adjusted for in data analyses. Additionally, self-reported sleep measures may pose a potential risk for recall bias. Thus, future research should employ an objective sleep measurement (e.g. actigraphy) and consider daytime naps and day-to-day variability in sleep patterns. Second, moderating and mediating effects may not be mutually exclusive. Due to the lack of statistical power,

https://doi.org/10.1017/S1368980021002329 Published online by Cambridge University Press

the regression models were not stratified by sleep groups to further examine the moderating effect of sleep quality. Future research is warranted to test more complex models, such as moderator mediation and mediator moderation. Third, the prevalence of low Zn in our sample may not represent the micronutrient status in Chinese children and adolescents[1]. Since prior epidemiological findings primarily relied on dietary Zn intake, the discrepancy may result from different indicators for micronutrient deficiencies at the population level. Due to the lack of dietary assessment, particularly Zn and Fe intake, we did not conduct sensitivity analysis using dietary Zn or Fe levels as independent variables or covary out dietary intake in the test of the associations of serum Zn and Fe with sleep and neurobehavioural function. Nevertheless, the associations between low Zn/Fe and neurobehavioural domains achieved statistical significance in this small sample, indicating a substantial difference in sleep quality and neurobehavioural function between adolescents with and without micronutrient deficiencies. Fourth, although participants were from a healthy school population, we did not assess their health status, especially emotional and behavioural health, which may potentially confound the results. Finally, the causal relationship among micronutrients, sleep and neurobehavioural function is not conclusive due to the observational design. Future experimental studies are needed to illustrate the nature of these relationships.

Conclusion

In summary, low serum Fe and Zn were not highly prevalent in our sample. Adolescents with low serum Fe and low serum Zn were more likely to have poor sleep quality and worse performance on selective neurobehavioural domains, including non-verbal reasoning, attention and spatial processing. Poor sleep quality partially mediates the association between low Zn/Fe and fast but error-prone performance on non-verbal reasoning. Our findings provide preliminary evidence to suggest that optimal sleep promotes brain health, and this process may be enhanced by adequate micronutrient status. The elucidation of the role of micronutrient deficiencies has significant public health implications for adolescents during developmental transitions. Community and public health providers may consider including micronutrient deficiencies in risk assessment, risk management and health promotion for poor sleep health and neurobehavioural dysfunction in adolescents. Further work in this area is needed to determine whether dietary micronutrient intake and biological micronutrient levels are predictive of objectively assessed sleep metrics in a diverse sample across countries and whether improving micronutrient status promotes sleep health and subsequent neurobehavioural function.

Acknowledgements

Acknowledgements: We would like to thank Dr. Ruben C. Gur and his research team for authorising and providing consultation on the usage of Penn's Computerized Neurocognitive Battery. We also extend our thanks to the participating children and their families from Jintan City and to the Jintan cohort study group. Finally, we want to thank Mr. Jesse L. Chittams for statistic consultation. *Financial support:* This study was supported by the U.S. National Institutes of Health (NIH), National Institute of Environment Health Sciences (NIEHS, R01-ES018858, K02-ES019878, K01-ES015877 and P30-ES013508) and by the Jintan City government as well as the Jintan Hospital, China. *Authorship:* X.J. conceptualised the research question, performed data analysis and interpretation, wrote the initial draft and critically revised the manuscript. J.L. conceptualised the research question, mentored the research process and critically revised the manuscript. All other authors contributed to the conceptualisation of the research question and critically revised the manuscript. *Conflicts of interest:* There are no conflicts of interest. *Ethics of human subject participation:* This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving research study participants were approved by the University of Pennsylvania and the ethical committee for research at Jintan Hospital in China. Written informed consent was obtained from all adolescents and their parents.

Supplementary material

For supplementary material accompanying this paper visit https://doi.org/10.1017/S1368980021002329

References

1. Wong AY, Chan EW, Chui CS *et al.* (2014) The phenomenon of micronutrient deficiency among children in China: a systematic review of the literature. *Public Health Nutr* **17**, 2605–2618.
2. Liu X, Piao J, Zhang Y *et al.* (2017) Assessment of zinc status in school-age children from rural areas in China Nutrition and Health Survey 2002 and 2012. *Biol Trace Elem Res* **178**, 194–200.
3. Bhatnagar S & Taneja S (2001) Zinc and cognitive development. *Br J Nutr* **85**, S139–S145.
4. Koshy B, Srinivasan M, Zachariah SM *et al.* (2020) Body iron and lead status in early childhood and its effects on development and cognition: a longitudinal study from urban Vellore. *Public Health Nutr* 1–11.
5. Ji X, Cui N & Liu J (2017) Neurocognitive function is associated with serum iron status in early adolescents. *Biol Res Nurs* **19**, 269–277.
6. More S, Shivkumar V, Gangane N *et al.* (2013) Effects of iron deficiency on cognitive function in school going adolescent females in rural area of central India. *Anemia* **2013**, 819136.

7. Falkingham M, Abdelhamid A, Curtis P *et al.* (2010) The effects of oral iron supplementation on cognition in older children and adults: a systematic review and meta-analysis. *Nutr J* **9**, 1e16.
8. Gogia S & Sachdev HS (2012) Zinc supplementation for mental and motor development in children. *Cochrane Libr* **12**, CD007991.
9. Sandstead HH (2012) Subclinical zinc deficiency impairs human brain function. *J Trace Elem Med Biol* **26**, 70–73.
10. Warthon-Medina M, Moran V, Stammers A *et al.* (2015) Zinc intake, status and indices of cognitive function in adults and children: a systematic review and meta-analysis. *Eur J Clin Nutr* **69**, 649.
11. Liang J, Matheson B, Kaye W *et al.* (2014) Neurocognitive correlates of obesity and obesity-related behaviors in children and adolescents. *Int J Obes* **38**, 494–506.
12. Slattery MJ, Grieve AJ, Ames ME *et al.* (2013) Neurocognitive function and state cognitive stress appraisal predict cortisol reactivity to an acute psychosocial stressor in adolescents. *Psychoneuroendocrinology* **38**, 1318–1327.
13. Owens EB & Hinshaw SP (2016) Pathways from neurocognitive vulnerability to co-occurring internalizing and externalizing problems among women with and without attention-deficit/hyperactivity disorder followed prospectively for 16 years. *Dev Psychopathol* **28**, 1013–1031.
14. Gradisar M, Gardner G & Dohnt H (2011) Recent worldwide sleep patterns and problems during adolescence: a review and meta-analysis of age, region, and sleep. *Sleep Med* **12**, 110–118.
15. Lovato N & Gradisar M (2014) A meta-analysis and model of the relationship between sleep and depression in adolescents: recommendations for future research and clinical practice. *Sleep Med Rev* **18**, 521–529.
16. de Bruin EJ, van Run C, Staaks J *et al.* (2017) Effects of sleep manipulation on cognitive functioning of adolescents: a systematic review. *Sleep Med Rev* **32**, 45–57.
17. Matthews KA & Pantesco EJ (2016) Sleep characteristics and cardiovascular risk in children and adolescents: an enumerative review. *Sleep Med* **18**, 36–49.
18. Ji X, Grandner MA & Liu J (2016) The relationship between micronutrient status and sleep patterns: a systematic review. *Public Health Nutr* **20**(4), 687–701.
19. Takeda A, Minami A, Seki Y *et al.* (2004) Differential effects of zinc on glutamatergic and GABAergic neurotransmitter systems in the hippocampus. *J Neurosci Res* **75**, 225–229.
20. Beard JL, Erikson KM & Jones BC (2002) Neurobehavioral analysis of developmental iron deficiency in rats. *Behav Brain Res* **134**, 517–524.
21. Kordas K, Siegel EH, Olney DK *et al.* (2008) Maternal reports of sleep in 6–18 month-old infants from Nepal and Zanzibar: association with iron deficiency anemia and stunting. *Early Hum Dev* **84**, 389–398.
22. Kordas K, Siegel EH, Olney DK *et al.* (2009) The effects of iron and/or zinc supplementation on maternal reports of sleep in infants from Nepal and Zanzibar. *J Dev Behav Pediatr: JDBP* **30**, 131.
23. Grandner MA, Jackson N, Gerstner JR *et al.* (2013) Dietary nutrients associated with short and long sleep duration. Data from a nationally representative sample. *Appetite* **64**, 71–80.
24. Ji X & Liu J (2015) Associations between blood zinc concentrations and sleep quality in childhood: a cohort study. *Nutrients* **7**, 5684–5696.
25. Liu J, McCauley LA, Zhao Y *et al.* (2010) Cohort profile: the China Jintan child cohort study. *Int J Epidemiol* **39**, 668–674.
26. Liu J, Cao S, Chen Z *et al.* (2015) Cohort profile update: the China Jintan child cohort study. *Int J Epidemiol* **44**(5), 1548–1548l.
27. Liu J, Ai Y-X, Hanlon A *et al.* (2011) Micronutrients deficiency and associated sociodemographic factors in Chinese children. *World J Pediatr* **7**, 217.
28. International Zinc Nutrition Consultative Group (IZiNCG), Brown KH, Rivera JA *et al.* (2004) International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr Bull* **25**(1 Suppl. 2), S99–S203.
29. Chao HC, Chang YJ & Huang WL (2018) Cut-off serum zinc concentration affecting the appetite, growth, and nutrition status of undernourished children supplemented with zinc. *Nutr Clin Pract* **33**, 701–710.
30. Buysse DJ, Reynolds CF, Monk TH *et al.* (1989) The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatr Res* **28**, 193–213.
31. John B, Bellipady SS & Bhat SU (2016) Sleep promotion program for improving sleep behaviors in adolescents: a randomized controlled pilot study. *Scientifica* **2016**, 8013431.
32. Zhou HQ, Shi WB, Wang XF *et al.* (2012) An epidemiological study of sleep quality in adolescents in South China: a school-based study. *Child: Care Health Dev* **38**, 581–587.
33. Gur RC, Richard J, Calkins ME *et al.* (2012) Age group and sex differences in performance on a computerized neurocognitive battery in children age 8–21. *Neuropsychology* **26**, 251.
34. McHale SM, Kim JY, Kan M *et al.* (2011) Sleep in Mexican-American adolescents: social ecological and well-being correlates. *J Youth Adolesc* **40**, 666–679.
35. Taji W, Mandell B & Liu J (2019) China's urban-rural childhood cognitive divide: evidence from a longitudinal cohort study after a 6-year follow up. *Intelligence* **73**, 1–7.
36. Blössner M, Siyam A, Borghi E *et al.* (2009) *WHO AnthroPlus for Personal Computers Manual: Software for Assessing Growth of the World's Children and Adolescents*. Geneva: World Health Organization.
37. Kuula L, Pesonen A-K, Martikainen S *et al.* (2015) Poor sleep and neurocognitive function in early adolescence. *Sleep Med* **16**, 1207–1212.
38. Rabe-Hesketh S, Skrondal A & Pickles A (2004) Generalized multilevel structural equation modeling. *Psychometrika* **69**, 167–190.
39. Piernas C, Wang D, Du S *et al.* (2015) The double burden of under-and overnutrition and nutrient adequacy among Chinese preschool and school-aged children in 2009–2011. *Eur J Clin Nutr* **69**, 1323–1329.
40. Lam LF & Lawlis TR (2017) Feeding the brain – the effects of micronutrient interventions on cognitive performance among school-aged children: a systematic review of randomized controlled trials. *Clin Nutr* **36**, 1007–1014.
41. Rozencwajg P & Corroyer D (2005) Cognitive processes in the reflective-impulsive cognitive style. *J Genet Psychol* **166**, 451–463.
42. Chiplonkar SA & Kawade R (2014) Linkages of biomarkers of zinc with cognitive performance and taste acuity in adolescent girls. *Int J Food Sci Nutr* **65**, 399–403.
43. Benton D (2001) Micro-nutrient supplementation and the intelligence of children. *Neurosci Biobehav Rev* **25**, 297–309.
44. Carpenter KL, Li W, Wei H *et al.* (2016) Magnetic susceptibility of brain iron is associated with childhood spatial IQ. *Neuroimage* **132**, 167–174.
45. Peirano P, Algarín C, Garrido M *et al.* (2007) Iron-deficiency anemia is associated with altered characteristics of sleep spindles in NREM sleep in infancy. *Neurochemical Res* **32**, 1665.
46. Song C-H, Kim Y-H & Jung K-I (2012) Associations of zinc and copper levels in serum and hair with sleep duration in adult women. *Biol Trace Elem Res* **149**, 16–21.

https://doi.org/10.1017/S1368980021002329 Published online by Cambridge University Press

47. Carskadon MA, Acebo C & Jenni OG (2004) Regulation of adolescent sleep: implications for behavior. *Ann NY Acad Sci* **1021**, 276–291.
48. Gobin CM, Banks JB, Fins AI *et al.* (2015) Poor sleep quality is associated with a negative cognitive bias and decreased sustained attention. *J Sleep Res* **24**, 535–542.
49. Hurley LS & Swenert'on H (1966) Congenital malformations resulting from zinc deficiency in rats. *Proc Soc Exp Biol Med* **123**, 692–696.
50. Wu L-l, Zhang L, Shao J *et al.* (2008) Effect of perinatal iron deficiency on myelination and associated behaviors in rat pups. *Behav Brain Res* **188**, 263–270.

https://doi.org/10.1017/S1368980021002329 Published online by Cambridge University Press