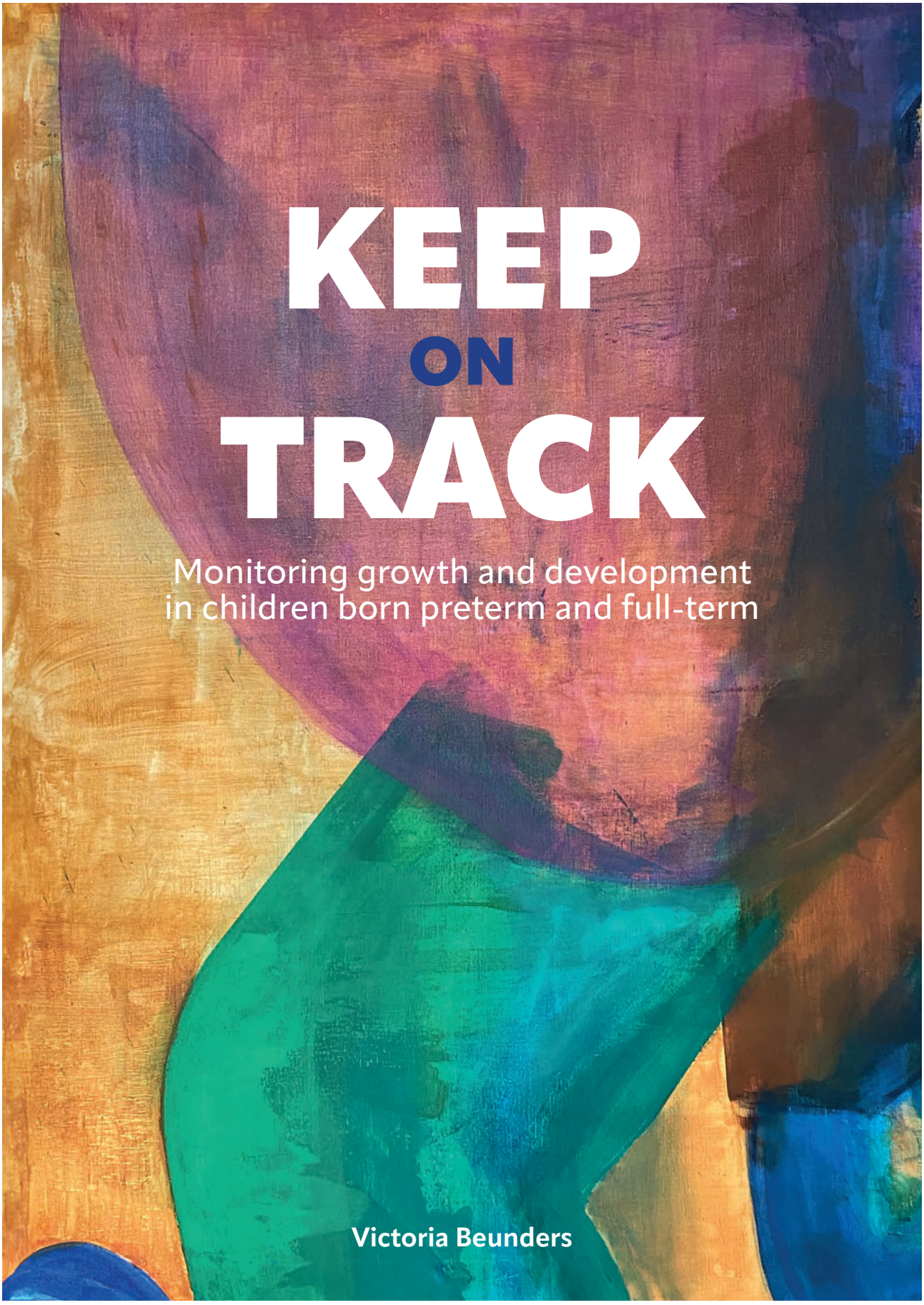
KEEP
ON
TRACK
Monitoring growth and development
in children born preterm and full-term
Victoria Beunders

KEEP on TRACK

Monitoring growth and development in children born preterm and full-term

VICTORIA BEUNDERS

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KEEP (ON) TRACK

Monitoring growth and development in
children born preterm and full-term

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Het monitoren van groei en ontwikkeling van
te vroeg en op tijd geboren kinderen

Proefschrift

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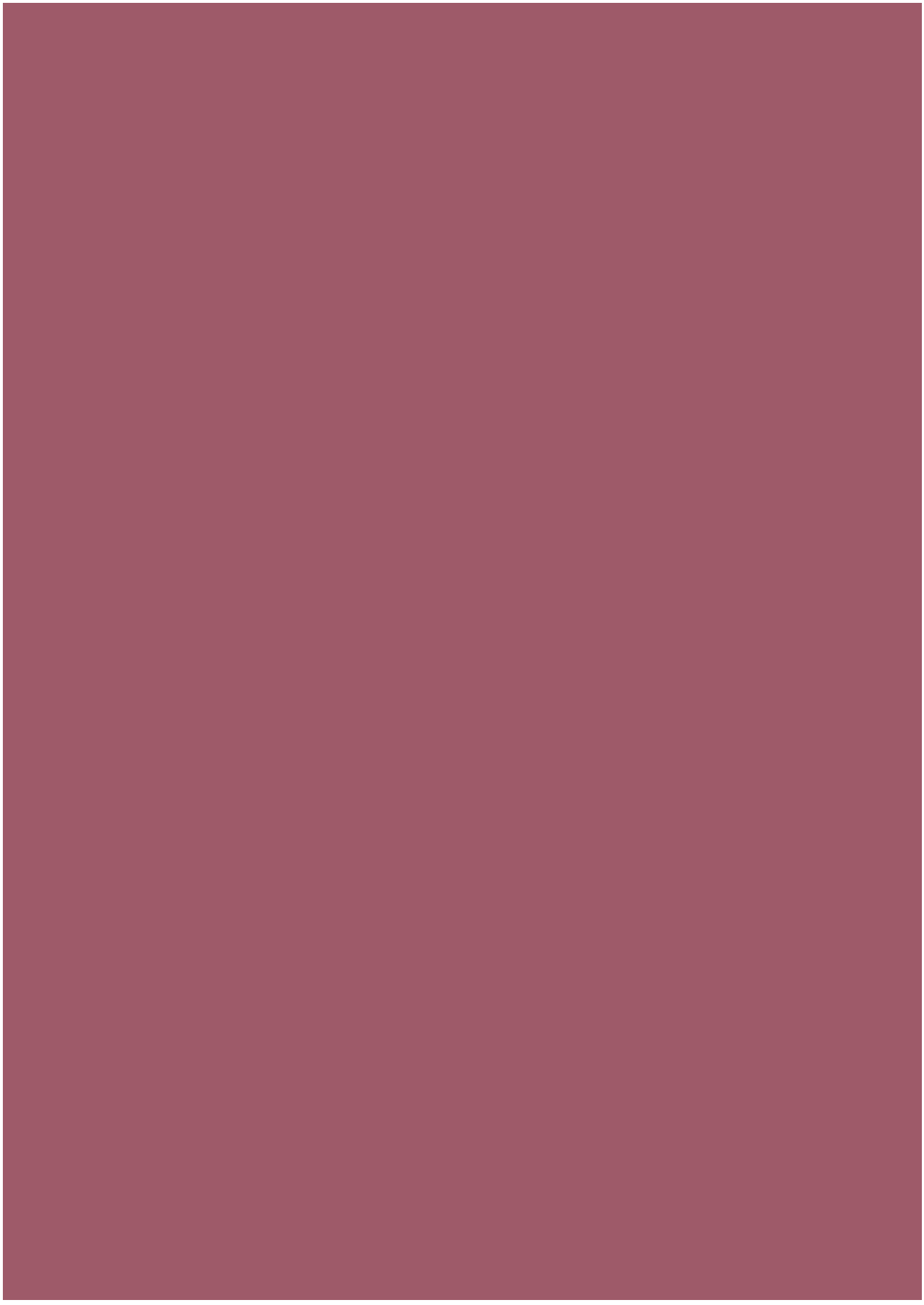

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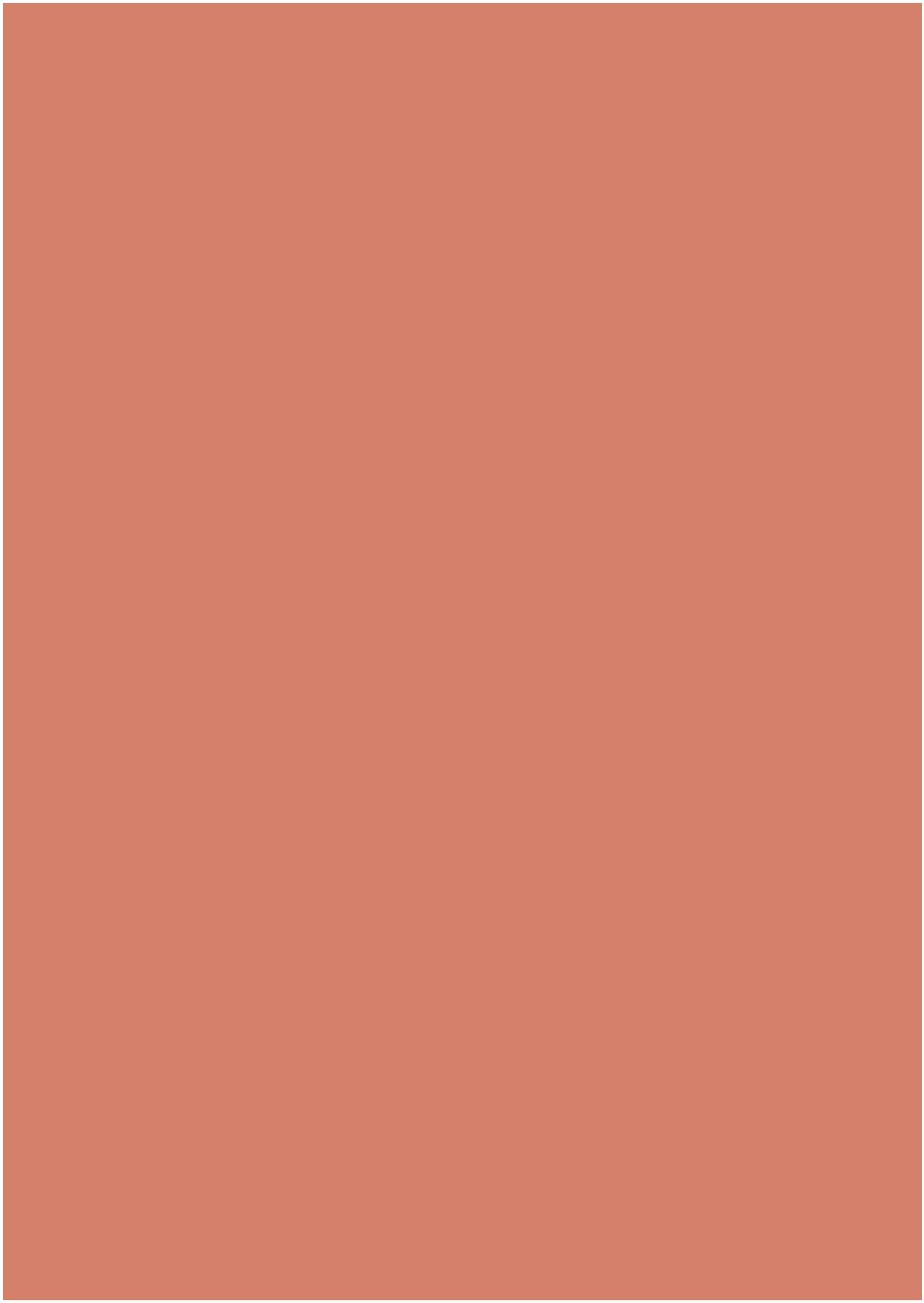


CHAPTER 1

General introduction

GENERAL INTRODUCTION

Early life as a crucial period for development

What happens during early life influences early development and growth, which in turn affects lifelong health. Adverse exposures or events during early fetal and infant life lead to internal adaptations to the changing environment, to increase chances of survival in that critical period. This hypothesis is incorporated in the Developmental Origins of Health and Disease (DOHaD) paradigm.[1] The DOHaD hypothesis states that those early critical developmental adaptations may have short or long-term consequences for disease later in life.[1,2] More recent studies have highlighted the crucial (mediating) role of epigenetic modifications such as DNA methylation in the process of this early life adaptive 'programming' in relation to later cardiovascular health.[3] The first 1000 days of life, calculated from conception to approximately 2 years of age, are considered most critical and sensitive to external influences.[4-6] Examples of adverse exposures during early life with increased risk of affecting the offspring's phenotype and health include impaired maternal health during pregnancy, fetal and infant growth restriction, and preterm birth. In this thesis, we will mainly focus on early fetal and infant growth and preterm birth, and their effects on child development up to school-age. We were particularly interested in the following developmental domains: neurodevelopment, sleep and 24-hour rhythms, and cardiometabolic health (e.g. adiposity and body composition), and their interdependence.

Preterm birth

Preterm birth is defined as birth before 37 weeks of gestation. In the Netherlands, approximately 7% of live born neonates are born preterm.[7] Preterm birth is considered the number one cause of perinatal morbidity and mortality in the developed world. Worldwide, approximately 15 million neonates are born preterm each year, with 1 million children dying due to preterm birth before the age of 5 years.[8] While improvements in care, such as better ventilation techniques and new pharmaceuticals, have greatly enhanced survival over the last decades, morbidity rates have only slightly decreased.[9-11] Most infants born preterm are admitted to a hospital for a prolonged time. Complications during hospital admission and thereafter include short-term effects such as impaired growth, infection, bronchopulmonary disease, patent ductus arteriosus, necrotizing enterocolitis, or retinopathy of prematurity. In the long term, lasting effects such as adverse cardiovascular health, behavior disorders or impaired academic performance are commonly seen in adulthood.[12,13] The growing health burden and increasing costs of healthcare for infants born preterm call for efficient and cost-effective follow-up programs and treatment, so targeted research is essential.[14,15]

Early fetal and infant growth

In infants born both full-term and preterm, optimal fetal and infant growth is fundamental for optimal development of the body and brain.[16-18] The most important condition for fetal growth is placental function, but also other environmental factors such as chronic inflammation (e.g. due to maternal health or lifestyle) can have an adverse effect on growth.[19] In the Netherlands, the prevalence of being born small for gestational age (SGA) is 10%.[7] Growth restriction often persists after the neonatal period and is associated with long-term adverse health issues comparable to those related to preterm birth.[20] These issues include impaired neurocognitive outcome, behavioral problems, obesity and increased cardiovascular risk.[18,21,22] Postnatal growth, of both body and brain, is dependent on both environmental exposures and infant nutrition.[23] In very low birthweight preterm infants, early feeding practices depend on parenteral nutrition and tube feeding.

There is an ongoing debate about what is considered 'optimal growth' for preterm infants and how to find the right balance between promoting growth for optimal neurodevelopment while preventing 'overnutrition'.[24] An important aspect is that excessive catch-up growth (accelerated growth) could lead to adverse cardiovascular outcome later in life.[25] For a long time, there was a consensus that intra-uterine growth rates should be continued in the postnatal period.[26] In clinical practice, this turns out to be very challenging as almost all preterm infants experience (physiological) weight loss during the first days of admission to the NICU.[27] In addition, most infants experience comorbidities along the way, which cost them energy and hampers adequate intake and absorption. More recent studies and guidelines have stepped away from the aim to mimic intra-uterine growth, and have advocated for adjusted growth goals and growth curves for very preterm infants.[27,28] For example, recent advises include not losing more than 1 standard deviation score in weight and head circumference from birth to discharge, or avoiding large weight loss after birth followed by stabile growth to gradually reach full-term growth trajectories at a later stage (around 44 weeks).[27,28]

Early detection of children at risk of adverse development

To improve child health in the general and preterm population, we need to identify infants and children at risk of adverse development as early as possible. In The Netherlands, perinatal care for preterm infants is distributed over different types of hospitals based on gestational age (GA). Worldwide, these different types of neonatal wards are generally classified as being part of a level 1, 2, 3 or 4 hospital according to the 2012 statement 'Levels of neonatal care' by the American Academy of Pediatrics Committee on Fetus And Newborn .[29] In the Netherlands, infants born between 32-36

weeks GA are born in a level 2 hospital with a regular neonatal ward, whereas infants born between 24-32 weeks GA are born in a Neonatal Intensive Care Unit (NICU) of a level 3 or 4 academic hospital. When infants born very preterm (<32 weeks) are stable and older than 30 weeks GA, they are usually transported to a level 2 hospital (and sometimes level 1 hospital after that) where they stay until discharge home.

General Dutch postnatal care for children aged 0-18 years is organized by the Dutch Youth Health Care (JGZ). Between 0-4 years old, regular visits are scheduled at one of the many Centers for Youth and Family (CJG) located in every neighborhood, to monitor growth and development and to offer vaccinations as part of the National Immunization Program. Between 4-18 years, children are screened during school visits. Children born preterm participate in the regular JGZ program but are also offered extra follow-up visits at the level 2 hospital they stayed in. In addition, children born very preterm who stayed at a NICU are invited for extra follow-up visits at the academic level 3 or 4 hospital of birth. A national consensus statement on this follow-up was defined in the 'Recommendation National Neonatal Follow-up - NICU Follow-up'.[30] It describes an extensive follow-up program at 6, 12 and 24 months, 5 years and, if feasible, also at 8 years corrected age (CA). The indication for and intensity of the NICU follow-up program is based on several factors. These include gestational age (GA) (e.g. <28 or <30 weeks GA at birth), birth weight (e.g. <1000 grams, or <1500 grams and below 10th percentile), and the presence of severe perinatal cerebral damage.[30] NICU follow-up visits are multidisciplinary and generally include a neurological examination by a neurologist or neonatologist, age-specific testing of motor function by a qualified physiotherapist, and testing of cognitive, (visual)motor, language, behavior and executive functions by a qualified psychologist.

The neurodevelopmental tests most commonly used are described in **Table 1**.

Table 1. Neurodevelopmental tests used in the neonatal follow-up program in The Netherlands.

	Neurological	Motor	Cognitive	Language	Behavior	Executive functions	Visual-motor
6M*	-Amiel Tison - Touwen	AIMS					
12M	- Amiel Tison - Touwen	AIMS					
24M	- Amiel Tison - Touwen - Hempel	Bayley-III-NL	Bayley-III-NL	Lexi list	CBCL 1.5-5yr	BRIEF-P	BEERY VMI
5Y	- Amiel Tison - Touwen	MABC-2-NL	WPPSI-III/ IV-NL	WPPSI-III/ IV-NL	CBCL 1.5-5yr	- NEPSY-II-NL - BRIEF-2	BEERY VMI
8Y		MABC-2-NL	WISC-V-NL	WISC-V-NL	CBCL 6-18yr TRF	- NEPSY-II-NL - BRIEF-2	BEERY VMI

The exact selection of tests used may differ per hospital. *All ages are corrected for prematurity. Abbreviations: M = months, Y = years, AIMS = Alberta Infant Motor Scale,[31] Bayley-III-NL = Bayley Scales of Infant and Toddler Development Third Edition[32] Dutch version, Lexi List[33], CBCL = Child Behavior Checklist,[34] BRIEF = Behavior Rating Inventory of Executive Function (BRIEF-P = toddler version,[35] BRIEF-2 = Second Edition for ages >5 years[36]), Beery VMI = Beery Visual Motor Integration,[37] MABC-2-NL = Movement ABC Second Edition[38] Dutch version, WPPSI-III/IV-NL = Wechsler Preschool and Primary Scale of Intelligence Third/Fourth Edition[39] Dutch version (Dutch 4th edition issued in 2020), WISC-V-NL = Wechsler Intelligence Scales for Children Fifth Edition[40] Dutch version, TRF = Teachers Report Form,[34] NEPSY-II-NL = A Developmental NEuroPSYchological Assessment Second Edition[41] Dutch version.

Neurodevelopment

Preterm birth is associated with impaired neurodevelopment, with its risk diminishing with increasing gestational age at birth.[11] Often, but not always, neurodevelopment is related to brain injury in the neonatal period.[42] Multiple domains of neurodevelopment may be impaired: neurosensory, cognitive, motor, behavior, and visuospatial. During fetal and infant life, brain volume and brain growth are important indicators of later neurodevelopment.[23,43-45] Several screening methods are available in the fetal and infant period, and in childhood. Tools to monitor brain growth postnatally are tape measurement of head circumference, cranial ultrasonography, or magnetic resonance imaging. All methods have advantages (easy to use, precise) and disadvantages (expensive, invasive, not bedside, or serially available).

After the neonatal period, head circumference and neurological examination are common methods to monitor brain growth at the outpatient clinic, and thereby

predicting neurodevelopment.[46,47] At the age of 2 years CA, more extensive neurodevelopmental testing is possible, with the validated Bayley Scales of Infant and Toddler Development, third edition Dutch version (Bayley-III-NL), most often used for cognitive and motor evaluation.[32] However, this test requires experienced staff, is quite demanding for the child and has limited predictive power for later IQ.[48] Other screening methods in Dutch follow-up programs include the Lexi-list for language development and the Child Behavior Checklist, a screening tool for behavior problems, which can be filled out by the parents or caregivers at the comfort of their own home.[34] Lastly, in recent years, a new eye tracking-based method for visuospatial function and processing has been developed which could potentially be used as a predictive instrument for later neurodevelopment.[49-52]

Body composition

Predisposition for overweight and obesity can be developed as early as fetal life.[53] Growth of the fetus is monitored by two-dimensional ultrasounds, with measurements of femur length, abdominal circumference and brain circumference being most often performed. Postnatal growth is measured by taking regular weight, length and head circumference as part of standard care. In recent years, body composition evaluation using weight quality or fat status are considered more reliable markers of adiposity than weight for length or body mass index alone.[54-56] Several techniques to measure body composition are available.[57] Two commonly used methods are Dual energy X-ray Absorptiometry (DXA) and Air Displacement Plethysmography (ADP).[58] ADP calculates body composition by measuring body volume, using the inverse pressure-volume-relation and can be applied either in infants ≤6 months old and ≤8 kg using PEAPOD,[59,60] or in children ≥2 years and ≥12 kg using BODPOD.[61]

Cardiometabolic health

Cardiometabolic risk factors can already be identified from infancy onwards and are described to track from childhood to adulthood, thereby increasing the risk of cardiovascular disease later in life.[62-66] Cardiometabolic risk factors include high blood pressure, increased adiposity, increased glucose levels, and an impaired lipid profile. Children born preterm are at particular risk of developing adverse cardiometabolic health later in life.[13]

Sleep and 24-hour activity rhythms

Adequate sleep and 24-hour rhythms are vital for optimal functioning of the human body, both for neurodevelopment and behavior, and cardiometabolic health.[67] Disturbed sleep has been associated with cardiovascular morbidity and mortality in adulthood.[68,69] Also, 24-hour activity rhythms, including a late chronotype (being

a 'night owl' instead of an 'early bird') and social jetlag (mismatch between your natural biological rhythm and socially determined sleep pattern), have been linked to obesity and elevated blood pressure in adults.[70] Although previous studies mainly focused on adults, there is increasing evidence suggesting that associations of sleep and 24-hour activity rhythms with cardiometabolic risk factors are already present earlier in life.[71,72] However, most pediatric studies used sleep diaries or questionnaires, instead of more direct measurements like actigraphy, or focused on only one sleep measure, mostly sleep duration. Development of sleep and 24-hour activity rhythm starts early in fetal life.[73] Following the DOHaD paradigm, early adverse events in life, such as preterm birth or growth restriction, may have long-lasting effects. Therefore, it would be interesting to study perinatal factors such as preterm birth in relation to later sleep in childhood.

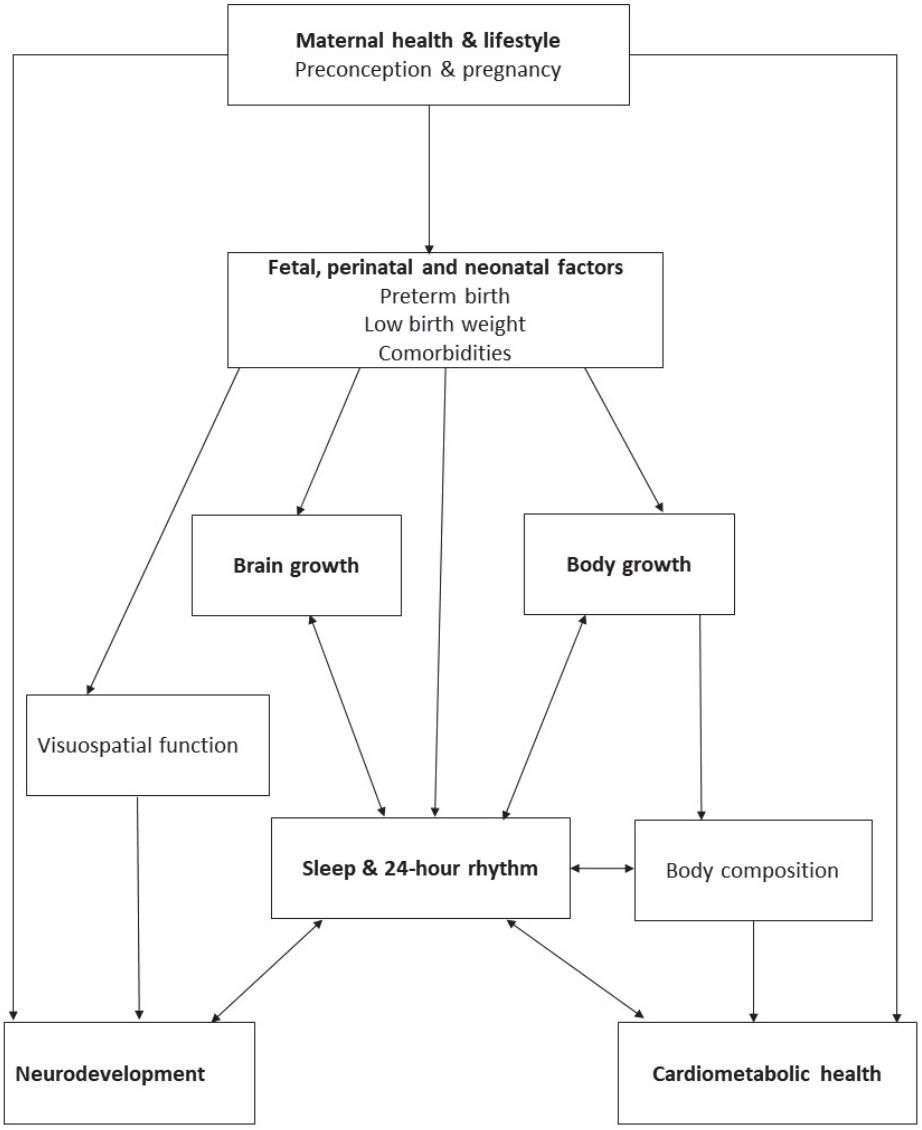
Challenges in pediatric screening

With an increasing number of tools and tests, it remains challenging which children to screen or test, and which not. The current neonatal follow-up programs are quite intensive yet still not complete in covering all developmental domains which could be affected by preterm birth. More studies are needed to work towards more individualized follow-up programs.

GENERAL AIMS OF THIS THESIS

A better understanding of the course, related risk factors and long-term consequences of child development would enable us to improve follow-up programs and standard of care for children born both preterm and full-term. We were specifically interested in three important domains of child development: 1) early brain growth and neurodevelopment, 2) early body growth, body composition and cardiometabolic health, and 3) sleep and 24-hour rhythms. The main aims of this thesis are to better understand the etiological and contributing factors to these three domains, and to evaluate the potential contribution of several (novel) monitoring techniques. An overview of the pathophysiological mechanisms and hypotheses studies within this thesis are shown in **Figure 1**.

Figure 1. Overview of the associations studied within this thesis.



GENERAL SETTING AND DESIGN

The reported studies in this thesis were performed within the BOND study and Generation R study. These studies were conducted at the department of Pediatrics, division of Neonatology, of the Erasmus MC Sophia Children's Hospital, and Generation R Study Group, respectively.

BOND study

The BOND study (BOdy composition and NeuroDevelopment in preterm infants) is an ongoing, prospective cohort study of children born very preterm.[74] Between September 2014 and September 2017, a total of 142 infants born <30 weeks gestation were included from the neonatal intensive care unit within 48 hours after birth and followed up into childhood. From birth until 5 years corrected age, growth, body composition, sleep, and brain and general neurodevelopment were monitored at multiple points in time. In this thesis, we focus on growth and development between birth and 2 years corrected age.

Generation R study

The Generation R study is a large, ongoing population-based prospective cohort study from early fetal life onwards in Rotterdam.[75] Almost 10.000 mothers and children born in Rotterdam between April 2002 and January 2006 participate(d) and are followed up from birth until 18 years old. In this thesis, we focus on sleep and 24-hour rhythm of children aged 10-15 years old in relation to sleep, 24-hour rhythm and early fetal/infant growth and cardiometabolic risk factors in childhood.

OUTLINE OF THIS THESIS

This thesis includes different aspects of child development in both the preterm and full-term born populations, with the focus on the brain, sleep and 24-hour rhythm, and cardiometabolic health.

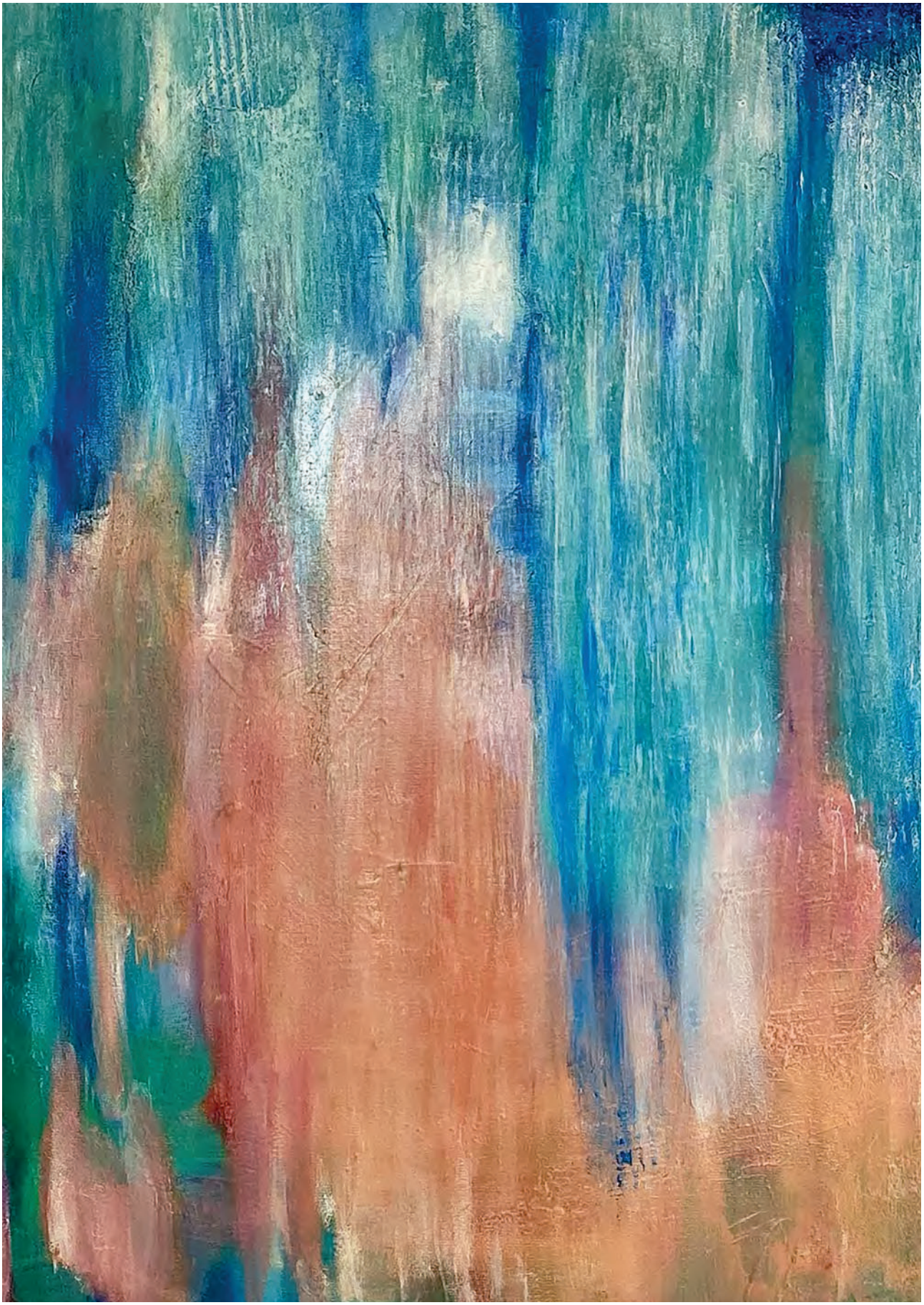
PART I focuses on different techniques to monitor brain growth and neuro-development in infants and children born very preterm. **Chapter 2** describes the association of a novel marker of brain growth in infancy, with neurodevelopment at 2 years. In **Chapter 3,** the use of an eye tracking-based test for visuospatial attention and processing at 1 and 2 years is evaluated, in relation to later neurodevelopment.

PART II comprises two projects on early growth and adiposity in infants and children born very preterm. In **Chapter 4,** the associations between four different early weight gain trajectories and body composition in infancy are presented. In **Chapter 5,** the use of two different techniques for body composition measurement in young children born full-term and preterm is compared: air-displacement plethysmography and dual-energy X-ray absorptiometry.

PART III describes the role of sleep and 24-hour activity rhythms in the development of school-age children in the general population. In **Chapter 6,** the associations of birth characteristics and early growth patterns with sleep and 24-hour rhythms in late childhood are investigated. **Chapter 7** assesses the associations between sleep and 24-hour rhythms and cardiometabolic risk factors at school-age.

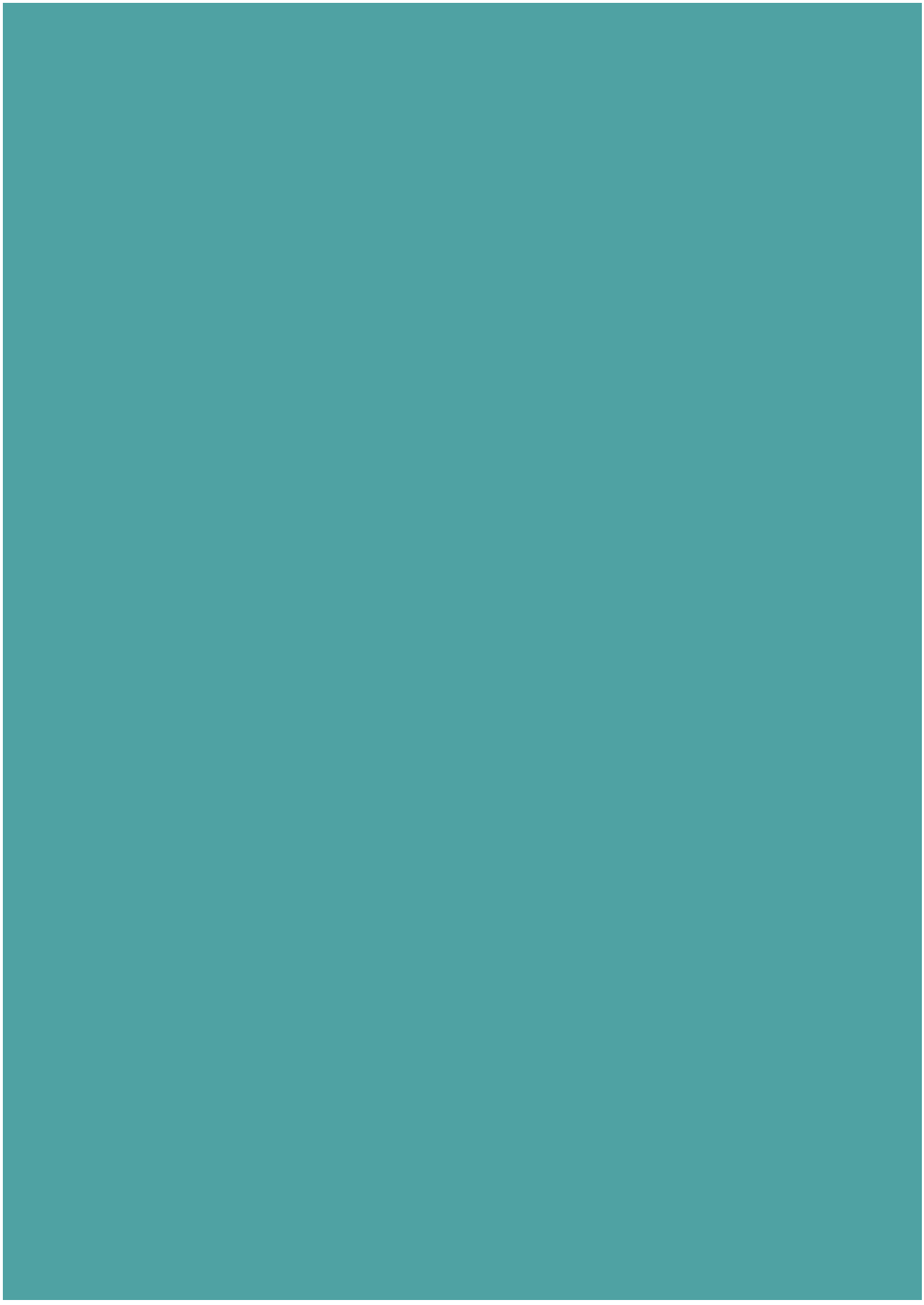
PART IV is dedicated to the discussion and summary of our findings. **Chapter 8** provides the general discussion and suggestions for future research. In **Chapter 9,** a summary of the main findings of this thesis is provided in English and Dutch.

1



PART I

The brain and neurodevelopment in children born preterm



CHAPTER 2

Early ultrasonic monitoring of brain growth and later neurodevelopmental outcome in very preterm infants

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ABSTRACT

Background and Purpose: In infants born very preterm, monitoring of early brain growth could contribute to prediction of later neurodevelopment. Therefore, our aim was to investigate 1) associations between two early cranial ultrasound (CUS) markers (corpus callosum–fastigium (CCF) and corpus callosum (CC) length) and neurodevelopmental outcome; and 2) the added value of both markers in prediction of neurodevelopmental outcome based on neonatal risk factors and head circumference (HC); in very preterm infants.

Materials and Methods: This prospective observational study included 225 infants born <30 weeks gestational age (GA), of whom 153 without any brain injury on CUS. CCF and CC length and HC were measured at birth, 29 weeks GA, transfer from neonatal intensive care unit to level-II hospital, and 2 months corrected age (CA). We analyzed associations of brain markers and their growth with cognitive, motor, language and behavioral outcome at 2 years CA.

Results: In infants without brain injury, greater CCF length at 2 months was associated with better cognitive outcome. CC length at 2 months was positively associated with cognitive, motor and language outcome. Faster growth of CC length between birth and 2 months was associated with better cognitive and motor function. Prediction of neurodevelopmental outcome based on neonatal risk factors with or without HC, significantly improved by adding CC length.

Conclusion: Both CCF and CC length on CUS are associated with neurodevelopmental outcome of very preterm infants without brain injury at 2 years, but only CC length shows added clinical utility in predicting neurodevelopmental outcome.

INTRODUCTION

In infants born very preterm, adverse brain growth is an important predictor for later neurodevelopmental impairment.[43,44] Therefore, monitoring early brain growth is important, and requires reliable and clinically applicable markers. The most commonly used marker in infancy is head circumference (HC), which is easily applicable in clinical care. In preterm infants however, head circumference often poorly reflects brain size due to head deformities and increased extracerebral fluid.[76,77] Brain imaging techniques can add valuable information on the actual size of the brain. MRI is considered the most reliable method but is not bed-side available and expensive, limiting the possibility of serially repeated imaging. Cranial ultrasound (CUS) can be applied more easily and therefore serially during stay on the neonatal intensive care unit (NICU).[78]

Several previous studies in preterm infants linked corpus callosum (CC) length at term equivalent age with neurodevelopmental outcome in childhood.[79-81] As CC length only reflects a small part of the brain, our study group introduced corpus callosum-fastigium (CCF) length as a new marker for brain growth.[82] CCF length is measured on CUS in a standard mid-sagittal plane and covers a larger part of the brain than CC length, including several important brain structures such as the thalamus. The measurement can be performed both pre- and postnatally. We previously showed that CCF length has high reproducibility and applicability for monitoring of brain growth during fetal life and NICU stay.[82,83] CCF length was found to be smaller in fetuses and neonates with fetal growth restriction as compared to those with normal growth.[83,84] However, the predictive value of CCF length for neurodevelopmental outcome needs further investigation.

In this study we explored the associations between length and growth of CCF and CC in early infancy, and neurodevelopmental outcome at 2 years corrected age (CA) in infants born very preterm, specifically in those without brain injury. We hypothesized that larger length and faster growth of CCF and CC are associated with improved neurodevelopmental outcome, and that both markers have added clinical value to prediction of neurodevelopment as compared to neonatal risk factors and head circumference.

METHODS

Participants

This study combined data of two comparable prospective observational cohort studies performed between 2010-2017 at the NICU of the Erasmus MC - Sophia Children's Hospital, Rotterdam, the Netherlands. All preterm infants born between 24 and 30 weeks gestational age (GA) and admitted to the NICU within 48 hours after birth were eligible for participation in Study A (Submarine study) or Study B (BOND study)(**Supplemental Figure S1**).[74,85] Infants with severe congenital or chromosomal abnormalities, perinatal asphyxia (cord blood/first postnatal PH <7.0 and APGAR score at 5 min <5), and congenital TORCHES infection (toxoplasmosis, rubella, cytomegalovirus, herpes simplex, and other organisms including syphilis, parvovirus and varicella zoster) were excluded. Parental informed consent was obtained for all participants. Both studies were approved by the medical ethical committee of the Erasmus Medical Center, Rotterdam.

Maternal, obstetric, and neonatal characteristics were collected prospectively from electronic medical records. Ethnicity was classified as non-Western if one or both parents were born in a non-Western country, and parental education level was based on both parents.[86] Brain injury was diagnosed on CUS and included subependymal and intraventricular hemorrhage (IVH grade I to II+), cerebellar hemorrhage, stroke and/or periventricular leukomalacia (PVL). Postnatal age (PA) was defined as days after birth, with day of birth as day 1.[87]

Markers of brain growth

CUS were routinely performed according to local clinical protocol by the attending neonatologist or an experienced researcher. The local protocol included CUS on PA day 1, 2, 3, and 7, followed by weekly measurements until transfer from the NICU to a level-II hospital. A MyLab 70 scanner (Esaote, Genoa, Italy) with a convex neonatal probe (7.5 MHz) was used. Off-line measurements of CC length and CCF length on a standard midsagittal plane were performed using MyLab software (Esaote) by one of the researchers. As described previously in detail, CCF length (cm) was measured from the genu of the corpus callosum (outer border) to the fastigium, and CC length (cm) from genu to the splenium (outer-outer border) (**Supplemental Figure S2**).[82]

Head circumference (cm) was measured during NICU stay as part of standard care using tape measure. Growth Z-scores were based on the Fenton growth charts from birth until discharge or 50 weeks GA, and on the World Health Organization growth charts thereafter.[88]

For this study, we used measurements of CCF length, CC length and HC assessed at (1) birth (PA day 1-3); (2) around 29 weeks GA (28-30 weeks), and (3) at NICU transfer to a level-II hospital (limited to 30-36 weeks GA). Growth rate (mm/week) of CCF length, CC length and HC was calculated between birth and NICU transfer. To increase homogeneity in timing and length of the growth periods, growth rate was only calculated when (1) the first CUS was performed in the first week of life, and (2) the period between two measurements covered at least 14 days.

In study B, CUS and HC measurements were also performed at the routine outpatient clinic visit at median 6.9 weeks CA (interquartile range, IQR 6.1;8.3), further referred to as the 2 months visit. In these infants, growth rate of each marker was also calculated between birth and 2 months.

Neurodevelopmental outcome

As part of the national neonatal follow-up program, all children were routinely invited to the outpatient clinic at 2 years CA for physical and neurological examination by a neonatologist or pediatric neurologist. Trained physiotherapists and psychologists performed extensive testing of psychomotor and cognitive development using the fine motor and gross motor (summarized in a total motor score) and cognitive tests of the Bayley Scales of Infant and Toddler Development-Third edition (Bayley-III, Dutch edition), expressed as standard scores adjusted for CA the moment of testing.[32] Following Dutch guidelines, the Lexi list was used to evaluate expressive language development. This validated questionnaire is completed by parents to quantify the child's vocabulary with scores adjusted for CA at assessment and sex.[33] For each child, parents were asked to complete the Child Behavior Checklist 1.5-5 years (CBCL 1.5-5); an internationally validated questionnaire examining behavioral and emotional problems.[34] For this study we used the CBCL total problems scale; expressed in T-scores adjusted for CA at assessment and sex. Assessors and parents were unaware of CC or CCF length measurements.

Statistical analysis

As the presence and severity of brain injury in the neonatal period can influence both brain growth and neurodevelopmental outcome disproportionately,[42] we mainly focused on the large group of infants without any brain injury on neonatal CUS. To explore the value of the brain growth markers in the presence of brain injury, additional exploratory analyses were done in the smaller and more heterogeneous group with any extent of neonatal brain injury on CUS. Relative risks (RR) for adverse outcomes were calculated comparing those with and without brain injury.

First, we used non-parametric statistical tests for non-response analyses. Second, we used linear regression models to study associations between length (at birth, 29 weeks GA, NICU transfer, and 2 months CA) and growth rate (between birth and NICU transfer, and between birth and 2 months) of CCF, CC and HC, and the four neurodevelopmental outcomes (motor, cognitive, language and behavior) at 2 years CA in both groups. In the basic models, we adjusted for GA or CA at CUS assessment. The adjusted models were additionally corrected for sex, GA at birth, birth weight Z-score, and parental education; based on relevance reported in literature.[23,78] These four covariates were tested and confirmed to either show a statistical association with at least one of the two ultrasonic brain markers at 2 months *and* cognitive outcome or change the effect size >10% after addition to the basic model. Given the number of participants and variables in our models and to limit type I or II error, we only performed analyses when at least 40 available measurements were available per analysis. For comparability of effect sizes, associations are reported by steps resembling the average IQR of each marker. We observed no significant interactions between any of the brain markers and sex.

Third, we evaluated the added clinical value of the brain markers in predicting neurodevelopmental outcome in infants without brain injury, as compared to prediction based on neonatal risk factors and head circumference only. As baseline, a 'basic neonatal' regression model was used for prediction of cognitive outcome. This model was recently created in a preterm population much overlapping this cohort.[89] This model included sex, GA at birth, combined parental education level, grade of bronchopulmonary dysplasia (BPD, no/mild/severe), treated patent ductus arteriosus (medically and surgically), brain injury and duration of hospital admission. As this analysis was done in the group of infants without any brain injury, we did not include 'brain injury' as a covariate in the 'basic neonatal' model of the current study. Using linear hierarchical regression models and explained variances (R^2), the 'basic neonatal' model (both with and without HC) was compared to models that additionally included any CUS marker(s) associated with neurodevelopmental outcome(s).

P-values (two-tailed) below 0.05 were considered statistically significant. We calculated 95% confidence intervals (CI) for all effect estimates. Correction for multiple testing was not deemed necessary given the step-based and exploratory character of the analyses. Data were analyzed using IBM SPSS Statistics, version 25.0 (IBM SPSS Statistics, Armonk, NY) and R statistical and computing software (R: A language and environment for Statistical Computing, version 3.5.1, 2018 for Windows, R Core Team, Vienna, Austria).

RESULTS

Study population

Out of 293 eligible children, 225 (77%) were included in this study (**Supplemental Figure S1**), of whom 153 (68%) showed no brain injury on CUS during the neonatal period**.** Nonresponse analyses showed that included children more often were Western, had slightly higher birth weights and encountered less complications during NICU-stay (data not shown). Parental, perinatal, and neonatal characteristics were mostly similar in infants with and without brain injury (**Table 1**).

Markers of brain growth

Length and growth rate of CC, CCF and HC are presented in **Table 2** and **Supplemental Figure S3**. The correlation of CC length and CCF length as compared to HC during NICU-stay is plotted in **Supplemental Figure S4**. At all four time points, absolute length of all three markers appeared to be (slightly) larger in infants without brain injury as compared to infants with brain injury. In both groups, length of CCF, CC and HC increased over time. Also, growth rate of CCF length, and even more so CC length, decreased after transfer from the NICU to a level-II hospital (median 31^{+5} – 32^{+1} weeks GA), while growth rate of HC increased.

Neurodevelopmental and neurologic outcome

Scores on the four neurodevelopmental tests, as well as the prevalence and relative risk of neurological complications are listed in **Supplemental Table S5**. In general, outcomes were less favorable in infants with brain injury, with 11% having cerebral palsy as compared to 3% (risk ratio (RR) 3.4, 95% CI 1.2;10.0), and 11% having visual disorders as compared to 5% in those without brain injury (RR 2.1, 0.8;5.4). In both groups, all four neurodevelopmental tests showed median scores within the normal range. However, moderate or severe motor impairment was more common in those with brain injury (14% versus 5%, RR 2.7, 1.1;6.5).

Table 1. Patient characteristics of children with and without brain injury

	No brain injury (n=153)	Brain injury (n=72)
Prenatal characteristics		
Parental education level		
Low	25 (16%)	15 (21%)
Middle	45 (29%)	22 (31%)
High	71 (46%), unknown 12 (8%)	30 (42%), unknown 5 (7%)
Multiplet pregnancy	22 (14%)	8 (11%)
Antenatal steroids	144 (94%), unknown 1 (0.7%)	63 (88%), unknown 1 (1%)
Infant characteristics		
Ethnicity		
Western	105 (69%)	49 (68%)
Non-Western	47 (31%), unknown 1 (0.7%)	23 (32%)
Female sex	54 (35%)	35 (49%)
GA at birth (weeks^{+days})	27^{+5} [26^{+2};28^{+5}]	26^{+6} [26^{+1};28^{+0}]
Birth weight (grams)	996 [813;1255]	960 [808;1119]
Birth weight Z-score (SD)	-0.1 [-0.8;0.7]	-0.1 [-1.0;0.6]
Apgar 5min (0-10)	8 [7;9], unknown 1 (0.7%)	8 [7;9], unknown 1 (1%)
IRDS, treated	100 (65%), unknown 1 (0.7%)	42 (58%)
Endotracheal intubation	107 (70%)	50 (69%)
Days on mechanical ventilation	2 [0;11]	3 [0;17]
Postnatal steroid therapy	26 (17%), unknown 1 (0.7%)	12 (17%), unknown 1 (1%)
BPD, of which:	47 (45%)	38 (53%)
Mild	71 (31%)	25 (35%)
Severe	22 (14%)	13 (18%)
PDA, treated	59 (39%)	25 (35%)
Use of inotropics	15 (10%)	13 (18%)
NEC	8 (5%)	5 (7%)
Culture proven sepsis	63 (41%)	24 (33%)
IVH, of which:	0 (0%)	54 (75%)
Grade I	0 (0%)	28 (39%)
Grade II	0 (0%)	25 (35%)
Grade II+	0 (0%)	1 (1%)
PVL	0 (0%)	6 (8%)
Stroke	0 (0%)	13 (18%), unknown 2 (3%)
Cerebellar bleeding	0 (0%)	8 (11%)
ROP, of which:	63 (41%)	30 (42%), unknown 1 (1%)
Grade I	50 (33%)	19 (26%)
Grade II	8 (5%)	3 (4%)
Grade III/IV	5 (3%)	8 (11%)
Duration of NICU-stay (days)	31 [17;58]	33 [21;65]
GA at NICU transfer to level-II hospital	32^{+1} [30^{+3};34^{+4}]	31^{+5} [30^{+3};35^{+1}]

Data are shown as median [interquartile range] or absolute numbers (percentage).
n = number of patients, GA = gestational age, IRDS = infant respiratory distress syndrome treated with surfactant, BPD = bronchopulmonary dysplasia, PDA = persistent ductus arteriosus treated medically or surgically, NEC = necrotizing enterocolitis ≥ stage 2, IVH = intraventricular hemorrhage, PVL = periventricular leukomalacia, ROP = retinopathy of prematurity, NICU = neonatal intensive care unit.

Table 2. Brain measurements from birth until 2 months corrected age in children with and without brain injury

No brain injury (n=153)								
Length (mm)		**Birth**		**GA 29wks**		**NICU transfer**		**2M-visit**
	n	**Median (IQR)**	**n**	**Median (IQR)**	**n**	**Median (IQR)**	**n**	**Median (IQR)**
Corpus callosum	115	36.5 [33.4;39.1]	126	38.2 [36.4;39.7]	82	40.5 [39.0;42.2]	72	52.8 [49.3;55.4]
Corpus callosum-fastigium	116	40.1 [38.5;42.3]	122	41.4 [40.0;43.0]	78	43.9 [42.6;45.4]	70	56.8 [55.1;58.4]
Head circumference	150	250 [235;265]	117	257 [242;265]	82	274 [265;283]	69	389 [375;400]
Growth (mm/wk)		**Birth-NICU transfer**				**Birth-2M**		
	n	**Median (IQR)**			**n**	**Median (IQR)**		
Corpus callosum	76	1.11 [0.83;1.37]			65	0.79 [0.66;0.88]		
Corpus callosum-fastigium	74	0.90 [0.61;1.13]			61	0.80 [0.74;0.89]		
Head circumference	71	5.39 [3.28;7.00]			68	6.92 [6.24;7.35]		
Brain injury (n=72)								
Length (mm)		**Birth**		**GA 29wks**		**NICU transfer**		**2M-visit**
	n	**Median (IQR)**	**n**	**Median (IQR)**	**n**	**Median (IQR)**	**n**	**Median (IQR)**
Corpus callosum	53	34.4 [33.1;36.8]	54	37.5 [35.1;38.8]	38	40.2 [38.2;43.3]	29	51.6 [50.0;54.0]
Corpus callosum-fastigium	52	39.0 [37.1;40.9]	58	41.0 [39.9;42.6]	36	43.86[41.4;46.1]	27	56.8 [54.1;58.5]
Head circumference	71	246 [235;258]	56	252 [243;262]	34	271 [259;284]	28	385 [366;395]
Growth (mm/wk)		**Birth-NICU transfer**				**Birth-2M**		
	n	**Median (IQR)**			**n**	**Median (IQR)**		
Corpus callosum	41	1.19 [0.96;1.36]			27	0.80 [0.65;0.89]		
Corpus callosum-fastigium	34	1.01 [0.73;1.36]			24	0.83 [0.73;0.91]		
Head circumference	32	5.84 [4.44;6.76]			27	6.59 [6.01;7.05]		

n = number of patients/measurements, GA = gestational age, wk = week, NICU = neonatal intensive care unit, M = month, IQR = interquartile range.

Associations between brain length or growth and neurodevelopmental outcome

In infants without neonatal brain injury, larger CCF *length* at 2 months was associated with better cognitive outcome: every IQR (5mm) increase in CCF length was associated with a 9.1 (95% CI 2.4;15.8) higher Bayley-III cognitive score (**Table 3**).As for CC length, we observed a 5.9 (2.8;9.1) point higher Bayley-III cognitive score, a 4.6 (1.3;8.0) higher total motor score, and a 6.5 (2.0;11.0) point higher language score for every IQR (5mm) increase at 2 months. In addition, a 5mm larger CC length at birth was associated with a 5.9 (0.4;11.4) higher motor score. HC was also positively associated with multiple neurodevelopmental outcomes: for every IQR (20mm) increase at 2 months we observed a 7.2 (2.9;11.6) points increase in cognitive score, and an 8.7 (2.7;14.7) points higher Lexi score.

Each IQR (0.25 mm/wk) increase in CC *growth rate* between birth and 2 months was associated with a 5.1 (0.9;9.4) point higher cognitive score, and a 4.5 (0.1;8.9) higher motor score. An IQR (1 mm/wk) faster HC growth in this period was associated with a 5.8 (0.9;10.7) point higher Lexi score. We did not observe any associations between growth rate of CCF length and neurodevelopmental outcomes. In the brain injury group, results were only available for the associations of absolute length of CC, CCF and HC at birth and 29 weeks GA and neurodevelopmental outcomes, due to too small group sizes (n<40) at the other time points. None of these associations were statistically significant (**Supplemental Table S6**). Results of the basic models, not corrected for sex, GA at birth, birth weight Z-score, and parental education, are presented in **Supplemental Table S7 and S8.**

Added value of CUS brain markers for outcome prediction

In **Table 4** we present the added values of CC and CCF length at 2 months to prediction of the three associated neurodevelopmental outcomes (cognitive, motor and language) by neonatal risk factors in infants without brain injury. Compared to the 'basic neonatal model' with or without HC, adding CC length led to an 8.8 to 9.8% increase in explained variance (R^2) of cognitive and language outcome ($p<0.05$). There was no added value of (additionally) adding CCF length to any of the models, nor for addition of any of the brain markers in predicting motor outcome.

Table 3. Associations between length and growth of CC, CCF and HC, and neurodevelopmental outcomes at 2 years in children without brain injury (n=153)

		Cognition		Motor		Language		Behavior
CC	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**
Length (per 5 mm)								
Birth	107	1.54 (-4.01;7.09)	94	**5.87 (0.38;11.36)**	105	5.49 (-1.51;12.48)	104	-0.65 (-5.29;3.99)
GA 29wks	116	2.37 (-2.49;7.24)	96	4.89 (-0.31;10.08)	114	2.81(-3.81;9.42)	115	0.15 (-3.89;4.19)
NICU-transfer	75	-1.13 (-7.36;5.10)	64	2.32 (-3.70;8.35)	74	0.31 (-7.29;7.89)	73	-1.48 (-6.17;3.21)
2M-visit	68	**5.93 (2.75;9.11)**	62	**4.64 (1.32;7.96)**	68	**6.48 (2.01;10.95)**	68	-1.96 (-4.70;0.78)
Growth (per 0.25 mm/wk)								
Birth-NICU transfer	72	0.07 (-1.39;1.53)	61	-0.96 (-2.50;0.59)	70	0.00 (-2.04;2.04)	71	-0.80 (-1.99;0.40)
Birth-2M	61	**5.11 (0.88;9.35)**	56	**4.52 (0.14;8.90)**	61	3.83 (-2.20;9.86)	61	-2.46 (-6.07;1.15)
		Cognition		**Motor**		**Language**		**Behavior**
CCF	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**
Length (per 5 mm)								
Birth	107	-3.71 (-9.88;2.46)	94	1.51 (-4.77;7.79)	105	-2.18 (-10.08;5.72)	104	-0.24 (-5.43;4.95)
GA 29wks	113	-2.13 (-7.80;3.54)	94	-3.39 (-9.48;2.70)	111	-3.08 (-10.72;4.56)	112	0.72 (-3.97;5.40)
NICU-transfer	72	-1.47 (-9.68;6.74)	61	-2.57 (-10.52;5.37)	72	1.67 (-8.09;11.44)	71	0.31 (-5.79;6.40)
2M-visit	66	**9.08 (2.38;15.78)**	60	0.04 (-7.14;7.22)	66	8.42 (-0.95;17.79)	66	-1.53 (-7.14;4.07)
Growth (per 0.25 mm/wk)								
Birth-NICU transfer	69	-0.15 (-1.71;1.42)	58	-0.29 (-1.97;1.40)	68	0.52 (-1.65;2.68)	69	0.44 (-0.85;1.72)
Birth-2M	57	5.52 (-0.27;11.30)	52	1.85 (-4.28;7.98)	57	6.45 (-1.57;14.46)	57	-2.70 (-7.56;2.17)

Table 3. Continued

	Cognition		Motor		Language		Behavior	
HC	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**
Circumference (per 20 mm)								
Birth	138	1.87 (-2.57;6.31)	117	0.39 (-4.35;5.12)	135	-1.84 (-8.01;4.33)	135	1.06 (-2.64;4.76)
GA 29wks	110	-3.36 (-6.87;0.14)	92	-1.93 (-5.91;2.06)	107	**-6.47 (-11.45;-1.50)**	108	0.23 (-2.84;3.31)
NICU-transfer	77	1.56 (-4.85;7.96)	65	0.42 (-6.01;6.85)	76	-1.57 (-9.40;6.26)	75	-2.74 (-7.51;2.04)
2M-visit	66	**7.22 (2.87;11.57)**	60	3.62 (-1.06;8.30)	66	**8.72 (2.73;14.72)**	66	-0.51 (-4.25;3.23)
Growth (per 1 mm/wk)								
Birth-NICU transfer	67	0.21 (-0.89;1.32)	56	0.06 (-1.12;1.23)	66	-0.13 (-1.64;1.37)	66	-0.53 (-1.42;0.36)
Birth-2M	65	2.56 (-1.09;6.21)	59	1.37 (-2.43;5.17)	65	**5.81 (0.94;10.66)**	65	-1.49 (-4.51;1.53)

Beta-coefficients (B) are shown per interquartile range of each marker, followed by 95% confidence intervals (CI). Significant associations are depicted in ***bold****. Neurodevelopmental outcomes were based on the cognitive and total motor score of the Bayley Scales of Infant and Toddler Development-Third edition (for cognition and motor outcome, respectively), Lexi-list (language) and total score of the Child Behavior Checklist (behavior). Models were adjusted for sex (female 0, male 1), gestational age at birth (weeks), birth weight Z-score and combined parental education (low/middle/high). Apart from these four covariates, the GA29wks and 2M-visit models were also adjusted for gestational age or corrected age at measurement, respectively. Results from similar analyses on infants with brain injury are shown in supplemental Table S6. CC = corpus callosum, n = number of patients/ultrasounds included, GA = gestational age, NICU = neonatal intensive care, M = months, CCF = corpus callosum-fastigium, HC = head circumference.*Added value of CUS brain markers for outcome prediction

Table 4. Added value of CC length, CCF length and/or HC at 2M-visit for prediction of neurodevelopmental outcome at 2 years in children without brain injury

N=72	Basic neonatal model		Neonatal + HC model		Neonatal + CC model		Neonatal + CCF model		Neonatal + HC + CC model		Neonatal + HC + CC + CCF model	
	R^2	P	ΔR^2	ΔP	ΔR^2	ΔP	ΔR^2	ΔP	ΔR^2	ΔP	ΔR^2	ΔP
Cognition	11.8	**0.040**	+1.4	0.248	**+9.8**	**0.013**	+2.2	0.108	**+8.8**	**0.036**	+6.2	0.108
Motor	6.6	0.155	-0.8	0.450	+6.5	0.083	-3.0	0.846	+4.1	0.170	+2.7	0.271
Language	8.4	0.085	+3.5	0.128	**+8.9**	**0.015**	-1.5	0.571	**+9.8**	**0.029**	+7.1	0.092

Explanatory value (adjusted R^2, as percentage) per outcome using the 'basic neonatal' model with only neonatal risk factors (sex, GA at birth, combined parental education level, BPD grade, treated PDA, and total days of hospital admission), and the difference in R^2 (ΔR^2) when CC length at 2 months, CCF length at 2 months, HC at 2 months, or a combination of those, are added to this model (all adjusted for CA at ultrasound). Significance levels (P) are given for R^2 of the 'basic neonatal model', as well as for the increase in explanatory value (ΔP) when brain growth markers are added. Neurodevelopmental outcomes were based on cognitive and total motor score of the Bayley Scales of Infant and Toddler Development-Third edition (for cognition and motor outcome, respectively) and the Lexi-list (language).

GA = gestational age, BPD = bronchopulmonary dysplasia, PDA = patent ductus arteriosus, CC = corpus callosum, CCF = corpus callosum-fastigium length, HC = head circumference, CA = corrected age.

DISCUSSION

In this longitudinal study of 225 infants born very preterm, larger corpus callosum-fastigium length at 2 months CA in infants without brain injury was associated with better cognitive outcome at 2 years CA . As for corpus callosum, larger length at 2 months CA, as well as faster growth rate from birth to 2 months, were associated with higher cognitive, motor and language scores at 2 years CA. These associations were similar to those observed for head circumference. Prediction of neurodevelopmental outcome based on neonatal risk factors and head circumference, significantly improved when CC length, but not CCF length, at 2 months was additionally taken into account.

CCF length is a new reliable marker of brain growth that captures a large part of the brain and is related to fetal growth restriction.[83,84] We showed that in infants without brain injury, CCF length was related to cognitive outcome, but had no added clinical value in prediction of neurodevelopment. As this is the first study to explore this association, there are no previous studies to compare with. The lack of predictive power may have different explanations. First, the anatomical structures that are covered by CCF length (diencephalon, thalamus, mesencephalon) are important areas of the brain, but the cerebellum and white matter (as reflected by the corpus callosum) are not incorporated in CCF length. Yet these parts of the brain may be more susceptible to external factors influencing brain growth and may therefore be more important for outcome in this specific patient group and time period after birth. [90,91] Second, measurement error may have played a role. However, we consider this explanation less likely as we previously showed adequate reproducibility and reliability of CCF length in a similar setting, and all measurements were performed by two experienced researchers.[82,83]

The positive associations of CC length at 2 months CA with cognitive, motor and language outcome, and CC growth until 2 months with cognitive and motor function, are in line with previous MRI and CUS studies, and reflect the importance of the corpus callosum as the major white matter pathway in the brain.[79-81,92-94] White matter is involved in different domains of neurodevelopment and is very susceptible to (micro) injury by external factors, including neonatal complications experienced after preterm birth.[93] Therefore, in this specific patient group, it is likely that CC size reflects the extent of injury of the white matter, which translates to later neurodevelopment. This may also explain why CC length and growth appear to be stronger associated with neurodevelopmental outcomes, than HC. Interestingly, apart from the association between CC length at birth and motor outcome, we only

observed associations with outcome when CC length was measured *after* NICU transfer (>30-32 weeks GA), and not during NICU stay. These findings are comparable with the CUS studies of *Anderson et al* who reported a relation with Bayley motor scores at 2 years for CC growth between 2-6 weeks after birth (30-34 weeks GA), but not for CC growth in the first 2 weeks after birth, in a similar preterm population.[93,94] We hypothesize that in infants without brain damage, the period after NICU stay may be more critical for neurodevelopment. This is supported by the decrease in CC growth rate after NICU transfer observed in this and other studies, likely due to the impact of more chronical complications like BPD.[78,94-96]

None of the brain markers were associated with behavioral outcome. This finding may reflect the complex and multifactorial origin of behavior development which hampers adequate prediction of later behavioral problems, especially at a young age. Furthermore, the CBCL 1.5-5 years used in this study is a screening questionnaire which roughly estimates problem behavior but is not suitable for diagnosis. In addition, underreporting of behavioral problems by parents might be an issue. Nonetheless, a very recent MRI study, linked global brain abnormalities at term age with the CBCL total problems score at 2 years CA.[97] An important difference with our study is that they used detailed and comprehensive Kidokoro scoring on MRI images as compared to one single CUS measure in our study.[98]

The observed associations of CC length at 2 months with neurodevelopmental outcomes were not stronger than those of HC. However, CC length at 2 months still showed significant added value in prediction of neurodevelopment, as compared to prediction based on neonatal risk factors and head circumference only. These findings are opposite to the conclusion that was drawn by *Perenyi et al* in a similar study of 87 very preterm infants, who stated that measuring CC length on CUS in early life had no additional clinical value.[80] To further explore and improve the potential clinical value of CUS at 2 months in neonatal follow-up programs, future studies could explore combining different CUS brain markers (e.g. CC length, CCF length, ventricular size, biparietal diameter, vermis length, and cerebellar width) with CUS injury scores to predict neurodevelopmental outcome.

Strengths and limitations

This study is unique in studying CCF length in relation to neurodevelopment in preterm infants. The availability of longitudinally performed CUS enabled us to study brain markers both during and after NICU admission. Another strength of this study is the relatively large cohort of preterm infants without brain damage, representing a part of the NICU population in whom neurodevelopment has always

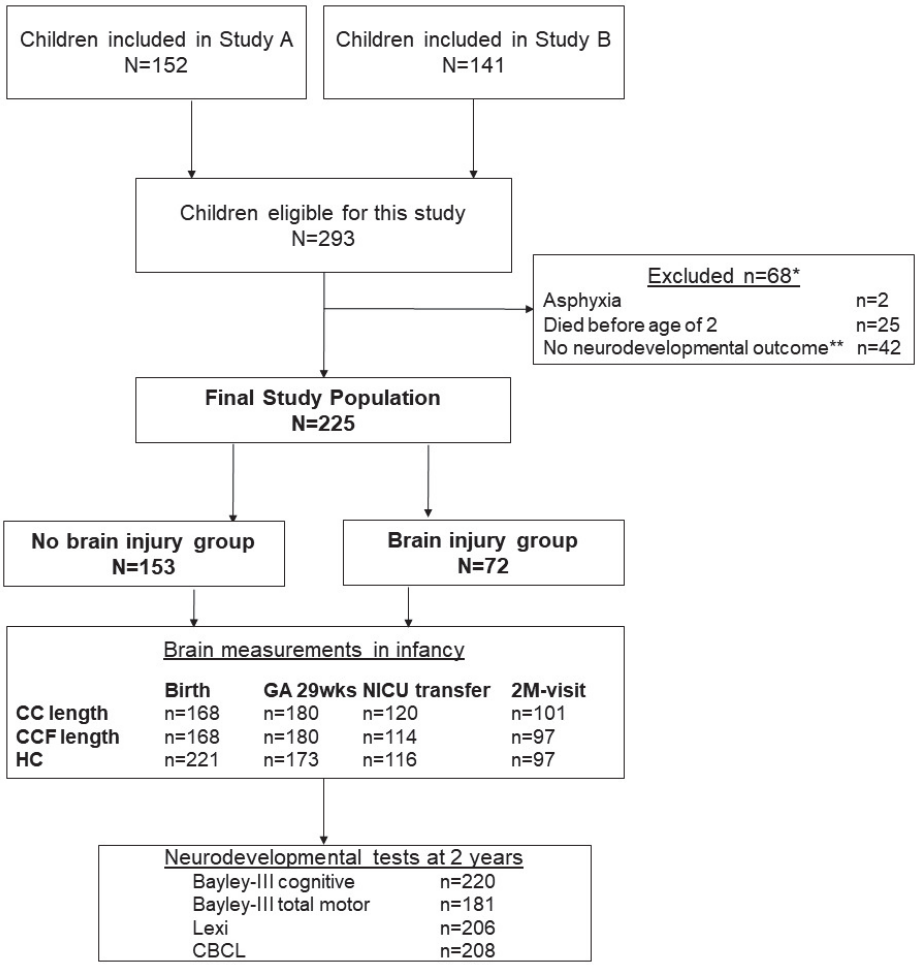
been difficult to predict. Our data confirm that in those *with* neonatal brain injury, neurodevelopmental outcomes are less favorable. Our study also has limitations. First, the group of infants with brain injury was too small and heterogeneous to perform reliable analyses at NICU transfer and 2 months, or on growth of CC and CCF length and HC. Also, the observed lack of associations at birth and 29 weeks GA in this group should be interpreted with caution as these analyses also contained small numbers of ultrasounds/infants. Future studies should explore how the observed associations in infants without any brain injury hold in a large cohort of children with brain injury. Larger cohorts are also needed to disentangle which types of brain injury affect brain growth and neurodevelopment most. Second, no CUS were performed around term equivalent age as, per national policy, infants were transferred to a level-II hospital when they were stable, most often around 30-32 weeks of gestation. Third, we were unable to correct for other psychological factors related to neurodevelopment, such as parenting or parental mental health. However, we believe that the most important perinatal, neonatal and sociodemographic confounders have been covered. Last, the Bayley-III test is a commonly used but rough estimate of global neurodevelopment with limited predictive value for later IQ performance.[48] Therefore, follow up of this cohort into school-age is needed.

CONCLUSION

This prospective study of infants born very preterm without asphyxia, severe congenital abnormalities, or infections, showed the clinical benefit of two brain growth markers, which can be easily measured on CUS. Especially corpus callosum (length and growth), but also corpus callosum-fastigium (length) at 2 months CA, was associated with various important neurodevelopmental outcomes at 2 years CA. Furthermore, corpus callosum length, but not corpus callosum-fastigium length, showed significant added clinical value to prediction of neurodevelopment based on neonatal risk factors and head circumference.

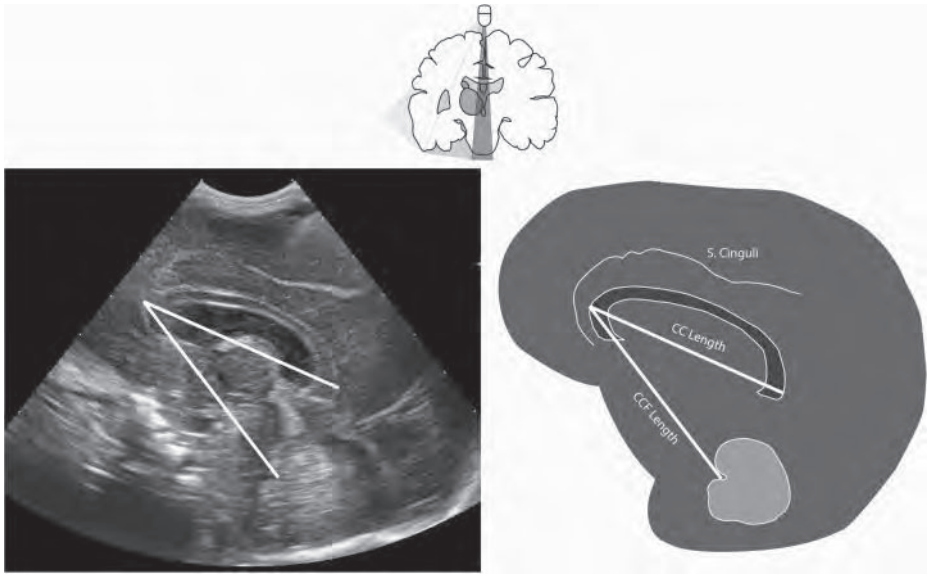
SUPPLEMENTARY FILES

Supplemental Figure S1. Flowchart of the study population



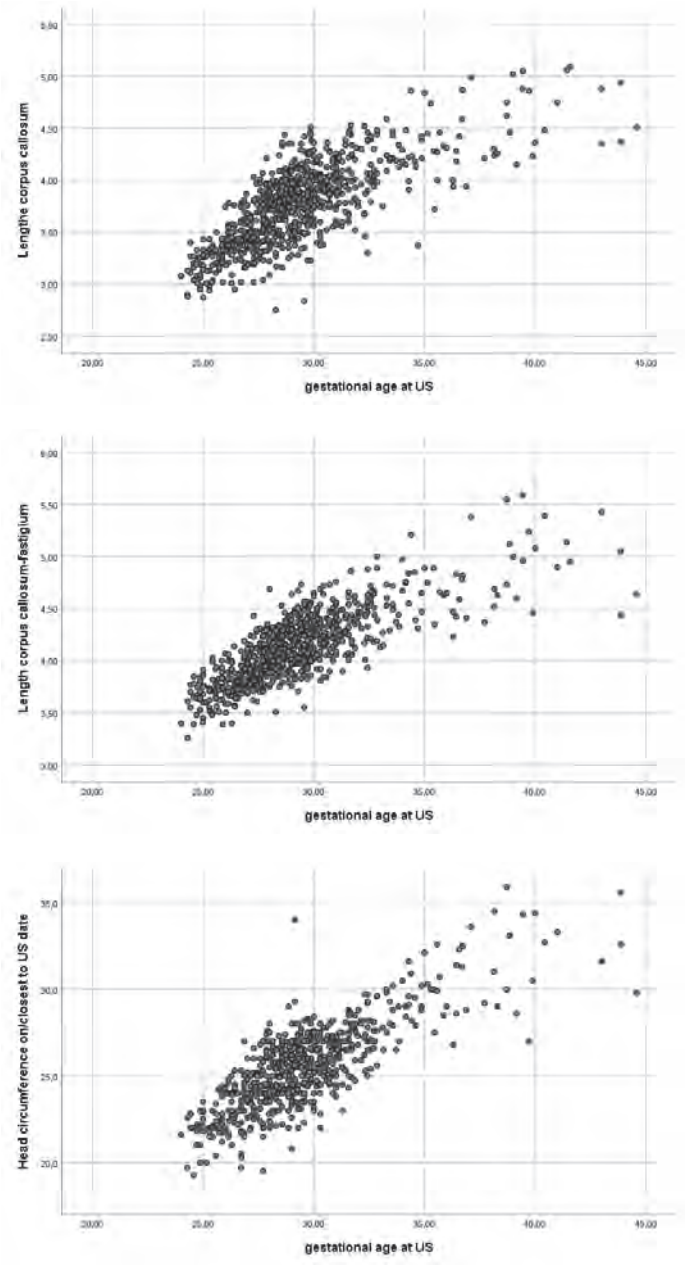
** Numbers per reason of exclusion exceed total number of children excluded as some children were excluded for multiple reasons. ** Possible reasons for absent neurodevelopmental test results: child refusal, not able to perform test (e.g. cerebral palsy), test performed elsewhere with unknown result, no appointment at outpatient clinic, no show at outpatient clinic visit. IVH = intraventricular hemorrhage, PHVD = post hemorrhagic ventricular dilation, CC = corpus callosum, CCF = corpus callosum-fastigium, HC = head circumference, GA = gestational age, wk = weeks, NICU = neonatal intensive care unit, M = months corrected age, Bayley-III = Bayley Scales of Infant and Toddler Development-Third edition; CBCL = Child Behavior Checklist.*

Supplemental Figure S2. Ultrasonic measurement of corpus callosum and corpus callosum-fastigium length[82]



*In the upper part, we show the coronal view of the brain and the position of the sonography probe for assessment of the corresponding correct sagittal plane below. Measurements of the corpus callosum–fastigium and corpus callosum length are displayed in the sagittal sonography view (*left*) and schematically (*right*). S. Cinguli indicates sulcus cinguli.*[82]

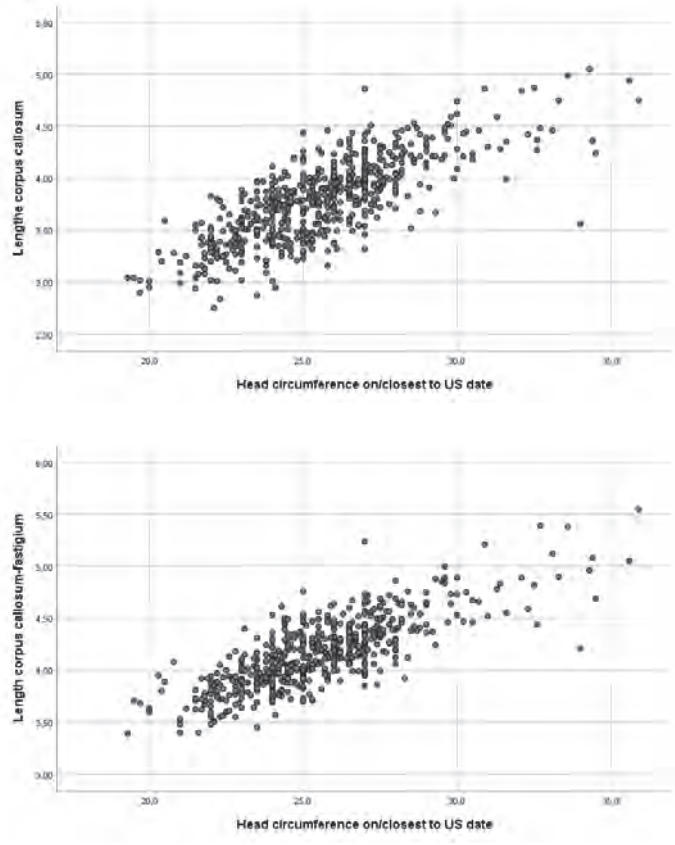
Supplemental Figure S3. CC length, CCF length and HC by gestational age during NICU-stay



Graphs consist of 808 ultrasonic measurements for CC length (in cm), 779 ultrasonic measurements for CCF length (in cm), and 637 tape measurements of HC (in cm), respectively.

NICU = neonatal intensive care unit, CC = corpus callosum, CCF = corpus callosum-fastigium, HC = head circumference, US = ultrasound (cranial), cm = centimeters.

Supplemental Figure S4. CC length and CCF length by HC during NICU-stay



Graphs consist of all available ultrasonic measurements for CC length (in cm, graph 1) and CCF length (in cm, graph 2), and tape measurements of HC (in cm) on or closest to date of ultrasound, during NICU-stay.
NICU = neonatal intensive care unit, CC = corpus callosum, CCF = corpus callosum-fastigium, HC = head circumference, cm = centimeters.

Supplemental Table S5.Neurodevelopmental outcome at 2 years in children with and without brain injury

	No injury (N=153)	Any brain injury (N=72)	Relative risk	95%-CI	p-value
Cerebral palsy, of which:	5 (3%), unknown 1 (0.7%)	8 (11%)	**3.40**	**1.15 - 10.03**	**0.03**
- GMFCS I	5 (3%)	7 (10%)			
- GMFCS II	0 (0%)	0 (0%)			
- GMFCS III	0 (0%)	1 (1%)			
Visual disorders,* of which:	8 (5%)	8 (11%)	2.13	0.83 - 5.44	0.12
- Wearing glasses	6 (4%)	8 (11%)			
- Strabismus	4 (3%)	4 (6%)			
- Nystagmus	1 (0.7%)	0 (0%)			
Bayley-III cognitive score, of which:	100 [90;105], unknown 3 (2%)	96 [90;105], unknown 2 (3%)			
- Moderate / severe impairment (score <85)	14 (9%)	8 (11%)	1.21	0.53 - 2.76	0.64
Bayley-III total motor score, of which:	98 [91;107], unknown 29 (19%)	94 [89;102], unknown 15 (21%)			
- Moderate / severe impairment (score <85)	8 (5%)	10 (14%)	**2.66**	**1.09 - 6.45**	**0.03**
Lexi test score, of which:	91 [81;100], unknown 12 (8%)	88 [81;99], unknown 7 (10%)			
- Moderate / severe impairment (score <81)	35 (23%)	16 (22%)	0.97	0.58 - 1.64	0.91
CBCL total score, of which:	45 [39;51], unknown 12 (8%)	50 [40;57], unknown 5 (7%)			
- Clinical problem behavior (score >63)	6 (4%)	5 (7%)	1.77	0.56 - .56	0.33

*Data are shown as median [interquartile range], absolute numbers (percentage) and relative risks with 95% confidence values and p-values (significant in **bold**). * Numbers of wearing glasses, strabismus and nystagmus exceed total number of visual disorders as some children had multiple diagnoses.*
N = number, CI = confidence interval, GMFCS = Gross Motor Function Classification System, Bayley-III = Bayley Scales of Infant and Toddler Development-Third edition; CBCL = Child Behavior Checklist.

Supplemental Table S6. Associations between length and growth of CC, CCF and HC, and neurodevelopmental outcomes at 2 years in children with brain injury (n=72)

		Cognition		Motor		Language		Behavior
CC	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**
Length (per 5 mm)								
Birth	50	2.66 (-5.52;10.83)	43	2.96 (-5.75;11.66)	49	6.17 (-4.81;17.14)	50	-1.20 (-7.93;5.52)
GA 29wks	49	2.73 (-4.52;9.98)	40	2.09 (-6.32;10.51)	46	-7.19 (-17.46;3.08)	48	1.43 (-5.09;7.96)
NICU-transfer	34	NA	32	NA	34	NA	34	NA
2M-visit	28	NA	21	NA	26	NA	27	NA
Growth (per 0.25 mm/wk)								
Birth-NICU transfer	39	NA	34	NA	37	NA	39	NA
Birth-2M	27	NA	20	NA	25	NA	26	NA
CCF	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**
Length (per 5 mm)								
Birth	48	-1.67 (-12.23;8.88)	42	3.36 (-7.74;14.46)	48	-0.29 (-14.44;13.87)	48	-6.58 (-14.99;1.84)
GA 29wks	51	3.77 (-5.52;13.06)	42	9.43 (-0.59;19.44)	48	0.42 (-12.67;13.51)	50	-6.15 (-13.88;1.58)
NICU-transfer	32	NA	31	NA	32	NA	32	NA
2M-visit	27	NA	21	NA	26	NA	26	NA
Growth (per 0.25 mm/wk)								
Birth-NICU transfer	31	NA	30	NA	31	NA	31	NA
Birth-2M	24	NA	18	NA	23	NA	23	NA

Supplemental Table S6. Continued

	Cognition		Motor		Language		Behavior	
HC	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**
Circumference (per 20 mm)								
Birth	65	6.12 (-0.08;12.32)	53	3.60 (-3.67;10.87)	61	5.56 (-3.46;14.58)	63	-1.13 (-7.20;4.95)
GA 29wks	50	1.16 (-5.33;7.64)	41	2.87 (-4.19;9.93)	47	-2.95 (-12.04;6.14)	49	0.10 (-5.46;5.66)
NICU-transfer	32	NA	30	NA	32	NA	32	NA
2M-visit	27	NA	21	NA	26	NA	27	NA
Growth (per 1 mm/wk)								
Birth-NICU transfer	30	NA	29	NA	30	NA	30	NA
Birth-2M	27	NA	20	NA	25	NA	26	NA

Beta-coefficients (B) are shown per interquartile range of each marker, followed by 95% confidence intervals (CI). To avoid type I or II error in subgroups too small for the number of covariates in the model, analyses were only performed when the number of patients/ultrasounds was 40 or higher. Significant associations are depicted in ***bold****. We used the cognitive and total motor score of the Bayley Scales of Infant and Toddler Development-Third edition (for cognition and motor outcome, respectively), Lexi-list (language) and total score of the Child Behavior Checklist (behavior). Models were adjusted for sex (female 0, male 1), gestational age at birth (weeks), birth weight Z-score and combined parental education (low/middle/high). Apart from these four covariates, the GA29wks and 2M-visit models were also adjusted for gestational age or corrected age at measurement, respectively. Results from similar analyses on infants with brain injury are shown in supplemental Table 3.*
CC = corpus callosum, n = number of patients/ultrasounds included, GA = gestational age, NICU = neonatal intensive care, NA = not applicable, M = months, CCF = corpus callosum-fastigium, HC = head circumference.

Supplemental Table S7. Associations between length and growth of CC, CCF and HC, and neurodevelopmental outcomes at 2 years in children without brain injury (n=153, basic model)

		Cognition		Motor		Language		Behavior
CC	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**
Length (per 5 mm)								
Birth	113	-0.45 (-5.2;4.30)	96	3.90 (-0.72;8.51)	106	2.31 (-3.75;8.37)	105	-0.71 (-4.63;3.21)
GA 29wks	124	1.90 (-2.04;5.83)	101	3.06 (-0.95;7.07)	118	2.81 (-2.48;8.10)	119	-1.64 (-4.87;1.59)
NICU-transfer	80	1.26 (-4.52;7.05)	68	1.78 (-3.32;6.88)	77	0.49 (-6.13;7.11)	76	-1.42 (-5.53;2.69)
2M-visit	70	**5.64 (2.45;8.83)**	62	**4.16 (1.00;7.32)**	70	**6.26 (2.04;10.48)**	70	-2.05 (-4.59;0.50)
Growth (per 0.25 mm/wk)								
Birth-NICU transfer	75	0.04 (-1.48;1.56)	61	-0.94 (-2.46;0.57)	70	-0.05 (-2.09;1.98)	71	-0.78 (-1.97;0.41)
Birth-2M	63	**5.40 (1.01;9.79)**	56	**4.58 (0.34;8.83)**	63	4.39 (-1.45;10.23)	63	-2.71 (-4.92;1.23)
		Cognition		**Motor**		**Language**		**Behavior**
CCF	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**
Length (per 5 mm)								
Birth [a]	114	-3.30 (-9.13;2.54)	97	1.54 (-4.24;7.32)	107	-0.99 (-8.50;6.52)	106	-1.35 (-6.19;3.50)
GA 29wks	120	-0.92 (-5.98;4.13)	98	-2.47 (-7.68;2.73)	114	-0.59 (-7.35;6.16)	115	-1.88 (-6.05;2.30)
NICU-transfer [a]	76	1.02 (-6.20;8.24)	64	-1.37 (-7.90;5.16)	74	3.08 (-5.14;11.30)	73	-0.68 (-5.82;4.47)
2M-visit [b]	68	5.66 (-0.71;12.03)	60	-0.93 (-7.21;5.35)	68	7.13 (-1.11;15.36)	68	-1.99 (-6.84;2.85)
Growth (per 0.25 mm/wk)								
Birth-NICU transfer	73	-0.84 (2.36;0.67)	58	-0.50 (-2.05;1.06)	68	-0.08 (-2.13;1.97)	69	0.65 (-0.55;1.86)
Birth-2M	55	3.39 (-2.56;9.33)	52	0.96 (-4.85;6.67)	59	5.14 (-2.51;12.79)	59	-2.35 (-6.94;2.25)

Supplemental Table S7. Continued

	Cognition		Motor		Language		Behavior	
HC	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**
Circumference (per 20 mm)								
Birth [a]	68	-0.17 (-3.19;2.84)	121	-0.15 (-3.27;2.98)	138	-0.16 (-4.31;3.99)	138	-0.73 (-3.20;1.73)
GA 29wks	116	-1.33 (-3.69;1.02)	95	-1.13 (-3.59;1.32)	109	-1.94 (-5.19;1.32)	111	-1.33 (-3.27;0.62)
NICU-transfer [a]	80	2.66 (-1.65;6.98)	67	0.31 (-3.63;4.25)	77	0.14 (-4.82;5.10)	76	-1.73 (-4.75;1.29)
2M-visit [b]	67	2.68 (-0.95;6.32)	60	1.15 (-2.37;4.67)	68	**5.33 (0.78;9.87)**-	68	-0.85 (-3.58;1.88)
Growth (per 1 mm/wk)								
Birth-NICU transfer	70	0.08 (-1.00;1.15)	55	0.08 (-1.01;1.17)	66	0.18 (-1.60;1.24)	66	-0.39 (-1.23;0.46)
Birth-2M	66	1.83 (-1.82;5.47)	59	0.99 (-2.53;4.50)	67	**5.71 (1.22;10.20)**	67	-1.58 (-4.37;1.20)

Beta-coefficients (B) are shown per interquartile range of each marker, followed by 95% confidence intervals (CI). We used the cognitive and total motor score of the Bayley Scales of Infant and Toddler Development-Third edition (for cognition and motor outcome, respectively), Lexi-test (language) and total score of the Child Behavior Checklist (behavior). [a] *Adjusted for GA at ultrasound,* [b] *adjusted for corrected age at ultrasound. Significant associations are depicted in* ***bold****. CC = corpus callosum, n = number of patients/ultrasounds included, GA = gestational age, NICU = neonatal intensive care, M = months, CCF = corpus callosum-fastigium, HC = head circumference.*

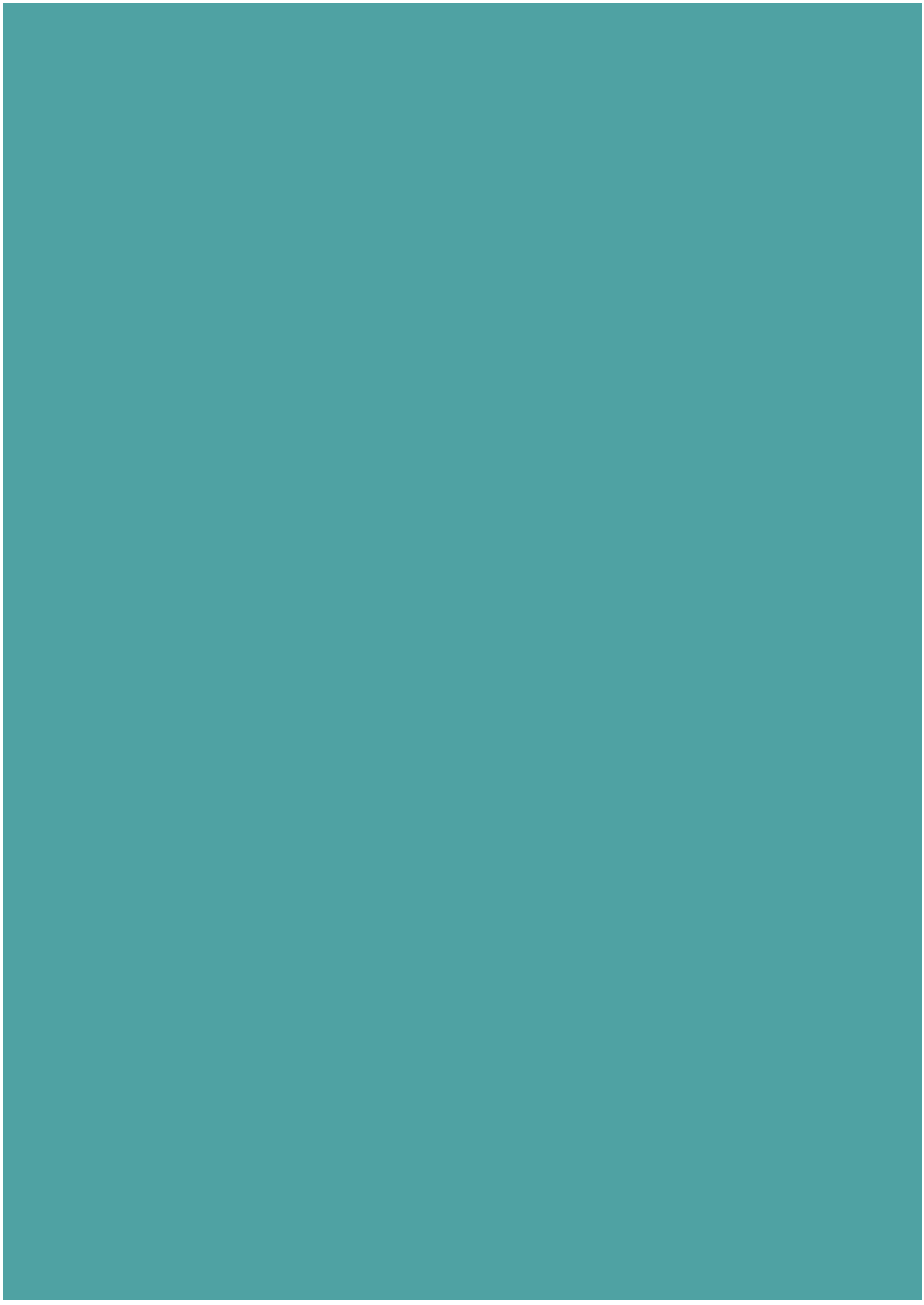
Supplemental Table S8.Associations between length and growth of CC, CCF and HC, and neurodevelopmental outcomes at 2 years in children with brain injury (n=72, basic model)

		Cognition		Motor		Language		Behavior
CC	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**
Length (per 5 mm)								
Birth	51	1.35 (-5.86;8.56)	44	3.64 (-3.82;11.10)	49	6.11 (-3.32;15.53)	51	-2.29 (-8.16;3.58)
GA 29wks	52	3.20 (-3.73;10.12)	43	2.12 (-5.91;10.15)	48	-2.81 (-12.25;6.64)	50	1.60 (-4.66;7.86)
NICU-transfer	38	NA	35	NA	37	NA	37	NA
2M-visit	28	NA	21	NA	26	NA	27	NA
Growth (per 0.25 mm/wk)								
Birth-NICU transfer	39	NA	34	NA	37	NA	39	NA
Birth-2M	27	NA	20	NA	25	NA	26	NA
CCF	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**
Length (per 5 mm)								
Birth [a]	51	0.43 (-9.39;10.26)	42	5.94 (-4.43;16.31)	49	2.81 (-10.23;15.84)	50	**-8.39 (-16.14;-0.65)**
GA 29wks	56	4.77 (-4.17;13.71)	46	**10.77 (1.04;20.50)**	52	3.38 (-8.73;15.50)	54	-6.02 (-13.90;1.87)
NICU-transfer [a]	36	NA	34	NA	35	NA	35	NA
2M-visit [b]	27	NA	21	NA	26	NA	26	NA
Growth (per 0.25 mm/wk)								
Birth-NICU transfer	33	NA	30	NA	32	NA	33	NA
Birth-2M	24	NA	18	NA	23	NA	23	NA

Supplemental Table S8. Continued

	Cognition		Motor		Language		Behavior	
HC	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**	**n**	**B (CI)**
Circumference (per 20 mm)								
Birth [a]	69	4.50 (-1.58;3.44)	56	4.61 (-0.74;9.96)	64	5.81 (-0.54;12.17)	66	-2.71 (-7.03;1.60)
GA 29wks	54	3.18 (-1.08;7.45)	44	4.04 (-0.72;8.81)	50	3.28 (-2.45;9.01)	52	1.02 (-2.66;4.70)
NICU-transfer [a]	34	NA	31	NA	34	NA	34	NA
2M-visit [b]	27	NA	21	NA	26	NA	27	NA
Growth (per 1 mm/wk)								
Birth-NICU transfer	32	NA	29	NA	32	NA	32	NA
Birth-2M	27	NA	20	NA	25	NA	26	NA

Beta-coefficients (B) are shown per interquartile range of each marker, followed by 95% confidence intervals (CI). We used the cognitive and total motor score of the Bayley Scales of Infant and Toddler Development-Third edition (for cognition and motor outcome, respectively), Lexi-test (language) and total score of the Child Behavior Checklist (behavior). [a] *Adjusted for GA at ultrasound,* [b] *adjusted for corrected age at ultrasound. Significant associations are depicted in* ***bold****. CC = corpus callosum, n = number of patients/ ultrasounds included, GA = gestational age, NICU = neonatal intensive care, NA = not applicable, M = months, CCF = corpus callosum-fastigium, HC = head circumference.*



CHAPTER 3

Early visuospatial attention and processing and related neurodevelopmental outcome at 2 years in children born very preterm

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ABSTRACT

Background: The ability to perceive and process visuospatial information is a condition for broader neurodevelopment. We examined the association of early visuospatial attention and processing with later neurodevelopmental outcome in very preterm infants.

Methods: Visuospatial attention and processing was assessed in 209 children (<30 weeks gestation) using an easy applicable eye tracking-based paradigm at 1 and 2 years. Average reaction times to fixation (RTF) on specific visual stimuli were calculated, representing time needed for overall attention (Cartoon stimuli) and processing (Motion and Form stimuli). Associations between RTFs and various measures of development at 2 years including cognitive and motor development (Bayley Scales of Infant and Toddler Development-Third edition; Bayley-III), language (Lexi test) and behavior (Child Behavior Checklist) were examined**.**

Results: At 1 year, 100msslower Cartoon and Motion RTFs were associated with lower cognitive Bayley-III scores (-4.4 points, 95% CI: -7.4;-1.5 and -1.0 points, -1.8;-0.2, respectively). A 100ms slower Cartoon RTF was associated with a 3.5 (-6.6;-0.5) point decrease in motor Bayley-III score.

Conclusions: Visuospatial attention and motion processing at 1 year is predictive of overall cognitive and motor development one year later. The nonverbal eye tracking-based test can assist in early detection of preterm children at risk of adverse neurodevelopment.

INTRODUCTION

Children born very preterm (gestational age <32 weeks) have an increased risk of neurodevelopmental impairment, which often presents in early childhood and lasts into adolescence and adulthood, as reflected by learning disabilities at school or work.[99-103] Early detection of probable neurodevelopmental impairment allows for timely interventions and individualized follow-up trajectories to prevent further delay. Standard neonatal follow-up programs mostly include preterm infants based on gestational age (GA, generally below 30 or 32 weeks) and/or birth weight (below 1000 or 1500 grams).[104,105] In this approach, not all children at risk of neurodevelopmental impairment are reached (e.g. children at risk but outside follow-up criteria), while redundant follow-up may take place in those who develop well. Current neurodevelopmental testing methods in young children are often lengthy (and thus demanding for the child) and costly (due to the need of trained personnel) and have limited predictive value for later IQ performance if used at an early age.[106,107] Therefore, there is a need for quick and easy tests which have a reliable predictive value that can be performed from an early age.

Neurodevelopmental impairment can be reflected in a broad spectrum of motor, cognitive, language, sensory and perceptual or behavioral problems.[108-111] An important conditional factor for both cognitive and motor development is visual (spatial) function, namely the ability to attend, perceive and process visual and spatial information in the environment.[112] Visuospatial attention and processing are vital functions that develop early in life and are regulated by an extensive cerebral network.[113] Visuospatial dysfunction is prevalent in preterm children, both with and without evident damage on brain imaging.[112,114-118] A recent cross-sectional study has linked delayed visual processing to impaired academic achievement in adolescents born extremely preterm.[119] As a result, they recommended testing of visual processing at a younger age: essential both to maximize early support and to study the predictive value of visual processing for later cognitive development.

Visual fixation (to a target moving horizontally, vertically and in an arc) has been tested at birth in full term infants,[120] and gaze gain (visual tracking through horizontal smooth pursuit, head movements and saccades) at four months in preterm infants.[121] Both measures showed a positive association with neurodevelopment at 2, 3 and/or 5 years. This suggests that early visuospatial testing could be predictive of later child development. Recently, a quantitative eye tracking-based method was developed to nonverbally assess visuospatial attention and processing.[49-51] During this assessment, a child is presented with specific visual stimuli on a computer screen,

while simultaneously eye movements are recorded using an integrated eye tracker. This way, reflexive viewing reactions to visual stimuli are quantified using reaction time and accuracy. This method can reliably detect abnormalities in visuospatial attention and various visual processing functions in children born very preterm at 1 year.[52,116] Whilst 'normal' development of visuospatial attention and processing is reflected by a significant decrease in viewing reaction times over age,[51] viewing reaction times do not always catch-up with this normative developmental trajectory in infants born preterm, resulting in a high prevalence of visuospatial delays at 1 and 2 years.[52,116,118] It is not yet known whether visuospatial attention and processing at 1 or 2 years, assessed using this eye tracking-based method, is associated with other neurodevelopmental domains.

In this study we hypothesized that delayed visuospatial attention and processing function at 1 and 2 years is related to neurodevelopmental impairment, and that these visuospatial functions can be used as early predictors of overall impaired neurodevelopment in children born very preterm. More specifically the aims of this study are:

1) to explore a possible association between visuospatial attention and processing at 1 and 2 years, and cognitive and motor development, expressive language and behavioral problems at 2 years; and

2) if an association is present, to evaluate whether there is added value in using these early measures of visuospatial attention and processing function for predicting neurodevelopmental outcome at 2 years compared to a prediction based on neonatal risk factors.

METHODS

Subjects

All preterm infants with a gestational age between 24 and 30 weeks who were admitted to the Neonatal Intensive Care Unit (NICU) of the Erasmus MC-Sophia Children's Hospital in Rotterdam within 48 hours after birth between 2011-2017 and who participated in the Blik Vooruit Study (Study A) and/or the BOND Study (Study B) were eligible for this study (n=283).[52,74] Combining these two cohorts was deemed suitable based on large similarity in source population, inclusion criteria, goals, methods and data collected.[52,74]

Infants were excluded from the study because ofsevere congenital or chromosomal abnormalities, perinatal asphyxia (cord blood/first postnatal PH <7.0 and APGAR score at 5min <5), an intraventricular hemorrhage (IVH, on cranial ultrasound in neonatal period) of grade III (with/without infarction), post hemorrhagic ventricular dilation (PHVD) requiring lumbar punctures, congenital TORCHES infection (toxoplasmosis, rubella, cytomegalovirus, herpes simplex, and other organisms including syphilis, parvovirus and varicella zoster) and those without any visuospatial assessment at 1 and 2 years CA. We also excluded children with retinopathy of prematurity (ROP, as assessed by a pediatric ophthalmologist) grade III or higher who received ophthalmic treatment (peripheral retinal laser photo-ablation or intravitreal bevacizumab injection) based on the association with impaired visual function which could influence eye tracking results.[122] In this study age refers to age corrected for prematurity.

Parental informed consent was obtained for all participants. Both Study A and B were approved by the medical ethical committee of the Erasmus Medical Center, Rotterdam.

Neonatal risk factors

Patient data were collected retrospectively (Study A) and prospectively (Study B) from the children's electronic medical records and regular follow-up questionnaires. These data consisted of parental characteristics (education level and ethnicity) and basic perinatal factors including antenatal steroids, sex, GA, birth weight, multiplet status**,** APGAR score and cord blood pH. From the neonatal period**,** information on respiratory (infant respiratory distress syndrome, bronchopulmonary dysplasia, mechanical ventilation, postnatal steroids), cardiac (inotropics, persistent ductus arteriosus), gastro-intestinal (necrotizing enterocolitis, abdominal surgery), infectious (sepsis), neurologic (IVH, periventricular leukomalacia, stroke, intracerebral bleeding) and ophthalmologic (ROP) factors, as well as data on general ill being (GA at discharge, duration of hospital admission) were explored.

Assessment and analysis of visuospatial attention and processing

All participants underwent visuospatial testing at 1 year and/or 2 years using the eye tracking-based method as previously described in more detail.[49,51,52,116] To guarantee sufficient visibility of the visuospatial assessment, a minimal visual acuity of 0.15 (Snellen equivalents, assessed with 4.8 cycles/cm Teller Acuity Card at 55 cm viewing distance) was ensured prior to the test. During the test the child was seated on the parent's lap at 60 cm distance from a 24-inch monitor with an integrated infrared eye tracking system sampling at 60 Hz (Tobii T60XL; Tobii Corporation, Danderyd,

Sweden). The system measures the gaze position of each eye separately with a latency of 30 ms. It also compensates for head movements within a range of 50-80 cm eye-monitor distance. After a standardized five-point calibration, children's viewing reactions were recorded during the presentation of a preferential looking paradigm on the monitor.[123] In the paradigm, various visual stimuli with distinctive target areas were randomly presented and used to assess visuospatial attention orienting and various types of visual processing.[52] To maintain the child's attention to the monitor, a standard set of short audiovisual movie clips was presented in between the test stimuli. During test administration basic oculomotor functions (saccades and smooth pursuit) were evaluated by observation. Total test duration was approximately 8 minutes. The assessment was repeated a second time in children who were able to maintain concentration.

Recorded eye movement data were analyzed offline using Matlab-based software (Mathworks Inc., Natick MA, USA), with a focus on reflexive, externally-triggered viewing reactions to the different visual stimuli (a more detailed description is described previously.[52,116] For each stimulus presentation, it was recorded whether the child detected the stimulus' target area and calculations regarding how fast the eyes reached the target were gathered (average reaction time to fixation; RTF).[50] RTF is a measure for the time needed to process presented visual information and execute an eye movement towards it. We analyzed viewing reactions to three stimuli that were previously found to be delayed in preterm children at 1 year including: Cartoon (a measure of general visuospatial attention orienting), Motion and Form (measures of motion and form processing).[52] To reach previously reported high reproducibility rates, strict criteria were used for inclusion of RTFs in further analyses (i.e. the child had to detect at least 20% of presentations per stimulus).[50]

For each child, the RTFs of all three stimuli were classified as either normal (within 95% confidence interval) or delayed (above the 95% confidence interval) based on a previously described normative reference sample of age-matched full term born controls.[51] Patterns of RTF delays (yes/no) from 1 to 2 years were categorized into four groups per stimulus: children with normal RTF at both ages (normal-stable), children with delayed RTF at both ages (abnormal-stable), children who changed from normal RTF at 1 year to delayed RTF at 2 years (deteriorated) and vice versa (normalized).[118]

Neurodevelopmental assessment

All children were routinely invited to the outpatient clinic at 2 years as part of the national neonatal follow-up program. During this visit, physical and neurological examination was done by a neonatologist or pediatric neurologist. Extensive testing

of psychomotor development was performed by a trained physiotherapist and psychologist using the fine motor and gross motor (summarized in a total motor score) and cognitive tests of the Bayley Scales of Infant and Toddler Development-Third edition (Bayley-III, Dutch edition: Bayley-III-NL).[32] In adherence to Dutch guidelines, expressive language development was evaluated by use of the Lexi test; a validated questionnaire completed by parents in order to quantify the child's vocabulary.[33] For each child, parents were asked to complete the Child Behavior Checklist for 1.5-5 years (CBCL); an internationally validated screening tool examining 13 domains of behavioral and emotional problems.[34] Neurodevelopmental outcomes were classified as moderately impaired when test scores were between 70-84 (cognitive and motor Bayley-III), 71-80 (Lexi test) or 60-63 (CBCL), whereas impairment was classified as severe for scores <70, <71 and >63 respectively. The neurodevelopmental assessors as well as the parents were not aware of the child's visuospatial test performance.

Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics, version 25.0 (IBM SPSS Statistics, Armonk, NY). P-values (two-tailed) below 0.05 were considered statistically significant. As most of the neonatal factors, neurodevelopmental outcomes and all visuospatial parameters were not normally distributed, medians and interquartile ranges were reported. Non-parametric statistical tests were used to explore selection bias, missing data, and group differences.

For the main analyses, linear regression models were used to explore associations between each of the three RTFs (Cartoon, Motion and Form stimuli) at 1 and 2 years and neurodevelopmental outcomes at 2 years. The primary focus was on four outcome measures: the cognitive and total motor scores of the Bayley-III, the total CBCL score and the Lexi score.To restrict multiple testing, additional analyses on the fine and/or gross motor subscales (Bayley-III) or the internalizing and externalizing subscales of the CBCL were conducted only if statistically significant associations were found between RTFs and the total motor and/or total CBCL score.Similarly, only if the RTF of a stimulus at 1 year showed a significant association with a certain outcome, further association of the patterns of delay from 1 to 2 years was explored for that outcome, given that the number of children within the delay pattern subgroup allowed for this. We evaluated effect size (β and adjusted R^2) and significance levels (p-values) of the models. Subgroup analyses on RTFs and the studied associations were performed in groups of children with or without ROP, with or without brain injury and below or above 28 weeks GA.

To evaluate the predictive value of visuospatial testing for neurodevelopmental outcome, a 'basic neonatal' multiple linear regression model was first devised. Out of all neonatal variables available, seven variables with low collinearity were selected based on their relevance reported in literature: sex, GA, combined parental education level, grade of bronchopulmonary dysplasia (BPD; 0: no BPD, 1: mild BPD, 2: severe BPD), treated patent ductus arteriosus (PDA; medical/surgical), brain injury (IVH grade II, stroke, cerebral bleeding or periventricular leukomalacia; PVL) and duration of hospital admission.[86,100,124-127]

Firstly, the RTFs that were associated with at least one of the neurodevelopmental outcomes and evaluated their predictive values (R^2) were selected. Secondly, the 'basic neonatal' multiple regression model was compared to a model that additionally included theRTFs ('neonatal and visuospatial' model). Using linear hierarchical regression models, the additional predictive value of visuospatial testing was expressed by the increase of predictive capacity (difference in adjusted R^2) by adding the RTFs. All residuals of the linear regression analyses were distributed fairly normally and there were no extreme outliers to exclude from analysis. Correction for multiple testing was not deemed necessary given the step-based and exploratory character of the analyses.

RESULTS

Participants

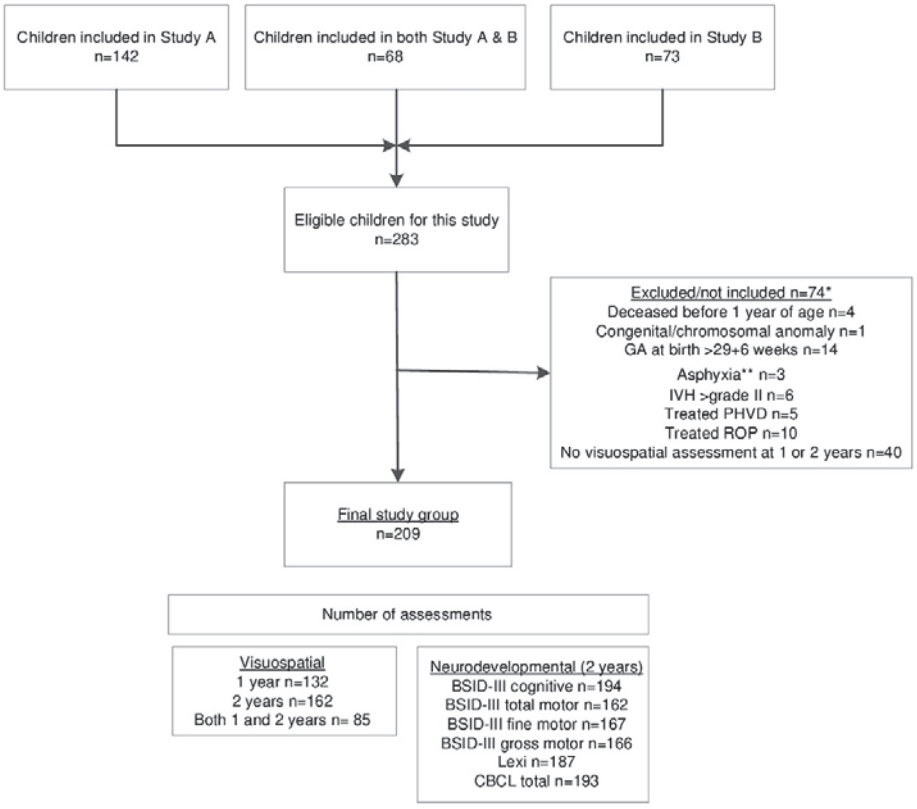
Figure 1 describes the inclusion of 209 children, with patient characteristics shown in **Table 1**. Comparisons with excluded children and by original study participation (Study A, B, or both) only showed differences that mirror our exclusion criteria or the time period of inclusion.

Visuospatial assessment

The visuospatial results for the three stimuli at 1 and 2 years are presented in **Table 2**, with rates of reliable tests ranging between 77-93%. The RTFs generally became faster between 1 and 2 years, as reflected by a decrease in RTFs for the Motion (-177ms, p=<0.001) and Form stimulus (-232ms, p=0.005). For all three stimuli, 66-69% of children had normal RTFs at both time points. Out of the children with reliable test results at 1 year, 34% showed delayed RTFs for at least one of the three stimuli presented. The prevalence of children with delayed RTFs increased from 16% at 1 year to 23% at 2 years for the Cartoon stimulus and from 12% to 33% (p=0.001) for the Motion stimulus, which translates to around 20% of children with a deteriorating

RTF pattern over time. When compared to the total study group, this group did not differ with respect to neonatal risk factors or neurodevelopmental outcome. In contrast, the rate of delayed response to the Form stimulus decreased from 19% at 1 year to 15% at 2 years. Subgroup analyses showed no significant differences in RTFs between children with or without ROP, with or without brain injury or born below or above 28 weeks of gestation.

Figure 1. Flowchart of the study group



** Numbers per reason of exclusion exceed total number of children excluded as some children were excluded for multiple reasons. ** Cord blood/first postnatal PH <7.0 & APGAR-score at 5min <5. GA = gestational age, IVH = intraventricular hemorrhage, PHVD = Post-Hemorrhagic Ventricular Dilation, ROP = retinopathy of prematurity, , BSID = Bayley, CBCL = Child Behavior Checklist.*

Table 1. Patient characteristics of the study population

	Study population (n=209)
Sex (female)	94 (45%)
Multiplet	63 (30%)
GA at birth (weeks)	27.7 [26.6;28.7]
Birth weight (grams)	1020 [868;1240]
Umbilical cord PH (mol/l)	7.31 [7.25;7.36], unknown 38 (18%)
Apgar5min (0-10)	8 [6;9], unknown 3 (1%)
Combined parental education level	
Low	29 (14%)
Middle	54 (26%)
High	103 (49%)
Unknown	23 (11%)
Ethnicity by country of birth parents	
Western European	151 (72%)
Non-Western European	50 (24%)
Unknown	8 (4%)
Inotropics	21 (10%)
Treated PDA	78 (37%)
Antenatal steroids	175 (84%), unknown 15 (7%)
IRDS (surfactant)	137 (66%)
Intubation	133 (64%)
Time on mechanical ventilation (days)	2 [0;7], unknown 4 (2%)
Postnatal steroids	38 (18%)
BPD, of which:	63 (30%)
Mild	44 (21%)
Severe	19 (9%)
NEC	10 (5%)
Culture proven sepsis	72 (34%)
IVH, of which:	53 (25%)
Grade I	22 (11%)
Grade II	31 (15%)
PVL	8 (4%)
Stroke	10 (5%)
Cerebellar bleeding	4 (2%)
ROP, of which:	67 (32%)
Grade I	52 (25%)
Grade II	11 (5%)
Grade III	4 (2%)
Surgery*, for reason:	39 (19%), unknown 1 (1%)
PDA	19 (9%), unknown 1 (1%)
NEC	3 (1%), unknown 1 (1%)
Other abdominal	13 (6%), unknown 1 (1%)
Other general (e.g. hernia inguinalis)	17 (8%), unknown 1 (1%)
Admission NICU (days)	29 [10;54], unknown 1 (1%)
Admission hospital (days)	84 [70;102], unknown 8 (4%)
GA at discharge (weeks)	39.6 [38.0;41.3], unknown 8 (4%)

*Data are shown as median [interquartile range] or absolute numbers (percentage). * Numbers per reason of surgery exceed total number of children with surgery as some children had multiple surgeries for varying reasons. GA = gestational age, PDA = persistent ductus arteriosus, BPD = bronchopulmonary dysplasia, NEC = necrotizing enterocolitis, IVH = intraventricular hemorrhage, PVL = periventricular leukomalacia, ROP = retinopathy of prematurity, NICU = neonatal intensive care unit.*

Table 2. Visuospatial attention and processing parameters at 1 and 2 years

	1 year (n=132)	2 years (n=162)	p-value
Age at measurement	1.00 [0.99; 1.03]	2.00 [1.99; 2.06]	
Cartoon			
Number of reliable tests	111 (84%)	141 (87%)	
% of stimuli detected	42% [29;58]	54% [33;75]	
Reaction Time to Fixation (ms)	275 [227;326]	251 [224;289]	0.83
Number delayed compared to term peers	18 (16%)	33 (23%)	0.17
Pattern of delay from 1 to 2 years	(n=67)		
Normal-stable	44 (66%)		
Abnormal-stable	4 (6%)		
Deteriorated	13 (19%)		
Normalized	6 (9%)		
Motion			
Number of reliable tests	113 (86%)	151 (93%)	
% of stimuli detected	50% [25;75]	50% [38;84]	
Reaction Time to Fixation (ms)	710 [571;853]	533 [456;642]	**<0.001**
Number delayed compared to term peers	14 (12%)	50 (33%)	**0.001**
Pattern of delay from 1 to 2 years	(n=75)		
Normal-stable	50 (67%)		
Abnormal-stable	6 (8%)		
Deteriorated	17 (23%)		
Normalized	2 (3%)		
Form			
Number of reliable tests	102 (77%)	145 (90%)	
% of stimuli detected	25% [13;50]	50% [25;75]	
Reaction Time to Fixation (ms)	1037 [810;1388]	805 [623;1016]	**0.005**
Number delayed compared to term peers	19 (19%)	22 (15%)	1.00
Pattern of delay from 1 to 2 years	(n=58)		
Normal-stable	40 (69%)		
Abnormal-stable	1 (2%)		
Deteriorated	8 (14%)		
Normalized	9 (16%)		

Count values are shown as absolute numbers (percentage), Reaction Times to Fixation and % of stimuli detected are shown as median [interquartile range]. Reaction Time to Fixation and number of delayed were compared within the subgroup with measurements at both time points (Wilcoxon signed ranks test and McNemar's test, respectively). Reaction Times to Fixation, number of delayed and patterns of delay were only calculated for reliable tests. Number and patterns of delay represent comparisons with the normative RTF references. Bold numbers indicate P<0.05.Normal-stable = no delay at 1 or 2 years, Abnormal-stable = delay at both 1 and 2 years, Deteriorated = no delay at 1 year but delay at 2 years; Normalized = delay at 1 year but no delay at 2 years.

Neurodevelopmental outcome

Table 3 shows the prevalence of neurological complications such as cerebral palsy and visual disorders (e.g. refractive error, strabismus, nystagmus), as well as median scores on the neurodevelopmental tests. Moderate to severe impairment for cognitive performance, motor functioning, language development and behavioral outcome was found in 8.2%, 7.4%, 24.1% and 7.3% of children, respectively.

Associations between visuospatial assessment and neurodevelopmental outcome

There were minimal associations between the seven neonatal risk factors and the RTF of any of the three stimuli at 1 or 2 years, although treated PDA and total days of hospital admission were associated with RTF Cartoon at 1 year, and sex with RTF Motion at 2 years, respectively (**Supplemental Table S1B**).

The associations between the RTFs of the three stimuli at 1 and 2 years and the four neurodevelopmental outcomes are shown in **Table 4**.

Table 3. Neurodevelopmental outcome of the study population at 2 years

	Study population (n=209)
CP, of which:	11 (5.3%), unknown 1 (0.5%)
GMFCS I	6 (2.9%)
GMFCS II	2 (1.0%)
GMFCS III	1 (0.5%)
GMFCS IV	2 (1.0%)
Visual disorders, of which:	13 (6.2%)
Wearing glasses	10 (4.8%)
Strabismus	6 (2.9%)
Nystagmus	1 (0.5%)
Bayley-III cognitive score, of which:	101 [91;105], unknown 15 (7.2%)
Moderate impairment (score 70-84)	14 (7.2%)
Severe impairment (score <70)	2 (1.0%)
Bayley-III total motor score, of which:	100 [92;109], unknown 47 (22.5%)
Moderate impairment (score 70-84)	11 (6.8%)
Severe impairment (score <70)	1 (0.6%)
Bayley-III fine motor score	11 [9;13], unknown 42 (20.1%)
Bayley-III gross motor score	9 [7;10], unknown 43 (20.6%)
Lexi test score, of which:	92 [82;102], unknown 22 (10.5%)
Moderate impairment (score 71-80)	28 (15.0%)
Severe impairment (score <71)	17 (9.1%)
CBCL total score, of which:	44 [38;53], unknown 16 (7.7%)
Borderline problem behavior (score 60-63)	5 (2.6%)
Clinical problem behavior (score >63)	9 (4.7%)
CBCL internalizing score	43 [37;51], unknown 16 (7.7%)
CBCL externalizing score	47 [41;55], unknown 16 (7.7%)

Data are shown as median [interquartile range] and absolute numbers (percentage).
CP = cerebral palsy, GMFCS = Gross Motor Function Classification System, Bayley-III = Bayley Scales of Infant and Toddler Development-Third edition; CBCL = Child Behavior Checklist.

Table 4. Associations between RTFs at 1 and 2 years, and neurodevelopmental outcomes at 2 years

	1 year					
	RTF Cartoon (n=111)		**RTF Motion (n=113)**		**RTF Form (n=102)**	
2 years	**B (CI)**	**R^2**	**B (CI)**	**R^2**	**B (CI)**	**R^2**
Bayley-III cognitive	-4.4 (-7.4;-1.5)	7.2*	-1.0 (-1.8;-0.2)	4.5**	0.2 (-0.2:0.6)	†
Bayley-III total motor	-3.5 (-6.6;-0.5)	4.4**	-0.5 (-1.5;0.5)	†	0.1 (-0.4;0.5)	†
Lexi	0.1 (-4.3;4.4)	†	-0.4 (-1.7;0.8)	†	-0.5 (-1.0;0.1)	†
CBCL total	-0.1 (-2.8;2.6)	†	0.3 (-0.5;1.1)	†	-0.0 (-0.4;0.4)	†
	2 years					
	RTF Cartoon (n=141)		**RTF Motion (n=151)**		**RTF Form (n=145)**	
2 years	**B (CI)**	**R^2**	**B (CI)**	**R^2**	**B (CI)**	**R^2**
Bayley-III cognitive	-1.3 (-4.8;2.1)	†	-0.6 (-1.5;0.3)	†	-0.1 (-0.5;0.3)	†
Bayley-III total motor	-0.6 (-5.0;3.7)	†	0.1 (-1.0;1.2)	†	-0.5 (-1.0;0.0)	†
Lexi	2.9 (-1.7;7.4)	†	-0.4 (-1.5;0.7)	†	-0.5 (-1.1;0.1)	†
CBCL total	0.9 (-2.2;4.0)	†	-0.1 (-0.8;0.6)	†	-0.1 (-0.5;0.3)	†

*Beta-coefficients (B) are shown per 100ms, followed by 95% confidence intervals (CI). R^2 is the adjusted proportion of the variance explained, shown as percentage. * = p-value<0.005, ** = p-value < 0.05, † = R^2 <3.0% and p-value>0.05. RTF = Reaction Time to Fixation, Bayley-III = Bayley Scales of Infant and Toddler Development-Third edition; CBCL = Child Behavior Checklist.*

Higher (slower) Cartoon and Motion RTFs at 1 year were significantly associated with a lower cognitive Bayley-III score at 2 years: a 100ms increase in RTF of the Cartoon stimulus was associated with a 4.4 point (95% CI: -7.4;-1.5) lower cognitive Bayley-III score, whereas a 100ms increase in Motion RTF resulted in a lowering of cognitive score by 1.0 point (95% CI: -1.8;-0.2). In addition, a 100ms higher RTF of the Cartoon stimulus at 1 year was associated with a 3.5 (95% CI: -6.6;-0.5) point lower total motor Bayley-III score, which was mainly explained by the gross motor score (β=-0.9, 95% CI: -1.5;-0.3, R^2=8.2%) but not by the fine motor score (β=-0.5, 95% CI: -1.2;0.3, R^2=0.6%). Subgroup analyses showed that the above significant associations were strongest in children born after 28 weeks GA, without brain injury or without ROP. There were no significant associations for the RTFs of the Form stimulus, nor for any of the RTFs with language and behavioral outcomes. Furthermore, none of the RTFs at 2 years were associated with any of the four outcomes at the same time point.

Results of the exploratory regression analyses on the patterns of delay in Cartoon RTF and cognitive and motor outcome should be interpreted with caution because of small sample sizes in three of the four pattern-subgroups. There was a trend showing that a normal-stable pattern (n=44) was regularly followed by higher Bayley-III motor scores (β=8.2, 95% CI: 1.9;14.4; median score 107). A normalized pattern (n=6) was

linked to lower cognitive scores (β=-5.95, 95% CI: -15.3;3.4; median score 96) as well as motor scores (β=-10.87, 95% CI: -20.6;-1.1; median score 93), when compared to the total study group.

Explanatory value of RTFs compared to neonatal risk factors

The explanatory values (R^2) of RTF of the Cartoon (7.2%) and Motion (4.5%) stimuli for the cognitive Bayley-III outcome were either in a similar range as explanatory values of the individual neonatal risk factors (e.g. 5.6% for BPD grade, 6.7% for total days of hospital admission or 8.9% for parental education), or higher (e.g. R^2 for GA, sex, treated PDA and brain injury were 0.3-1.5%). Similarly, for the motor Bayley-III score, the variance explained by the Cartoon RTF (4.4%) was within the range of variances explained by the individual neonatal risk factors (0.0-8.9%) (**Table 4** and **Supplemental Table S1A***).*

In **Table 5** we show the effect sizes of the combined 'basic neonatal' model and the 'neonatal and visuospatial' model. There was a small but significant increase in explained variance of the cognitive Bayley-III score when RTFs of the Cartoon and Motion stimuli at 1 year were added to the 'basic neonatal' model (R^2=3.9%, p=0.04). Adding the Cartoon and Motion RTFs to the 'basic neonatal' models for motor Bayley-III, Lexi or CBCL scores did not increase the effect size of these models.

Table 5. Added explanatory value of Cartoon and Motion RTFs to a basic neonatal model for prediction of neurodevelopmental outcome at 2 years.

n=101	Basic neonatal model		Neonatal and visuospatial model		Difference	
	R^2	P	R^2	P	ΔR^2	ΔP
Bayley-III cognitive	34.5	**<0.001**	38.4	**<0.001**	3.9	**0.04**
Bayley-III total motor	12.9	**0.02**	12.7	**0.03**	*	0.39
Lexi	14.5	**0.01**	12.8	**0.03**	*	0.75
CBCL total	19.9	**0.001**	18.2	**0.004**	*	0.79

*Explanatory value per outcome of the 'basic neonatal' model with only neonatal risk factors (sex, GA, combined parental education level, BPD grade, treated PDA, brain injury en total days of hospital admission) and the 'neonatal and visuospatial' model (same neonatal risk factors + RTFs for the Cartoon and Motion stimulus at 1y CA), and the difference (Δ) between the two models. R^2 is the adjusted proportion of the variance explained by each model, shown as percentage. ΔR^2 depicts how much more of outcome variance is explained through adding RTF for both the Cartoon and Motion stimulus at 1 year to the model. Significance levels (P) are given for the proportion of variance explained as well as for the increase in explanatory value (ΔP). Bold numbers indicate P<0.05. * = R^2 <1.0%. RTF = Reaction Time to Fixation, Bayley-III = Bayley Scales of Infant and Toddler Development-Third edition; CBCL = Child Behavior Checklist, GA = gestational age, BPD = bronchopulmonary dysplasia, PDA = persistent ductus arteriosus, brain injury = intraventricular hemorrhage grade I or II, stroke, cerebral bleeding, or periventricular leukomalacia*

DISCUSSION

Our study showed that delays in visuospatial attention and motion processing at 1 year CA are associated with lower Bayley-III cognitive and motor scores at 2 years CA. The individual explanatory values (R^2) of these visuospatial factors (i.e. viewing reaction times to the Cartoon and Motion stimuli) for the cognitive and motor Bayley-III outcome are similar to or higher than explanatory values of known important neonatal risk factors such as sex, gestational age, BPD or parental education in our study. Adding the visuospatial factors at 1 year to a prediction model with a combined set of neonatal risk factors leads to a modest but significant increase in explanatory value for cognitive neurodevelopmental outcome at 2 years.

The proportion of reliable assessments (77-93%) and prevalence of delayed visuospatial attention and processing (12-33%) and a deteriorating delay pattern (20%) in this study are comparable to previous reports in (preterm) children using the same eye tracking-based method at the same ages.[52,116,118,128] However, in the current, larger, study we found slightly fewer children with delayed RTF for the Cartoon stimulus at 1 year (16%, compared to 19-23% in previous studies).[52,118] This difference may be due to the low rate of severe brain damage in thestudy group following the exclusion of children with IVH grade III (with or without infarction) and treated PHVD who are more likely to have a complicated neonatal course and impaired neurodevelopmental outcome. Although not all children with severe brain injury were excluded (the cohort still contained some children with stroke, PVL or cerebral bleeding), this may also explain therelatively highneurodevelopmental scores and normal rates of impaired expressive language development and behavioral problems in our study group when compared to previous literature.[129-134]

The strongest association between visuospatial attention and processing function at 1 year and neurodevelopmental outcomes at 2 years was found for visuospatial attention orienting, measured with the highly salient Cartoon stimulus.[51] It seems plausible that the general ability to orient visual attention is closely related to the relatively broad measures of cognitive and motor development. In addition, we found an association between viewing reaction times to the Motion stimulus and cognitive outcome. Reacting to this stimulus requires the detection and processing of movement, which typically starts developing around 3 months of age and is regulated by the so-called *dorsal* visual processing pathway.[112] This dorsal pathway is also involved in attentional capabilities and is therefore likely to be implicated in viewing reactions to the Cartoon stimulus as well. Disturbance of this *dorsal* pathway can be present irrespective of evidence for brain damage, which suggests

compromised cerebral connectivity on a more microstructural level.[116,117] On the other hand, detecting and processing of Form information is regulated by the so-called *ventral* visual processing pathway, which is more often related to periventricular brain damage and starts developing after 4-6 months of age.[112,117] This differential maturation process may translate to the larger intra-individual variation in RTFs for the Form stimulus at early ages in both preterm and full term born children.[52,118] This less reliable and discriminativenature of RTF of the Form stimulus may explain why it was not associated with any of the outcomes.

Importantly, the measurement of visuospatial attention and processing as employed in the present study revolves around reflexive viewing reactions to visual input. These reactions are indicative of the efficiency with which visual input is detected, processed, and responded to by means of an eye movement. Given that visuospatial orienting is a relatively low-level function (i.e., *acknowledging* to see something yes/no) with limited cognitive involvement (i.e., it does not involve *understanding* what you see), it is unlikely that delayed viewing reactions are directly related to cognitive dysfunction.[135] Instead, this association may be mediated by top-down or executive attentional functions that are more directly related to general cognitive development. Alternatively, viewing reactions may be a qualitative marker of visual information conduction, in the sense that better-developed cerebral connectivity could allow for faster viewing reactions but also for faster cognitive processing.

Using the eye tracking-based method at 1 year significantly added to the prediction of neurodevelopmental outcome at 2 years. However, the added explanatory value to our 'basic neonatal' prediction model was small (R^2 3.9%) and only present for cognitive Bayley-III score. This added value is lower than the 11.4% increase in predictive ability *Kaul et al* found for cognitive Bayley-III outcome at 3 years after adding visual tracking function to their –slightly different- neonatal model.[121] However, direct comparison of these percentages is complicated by important differences in type of visual function tested (i.e. visual tracking of the eyes versus processing functions) and exclusion criteria. Moreover**,** our well performing 'basic neonatal' model for cognitive outcome, with an R^2 of 35%, may have left less room for improvement.

Language and behavioral outcomes at 2 years had no significant relation with visuospatial attention and orienting at 1 or 2 years, and low or even absent associations with neonatal risk factors. These findings illustrate the complex and multifactorial origin of language and behavior which likely make them more difficult to predict. Very little is known about the relation between language and visuospatial

function. *Geldof et al* found that visual perceptive dysfunction explained small amounts of variance in verbal IQ (VIQ) when compared to performance IQ (PIQ) at 5 years (13% vs 35%, respectively), and that children with cerebral visual impairment had significantly lower PIQ but not VIQ, as compared to those without cerebral visual impairment.[136,137] This could be explained by the fact that visuospatial function is believed to share neural networks and visual abilities with cognitive performance, but less so with expressive language development.[136] With regard to behavioral outcome, previous eye tracking-based studies in preterm children at an older age showed associations between aberrant gaze patterns or other eye movement errors or delays and psychiatric disorders, diagnosed with the Diagnostic and Statistical Manual of Mental Disorders (DSM).[138,139] However, the CBCL for 1.5-5 years used in the present study is a screening questionnaire with questionable predictive value for later DSM-related pathology.[140-143] Given these challenges in diagnosing behavioral disorders at the age of 2 years, it would be interesting to follow behavioral performance up to a later age.

No associations between visuospatial attention and processing at 2 years and neurodevelopmental outcome were found. Exploration of the delay patterns suggests that it is mainly the measurement at 1 year that drives the predictive effect. In particular, visuospatial function could be considered an essential factor to normal cognitive development in the following year(s). This implies that visuospatial dysfunction at 2 years mayin fact be associated with impaired neurodevelopment later in childhood. Hence, follow-up studies are needed to investigate how the current associations evolve over the course of childhood, especially given the before mentioned limited predictive value of early Bayley-III testing for later IQ performance.[106,107] Another explanation for the absent association at 2 years might be the smaller intra-individual variation in RTFs at 2 years as compared to 1 year. A larger sample size might therefore be needed to reveal subtle associations.

A strength of this study is the large cohort of young preterm children that constitutes a representative sample of the broader preterm population, namely with no, mild or moderate brain damage, in which neurodevelopment has always been difficult to predict. Another strength of this study is the extensive information on perinatal and neonatal risk factors which allowed for a 'basic neonatal' model with high predictive ability. In addition, the reflexive nature of the eye tracking-based paradigm and the fact that results are only obtained when a child actually attends the paradigm, means that its parameters (RTFs) are not likely to be influenced by loss of attention, fatigue or lack of motivation. This is an important characteristic because conventional neurodevelopmental test results are generally hampered by such factors.

A limitation of this study is that due to practical reasons, only 85 (41%) of the 209 children underwent visuospatial testing at both 1 and 2 years. This resulted in insufficient statistical power to investigate the visuospatial delay patterns over time in more detail. In addition, translating results into clinical practice requires further research. However, our study may be a steppingstone towards more individualized follow-up programs, needed in times of health care cuts and development of personalized medicine. The study showed that adding visuospatial attention and processing dysfunction to current criteria for inclusion in neonatal follow-up programs could improve detection of children at risk of adverse neurodevelopment rather than using cut-offs based on neonatal factors alone. In addition to its potential predictive value for general adverse neurodevelopment, adding this quick and easy visuospatial test as a screening tool to neonatal follow-up programs allows for detection of preterm children at risk of (cerebral) visuospatial dysfunction, a neurodevelopmental domain that is currently not incorporated in follow-up programs. Including this domain is of importance, given that the prevalence of children born preterm showing signs of (cerebral) visual (spatial) impairment in the first 5 years of life is high (20-45%).[52,118,137,144]

CONCLUSION

This study showed that visuospatial attention and motion processing function at 1 year is a predictive factor for overall cognitive and motor development 1 year later. This suggests that a quick and easy eye tracking-based assessment can help to identify preterm children at risk of adverse neurodevelopment. Although follow-up studies are needed to investigate how these associations evolve over the course of childhood, this visuospatial method could be a valuable addition to neonatal follow-up programs in the future.

Supplemental Table S1A. Associations between neonatal risk factors and neurodevelopmental outcomes at 2 years

	Sex (n=209)		GA (n=209)		Combined parental education (n=186)		BPD grade (n=209)		Treated PDA (n=78)		Brain injury (n=61)		Total days of hospital admission (n=201)	
	B (CI)	**R^2**	**B (CI)**	**R^2**	**B (CI)**	**R^2**	**B (CI)**	**R^2**	**B (CI)**	**R^2**	**B (CI)**	**R^2**	**B (CI)**	**R^2**
Bayley cogn	-3.4 (-6.7;-0.0)	1.5**	1.1 (-0.0;2.2)	†	4.9 (2.7;7.2)	8.9*	-4.6 (-7.1;-2.0)	5.6*	-2.2 (-5.6;1.3)	†	-3.7 (-7.4-0.1)	1.5**	-0.1 (-0.2;-0.1)	6.7*
Bayley motor	-2.4 (-6.2;1.3)	†	0.6 (-0.6;1.8)	†	5.2 (2.6;7.8)	8.9*	-2.4 (-5.3;0.5)	†	-2.0 (-5.9;1.9)	†	-6.3 (-10.5;-2.2)	4.9*	-0.1 (-0.2;-0.0)	2.6**
Bayley fine mot	-	-	-	-	1.1 (0.5;1.7)	7.8*	-	-	-	-	-1.0 (-2.0;-0.1)	2.4**	-0.0 (-0.0;0.0)	†
Bayley gross mot	-	-	-	-	0.7 (0.1;1.3)	3.3**	-	-	-	-	-1.1 (-2.0;-0.2)	3.2**	-0.0 (-0.0;-0.0)	2.5**
Lexi	-0.0 (-4.6;4.6)	†	0.2 (-1.3;1.7)	†	2.2 (-1.1;5.6)	†	-3.8 (-7.3;-0.3)	1.9**	-0.4 (-5.1;4.4)	†	2.0 (-3.0;7.0)	†	-0.1 (-0.2;-0.0)	1.7**
CBCL total	-3.2 (-6.1;-0.4)	2.1**	0.1 (-0.8;1.1)	†	-3.1 (-5.1;-1.1)	4.6*	-0.3 (-2.5;1.9)	†	-1.4 (-4.3;1.5)	†	2.4 (-0.8;5.6)	†	-0.0 (-0.1;0.1)	†
CBCL int	-3.4 (-6.4;-0.3)	1.9**	-	-	-3.9 (-6.0;-1.8)	6.5*	-	-	-	-	-	-	-	-
CBCL ext	-2.3 (-5.1;0.5)	†	-	-	-3.0 (-4.9;-1.1)	4.5*	-	-	-	-	-	-	-	-

*Beta-coefficients (B) are followed by 95% confidence intervals (CI). R^2 is the adjusted proportion of the variance explained, shown as percentage. Sex: female = 0, male = 1; GA = gestational age in weeks, combined parental education: 1 = low, 2 = middle, 3 = high; BPD = bronchopulmonary dysplasia (0 = no BPD, 1 = mild BPD, 2 = severe BPD), PDA = persistent ductus arteriosus, brain injury = intraventricular hemorrhage grade II, stroke, cerebral bleeding or periventricular leukomalacia; Bayley = Bayley Scales of Infant and Toddler Development-Third edition (cogn = cognitive, mot = motor); CBCL = Child Behavior Checklist (int = internalizing, ext = externalizing). * = p-value<0.005, ** = p-value < 0.05, † = R^2 <1.5% and p-value>0.05, - = test on subscale not performed because total scale was not significantly associated.*

Supplemental Table S1B.Associations between neonatal risk factors and RTFs at 1 and 2 years

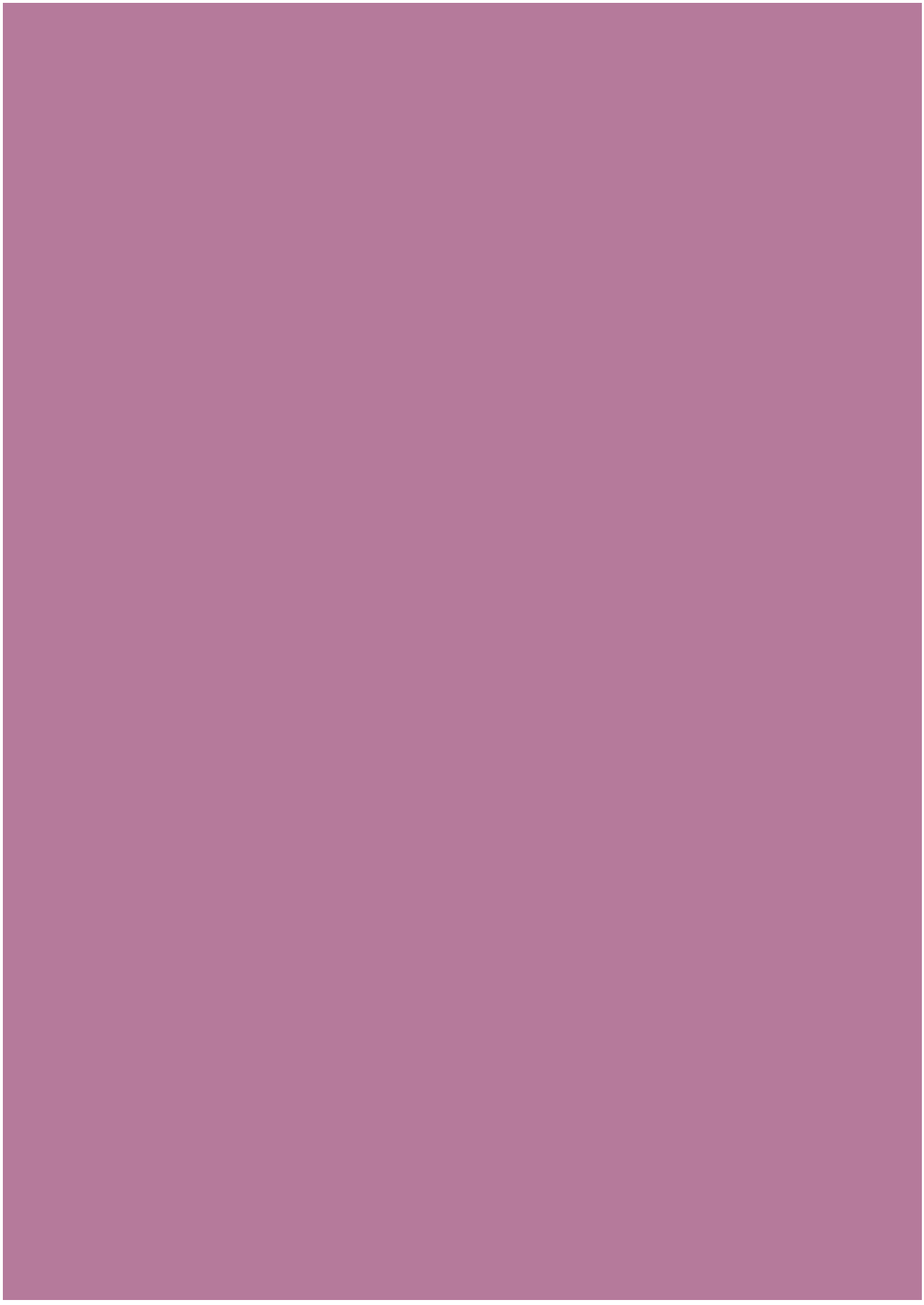
		Sex (n=209)		GA (n=209)		Combined parental education (n=186)		BPD grade (n=209)		Treated PDA (n=78)		Brain injury (n=61)		Total days of hospital admission (n=201)	
		B (CI)	R^2	B (CI)	R^2	B (CI)	R^2	B (CI)	R^2	B (CI)	R^2	B (CI)	R^2	B (CI)	R^2
1 year	**Cartoon**	0.1 (-0.1;0.4)	†	-0.1 (-0.2;0.0)	2.4¥	-0.1 (-0.3;0.1)	†	0.0 (-0.2;0.2)	†	0.4 (0.1;0.6)	4.7*	0.1 (-0.2;0.4)	†	0.0 (0.0;0.0)	2.7*
	Motion	0.7 (-0.2;1.6)	†	-0.1 (-0.4;0.2)	†	-0.3 (-0.9;0.4)	†	0.2 (-0.5;0.9)	†	0.6 (-0.3;1.5)	†	0.1 (-0.9;1.1)	†	0.0 (-0.0;0.0)	†
	Form	-1.0 (-3.2;1.1)	†	-0.4 (-1.1;0.3)	†	0.2 (-1.3;1.7)	†	1.1 (-0.7;3.0)	†	-0.2 (-2.4;2.0)	†	0.3 (-2.1;2.7)	†	-0.0 (-0.0;0.0)	†
2 years	**Cartoon**	0.0 (-0.1;0.2)	†	0.0 (-0.1;0.1)	†	-0.1 (-0.2;0.0)	†	-0.0 (-0.2;0.1)	†	0.2 (-0.0;0.4)	†	-0.0 (-0.2;0.2)	†	-0.0 (-0.0;0.0)	†
	Motion	-0.7 (-1.5;-0.0)	1.9*	0.0 (-0.3;0.3)	†	0.1 (-0.5;0.6)	†	0.1 (-0.5;0.6)	†	-0.2 (-1.0;0.6)	†	0.2 (-0.6;1.1)	†	-0.0 (-0.0;0.0)	†
	Form	-0.9 (-2.4;0.7)	†	0.0 (-0.5;0.6)	†	0.5 (-0.4;1.5)	†	0.1 (-1.0;1.2)	†	0.2 (-1.4;1.8)	†	-1.2 (-2.8;0.4)	†	-0.0 (-0.1;0.0)	†

*Beta-coefficients (B) are shown per 100ms and followed by 95% confidence intervals (CI). R^2 is the adjusted proportion of the variance explained, shown as percentage. RTF = Reaction Time to Fixation per 100ms, Sex: female = 0, male = 1; GA = gestational age in weeks, combined parental education: 1 = low, 2 = middle, 3 = high; BPD = bronchopulmonary dysplasia (0 = no BPD, 1 = mild BPD, 2 = severe BPD), PDA = persistent ductus arteriosus, brain injury = intraventricular hemorrhage grade II, stroke, cerebral bleeding or periventricular leukomalacia. * = p-value < 0.05, ¥ = p-value > 0.05, † = R^2 <1.5% and p-value>0.05.*

3

PART II

Early growth and body composition in children born preterm



CHAPTER 4

Early weight gain trajectories and body composition in infancy in infants born very preterm

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ABSTRACT

Background: Concerns are raised about the influence of rapid growth on excessive fat mass (FM) gain in early life and later cardiometabolic health of infants born preterm.

Objectives: To study the association between postnatal weight gain trajectories and body composition in infancy in infants born very preterm.

Methods: In infants born <30 weeks gestation, we evaluated associations between weight Z-score trajectories for three consecutive timeframes (NICU stay, level-II hospital stay and at home) and body composition, measured at 2 and 6 months corrected age by air-displacement plethysmography.

Results: Of 120 infants included, median gestational age at birth was 27^{+5} (interquartile range 26^{+1};28^{+5}) and birth weight 1015 grams (801;1250). The majority of infants did not make up for their initial loss of weight Z-score, but growth and later body composition were within term reference values. Weight gain during NICU stay was not associated with fat mass (absolute, %FM or FM index) in infancy. Weight gain during NICU and level II hospital stay was weakly associated with higher absolute lean mass (LM), but not after adjustment for length (LM index). Weight gain in the level-II hospital was positively associated with fat mass parameters at 2 months but not at 6 months. Strongest associations were found between weight gain at homeand body composition (at both time points), especially fat mass.

Conclusions: Weight gain in different timeframes after preterm birth is associated with distinct parameters of body composition in infancy, with weight gain at home being most strongly related to fat mass.

INTRODUCTION

Infants who are born prematurely start their extra-uterine life in a critical period for growth and development.[145,146] They are at high risk for postnatal growth restriction, associated with long term neurodevelopmental problems.[16,17] To improve neurodevelopmental outcome, pro-active nutritional treatment in early life has been recommended by the American Academy of Pediatrics and European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN).[147,148] However, concerns have been raised about the adverse influence of high nutritional intake and rapid growth during the first months of life on later cardiometabolic health.[149,150] The underlying theory is covered in the Developmental Origins of Health and Disease (DOHaD) paradigm. The DOHaD hypothesis postulates that after a period of nutritional deprivation, stress, or inflammation (e.g. fetal growth restriction (FGR), preterm birth or stay on the neonatal intensive care unit (NICU)), an environment with a relative excess of oxygen (radicals) and nutrients can result in an increased risk of adverse cardiometabolic health.[151]

In infants born at term, higher protein intake during the first year of life was found to be associated with an increased risk of obesity at school age.[152] In infants born preterm, however, recent studies show contradictive effects of enhanced early nutrition and growth during the first year of life on long-term cardiometabolic outcome.[55,153-158] The question therefore remains which growth pattern is most beneficial for long-term outcome: do the known neurodevelopmental benefits of early rapid weight gain outweigh the potential risk of adverse cardiometabolic health in child- and adulthood?[25]

To answer these questions, it is essential to determine whether early rapid weight gain in infants born preterm is indeed harmful, and to identify critical periods. This is important as specifically in preterm born infants, nutritional practices change heavily during the first months of life, and even short periods of altered growth may have great impact on later health and development.[155] Also, a reliable early marker of cardiometabolic health is needed. Body composition, which can be measured patient-friendly in the outpatient setting, has been used in earlier studies showing different trajectories in infants born preterm and full term.[54,55]

In term born infants, the first three months of life are identified as most critical for the development of cardiometabolic risk factors during infancy such as overall fat and visceral fat.[159,160] Unfortunately, in infants born preterm, studies assessing growth over multiple timeframes are scarce, so a critical growth period has not

been identified yet.[155] In The Netherlands, national policy is to transfer preterm born infants from the NICU (a level-III or level-IV hospital) to a level-II hospital as soon as they are stable, usually between 30-32 weeks of gestation, to stay there until discharge home. These clear cut offs between an early 'critical' neonatal phase and a more 'stable' phase facilitate studying growth over different time frames.

In this study, we aimed to study the association between postnatal weight gain during three different timeframes (NICU-stay, level-II hospital stay and at home) and body composition at 2 and 6 months corrected age in infants born very preterm (< 30 weeks of gestation). We hypothesized that associations are timeframe specific, with greater postnatal weight gain being associated with both higher lean and fat mass in infancy.

METHODS

This study is part of an ongoing prospective observational cohort study (BOND Study), conducted at the level IV NICU and the outpatient clinic of the Erasmus MC – Sophia Children's Hospital, Rotterdam, the Netherlands. Infants born before 30 weeks of gestation, admitted within 48 hours after birth, were eligible for inclusion in the study. Exclusion criteria included congenital anomalies (including chromosomal defects) that may interfere with growth, severe brain injury (i.e., intraventricular hemorrhage grade III/IV and post-hemorrhagic ventricular dilatation requiring lumbar or ventricular reservoir punctures), congenital infections, and perinatal asphyxia (umbilical cord pH < 7.00 and APGAR score at 5 min < 5). Data were collected between September 2014 and January 2018. The study is registered in the Netherlands Trial Register (NTR6024) and approved by the local ethical review board. Written parental informed consent was obtained before enrollment in the study.

Local nutrition protocol

During NICU stay, all infants were fed according to the parenteral and enteral ESPGHAN guidelines.[26,74,148] In short, parenteral glucose administration was started directly after birth, with a minimum of 4 and maximum of 12 mg/kg/min. Amino acid administration was also started directly after birth at 2.4 g/kg/d and gradually increased to a target dose of 3.5 – 4.0 g/kg/d. Lipids were started the day after birth at 2.4 g/kg/d and gradually increased to a target dose of 2.5 – 3 g/kg/d. Enteral bolus feeding was started on the day of birth and increased daily.[161] With this increasing daily enteral intake, parenteral nutrition was stepwise decreased and ceased at an enteral intake of 130 ml/kg/d.

Expressed breast milk was the first choice of enteral feeding. If not (sufficiently) available, preterm formula was supplemented (Nenatal start®, Nutricia Advanced Medical Nutrition, Zoetermeer, the Netherlands), as donor milk was not available. Breast milk fortification was started at an enteral intake of 100 ml/kg/d (Breast Milk Fortifier®, Nutricia Advanced Medical Nutrition, Zoetermeer, the Netherlands).

After transfer from the NICU, feeding regimens, including fortification and post-discharge feeding, were determined according to local hospital guidelines. Dependent on the developmental stage and health status of the child, scheduled nasogastric tube feeding was gradually decreased, to reach breastfeeding or bottle feeding on demand at home. After discharge home, post-discharge formula or prolongation of fortification was not given routinely. The treating neonatologist set the indication for fortification and post-discharge feeding, based on individual growth trajectories, taking feeding mode, tolerance, and parental preferences into account.

Clinical data

Maternal characteristics and obstetrical and neonatal data were prospectively collected. In this study unlabeled use of age refers to age corrected for prematurity. Postnatal age was defined as days after birth, with the day of birth corresponding with day 1. At each study visit, parents filled out a questionnaire on their infants' feeding practices to collect data on type of feeding, fortification, and complementary feeding. Estimation of socio-economic status was based on home address, using Z-scores that summarize the local average income, low-income rate, low educational level rate, and unemployment rate in the ZIP code area.[162]

Growth

Body weight, head circumference, and length measurements were performed as part of standard care according to local protocols. Length was not routinely measured during NICU and level-II hospital stay, but was part of follow-up anthropometry. Gestational age (GA) and sex-corrected Z-scores for weight were calculated at the following time points: birth, postnatal weight nadir (day with lowest postnatal weight), 30 weeks GA, transfer from NICU to level-II hospital, discharge home, and at both outpatient clinic visits (2 and 6 months). Z-scores were based on the Fenton growth charts from birth until discharge or 50 weeks GA, and on the World Health Organization (WHO) growth charts thereafter.[88,163,164]

Outpatient clinic visits

All patients attended the standard national neonatal follow-up program for medical and neurodevelopmental assessment. Visits were planned around 2 months

and 6 months corrected age. At both visits, body composition was measured for research purposes using air-displacement plethysmography (PEA POD®, Infant Body Composition System, COSMED). This validated method estimates fat mass as percentage of total body weight (%FM), absolute fat mass (FM) and lean mass (LM) by direct measurements of body volume and mass.[59,165] To correct body composition variables for small body size (such as expected in our study group), FM index (FMI; FM (kg) / length $(m)^2$) and LM index (LMI; lean mass (kg) / length $(m)^2$) were calculated by dividing the FM and LM by squared length.[166] Age and sex corrected Z-scores for body composition parameters were calculated based on average values from a large group of term born infants measured at our research center within the same time period.[167] The body composition data contained no extreme outliers to exclude from analysis.

Statistical analyses

The first step was to model individual weight gain trajectories based on weight Z-score within different time frames using linear mixed models. Because infants experience physiologic weight loss in the first days of life, the first weight gain trajectory was estimated from the moment of maximum postnatal weight loss (weight nadir, median day 5).[27] Weight gain trajectories were studied within the following timeframes: (1) NICUstay: from initial postnatal weight nadir until transfer from NICU to level-II hospital; (2) level-II hospitalstay: from admission to level-II hospital until discharge home; (3) homestay: from discharge home until body composition measurement at 2 or 6 months at the outpatient clinic. The weight gain trajectories were modelled by using weight Z-score (dependent variable) as the response over time with postnatal age at weight measurement as covariate (independent). Fit of the level-II hospitalandhome weight gain statistical model was optimal with a random intercept and slope, and for the NICUweight gain model with addition of a quadratic slope. Subject-specific weight Z-score trajectories were then expressed by the individual intercept and (quadratic) slope. In the second step of the analysis, these subject-specific weight Z-score indicators were used as covariates (independent) in the linear regression analyses with %FM, FM, FMI, LM and LMI as outcome measures (dependent). The basic regression model included the subject-specific weight Z-score indicators, and sex, gestational age at birth, birth weight Z-score, and corrected age at body composition measurement. The adjusted model included the covariates of the basic model plus days on parenteral nutrition during NICU stay, days on mechanical ventilation, socio-economic status, and the use of any breast milk at the 2 months-visit.

To evaluate the contribution of the weight Z-score trajectories to body composition, we compared the explained variances of the model with and without the subject

specific weight Z-score indicators included, using the Likelihood Ratio Test. The difference in explained variance of the models could be contributed to the subject-specific effects of weight gain.

Explorative analysis

To date, no strict values for body composition parameters are known to be considered harmful for long-term health. Therefore, we divided our study population in three tertiles for FMI at 6 months. We visually explored the weight Z-scores trajectories of these tertiles between birth and 6 months.

A 2-tailed *P* value <0.05 was considered statistically significant. Analyses were performed using SPSS package 25.0 (IBM SPSS Statistics, Armonk, NY) and R (R: A language and environment for Statistical Computing, version 3.5.1, 2018 for Windows, R Core Team, Vienna, Austria).

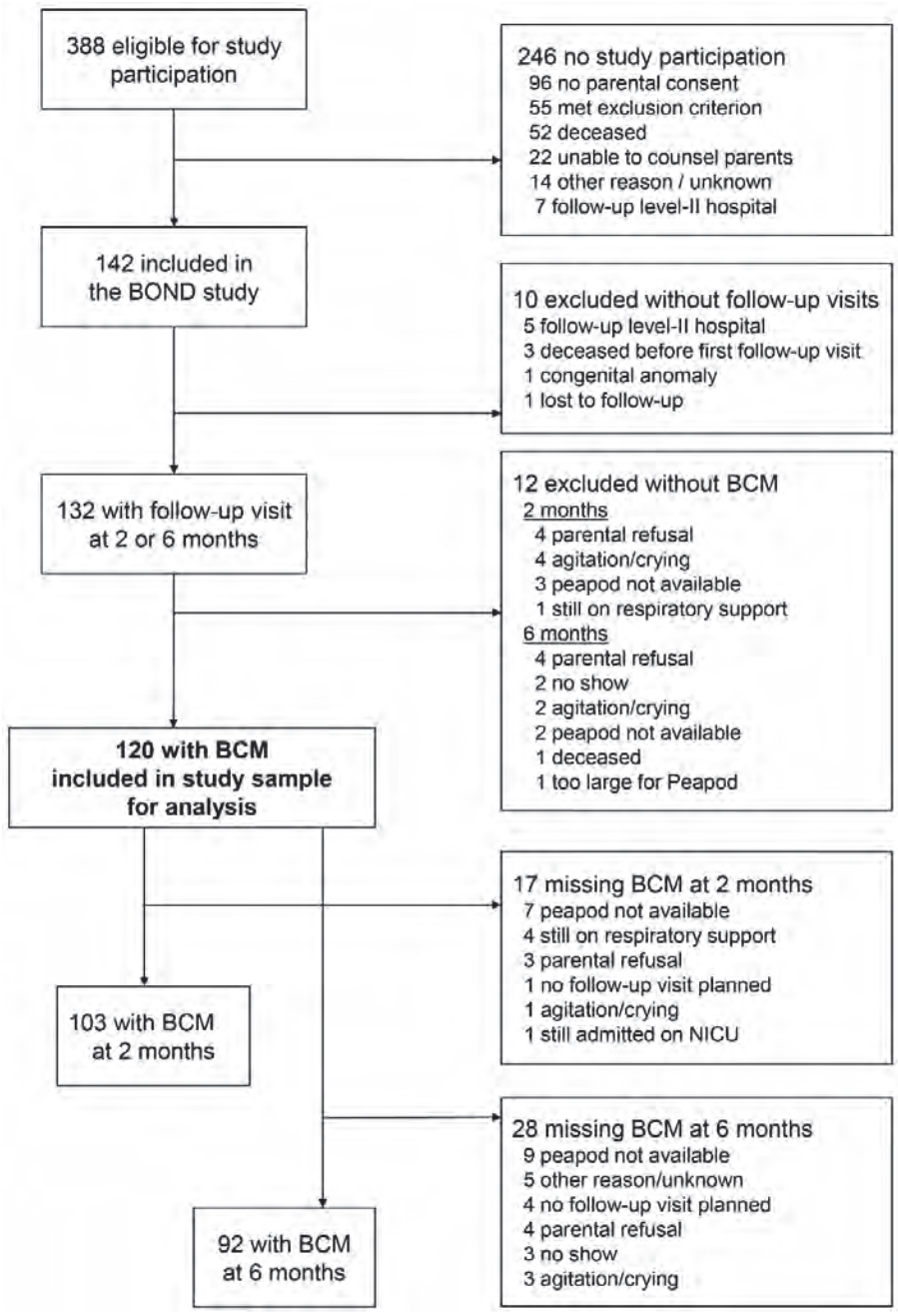
RESULTS

In total, 142 infants were enrolled in the BOND Study. After excluding one infant with a congenital anomaly interfering with growth (panhypopituitarism), three infants who deceased before discharge home, six without follow-up visits at the outpatient clinic, and 12 without any body composition measurements, the study population included for analysis consisted of 120 infants **(Figure 1)**.

Baseline characteristics

The baseline characteristics of the study population are provided in **Table 1**. Median GA at birth was 27^{+5} weeks (interquartile range (IQR) 26^{+1};28^{+5}) with a birth weight of 1015 g (IQR 801;1250), 0.1 SD (-0.4;0.7). Median duration of parenteral amino acid or lipid administration during NICU stay was 10 days (8;16). The majority of the infants (*n*=90, 75%) received both own mothers' milk and formula during NICU stay. The infants were transferred from the NICU to a level-II hospital at a median postnatal age of 29 (17;67) days, corresponding with 32^{+0} (30^{+3};36^{+1}) weeks of gestation. They were discharged home at a postnatal age of 84 (70;104) days, corresponding with 39^{+5} (38^{+0};41^{+5}) weeks of gestation.

Figure 1. Flowchart of the study population



Flowchart of the study population and number of body composition measurements at the outpatient clinic visits at 2 and 6 months. Abbreviations: BCM, body composition measurement; NICU, neonatal intensive care unit.

Table 1. Maternal and infant characteristics (n=120)

Maternal characteristics		
Age at delivery	*years*	30 (27;34)
Pre-pregnancy BMI	*kg/m²*	24.7 (21.8;29.1)[1]
Pregnancy complications	*(G)DM*	7 (6%)
	Hypertension [a]	8 (7%)
	PE/HELLP	20 (17%)
	FGR [b]	22 (18%)[2]
	PPROM	25 (21%)
Singleton pregnancy		94 (78%)
Antenatal corticosteroids	*0/1/2 doses*	8/33/79 (7/28/66%)
Caesarean section		70 (58%)
Infant characteristics		
Sex	*male*	75 (63%)
GA at birth	*weeks*$^{+days}$	27^{+5} (26^{+1};28^{+5})
Birth weight	*gram*	1015 (801;1250)
	Z-score	0.1 (-0.4;0.7)
Apgar	*5 min*	8 (6;9)[3]
Culture-proven sepsis	*early onset* [c]	4 (3%)
	late onset	39 (33%)
NEC	*Bell stage ≥2*	5 (4%)
Treated PDA		39 (33%)
BPD [d]	*mild*	28 (23%)
	severe	17 (14%)
Postnatal steroid use		20 (17%)[2]
Brain injury [e]		36 (30%)
Treated ROP		6 (5%)
Mechanical ventilation	*days*	2 (0;11)
NICU stay	*days*	29 (17;67)
GA at NICU transfer to level-II hospital	*weeks*$^{+days}$	32^{+0} (30^{+3};36^{+1})
Total hospital stay [f]	*days*	84 (68;104)[3]
GA at discharge home	*weeks*$^{+days}$	39^{+5} (38^{+0};41^{+5})[3]

All data are expressed in median (interquartile range) or number (percentages). [a] *Either pre-existent or pregnancy induced;* [b] *Estimated fetal weight or abdominal circumference below 10th percentile on Robinson curve;* [c] *Positive blood culture within 72h after birth;* [c] *BPD: >28 days O2 + X-ray abnormalities, severe BPD: endotracheal or CPAP at 36 weeks of gestation or >30% fiO2 or >1L/min flow via nasal prongs;* [e] *Brain injury includes IVH gr I/II, cerebellar bleeding, arterial/venous stroke, periventricular leukomalacia and convulsions;* [f] *NICU + level-II hospital. Missing data:* [1] *14 infants,* [2] *4 infants,* [3] *1 infant. Abbreviations: n, number; BMI, body mass index; (G)DM, (gestational) diabetes mellitus; PE, pre-eclampsia, HELLP, hemolysis, elevated liver enzymes, and a low platelet count; FGR, fetal growth restriction; PPROM, preterm prelabour rupture of membranes; GA, gestational age; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; BPD, bronchopulmonary dysplasia; ROP, retinopathy of prematurity; NICU, neonatal intensive care unit.*

Growth

Growth data are shown in **Table 2**. Maximum initial weight loss of birth weight was median 10.4% (IQR 7.6;13.6) which was reached on median day five (3;6), resulting in a weight Z-score decrease of -0.8 SD. Overall, infants were unable to make up for this early loss of weight Z-score during the study period. Median length and head circumference did show an increase in Z-score from -1.6 SD and -0.8 SD at transfer to the level-II hospital to -0.2 SD and +0.3 SD at 6 months, respectively.

Body composition

Of the 120 infants enrolled in the study, body composition was measured in 103 (86%) at 2 months and in 92 (77%) at 6 months (**Table 2)**.In 76 infants (63%) body composition was measured at both time points. Reasons for missing body composition measurements are presented in **Figure 1**.

Median %FM at 2 months was 21.9% (0.5 SD, 17.8;23.9) and 20.4% (-0.6SD, 18.0;23.3) at 6 months, with similar values in boys and girls (**Supplemental Table S1**). Between 2 and 6 months, median FMI of all infants measured decreased whereas median LMI increased. Clinical and nutritional characteristics of the infants during the outpatient visits can be read from **Table 3**.

Associations between weight gain and body composition

The results of the fully adjusted regression analyses are presented in **Table 4.** The basic model generally showed the same effects and similar effect sizes as the fully adjusted model (data not shown). NICU weight gain was weakly associated with higher LM at 2 months: weight gain (Z-score) trajectories during NICU stay explained 3.3% of the variance in LM at 2 months, with the association losing significance when LM was adjusted for length (LMI). No association was found for NICU weight gain and FM, %FM or FMI at 2 months, or with any of the body composition parameters at 6 months.

Weight gain in the level-II hospital was positively associated with all components of body composition at 2 months, except LMI. At 6 months, it was only positively associated with absolute LM, not with LMI or any of the fat parameters. Weight gain at home was strongly positively associated with all measures of body composition at both 2 and 6 months, especially fat parameters. For FMI, the variance explained by weight gain at home was 46.3% at 2 months, and 36.5% at 6 months (both $p<0.001$).

Table 2. Growth and body composition parameters from birth to 6 months

		Birth	Weight nadir	Transfer NICU to level-II hospital	Discharge home	2M-visit	6M-visit
Growth[1]	**n**	120	120	120	119	119	113
Gestational/corrected age[2]	*weeks*$^{+days}$	27^{+5} (26^{+1};28^{+5})	28^{+2} (26^{+5};29^{+1})	32^{+0} (30^{+3};36^{+1})	39^{+5} (38^{+0};41^{+5})[a]	7^{+4} (6^{+4};9^{+6})	26^{+3} (25^{+2};27^{+5})
Postnatal age	*days*	1	5 (3;6)	29 (17;67)	84 (68;104)[a]	138 (128;163)	273 (261;286)
Weight	*kg*	1.02 (0.80;1.25)	0.89 (0.73;1.10)	1.45 (1.25;2.04)	3.11 (2.71;3.59)[a]	4.73 (4.15;5.30)	7.19 (6.42;7.73)
	Z-score	0.1 (-0.4;0.7)	-0.7 (-1.2;-0.3)	-0.9 (-1.5;-0.4)	-0.7 (-1.7;0.1)[a]	-0.8 (-1.6;-0.1)	-0.7 (-1.5;0.1)
Head circumference	*cm*	24.8 (23.7;26.6)[b]	NA	28.0 (26.9;31.4)[c]	34.5 (33.2;36.3)[d]	38.7 (37.5;40.0)[b]	43.4 (42.0;44.2)[b]
	Z-score	0.1 (-0.5;0.6)[b]	NA	-0.8 (-1.4;-0.3)[c]	-0.1 (-1.1;0.6)[d]	0.2 (-0.7;1.0)[b]	0.3 (-0.5;1.0)[b]
Length	*cm*	NA	NA	38.0 (36.6;42.4)[e]	48.0 (46.0;50.3)[f]	55.2 (53.6;57.2)[a]	66.4 (64.0;68.5)[g]
	Z-score	NA	NA	-1.6 (-2.6;-1.0)[e]	-1.0 (-2.1;-0.6)[f]	-1.1 (-1.9;-0.3)[a]	-0.2 (-1.2;0.5)[g]
Body composition	**n**					103	92
Relative fat mass	*%*	NA	NA	NA	NA	21.9 (17.8;23.9)	20.4 (18.0;23.3)
	Z-score	NA	NA	NA	NA	0.5 (-0.4;1.1)	-0.6 (-1.3;-0.1)
Absolute fat mass	*kg*	NA	NA	NA	NA	0.98 (0.78;1.30)	1.44 (1.12;1.70)
	Z-score	NA	NA	NA	NA	0.0 (-0.8; 0.8)	-0.8 (-1.5;-0.2)
Fat mass index	*kg/m*^{2}	NA	NA	NA	NA	3.42 (2.66;3.95)	3.25 (2.76;3.87)
	Z-score	NA	NA	NA	NA	0.3 (-0.4;1.1)	-0.6 (-1.2;-0.1)
Absolute lean mass	*kg*	NA	NA	NA	NA	3.70 (3.33;4.04)	5.65 (5.08;6.08)
	Z-score	NA	NA	NA	NA	-0.9 (-1.6;-0.3)	-0.4 (-1.2;0.3)
Lean mass index	*kg/m*^{2}	NA	NA	NA	NA	12.04 (11.32;12.82)	12.67 (12.0;13.4)
	Z-score	NA	NA	NA	NA	-0.1 (-0.8;0.8)	0.1 (-0.6;0.6)

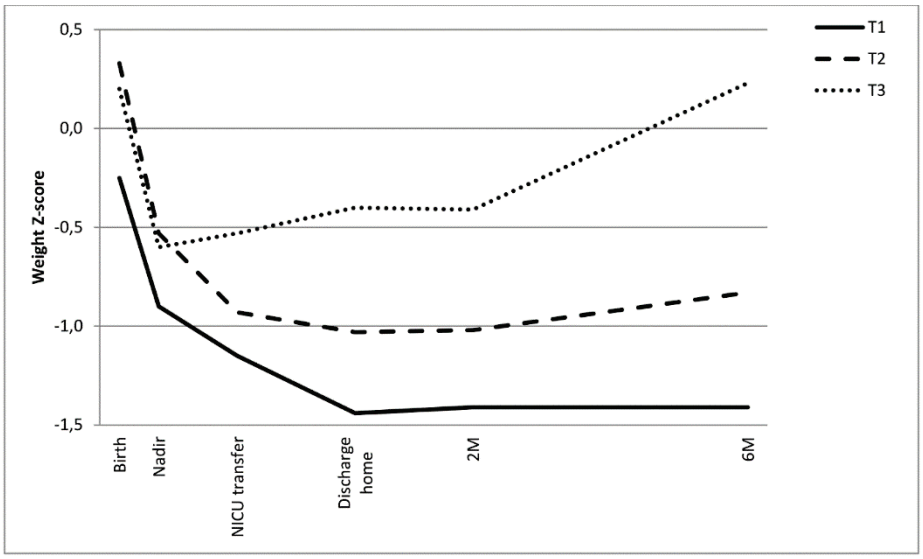
All data are expressed in median (interquartile range). Length measurement was not routinely collected during NICU and level-II hospital stay, leading to many missing values. Z-scores for body composition parameters were calculated based on average values from a large group of term born infants assessed at our research center within the same time period. [1] *Missing growth data of 1 infant at discharge home, 1 infant at 2M-visit (no appointment at outpatient clinic) and 7 infants at 6M-visit (4 no appointment at outpatient clinic, 3 no show for appointment).* [2] *Gestational age for birth until discharge home, corrected age for 2M- and 6M-visits. Missing data:* [a] *2 infants,* [b] *3 infants,* [c] *14 infants,* [d] *44 infants,* [e] *64 infants,* [f] *71 infants,* [g] *1 infant.Abbreviations: NICU, neonatal intensive care unit; M, month; n, number; kg, kilograms; cm, centimeter; m, meter.*

Table 3. Clinical characteristics at the outpatient clinic visits

		2 months	6 months
n		119 (99%)	113 (94%)
Corrected age	*weeks*	7.6 (6.6;9.9)	26.4 (25.3;27.7)
Feeding type [a]	*Own mothers milk*	19 (16%)	5 (4%)
	Formula feeding	78 (66%)	101 (89%)
	Mixed feeding	22 (19%)	4 (4%)
Enriched nutrition [b]		23 (20%)	4 (4%)
Tube feeding		16 (13%)	4 (4%)
Parenteral nutrition		2 (2%)	1 (1%)
Oxygen supply		9 (8%)	4 (4%)

All data are expressed in median (interquartile range) or number (percentages). [a] *Data missing for 3 infants at 6 months,* [b] *Either preterm formula or fortified human milk enriched with extra protein, or with fat or carbohydrates (rare); data missing for 1 infant at 2 months and 2 infants at 6 months. Abbreviations: n; number.*

Figure 2. Weight Z-score trajectories grouped for each FMI tertile at 6 months



Abbreviations FMI, fat mass index; T1, lowest FMI tertile (FMI ≤2.93 kg/m²); T2, middle FMI tertile (FMI 2.93-3.71 kg/m²); T3, highest FMI tertile (FMI ≥3.72 kg/m²); Nadir, day with lowest postnatal weight; NICU, neonatal intensive care unit; M, months.

Table 4. Associations between weight gain trajectories and body composition at 2 months and 6 months

	2 months (n=103)					6 months (n=92)				
	%FM	**FM**	**FMI**	**LM**	**LMI**	**%FM**	**FM**	**FMI**	**LM**	**LMI**
Weight gain trajectory										
NICU	¥	¥	¥	3.3%*	¥	¥	¥	¥	¥	¥
Level-II hospital	6.5%*	11.9%**	7.9%*	10.8%**	¥	¥	¥	¥	4.6%*	¥
Home	32.6%**	49.8%**	46.3%**	24.6%**	6.0%*	24.6%**	46.8%**	36.5%**	31.8%**	8.6%*

*The values represent the variance (R^2) in body composition explained by the weight gain trajectories (Z-score), computed as the change in R^2 by adding the timeframe specific weight gain trajectory indicators (intercept and/or slope) to the linear regression model. Covariates included gestational age at birth, birth weight Z-score, sex, corrected age at body composition measurement, days on parenteral nutrition during NICU stay, days on invasive respiratory support, socio-economic status, and breast milk at two months CA (any/no). Abbreviations: %FM, percentage fat mass relative to weight; FM, absolute fat mass in kilograms; FMI, fat mass index (FM/length(m)^2); LM, absolute lean mass in kilograms; LMI, lean mass index (LM/length(m)^2); NICU, neonatal intensive care unit. ¥ R^2 <3% and p-value>0.05, * p-value<0.05, **p-value<0.001*

Explorative analyses

Median weight Z-score trajectories from birth to 6 months of the three FMI tertiles at 6 months are shown in **Figure 2,** with infant characteristics and growth parameters of each subgroup in **Supplemental Table S2**. In all groups, around 50% was being exclusively breastfed and 55% used fortification at discharge home. Infants in the lowest tertile started at a 0.5 SD lower mean birth weight Z-score as compared to the middle and highest tertile, and overall lost another 1 SD during hospital stay, to remain stable after discharge home until 6 months. Their relatively lower weight at 6 months (-1.4 SD) is mainly explained by low fat (FMI -1.6 SD), at a normal lean mass for length (LMI 0.0 SD). Infants in the middle FMI tertile started at a higher birth weight Z-score than infants in the lowest tertile but showed a similar pattern with an initial drop to -1 SD in weight Z-score and little change after NICU transfer. Their body composition measures suggest a more balanced fat and lean mass acquisition, with a FMI at -0.7 SD and LMI at -0.1 SD. Only the highest tertile showed a gradual increase of weight Z-score after the initial dip in the NICU, with the steepest increase in the period between 2 and 6 months. Their growth and body composition measures were very close to the median of the healthy term born references, suggesting proportionate growth.

DISCUSSION

In this prospective cohort study we studied the associations between postnatal weight gain trajectories during different timeframes, and body composition in infancy in infants born very preterm. We found that weight gain in the postnatal period was associated with an increase in both lean and fat mass in infancy. In line with the hypothesis, the associations with body composition were timeframe specific. Greater weight gain during NICU and level II hospital stay was (weakly) associated with higher absolute lean mass in infancy, but not after correction for length (LMI). Weight gain during NICU stay was not associated with any of the fat mass parameters (absolute, %FM or FMI), though weight gain in the level-II hospital was positively associated with all three fat mass parameters at 2 months. Weight gain at homewas most strongly associated with body composition, especially fat mass, at both 2 and 6 months, also when adjusted for length.

Our findings are largely in line with previous studies, reporting positive associations between in-hospital weight gain and lean mass and %FM at discharge.[168,169] With regard to body composition later in infancy, previous studies showed that early weight gain (from birth until body composition measurement) was associated with

%FM at 3, 6 and 12 months,[170] whereas insufficient weight gain before and after 36 weeks GA was associated with lower lean mass, fat mass and %FM at 6 months.[171] What our study adds is more insight into effects of weight gain over different (critical and stable) timeframes in infancy on body composition. In addition, by using FMI and LMI, we took length into account, which is important in a cohort of preterm born children at risk of restricted growth.

Our longitudinal data showed that the postnatal decrease in median weight Z-score from 0.1 SD to -0.7 SD in the first days of life, in clinical practice considered as physiological, was not recovered at 6 months. Head circumference and length increased in Z-score after NICU discharge, reaching values similar to healthy term born infants at 6 months. The increase in length and head circumference Z-scores corresponded with a similar increase in lean mass between 2 and 6 months, also reflected by a stable lean mass index.

We found that %FM and FMI were above average values of term born infants at 2 months (+0.5 SD and +0.3 SD) but decreased to below average term values at 6 months (both -0.6 SD). This decreasing relative fat mass during infancy corresponds with previous findings showing that %FM is higher in infants born preterm around term equivalent age, and decreases to levels below those of infants born full term three to four months after term age.[55,129,172-175] It seems that the peak in fat mass presents earlier in infants born preterm (at around 3 months corrected age) than in infants born full term (at around 6 months).[167,176,177] The mechanism of this altered fat trajectory in infants born preterm is not yet understood. Recent studies suggest that the rise in fat mass in the first months after birth is the result of adaptation to challenges of ex utero life, and may therefore be physiological in both infants born at term and preterm.[55,168,176] Following this hypothesis, infants born preterm would simply start this transition 'earlier' but at a comparable postnatal age as infants born at term.[55] Two studies comparing infants born 'late' and 'early' preterm indeed showed that infants born 'early' preterm had a higher %FM around 32-36 weeks GA and at term equivalent age than infants born 'later' preterm.[178,179] In addition to early adaptation, differences in nutrition and early feeding practices between term infants ('natural' ad lib oral feeding) and preterm infants ('artificial' parenteral and tube feeding) will likely play a role in the altered fat trajectory and earlier fat peak seen in preterm infants. Body composition measurements closer to birth are needed to further explore the exact onset of rapid fat accumulation in infants born preterm. In practice, this is complicated as body composition measurements using the PEA POD® can only be performed when the infant is weaned from respiratory support.

Our findings on the association between weight gain during NICU stay and body composition in infancy can only cautiously be extrapolated to clinical practice. Based on earlier reports, the observed lean mass gain without an effect on fat mass may be beneficial for both neurodevelopmental outcome and cardiometabolic health.[129,180-182] This supports recent adaptations in nutritional policy, including early high parenteral amino acid provision. However, the lack of an association between NICU weight gain and fat mass in the first 6 months could also reflect malnourishment during NICU stay. In the acute phase after birth, preterm born infants may need all caloric intake for vital energy expenditure, with little to none left for fat storage or catch up growth.[74] Altogether, our data suggest that during the first critical period, focus should still be on further optimizing nutrition to prevent growth restriction and lean mass deficit, without major concerns about adverse effects on cardiometabolic health in the first months of life.[168]

Greater weight gain after NICU stay, especially athome, was strongly related to higher fat mass levels in infancy. For example, infants in the highest tertile of FMI at 6 months showed the greatest increase in weight Z-score between 2 and 6 months, mostly explained by fat mass gain. Interestingly, this did not result in high fat mass parameters as compared to term born infants. Therefore, it is unclear whether this weight gain is an actual risk factor for long term cardiometabolic health.[55] On one hand, the trajectory with rapid fat accumulation may program the body for an increased risk of metabolic complications in adult life. On the other hand, this rapid fat accumulation may be protective for later cardiometabolic health by compensating for the growth restriction which developed during the critical phase of NICU stay. It may even be the infants in the lowest FMI tertile that are most at risk, as their weight Z-score trajectory is most deviant from infants born full term. Therefore, follow-up of this cohort into school age and adulthood is warranted to provide a complete view on the influence of early postnatal growth on cardiometabolic health, and to link the growth trajectories with neurodevelopmental outcome. Definitions of optimal growth and body composition trajectories in infants born preterm are needed for a next step in clinical nutritional care: using outcome-based targets for growth and body composition could be preferred over the current practice of targeting on reference values based on the distribution in the general population.

The strengths of our study include the prospective design and longitudinal growth measurements, which enabled us to model individual weight Z-score trajectories, rather than only cross-sectional growth measures. Furthermore, the national policy of transferring infants born preterm to a level-II hospital at around 32 weeks GA facilitated studying growth over different timeframes (critical versus stable hospital phase versus at home) which turned out to have distinct effects on body

composition. Lastly, comparison of body composition measures with healthy term born infants was possible by the availability of locally generated reference values. A few considerations should be taken into account when interpreting our study results. First, only in 63% of the infants' body composition measurement was measured twice, which might hamper the comparability of the group analyses at 2 and 6 months. Second, we used the PEA POD® to measure body composition, because it is a validated device that is feasible in clinical and research settings and is most patient-friendly.[59,60,165] A drawback of this method however, is the lack of information on fat distribution (e.g. subcutaneous versus visceral), while excessive visceral fat is considered an important risk factor of adverse cardiometabolic health.[183] Third, this study was not designed to elucidate the underlying mechanisms and risk factors explaining the differences in early growth trajectories. The sample size did not allow for subgroup analysis to study, for example, the effects of sex, FGR or type of nutrition on the associations between weight gain and body composition. We were also not able to enter more potential confounders to the models, such as specific medication use (e.g. steroids) or nutritional intake during each timeframe. However, by correcting for several perinatal, neonatal and sociodemographic factors in our models, we assume that the most important confounders have been covered. This is supported by the descriptive data, which do not suggest an important role of sex, breastfeeding or fortification in the association between weight gain and body composition. To further explore the role of these factors in future large cohort studies, requires more detailed longitudinal data and complex statistical models, preferably incorporating fetal growth, longitudinal type of feeding, total daily caloric intake (dependent on diet and appetite), physical activity, health status, and genetic factors. The only proper way to disentangle the complex causal relation between nutrition and growth, is to randomize between different strategies in intervention studies.

CONCLUSION

In this prospective cohort study of infants born very preterm, we found that weight gain in different timeframes after preterm birth was associated with distinct parameters of body composition in infancy. When adjusted for length, NICU weight gain was not associated with body composition parameters in the first months of life. In contrast, weight gain after NICU stay, especially at home, was associated with an increase in lean mass and, most strongly, fat mass. However, as fat mass parameters in infancy were still below average values of infants born full term, further research is needed to explore the association between early postnatal growth and cardiometabolic outcome later in life.

Supplemental Table S1. Growth and body composition parameters per sex from birth to 6 months

		Hospital stay							
		GIRLS				BOYS			
		Birth	Weight nadir	Transfer from NICU	Discharge home	Birth	Weight nadir	Transfer from NICU	Discharge home
	n	**45**	**45**	**45**	**45**	**75**	**75**	**75**	**72**
Weight	*kg*	0.90 (0.74;1.12)	0.80 (0.69;1.02)	1.30 (1.15;1.93)	2.91 (2.55;3.41)	1.09 (0.85;1.29)	0.94 (0.77;1.13)	1.54 (1.30;2.28)	3.19 (2.89;3.66)
	Z-score	-0.3 (-0.9;0.4)	-0.9 (-1.4;-0.4)	-1.0 (-1.7;-0.5)	-1.2 (-2.0;-0.1)	0.3 (-0.1;0.7)	-0.6 (-1.0;-0.2)	-0.7 (-1.4;-0.4)	-0.6 (-1.6;0.2)
Head circumference	*cm*	24.0 (23.3;26.4)	NA	27.6 (26.0;30.3)[a]	34.4 (33.0;36.2)[b]	25.7 (24.0;27.0)[c]	NA	28.4 (27.0;32.0)[d]	35.0 (33.9;36.3)[e]
	Z-score	-0.1 (-0.8;0.5)	NA	-0.9 (-1.7;-0.4)[a]	-0.5 (-1.6;0.4)[b]	0.2 (-0.4;0.6)[c]	NA	-0.6 (-1.2;-0.3)[d]	-0.7 (-1.7;0.1)[e]
Length	*cm*	NA	NA	38.0 (37.0;42.2)[e]	48.0 (46.0;51.0)[f]	NA	NA	39.0 (36.5;42.5)[g]	48.0 (45.0;50.0)[h]
	Z-score	NA	NA	-1.3 (-2.8;-0.6)[e]	-1.0 (-2.5;-0.7)[f]	NA	NA	-1.7 (-2.3;-1.2)[g]	-1.0 (-2.0;-0.5)[h]
		Out-patient clinic							
		GIRLS				BOYS			
		2 months CA	6 months CA			2 months CA	6 months CA		
	n	**45**	**44**			**74**	**69**		
Weight	*kg*	4.39 (3.86;4.86)	6.74 (6.06;7.47)			4.88 (4.35;5.52)	7.38 (6.62;8.09)		
	Z-score	-1.2 (-1.7;-0.2)	-0.7 (-1.6;0.1)			-0.7 (-1.5;0.0)	-0.7 (-1.4;0.1)		
Head circumference	*cm*	37.5 (36.5;39.0)[i]	42.4 (41.0;43.6)[j]			39.1 (38.2;40.3)[j]	43.8 (42.7;44.8)[i]		
	Z-score	-0.2 (-1.0;0.9)[i]	0.1 (-0.9;1.0)[j]			0.4 (-0.6;1.2)[j]	0.4 (-0.5;1.2)[i]		
Length	*cm*	54.5 (52.0;56.4)[i]	65.4 (62.3;67.2)			56.1 (54.0;57.4)	67.1 (64.7;68.9)[j]		
	Z-score	-1.3 (-2.3;-0.3)[i]	-0.2 (-1.4;0.5)			-1.0 (-1.8;-0.2)	-0.4 (-1.0;0.5)[j]		
	n	**38**	**41**			**65**	**51**		
Relative fat mass	*%*	21.9 (17.6;23.5)	20.5 (18.2;23.7)			22.0 (18.3;24.0)	20.2 (17.9;23.2)		
	Z-score	0.4 (-0.6;1.0)	-0.8 (-1.3;-0.3)			0.6 (-0.2;1.2)	-0.5 (-1.0;0.0)		
Absolute fat mass	*kg*	0.95 (0.75;1.13)	1.43 (1.07;1.69)			1.06 (0.78;1.35)	1.45 (1.24;1.73)		
	Z-score	-0.3 (-0.9; 0.7)	-0.8 (-1.6;-0.3)			0.1 (-0.7; 0.9)	-0.7 (-1.2;-0.2)		

Supplemental Table S1. Continued

Fat mass index	*kg/m²*	3.15 (2.66;3.79)	3.08 (2.69;3.97)	3.46 (2.56;4.06)	3.39 (2.76;3.77)
	Z-score	0.2 (-0.5;1.1)	-1.0 (-1.4;-0.2)	0.4 (-0.4;1.3)	-0.5 (-1.1;-0.0)
Absolute lean mass	*kg*	3.46 (3.25;3.78)	5.25 (4.80;5.96)	3.82 (3.42;4.20)	5.85 (5.40;6.31)
	Z-score	-0.8 (-1.5;-0.1)	-0.5 (-1.6;0.3)	-1.0 (-1.7;-0.3)	-0.4 (-0.8;0.2)
Lean mass index	*kg/m²*	11.91 (11.14;12.61)	12.27 (11.81;12.97)	12.32 (11.56;13.10)	12.67 (12.0;13.4)
	Z-score	0.0 (-0.8;0.9)	0.0 (-0.6;0.8)	-0.1 (-0.9;0.8)	0.2 (-0.6;0.6)

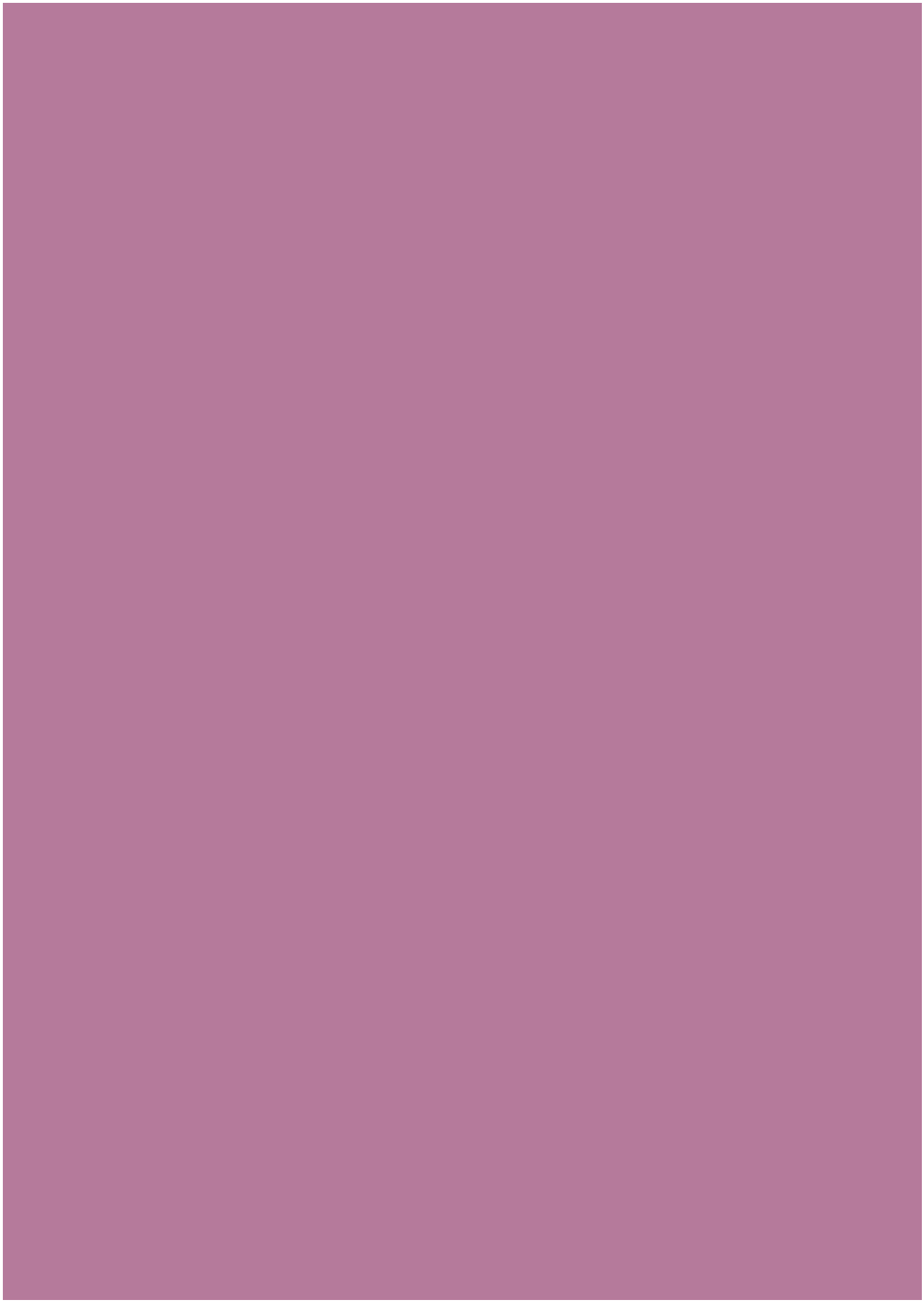
All data are expressed in median (interquartile range). Length measurement was not routinely collected during NICU and level-II hospital stay, leading to incomplete data. Z-scores for body composition parameters were calculated based on average values from a large group of term born infants assessed at our research center within the same time period. Number of infants with missing data: [a] 5, [b] 20, [c] 3, [d] 9, [e] 22, [f] 27, [g] 42, [h] 41, [i] 2, [j] 1.
Abbreviations: NICU, neonatal intensive care unit; CA, corrected age; n, number; kg, kilograms; cm, centimeter; m, meter.

Supplemental Table S2. Infant characteristics per tertile of FMI at 6 months

	T1 (FMI <2.93 kg/m²) (n=30)	T2 (FMI 2.93-3.71 kg/m²) (n=31)	T3 (FMI ≥3.72 kg/m²) (n=31)
Hospital stay			
Sex (female)	16 (53%)	10 (32%)	15 (48%)
GA at birth (weeks)	27.6 [26.2;28.3]	27.7 [27.3;28.9]	28.0 [26.1;29.0]
Birth weight (grams)	880 [790;1093]	1080[965;1250]	1058 [750;1315]
Birth weight SD	-0.3 [-0.9;0.7]	0.3 [-0.3;0.6]	0.2 [-0.3;0.9]
Birth head circumference SD			
Culture-proven sepsis [a]	10 (33%)	11 (36%)	12 (39%)
NEC	2 (7%)	2 (7%)	2 (7%)
Treated PDA	10 (33%)	9 (29%)	10 (32%)
BPD [b], of which	15 (50%)	12 (39%)	7 (23%)
Mild	8 (30%)	9 (24%)	5 (19%)
Severe	7 (27%)	3 (3%)	2 (9%)
Postnatal steroid use	7 (23%)	6 (19%)	4 (13%)
Brain injury [c]	9 (30%)	9 (29%)	12 (39%)
Mechanical ventilation (days)	2 [0;7]	0 [2;11]	0 [0;4]
Parental nutrition (days)	11 [9;17]	10 [8;14]	9 [7;14]
Total hospital stay (days) [d]	84 [68;104]	85 [70;100]	75 [61;100]
NICU transfer weight SD	-1.2 [-1.6;-0.5]	-0.9 [-1.6;-0.5]	-0.5 [-1.2;-0.2]
NICU transfer head circumference SD	-1.0 [-1.6;-0.6]	-0.7 [-1.0;-0.3]	-0.4 [-1.5;0.1]
NICU transfer length SD	-1.4 [-2.3;-0.5]	-1.6 [-3.1;-1.1]	-1.2 [-2.7;-0.2]
Discharge home			
Feeding type			
Only MM	13 (48%)	14 (50%)	13 (48%)
Only Formula	9 (33%)	8 (29%)	7 (26%)
Mix MM/Formula	5 (19%)	6 (21%)	7 (26%)
Fortification	14 (56%)	15 (54%)	13 (54%)
Tube feeding	17 (63%)	10 (35%)	10 (36%)
Oxygen	6 (20%)	2 (7%)	1 (3%)
Weight SD	-1.4 [-1.8;-0.3]	-1.0 [-1.9;-0.1]	-0.4 [-1.5;0.3]
Head circumference SD	-0.7 [-1.8;0.7]	-0.2 [-1.1;0.8]	-0.1 [-1.1;0.6]
Length SD	-1.0 [-2.0;-0.6]	-0.9 [-3.0;-0.5]	-1.1 [-2.1;-0.3]
2-month visit			
Feeding type			
Only MM	3 (10%)	8 (26%)	3 (10%)
Only Formula	20 (67%)	18 (58%)	21 (70%)
Mix MM/Formula	7 (23%)	5 (16%)	6 (20%)
Fortification	8 (27%)	5 (16%)	5 (17%)
Tube feeding	7 (23%)	5 (16%)	3 (10%)
Oxygen	5 (17%)	3 (10%)	1 (3%)
Weight SD	-1.4 [-2.1;-0.7]	-1.0 [-1.8;0.0]	-0.4 [-1.1;0.4]
Head circumference SD	-0.3 [-1.2;1.0]	0.6 [-0.6;1.0]	0.2 [-0.8;1.3]
Length SD	-1.2 [-2.1;-0.2]	-1.3 [-2.1;-0.3]	-0.7 [-1.9;-0.3]
FMI SD	-0.4 [-1.1;0.3]	0.4 [-0.4;1.1]	0.8 [-0.1;1.8]
LMI SD	-0.7 [-1.3;0.4]	-0.1 [-0.9;0.8]	0.4 [-0.5;1.1]

	T1 (FMI <2.93 kg/m²) (n=30)	T2 (FMI 2.93-3.71 kg/m²) (n=31)	T3 (FMI ≥3.72 kg/m²) (n=31)
6-month visit			
Feeding type			
Only MM	1 (3%)	3 (10%)	1 (3%)
Only Formula	29 (97%)	27 (87%)	26 (90%)
Mix MM/Formula	0 (0%)	1 (3%)	2 (7%)
Fortification	1 (4%)	2 (7%)	1 (4%)
Tube feeding	0 (0%)	1 (3%)	2 (7%)
Oxygen	3 (10%)	1 (3%)	0 (0%)
Weight SD	-1.4 [-2.1;-0.8]	-0.8 [-1.6;-0.3]	0.2 [-0.5;0.7]
Head circumference SD	-0.1 [-1.2;0.8]	0.7 [-0.2;1.2]	0.5 [-0.4;1.4]
Length SD	-0.5 [-1.3;0.3]	-0.5 [-1.0;0.5]	0.0 [-1.2;0.7]
FMI SD	-1.6 [-2.0;-1.2]	-0.7 [-0.9;-0.4]	0.2 [-0.1;0.7]
LMI SD	0.0 [-0.7;0.5]	-0.1 [-1.0;0.6]	0.3 [-0.4;1.4]

All data are expressed in median (interquartile range) or number (percentages). [a] Positive blood culture within 72h after birth; [b] BPD: >28 days O2 + X-ray abnormalities, severe BPD: endotracheal or CPAP at 36 weeks of gestation or >30% fiO2 or >1L/min flow via nasal prongs; [c] Brain injury includes IVH gr I/II, cerebellar bleeding, arterial/venous stroke, periventricular leukomalacia and/or convulsions; [d] NICU + level-II hospital. Abbreviations: FMI, fat mass index (FM/height² in meters); n, number; GA, gestational age; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; BPD, bronchopulmonary dysplasia; NICU, neonatal intensive care unit; MM, mothers milk.



CHAPTER 5

Body composition assessment by Air Displacement Plethysmography compared to Dual energy X-ray Absorptiometry in full-term and preterm aged three to five years

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ABSTRACT

It is important to monitor body composition longitudinally, especially in children with atypical body composition trajectories. Dual-energy-X-ray-absorptiometry (DXA) can be used, and reference values are available. Air-displacement-plethysmography (ADP) is a relatively new technique, but reference values are lacking. Besides, estimates of fat-free-mass-density (Dffm), needed in ADP-calculations, are based on children aged >8 years and may not be valid for younger children. We, therefore, aimed to investigate whether DXA and ADP results were comparable in young children aged 3-5 years, either born full-term or preterm, and if Dffm-estimates in the ADP algorithm could be improved.

In 154 healthy children born full-term and 67 born <30 weeks of gestation, aged 3-5 years, body composition was measured using ADP (BODPOD, with default Lohman Dffm-estimates) and DXA (Lunar Prodigy). We compared fat-mass (FM), fat-mass-percentage (FM%) and fat-free-mass (FFM), between ADP and DXA using Bland-Altman-analyses, in both groups. Using a 3-compartment-model as reference method, we revised the Dffm-estimates for ADP.

In full-term born children, Bland-Altman analyses showed considerable fixed and proportional bias for FM, FM% and FFM. After revising the Dffm-estimates, agreement between ADP and DXA improved, with mean differences [LoA] for FM, FM% and FFM of -0.67kg [-2.38;1.04], -3.54% [-13.44;6.36], and 0.5kg [-1.30;2.30], respectively, but a small fixed and proportional bias remained. The differences between ADP and DXA were larger in preterm born children, even after revising Dffm-estimates.

So, despite revised and improved sex and age-specific Dffm-estimates, results of ADP and DXA remained not comparable and should not be used interchangeably in the longitudinal assessment of body composition in children aged 3-5 years, and especially not in very preterm-born children of that age.

INTRODUCTION

Childhood obesity tracks into adulthood and has been linked to both short-term and long-term morbidity.[184,185] Consequently, it is important to identify children at risk of excess adiposity early in childhood in order to start preventive and therapeutic strategies as early as possible. Body composition is a more adequate indicator of adiposity than standard anthropometric measures such as weight or body mass index (BMI), especially in infants and young children.[56,160] Therefore, reliable methods to longitudinally assess body composition from early childhood onwards are needed. Specific attention should be given to children at risk of altered adiposity trajectories, such as children born preterm.[55]

Multiple tools are available for measuring body composition during childhood, with Dual energy X-ray Absorptiometry (DXA) and Air Displacement Plethysmography (ADP) being most frequently used.[58,186] DXA is often used as a reference method to determine body composition in research and clinical practice.[58] Longitudinal reference values are available for infants and young children from birth until age 5 years, showing slightly higher fat mass in girls as compared to boys.[167,187] However, DXA uses a very small dose of radiation (0.0002 mSv). ADP calculates body composition by measuring body volume, using the inverse pressure-volume-relation[188] and can be applied in infants ≤6 months old and/or ≤8 kg using PEAPOD,[59,60] and in children ≥2 years and ≥12 kg using BODPOD.[61] ADP is, however, currently more costly than DXA and requires cooperation of the child, as movement and crying influence results.[61] It is our experience that BODPOD is feasible in children ≥3 years of age. Importantly, ADP uses multiple assumptions to calculate body composition parameters from measured body volume. These assumptions include estimates for fat free mass (FFM) density (Dffm). The default estimates in BODPOD software are based on outdated, small studies in which results of healthy older children and adults were extrapolated to children aged <8 years, per 2-year-intervals.[189,190] These Dffm-estimates may, therefore, not be valid in young children. In fact, especially in young children with deviant body composition, such as preterm born children, we noticed that ADP results are often clinically questionable (e.g. extremely low values of fat mass percentage (FM%), <5%). Wells *et al* developed novel Dffm-estimates for healthy children aged ≥ 5 years,[191] which have been reported to be superior to the default estimates in ADP for children aged 5 years.[192] However, improved Dffm-estimates for children aged <5 years are not yet available.

In order to monitor body composition longitudinally in infants and young children, it would be favorable if ADP and DXA could be used interchangeably. Our research group reported that results from ADP (PEAPOD) were comparable with DXA in

infants aged 6 months.[167] Studies in healthy schoolchildren, adolescents and adults, however, showed conflicting results on comparability.[188,193-195] In young children, aged 3-5 years, comparison between ADP and DXA has not yet been described.

The primary aim of our study was to compare fat mass (FM), FM% and FFM results assessed by ADP with DXA, in a cohort of healthy full-term born children aged 3 to 5 years. Secondly, we aimed to explore potential improvements to the default Dffm-estimates in the ADP algorithm for full-term born children in this age category. Furthermore, we evaluated body composition based on the default and revised Dffm-estimates in a group of very preterm born children aged 3-5 years. We hypothesized that ADP and DXA are both reliable methods to estimate body composition in young children, but may not be used interchangeably, especially not in very preterm born children aged 3-5 years.

MATERIAL AND METHODS

Study setting and subjects

The current cross-sectional study included subjects of two ongoing prospective birth cohort studies on growth and body composition which started from 2012 at the Erasmus MC Sophia Children's Hospital in Rotterdam, The Netherlands. The Sophia Pluto study included healthy full-term born infants,[196,197] whereas the BOND study included infants born very preterm (<30 weeks gestation).[74] The full-term born infants (≥37 weeks) were recruited from all seven maternity wards in Rotterdam and experienced an uncomplicated neonatal period. Infants with a *complicated* perinatal or neonatal period were excluded: in case of maternal disease or medication that could interfere with growth and development, perinatal asphyxia, neonatal sepsis, neonatal respiratory ventilation and significant congenital or intrauterine disease.[196] The very preterm born children were admitted to our level IV neonatal intensive care unit within 48 hours after birth. Exclusion criteria for this group included congenital and chromosomal anomalies that could interfere with growth, severe brain injury, congenital infection, or perinatal asphyxia.[74]

The present analyses were based on a subgroup of children from both cohorts whose body composition was, per study protocol, measured by both ADP and DXA at the age of 3-5 years, between April 2019 and November 2021. All participants of both cohorts who were in this age range within this timeframe were eligible. The Medical Ethics Committee of the Erasmus MC approved both studies (MEC-2012-164 and MEC-2014-379). We obtained written informed consent of all parents/caregivers.

Data collection and measurements

For full-term born subjects, outpatient clinic visits were scheduled at 3, 4 and 5 years, and for preterm-born subjects at 3 and 5.5 years corrected age. Data on child ethnicity were derived from parental questionnaires.

Anthropometrics

Weight was measured without heavy clothing to the nearest 5 grams using a flat scale (Seca, Hamburg, Germany). Height was measured twice to the nearest 0.1 cm in upright position by a stadiometer (Seca), with the average of both measurements used in the analyses. Age and sex-corrected SD-scores for weight and length at birth and at 3-5 years were calculated using Dutch reference values, and Fenton charts at preterm birth.[88,198]

Body composition

In 154 healthy full-term born children and 67 very preterm children, body composition was measured by ADP and DXA within one hour. For ADP, we used BODPOD (COSMED) with pediatric hardware and software, including the default Lohman density model.[189] Children wore tight underwear (without diaper) and a Lycra cap covering all scalp hair.[189] The DXA (Lunar Prodigy, GE Healthcare) was used with Encore v14.1 software. During DXA-scan, children wore light clothing. FFM was calculated as the sum of lean body mass and bone mineral content.

The same ADP and DXA devices were used during the entire study period. Both devices were calibrated daily and used and maintained according to the supplier's manuals.[61] During measurements, children were instructed not to move. We excluded measurements if the supplier's terms of use were not met, or when the child cried. To test reliability, a random sample of full-term born children was measured twice, after repositioning, with the same device (13 with ADP and 16 with DXA). Intra-class-correlation-coefficients for FM, FM% and FFM for ADP were 0.980, 0.978 and 0.994, and for DXA 0.991, 0.985 and 0.994 (all $p<0.001$), respectively.

The BODPOD calculates FM% using two constants (C1 and C2), derived from the programmed, sex-specific density models for Dffm and FM density (Dfm), and measured body density (BD (kg/L)), as expressed in Formula 1:[189]

$$C1 = (Dffm * Dfm) / (Dffm - Dfm)$$
$$C2 = Dfm / (Dffm - Dfm)$$
$$FM\% = (C1 / BD - C2) * 100\%$$

The standard algorithm in the BODPOD software follows the assumption that Dfm remains stable during life at 0.9007 kg/L.[189-191] Consequently, Formula 1 can be rewritten as Formula 2:

$$Dffm = ((0.9007 * FM\% - 90.07) * BD) / (FM\% * BD - 90.07)$$

Statistical analysis

Children born full-term and preterm were analyzed as separate groups. Independent sample t-tests were used to compare group characteristics. Paired sample t-tests were used to compare ADP and DXA results for each group at all ages. Bland-Altman analyses were used to test agreement between ADP and DXA results. Fixed bias was determined by one sample t-test, and proportional bias by linear regression. As body composition, like anthropometrics, differs per sex, we analyzed boys and girls separately.[167,187]

As the current algorithms used in ADP are based on Dffm's of children aged > 8 years, which are extrapolated for younger ages,[189] we re-calculated Dffm for each included full-term born child. We used Formula 2 with body density (BD) as measured by ADP, and FM% as derived from the 3-compartment model.[199] For the 3 compartments, we entered body volume (BV) measured by ADP (BODPOD), bone mineral content (BMC) measured by DXA and body weight (BW) measured by scale, as follows:

$$FM\% = \frac{(6.386 * BV + 3.961 * BMC - 6.09 * BW)}{BW} * 100\%$$

We used the re-calculated Dffm-values to create sex-specific curves by age, using generalized additive models for location, scale, and shape (GAMLSS).[200,201] Box-Cox-Cole and Green distribution (BCCG) was applied to fit the three parameters of mu (μ), sigma (σ) and nu (ν). The distribution expresses the mean (μ), variance (σ) and skewness (ν) that change as a function of age. Median Dffm was then assessed for ages between 3 and 5 years, using 0.25-year time-intervals. Based on these new sex- and age-specific median Dffm-estimates, we re-calculated FM% for each ADP-measurement using Formula 1. For children with age > 5 years, which was above the modelled age range, we used Wells *et al* Dffm-estimates.[192]

A 2-tailed *p*-value <0.05 was considered statistically significant. Analyses were performed using SPSS-package 25.0 (IBM SPSS Statistics, Armonk, NY) and R with GAMLSS-package v.5.2.0 (V 4.0.0 for MacOS, R Core Team, Vienna, Austria).

RESULTS

Clinical characteristics of the full-term and preterm born children are shown in **Table 1**. SD-scores for weight-for-height and height were lower in the preterm compared to the full-term group at 3 and 5 years corrected age. Body composition parameters assessed by ADP and DXA are presented in Table 2. FM and FM%, assessed by DXA, were higher in full-term compared to preterm born children at each time point (all $p \leq 0.001$). In both groups, FM and FFM increased with age and body-size corrected FM% decreased with age (**Supplemental Table S1**).

Comparison between ADP and DXA in full-term born children

Absolute results of FM, FM% and FFM by ADP and DXA were significantly different (all, $p<0.001$) (**Table 2**).

Table 1. Clinical characteristics

	Full-term		Very preterm		*p*-value
	Boys	**Girls**	**Boys**	**Girls**	
Birth	N=79	N=75	N=39	N=28	
Gestational age (weeks)	39.47 (1.29)	39.77 (1.24)	27.50 (1.55)	27.44 (1.55)	**<0.001**
Birth weight SDS	0.39 (1.00)	0.19 (1.09)	0.27 (0.68)	0.05 (0.76)	0.416
BPD (%)	*NA*	*NA*	12 (30.8%)	5 (17.9%)	
Ethnicity (%)					**<0.020**
White	54 (68.4%)	45 (60.0%)	30 (76.9%)	23 (82.1%)	
Non-white	25 (31.6%)	30 (40.0%)	9 (23.1%)	5 (17.9%)	
All visits, total group	N=97	N=89	N=39	N=28	
Weight-for-height SDS	0.07 (1.11)	0.40 (0.91)	-0.55 (1.10)	-0.51 (1.13)	
Height SDS	-0.26 (0.79)	-0.20 (1.02)	-0.87 (0.76)	-0.70 (1.11)	
Age 3 years	N=18	N=24	N=13	N=10	
Age (years)	3.06 (0.11)	3.08 (0.10)	3.44 (0.15)	3.46 (0.18)	**<0.001**
Weight-for-height SDS	0.31 (1.08)	0.51 (1.02)	-0.43 (0.94)	-0.47 (0.95)	**0.001**
Height SDS	-0.13 (0.72)	0.09 (0.93)	-0.69 (0.65)	-0.58 (1.34)	**0.008**
Age 4 years	N=33	N=24			
Age (years)	4.11 (0.13)	4.15 (0.15)	*NA*	*NA*	
Weight-for-height SDS	-0.13 (1.21)	0.45 (0.83)	*NA*	*NA*	
Height SDS	-0.32 (0.89)	0.02 (1.08)	*NA*	*NA*	
Age 5 years	N=46	N=41	N=26	N=18	
Age (years)	5.11 (0.14)	5.08 (0.13)	5.97 (0.17)	5.94 (0.12)	**<0.001**
Weight-for-height SDS	0.13 (1.05)	0.31 (0.14)	-0.61 (1.18)	-0.53 (1.25)	**<0.001**
Height SDS	-0.27 (0.75)	-0.51 (0.98)	-0.96 (0.80)	-0.77 (1.00)	**0.002**

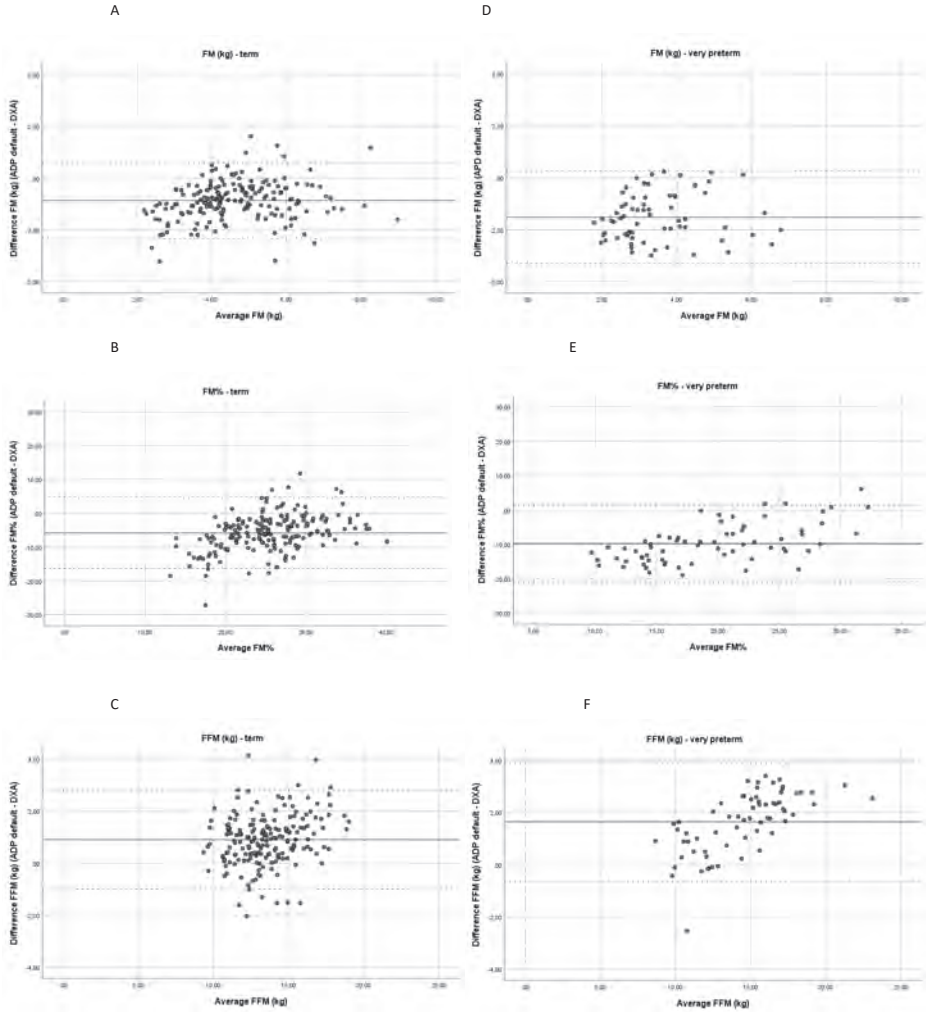
Data are expressed as absolute numbers (percentage) or mean (SD). P-values represent the differences between full-term and very preterm born children (both sexes combined), analyzed with independent t-test. Significant p-values are boldfaced. Abbreviations: n, number; SDS, standard deviation score, NA, not applicable; BPD, bronchopulmonary dysplasia.

Table 2. Body composition parameters assessed by ADP and DXA

	Full-term N=186#	Very preterm N=67	*p*-value
FM (kg)			
DXA	5.16 (1.26)	4.43 (1.26)	**<0.001**
ADP default	4.09 (1.45)	2.54 (1.35)	**<0.001**
ADP revised	4.47 (1.40)	2.98 (1.73)	**<0.001**
Mean difference [LoA] ADP default –DXA	-1.08 * [-2.92;0.76]	-1.89 * [-4.10;0.32]	**< 0.001**
Mean difference [LoA] ADP revised –DXA	-0.67 * [-2.38;1.04]	-1.45 * [-3.53;0.63]	**<0.001**
FM%			
DXA	28.26 (4.88)	24.39 (4.76)	**<0.001**
ADP default	22.47 (6.91)	14.60 (7.88)	**<0.001**
ADP revised	24.90 (6.64)	17.07 (7.93)	**<0.001**
Mean difference [LoA] ADP default –DXA	-5.78 * [-16.25;4.69]	-9.79 * [-20.92;1.34]	**<0.001**
Mean difference [LoA] ADP revised –DXA	-3.54 * [-13.44;6.36]	-7.32 * [-18.26;3.62]	**<0.001**
FFM (kg)			
DXA	13.06 (2.01)	13.72 (2.59)	0.064
ADP default	13.96 (2.25)	15.36 (3.36)	**0.002**
ADP revised	13.41 (2.13)	14.91 (3.28)	**0.001**
Mean difference [LoA] ADP default –DXA	0.90 * [-1.00;2.80]	1.64 * [-0.63;3.91]	**<0.001**
Mean difference [LoA] ADP revised –DXA	0.50 * [-1.30;2.30]	1.20 * [-0.92;3.32]	**<0.001**

*Data are expressed as mean (SD). P-value term vs preterm is difference between mean difference in term and very preterm born children. # In the term-born group, some children were measured at more than 1 age visit. * indicates differences between ADP and DXA p<0.001. Abbreviations: ADP, air-displacement plethysmography; DXA, dual energy X-ray absorptiometry; N = number; FM fat mass; FM% = fat mass percentage; FFM, fat-free mass; LoA, limits of agreement (95% CI).*

Figure 1. Bland-Altman plots for FM, FM% and FFM measured by ADPand DXA in full-term (A, B, C) and very preterm born children (D, E and F) aged 3-5 years



Continuous line represents the mean difference between ADP and DXA. The dashed lines represent the limits of agreement. Abbreviations: FM, fat mass, FM%, fat mass percentage, FFM, fat-free mass; kg, kilograms; DXA, dual energy X-ray absorptiometry; ADP, air-displacement plethysmography

Bland-Altman analyses (**Figure 1**) showed that mean differences [limits of agreement (LoA)] for FM, FM% and FFM between ADP and DXA were -1.08 [-2.92;0.76], -5.78% [-16.25;4.69] and 0.90 kg [-1.00;2.80], respectively. For all three parameters, a fixed bias (all, p<0.001) and a proportional bias for FM (β: 0.135, p=0.014), FM% (β: 0.396, p<0.001) and FFM (β: 0.109, p=0.002) were observed. Proportional bias indicates that the difference between ADP and DXA increased when the result deviated more from the mean.

Revised FFM density model

Table 3 presents the revised, sex-specific estimates for Dffm for full-term children aged 3-5 years. Dffm increased between age 3 and 5 years. Compared to the default Lohman Dffm model, the revised Dffm estimates are higher at all ages. At age 5 years, they were in the range of the Wells *et al*[191] estimates (**Figure 2**).

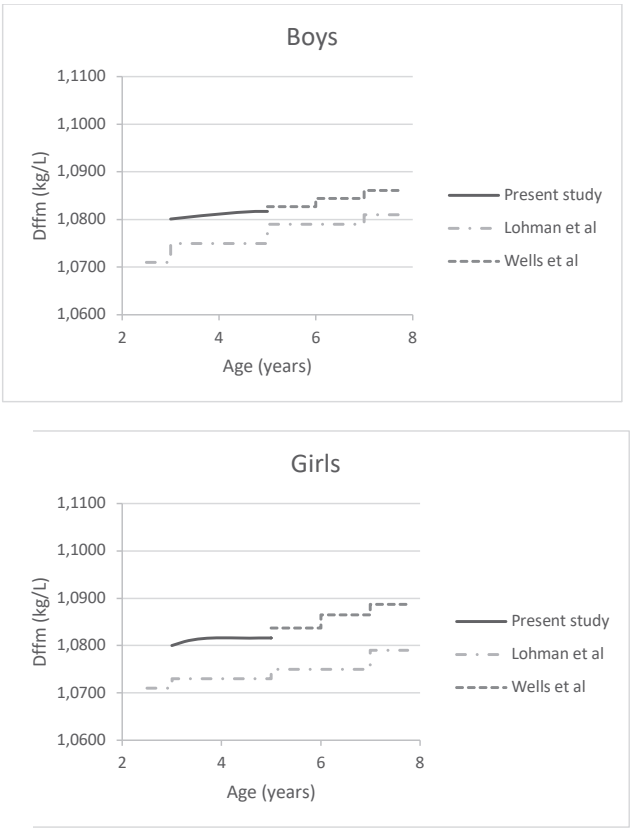
The agreement with DXA improved when using the revised Dffm-estimates for ADP, with mean differences [LoA] for FM, FM% and FFM of -0.67kg [-2.38;1.04], -3.54% [-13.44;6.36] and 0.50 kg [-1.30;2.30], respectively (**Table 2**). Although smaller, a fixed (all, p<0.001) and proportional bias remained, for FM (β: 0.135, p=0.010), FM% (β: 0.374, p<0.001) and FFM (β: 0.106, p=0.002) .

Table 3. Revised fat-free mass density models for children aged 3-5 years

Age (years)	Boys			Girls		
	C1	C2	Dffm	C1	C2	Dffm
2.75	5.432	5.031	1.0797	5.449	5.050	1.0790
3	5.424	5.022	1.0801	5.426	5.025	1.0800
3.25	5.416	5.013	1.0804	5.405	5.001	1.0808
3.5	5.409	5.005	1.0807	5.393	4.987	1.0813
3.75	5.402	4.998	1.0809	5.386	4.980	1.0816
4	5.395	4.990	1.0812	5.384	4.978	1.0816
4.25	5.390	4.984	1.0814	5.384	4.978	1.0816
4.5	5.386	4.980	1.0816	5.384	4.978	1.0816
4.75	5.384	4.978	1.0817	5.384	4.978	1.0816
5	5.384	4.977	1.0817	5.384	4.978	1.0816

*Median Dffm and C1 and C2 predicted in 0.25-year intervals for children aged 3-5 years. C1 = (Dffm * Dfm)/ (Dffm-Dfm). C2 = Dfm/ (Dffm-Dfm)) Abbreviations: Dffm= fat-free mass density. Dfm= fat mass density = 0.9007 kg/L.*

Figure 2. Dffm-estimates plotted against age for boys and girls separately



Presented are the revised Dffm-estimates from present study and those of Lohman et al[189] *and Wells et al.*[191]
Abbreviation: Dffm= fat-free mass density.

Comparison between ADP and DXA in very preterm born children

Using the default Dffm-estimates in children born very preterm, absolute results of ADP and DXA were very different (all p<0.001)(**Table 2**). In fact, differences in FM, FM% and FFM results between both methods were significantly larger in preterm compared to full-term born children (all, p<0.001), with mean differences [LoA] of -1.89 kg [-4.10;0.32] for FM, -9.79% [-20.92;1.34] for FM% and 1.64 kg [-0.63;3.91] for FFM (**Figure 1**). Similar to the full-term group, a fixed bias (p<0.001) was observed for all three parameters, and a proportional bias for FM% (β: 0.575, p<0.001) and FFM (β: 0.264, p=0.001), but not for FM (β: 0.080, p=0.504).

When using the revised Dffm-estimates, comparison of ADP and DXA showed smaller fixed bias, with mean differences [LoA] for FM: -1.45 kg [-3.53;0.63], FM%: -7.32% [-18.26;3.62], FFM: 1.20kg [-0.92;3.32] (**Table 2**), but the proportional bias remained similar.

DISCUSSION

To our knowledge, this is the first study to compare ADP with DXA in a relatively large group of young children aged 3–5 years who underwent both ADP and DXA. We observed significant differences in FM, FM% and FFM results derived with both techniques. Based on our cohort of healthy full-term born children, we provide a revised Dffm-model to be used with ADP in children aged 3-5 years. Furthermore, differences between ADP, using default or revised Dffm-estimates, and DXA were significantly larger in very preterm compared to full-term born children. Although our revised Dffm-estimates improved agreement between ADP and DXA, we have to conclude that results of both techniques are not comparable and should thus not be used interchangeably in the longitudinal assessment of body composition in children aged 3-5 years.

Literature on comparison of ADP and DXA in the pediatric population is limited but shows similarities with our findings. Two studies in infants aged 0-6 months observed that both methods generated highly correlated but significantly different absolute results.[202,203] In particular, FM and FM% estimates by ADP were significantly lower compared to DXA, while FFM results were higher; as also observed in present study. In adolescence, ADP and DXA results were reported to be strongly correlated.[195,204] However, FM% results were not comparable in subjects with more deviant body composition, such as individuals with severe under- or overweight.[195,204] These findings correspond with the observed proportional bias, as well as the larger inter-method differences in very preterm born children. We extend the previous literature by adding data on ADP and DXA comparison in young children aged 3–5 years, in whom comparative studies were lacking.

Although DXA and ADP have been validated against 4-component models in small samples of healthy children with normal weight,[61] both machines use different techniques with limitations that could explain the observed differences. ADP has several limitations. It measures body volume using the inverse pressure-volume-relation, which is sensitive for environmental factors that influence air pressure and density, such as crying of the subject or fluctuations in room temperature.[61,188] Besides, in order to calculate body composition parameters based on body volume, it uses density models that are based on multiple assumptions.[188] First, FM is thought to contain no water and have a constant density throughout life, whereas Dffm is considered to increase with age, as FFM hydration decreases throughout life.[189-191] Other assumptions include the content of bone mineral constituents and the amount of fat in the bones, as well as lung volume.[188] The Lohman Dffm-model, used as

default in the ADP-software, was extrapolated from data of small populations of subjects aged 0-1 and 8-30 years measured in the 1980's.[189-191] Dffm-estimates were then extrapolated to other pediatric age categories per 2-year-intervals.[189] Also, Dffm can vary in children with different nutritional status (e.g. hydration status), physical activity level, ethnicity and disease status, but these variables were not included in the density models.[189,205] All these factors could have added to the inter-method differences observed in present study. DXA is based on a 3-compartment-model and uses the attenuation of X-ray-energy passing different types of tissue.[206,207] DXA-software differentiates bone, fat and other tissues. For pixels that contain mixed tissues, the software calculates the three parameters based on fixed algorithms using bone-edge-detection.[206] These tools are based on a constant hydration status of FFM, but it is known that the hydration status of FFM in children decreases with age.[208] Furthermore, it has been reported that DXA-software encounters difficulties differentiating tissues in objects with a smaller body size.[209] DXA-software might, therefore, be less accurate in young children, despite pediatric software options.

Our revised Dffm-estimates are higher compared to those of Lohman *et al*,[189] which are used as default in the BODPOD machine for age 3-5 years. Our estimates are in line with a study from Wells *et al*,[191] who revised Dffm-estimates for children aged ≥5 years using a 4-compartment model. The Wells *et al* estimates were more accurate compared to Lohmans estimates in healthy 5.5-year-old children, when validated against a 3-compartment model, including isotope dilution.[192] We have now added revised Dffm estimates for younger children, aged 3-5 years.

We observed that the inter-method differences were significantly larger in children born preterm compared to full-term children. Although using our revised Dffm-estimates improved ADP-results, considerable bias, fixed and proportional, remained present. This could have several explanations. First, very preterm-born children are prone to experience impaired growth resulting in smaller body size as compared to full-term born peers,[210] as also seen in our cohort. The aforementioned limitations of DXA-software in subjects with small body size may, therefore, hamper accurate assessment of body composition in this group.[209] More importantly, children born very preterm show a different pattern of body composition and Dffm over childhood. While FM in preterm children was observed to be higher around term age, studies later in childhood reported lower FM and FFM as compared to full-term born children.[55,211] Furthermore, recent studies showed that bone mineral content and density were also lower in preterm born children at the age of 5-9 years as compared to full-term born children.[212,213] Besides, incorrect assumptions about thoracic gas volume could lead to incorrect body composition estimates by ADP.[188]

Preterm born children, with or without bronchopulmonary dysplasia (BPD), more often have reduced lung volumes or impaired lung function in mid childhood.[214] All these variables may complicate accurate assessment of body composition by ADP in the preterm population, in which accurate information on body composition is important for long-term health. Given the observed proportional bias between ADP and DXA, a low FM% will lead to greater inter-method differences. Moreover, a recent study in over 900 subjects aged 4-22 years showed that leaner body types have lower FFM hydration and consequently higher Dffm.[205] Altogether, we suggest that caution is needed when interpreting ADP-results of this specific patient group. In fact, it warrants further research to compose separate Dffm-estimates for children with deviant body composition trajectories, such as preterm born children.

Strength of this study is the relatively large number of healthy full-term born children who underwent an ADP and DXA assessment within one hour. To our knowledge, this is the first study to provide revised Dffm-models for ADP in full-term born children aged 3-5 years. Furthermore, comparing results with a group of very preterm born children emphasizes the challenges of assessing body composition in children at risk for deviant growth patterns. We also acknowledge several limitations. We revised the Dffm-model using FM% prediction not from a 4-compartment but from a 3-compartment model as reference method. Yet, a 4-compartment model has not been investigated in children below the age of 5.5 years.[207] Although we observed improvement of ADP results using our revised Dffm-estimates, future studies should explore how the revised estimates hold in pediatric populations elsewhere. In particular, including sufficient numbers of children from different ethnical groups would increase external generalizability. Moreover, our findings suggest the need for specific Dffm-models for different patient groups with deviant body composition trajectories, such as preterm born children. Because the 3-compartment model was validated for healthy subjects, it proved not suitable as a reference for the very preterm group in our study. Further research, using a 4-compartment model including isotope dilution, in larger cohorts is needed to calculate and validate Dffm-estimates for particular patient groups (e.g. other growth disorders). Lastly, development of cheaper but reliable methods also applicable in lower-resource settings could improve body composition measurement in a broader sense.

CONCLUSION

Despite revised and improved age and sex-specific Dffm-estimates for ADP, results of ADP and DXA remained not comparable and should not be used interchangeably in the longitudinal assessment of body composition in children aged 3-5 years, especially not in very preterm born children of that age.

Supplement Table S1. Body composition parameters assessed by ADP and DXA between age 3-5 years

	3 years			4 years	5 years		
	Full-term	**Very preterm**	***p*-value**	**Full-term**	**Full-term**	**Very preterm**	***p*-value**
FM (kg)							
DXA	4.86 (1.20)	3.92 (0.79)	**0.001**	5.10 (1.21)	5.35 (1.30)	4.70 (1.39)	**0.009**
ADP default	3.67 (1.47)	3.34 (1.04)	0.344	4.16 (1.39)	4.24 (1.46)	2.12 (1.31)	**<0.001**
ADP revised	4.04 (1.46)	3.71 (1.03)	0.341	4.59 (1.42)	4.64 (1.32)	2.60 (1.39)	**<0.001**
FM%							
DXA	30.30 (4.76)	26.06 (4.01)	**0.001**	28.70 (4.86)	26.98 (4.60)	23.52 (4.93)	**<0.001**
ADP default	23.06 (7.65)	22.30 (5.69)	0.677	23.50 (6.66)	21.52 (6.65)	10.58 (5.52)	**<0.001**
ADP revised	25.53 (8.37)	25.05 (6.86)	0.815	26.01 (7.41)	22.87 (7.78)	10.96 (5.93)	**<0.001**
FFM (kg)							
DXA	11.07 (1.15)	11.11 (1.48)	0.893	12.56 (1.51)	14.35 (1.68)	15.08 (1.19)	**0.028**
ADP default	12.00 (1.32)	11.52 (1.36)	0.169	13.35 (1.67)	15.31 (2.05)	17.36 (2.10)	**<0.001**
ADP revised	11.63 (1.27)	11.15 (1.34)	0.157	12.92 (1.61)	14.73 (1.99)	16.88 (2.02)	**<0.001**

Data are expressed as mean (SD). P-value term vs preterm is difference between mean difference in term and very preterm born children. Abbreviations: ADP, air-displacement plethysmography; DXA, dual energy X-ray absorptiometry; FM, fat mass; FM% = fat mass percentage; FFM, fat-free mass; LoA, limits of agreement (95% confidence interval)

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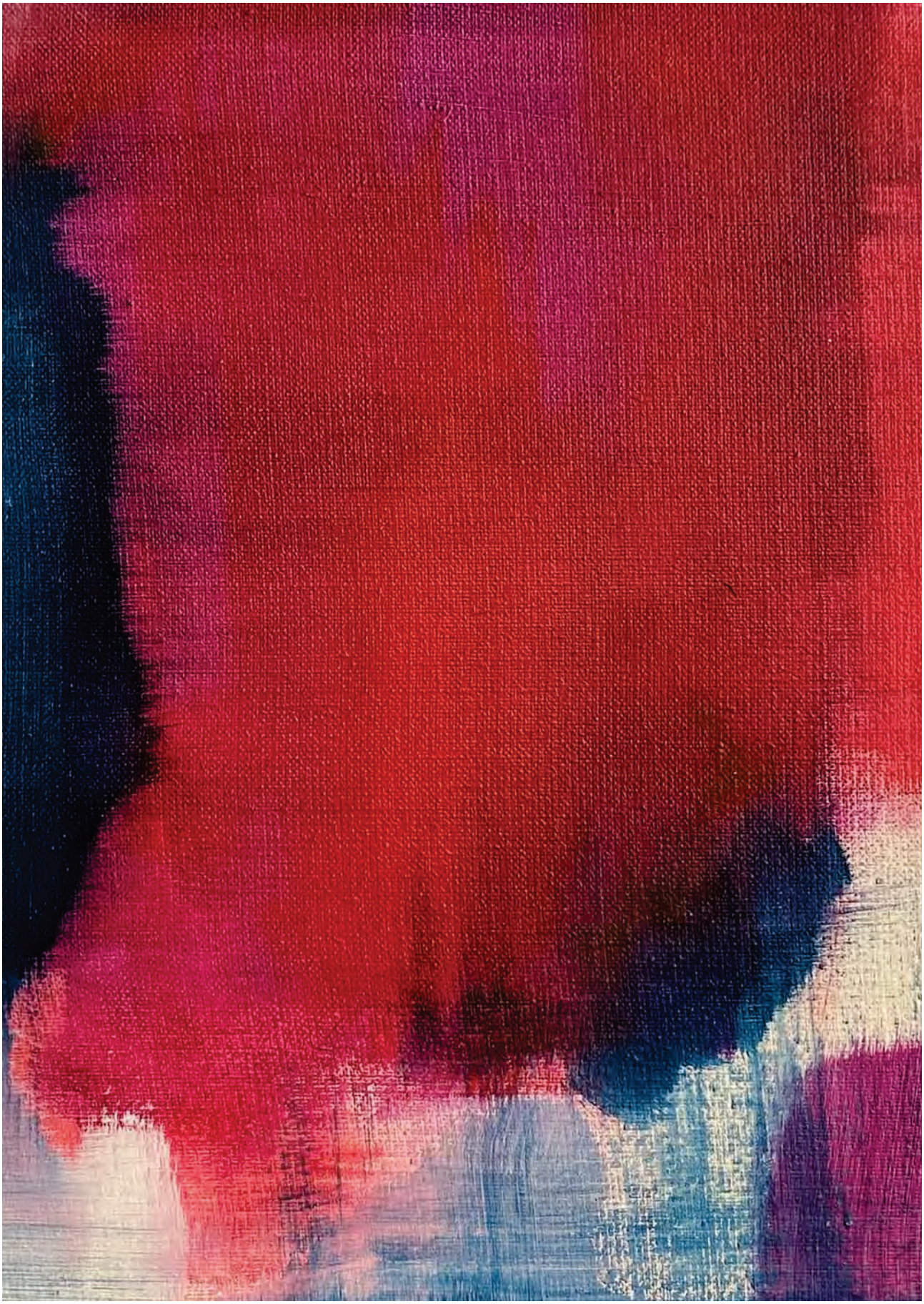
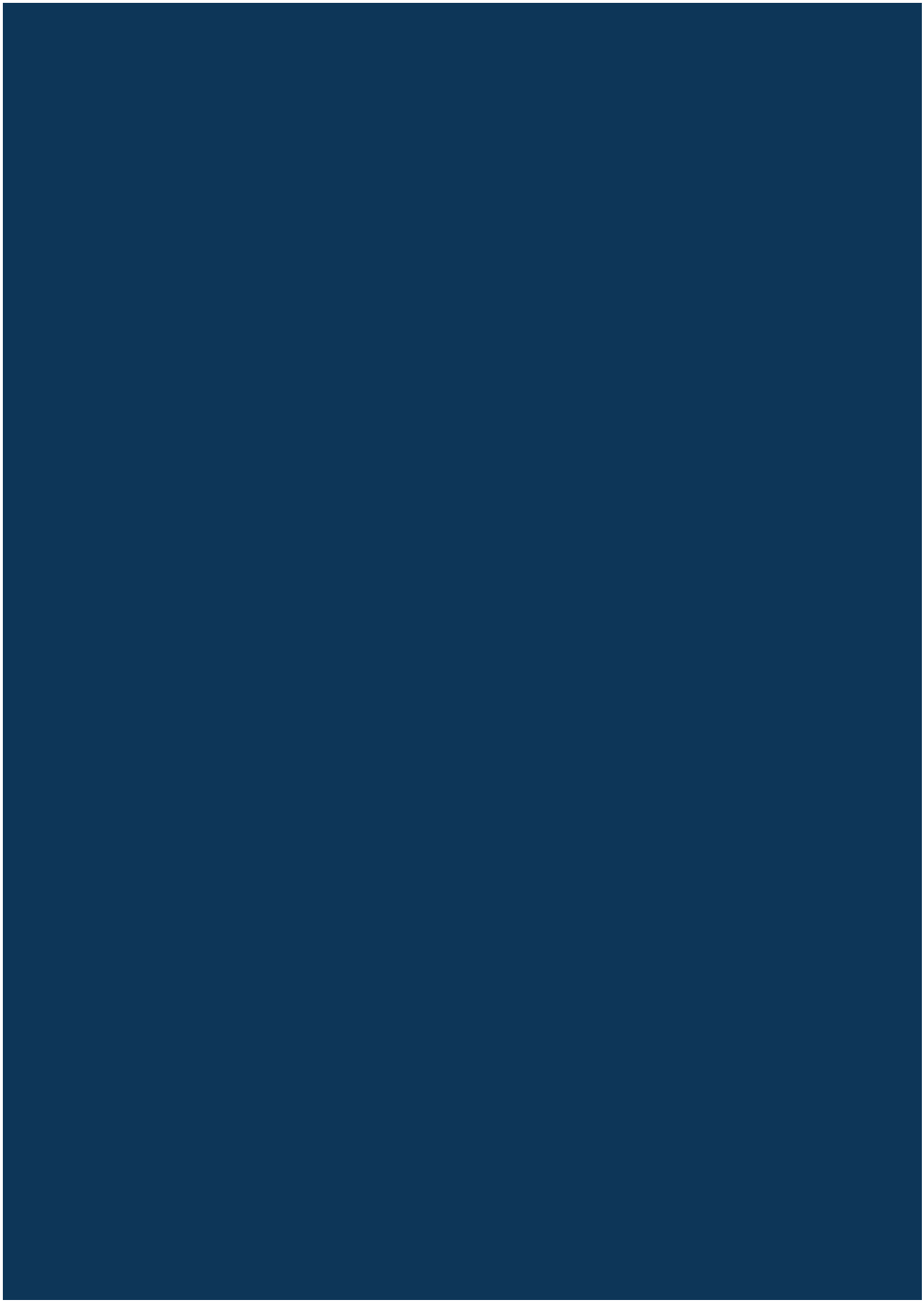


PART III

Sleep and 24-hour activity rhythms in preterm preschooler and healthy school-age children



CHAPTER 6

Sleep and 24-hour rhythm characteristics in preschool children born very-preterm and full-term

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ABSTRACT

Study Objectives: Sleep impacts the quality of life and is associated with cardiometabolic and neurocognitive outcomes. Little is known about sleep of preterm-born children at pre-school age. We, therefore, studied sleep and 24-hour rhythms of pre-school children born very-preterm compared to full-term children.

Methods: Prospective cohort study comparing sleep quality and quantity of children born very-preterm (gestational age (GA) <30 weeks) with full-term children at the (corrected) age of 3 years, using: 1) two parent-reported questionnaires (Brief Infant Sleep Questionnaire and The Munich Chronotype Questionnaire), and 2) at least 3-day days tri-axial wrist actigraphy combined with sleep diary. We performed regression analyses with adjustment for sex, (corrected) age and birthweight SD-score.

Results: Ninety-seven very-preterm-born (median GA 27+5 (interquartile range 26+3;29+0)) and 92 full-term children (GA 39+3;38+4;40+4) were included. Sleep problems and other reported sleep parameters were not different between groups. As measured with actigraphy, sleep and 24-hour rhythm were similar between groups, except for very-preterm born children waking up 21 minutes (4;38) minutes later than full-term children (adjusted p=0.001).

Conclusions: Based on parent reports and actigraphy, very-preterm-born children sleep quite similar to full-term controls at the corrected age of three years. Reported sleep problems were not different between groups. Actigraphy data suggest that preterm born children may wake up later than children born full-term. Further studies are needed to explore how sleep relates to cardiometabolic and neurodevelopmental outcomes after preterm birth and whether early interventions are useful to optimize 24-h rhythm and sleep.

INTRODUCTION

Sleep and 24-hour rhythm are vital for development and physiological function in children.[67] About a quarter of parents, however, report sleep problems in their children.[215] Sleep problems are most prevalent in infancy, declining towards middle childhood.[216] Insufficient or disturbed sleep is not only associated with decreased neurocognitive functioning, but also with an increased risk of obesity and cardio-metabolic diseases.[217] Preterm born children are at increased risk for these adverse outcomes, potentially creating opportunities to improve long-term health and well-being by improving their sleep patterns.

Sleep problems may be more common in children born preterm, due to disturbance of the fetal development of the 24-hour rhythm and sleep.[73] Possible mechanisms include premature disconnection to maternal circadian cues, impaired growth, neonatal morbidities, and adverse environmental factors, all in a critical period of development of the immature nervous system.[218] Most studies on sleep patterns after preterm birth are based on parental reports and describe sleep at various ages between 3 and 18 years. They generally showed lower sleep quality, more nocturnal awakenings and daytime sleepiness, than after full-term birth.[219-223] The few studies using objective measurements, like actigraphy or polysomnography,[224] in children at school-age, reported inconclusive results based on small populations.[225,226] Studies using polysomnography suggest that preterm born children have more nocturnal awakenings at school age.[222] Overall, very little is known on sleep problems and 24-hour rhythm in very-preterm born children in the preschool period.

Therefore, the aim of this study was to compare sleep and 24-hour activity rhythm, between very-preterm and full-term born children at the age of 3 years, using parent reports and actigraphy. We hypothesized that very-preterm birth is associated with more reported sleep problems and with lower quantity and quality of sleep, including shorter sleep duration, lower sleep efficiency and more fragmented 24-hour activity rhythms than those born full-term. We expected to find this both in parent reports and actigraphy data.

METHODS

Study population

This study was nested in two ongoing prospective observational birth cohort studies at the Erasmus MC Sophia Children's hospital in Rotterdam, the Netherlands. The first

study (the BOND Study) included 142 very-preterm infants born at less than 30 weeks gestational age (GA) and admitted within 48 hours after birth to the level-IV Neonatal Intensive Care Unit (NICU) between 2014 and 2017. This study excluded congenital anomalies, early severe brain injury (IVH grade >II or posthemorrhagic ventricular dilatation (PHVD)), congenital infection or perinatal asphyxia (umbilical cord pH <7.00 and Apgar score below 5 after 5 minutes).[74] The second study (the Sophia Pluto Study) provided the full-term (≥ 37 weeks GA) participants, which included 1012 healthy infants born on several maternity wards in Rotterdam between 2013-2021 with an uncomplicated neonatal period. It excluded severe asphyxia (Apgar score below 3 after 5 minutes), sepsis or respiratory ventilation in the neonatal period, confirmed intrauterine infection, and known congenital, postnatal, or maternal disease or medication that could interfere with the child's growth and development, including maternal corticosteroid use.[227]

For the current study, participants of both studies, with a study visit at 3 years of (corrected) age between June 2019 and May 2022 were eligible. In the very-preterm cohort, sleep measurements were part of the general study protocol applying to all participants, while in the full-term cohort parents were asked to opt-in for the additional sleep measurements during the study period. The Medical Ethics Committee of the Erasmus Medical Centre approved both studies (MEC-2014-379, MEC-2012-164). Written informed consent was obtained from all parents/caregivers.

Data collection

Prenatal and neonatal factors were collected from hospital and midwife records and parental questionnaires. Retrieved from questionnaires, ethnicity was classified as 'Western-European' or 'non-Western' if one or both parents were born in a non-Western country, and level of parental education level was based on both parents.[86] Age- and sex-adjusted SD-scores (SDS) for birth weight were calculated with the Fenton Growth Chart Calculator. Small for gestational age (SGA) was defined as < 10th percentile for weight.[88] Age and sex-corrected SDS for weight and weight-for-height SDS were calculated using Dutch reference values.[198]

Sleep questionnaires

Parents were asked to complete the following paper sleep questionnaires:

(1) Brief Infant Sleep Questionnaire (BISQ).[228] The BISQ aims to evaluate sleep patterns and habits and is composed of questions related to the following areas: (a) bedtime, (b) nocturnal sleep duration (between the hours of 7 PM and 7 AM), (c) daytime sleep duration (between the hours of 7 AM and 7 PM), (d) number of

nightly awakenings, (e) sleep onset latency (SOL) at night, (f) method of falling asleep, (g) location of sleep, (h) preferred body position and (i) parental rating of sleep problems, in children aged 0-3 years old.

(2) The Munich Chronotype Questionnaire (MCTQ).[229] This questionnaire documents sleep times, sleep duration, SOL and self-reported exposure to daylight on weekdays and weekend days. We computed midpoint sleep, defined as the middle time point between sleep onset time and wakeup time. If midpoint sleep was different for week- and weekend days, sleep and wake data would be presented separately.

Actigraphy

Sleep was assessed using a tri-axial actigraph (GENEActiv; Activinsights, UK) for at least five consecutive nights (three week nights and two weekend nights). The actigraph is a wristwatch-like device that monitors activity levels for extended continuous periods.[224] As in preschool children the non-dominant and dominant wrist yield similar results, children were free to use their preferred wrist.[230] Additionally, parents were asked to complete a paper sleep diary daily for each day and night the actigraph was worn. In the sleep diary parents filled in the child's nocturnal sleep duration, as well as any 'daytime naps' if the child slept more than 15 minutes during daytime.

Actigraphs were set at a frequency of 50 Hz. To be included in the actigraphy analyses, a child should have worn the actigraph for 16 hours or more per day capturing at least 4 hours sleep time per night, for a minimum of 3 days. Raw sleep data (.bin files) were analyzed with the R-packageGGIR version 2.6.0., using an algorithm with 5-s epochs and the reported bedtime and wake up times from the sleep diaries as guiders.[231] Actigraphy sleep measures were calculated using this script, and defined as follows.[231]

1. 24-hour sleep duration: total duration of estimated sleep per 24 hours, in hours:minutes. This measure was calculated by combining nocturnal sleep duration with the registered "accumulated sustained inactivity bouts during the day".[232] Sustained inactivity bouts are periods labeled as sleep during the night, but as 'inactivity' during the day. These 'inactivity periods' during the day may represent daytime sleep or wakefulness while being motionless for a sustained period of time. We only used sustained inactivity bouts that lasted at least 15 minutes.

2. Nocturnal sleep duration: total duration of estimated sleep between sleep onset in the evening and final wakening in the morning, in hours:minutes.
3. Daytime sleep duration: accumulated sustained inactivity bouts, based on bouts that lasted at least 15 minutes, in hours:minutes.
4. Sleep efficiency (percentage of time spent asleep between sleep onset at night and final waking time in the morning).
5. Wake after sleep onset (WASO, number of minutes scored as wake during the nightly sleep period).
6. Sleep onset latency (SOL, time between bedtime and sleep onset at night, in hours: minutes).

The following 24-hour activity rhythm parameters were calculated from the actigraphy data, using the GGIR script:[231]

1. Intradaily variability (indication of fragmentation of the sleep rhythm, ranging from 0 to 2, with higher scores indicating more fragmentation).
2. Interdaily stability (indicating the stability of the 24-hour activity rhythm across days, ranging from 0 to 1, with higher scores indicating more stable rhythms.[232-234]

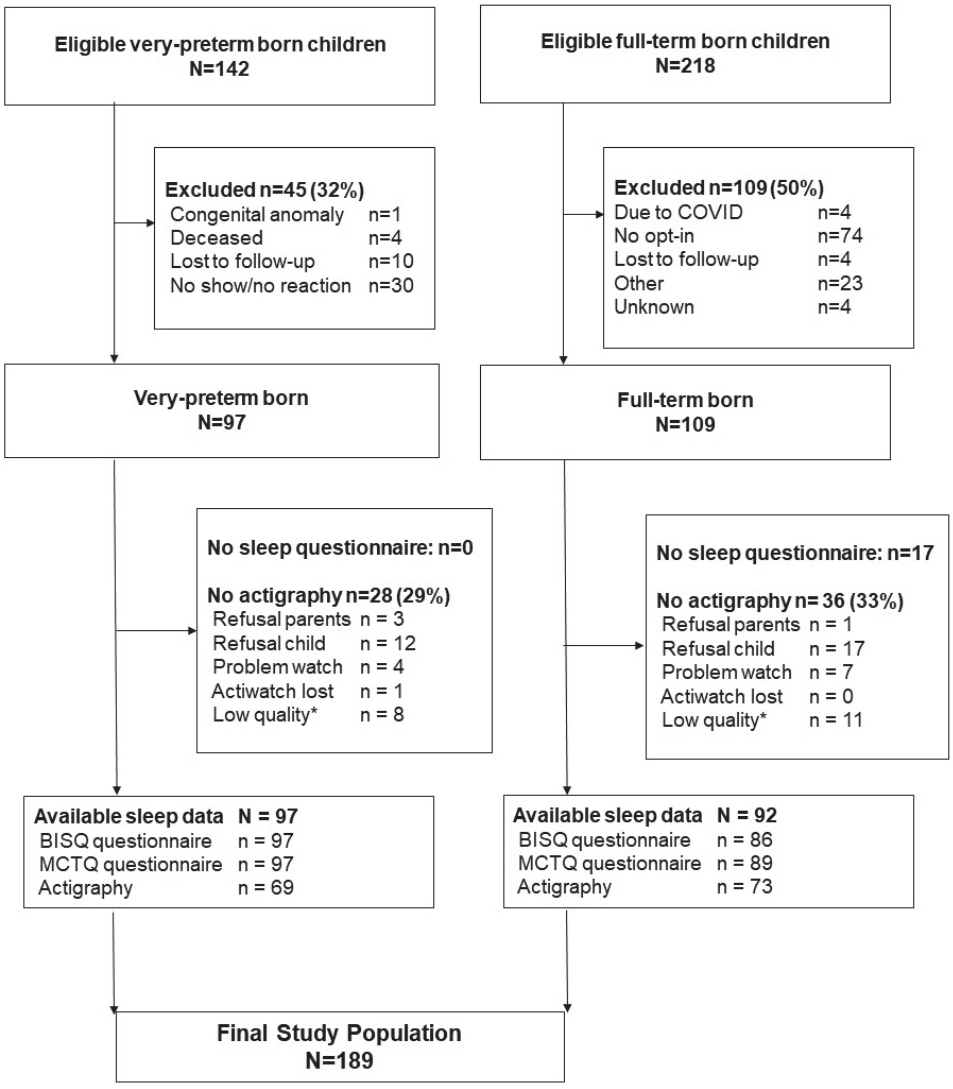
Statistical analysis

Characteristics and outcome data were described for very-preterm and full-term born children separately.Parametric and non-parametric tests were used for comparison of group characteristics between full-term and very-preterm born children, as well as those who opted-in and out in the full-term group, as appropriate. The primary analyses were based on linear and logistic regression models to compare sleep characteristics and 24-hour activity rhythms between the two groups, with a Poisson distribution for count data (number of awakenings). All models were adjusted for potential confounders selected based on literature. These included sex, age, and birth weight-SDS; as boys, younger age and low birth weight-SDS were previously associated with lower quality or quantity of sleep.[235,236] Reported results refer to adjusted analyses unless stated otherwise, unadjusted results are shown in the supplemental material. *P*-values of <0.05 (2-sided) were considered statistically significant. Data was analyzed using SPSS version 25.0 (IBM SPSS Statistics, Chicago, IL) and R-packageGGIR version 2.6.0.

RESULTS

In total, 97 very-preterm born (42% female), and 92 full-term born children (59% female) were included at three years of (corrected) age **(Figure 1).**

Figure 1. Flowchart of study population



**Actigraphy data were defined as 'low quality' as the following criteria were met: < 16 hours wearing time per day capturing < 4 hours sleep time with less than three such days per participant. BISQ: Brief Infant Sleep Questionnaire, MCTQ: Munich Chronotype Questionnaire*

Table 1. Baseline characteristics of preterm and full-term group

	Very-preterm (N = 97)	Full-term (N = 92)	P
Demographic Characteristics			
Gestational age (weeks)	27+5 (26+3;29+0)	39+3 (38+4;40+4)	**0.00**
Birth weight (grams)	1020 (828;1250)	3285 (2923;3708)	**<0.001**
Birth weight SDS	0.14 (-0.40;0.70)	-0.28 (-0.79;0.49)	**0.02**
SGA*	8 (8)	10 (11)	0.54
Sex Girl	41 (42)	54 (59)	**0.02**
Apgar 5 min *Missing*	8 (6;9) 1 (1)	10 (9;10) 1(1)	**0.00**
Family background			
Education level			**0.02**
Low	12 (12)	5 (6)	
Middle	28 (29)	13 (14)	
High	51 (53)	59 (64)	
Missing	6 (6)	15 (16)	
Ethnicity			**0.01**
Western European	76 (78)	58 (63)	
Non-Western	21 (22)	29 (32)	
Unknown	0 (0)	5 (5)	
Neonatal morbidity			
IVH			
No IVH	77 (80)	NA	
IVH grade 1	11 (11)		
IVH grade 2	9 (9)		
BPD			
No BPD	59 (61)	NA	
Mild BPD	22 (23)		
Severe BPD	16 (16)		
3 years visit			
(Corrected) Age (in years)	3.22 (3.10;3.40)	3.05 (3.01;3.16)	**<0.001**
Weight SDS	-0.99 (-1.84;-0.17	-0.02 (-0.90;0.52)	**<0.001**
Weight-for-height SDS	-0.68 (-1.50;0.14)	0.14 (-0.63;0.96)	**<0.001**

*Data is presented as Median (25th-75th percentile) or n (%). P-values for comparisons using Mann Whitney U or Chi-Square tests. * Small for gestational age (SGA) is defined as < 10th percentile for weight. Abbreviations: n: number, SDS: standard deviation score; BPD: bronchopulmonary dysplasia; IVH: intraventricular hemorrhage; NA: not applicable.*

In the very-preterm group median GA was 27+5 (interquartile range 26+3;29+0) weeks and birth weight-SDS was 0.14 (-0.40;0.70), versus 39+3 (38+4;40+4) weeks and -0.28 (-0.79;0.49), respectively, in the full-term group **(Table 1).** Comprehensive descriptions of the population characteristics have been described previously.[227,237].

Except for ethnicity (participants more often reported a non-Western ethnicity), no differences were found in population characteristics of the full-term group between those who opted in and out of the sleep measures **(Supplemental Table S4)**.

Sleep questionnaires

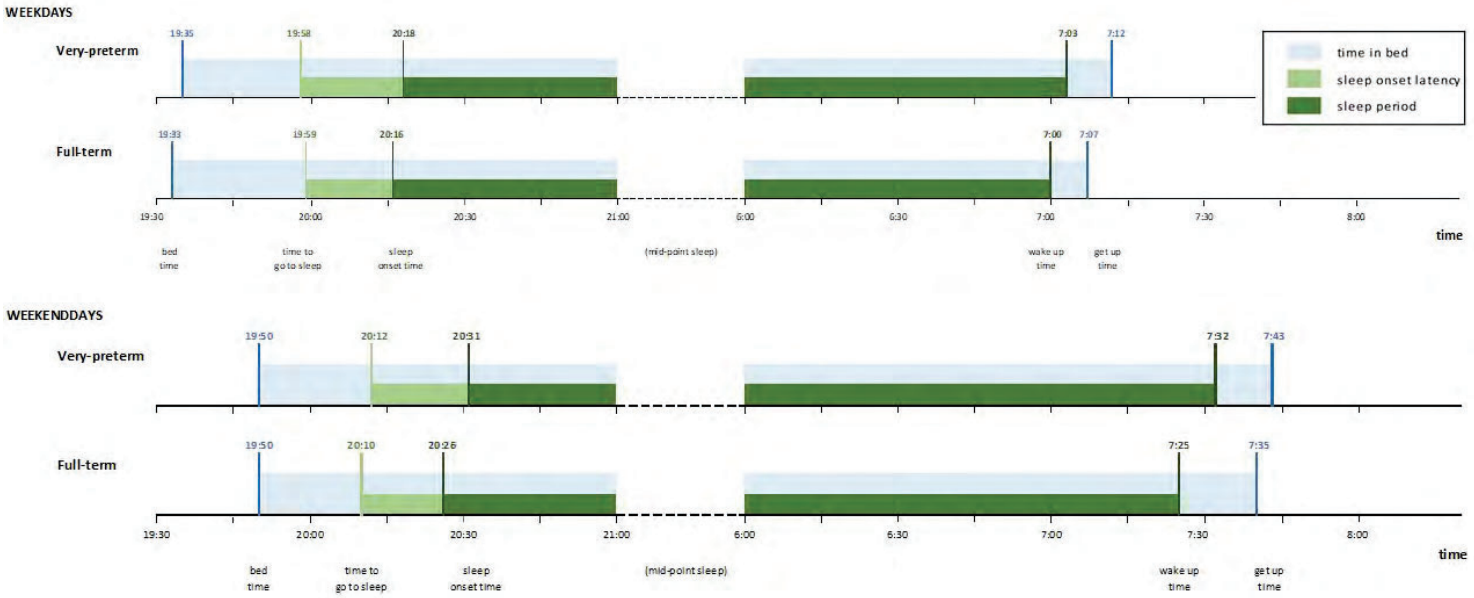
Parents reported on the BISQ questionnaire that very-preterm born children slept 23 minutes (95% confidence interval (CI) 5;42) longer during the night than those born full-term (p =0.01) **(Supplemental Table S1)**. After correction for sex, age, and birth weight-SDS, this difference was not significant (19 minutes, 95% CI -1;39 minutes, p=0.07) **(Table 2).** Sleep problems were reported in 26% of the very-preterm and in 20% of the full-term group (p=0.44). Daytime sleep duration, 24-h sleep duration, number of nighttime awakenings, method of falling asleep, sleep location, sleeping position and sleep onset time were not different between the very-preterm and full-term born children. In 20 (31%) and 31 (37%) of very-preterm and full-term children respectively, parents reported no daytime sleep.

Table 2. Parent reported sleep characteristics in preterm and full-term children at the age of three years

	Very-preterm (n = 97)	n	Full-term (n = 92)	n	β	95% CI	P
Nocturnal sleep duration (hh:mm)	10:56 (00:58)	97	10:32 (01:06)	85	00:19	-00:01;00:39	0.07
Daytime sleep duration (hh:mm)*	00:51 (00:58)	90	00:58 (00:53)	85	00:03	-00:16;00:21	0.76
24h sleep duration (hh:mm)	11:45 (01:06)	91	11:30 (01:17)	85	00:20	-00:03;00:44	0.09
No. nighttime awakenings	1 (0;1)	97	0.5 (0;1)	86	-0.26	-0.69;0.18	0.25
Sleep problem yes N (%)	25 (26)	97	17 (20)	87	NA	NA	0.44

*Shown are group mean (±SD) or median (IQR), numbers of participants with data, and effect estimates of the comparison between the very-preterm (1) and term group (0) based on linear and logistic regression analysis adjusted for sex, age and birth weight SD-score (SDS). Unadjusted analyses are shown in Supplemental Table S1. n: number, BISQ: Brief Infant Sleep Questionnaire, NA: not applicable, 95% CI: 95% Confidence Interval. *n = 20 (31 %) and n = 31 (37 %) of parents reported zero daytime sleep in very-preterm and full-term children, respectively.*

Figure 2. Parent reported sleep/wake timelines for the very-preterm and full-term group



Time line with mean parent reported sleep/wake time points based on the Munich Chronotype Questionnaire (MCTQ) for the very-preterm and full-term group, for weekdays and weekend days, after adjustment for sex, age and birth weight SD-score (SDS), as shown in ***Supplemental Table S2****.*

Parent-reported sleep/wake times on week- and weekend days, were not different between very-preterm and full-term children **(Figure 2, Supplemental Table S2)**. In both groups, midpoint sleep was earlier on weekdays (mean 01:40 and 01:38 hh:mm) than on weekend days (mean 02:02 and 01:55 hh:mm) in very-preterm and full-term children respectively (p<0.001 in both groups).

Actigraphy

Actigraphy data were available for 69 (71%) of the very-preterm and 73 (67%) of the full-term children **(Figure 1).** Actigraph wearing time varied between 3 to 7 days and was comparable between the very-preterm (mean 4.94) and full-term children (mean 5.04, p=0.50).

Table 3. Sleep and 24-hour activity rhythm by actigraphy in very-preterm and full-term born children at the age of 3 years

	Very-preterm (n=69)	Full-term (n=73)	β	95% CI	P
Sleep onset time (hh:mm)	20:32	20:44	-00:22	-01:06;00:22	0.33
Wake-up time (hh:mm)	07:11	06:50	00:21	0:04;0:38	**0.01**
24h sleep duration (hh:mm)	08:58	09:16	-00:07	-00:30;00:16	0.53
Nocturnal sleep duration (hh:mm)	07:50	07:50	00:03	-00:19;00:25	0.80
Daytime sleep duration (hh:mm)	01:10	01:25	-00:10	-00:27;00:07	0.26
SOL (hh:mm) *Missing*	0:41	0:44 *1*	-0:03	-0:13;0:05	0.44
WASO (minutes)	148	137	13	-2;28	0.08
Sleep efficiency (%)	67	69	- 1	- 4;2	0.50
Interdaily stability	0.71	0.73	-0.04	-0.11;0.03	0.23
Intradaily variability	0.34	0.42	-0.08	-0.18;0.02	0.10

Shown are group means, numbers of participants with data, and effect estimates of the comparison between the very-preterm (1) and term group (0) based on linear regression analysis adjusted for sex, age and birth weight SD-score. n: number, SOL: sleep onset latency, WASO: wake after sleep onset, IS: interdaily stability, range 0-1 with higher values indicating more stability; IV: intradaily variability, range 0-2 with higher values indicating more fragmentation. Unadjusted comparisons are shown in ***Supplemental Table S3***

The very-preterm born children woke up 21 minutes (95% CI 4;38, $p_{adjusted}$=0.01) later than the full-term born children **(Table 3 and Supplemental Table S3).** Mean daytime sleep duration was 16 minutes (95% CI -00:31;00:00, p=0.04) shorter in the very-preterm born children compared to the full-terms (**Supplemental Table S3)**. After correction for confounders, this difference was not significant (10 minutes, 95%

CI -00:27;00:07, p=0.26) **(Table 3).** No differences were observed in sleep onset time, 24-hour and nocturnal sleep duration, SOL, WASO or sleep efficiency between the very-preterm and full-term born children. Also, comparison of the 24-hour activity rhythm variables showed no differences in either intradaily variability (mean 0.34 and 0.42, p=0.10) or interdaily stability (mean 0.71 and 0.73, p=0.23) between the very-preterm and full-term born children (**Table 3**).

Both in the very-preterm and full-term children, sleep data reported by parents were different from data measured by actigraphy. Specifically, the mean 24-h sleep duration reported by parents was 02:46 (95% CI: 02:27;03:05; p <0.001) and 02:19 (95% CI: 01:55;02:44, p <0.001) hh:mm longer in the very-preterm and full-term born children respectively, as compared to 24-h sleep duration as measured by actigraphy. Likewise, parents reported shorter SOL, earlier sleep onset time and later wake-up time (**Supplemental Table S5**).

DISCUSSION

In this prospective observational study, we compared sleep and 24-hour activity rhythms between very-preterm (corrected) 3-year-olds and their full-term born peers, while taking sex, age at assessment and birth weight SDS into account. We observed a trend of longer parent reported nocturnal sleep in children born very-preterm, but this association attenuated after adjustment. Contrary to our hypothesis, we found no large differences in sleep problems, quality of sleep, 24-hour sleep duration or activity rhythms between the groups. With actigraphy, children born very-preterm appeared to wake up 21 minutes later than their term-born peers. Although other actigraphy variables showed no significant differences, there may be a trend of lower sleep efficiency, higher WASO and spending more time in bed in preterm born children. The 21-26% incidence of parent reported sleep problems in the groups was comparable to data from a large Dutch population-based cohort of children aged 2 to 14-years.[215] The 23-minutes longer parent reported 24h-sleep duration in the preterm group was no longer significant after correction for confounders, nor was it observed with actigraphy. Based on previous literature, we expected parents of very-preterm children to report longer sleep duration.[219,238] Although not significant, the observed effect size of 23 minutes was in line with three earlier population-based cohort studies using BISQ questionnaires, reporting that in children of 3-36 months of age, each week of shorter GA was associated with 3 minutes longer sleep duration.[238] In another study, at a much later age of 11 years, parents reported 18 minutes longer sleep (9.6 versus 9.3 hours) in extremely preterm born children (<28

weeks of GA) compared to full-terms.[219] Although the mechanisms are still unclear, longer sleep duration of preterm born children in the first years of life, might be explained as catch up sleep needed for maturational processes after shorter gestation and a neonatal period complicated with persistent disturbed sleep.[238,239] However, the difference in sleep duration was not observed with actigraphy.

Our actigraphy data showed that very-preterm children woke up later than full-term born children. On this topic, literature is not consistent. In older preterm born children aged 6 to 12-year, no difference was found in wake-up time using polysomnography.[240,241] Whereas in adolescents, using questionnaires and actigraphy, an earlier wake up time was found in those who were born preterm.[219,242] It is unclear whether the previous findings are explained by lack of power in these studies, the use of different techniques, selection of different study populations, or reflect real changes in sleep patterns and chronotype over time at increasing age. We cannot rule out that later wake-up time in our very-preterm group may (partly) be explained by unmeasured social factors influencing the family schedules and waking times. Also data on family size, sleep problems of family members, behavior problems and parenting style may be relevant.[243]

This study provides unique actigraphy data on 24-hour rhythms in 3-year-old children born very-preterm compared to peers born full-term, and found that interdaily stability and intradaily variability were not different between the groups. We found no previous studies with stability and variability data in preterm children in the preschool period. In a Dutch cohort of term-born children at preschool age, interdaily stability was 0.72, which is comparable to the values in both our groups.[244] That earlier study showed a higher intradaily variability of 0.60 (i.e. more fragmented 24-hour activity rhythms) than observed in our study groups.[244] This may be explained by more fragmentation of 24-hour activity rhythms in older children (6-12 years and 13-18 years) compared to younger children (aged 2-5 years). Their higher intradaily variability may simply be the result of older children in their cohort, reflecting normal development of sleep-wake patterns during childhood.[244] To our knowledge, only one study compared sleep fragmentation in preterm versus term children, reporting more fragmented sleep patterns in children born preterm aged 5-12 years (mean age 9 years), based on polysomnography.[236] We could not confirm their findings, likely since we used different measurement technique, and our study cohorts consists of children of much younger age.

Parental reports and actigraphy showed large differences in sleep duration, which is in line with previous studies in older children and adults.[224] In BISQ questionnaires,

parents reported 2 to 3 hours longer sleep than was measured by actigraphy. A Portuguese study in 3-6 year-olds, showed a 159 min longer total sleep time reported by parents (Children's Sleep Habits Questionnaire) as compared to actigraphy.[245] Possible explanations for parents reporting longer sleep, may reflect parents being not aware of their children being awake for periods, when lying quietly in bed.[245] Possible explanations for actigraphy measuring shorter sleep time, may be found in falsely classifying movements during restless sleep episodes (typical in young children) as awakenings.[246-249] Similarly, daytime sleeping time may be misclassified as wake time in conditions with external movement, for example when a child is sleeping in a pushchair or car seat.[250] As both methods have their strengths and shortcomings, and superiority of one method over the other is not clear in this population, we considered both methods relevant.[245] Future studies using gold standard methods, such as polysomnography, are needed to further evaluate both methods.

Quantity and quality of sleep are important for physical and mental health and child development. Sleep has been acknowledged as one of the most important newborn health outcomes by the International Consortium for Health Outcomes Measurement (ICHOM) group of leading physicians, measurement experts and patients.[251] The differences in sleep between very-preterm and full-term children found in our study may appear small. And needing more time in bed to reach the same amount of nocturnal sleep, may not seem problematic. However, we cannot rule out that in families who experienced preterm birth, even small differences may have large impact on quality of life. We have no data available on the impact of sleep on the quality of life in our groups, except that sleep problems were not reported significantly more often by parents of very-preterm born children, and that reported sleep duration in both our study groups was within the recommended range of 10-13 hours per 24 hours for children 3 - 5 years.[238] Future studies would benefit from taking more patient reported outcomes into account.

We found unexpectedly small or lack of associations between very-preterm birth and sleep, which may have different explanations. The size of our study population limited power, which may have led to false negative findings. For example, the reported sleep problems in 26% of very-preterm born children may be significantly higher than the 20% in the full-term born children, if measured in a large cohort. The sample size also limited our possibilities to study the role of socio-economic factors, such as ethnical background (more Western), parental education (lower) or neonatal complications, in the very-preterm born children. Assuming that our findings are valid, the very-preterm born children in our study either had less sleep problems or the full-terms

had more problems, than expected. The latter is unlikely as the values found were all within normal ranges of previous cohort studies. Preterm born children having unexpected normal sleep may cautiously suggest that the postnatal care and home environment were adequately adjusted to preserve or restore the development of the 24-hour rhythm and sleep. Possible protective factors may include our NIDCAP (Newborn Individualized Development Care and Assessment Program), which is associated with increased time in quiet and active sleep states.[252] Skin-to skin contact or kangaroo care may positively affected the state organization, sleep and wake states and brain maturation.[253] In addition, post-discharge interventions, such as the TOP program (Transmural developmental support for very-preterm infants and their parents), may have had beneficial effects.[254]

Strengths and limitations

As compared to previous studies, the strengths of this study include the relatively large number of very-preterm (<30 weeks GA) and full-term born children at the same age, born in the same period and living in the same geographic area. We used both subjective and objective sleep measurement methods, including parental perspectives. We measured sleep with actigraphy for multiple days and included 'daytime naps', which are still relevant for the 24-hour sleep duration in the majority of preschool children. We were able to report unique data on intradaily variability and interdaily stability in children born very-preterm at this age. In line with national policy and the literature, we adjusted for corrected age, to account for a prolonged effect of low gestational age at birth.[255]

One of the main limitations of this study is non-adherence, as 12 very-preterm (12%) and 17 full-term (16%) children refused to wear the actigraph. This may have resulted in selection bias if children with behavioral problems (related to sleep problems) were more likely to refuse participation. Furthermore, in a total of 12 children there was a problem with the watch (broken or lost) resulting in loss of data. However, bias is likely limited, as the non-adherence percentages and material problems were comparable in both groups (child refusal in 12% and 16%, watch problems in 5% and 6% of very-preterm and full-term born children, respectively). Only if reporting and awareness differed between parents of very-preterm and full-term children, this may have biased our results. As all questionnaires were fully completed by the parents of the very-preterm born children, but incomplete or missing in 16% of the controls, bias cannot be ruled out. Dilution of our results may have been introduced by the above issues, as well as by excluding infants with severe early brain injury (IVH grade >II or PHVD), who may have more severe sleep problems. This might also limit external generalizability to other very-preterm cohorts.

To the best of our knowledge, no questionnaires on chronotype are validated for use below the age of 4 years. We used the short and simple MCTQ, which is validated starting from 6 years and can be used into adulthood.[229] Using questionnaires and diaries always creates a risk of recall or response bias. We noticed that some parents perceived difficulties in reporting their child's exact sleep and wake-up times. Parents also acknowledged that sleep may have been over reported as they may not be fully aware of their children's behaviors during the night, for example when lying quietly awake in bed. One of the main limitations of actigraphy compared to polysomnography is the poor agreement of total sleep time and WASO, while estimates of total bedtime often show satisfactory agreement.[256] Furthermore, due to lack of access to (home) polysomnography and data on upper airway obstruction, we have no information about sleep-disordered breathing.[257] As preterm birth is associated with obstructive sleep apnea syndrome, this may have influenced sleep measures in this group. For future studies, we would recommend a multimodal sleep assessment to be able to study the duration of all sleep states and true awakenings. To disentangle the role of the earlier mentioned social and socio-demographic factors as well as intervention programs, larger cohorts are needed.

In conclusion, we found that very-preterm born children at three years of age sleep quite similar to their term-born peers while taking sex, (corrected) age at assessment and birth weight SDS into account. Sleep problems were common, but not more prevalent than in full-terms. As sleep patterns evolve over a lifetime, this does not rule out that more serious sleep problems may occur later in life. Actigraphy data suggest that preterm born children may wake up later than children who are born full-term, although this was not reported by parents. Further studies are needed to explore how sleep relates to cardiometabolic and neurodevelopmental outcomes after preterm birth and whether interventions to minimalize disturbance of rhythm and sleep should start the early neonatal and infant period.

Supplemental Table S1. Parent reported sleep characteristics from the BISQ between very-preterm and full-term children (unadjusted)

	Very-preterm (n = 97)	n	Full-term (n = 92)	n	β	95% CI	P
Nocturnal sleep duration (hh:mm)	10:56 (00:58)	97	10:32 (01:06)	85	00:23	00:05;00:42	**0.01**
Daytime sleep duration (hh:mm)	00:51 (00:58)	90	00:58 (00:53)	85	-00:07	-00:24;00:10	0.40
24h sleep duration (hh:mm)	11:45 (01:06)	91	11:30 (01:17)	85	00:15	-00:06;00:36	0.17
No. nighttime awakenings	1 (0;1)	97	0.5 (0;1)	86	-0.18	-0.57;0.21	0.36
Sleeping problem yes N (%)	25 (26)	97	17 (20)	87	NA	NA	0.32

*Shown are group mean (±SD) or median (IQR), numbers of participants with data, and effect estimates of the comparison between the very-preterm (1) and term group (0) based on unadjusted linear and logistic regression analysis. Abbreviations: n: number, BISQ: Brief Infant Sleep Questionnaire, NA: not applicable. Adjusted comparisons are shown in **Table 2**.*

Supplemental Table S2. Parent reported sleep/wake parameters from the MCTQ in very-preterm and full-term children (adjusted)

	Very-preterm (n=97)	n	Full-term (n=92)	n	β	95 % CI	P
WEEKDAYS							
Bedtime (hh:mm)	19:35 (00:37)	97	19:33 (00:35)	88	00:02	-00:09;00:14	0.67
Sleeptime (hh:mm)	19:58 (00:45)	97	19:59 (00:39)	87	00:02	-00:11;00:16	0.72
SOL (min)	20 (17)	96	17 (15)	86	4	-1;9	0.14
Wake up time (hh:mm)	07:03 (00:33)	97	07:00 (00:36)	89	00:01	-00:10;00:12	0.82
Minutes to get up (min)	9 (20)	96	7 (9)	82	2	-3;7	0.34
Mid-point sleep (hh:mm)	01:40 (00:35)	97	01:38 (00:33)	86	00:03	-00:07;00:14	0.52
WEEKENDDAYS							
Bedtime (hh:mm)	19:50 (00:46)	97	19:50 (00:44)	81	-00:01	-00:16;00:13	0.83
Sleeptime (hh:mm)	20:12 (00:52)	97	20:10 (0:47)	79	00:02	-00:14;00:18	0.81
SOL (min)	19 (17)	97	16 (15)	81	4	-1;10	0.13
Wake up time (hh:mm)	07:32 (00:48)	96	07:25 (00:53)	80	00:05	-00:11;00:22	0.55
Minutes to get up (min)	11 (19)	94	10 (18)	82	1	-5;8	0.74
Mid-point sleep (hh:mm)	02:02 (00:44)	96	01:55 (00:44)	77	00:05	-00:08;00:20	0.44

*Shown are group mean (±SD), numbers of participants with data, and effect estimates of the comparison between the very-preterm (1) and term group (0) based on linear regression analysis adjusted for sex, age and birth weight SD-score (BW SDS). Abbreviations: MCTQ: Munich Chronotype Questionnaire, n: number, SOL: sleep onset latency. 95% CI: 95% Confidence Interval, B: Unstandardized β. Time line is shown in **Figure 2**.*

Supplemental Table S3. Sleep and 24-hour activity rhythm by actigraphy between very-preterm and full-term born children (unadjusted)

	Very-preterm (n=69)	Full-term (n=73)	β	95% CI	P
Sleep onset time (hh:mm)	20:32	20:44	-00:11	-00:50;00:27	0.56
Wake-up time (hh:mm)	7:11	06:50	00:25	0:06;0:43	**0.008**
24h sleep duration (hh:mm)	08:58	09:15	-00:17	-00:37;00:03	0.09
Nocturnal sleep duration (hh:mm)	07:50	07:50	-00:01	-00:21;00:18	0.90
Daytime sleep duration (hh:mm)	01:10	01:25	-00:16	-00:31;00:00	**0.04**
SOL (hh:mm) *Missing*	0:41	0:44 *1*	-00:02	-00:11;00:05	0.56
WASO (minutes)	148	137	11	-2;24	0.08
Sleep efficiency (%)	67	69	-1	-4;1	0.22
Interdaily stability	0.71	0.73	-0.03	-0.09;0.03	0.38
Intradaily variability	0.34	0.42	-0.08	-0.17;0.003	0.06

Shown are group means, numbers of participants with data, and effect estimates of the unadjusted comparison between the very-preterm (1) and term group (0) based on linear regression analysis. Abbreviations: n: number, SOL: sleep onset latency, WASO: wake after sleep onset, 95% CI: 95% Confidence Interval, B: Unstandardized β. Interdaily stability, range 0-1 with higher values indicating more stability; Intradaily variability, range 0-2 with higher values indicating more fragmentation. Adjusted comparisons are shown in ***Table 3.***

Supplemental Table S4. Full-term born children: opt-in versus opt-out characteristics

	Full-term Opt-In (N = 92)	Full-term Opt-Out (N = 71)	P
Demographic Characteristics			
Gestational age (weeks)	39+3 (38+4;40+4)	39+6 (39+0;40+5)	0.14
Birth weight (grams)	3285 (2923;3708)	3379 (3113;3757)	0.11
Birth weight SDS	-0.28 (-0.79;0.49)	-0.34 (-0.89;0.57)	0.77
Small for gestational age*	10 (11)	9 (13)	0.70
Sex Girl	54 (59)	34 (48)	0.17
Family background			
Education level			0.33
Low	5 (6)	8 (11)	
Middle	13 (14)	10 (14)	
High	59 (64)	39 (55)	
Missing	15 (16)	14 (20)	
Ethnicity			**<0.001**
Western European	58 (63)	54 (76)	
Non-Western	29 (32)	4 (6)	
Unknown	5 (5)	13 (18)	

Supplemental Table S4. Continued

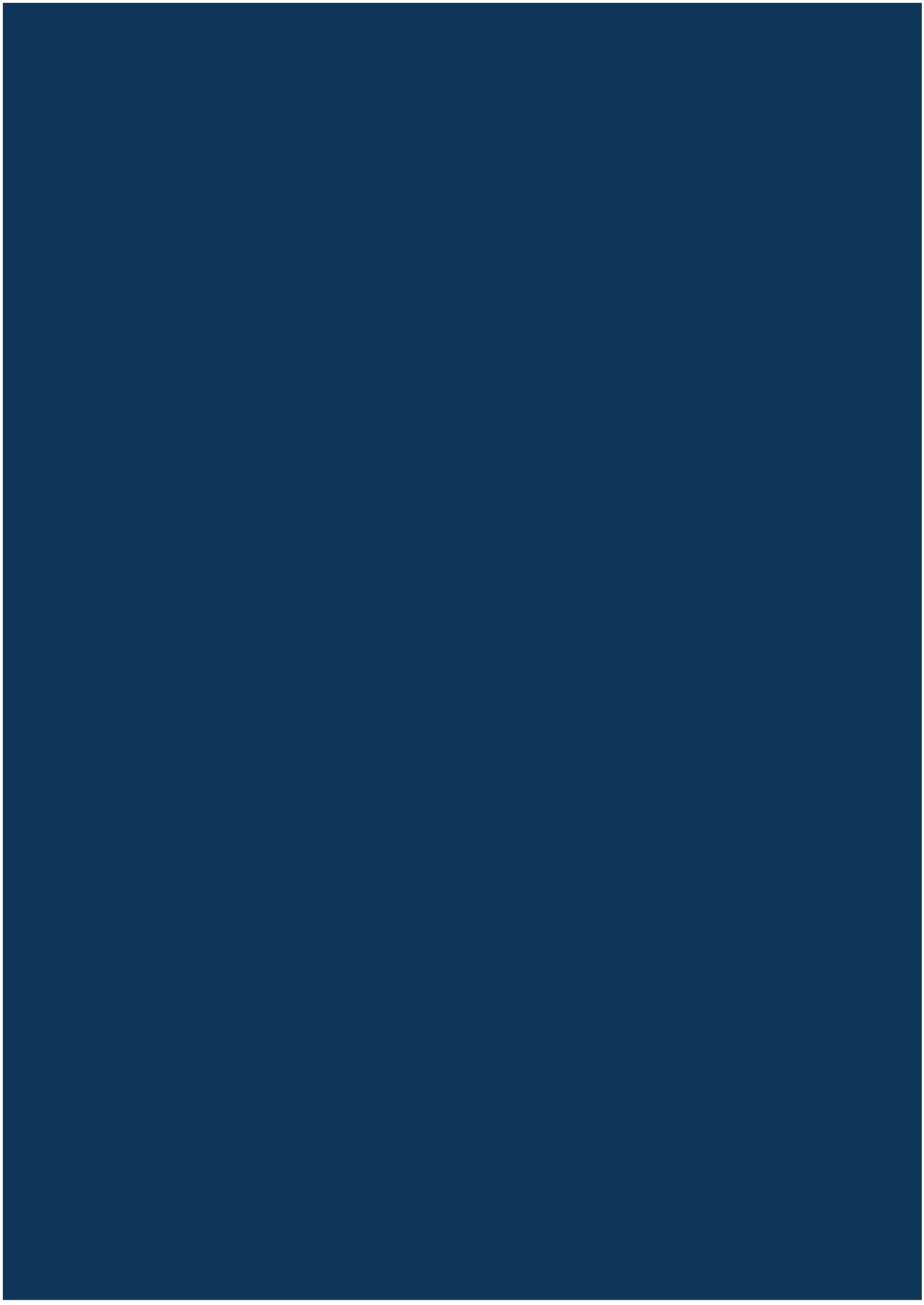
	Full-term Opt-In (N = 92)	Full-term Opt-Out (N = 71)	P
3 years visit			
Age (in years) *Missing*	3.05 (3.01;3.16)	3.04 (2.99;3.14) 7 (10)	0.60
Weight SDS *Missing*	-0.02 (-0.90;0.52)	-0.30 (-1.04;0.41) 7 (10)	0.14
Weight-for-height SDS *Missing*	0.14 (-0.63;0.96)	0.13 (-0.61;0.73) 10 (14)	0.50

*Data is presented as median (25th-75th percentile) or n (%). P-values for comparisons using Mann Whitney U or Chi-Square tests. * Small for gestational age was defined as < 10th percentile for weight. Abbreviations: n: number, SDS: standard deviation score.*

Supplemental Table S5. Comparison of questionnaire and actigraphy data

	Questionnaire mean (SD)	Actigraphy mean (SD)	*N*	Mean difference (95 % CI)	P
Preterm (N=67)					
24-hour sleep duration (min)	708 (70)	541 (54)	63	166 (147;185)	**<0.001**
SOL (hh:mm)	00:22 (00:19)	00:41 (00:24)	67	-00:19 (-00:26;-00:11)	**<0.001**
Sleep onset time (hh:mm)	19:58 (00:47)	20:33 (02:38)	68	-00:35 (-01:17;00:08)	0.11
Wake-up time (hh:mm)	07:31 (00:41)	07:15 (00:46)	65	00:16 (00:05;00:27)	**0.005**
Full-term (N=65)					
24-hour sleep duration (min)	696 (80)	557 (65)	65	139 (115;164)	**<0.001**
SOL (hh:mm)	00:24 (00:22)	00:45 (00:27)	65	-00:21 (-00:28;-00:15)	**<0.001**
Sleep onset time (hh:mm)	20:03 (00:40)	20:44 (00:53)	65	-00:40 (-00:49;-00:30)	**<0.001**
Wake-up time (hh:mm)	07:20 (00:44)	06:45 (00:53)	60	00:36 (00:24;00:49)	**<0.001**

Shown are group means (±SD) and mean difference (95%CI) of the comparison between questionnaires and actigraphy in the preterm group and full-term group based on Paired T-Test. Abbreviations: min: minutes, CI: confidence interval, SD: standard deviation. A variable was selected for comparison if it was measured with both actigraphy and at least one of the questionnaires.



CHAPTER 7

Fetal and infant growth patterns, sleep, and 24-hour activity rhythms: a population-based prospective cohort study in school-age children.

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ABSTRACT

The study objective was to exploreassociations of fetal and infant weight patterns and preterm birth with sleep and 24-hour activity rhythm parameters at school-age. In our prospective population-based study, 1327 children were followed from birth to age 10-15 years. Fetal weight was estimated using ultrasound in the second and third trimester of pregnancy. Birth weight and gestational age were available from midwife registries. Infant weight was measured at 6, 12 and 24 months. Fetal and infant weight acceleration or deceleration were defined as a change of >0.67 SD between the corresponding age intervals. At school-age, sleep duration, sleep efficiency, wake after sleep onset (WASO), social jetlag, interdaily stability, and intradaily variability were assessed using tri-axial wrist actigraphy for 9 consecutive nights. We observed that low birth weight (<2500 grams) was associated with 0.24 SD (95% confidence interval 0.04;0.43) longer sleep duration compared to normal weight. Compared to normal growth, growth deceleration in fetal life and infancy was associated with 0.40 SD (0.07;0.73) longer sleep duration, 0.44 SD (0.14;0.73) higher sleep efficiency, and -0.41 SD (-0.76;-0.07) shorter WASO. A pattern of normal fetal growth followed by infant growth acceleration was associated with -0.40 SD (-0.61;-0.19) lower interdaily stability. Preterm birth was not associated with any sleep or 24-hour rhythm parameters. Our findings showed that children with fetal and infant growth restriction had longer and more efficient sleep at school-age, which may be indicative of an increased need for sleep for maturational processes and development after a difficult start in life.

INTRODUCTION

Sleep development starts during fetal life and is associated with the development, maturation and connectivity of neural networks in the brain.[73] Adverse events in fetal life or infancy can disturb this development and may have persistent effects on sleep and 24-hour rhythm patterns in childhood.[221,258] Studies in preterm born children suggest that preterm birth itself as well as related comorbidities, such as being born small for gestational age (SGA) and cerebral hemorrhage, are associated with disturbed sleep (e.g. difficulties falling asleep and frequent awakenings) at child age.[218,259] Apart from neonatal comorbidities, the premature disconnection to maternal circadian cues and adverse environmental factors (e.g. during hospital admission) may also influence sleep development.[218]

A previous study among 52 children using polysomnography, showed that fetal growth restricted (FGR) and preterm born children had reduced sleep duration and efficiency, and altered rapid eye movement (REM)-sleep at the age of 5-12 years.[258] A larger study (n=787) using parental questionnaires, reported different sleep habits (e.g. earlier bed times and longer sleep duration) and more sleep problems in children aged 11 years born preterm as compared to children born full-term.[219] Studies of fetal or infant determinants of 24-hour activity rhythms in childhood are scarce. Findings from two Finnish actigraphy studies among young adults born preterm and full-term suggest that very low birth weight children might show an earlier chronotype in adult life.[260,261] Possible mechanisms include a longer period of melatonin deficiency after birth and adverse effects on circadian rhythm programming by prenatal hypoxia, and protein malnutrition and environmental factors in the early postnatal period.[260] The age of onset of developing the suggested earlier chronotype as well as its relation with cardiometabolic health require further investigation. Most previous studies are based on specific clinical neonatal populations, exposed to extreme circumstances before or after birth. To the best of our knowledge, the associations of fetal and infant growth patterns and gestational age across the full range, with sleep and 24-hour activity rhythms in childhood, have not been studied yet.

We hypothesized that altered fetal and infant developmental patterns, reflected by gestational age and weight growth are associated with disturbed sleep and 24-hour activity rhythms at school-age in a population-based sample. In a birth cohort including 1327 mothers and children, we examined the associations of preterm birth and growth in early life with actigraphy estimated measures of sleep and 24-hour activity rhythms at the age of 10 to 15 years.

METHODS

Study design and participants

This analysis was performed in the Generation R Study, a prospective population-based cohort study from early fetal life onward in Rotterdam, The Netherlands.[75] The study has been approved by the Medical Ethical Committee of the Erasmus MC Rotterdam (MEC 198.782/2001/31). Written informed parental consent was obtained for all participants. Pregnant women living in Rotterdam with an expected delivery between April 2002 and January 2006 were eligible for study participation (61% included, n=9778). We had information on fetal or infant growth of 9257 singleton births.[262] Between September 2015 and June 2018, a subsample of 1910 children who attended the regular 11-years (n=1152, median age 11.7 years) or 14-years study visit (n=758, median age 14.7 years) was asked to participate in an actigraphy substudy, of whom 1483 (77.6%) gave consent. In this subgroup, children born preterm were oversampled because of specific interest in the long-term consequences of preterm birth. We excluded children from multiple pregnancies, children without any data on weekday sleep and children in whom none of the recorded nights passed standard quality control (>6 hours wear time, >4 hours detected sleep time).[263,264]

Fetal and infant growth measures

Fetal ultrasound examinations were performed in all three trimesters by well-trained researchers according to clinical standards, as described previously.[75,262] Last menstrual period or first-trimester ultrasonography was used to establish gestational age.[265] Fetal ultrasounds in the second trimester were performed at median 20.5 (interquartile range (IQR) 20.0; 21.2) weeks, and in the third trimester at median 30.4 (IQR 29.8; 30.9) weeks. Head circumference, abdominal circumference, and femur length were measured to the nearest millimeter. Fetal weight was estimated by measuring head circumference, abdominal circumference, and femur length (to the nearest millimeter) using the formula by *Hadlock et al.*[266] We calculated sex-adjusted standard deviation scores (SDS) for estimated fetal weight. Gestational age at birth was divided in categories of preterm birth (<37 weeks), normal term birth (37-41 weeks) and late term birth (>41 weeks). Birth weight was obtained from community midwife and hospital registries, and divided in categories <2500 grams, 2500-4250 grams and >4250 grams. We calculated sex- and gestational age-adjusted SDS for birth weight within our study population using the Growth Analyzer 3.5 (Dutch Growth Research Foundation), based on North European reference charts.[267] Children born SGA were defined as sex- and gestational age–adjusted SDS for birth weight below the tenth percentile, and those born large for gestational age as sex- and gestational age–adjusted SDS for birth weight above the 90th percentile.[262]

Infant weight was measured in community health centers with a mechanical personal scale at median 6.2 (IQR 6.0;6.4) months, 11.0 (IQR 10.7;11.4) months and 24.8 (IQR 24.2;25.6) months, further referred to as the 6, 12, and 24 month visits.[75,262] Age- and sex-adjusted SD scores were created using Dutch reference growth charts.[268] Fetal growth was defined as growth between the second trimester and birth, and infant growth as growth from birth to 24 months. For both fetal and infant growth, we defined a decrease of >0.67 SD between time points as growth deceleration, an increase of >0.67 SD between time points as growth acceleration, and growth in between (-0.67 to +0.67 SD) as normal growth.[262] Combining the growth categories of the fetal period with the growth categories of the infant period, yielded 9 different growth patterns for the total period of second trimester till 24 months.

Sleep and 24-hour activity rhythm measures

At both the 11- and 14-year visit, we assessed sleep using a tri-axial wrist actigraph (GENEActiv; Activinsights, UK), as described previously.[264] In short, children wore the device for 9 consecutive nights (f5 school nights and 4 weekend nights) on their non-dominant wrist. A recent study showed acceptable reliability of the GENEActiv actigraph to estimate sleep in children aged 7-12 years when a minimum of 3-5 nights were measured.[269] Additionally, each morning children filled out a sleep diary with questions about their previous night's sleep. No sleep measurements were performed during school holidays or within 7 days after the start or end of daylight saving time. Actigraphs were set at a frequency of 50 Hz and raw sleep data were processed with the R-package GGIR using an algorithm with 5-s epochs.[270] All days with >16 hour wear time per 24 hours, were included in the analyses. This procedure generated the following sleep measures: 1) sleep duration (total duration of estimated sleep between sleep onset and final wakening in minutes); 2) sleep efficiency (percentage of time spent asleep between sleep onset and final waking time); and 3) wake after sleep onset (WASO, total time awake between sleep onset and final awakening). Additionally, we calculated 24-hour activity rhythm parameters: 4) social jetlag (average midpoint sleep during the weekend subtracted by the average midpoint sleep during the week, in hours), 5) intradaily variability (indication of fragmentation of the sleep rhythm, ranging from 0 to 2, with higher scores indicating more fragmentation), and 6) interdaily stability (indicating the stability of the 24-hour activity rhythm across days, ranging from 0 to 1, with higher scores indicating more stable rhythms).[232-234] Intradaily variability and interdaily stability were selected as non-parametric indicators of the 24-hour activity rhythm as previous research showed that these measures correlated highly with the relative amplitude but were more specific.[271] For homogeneity, we only used school days for the measures of sleep duration, sleep efficiency, and WASO, to minimize the

influence of atypical weekend events.[263,264] We included weekend sleep in a separate sensitivity analysis to test the robustness of this assumption.

Covariates

Information on maternal factors included educational level (based on highest attained educational level and categorized as low, middle, or high), pre-pregnancy BMI, folic acid use during pregnancy (yes/no), smoking and alcohol use during pregnancy (continued/no) and breastfeeding at 2 months (yes/no) were assessed by questionnaires. Sex and age of the child were obtained from medical records. Child ethnicity was based on country of birth of the parents (Dutch or non-Dutch). Season of sleep assessment was defined as 'Spring', 'Summer', 'Autumn', or 'Winter'.

Statistical analysis

First, we checked the correlations of all fetal and infant growth exposure measures with all sleep and 24-hour activity rhythm outcome variables. Second, we used linear regression to estimate the associations of the main birth outcomes (gestational age, birth weight, size for gestational age; both continuous and per category) with sleep outcomes (sleep duration, sleep efficiency, WASO) and 24-hour activity rhythm outcomes (social jetlag, intradaily variability, interdaily stability). Third, we used linear regression to estimate the associations of the 9 different growth patterns of combined fetal and infant growth (with the pattern of normal fetal growth-normal infant growth as the reference period), with sleep and 24-hour activity rhythms. All assumptions of linear regression were met for all analyses. We checked whether covariates were associated with both exposures and main outcomes (sleep duration, WASO, social jetlag, intradaily variability), or changed the effect estimate >10% when added to the models. We used three models: a 'basic model' adjusted for season and age at sleep assessment; a main 'confounder' model that was additionally adjusted for maternal education, pre-pregnancy BMI, folic acid use, smoking and alcohol during pregnancy, as well as child sex, ethnicity and breastfeeding at 2 months; and a 'BMI' model in which we additionally adjusted for child BMI at sleep assessment. Because of skewed distributions of sleep duration, WASO and interdaily stability, we used natural logged values for sleep duration and interdaily stability, and square root values for WASO, in all linear regression analyses. For comparison of effect estimates, we calculated SDS (observed value–mean/SD) for all outcome measures. To take into account multiple testing, we present results based on statistical significance on p-value < 0.05 and p-value <0.025 (based on 2 main outcomes groups: sleep and 24-hour activity rhythm). We considered more extensive Bonferroni correction too strict because of several intercorrelated exposures and outcomes.

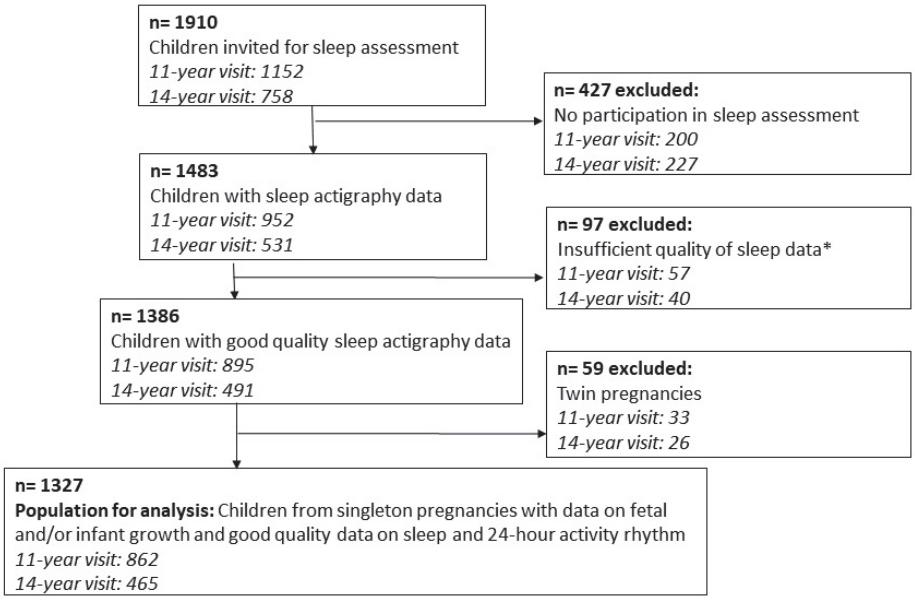
As the amount and timing of sleep changes during adolescence,[272] we first explored whether associations were different between those measured at the 11- and 14-year visit. We observed statistically significant interactions of timing of visit with gestational age at birth and multiple weight parameters (birth weight; weight SDS at birth, third trimester, 24 months), for sleep efficiency, WASO, social jetlag and interdaily stability. We therefore performed additional stratified analyses on study visit group as sensitivity analyses. No interactions were observed between sex and gestational age or weight parameters in the associations with sleep or 24-hour rhythm. Second, to test the robustness of associations, we reran the confounder models using combined weekend and weekday sleep. For all analyses, we used multiple imputations for missing covariates using the Markov chain Monte Carlo approach. Five datasets were created and pooled results were reported. Statistical analysis was carried out using IBM SPSS Statistics, version 25.0 (IBM SPSS Statistics, Armonk, NY).

RESULTS

Subject characteristics

The final population for analysis of this study comprised 1327 children (**Figure 1**).

Figure 1. Flowchart of the study population.



** No data available on weekday sleep, or none of the night data passing standard quality control (>6 hours wear time, >4 hours detected sleep time).*

Table 1 shows that 10% of children were born preterm (<37 weeks) and 7% with low birth weight (<2500 grams). In all, 95% of the children wore the device ≥7 days. Median (IQR) sleep duration was 7.5 (6.9; 8.0) hours and mean (SD) social jetlag 1.0 (0.7) hours. The correlations between exposures and outcomes are presented in **Supplemental Table S1**.

Table 1. Baseline characteristics of the study population

	n	Total population (n=1327)
Maternal characteristics		
Age at intake (yrs), mean (SD)	1327	32.1 (4.1)
Pre-pregnancy BMI (kg/m^2), median (IQR)	1086	22.5 (20.8;24.9)
Education, n (%)	1291	
Low/middle		487 (37.7)
High		804 (62.3)
Folic acid use during pregnancy (yes), n (%)	1027	925 (90.1)
Smoking during pregnancy (continued: yes), n (%)	1196	147 (12.3)
Alcohol use during pregnancy (continued: yes), n (%)	1147	611 (53.3)
Fetal and child characteristics		
Sex (female), n (%)	1327	707 (53.3)
Ethnicity, n (%)	1326	
Dutch		1093 (82.4)
Non-Dutch		233 (17.6)
Fetal period		
Second trimester, median (IQR)		
Gestational age (wks)	1240	20.5 (20.0;21.2)
Estimated fetal weight (gr)	1221	367 (326;420)
Third trimester, median (IQR)		
Gestational age (wks)	1245	30.4 (29.8;30.9)
Estimated fetal weight (gr)	1234	1619 (1462;1771)
Birth		
Gestational age at birth (wks), median (IQR)	1325	40.1 (39.0;41.0)
Preterm birth (<37wks gestational age), n (%)		133 (10.0)
Late birth (>41 wks gestational age), n (%)		327 (24.7)
Birth weight (gr), median (IQR)	1327	3500 (3100;3860)
Low birth weight (< 2500 gr), n (%)		93 (7.0)
High birth weight (> 4250 gr), n (%)		97 (7.3)
Birth weight SD-score (SD), mean (SD)	1325	0.1 (1.0)
Birth size	1325	
Small for gestational age (<10th percentile), n (%)		102 (7.7)
Large for gestational age (>90th percentile), n (%)		167 (12.6)

Table 1. Continued

	n	Total population (n=1327)
Infancy		
Breastfeeding at 2 months (yes), n (%)	1145	798 (69.7)
At 6 months, median (IQR)		
Age at visit (months)	1129	6.2 (6.0;6.4)
Weight (kg)	1126	7.8 (7.2;8.4)
At 12 months, median (IQR)		
Age at visit (months)	1059	11.0 (10.7;11.4)
Weight (kg)	1052	9.6 (8.9;1.0)
At 24 months, median (IQR)		
Age at visit (months)	1025	24.8 (24.2;25.6)
Weight (kg)	1024	12.8 (12.0;13.8)
Childhood (10-15yrs)		
Age at sleep assessment (yrs), median (IQR)	1327	11.8 (11.6;14.5)
BMI at center visit (kg/m2), mean (SD)	1322	17.9 (2.9)
Overweight/obesity, n (%)		160 (12.1)
Season of sleep assessment, n (%)	1325	
Winter		402 (30.3)
Spring		485 (36.6)
Summer		387 (29.2)
Autumn		51 (3.8)
Actigraphy		
Sleep duration (hrs), median (IQR)	1325	7.5 (6.9;8.0)
Sleep efficiency (%), mean (SD)	1325	84.6 (5.5)
Wake after sleep onset (min), median (IQR)	1325	79 (60;100)
Social jetlag (hrs), mean (SD)	1321	1.0 (0.7)
Sleep midpoint weekdays (time), mean (SD in min)	1325	02:51 (42)
Sleep midpoint weekends (time), mean (SD in min)	1324	03:52 (59)
Intradaily variability, mean (SD)	1324	0.56 (0.10)
Interdaily stability, median (IQR)	1324	0.17 (0.14;0.20)
Sleep diary		
Sleep duration (hrs), mean (SD)	1258	9.1 (1.1)
Nightly awakenings (n), mean (SD)	1277	0.5 (0.7)

Abbreviations: n, number; yrs, years; SD, standard deviation; BMI, body mass index; IQR, interquartile range; wks, weeks; gr, grams; hrs, hours; kg, kilograms; m, meter; min, minutes.

Birth outcomes, sleep and 24-hour rhythms

Low birth weight was associated with 0.24 SDS (95% confidence interval (CI) 0.04;0.43) longer sleep duration (**Table 2**). The associations between preterm birth and higher intradaily variability, between low birth weight and shorter WASO, and between small birth size for gestational age and longer social jetlag, all attenuated

to non-significance after correction for multiple testing. Gestational age, birth weight or birth size across the full range were not associated with sleep or 24-hour activity rhythms.

Fetal and infant growth patterns, sleep and 24-hour rhythms

As compared to normal fetal and infant growth, a pattern of continued growth deceleration in both fetal life and infancy was associated with 0.40 SDS (95% CI 0.07;0.73) longer sleep duration, 0.44 SDS (95% CI 0.14;0.73) higher sleep efficiency and -0.41 SDS (95% CI -0.76;-0.07) shorter WASO **(Table 3)**. Furthermore, a pattern of normal fetal growth followed by infant growth acceleration was associated with a -0.40 SDS (95% CI -0.61;-0.19) lower interdaily stability. Results from the basic models are presented in **Supplemental Tables S2 and S3**. **Supplemental Tables S4 and S5** show that findings were similar after additional adjustment for BMI.

Sensitivity analyses

Stratified analyses per study visit revealed that the associations of low birth weight with sleep duration and WASO, and of small birth size with social jetlag were similar but stronger at the 14-year visit (**Supplemental Table S6**). The associations of the growth patterns of continued fetal and infant growth deceleration with sleep duration, sleep efficiency and WASO, and of normal fetal growth with infant growth acceleration with interdaily stability, were in the same direction at both visits (**Supplemental Table S7**). However, the smaller group sizes in the subgroup analyses yielded small differences in effect estimates and reaching significance. Results were very similar when we included weekend sleep in the sleep measures (**Supplemental Table S8 and S9**).

Table 2. Associations of birth outcomes with childhood sleep and 24-hour activity rhythms (confounder model)

		Difference (95% Confidence Interval) in SDS					
Birth outcomes	**n**	**Sleep duration**	**Sleep efficiency**	**WASO**	**Social jetlag**	**Intradaily variability**	**Interdaily stability**
Gestational age							
< 37 weeks	133	0.15 (-0.01;0.32)	0.08 (-0.10;0.26)	-0.10 (-0.28;0.07)	-0.02 (-0.20;0.16)	**0.20 (0.02;0.38)**	-0.11 (-0.29;0.06)
37-41 weeks	863	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
>41 weeks	327	-0.04 (-0.15;0.08)	0.01 (-0.12;0.13)	-0.01 (-0.13;0.11)	0.08 (-0.04;0.20)	-0.05 (-0.17;0.07)	0.07 (-0.05;0.19)
Trend (per week)	1323	-0.02 (-0.04;0.00)	-0.02 (-0.04;0.01)	0.02 (-0.00;0.05)	0.01 (-0.02;0.03)	-0.02 (-0.04;0.01)	0.02 (-0.01;0.04)
Birth weight							
<2500 gr	93	**0.24 (0.04;0.43)***	0.17 (-0.04;0.38)	**-0.21 (-0.42;-0.01)**	0.04 (-0.17;0.25)	0.09 (-0.12;0.30)	-0.12 (-0.32;0.08)
2500-4250 gr	1135	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
>4250 gr	97	-0.02 (-0.20;0.17)	0.20 (-0.01;0.40)	-0.20 (-0.39;0.00)	-0.01 (-0.21;0.19)	0.09 (-0.11;0.29)	-0.02 (-0.21;0.18)
Trend (per 500gr)	1325	-0.04 (-0.08;0.00)	-0.01 (-0.06;0.03)	0.02 (-0.03;0.06)	-0.02 (-0.06;0.03)	-0.01 (-0.05;0.03)	0.02 (-0.02;0.06)
Size for gestational age							
Small (<p10)	102	-0.01 (-0.20;0.17)	-0.02 (-0.22;0.18)	0.05 (-0.14;0.24)	**0.22 (0.02;0.41)**	0.08 (-0.12;0.27)	0.03 (-0.16;0.22)
Appropriate (p10-p90)	1054	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Large (>p90)	167	-0.06 (-0.20;0.09)	0.08 (-0.09;0.24)	-0.07 (-0.22;0.09)	0.02 (-0.14;0.21)	0.05 (-0.11;0.20)	-0.00 (-0.15;0.15)
Trend (per SDS)	1323	-0.02 (-0.06;0.03)	0.02 (-0.03;0.07)	-0.03 (-0.08;0.02)	-0.04 (-0.09;0.01)	0.02 (-0.03;0.07)	0.01 (-0.04;0.06)

Abbreviations: SDS, standard deviation score; n, number; WASO, wake after sleep onset (minutes); gr, grams; p, percentile. Values are linear regression coefficients (95% confidence intervals) and reflect the change in sleep and 24-hour activity rhythm parameters per birth outcome. Models are adjusted for season and age at sleep assessment; child sex and ethnicity; maternal pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy; and breastfeeding at 2 months. Bold=p<0.05, bold=p<0.025.*

Table 3. Associations of fetal and infant growth patterns with childhood sleep and 24-hour activity rhythms (confounder model)

		Difference (95% Confidence Interval) in SDS					
Growth patterns	**n**	**Sleep duration**	**Sleep efficiency**	**WASO**	**Social jetlag**	**Intradaily variability**	**Interdaily stability**
Fetal growth deceleration							
Infant growth deceleration	30	**0.40 (0.07;0.73)***	**0.44 (0.14;0.73)***	**-0.41 (-0.76;-0.07)***	-0.08 (-0.43;0.27)	-0.08 (-0.44;0.28)	-0.22 (-0.57;0.13)
Infant normal growth	113	-0.02 (-0.21;0.18)	-0.05 (-0.27;0.16)	0.01 (-0.19;0.22)	0.02 (-0.19;0.22)	-0.04 (-0.25;0.17)	-0.20 (-0.40;0.00)
Infant growth acceleration	115	-0.01 (-0.20;0.19)	0.12 (-0.09;0.33)	-0.12 (-0.32;0.09)	0.03 (-0.18;0.23)	-0.16 (-0.37;0.06)	0.09 (-0.11;0.30)
Fetal normal growth							
Infant growth deceleration	113	-0.07 (-0.26;0.13)	-0.03 (-0.24;0.19)	-0.04 (-0.24;0.16)	-0.04 (-0.24;0.17)	0.02 (-0.19;0.24)	0.01 (-0.19;0.21)
Infant normal growth	258	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Infant growth acceleration	104	-0.07 (-0.27;0.13)	-0.04 (-0.26;0.18)	-0.01 (-0.22;0.20)	-0.03 (-0.24;0.18)	-0.00 (-0.22;0.21)	**-0.40 (-0.61;-0.19)***
Fetal growth acceleration							
Infant growth deceleration	163	-0.03 (-0.20;0.15)	-0.02 (-0.20;0.17)	0.01 (-0.17;0.19)	-0.03 (-0.22;0.15)	0.01 (-0.17;0.20)	-0.15 (-0.33;0.03)
Infant normal growth	164	-0.13 (-0.30;0.04)	0.05 (-0.13;0.24)	-0.10 (-0.28;0.08)	-0.04 (-0.23;0.14)	-0.11 (-0.30;0.08)	-0.15 (-0.33;0.03)
Infant growth acceleration	41	0.07 (-0.22;0.36)	0.13 (-0.19;0.45)	-0.10 (-0.41;0.20)	-0.09 (-0.39;0.22)	0.09 (-0.22;0.41)	-0.28 (-0.58;0.02)

Abbreviations: SDS, standard deviation score; n, number; WASO, wake after sleep onset (minutes). Values are linear regression coefficients (95% confidence intervals) and reflect the difference in sleep and 24-hour activity rhythm parameters compared to children with normal fetal and infant growth. Models are adjusted for season and age at sleep assessment; child sex and ethnicity; maternal pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy; and breastfeeding at 2 months. Bold=p<0.05, bold=p<0.025.*

DISCUSSION

In this large prospective population-based birth cohort, we observed that both low birth weight and a pattern of continued fetal and infant growth deceleration were associated with longer sleep duration at the age of 10-15 years. The pattern of continued growth deceleration was also associated with higher sleep efficiency and shorter WASO, whereas a pattern of normal fetal growth followed by infant growth acceleration was associated with lower interdaily stability. Subgroup analyses per study visit showed that associations of gestational age across the full range and low birth weight with WASO were stronger and only significant in children aged 14-15 years old, as compared to children aged 10-11 years. The same observation applied to the association of birth size, both small and across the full range, with social jetlag. We observed no interactions with child sex.

Our findings that children with low birth weight or continued fetal and infant growth restriction showed more favorable sleep outcomes, as expressed by longer sleep duration, higher sleep efficiency and shorter WASO, partly aligned with previous studies.[236,258] Only a limited number of studies have investigated the effects of fetal growth restriction on childhood sleep, showing conflicting results.[258,273,274] Using polysomnography in children aged 5-12 years, *Yiallourou et al* presented comparable results to ours by demonstrating that FGR children born preterm showed longer sleep duration, higher sleep efficiency and shorter WASO than preterm children who were born with appropriate birth weight for gestational age (AGA), but not as compared to their term AGA peers.[236,258] By contrast, actigraphy studies of *Leitner et al* and *Pesonen et al* showed shorter sleep, lower sleep efficiency and/or more awakenings in children aged 4-8 years with fetal growth restriction or low birth weight, respectively, as compared to children with normal birth weight.[273,274] A possible explanation for these remarkable conflicting results is that in FGR children, sleep characteristics evolve differently over the course of childhood as compared to their normal birth weight peers; but further research at school-age is required. Although there is overlap between children born preterm and children with low birth weight or fetal growth restriction, we did not observe any associations with preterm birth or gestational age across the full range in our full group analyses. These findings only partly agree with results of previous studies on sleep duration in school-age children born preterm, which are also not consistent.[73,218,222] Some studies reported longer sleep duration,[219] while others reported no differences,[221,275,276] or shorter sleep in those born preterm.[258,277]

As for 24-hour rhythms, we showed that at the 14-year visit, birth size was linked to social jetlag, with children born SGA having greater misalignment in sleep/wake timing between weekdays and weekends than children with normal size at birth. The rapid changes of the sleep-wake rhythms during adolescence may explain why we

did not observe this association at the 11-year visit, as social jetlag may become more prominent at an older age.[272] To the best of our knowledge, this is the first study to investigate early growth and social jetlag in childhood, which hampers comparison of our results with previous research. A suggested risk factor for social jetlag is a late(r) chronotype, when sleep debt builds up during the week to be compensated during the weekend.[278] Chronotype has previously been investigated in relation to birth outcomes. However, those studies have not described a later but in fact an earlier chronotype (earlier bed and waking times) in young adults and adolescents born preterm, especially in those born SGA.[242,261,279] This earlier chronotype may reflect developmental influences, as well as genetic alterations and/or parenting styles, and may be protective for later mental and physical health.[242,280] Based on these findings, the association of birth size and social jetlag would have been expected in the opposite direction as observed in our study. Interestingly, a growth pattern of normal fetal growth and accelerated infant growth was associated with a decrease in interdaily stability. This may suggest that catch-up growth might affect the consolidation of a stable 24-hour activity rhythm at school-age. However, as there are no studies for comparison, further research on perinatal and infant growth influences on 24-hour rhythms in childhood is essential.

The mechanisms underlying the associations of early growth, sleep and 24-hour rhythms are not fully understood and are likely multifactorial. Development of sleep and 24-hour rhythms already starts during the early fetal period when brain maturation commences, and neural networks become more coherent.[73] As from 32 weeks gestational age, four different sleep-wake states can be distinguished. Preterm birth affects this developmental process of sleep and 24-hour rhythm, likely mostly as a result of adverse brain growth.[44,73,239] This is even more prominent in FGR infants, in whom even lower neural myelination and a larger reduction in structure and organization of neural connections between brain regions have been observed.[73] Other mediating factors are thought to be fetal and neonatal hypoxia, the loss of placental steroids and hormones, inflammation, genetic alterations and environmental factors during the neonatal period.[73] Previous research in infants 0-6 months corrected age suggested that those born preterm show an earlier emergence of a circadian 24-hour rhythm than their full-term peers, potentially due to longer exposition to external time cues such as light.[281] How this finding relates to the previously described 'early' chronotype later in life in this population, requires further investigation.

The higher sleep quantity and quality observed in children with low birth weight and early growth restriction could be the result of earlier bed times (possibly related to more protective parenting) and/or an increased need for restorative sleep to benefit

(catch-up) growth, but could also be related to certain behavioral traits more often seen in preterm born or fetal growth restricted children, such as requiring extra time for processing of stimuli experienced during the day.[282] Although further research is needed to understand their increased sleep (need) and its consequences for future health, our findings emphasize the importance of sleep and 24-hour rhythm at school-age in this vulnerable group of children. Therefore, exploration of sleep and 24-hour rhythms should be integrated into neonatal follow-up of children born preterm and SGA.

Strengths and limitations

Major strengths of this study are the prospective analysis in an ongoing birth cohort study with a large sample size and extensive information on perinatal and sociodemographic risk factors, which permitted correction for multiple confounders. Using weight measurements at six different time points from second trimester till 24 months of age enabled us to construct and study nine different growth patterns of combined fetal and infant growth. Another strength is the use of objectively measured sleep and 24-hour rhythms using actigraphy rather than subjective (parental) report.[247] Lastly, this is, to our knowledge, the first study to investigate how pre-, peri- and postnatal growth associates to 24-hour activity rhythms in school-age children.

Some study limitations should also be considered. Being a population-based study, the number of children born preterm in this study (10%) was a fairly good representation of the national prevalence of preterm birth (7%).[283] However, the proportion of children born very preterm (<30 weeks gestation) in our study was low (n=11, 0.8%). This might have limited statistical power to detect significant associations for preterm birth/gestational age and affects the generalizability of our findings to this specific group, in which sleep problems were described to occur more frequently.[219] Furthermore, the observed differences in associations between the 11- and 14-year visit suggest that the onset of puberty may play a role. However, we were unable to correct for pubertal status as insufficient information on Tanner stages was available. Follow-up studies are needed to assess the observed differences between late childhood and (early) adolescence. Finally, although many covariates were included, residual confounding might still be an issue, as in any observational study. For instance, the influence of parenting and other socio-ecological factors was not included in this study but could have confounded our results.[284] Second, although we corrected for BMI at sleep assessment, future research should further explore the possible mediating role of physical activity and adiposity in childhood in the association of early growth, sleep and 24-hour rhythms.

CONCLUSION

We observed that children with fetal and/or infant growth restriction showed better sleep quantity and quality at school-age. These findings may be indicative of an increased need for sleep in these children, which they may use for maturational processes and development after a more complex start in life.

Supplemental Table S1A. Correlations between fetal and infant growth measures

	Gestational age at birth	Birth weight	Birth weight SDS	EFW 2nd Trim	EFW 3rd Trim	Weight SDS 6M	Weight SDS 12M	Weight SDS 24M
Gestational age at birth	1.00	0.694**	0.069*	-0.075**	0.010	0.293**	0.207**	0.135**
Birth weight	0.694**	1.00	0.748**	0.171**	0.456**	0.457**	0.399**	0.348**
Birth weight SDS	0.069*	0.748**	1.00	0.276**	0.568**	0.386**	0.381**	0.373**
EFW 2nd Trim	-0.075**	0.171**	0.276**	1.00	0.479**	0.084**	0.135**	0.129**
EFW 3rd Trim	0.010	0.456**	0.568**	0.479**	1.00	0.236**	0.277**	0.272**
Weight SDS 6M	0.293**	0.457**	0.386**	0.084**	0.236**	1.00	0.841**	0.686**
Weight SDS 12M	0.207**	0.399**	0.381**	0.135**	0.277**	0.841**	1.00	0.832**
Weight SDS 24M	0.135**	0.348**	0.373**	0.129**	0.272**	0.686**	0.832**	1.00

*Abbreviations: SDS, standard deviation score; EFW, estimated fetal weight; trim, trimester; M, months. Correlations are shown as Pearson correlation coefficients. * P <0.05, ** P <0.01.*

Supplemental Table S1B. Correlations between sleep and 24-hour activity rhythm measures

	Sleep duration	Sleep efficiency	WASO	Social jetlag	Intradaily variability	Interdaily stability
Sleep duration	1.00	0.416**	-0.177**	-0.159**	-0.071**	0.176**
Sleep efficiency	0.416**	1.00	-0.961**	-0.003	-0.053	-0.013
WASO	-0.177*	-0.961**	1.00	-0.041	0.075**	0.058*
Social jetlag	-0.159**	-0.003	-0.041	1.00	-0.079**	-0.080**
Intradaily variability	0.071**	-0.053	0.075**	-0.079**	1.00	-0.134**
Interdaily stability	0.176**	-0.013	0.058*	-0.080**	-0.134**	1.00

*Abbreviations: WASO = wake after sleep onset (minutes). Correlations are shown as Pearson correlation coefficients. * P <0.05, ** P <0.01.*

Supplemenal Table S2. Associations of birth outcomes with childhood sleep and 24-hour activity rhythms (basic model)

		Difference (95% Confidence Interval) in SDS					
Birth outcomes	**n**	**Sleep duration**	**Sleep efficiency**	**WASO**	**Social jetlag**	**Intradaily variability**	**Interdaily stability**
Gestational age							
< 37 weeks	133	0.07 (-0.09;0.24)	0.03 (-0.15;0.21)	-0.08 (-0.26;0.09)	0.02 (-0.16;0.20)	**0.24 (0.06;0.42)***	-0.11 (-0.29;0.06)
37-41 weeks	863	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
>41 weeks	327	-0.03 (-0.14;0.09)	-0.00 (-0.13;0.12)	0.00 (-0.12;0.13)	0.05 (-0.08;0.17)	-0.06 (-0.19;0.06)	0.05 (-0.07;0.17)
Trend (per week)	1323	-0.01 (-0.03;0.01)	-0.01 (-0.03;0.01)	0.02 (-0.01;0.04)	-0.01 (-0.03;0.02)	**-0.02 (-0.05;-0.00)**	0.01 (-0.01;0.04)
Birth weight							
<2500 gr	93	0.14 (-0.05;0.33)	0.12 (-0.09;0.32)	-0.19 (-0.39;0.02)	0.08 (-0.12;0.29)	0.14 (-0.06;0.35)	-0.12 (-0.32;0.08)
2500-4250 gr	1135	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
>4250 gr	97	-0.03 (-0.22;0.16)	0.16 (-0.05;0.36)	-0.16 (-0.36;0.03)	-0.07 (-0.27;0.14)	0.09 (-0.11;0.29)	-0.05 (-0.25;0.15)
Trend (per 500gr)	1325	-0.03 (-0.07;0.01)	-0.02 (-0.06;0.03)	0.02 (-0.02;0.06)	**-0.04 (-0.09;-0.00)**	-0.02 (-0.06;0.02)	0.01 (-0.04;0.05)
Size for gestational age							
Small (<p10)	102	-0.04 (-0.22;0.15)	-0.02 (-0.22;0.18)	0.04 (-0.15;0.24)	**0.24 (0.04;0.44)***	0.08 (-0.11;0.28)	0.04 (-0.15;0.24)
Appropriate (p10-p90)	1054	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Large (>p90)	167	-0.05 (-0.19;0.10)	0.09 (-0.07;0.25)	-0.08 (-0.23;0.08)	0.01 (-0.15;0.17)	0.04 (-0.11;0.20)	0.02 (-0.14;0.17)
Trend (per SDS)	1323	-0.01 (-0.06;0.04)	0.03 (-0.03;0.08)	-0.03 (-0.08;0.02)	-0.05 (-0.10;0.01)	0.02 (-0.03;0.07)	0.01 (-0.04;0.06)

Abbreviations: SDS, standard deviation score; n, number; WASO, wake after sleep onset (minutes); gr, grams; p, percentile. Values are linear regression coefficients (95% confidence intervals) and reflect the change in sleep and 24-hour activity rhythm parameters per birth outcome. Models are adjusted for season and age at sleep assessment; child sex and ethnicity; maternal pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy; and breastfeeding at 2 months. Bold=p<0.05, bold=p<0.025.*

Supplemental Table S3. Associations of fetal and infant growth patterns with childhood sleep and 24-hour activity rhythms (basic model)

		Difference (95% Confidence Interval) in SDS					
Growth patterns	**n**	**Sleep duration**	**Sleep efficiency**	**WASO**	**Social jetlag**	**Intradaily variability**	**Interdaily stability**
Fetal growth deceleration							
Infant growth deceleration	30	**0.44 (0.10;0.77)***	**0.47 (0.11;0.84)***	**-0.44 (-0.79;-0.09)***	-0.04 (-0.40;0.31)	-0.09 (-0.45;0.28)	-0.19 (-0.54;0.16)
Infant normal growth	113	-0.01 (-0.20;0.19)	-0.07 (-0.29;0.15)	0.04 (-0.17;0.24)	0.05 (-0.16;0.26)	-0.03 (-0.24;0.18)	-0.19 (-0.40;0.01)
Infant growth acceleration	115	0.01 (-0.18;0.21)	0.16 (-0.06;0.37)	-0.15 (-0.36;0.05)	0.07 (-0.14;0.27)	-0.16 (-0.37;0.06)	0.13 (-0.08;0.33)
Fetal normal growth							
Infant growth deceleration	113	-0.05 (-0.24;0.15)	-0.01 (-0.22;0.21)	-0.06 (-0.26;0.15)	-0.05 (-0.26;0.16)	0.02 (-0.20;0.23)	0.01 (-0.20;0.21)
Infant normal growth	258	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Infant growth acceleration	104	-0.06 (-0.26;0.14)	-0.06 (-0.28;0.16)	0.02 (-0.20;0.23)	-0.02 (-0.24;0.19)	-0.00 (-0.22;0.22)	**-0.41 (-0.62;-0.20)***
Fetal growth acceleration							
Infant growth deceleration	163	-0.02 (-0.19;0.16)	-0.02 (-0.21;0.18)	0.01 (-0.17;0.20)	-0.02 (-0.21;0.16)	0.02 (-0.17;0.21)	-0.15 (-0.34;0.03)
Infant normal growth	164	-0.08 (-0.26;0.09)	0.09 (-0.10;0.28)	-0.13 (-0.31;0.06)	-0.03 (-0.22;0.15)	-0.13 (-0.31;0.06)	-0.12 (-0.30;0.06)
Infant growth acceleration	41	0.08 (-0.21;0.37)	0.15 (-0.17;0.47)	-0.12 (-0.43;0.18)	-0.05 (-0.36;0.26)	0.09 (-0.23;0.41)	-0.26 (-0.56;0.05)

Abbreviations: SDS, standard deviation score; n, number; WASO, wake after sleep onset (minutes). Values are linear regression coefficients (95% confidence intervals) and reflect the difference in sleep and 24-hour activity rhythm parameters compared to children with normal fetal and infant growth. Models are adjusted for season and age at sleep assessment; child sex and ethnicity; maternal pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy; and breastfeeding at 2 months. Bold=p<0.05, bold=p<0.025.*

Supplemental Table S4. Associations of birth outcomes with childhood sleep and 24-hour activity rhythms (BMI model)

		Difference (95% Confidence Interval) in SDS					
Birth outcomes	**n**	**Sleep duration**	**Sleep efficiency**	**WASO**	**Social jetlag**	**Intradaily variability**	**Interdaily stability**
Gestational age							
< 37 weeks	133	0.16 (-0.01;0.32)	0.07 (-0.11;0.26)	-0.10 (-0.28;0.08)	-0.03 (-0.21;0.15)	**0.19 (0.01;0.37)**	-0.11 (-0.29;0.06)
37-41 weeks	863	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
>41 weeks	327	-0.04 (-0.15;0.08)	0.01 (-0.12;0.13)	-0.01 (-0.13;0.11)	0.08 (-0.04;0.20)	-0.06 (-0.18;0.07)	0.07 (-0.05;0.19)
Trend (per week)	1323	-0.02 (-0.04;0.00)	-0.02 (-0.04;0.01)	0.02 (-0.00;0.05)	0.01 (-0.02;0.03)	-0.02 (-0.04;0.01)	0.02 (-0.01;0.04)
Birth weight							
<2500 gr	93	**0.25 (0.06;0.44)***	0.16 (-0.05;0.38)	-0.20 (-0.41;0.01)	0.02 (-0.19;0.23)	0.08 (-0.13;0.29)	-0.13 (-0.33;0.08)
2500-4250 gr	1135	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
>4250 gr	97	-0.00 (-0.19;0.19)	0.19 (-0.01;0.40)	-0.19 (-0.38;0.01)	-0.00 (-0.20;0.20)	0.08 (-0.12;0.28)	-0.03 (-0.22;0.16)
Trend (per 500gr)	1325	-0.04 (-0.08;0.00)	-0.01 (-0.06;0.03)	0.02 (-0.03;0.06)	-0.01 (-0.06;0.03)	-0.01 (-0.05;0.03)	0.02 (-0.02;0.06)
Size for gestational age							
Small (<p10)	102	-0.01 (-0.19;0.17)	-0.02 (-0.21;0.18)	0.05 (-0.14;0.24)	**0.22 (0.02;0.41)**	0.07 (-0.13;0.27)	0.03 (-0.16;0.22)
Appropriate (p10-p90)	1054	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Large (>p90)	167	-0.05 (-0.19;0.10)	0.07 (-0.09;0.23)	-0.05 (-0.21;0.10)	0.02 (-0.13;0.18)	0.04 (-0.12;0.20)	-0.02 (-0.17;0.14)
Trend (per SDS)	1323	-0.01 (-0.06;0.03)	0.02 (-0.03;0.07)	-0.02 (-0.07;0.03)	-0.04 (-0.09;0.02)	0.02 (-0.04;0.07)	0.00 (-0.05;0.05)

Abbreviations: SDS, standard deviation score; n, number; WASO, wake after sleep onset (minutes); gr, grams; p, percentile. Values are linear regression coefficients (95% confidence intervals) and reflect the change in sleep and 24-hour activity rhythm parameters per birth outcome. Models are adjusted for season and age at sleep assessment; child sex and ethnicity; maternal pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy; and breastfeeding at 2 months. Bold=p<0.05, bold=p<0.025.*

Supplemental Table S5. Associations of fetal and infant growth patterns with childhood sleep and 24-hour activity rhythms (BMI model)

		Difference (95% Confidence Interval) in SDS					
Growth patterns	**n**	**Sleep duration**	**Sleep efficiency**	**WASO**	**Social jetlag**	**Intradaily variability**	**Interdaily stability**
Fetal growth deceleration							
Infant growth deceleration	30	**0.38 (0.05;0.71)***	**0.44 (0.11;0.78)***	**-0.42 (-0.77;-0.08)***	-0.07 (-0.42;0.28)	-0.07 (-0.44;0.29)	-0.21 (-0.55;0.14)
Infant normal growth	113	-0.02 (-0.21;0.17)	-0.05 (-0.26;0.16)	0.01 (-0.19;0.22)	0.02 (-0.19;0.22)	-0.04 (-0.25;0.17)	-0.19 (-0.40;0.01)
Infant growth acceleration	115	0.01 (-0.19;0.20)	0.12 (-0.10;0.33)	-0.11 (-0.31;0.10)	0.03 (-0.18;0.23)	-0.16 (-0.37;0.05)	0.09 (-0.12;0.29)
Fetal normal growth							
Infant growth deceleration	113	-0.08 (-0.27;0.12)	-0.02 (-0.24;0.19)	-0.04 (-0.25;0.16)	-0.03 (-0.24;0.17)	0.03 (-0.18;0.24)	0.02 (-0.18;0.23)
Infant normal growth	258	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Infant growth acceleration	104	-0.07 (-0.27;0.13)	-0.05 (-0.27;0.17)	0.00 (-0.21;0.21)	-0.03 (-0.25;0.18)	0.03 (-0.19;0.25)	**-0.42 (-0.63;-0.21)***
Fetal growth acceleration							
Infant growth deceleration	163	-0.03 (-0.20;0.14)	-0.02 (-0.21;0.17)	0.01 (-0.17;0.19)	-0.03 (-0.21;0.15)	0.02 (-0.17;0.21)	-0.15 (-0.33;0.03)
Infant normal growth	164	-0.12 (-0.29;0.05)	0.04 (-0.15;0.23)	-0.08 (-0.26;0.10)	-0.03 (-0.22;0.15)	-0.11 (-0.30;0.08)	-0.14 (-0.32;0.04)
Infant growth acceleration	41	0.11 (-0.18;0.40)	0.12 (-0.20;0.44)	-0.08 (-0.38;0.23)	-0.08 (-0.39;0.22)	0.09 (-0.23;0.40)	-0.30 (-0.61;0.00)

Abbreviations: SDS, standard deviation score; n, number; WASO, wake after sleep onset (minutes). Values are linear regression coefficients (95% confidence intervals) and reflect the difference in sleep and 24-hour activity rhythm parameters compared to children with normal fetal and infant growth. Models are adjusted for season and age at sleep assessment; child sex and ethnicity; maternal pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy; and breastfeeding at 2 months. Bold=p<0.05, bold=p<0.025.*

Supplemental Table S6. Associations of birth outcomes with childhood sleep and 24-hour activity rhythms (confounder model, stratified per study visit)

	Difference (95% Confidence Interval) in SDS					
11-YEAR VISIT (n=862)	**Sleep duration**	**Sleep efficiency**	**WASO**	**Social jetlag**	**Intradaily variability**	**Interdaily stability**
Gestational age						
< 37 weeks	**0.18 (0.00;0.36)**	0.07 (-0.15;0.28)	-0.03 (-0.23;0.17)	-0.04 (-0.25;0.17)	0.19 (0.02;0.40)	-0.04 (-0.24;0.15)
37-41 weeks	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
>41 weeks	-0.03 (-0.16;0.09)	0.04 (-0.10;0.18)	-0.05 (-0.19;0.08)	0.02 (-0.12;0.16)	-0.10 (-0.24;0.04)	0.05 (-0.08;0.19)
Trend (per week)	-0.02 (-0.04;0.00)	0.00 (-0.03;0.03)	-0.01 (-0.03;0.02)	0.00 (-0.03;0.03)	-0.02 (-0.04;0.01)	0.02 (-0.01;0.04)
Birth weight						
<2500 gr	0.18 (-0.03;0.39)	0.10 (-0.15;0.35)	-0.06 (-0.29;0.17)	0.02 (-0.17;0.25)	0.02 (-0.22;0.27)	-0.08 (-0.30;0.15)
2500-4250 gr	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
>4250 gr	0.00 (-0.19;0.19)	**0.23 (0.01;0.45)**	**-0.24 (-0.45;-0.03)***	0.02 (-0.22;0.26)	0.03 (-0.19;0.25)	0.04 (-0.17;0.24)
Trend (per 500gr)	-0.04 (-0.08;0.01)	0.01 (-0.04;0.06)	-0.03 (-0.07;0.02)	0.01 (-0.04;0.05)	-0.00 (-0.05;0.05)	0.01 (-0.04;0.06)
Size for gestational age						
Small (<p10)	-0.03 (-0.20;0.17)	-0.06 (-0.29;0.18)	0.07 (-0.15;0.29)	0.04 (-0.19;0.27)	0.02 (-0.22;0.25)	**0.23 (0.02;0.45)**
Appropriate (p10-p90)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Large (>p90)	-0.01 (-0.23;0.17)	0.14 (-0.05;0.32)	-0.14 (-0.31;0.03)	0.06 (-0.12;0.24)	0.02 (-0.16;0.21)	0.04 (-0.12;0.21)
Trend (per SDS)	-0.02 (-0.07;0.04)	0.03 (-0.03;0.09)	-0.04 (-0.09;0.02)	0.01 (-0.05;0.07)	0.03 (-0.03;0.09)	-0.01 (-0.07;0.04)
14-YEAR VISIT (n=465)	**Sleep duration**	**Sleep efficiency**	**WASO**	**Social jetlag**	**Intradaily variability**	**Interdaily stability**
Gestational age						
< 37 weeks	0.14 (-0.19;0.47)	0.14 (-0.21;0.48)	-0.29 (-0.64;0.06)	-0.07 (-0.41;0.26)	0.16 (-0.18;0.49)	-0.23 (-0.57;0.11)
37-41 weeks	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
>41 weeks	0.00 (-0.23;0.23)	-0.02 (-0.26;0.22)	0.06 (-0.19;0.30)	0.17 (-0.06;0.41)	0.04 (-0.20;0.28)	0.11 (-0.13;0.35)
Trend (per week)	-0.02 (-0.07;0.02)	-0.05 (-0.09;0.00)	**0.08 (0.03;0.12)***	0.02 (-0.03;0.07)	-0.01 (-0.06;0.04)	0.02 (-0.03;0.07)

Supplemental Table S6. Continued

	Difference (95% Confidence Interval) in SDS					
Birth weight						
<2500 gr	0.37 (-0.01;0.75)	0.32 (-0.08;0.72)	**-0.51 (-0.92;-0.11)***	0.01 (-0.38;0.40)	0.17 (-0.22;0.57)	-0.19 (-0.59;0.21)
2500-4250 gr	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
>4250 gr	-0.06 (-0.47;0.37)	0.12 (-0.32;0.56)	-0.09 (-0.53;0.36)	-0.13 (-0.56;0.29)	0.24 (-0.20;0.67)	-0.13 (-0.56;0.31)
Trend (per 500gr)	-0.04 (-0.12;0.04)	-0.06 (-0.14;0.03)	**0.09 (0.01;0.18)**	-0.05 (-0.13;0.04)	-0.01 (-0.09;0.07)	0.04 (-0.04;0.13)
Size for gestational age						
Small (<p10)	0.01 (-0.35;0.36)	0.05 (-0.32;0.42)	-0.01 (-0.38;0.37)	**0.50 (0.15;0.86)***	0.12 (-0.24;0.47)	-0.27 (-0.63;0.10)
Appropriate (p10-p90)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Large (>p90)	-0.16 (-0.45;0.14)	-0.05 (-0.36;0.26)	0.09 (-0.23;0.40)	-0.08 (-0.38;0.22)	0.09 (-0.21;0.39)	-0.07 (-0.37;0.24)
Trend (per SDS)	-0.02 (-0.12;0.08)	0.00 (-0.10;0.10)	-0.00 (-0.11;0.10)	**-0.13 (-0.22;-0.03)***	0.01 (-0.09;0.11)	0.04 (-0.06;0.14)

Abbreviations: SDS, standard deviation score; n, number; WASO, wake after sleep onset (minutes); gr, grams; p, percentile. Values are linear regression coefficients (95% confidence intervals) and reflect the change in sleep and 24-hour activity rhythm parameters per birth outcome. Models are adjusted for season and age at sleep assessment; child sex and ethnicity; maternal pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy; and breastfeeding at 2 months. Bold=p<0.05, bold=p<0.025.*

Supplemental Table S7. Associations of fetal and infant growth patterns with childhood sleep and 24-hour activity rhythms (confounder model, stratified per study visit)

Growth patterns	Difference (95% Confidence Interval) in SDS					
11-YEAR VISIT (n=776)	**Sleep duration**	**Sleep efficiency**	**WASO**	**Social jetlag**	**Intradaily variability**	**Interdaily stability**
Fetal growth deceleration						
Infant growth deceleration	**0.51 (0.13;0.89)***	**0.46 (0.02;0.90)**	-0.34 (-0.76;0.07)	-0.17 (-0.59;0.25)	-0.02 (-0.46;0.41)	-0.00 (-0.40;0.39)
Infant normal growth	-0.10 (-0.30;0.11)	-0.09 (-0.33;0.15)	0.06 (-0.17;0.28)	0.06 (-0.17;0.29)	-0.03 (-0.27;0.20)	-0.21 (-0.42;0.01)
Infant growth acceleration	0.05 (-0.17;0.28)	0.06 (-0.21;0.32)	-0.03 (-0.28;0.21)	0.00 (-0.25;0.25)	-0.12 (-0.38;0.14)	0.09 (-0.15;0.32)
Fetal normal growth						
Infant growth deceleration	-0.03 (-0.24;0.18)	0.07 (-0.17;0.32)	-0.11 (-0.34;0.12)	-0.02 (-0.25;0.22)	-0.04 (-0.28;0.20)	-0.02 (-0.24;0.20)
Infant normal growth	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Infant growth acceleration	-0.07 (-0.28;0.14)	-0.01 (-0.26;0.23)	-0.01 (-0.24;0.22)	0.02 (-0.21;0.26)	0.14 (-0.10;0.38)	**-0.23 (-0.45;-0.01)**
Fetal growth acceleration						
Infant growth deceleration	-0.03 (-0.21;0.14)	-0.02 (-0.22;0.19)	0.01 (-0.18;0.21)	-0.01 (-0.21;0.18)	0.11 (-0.09;0.31)	-0.10 (-0.28;0.08)
Infant normal growth	**-0.19 (-0.37;-0.01)**	-0.05 (-0.26;0.16)	-0.01 (-0.20;0.19)	-0.01 (-0.21;0.19)	-0.11 (-0.31;0.10)	-0.16 (-0.35;0.03)
Infant growth acceleration	0.03 (-0.28;0.33)	0.02 (-0.34;0.37)	-0.00 (-0.34;0.33)	-0.06 (-0.40;0.28)	0.06 (-0.29;0.41)	**-0.45 (-0.77;-0.13)***
14-YEAR VISIT (n=324)	**Sleep duration**	**Sleep efficiency**	**WASO**	**Social jetlag**	**Intradaily variability**	**Interdaily stability**
Fetal growth deceleration						
Infant growth deceleration	0.17 (-0.47;0.81)	0.37 (-0.28;1.03)	-0.48 (-1.14;0.17)	0.02 (-0.63;0.68)	-0.18 (-0.85;0.50)	**-0.68 (-1.38;-0.01)**
Infant normal growth	0.18 (-0.26;0.63)	0.12 (-0.33;0.58)	-0.19 (-0.64;0.27)	-0.11 (-0.57;0.35)	-0.21 (-0.68;0.25)	-0.05 (-0.52;0.41)
Infant growth acceleration	-0.05 (-0.43;0.34)	0.30 (-0.09;0.69)	-0.32 (-0.71;0.07)	0.01 (-0.38;0.40)	-0.26 (-0.66;0.14)	0.07 (-0.33;0.48)
Fetal normal growth						
Infant growth deceleration	-0.06 (-0.46;0.35)	-0.16 (-0.58;0.25)	0.06 (-0.36;0.47)	-0.16 (-0.58;0.25)	0.06 (-0.37;0.48)	0.04 (-0.39;0.47)
Infant normal growth	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Infant growth acceleration	-0.04 (-0.50;0.43)	-0.07 (-0.54;0.40)	-0.05 (-0.52;0.43)	-0.07 (-0.54;0.40)	-0.42 (-0.91;0.07)	**-0.94 (-1.42;-0.45)***

Supplemental Table S7. Continued

Growth patterns	Difference (95% Confidence Interval) in SDS					
Fetal growth acceleration						
Infant growth deceleration	-0.13 (-0.56;0.30)	-0.05 (-0.49;0.39)	0.01 (-0.43;0.45)	-0.09 (-0.53;0.35)	-0.35 (-0.80;0.10)	-0.36 (-0.81;0.10)
Infant normal growth	0.06 (-0.35;0.46)	0.40 (-0.02;0.81)	-0.39 (-0.80;0.03)	-0.23 (-0.64;0.19)	-0.15 (-0.58;0.28)	-0.05 (-0.48;0.37)
Infant growth acceleration	0.12 (-0.52;0.76)	0.50 (-0.16;1.16)	-0.43 (-1.08;0.23)	-0.19 (-0.85;0.46)	0.08 (-0.60;0.76)	-0.18 (-0.50;0.86)

Abbreviations: SDS, standard deviation score; n, number; WASO, wake after sleep onset (minutes). Values are linear regression coefficients (95% confidence intervals) and reflect the difference in sleep and 24-hour activity rhythm parameters compared to children with normal fetal and infant growth. Models are adjusted for season and age at sleep assessment; child sex and ethnicity; maternal pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy; and breastfeeding at 2 months. Bold=p<0.05, bold=p<0.025.*

Supplemental Table S8. Associations of birth outcomes with childhood sleep (week and weekend days, confounder model)

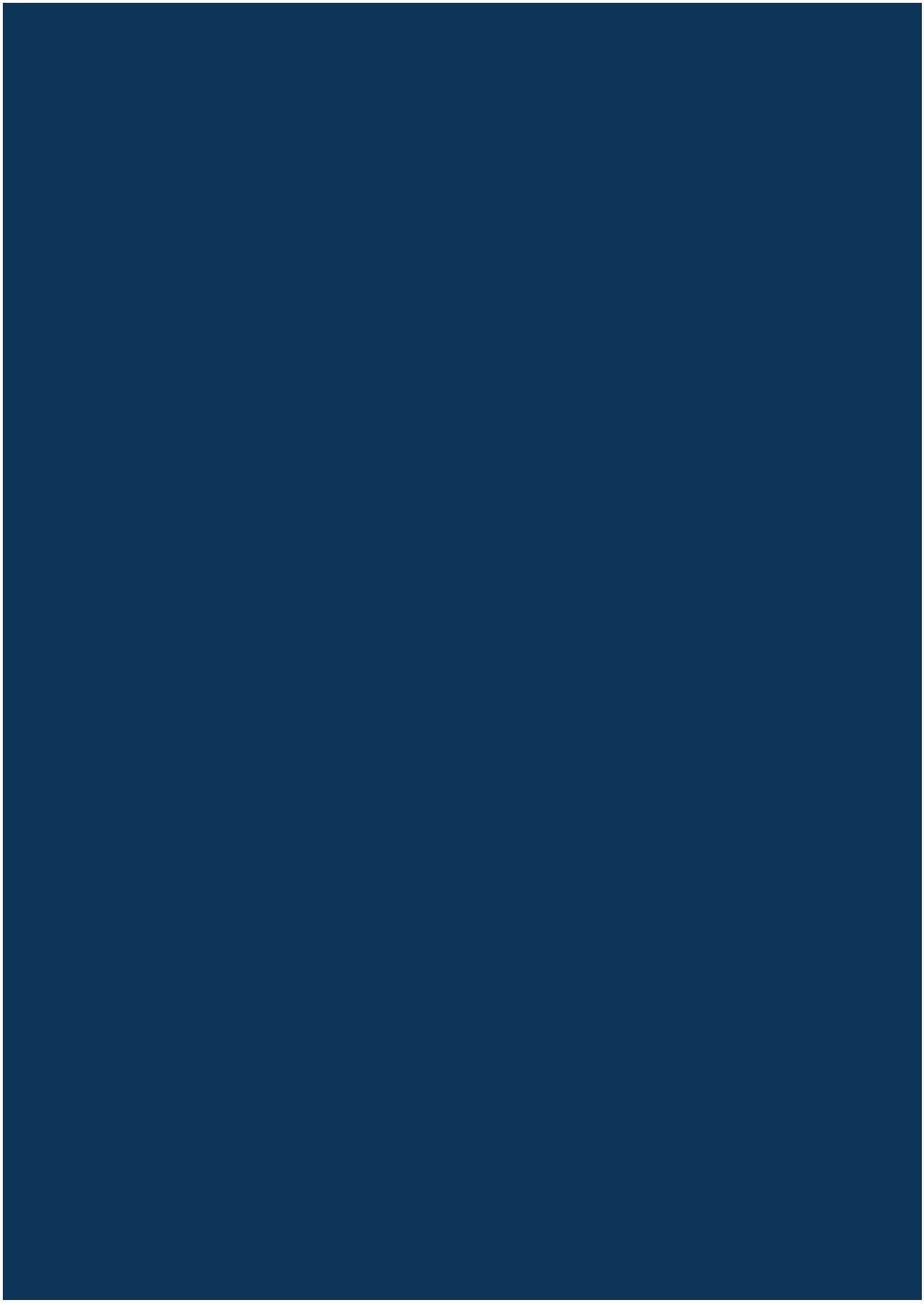
		Difference (95% Confidence Interval) in SDS		
Birth outcomes	**n**	**Sleep duration**	**Sleep efficiency**	**WASO**
Gestational age				
< 37 weeks	133	0.15 (-0.03;0.32)	0.08 (-0.10;0.27)	-0.08 (-0.25;0.10)
37-41 weeks	863	[Reference]	[Reference]	[Reference]
>41 weeks	327	-0.01 (-0.13;0.11)	0.02 (-0.10;0.15)	-0.02 (-0.14;0.10)
Trend (per week)	1323	-0.02 (-0.04;0.01)	-0.01 (-0.04;0.01)	0.01 (-0.01;0.04)
Birth weight				
<2500 gr	93	**0.25 (0.05;0.44)***	0.21 (-0.00;0.42)	-0.20 (-0.40;0.01)
2500-4250 gr	1135	[Reference]	[Reference]	[Reference]
>4250 gr	97	-0.09 (-0.28;0.10)	0.20 (-0.01;0.40)	**-0.21 (-0.39;-0.01)**
Trend (per 500gr)	1325	-0.04 (-0.08;0.00)	-0.00 (-0.05;0.04)	0.00 (-0.05;0.04)
Size for gestational age				
Small (<p10)	102	-0.01 (-0.20;0.17)	-0.05 (-0.24;0.15)	0.06 (-0.14;0.25)
Appropriate (p10-p90)	1054	[Reference]	[Reference]	[Reference]
Large (>p90)	167	-0.10 (-0.25;0.05)	0.11 (-0.05;0.27)	-0.11 (-0.26;0.05)
Trend (per SDS)	1323	-0.02 (-0.07;0.03)	0.03 (-0.02;0.08)	-0.04 (-0.09;0.02)

Abbreviations: SDS, standard deviation score; n, number; WASO, wake after sleep onset (minutes); gr, grams; p, percentile. Values are linear regression coefficients (95% confidence intervals) and reflect the change in sleep and 24-hour activity rhythm parameters per birth outcome. Models are adjusted for season and age at sleep assessment; child sex and ethnicity; maternal pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy; and breastfeeding at 2 months. Bold=p<0.05, bold=p<0.025.*

Supplemental Table S9. Associations of fetal and infant growth patterns with childhood sleep (week and weekend days, confounder model)

		Difference (95% Confidence Interval) in SDS		
Growth patterns	**n**	**Sleep duration**	**Sleep efficiency**	**WASO**
Fetal growth deceleration				
Infant growth deceleration	30	0.30 (-0.03;0.63)	**0.43 (0.08;0.78)***	**-0.42 (-0.71;-0.13)***
Infant normal growth	113	-0.02 (-0.22;0.17)	-0.05 (-0.26;0.16)	0.02 (-0.18;0.23)
Infant growth acceleration	115	0.06 (-0.14;0.25)	0.12 (-0.08;0.33)	-0.15 (-0.35;0.06)
Fetal normal growth				
Infant growth deceleration	113	-0.03 (-0.22;0.17)	-0.03 (-0.23;0.18)	-0.00 (-0.21;0.20)
Infant normal growth	258	[Reference]	[Reference]	[Reference]
Infant growth acceleration	104	-0.09 (-0.29;0.11)	-0.11 (-0.32;0.11)	0.09 (-0.12;0.30)
Fetal growth acceleration				
Infant growth deceleration	163	-0.02 (-0.19;0.15)	0.03 (-0.15;0.22)	-0.03 (-0.21;0.15)
Infant normal growth	164	-0.12 (-0.30;0.05)	0.07 (-0.12;0.25)	-0.13 (-0.31;0.05)
Infant growth acceleration	41	-0.02 (-0.31;0.27)	0.08 (-0.23;0.38)	-0.07 (-0.37;0.24)

Abbreviations: SDS, standard deviation score; n, number; WASO, wake after sleep onset (minutes). Values are linear regression coefficients (95% confidence intervals) and reflect the difference in sleep and 24-hour activity rhythm parameters compared to children with normal fetal and infant growth. Models are adjusted for season and age at sleep assessment; child sex and ethnicity; maternal pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy; and breastfeeding at 2 months. Bold=p<0.05, bold=p<0.025.*



CHAPTER 8

Sleep, 24-hour activity rhythms, and cardiometabolic risk factors in school-age children

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ABSTRACT

Study objectives: Disturbed sleep and 24-hour activity rhythms are linked to adverse cardiometabolic profiles in adults and adolescents, and these associations may originate in early life. We aimed to study associations of sleep and 24-hour rhythms with cardiometabolic risk factors in school-age children.

Methods: This cross-sectional population-based study comprised 894 children aged 8-11 years from the Generation R Study. Sleep (duration, efficiency, number of awakenings, time awake after sleep onset) and 24-hour activity rhythms (social jetlag, interdaily stability, intradaily variability) were assessed using tri-axial wrist actigraphy for 9 consecutive nights. Cardiometabolic risk factors included adiposity (body mass index Z-score, fat mass index using dual-energy-X-ray-absorptiometry, visceral fat mass and liver fat fraction using magnetic resonance imaging), blood pressure and blood markers (glucose, insulin, lipids). We adjusted for season, age, sociodemographics, and lifestyle factors.

Results: Each increase in interquartile range (IQR) of nightly awakenings (2 times) was associated with -0.12 SD (95% CI -0.21;-0.04) lower body mass index and 0.15 mmol/L (0.10;0.21) higher glucose. Among boys, an increase in IQR of intradaily variability (0.12) was associated with higher fat mass index (+0.07 kg/m2, 95% CI 0.03;0.11) and visceral FM (+0.08 gr, 0.02;0.15). We observed no associations with blood pressure or clustering of cardiometabolic risk factors.

Conclusions: Already at school-age, greater fragmentation of the 24-hour activity rhythm is associated with general and organ adiposity. In contrast, more nightly awakenings were associated with lower BMI. Future research should bring clarity to these disparate observations in order to create potential targets for obesity prevention programs.

INTRODUCTION

Disturbed sleep has been associated with cardiovascular morbidity and mortality in adulthood.[68,69] In recent years, an increasing number of studies has shown that not only sleep but also 24-hour activity rhythms, an indicator of the circadian organization of the sleep-wake cycle, are important for cardiometabolic health. For example, a late chronotype and social jetlag have been linked to obesity and elevated blood pressure in adults.[70] These associations may be explained by both behavior and neuroendocrine pathways.[285] Disrupted sleep or 24-hour rhythms may have adverse effects on diet choices and physical activity levels, and may directly or indirectly dysregulate the hypothalamic–pituitary–adrenal axis (HPA), resulting in a misbalance in energy intake and expenditure.[285,286]

Although previous studies mainly focused on adults, there is growing evidence suggesting that associations of sleep and 24-hour activity rhythms with cardiometabolic risk factors are already present earlier in life.[71,72,287] However, most pediatric studies used sleep diaries or questionnaires, instead of more direct measurements like actigraphy, or focused on only one sleep measure, mostly sleep duration.[217,288-290] Two recent actigraphic studies reported that shorter sleep duration and lower sleep efficiency in boys and girls, and late chronotype and social jet lag only in girls, were associated with less favorable cardiometabolic profiles such as higher fat mass index (FMI) and blood pressure in early adolescence.[291,292] The underlying mechanism for potential sex-specific effects are unknown but might be explained by developmental and hormonal differences. Identification of sleep and 24-hour activity rhythms associated with cardiovascular health earlier in childhood may help to improve future preventive strategies.

In this study, we aimed to assess the associations of actigraphically estimated sleep and 24-hour activity rhythms with cardiometabolic risk factors in children aged 8-11 years. We hypothesized that both disturbed sleep and 24-hour activity rhythms are associated with cardiometabolic risk factors from childhood onwards and that these associations are sex-specific.

MATERIAL AND METHODS

Study design and participants

This cross-sectional analysis was embedded in the Generation R Study, a prospective population-based cohort from early fetal life onward in Rotterdam, The Netherlands.[75] The study has been performed in accordance with the Declaration of Helsinki and has been approved by the Medical Ethical Committee of the Erasmus MC Rotterdam (MEC 198.782/2001/31). Written informed parental consent was obtained for all participants. Pregnant women living in Rotterdam with an expected delivery between April 2002 and January 2006 were eligible for study participation (61% included, n=9778). Between September 2015 and May 2017, a subsample of 1152 children who participated in the 10-year study visit was asked to participate in an actigraphy substudy on sleep, of which 952 consented (82%). In this subgroup, children born preterm were oversampled because of specific interest in the long term consequences of preterm birth. The children participating in the sleep subsample were more often of Dutch nationality and had mothers with higher educational levels. The study sample has been described previously in more detail.[264] We excluded children without data on weekday sleep or when night data did not pass standard quality control (<6 hours wear time, <4 hours detected sleep time).

Sleep and 24-hour activity rhythm measures

Sleep was assessed using a tri-axial wrist actigraph (GENEActiv; Activinsights, UK), as described previously.[264] In short, children wore the device for nine subsequent nights (five school nights and four weekend nights) on their non-dominant wrist. Additionally, each morning children filled out a sleep diary with questions about their previous night's sleep. No sleep measurements were performed during school holidays or within seven days after the start or end of daylight saving time. Actigraphs were set at a frequency of 50 Hz and raw sleep data were processed with the R-package GGIR using an algorithm with 5-s epochs.[270] All days with >16 hour wear time per 24 hours, as well as >6 hours nightly wear time and >4 hours detected sleep, were included in the analyses. This procedure generated the following sleep measures: 1) sleep duration (total duration of estimated sleep between sleep onset and final wakening in minutes); 2) sleep efficiency (percentage of time spent asleep between sleep onset and final waking time); 3) number of nightly awakenings (movement detected for at least five consecutive minutes between two sleep periods); and 4) wake after sleep onset (WASO, total time awake between sleep onset and final awakening). Additionally, we calculated 24-hour activity rhythm parameters: 5) social jetlag (average midpoint sleep during the weekend subtracted by the average midpoint sleep during the week, in hours), and 6) intradaily variability

(IV), which indicates the variability of the periods of rest and activity within a 24-hour time period and reflects the fragmentation of the 24-hour rhythm. IV ranges from 0 to 2 where higher scores indicate more fragmented and poorer rhythms. IV indicates *rhythm* fragmentation, so not only *sleep* fragmentation. Nightly awakenings are partly included in the rhythm fragmentation measure IV, because nighttime awakenings can only occur during the *night* part of the 24-hour day, where rhythm fragmentation (IV) indicates transitions throughout the *full* 24-hour period, and 7) interdaily stability (IS), which indicates the stability of the 24-hour activity rhythm across the days, therefore reflecting the similarity of the rhythm between the days with a value ranging from 0 to 1, where higher scores indicate more stable and better rhythms).[232-234] The variables IV and IS were selected as previous research described them as accurate non-parametric indicators of the 24-hour activity rhythm without assuming a waveform.[233,244,271] The exact formulae for calculating IV and IS have been described previously.[233,234] For homogeneity, we only used school days in our main analyses of the measures of sleep duration, sleep efficiency, awakenings, and WASO, to represent the typical pattern of weekday sleep and minimize the influence of atypical weekend events.[264] However, we did include weekend sleep in a separate sensitivity analysis to test the robustness of this assumption.

Cardiometabolic risk factors

At the 10-year visit, we measured height and weight without shoes or heavy clothing and calculated body mass index (BMI) with sex- and age-adjusted BMI Z-scores based on Dutch reference growth charts (Growth Analyzer 4.0 Dutch Growth Research Foundation).[268] We created BMI categories (underweight, normal, overweight, and obese) using the International Obesity Task Force cutoffs.[293] Body composition was measured with a dual-energy X-ray absorptiometry (DXA, 2008, GE-Lunar, Madison, WI, USA). FMI was calculated as total body fat mass (kilograms)/height(meters)^2. Liver fat was measured with a 3.0 Tesla MRI (Discovery MR750w, GE Healthcare, Milwaukee, WI, USA) using standard protocols, during a second center visit approximately one month later.[294,295] Liver fat fraction was determined by the average mean signal intensities from four samples of at least 4 cm^2 from the central portion of the hepatic volume.[295] Nonalcoholic fatty liver disease was defined as liver fat fraction ≥ 5.0%.

We measured blood pressure at the right brachial artery four times with 1-minute intervals using the automatic sphygmomanometer Datascope Accutor Plus (Paramus, NJ). We calculated the mean systolic and diastolic blood pressure from the last three measurements. Non-fasting venous blood samples were collected to measure insulin, glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides concentrations using the Coba 8000 analyzer (Roche, Almere, the

Netherlands). Concentrations of low-density lipoprotein (LDL) cholesterol were calculated using the Friedewald formula.[296] Clustering of cardiometabolic risk factors (yes/no) was defined following the definition of childhood metabolic syndrome phenotype as issued by the International Diabetes Federation and American Heart Association, which includes having three or four of the following risk factors: visceral fat mass >75th percentile, systolic or diastolic blood pressure >75th percentile, HDL-cholesterol <25th percentile or triglycerides >75th percentile, and insulin level >75th percentile.[297] Percentiles were based on the entire study population in which these measures were assessed at the 10-year visit.

Covariates

Potential confounders were selected based on previous studies.[217,288,291,298,299] In addition, gestational age at birth was included as children born preterm were oversampled. Sex of the child and gestational age at birth were obtained from medical records. Maternal education was based on highest attained educational level (low/middle or high). Child ethnicity was based on country of birth of the parents (Dutch or non-Dutch). Season of sleep assessment was defined as 'spring', 'summer', 'autumn', or 'winter'. We used parental questionnaires on the child's television viewing (hours per day), sports (0-2, 2-4 and >4 hours per week), and high sugar consumptions (0-1, 2, 3, 4, or >4 high-sugar snacks and/or drinks per day) at 10 years.[300]

Statistical analysis

First, we checked normality of all variables and assessed differences in characteristics by sex with Student *t* tests, Mann-Whitney U-tests, and chi-square tests. We checked the correlations between all sleep and 24-hour activity rhythm exposure variables and all cardiometabolic risk factors. Second, we used linear regression models to assess the associations of sleep and 24-hour activity rhythm measures (independent variables) with continuously measured cardiometabolic risk factors (BMI Z-score, FMI, visceral fat mass, liver fat fraction, systolic and diastolic blood pressure, glucose, insulin, total/HDL/LDL cholesterol, and triglycerides concentrations) as dependent variables. We used logistic regression models to assess the associations of sleep and 24-hour activity rhythm measures (independent variables) with the odds of being overweight and having clustering of cardiometabolic risk factors as dependent variables. We performed both full group and sex-stratified analyses, because previous studies showed associations between sleep and 24-hour rhythms and cardiometabolic risk factors differed by sex.[291,292] We checked whether covariates were associated with both the exposures and one of the main outcomes (BMI Z-score, FMI, systolic blood pressure), and/or changed the effect estimate >10% when added to the models. We used three models: a 'basic' model adjusted for season at sleep assessment, and child

ages at sleep and cardiometabolic assessments (as we did not consider these two variables as confounders); a main 'confounder' model which was additionally adjusted for potential nonmodifiable confounders sex, gestational age at birth, ethnicity and maternal education; and a 'lifestyle' mediator model in which we additionally adjusted for sports, television viewing, and high sugar intake as modifiable confounders or potential mediators. We examined the influence of lifestyle factors separately because these factors could be either confounders or mediators.

Residuals of the linear regression analyses were checked for independence**,** homoscedasticity, and normality. For comparison of effect estimates, we used IQRs (difference between 25th and 75th percentile) instead of absolute values for all seven sleep and 24-hour rhythm exposure variables. Because the distributions of FMI, visceral fat mass, liver fat fraction, and insulin and triglycerides concentrations were skewed, we used their natural logged values in all linear regression analyses. To take into account multiple testing using a Bonferroni correction, we present results based on statistical significance on p-value < 0.05 and p-value <0.017 (0.05/3; based on 3 main outcomes groups: body fat, blood pressure, and metabolic markers). We considered a Bonferroni correction based on all separate determinants and outcomes to be too strict because several exposures and outcomes were correlated. As sensitivity analyses, we first explored whether associations were different between children born full-term or preterm. Because we observed interactions between preterm birth (gestational age <37 weeks) and awakenings for liver fat fraction, and between preterm birth and IV for BMI Z-score, FMI, and liver fat fraction, we performed additional stratified analyses. Second, to test the robustness of associations, we reran the confounder models using combined weekend and weekday sleep. For all analyses, we used multiple imputations for missing covariates using the Markov chain Monte Carlo approach. The total amount of missing values was 3.4% and data were most commonly observed missing for the three lifestyle factors that were derived from questionnaires. The pattern of the missing values was assumed to be random. Five datasets were created and pooled results were reported. Statistical analysis was carried out using IBM SPSS Statistics, version 25.0 (IBM SPSS Statistics, Armonk, NY).

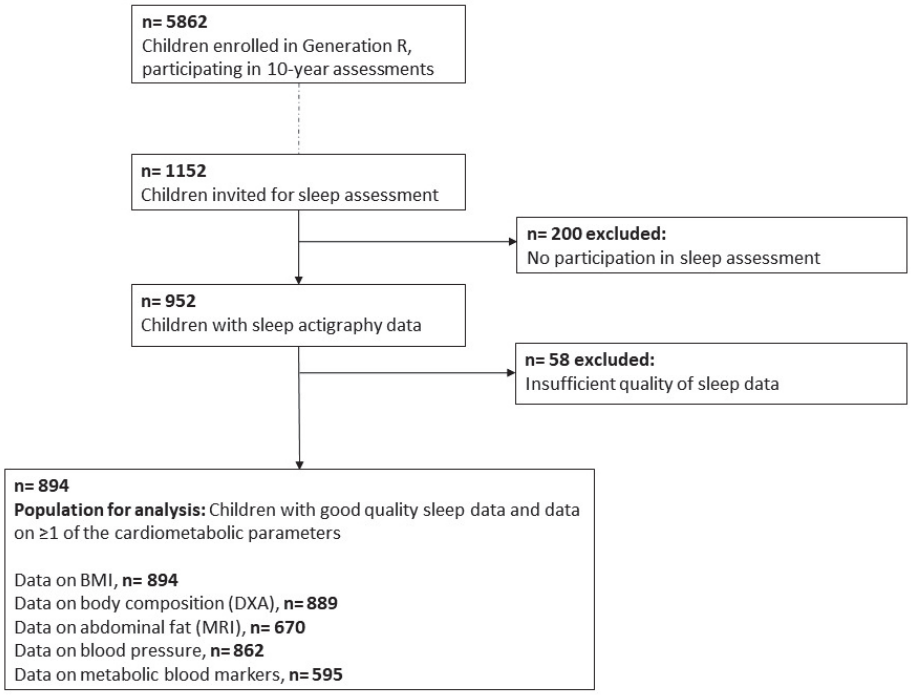
RESULTS

Subject characteristics

The final population for analysis of this study comprised 894 children (**Figure 1**). Ninety-six percent of the children wore the device 7 days or more. **Table 1** shows

that mean sleep duration (SD) was 7.7 (0.7) hours per day as measured by actigraphy, both on weekdays and weekends. Compared to boys, girls had a slightly longer sleep duration, higher sleep efficiency, fewer awakenings, larger social jetlag, and higher IV and IS (all p-value <0.05). The correlations between exposures and outcomes are presented in **Supplemental Table S1**.

Figure 1. Flowchart of the study population



Abbreviations: n, number; BMI, body mass index; DXA, dual-energy X-ray absorptiometry; MRI, magnetic resonance imaging.

Table 1. Baseline characteristics of the study population

	n	Total population (n=894)	Girls (n=471)	Boys (n=423)	p-value
Maternal characteristics					
Age intake (yrs), mean (SD)	894	32.2 (3.9)	32.2 (4.0)	32.3 (3.7)	0.240
Pre-pregnancy BMI (kg/m^2), median (IQR)	708	22.3 (20.8;24.7)	22.3 (20.7;24.4)	22.3 (20.9;24.8)	0.664
Education, n (%)	863				0.462
Low/middle		302 (35.0)	170 (33.7)	163 (36.4)	
High		561 (65.0)	301 (66.3)	260 (63.6)	
Child characteristics					
Sex (female), n (%)	894	471 (52.7)	471 (100.0)	0 (0.0)	1.00
Ethnicity, n (%)	893				0.552
Dutch		745 (83.4)	397 (84.5)	348 (82.3)	
Non-Dutch		148 (16.6)	74 (15.5)	75 (17.7)	
Gestational age at birth (wk), median (IQR)	894	40.1 (38.7;41.0)	40.1 (38.6;41.0)	40.1 (39.0;41.1)	0.223
Preterm birth (<37wks gestational age), n (%)	894	113 (12.6)	63 (13.4)	50 (11.8)	0.485
Birth weight (gr), mean (SD)	894	3411 (660)	3330 (676)	3502 (630)	0.198
Low birth weight (< 2500 grams, n (%)	894	81 (9.1)	48 (10.2)	33 (7.8)	0.214
Lifestyle factors					
Television viewing (hrs/day), median (IQR)	793	1.29 (0.93;1.93)	1.25 (0.89;1.89)	1.36 (0.93;1.96)	0.069
High sugar drinks and/or snacks (n/day)	677				0.125
0-1		129 (19.1)	69 (19.7)	60 (18.4)	
2		139 (20.5)	77 (21.9)	62 (19.0)	
3		141 (20.8)	64 (18.2)	77 (23.6)	
4		137 (20.2)	71 (20.2)	66 (20.2)	
>4		131 (19.4)	70 (20.0)	61 (18.8)	
Sports (hrs/wk)	770				**0.044**
0-2hrs/wk		218 (28.3)	131 (32.7)	7 (23.6)	
2-4hrs/wk		376 (48.8)	186 (46.4)	190 (51.5)	
>4hrs/wk		176 (22.9)	84 (20.9)	92 (24.9)	
Sleep					
Age at assessment (yrs), median (IQR)	894	11.7 (11.6;11.8)	11.7 (11.6;11.8)	11.7 (11.6;11.8)	0.992
Season of assessment, n (%)	892				0.954
Winter		306 (34.3)	163 (34.8)	143 (33.8)	
Spring		328 (36.8)	170 (36.2)	158 (37.4)	
Summer		215 (24.1)	112 (23.9)	103 (24.3)	
Fall		43 (4.8)	24 (5.1)	19 (4.5)	

Table 1. Continued

	n	Total population (n=894)	Girls (n=471)	Boys (n=423)	p-value
Actigraphy					
Sleep duration (min), mean (SD)	892	462 (43)	466 (43)	457 (44)	0.298
Sleep duration (hrs), mean (SD)	892	7.7 (0.7)	7.8 (0.7)	7.6 (0.7)	0.298
Sleep efficiency (%), mean (SD)	892	84.0 (5.0)	85.0 (4.6)	83.0 (4.7)	**0.005**
Nightly awakenings (n), mean (SD)	892	3.0 (1.5)	2.8 (1.4)	3.3 (1.6)	**0.009**
Wake after sleep onset (min), mean (SD)	892	88 (29)	83 (27)	94 (31)	0.172
Social jetlag (hrs), mean (SD)	891	0.90 (0.6)	1.03 (0.6)	0.76 (0.6)	**<0.001**
Sleep midpoint weekdays (time), mean (SD in min)	892	02:40 (37)	02:40 (39)	02:40 (35)	0.704
Sleep midpoint weekends (time), mean (SD in min)	893	03:34 (52)	03:41 (52)	03:26 (50)	0.267
Intradaily variability, mean (SD)	891	0.58 (0.09)	0.58 (0.09)	0.57 (0.09)	0.270
Interdaily stability, mean (SD)	891	0.18 (0.04)	0.18 (0.04)	0.17 (0.04)	0.165
Sleep diary					
Sleep duration (hrs), mean (SD)	839	9.6 (0.8)	9.6 (0.8)	9.6 (0.8)	0.777
Nightly awakenings (n), mean (SD)	853	0.6 (0.7)	0.6 (0.8)	0.5 (0.6)	0.194
Cardiometabolic outcomes					
Age at assessment (yrs), mean (SD)	894	9.8 (0.3)	9.8 (0.2)	9.8 (0.3)	0.565
BMI, median (IQR)	894	16.6 (15.5;18.0)	16.6 (15.5;18.0)	16.6 (15.6;18.0)	0.690
BMI Z-score, mean (SD)	894	0.09 (0.96)	0.02 (0.93)	0.17 (0.99)	**0.012**
BMI category, n (%)	894				0.684
Underweight		72 (8.0)	40 (8.4)	32 (7.6)	
Normal weight		727 (81.3)	378 (80.3)	349 (82.5)	
Overweight		81 (9.1)	47 (10.0)	34 (8.0)	
Obesity		14 (1.6)	6 (1.3)	8 (1.9)	
Fat mass index (kg/m^2), median (IQR)	889	3.97 (3.28;5.18)	4.35 (3.63;5.46)	3.64 (2.94;4.65)	**<0.001**
MRI visceral fat mass (gr), median (IQR)	606	370 (273;495)	385 (285;518)	355 (257;458)	**0.005**
MRI liver fat fraction (%), median (IQR)	670	1.9 (1.6;2.3)	2.0 (1.6;2.4)	1.9 (1.6;2.3)	0.134
Nonalcoholic fatty liver disease, n (%)	670	8 (1.2)	5 (1.4)	3 (0.9)	0.559
Systolic blood pressure (mm Hg), mean (SD)	862	102.8 (7.7)	103.2 (7.9)	102.2 (7.5)	0.188
Diastolic blood pressure (mm Hg), mean (SD)	862	57.8 (6.2)	58.5 (6.2)	57.0 (6.1)	0.983
Glucose (mmol/L), mean (SD)	595	5.4 (0.9)	5.3 (0.9)	5.4 (1.0)	0.785
Insulin (pmol/L), median (IQR)	595	186 (107;291)	180 (106;284)	194 (107;299)	0.800

Table 1. Continued

	n	Total population (n=894)	Girls (n=471)	Boys (n=423)	p-value
Total cholesterol (mmol/L), mean (SD)	595	4.3 (0.7)	4.4 (0.7)	4.2 (0.6)	0.171
HDL cholesterol (mmol/L), mean (SD)	595	1.5 (0.3)	1.5 (0.3)	1.5 (0.3)	0.183
LDL cholesterol (mmol/L), mean (SD)	593	2.3 (0.6)	2.4 (0.6)	2.2 (0.6)	0.725
Triglycerides (mmol/L), median (IQR)	593	0.9 (0.7;1.3)	0.9 (0.7;1.3)	0.9 (0.6;1.2)	0.384
Cardiometabolic risk clustering (score ≥ 3), n (%)	397	40 (10.1)	25 (11.5)	15 (8.4)	0.309

Abbreviations: n, number; SD, standard deviation; IQR, interquartile range; BMI, body mass index; yrs, years; gr, grams; wk, weeks; hrs, hours; DXA, dual-energy X-ray absorptiometry; MRI, magnetic resonance imaging; HDL, high-density-lipoprotein; LDL, low-density-lipoprotein.

Sleep, 24-hour activity rhythm and adiposity

Table 2 presents the results of the confounder models andshows that in the full group, each IQR increase in awakenings (2 times) was associated with -0.12 SD (95% Confidence Interval (CI) -0.21;-0.04) lower BMI. The associations of an IQR increase in sleep duration (59min) with BMI (-0.14 SD, 95% CI -0.27;-0.02) and log visceral fat mass (-0.08 gr, 95% CI -0.15;-0.01) were only present in girls but attenuated after correction for multiple testing. Sleep efficiency and WASO were not associated with any of the adiposity measures. As for 24-hour activity rhythm, each increase in IQR of IV (0.12) was associated with higher log FMI (0.07 kg/m^2, 95% CI 0.03;0.11) and log visceral fat mass (0.08 gr, 95% CI 0.02;0.15) among boys. Also, each increase in IQR of IV (0.12) was associated with higher odds of overweight (OR 1.58, 95% CI 1.03;2.43) (**Table 3**), but this association attenuated into nonsignificant after correction for multiple testing. Social jetlag and IS were not associated with adiposity.

Sleep, 24-hour activity rhythm and cardiometabolic risk factors

The associations of sleep and 24-hour activity rhythm with blood pressure, metabolic blood markers, and clustering of cardiometabolic risk factors are presented in **Table 3**, **Table 4**, and **Supplemental Table S2**. There were no robust associations between sleep or 24-hour activity rhythms and blood pressure. As for metabolic blood markers, each IQR increase in awakenings (2 times) was associated with 0.19 mmol/L (95% CI 0.04;0.35) higher glucose concentrations among boys. Among girls, an IQR increase in sleep duration (59min) was associated with lower odds of cardiometabolic clustering (OR 0.50, 95% CI 0.26;0.93), but this association attenuated after multiple testing correction.

Table 2. Associations of sleep and 24-hour rhythm measures with body fat

Total population (n=894)	**BMI Z-score**	**Log DXA fat mass index (kg/m²)**	**Log MRI visceral fat mass (gr)**	**Log MRI liver fat fraction (%)**
Sleep duration (per 59 min)	**-0.09 (-0.18;-0.00)**	**-0.03 (-0.06;-0.00)**	-0.04 (-0.09;0.01)	-0.02 (-0.06;0.01)
Sleep efficiency (per 7%)	0.05 (-0.03;0.13)	0.01 (-0.01;0.04)	0.03 (-0.02;0.07)	0.01 (-0.02;0.05)
Awakenings (per 2)	**-0.12 (-0.21;-0.04)***	-0.03 (-0.06;0.00)	-0.03 (-0.08;0.02)	-0.02 (-0.06;0.01)
WASO (per 39min)	-0.08 (-0.16;0.00)	-0.02 (-0.05;0.01)	-0.04 (-0.08;0.01)	-0.02 (-0.05;0.02)
Social jetlag (per 48 min)	0.04 (-0.04;0.12)	0.01 (-0.02;0.04)	-0.00 (-0.05;0.04)	0.02 (-0.01;0.06)
Intradaily variability (per 0.12)	**0.09 (0.01;0.17)**	**0.05 (0.02;0.07)***	**0.06 (0.02;0.11)***	0.02 (-0.01;0.05)
Interdaily stability (per 0.05)	-0.01 (-0.09;0.07)	-0.01 (-0.04;0.02)	0.02 (-0.03;0.07)	0.01 (-0.03;0.04)
Boys (n=423)	**BMI Z-score**	**Log DXA fat mass index (kg/m²)**	**Log MRI visceral fat mass (gr)**	**Log MRI liver fat fraction (%)**
Sleep duration (per 59 min)	-0.03 (-0.17;0.10)	-0.02 (-0.07;0.03)	0.00 (-0.07;0.08)	-0.01 (-0.06;0.04)
Sleep efficiency (per 7%)	0.07 (-0.05;0.18)	0.01 (-0.04;0.05)	0.03 (-0.04;0.10)	-0.01 (-0.06;0.04)
Awakenings (per 2)	**-0.14 (-0.26;-0.01)**	-0.02 (-0.06;0.02)	-0.01 (-0.09;0.06)	0.01 (-0.04;0.05)
WASO (per 39min)	-0.09 (-0.20;0.03)	-0.01 (-0.05;0.03)	-0.03 (-0.10;0.05)	0.01 (-0.04;0.06)
Social jetlag (per 48 min)	0.03 (-0.09;0.15)	0.01 (-0.04;0.05)	-0.01 (-0.07;0.06)	0.02 (-0.02;0.07)
Intradaily variability (per 0.12)	0.11 (-0.01;0.22)	**0.07 (0.03;0.11)***	**0.08 (0.02;0.15)***	0.02 (-0.02;0.07)
Interdaily stability (per 0.05)	-0.06 (-0.19;0.07)	-0.04 (-0.08;0.01)	-0.01 (-0.08;0.06)	-0.01 (-0.06;0.03)
Girls (n=471)	**BMI Z-score**	**Log DXA fat mass index (kg/m²)**	**Log MRI visceral fat mass (gr)**	**Log MRI liver fat fraction (%)**
Sleep duration (per 59 min)	**-0.14 (-0.27;-0.02)**	-0.04 (-0.08;-.00)	**-0.08 (-0.15;-0.01)**	-0.04 (-0.09;0.02)
Sleep efficiency (per 7%)	0.04 (-0.07;0.16)	0.03 (-0.01;0.07)	0.02 (-0.04;0.09)	0.03 (-0.02;0.08)
Awakenings (per 2)	-0.11 (-0.23;0.02)	-0.04 (-0.08;0.00)	-0.03 (-0.10;0.04)	-0.04 (-0.09;0.01)
WASO (per 39min)	-0.08 (-0.20;0.04)	-0.04 (-0.08;0.00)	-0.04 (-0.11;0.02)	-0.04 (-0.09;0.01)
Social jetlag (per 48 min)	0.05 (-0.07;0.16)	0.01 (-0.03;0.05)	0.00 (-0.06;0.07)	0.02 (-0.03;0.07)
Intradaily variability (per 0.12)	0.08 (-0.03;0.19)	0.03 (-0.01;0.06)	0.04 (-0.02;0.11)	0.02 (-0.03;0.07)
Interdaily stability (per 0.05)	0.04 (-0.07;0.15)	0.01 (-0.02;0.05)	0.05 (-0.01;0.11)	0.03 (0.00;0.05)

*Abbreviations: BMI, body mass index; kg, kilogram; m, meter, gr, grams; DXA, dual-energy X-ray absorptiometry; MRI, magnetic resonance imaging; min, minutes, WASO, wake after sleep onset. Values are regression coefficients (95% confidence interval) from multiple linear regression models and reflect the differences in body fat per interquartile range (difference between 25th and 75th percentile) in sleep duration, sleep efficiency, number of nightly awakenings, WASO, social jetlag, intradaily variability and interdaily stability. Results are from the 'confounder models' which are adjusted for season at sleep assessment, child ages at sleep and cardio-metabolic assessments, sex, gestational age, child ethnicity, and maternal education. Bold numbers have P-value <0.05; * P-value <0.017.*

Table 3. Associations of sleep and 24-hour rhythm measures with overweight and cardiometabolic clustering

Total population	**Overweight**	**Cardiometabolic clustering**
	OR (95% CI) n=894	**OR (95% CI) n=397**
Sleep duration (per 59 min)	1.00 (0.74;1.37)	0.79 (0.49;1.27)
Sleep efficiency (per 7%)	1.20 (0.89;1.61)	0.84 (0.54;1.32)
Awakenings (per 2)	0.79 (0.58;1.08)	1.07 (0.68;1.69)
WASO (per 39min)	0.81 (0.60;1.10)	1.17 (0.74;1.84)
Social jetlag (per 48 min)	1.12 (0.85;1.48)	0.95 (0.60;1.50)
Intradaily variability (per 0.12)	**1.41 (1.06;1.89)**	1.14 (0.71;1.83)
Interdaily stability (per 0.05)	0.96 (0.72;1.28)	1.51 (0.97;2.36)
Boys	**Overweight**	**Cardiometabolic clustering**
	OR (95% CI) n=423	**OR (95% CI) n=176**
Sleep duration (per 59 min)	1.17 (0.73;1.86)	1.73 (0.76;3.93)
Sleep efficiency (per 7%)	1.16 (0.76;1.76)	0.95 (0.45;1.99)
Awakenings (per 2)	0.77 (0.49;1.22)	1.47 (0.99;2.19)
WASO (per 39min)	0.86 (0.56;1.32)	1.20 (0.57;2.50)
Social jetlag (per 48 min)	1.22 (0.81;1.83)	0.83 (0.39;1.76)
Intradaily variability (per 0.12)	**1.58 (1.03;2.43)**	0.98 (0.46;2.08)
Interdaily stability (per 0.05)	0.79 (0.49;1.25)	1.64 (0.80;3.38)
Girls	**Overweight**	**Cardiometabolic clustering**
	OR (95% CI) n=471	**OR (95% CI) n=214**
Sleep duration (per 59 min)	0.86 (0.57;1.32)	**0.50 (0.26;0.93)**
Sleep efficiency (per 7%)	1.22 (0.80;1.86)	0.75 (0.42;1.35)
Awakenings (per 2)	0.84 (0.54;1.32)	0.96 (0.52;1.76)
WASO (per 39min)	0.78 (0.50;1.22)	1.22 (0.67;2.23)
Social jetlag (per 48 min)	1.09 (0.75;1.59)	1.16 (0.63;2.14)
Intradaily variability (per 0.12)	1.32 (0.88;2.00)	1.14 (0.59;2.21)
Interdaily stability (per 0.05)	1.09 (0.75;1.58)	1.63 (0.91;2.93)

*Abbreviations: OR, odds ratio; CI, confidence interval; n, number; min, minutes, WASO, wake after sleep onset. Values are odds ratios (95% confidence interval) from multiple logistic regression models and reflect the risk of having overweight/obesity as compared with having normal weight, and the risk of cardiometabolic clustering per interquartile range (difference between 25th and 75th percentile) in sleep duration, sleep efficiency, number of nightly awakenings, WASO, social jetlag, intradaily variability and interdaily stability. Cardiometabolic clustering was defined as having three or four risk factors (>75th percentile visceral fat mass, >75th percentile systolic or diastolic blood pressure, <25th percentile HDL cholesterol or >75th percentile triglycerides, and >75th percentile insulin. Results are from the 'confounder models' which are adjusted for season at sleep assessment, child ages at sleep and cardio-metabolic assessments, sex, gestational age, child ethnicity, and maternal education. Bold numbers have P-value <0.05; * P-value <0.017.*

Table 4. Associations of sleep and 24-hour rhythm measures with blood pressure and metabolic blood markers

Total population (n=594)	**Systolic blood pressure (mm Hg)**	**Diastolic blood pressure (mm Hg)**	**Glucose (mmol/L)**	**Log Insulin (pmol/L)**	**Total cholesterol (mmol/L)**
Sleep duration (per 59 min)	0.06 (-0.69;0.81)	0.57 (-0.03;1.16)	-0.02 (-0.13;0.09)	-0.07 (-0.15;0.02)	-0.00 (-0.08;0.08)
Sleep efficiency (per 7%)	0.23 (-0.45;0.91)	0.35 (-0.19;0.89)	-0.01 (-0.11;0.09)	-0.02 (-0.09;0.06)	-0.02 (-0.09;0.05)
Awakenings (per 2)	-0.11 (-0.83;0.61)	-0.15 (-0.72;0.42)	**0.15 (0.10;0.21)***	0.04 (-0.04;0.20)	0.03 (-0.01;0.07)
WASO (per 39min)	-0.26 (-0.96;0.44)	-0.27 (-0.83;0.28)	0.00 (-0.10;0.11)	0.00 (-0.08;0.08)	0.02 (-0.05;0.09)
Social jetlag (per 48 min)	0.11 (-0.59;0.80)	-0.40 (-0.95;0.15)	-0.08 (-0.18;0.03)	0.05 (-0.03;0.13)	0.03 (-0.04;0.10)
Intradaily variability (per 0.12)	-0.25 (-0.92;0.42)	-0.19 (-0.72;0.35)	-0.04 (-0.14;0.07)	0.01 (-0.08;0.09)	-0.01 (-0.09;0.06)
Interdaily stability (per 0.05)	0.04 (-0.65;0.73)	0.03 (0.52;0.58)	-0.06 (-0.16;0.05)	-0.02 (-0.10;0.07)	-0.00 (-0.08;0.07)
Boys (n=278)	**Systolic blood pressure (mm Hg)**	**Diastolic blood pressure (mm Hg)**	**Glucose (mmol/L)**	**Log Insulin (pmol/L)**	**Total cholesterol (mmol/L)**
Sleep duration (per 59 min)	0.03 (-1.02;1.08)	0.28 (-0.58;1.15)	-0.07 (-0.23;0.09)	-0.11 (-0.23;0.02)	0.00 (-0.10;0.11)
Sleep efficiency (per 7%)	-0.14 (-1.06;0.78)	-0.05 (-0.80;0.70)	-0.01 (-0.15;0.13)	-0.05 (-0.16;0.06)	-0.02 (-0.11;0.08)
Awakenings (per 2)	0.32 (-0.65;1.29)	0.11 (-0.68;0.90)	**0.19 (0.04;0.35)***	0.05 (-0.07;0.17)	0.04 (-0.07;0.14)
WASO (per 39min)	0.14 (-0.80;1.08)	0.16 (-0.61;0.92)	-0.01 (-0.15;0.14)	0.04 (-0.08;0.15)	0.02 (-0.08;0.11)
Social jetlag (per 48 min)	0.16 (-0.81;1.12)	0.33 (-0.46;1.13)	-0.12 (-0.28;0.04)	0.04 (-0.08;0.16)	0.04 (-0.06;0.15)
Intradaily variability (per 0.12)	0.51 (-0.42;1.45)	0.35 (-0.41;1.12)	-0.06 (-0.22;0.10)	0.02 (-0.11;0.14)	-0.01 (-0.12;0.09)
Interdaily stability (per 0.05)	-0.54 (-1.54;0.47)	-0.35 (-1.18;0.47)	0.04 (-0.12;0.21)	0.00 (-0.06;0.07)	0.02 (-0.03;0.08)
Girls (n=316)	**Systolic blood pressure (mm Hg)**	**Diastolic blood pressure (mm Hg)**	**Glucose (mmol/L)**	**Log Insulin (pmol/L)**	**Total cholesterol (mmol/L)**
Sleep duration (per 59 min)	0.19 (-0.88;1.25)	**0.90 (0.07;1.72)**	0.02 (-0.14;0.17)	-0.02 (-0.14;0.10)	0.00 (-0.11;0.12)
Sleep efficiency (per 7%)	0.76 (-0.25;1.77)	**0.83 (0.05;1.62)**	-0.00 (-0.14;0.13)	0.03 (-0.08;0.14)	-0.01 (-0.12;0.10)
Awakenings (per 2)	-0.61 (-1.68;0.45)	-0.41 (-1.24;0.42)	0.11 (-0.04;0.26)	0.04 (-0.08;0.15)	0.02 (-0.09;0.13)
WASO (per 39min)	-0.82 (-1.87;0.22)	-0.78 (-1.59;0.03)	0.01 (-0.14;0.16)	-0.04 (-0.15;0.08)	0.01 (-0.10;0.12)
Social jetlag (per 48 min)	0.12 (-0.87;1.11)	-1.06 (-1.82;0.30)	-0.03 (-0.17;0.11)	0.07 (-0.04;0.17)	0.02 (-0.08;0.12)
Intradaily variability (per 0.12)	-1.04 (-2.01;0.08)	-0.72 (-1.48;0.03)	-0.02 (-0.16;0.13)	0.00 (-0.11;0.12)	-0.03 (-0.14;0.08)
Interdaily stability (per 0.05)	0.58 (-0.37;1.53)	0.42 (-0.32;1.16)	-0.13 (-0.27;0.00)	-0.03 (-0.14;0.07)	-0.03 (-0.13;0.07)

*Abbreviations: n, number; min, minutes, WASO, wake after sleep onset. Values are regression coefficients (95% confidence interval) from multiple linear regression models and reflect the differences for blood pressure and metabolic blood markers per interquartile range (difference between 25th and 75th percentile) in sleep duration, sleep efficiency, number of nightly awakenings, WASO, social jetlag, intradaily variability and interdaily stability. Results are from the 'confounder models' which are adjusted for season at sleep assessment, child ages at sleep and cardio-metabolic assessments, sex, gestational age, child ethnicity, and maternal education. Bold numbers have P-value <0.05; * P-value <0.017.*

Results from all basic models are presented in **Supplemental Tables S3** and **S4**. **Supplemental Table S5** shows thatthe associations of awakenings with BMI and glucose, and of IV with FMI and visceral fat mass were independent of lifestyle factors.

Sensitivity analyses

The associations of awakenings and IV with adiposity measures appeared to be stronger in children born preterm than in those born at term, but small sample size likely hampered reaching statistical significance in the preterm group (**Supplemental Table S6**). Results were very similar when we included weekend sleep in the sleep measures (**Supplemental Table S7**).

DISCUSSION

In this large population-based cross-sectional study we observed that nightly awakenings and intradaily variability of the 24-hour activity rhythm were associated with body fat and glucose concentrations already at school age. The associations of fewer awakenings with lower glucose, and higher IV with higher FMI and visceral fat mass, were only prevalent among boys. Sleep and 24-hour rhythm patterns were not associated with other cardiometabolic risk factors.

For sleep measures, we observed associations of more awakenings with higher glucose concentrations, but with lower BMI. Although there are very few studies that included nightly awakenings to make comparisons, the observed association with BMI was in the opposite direction to that expected and compared to a recent actigraphic study in 676 children aged 5-6 years in Poland.[301] These conflicting results might be explained by the different type and location of actigraph used (ActiGraph on hip), younger age, or higher prevalence of overweight. Moreover, the number of awakenings in our study may be overestimated: actigraphs are known to have poor specificity to detect wake periods, because data on sleep/wake are based on movement only.[247,248] Therefore, we chose to only assess periods containing at least 5 consecutive minutes of movement as an awakening. However, the observed negative association between awakenings and BMI might still (partly) be driven by increased physical movement during sleep rather than actual sleep disruptions. This may explain why we, similar to previous pediatric studies, did not observe any association between WASO (minutes) and adiposity.[244,291] Recent studies proposed including parameters of nightly movement in pediatric sleep actigraphy studies, such as mean activity count or activity index.[302] The observed discrepancy in association of more nightly awakenings with lower BMI but higher glucose concentrations is

remarkable and needs further investigation. We observed suggestive evidence, which attenuated after multiple testing adjustment, for the associations of sleep duration with BMI and visceral fat mass among girls. This finding is consistent with previously reported associations of shorter sleep duration with increased BMI and FMI (DXA) in adolescents of both sexes.[289,291,303] Yet, associations are not as strong as reported in adults, suggesting the association of sleep duration with adiposity might become stronger at older ages. Further follow-up studies are needed to assess this difference between childhood and adolescence and between sexes.

For 24-hour activity rhythms, we observed positive associations of IV, but not IS, with FMI and visceral fat mass among boys. Previous studies linked higher IV to obesity in adolescents and increased mortality risk in adults.[304,305] A previous study among 94 Dutch children aged 2-18 years reported no associations of IV or IS with BMI; however, sample size was small.[244] Because IV is a measure of fragmentation of the 24-hour activity rhythm, our findings may imply that at school age adiposity in boys could benefit from more regular 24-hour rhythms. However, from a clinical point of view, observed effect estimates were small. Future studies should explore the underlying mechanism of the observed sex-differences, as well as potential intervention methods to increase regularity of the 24-hour activity rhythm. In contrast to a recent large study in children aged 13-14 years, we did not find an association of social jetlag with cardiometabolic risk factors.[292] Although the amount of social jetlag was very similar in both studies, our null findings might be a result of the younger age of the study population; the cardiometabolic effects of social jetlag may become more prominent during adolescence.[272] We did not find any associations of sleep or 24-hour activity rhythms with blood pressure, blood lipids and cardiometabolic clustering. This is in contrast with other actigraphic studies in school-aged children, and even more so in adolescents.[291,303,306-309] Possible explanations for the lack of associations in our study include our relatively young and healthy study population, smaller sample size for metabolic blood markers, and set of confounders included.

The observational and cross-sectional nature of our study precludes any causal or mechanistic conclusion. The associations were largely independent from lifestyle related factors, suggesting that other pathways are involved. Previous studies suggest that the mechanisms underlying the associations of sleep and 24-hour rhythms patterns with cardiometabolic health are not fully understood but may include neuroendocrine, behavioral and genetic pathways.[285] Suboptimal sleep duration, sleep quality and 24-hour rhythms might dysregulate the HPA-axis, resulting in systemic inflammation, endothelial dysfunction, decreased fat oxidation, and abnormalities in the autonomic nervous system.[285,310,311] The observed sex differences

might be explained by developmental differences between boys and girls of the same age (e.g. more/less sedentary behavior) or by sex hormones.[292,312] The observed trend of stronger associations in children born preterm could be related to differences in body composition, activity level, and sleep characteristics in this group, [211,222] but further research with larger study samples is needed.

Further follow-up studies are needed to disentangle the direction, causality, and underlying mechanisms of the associations observed. Our findings suggest that 24-hour activity rhythm may be an important determinant of cardiometabolic health in childhood, independent of other obesity-related lifestyle factors, and might be a target for future strategies for prevention of obesity later in life.

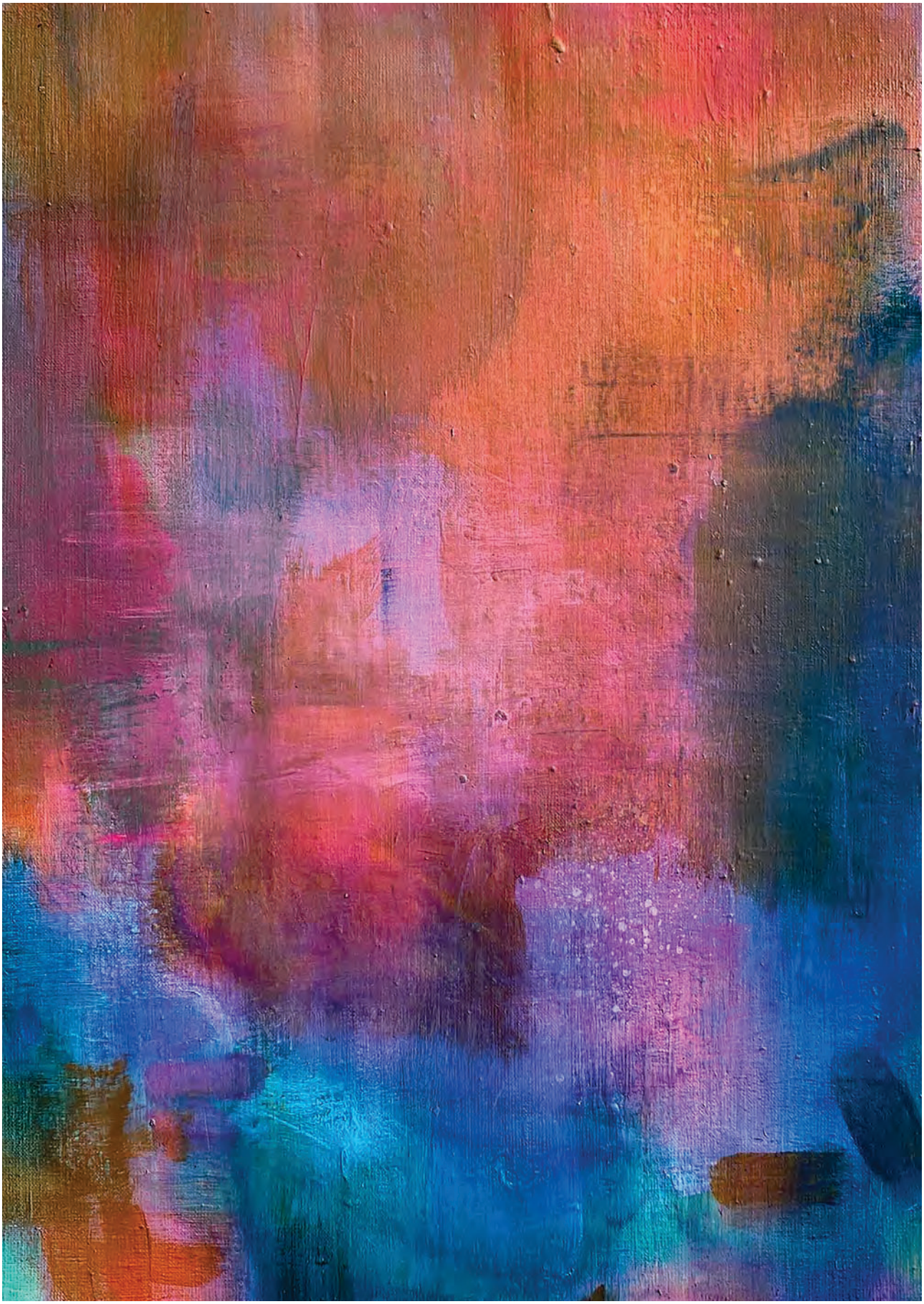
Strengths and limitations

To our knowledge, this is one of the largest studies to investigate associations of multiple sleep and 24-hour activity rhythm parameters with an extensive set of accurately measured adiposity and cardiometabolic risk factors in school-age children. We used actigraphy, a reliable, practical, and non-invasive method to estimate objective sleep in children.[247] Furthermore, this study appears to be the first on this subject to include visceral and liver fat measured with MRI as well as IV and IS as novel parameters of 24-hour activity rhythms. This study also has some limitations. First, our study population consisted of mostly Dutch children from high socio-economic backgrounds, with relatively good sleep (quality) and a low prevalence of overweight and clustering of cardiometabolic risk factors. This might have limited statistical power to detect significant associations for these outcomes and may have affected the generalizability of our findings. Second, due to study logistics, assessment of sleep and 24-hour rhythm variables took place approximately 1.5 year after the cardiometabolic risk factors were measured. However, previous studies suggest that sleep and cardiometabolic risk factors such as body composition are relatively stable in school-age children (6-12 years) until the onset of puberty (13-17 years)[249,313,314]. As all participants were <12 years old and almost all of White ethnicity, we assume that similar sleep patterns were present when the cardiometabolic risk factors were measured. In addition, we corrected for age at both sleep and cardiometabolic assessment. Third, we did not include sleep apnea as a possible confounder, but the prevalence of apnea-related symptoms (breathing difficulties/stops or frequent snoring) in our cohort was very low. Finally, as in any observational study, residual confounding might be an issue.

CONCLUSION

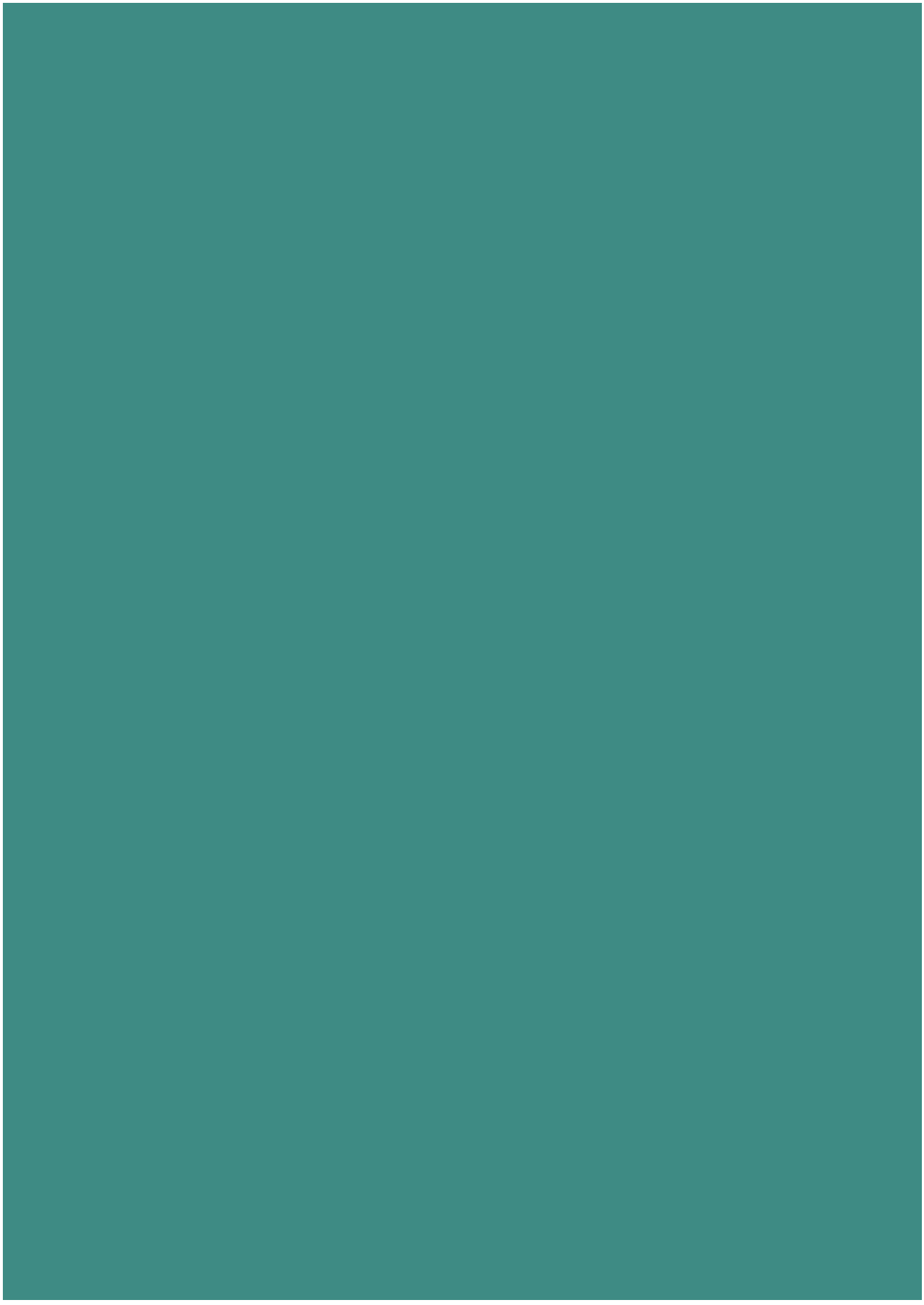
Out of seven sleep and 24-hour rhythm variables and multiple cardiometabolic risk factors studied, we observed that not so much sleep, but rather two specific 24-hour activity rhythm measures (i.e. higher rhythm fragmentation and less nightly awakenings) are associated with general and organ adiposity in children of school age. Although our findings on nightly awakenings are conflicting and need further research, our findings suggest that obesity prevention later in life may benefit from optimizing 24-hour rhythms from childhood onwards. However, follow-up studies are needed to assess the direction and causality of the observed associations.

8



PART IV

Discussion and summary



CHAPTER 9

GENERAL DISCUSSION

INTRODUCTION

Early life environmental factors influence early development and growth, affecting lifelong health. Perinatal and infant factors such as preterm birth, low birth weight and postnatal growth restriction have been linked to adverse cognitive (e.g. poorer academic achievement) and cardiometabolic outcomes in adulthood.[12,13] Adverse cardiometabolic health may include increased adiposity or obesity, hypertension, dyslipidemia, diabetes, or metabolic syndrome. With the increasing burden of cardiometabolic disease on healthcare systems worldwide, it is important to identify those at high risk as early as possible and to develop appropriate interventions. From a practical point of view, a better understanding of the course, related risk factors and long-term consequences of adverse early development of children born preterm and full-term would enable us to improve pediatric follow-up programs and standards of care.

In this thesis, we presented seven research projects within three important domains of child development: 1) early brain growth and neurodevelopment, 2) early body growth, body composition and cardiometabolic health, and 3) sleep and 24-hour rhythms. The main aims of this thesis were to better understand the etiological and contributing factors to these three domains, and to evaluate the potential contribution of several (novel) monitoring techniques. In this chapter, I will discuss our findings and address critical methodological considerations of the studies involved. Furthermore, I will deliberate on clinical implications and future studies to be conducted on these topics, as well as on how current neonatal follow-up programs could be optimized.

Detection of children born preterm at risk of adverse development

In The Netherlands, almost all children born very preterm (<32 weeks gestation) who were admitted to a neonatal intensive care unit (NICU) are invited for follow-up visits at the academic level 3 or 4 hospital of birth.[29] Due to the large number of (lengthy) tests included, these follow-up visits are quite intensive yet still not complete in covering all developmental domains which could be affected by preterm birth. Among others, visuospatial processing, socioemotional functioning, and quality of life are not routinely included. Therefore, the challenge lies in designing a neonatal follow-up program that is both complete and cost- and time-efficient for healthcare and valuable for the patients and their families. Within this thesis, we investigated multiple promising screening methods that could be useful in detecting preterm born children at risk of developmental impairment.

INTERPRETATION OF MAIN FINDINGS

Neurodevelopment

Children born very preterm have an increased risk of neurodevelopmental impairment, of which adverse early brain growth is regarded as an important predictor.[11,42-44] Therefore, monitoring early brain growth is important, and requires reliable and clinically applicable markers. In **Chapter 2,** we examined the predictive value and added clinical utility of two early cranial ultrasound (CUS) markers, corpus callosum (CC) length and corpus callosum-fastigium (CCF) length, with neurodevelopmental outcome in preterm infants without brain injury.[315] As CCF length covers a larger part of the brain than CC length and has been proven a reliable marker of brain growth,[82,83] we hypothesized that it would benefit the prediction of neurodevelopment. As both markers can be measured both prenatally and postnatally, even into adulthood, they could be highly suitable for serial follow-up. We observed that most strongly CC length and growth, but also CCF length, at 2 months were associated with cognitive, motor and/or language outcome at 2 years age (CA). However, prediction of neurodevelopmental outcome based on neonatal risk factors and head circumference, only significantly improved when CC length was additionally taken into account. Adding CCF length did not improve the prediction model. A possible explanation could be that CCF length does not incorporate the cerebellum and the corpus callosum itself, the largest white matter structure in the brain.[90,91]

Our findings add to a previous study in preterm infants, which stated that measuring CC length by CUS in early life had no additional clinical value.[80] At this stage, further research into the practical clinical value is needed before we can recommend CC or CCF length to be included in routine cranial ultrasound protocols for infants born preterm. Such future studies should explore combining different CUS brain markers (e.g. CC length, CCF length, ventricular size, biparietal diameter, and cerebellar width and vermis length) into CUS injury scores (comparable to e.g. Kidokoro scores on MRI imaging) to predict neurodevelopmental outcome.[98,316-320] Another interesting addition to current ultrasonic practices for monitoring brain growth could be the addition of temporal and mastoid windows to include measurements in the posterior fossa such as the cerebellum.[321-323] Furthermore, the role of MRI in the follow-up of very preterm infants is still under debate but could further improve prediction of neurodevelopmental outcome.[324-327] For example, increasing evidence links cranial and cerebellar volume to later neurodevelopment.[90,328-331] Also, as the quality of prenatal ultrasonic 3D imaging techniques continues to improve, further exploring the opportunities of 3D ultrasound imaging in the postnatal period, e.g. to measure volumes of the brain or separate structures like the corpus callosum, is warranted.[81,332,333]

Although early brain growth is an important predictor of later neurodevelopment, the actual neurological outcome or phenotype of each individual infant who suffered from early brain damage remains difficult to predict. In addition, it takes some time before impaired neurodevelopment becomes apparent ('growing into deficit') and can be tested reliably. Therefore, also after infancy early detection of probable neurodevelopmental impairment with quick and easy tests remains important. Early detection may allow for timely interventions and individualized follow-up trajectories to prevent further delay. CUS is generally not possible after the age of 6 months CA due to the closing of the anterior fontanelle, so other screening techniques are needed. In **Chapter 3,** we investigated another potential predictive screening instrument of later neurodevelopment: a quantitative eye tracking-based method developed to nonverbally assess visuospatial attention and processing in children aged 1 year or older.[49-51,89,116] Visuospatial attention and processing are vital functions that develop early in life and are considered conditional factors for broader neurodevelopment.[112,113] We showed that visuospatial attention and motion-processing function at 1 year is a predictive factor for overall cognitive and motor development 1 year later. In our study, its individual explanatory values (R^2) for cognitive and motor outcome were similar to or even higher than explanatory values of known important neonatal risk factors such as sex, gestational age, BPD or parental education. This suggests that a quick and easy eye-tracking-based assessment can help to identify preterm children at risk of adverse neurodevelopment. Although follow-up studies at older ages are needed, it could be a valuable addition to neonatal follow-up programs in the future.

An interesting question is what the best way would be to implement this new eye-tracking tool into neonatal follow-up programs. Apart from being a predictive factor for later *overall* neurodevelopment, eye-tracking assessment can also be used as a specific screening method for visual (spatial) dysfunction. This may be especially valuable as the prevalence of (cerebral) visuospatial impairment in infants born very preterm is high (20-45%), especially in those who suffered brain injury, and testing of visuospatial function is currently not included in neonatal follow-up programs.[118,334] Another option is to use eye-tracking as an extension to the current follow-up program in order to make it more personalized (and eventually more budget-friendly). For example, preterm infants with good scores on the eye-tracking test, neurological examination and developmental tests/questionnaires may be invited back less frequently. Alternatively, the quick and easy eye-tracking tool could be used in preterm infants who are *not* yet included in the specialized follow-up programs at level 3 or 4 NICU centers based on current cut-offs. In case of signs of visuospatial impairment on the eye-tracking test, more extensive developmental testing or more intensive follow-up could be offered.

Both studies in **Chapter 2** and **Chapter 3** linked early screening methods with various domains of neurodevelopment at the age of 2 years CA. We used the Bayley Scales of Infant and Toddler Development-Third edition (Bayley-III, Dutch edition: Bayley-III-NL) for cognitive and motor outcome,[32] the Lexi list for language outcome,[33] and the Child Behavior Checklist for 1.5-5 years (CBCL) for behavior problems.[34] Whereas CC length on CUS at 2 months was associated with language outcome at 2 years, CCF length at 2 months and visuospatial function at 1 year were not. In addition, none of the brain markers (CC or CCF length/growth) or eye-tracking parameters were related to behavior outcome, as measured by the total problem scale of the CBCL. These findings may reflect the complex and multifactorial origin of language and behavior, which may need more extensive testing than a parent-report, based questionnaire only.[335] Another important explanation is the young age of the participants, as many developmental disorders only become apparent at later ages. The CBCL used is a screening questionnaire which roughly estimates problem behavior but is not suitable for diagnosis, given its questionable predictive value for later pathology as classified by the Diagnostic and Statistical Manual of Mental Disorders.[140,143] Therefore, it would be interesting to follow behavioral performance up to a later age, ideally into adulthood. Likewise, the predictive value of early cognitive testing using the Bayley-III for later IQ performance is limited due to the young age of testing.[48,106,107] A recent study investigated the ability of the Bayley-III at 2 years to predict performance on the Wechsler Intelligence Scale for Children—Fourth Edition (WISC-IV) at 6.5 years CA in extremely preterm born children.[336] They observed that sensitivity of the Bayley-III to detect children with IQ<70 on the WISC-IV 4 years later was only 18% when a cut-off of <70 was used for the Bayley cognitive index score, and 44% with a cut-off of <85. Therefore, the associations described in **Chapter 2** and **Chapter 3** should be studied at later ages (e.g. at 5 and/or 8 years CA, when the Wechsler Preschool and Primary Scale of Intelligence (WPPSI), WISC and Movement-ABC could be used). Lastly, as no associations were observed between visuospatial attention and processing at 2 years and neurodevelopmental outcome at the same age, future studies should explore the relation of visuospatial function at 2 years with neurodevelopment at later ages.

Both studies were performed within the BOND study, a prospective cohort study of 142 infants born <30 weeks gestation.[74] One of the exclusion criteria of the BOND study was severe brain injury, defined as intraventricular hemorrhage grade III (with/without infarction) or post hemorrhagic ventricular dilation requiring treatment. This exclusion criterion was created because the impact of severe brain damage on neurodevelopmental outcome is usually so large that effects of early environmental factors under study (such as growth and nutrition) are overshadowed. Furthermore,

they more often experience comorbidities (e.g. feeding problems, cerebral palsy, epilepsy, etc.) which hampers accurate developmental testing. As a result, our findings are mostly generalizable to the NICU population with no or mild brain injury. Therefore, future studies should explore how the observed associations hold in a large cohort of children *with* (severe) brain injury. Larger cohorts are also needed to disentangle which types of brain injury affect brain growth and neurodevelopment most. Last, in studies of neurodevelopment it's important to incorporate environmental factors. In our studies, we adjusted for 2 important confounders: parental education level and ethnicity.[337] Future research on these topics could benefit from also including psychological variables such as parenting style or parental mental health.[338]

Early growth and body composition

Infants born preterm are at high risk for postnatal growth restriction, which is associated with long-term neurodevelopmental problems.[16-18] On the other hand, high nutritional intake and rapid growth during the first months of life may in turn lead to increased adiposity and adverse cardiometabolic health.[25] Therefore, it is essential to determine whether early rapid weight gain in infants born preterm is indeed harmful, and to identify critical periods. In **Chapter 4,** we studied the association between postnatal weight gain during three different timeframes (NICU-stay, level-II hospital stay and at home) and body composition at 2 and 6 months CA in infants born very preterm (< 30 weeks of gestation).[237] In line with our hypothesis, associations of early weight gain with body composition were timeframe specific. Greater weight gain during NICU and level II hospital stay was (weakly) associated with higher absolute lean mass in infancy, but not after correction for length (as expressed in the 'lean mass index', which reflects lean mass in kilograms per body surface m^2). This finding emphasizes the importance of including length into body composition studies of preterm infants, as both length and body size are generally different than in full-term infants resulting in a different distribution of lean and fat mass per m^2. Weight gain during NICU stay was not associated with any of the fat mass parameters, though weight gain in the level-II hospital was positively associated with all fat mass parameters at 2 months. Weight gain at homewas most strongly associated with body composition, especially fat mass, at both 2 and 6 months, also when adjusted for length. Our data suggest that there is room for further optimizing neonatal nutrition during NICU admission to prevent growth restriction and lean mass deficit. Although our data don't raise major concerns about adverse effects of increased early weight gain on adiposity in the first weeks to months of life after preterm birth, further research is needed at later ages as these first 3 months have been described as crucial for body composition later in life.[159,160,197,339,340]

Although we did not observe any adverse effects of early weight gain on fat mass in infancy, it is still arguable whether high-caloric early nutrition and mimicking intra-uterine growth is desirable. One important question is which growth curve and postnatal growth aims should be used for infants born very preterm. In our study, the postnatal decrease in median weight Z-score from 0.1 SD to -0.7 SD in the first days of life, in clinical practice considered as physiological, was not recovered at 6 months CA. This is in line with previous studies and the reason why a study of *Landau-Crangle et al* created an adapted version of the commonly used Fenton growth curve.[27] This adapted version included a 'Fetal-Median-Growth factor' to reflect the transition to extra-uterine conditions within the first weeks after birth. This shift in opinion on optimal postnatal growth (curves) for preterm infants is also reflected by the very recently published new guideline for enteral nutrition in preterm infants by the European Society of Pediatric Gastroenterology, Hepatology and Nutrition.[28] In this guideline, it is acknowledged that the optimal growth velocity that optimizes outcomes in preterm infants remains unclear and should not necessarily mimic intra-uterine growth or birth weight data of full-term infants. Our data support the development of such new, more personalized growth curves for monitoring growth of preterm born infants in infancy.

Also later in infancy, after hospital discharge, it is still unclear whether preterm infants should follow growth patterns of their full-term born peers. In our study, weight gain in the first months at home was associated with higher fat mass in infancy but did not result in high fat mass parameters as compared to term born infants. Therefore, it is unclear whether this early weight gain is a *protective* factor (the observed higher fat mass brings them closer to their full-term born peers) or a *risk* factor (due to the growth in fat mass) for later cardiometabolic health and neurodevelopment. In addition, it is important to keep in mind that risk factors for cardiovascular disease such as high blood pressure, overweight or dyslipidaemia may need time to become apparent. Therefore, follow-up of this cohort into school age and adulthood is warranted. With this goal in mind, our research group of the BOND study has recently completed all follow-up visits at 5 years CA and has just started with an extra research visit at 8 years CA. Furthermore, longer follow-up time also allows for studying associations of longer growth trajectories of body composition. Lastly, definitions of optimal growth and body composition trajectories in infants born preterm are needed for a next step in clinical nutritional care: using outcome-based targets for growth and body composition could be preferred over the current practice of targeting on reference values based on the distribution in the general population.

The observed decrease in relative fat mass from above reference values of full-term born infants at 2 months to below the reference values at 6 months suggests that the peak in fat mass presents earlier in infants born preterm (at around 3 months CA) than in infants born full term (at around 6 months).[167,176,177] The mechanism of this altered trajectory of fat mass accumulation in infants born preterm is not yet understood. Recent studies suggest that the rise in fat mass in the first months after birth is the result of adaptation to ex utero life, and may therefore be physiological in both infants born at term and preterm.[55,168,176] Following this hypothesis, infants born preterm would simply start this transition and adaptation 'earlier' but at a comparable postnatal age (6 months after birth) as infants born at term. Body composition measurements closer to birth (with the possibility to measure when still on respiratory support) are needed to further explore the exact onset of the accumulation of fat and lean mass in infants born preterm.[55] An interesting new topic is the role of epigenetics in the extra-uterine adaptation in adiposity. Epigenetic mechanisms acquired during intrauterine life and the first weeks postnatally are thought to be the mechanistic link between environmental factors and long-lasting phenotype.[55,341] A recent study showed that both decreased weight growth and reduced intake of protein and lipids were associated with methylation of a specific imprinting center (IC1) that regulates the expression of insulin-like growth factor 2.[341] The associations between epigenetics and early growth of preterm infants warrant further study, especially because nutritional intake is highly modifiable in the early phase. In addition, future studies using larger longitudinal cohorts should investigate the effects of sex, fetal growth restriction, type of nutrition and specific medication use (e.g. steroids) on the associations between early weight gain and body composition. Randomized controlled nutritional intervention studies are needed to disentangle the complex causal underlying mechanisms between nutrition and growth and later health.

Body composition is regarded a reliable early marker of cardiometabolic health and has been used in several earlier studies showing different trajectories in infants born preterm and full-term.[54,55,58,186]Body composition can be measured using different methods.[57] Within the BOND-study we used the relatively new method of air-displacement-plethysmography (ADP). ADP has been described as an easy to use, non-invasive and patient-friendly method for both adults and children.[59-61,165,188,342] A shortcoming of ADP is that, contrary to the other commonly used method of Dual-energy-X-ray-absorptiometry (DXA), reference values for ADP in children are lacking after 6 months of age.[343] Furthermore, the BODPOD calculates fat mass percentage using a programmed algorithm including sex-specific estimates of fat mass and fat-free mass density (Dffm) and measured body density.[189] These Dffm estimates

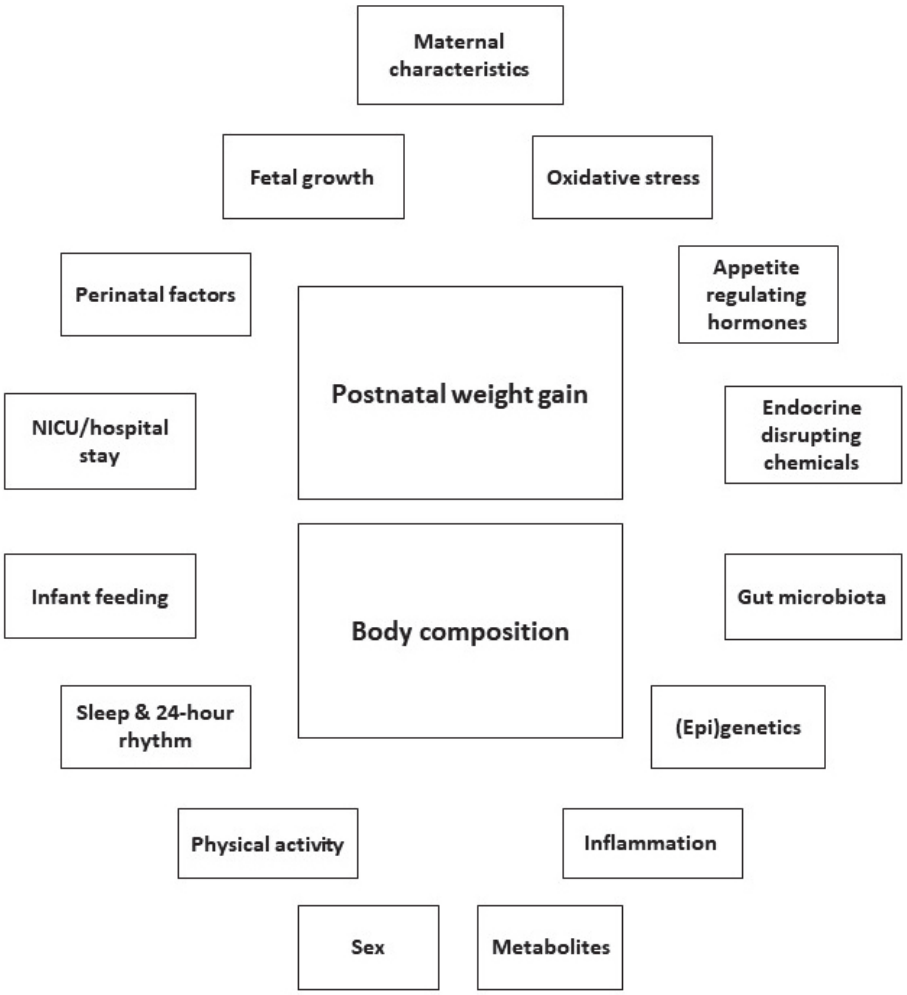
however, were based on children aged >8 years and may not be valid for younger children.[190] We, therefore, investigated in **Chapter 5** whether DXA and ADP results were comparable in young children aged 3-5 years, either born full-term or preterm, and if Dffm-estimates in the ADP algorithm could be improved.[344] Based on our data, we re-calculated age and sex-specific Dffm values for each included full-term born child. We observed that despite these revised and improved Dffm-estimates for ADP, results of ADP and DXA remained not comparable and should not be used interchangeably in the longitudinal assessment of body composition in children aged 3-5 years, especially not in very preterm born children of that age. Possible explanations for the observed larger differences in children born preterm could be their smaller body size, smaller lung volumes, lower fat and lean mass, and lower bone mineral content and density.[210,213,214]

Altogether, we recommend caution when interpreting ADP-results of children born preterm. We also advocate the development of specific Dffm-estimates for these children. Given the current limitations, we conclude that ADP is not the ideal method for longitudinal assessment of body composition in preterm born children and therefore cannot recommend ADP to be included in routine neonatal follow-up programs. Acknowledging that body composition is an important proxy for cardiometabolic health, especially in preterm born children, the question remains how best to incorporate that in routine NICU follow-up (i.e. which method should be used). To answer this question, it is crucial to collect more data from measurements in study context using different measuring techniques, ideally longitudinal studies up to adolescence/adulthood. That way, definitions of optimal body composition trajectories for preterm born children can be developed. On top of collecting descriptive data and constructing trajectories, future studies should also extend recent research on the influence of hormones (e.g. leptin, IGF-1), inflammation (e.g. C-reactive protein, procalcitonin), genetics (e.g. telomere growth), metabolites and endocrine disrupting chemicals (e.g. PFAS) on body composition.[227,345-350] Furthermore, it is important not only to study general adiposity (e.g. fat mass as kilograms/percentage/index) but also to look at the distribution of fat.[351] In **Chapter 8,** we included visceral fat mass and liver fat fraction as indicators of cardiometabolic health in relation to sleep and 24-hour rhythm in healthy children of the general population at the age of 8-11 years old.[352] Several studies have shown that the proportion of abdominal (mostly visceral) fat is most strongly associated with later cardiometabolic morbidity such as hypertension and dyslipidemia.[351] More specifically, recent studies in the general pediatric population described that increased liver fat and non-alcoholic fatty liver disease (liver fat fraction ≥ 5.0%), as measured on magnetic resonance imaging (MRI), are already present in children with

and without obesity[295]. These findings warrant further research on liver fat and the prevalence of non-alcoholic fatty liver disease in the preterm population, already at risk of increased adiposity in adult life. To include this measurement in neonatal follow-up programs, faster, cheaper and less invasive alternatives to MRI should be evaluated first (e.g. abdominal ultrasound). Lastly, from a psychosocial point of view, the associations of stress, physical activity, sleep and neurodevelopment with body composition in childhood call for further research.[182,301,353]

The potential factors influencing early postnatal growth and body composition are depicted in **Figure 1**.

Figure 1. Visual overview of factors (potentially) influencing early infant growth and body composition



Cardiometabolic health

In **Chapter 8** we studied children aged 8-11 years old, participating in the population-based cohort of Generation R, to evaluate diastolic and systolic blood pressure, several blood lipids (glucose, insulin, HDL/LDL/total cholesterol and triglycerides) and clustering of cardiometabolic risk factors in relation to sleep and 24-hour activity rhythms.[352] We observed several associations between disturbed sleep and 24-hour rhythm with increased adiposity and glucose levels. Interestingly, and contrary to previous studies, we did not observe any associations of sleep and 24-hour rhythm with blood pressure, clustering of cardiometabolic risk factors or any of the other blood markers.[291,303,306-309] Possible explanations could be our relatively healthy study population and/or that we measured too early in childhood, while these associations need time to become more apparent in adolescence and adulthood.

With the increasing burden of cardiometabolic disease worldwide and cardiometabolic risk factors already being identifiable in early childhood, it is important to establish which cardiometabolic markers are preferred for use in the pediatric population. In addition to body composition, also other cardiometabolic risk factors such as blood pressure and blood lipids are considered important indicators of cardiometabolic health in childhood and adult life. As for blood markers, in our studies we used the commonly used markers glucose, insulin, cholesterol and triglycerides. Recent studies however, have introduced several other blood markers (e.g. functional or inflammatory), such as insulin sensitivity (e.g. quantitative insulin sensitivity check index 'QUICKI' of Homeostatic model assessment of insulin resistance 'HOMA-IR') or circulating adipokine levels.[354,355] In addition, other promising new markers in childhood include specific cardio*vascular* parameters such as carotid intima media thickness, stiffness (arterial pulse wave velocity) and distensibility as measured by carotic ultrasound.[356,357] These measures have been strongly associated with systemic atherosclerosis, which in turn is related to high blood pressure and cardiovascular mortality in adulthood.[358,359] Although all these different markers contribute to a better understanding of the complex mechanism of cardiovascular disease development through life, the next step should be to distill which (set of) markers are preferred for use in prevention programs, and consequently are most meaningful for patient outcome in the long term.

Sleep and 24-hour rhythms

The development of sleep patterns and 24-hour rhythm starts during fetal life and is vital for neurodevelopment and physiological function in children.[73,221] Disturbed sleep and 24-hour rhythms have been associated with impaired neurodevelopment and adverse cardiometabolic health in adults and adolescents.[217,360] Over the course

of childhood several factors can influence sleep and 24-hour rhythms, such as growth, disease, socioeconomic status (e.g. family structure and housing), school and social activities (social jetlag). Specific pediatric patient populations may experience specific sleep disturbances. In children born preterm for example, preterm birth itself and stay on the neonatal intensive care may disturb the normal development of sleep, leading to a disturbed rhythm and quantitative and qualitative sleep problems in childhood. Therefore, it is important to study sleep and 24-hour rhythms in infants and children of the general population, but also in those born preterm, who are already at increased risk for adverse neurodevelopmental and cardiometabolic outcomes. In recent years, the number of studies on sleep and 24-hour rhythms in the pediatric population has increased. However, most pediatric studies only used sleep diaries or questionnaires, and lacked more direct measurements like polysomnography and actigraphy, or focused on a single sleep measure such as sleep duration. Furthermore, objective sleep studies on preterm children at pre-school age were lacking. Therefore, in **Chapter 6** we compared sleep and 24-hour activity rhythm, between very preterm (BOND-study) and full-term born (PLUTO-study) children at the age of 3 years, using both parent reports and actigraphy.[361] Previous studies on sleep and 24-hour rhythms in preterm children showed lower sleep quality, more nocturnal awakenings and daytime sleepiness and greater fragmentation of the 24-hour rhythm, than after full-term birth.[218-223,258,259] Contrary to our hypothesis, we did not observe large differences in sleep problems, quality of sleep, 24-hour sleep duration or activity rhythms between the groups. With actigraphy, children born very preterm appeared to wake up 21 minutes later than their term-born peers did. On the latter topic, literature is not consistent, showing both earlier as well as similar waking times in preterm children as compared to full-term born children.[219,240-242] Waking times in our study may have been influenced by unmeasured social factors related to the family schedules such as family size, sleep problems of family members, behavior problems and parenting style. Therefore, future sleep studies in children born preterm should include more (psycho)social parameters. Another interesting mechanistic factor may be found in the environment of the NICU. Stay on the NICU involves many environmental factors that may disturb sleep and 24-hour rhythms, such as noise of alarms, medical interventions, exposure to light and dark, etc. Therefore, a relevant topic for future research is the influence of open bay versus single room NICU care on the development and characteristics of sleep and 24-hour rhythm of preterm born children.

In **Chapter 7,** we described the associations of fetal and infant growth and gestational age with sleep and 24-hour rhythms in children aged 10-15 years old of the population-based cohort of the Generation R study.[362] Similar to our findings

in the preterm population of the BOND-study in **Chapter 6**, we showed that in the Generation R cohort, preterm birth (n=113) was not associated with any sleep or 24-hour rhythm parameters. Low birth weight (<2500 grams) and a pattern of growth deceleration in fetal life and infancy were associated with both sleep quantity (longer sleep duration) and sleep quality (higher sleep efficiency and shorter WASO). Previous other studies in fetal growth-restricted infants showed conflicting results reporting either longer or shorter sleep duration, as well as either lower or higher sleep efficiency, at different ages in childhood.[236,258,273,274] The mechanisms underlying the observed associations are not fully understood and are most likely multifactorial. Possible mediating factors are thought to be adverse brain growth, fetal and neonatal hypoxia, the loss of placental steroids and hormones, inflammation, genetic alterations and environmental factors during the neonatal period.[44,73,239] In addition, just like in our preterm study (**Chapter 6)** unmeasured social factors could play a role. Lastly, two recent studies have highlighted the role of maternal health in relation to child sleep. They showed associations of maternal pre-pregnancy BMI, anxiety or depression in pregnancy or infancy, and use of alcohol use and tobacco smoking with shorter sleep duration and more sleep problems at 7-8 years.[363,364] As all these four maternal factors are also associated with fetal and infant growth, future research should further explore the possible mediating role of early growth in the association of maternal (pre)pregnancy health, sleep and 24-hour rhythms.

In **Chapter 8**, we observed that in children aged 8-11 years of the same Generation R cohort, more nightly awakenings were associated with lower BMI but higher glucose.[352] The association of more awakenings with lower BMI was in the opposite direction as expected. In addition, the discrepancy in association with lower BMI and higher glucose is remarkable and needs further investigation. A possible explanation for our disparate findings may be an overestimation of the number of awakenings by actigraphy.[247,248] To reduce this, we limited the definition of an 'awakening' to at least five consecutive minutes of movement. We hypothesized that the observed negative association between awakenings and BMI might still (partly) be driven by increased physical movement during sleep rather than actual sleep disruptions. This may explain why we, similar to previous pediatric studies, did not observe any association between WASO (minutes) and adiposity. Recent studies proposed including parameters of nightly movement in pediatric sleep actigraphy studies, such as mean activity count or activity index. As for 24-hour rhythms, we observed that higher intradaily variability (rhythm fragmentation) was associated with increased general and organ adiposity already at school age, especially in boys. Our findings may imply that at school age, body composition in boys may benefit from optimizing 24-hour rhythms from childhood onwards. However, effect estimates were small and

possible interventions to achieve more regular rhythms in clinical practice require further investigation. Furthermore, as less rhythm fragmentation and more nightly awakenings yield conflicting recommendations, more research is needed before our findings can be translated into specific targets for obesity prevention programs.

Both in the very preterm and full-term children (**Chapters 6, 7, 8**), sleep data reported by children or parents were remarkably different from data measured by actigraphy. Using the Brief Infant Sleep Questionnaires in children aged 3 years in **Chapter 6**, parents reported 2 to 3 hours longer sleep than was measured by actigraphy. The difference in sleep duration as documented in the sleep diary and by actigraphy by children aged 10-15 years in **Chapters 7 and 8** was 1.6-1.9 hours, respectively. These differences have been described previously and are explained by the poor specificity of actigraphs to detect wake periods because they base data on sleep/wake on movement only.[247,248] This leads to an overestimation of wake periods and consequently shorter sleep duration using actigraphy. Therefore, it is important to acknowledge this difference when comparing sleep studies using different measurement techniques.

Several factors in early life can influence the development of 'chronotypes': the natural inclination of the body to sleep at a certain time (i.e. so called 'early birds' versus 'night owls'). Previous studies have shown an earlier chronotype (earlier bed and waking times) in young adults and adolescents born preterm, especially in those born small for gestational age.[242,261,279] Furthermore, 'late' chronotypes and have been associated with adverse cardiometabolic risk profiles.[292] An easy tool to determine chronotype is the Munich Chronotype questionnaire (MCTQ).[229] This self-rated scale uses the midpoint between sleep on- and offset on free days to assess chronotype, and can be used in adults and children >6 years of age. While we used the MCTQ to collect sleep and wake times in the preschool preterm population in **Chapter 6**, we did not determine actual chronotypes in any of the described studies in **Chapter 6-8**. Future studies should further explore the role of preterm birth in the development of chronotypes from infancy until adolescence, as well as its relation to neurodevelopment and cardiometabolic health. Ideally, these chronotype studies should include both subjective and objective measurements. However, as there are currently no chronotype questionnaires available for ages 0-4 years old, further research is needed to develop a specific preschooler version, as the situation of this age group does not match the pediatric or adult version of the MCTQ. This new version may need to include the timing and structure of (daytime) naps.

Social jetlag occurs when there is a discrepancy between biological and social rhythms and is measured as the difference in sleep midpoint on work/school days

and free days. Within this thesis, we observed that social jetlag played less of a role in the children studied than expected. In **Chapter 7,** we showed that small size at birth (SGA, sex- and gestational age–adjusted SDS for birth weight below the tenth percentile) was associated with greater social jetlag in childhood, but only in the older children (14-15 years). In **Chapter 8,** we did not find an association of social jetlag with cardiometabolic risk factors. We only found suggestive evidence for an association between shorter sleep on weekdays and increased adiposity, whereas this relation has reportedly been more prominent in adolescent and adult studies.[272,292,365-369] As sleep and 24-hour rhythms change rapidly during adolescence, social jetlag may become more prominent at an older age.[272] Similarly, adiposity and other cardiometabolic parameters also change during puberty which suggests that the described associations between sleep, 24-hour rhythms and cardiometabolic risk factors may become more evident at older ages. Therefore, it is recommended that studies on these topics during adolescence take stages of puberty into account.

In our sleep studies, we focused on social jetlag, intradaily variability and interdaily stability as measures of the 24-hour activity rhythm, which is an indicator of the circadian organization of the *sleep-wake cycle*. The biological clock, however, embraces more than just the actual sleep-wake cycle. It has an important influence on our daily lives by regulating the day-night rhythms of cells, (epi)genes, hormones (among others melatonin and cortisol) and organs.[285,310-312] Future studies should further explore how these parameters of the circadian rhythm influence the neuro- and cardiometabolic development of children born preterm and full-term. Furthermore, more research is needed on how neonatal care, including cycled light and noise exposure, as well as timing of drugs and nutrition can be better aligned with the neonatal circadian rhythm during admission on the NICU.

The role of nutrition

Although not specifically studied in this thesis, nutrition has been known to play an important role in three domains described in this thesis: growth, body composition and neurodevelopment. In preterm infants, early nutrition (during the first 3 months, both parenteral and enteral) has been linked to better short-term outcomes such as survival.[370] For a long time, it was believed that more aggressive (protein-rich) nutrition in the first days of life was related to a more lean body composition type and better brain growth and development of preterm infants.[148,370] However, two very recent large randomized placebo-controlled trials raised debate, by describing how early administration of extra high amounts of amino acids did not improve neurodevelopmental outcome, and may even be harmful by an increased risk of developing refeeding syndrome.[371,372]

The existing data suggest that the relation between nutrition, cardiometabolic health and sleep and 24-hour rhythm is multidirectionally interrelated.[373,374] On one hand the timing and content of food influences sleep onset time, sleep duration and sleep efficiency.[375] On the other hand, good sleep hygiene influences eating behavior and healthy food choices.[376,377] Lastly, breast milk may have circadian properties which may affect later circadian rhythm, but this topic requires further research (currently performed by our research group on the NICU in Rotterdam).

METHODOLOGICAL CONSIDERATIONS

In the specific chapters, strengths and limitations of all studies have been addressed. Here I will describe more general methodological considerations in terms of different types of bias, causality and methods used. The studies described in **Chapters 2**-**6** have been conducted in the BOND-study. There are some limitations to the interpretation of our findings. The BOND cohort is a very specific population of very preterm infants <30 weeks gestation admitted to the NICU of a large level 4 academic hospital. The Dutch policy of transporting very preterm infants to a level 2 hospital as soon as they are stable is different from most other countries. Therefore, generalizing our findings to other settings, such as more basic hospitals or later preterm infants, requires caution. In addition, selection bias may have played a role as study participation in general correlates with social, educational and health conditions.[378,379] Within the BOND study, we indeed observed some over-presentation of highly educated parents (45%) as compared to middle (30%) or low (18%) education; a common phenomenon in observational studies.[380] We corrected for combined parental education level in all analyses in **Chapters 2-4**. For an observational study with relatively long follow-up, the BOND-study showed limited loss to follow-up (two children out of 142). Moreover, incomplete study measurements were mainly due to logistic problems, rather than patient characteristics.

In general, no measuring method is ideal. Validity, but also practical aspects and availability of tools were taken into account when designing our study. In **Chapter 3** and **4**, we used ADP and DXA to measure body composition. A three or four compartment model including isotope dilution or MRI could have provided more detailed and accurate measurements than ADP or DXA, but both were not feasible in our setting. As for measurement of sleep and 24-hour rhythm, we relied on actigraphy and questionnaires. Level 1 polysomnography, which includes electroencephalography (EEG) registration, is considered the golden standard, but requires 24-hour recording in a hospital setting. Using actigraphy, we were able to provide unique 24-hour rhythm profiles in home settings of preterm born children at early ages.[224,247]

The studies described in **Chapters 7 and 8** were conducted within the Generation R Study. This is a large, ongoing population-based study of almost 10.000 parents and children born in Rotterdam between April 2002 and January 2006 who are followed from early fetal life until 18 years old. Of all children eligible at birth, participation rate of the Generation R Study was 61%.[75] Study participants and their mothers were mostly of white ethnicity and higher socioeconomic status. This might have led to a lower prevalence of cardiometabolic risk factors and more favorable sleep and 24-hour rhythm profiles and subsequently reduced statistical power for these outcomes. This may affect the generalizability of our findings to higher-risk populations. Furthermore, the sleep study was a sub-study within the larger Generation R Study for which mostly children with good follow-up rates were invited. However, studies have shown that in large prospective studies selection at baseline may lead to some bias in prevalence estimates, but not to bias in estimates of exposure-outcome associations.[380] It is possible that selective loss to follow-up may have led to biased results, but it is difficult to quantify its magnitude and direction. Multiple imputation was used to reduce the risk of selection bias due to missing values. It is unlikely that differential misclassification has occurred in any of the described Generation R studies because 1) data on exposures were gathered before assessment of the outcomes using standardized measurement protocols, 2) data collectors were unaware of the exposure status while assessing the outcomes, and 3) children/parents as well as data collectors were uninformed about the specific research questions.

In both the BOND and Generation R Study, the risk of confounding bias was diminished by adjusting for multiple confounders. Confounders were selected based on literature, on their association with the exposure and outcome, or a change in effect estimate of more than 10%. Still, as in any observational study, residual confounding might be an issue due to unknown or unmeasured confounding variables.

FUTURE PERSPECTIVES

Based on this thesis, some general directions for further research can be formulated. Several specific suggestions per research topic have already been mentioned earlier in this discussion.

We can conclude that early factors in life such as birth weight and preterm birth can lead to a wide range of long-term effects. These effects do not only include neurodevelopment and cardiometabolic health, but also other domains like sleep,

24-hour activity rhythm, visuospatial function and quality of life. We studied several associations between domains, but due to the observational nature of our studies, more research is needed on establishing causality for the observed associations, and to further disentangle the (complex) underlying mechanisms. Although I am not yet able to present the perfect neonatal follow-up program which completely fits the children's needs, the studies in this thesis may serve as puzzle pieces that push research forward in reaching this goal.

In children born preterm, future studies should focus on further developing the screening tools to monitor brain growth and predict later neurodevelopment, as well as how to best make use of them. These tools will most probably include neuroimaging by CUS or MRI, but additional research is needed to determine which exact (combination of) parameters should be used. An interesting new topic is the potential added value of artificial intelligence in prediction of neurodevelopmental outcomes.[381] However, the actual implementation of artificial intelligence in healthcare is challenging requiring further development, including a multidisciplinary team and a clear division of roles of product owners and domain experts.[382]

Further exploration of how eye-tracking assessment could be best incorporated into neonatal follow-up programs is needed. Following our research at ages 1 and 2 years CA, tracking of visuospatial function in children born preterm should be extended to ages 2-18 years to create longitudinal eye-tracking trajectories. From my experience, this should be easy to implement as parents and children describe this test as a short, fun, low-threshold and patient-friendly experience. In addition, it should be investigated if and how the observed associations of visuospatial function with neurodevelopment from this thesis hold at older ages. As a start, our research group is currently looking at the associations between visuospatial function and executive functions at 5 years CA.

As for early growth in relation to body composition, future studies should focus on the 'optimal nutrition' for preterm infants during NICU stay. Aiming for a transition towards more personalized nutrition requires additional research on (epi)genetics and the development of more detailed risk profiles focused on both neurodevelopment and cardiometabolic health, necessitating longer follow-up.

Following our studies on ADP in children aged 3-5 years, additional pediatric reference curves for ADP should be established for ages >5 years old. Furthermore, separate Dffm measures and reference curves should be constructed for children

with altered body composition trajectories such as fetal restricted or preterm born children. When the ADP paradigm is optimized for the preterm population, the next step would be to extend body composition assessments in children 2-18 years to create longer trajectories. Then, different growth and body composition trajectories should be related to neurodevelopmental and cardiometabolic outcomes. This would allow for development of new reference curves for growth and body composition based on short and long-term outcomes rather than on trajectories in the general population as comparison.

As for sleep and 24-hour rhythm, we need additional sleep studies at different ages of development (including adolescence) using both subjective (questionnaires) and objective (actigraphy) methods. Special attention should not only be given to children from the preterm population, but also those who were born small for gestational age and otherwise critically ill neonates. In these groups with a high risk of early disturbance of the circadian rhythm, the complex interaction of sleep and 24-hour rhythm with growth (in brain, weight and height), body composition and neurodevelopment needs further exploration. Furthermore, it would be interesting to study sleep and 24-hour rhythms (e.g. shift work) of future mothers in the preconceptional period and in pregnancy, in relation to perinatal outcomes and later child growth and development.

Clinical implications

Although the described associations in the studies from this thesis do not prove causality, they have the potential to serve public health and clinical practice for children born both preterm and full-term. First, we evaluated two different ultrasound markers for monitoring brain growth in preterm infants in the light of prediction of later neurodevelopment. These findings may be used as a stepping-stone in the search for the optimal neuroimaging screening tool for this population. Second, we showed that a new eye-tracking based method could be a valuable screening method for both visuospatial and more general neurodevelopmental impairment. Being a quick, easy and non-invasive test (even the favorite test of many of our study participants) makes it practically suitable for implementation in follow-up programs.

Our findings on weight gain during NICU stay and body composition during infancy suggest that there is room for further optimizing neonatal nutrition to prevent growth restriction and lean mass deficit. Although further research is needed on more long-term outcomes, we observed no major concerns on adverse effects of increased early weight gain on cardiometabolic health in the first months of life. With our new Dffm-estimates for ADP in children 3-5 years old, more accurate body

composition results can be retrieved. Our message is not to use results of DXA and ADP interchangeably, and to interpret ADP results in preterm infants with caution.

This thesis shows potential benefits of monitoring sleep and 24-hour rhythms in children, especially in children with fetal growth restriction or low birth weight. Parents, children and clinicians could be better educated on the role of sleep and 24-hour rhythm in health and functioning. In particular, our data suggest that children born preterm have a need for longer, more efficient sleep. Although effect estimates were small, advising on a more regular 24-hour activity rhythm, especially in boys, may be beneficial in obesity prevention programs.

Optimizing neonatal follow-up programs

Ideal neonatal follow-up programs are more personalized and consequently more cost-efficient. The latter has become increasingly important in times of increasing healthcare costs and general health staff shortage. This thesis emphasizes the importance of longer follow-up periods of very preterm born infants. For example, we observed associations of low birth weight and sleep at 10-15 years old, an age category currently not included in neonatal follow-up programs. Longer follow-up is only future proof, if the follow-up programs are more carefully tailored to the patient's needs and health care resources available. For more personalized programs, more extensive or detailed risk profiles are needed to decide which children should be followed for which specific time period and with which specific tests. In the future, blood tests for (epi)genetics, metabolomics and/or inflammation markers could potentially add to developing more personalized risk profiles, but more research is needed on these topics.

Based on this thesis, I would advise to start with intensive early neonatal follow-up, including screening on several neurodevelopmental domains. I recommend considering additional screening for adverse brain growth, visuospatial dysfunction, altered body composition and/or disturbed sleep or 24-hour rhythm into the current neonatal follow-up programs. After this early screening period, more personalized follow-up trajectories with tailored intensity and duration should be created, with specific attention to those domains affected. Ideally, those children requiring prolonged follow-up should have regular visits to the neonatal outpatient clinic (e.g. every 3 to 4 years) up into adulthood. As the availability of screening tests depends on the hospital's facilities, it would be interesting to explore the option of centralizing NICU follow-up to certain follow-up expertise centers in the future, which could have both patient and financial benefits.

It is important to create individual follow-up trajectories not only based on the results of screening tests. As a first step towards more value-based and patient-oriented neonatal follow-up, more attention should be given to the parental perspective of what follow-up is needed or preferred. Their opinion and rating of the burden and relevance of the different components of follow-up should be taken into account and respected. Within the BOND-study parental input has already generated interesting new research topics such as sleep, 24-hour rhythm and dental health. Multiple parents had informed us that their very preterm born children seemed to have more issues with sleep and problems such as caries. These subjective observations led to the introduction of objective measurements such as actigraphy, sleep questionnaires and an extra dental exam by a qualified dentist at the age (of three and) five years for research purposes. Short questionnaires on the child's quality of life such as the 'Patient Reported Outcomes Measurement Information System' (PROMIS) and 'Pediatric Quality of Life Inventory' (PedsQL) could help structure parental input.[383,384] Within the BOND-study, both the PROMIS and PedsQL will be used at the upcoming follow-up visits at 8 years CA. In addition, playful and easy evaluation forms were designed for children and parents to evaluate the items of standard neonatal follow-up program as well as those included in the study based on relevance and burden. I believe that a multidisciplinary approach combining the expertise of physicians, psychologists, physiotherapists, ergo therapists and visuospatial specialists, in co-creation with the parents and children, is essential for future development of personalized follow-up programs.

CONCLUSIONS

In conclusion, in **Part I** of this thesis we showed that in preterm infants, length and growth of the corpus callosum, but not so much corpus callosum-fastigium, are good markers of brain growth and predictors of later neurodevelopment. At the age of 1 year CA, we showed that visuospatial attention and motion-processing function, as measured by a novel eye-tracking based method, is a predictive factor for overall cognitive and motor development 1 year later.

In **Part II,** we showed that weight gain in different timeframes after preterm birth is associated with distinct parameters of body composition in infancy. We observed that not NICU weight gain but weight gain at home is most strongly associated with lean and especially fat much later in infancy. We found that results of fat mass (percentage) and lean mass at 3-5 years old are significantly different between DXA and ADP, and that these differences are larger in children born very preterm

as compared to those born full-term. We created improved, sex and age-specific estimates for lean mass density to be used in the ADP algorithm.

In **Part III,** we demonstrated that parent-reported sleep characteristics and problems are similar between very preterm and full-term born children at the age of 3 years CA. The only observed difference was a 21-minute later wake-up time in preterm children as compared to their full-term peers as measured with actigraphy. In the general population, we showed that low birth weight (<2500 grams) and growth deceleration in fetal life and infancy are associated with longer sleep duration, higher sleep efficiency and shorter 'wake after sleep onset'-time at the age of 10-15 years. In addition, at the same school age, more nightly awakenings are associated with lower body mass index and higher blood glucose, whereas greater intradaily variability (fragmentation of the 24-hour activity rhythm) is associated with higher fat mass index and visceral fat mass in boys.

Altogether, this thesis adds 7 extra puzzle pieces that will hopefully bring us closer to collectively solving the complex puzzle of which and how 'Early life environmental factors influence early development and growth, impacting lifelong health'.

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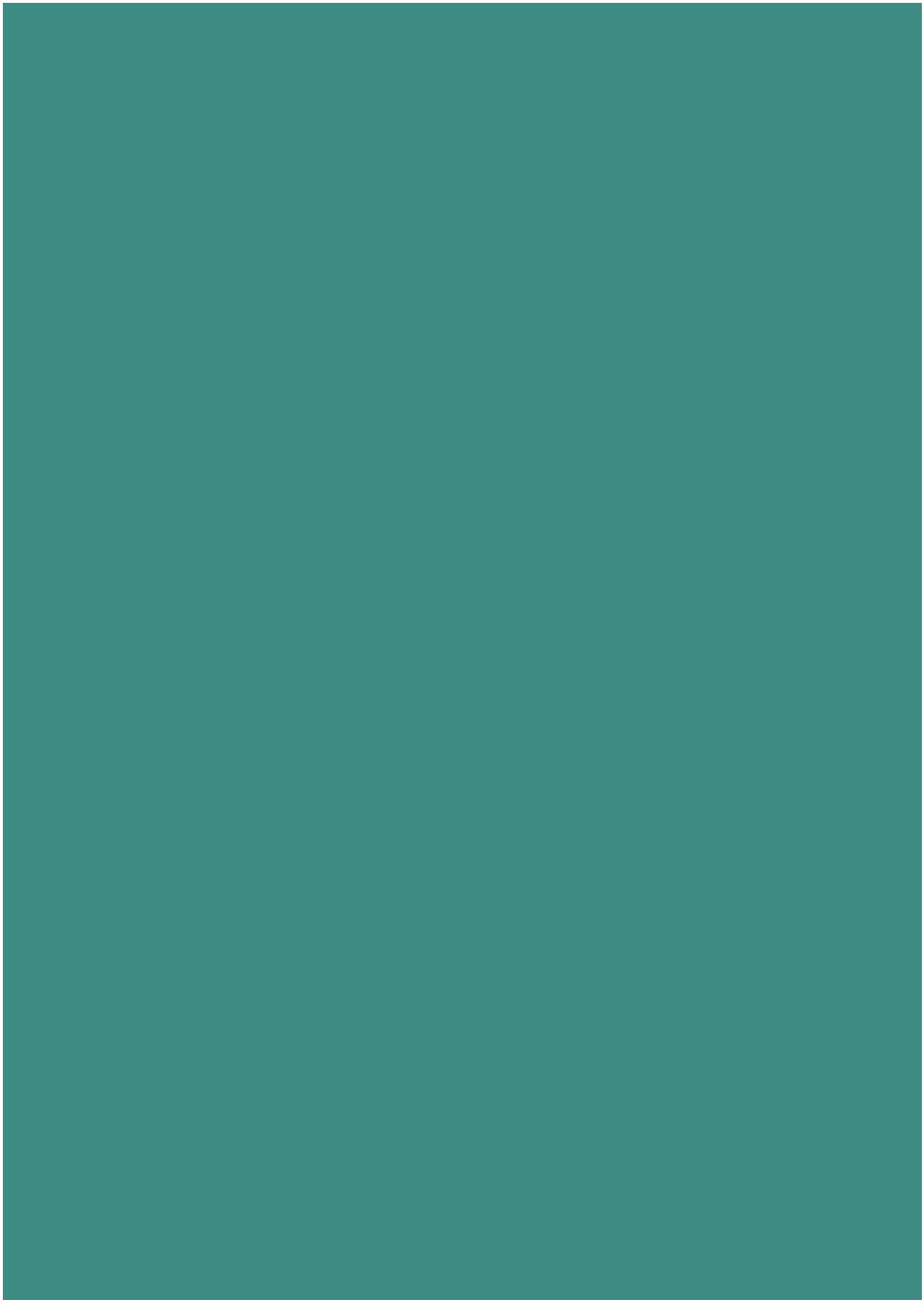


CHAPTER 10

SUMMARY

SUMMARY

The road from fetus to healthy adulthood is long, complex and full of challenges. Essential elements of a healthy childhood are growth, development and the absence of disease. These elements can in turn be influenced by both internal (i.e. genetics, hormones) and external (e.g. environment, lifestyle, parenting) factors. In this thesis, we focused on three important domains of pediatric development: 1) brain growth and neurodevelopment, 2) body growth, body composition and cardiometabolic health, and 3) sleep and 24-hour rhythms. We studied children born very preterm (<30 weeks of gestation) and full-term, from fetal life until school age.

In **Chapter 1,** I introduce the topics that are presented in the following chapters. I describe the DOHaD paradigm (Developmental Origins of Health and Disease) which considers the first 1000 days of life (calculated from conception to approximately 2 years of age) as a crucial period for development. I depict how adverse events in early life such as fetal growth retardation and preterm birth can negatively influence this development. I discuss several screening methods and tests to detect children at risk of adverse development of brain growth, neurodevelopment, body composition, cardiometabolic health, sleep, and 24-hour rhythm. Last, I discuss the general aims of this thesis, as well as the general setting and design of the two prospective cohort studies that form the basis of this thesis: the BOND study in children born very preterm aged 0-5 years old, and the large population-based Generation R study in healthy children aged 0-18 years old.

Part I – The brain and neurodevelopment

In preterm infants, there is a need for reliable markers of early brain growth to contribute to the prediction of later neurodevelopment. A relatively new measure of brain growth on cranial ultrasound is corpus callosum-fastigium (CCF) length, which covers a larger part of the brain than corpus callosum (CC) length alone. In **Chapter 2,** we investigated whether these two markers are clinically relevant by studying their associations with neurodevelopment at two years corrected age. We observed that in 153 infants without brain injury, greater CCF length at 2 months was associated with better cognitive outcome. CC length at 2 months was positively associated with cognitive, motor and language outcome. Faster growth of CC length between birth and 2 months was associated with better cognitive and motor function. Prediction of neurodevelopmental outcome based on neonatal risk factors and head circumference, significantly improved by adding CC length. These findings demonstrate that both markers are associated with neurodevelopmental outcome at 2 years, but only CC length showed added clinical utility in predicting neurodevelopmental outcome.

Also after infancy, early detection of probable neurodevelopmental impairment with quick and easy tests remains important. It allows for timely interventions and individualized follow-up trajectories to prevent further delay. A potential new screening test could be a nonverbal eye tracking-based test to assess visuospatial attention and processing: the ability to perceive and process visuospatial information, a condition for broader neurodevelopment (**Chapter 3**). In 209 children born very preterm at the age of 1 and 2 years, we examined the association of early visuospatial attention and processing (calculated as average reaction times to fixation on specific visual stimuli of attention, motion and form) with later neurodevelopmental outcome. Visuospatial attention and motion processing at 1 year was predictive of overall cognitive and motor development one year later. We stated that this nonverbal eye tracking-based test could assist in early detection of preterm children at risk of adverse neurodevelopment.

Part II – Early growth and body composition

In **Chapter 4,** we studied the association between postnatal weight gain and body composition in infancy (measured at 2 and 6 months corrected age using the PEAPOD) in 120 infants born very preterm. We observed that weight gain in different timeframes after preterm birth was associated with distinct parameters of body composition in infancy. When adjusted for length, weight gain during stay in the neonatal intensive care unit (NICU) was not associated with body composition parameters in the first months of life. In contrast, weight gain after NICU stay, especially at home, was associated with an increase in lean mass and, most strongly, fat mass. However, as fat mass parameters in infancy were still below average values of infants born full term, further research is needed to explore the association between early postnatal growth and cardiometabolic outcome later in life.

It is important to monitor body composition longitudinally, especially in children with atypical body composition trajectories. The above-mentioned PEAPOD uses the relatively new technique of Air Displacement Plethysmography (ADP) to estimate body composition in infants ≤6 months old and/or ≤8 kg. For older children with a higher weight, the BODPOD is available, using the same technique. Another method for measuring body composition in children is Dual energy X-ray Absorptiometry (DXA). However, both methods have advantages and disadvantages, and reliable pediatric reference values for ADP were lacking. We, therefore, aimed to investigate in **Chapter 5** whether DXA and ADP results were comparable in 154 healthy full-term and 67 very preterm born children aged 3-5 years. We found that results of fat mass (percentage) and lean mass were significantly different between the two methods, and that these differences were larger in children born very preterm as compared

to those born full-term. We created improved, sex and age-specific estimates for lean mass density to be used in the ADP algorithm. However, despite these revised estimates, results of ADP and DXA remained not comparable and should not be used interchangeably in the longitudinal assessment of body composition in children aged 3-5 years; especially not in very preterm-born children of that age.

Part III – Sleep ad 24-hour activity rhythms

Disturbed sleep and 24-hour rhythms have been linked to adverse cardiometabolic health in adulthood and these associations may originate in early life. However, few studies used objective methods like actigraphy that evaluated these associations at school age. Furthermore, the connection between perinatal factors such as preterm birth and fetal and infant growth retardation, and child sleep and 24-hour rhythms remains not fully understood. In **Chapter 6,** we studied sleep and 24-hour rhythms of pre-school children born very preterm compared to full-term children, using both parent-reported questionnaires and actigraphy combined with a sleep diary. In 97 very preterm born and 92 full-term children at the (corrected) age of 3 years, we observed that parent-reported sleep characteristics and problems were similar between both groups. The only significant difference was measured by actigraphy, which detected a 21-minute later wake-up time in preterm children as compared to their full-term peers.

In **Chapters 7** and **8,** we focused on early determinants of sleep and 24-hour rhythm (as measured with actigraphy) in childhood, as well as their association with cardiometabolic health. In **Chapter 7,** we exploredassociations of fetal and infant weight patterns and preterm birth with sleep and 24-hour activity rhythm parameters in 1327 children aged 10-15 years old. We observed that low birth weight (<2500 grams) was associated with longer sleep duration compared to normal weight. Compared to normal growth, growth deceleration in fetal life and infancy was associated with longer sleep duration, higher sleep efficiency and shorter 'wake after sleep onset'-time. A pattern of normal fetal growth followed by infant growth acceleration was associated with lower interdaily stability of the 24-hour activity rhythm. Preterm birth was not associated with any sleep or 24-hour rhythm parameters. Our findings show that children with fetal and infant growth restriction had longer and more efficient sleep at school age, which may be indicative of an increased need for sleep for maturational processes and development after a difficult start in life.

In **Chapter 8,** we aimed to study associations of sleep and 24-hour rhythms with cardiometabolic risk factors in 894 children aged 8-11 years. More nightly awakenings were associated with lower body mass index and higher glucose. Among

boys, greater intradaily variability (fragmentation of the 24-hour activity rhythm) was associated with higher fat mass index and visceral fat mass, as measured by DXA and MRI-scan. We observed no associations of sleep and rhythm with blood pressure or clustering of cardiometabolic risk factors. In conclusion, already at school age, greater fragmentation of the 24-hour activity rhythm is associated with general and organ adiposity. In contrast, more nightly awakenings were associated with lower BMI. Therefore, optimizing 24-hour activity rhythms may help to reduce obesity from childhood onwards, particularly in boys.

Part IV – Discussion

In the general discussion (**Chapter 9**), I discuss the main findings of all seven studies described in this thesis. I address methodological considerations and discuss their clinical implications. Last but not least, I deliberate on future studies to be conducted on these topics, as well as how current neonatal follow-up programs could be optimized.

NEDERLANDSE SAMENVATTING

De weg van foetus naar gezonde volwassenheid is lang, complex en vol uitdagingen. Bepalend voor een gezonde kindertijd zijn groei, ontwikkeling en de afwezigheid van ziekte. Deze factoren kunnen echter op hun beurt weer worden beïnvloed door zowel interne (o.a. genetica, hormonen) als externe factoren (bijv. omgeving, leefstijl, opvoedstijl). In deze thesis ligt de nadruk op drie belangrijke bouwstenen voor de ontwikkeling van het kind: 1) hersengroei en neurologische ontwikkeling, 2) lichaamsgroei, lichaamssamenstelling en cardiometabole gezondheid, en 3) slaap en het 24-uurs ritme. We hebben zowel zeer vroeggeboren (<30 weken zwangerschap) als op tijd geboren kinderen onderzocht, vanaf het foetale leven tot aan de schoolleeftijd.

In **Hoofdstuk 1** introduceer en definieer ik de onderwerpen die in de daaropvolgende hoofdstukken aan bod komen. Ik beschrijf onder andere het DOHaD paradigma (Developmental Origins of Health and Disease) dat stelt dat de eerste 1000 dagen van het leven (gerekend vanaf de bevruchting tot ongeveer 2-jarige leeftijd) een cruciale periode vormen voor de ontwikkeling van een kind. Ik schets hoe nadelige gebeurtenissen in het vroege leven zoals foetale groeivertraging en vroeggeboorte negatieve gevolgen kunnen hebben op deze ontwikkeling. Ook beschouw ik verschillende screeningsmethoden en testen om kinderen op te sporen die risico lopen op een ongunstige ontwikkeling van hersengroei, neurologische status, lichaamssamenstelling, cardiometabole gezondheid, slaap en 24-uurs ritme. Als laatste bespreek ik de algemene doelen van deze thesis, evenals de algemene setting en ontwerp van de twee prospectieve studies die aan de basis liggen van deze thesis: de BOND-studie in zeer vroeggeboren kinderen van 0-5 jaar oud en de Generation R-studie met gezonde kinderen van 0-18 jaar uit de algemene populatie.

Deel I – De hersenen en neurologische ontwikkeling

Bij vroeggeboren baby's is er behoefte aan betrouwbare markers van vroege hersengroei die kunnen bijdragen aan het voorspellen van hun latere neurologische ontwikkeling. Een relatief nieuwe meetwaarde van hersengroei op een echo van het hoofd is corpus callosum-fastigium (CCF) lengte. De CCF-lengte beslaat een groter gedeelte van de hersenen dan de lengte van het corpus callosum (CC) alleen. **Hoofdstuk 2** bevat de weergave van onze zoektocht of deze twee markers klinisch relevant zijn door hun verband met neurologische ontwikkeling op 2-jarige leeftijd te onderzoeken. We vonden bij 153 zuigelingen zonder hersenschade dat de CCF-lengte op de leeftijd van 2 maanden geassocieerd was met een betere cognitieve uitkomst. Ook bleek er op deze leeftijd een positieve relatie te bestaan tussen

CC-lengte en cognitieve, motorische en taaluitkomsten. Snellere groei van de CC-lengte tussen geboorte en 2 maanden was gerelateerd aan een betere cognitieve en motorische functie. De voorspelling van neurologische uitkomst door middel van neonatale risicofactoren en hoofdomtrek verbeterde significant door het toevoegen van CC-lengte. Deze bevindingen laten zien dat beide markers geassocieerd zijn met de neurologische uitkomst op 2-jarige leeftijd, maar dat alleen CC-lengte klinisch toegevoegde waarde heeft in het voorspellen van de neurologische uitkomst.

Ook na de zuigelingentijd blijft vroege opsporing van een mogelijke ontwikkelingsstoornis door middel van snelle en gemakkelijke testen belangrijk. Het biedt mogelijkheden voor tijdige interventies en geïndividualiseerde follow-up trajecten om verdere achterstand te voorkomen. Een zogenoemde 'eye tracking'-test zou een mogelijk nieuw non-verbaal screeningsinstrument kunnen zijn voor het in kaart brengen van de visueel-ruimtelijke aandacht en verwerking: het vermogen visueel-ruimtelijke informatie te ontvangen en verwerken, een voorwaarde voor bredere neurologische ontwikkeling (**Hoofdstuk 3**). Bij 209 zeer vroeggeboren kinderen onderzochten we op de leeftijd van 1 en 2 jaar het verband tussen vroege visueel-ruimtelijke aandacht en verwerking (berekend als de gemiddelde reactietijd tot het fixeren op specifieke visuele stimuli van aandacht, beweging en vorm) en latere neurologische uitkomst. Visueel-ruimtelijke aandacht en het verwerken van beweging op 1-jarige leeftijd was voorspellend voor algehele cognitieve en motorische ontwikkeling een jaar later. Wij stelden vast dat deze non-verbale eye tracking-test een bijdrage zou kunnen leveren aan de vroege opsporing van vroeggeboren kinderen met een verhoogd risico op een ongunstige neurologische ontwikkeling.

Deel II – Vroege groei en lichaamssamenstelling

In **Hoofdstuk 4** onderzochten wij de associatie tussen gewichtstoename na de geboorte en lichaamssamenstelling in de zuigelingentijd (gemeten op de leeftijd van 2 en 6 maanden met de PEAPOD) bij 120 zeer vroeggeboren kinderen. We zagen dat het gewichtsverlies in verschillende tijdsperioden na de vroeggeboorte verband hield met specifieke parameters van lichaamssamenstelling in de zuigelingentijd. Als gewichtstoename tijdens verblijf op de neonatale intensive care (NICU) gecorrigeerd werd voor lengte, was het niet geassocieerd met lichaamssamenstelling in de eerste maanden van het leven. In tegenstelling tot *tijdens* de NICU-periode, bleek gewichtstoename *ná* NICU-opname, vooral thuis, verband te houden met een toename in vetvrije massa en, in nog grotere mate, vetmassa. Echter, aangezien de vetmassa-parameters in de zuigelingentijd lager waren dan de gemiddelde waardes van op tijd geboren baby's, is verder onderzoek nodig om uit te zoeken of er een verband is met vroege groei na de geboorte en cardiometabole uitkomsten later in het leven.

Het is belangrijk om lichaamssamenstelling longitudinaal te monitoren, vooral in kinderen met een atypisch beloop van de lichaamssamenstelling. Bij de hierboven genoemde PEAPOD wordt gebruikt gemaakt van een relatief nieuwe techniek genaamd Air Displacement Plethysmography (ADP), om een schatting te maken van de lichaamssamenstelling van zuigelingen tot 6 maanden en/of 8 kilogram. Voor oudere kinderen met een hoger gewicht bestaat de BODPOD, die gebruik maakt van dezelfde techniek. Een andere methode om de lichaamssamenstelling bij kinderen te meten is Dual energy X-ray Absorptiometry (DXA). Beide methodes hebben echter voor- en nadelen; bij ADP ontbreken betrouwbare referentiewaarden voor kinderen. Daarom was ons doel in **Hoofdstuk 5** bij 154 gezonde op tijd geboren en 67 zeer vroeggeboren kinderen van 3-5 jaar oud uit te zoeken of de resultaten van DXA en ADP vergelijkbaar waren. We zagen dat de gemeten waardes van vetmassa (percentage) en vetvrije massa bij beide methodes significant anders waren en dat deze verschillen groter waren bij zeer vroeggeboren kinderen dan bij op tijd geboren kinderen. We creëerden verbeterde, geslacht- en leeftijdsgebonden schattingswaardes voor de dichtheid van de vetvrije massa die gebruikt konden worden in het algoritme van ADP. Maar de resultaten van ADP en DXA bleven ondanks deze gereviseerde schattingswaardes niet vergelijkbaar. Zij kunnen dus niet door elkaar worden gebruikt in longitudinaal onderzoek van lichaamssamenstelling bij kinderen van 3-5 jaar oud en zeker niet bij te vroeggeboren kinderen van die leeftijd.

Deel III – Slaap en het 24-uurs activiteitenritme

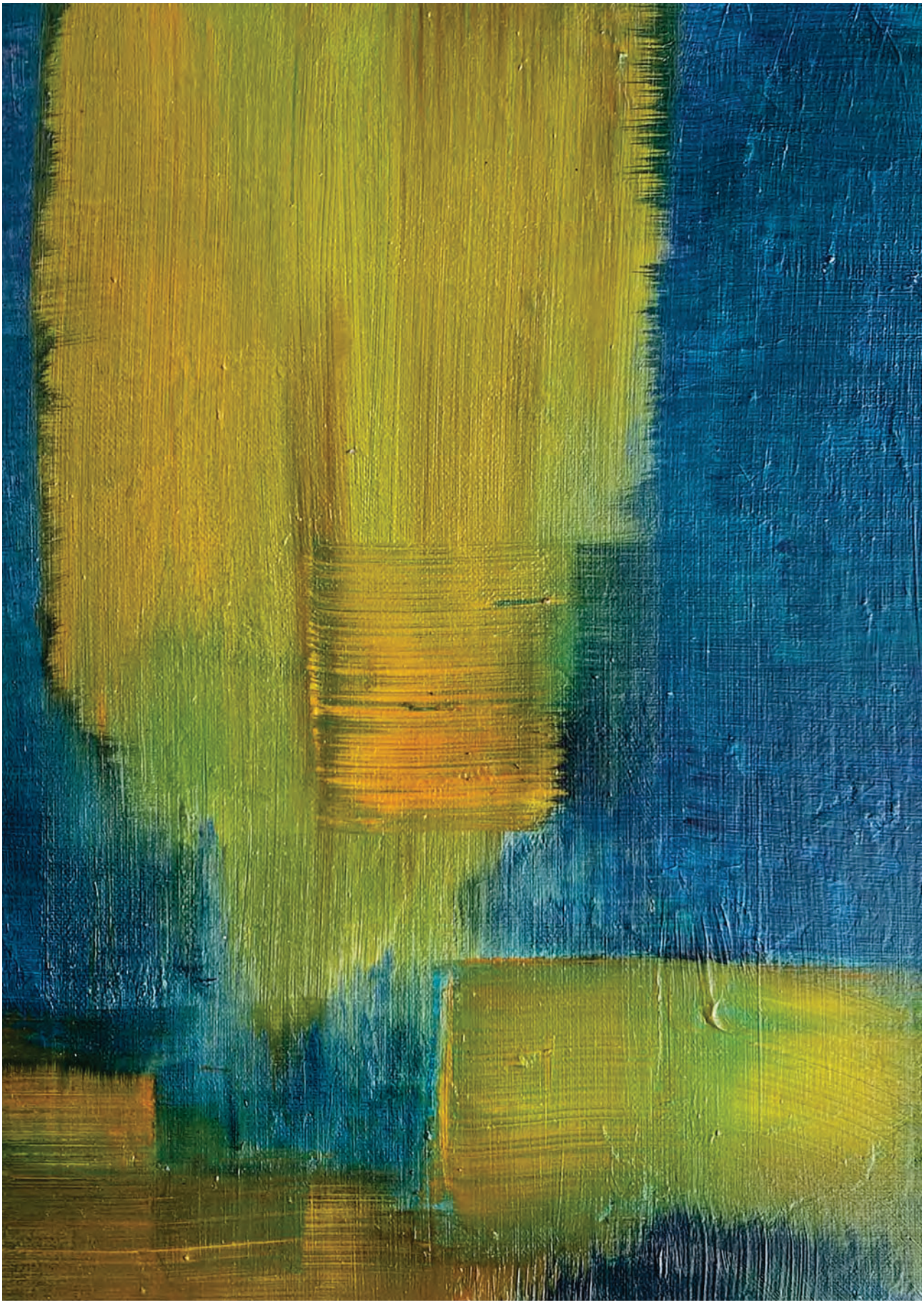
Verstoorde slaap en 24-uurs ritmes zijn in verband gebracht met ongunstige cardiometabole gezondheid in het volwassen leven. Dit verband vindt zijn oorsprong misschien al in het vroege leven. Er bestaan echter weinig studies die objectieve methoden zoals actigrafie gebruiken om deze associatie op de kinderleeftijd te onderzoeken. Ook wordt het verband tussen perinatale factoren zoals vroeggeboorte en groeivertraging in de foetale en zuigelingenperiode en slaap en 24-uurs ritme op de kinderleeftijd nog steeds niet goed begrepen. In **Hoofdstuk 6** beschrijven wij ons onderzoek naar slaap en 24-uurs ritme van vroeg- en op tijd geboren kinderen op de peuterleeftijd waarbij wij gebruik hebben gemaakt van zowel vragenlijsten voor de ouders als actigrafie gecombineerd met een slaapdagboek. Wij lieten zien dat de ouders van 97 zeer vroeggeboren en 92 op tijd geboren kinderen vergelijkbare slaapgegevens en slaapproblemen rapporteerden op de (gecorrigeerde) leeftijd van 3 jaar. Het enige significante verschil werd gemeten met actigrafie; dat toonde aan dat vroeggeboren kinderen gemiddeld 21 minuten later wakker werden dan leeftijdsgenoten die op tijd geboren waren.

In de **Hoofdstukken 7** en **8** richtten wij ons op vroege determinanten van slaap en 24-uurs ritme op de kinderleeftijd en op hun relatie met cardiometabole gezondheid. In **Hoofdstuk 7** zochten wij onder 1327 kinderen van 10-15 jaar oud naar mogelijke associaties tussen groeipatronen in de foetale en zuigelingenperiode en vroeggeboorte met kenmerken van slaap en 24-uurs ritme. Wij vonden dat vergeleken met een normaal geboortegewicht, een laag geboortegewicht (<2500 gram) geassocieerd was met een langere slaapduur. Vergeleken met normale groei, bleek groeivertraging in de foetale periode en zuigelingentijd verband te houden met een langere slaapduur, hogere slaapefficiëntie en kortere 'wake after sleep onset'-tijd. Een groeipatroon waarbij normale foetale groei gevolgd werd door een groeiversnelling in de zuigelingenperiode was geassocieerd met een lagere interdagelijkse stabiliteit van het 24-uurs activiteitenritme. Er was geen verband tussen vroeggeboorte en de slaap- en ritmeparameters. Onze bevindingen laten zien dat kinderen met groeivertraging in de foetale- en zuigelingenperiode in de schoolleeftijd langer en efficiënter slapen. Dit zou kunnen duiden op een grotere behoefte aan slaap die zij nodig zouden kunnen hebben voor rijpingsprocessen en ontwikkeling na een moeilijke start van het leven.

Het doel van **Hoofstuk 8** was het bij 894 kinderen van 8-11 jaar oud in kaart brengen van mogelijke associaties tussen slaap en 24-uurs ritmes met cardiometabole risicofactoren. Vaker 's nachts wakker worden bleek geassocieerd met een lager BMI en hogere glucosewaardes. Bij jongens zagen wij dat een grotere variabiliteit (fragmentatie) van het 24-uurs ritme geassocieerd was met een hogere vetindex en viscerale vetmassa gemeten met DXA- en MRI-scan. We vonden geen verband tussen slaap en ritme met bloeddruk of clustervorming van cardiometabole risicofactoren. Concluderend kunnen wij zeggen dat grotere fragmentatie van het 24-uurs ritme al op de schoolleeftijd geassocieerd is met algemene en orgaan-specifieke adipositas. Hiertegenover stond dat vaker 's nachts wakker worden geassocieerd was met een lager BMI. Het optimaliseren van het 24-uurs activiteitenritme zou dus een rol kunnen spelen in het verminderen van obesitas vanaf de kinderleeftijd, vooral bij jongens.

Deel IV – Discussie

In de algemene discussie (**Hoofdstuk 9**) bespreek ik de belangrijkste bevindingen van de zeven studies die beschreven staan in deze thesis. Ik benoem methodologische overwegingen en bespreek de klinische implicaties. Als laatste bespreek ik toekomstige studies die uitgevoerd zouden kunnen worden naar deze onderwerpen en geef ik aan hoe de huidige follow-up programma's voor vroeggeboren kinderen verbeterd zouden kunnen worden.



PART V

Appendices

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ABBREVIATIONS

ADP	Air-displacement-plethysmography
AGA	Appropriate (birth weight) for gestational age
AIMS	Alberta Infant Motor Scale
BAYLEY-III-NL	Bayley Scales of Infant and Toddler Development Third Edition Dutch version
Beery VMI	Beery Visual Motor Integration
BPD	Bronchopulmonary dysplasia
BISQ	Brief Infant Sleep Questionnaire
BMI	Body Mass Index
BRIEF	Behavior Rating Inventory of Executive Function (-P toddler version, -2 for >5 yrs old)
BW	Birth weight
CA	Corrected age
CBCL	Child Behavior Checklist
CC	Corpus callosum
CCF	Corpus callosum-fastigium
CI	Confidence interval
CJG	Centrum Jeugd en Gezin (Centers for Youth and Family)
CUS	Cranial ultrasound
Dffm	Fat-free-mass-density
DOHaD	Developmental Origins of Health and Disease
DSM	Diagnostic and Statistical Manual of Mental Disorders
DXA	Dual-energy X-ray Absorptiometry
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology and Nutrition
FGR	Fetal growth restricition
FM(I)	Fat mass (index)
FFM(I)	Fat free mass (index)
GA	Gestational age
HC	Head circumference

HDL	High-Density Lipoprotein
HPA	Hypothalamic–Pituitary–Adrenal
IRDS	Infant respiratory distress syndrome
IQR	Interquartile range
IVH	Intraventricular hemorrhage
JGZ	Jeugdgezondheidszorg (Dutch Youth Health Care)
LDL	Low-Density Lipoprotein
LM(I)	Lean mass (index)
MABC-2-NL	Movement ABC Second Edition Dutch version
MCTQ	Munich Chronotype Questionnaire
NICU	Neonatal Intensive Care Unit
MRI	Magnetic Resonance Imaging
NEPSY-II-NL	A Developmental NEuroPSYchological Assessment Second Edition Dutch version
OR	Odds ratio
PA	Postnatal age
PDA	Patent ductus arteriosus
PHVD	Posthemorrhagic ventricular dilatation
PVL	Periventricular leukomalacia
REM	Rapid eye movement
ROP	Retinopathy of prematurity
RR	Risk ratio
RTF	Reaction rime to fixation
SD(S)	Standard deviation (score)
SGA	Small (birth weight) for gestational age
SOL	Sleep onset latency
TRF	Teachers Report Form
WASO	Wake after sleep onset
WISC-V-NL	Wechsler Intelligence Scales for Children Fifth Edition Dutch version
WPPSI-IV-NL	Wechsler Preschool and Primary Scale of Intelligence Fourth Edition Dutch version

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V.A.A. Beunders, M.E. Koopman-Verhoeff, M.J. Vermeulen, et al. Sleep, 24-hour activity rhythms and cardiometabolic risk factors in school-age children. J Clin Sleep Med. 2023 Jul 1;19(7):1219-1229.

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V.A.A. Beunders, M.J. Vermeulen, J.A. Roelants, et al. Early visuospatial attention and processing and related neurodevelopmental outcome at 2 years in children born very preterm. Pediatr Res. 2021 Sep;90(3):608-616.

Outside this thesis

A. Bijlsma, I.A.L.P van Beijsterveldt, M.J. Vermeulen, **V.A.A. Beunders**, et al. Challenges in body composition assessment using air-displacement plethysmography by BOD POD in pediatric and young adult patients. Clin Nutr. 2023 Sep;42(9):1588-1594.

J. Minderhoud, R.M. van Nispen, **V.A.A. Beunders**, et al. Epidemiology and aetiology of childhood ocular trauma in the Republic of Suriname. Acta Ophthalmology. 2016 Aug;94(5):479-84.

A.A.M Heijthuijsen, **V.A.A. Beunders**, D. Jiawan, et al. Causes of severe visual impairment and blindness in children in the Republic of Suriname. Br J Ophthalmol. 2013 Jul;97(7):812-5.

PHD PORTFOLIO

PHD TRAINING	YEAR	ECTS
General courses		
BROK-course (NFU BROK academy)	2017	1.5
Systematic Literature Retrieval in multiple databases (Erasmus MC)	2017	1.0
Scientific Integrity (Erasmus MC)	2018	0.3
CPO-mini-course (Erasmus MC)	2018	0.3
SPSS-course (MolMed)	2018	1.0
Basic course 'R' (MolMed)	2018	1.8
Biomedical English Writing, Erasmus MC	2019	1.0
Re-registration BROK (NFU BROK academy)	2022	0.5
Specific courses & workshops		
Master of Science in Health Sciences, specialization 'Clinical Epidemiology' (NIHES)	2018-2020	70
Local research meetings		
Weekly Neonatology research meeting	2017 – 2021	0.5
Weekly Generation R research meeting	2018 – 2021	0.5
Quarterly 'Metabolism, endocrinology and nutrition' research meeting	2017 – 2021	0.3
Bi-annual Nutricia Research meeting	2017 – 2021	0.3
Annual PhD Day (Promeras/Erasmus MC)	2018 – 2020	0.3
Annual Sophia Research Day (Sophia Child's Hospital)	2019 – 2021	0.3
Conferences attended		
Healthy Food Congress, Rotterdam, The Netherlands	2017	0.3
Child Health Symposium (Tulips), Noordwijk, The Netherlands	2018	0.3
Young Researchers Day (Tulips), Utrecht, The Netherlands	2018	0.3
Kempenhaeghe Clinical Symposium, Heeze, The Netherlands	2019	0.3
jENS, Maastricht, The Netherlands	2019	0.5
SLAAP2019, Ermelo, The Netherlands	2019	0.3

Presentations at conferences		
ESPEN 2020, virtual [poster]	2020	1.0
EAPS 2020, virtual [poster presentation]	2020	1.0
Fetal & Neonatal Neurology, virtual [oral presentation]	2021	1.0
WEON 2021, virtual [oral presentation]	2021	1.0
SPER 2021, virtual [oral presentation]	2021	1.0
Sophia Research Day 2021, virtual [oral presentation]	2021	1.0
EAPS 2022, Barcelona [poster presentation, oral presentation]	2022	1.0
Other		
Peer review of articles for international scientific journals	2017 – 2021	0.5
Participation in TULIPS PhD program	2019 – 2021	0.5
Sophia Research Representation (SOV), member of organizing committees: Annual SOV dinner 2018, SOV weekend 2019, Sophia Research Day 2019	2017 - 2019	0.5

ABOUT THE AUTHOR

Victoria Beunders was born on September 13th 1988 in the Amsterdam University Medical Center (AUMC), location VUmc, The Netherlands. She completed her high school at the Vossius Gymnasium in Amsterdam in 2006 (cum laude). During high school she developed a passion for studying languages and took extra-curricular classes in Italian language and culture. It was this same period that her interest for the academic world started, and during the last two years of high school she enrolled into the Pre-University College project at Leiden University. After she finished her school research project on the physical and psychological effects of kidney disease on children, she slowly started to think about studying medicine to become a pediatrician. After high school she was privileged to take a year off to study Italian in Perugia and Rome for 6 months, as well English on Malta and in Brighton for 3 months. In 2007 she started her medical education at the University of Amsterdam, location AMC. During her medical education she was inspired to learn more about global health, and she was given the chance to do internships in Surinam (both nursing and research), Benin and Zambia. During her clinical internships, Victoria's interest and talent for pediatrics was confirmed.

In May 2015 she completed her studies in Medicine. One month later, she started her residency (ANIOS) period in pediatrics at the Zaans Medical Center in Zaandam. After one year she switched to the larger teaching hospital OLVG in Amsterdam. In September 2017 she started as a PhD student at the Erasmus MC Sophia Children's Hospital in Rotterdam under supervision of prof. dr. I.K.M. Reiss, prof. dr. K.F.M. Joosten, and dr. M.J. Vermeulen. Within the BOND study, she focused on growth, body composition, neurodevelopment, sleep, and 24-hour rhythms in children born very preterm. In 2018 she started a second research project on growth, body composition and sleep in healthy, older children within the population-based Generation R Study at the Erasmus MC. For this project she was supervised by prof. dr. V.W.V. Jaddoe, who replaced prof. dr. I.K.M Reiss as Victoria's second promotor.

During her PhD she also obtained her master's in clinical Epidemiology at the Netherlands Institute of Health Sciences and participated in the 2-year TULIPS curriculum. During the last phase of finishing her PhD (December 2021 till present), she started working again as an ANIOS in pediatrics at the OLVG Hospital in Amsterdam. In January 2024 Victoria will start her training to become a pediatrician at the AUMC under supervision of prof. dr. D.K. Bosman and prof. dr. H.M.A. de Bie. She currently still lives in Amsterdam with her fiancé Paul and their cat Mei-Li.

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