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Sleep and the gut microbiota in preschool-aged children

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Abstract

Sleep plays a significant role in the mental and physical development of children. Emerging evidence in animals and human adults indicates a relationship between sleep and the gut microbiota; however, it is unclear whether the sleep of preschoolers during a key developmental period, associates with features of their gut microbiota. The objective of this study was to assess the relationship between sleep and gut microbiota in preschool-aged children (4.37 ± 0.48 years, $n = 143$). Sleep measures included total night-time sleep (TST), sleep efficiency (SE), and wake-time after sleep onset (WASO) assessed using actigraphy. Beta-diversity differences between children with low and high TST ($p = .048$) suggest gut microbiota community differences. Particularly, relative abundance of *Bifidobacterium* was higher in the high TST group and *Bacteroides,* was higher in children who had greater SE and less WASO (LDA score >2). In contrast, some *Lachnospiraceae* members including *Blautia* and *Coprococcus* 1 were associated with shorter night-time sleep duration and less efficiency, respectively. We also found a group of fecal metabolites, including specific neuroactive compounds and immunomodulating metabolites were associated with greater sleep efficiency and less time awake at night. Notably, tryptophan and its metabolizing products were higher in children who had higher SE or lower WASO (LDA score >2); concentration of propionate was higher in children with less WASO ($p = .036$). Overall, our results reveal a novel association between sleep and gut microbiota in preschool-aged children. Longer night-time sleep and greater sleep efficiency were associated with specific commensal bacteria that may regulate sleep through modulating neurotransmitter metabolism and the immune system.

Statement of Significance

Our study assessed the association between sleep and gut microbiota in preschool-aged children, a developmental period when sleep problems are common. An association between sleep and specific gut microbial taxa including *Bifidobacterium* and *Bacteroides* was observed in this preschool cohort; the findings suggest that sleep and gut microbiota crosstalk in preschoolers may be mediated by microbial metabolites such as tryptophan and its derivatives and propionate. Our results warrant future studies to explore the potential of gut microbiota alteration as a strategy to improve sleep in young children.

Key words: actigraphy; Alberta Pregnancy Outcomes and Nutrition (APrON) study; gut microbiota; preschoolers; short-chain fatty acids; sleep; tryptophan

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Introduction

Sleep is essential for child growth and development [1]. Unfortunately, sleep problems, short sleep duration, and night waking are common in children [2–5]. Among children under 5 years of age, sleep problems have been reported by a quarter of parents [1]. Persistent child sleep problems and short sleep duration are associated with important outcomes, including impaired cognitive development, risk of obesity, poor mental health, poor academic performance, and poor social adjustment [6–9].

Recent evidence suggests that the composition and function of the gut microbiota contribute to regulation of sleep [10, 11]. Gut microbiota plays important roles in modulating fundamental processes, such as metabolism and immune system function [12–15], with downstream effects on circadian patterns and sleep. In addition, extensive bi-directional communication between the gut and brain suggests a role for the gut microbiota on sleep regulation [16, 17]. Specifically, the gut bacteria produce a wide range of neurochemicals that can directly and indirectly stimulate the nervous system [18], and potentially affect sleep. For example, serotonin is one of the key neurotransmitters that controls circadian rhythm, sleep, and waking [19, 20], and it is known that the gut microbiota plays an important role in serotonin biosynthesis and regulation [21].

The interrelationship between gut microbiota, microbial metabolites, and sleep has been observed in both animal and human studies. A recent murine study reported that mice whose gut bacteria have been depleted showed significant sleep disturbances (spending less time in non-REM sleep during the light phase and spending more time in non-REM and REM sleep during the dark phase), and they have reduced gut serotonin concentrations [22]. In human adults (males), sleep efficiency and total sleep time were positively correlated with the diversity of the gut microbiota (the complexity or variety of bacterial taxa present in the gut) [23]. The importance of gut-brain connections for sleep is underscored by evidence that perturbing sleep in rodents and human adults also perturbs the composition of the gut microbiota [24, 25].

Despite a small body of evidence supporting a connection between sleep duration and efficiency and gut microbiota in human adults, no study has determined whether sleep is associated with gut microbiota in children at preschool age. The preschool years are a period when both sleep patterns and gut microbiota undergo transition toward an adultlike pattern, yet not completely mature [26, 27]. Characteristics of sleep patterns at preschool age include discontinuation of daytime naps, the consolidation of sleep to the night-time period, and a gradual decrease in sleep duration [28, 29]. Similarly, the gut microbiota reaches a relatively stable stage after three years age, with the presence of adult-like phylum members [27]. However, some characteristics that strongly resemble an earlier age remain, such as enrichment of oligosaccharides degrader *Bifidobacterium* [30] and lower diversity compared to adults [31]. Understanding the relationship between sleep and gut microbiota during this transitional period may allow development of targeted intervention to promote sleep health through reshaping the gut microbiota. The objective of the study was to assess the association between actigraphy sleep measures and the fecal microbiota composition and metabolites in young children at preschool age.

We hypothesized that sleep duration, sleep efficiency, and wake after sleep onset in preschoolers are associated with gut microbiota profile and function.

Methods

Study design

This is a subanalysis of the Alberta Pregnancy Outcomes and Nutrition (APrON) prospective cohort study [32] that recruited pregnant individuals between 2009 and 2012 in Alberta, Canada. Data in this subanalysis are from a follow-up study conducted at child ages 3–4 years. Ethics approval for this study was obtained from the Health Research Ethics Boards at the University of Calgary (REB14-1702 and REB15-1200). Informed written consent was obtained from the parent or legal guardian and verbal assent was obtained from children prior to data collection.

Participants

Complete details of participants in the APrON study have been reported [32]. Briefly, at the time of recruitment, pregnant women were ≥16 years, <27 weeks of gestation, and planned to deliver their babies within the recruitment region. Women were excluded if they were unable to complete questionnaires in English. The sample is largely White, well educated, with average income for the recruitment region. The sample somewhat underrepresents young, low income, and low education mothers, relative to nationally representative data from pregnant women in Canada [33]. For the current sample, preschool children born to APrON mothers were invited to participate in a laboratory-based neurocognitive assessment (not reported here) and objective measures of child sleep (actigraphy) and provide a fecal sample. Children with antibiotic exposure 2 weeks before fecal sample collection were excluded. The current sample comprises the 143 children who provided both a fecal sample and actigraphy data

Actigraphy measures

Wrist actigraphy data were collected using the Motionlogger Actigraph (Ambulatory Monitoring, Inc.). Children were asked to wear the actigraph on their nondominant wrist for 48 hours, capturing two nights of sleep. Data analysis was completed within the ActionW software using the Cole-Kripke algorithm [34] and a 5-minute threshold for sleep and wake periods [35]. For each participant, we calculated: 1) total night-time sleep (the hours/night spent sleeping while in bed); 2) the total duration of awakenings after sleep onset (WASO); and 3) sleep efficiency (percentage of time in bed/ total time spent sleeping). Parents were asked to keep a diary of night-time sleep. Daytime quiescence was recorded by actigraph; however, a daytime diary was not taken therefore it was not possible to determine if these periods of quiescence were sleep periods.

Microbiota analysis

Fecal samples were collected in the same week as actigraphy measures with assistance of parents at home using a toilet cover along with a sterile 50-ml plastic conical collection tube and plastic applicator. Time of stool sample collection was recorded. Information on usual dietary intake was collected at 3 years of age (approximately 1 year prior to stool sample collection) using a semi-quantitative food frequency questionnaire. Overall diet quality was estimated using a previously published dietary quality score that consists of six diet components (vegetables and fruit, grain products, milk and alternatives, meat and alternatives, candy/snacks, and sugar-sweetened beverages) [36].

Collected fecal samples were temporarily stored in a home freezer for up to 24 h before transport to the study lab in a cooler surrounded by freezer packs. Fecal samples were stored at −80°C until further processing. Fecal DNA was extracted using FastDNA® Spin Kit for Feces (MP Biomedicals, Santa Ana, California, USA) following manufacture's instruction. 16S rRNA gene amplicon sequencing was performed using the MiSeq platform at the Centre for Health Genomics and Informatics (University of Calgary, Calgary, Canada). The PCR amplification of the V3 and V4 region of the 16S rRNA gene was performed using manufacturer-recommended primers (Forward: 5'-TCG TCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCW GCAG-3'; Reverse: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGA GACAGGACTACHVGGGT ATCTAATCC-3'). Paired-end16S rRNA gene amplicon sequence reads were analyzed with QIIME 2 (2018.11.0) [37]. Raw sequence data were demultiplexed and quality filtered using the q2-demux plugin followed by denoising with DADA2 [38] (via q2-dada2). All amplicon sequence variants (ASVs) were aligned with mafft [39] (via q2-alignment) and used to construct a phylogeny with fasttree2 [40] (via q2-phylogeny). Alpha-diversity metrics (a measure of diversity within a gut microbiota community) and beta-diversity metrics (a measure of similarity of two microbiota communities, weighted UniFrac, and unweighted UniFrac) were estimated using q2-diversity. The sampling depth used in diversity analyses was 11 896. Taxonomy was assigned to ASVs using the q2-feature-classifier [41] classify-sklearn naive Bayes taxonomy classifier against the Silva (132) 99% OTUs reference sequences [42].

Fecal metabolomic analysis

Fecal metabolomic analysis was performed at the Calgary Metabolomics Research Facility. Briefly, 100–200 mg of fecal samples were diluted 5 times (w/v) into 50% methanol/water solution. The diluted samples were homogenized using Tissue Lyser II (QIAGEN) and then incubated on ice for 30 minutes for full extraction. After incubation, samples were centrifuged at approximately 13 000 rpm and 500 µl of supernatant were collected for metabolomic analysis. Metabolomics runs were performed on a Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (Thermo-Fisher) coupled to a Vanquish™ UHPLC System (Thermo-Fisher). Chromatographic separation was achieved on a Syncronis HILIC UHPLC column (2.1 mm x 100 mm x 1.7 µm, Thermo-Fisher) using a binary solvent system at a flow rate of 600 µL/min. Metabolite data were analyzed by El-MAVEN software package. Metabolites were identified by matching observed m/z signals (+/−10 ppm) and chromatographic retention times to those observed from commercial metabolite standards (Sigma-Aldrich). Raw untargeted data was generated using the automated feature detection function in EL-MAVEN with a minimum signal intensity threshold of 50 000 signal intensity.

Short-chain fatty acids (SCFAs) were extracted from fecal samples with ice-cold extraction solvent (50% water/acetonitrile, v/v) spiked with stable isotope-labeled internal standards (acetic acid-1,2-13C2, propionic acid-13C3, 1 mM; butyric acid-1,2-13C2, 1 mM) before submitted to LC-MS/MS-based analysis. More details of SCFA sample preparation and analyzing methods were described in our previous study [43].

Statistical analysis

To assess the association between sleep and the gut microbiota, participants were divided into "low" and "high" groups based on the median of TST, SE, and WASO.

Differences in alpha-diversity (Observed OTUs, Shannon, and Faith PD) and SCFAs between low and high sleep groups were assessed using Mann-Whitney test (GraphPad Prism version 5.00). Group differences in beta-diversity of microbiota were assessed using the permutational analysis of variance (PERMANOVA) model with 9999 permutations based on the parameters permutation of residuals under a reduced model and a type III sum of squares (Primer-E v.7; Primer-E Ltd.). Taxonomic differences at genus level and metabolite differences between low and high sleep groups were analyzed using LEfSe (linear discriminant analysis effect size) [44] with LDA score > 2 as significance cut-off.

Results

Demographic characteristics and sleep measures

Participating children have an average age of 4.37 ± 0.48 years old and with approximately equal sex distribution (males, 47.6%). The majority of children were White (88.1%) and from a family with household income > $100 000 CAD per year (63.6%), which was the median household income for the recruitment region.

Children in this study had an average TST of 9.48 hours per night (568.6 ± 51.70 min), SE of 94.43 ± 3.78%, and WASO of 32.97 ± 22.04 min (Table 1). Children were categorized to low and high sleep groups using median split (values above the median were classified as "high"; values equal to and smaller than median were classified as "low"). The majority of children who had longer night-time sleep (high TST) also had high SE (overlap rate 69%) and low WASO (overlap rate 67%), and almost all children who had high SE also had low WASO (overlap rate 99%). The majority of sleep data were collected on consecutive days (57%) during a weekday (75%). Sleep measures collected on weekdays were not different from these collected on weekends and holidays (data not shown). A small proportion of studied children (7.8%) had periods of quiescence during the day (62.4 ± 17.7 min), however, we could not determine if these were naps because we did not have a daytime napping diary.

Sleep and the fecal microbiota

The gut microbial communities (weighted UniFrac distance of beta-diversity, which takes into account relative abundance of taxa) were different between children who had low and high TST (p = .048, Figure 1). Similarly, SE showed a trend toward significant difference with a p value of .07 however beta-diversity was not different for low and high WASO (p = .11). Unweighted

Table 1. Participant characteristics and sleep measures (*n* = 143)

Age (year), mean (SD, range)	4.37 (0.48, 3.06–5.00)
Sex -male, *n* (%)	68 (47.6%)
BMI z-score, mean (SD, range)	0.29 (0.80, –3.00 to 1.98)
Ethnicity	
Caucasian	126 (88.1%)
Chinese	6 (4.2%)
Latin American	4 (2.8%)
Other	7 (4.9%)
Preterm (*n*, %)	4 (2.8%)
Delivery mode-Cesarean, *n*,(%)	33 (23.1%)
Mother's education level, *n*, (%)	
Completed high school diploma	6 (4.2%)
Completed trade, technical diploma	22 (15.4%)
Completed university degree	83 (58%)
Completed postgraduate degree	32 (22.4%)
Annual household income (CAD), *n* (%)†	
<$20 000	2 (1.4%)
$20 000 – $39 999	2 (1.4%)
$40 000 – $69 9999	11 (7.7%)
$70 000 – $999 999	37 (35.9%)
>$100 000	91 (63.6%)
Actigraphy measures	
TST (min), mean (SD, range)	568.57 (51.70, 369.00–684.50)
SE (%), mean (SD, range)	94.43 (3.78, 79.03–99.92)
WASO (min), mean (SD, range)	32.97 (22.04, 0.50–101.50)

BMI, body mass index; SE, sleep efficiency; TST, total night sleep time; WASO, wake-time after sleep onset.

UniFrac distance (which takes into account only presence/abundance of taxa) was not different between any sleep measures. No differences in alpha-diversity (Observed OTUs, Shannon and Faith PD) of the fecal microbiota were observed between children who were classified in the low and high groups of TST, SE, and WASO (Supplementary Figure 1).

Some taxa differed between the low and high groups for TST, SE, and WASO (Figure 2). Specifically, children who recorded more total night-time sleep (high TST) had higher relative abundance of *Bifidobacterium* (8.06 ± 5.01% vs. 6.26% ± 4.24%, LDA = 4.04), *Parabacteroides* (0.91 ± 1.04% vs. 0.68 ± 1.03%, LDA =3.76), and *Turicibacter* (0.47 ± 0.60% vs. 0.45 ± 0.97%, LDA = 3.51); children who had higher SE showed higher relative abundance of *Ruminiclostridium* 9 (1.03 ± 0.21% vs. 0.07 ± 1.23%, LDA = 3.73) and *Bacteroides* (13.25 ± 8.75% vs. 10.08 ± 7.94%, LDA = 4.18). Relative abundance of *Bacteroides* was also higher in children who had lower WASO scores (10.08 ± 7.94% vs. 9.94 ± 8.03%, for low and high WASO, LDA = 4.29). In contrast, children with low SE or high WASO had lower relative abundance of taxa belonging to *Eubacterium ruminantium* group compared to their corresponding high or low groups (0.11 ± 0.40% vs. 0.26 ± 0.54%, for low and high SE, LDA = 3.32; 0.074 ± 0.33% vs. 0.28 ± 0.58%, for high and low WASO, LDA = 3.94). Other taxa that were associated with sleep groups included *Blautia* (9.75 ± 4.32% vs. 10.99 ± 4.43%, for high and low TST, LDA = 5.05) and *Lachnospiraceae* unclassified (5.21 ± 3.07% vs. 6.09 ± 3.04%, for high and low TST, LDA = 3.73), *Coprococcus* 1 (0.18 ± 0.22% vs. 0.27 ± 0.29%, for high and low SE, LDA = 3.58), and a taxon belonging to Coriobacteriales (Coriobacteriales Incertae Sedis, 0.12 ± 0.21% vs. 0.074 ± 0.16%, for high and low WASO, LDA = 3.89). Overall diet quality and time of fecal sample collection were not different between low and high sleep groups (Supplementary Table 1).

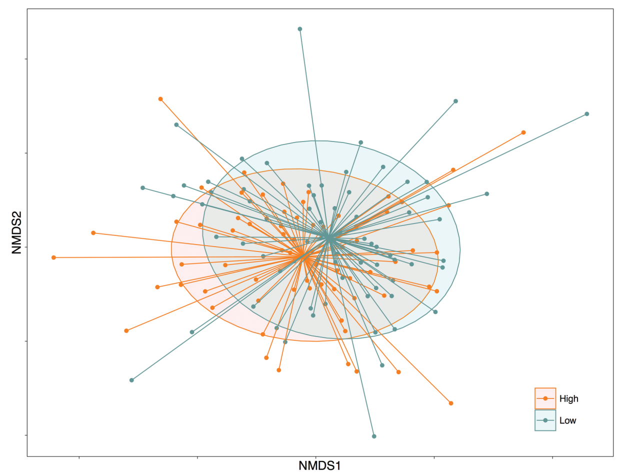

Figure 1. Nonmetric multidimensional scaling plot derived from weighted UniFrac distance showing difference in gut microbiota in children with low and high total night-time sleep (PERMANOVA, *p* = .048).

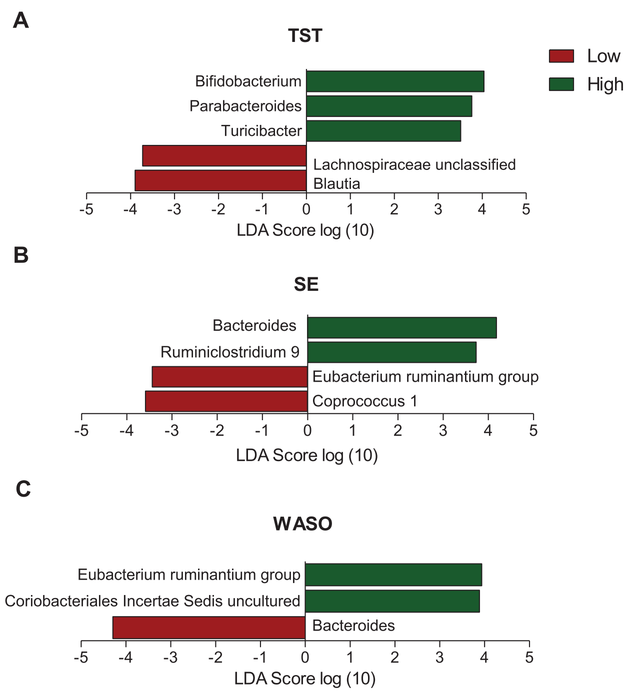

Figure 2. Relative abundance of the most discriminant bacterial groups according to LEfSe in children who had low and high total night-time sleep (TST, A), sleep efficiency (SE, B), and wake-time after sleep onset (WASO, C).

Sleep and the fecal metabolites

We analyzed the fecal metabolites of the preschoolers to further assess the association between sleep and the function of the gut microbiota. In total, 141 metabolites were detected and 23 metabolites differed as a function of sleep groups, including amino acids, vitamins, monosaccharides, and sugar alcohol (Figure 3). Signal intensity of N-Acetyl-L-aspartic acid (LDA = 2.16) and D-sorbitol (LDA = 2.32) were greater in children with high TST. Moreover, a group of detected metabolites were greater in both high SE and low WASO, including nicotinate (LDA = 3.23 for SE; LDA = 3.32 for WASO), glycine (LDA = 2.41 for SE; LDA = 2.39 for WASO), tryptophan (LDA = 2.26 for SE; LDA = 2.15 for WASO), tyrosine (LDA = 2.41

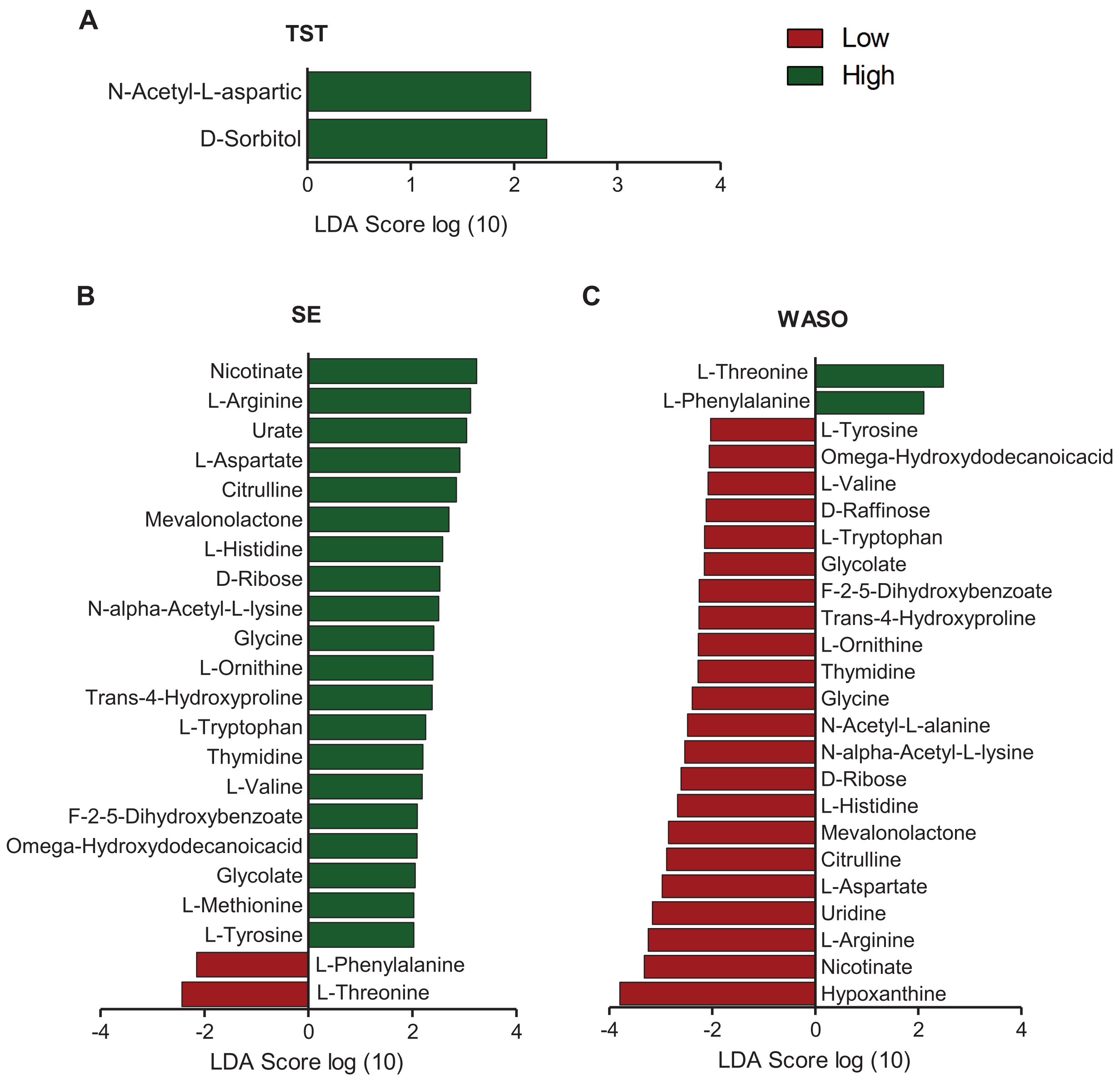

Figure 3. Relative abundance of the most discriminant fecal metabolites according to LEfSe in children who had low and total night-time sleep (TST, A), sleep efficiency (SE, B), and wake-time after sleep onset (WASO, C).

for SE; LDA = 2.39 for WASO) and L-ornithine (LDA = 2.27 for WASO). In contrast, L- phenylalanine (LDA = 2.15 for SE; LDA = 2.11 for WASO) and L-threonine (LDA = 2.43 for SE; LDA = 2.49 for WASO) were associated with less sleep efficiency and more disruption and they were higher in both low SE and high WASO groups.

Furthermore, fecal propionate concentrations were higher in children with low WASO compared to the children who had longer wake time (high WASO). Differences in SCFA concentrations were not observed between low and high TST and SE groups (Figure 4).

Discussion

This is the first study, to our knowledge, investigating the association between objectively assessed sleep and the gut microbiota in children at preschool age. Our results show key differences in gut microbiota composition and metabolites between children with low and high TST, SE, and WASO measures and, therefore, demonstrate the association between sleep and the gut microbiota in preschoolers.

Our results show that children who had a relatively longer night sleep had a significantly different gut microbiota community structure (beta-diversity, weighted UniFrac distance) compared to children who had shorter night sleep. Beta-diversity differences were only significant for TST not for SE or WASO indicating that night sleep duration may have a stronger influence on the overall gut microbiota community than sleep efficiency. The discriminate analysis (LEfSe) further determined which specific microbial members are most likely to explain differences between the low and high sleep groups. We found a group of commensal gut bacteria, including *Bifidobacterium* and *Bacteroides*, were separately associated with longer night-time sleep duration, greater sleep efficiency, and less time awake at night in preschoolers. Most of these taxa have been linked with sleep-related neurochemicals in previous studies,

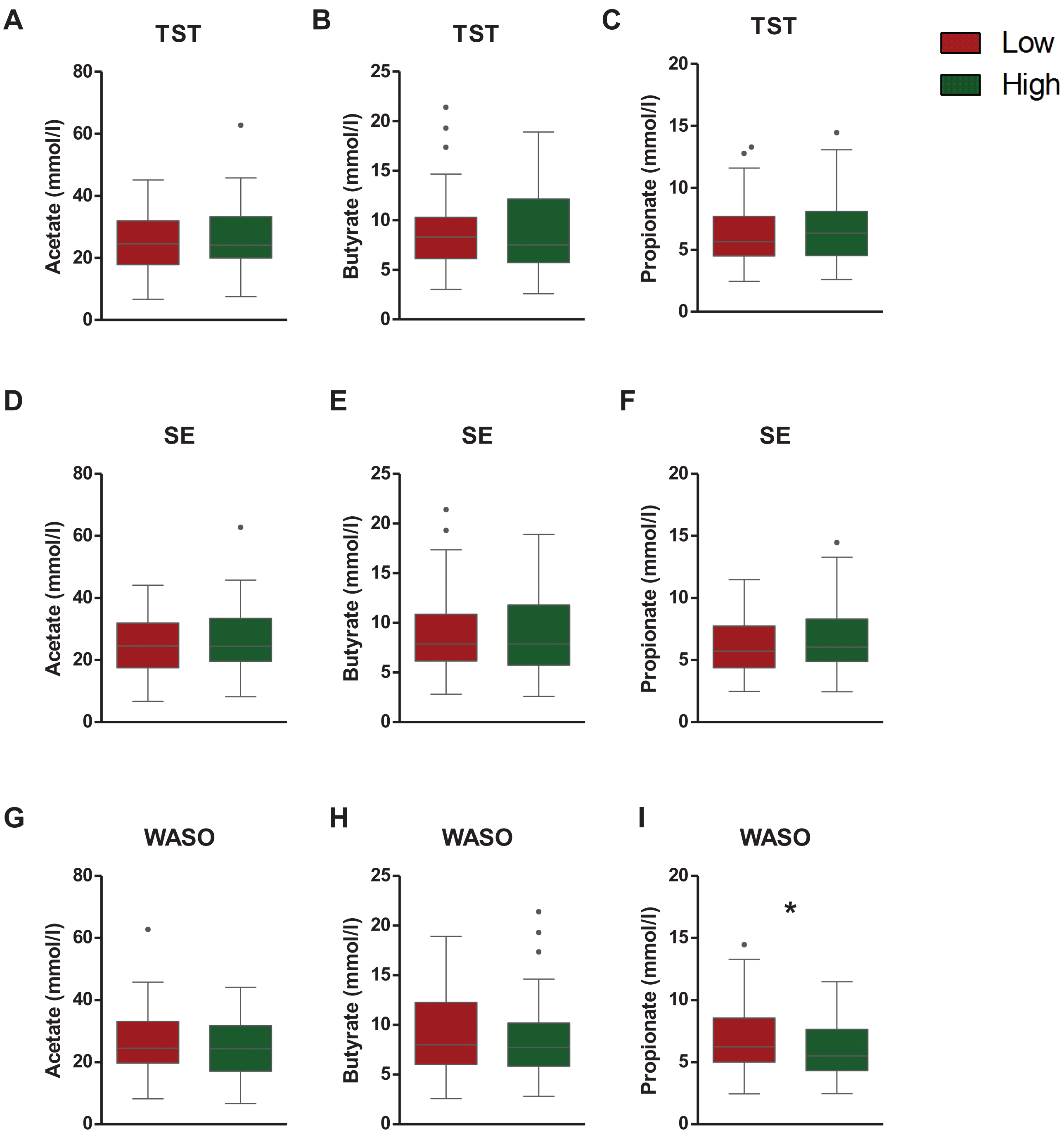

Figure 4. Differences of fecal SCFA concentrations in children with low and high total night-time sleep (TST, A-C), sleep efficiency (SE, D-F) and wake-time after sleep onset (WASO, G-I). Mann-Whitney U test; *, $p < .05$.

such as serotonin [45, 46] and its precursors (e.g. tryptophan and gamma-aminobutyric acid) [47]. This suggests the potential mediating roles of neurotransmitters between gut microbiota and sleep. Importantly, studies in human adults also demonstrated the effect of *Bifidobacterium*, either administrated as a single probiotic or mixed with other probiotics, in regulating mood [47, 48] and improving sleep quality [49]. In addition to the impact on neurotransmission, the immunoregulating properties of specific taxa may also contribute to regulating sleep. The sleep-immune interaction is a well-established phenomenon [50] and the immunomodulating effect of both *Bifidobacterium* and *Bacteroides*, the two relatively abundant taxa in children, are well demonstrated in both *in vitro* and *in vivo* studies [51]. Additionally, *Bacteroides* is one of the key taxa producing the SCFA propionate via the succinate pathway [52]; a high proportion of propionate in fecal samples is linked with longer uninterrupted sleep in infants [53] and propionate was also associated with low WASO in our study. Therefore, the SCFA producing capability may be another factor contributing to the connection of specific taxa and fewer night-time sleep disruptions.

Taken together, the association between the five specific taxa and longer night-time sleep duration, greater sleep efficiency, and fewer night-time sleep disruptions may be due to their functional properties in neurotransmission regulation, immunomodulation, and SCFA production.

On the other hand, we also found five taxa that showed greater abundance in children who had shorter night-time sleep duration, less sleep efficiency, and longer waking time (Figure 2), of which *Blautia* is the second most abundant genera in the studied children. Taxa associated with shorter night-time sleep duration and sleep efficiency in this study are highly consistent with a recent study in healthy adults, where Smith et al. reported that both *Blautia* and other *Lachnospiraceae* members were negatively correlated with SE and TST, whereas *Coprococcus* was positively correlated with the number of night awakenings [23]. These findings suggest that taxa contributing to shorter sleep duration in adults may also do so in young children. Notably, three of the taxa associated with sleep disruptions in adults, including *Blautia*, *Coprococcus*, and the *Lachnospiraceae* unclassified, belong to the *Lachnospiraceae* family. Despite the SCFA producing ability of many *Lachnospiraceae* members [52], the impact of *Lachnospiraceae* on host physiology is in fact controversial as *Lachnospiraceae* prevalence is associated with intra- and extraintestinal diseases [54] such as metabolic disorders [55, 56]. Although it is difficult to formulate a plausible mechanism between identified taxa and sleep disruption based on available literature, the consistency of identified taxa related to shorter sleep duration and more night-time sleep disruption in the current and previous studies encourage further investigation on the negative impact of these specific microbial members on sleep.

The differences of fecal metabolites observed between low and high sleep groups provided further insight into the links between sleep and gut microbiota. Several metabolites associated with greater sleep efficiency and less WASO are involved in tryptophan metabolism, including nicotinate, arginine, glycine, tryptophan, and ornithine (KEGG, pathway reference, map00260, map00380, map00400). Tryptophan is an essential aromatic amino acid and a precursor to many microbial and host metabolites including serotonin [57]. Although it is unclear whether gut microbiota-produced tryptophan makes a significant contribution to host physiology [57], tryptophan metabolites generated from direct metabolism by microbes and kynurenine pathway are known to play critical roles in immune responses and neurotransmission [57]. Moreover, effects of some of the metabolites, such as glycine and ornithine, in improving sleep quality have been shown in human trials [58, 59]. On the other hand, the relatively higher level of metabolites associated with low SE and high WASO may be associated with less efficient microbial metabolism. Phenylalanine and threonine are precursors of tyrosine and glycine, respectively. As tyrosine and glycine were higher in the high SE and low WASO groups, it is reasonable to observe that phenylalanine and threonine showed relatively greater signal intensity in low SE and high WASO groups.

The link between sleep and SCFAs is thought to derive mainly from their roles in the neuro-immunoendocrine regulation [60]. Yet, studies investigating the association between sleep and SCFAs are few and the outcomes are inconsistent [61, 62]. The higher level of propionate in low WASO can be explained by the higher relative abundance of *Bacteroides* in the same group of children because *Bacteroides* is known to be one of the main propionate producers [52]. The connection of propionate and sleep is supported by previous studies revealing the modulating roles of propionate in gut-brain communication, although the mechanisms of how propionate influences sleep are unknown. Propionate can initiate a gut-brain neural circuit [63] and interact with endothelium of the blood-brain barrier *in vitro* [64]; effect of propionate reducing anticipatory reward responses was reported in healthy adult men [65]. Our results for propionate are highly consistent with a recent study in infants showing that a higher proportion of propionate in fecal samples was associated with longer uninterrupted sleep [53].

Gut microbiota profile of preschoolers can be affect by diet [66, 67] and even perinatal factors such as gestational age and delivery mode [68]. Although our findings cannot describe the factors that contribute to development of the gut microbiota community, our study showed a clear difference in gut microbiota profile between children who had longer sleep duration and higher efficiency compared to those who shorter sleep duration and lower efficiency. Furthermore, overall dietary quality, gestational age, and delivery mode were not different between low and high sleep groups reported in this study. As such, it seems unlikely that any of these factors influence the observed association between gut microbiota and sleep, and specifically that they are not driving the gut microbiota characteristics in children who showed “high” and “low” sleep measures.

Using a combined technology of 16S rRNA amplicon sequencing and metabolomics, our study not only revealed the relationship between sleep duration and gut microbiota members but also their activities. These findings generate new insights into previously unidentified causes of sleep problems in children at preschool age and may allow the development of a more precise gut microbiota manipulation strategy to improve sleep duration and decrease night-time sleep disruption in children. Yet, this study also has several limitations that should be kept in mind. For instance, only two nights of actigraphy data were collected to assess sleep. This time window reflects a pragmatic choice geared toward encouraging our young participants to complete the study but is shorter than the recommended five or more nights, although pediatric studies with two nights have shown acceptable reliability [69]. Future studies should aim to collect sleep measures over a longer period of time to confirm our findings. Our study is also limited by its focus on night-time sleep. Because participants in the current study were asked to perform many additional data collection steps (not reported here), we decided to not obtain daytime napping diaries from parents (parents did keep a diary of night-time sleep). We were able to detect periods of quiesence consistent with napping in the actigraph record, but we did not include these potential napping periods in the total sleep time beucase we could not be sure that the children were asleep durng this time. Consistent with previously reports [28, 29], the majorty of children in our study did not have any periods of quiesence that could have been a nap (92.2%). Nevertheless, a small number of children may have continued to nap at this age and the data we collected for them reflects only the night-time portion of their sleep and not their total sleep. Additionaly, only one fecal sample was collected in our study. Feacal microbiota compostion and function may have cyclic dynamics and daily variation [70]. However, previous studies have shown that the intraindividual gut microbiome signature is largely stable over different time points [71], indicating that a single gut microbiome time point can still provide valuable insights into the associations between the gut microbiota and sleep assessed in this study. Moreover, the time of fecal sample collection was not different between sleep groups and none of the fecal metabolites associated with sleep differed as a function of stool collection time. Nevertheless, this is the first study investigating the association between sleep and gut microbiota in preschool-aged children and a significant part of the outcomes are consistent with previously reported microbiota-sleep associations in adults.

In conclusion, our results demonstrated that sleep duration, efficiency, and wake after sleep onset in young children are associated with gut microbiota and microbial metabolites such as tryptophan, tryptophan derivatives, and propionate. These microbial metabolites may be key mediators for the sleep and gut microbiota connection. Although the underlying mechanisms of the connection between sleep and gut microbiota remain unclear, our study suggests that further investigation is warranted to elucidate the connection between sleep and specific microbial members and metabolites and to determine if these factors can be harnessed to improve sleep in young children.

Supplementary material

Supplementary material is available at *SLEEP* online.

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Disclosure Statement

The authors declare no conflicts of interest.

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