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Sleep duration trajectories associated with levels of specific serum cytokines at age 5: A longitudinal study in preschoolers from the EDEN birth cohort

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ABSTRACT

Sleep is essential for optimal child development and health during the life course. However, sleep disturbances are common in early childhood and increase the risk of cognitive, metabolic and inflammatory disorders throughout life. Sleep and immunity are mutually linked, and cytokines secreted by immune cells could mediate this interaction. The sleep modulation of cytokines has been studied mostly in adults and adolescents; few studies have focused on school-aged children and none on preschoolers. We hypothesized that night sleep duration affects cytokine levels in preschoolers. In a sample of 687 children from the EDEN French birth cohort, we studied the associations between night sleep duration trajectories from age to 2–5 years old and serum concentrations of four cytokines (Tumor necrosis factor α [TNF-α], Interleukin 6 [IL-6], IL-10, Interferon γ [IFN)-γ] at age 5, adjusting for relevant covariates. As compared with the reference trajectory (≈11h30/night sleep, 37.4% of children), a shorter sleep duration trajectory (<10 h/night, 4.5% of children), and changing sleep duration trajectory (≥11h30/night then 10h30/night, 5.6% of children) were associated with higher serum levels of IL-6 and TNF-α, respectively at age 5. We found no associations between sleep duration trajectories and IL-10 or IFN-γ levels. This first longitudinal study among children aged 2–5 years old suggests an impact of sleep duration on immune activity in early childhood. Our study warrants replication studies in larger cohorts to further explore whether and how immune activity interacts with sleep trajectories to enhance susceptibility to adverse health conditions.

1. Introduction

Sleep is a vital function characterized by a recurring prolonged naturally quiescent state with an altered but reversible consciousness, reduced responsiveness to external stimuli, and a reduced motor activity (Keene and Duboue 2018). Optimal sleep is essential for a child's development because it contributes to physical and mental health as well as immune function (Chaput et al. 2016, 2017; Besedovsky et al., 2019; Cook et al., 2020). Sleep issues track from early childhood to adulthood (Al Mamun et al., 2012) and have been associated with poor behavior, worse school performance, and obesity in childhood/adolescence (Chaput et al. 2016, 2017). Finally, sleep problems are significant predictors of later adult health issues (Mamun et al., 2007; Landhuis et al., 2008; Wong et al., 2010; Bilgin et al., 2020).

Sleep and immunity reciprocally influence each other, both in physiological conditions and in the context of infectious diseases and inflammation (Besedovsky et al., 2019). Preclinical and clinical studies of the manipulation of sleep or the immune system in infectious or inflammatory contexts support that cytokines secreted by immune cells could mediate this interaction (Besedovsky et al., 2019). Also, the sleep-wake cycle regulates circadian variations in immune cell populations frequencies and cytokine secretion, whereas cytokines such as tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) are involved in the homeostatic regulation of sleep (Besedovsky et al., 2019).

Most of our understanding of sleep-immune interactions in humans arises from studies performed in adult populations. A meta-analysis based on 72 studies (70 studies of adults 20–90 years old and 2 studies of adolescents 13–18 years old) focused on the associations between sleep disturbances (both in naturalistic and experimental settings) and levels of inflammatory markers: C-reactive protein (CRP), IL-6, and TNF-α (Irwin et al., 2016). The authors concluded that shorter sleep duration was associated with increased levels of CRP, but not IL-6. Extremely long sleep duration was associated with increased levels of CRP and IL-6. This meta-analysis suggests that sleep disturbance and sleep duration are

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associated with increased levels of markers of systemic inflammation, at least in adolescents and adults.

Sleep patterns show important changes during childhood (Bathory and Tomopoulos 2017), and findings for the sleep-immune relationship in adults are not transferable to children. Few studies have focused on adolescents and school-aged children. In a large sample of adolescents (12.5–17.5 years old; N = 933), Perez de Heredia et al. showed higher IL-4 serum levels in adolescents sleeping 8–9 h per night than those sleeping shorter (≤8 h/night) and longer (≥9 h/night) hours. Moreover, adolescent girls with self-reported long sleep duration (≥9 h/night) had low serum levels of IL-5 and IL-6, whereas for adolescent boys (≤8 h/night), long sleep was correlated with increased IL-10 levels. No association was reported with CRP, IL-1, IL-2, TNF-α, or INF-γ level (Perez de Heredia et al., 2014). Three studies, albeit of much smaller study samples, reported no association between sleep duration and IL-6 levels in plasma or saliva from children and adolescents (13–17, 16.9 and 8–16 years old, respectively) (Martinez-Gomez et al., 2011; Fernandez-Mendoza et al., 2017; LaVoy et al., 2020), but long sleep duration was associated with increased IL-1β level (LaVoy et al., 2020). In 8- to 9-year--old children (N = 64), long sleep duration and better sleep quality were associated with increased salivary IL-6 level after moderate stress in boys, but not in girls (El-Sheikh et al., 2007). Finally, one longitudinal study of a cohort of 652 children showed that levels of IL-6 and CRP levels were increased, although not significantly, at age 8 years with sleep curtailment from 6 months to 8 years (Cespedes et al., 2014).

Altogether, these elements suggest that sleep duration affects cytokine secretion. Sleep troubles, including shortened sleep duration, affect up to 30% of children under the age of 3 (Al Mamun et al., 2012; Bruni et al., 2014; Messayke et al., 2020). This finding suggests that sleep alterations in children could lead to changes in cytokine secretion very early in life, possibly priming the later development of mental and physical health issues. To motivate preventive early sleep interventions, studying the associations between sleep duration and cytokines levels is warranted in children. However, no study has addressed the longitudinal associations between sleep duration and cytokine levels in preschoolers (age <6 years) sampled from the general population.

Here, we hypothesized that sleep duration trajectories between age 2 to 5 are associated with altered serum levels of cytokines at age 5. Therefore, we tested the associations between sleep duration trajectories and serum levels of four cytokines (IL-6, IL-10, INF-γ, TNF-α), adjusting for relevant covariates, In children from the EDEN birth cohort study.

2. Materials and methods

2.1. Study design

The EDEN (Etude sur les Déterminants pré-et postnataux du développement psychomoteur et de la santé de l'ENfant) mother–child cohort was set up to assess the effect of pre- and post-natal determinants on child development and health (Heude et al., 2016). Pregnant women were recruited before 24 weeks' gestation at the Obstetrics and Gynecology department of the French University Hospitals of Nancy and Poitiers between 2003 and 2006. Exclusion criteria included age <18 years, multiple pregnancies, a known history of diabetes, the inability to speak and read French or plans to move out of the study region in the following 3 years. A total of 2002 women were enrolled in the study and 1899 children were eligible for follow-up from birth. During pregnancy and after birth (4, 8, 12, 24 months, 2, 3 and 5 years), sociodemographic and biomedical data on the mother and child were gathered from medical records, face-to-face interviews with the mother, and mother's self-completed questionnaires.

Written informed consent was obtained twice from parents, once at enrollment and once after the child's birth. The study was approved by the ethics research committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale) of the Bicêtre Hospital and the Data Protection Authority (Commission Nationale de l'Informatique et des Libertés). All research was performed in accordance with relevant guidelines and regulations.

2.2. Measurement of serum cytokine levels

At age 5-years, a venous blood sample was obtained from 786 children and allowed to clot for 1h at room temperature before centrifugation (1500g, 10 min). Serum samples were stored at −80 °C. Serum samples were thawed on ice only once, and assayed by using kits and reagents from two batches. All assays for determining serum concentration of cytokines were completed within a 4-week period by the same investigator, blind to the sample class. All assays were performed according to the manufacturer's instructions. Concentrations of IL-6, IL-10, TNF-α and IFN-γ were measured by using the Proinflammatory Panel 1 v-plex multiplex immunoassay (Meso Scale Diagnostics, Rockville, MD, USA). On each of the 96-well plates, seven serial dilutions of standards and buffer only (in duplicates) were run together with 80 samples (in singlicate) on the Sector Imager 2400 plate reader (Meso Scale Diagnostics, Rockville, MD, USA). Concentrations of cytokines in each sample were interpolated from standard curves generated with a five-parameter logistic regression equation in Discovery Workbench 3.0 software (Meso Scale Diagnostics, Rockville, MD, USA). Intra-run and inter-runs coefficients of variations, based on the concentrations obtained for each of the 7 serially diluted standards across the plates used to analyze the samples, were <15% (Supplementary Table 1). The lower limits of detection (LLOD) indicated by the manufacturer for IL-6, IL-10, TNF-α and IFN-γ were 0.06, 0.03, 0.04 and 0.2 pg/ml, respectively. For sample with cytokine levels < LLOD, we imputed a value equal to half the LLOD value, as recommended. This corresponds to N = 1 for IL-10, TNF-α and IFN-γ, and N = 7 for IL-6 in our study sample (N = 687).

2.3. Sleep trajectories

Night-sleep duration was collected at age 2, 3 and 5.5 years by using parental self-administered questionnaires and was calculated on the basis of the answers to the following ad-hoc questions: "Usually, at what time does your child go to bed?" and "Usually, at what time does your child wake up?" Responses were recorded in hours and minutes (e.g., 20h30 and 6h45 corresponding to a sleep duration of 10h15). To identify night-sleep duration trajectories among the 1205 children whose parents had answered the questions regarding night-sleep durations for at least two of the three age points, we used the data-driven "Group-based trajectory modeling" developed by Nagin et al. (Nagin 2005), implemented under SAS 9.4 (PROC TRAJ). The method is based on the underlying hypothesis that, there are inherent groups within a population that evolve according to different sleep patterns. The groups are not directly identifiable or pre-established by sets of characteristics but rather are statistically determined by each series of responses by using maximum likelihood.

Five night-sleep duration trajectories were previously established for 1205 children of the EDEN cohort (Plancoulaine et al., 2018); the naming of the trajectories was based on the global level of both the sleep duration and the trajectory direction (flat, up, down). (Fig. 1): Short Sleep (SS, <10h30/night, 4.9% of sample), Medium-Low Sleep (MLS, 10h30-11h00/night, 47.8%), Medium-High Sleep (MHS, about 11h30/night, 37.2%), Long Sleep (LS, ≥11h30/night, 4.5%) and Changing Sleep (CS, i.e., LS then MLS, 5.6%). Each child was assigned to the trajectory to which they belonged with the highest probability. Day-sleep durations did not differ according to trajectories at both age 2 and 3.5 years (p = 0.84 and p = 0.35, respectively). Children with both an assigned sleep trajectory and serum cytokine measures were included; our study sample therefore consisted of N = 687 children.

2.4. Covariates

Covariates were selected based on literature review and previous studies in the EDEN cohort (O'Connor et al., 2009; Plancoulaine et al.,

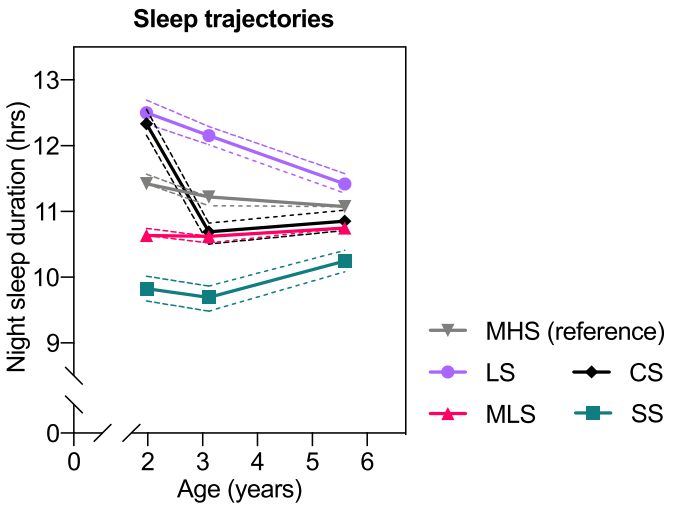

Fig. 1. Night sleep trajectories for children from age 2 to 5-years in the EDEN population.
SS: Short Sleep, MLS: Medium-Low Sleep, MHS: Medium-High Sleep, LS: Long Sleep, and CS: Changing Sleep. Dashed lines: 95% mean confidence interval.

2018; Barbosa et al., 2020) and by using a Directed Acyclic Graph (http://dagitty.net). They concerned maternal, perinatal, and child's characteristics that could affect serum cytokine levels and/or child's sleep. Maternal characteristics, collected at inclusion, were: age at birth (years), pre-pregnancy body mass index (BMI, $kg.m^{-2}$), smoking throughout pregnancy (never; <10 cigarettes/day; ≥10 cigarettes/day), education duration (years), monthly family income (<1500 €; 1500–3000 €; >3000 €), and recruitment center (Nancy/Poitiers). Child variables were: sex, gestational age at birth (weeks), breastfeeding duration (months) and variables collected at age 5: exact age at time of sampling, BMI, medical diagnosis of allergy (asthma and/or eczema/-dermatitis and/or food allergy since birth: yes/no), use of antibiotics since age 3 years (yes/no), and number of children in the household. We also included the child's physical activity and dietary habits at age 5. Child's physical activity (walking, playing outside) in h/day significantly depended on the season of questionnaire completion, and therefore physical activity data were split into quartiles for each season. Specific child dietary patterns were previously identified by principal component analyses of data from a food frequency questionnaire adapted for children (Lioret et al., 2015). The first pattern was named "protein-rich and diversified", which was positively correlated with eating foods of animal origin (meat, processed meat, ham, fish and eggs), starchy foods (rice, potatoes, bread and legumes), vegetables (cooked and raw), pizza and fresh fruit; and the second pattern was named "processed and fast foods", which was positively correlated with eating cookies, chips, soft drinks, chocolate, dairy desserts, ice cream and processed meat and was inversely correlated with eating fresh fruits and cooked and raw vegetables. We also included the time of blood sampling, which could affect cytokine levels.

2.5. Statistical analyses

All analyses were performed with SAS 9.4. Comparison of the included versus excluded population was performed by Chi-square test for categorical variables, Student *t*-test for continuous variables with normal distribution and Mann-Whitney *U* test for continuous variables with non-normal distribution. Cytokine serum levels were log-transformed and standardized.

Multiple imputation (MI) was performed for covariate missing data (1.7%) by using the fully conditional specification method (SAS 9.4: MI procedure, FCS statement, NIMPUTE option). We assumed that data were missing at random and generated 20 imputed datasets. Pooled effect estimates were calculated (SAS 9.4: MIANALYSE procedure) based on Rubin's rules (Rubin 1987). This method assigned data to missing measurements based on the measurements in children with similar profiles. In imputation models, we included all variables of interest after ranking them in ascending order of missing data. Categorical variables were imputed with a multinomial model, ordinal or binary variables with logistic regression, and continuous variables with linear regression.

Associations between sleep duration trajectories and cytokine levels at age 5 were modeled by multivariable linear regression separately for each cytokine. The MHS trajectory was used as the reference category because this is the trajectory closest to the recommended sleep duration between age 2 and 5 (Paruthi et al., 2016). Model 1 presents the raw associations (unadjusted). In model 2, we adjusted for maternal variables (age at delivery, pre-pregnancy BMI, smoking throughout pregnancy, education duration, monthly family income, and recruitment center) and child variables (sex, gestational age at birth, breastfeeding duration, exact age at time sampling, and time of blood sampling). In a model 3, we additionally adjusted for data collected at age 5: BMI, medical diagnosis of allergy, use of antibiotics since the age of 3 years, number of children in the household, physical activity duration, dietary habits. Statistical significance was set at a $p < 0.05$.

3. Results

Study sample characteristics are presented in Table 1. Mothers of children included in this study (N = 687) were older, had more years of education, higher family income, and smoked less during pregnancy than mothers of children included at birth (N = 1899). In addition, included children were more frequently boys.

The distribution of sleep trajectories of the included children was similar to that in the total sample reported previously (cf 2.1., Fig. 1 (Plancoulaine et al., 2018),): short sleep (SS, <10h30/night, 4.5%, medium-low sleep (MLS, 10h30-11h00/night, 48.5%), medium-high sleep (MHS, about 11h30/night, 37.4%), long sleep (LS, ≥11h30/night, 4.9%) and changing sleep (CS, i.e., LS then MLS, 4.7%).

Serum cytokine levels for the study sample are presented in Table 2.

Raw and adjusted associations between 2- to 5 years sleep duration trajectories and serum cytokines levels at age 5 are presented in Table 3. Positive associations were observed between selective sleep duration trajectories and levels of specific cytokines. As compared with MHS, SS (<10h30/night) was associated with increased IL-6 level and CS (≥11h30 then 10h30-11h00/night) with increased TNF-α level. Adjustment on all covariates had limited impact on effect sizes and nominative p-values. We found no association between sleep duration trajectories and both IL-10 or IFN-γ level. Further adjustment on data collected at age 5 i.e., BMI, physical activity duration, score for the "diversified food" and "processed food" patterns, antibiotic use since age of 3 years, medical diagnosis of allergy since birth, number of children in the household did not change the results (Supplementary Table 2).

4. Discussion

Here we show that the shorter sleep duration and changing sleep duration trajectories were associated with increased levels of IL-6 and TNF-α, respectively, but none of the sleep duration trajectories was associated with IL-10 and IFN-γ levels. This observation suggests that sleep patterns can selectively impact the serum levels of specific cytokines as early as in the preschool age, possibly priming health disorders later in life.

4.1. Comparison with other studies

Comparison of our results with the literature seems challenging given that associations between cytokines levels and sleep duration are influenced by: 1) the biological matrix used (Cullen et al., 2015; Gong et al., 2019), 2) the immunoassay used (Belzeaux et al., 2017), 3) the study

Table 1
Mother and child characteristics for children included at birth and children included in this study.

	Children included at birth (N = 1899)		Children included in this study (N = 687)	
	% (N) or Mean (±SD) or median (range)	Missing (N)	% (N) or Mean (±SD) or median (range)	Missing (N)
Maternal and socioeconomic characteristics				
Pre-pregnancy BMI ($kg.m^{-2}$)	23.2 (±4.6)	39	23.5 (±4.5)	11
Smoking during pregnancy (n cigarettes/day)		52		18
Never	73.8% (1363)		78.0% (522)	
<10	21.2% (391)		18.5% (124)	
≥10	5.0% (256)		3.5% (23)	
Age at delivery (years)	29.5 (±4.9)	0	30.2 (±4.7)	0
Education (years)	13.6 (±2.7)	15	14.8 (±2.5)	0
Monthly income (€)		12		2
<1500	16.7% (315)		11.7% (80)	
1500-3000	56.1% (1059)		64.1% (439)	
>3000	27.2% (513)		24.2% (166)	
Number of children in household at age 5		0		34
1			11.8% (77)	
2			54.4% (355)	
≥3			33.8% (221)	
Child characteristics at birth and at age 5y				
Sex (males)	52.6% (998)	0	56.3% (387)	0
Gestational age (weeks)	39.2 (±1.7)	0	39.3 (±1.6)	0
Breastfeeding duration (months)			3.1 (±3.7)	2
Age (years)			5.6 (±0.1)	0
Medical diagnosis of allergy (yes)			33.6% (230)	2
Antibiotic use since age 3 (yes)			86.2% (575)	20
Physical activity in quartiles at age 5				91
Q1			23.0% (137)	
Q2			24.8% (148)	
Q3			26.3% (157)	
Q4			25.9% (154)	
BMI at age 5 ($kg.m^{-2}$)			15.4 (±1.3)	1
Processed food pattern PCA loading			0.04 (−2,8; 7,36)	6
Protein rich and diversified food pattern PCA loading			−0,11 (−2,20; 7,43)	6

BMI, body mass index; PCA, principal component analysis.

Table 2
Serum cytokine levels (pg/mL) in the study sample (N = 687). Mean and standard deviation (SD), median, minimum (Min.), maximum (Max.), and 5th/95th values are indicated.

	Mean (SD)	Median	Min.	Max.	5th percentile	95th percentile
IL-6	0.70 (0.99)	0.42	0.03	16.11	0.11	2.32
TNF-α	3.12 (1.10)	2.96	0.34	15.80	1.99	4.88
IL-10	0.72 (2.06)	0.43	0.02	50.85	0.18	1.89
IFN-γ	15.64 (34.64)	6.78	0.10	471.72	3.10	56.80

design (longitudinal vs cross-sectional), 4) the age group (preschoolers vs school-aged, adolescent or adults), and 5) the confounders accounted for. Only two studies were of school-aged children but used distinct matrices: saliva (El-Sheikh et al., 2007) and plasma (Cespedes et al., 2014), which limits possible comparisons. To the best of our knowledge, this is the first longitudinal study looking at the associations between sleep duration and immune markers in preschoolers.

Inflammation is characterized by the joint secretion of pro-inflammatory cytokines (e.g., TNF-α, IL-6) and the anti-inflammatory cytokine IL-10 (Rose-John 2020). Therefore, the discrete associations (IL-6 with short-sleep duration trajectory, TNF-α with changing sleep duration trajectory compared to medium-high sleep duration trajectory) we report here do not enable interpretation in terms of specific sleep trajectories associated with more or less systemic inflammation but rather suggest that sleep patterns during childhood selectively impact each of these cytokines.

IL-6 is mainly secreted by activated macrophages in response to infection and a is an important mediator of fever and of the acute phase response (Rose-John 2020). IL-6 also plays a role in the resolution of inflammatory processes due to its anti-inflammatory properties, as it represses the pro-inflammatory cytokines TNF-α and IL-1β, and promotes expression of the anti-inflammatory cytokine IL-10 (Rose-John 2020). IL-6 is also secreted by non-immune cells, and notably myocytes during exercise, adipocytes and osteoblasts (Rose-John 2020). IL-6 is additionally expressed both by neurons and glia and modulates neurodevelopment and brain function (Rose-John 2020). Furthermore, IL-6 is involved in the homeostatic regulation of sleep (Krueger and Majde 1995; Opp 2005). We showed increased in IL-6 serum level in children exhibiting a trajectory of persistent short sleep between age 2 and 5 years (<10h30/night), which suggests that sleep duration is negatively associated with IL-6 level in children. Previous studies of pediatric/adolescent populations did not report such association. However, these studies were generally cross-sectional and involved smaller samples (Martinez-Gomez et al., 2011; Fernandez-Mendoza et al., 2017). In one longitudinal study of 652 children, IL-6 plasma level was increased, although not significantly, at age 7 in children with the highest sleep curtailment (Cespedes et al., 2014), which supports our findings. Our data are also in agreement with: 1) a recent meta-analysis highlighting a negative cross-sectional association between sleep duration and IL-6 level in adults (Irwin et al., 2016) and 2) experimental studies showing that chronic sleep restriction increases IL-6 serum level (Moldofsky et al., 1989; Meier-Ewert et al., 2004; Vgontzas et al., 2004; van Leeuwen et al., 2009; Chennaoui et al., 2011). Altogether, these data suggest that short sleep durations are linked to elevated IL-6 level. In adults, common chronic disorders (e.g., diabetes, atherosclerosis, cancer, rheumatoid arthritis, depression) are associated with elevated circulating levels of IL-6 (Barton 2005; Nishimoto 2006; Dowlati et al., 2010; Chung and Choi 2018; Rose-John 2020). This suggests that elevated IL-6 levels could prime adverse somatic and/or psychiatric health outcomes later in life in children with short sleep trajectories.

Like IL-6, TNF-α is classified as a pro-inflammatory cytokine. Notably, activated macrophages secrete massively TNF-α to alert other immune cells and orchestrate the immune response to pathogens (Besedovsky et al., 2019). Under physiological conditions, TNF-α is also constitutively secreted in minute amounts by non-immune cell types both in the periphery (myocytes, adipocytes), and in the brain (neurons, glia) (Probert 2015; Chung and Choi 2018). In these conditions, TNF-α is required for organ maintenance and homeostasis (Probert 2015; Chung and Choi 2018). In the current study, we showed increased TNF-α serum level at age 5 in children with a changing sleep trajectory (i.e., ≥11h30/night at age 2 and 10h30-11 h/night from age of 3–5 years). Most previous studies of children or adolescent population did not report associations between sleep curtailment or duration and TNF-α level (Cespedes et al., 2014; Perez de Heredia et al., 2014; Fernandez-Mendoza et al., 2017). Results of a meta-analysis of adult studies showed no association between sleep duration and TNF-α level (Irwin et al., 2016). However, one experimental study of adults showed higher plasma TNF-α levels in a group of chronically sleep-deprived adults with circadian misalignment than in the group without circadian misalignment (Wright et al., 2015). Thus, a changed sleep duration between age 2 and 5 may reflect a

Table 3
Associations between sleep trajectories between age 2 to 5 years and cytokine serum levels at age 5. SS: short sleep (<10h30/night), MLS: medium-low sleep (10h30-11h00/night), MHS: medium-high sleep (about 11h30/night, reference (Ref.) trajectory), CS: changing sleep (LS then MLS) and LS: long sleep (≥11h30/night). MHS trajectory was used as the reference. SD: standard deviation.

	IL-6		TNF-α		IL-10		IFN-γ	
	β (SD)	**p-val.**	**β (SD)**	**p-val.**	**β (SD)**	**p-val.**	**β (SD)**	**p-val.**
Model 1[a]								
SS	**0.40 (0.19)**	**0.03**	−0.18 (0.19)	0.35	−0.16 (0.19)	0.41	−0.17 (0.19)	0.37
MLS	−0.04 (0.08)	0.62	−0.05 (0.21)	0.56	−0.09 (0.08)	0.25	−0.09 (0.08)	0.27
MHS	Ref.		Ref.		Ref.		Ref.	
CS	0.06 (0.18)	0.74	**0.45 (0.18)**	**0.01**	0.17 (0.18)	0.34	0.27 (0.18)	0.13
LS	0.05 (0.18)	0.81	0.09 (0.19)	0.61	0.35 (0.19)	0.06	0.17 (0.19)	0.35
Model 2[b]								
SS	**0.39 (0.19)**	**0.03**	−0.26 (0.19)	0.17	−0.17 (0.19)	0.36	−0.16 (0.19)	0.39
MLS	−0.02 (0.08)	0.82	−0.09 (0.08)	0.25	−0.10 (0.08)	0.22	−0.08 (0.08)	0.32
MHS	Ref.		Ref.		Ref.		Ref.	
CS	0.06 (0.18)	0.74	**0.45 (0.18)**	**0.01**	0.21 (0.18)	0.25	0.26 (0.18)	0.15
LS	0.05 (0.19)	0.80	0.13 (0.18)	0.49	0.32 (0.19)	0.08	0.18 (0.19)	0.33

[a] Model 1: Raw model.
[b] Model 2: Model adjusted for maternal recruitment center, income and education, smoking during pregnancy, maternal age at delivery, maternal pre-pregnancy body mass index (BMI), breastfeeding duration, child sex, age, gestational age, and time of blood sampling.

circadian misalignment for these children. In adults, elevated TNF-α levels are associated with a variety of conditions, including obesity, type 2 diabetes, multiple sclerosis, and depression (Chung and Choi 2018; Pegoretti et al., 2018). This suggests a possible early priming to adverse health outcomes later in life in children with changing sleep trajectories presenting elevated TNF-α levels.

We did not observe an association between sleep duration trajectories and IFN-γ or IL-10 level. The lack of association with IFN-γ is in line with a cross-sectional study in adolescents which showed no association between sleep duration and IFN-γ levels (Perez de Heredia et al., 2014). IFN-γ is a pro-inflammatory cytokine involved in antiviral responses and known to modulate sleep in this context, possibly explaining the lack of association in the general population (Besedovsky et al., 2019). The anti-inflammatory cytokine IL-10 is known to antagonize the effects of the chief pro-inflammatory cytokines IL-6 and TNF-α by downregulating their signaling and contributing to resolve inflammation (Besedovsky et al., 2019). Long sleep was previously found correlated with increased IL-10 level, but only in adolescent boys (Perez de Heredia et al., 2014). In contrast, one experimental study of chronically sleep-deprived adults showed higher plasma IL-10 level in a group with circadian misalignment than in the group without circadian misalignment (Wright et al., 2015), which suggests that circadian misalignment rather than sleep duration might modulate IL-10 secretion. Further studies are required to determine the impact of both sleep duration and circadian misalignment on the secretion of this anti-inflammatory cytokine.

4.2. Possible underlying mechanisms

The sleep-wake cycle and immunity are linked by the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis (Irwin 2019). Via cortisol, the HPA axis downregulates the expression of pro-inflammatory immune response genes *IL1B*, *IL6*, and *TNF* (Irwin and Cole 2011). Conversely, via noradrenaline and adrenaline, the SNS skews the basal gene expression profile toward a proinflammatory profile (Irwin and Cole 2011). Sleep disturbance can be linked to alterations in both systems and can therefore perturb the circadian regulation of immune cell abundance in the blood and therefore impact circulating cytokine levels (Besedovsky et al., 2019). In adults, long sleep duration was repeatedly found to decrease the blood cell counts of monocytes and lymphocytes, which are important producers of IL-6 and TNF-α (Besedovsky et al., 2019). In adolescents, short sleep duration was associated with increased leukocyte, neutrophil, monocyte, $CD4^+$, and $CD4^+CD45RO^+$ T lymphocytes counts (Perez de Heredia et al., 2014). These findings may explain why IL-6 and TNF-α levels are elevated in children with a short sleep duration trajectory and a sleep duration trajectory changing from long to short, respectively.

4.3. Study strengths and limitations

The strengths of the current study include the novelty of the finding that longitudinally assessed sleep trajectories are associated with altered levels of IL-6 and TNF-α in preschoolers. It also has methodological strengths: the measurement of serum cytokines in a population-based sample of preschoolers, the use of sleep duration trajectory modeling to reflect the long-term effects of sleep, and the robustness of the results in sensitivity analyses. However, our study has some limitations. First, although sleep data were collected prospectively, limiting memory bias, sleep duration might be overestimated because it was assessed by self-administered parental questionnaires and reflects more the time spent in bed than the effective sleep duration. Nevertheless, self-administered sleep questionnaires are still valid and widely used in large epidemiological studies (Galland et al., 2012; Hublin et al., 2020; Tham et al., 2021). Second, we focused on night sleep duration trajectories as did others (Touchette et al., 2007; Zheng et al., 2020), but day sleep duration still varies in this age range. However, mean day sleep duration did not differ according to trajectories at each age. Third, the current study relies on serum samples collected at the age of 5 years and is therefore based on cytokines measured at a single time point and only in serum. Therefore, even though the time of blood sampling accounted for the circadian variation of cytokine secretion, we could not investigate the stability of our results overtime and over other blood-derived matrices such as plasma. In addition, we cannot exclude a reverse causation bias (i.e., that our results reflect a pre-existent elevation of IL-6 or TNF-α in children with distinct sleep trajectories before age 2 years). Fourth, the study sample size was limited, which implies possible power issues to detect associations. Fifth, although data from our study sample were within the national estimates for most socioeconomic factors (Heude et al., 2016; Blondel et al., 2017), the included children in the analysis, relative to the original sample, were more frequently from mothers with higher socioeconomic status and less exposed to smoking during pregnancy. Consequently, included children might have healthier lifestyle habits in a healthier environment and be then less exposed to factors associated with changes in immune marker levels than children from the original sample, suggesting that the observed associations might be underestimated. Also, our socially advantaged population sample may preclude generalization of our results. To ascertain our results, further studies in independent mother-child cohort are warranted.

4.4. General conclusions

In this longitudinal study, we found associations between sleep duration trajectories and immune markers (IL-6 and TNF-α) as early as the preschool age. Future longitudinal studies in larger cohorts will allow for exploring the associations between sleep patterns and cytokine levels in children and possibly to evaluate the mediating effect of cytokines between sleep duration and subsequent disease development. In this context, sleep could be a valid target for preventive interventions.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2022.100429.

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