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NapBiome trial: Targeting gut microbiota to improve sleep rhythm and developmental and behavioural outcomes in early childhood in a birth cohort in Switzerland – a study protocol

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ABSTRACT

Introduction The gut–brain axis plays a crucial role in the regulation and development of psychological and physical processes. The first year of life is a critical period for the development of the gut microbiome, which parallels important milestones in establishing sleep rhythm and brain development. Growing evidence suggests that the gut microbiome influences sleep, cognition and early neurodevelopment. For term-born and preterm-born infants, difficulties in sleep regulation may have consequences on health. Identifying effective interventions on the gut–brain axis in early life is likely to have long-term implications for the health and development of at-risk infants.

Methods and analyses In this multicentre, four-group, double-blinded, placebo (PLC)-controlled randomised trial with a factorial design, 120 preterm-born and 260 term-born infants will be included. The study will investigate whether the administration of daily synbiotics or PLC for a duration of 3 months improves sleep patterns and neurodevelopmental outcomes up to 2 years of age. The trial will also: (1) determine the association between gut microbiota, sleep patterns and health outcomes in children up to 2 years of age; and (2) leverage the interactions between gut microbiota, brain and sleep to develop new intervention strategies for at-risk infants.

Ethics and dissemination The NapBiome trial has received ethical approval by the Committee of Northwestern and Central Switzerland and Canton Vaud, Switzerland (#2024–01681). Outcomes will be disseminated through publication and will be presented at scientific conferences. Metagenomic data will be shared through the European Nucleotide Archive.

Trial registration number The US National Institutes of Health NCT06396689.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ A double-blinded and placebo-controlled design ensures the reliability and validity of the findings.
⇒ Comprehensive data collection using various physiological as well as subjective measures (eg, high-density electroencephalogram, microbiota composition, wearable actimetry, questionnaires) to assess outcomes, provides a comprehensive evaluation of the intervention's impact.
⇒ Inclusion criteria restricting to partially breastfed preterm-born and term-born infants may limit applicability to other groups.
⇒ The 2-year follow-up may not capture potential longer-term effects.

INTRODUCTION

The gut–brain axis plays a crucial role in the regulation and development of psychological and physical processes. Growing evidence suggests that the gut microbiome also influences sleep and cognition.[1] The first year of life is a critical period for the development and stabilisation of the gut microbiome,[2–4] paralleling important milestones in establishing sleep rhythm and neurodevelopment.[5 6] Difficulties in sleep regulation can have significant consequences for infants' health and attachment between infants and their caregivers.[7] Preterm-born infants are at higher risk of sleep and neurodevelopmental problems,[8 9] and 20–30% suffer from severe long-term cognitive, motor or visual impairments.[10 11] Although neonatal care has improved over recent decades, different situations such as preterm birth, disturbed postnatal adaptation, infections and their management strategies such as antibiotic exposure, parenteral nutrition and enteral feeding strategies have an impact on the developing brain and disrupt the development of the microbiome. Both pathways may lead to a wide range of neurodevelopmental changes and disabilities that are unaddressed with current therapies.[12–14] Given the proposed importance of both sleep and the gut microbiome for brain maturation

and behaviour,[15–24] identifying effective therapies is likely to have long-term implications for the health and development of at-risk infants. We hypothesise that the establishment of a healthy gut microbiome during early life is vital for short- and long-term child health, as dysbiosis may harm sleep regulation, brain connectivity and neurobehavioural development. We hypothesise that the administration of synbiotics (SYN) improves microbiota establishment, sleep and neurodevelopmental outcomes.

The gut microbiome consists of trillions of microbes inhabiting the gastrointestinal tract and provides the most significant interface for host-microbial interaction.[25] During infancy, the microbiome undergoes its most dramatic period of development, mirroring the critical stages of physiological and immunological establishment.[26 27] Exposure to the mother's microbiome during and following birth is an essential source of microbiota for the infant, and early disruptions, for example, from caesarean section and antibiotic use disrupt microbiome establishment.[3 28] Similarly, the early gut microbiota of preterm infants is altered, characterised by low microbial diversity and delayed colonisation with *Bifidobacterium* and *Lactobacillus*,[4] genera often associated with health benefits.[29] Dysbiosis during infancy has been implicated in increased risk for metabolic and immune diseases, including diabetes,[30] eczema,[31] asthma[30 32 33] and allergic disorders.[29 34] Hence, early life represents a critical 'window of opportunity' for supporting the healthy establishment of the gut microbiome and later health outcomes. However, despite promising correlations to sleep, neurodevelopmental and behavioural outcomes,[35 36] causative links must be established to enable translational strategies for improving health in children.

The benefits of probiotics (live microorganisms that can provide health benefits when consumed in adequate amounts) and SYN (combinations of probiotics and prebiotics, which are non-digestible food ingredients that stimulate the growth and activity of beneficial microorganisms) for infant health is an area of active research. Probiotics reduce the risk of necrotising enterocolitis in both preterm-born and low birth weight infants.[37–39] Furthermore, probiotics also reduce the length of hospital stay of preterm infants[38] and have been associated with a reduction in infant crying.[40] Supplementation of infants with prebiotics and probiotics has been associated with improving intestinal health markers,[41] such as short-chain fatty acid (SCFA) production, decreased gut pH,[42 43] colonisation resistance to pathogens,[44] improved vaccine responses,[45] cognition,[46] sleep patterns,[47] sleep architecture and brain activity.[48] In one small randomised controlled trial, the administration of *Bifidobacterium animalis subsp. lactis* BB-12 led to an increase in sleep duration in infants.[49] Although a few small studies have found no association between the administration of probiotics and cognitive outcomes,[50] they have mainly used single-bacteria probiotics instead of SYN containing multiple species and prebiotics.

Gut microbiota modulate various neurophysiological functions in the host, including cognitive function, through the secretion of neuroactive molecules and immunomodulation.[20 51] It is becoming increasingly clear that the gut–brain axis also (in)directly impacts sleep. The gut microbiome exhibits cyclic, diurnal regulation[52–54] and dysregulated sleep alters gut microbiota composition,[52 55 56] metabolism and translocation.[57 58] Development of the gut microbiome in early life parallels the establishment of the serotonergic and stress systems, suggesting possible interactions with long-term effects.[59–61] In infants, nighttime sleep fragmentation and duration are associated with SCFA-producing gut microbiota[62] and faecal propionate levels.[63] SCFA concentrations fluctuate rhythmically over the course of a day[64] and can influence both circadian rhythm-related genes and sleep.[65 66] Thus, interest in SCFA-targeted therapies is increasing due to their close connection with sleep and associated psychological and metabolic health.[67]

In this multicentre, four-group, double-blinded, placebo (PLC)-controlled randomised trial with a factorial design, 120 preterm-born and 260 term-born infants will be included. The study will investigate whether the administration of daily SYN or PLC for a duration of 3 months improves sleep patterns and neurodevelopmental outcomes up to 2 years of age. The trial will also: (1) demonstrate the association between gut microbiota, sleep patterns and health outcomes in children up to 2 years of age; and (2) leverage the interactions between gut microbiota, brain and sleep to develop new intervention strategies for at-risk infants.

Breath metabolomics has gained attention as a non-invasive and sensitive method for detecting biomarkers, offering a promising alternative to traditional, more invasive techniques like blood and urine analyses. Exhaled breath contains volatile organic compounds and aerosol particles that reflect metabolic processes, making it useful for diagnosing diseases, monitoring treatment responses and assessing nutritional intake. Recent advances in chemical sensing technologies and mass spectrometry (MS) have enhanced the ability to analyse breath in real-time, allowing for the detection of health-related biomarkers. Understanding breath metabolomics could improve diagnostic precision, offering insights into health and metabolism, and paving the way for personalised health and nutritional strategies.

Determining the relationship between preterm birth, gut microbiota, infant sleep patterns and their potential association with neurodevelopmental outcomes will provide stronger evidence for targeted early interventions. Additionally, this study will form the basis for developing strategies to support neurodevelopmental health in preterm and term-born infants, including tailored SYN and the potential development of postbiotics. By identifying microorganisms that regulate host circadian rhythms and investigating biomarkers for neurodevelopment, we aim to advance

early prevention methods for developmental and mental illnesses.

Hypothesis and objectives

Hypothesis

In both preterm-born and term-born infants, the composition of the gut microbiota is associated with the establishment of sleep rhythmicity, neuronal connectivity, neurobehavioural development and various other health outcomes, all of which can be influenced by the administration of SYN.

Primary objectives

This study aims to investigate how exposure to SYN influences the gut microbiome composition (metagenomes) and metabolomes, as well as (1) the development of sleep patterns and physiological markers of circadian rhythm (ie, diurnal breath metabolome profiling), (2) neurophysiology and (3) neurobehavioural development.

METHODS AND ANALYSIS

Study design

Multicentre, four-group, double-blinded, PLC-controlled randomised trial with a factorial design including 120 preterm-born and 260 term-born infants born at the hospitals in Fribourg and Lucerne. Infants will be randomised at birth (up to a maximum of 7 days of life) to either receive daily SYN or a PLC for a duration of 3 months (n=60 preterm-born infants assigned to 'synbiotics' (PRET-SYN), n=60 preterm-born infants to 'placebo' (PRET-PLC), n=130 term-born infants to 'synbiotics' (TERM-SYN) and n=130 term-born infants to 'placebo' (TERM-PLC) (figure 1). Infants will be followed longitudinally until 2 years of age with an assessment of microbial, sleep, breath, health and developmental parameters with methodologies aligned with previous and ongoing studies.[68 69] The study period will run from 1 March 2025 to 28 February 2029.

Eligibility criteria

Inclusion criteria

For the preterm group, neonates born between a gestational age of 34 0/7 to 36 6/7 weeks and, for the term group, neonates born at a gestational age of ≥37 0/7 weeks will be included. At inclusion, infants need to be at least partially breastfed.

Exclusion criteria

Infants who (1) receive probiotics outside the trial design, (2) birth weight<1500 g, (3) were prenatally drug-exposed (cannabis, cocaine, heroin, opiates and alcohol), (4) have suspected or confirmed immunodeficiency or (5) have an underlying disease (excluding transient conditions such as alimentation problems, hyperbilirubinaemia, hypoglycaemia, anaemia, respiratory distress syndrome or apnoea–bradycardia syndrome), congenital malformations, central nervous system disease or injury or congenital infections.

Justification

There is an early life 'critical window' during which the microbiota and many physiological processes develop concurrently. Only by studying infants can this complex interplay be investigated. The research questions cannot be answered in older children or adults. As the intervention has already proven safe and is used in routine care (prevention of necrotising enterocolitis), and no invasive procedures are necessary, the study poses an acceptable burden on participants.

Outcomes

Primary endpoints

- Sleep-wake behaviour: Brief Infant Sleep Questionnaire; actimetry and sleep-wake diary.
- Neuronal connectivity: high-density electroencephalogram (hdEEG) assessed during sleep.
- Neurobehavioural development: Bayley Scales of Infant Development (BSID).

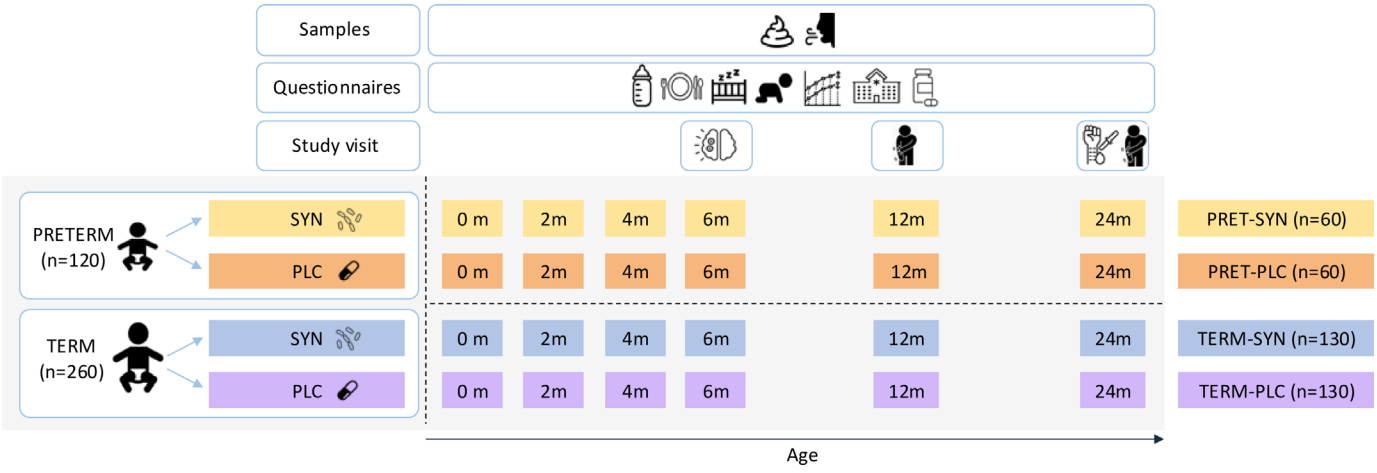

Figure 1 Assessment overview. SYN refers to the synbiotic intervention and PLC to placebo, PRET refers to preterm-born infants, TERM to term-born infants. Ages are given in months (m).

- Behaviour: Infant Behaviour Questionnaire, eye-tracking.
- Gut microbiota: composition of stool microbiota.
- Stool metabolome: composition of stool metabolites.
- Breath metabolome: composition of breath metabolites (exhalome).

Secondary endpoints

- Eczema: Patient-Oriented Eczema Measure (POEM) score and SCORing Atopic Dermatitis scoring system (SCORAD).
- Food allergy: skin prick test.
- Rates of infection: number of episodes.
- Microbiota: breast milk, nasal, oral.

Study intervention

SYN intervention

The two SYN infant groups will receive one capsule of the dietary supplement daily. The capsule contains *Lactobacillus helveticus* R0052, *Bifidobacterium infantis* R0033 and *Bifidobacterium bifidum* R0071 (3 billion bacteria per capsule), as well as zinc oxide, potato starch fructooligosaccharides, coating agent, methyl hydroxypropyl cellulose, anticaking agent and magnesium stearate. The supplement is used in routine care in preterm infants in Switzerland to prevent necrotising enterocolitis, late-onset and death.

PLC intervention

The PLC infant group will receive capsules containing the same ingredients but without bacteria and potato starch fructooligosaccharides.

The content of the SYN and PLC capsule will be diluted in 3.6 mL water and given orally in the morning. The study nurse or the local investigators will explain the administration of the SYN or PLC to the parents.

Recruitment, screening, informed consent procedure and discontinuation

Parents or caregivers of newborns fulfilling the inclusion criteria will be approached by a study nurse or doctor and asked to consider enrolling their infant in the study. The investigators will explain the nature of the study, its purpose, procedures, expected duration, potential risks and benefits. Parents will be informed that participation in the study is voluntary, that they may withdraw their infant's participation at any time, and that withdrawal of consent will not affect the infant's subsequent medical assistance and treatment. Parents will be informed that their infant's medical records may be examined by authorised individuals other than their treating physician.

Parents will be provided with participant information and a consent form describing the study and providing sufficient information to make an informed decision about their infant's participation in the study (online supplemental data). They will be given ample time to decide whether or not to participate. If subjects are eligible and families are willing to participate, the consent form will be signed by the parents and the sponsor–investigator or the designated representative or completed online with a digital, dated signature. The consent form will be retained as part of the study records. Formal written consent of parents will be obtained before the infant is submitted to any study procedure. An electronic copy of the signed informed consent will be provided to the parents.

If an infant becomes seriously ill during the study (eg, requires major surgery or develops sepsis), they will be excluded, and the study intervention will be unblinded.

Patient and public involvement

Patients and the public were not involved in the design of this study. The results of this study will be disseminated to parents of the study participants via a participant newsletter distributed by email.

Randomisation

Participants within the preterm and term groups will be randomly allocated to intervention or control groups, using a web-based randomisation procedure, stratified by study centre, delivery mode and gestational age in randomly permuted blocks of variable length (4 or 8), enabling the prevention of predictability of intervention assignments. The randomisation lists will be generated prior to the initiation of the study by someone not involved in the study analysis and will be integrated into Research Electronic Data Capture (REDCap).

Blinding

Parents will be blinded to the intervention (SYN or PLC). The research team will be blinded to the group of infants when outcomes are measured.

Data statement section

All data collected by study personnel will be directly entered into the REDCap. Standard protocols will be used to collect data to ensure they are reliable and consistent. Source data will be collected from medical records (routinely collected data before and during delivery). All study personnel will be instructed, trained and supervised on a regular basis to correctly collect and enter data into the electronic database.

Participants are only identified by a unique participant number. The participant identification list will be stored by the principal investigators on the server of the university and will be protected from unauthorised or accidental disclosure by password.

Further, data will then be generated by parents through computer-assisted questionnaires. Answers from parents' questionnaires are directly linked to the electronic database which will minimise errors in data collection. The data is saved on a server provided by the university, which has adequate data protection in place. The final trial data set will be accessible to the principal investigators, co-investigators and designated members of the research team who are directly involved in the analysis and interpretation of the study results. Access to the data set will be governed by the study's data management plan, ensuring

that only authorised personnel can view or analyse the data.

An independent Data Safety and Monitoring Committee will be formed before the study begins, consisting of researchers who are independent of the sponsors and have no competing interests.

Metagenomic data will be shared through the European Nucleotide Archive (ENA).

Interim analyses and safety

Interim analyses will be conducted at predetermined intervals to assess safety, efficacy and overall trial progress. The first interim analysis will occur after 25% of participants have completed the study protocol, and subsequent analyses will be conducted after 50% and 75% of participants have completed the protocol. The purpose of these interim analyses is to identify any significant safety concerns, evidence of overwhelming efficacy or futility in continuing the trial.

Adverse events (AEs) will be actively monitored and collected throughout the study period. Investigators will record all solicited AEs as specified in the study protocol. Additionally, any spontaneously reported AEs, unexpected symptoms or unintended effects observed by investigators or reported by participants will also be documented. All AEs will be recorded in the case report forms and entered into the study database. Serious AEs will be reported to the study sponsor, ethics committee and regulatory authorities within 24 hours of becoming aware of the event. Regular summary reports of AEs will be provided to the Data Safety and Monitoring Committee for ongoing safety evaluation.

Study outcome measures

Outcome measures

All infants will undergo a set of basic assessments (details follow), while two subgroups will receive additional exploratory and more comprehensive evaluations: hdEEG during sleep (n=40) and exhalomics (n=40) (ie, n=10 per treatment group), each supplemented by ankle/wrist actimetry measures. We will use internationally accepted validated measures for clinical outcomes (table 1). The research team will be blinded to group allocation when outcomes are measured.

Questionnaires

We will distribute online surveys to record parental ancestry, sociodemographic data, smoking habits, family history of allergies, eczema, asthma and other immune disorders, and antenatal variables such as maternal age, diet, weight, underlying diseases, medication and supplementation use (eg, probiotics and vitamins). We will also collect perinatal (gestational age at birth, delivery mode, birth weight, height and cranial perimeter, duration of hospitalisation, oxygen administration, infections, with particular attention to the use of antibiotics and other medication), dietary (breastfeeding, age at introduction of formula and new foods, use of probiotics and vitamins), medical (vaccinations, use of antibiotics and other medications, medical visits, illnesses including infections, allergies, eczema and hospital admissions) and environmental data (number of siblings, child care attendance, pets and farm animals). Specific questionnaires focusing on the child's sleep behaviour and attitude towards parenting (schedules) will be used. Data will be stored using the REDCap database.[70]

Sleep

Sleep neurophysiology (hdEEG) will be assessed during home visits (sponge electrode nets, 124 electrodes, Electrical Geodesics Sensor Net, Electrical Geodesics; amplifier and software MES, Brain Products) following infants' habitual timing of evening sleep.[71] Impedances will be kept below 50 kΩ, and data will be referenced to the vertex and sampled at 500 Hz (filtered 0.01–200 Hz). Electrodes will be removed after a maximum of 120 min, or sooner if the infant remains unsettled after waking. Actimetry (GENEActiv) will be used to assess habitual sleep–wake behaviour based on infant leg (or arm) movement, in conjunction with a 24-hour sleep-wake protocol conducted over up to 10 days.[72] This method has good agreement with gold-standard sleep parameters[73] (sensitivity 74–99%, specificity 59–77%).[74]

Cognitive development

Neurobehaviour. During the visit at the clinic, trained team members will use BSID[75] to capture motor, language and cognitive development (BSID-III, 45–60 min per session; video recordings will record different angles to code infant behaviour off-line). Eye tracking. A software using a webcam-linked eye tracker will be implemented to explore and assess visual attention at home through computer vision and machine learning.[76]

Secondary outcomes

Eczema: the presence of eczema will be evaluated using the UK diagnostic tool on the online questionnaires and at clinical assessments during study visits.[77–79] Age of onset, use of topical steroids, and severity will be evaluated (POEM score[80] in questionnaires, SCORAD during study visits).[81] Food allergy: food allergies will be evaluated as a parent report of a doctor-diagnosed food allergy with or without a positive skin prick test to the relevant food allergen to capture both immunoglobulin (Ig)E and non-IgE–mediated food allergy phenotypes. Acute otitis media (AOM), lower respiratory tract infection (LRTI), urinary tract infection (URTI): Number of episodes of AOM and URTI will be evaluated by parent report of doctor-diagnosed episodes. Symptoms of LRTI (such as cough and wheezing with or without fever)[82 83] will be recorded by parents in the questionnaires. Antibiotic exposure: antibiotic intake, including the name of the drug, dose, administration route, interval and a number of doses, will be recorded by parents and verified by patients' records if necessary. Hospitalisation for infection: number and reason for hospitalisation will be

Table 1 Primary outcome measures for infant cohorts (m=age in months)

Time (chronological age)		0 m	2 m	4 m	6 m	12 m	24 m
Assessments for primary outcomes							
Questionnaires	Sociodemographic, perinatal, dietary and medical data		✓	✓	✓	✓	✓
Sleep	Brief Infant Sleep Questionnaire (BISQ)[125]		✓	✓	✓	✓	✓
	Sleep hdEEG, home visit (subgroupEEG)[68]				(✓)		
	Actimetry and sleep-wake diary (subgroupEEG, subgroupBreath already at 6 m)[5]				(✓)	(✓)	(✓)
Neurobehaviour	Bayley Scales of Infant Development[75]						✓
	Infant Behaviour Questionnaire (IBQ)[126]				✓	✓	✓
	Eye tracking (subgroupEEG, subgroupBreath)[76]				(✓)		
Microbiota and metabolome	Stool sample[69]	✓	✓	✓	✓	✓	✓
Breath metabolome	Exhalomics (subgroupBreath)[81]	(✓)			(✓)	(✓)	(✓)
Assessments for secondary outcomes							
Questionnaires	Eczema[77–79]		✓	✓	✓	✓	✓
	Food allergy		✓	✓	✓	✓	✓
	Acute otitis media, lower respiratory tract infection, urinary tract infection, antibiotic intake (drug, dose and length), hospitalisations for infection[82 83]	✓	✓	✓	✓	✓	✓
	Weight, height and head circumference[84]	✓	✓	✓	✓	✓	✓
Clinical examination	Structured interview, clinical eczema assessment[80 81]					✓	✓
Skin prick test	Allergic sensitisation (optional)[85]						(✓)
Microbiota	Breast milk sample for biobank (optional)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)
	Nasal swab for biobank (optional)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)
	Oral swab for biobank (optional)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)
Blood	Serum for biobank (optional)					(✓)	(✓)

The 6-month assessment will be done in a home visit, the 12-month and 24-month assessments during a hospital visit.
hdEEG, high-density electroencephalogram.

recorded by parents and verified by patients records if necessary. Growth: weight, height and head circumference will be recorded by parents and assessed during the clinical examination. The WHO Child Growth Standards will be used as a reference for percentiles.[84] Skin prick tests: sensitisation to the following panel of allergens will be in children whose parents consent to this component of the study: cow's milk, egg, peanut, sesame, house dust mite (*Dermatophagoides pteronyssinus 1*), cat, dog and grass pollen. Skin prick allergy testing will be performed according to standard guidelines.[85]

Clinical examination

Children will be reviewed in a specially designated clinic by a study nurse or doctor using a structured interview and clinical eczema assessment.

Sample collection and storage

Stool: a meconium sample (5–10 g) will be collected from infants as soon as possible after birth by a study nurse. Parents will then be asked to collect stool samples from their infants at the remaining time points and freeze immediately. To minimise variation, parents are asked to collect stool from the first bowel movement of the day (with date and time recorded). Nasal and oral swabs, breast milk: study nurses will collect nasal and oral swabs and 1–2 mL of breast milk as soon as possible after birth. Mothers will be instructed to collect nasal and oral swabs and approximately 5 mL of breast milk before the first feed of the day (a minimum of 2 hours required to the previous feed) when infants are 2, 4 and 6 months old. Mothers will be instructed to meticulously wash their hands and breasts and manually extract breast milk without touching the areola. The first few drops will be discarded. eNAT tubes (Copan, Italy) for the swabs and sterile containers for stool and milk will be provided. Storage of samples: parents will be instructed to freeze stool, breast milk and nasal and oral swabs in their domestic freezer at approximately −20°C. Samples will be collected by the research

team, kept frozen during transportation to the laboratory where they will be aliquoted and stored at −80°C. Blood: if blood is drawn during routine care in the first week of life, we will ask for the parent's permission to store 1–2 mL. During the clinical examination at 12 and 24 months of age, blood will be collected by fingertip puncture from children whose parents consent to this component of the study. Biobank: nasal and oral swabs and breast milk will be stored in the Swiss Biobanking Platform (VITA label) for future projects to evaluate the effect of the microbiota on the immune system.

EEG data preprocessing, sample preparation, variable extraction

Sleep

Basic variables quantifying sleep rhythm will be computed from surveys.[86] EEG data will be preprocessed according to in-laboratory standards, including bandpass filtering 0.5–50 Hz, downsampling, sleep stage scoring,[87] semi-automatic artefact rejection, data re-referencing; and hdEEG processing will be completed[71 88] to compute sleep architecture, spectral analysis, slow wave activity and wave morphology. Neurodevelopmental markers will be derived on a topographical dimension.[89] Actigraphy will be calibrated according to in-laboratory standards[5 72 90] to compute markers of rhythm, for e.g., circadian function index.[91]

Stool DNA extraction

DNA from stool samples (approximately 200 mg) will be extracted using the MagMAX Microbiome Nucleic Acid Isolation Kit (including bead beating). Concentrations will be quantified using a Qubit 4.0 fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and high-sensitivity reagents. Bacteria and fungi will be quantified by broad-range quantitative PCR.

Shotgun metagenome sequencing

Library preparation will be done using Nextera DNA Flex Library Preparations Kits. Extracted DNA will be indexed with IDT Illumina Nextera DNA Unique Dual Indexes to allow analysis of pooled samples. 150 bp pair-end sequencing will be done using an Illumina NextSeq or NovaSeq. The required sequencing depth to provide adequate coverage of microbial communities for taxonomic profiling will be determined by rarefaction curves. We will aim for a minimum yield of 5×10^7 read pairs per sample. Appropriate negative controls (including sampling and extraction blanks, etc) and positive controls of mock communities will be included. These controls will be sequenced with the samples to identify potential environmental and laboratory contaminants.

Exhalomics

Breath samples from a subset of infants (n=40 taken up to five times within a 24-hour day and across four time points 0, 6, 12, 24 months) will be analysed using offline metabolomics analysis using MS to capture diurnal compound dynamics using a secondary electrospray ionisation (SESI) source purchased from Fossil Ion Technology SL, coupled with a high-resolution MS system (Q Exactive Orbitrap, Thermo Fisher Scientific). SESI-MS is a well-established and robust analytical technology that was especially developed for in-depth breath characterisation, with demonstrated applications in adults and infants.[92–100] 1 L sampling bags of gas molecules obtained with a paediatric non-invasive face mask are shipped/transported to the laboratory for analysis SESI-MS; a sampling bag desampling system allows collected breath molecules to pass through a short-length heated sample transfer line into the ionisation chamber for charge transfer and ionisation. The produced charged breath molecules are then introduced into the mass analyser for mass-to-charge separation and detection. Measurements are carried out both in the positive and negative ion mode, which facilitates the determination of protonated and deprotonated species. Mass calibration of the system in both ionisation modes is performed once per week or more often if required. Mass spectra are recorded in the full mass range of interest (m/z 50–500). SESI-MS operates at ambient pressure and does not suffer from fragmentation issues, enabling tandem MS^2 analysis and reliable compound identification. It is characterised by operational simplicity, high resolving power (240 000), high mass accuracy, low limits of detection (sub ppt_v) and low limits of quantification.[97] In addition, SESI allows the analysis of high molecular weight compounds (up to 1000 Da) with high polarity, delivering clear molecular discrimination and identification in the mass region of our interest.

Targeted and untargeted metabolomics analysis

Stool and blood samples will be analysed with standardised Liquid Chromatography-Mass Spectrometry (LC-MS) methods (reversed-phase and Hydrophilic Interaction Liquid Chromatography (HILIC)) for orthogonal metabolite coverage and chemical identification using spectral libraries. For LC-MS measurements, the sample preparation and analysis order will be randomised to avoid drifts due to preparatory artefacts. Quality control samples and blanks will also be injected with the study samples and used for the filtering and quality-control steps of the data analysis.

Metagenome data analysis

Data will be analysed and processed (quality control, denoising) and analysed using the QIIME 2[101] microbiome bioinformatics platform and MOSHPIT (MOdular SHotgun metagenome Pipelines with Integrated provenance Tracking) metagenomics toolkit (https://moshpit.readthedocs.io/). Host sequences will be removed by mapping against the Human genome with Bowtie2[102] and Kraken2.[103] Metagenome data will be filtered and trimmed using Trimmomatic,[104] assembled using metaSPAdes,[105] binned with metaBat2,[106] annotated with metaProdigal[107] and eggNOG[108] and taxonomically binned with Kraken2[103] and Genome Taxonomy Database (GTDB) taxonomy.[109]

Data (excluding any human reads) will be released on publication through the ENA. Shotgun sequencing is chosen here to profile broad-spectrum microbiota composition as well as genome content, enabling assessment of microbe-health interactions at the level of individual microbial genes and genomes. Viral metagenomes will be analysed using VirSorter2[110] for viral sequence discovery, quality control with ViromeQC,[111] genome assembly with MetaviralSPAdes,[112] and host range prediction with Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) spacer matching,[113] the BacteriophageHost-Prediction tool[114] and Virus-host-DB.[115] Diversity analysis will use the QIIME 2[101] microbiome bioinformatics platform. Differences in beta diversity will be compared using permutational analysis of variance (PERMANOVA), permdisp and variance homogeneity will be tested by analysis of variance to centroids. Beta diversity differences will be visualised using principal coordinates analysis and t-SNE clustering. Differential abundance testing will use specialised tests that account for the high dimensionality, sparsity and compositionality of metagenome data (Aldex2[116] and ANCOM-BC).[117]

Statistical power calculation

The required sample size and effect size were calculated using the software package evident[118] based on independent t-tests and Cohen's D test, respectively, to determine a significant difference in beta diversity (Bray Curtis dissimilarity). An effect size of 0.36 was estimated using data from a previous study of SYN treatment in European infants.[119] Assuming a power of 0.8, alpha=0.05 and effect size of 0.36, our sample size (n=380 infants) exceeds the predicted minimum sample size (~240), and that of previous infant cohort studies (both observational and those with prebiotic/probiotic interventions) in which significant differences in microbiota were observed (eg,[3 35 120–123]). Subgroup sample size for EEG assessment was estimated in relation to feasibility and existing at-home infant sleep hdEEG assessments with healthy infants.[68]

Statistical analysis

Microbial variables will be examined in relation to infant sleep and behavioural/health outcomes using PERMANOVA, linear mixed effect models (with individual and time as random effects to account for interindividual variations) and random forest models to examine predictive links between longitudinal microbiome maturation and health outcomes.[3] Gestational age, delivery mode and SYN/PLC will be used for grouping, and comparisons are performed for all time points. A factorial design will be used to evaluate outcomes of the four intervention groups and quantify the effects for each intervention separately or in combination, that is, PRET-SYN, PRET-PLC, TERM-SYN, TERM-PLC. Outcomes will be analysed using multivariable regression and binary regression models, adjusted for the stratification factor of birth age and reported with 95% CIs. Analyses will follow the intention-to-treat principle, ensuring that all randomised participants are analysed in their assigned intervention groups, regardless of adherence, to maintain the benefits of randomisation and minimise bias. The average of the two interventions will be weighted according to the sample sizes and adjusted for the other intervention as well as covariates. Age-normative microbiota composition will be assessed using supervised learning models trained on external, public reference data sets that predict an infant's age based on microbiota composition and calculate a Z-score or similar metric to report deviation in predicted age or maturity, similar to previously reported methods.[124]

Missing data

If the fraction of missing data is less than 5%, the primary analysis will be a complete case analysis. If not, the rate and patterns of missing data will be examined and, if appropriate, multiple imputation models will be applied for the outcome variables.

Drop-outs

We expect high compliance and minimal missing data with maximal participant retainment across the 24-month data collection, supported through user-friendly online questionnaires, email reminders and only two study visits per participant. Study personnel will be thoroughly trained and will follow-up with parents to provide data and samples. Missing data, if any, will be assumed random. Depending on the extent of missing data, case deletion or multiple imputation will be used, and reasons for missing data will be reported in the study results.

Ethics and dissemination

Ethics approval

The NapBiome trial has received ethical approval by the Committee of Northwestern and Central Switzerland and Canton Vaud, Switzerland (#2024–01681). The findings of the study will be disseminated through multiple channels. The primary results will be submitted for publication in peer-reviewed scientific journals and presented at national and international conferences related to paediatrics, infectious diseases, microbiome research and sleep. Additionally, findings will be communicated to healthcare professionals and policymakers through targeted presentations and workshops. Public engagement will be facilitated through media releases and outreach activities to promote awareness of the study's findings.

Metagenomic sequencing data generated from the study will be shared in an open-access format through the ENA to support transparency and further research in the field. Additional de-identified data sets will be available on reasonable request to the study investigators, following institutional and ethical guidelines.

DISCUSSION

The NapBiome trial is designed to address the gap in understanding the relationship between gut microbiota, sleep patterns and neurodevelopment in early childhood. By leveraging a rigorous, multicentre, double-blinded, PLC-controlled randomised trial with a factorial design, this study aims to provide insights into how SYN supplementation influences these interconnected biological processes. While existing literature suggests a role for the gut microbiome in neurodevelopment and sleep regulation, causality remains difficult to establish due to confounding factors and the complex interplay between host and microbial factors. Our study aims to disentangle these relationships through longitudinal assessments and multiomics integration, thereby contributing valuable data to this emerging field.

One of the major strengths of this study is its robust methodological design. The double-blinded, PLC-controlled nature of the trial minimises bias and enhances the reliability of findings. The inclusion of both preterm-born and term-born infants allows for the examination of differential effects across these populations, specifically testing a targeted intervention for an at-risk group. Furthermore, the integration of multiomics approaches, including metagenomics, metabolomics and exhalomics, enables a comprehensive assessment of gut microbiota composition, functional metabolic outputs and their associations with sleep regulatory and neurodevelopmental parameters.

Another strength is the extensive and longitudinal data collection. The use of hdEEG, actigraphy, (neuro)developmental assessments and detailed parental questionnaires provides a unique multidimensional view of the intervention's effects. The inclusion of a 2-year follow-up period allows for the observation of sustained impacts beyond the immediate intervention phase, offering a complete picture of developmental trajectories.

Despite these strengths, there are inherent limitations to the study. First, the restriction of eligibility to partially breastfed infants may limit the generalisability of findings to exclusively formula-fed or exclusively breastfed infants, who may have different gut microbiota profiles and responses to SYN supplementation. Additionally, while the 2-year follow-up is valuable, it may not capture potential developmental effects beyond this window, necessitating future studies with extended follow-up periods into school age and adolescence.

Another limitation is the complexity of gut microbiota interactions, which are influenced by numerous environmental and genetic factors. While we attempt to account for confounders through extensive metadata collection and stratification, residual confounding remains a possibility. Additionally, while metagenomic sequencing provides comprehensive taxonomic and functional insights, it cannot establish causal mechanisms on its own, for which follow-up targeted intervention trials will be required.

The expected outcomes of this study include a better understanding of how SYN supplementation influences gut microbiota composition and function, sleep/circadian regulation and neurodevelopment in early childhood. We anticipate identifying key microbial taxa and metabolites associated with improved sleep regulation and neurodevelopmental outcomes, which could inform future targeted interventions. Furthermore, the study's findings may contribute to the development of early-life microbiome-based strategies aimed at cognitive development, particularly in vulnerable populations such as preterm-born infants.

By elucidating the role of gut microbiota in early neurodevelopment, this study has the potential to inform clinical guidelines and public health strategies. If SYN supplementation is found to have beneficial effects, it could pave the way for larger-scale trials and implementation in neonatal care practices.

In conclusion, while this study is subject to certain limitations, its strengths in design, data collection and multiomics integration position it to make substantial contributions to understanding the gut–brain-sleep axis in early life. The insights gained from this research have the potential to shape future interventions aimed at supporting infant sleep regulation and (neuro)development through microbiota-targeted strategies.

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REFERENCES

1 Sen P, Molinero-Perez A, O'Riordan KJ, *et al*. Microbiota and sleep: awakening the gut feeling. *Trends Mol Med* 2021;27:935–45.
2 Schneider N, Mutungi G, Cubero J. Diet and nutrients in the modulation of infant sleep: A review of the literature. *Nutr Neurosci* 2018;21:151–61.
3 Bokulich NA, Chung J, Battaglia T, *et al*. Antibiotics, birth mode, and diet shape microbiome maturation during early life. *Sci Transl Med* 2016;8:343ra82.
4 Zimmermann P, Curtis N. Factors Influencing the Intestinal Microbiome During the First Year of Life. *Pediatr Infect Dis J* 2018;37:e315–35.
5 Schoch SF, Huber R, Kohler M, *et al*. Which are the Central Aspects of Infant Sleep? The Dynamics of Sleep Composites across Infancy. *Sensors (Basel)* 2020;20:7188.
6 Meltzer LJ, Williamson AA, Mindell JA. Pediatric sleep health: It matters, and so does how we define it. *Sleep Med Rev* 2021;57:101425.
7 Beijers R, Jansen J, Riksen-Walraven M, *et al*. Attachment and infant night waking: a longitudinal study from birth through the first year of life. *J Dev Behav Pediatr* 2011;32:635–43.
8 You J, Shamsi BH, Hao M-C, *et al*. A study on the neurodevelopment outcomes of late preterm infants. *BMC Neurol* 2019;19:108.
9 Durankus F, Aladag Ciftdemir N, Vatansever Ozbek U, *et al*. Comparison of sleep problems between term and preterm born preschool children. *Sleep Med* 2020;75:484–90.
10 Johnson S, Fawke J, Hennessy E, *et al*. Neurodevelopmental disability through 11 years of age in children born before 26 weeks of gestation. *Pediatrics* 2009;124:e249–57.
11 Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. *Lancet* 2008;371:261–9.
12 Brydges CR, Landes JK, Reid CL, *et al*. Cognitive outcomes in children and adolescents born very preterm: a meta-analysis. *Dev Med Child Neurol* 2018;60:452–68.
13 Franz AP, Bolat GU, Bolat H, *et al*. Attention-Deficit/Hyperactivity Disorder and Very Preterm/Very Low Birth Weight: A Meta-analysis. *Pediatrics* 2018;141:e20171645.
14 Pascal A, Govaert P, Oostra A, *et al*. Neurodevelopmental outcome in very preterm and very-low-birthweight infants born over the past decade: a meta-analytic review. *Dev Med Child Neurol* 2018;60:342–55.
15 Garrison MM. The feedback whirlpool of early childhood sleep and behavior problems. *JAMA Pediatr* 2015;169:525–6.
16 Touchette E, Dionne G, Forget-Dubois N, *et al*. Genetic and environmental influences on daytime and nighttime sleep duration in early childhood. *Pediatrics* 2013;131:e1874–80.
17 Taveras EM, Rifas-Shiman SL, Oken E, *et al*. Short sleep duration in infancy and risk of childhood overweight. *Arch Pediatr Adolesc Med* 2008;162:305–11.
18 Gregory AM, Caspi A, Eley TC, *et al*. Prospective longitudinal associations between persistent sleep problems in childhood and anxiety and depression disorders in adulthood. *J Abnorm Child Psychol* 2005;33:157–63.
19 Simola P, Liukkonen K, Pitkäranta A, *et al*. Psychosocial and somatic outcomes of sleep problems in children: a 4-year follow-up study. *Child Care Health Dev* 2014;40:60–7.
20 Mayer EA, Knight R, Mazmanian SK, *et al*. Gut microbes and the brain: paradigm shift in neuroscience. *J Neurosci* 2014;34:15490–6.
21 Jha SK, Jones BE, Coleman T, *et al*. Sleep-dependent plasticity requires cortical activity. *J Neurosci* 2005;25:9266–74.
22 Yano JM, Yu K, Donaldson GP, *et al*. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* 2015;161:264–76.
23 Carlson AL, Xia K, Azcarate-Peril MA, *et al*. Infant Gut Microbiome Associated With Cognitive Development. *Biol Psychiatry* 2018;83:148–59.
24 Buffington SA, Di Prisco GV, Auchtung TA, *et al*. Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. *Cell* 2016;165:1762–75.
25 Helander HF, Fändriks L. Surface area of the digestive tract - revisited. *Scand J Gastroenterol* 2014;49:681–9.
26 Robertson RC, Manges AR, Finlay BB, *et al*. The Human Microbiome and Child Growth - First 1000 Days and Beyond. *Trends Microbiol* 2019;27:131–47.
27 Renz H, Brandtzaeg P, Hornef M. The impact of perinatal immune development on mucosal homeostasis and chronic inflammation. *Nat Rev Immunol* 2011;12:9–23.
28 Zimmermann P, Curtis N. Prophylactic antibiotics after operative vaginal delivery. *Lancet* 2020;395.
29 Zimmermann P, Messina N, Mohn WW, *et al*. Association between the intestinal microbiota and allergic sensitization, eczema, and asthma: A systematic review. *J Allergy Clin Immunol* 2019;143:467–85.
30 Kilkkinen A, Virtanen SM, Klaukka T, *et al*. Use of antimicrobials and risk of type 1 diabetes in a population-based mother-child cohort. *Diabetologia* 2006;49:66–70.
31 Leo S, Cetiner OF, Pittet LF, *et al*. The association between the composition of the early-life intestinal microbiome and eczema in the first year of life. *Front Microbiomes* 2023;2.
32 Boutin RCT, Sbihi H, McLaughlin RJ, *et al*. Composition and Associations of the Infant Gut Fungal Microbiota with Environmental Factors and Childhood Allergic Outcomes. *MBio* 2021;12:e0339620.
33 Loewen K, Monchka B, Mahmud SM, *et al*. Prenatal antibiotic exposure and childhood asthma: a population-based study. *Eur Respir J* 2018;52:1702070.
34 Metsälä J, Lundqvist A, Virta LJ, *et al*. Mother's and offspring's use of antibiotics and infant allergy to cow's milk. *Epidemiology* 2013;24:303–9.
35 Fox M, Lee SM, Wiley KS, *et al*. Development of the infant gut microbiome predicts temperament across the first year of life. *Dev Psychopathol* 2022;34:1914–25.
36 Rothenberg SE, Chen Q, Shen J, *et al*. Neurodevelopment correlates with gut microbiota in a cross-sectional analysis of children at 3 years of age in rural China. *Sci Rep* 2021;11:7384.
37 Agha L, Staiger D, Brown C, *et al*. Association of Hospital Adoption of Probiotics With Outcomes Among Neonates With Very Low Birth Weight. *JAMA Health Forum* 2023;4:e230960.
38 Morgan RL, Preidis GA, Kashyap PC, *et al*. Probiotics Reduce Mortality and Morbidity in Preterm, Low-Birth-Weight Infants: A Systematic Review and Network Meta-analysis of Randomized Trials. *Gastroenterology* 2020;159:467–80.
39 Sharif S, Meader N, Oddie SJ, *et al*. Probiotics to prevent necrotising enterocolitis in very preterm or very low birth weight infants. *Cochrane Database Syst Rev* 2020;10:CD005496.
40 Ong TG, Gordon M, Banks SS, *et al*. Probiotics to prevent infantile colic. *Cochrane Database Syst Rev* 2019;3:CD012473.
41 Radke M, Picaud J-C, Loui A, *et al*. Starter formula enriched in prebiotics and probiotics ensures normal growth of infants and promotes gut health: a randomized clinical trial. *Pediatr Res* 2017;81:622–31.
42 Bertelsen RJ, Jensen ET, Ringel-Kulka T. Use of probiotics and prebiotics in infant feeding. *Best Pract Res Clin Gastroenterol* 2016;30:39–48.
43 Vandenplas Y, Zakharova I, Dmitrieva Y. Oligosaccharides in infant formula: more evidence to validate the role of prebiotics. *Br J Nutr* 2015;113:1339–44.
44 Sassone-Corsi M, Raffatellu M. No vacancy: how beneficial microbes cooperate with immunity to provide colonization resistance to pathogens. *J Immunol* 2015;194:4081–7.
45 Zimmermann P, Curtis N. The influence of probiotics on vaccine responses - A systematic review. *Vaccine (Auckl)* 2018;36:207–13.
46 Cho S, Samuel TM, Li T, *et al*. Interactions between *Bifidobacterium* and *Bacteroides* and human milk oligosaccharides and their associations with infant cognition. *Front Nutr* 2023;10:1216327.
47 Colombo J, Carlson SE, Algarín C, *et al*. Developmental effects on sleep-wake patterns in infants receiving a cow's milk-based infant formula with an added prebiotic blend: a Randomized Controlled Trial. *Pediatr Res* 2021;89:1222–31.

48 Nakakita Y, Tsuchimoto N, Takata Y, *et al*. Effect of dietary heat-killed Lactobacillus brevis SBC8803 (SBL88™) on sleep: a non-randomised, double blind, placebo-controlled, and crossover pilot study. *Benef Microbes* 2016;7:501–9.
49 Nocerino R, De Filippis F, Cecere G, *et al*. The therapeutic efficacy of Bifidobacterium animalis subsp. lactis BB-12® in infant colic: A randomised, double blind, placebo-controlled trial. *Aliment Pharmacol Ther* 2020;51:110–20.
50 Rianda D, Agustina R, Setiawan EA, *et al*. Effect of probiotic supplementation on cognitive function in children and adolescents: a systematic review of randomised trials. *Benef Microbes* 2019;10:873–82.
51 Boehme M, Guzzetta KE, Bastiaanssen TFS, *et al*. Microbiota from young mice counteracts selective age-associated behavioral deficits. *Nat Aging* 2021;1:666–76.
52 Thaiss CA, Zeevi D, Levy M, *et al*. Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell* 2014;159:514–29.
53 Malloy JN, Paulose JK, Li Y, *et al*. Circadian rhythms of gastrointestinal function are regulated by both central and peripheral oscillators. *Am J Physiol Gastrointest Liver Physiol* 2012;303:G461–73.
54 Heddes M, Altaha B, Niu Y, *et al*. The intestinal clock drives the microbiome to maintain gastrointestinal homeostasis. *Nat Commun* 2022;13:6068.
55 Voigt RM, Forsyth CB, Green SJ, *et al*. Circadian disorganization alters intestinal microbiota. *PLoS ONE* 2014;9:e97500.
56 Poroyko VA, Carreras A, Khalyfa A, *et al*. Chronic Sleep Disruption Alters Gut Microbiota, Induces Systemic and Adipose Tissue Inflammation and Insulin Resistance in Mice. *Sci Rep* 2016;6:35405.
57 Benedict C, Vogel H, Jonas W, *et al*. Gut microbiota and glucometabolic alterations in response to recurrent partial sleep deprivation in normal-weight young individuals. *Mol Metab* 2016;5:1175–86.
58 Everson CA, Toth LA. Systemic bacterial invasion induced by sleep deprivation. *Am J Physiol Regul Integr Comp Physiol* 2000;278:R905–16.
59 O'Mahony SM, Clarke G, Borre YE, *et al*. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behav Brain Res* 2015;277:32–48.
60 Borre YE, O'Keeffe GW, Clarke G, *et al*. Microbiota and neurodevelopmental windows: implications for brain disorders. *Trends Mol Med* 2014;20:509–18.
61 Clarke G, O'Mahony SM, Dinan TG, *et al*. Priming for health: gut microbiota acquired in early life regulates physiology, brain and behaviour. *Acta Paediatr* 2014;103:812–9.
62 Matsuda Y, Ozawa N, Shinozaki T, *et al*. Ergothioneine, a metabolite of the gut bacterium Lactobacillus reuteri, protects against stress-induced sleep disturbances. *Transl Psychiatry* 2020;10:170.
63 Heath A-LM, Haszard JJ, Galland BC, *et al*. Association between the faecal short-chain fatty acid propionate and infant sleep. *Eur J Clin Nutr* 2020;74:1362–5.
64 Kaczmarek JL, Musaad SM, Holscher HD. Time of day and eating behaviors are associated with the composition and function of the human gastrointestinal microbiota. *Am J Clin Nutr* 2017;106:1220–31.
65 Szentirmai É, Millican NS, Massie AR, *et al*. Butyrate, a metabolite of intestinal bacteria, enhances sleep. *Sci Rep* 2019;9:7035.
66 Leone V, Gibbons SM, Martinez K, *et al*. Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. *Cell Host Microbe* 2015;17:681–9.
67 Yan R, Murphy M, Genoni A, *et al*. Does Fibre-fix provided to people with irritable bowel syndrome who are consuming a low FODMAP diet improve their gut health, gut microbiome, sleep and mental health? A double-blinded, randomised controlled trial. *BMJ Open Gastroenterol* 2020;7:e000448.
68 Schoch SF, Castro-Mejía JL, Krych L, *et al*. From Alpha Diversity to Zzz: Interactions among sleep, the brain, and gut microbiota in the first year of life. *Prog Neurobiol* 2022;209:102208.
69 Volery M, Scherz V, Jakob W, *et al*. Study protocol for the ABERRANT study: antibiotic-induced disruption of the maternal and infant microbiome and adverse health outcomes - a prospective cohort study among children born at term. *BMJ Open* 2020;10:e036275.
70 Harris PA, Taylor R, Minor BL, *et al*. The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform* 2019;95:103208.
71 Kurth S, Ringli M, Geiger A, *et al*. Mapping of cortical activity in the first two decades of life: a high-density sleep electroencephalogram study. *J Neurosci* 2010;30:13211–9.
72 Schoch SF, Jenni OG, Kohler M, *et al*. Actimetry in infant sleep research: an approach to facilitate comparability. *Sleep* 2019;42:zsz083.
73 te Lindert BHW, Van Someren EJW. Sleep Estimates Using Microelectromechanical Systems (MEMS). *Sleep* 2013;36:781–9.
74 Meltzer LJ, Montgomery-Downs HE, Insana SP, *et al*. Use of actigraphy for assessment in pediatric sleep research. *Sleep Med Rev* 2012;16:463–75.
75 McKee MLea. Bayley Scales of Infant Development: Third Edition. Springer, 2011.
76 Werchan DM, Thomason ME, Brito NH. OWLET: An automated, open-source method for infant gaze tracking using smartphone and webcam recordings. *Behav Res* 2023;55:3149–63.
77 Williams HC, Burney PG, Hay RJ, *et al*. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. I. Derivation of a minimum set of discriminators for atopic dermatitis. *Br J Dermatol* 1994;131:383–96.
78 Williams HC, Burney PG, Pembroke AC, *et al*. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. III. Independent hospital validation. *Br J Dermatol* 1994;131:406–16.
79 Pittet LF, Messina NL, Gardiner K, *et al*. Prevention of infant eczema by neonatal Bacillus Calmette-Guérin vaccination: The MIS BAIR randomized controlled trial. *Allergy* 2022;77:956–65.
80 Charman CR, Venn AJ, Williams HC. The patient-oriented eczema measure: development and initial validation of a new tool for measuring atopic eczema severity from the patients' perspective. *Arch Dermatol* 2004;140:1513–9.
81 Schmitt J, Langan S, Williams HC, *et al*. What are the best outcome measurements for atopic eczema? A systematic review. *J Allergy Clin Immunol* 2007;120:1389–98.
82 Kusel MMH, de Klerk NH, Holt PG, *et al*. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life: a birth cohort study. *Pediatr Infect Dis J* 2006;25:680–6.
83 Oddy WH, de Klerk NH, Sly PD, *et al*. The effects of respiratory infections, atopy, and breastfeeding on childhood asthma. *Eur Respir J* 2002;19:899–905.
84 The WHO child growth standards. Available: https://www.who.int/childgrowth/standards/en/ [Accessed 02 Aug 2024].
85 Bernstein IL, Storms WW. Practice parameters for allergy diagnostic testing. Joint Task Force on Practice Parameters for the Diagnosis and Treatment of Asthma. The American Academy of Allergy, Asthma and Immunology and the American College of Allergy, Asthma and Immunology. *Ann Allergy Asthma Immunol* 1995;75:543–625.
86 Markovic A, Mühlematter C, Beaugrand M, *et al*. Severe effects of the COVID-19 confinement on young children's sleep: A longitudinal study identifying risk and protective factors. *J Sleep Res* 2021;30:e13314.
87 Iber C, Ancoli-Israel S, Chesson AL, *et al*. The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. Westchester, IL: American Academy of Sleep Medicine, 2007.
88 Kurth S, Riedner BA, Dean DC, *et al*. Traveling Slow Oscillations During Sleep: A Marker of Brain Connectivity in Childhood. *Sleep* 2017;40:zsx121.
89 Jaramillo V, Schoch SF, Markovic A, *et al*. An infant sleep electroencephalographic marker of thalamocortical connectivity predicts behavioral outcome in late infancy. *Neuroimage* 2023;269.
90 Sadeh A, Acebo C, Seifer R, *et al*. Activity-based assessment of sleep-wake patterns during the 1st year of life. *Infant Behavior and Development* 1995;18:329–37.
91 Ortiz-Tudela E, Martinez-Nicolas A, Campos M, *et al*. A new integrated variable based on thermometry, actimetry and body position (TAP) to evaluate circadian system status in humans. *PLoS Comput Biol* 2010;6:e1000996.
92 He J, Sinues PM-L, Hollmén M, *et al*. Fingerprinting breast cancer vs. normal mammary cells by mass spectrometric analysis of volatiles. *Sci Rep* 2014;4:5196.
93 Tejero Rioseras A, Singh KD, Nowak N, *et al*. Real-Time Monitoring of Tricarboxylic Acid Metabolites in Exhaled Breath. *Anal Chem* 2018;90:6453–60.
94 García-Gómez D, Martínez-Lozano Sinues P, Barrios-Collado C, *et al*. Identification of 2-alkenals, 4-hydroxy-2-alkenals, and 4-hydroxy-2,6-alkadienals in exhaled breath condensate by UHPLC-HRMS and in breath by real-time HRMS. *Anal Chem* 2015;87:3087–93.
95 García-Gómez D, Gaisl T, Bregy L, *et al*. Secondary electrospray ionization coupled to high-resolution mass spectrometry reveals tryptophan pathway metabolites in exhaled human breath. *Chem Commun (Camb)* 2016;52:8526–8.

96 Gaugg MT, Gomez DG, Barrios-Collado C, *et al*. Expanding metabolite coverage of real-time breath analysis by coupling a universal secondary electrospray ionization source and high resolution mass spectrometry--a pilot study on tobacco smokers. *J Breath Res* 2016;10:016010.
97 Bruderer T, Gaugg MT, Cappellin L, *et al*. Detection of Volatile Organic Compounds with Secondary Electrospray Ionization and Proton Transfer Reaction High-Resolution Mass Spectrometry: A Feature Comparison. *J Am Soc Mass Spectrom* 2020.
98 Nowak N, Engler A, Thiel S, *et al*. Validation of breath biomarkers for obstructive sleep apnea. *Sleep Med* 2021;85:75–86.
99 Bregy L, Müggler AR, Martinez-Lozano Sinues P, *et al*. Differentiation of oral bacteria in in vitro cultures and human saliva by secondary electrospray ionization - mass spectrometry. *Sci Rep* 2015;5:15163.
100 Decrue F, Singh KD, Gisler A, *et al*. Combination of Exhaled Breath Analysis with Parallel Lung Function and FeNO Measurements in Infants. *Anal Chem* 2021;93:15579–83.
101 Bolyen E, Rideout JR, Dillon MR, *et al*. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 2019;37:852–7.
102 Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 2012;9:357–9.
103 Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. *Genome Biol* 2019;20:257.
104 Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;30:2114–20.
105 Nurk S, Meleshko D, Korobeynikov A, *et al*. metaSPAdes: a new versatile metagenomic assembler. *Genome Res* 2017;27:824–34.
106 Kang DD, Li F, Kirton E, *et al*. MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. *PeerJ* 2019;7:e7359.
107 Hyatt D, LoCascio PF, Hauser LJ, *et al*. Gene and translation initiation site prediction in metagenomic sequences. *Bioinformatics* 2012;28:2223–30.
108 Huerta-Cepas J, Szklarczyk D, Heller D, *et al*. eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Res* 2019;47:D309–14.
109 Parks DH, Chuvochina M, Waite DW, *et al*. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. *Nat Biotechnol* 2018;36:996–1004.
110 Guo J, Bolduc B, Zayed AA, *et al*. VirSorter2: a multi-classifier, expert-guided approach to detect diverse DNA and RNA viruses. *Microbiome* 2021;9:37.
111 Zolfo M, Pinto F, Asnicar F, *et al*. Detecting contamination in viromes using ViromeQC. *Nat Biotechnol* 2019;37:1408–12.
112 Antipov D, Raiko M, Lapidus A, *et al*. Metaviral SPAdes: assembly of viruses from metagenomic data. *Bioinformatics* 2020;36:4126–9.
113 Edwards RA, McNair K, Faust K, *et al*. Computational approaches to predict bacteriophage-host relationships. *FEMS Microbiol Rev* 2016;40:258–72.
114 Boeckaerts D, Stock M, Criel B, *et al*. Predicting bacteriophage hosts based on sequences of annotated receptor-binding proteins. *Sci Rep* 2021;11:1467.
115 Mihara T, Nishimura Y, Shimizu Y, *et al*. Linking Virus Genomes with Host Taxonomy. *Viruses* 2016;8:66.
116 Fernandes AD, Reid JN, Macklaim JM, *et al*. Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. *Microbiome* 2014;2:15.
117 Lin H, Peddada SD. Analysis of compositions of microbiomes with bias correction. *Nat Commun* 2020;11:3514.
118 Rahman G, McDonald D, Gonzalez A, *et al*. Determination of Effect Sizes for Power Analysis for Microbiome Studies Using Large Microbiome Databases. *Genes (Basel)* 2023;14:1239.
119 Lagkouvardos I, Intze E, Schaubeck M, *et al*. Early life gut microbiota profiles linked to synbiotic formula effects: a randomized clinical trial in European infants. *Am J Clin Nutr* 2023;117:326–39.
120 Chichlowski M, Bokulich N, Harris CL, *et al*. Effect of Bovine Milk Fat Globule Membrane and Lactoferrin in Infant Formula on Gut Microbiome and Metabolome at 4 Months of Age. *Curr Dev Nutr* 2021;5:nzab027.
121 Shulman RJ, Chichlowski M, Orozco FG, *et al*. Infant behavioral state and stool microbiome in infants receiving Lactocaseibacillus rhamnosus GG in formula: randomized controlled trial. *BMC Pediatr* 2022;22:580.
122 Kaelin EA, Rodriguez C, Hall-Moore C, *et al*. Longitudinal gut virome analysis identifies specific viral signatures that precede necrotizing enterocolitis onset in preterm infants. *Nat Microbiol* 2022;7:653–62.
123 Samara J, Moossavi S, Alshaikh B, *et al*. Supplementation with a probiotic mixture accelerates gut microbiome maturation and reduces intestinal inflammation in extremely preterm infants. *Cell Host Microbe* 2022;30:696–711.
124 Subramanian S, Huq S, Yatsunenko T, *et al*. Persistent gut microbiota immaturity in malnourished Bangladeshi children. *Nature New Biol* 2014;510:417–21.
125 Sadeh A. A brief screening questionnaire for infant sleep problems: validation and findings for an Internet sample. *Pediatrics* 2004;113:e570–7.
126 Gartstein MA, Rothbart MK. Studying infant temperament via the Revised Infant Behavior Questionnaire. *Infant Behav Dev* 2003;26:64–86.