Nutrition's Route to Behaviour and Vice Versa: Longitudinal Links from Early Life to Adolescence

Yvonne Willemsen

DONDERS SERIES

RADBOUD UNIVERSITY PRESS

Radboud Dissertation Series

Nutrition's Route to Behaviour and Vice Versa: Longitudinal Links from Early Life to Adolescence

Yvonne Willemsen

This publication has been made possible by the European Union's Horizon 2020 research and innovation programme.

Author: Yvonne Willemsen
Title: Nutrition's Route to Behaviour and Vice Versa: Longitudinal Links from Early Life to Adolescence

Radboud Dissertations Series ISSN: 2950-2772 (Online); 2950-2780 (Print)

Published by RADBOUD UNIVERSITY PRESS
Postbus 9100, 6500 HA Nijmegen, The Netherlands
www.radbouduniversitypress.nl

Design: Yvonne Willemsen
Cover: MaggieW
Printing: DPN Rikken/Pumbo

ISBN: 9789493296374
DOI: 10.54195/ 9789493296374
Free download at: www.boekenbestellen.nl/radboud-university-press/dissertations

RADBOUD
UNIVERSITY
PRESS

Nutrition's Route to Behaviour and Vice Versa: Longitudinal Links from Early Life to Adolescence

Proefschrift ter verkrijging van de graad van doctor

aan de Radboud Universiteit Nijmegen

op gezag van de rector magnificus prof. dr. J.M. Sanders,

volgens besluit van het college voor promoties

in het openbaar te verdedigen op

maandag 15 april 2024

om 16:30 uur precies

door

Yvonne Willemsen

geboren op 6 december 1992

te Wageningen

Promotor:

Prof. dr. C. de Weerth

Copromotoren:

Dr. R. Beijers

Dr. A. Arias Vásquez

Manuscriptcommissie:

Prof. dr. E. Aarts (voorzitter)

Prof. dr. R.F. Witkamp (Wageningen University & Research)

Prof. dr. H. van Goudoever (Amsterdam UMC)

Dr. J. Penders (Maastricht University)

Dr. J.K. Larsen

Funding

This research was supported by the European Union's Horizon 2020 Eat2beNice grant (728018 to C. de Weerth and A. Arias Vásquez), a Jacobs Foundation Advanced Research Fellowship (to C. de Weerth), and a Netherlands Organization for Scientific Research VENI grant (016.195.197 to R. Beijers), VIDI grant (575-25-009 to C. de Weerth), and VICI grant (016.Vici.185.038 to C. de Weerth).

Table of Contents

Chapter 1

General Introduction

The general goal of the current thesis is to uncover unknown cross sectional and longitudinal links between nutrition and behaviour from early life until adolescence. This goal is reached through three aims that are explained in this first section. In the second section, the theory behind these aims is elaborated on in more detail.

Executive functions (EF) are cognitive processes that allow for planning, monitoring, and executing goal-directed behaviours (Diamond, 2013). As such, EF are important for many aspects of life. The current thesis focuses on EF in general as well as on a specific aspect of EF, namely inhibitory control (IC). IC represents the ability to suppress impulsive behaviour and is an integral part of EF and cognition (Diamond, 2013). The development of EF and IC starts and progresses rapidly in the early ages (Diamond, 2013). Especially in toddlerhood, EF and IC are important behavioural indicators of future problem behaviour and poor developmental outcomes (i.e., externalizing behavioural problems and attention deficit hyperactivity disorder (ADHD) related problems, accounting for 23% of the variance) (Gagne et al., 2011). The factors influencing EF and IC are not yet fully identified. However, nutrition may play a pivotal role in the inter-individual variability of EF and IC (see review by Costello et al. (2020)). Specifically, because early life is shown to be a vulnerable and susceptible life phase for predicting future health (Robertson et al., 2019), early life nutrition is a vital factor when researching predictors for cognitive outcomes (Moody et al., 2017; Prado and Dewey, 2014). Therefore, the **first aim** of this dissertation was to investigate the relations between breastfeeding factors and later child EF and IC.

But how could nutrition be related to the development of EF and IC? The microbiota-gut-brain axis, the bi-directional communication route between the microbiota in the gut and the brain, is considered an important biological mechanism for explaining the relations between nutrition, the brain, and behavioural development (Cryan and O'Mahony, 2011; Cryan et al., 2019). Gut microbiota are affected by nutritional intake and can therefore vary from day-to-day (Oriach et al., 2016; Vandeputte et al., 2021).

Furthermore, the gut microbiota and the brain are able to communicate through different pathways, such as the vagal nerve, impacting brain activity (Cryan et al., 2019). Subsequently, altered brain activity is linked to altered behaviour (Chye et al., 2021). As both the gut microbiota and the brain develop rapidly in early life (de Weerth, 2017; Rice and Barone, 2000; Wang et al., 2018), a possible interplay early in life is suggested (Robertson et al., 2019). The **second aim** was therefore to investigate early life gut microbiota associations with toddler EF and IC.

Next to examining healthy (early life) nutrition as predictor of EF and IC, healthy nutrition also plays an important role in the development of the immune system, muscle growth, and neurodevelopment, as well as prevention of non-communicable diseases, etc. (Norris et al., 2022). It is therefore important to investigate the determinants of dietary intake. One developmental stage in which healthy nutritional behaviours are at risk is adolescence (Limbers et al., 2021; World Health Organization, 2005). Parents are an important predictor of a range of child developmental outcomes (Albanese et al., 2019; Delvecchio et al., 2020; Khozaei and Carbon, 2022). Therefore, the **third aim** of this thesis was to investigate the role of maternal caregiving behaviour on adolescent IC and healthy nutritional behaviours. The following paragraphs explain this thesis' aims in more detail. Box 1 shows the aims of the current thesis, and Figure 1.1 shows the overview of this thesis including the research aims and chapters.

Box 1
Research aims

Aim 1: investigate the relations between breastfeeding factors and toddler executive functions and inhibitory control.

Aim 2: investigate early life gut microbiota associations with toddler executive functions and inhibitory control.

Aim 3: investigate the role of maternal caregiving behaviour on adolescent inhibitory control and healthy nutritional behaviours.

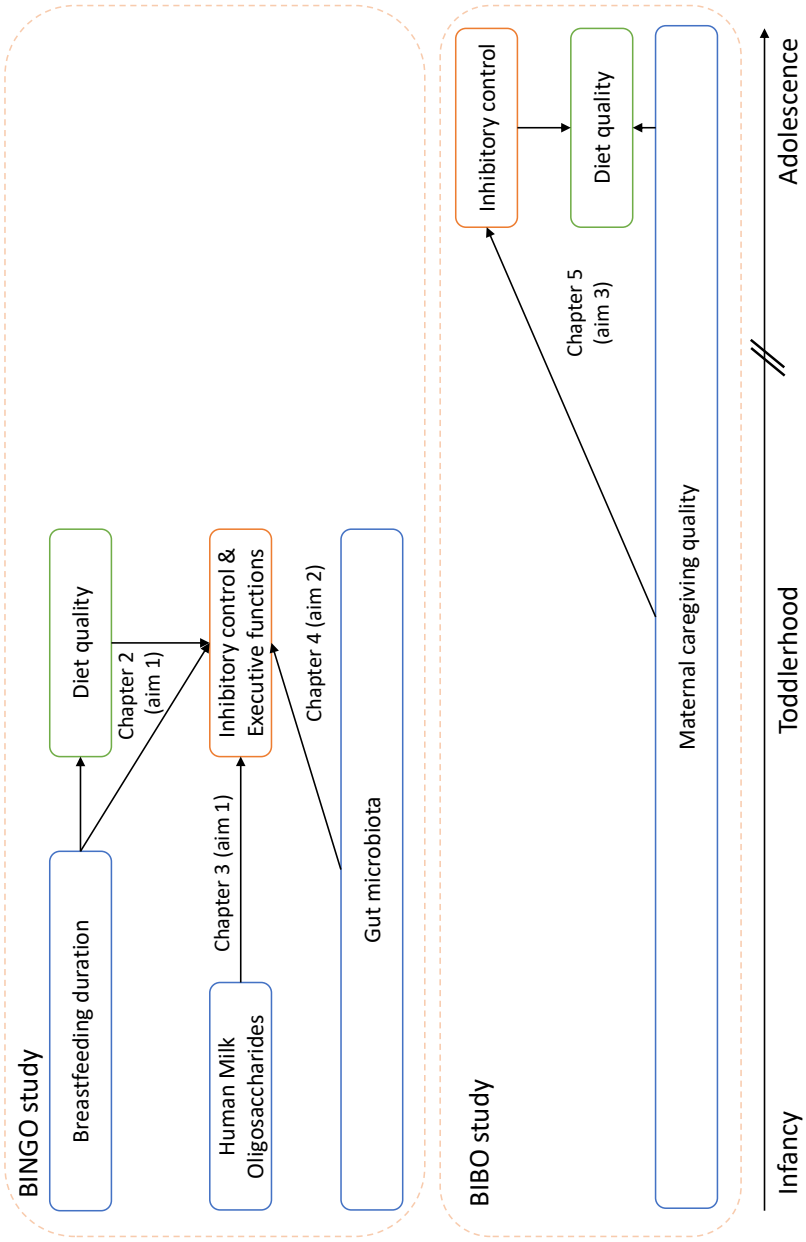


Figure 1.1. Overview of the research topics per chapter. Blue boxes indicate the expected predictors. The orange and green boxes indicate the outcome measures. Light orange dotted lines indicate the cohorts used. The black arrows indicate the expected direction of the association, as causality could not be determined in the present data.

Executive functions and inhibitory control

Executive functions (EF) can be divided into several constructs: working memory, cognitive flexibility, and inhibitory control (IC) (Diamond, 2013). Working memory is the ability to remember information and mentally work with it, cognitive flexibility is the ability to maintain information and switch perspectives towards it, and IC is the ability that helps us control our impulses in order to make rational decisions (American Psychological Association, 2023). Working memory and cognitive flexibility both require IC, making IC a vital adaptive human ability that is an integral part of higher-order EF (Diamond, 2013). IC helps us consider consequences of our actions and dominates impulsive urges that satisfy short term rewards, thereby protecting us against harm and stimulating behavioural patterns that allow for long term larger rewards (Allom et al., 2015; Cappelli et al., 2019; Li et al., 2022). For example, one can refrain from eating in the absence of hunger to maintain a healthy weight. Higher levels of EF and IC around preschool age and early school years are associated with positive future outcomes including higher academic performance (Cortés Pascual et al., 2019), higher socioemotional competence (Ayduk et al., 2000; Duckworth et al., 2013; Eisenberg et al., 2007; Munakata and Michaelson, 2021; Shoda et al., 1990), and better general health (Robson et al., 2020). Contrarily, uncontrolled impulsivity could manifest into psychopathology such as ADHD, and internalizing and externalizing problems (Gagne et al., 2011, 2019; Lipszyc and Schachar, 2010). Taken together, EF and IC predict many health outcomes making it important to investigate the development of these cognitive functions.

The development of EF and IC starts in the first few years (Best and Miller, 2010; Garon et al., 2008; Swingler et al., 2011), making the period from early life to toddlerhood a crucial age to investigate. Early life factors that predict EF and IC have received attention from researchers. Various factors seem to predict EF and IC, includ-

ing genetics, epigenetics, and nutrition (Coda and Gräff, 2020; Costello et al., 2020). Specifically nutrition, and especially early life nutrition, may play an essential role in the development of EF and IC (Costello et al., 2020).

Early life nutrition

Early life refers to the first 1000 days from the moment of conception, and is known as a vulnerable and susceptible life phase which is subject to external factors that could impact many developmental processes with long-lasting effects (Robertson et al., 2019). Importantly, the brain develops most rapidly in the first 1000 days of life (Moore, 2016). As early life nutrition provides the building blocks for the development and refinement of the brain (Prado and Dewey, 2014), early life nutrition is an important topic of interest for investigating future brain and behavioural outcomes.

Breastfeeding duration

For many infants, breastfeeding is the primary nutrition during the first period after birth (Theurich et al., 2019). The World Health Organization recommends exclusive breastfeeding for the first six months after birth, and continued breastfeeding until two years of age or beyond for optimal child development (World Health Organization, 2017). Indeed, different breastfeeding parameters, such as initiation and duration, have been related to fewer illnesses, lower risk of obesity, higher diet quality (Pattison et al., 2019; Ventura, 2017), and possibly improved child cognition and behaviour (Hou et al., 2021; Lucas, 2005; Moody et al., 2017; Prado and Dewey, 2014; Victora et al., 2016). However, research on breastfeeding and toddler EF and IC has been scarce, with divergent results (Belfort et al., 2016; Julvez et al., 2007; Lopez et al., 2021). Therefore, the first aim was to investigate the relations between early life nutritional factors and later child EF and IC. Specifically, in **Chapter 2**, the duration of breastfeeding was investigated in relation to EF and IC. In addition, there is evidence that early life nutrition

predicts toddler diet quality (Ventura, 2017), and diet quality may relate to IC (Cohen et al., 2016). Hence, we also investigated whether toddler diet quality mediated the relation between breastfeeding duration and toddler EF and IC.

Breast milk content

Next to discovering links between breastfeeding duration and toddler EF and IC, the constituents of human milk are also of interest. This is underpinned by the Lactocrine Programming hypothesis, which is the theory that non-nutritive bioactive components in milk contribute to the infants' developmental trajectory with long-term consequences in adulthood (Bartol et al., 2008; Hinde et al., 2013). Human milk contains multiple solid components which provide the infant with building blocks to develop properly, such as hormones, microbiota, growth factors, and nutrients (Kim and Yi, 2020). Evidence is accumulating that non-nutritive milk constituents predict cognitive outcomes (de Weerth et al., 2022). One of the most abundant solid components in human milk are human milk oligosaccharides (HMOs) (Bode, 2012). Interestingly, HMOs serve as nutrition for the intestinal gut microbiota rather than for the infant (Bode, 2015). HMO composition changes throughout lactation, and differs greatly between women (Borewicz et al., 2020; Soyyilmaz et al., 2021; Thum et al., 2021). Animal studies have confirmed a causal association between specific HMOs and animal cognition (Docq et al., 2020). However, this relation has been less well established in humans. Hence, in **Chapter 3**, we investigated relations between HMO exposure in early life (at two, six, and 12 weeks of age) and toddler EF and IC. The microbiota-gut-brain axis is hypothesized to be central to understanding nutrition-behaviour associations and will be explained in the next paragraph.

Microbiota-gut-brain axis

A bi-directional communication route exists between the gastrointestinal tract and the brain, also known as the gut-brain axis (Dinan and Cryan, 2017). There are several pathways through which this communication takes place, including immunological, endocrine, and neurological pathways (Cryan et al., 2019; Cryan and Dinan, 2012). Indeed, studies show that the psychological state (e.g., stress) can affect digestive function (e.g., gut permeability), and reversely, that the digestive function drives the psychological state (Borre et al., 2014; Collins, 2001; Cryan and Dinan, 2012). In addition to this, another important key regulator in this bi-directional communication route was discovered, namely the gut microbiota.

The gut microbiota are a community of microorganisms, including bacteria and fungi, that are present in the gut (Thursby and Juge, 2017). The gut microbiota live in symbiosis with the host by fermenting dietary fibres, and endogenous intestinal mucus, resulting in production of substrates that impact host phenotype, fitness, and health (Liu et al., 2022). Just like the brain, the gut microbiota also develop rapidly in the first 1000 days of life (de Weerth, 2017; Wang et al., 2018). Infants are born with virtually sterile intestines, with the birth mode importantly affecting the initial gut microbiota colonisation (Korpela, 2021). Vaginally born infants have similar gut microbiota composition to their mother's vaginal microbiota (i.e., high abundance of *Bifidobacteria*, *Bacteroides*, and *Lactobacillus*), while infants born via C-section have a gut microbiota composition similar to the mother's skin and environment they were born in (i.e., high abundance of *Staphylococcus*, *Streptococcus*, and *Clostridium*) (Coelho et al., 2021). After birth, many environmental factors will influence how the gut microbiota colonisation proceeds. Examples are the living environment, antibiotics use, medication, genetics, and nutrition (Wen and Duffy, 2017).

As part of the microbiota-gut-brain axis, the microbiota are an important regu-

lator between the gut and the brain (Cryan et al., 2019). Gut microbiota produce metabolites, and neurotransmitters that affect the pathways involved in the interaction between the gut and brain (Cryan et al., 2019; Gacias et al., 2016), see Figure 1.2. Mechanistic explanations for how the gut microbiota can affect the brain and behaviour are derived from rodent studies, as causal relations are difficult to determine in humans. Robust causal results from rodent studies were found for specific bacteria in relation to social behaviour and anxiety (Cowan et al., 2016; Li et al., 2018; Sgritta et al., 2019; Tabouy et al., 2018). As a result, the microbiota-gut-brain axis is considered an important biological mechanism contributing to brain and behavioural development (Cryan and O'Mahony, 2011; Cryan et al., 2019; Dinan and Cryan, 2015). Specifically, because early life has been shown to be a critical developmental phase where the brain develops rapidly (Moore, 2016), the focus on early life gut microbiota development in relation to brain and cognition has been receiving increasing attention.

Several studies investigated the relations between gut microbiota and cognitive outcomes, but results have been divergent so far (Aatsinki et al., 2020; Carlson et al., 2017; Rothenberg et al., 2021; Streit et al., 2021; Tamana et al., 2021). Because nutrition affects the composition of the gut microbiota, nutrition may be able to indirectly affect brain and behaviour through the microbiota-gut-brain axis. Thus, to discover how nutrition, such as breastfeeding duration and HMOs, predicts behaviour, we investigated a potential underlying mechanism behind the relations between nutrition and cognitive and behavioural outcomes (aim 2). Specifically, in **Chapter 4**, we investigated whether gut microbiota composition at ages two, six, and 12 weeks, and one and three years, relates to toddler EF, IC, and problem behaviour.

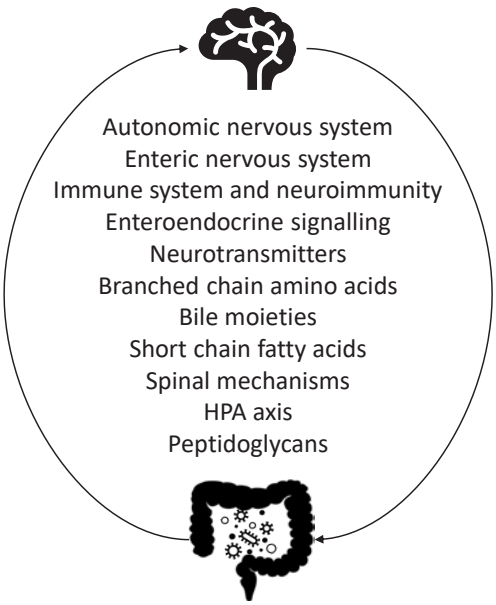


Figure 1.2. Schematic view of the microbiota gut brain axis, and the pathways involved (based on Cryan et al. (2019)). Some icons are obtained from thenounproject.com (Maxim Kulikov, Peter van Driel).

Adolescent nutrition and its predictors

Nutrition plays an important role in brain development. However, nutrition is also involved in many other bodily processes that stimulate development of the immune system, muscle growth, and neurodevelopment, as well as prevention of non-communicable diseases, etc. (Das et al., 2017; Norris et al., 2022). It is therefore extremely valuable to determine how healthy dietary habits are established. Especially, adolescence is a developmental phase where the risk for unhealthy nutritional behaviours is increased (Limbers et al., 2021; World Health Organization, 2005). Moreover, dietary habits during adolescence are shown to last until adulthood, with profound psychological and physical implications (Movassagh et al., 2017). Despite knowing this, the possible factors involved in the development and variability of adolescent nutritional behaviours

are under-investigated.

One of the important factors predicting healthy adolescent nutritional behaviours may be maternal caregiving quality, as it is closely related to child development and health. Caregiving quality is characterised by how sensitively, responsively, and consistently the mother attends to the needs of her child (Layzer and Goodson, 2006). Studies have shown that higher maternal caregiving quality is related to better child and adolescent behaviour, like educational performance and psychosocial development (Guyon-Harris et al., 2021; Raby et al., 2015; Scherer et al., 2019; Sroufe et al., 2010). However, it is unclear whether maternal caregiving quality predicts adolescent nutritional behaviours, though there is some evidence suggesting that caregiving quality independently relates to toddler and child diet quality (see reviews by Hughes and Papaioannou (2018) and Sleddens et al. (2011)). Whether this relation holds in adolescence is unclear as studies have shown inconsistent results, either finding positive relations between higher parental caregiving quality and higher intake of healthy foods, and lower intake of unhealthy foods (Kim, 2007; Kim et al., 2008; Kremers et al., 2003; Lytle et al., 2003; Pearson et al., 2010; Zietz et al., 2022), or null results (Kim, 2007; Kim et al., 2008; Vereecken et al., 2009).

If maternal caregiving quality were indeed to predict adolescent nutritional behaviours, the mechanism that would underlie this potential association is as yet unclear. A candidate mechanism may be adolescent IC. Indeed, many studies have shown that caregiving quality, both in the short and long term, is of major importance for child self-regulation and IC (Bernier et al., 2010; Bosquet Enlow et al., 2019; Bravo et al., 2023; Brophy-Herb et al., 2012; Frick et al., 2019a,b; Geeraerts et al., 2021; Jennings et al., 2008; Kochanska et al., 2000; Kok et al., 2013; Spinrad et al., 2012; von Suchodoletz et al., 2011; Vrijhof et al., 2020; Wu et al., 2022). To our knowledge, only one study was performed at adolescent age, and found that higher maternal caregiving quality at child age seven years predicted less over-inhibition in adolescence (van der Voort et al.,

2014). In addition, some studies have found that high levels of adolescent inhibitory control contribute to making healthier food choices during adolescence, subsequently improving adolescents' diet quality (Ames et al., 2014; Byrne et al., 2021). Hence, in **Chapter 5** (aim 3), we investigated whether maternal caregiving quality throughout childhood, from child age of five weeks to 14 years, predicted adolescent diet quality and emotional eating behaviour, and whether adolescent inhibitory control mediated this relation.

Studies involved in this thesis

All aims of this thesis were addressed in healthy Dutch children, from birth till 14 years of age, and their parents. To investigate the three research aims of the current thesis, data from the BINGO and BIBO studies were used.

BINGO study

The BINGO study, Dutch acronym for Biological Influences on Baby's Health and Development (in Dutch: Biologische INvloeden op baby's Gezondheid en Ontwikkeling), is a prospective longitudinal study that included and followed 88 pregnant mothers, 57 partners, and their children from pregnancy until the child was three years old (for details, see Hechler et al. (2018), and https://dpblab.org/projects/bingo). The main purpose of the BINGO study was to investigate the relations between prenatal and early postnatal factors on the psychobiological development of the child.

In the current thesis, data from multiple assessments within the three postnatal years were used. At two, six, and 12 weeks, maternal milk samples and child stool samples were collected for determination of human milk oligosaccharides and gut microbiota composition, respectively. In addition, child stool samples were collected at one and three years of age. Furthermore, mothers prospectively reported whether and how (i.e., exclusively, or partially) they were breastfeeding in the first three postnatal

years. At three years of age, mothers digitally reported their child's nutritional intake for three random days (two weekdays, and one weekend day). Furthermore, both parents filled in questionnaires about their child's EF and IC. In addition, child IC was assessed with six behavioural tasks.

BIBO study

The BIBO study, Dutch acronym for Basal Influences on Baby Development (in Dutch: Basale Invloeden op de Baby Ontwikkeling), is an ongoing prospective longitudinal study that included and followed 193 healthy mothers and their healthy children from pregnancy onwards (for details, see Beijers et al. (2010, 2013), and https://dpblab.org/projects/bibo). The main purpose of the BIBO study is to investigate the relations between prenatal and early life environment on the psychobiological development of children.

In the current thesis, data from multiple assessments within the 14 postnatal years were used. At the age of five weeks, 12 months, 2.5 years, 10 years, and 14 years, mothers and their children performed different age-appropriate naturalistic interaction tasks from which quality of maternal caregiving behaviour was observed (Beijers et al., 2020). Furthermore, at age 14 years, adolescents reported their habitual nutritional intake and emotional eating behaviour, online and on paper, respectively. Lastly, also at age 14, adolescent inhibitory control was assessed with three behavioural tasks during a home visit and an online maternal report.

Thesis outline

This thesis consists of four empirical observational studies that are presented in four chapters (Chapter 2–5). Chapter 2 is the study on breastfeeding duration in relation to toddler EF and IC, including diet quality as a mediator. Chapter 3 is the study on mother milk constituents, namely HMOs, in relation to toddler EF and IC. Chapter 4

is the study on gut microbiota composition in relation to toddler EF and IC, as well as problem behaviour. Chapter 5 is the study on maternal caregiving quality throughout childhood in relation to adolescent diet quality and emotional eating behaviour, and the potential mediating role of adolescent inhibitory control in these relations. Chapter 6 contains a general discussion, and a conclusion. Chapter 7 contains supplementary materials of Chapter 2-5. Chapter 8 contains the appendices of this thesis. Figure 1.1 shows the overview of this thesis including the research aims and chapters.

This thesis contributes to the understanding of how nutrition plays a role in cognition, and how nutrition is shaped through maternal caregiving. Increasing this body of literature aids in the possible future development of interventions to improve toddler and adolescent cognition and nutritional behaviours.

References

Aatsinki, A.-K., Kataja, E.-L., Munukka, E., Lahti, L., Keskitalo, A., Korja, R., Nolvi, S., Häikiö, T., Tarro, S., Karlsson, H., and Karlsson, L. (2020). Infant fecal microbiota composition and attention to emotional faces. *Emotion*, 22(6):1159.

Albanese, A. M., Russo, G. R., and Geller, P. A. (2019). The role of parental self-efficacy in parent and child well-being: A systematic review of associated outcomes. *Child: Care, Health and Development*, 45(3):333–363.

Allom, V., Mullan, B., and Hagger, M. (2015). Does inhibitory control training improve health behaviour? A meta-analysis. *Health Psychology Review*, 10(2):168–186.

American Psychological Association (2023). Inhibition –APA Dictionary of Psychology.

Ames, S. L., Kisbu-Sakarya, Y., Reynolds, K. D., Boyle, S., Cappelli, C., Cox, M. G., Dust, M., Grenard, J. L., Mackinnon, D. P., and Stacy, A. W. (2014). Inhibitory control effects in adolescent binge eating and consumption of sugar-sweetened beverages and snacks. *Appetite*, 81:180–192.

Ayduk, O., Mendoza-Denton, R., Mischel, W., Downey, G., Peake, P. K., and Rodriguez, M. (2000). Regulating the interpersonal self: Strategic self-regulation for coping with rejection sensitivity. *Journal of Personality and Social Psychology*, 79(5):776–792.

Bartol, F. F., Wiley, A. A., and Bagnell, C. A. (2008). Epigenetic Programming of Porcine Endometrial Function and the Lactocrine Hypothesis. *Reproduction in Domestic Animals*, 43:273–279.

Beijers, R., Hartman, S., Shalev, I., Hastings, W., Mattern, B. C., de Weerth, C., and Belsky, J. (2020). Testing three hypotheses about effects of sensitive-insensitive parenting on telomeres. *Developmental Psychology*, 56(2):237–250.

Beijers, R., Jansen, J., Riksen-Walraven, M., and de Weerth, C. (2010). Maternal Prenatal Anxiety and Stress Predict Infant Illnesses and Health Complaints. *Pediatrics*, 126(2):e401–e409.

Beijers, R., Riksen-Walraven, M., Putnam, S., Jong, M. d., and de Weerth, C. (2013). Early non-parental care and toddler behaviour problems: Links with temperamental negative affectivity and inhibitory control. *Early Childhood Research Quarterly*, 28(4):714–722.

Belfort, M. B., Rifas-Shiman, S. L., Kleinman, K. P., Bellinger, D. C., Harris, M. H., Taveras, E. M., Gillman, M. W., and Oken, E. (2016). Infant Breastfeeding Duration and Mid-Childhood Executive Function, Behavior, and Social-Emotional Development. *Journal of developmental and behavioral pediatrics*, 37(1):43–52.

Bernier, A., Carlson, S. M., and Whipple, N. (2010). From external regulation to self-regulation: early parenting precursors of young children's executive functioning. *Child Development*, 81(1):326–339.

Best, J. R. and Miller, P. H. (2010). A Developmental Perspective on Executive Function. *Child Development*, 81(6):1641–1660.

Bode, L. (2012). Human milk oligosaccharides: Every baby needs a sugar mama. *Glycobiology*, 22(9):1147–1162.

Bode, L. (2015). The functional biology of human milk oligosaccharides. *Early Human Development*, 91(11):619–622.

Borewicz, K., Gu, F., Saccenti, E., Hechler, C., Beijers, R., de Weerth, C., van Leeuwen, S. S., Schols, H. A., and Smidt, H. (2020). The association between breastmilk oligosaccharides and faecal microbiota in healthy breastfed infants at two, six, and twelve weeks of age. *Scientific Reports 2020 10:1*, 10(1):1–12.

Borre, Y. E., Moloney, R. D., Clarke, G., Dinan, T. G., and Cryan, J. F. (2014). The impact of microbiota on brain and behavior: mechanisms & therapeutic potential. *Advances in experimental medicine and biology*, 817:373–403.

Bosquet Enlow, M., Petty, C. R., Svelnys, C., Gusman, M., Huezo, M., Malin, A., and Wright, R. J. (2019). Differential Effects of Stress Exposures, Caregiving Quality, and Temperament in Early Life on Working Memory versus Inhibitory Control in Preschool-Aged Children. *Developmental Neuropsychology*, 44(4):339–356.

Bravo, P., Cárcamo, R., Santos, S., Ringoot, A., IJzendoorn, M. H. v., Hillegers, M. H. J., and Jansen, P. W. (2023). Maternal sensitivity in early childhood and body mass index in adolescence: A population-based study on the role of self-regulation as a mediator. *Appetite*, 182:106418.

Brophy-Herb, H. E., Stansbury, K., Bocknek, E., and Horodynski, M. A. (2012). Modeling maternal emotion-related socialization behaviors in a low-income sample: Relations with toddlers' self-regulation. *Early Childhood Research Quarterly*, 27(3):352–364.

Byrne, M. E., Shank, L. M., Altman, D. R., Swanson, T. N., Ramirez, E., Moore, N. A., Rubin, S. G., LeMay-Russell, S., Parker, M. N., Kaufman, R. E., Yang, S. B., Torres, S. L., Brady, S. M., Kelly, N. R., Tanofsky-Kraff, M., and Yanovski, J. A. (2021). Inhibitory control and negative affect in relation to food intake among youth. *Appetite*, 156:104858.

Cappelli, C., Pike, J. R., Riggs, N. R., Warren, C. M., and Pentz, M. A. (2019). Executive function and probabilities of engaging in long-term sedentary and high calorie/low nutrition eating behaviors in early adolescence. *Social science & medicine*, 237:112483.

Carlson, A. L., Xia, K., Azcarate-Peril, M. A., Goldman, B. D., Ahn, M., Styner, M. A., Thompson, A. L., Geng, X., Gilmore, J. H., and Knickmeyer, R. C. (2017). Infant gut microbiome associated with cognitive development. *Biological Psychiatry*, 83(2):148–159.

Chye, Y., Suo, C., Romero-Garcia, R., Bethlehem, R. A. I., Hook, R., Tiego, J., Goodyer, I., Jones, P. B., Dolan, R., Bullmore, E. T., Grant, J. E., Yücel, M., and Chamberlain, S. R. (2021). Examining the relationship between altered brain functional connectome and disinhibition across 33 impulsive and compulsive behaviours. *The British journal of psychiatry : the journal of mental science*, 220(2):76–78.

Coda, D. M. and Gräff, J. (2020). Neurogenetic and Neuroepigenetic Mechanisms in Cognitive Health and Disease. *Frontiers in molecular neuroscience*, 13:205.

Coelho, G. D. P., Ayres, L. F. A., Barreto, D. S., Henriques, B. D., Prado, M. R. M. C., and Passos, C. M. D. (2021). Acquisition of microbiota according to the type of birth: an integrative review. *Revista latino-americana de enfermagem*, 29.

Cohen, J. F. W., Gorski, M. T., Gruber, S. A., Kurdziel, L. B. F., and Rimm, E. B. (2016). The effect of healthy dietary consumption on executive cognitive functioning in children and adolescents: a systematic review. *British Journal of Nutrition*, 116(06):989–1000.

Collins, S. M. (2001). Stress and the gastrointestinal tract IV. Modulation of intestinal inflammation by stress: Basic mechanisms and clinical relevance. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, 280(3):43–3.

Cortés Pascual, A., Moyano, N., and Robres, A. Q. (2019). The relationship between executive functions and academic performance in primary education: Review and meta-analysis. *Frontiers in psychology*, 10:1582.

Costello, S. E., Geiser, E., and Schneider, N. (2020). Nutrients for executive function development and related brain connectivity in school-aged children. *Nutrition Reviews*, 79(12):1293–1306.

Cowan, C. S. M., Callaghan, B. L., and Richardson, R. (2016). The effects of a probiotic formulation (Lactobacillus rhamnosus and L. helveticus) on developmental trajectories of emotional learning in stressed infant rats. *Translational Psychiatry*, 6(5):e823–e823.

Cryan, J. F. and Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience*, 13(10):701.

Cryan, J. F. and O'Mahony, S. M. (2011). The microbiome-gut-brain axis: from bowel to behavior. *Neurogastroenterology & Motility*, 23(3):187–192.

Cryan, J. F., O'riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., Codagnone, M. G., Cussotto, S., Fulling, C., Golubeva, A. V., Guzzetta, K. E., Jaggar, M., Long-Smith, C. M., Lyte, J. M., Martin, J. A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., O'connor, R., Cruz-Pereira, J. S., Peterson, V. L., Rea, K., Ritz, N. L., Sherwin, E., Spichak, S., Teichman, E. M., Wouw, M. v. d., Ventura-Silva, A. P., Wallace-Fitzsimons, S. E., Hyland, N., Clarke, G., and Dinan, T. G. (2019). The microbiota-gut-brain axis. *Physiological Reviews*, 99(4):1877–2013.

Das, J. K., Salam, R. A., Thornburg, K. L., Prentice, A. M., Campisi, S., Lassi, Z. S., Koletzko, B., and Bhutta, Z. A. (2017). Nutrition in adolescents: physiology, metabolism, and nutritional needs. *Annals of the New York Academy of Sciences*, 1393(1):21–33.

de Weerth, C. (2017). Do bacteria shape our development? Crosstalk between intestinal microbiota and HPA axis. *Neuroscience & Biobehavioral Reviews*, 83:458–471.

de Weerth, C., Aatsinki, A.-K., Azad, M. B., Bartol, F. F., Bode, L., Collado, M. C., Dettmer, A. M., Field, C. J., Guilfoyle, M., Hinde, K., Korosi, A., Lustermans, H., Husna, N., Shukri, M., Moore, S. E., Pundir, S., Rodriguez, J. M., Slupsky, C. M., Turner, S., Goudoever, J. B. V., Ziomkiewicz, A., and Beijers, R. (2022). Human milk: From complex tailored nutrition to bioactive impact on child cognition and behavior. *Critical reviews in food science and nutrition*, pages 1–38.

Delvecchio, E., Germani, A., Raspa, V., Lis, A., and Mazzeschi, C. (2020). Parenting Styles and Child's Well-Being: The Mediating Role of the Perceived Parental Stress. *Europe's journal of psychology*, 16(3):514–531.

Diamond, A. (2013). Executive functions. *Annual review of psychology*, 64:135–68.

Dinan, T. G. and Cryan, J. F. (2015). The impact of gut microbiota on brain and behaviour. *Current Opinion in Clinical Nutrition and Metabolic Care*, 18(6):552–558.

Dinan, T. G. and Cryan, J. F. (2017). Brain–gut–microbiota axis —mood, metabolism and behaviour. *Nature Reviews Gastroenterology & Hepatology*, 14(2):69–70.

Docq, S., Spoelder, M., Wang, W., and Homberg, J. R. (2020). The Protective and Long-Lasting Effects of Human Milk Oligosaccharides on Cognition in Mammals. *Nutrients*, 12(11):3572.

Duckworth, A. L., Tsukayama, E., and Kirby, T. A. (2013). Is It Really Self-Control? Examining the Predictive Power of the Delay of Gratification Task. *Personality and Social Psychology Bulletin*, 39(7):843–855.

Eisenberg, N., Hofer, C., and Vaughan, J. (2007). Effortful control and its socioemotional consequences. *Handbook of emotion regulation*, 2:287–288.

Frick, M. A., Forslund, T., and Brocki, K. C. (2019a). Can reactivity and regulation in infancy predict inattentive and hyperactive/impulsive behavior in 3-year-olds? *Development and Psychopathology*, 31(2):619–629.

Frick, M. A., Forslund, T., and Brocki, K. C. (2019b). Does child verbal ability mediate the relationship between maternal sensitivity and later self-regulation? A longitudinal study from infancy to 4 years. *Scandinavian journal of psychology*, 60(2):97–105.

Gacias, M., Gaspari, S., Santos, P. M. G., Tamburini, S., Andrade, M., Zhang, F., Shen, N., Tolstikov, V., Kiebish, M. A., Dupree, J. L., Zachariou, V., Clemente, J. C., and Casaccia, P. (2016). Microbiota-driven transcriptional changes in prefrontal cortex override genetic differences in social behavior. *eLife*, 5:e13442.

Gagne, J. R., Chang, C.-N., Fang, H., Spann, C., and Kwok, O.-M. (2019). A multimethod study of inhibitory control and behavioural problems in preschoolers. *Infant and Child Development*, 28(1):e2115.

Gagne, J. R., Saudino, K. J., and Asherson, P. (2011). The genetic etiology of inhibitory control and behavior problems at 24 months of age. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, 52(11):1155–1163.

Garon, N., Bryson, S. E., and Smith, I. M. (2008). Executive Function in Preschoolers: A Review Using an Integrative Framework. *Psychological bulletin*, 134(1):31.

Geeraerts, S. B., Endendijk, J. J., Deković, M., Huijding, J., Deater-Deckard, K., and Mesman, J. (2021). Inhibitory Control Across the Preschool Years: Developmental Changes and Associations with Parenting. *Child Development*, 92(1):335.

Guyon-Harris, K. L., Humphreys, K. L., Miron, D., Tibu, F., Fox, N. A., Nelson, C. A., and Zeanah, C. H. (2021). Early caregiving quality predicts consistency of competent functioning from middle childhood to adolescence following early psychosocial deprivation. *Development and psychopathology*, 33(1):18–28.

Hechler, C., Beijers, R., Riksen-Walraven, J. M., and de Weerth, C. (2018). Are cortisol concentrations in human breast milk associated with infant crying? *Developmental Psychobiology*, 60(6):639–650.

Hinde, K., Foster, A. B., Landis, L. M., Rendina, D., Oftedal, O. T., and Power, M. L. (2013). Daughter dearest: Sex-biased calcium in mother's milk among rhesus macaques. *American Journal of Physical Anthropology*, 151(1):144–150.

Hou, L., Li, X., Yan, P., Li, Y., Wu, Y., Yang, Q., Shi, X., Ge, L., and Yang, K. (2021). Impact of the Duration of Breastfeeding on the Intelligence of Children: A Systematic Review with Network Meta-Analysis. *Breastfeeding Medicine*, 16(9):687–696.

Hughes, S. O. and Papaioannou, M. A. (2018). Maternal Predictors of Child Dietary Behaviors and Weight Status. *Current nutrition reports*, 7(4):268–273.

Jennings, K. D., Sandberg, I., Kelley, S. A., Valdes, L., Yaggi, K., Abrew, A., and Macey-Kalcevic, M. (2008). Understanding of self and maternal warmth predict later self-regulation in toddlers. *International Journal of Behavioral Development*, 32(2):108–118.

Julvez, J., Ribas-Fitó, N., Forns, M., Garcia-Esteban, R., Torrent, M., and Sunyer, J. (2007). Attention behaviour and hyperactivity at age 4 and duration of breast-feeding. *Acta Paediatrica*, 96(6):842–847.

Khozaei, F. and Carbon, C. C. (2022). On the Parental Influence on Children's Physical Activities and Mental Health During the COVID-19 Pandemic. *Frontiers in Psychology*, 13:1197.

Kim, M. J. (2007). *Parenting style and older children's and young adolescents' dietary intake and nutritional status*. PhD thesis, Texas A&M University.

Kim, M. J., McIntosh, W. A., Anding, J., Kubena, K. S., Reed, D. B., and Moon, G. S. (2008). Perceived parenting behaviours predict young adolescents' nutritional intake and body fatness. *Maternal & child nutrition*, 4(4):287–303.

Kim, S. Y. and Yi, D. Y. (2020). Components of human breast milk: from macronutrient to microbiome and microRNA. *Clinical and Experimental Pediatrics*, 63(8):301.

Kochanska, G., Murray, K. T., and Harlan, E. T. (2000). Effortful control in early childhood: continuity and change, antecedents, and implications for social development. *Developmental psychology*, 36(2):220–232.

Kok, R., Lucassen, N., Bakermans-Kranenburg, M. J., Ijzendoorn, M. H. V., Ghassabian, A., Roza, S. J., Govaert, P., Jaddoe, V. W., Hofman, A., Verhulst, F. C., and Tiemeier, H. (2013). Parenting, corpus callosum, and executive function in preschool children. *Child neuropsychology : a journal on normal and abnormal development in childhood and adolescence*, 20(5):583–606.

Korpela, K. (2021). Impact of Delivery Mode on Infant Gut Microbiota. *Annals of nutrition & metabolism*, 77:11–19.

Kremers, S. P. J., Brug, J., Vries, H. D., and Engels, R. C. M. E. (2003). Parenting style and adolescent fruit consumption. *Appetite*, 41(1):43–50.

Layzer, J. I. and Goodson, B. D. (2006). The "Quality " of Early Care and Education Settings. *Evaluation review*, 30(5):556–576.

Li, N., Wang, Q., Wang, Y., Sun, A., Lin, Y., Jin, Y., and Li, X. (2018). Oral probiotics ameliorate the behavioral deficits induced by chronic mild stress in mice via the gut microbiota-inflammation axis. *Frontiers in Behavioral Neuroscience*, 12:266.

Li, W., Shang, Y., Zhuang, W., Mai, W., Cheng, W., and Chen, Z. (2022). Effectiveness of Response Inhibition Training and Its Long-Term Effects in Healthy Adults: A Systematic Review and Meta-Analysis. *Frontiers in neuroscience*, 16.

Limbers, C. A., Summers, E., Smith, K., and Lavender, J. (2021). Emotional Eating and Weight Status in Adolescents: A Systematic Review. *International Journal of Environmental Research and Public Health*, 18(3):991.

Lipszyc, J. and Schachar, R. (2010). Inhibitory control and psychopathology: A meta-analysis of studies using the stop signal task. *Journal of the International Neuropsychological Society*, 16(6):1064–1076.

Liu, J., Tan, Y., Cheng, H., Zhang, D., Feng, W., and Peng, C. (2022). Functions of Gut Microbiota Metabolites, Current Status and Future Perspectives. *Aging and Disease*, 13(4):1106.

Lopez, D. A., Foxe, J. J., Mao, Y., Thompson, W. K., Martin, H. J., and Freedman, E. G. (2021). Breastfeeding Duration Is Associated With Domain-Specific Improvements in Cognitive Performance in 9–10-Year-Old Children. *Frontiers in Public Health*, 9:657422.

Lucas, A. (2005). Long-Term Programming Effects of Early Nutrition —Implications for the Preterm Infant. *Journal of Perinatology*, 25(2):S2–S6.

Lytle, L. A., Varnell, S., Murray, D. M., Story, M., Perry, C., Birnbaum, A. S., and Kubik, M. Y. (2003). Predicting adolescents' intake of fruits and vegetables. *Journal of nutrition education and behavior*, 35(4):170–178.

Moody, L., Chen, H., and Pan, Y. X. (2017). Early-Life Nutritional Programming of Cognition-The Fundamental Role of Epigenetic Mechanisms in Mediating the Relation between Early-Life Environment and Learning and Memory Process. *Advances in nutrition*, 8(2):337–350.

Moore, S. E. (2016). Early life nutritional programming of health and disease in The Gambia. *Journal of Developmental Origins of Health and Disease*, 7(2):123–131.

Movassagh, E. Z., Baxter-Jones, A. D. G., Kontulainen, S., Whiting, S. J., and Vatanparast, H. (2017). Tracking Dietary Patterns over 20 Years from Childhood through Adolescence into Young Adulthood: The Saskatchewan Pediatric Bone Mineral Accrual Study. *Nutrients*, 9(9):1–14.

Munakata, Y. and Michaelson, L. E. (2021). Executive Functions in Social Context: Implications for Conceptualizing, Measuring, and Supporting Developmental Trajectories. *Developmental Psychology*, 3(1):139–163.

Norris, S. A., Frongillo, E. A., Black, M. M., Dong, Y., Fall, C., Lampl, M., Liese, A. D., Naguib, M., Prentice, A., Rochat, T., Stephensen, C. B., Tinago, C. B., Ward, K. A., Wrottesley, S. V., and Patton, G. C. (2022). Nutrition in adolescent growth and development. *The Lancet*, 399(10320):172–184.

Oriach, C. S., Robertson, R. C., Stanton, C., Cryan, J. F., and Dinan, T. G. (2016). Food for thought: The role of nutrition in the microbiota-gut-brain axis. *Clinical Nutrition Experimental*, 6:25–38.

Pattison, K. L., Kraschnewski, J. L., Lehman, E., Savage, J. S., Downs, D. S., Leonard, K. S., Adams, E. L., Paul, I. M., and Kjerulff, K. H. (2019). Breastfeeding Initiation and Duration and Child Health Outcomes in the First Baby Study. *Preventive medicine*, 118:1.

Pearson, N., Atkin, A. J., Biddle, S. J. H., Gorely, T., and Edwardson, C. (2010). Parenting styles, family structure and adolescent dietary behaviour. *Public health nutrition*, 13(8):1245–1253.

Prado, E. L. and Dewey, K. G. (2014). Nutrition and brain development in early life. *Nutrition Reviews*, 72(4):267–284.

Raby, K. L., Roisman, G. I., Fraley, R. C., and Simpson, J. A. (2015). The Enduring Predictive Significance of Early Maternal Sensitivity: Social and Academic Competence Through Age 32 Years. *Child Development*, 86(3):695–708.

Rice, D. and Barone, S. (2000). Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environmental health perspectives*, 108(suppl 3):511–533.

Robertson, R. C., Manges, A. R., Finlay, B. B., and Prendergast, A. J. (2019). The Human Microbiome and Child Growth - First 1000 Days and Beyond. *Trends in microbiology*, 27(2):131–147.

Robson, D. A., Allen, M. S., and Howard, S. J. (2020). Self-Regulation in Childhood as a Predictor of Future Outcomes: A Meta-Analytic Review. *Psychological Bulletin*, 146(4).

Rothenberg, S. E., Chen, Q., Shen, J., Nong, Y., Nong, H., Trinh, E. P., Biasini, F. J., Liu, J., Zeng, X., Zou, Y., Ouyang, F., and Korrick, S. A. (2021). Neurodevelopment correlates with gut microbiota in a cross-sectional analysis of children at 3 years of age in rural China. *Scientific Reports*, 11(1):1–11.

Scherer, E., Hagaman, A., Chung, E., Rahman, A., O'Donnell, K., and Maselko, J. (2019). The relationship between responsive caregiving and child outcomes: evidence from direct observations of mother-child dyads in Pakistan. *BMC public health*, 19(1).

Sgritta, M., Dooling, S. W., Buffington, S. A., Momin, E. N., Francis, M. B., Britton, R. A., and Costa-Mattioli, M. (2019). Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in Mouse Models of Autism Spectrum Disorder. *Neuron*, 101(2):246–259.e6.

Shoda, Y., Mischel, W., and Peake, P. K. (1990). Predicting Adolescent Cognitive and Self-Regulatory Competencies From Preschool Delay of Gratification: Identifying Diagnostic Conditions. *Developmental Psychology*, 26(6):978–986.

Sleddens, E. F. C., Gerards, S. M. P. L., Thijs, C., Vries, N. K. D., and Kremers, S. P. J. (2011). General parenting, childhood overweight and obesity-inducing behaviors: a review. *International journal of pediatric obesity*, 6(sup3):e12–27.

Soyyilmaz, B., Mikš, M. H., Röhrig, C. H., Matwiejuk, M., Meszaros-matwiejuk, A., and Vigsnæs, L. K. (2021). The mean of milk: A review of human milk oligosaccharide concentrations throughout lactation. *Nutrients*, 13(8).

Spinrad, T. L., Eisenberg, N., Silva, K. M., Eggum, N. D., Reiser, M., Edwards, A., Iyer, R., Kupfer, A. S., Hofer, C., Smith, C. L., Hayashi, A., and Gaertner, B. M. (2012). Longitudinal Relations Among Maternal Behaviors, Effortful Control and Young Children's Committed Compliance. *Developmental Psychology*, 48(2):552.

Sroufe, L. A., Coffino, B., and Carlson, E. A. (2010). Conceptualizing the Role of Early Experience: Lessons from the Minnesota Longitudinal Study. *Developmental review*, 30(1):36.

Streit, F., Prandovszky, E., Send, T., Zillich, L., Frank, J., Sabunciyan, S., Foo, J., Sirignano, L., Lange, B., Bardtke, S., Hatfield, G., Witt, S. H., Gilles, M., Rietschel, M., Deuschle, M., and Yolken, R. (2021). Microbiome profiles are associated with cognitive functioning in 45-month-old children. *Brain, behavior, and immunity*, 98:151–160.

Swingler, M. M., Willoughby, M. T., and Calkins, S. D. (2011). EEG power and coherence during preschoolers' performance of an executive function battery. *Developmental Psychobiology*, 53(8):771–784.

Tabouy, L., Getselter, D., Ziv, O., Karpuj, M., Tabouy, T., Lukic, I., Maayouf, R., Werbner, N., Ben-Amram, H., Nuriel-Ohayon, M., Koren, O., and Elliott, E. (2018). Dysbiosis of microbiome and probiotic treatment in a genetic model of autism spectrum disorders. *Brain, Behavior, and Immunity*, 73:310–319.

Tamana, S. K., Tun, H. M., Konya, T., Chari, R. S., Field, C. J., Guttman, D. S., Becker, A. B., Moraes, T. J., Turvey, S. E., Subbarao, P., Sears, M. R., Pei, J., Scott, J. A., Mandhane, P. J., and Kozyrskyj, A. L. (2021). Bacteroides-dominant gut microbiome of late infancy is associated with enhanced neurodevelopment. *Gut Microbes*, 13(1):1–17.

Theurich, M. A., Davanzo, R., Busck-Rasmussen, M., Díaz-Gómez, N. M., Brennan, C., Kylberg, E., Bærug, A., McHugh, L., Weikert, C., Abraham, K., and Koletzko, B. (2019). Breastfeeding Rates and Programs in Europe: A Survey of 11 National Breastfeeding Committees and Representatives. *Journal of pediatric gastroenterology and nutrition*, 68(3):400–407.

Thum, C., Wall, C. R., Weiss, G. A., Wang, W., Szeto, I. M. Y., and Day, L. (2021). Changes in hmo concentrations throughout lactation: Influencing factors, health effects and opportunities. *Nutrients*, 13(7).

Thursby, E. and Juge, N. (2017). Introduction to the human gut microbiota. *Biochemical Journal*, 474(11):1823.

van der Voort, A., Linting, M., Juffer, F., Bakermans-Kranenburg, M. J., Schoenmaker, C., and IJzendoorn, M. H. v. (2014). The development of adolescents' internalizing behavior: longitudinal effects of maternal sensitivity and child inhibition. *Journal of youth and adolescence*, 43(4):528–540.

Vandeputte, D., Commer, L. D., Tito, R. Y., Kathagen, G., Sabino, J., Vermeire, S., Faust, K., and Raes, J. (2021). Temporal variability in quantitative human gut microbiome profiles and implications for clinical research. *Nature Communications 2021 12:1*, 12(1):1–13.

Ventura, A. K. (2017). Does Breastfeeding Shape Food Preferences? Links to Obesity. *Annals of Nutrition and Metabolism*, 70(3):8–15.

Vereecken, C., Legtest, E., Bourdeaudhuij, I. D., and Maes, L. (2009). Associations between General Parenting Styles and Specific Food-Related Parenting Practices and Children's Food Consumption. *American Journal of Health Promotion*, 23(4):233–240.

Victora, C. G., Bahl, R., Barros, A. J. D., França, G. V. A., Horton, S., Krasevec, J., Murch, S., Sankar, M. J., Walker, N., Rollins, N. C., and Group, L. B. S. (2016). Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. *The Lancet*, 387(10017):475–90.

von Suchodoletz, A., Trommsdorff, G., and Heikamp, T. (2011). Linking Maternal Warmth and Responsiveness to Children's Self-regulation. *Social Development*, 20(3):486–503.

Vrijhof, C. I., Euser, S., Bakermans-Kranenburg, M. J., Bulk, B. G. v. d., Bosdriesz, J. R., Linting, M., Wijk, I. C. v., Visser, I. d., and IJzendoorn, M. H. v. (2020). Effects of parental sensitivity in different contexts on children's hot and cool effortful control. *Journal of family psychology*, 34(4).

Wang, S., Harvey, L., Martin, R., Beek, E. M. v. d., Knol, J., Cryan, J. F., and Renes, I. B. (2018). Targeting the gut microbiota to influence brain development and function in early life. *Neuroscience & Biobehavioral Reviews*, 95:191–201.

Wen, L. and Duffy, A. (2017). Factors Influencing the Gut Microbiota, Inflammation, and Type 2 Diabetes. *The Journal of Nutrition*, 147(7):1468S–1475S.

World Health Organization (2005). Nutrition in adolescence: issues and challenges for the health sector: issues in adolescent health and development.

World Health Organization (2017). WHO recommendations on child health: guidelines approved by the WHO Guidelines Review Committee.

Wu, Q., Jalapa, K., Han, S. J., Tawfiq, D., and Cui, M. (2022). A dynamic systems perspective towards executive function development: Susceptibility at both ends for inhibitory control. *Development and Psychopathology*, pages 1–9.

Zietz, S., Cheng, E., Lansford, J. E., Deater-Deckard, K., Giunta, L. D., Dodge, K. A., Gurdal, S., Liu, Q., Long, Q., Oburu, P., Pastorelli, C., Skinner, A. T., Sorbring, E., Steinberg, L., Tapanya, S., Tirado, L. M. U., Yotanyamaneewong, S., Alampay, L. P., Al-Hassan, S. M., Bacchini, D., Chang, L., and Bornstein, M. H. (2022). Positive parenting, adolescent adjustment, and quality of adolescent diet in nine countries. *Journal of adolescence*, 94(8):1130–1141.

Chapter 2

Do Breastfeeding History and Diet Quality Predict Inhibitory Control at Preschool Age?

Based on: Willemsen, Y., Beijers, R., Arias Vásquez, A., de Weerth, C., Do Breastfeeding History and Diet Quality Predict Inhibitory Control at Preschool Age? *Nutrients*, 2021, 13, 2752.

Abstract

Inhibitory control is the ability to control impulsive behaviour. It is associated with a range of mental and physical health outcomes, including attention deficit hyperactivity disorder and substance dependence. Breastfeeding and healthy dietary patterns have been associated with better executive functions, of which inhibitory control is part. Additionally, breastfeeding has been associated with healthy dietary patterns. Following our preregistration in the Open Science Framework, we investigated the associations between breastfeeding history and inhibitory control at preschool age, with habitual diet quality as a potential mediating factor. A total of 72 families from a longitudinal study participated at child age three. Breastfeeding questionnaires were administered at two, six, and 12 weeks, and at 12 and 36 months. Six inhibitory control tasks were performed during a home visit, and questionnaires were filled in by both parents. Diet quality at age three was assessed via three unannounced 24-h recalls. Structural equation modelling was performed in R. This study did not provide evidence that breastfeeding history is associated with inhibitory control in three-year-old children. Furthermore, diet quality at age three did not mediate the link between breastfeeding history and inhibitory control. Previous studies have investigated broader aspects of inhibitory control, such as executive functions, and used different methods to assess nutritional intake, which might explain our differential findings. Our findings contribute to the growing literature on associations between nutrition and behaviour. Future replications with larger and more diverse preschool samples are recommended.

Introduction

Inhibitory control is the ability to control impulsive behaviour (American Psychological Association, 2019), and is an integral part of higher-order executive functioning (EF) (Diamond, 2013). Inhibitory control has been shown to predict a range of health, wealth, and public safety outcomes including physical health, substance dependence, personal finances, and criminal offending outcomes (Moffitt et al., 2011). Furthermore, studies have associated poor inhibitory control with psychopathology, such as attention deficit hyperactivity disorder (ADHD), and child internalizing and externalizing problems (Gagne et al., 2011, 2019; Lipszyc and Schachar, 2010). Several factors have been associated with higher levels of inhibitory control, including high socioeconomic status, high parenting quality, and genetic factors (Cheng et al., 2018; Engelhardt et al., 2015; St. John et al., 2019). Nutrition has also been associated with inhibitory control, though its exact role remains unclear (Egbert et al., 2019). One biological pathway hypothesized to be involved in the association between breastfeeding, diet, and inhibitory control is the microbiota-gut-brain axis, which is a bidirectional communication route between bacteria in the gut and the brain (Cryan et al., 2019). Gut microbiota are in constant interplay with diet; thus it is possible that the gut microbiota moderate the effects of diet on the brain and potentially affect behaviour (Cryan et al., 2019; Vernocchi et al., 2020). As inhibitory control develops quickly early in life, early life nutrition might be especially important. This paper investigated the associations between breastfeeding history, habitual diet, and preschoolers' inhibitory control.

After birth, breast milk is often the first source of nutrition for infants. A continuously increasing body of research shows that early life nutrition shapes the brain, hence affecting its function throughout development, and having major lifelong effects on cognition and behaviour (Georgieff et al., 2015). Thus, breastfeeding plays an important role in health and early development (Duijts et al., 2009; Victora et al., 2016).

For example, Bar et al. (2016) reviewed studies investigating benefits of breastfeeding in relation to cognitive development, and ADHD in preschool and school-age children. They found that children who were breastfed longer than six months had better cognitive outcomes compared to children who were breastfed for shorter times (Bar et al., 2016). Furthermore, a meta-analysis concluded that breastfeeding may reduce the risk of ADHD in 3- to 17-year-old children (Zeng et al., 2018). However, while one prospective longitudinal study including 500 preschoolers found that EF (assessed by validated questionnaires) was better when they were breastfed longer (Julvez et al., 2007), another prospective longitudinal study including 180 children found no relation between breastfeeding and EF (assessed with cognitive tests) in 6- to 7-year-old children (Belfort et al., 2016). Additionally, a recent cross sectional study by Lopez et al. (2021) also found no evidence for an association between breastfeeding and executive functioning (assessed with cognitive tests) in 9,116 9-10-year-old children (Lopez et al., 2021). To our knowledge, no study has specifically examined associations between breastfeeding history and inhibitory control in three-year-olds.

The association between habitual diet and child EF, including inhibitory control, is equivocal (see review by Egbert et al. (2019)). For example, the two cross-sectional studies by Levitan et al. (2014) (n=193) and Pieper and Laugero (2013) (n=29) performed in preschool-aged children, who assessed spontaneous food choices in an experimental setting, found no relation between inhibitory control and calories or protein consumed. Note however, that Levitan et al. (2014) found that higher intake of sugars was associated with worse inhibitory control in preschoolers. Additionally, a review by Cohen and colleagues (2016) including 21 studies, showed positive associations between overall higher diet quality and executive functioning in studies examining the effects of long term diet on executive functioning in children aged 5–17 years (Cohen et al., 2016). Intake of fruits, vegetables, fish, and whole grain products were associated with improved executive functions, while higher intake of snacks and sugar-sweetened

beverages were associated with worse executive functions (Cohen et al., 2016). As the development of problem behaviour typically starts around preschool age, and behaviour problems at this early stage increase the risk for poor developmental outcomes (Gagne et al., 2011), it is important to investigate the early determinants of preschoolers' inhibitory control.

While breastfeeding might play a role in the development of child inhibitory control, its role has only been investigated in relation to ADHD and EF, to our knowledge. Moreover, the role of habitual diet predicting preschoolers' inhibitory control received little attention. Since breastfeeding is predictive of better diet quality (Ventura, 2017), and better diet quality is suggested to be related to higher levels of EF, it is possible that the relation between breastfeeding and inhibitory control is (partially) mediated by habitual diet quality. Therefore, our goal was to investigate whether breastfeeding history (i.e., exclusive breastfeeding duration and breastfeeding cessation age) is predictive of inhibitory control at age three years, and if this association is (partially) mediated by habitual diet quality. As most previous studies investigated EF (Belfort et al., 2016; Hayatbakhsh et al., 2012; Julvez et al., 2007), for comparability purposes, this study investigated EF as a secondary outcome.

Materials and methods

Participants

This study is part of the ongoing longitudinal BINGO study investigating early predictors of child development. Participants were healthy children living in the Netherlands, whose parents were recruited during pregnancy in 2014/2015. Prenatal exclusion criteria were: excessive alcohol use, drug use, health problems or pregnancy complications, and insufficient knowledge of the Dutch language. At baseline, 88 pregnant women joined the BINGO study. Postnatal exclusion criteria were: complications dur-

ing pregnancy (after initial contact), gestational age at birth <37 weeks, birth weight <2500 grams, 5-minute Apgar score <7, and congenital malformations. After postnatal exclusion, 77 mothers were followed up (Hechler et al., 2018). At the 36-month measurement round (2017/2018), 76 families were contacted, as one drop-out occurred during the previous measurement rounds. Six families did not participate due to time constraints, and one family dropped out due to personal reasons. Two families could not be contacted. There were no differences in parental demographics between participating and non-participating families. In total, 67 families participated. In 54 families (81%), both parents participated, and in 13 families (19%), only mothers participated. Four families were too busy to participate in a home visit and only filled in questionnaires. See Figure 2.1 for the flow chart of the BINGO study, leading to participants of the current paper. The BINGO study was approved by the Ethical Committee of the Faculty of Social Sciences of Radboud University [ECSW2014-1003-189 and amendment: ECSW-2018-034]. The current study was preregistered on the Open Science Framework: https://osf.io/5mgnf and amendment: https://osf.io/35tg6.

Data collection procedure

Information on exclusive breastfeeding duration (no additional solids or formula feeding next to breastfeeding) and breastfeeding cessation age (age when child stopped receiving breastfeeding) was collected via maternal questionnaires at two, six, and 12 weeks, and at 12 and 36 months of age. At 36 months, a home visit took place in either the morning or afternoon. Children were fed before the home visit took place. The home visit consisted of: two inhibitory control tasks (Snack Delay and Flanker), saliva collection, prosocial task, mother-child interaction tasks, two inhibitory control tasks (Whisper and Bear Dragon), saliva collection, partner-child interaction tasks, prosocial task, and two inhibitory control tasks (Gift Wrap and Gift Delay). Only the inhibitory control tasks are part of the current study. Distractions, such as siblings,

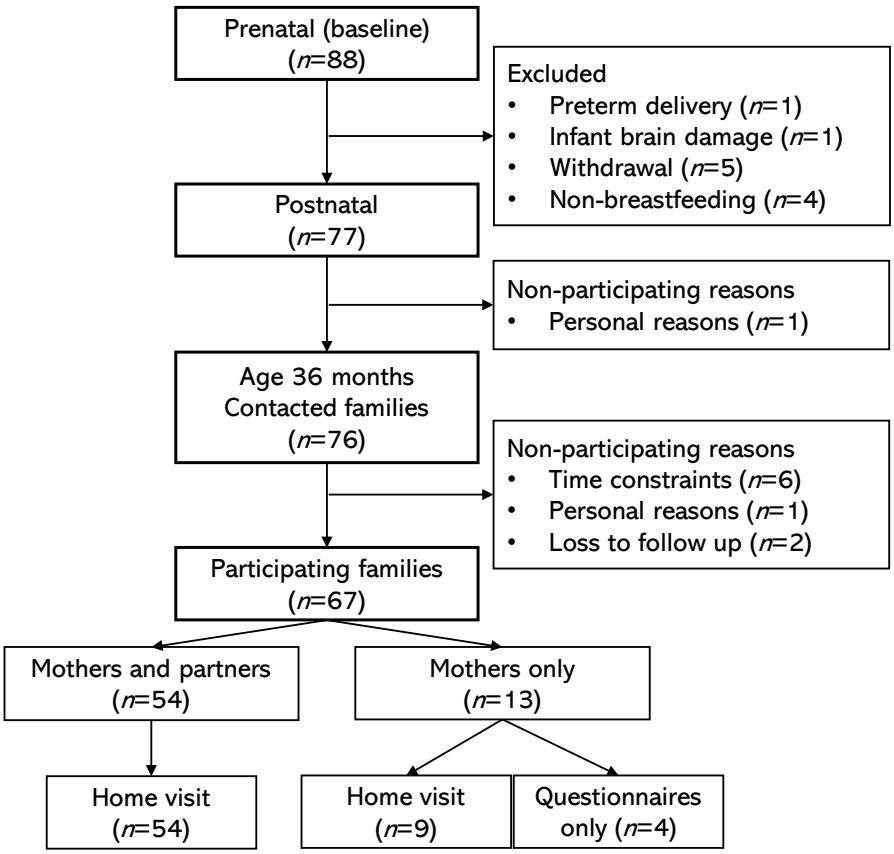


Figure 2.1. Flow chart of participating families.

doorbells ringing, or other factors were handled by the parent who was available (i.e., not performing a task with the child), or the student assistant who joined every home visit. Tasks were video recorded and afterwards rated by two trained observers. Additionally, mothers and partners independently filled in digital questionnaires about their child's and their own inhibitory control and EF. Lastly, parents received three unannounced online 24 hour (24-h) recalls before the home visit to assess their child's habitual nutritional intake. If parents had missed a 24-h recall, a recall was performed during the home visit, or was sent after the home visit.

Measures

Breastfeeding history

At age two, six, and 12 weeks, mothers were asked if they exclusively breastfed the child. Water intake during exclusive breastfeeding was not asked. Note that in the Netherlands it is very uncommon to give exclusively breastfeeding infants water. At age 12 months, mothers reported their child's feeding history: (1) are you breastfeeding? (yes or no; if no, breastfeeding cessation age was asked), (2) age of formula introduction, (3) age of solids introduction (i.e., fruits, vegetables, porridge). At age 36 months, mothers were again asked about the child's breastfeeding history and to describe the child's solid food intake. To accurately determine breastfeeding history, the breastfeeding data from the 36-months measurement follow-up was compared to that of the previous assessments. If data between the time points were conflicting (e.g., three months of breastfeeding reported at 12 weeks versus two months of breastfeeding reported at 36 months), the data with the shortest recall time were used, as retrospective data are more susceptible to recall bias (Horta and Victora, 2013). Breastfeeding cessation age correlated significantly with exclusive breastfeeding duration (r=0.64, p<0.001). In line with earlier studies (Belfort et al., 2016; Julvez et al., 2007), the main analyses were run separately for both variables.

Diet quality

Three online 24-h food intake recalls, using Compl-eat™ (Meijboom et al., 2017), were used to measure children's nutritional intake. Mothers were asked to report the dietary intake of their child from the previous day on two unannounced random weekdays and one unannounced random weekend day. Scores were given to each 24-h recall according to a diet quality score for preschool children (Voortman et al., 2015). The diet quality score is determined by the intake and amount of 10 dietary food groups

(see Table 2.1). A score between 0 and 1 was given to each food group. The ratio of the reported intake and the cut off level was calculated (scores were truncated at 1). For example, vegetable intake of 80 g per day was assigned a score of 0.8 (80/100 g/d). This was reverse-scored for intake of candy and snacks, and sugar-sweetened beverages. Scores for the 10 food groups were summed to create a diet quality score for each 24-h recall, and subsequently averaged to determine the overall diet quality score. A higher score corresponds with higher diet quality.

Table 2.1. Food groups and cut off levels.

Food Group	Cut-off Level
Vegetables	≥100 g/d
Fruit	≥150 g/d
Bread and cereals	≥70 g/d
Rice, pasta, potatoes, and legumes	≥70 g/d
Dairy	≥350 g/d
Meat, eggs, and meat substitutes	≥35 g/d
Fish	≥15 g/d
Oils and fats	≥25 g/d
Candy and snacks	≤20 g/d
Sugar-sweetened beverages	≤100 g/d

Cut off levels are determined by Voortman et al. (2015); g/d: grams per day.

Inhibitory control tasks

Behavioural tasks were chosen according to five categories of inhibitory control classified by Anderson and Reidy (2012): Delay of gratification (i.e., resist direct temptation to receive a bigger reward after the delay), Verbal inhibition (i.e., inhibit verbal responses), Go/No-go (i.e., perform certain behaviour after being shown a stimulus and to inhibit that behaviour after being shown a different stimulus), Motoric inhibition (i.e., learn response sets that conflict with an established behaviour) and Impulse control (i.e., inhibit an instinctive response).

Snack Delay (Beijers et al., 2013; Kochanska et al., 1996). To measure delay

of gratification, Snack Delay was used. Children were asked to put their hands on a placemat. Then, a self-chosen snack was placed at the top-center of the mat, and was covered with a transparent cup. Children were instructed to take the snack after the experimenter rang a bell. After a maximum of three practice trials, three consecutive trials were conducted with delays for ringing the bell of 20, 30, and 50 seconds, respectively. Children's waiting behaviour was coded every five seconds with a score ranging from 0 to 4 (0 = eats snack before the bell rings; 1 = touches/grasps snack before the bell rings; 2 = touches/grasps cup before the bell rings; 3 = waits for the bell to ring without hands on the placemat; 4 = waits for the bell to ring with hands on the placemat), and summed. Due to insufficient variation (no child ate the snack or touched the cup), this task was excluded from analyses.

Flanker (Eriksen and Eriksen, 1974). Flanker was used to measure motoric inhibition. The Flanker task showed excellent test-retest reliability and excellent convergent validity in preschool aged children (Zelazo et al., 2013). Children were asked to point in the same direction of where a centrally located target fish was swimming towards, ignoring the presence of interfering stimuli (i.e., flanking fish oriented in the same or opposite directions). After four practice trials, children were presented seven congruent trials and three incongruent trials. Accuracy in the incongruent trials was scored between 0 and 3 (0 = pointing in the wrong direction; 1 = first pointing correctly, then pointing in the wrong direction; 2 = first pointing wrongly, then pointing in the correct direction; 3 = pointing in the correct direction), and averaged. Out of 63 children tested, 49 passed the practice trial.

Whisper (Beijers et al., 2013; Kochanska et al., 1996). To measure verbal inhibition, Whisper was used. After two practice trials, children were asked to whisper the names of 12 presented animal pictures. Responses were coded 0 to 2 for every picture (0 = shout; 1 = normal or mixed tone; 2 = whisper), and averaged.

Bear Dragon (Kochanska et al., 1996; Reed et al., 1984). To measure go/no-go,

Bear Dragon was used. The experimenter introduced a "nice" bear puppet (using a soft, high-pitched voice) and a "naughty" dragon puppet (using a gruff, low-pitched voice). Children were told to obey the bear's commands and ignore the dragon's commands. After a maximum of three practice trials, 10 test trials followed. Child behaviour was scored per dragon command, ranging from 0 to 2 (0 = obeying the dragon's command; 1 = corrected movement to the dragon's command; 2 = ignoring the dragon's command), and averaged. Due to the low number of children that passed the practice trials (n=31), this task was excluded from the analyses. This low number is similar to a study by Kloo and Sodian (2017), where more than 50% of the preschoolers failed the practice trials.

Gift Wrap (Beijers et al., 2013; Kochanska et al., 1996). To measure motoric inhibition, Gift Wrap was used. Children were asked to cover their eyes with their hands and not peek while their gift, in front of them, was being wrapped for one minute. Children's waiting behaviour was coded every five seconds with a score ranging from 0 to 3 (0 = watches wrapping/gift; 1 = peeks; 2 = looks away from wrapping/gift; 3 = closed eyes and/or hands in front of the eyes), and averaged. One child was unable to follow instructions for this task.

Gift Delay (Kochanska et al., 1996). To measure impulse control, Gift Delay was used. Children were asked to not touch and unwrap the present, placed in front of the child, while the examiner left the room for one-and-a-half minutes. Latency (measured in seconds) until touching the present was used as a measure of impulse control. A higher score indicated better inhibitory control.

Reliability of coding

Recordings were observed by two observers independently. A codebook was made to set the rules for coding. The first five recordings were coded by both observers independently and immediately checked for agreement. Disagreements were discussed

and adjusted in the codebook. Thereafter, the observers continued and only discussed recordings in case of insecurities. To determine inter-rater reliability, 30 out of 63 recordings were scored by both observers. Reliability was quantified by the Intraclass Correlation Coefficient (ICC) relying on absolute agreement. The ICC's for the inhibitory control tasks were good, ranging from r=0.84 to r=0.96 (p<0.001): 0.95 for the Flanker, 0.86 for the Whisper, 0.96 for the Bear Dragon, 0.88 for the Gift Wrap, and 0.84 for the Gift Delay.

Parental questionnaires on inhibitory control and executive runctioning

ECBQ (Putnam et al., 2006). The Early Child Behavior Questionnaire (ECBQ) is a 107-item questionnaire of child temperament that was filled in by mothers only. The ECBQ contains a 6-item inhibition subscale, scored on a 7-point scale. A higher score indicates better inhibitory control. Because the Cronbach's alpha was 0.59 for the inhibition subscale, this subscale was removed from further analyses.

BRIEF-P (Sherman and Brooks, 2010). The Behavior Rating Inventory of Executive Function-Preschool Version (BRIEF-P) is a 63-item questionnaire of EF in preschool age, and contains a 16-item inhibition subscale, scored on a 3-point scale. As higher scores indicate worse EF and inhibitory control, and to align with our other inhibition and EF measures, the outcome of the BRIEF-P was reverse-coded. Consequently, higher scores on the BRIEF-P indicated better inhibitory control and EF. The Cronbach's alphas were good for the inhibition subscale and the total EF score (mothers: α=0.89 and α=0.94, respectively, and partners: α=0.84 and α=0.96, respectively).

REEF (Nilsen et al., 2017). The Ratings of Everyday Executive Functioning (REEF) is a 77-item questionnaire of EF in preschool age, using a 4-point scale. The REEF contains an inhibition subscale, but usage of separate subscales appeared unreliable (Nilsen et al., 2017). Therefore, only the total EF score was calculated. A higher score

indicates better EF. The Cronbach's alpha was 0.96 for mothers and 0.95 for partners.

Confounding

The following confounding variables were taken into account: maternal educational level (ranging from 1, primary education, to 8, university education) (Ardila et al., 2005; Kao et al., 2018), child gender (1 = boy, 2 = girl), and parental inhibitory control (Kao et al., 2018). For this last confounder, parental inhibitory control was assessed with the Behavior Rating Inventory of Executive Function-Adult (BRIEF-A) (Roth et al., 2005; Stewart et al., 2018). The BRIEF-A is a 75-item self-report questionnaire of EF in adults, and contains an eight-item inhibition subscale, scored on a 3-point scale. We reverse-coded the BRIEF-A outcome for interpretation purposes, so that higher scores indicate better inhibitory control and EF. The Cronbach's alphas for the inhibition subscale were insufficient for mothers (α=0.57) and partners (α=0.54). Therefore, the parental total score of the BRIEF-A was used as a confounder (Cronbach's alpha: 0.96 for mothers, and 0.93 for partners). To preserve power, confounding variables were only included if they significantly correlated with the independent variables or the outcome variables (Jager et al., 2008).

Statistical analyses

Descriptive analyses and normality analyses were computed. As not all variables were normally distributed, robust estimators were used in our main analyses. Furthermore, the data were inspected for outliers. Two variables contained one outlier each (breastfeeding cessation age, and the maternal BRIEF-P), and were subsequently winsorized (Blaine and Fisher, 2018). Results were similar with and without winsorizing. Pearson and Spearman correlations were performed to correlate (non-)normally distributed variables. Sample size could not be adjusted due to the longitudinal nature of our study. According to Fritz and MacKinnon (2007), with a power of 0.8, our study

was able to detect medium to large mediation effects ($\beta \geq 0.39$ and $\beta \geq 0.59$). Robust estimators were used to account for small sample size (Tabachnick and Fidell, 2014).

Missing data

The following 24-h recall data were missing: day one (n=1), day two (n=6), and day three (n=12). Missing 24-h recall data were imputed by means of expected maximization to allow for calculation of the average diet quality score. The following behavioural data were missing: Whisper (n=4), Flanker (n=18, of which 15 were missing because children did not pass the practice trial), Gift Wrap (n=5), and Gift Delay (n=4). The following maternal questionnaire data were missing: REEF (n=1). The following partner questionnaire data were missing: REEF (n=16), BRIEF-P (n=15), and BRIEF-A (n=21) (of which 15 of each questionnaire were missing because partners did not join this study from the start). The LittleMCAR test from the BaylorEdPsych package indicated that data were missing completely at random (χ^2=272.547, p=0.445). Missing data were accounted for by means of Full Information Maximum Likelihood (FIML) in the analyses.

Latent variable and composite score creation

Latent variable. Latent variables were computed when the following assumptions were met: Kaiser-Meyer-Olkin (KMO)>0.6 (Cerny and Kaiser, 1977), Bartlett's test of sphericity $p<0.05$ (Bartlett, 1951), and linear independency $p>0.00001$.

Composite score. Since we assume that the different inhibitory control tasks measure different forms of the same overarching construct, "lumping" was preferred over "splitting". Therefore, and following our preregistration, if a latent variable could not be created due to violations of the assumptions, a composite score was made using z-scores.

Main analyses

Structural Equation Modelling (SEM) was employed using the Lavaan package (Rosseel, 2012). SEM has several advantages over standard regression models such as allowing for multiple independent variables to be added in one model and handling missing data (Gunzler et al., 2013). The models were adjusted based on corresponding modification indices. For each modification, the covariance with the highest modification index was included in the previous model if it was theoretically logical. Models were adjusted handling the goodness of fit indices: Comparative Fit Index (CFI)$>$0.95, Root Mean Square Error of Approximation (RMSEA)$<$0.05, Standardized Root Mean Square Residual (SRMR)$<$0.05, and a non-significant χ^2 ($p>0.05$) (Tabachnick and Fidell, 2014). Bias-corrected confidence intervals were obtained by use of bootstrapping, as this it is recommended to check bootstrapped confidence intervals as well as significance when drawing conclusions (Feng et al., 2020).

To test whether breastfeeding history was predictive of inhibitory control, and if diet quality at age three mediated this association, four SEM models were run (see Figure 2.2 for a general path diagram illustrating our SEM models). For the first two models, we tested if exclusive breastfeeding duration (model 1) and breastfeeding cessation age (model 2) were predictive of observed inhibitory control determined by behavioural tests. In model 3 and 4, reported inhibitory control (BRIEF-P inhibition subscale) was used as the outcome measure.

Comparability analyses

For comparability purposes, we investigated whether exclusive breastfeeding duration (model 5) and breastfeeding cessation age (model 6) were related to EF at age three years, determined by a latent score of the BRIEF-P and the REEF. Because models 5 and 6 could not be fitted in the SEM model, an additional four models (models 7 to 10) were tested. We tested if exclusive breastfeeding duration (model 7) and

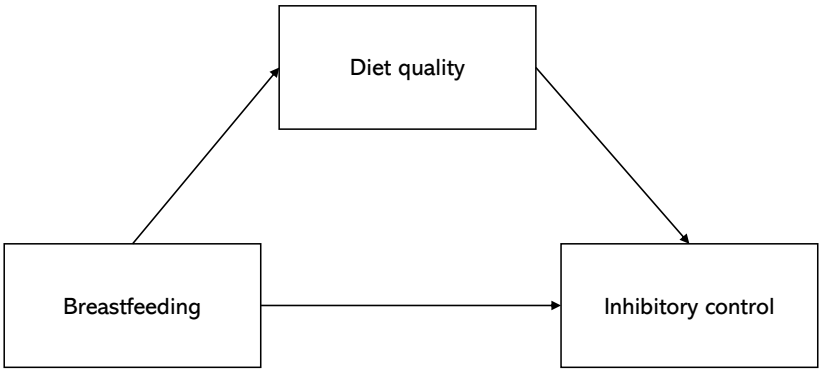


Figure 2.2. General path diagram of the SEM models.

breastfeeding cessation age (model 8) were predictive of reported EF determined by the BRIEF-P composite score, and whether exclusive breastfeeding duration (model 9) and breastfeeding cessation age (model 10) were predictive of reported EF determined by the REEF composite score. In model 5 to 10, the mediating role of diet quality at age three was also assessed.

Exploratory analyses

Exploratorily, we tested if exclusive breastfeeding duration and breastfeeding cessation age were predictive of individual inhibitory control tasks, and whether diet quality at age three mediated these associations. Furthermore, we investigated the mediating role of three different food groups (vegetable, fruit, and snacks and candy) in the association between exclusive breastfeeding duration and breastfeeding cessation age, and inhibitory control and EF. Fish was not investigated because of low variation of fish intake.

Results

Preliminary analyses

Descriptives

Table 2.2 presents descriptive statistics of the study population. The study population is mostly highly educated. Scores on questionnaires were similar between mothers and partners for the BRIEF-P and the REEF. Average diet quality score was a 3.9, which is similar to the diet quality score from a previous study in Dutch children between 12–28 months (i.e., 4.1; Voortman et al. (2015)).

Table 2.3 shows correlations between the study variables. Longer duration of exclusive breastfeeding is significantly correlated with age of breastfeeding cessation (r=0.638, p<0.001), and with better diet quality at age three (r=0.321, p=0.009). Scores of the inhibitory control subscale from the BRIEF-P correlated significantly between mother and partner (r=0.415, p=0.002). There were no significant correlations between behavioural tasks. Supplementary Table 7.1 shows the correlations between mother and partner reports.

Table 2.2. Characteristics of the study population.

	Mean ± SD	Range	*n*	
Maternal characteristics				
Age (years)	34.4 ± 3.7	28–44	67	
Educational level			65	
Low	0%			
Middle	13.8%			
High	86.2%			
Partner characteristics				
Gender			51	
Man	96.1%			
Woman	3.9%			
Age (years)	35.8 ± 4.1	28–50	51	
Educational level[a]			48	
Low	4.2%			
Middle	16.6%			
High	79.2%			
Child characteristics				
Gender			67	
Boy	47.8%			
Girl	52.2%			
Child birth weight (grams)	3531.8 ± 420.0	2570–4445	63	
Child gestational age (weeks)	39.8 ± 1.6	35.6–42.1	67	
Child age (months)	37.7 ± 1.2	36–47	67	
Study variables				
Breastfeeding				
Exclusive breastfeeding duration (months)	3.5 ± 1.9		67	
Breastfeeding cessation age (months)	9.5 ± 8.0		67	
Diet quality	3.9 ± 1.1			
Behavioural tests				
Flanker	1.2 ± 0.7		49	
Whisper	1.8 ± 0.3		63	
Gift Wrap	2.1 ± 0.9		62	
Gift Delay (seconds)	77.9 ± 27.5		63	
Questionnaires	Mother	Partner	*n* (Mother)	*n* (Partner)
BRIEF-P inhibitory control subscale	23.1 ± 5.8	22.8 ± 4.6	67	52
BRIEF-P	94.6 ± 15.6	92.4 ± 18.0	67	52
REEF	147.8 ± 33.1	146.6 ± 28.8	66	51

SD: Standard deviation; [a]: assessed during pregnancy.

Table 2.3. Correlations between questionnaires, breastfeeding history, and diet score.

	Exclusive BF Duration	Age of BF Cessation	Diet Quality Score	Flanker	Whisper	Gift Wrap	Gift Delay	BRIEF-P inh-M
Exclusive BF duration	-							
Age of BF cessation	0.638**	-						
Diet quality score	0.321**	0.117	-					
Flanker	0.161	0.341*	-0.105	-				
Whisper	-0.117	-0.107	0.063	-0.069	-			
Gift Wrap	-0.137	0.029	0.177	0.134	-0.117	-		
Gift Delay	0.123	0.212	-0.036	0.137	0.135	0.211	-	
BRIEF-P inh-M	0.115	0.139	0.056	-0.065	0.250*	0.052	0.152	-
BRIEF-P inh-P	0.219	0.078	0.196	0.033	0.051	- 0.081	-0.012	0.415**

Correlations are denoted as *r*. BF: Breastfeeding; BRIEF-P-inh-M: Score of the inhibitory control scale of the BRIEF-P filled in by mother; BRIEF-P-inh-P: Score of the inhibitory control scale of the BRIEF-P filled in by partner.*$p<0.05$. **$p<0.01$.

Latent variable and composite score creation

For the main analyses. Assumption testing for latent variable creation was performed for the behavioural tasks: Flanker, Whisper, Gift Wrap, and Gift Delay. KMO values ranged between 0.41 and 0.55. Bartlett's test did not reach significance, and determination testing yielded p=0.934. Because KMO values and Bartlett's test did not meet the cut off values, a latent variable for the behavioural tasks was not created. Instead, a composite score was made by averaging z-scores of the inhibitory control tasks. An average score was calculated only if maximally one test score was missing. In total, four children had more than one test score missing.

Next, we performed assumption testing for the BRIEF-P inhibition subscale filled in by mother and partner. KMO values were 0.5 for maternal and partner reports. Bartlett's test reached significance, and determinant testing yielded results p>0.0001. Because KMO values were lower than 0.6, a latent variable was not created. Instead, a composite score was made for the BRIEF-P inhibitory control score by averaging parental scores.

For the comparability analyses. To define reported EF for the comparability analyses, assumption testing for latent variable creation was performed from the four EF questionnaires (BRIEF-P and the REEF, filled in by mother and partner). KMO values were: BRIEF-P of mother: 0.59, and partner: 0.62, REEF of mother: 0.62, and partner: 0.61. Bartlett's test reached significance (p<0.001), and the data was linearly independent (p=0.488). The KMO value for the maternal BRIEF-P was 0.59, indicating borderline acceptable sampling adequacy. However, because the KMO value for the other three questionnaires was acceptable, and creating a latent variable was preferred over a composite score, we created a latent variable from the four parental EF questionnaires. Nonetheless, the models with the latent variable for EF could not be fitted (models 5 and 6). Therefore, assumption testing for latent variable creation

was performed to combine mother and partner scores of the BRIEF-P, and to combine mother and partner scores of the REEF. The KMO value was 0.5 for all questionnaires, Bartlett's test reached significance for all analyses, and data were linearly independent (p>0.0001). Because the KMO values were lower than 0.6, latent variables were not created. Instead, composite scores of mother and partner questionnaires were made for the BRIEF-P and the REEF, which were used as outcome measures (models 7 to 10). Goodness of fit measures showed an adequate fit for models utilizing the BRIEF-P composite score as outcome variable (models 7 and 8), but not for models utilizing the REEF composite score as outcome variable (models 9 and 10).

Confounders

Maternal educational level correlated significantly with breastfeeding cessation age (r=0.290, p=0.019), and was added as a confounder in the models where breastfeeding cessation age was used as predictor. The BRIEF-A positively correlated with the BRIEF-P inhibition subscale (r=0.351, p=0.004) and the BRIEF-P total questionnaire (r=0.437, p<0.001), and negatively correlated with observed inhibitory control (r=-0.250, p=0.048). The BRIEF-A was added to the model as a confounder when the BRIEF-P inhibition subscale, BRIEF-P total questionnaire, and observed inhibitory control were used as outcome variable. An overview of all correlations with potential confounding variables, including correlations with exploratory outcome measures, is shown in the Supplementary Materials in Table 7.2.

Main analyses

Goodness of fit measures showed an adequate fit of final models 1 through 4. Parameter estimates and bootstrapped confidence intervals per model are shown in Table 2.4. Exclusive breastfeeding duration (model 1) and breastfeeding cessation age (model 2) did not predict observed inhibitory control. However, longer exclusive breastfeeding

duration was associated with better diet quality at age three (β=0.173, 95% CI [0.035, 0.310]). Next, exclusive breastfeeding duration (model 3) and breastfeeding cessation age (model 4) did not predict reported inhibitory control. Additionally, no significant mediation effect of diet quality score was found for models 1 to 4. Furthermore, better parental EF was associated with low levels of observed child inhibitory control at age three (β=-0.009, 95% CI [-0.017, -0.001]). Contrarily, better parental EF was associated with high levels of child inhibitory control reported by both parents at age three (β=0.091, 95% CI [0.034, 0.148]).

Comparability analyses

Results showed that exclusive breastfeeding duration (model 7) and breastfeeding cessation age (model 8) were not predictive of EF as reported by the BRIEF-P. Diet quality had no mediating effect in both models. Parameter estimates and their respective confidence intervals are shown in the Supplementary Materials in Table 7.3.

Exploratory analyses

Breastfeeding cessation age was significantly associated with the Flanker task (β=0.026, 95% CI [0.003, 0.048]). No other significant relations were found between breastfeeding history and the behavioural tasks. Parameter estimates and bootstrapped confidence intervals are shown in Supplementary Table 7.4. With respect to the food groups (vegetable, fruit, and snacks and candy), because all exploratory models could not be fitted using the latent variable for EF, the BRIEF-P composite score and the REEF composite score were used as outcome variables in separate models. No significant mediation effects of food groups were found (see Supplementary Materials Tables 7.5 and 7.6 for the parameter estimates).

Table 2.4. Parameter estimates and bootstrapped confidence intervals for Model 1, 2, 3, and 4.

	B	SE	Lower CI	Upper CI
	Model 1: Exclusive breastfeeding duration → Diet quality score → Observed inhibitory control			
Regression paths				
Observed inhibitory control (composite)				
Exclusive breastfeeding duration	-0.016	0.035	-0.085	0.052
Diet quality score	0.065	0.066	-0.063	0.194
Parental executive functioning	-0.009	0.004	-0.017	0.000
Diet quality score				
Exclusive breastfeeding duration	0.173*	0.070	0.035	0.311
Mediation effect	0.011	0.012	-0.013	0.035
Total effect	-0.013	0.033	-0.078	0.051
	Model 2: Breastfeeding cessation age → Diet quality score → Observed inhibitory control			
Regression paths				
Observed inhibitory control (composite)				
Breastfeeding cessation age	0.008	0.011	-0.013	0.029
Diet quality score	0.024	0.057	-0.088	0.136
Parental executive functioning	-0.009*	0.004	-0.017	-0.001
Maternal education	0.089	0.048	-0.004	0.182
Diet quality score				
Breastfeeding cessation age	0.024	0.016	-0.008	0.055
Mediation effect	0.001	0.001	-0.002	0.003
Total effect	0.088	0.047	-0.003	0.180
	Model 3: Exclusive breastfeeding duration → Diet quality score → Reported inhibitory control			
Regression paths				
Reported inhibitory control				
Exclusive breastfeeding duration	0.296	0.339	-0.368	0.961
Diet quality score	-0.167	0.469	-1.086	0.751
Parental executive functioning	0.086**	0.030	0.028	0.144
Diet quality score				
Exclusive breastfeeding duration	0.172*	0.070	0.035	0.310
Mediation effect	-0.029	0.0832	-0.190	0.132
Total effect	0.354	0.309	-0.252	0.959
	Model 4: Breastfeeding cessation age → Diet quality score → Reported inhibitory control			
Regression paths				
Reported inhibitory control				
Breastfeeding cessation age	0.114	0.070	-0.024	0.252
Diet quality score	-0.125	0.443	-0.992	0.743
Parental executive functioning	0.091**	0.029	0.034	0.148

Table 2.4 (continued).

Maternal educational level	-0.052	0.389	-0.815	0.711
Diet quality score				
Breastfeeding cessation age	0.024	0.016	-0.008	0.055
Mediation effect	-0.003	0.011	-0.025	0.019
Total effect	0.150	0.369	-0.573	0.873

MLR estimator used to calculate parameter estimates, bootstrapping used to calculate bias-corrected confidence intervals. Model 1: $\chi^2(3)$ =0.919, p=0.338; CFI=1.000, RMSEA=0.000, SRMR=0.029, n=66. Model 2: $\chi^2(4)$=1.804, p=0.614; CFI=1.000, RMSEA=0.000, SRMR=0.045, n=66. Model 3: $\chi^2(3)$=0.996, p=0.307; CFI=0.996, RMSEA=0.026, SRMR=0.031, n=67. Model 4: $\chi^2(4)$=1.779, p=0.619; CFI=1.000, RMSEA=0.000, SRMR=0.044, n=67. *p<0.05. **p<0.01.

Discussion

This study did not provide evidence that breastfeeding history (i.e., exclusive breastfeeding duration and breastfeeding cessation age) is associated with inhibitory control and EF in three-year-old children. Furthermore, diet quality at age three was not associated with inhibitory control and EF in three-year-old children, and as such did not mediate a link between these child outcomes and breastfeeding history. Longer exclusive breastfeeding duration, but not breastfeeding cessation age, was predictive of higher diet quality at age three.

Contrary to our hypotheses, breastfeeding history did not predict inhibitory control or EF in three-year-olds. These results are in line with the results from Belfort et al. (2016), and Lopez et al. (2021), who also found no evidence for an association between breastfeeding and executive functioning. Contrarily, Julvez et al. (2007) found that breastfeeding was associated with high levels of observed EF in a sample of 500 4-year-old children. The observational tasks used in Julvez et al.'s (2007) study focused on other aspects of EF, such as working memory and attention, rather than inhibitory control specifically. Although we did not find an association between breastfeeding history and inhibitory control or the more general measures of EF, it remains possible

that breastfeeding history is related to other specific aspects of EF.

Another explanation for our null-results regarding breastfeeding history might be that small amounts of breastfeeding are already beneficial for developing inhibitory control. Of the 500 mothers in Julvez et al.'s (2007) study, 196 breastfed their infants for less than 12 weeks. As our sample contained many mothers breastfeeding for a long period, and few mothers breastfeeding for less than 12 weeks (n=7) or not breastfeeding (n=5), future studies investigating inhibitory control in a sample with more variation in breastfeeding duration may help shed light on these differences in results.

No evidence was found that habitual diet quality predicted inhibitory control and EF, or mediated the relation between breastfeeding history and these outcomes. Research on habitual diet has been performed more often in older children (6–18 year) in relation to ADHD and EF (Del-Ponte et al., 2019; Egbert et al., 2019). Moreover, of the two studies that assessed nutritional intake in relation to inhibitory control in preschool-aged children, one found that higher intake of sugars was associated with worse inhibitory control (Levitan et al., 2014), while the other found no association between nutritional intake and inhibitory control (Pieper and Laugero, 2013). Both these studies assessed spontaneous food choices in an experimental setting, while our study is the first to study habitual diet and inhibitory control in preschool age. This makes comparisons difficult and stresses the need for further studies in preschool-aged children, preferably replication studies using the same measurement methods to assess dietary intake, inhibitory control, and EF.

It might also be possible that the effects of breastfeeding and diet quality are different between children due to moderating effects by individual differences. One such factor that could moderate the association between diet and child outcomes, that has recently been receiving attention, is the gut microbiota. The gut microbiota are a collection of bacteria, archaea, and eukarya colonizing the gastrointestinal tract of the human (Thursby and Juge, 2017). Recent studies showed that individuals with

ADHD -who have lower levels of inhibitory control- have different bacteria present in their gut compared to individuals without ADHD (Bull-Larsen and Mohajeri, 2019; Szopinska-Tokov et al., 2020). Since gut microbiota are in constant interplay with diet, it is possible that the gut microbiota moderate the effects of diet on the brain (Cryan et al., 2019; Vernocchi et al., 2020). As gut microbiota composition differs between three-year-old children (Stewart et al., 2018), it may affect the way nutrition impacts brain development in individual children (Oriach et al., 2016).

Furthermore, our results indicated that longer exclusive breastfeeding duration is predictive of better diet quality at preschool age. This is consistent with Ventura's (2017) review which included studies investigating food groups and showed that longer exclusive and total breastfeeding duration is associated with higher intake of vegetables and fruits in young children, contributing to better diet quality (Ventura, 2017). Note, however, that we did not find an association between breastfeeding cessation age and diet quality. Potentially, exclusive breastfeeding duration is a better indicator of maternal investment in the quality of offspring nutrition than breastfeeding cessation age. Because high diet quality is also associated with different child outcomes, including improved physical, school, and emotional functioning, and psychosocial quality of life (Wu et al., 2019), we recommend future studies to consider the role of diet quality when studying relations between breastfeeding and child outcomes.

Lastly, we found that the behavioural tasks in our study did not correlate. Previous studies also found no consistent correlations between multiple inhibitory control tasks (Beijers et al., 2013; Carlson and Wang, 2007). Following the categorization of Anderson and Reidy (2012), who divided inhibitory control into motoric inhibition, verbal inhibition, impulse control, go/no-go, and delay of gratification, we deliberately choose our tasks to measure each of these components. Though this might explain the absence of correlations to some part, if all components tap onto the same overarching construct, some correlations would have been expected. The question arises whether

the four tasks that we used accurately measure inhibitory control, and how other factors play a role, such as age, attention, and cognitive functioning (Aatsinki et al., 2020; Friedman and Miyake, 2004). Therefore, we encourage future research to reinvestigate tasks to be able to accurately measure inhibitory control in preschool aged children.

Our study has several strengths including the longitudinal nature, covering the period from pregnancy and birth until three years and allowing for frequent maternal reports on breastfeeding status, the assessment of inhibitory control using multiple behavioural tasks and reports by mother and partner, and the assessment of habitual diet with three 24-h recalls (i.e., considered the least biased self-report instrument; Thompson and Subar (2017)). The current study also has limitations. The generalizability of our findings is limited by our mostly highly educated sample and low variation in breastfeeding duration. Furthermore, the relatively small sample size reduced our statistical power. We preserved power by using composite scores and performing exploratory analyses for individual scores, but replications in larger and more varied study populations are highly recommended.

To conclude, no evidence was found for a relation between breastfeeding history and inhibitory control or EF, and no evidence was found for a mediating effect of diet quality. Nonetheless, we found that longer exclusive breastfeeding duration predicted better diet quality at age three, which is similar to previous literature. The fact that we found no evidence for the expected association between breastfeeding history, habitual diet, and inhibitory control/EF, is not equivalent to confirming the null hypothesis, especially considering our study limitations. Given our results and the inconclusive nature of previous literature, as well as the role of low inhibitory control in psychopathology and child behaviour problems, we recommend future studies to continue investigating nutrition and inhibitory control in the toddler to preschool ages.

References

Aatsinki, A.-K., Kataja, E.-L., Munukka, E., Lahti, L., Keskitalo, A., Korja, R., Nolvi, S., Häikiö, T., Tarro, S., Karlsson, H., and Karlsson, L. (2020). Infant fecal microbiota composition and attention to emotional faces. *Emotion*, 22(6):1159.

American Psychological Association (2019). Inhibition –APA Dictionary of Psychology.

Anderson, P. J. and Reidy, N. (2012). Assessing executive function in preschoolers. *Neuropsychology Review*, 22(4):345–360.

Ardila, A., Rosselli, M., Matute, E., and Guajardo, S. (2005). The influence of the parents' educational level on the development of executive functions. *Developmental Neuropsychology*, 28(1):539–560.

Bar, S., Milanaik, R., and Adesman, A. (2016). Long-term neurodevelopmental benefits of breastfeeding. *Current Opinion in Pediatrics*, 28(4):559–566.

Bartlett, M. S. (1951). The effect of standardization on a χ 2 approximation in factor analysis. *Biometrika*, 38(3/4):337–344.

Beijers, R., Riksen-Walraven, M., Putnam, S., Jong, M. d., and de Weerth, C. (2013). Early non-parental care and toddler behaviour problems: Links with temperamental negative affectivity and inhibitory control. *Early Childhood Research Quarterly*, 28(4):714–722.

Belfort, M. B., Rifas-Shiman, S. L., Kleinman, K. P., Bellinger, D. C., Harris, M. H., Taveras, E. M., Gillman, M. W., and Oken, E. (2016). Infant Breastfeeding Duration and Mid-Childhood Executive Function, Behavior, and Social-Emotional Development. *Journal of developmental and behavioral pediatrics*, 37(1):43–52.

Blaine, B. E. and Fisher, S. J. (2018). Winsorizing. *The SAGE Encyclopedia of Educational Research, Measurement, and Evaluation*, page 1817.

Bull-Larsen and Mohajeri (2019). The potential influence of the bacterial microbiome on the development and progression of ADHD. *Nutrients*, 11(11):2805.

Carlson, S. M. and Wang, T. S. (2007). Inhibitory control and emotion regulation in preschool children. *Cognitive Development*, 22(4):489–510.

Cerny, B. A. and Kaiser, H. F. (1977). A study of a measure of sampling adequacy for factor-analytic correlation matrices. *Multivariate Behavioral Research*, 12(1):43–47.

Cheng, N., Lu, S., Archer, M., and Wang, Z. (2018). Quality of maternal parenting of 9-month-old infants predicts executive function performance at 2 and 3 years of age. *Frontiers in Psychology*, 8:2293.

Cohen, J. F. W., Gorski, M. T., Gruber, S. A., Kurdziel, L. B. F., and Rimm, E. B. (2016). The effect of healthy dietary consumption on executive cognitive functioning in children and adolescents: a systematic review. *British Journal of Nutrition*, 116(06):989–1000.

Cryan, J. F., O'riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., Codagnone, M. G., Cussotto, S., Fulling, C., Golubeva, A. V., Guzzetta, K. E., Jaggar, M., Long-Smith, C. M., Lyte, J. M., Martin, J. A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., O'connor, R., Cruz-Pereira, J. S., Peterson, V. L., Rea, K., Ritz, N. L., Sherwin, E., Spichak, S., Teichman, E. M., Wouw, M. v. d., Ventura-Silva, A. P., Wallace-Fitzsimons, S. E., Hyland, N., Clarke, G., and Dinan, T. G. (2019). The microbiota-gut-brain axis. *Physiological Reviews*, 99(4):1877–2013.

Del-Ponte, B., Quinte, G. C., Cruz, S., Grellert, M., and Santos, I. S. (2019). Dietary patterns and attention deficit/hyperactivity disorder (ADHD): A systematic review and meta-analysis. *Journal of Affective Disorders*, 252:160–173.

Diamond, A. (2013). Executive functions. *Annual review of psychology*, 64:135–68.

Duijts, L., Ramadhani, M. K., and Moll, H. A. (2009). Breastfeeding protects against infectious diseases during infancy in industrialized countries. a systematic review. *Maternal & Child Nutrition*, 5(3):199–210.

Egbert, A. H., Creber, C., Loren, D. M., and Bohnert, A. M. (2019). Executive function and dietary intake in youth: A systematic review of the literature. *Appetite*, 139:197–212.

Engelhardt, L. E., Briley, D. A., Mann, F. D., Harden, K. P., and Tucker-Drob, E. M. (2015). Genes unite executive functions in childhood. *Psychological Science*, 26(8):1151–1163.

Eriksen, B. A. and Eriksen, C. W. (1974). Effects of noise letters upon the identification of a target letter in a nonsearch task. *Perception & Psychophysics*, 16(1):143–149.

Feng, Q., Song, Q., Zhang, L., Zheng, S., and Pan, J. (2020). Integration of moderation and mediation in a latent variable framework: A comparison of estimation approaches for the second-stage moderated mediation model. *Frontiers in Psychology*, 11:2167.

Friedman, N. P. and Miyake, A. (2004). The relations among inhibition and interference control functions: A latent-variable analysis. *Journal of Experimental Psychology: General*, 133(1):101–135.

Fritz, M. S. and MacKinnon, D. P. (2007). Required sample size to detect the mediated effect. *Psychological Science*, 18(3):233–239.

Gagne, J. R., Chang, C.-N., Fang, H., Spann, C., and Kwok, O.-M. (2019). A multimethod study of inhibitory control and behavioural problems in preschoolers. *Infant and Child Development*, 28(1):e2115.

Gagne, J. R., Saudino, K. J., and Asherson, P. (2011). The genetic etiology of inhibitory control and behavior problems at 24 months of age. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, 52(11):1155–1163.

Georgieff, M. K., Brunette, K. E., and Tran, P. V. (2015). Early life nutrition and neural plasticity. *Development and Psychopathology*, 27(2):411–423.

Gunzler, D., Chen, T., Wu, P., and Zhang, H. (2013). Introduction to mediation analysis with structural equation modeling. *Shanghai Archives of Psychiatry*, 25(6):390.

Hayatbakhsh, M. R., O'Callaghan, M. J., Bor, W., Williams, G. M., and Najman, J. M. (2012). Association of breastfeeding and adolescents' psychopathology: A large prospective study. *Breastfeeding Medicine*, 7(6):480–486.

Hechler, C., Beijers, R., Riksen-Walraven, J. M., and de Weerth, C. (2018). Are cortisol concentrations in human breast milk associated with infant crying? *Developmental Psychobiology*, 60(6):639–650.

Horta, B. L. and Victora, C. G. (2013). Long-term effects of breastfeeding: a systematic review. *World Health Organization*.

Jager, K., Zoccali, C., MacLeod, A., and Dekker, F. (2008). Confounding: What it is and how to deal with it. *Kidney International*, 73(3):256–260.

Julvez, J., Ribas-Fitó, N., Forns, M., Garcia-Esteban, R., Torrent, M., and Sunyer, J. (2007). Attention behaviour and hyperactivity at age 4 and duration of breast-feeding. *Acta Paediatrica*, 96(6):842–847.

Kao, K., Nayak, S., Doan, S. N., and Tarullo, A. R. (2018). Relations between parent EF and child EF: The role of socioeconomic status and parenting on executive functioning in early childhood. *Translational Issues in Psychological Science*, 4(2):122–137.

Kloo, D. and Sodian, B. (2017). The developmental stability of inhibition from 2 to 5 years. *British Journal of Developmental Psychology*, 35(4):582–595.

Kochanska, G., Murray, K., Jacques, T. Y., Koenig, A. L., and Vandegeest, K. A. (1996). Inhibitory control in young children and its role in emerging internalization. *Child Development*, 67(2):490–507.

Levitan, R. D., Rivera, J., Silveira, P. P., Steiner, M., Gaudreau, H., Hamilton, J., Kennedy, J. L., Davis, C., Dube, L., Fellows, L., Wazana, A., Matthews, S., and and, M. J. M. (2014). Gender differences in the association between stop-signal reaction times, body mass indices and/or spontaneous food intake in pre-school children: an early model of compromised inhibitory control and obesity. *International Journal of Obesity*, 39(4):614–619.

Lipszyc, J. and Schachar, R. (2010). Inhibitory control and psychopathology: A meta-analysis of studies using the stop signal task. *Journal of the International Neuropsychological Society*, 16(6):1064–1076.

Lopez, D. A., Foxe, J. J., Mao, Y., Thompson, W. K., Martin, H. J., and Freedman, E. G. (2021). Breastfeeding Duration Is Associated With Domain-Specific Improvements in Cognitive Performance in 9–10-Year-Old Children. *Frontiers in Public Health*, 9:657422.

Meijboom, S., van Houts-Streppel, M. T., Perenboom, C., Siebelink, E., van de Wiel, A. M., Geelen, A., Feskens, E. J. M., and de Vries, J. H. M. (2017). Evaluation of dietary intake assessed by the dutch self-administered web-based dietary 24-h recall tool (compl-eat™) against interviewer-administered telephone-based 24-h recalls. *Journal of Nutritional Science*, 6:e49.

Moffitt, T. E., Arseneault, L., Belsky, D., Dickson, N., Hancox, R. J., Harrington, H., Houts, R., Poulton, R., Roberts, B. W., Ross, S., Sears, M. R., Thomson, W. M., and Caspi, A. (2011). A gradient of childhood self-control predicts health, wealth, and public safety. *Proceedings of the National Academy of Sciences*, 108(7):2693–2698.

Nilsen, E. S., Huyder, V., McAuley, T., and Liebermann, D. (2017). Ratings of everyday executive functioning (REEF): A parent-report measure of preschoolers' executive functioning skills. *Psychological Assessment*, 29(1):50–64.

Oriach, C. S., Robertson, R. C., Stanton, C., Cryan, J. F., and Dinan, T. G. (2016). Food for thought: The role of nutrition in the microbiota-gut-brain axis. *Clinical Nutrition Experimental*, 6:25–38.

Pieper, J. R. and Laugero, K. D. (2013). Preschool children with lower executive function may be more vulnerable to emotional-based eating in the absence of hunger. *Appetite*, 62:103–109.

Putnam, S. P., Gartstein, M. A., and Rothbart, M. K. (2006). Measurement of fine-grained aspects of toddler temperament: The early childhood behavior questionnaire. *Infant Behavior and Development*, 29(3):386–401.

Reed, M. A., Pien, D. L., and Rothbart, M. K. (1984). Inhibitory Self-Control in Preschool Children. *Merrill-Palmer Quarterly*, pages 131–147.

Rosseel, Y. (2012). lavaan: An r package for structural equation modeling. *Journal of Statistical Software*, 48(2).

Roth, R. M., Isquith, P. K., and Gioia, G. A. (2005). Behavior rating inventory of executive function®–adult version.

Sherman, E. M. S. and Brooks, B. L. (2010). Behavior rating inventory of executive function – preschool version (BRIEF-p): Test review and clinical guidelines for use. *Child Neuropsychology*, 16(5):503–519.

St. John, A. M., Kibbe, M., and Tarullo, A. R. (2019). A systematic assessment of socioeconomic status and executive functioning in early childhood. *Journal of Experimental Child Psychology*, 178:352–368.

Stewart, C. J., Ajami, N. J., O'Brien, J. L., Hutchinson, D. S., Smith, D. P., Wong, M. C., Ross, M. C., Lloyd, R. E., Doddapaneni, H., Metcalf, G. A., Muzny, D., Gibbs, R. A., Vatanen, T., Huttenhower, C., Xavier, R. J., Rewers, M., Hagopian, W., Toppari, J., Ziegler, A.-G., She, J.-X., Akolkar, B., Lernmark, A., Hyoty, H., Vehik, K., Krischer, J. P., and Petrosino, J. F. (2018). Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature*, 562(7728):583–588.

Szopinska-Tokov, J., Dam, S., Naaijen, J., Konstanti, P., Rommelse, N., Belzer, C., Buitelaar, J., Franke, B., Bloemendaal, M., Aarts, E., and Vasquez, A. A. (2020). Investigating the gut microbiota composition of individuals with attention-deficit/hyperactivity disorder and association with symptoms. *Microorganisms*, 8(3):406.

Tabachnick, B. G. and Fidell, L. S. (2014). Structural Equation Modeling. In Pearson, E., editor, *Using Multivariate Statistics*, pages 731–836. Pearson Education Inc Boston, MA, 6 edition.

Thompson, F. E. and Subar, A. F. (2017). Dietary assessment methodology. In *Nutrition in the Prevention and Treatment of Disease*, pages 5–48. Elsevier.

Thursby, E. and Juge, N. (2017). Introduction to the human gut microbiota. *Biochemical Journal*, 474(11):1823.

Ventura, A. K. (2017). Does Breastfeeding Shape Food Preferences? Links to Obesity. *Annals of Nutrition and Metabolism*, 70(3):8–15.

Vernocchi, P., Chierico, F. D., and Putignani, L. (2020). Gut microbiota metabolism and interaction with food components. *International Journal of Molecular Sciences*, 21(10):3688.

Victora, C. G., Bahl, R., Barros, A. J. D., França, G. V. A., Horton, S., Krasevec, J., Murch, S., Sankar, M. J., Walker, N., Rollins, N. C., and Group, L. B. S. (2016). Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. *The Lancet*, 387(10017):475–90.

Voortman, T., de Jong, J. C. K., Geelen, A., Villamor, E., Moll, H. A., de Jongste, J. C., Raat, H., Hofman, A., Jaddoe, V. W., Franco, O. H., and van den Hooven, E. H. (2015). The development of a diet quality score for preschool children and its validation and determinants in the generation r study. *The Journal of Nutrition*, 145(2):306–314.

Wu, X. Y., Zhuang, L. H., Li, W., Guo, H. W., Zhang, J. H., Zhao, Y. K., Hu, J. W., Gao, Q. Q., Luo, S., Ohinmaa, A., and Veugelers, P. J. (2019). The influence of diet quality and dietary behavior on health-related quality of life in the general population of children and adolescents: a systematic review and meta-analysis. *Quality of Life Research*, 28(8):1989–2015.

Zelazo, P. D., Anderson, J. E., Richler, J., Wallner-Allen, K., Beaumont, J. L., and Weintraub, S. (2013). II. NIH TOOLBOX COGNITION BATTERY (CB): MEASURING EXECUTIVE FUNCTION AND ATTENTION. *Monographs of the Society for Research in Child Development*, 78(4):16–33.

Zeng, Y., Tang, Y., Tang, J., Shi, J., Zhang, L., Zhu, T., Xiao, D., Qu, Y., and Mu, D. (2018). Association between the different duration of breastfeeding and attention deficit/hyperactivity disorder in children: a systematic review and meta-analysis. *Nutritional Neuroscience*, 23(10):811–823.

Chapter 3

Fucosylated Human Milk Oligosaccharides during the First 12 Postnatal Weeks are Associated with Better Executive Functions in Toddlers

Based on: Willemsen, Y., Beijers, R., Gu, F., Arias Vásquez, A., Schols, H.A., de Weerth, C. Fucosylated Human Milk Oligosaccharides during the First 12 Postnatal Weeks are Associated with Better Executive Functions in Toddlers. *Nutrients*, 2023, 15, 1463.

Abstract

Human milk oligosaccharides (HMOs) are one of the most abundant solid components in a mother's milk. Animal studies have confirmed a link between early life exposure to HMOs and better cognitive outcomes in the offspring. Human studies on HMOs and associations with later child cognition are scarce. In this preregistered longitudinal study, we investigated whether human milk 2'-fucosyllactose, 3'-sialyllactose, 6'-sialyllactose, grouped fucosylated HMOs, and grouped sialylated HMOs, assessed during the first 12 postnatal weeks, are associated with better child executive functions at age three years. At infant age two, six, and 12 weeks, a sample of human milk was collected by mothers who were exclusively (n=45) or partially breastfeeding (n=18). HMO composition was analysed by use of porous graphitized carbon-ultra high-performance liquid chromatography–mass spectrometry. Executive functions were assessed at age three years with two executive function questionnaires independently filled in by mothers and their partners, and four behavioural tasks. Multiple regression analyses were performed in R. Results indicated that concentrations of 2'-fucosyllactose and grouped fucosylated HMOs were associated with better executive functions, while concentrations of grouped sialylated HMOs were associated with worse executive functions at age three years. Future studies on HMOs that sample frequently during the first months of life and experimental HMO administration studies in exclusively formula-fed infants can further reveal associations with child cognitive development and uncover potential causality and sensitive periods.

Introduction

Human milk is considered the best nutrition for the infant because of its beneficial effects on child development and on maternal and child health (de Weerth et al., 2022). For example, human milk is shown to protect against infections during infancy and metabolic diseases in later life (Victora et al., 2016). Moreover, breastfeeding parameters, such as the initiation and duration of breastfeeding, have been related to improved child neurodevelopment and cognition (Hou et al., 2021; Victora et al., 2016). Increasing evidence also shows that not only breastfeeding parameters, but also specific constituents of human milk, are related to child behavioural and cognitive outcomes (for a review see de Weerth et al. (2022)). This concept of human milk determining the trajectory of development with long-term consequences for the phenotype, is also known as Lactocrine Programming (Bartol et al., 2008, 2017; Liu et al., 2014). Human milk oligosaccharides (HMOs) are the most abundant solid component in human milk after lipids and lactose (Bode, 2012). With respect to Lactocrine Programming, HMOs are of interest because of their potential beneficial effects on child neurodevelopment (Bode, 2012; Wang, 2012). However, to date, the few studies that investigated HMOs' associations with child cognition focused on general cognition and language, and reached until the age of 24 months (Berger et al., 2020b; Cho et al., 2021; Jorgensen et al., 2021; Oliveros et al., 2021). The current study extends these first findings by investigating, in a healthy community sample, the longitudinal associations between HMOs in the first weeks of life and executive functions, which represent higher cognitive abilities, at child age three years.

HMOs are complex carbohydrates made up of various combinations of five monosaccharides (i.e., galactose, glucose, N-acetylglucosamine, fucose, and sialic acid). Based on combinations of these monosaccharides, HMOs can be divided in three groups: neutral core HMOs (containing only glucose, galactose, and N-acetylglucosamine residues),

fucosylated HMOs (containing a lactose or neutral core backbone, with one or more fucose units), and sialylated HMOs (containing a lactose or neutral core backbone with one or more sialic acid units) (Tonon et al., 2019). The HMO structure determines the HMO function and influence on the body (Bode and Jantscher-Krenn, 2012). Women can secrete about 200 different structures of HMOs, though 10 individual HMOs make up over 70% of the total HMO concentration (Soyyilmaz et al., 2021). While HMO composition remains mainly constant during the day and week, HMO composition does change throughout lactation and varies greatly between women (Borewicz et al., 2020; Soyyilmaz et al., 2021; Thum et al., 2021). One of the most important factors explaining variance in milk HMO composition is secretor status. Secretor status is controlled by the FUT2 gene and refers to the presence or absence of water-soluble ABO blood group antigens in a person's bodily fluids, including breast milk. People who secrete these antigens in their bodily fluids are referred to as secretors, while people who do not are termed non-secretors. Maternal secretor status has been shown to affect levels of HMOs with fucose-containing structures (Thurl et al., 2017). Mothers who are secretor-negative usually produce none, or very low levels of 2'-fucosyllactose (2'FL), as opposed to secretor-positive mothers (Thurl et al., 2017).

Because the most important function of HMOs is to provide nutrients to specific gut bacteria, the microbiota-gut-brain axis is a likely pathway through which HMOs can ultimately exert effects on brain and behaviour. Rodent and human studies have shown that the microorganisms in our gut, or gut microbiota, are able to communicate with the brain via the microbiota-gut-brain axis, which is the bi-directional communication route between the gut microbiota and the brain (Cryan et al., 2019; de Weerth, 2017). Mainly *Bifidobacteria* benefit from HMOs, for example *Bifidobacterium longum* subspecies *infantis* uses HMOs as metabolic substrates (Bode, 2015; Totten et al., 2012; Underwood et al., 2014). The exact mechanism of how *Bifidobacteria* can subsequently affect brain development is still unclear. However, *Bifidobacteria* are able to produce

short chain fatty acids, which are able to cross the blood brain barrier and exert positive effects on the brain (Dalile et al., 2019). This proposed mechanism may hence explain associations between HMOs and cognitive development. HMOs have been causally related to long term cognition in animal studies (for a review on HMO administration in animal studies, see Docq et al. (2020)). For example, 2'FL administered at early ages (in combination with other components or HMOs) until the end of the experiment (postnatal day 33), contributed to improved memory performance and faster learning speed in adult pigs, compared to control pigs (Fleming et al., 2020a,b). Furthermore, sialylated HMOs, mainly 3'-sialyllactose (3'SL) and 6'-sialyllactose (6'SL), administered at an early age until the end of the experiment (postnatal day 19 and 35) contributed to better performance of rats and piglets in memory and learning tasks in adolescence and older adulthood, compared to control animals (Obelitz-Ryom et al., 2019; Wang et al., 2007). In addition, Oliveros et al. (2016, 2018) administered 2'FL and 6'SL in rats in early life only and found an association with better performance on learning tasks in adulthood. These positive effects of early life HMO administration on memory and learning in adulthood indicate that HMO consumption in early life can exert lifelong effects on the cognition of mammals.

Mechanisms have been explored for several HMOs. The HMO 2'FL is known for its specific stimulation of the growth of *Bifidobacteria* in the gut (Berger et al., 2020a). Additionally, in rat brains, 2'FL induced long-term potentiation (LTP) which is involved in learning and memory (Krug et al., 1994; Matthies et al., 1996; Vázquez et al., 2015). Next to that, deprivation of 6'SL affected cognitive functions, as seen by reduced expression of important genes in the prefrontal cortex, a brain region that mediates executive functions and memory (Hauser et al., 2021). Lastly, sialic acid has been suggested to play a key role in neurodevelopment during the early postnatal stages, as it provides the building blocks for brain gangliosides (Wang, 2009), which have been related to neurophysiological outcomes, such as memory formation (Schnaar et al.,

2014; Wang, 2009, 2012).

To our knowledge, no studies on the effects of HMO supplementation on cognition have been performed in humans. However, observational human studies are emerging that investigate longitudinal relations between HMOs, cognition, and related constructs. One study found that 2'FL concentrations in milk samples collected at one month postpartum were associated with better cognitive development at 24 months of age, as measured with the Bayley Scales of Infant and Toddler Development (Berger et al., 2020b). Another longitudinal human study found a positive association between 6'SL and better cognitive development scores at 18 months of age, also measured with the Bayley Scales (Oliveros et al., 2021). In addition, Cho et al. (2021) found positive links between 3'SL concentration and a composite of cognition at multiple ages (language in particular) in human infants (Cho et al., 2021). Lastly, a large study in Malawi participants found a positive link between grouped fucosylated and grouped sialylated HMO concentrations in mothers milk collected at six months postpartum, and language at child age 18 months (Jorgensen et al., 2021). To sum, most human studies assessed HMO concentrations only at one time point or at older ages (i.e., six months). Because HMO composition changes over lactation, and early life is known to be a sensitive period for future child development, multiple samples in early life are required to obtain a more reliable picture of an infant's exposure to HMOs during the first months of life and its association with later child cognitive development.

This prospective, longitudinal study investigated, in a healthy Dutch community sample, the associations between HMOs measured at infant age two, six, and 12 weeks, and executive functions, at child age three years. As inhibitory control is an important aspect of executive functions and essential for child mental health development (Cook et al., 2019; Montroy et al., 2016), we also included behavioural measures of inhibitory control. The study was preregistered on the Open Science Framework (https://osf.io/h4ztw). Based on the literature mentioned above, we selected specific

HMOs (2'FL, 3'SL, and 6'SL) and composed two HMO groups (fucosylated-, and sialylated HMOs) for our study. We hypothesized that 2'FL, 3'SL, 6'SL, grouped fucosylated HMOs, and grouped sialylated HMOs would be positively associated with better executive functions and inhibitory control. With the goal of expanding our knowledge on HMOs, we exploratorily investigated associations between child cognition and 21 other HMOs: 3'-fucosyllactose (3-FL), difucosyllactose (DFL), di-/tri-fucosyllacto-N-hexaose (DF-/TF-LNH), four different isomers of fucosyllacto-N-hexaose (F-LNH), isofucosyl-Lacto-N-hexaose I (IF-LNH-I), lacto-N-difucohexaose I (LNDFH-I), lacto-N-difucosylhexaose II (LNDFH-II), lacto-N-fucopentaose I (LNFP-I), lacto-N-fucopentaose II (LNFP-II), lacto-N-fucopentaose III (LNFP-III), lacto-N-fucopentaose V (LNFP-V), lacto-N-tetraose (LNT) and lacto-N-neotetraose (LNnT) combined, lacto-N-hexaose (LNH), lacto-N-neohexaose (LNnH), para-lacto-N-hexaose (pLNH), sialyllacto-N-tetraose-a (LSTa), sialyllacto-N-tetraose-b (LSTb), sialyllacto-N-tetraose-c (LSTc), and three more HMO groups: non-fucosylated and non-sialylated HMOs, mono-fucosylated HMOs, and di- and tri-fucosylated HMOs.

Materials and methods

Participants

This study is part of the longitudinal BINGO study investigating early prenatal and postpartum predictors of child development (Hechler et al., 2018, 2019). Participants were parents and their children recruited in the Netherlands during pregnancy in 2014/2015 (n=88). Postnatal exclusion criteria were pregnancy complications, birth weight <2500 g, gestational age at birth <37 weeks, 5-min Apgar score <7, and congenital malformations (Hechler et al., 2018). After birth, eleven participating families were excluded, either because inclusion criteria were not met, or because of withdrawal due to personal circumstances. After one drop-out during the first postnatal year, 76

families were contacted at the 36-month measurement round (2017/2018) and 67 agreed to participate (see participant flowchart in Figure 3.1). Of these 67 participants, four mothers did not breastfeed during the first postnatal weeks and thus did not provide milk samples, resulting in a total number of 63 participants for the current study. All the analyses were first performed on data of exclusively breastfed children during the first 12 postnatal weeks (n=45, including two infants who received one formula feeding a week), to avoid potential effects of formula feeding on behaviour. Subsequently, the analyses were repeated with the whole group of infants (n=63), correcting for percentage breastfeeding (see HMOs section).

The BINGO study was independently reviewed by the Ethics Committee of Social Sciences (ECSW) of Radboud University, and no formal objection to this research was made [ECSW2014-1003-189 and amendment: ECSW-2018-034]. The current study was preregistered on the Open Science Framework: https://osf.io/h4ztw.

Procedure

Mothers collected milk samples at two, six, and 12 weeks after delivery. Mothers collected these samples (approximately 20 mL) into sterile collection cups by hand expression, in the morning, before feeding the infant. Mothers were asked to wash their hands before collection. In case breast pads or cream had been used, mothers were asked to also wash their breasts before collection. The three mothers who collected milk via a pump were asked to first boil the parts that come into contact with the milk. Samples were stored in the participant's freezer before they were collected with a portable freezer when the infant was around 12-14 weeks. The samples were subsequently stored at -80°C at the Radboud University, and afterwards delivered to the lab of Food Chemistry of Wageningen University for HMO content analysis.

At three years of age, mothers and their partners independently filled in online questionnaires about their child's executive functioning. In addition, home visits took

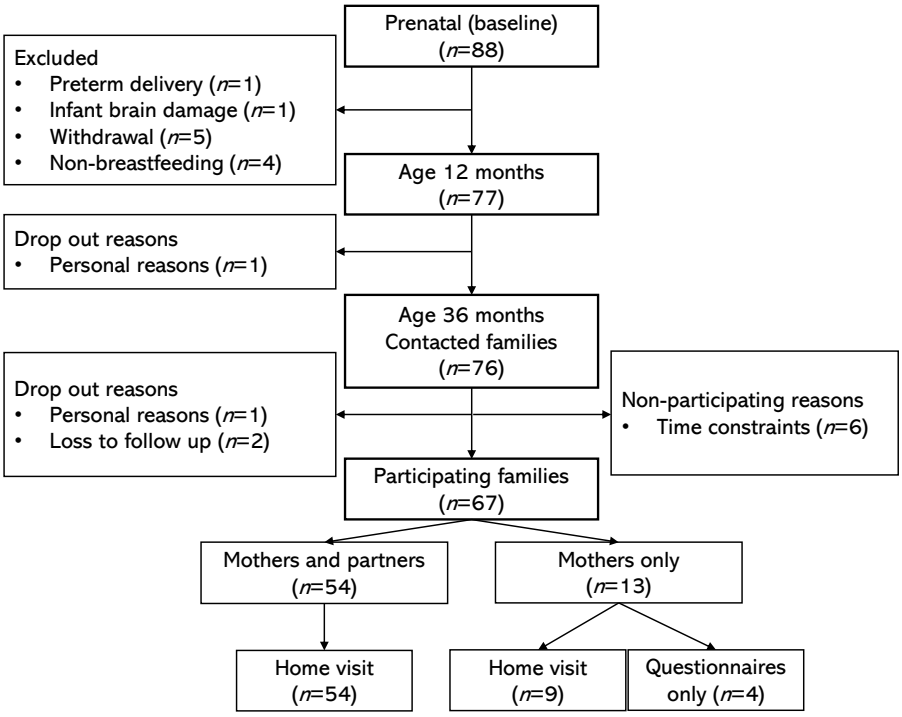


Figure 3.1. Flowchart of participant follow-up. Drop outs are shown on the left side of the flowchart. Participants who skipped the assessment round are shown on the right side of the flowchart.

place where child inhibitory control was assessed by a trained examiner through six inhibitory control tasks. For more information on the procedure and content of the visit, see Willemsen et al. (2021).

HMOs

The HMOs were extracted, purified by solid phase extraction, and quantified by using porous graphitized carbon-ultra high-performance liquid chromatography - mass spectrometry (PGC-UPLC-MS) and high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Gu et al., 2021a). The following 24 different HMO structures were determined: 2'FL, 3-FL, 3'SL, 6'SL, DF-TF-LNH, DFL, four different isomers of F-LNH, IF-LNH-I, LNDFH-I, LNDFH-II, LNFP-I, LNFP-

II, LNFP-III, LNFP-V, LNH, LNnH, pLNH, LNT and LNnT combined, LSTa, LSTb, LSTc (see also Borewicz et al. (2020) and Gu et al. (2021b) for other analyses with these data).

The group of fucosylated HMOs consisted of: 2'FL, 3-FL, LNFP-I, LNFP-II, LNFP-III, LNFP-V, four isomers of F-LNH, IF-LNH-I, LNDFH-I, LNDFH-II, DFL, and DF-TF-LNH. The group of sialylated HMOs consisted of 3'SL, 6'SL, LSTa, LSTb and LSTc. The group of non-fucosylated and non-sialylated HMOs consist of: LNT and LNnT combined, LNH, LNnH, and pLNH. The group of mono-fucosylated HMOs consists of: 2'FL, 3-FL, LNFP-III, LNFP-II, LNFP-I, LNFP-V, four different isomers of F-LNH, and IF-LNH-I. The di- and tri-fucosylated HMOs consist of: LNDFH-I, LNDFH-II, DFL, and DF-TF-LNH. The identification of different LNH isomers was not possible as the pure substances for identifying LNH isomers were not commercially available during the time of wet analyses. However, identification of LNH isomers was achieved based on mass-to-charge ratios of peaks and was then compared to retention times in the literature. This allowed for relative comparisons between the HMOs.

Due to naturally occurring differences in milk dilutions, HMO concentrations were corrected for sample-to-sample variability by normalizing readout values for each time point separately using the Probabilistic Quotient Normalization (PQN) method in R, as performed by Borewicz et al. (2020). Furthermore, corrections for estimated daily milk intake were based on previous literature (480g, 580g, and 630g at weeks two, six, and 12, respectively) (Institute of Medicine (US). Subcommittee on Nutrition during Lactation and United States. Health Resources and Services Administration, 1991). The resulting variables were used as such for the exclusive breastfeeding group (n=45). For the total group (n=63), in which also mothers were included who were partially breastfeeding, these corrected values were further adjusted for the proportion of human milk feedings. E.g., if the infant at 12 weeks received 30% formula and 70% human milk, the corrected HMO concentrations at that time point were further

adjusted by multiplying by 0.7.

Executive functions

Two questionnaires were used to measure child executive functioning. The Behavior Rating Inventory of Executive Function-Preschool Version (BRIEF-P) is a commonly used executive functions questionnaire that measures general child executive functions and does not differentiate between different situations. The Ratings of Everyday Executive Functioning (REEF) is less commonly used and rates child executive functions in different situations (e.g., executive functions around friends, during grocery shopping, or in the community). Because of their different assessment methods, and because previous literature showed different outcomes between the two questionnaires (Willemsen et al., 2021), we included both in our research.

The **BRIEF-P** (Sherman and Brooks, 2010) is a 63-item questionnaire that assesses preschool aged executive functioning, using a 3-point scale (answer options: 'Never', 'Sometimes', 'Often'). Example items are: 'Overreacts to small problems' and 'Is easily overwhelmed or overstimulated by typical daily activities'. Higher scores indicate worse executive functioning. To align with our other executive functioning and inhibition measures, the outcome of the BRIEF-P was reverse-coded. Consequently, higher scores on the BRIEF-P indicated better executive functioning. The Cronbach's alpha was 0.94 for mothers, and 0.96 for partners.

The **REEF** (Nilsen et al., 2017) is a 77-item questionnaire that assesses preschool age executive functions, using a 4-point scale (answer options: 'Is not able', 'Never or almost never', 'Sometimes', 'Always or almost always'). This questionnaire assesses the child's behaviour in eight different scenarios, namely: how the child plays games, how the child plays games with others, how the child interacts with others, around the house, in the community, out shopping, story time, and general skills and behaviours. Example items are: 'Plays "Hide and Go Seek" without cheating (e.g., does not peek when

counting)' and 'Waits to pay for items without complaint'. A higher score indicates better executive functioning. The Cronbach's alpha was 0.96 for mothers and 0.95 for partners.

As some partner reports were missing (n=14 for the BRIEF-P and n=15 for the REEF), and they correlated significantly with maternal reports (r=0.51 for the BRIEF-P and r=0.30 for the REEF), maternal reports were used in the main analyses. Partner reports of the BRIEF-P and the REEF were used as sensitivity measures.

Inhibitory control tasks

Behavioural tasks were chosen according to five categories of inhibitory control classified by Anderson and Reidy (2012): Motor inhibition (i.e., inhibit motor behaviour at specific moments after learning it), Verbal inhibition (i.e., inhibit verbal responses), Impulse control (i.e., inhibit an instinctive response), Delay of gratification (i.e., resist direct temptation to receive a larger reward after the delay) and Go/No-go (i.e., perform certain behaviour after being shown a stimulus and to inhibit that behaviour after being shown a different stimulus). A higher score on the tasks indicates better inhibitory control.

The **Flanker** task (Eriksen and Eriksen, 1974) was used to measure motor inhibition. Children were asked to point in the same direction of where a centrally located target fish was swimming towards, ignoring the presence of interfering stimuli (i.e., flanking fish oriented in the same or opposite directions). Children who passed the four practice trials, were presented another 10 trials of which three were incongruent trials. Accuracy of the incongruent trials was scored between 0 and 3 (0=pointing in the wrong direction; 1=first pointing in the correct direction, then pointing wrongly; 2=first pointing in the wrong direction, then pointing correctly; 3=pointing in the correct direction), and subsequently averaged. Forty-nine out of 63 children passed the practice trial.

The **Whisper** task (Beijers et al., 2013; Kochanska et al., 1996) was used to measure verbal inhibition. Children who passed the two practice trials, were asked to whisper the names of another 12 animal pictures. Answers were coded 0 to 2 for every picture (0=shout; 1=normal or mixed tone; 2=whisper), and averaged. All children passed the practice trial.

The **Gift Wrap** task (Beijers et al., 2013; Kochanska et al., 1996) was used to measure motor inhibition. Before the gift in front of them was wrapped, the children were asked to cover their eyes with their hands and not peek. Wrapping lasted for one minute. Children's waiting behaviour was coded every five seconds with a score ranging from 0 to 3 (0=watches wrapping/gift; 1=peeks; 2=looks away from wrapping/gift; 3=closed eyes and/or hands in front of the eyes), and averaged. One child did not understand the task and was therefore excluded from the analysis.

The **Gift Delay** task (Kochanska et al., 1996) was used to measure impulse control. Children were asked to refrain from touching and unwrapping the present, placed in front of the child, when the examiner left the room for one-and-a-half minute. Impulse control was measured as latency (measured in seconds) until touching the present.

Due to insufficient variation and low number of children that passed the practice trials in the Snack Delay task (to measure delay of gratification) (Beijers et al., 2013; Kochanska et al., 1996), and the Bear Dragon task (to measure go/no-go) (Kochanska et al., 1996; Reed et al., 1984), respectively, these tasks were excluded from the analyses (Willemsen et al., 2021).

Scoring of inhibitory control tasks

Video recordings of the inhibitory control tasks were observed and scored independently by two observers. The first five recordings were scored by both observers independently and checked for agreement. Disagreements were discussed and adjusted in the scoring book. Thereafter, the observers only discussed recordings in case of un-

certainties. Thirty out of 63 recordings were scored by both observers to determine inter-rater reliability. Reliability was quantified by the Intraclass Correlation Coefficient (ICC) relying on absolute agreement. The ICC's for the inhibitory control tasks were good: 0.95 for the Flanker, 0.86 for the Whisper, 0.88 for the Gift Wrap, and 0.84 for the Gift Delay. Because the tasks measured different forms of inhibitory control as part of the same overarching construct, "lumping" was preferred over "splitting". Following Willemsen et al. (2021), a composite score was created for the inhibitory control tasks by averaging the z-scores. Note that a latent variable could not be created due to violations of the assumptions (Willemsen et al., 2021).

Confounders

Potential confounding variables were based on previous literature and plotted in a directed acyclic graph (DAG) (see Figure 3.2 for the DAG) (Cinelli et al., 2022), to determine their inclusion in the main analysis. Based on the DAG, the following confounding variables were considered for the main analyses: gestational age at birth (Han et al., 2021; Yang et al., 2010), maternal educational level (ranging from 1, primary education, to 8, university education) (Ardila et al., 2005; Kao et al., 2018), and executive functioning of the parent(s) (Kao et al., 2018). For this last confounder, parental executive functioning was assessed with the Behavior Rating Inventory of Executive Function-Adult (BRIEF-A) (Roth et al., 2005). The BRIEF-A is a 75-item self-report questionnaire of executive functioning in adults, scored on a 3-point scale. We reverse-coded the BRIEF-A outcome for interpretation purposes, so that higher scores indicate better executive functioning. The Cronbach's alphas were good for mothers (α=0.96), and partners (α=0.93). Similar to the BRIEF-P, some partner reports were missing (n=20), and they correlated significantly with maternal reports (r=0.54). Therefore, only maternal reports were used as confounder and partner reports of the BRIEF-A were used as sensitivity measures. These potential confounders were

correlated with the executive functions and inhibitory control variables to determine their inclusion as confounders in the analyses. Other potential confounders that were considered but eventually excluded based on the DAG were: maternal age (Han et al., 2021), secretor status (Thurl et al., 2017), mode of delivery (Ahlqvist et al., 2021; Samuel et al., 2019), and parity (Samuel et al., 2019). While low infant birthweight has been associated with HMO composition and cognitive functioning (Cheng et al., 2014; Hack et al., 1995), the current study only included infants with healthy birthweight; therefore, infant birthweight was not included as confounder. Moreover, breastfeeding duration had previously been assessed in the same cohort with the same outcome variables (i.e., BRIEF-P, REEF, Whisper, Flanker, Gift Wrap, and Gift Delay), and no significant associations were found between breastfeeding duration and these outcomes (Willemsen et al., 2021). Hence, breastfeeding duration was not included as confounder. The following potential confounders were not considered in the DAG, as no data had been collected: gestational weight gain and maternal body mass index (Han et al., 2021).

Missing data

Excluding the sum variables derived from the original data (e.g., grouped fucosylated HMOs are the sum of specific individual HMOs), 9% of the data were missing. The following milk data was missing: one sample at two weeks and one sample at six weeks. Additional missing milk data was due to some mothers not breastfeeding and therefore unable to provide a sample (two samples at two weeks, four samples at six weeks, and nine samples at 12 weeks). The following questionnaire data were missing: for mothers, REEF (n=1) and BRIEF-A (n=1), and for partners, BRIEF-P (n=14), REEF (n=15), and BRIEF-A (n=20) (of which 14 of each questionnaire were missing because these partners did not join this study at baseline). The following behavioural data were missing: Whisper (n=4), Gift Wrap (n=4), Gift Delay (n=4), and Flanker

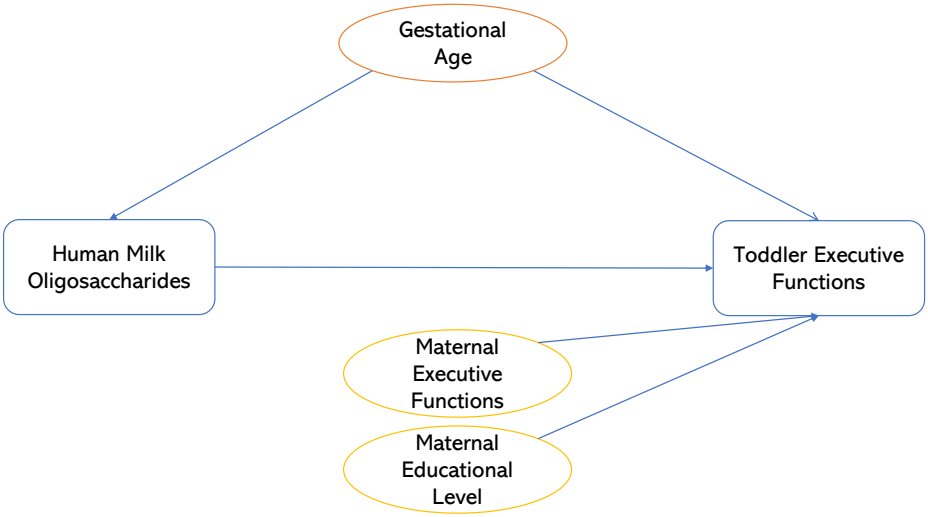


Figure 3.2. Directed Acyclic Graph for determining confounders. Blue colours represent the predictor and outcome variable. Orange represents the potential confounder related to both the predictor and the outcome, and yellow represents the potential confounder related to the outcome variable.

(n=18, of which 15 were missing because children did not pass the practice trial). The LittleMCAR test from the 'BaylorEdPsych' package indicated that data were missing completely at random (χ^2=153.173, p=0.549). Missing data were imputed by means of multiple imputation according to van Buuren and Groothuis-Oudshoorn (2011) using the 'mice' package. Data was imputed 20 times and analyses were thus run 20 times. Results of these analyses were pooled using the pool function of the 'mice' package, which averages the estimates of the 20 analyses.

Statistical analyses

All analyses were performed in R version 4.0.2. A 95% confidence interval that does not contain a 0, and a p-value of $<$0.05, were considered statistically significant. Variables were checked for normality, and the following were not normally distributed: 2'FL (at two, six, and 12 weeks), 3'SL (at two, six, and 12 weeks), 6'SL (at two, six, and 12 weeks), grouped fucosylated HMOs (at six and 12 weeks), grouped sialylated

HMOs (at two, six, and 12 weeks), inhibitory control composite score, gestational age, partner executive functions, and maternal educational level. Pearson and Spearman correlations were performed to correlate normally and non-normally distributed variables, respectively. Furthermore, the area under the curve (AUC) with respect to the ground was calculated for the HMOs of the three milk assessment time points to create one variable that reflects infant exposure to HMOs during the first 12 postnatal weeks (Pruessner et al., 2003).

Next, the data used for the final analyses were inspected for outliers. The following variables contained outliers: the AUC of 3'SL (two outliers), the AUC of 6'SL (two outliers), BRIEF-P filled in by the mother (one outlier), and inhibitory control composite score (one outlier). The outliers were winsorized (Blaine, 2018). Results of the analyses were similar with and without winsorizing (i.e., including the outliers).

For the main analyses, multiple regression analyses were performed to assess the association between the HMOs (i.e., 2'FL, 3'SL, 6'SL, grouped fucosylated, and grouped sialylated HMOs) and the outcome variables (i.e., executive functions as assessed by the BRIEF-P, REEF, and the inhibitory control composite score). Six models were run, two per outcome variable. The three separate HMOs (2'FL, 3'SL, and 6'SL) were added to three models as predictors of the outcome variables. Because the two HMO groups (fucosylated and sialylated HMOs) are partly derived from the separate HMOs in the first three models, we analysed the HMOs separately. The two HMO groups were added to the three models as predictors for the three outcome variables. These six models were run twice, once including data of exclusively breastfed infants, and once including data of partially breastfed infants.

Sample size could not be adjusted due to the longitudinal nature of our study. We determined the power of our analyses depending on the effect size and sample size, using G*Power. According to Cohen's (1988) guidelines for multiple regression analyses $f^2 \geq 0.02$, $f^2 \geq 0.15$, and $f^2 \geq 0.35$ represent small, medium, and large effect sizes,

respectively (Cohen, 1988). We entered an alpha error probability of 0.05, and six predictors (i.e., the three individual HMOs, and the three confounders) for the model with individual HMOs. Five predictors were entered for the model with grouped HMOs. When including exclusively breastfed infants only (n=45), our power is 0.15, 0.72, and 0.97 for detecting small, medium, and large effect sizes, respectively. When including partially breastfed infants (n=63), our power is 0.20, 0.86, and >0.99, for detecting small, medium, and large effect sizes, respectively. Log likelihood tests were performed to check which model fits the data best. Models including the confounders fit significantly better than models without confounders. Hence only results from the models with the confounders were interpreted for the results.

Exploratory analyses

Clinically relevant executive function problems. Multivariate logistic regression analysis was performed to assess the differences between the group of children who scored above the (sub)clinical cut-off of the BRIEF-P (i.e., indicating that these children experience clinically relevant executive function problems), and a group of children without such problems (Sherman and Brooks, 2010). Seventeen participants scored above the subclinical cut off of the BRIEF-P (i.e., a t-score of 60 or higher). For the high-low comparison analyses, a contrast group was made by taking the 19 participants who scored the best on executive functions of the BRIEF-P (i.e., t-score of 48 or lower). The dummy outcome variable was being in the group with low executive functions (0) or in the group with high executive functions (1). The predictors were the AUCs of the separate HMOs (2'FL, 3'SL, and 6'SL). The same analyses were performed with the AUCs of the HMO groups as predictors (fucosylated and sialylated HMOs). Note that the BRIEF-P was the only outcome measure in which clinical cut-off values are available.

Individual HMOs and individual time points. To investigate the effects of other

HMOs on executive functions and inhibitory control, we added all HMOs from all time points to a random forest model. In total, 24 HMOs (2'FL, 3-FL, 3'SL, 6'SL, DF-TF-LNH, DFL, four different isomers of F-LNH, IF-LNH-I, LNDFH-I, LNDFH-II, LNFP-I, LNFP-II, LNFP-III, LNFP-V, LNH, LNnH, pLNH, LNT and LNnT combined, LSTa, LSTb, LSTc) were added to a Random Forest model to assess which HMO had the highest predictive value for the BRIEF-P, the REEF, and the inhibitory control composite. We ran the same analyses for the five HMO groups (grouped fucosylated HMOs, grouped sialylated HMOs, grouped non-fucosylated and grouped non-sialylated HMOs, grouped mono fucosylated HMOs, and grouped di- and tri-fucosylated HMOs). One random forest model was run per outcome variable. Thus, three models were run for the exploration of separate HMOs and three models were run for the exploration of HMO groups. The 'randomForest' package was used to run the random forest analyses. We fitted a random forest models using the 'Tuneranger' package (Wright and Ziegler, 2017). After that, the HMOs and HMO groups from all time points were added separately to a random forest model to assess which HMO at what time point had the most predictive value for the BRIEF-P, the REEF, and the inhibitory control composite (e.g., all HMOs at age 2 weeks were added in one model with the BRIEF-P).

Results

Descriptives of study population characteristics and study variables

Table 3.1 shows the descriptive statistics of the study population. Table 3.2 shows the descriptives of the measured variables including the percentages of exclusive breastfeeding mothers, the measured concentrations of the main HMOs and HMO groups of interest, and the scores on executive functions and inhibitory control tasks. Differences in concentrations over time were tested with a One-Way ANOVA test. Figure

3.3 shows the significant changes in HMO concentration over time. Concentrations of 6'SL, grouped fucosylated HMOs, and grouped sialylated HMOs decreased significantly over time. Concentrations of 2'FL significantly differed between two weeks and 12 weeks, but not for six weeks. Concentrations of 3'SL at two weeks significantly differed from concentrations at six and 12 weeks. However, concentrations of 3'SL at six weeks did not differ from concentrations at 12 weeks. After adjustment for estimated daily intake, 6'SL and grouped sialylated HMOs decreased significantly over time. Estimated intake of 2'FL, 3'SL, and grouped fucosylated HMOs did not change significantly over time. Scores on the BRIEF-P, the REEF, and the BRIEF-A did not differ significantly between mother and partner.

Table 3.1. Study population characteristics.

Characteristics	%	*n*
Child sex		
Girl	49.2	31
Boy	50.8	32
Maternal educational level		
Low	0	0
Middle	14.5	9
High	85.5	53
Missing	1.6	1
	Age (±SD)	*n*
Gestational age (weeks)	39.8 (±1.6)	63
Child age (months)	37.6 (±1.1)	63
Maternal age (years)[1]	34.5 (±3.6)	63
Partner age (years)[1]	35.9 (±4.1)	47

[1]Ages at child age three years (date of the home visit).

Table 3.2. Descriptive statistics of measured variables: breastfeeding, HMO levels, executive functions, and inhibitory control.

	%	*n*		
Exclusive breastfeeding (2 weeks)	86	54		
Exclusive breastfeeding (6 weeks)	89	56		
Exclusive breastfeeding (12 weeks)	78	49		
HMO levels	Mean concentration (g/L) (±SD)[1]	*n*	Estimated daily intake for exclusively breastfed infants (g) (±SD)[2]	*n*
2'FL 2 weeks	0.95 (±0.52)^{a}	60	0.45 (±0.24)	43
2'FL 6 weeks	0.82 (±0.41)^{ab}	58	0.47 (±0.24)	44
2'FL 12 weeks	0.67 (±0.35)^{b}	54	0.41 (±0.23)	45
3'SL 2 weeks	0.18 (±0.03)^{a}	60	0.08 (±0.01)	43
3'SL 6 weeks	0.17 (±0.01)^{b}	58	0.10 (±0.01)	44
3'SL 12 weeks	0.16 (±0.02)^{b}	54	0.10 (±0.01)	45
6'SL 2 weeks	0.38 (±0.09)^{a}	60	0.18 (±0.04)	43
6'SL 6 weeks	0.18 (±0.02)^{b}	58	0.11 (±0.01)	44
6'SL 12 weeks	0.07 (±0.02)^{c}	54	0.04 (±0.01)	45
Fucosylated HMOs 2 weeks	4.84 (±0.46)^{a}	60	2.31 (±0.18)	43
Fucosylated HMOs 6 weeks	4.31 (±0.32)^{b}	58	2.49 (±0.19)	44
Fucosylated HMOs 12 weeks	3.88 (±0.42)^{c}	54	2.45 (±0.29)	45
Sialylated HMOs 2 weeks	1.10 (±0.19)^{a}	60	0.52 (±0.07)	43
Sialylated HMOs 6 weeks	0.66 (±0.06)^{b}	57	0.38 (±0.04)	44
Sialylated HMOs 12 weeks	0.43 (±0.06)^{c}	54	0.27 (±0.03)	45
Behaviour	Score (±SD)	*n*		
Executive functions Questionnaires				
BRIEF-P mother	95.0 (±15.8)	63		
BRIEF-P partner	97.4 (±18.1)	49		
REEF mother	146.4 (±32.6)	62		
REEF partner	144.9 (±28.0)	48		
BRIEF-A mother	108.2 (±19.7)	62		
BRIEF-A partner	108.3 (±16.0)	43		
Inhibitory control tasks				
Flanker	1.3 (±0.7)	45		
Whisper	1.8 (±0.3)	59		
Gift Wrap	2.1 (±0.9)	59		
Gift Delay (seconds)	77.0 (±28.2)	59		

[1] Adjusted for sample to sample variability (with probabilistic quotient normalization). [2] Adjusted for and daily intake volumes of 480g, 580g, and 630g at weeks two, six, and 12, respectively, based on previous literature (Institute of Medicine (US). Subcommittee on Nutrition during Lactation and United States. Health Resources and Services Administration, 1991). ^{a},^{b},^{c} indicate significant differences ($p<0.05$) between time points (i.e., ^{a} differs from ^{b} and ^{c}, but not from ^{a}).

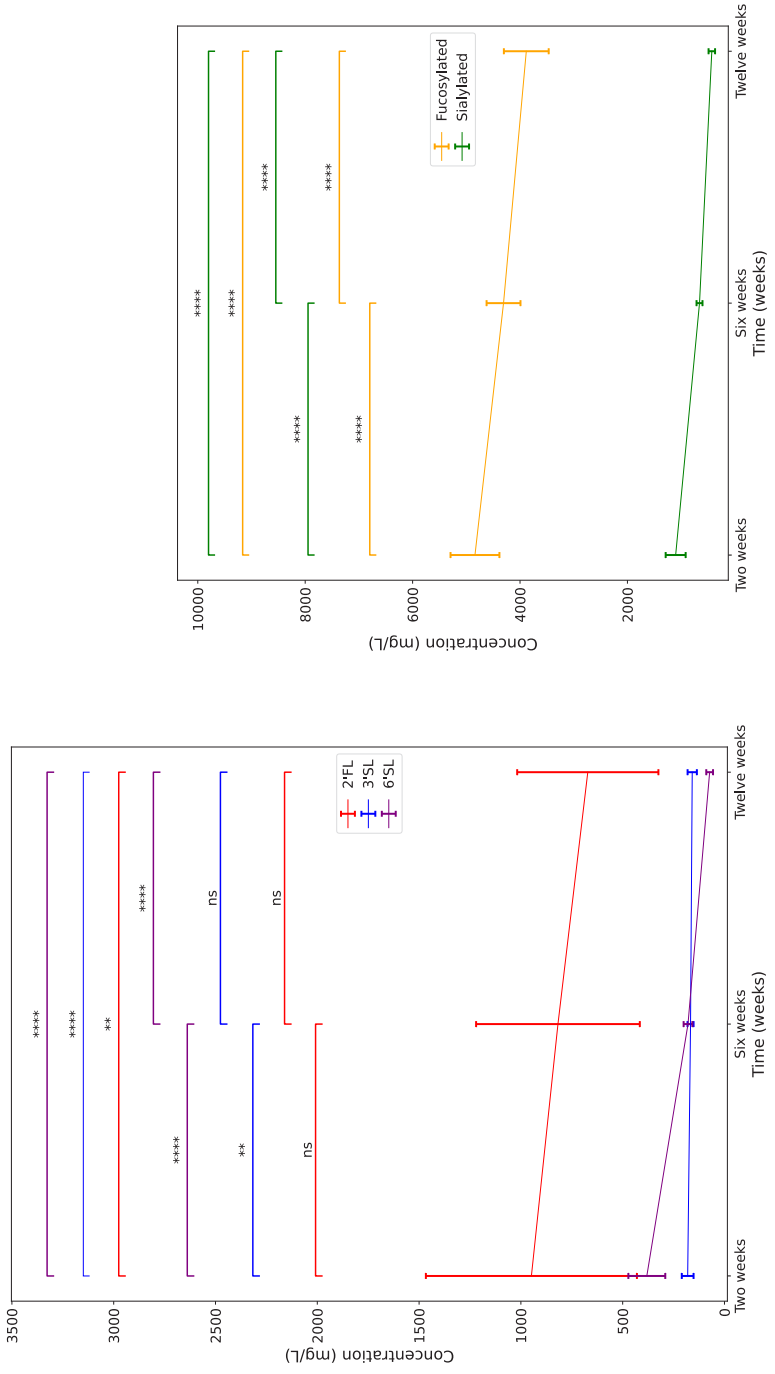


Figure 3.3. Change in HMO concentrations over the first three months. Left graph shows the change of individual HMO concentrations. Right graph shows the change of grouped HMO concentrations. Error bars represent 1 SD of the mean. ns: non-significant; **$p<0.01$, ****$p<0.0001$.

Correlations

Correlations between executive function and inhibitory control measures

Correlations between executive function questionnaires and inhibitory control tasks are shown in Table 3.3. The BRIEF-P and the REEF correlated significantly for mothers (r=0.38), but not for partners. Both the BRIEF-P and the REEF correlated between mother and partner (r=0.51 and r=0.30, respectively). The BRIEF-A (reflecting parent's executive functions) and the BRIEF-P (reflecting toddler's executive functions) correlated significantly for mother and for partner (r=0.34 and r=0.50, respectively). In addition, the inhibitory control tasks did not intercorrelate. Better performance on the Gift Wrap and the Gift Delay correlated positively with better executive functions as measured by the REEF filled in by the mother (r=0.29 and r=0.37, respectively).

Correlations between main HMOs of interest and behavioural measures

Next, correlations between the concentrations of HMOs of main interest are shown in Table 3.4. Concentrations of 2'FL correlated significantly between two and 12 weeks (r=0.30). All concentrations of 2'FL correlated significantly with concentrations of grouped fucosylated HMOs at all time points (r ranging from 0.29 to 0.85), except for 2'FL at six weeks and grouped fucosylated HMOs at 12 weeks. Furthermore, 3'SL, 6'SL, and grouped sialylated HMOs correlated negatively over time (r ranging from -0.27 to -0.64). After removal of outliers in these measures, the correlations remained mostly similar.

Correlations between the predictor and outcome variables used in the main models (i.e., AUC of the HMOs, maternal reports of executive functions, and inhibitory control composite) are shown in Table 3.5. Only the AUC of grouped sialylated HMOs was negatively correlated with the BRIEF-P (r=-0.31). No other HMOs were significantly correlated with the executive function and inhibitory control measures.

Table 3.3. Correlations between executive function measures and inhibitory control measures.

	BRIEF-P Mother	BRIEF-P Partner	REEF Mother	REEF Partner	BRIEF-A Mother	BRIEF-A Partner	Flanker	Whisper	Gift Wrap	Gift Delay
BRIEF-P Mother	-									
BRIEF-P Partner	0.51***	-								
REEF Mother	0.38**	0.23	-							
REEF Partner	0.03	0.08	0.30*	-						
BRIEF-A Mother	0.34**	0.26	-0.14	-0.06	-					
BRIEF-A Partner	0.34*	0.50**	0.13	0.05	0.54***	-				
Flanker	0.07	0.23	0.15	0.23	-0.02	0.25	-			
Whisper	0.00	-0.17	0.00	0.04	-0.06	-0.21	0.04	-		
Gift Wrap	-0.03	-0.02	0.29*	0.18	-0.24	-0.02	0.10	-0.07	-	
Gift Delay	0.10	0.00	0.37**	0.15	-0.32*	-0.23	0.19	0.12	0.21	-

Note: BRIEF-P and BRIEF-A are reverse-scored (i.e., higher scores indicate better executive functions). *$p<0.05$. **$p<0.01$. ***$p<0.001$. $n=63$.

Table 3.4. Correlations between concentrations of individual HMOs and grouped HMOs.

	2'FL (2w)	2'FL (6w)	2'FL (12w)	3'SL (2w)	3'SL (6w)	3'SL (12w)	6'SL (2w)	6'SL (6w)	6'SL (12w)	Fuc HMOs (2w)	Fuc HMOs (6w)	Fuc HMOs (12w)	Sial HMOs (2w)	Sial HMOs (6w)
2'FL (2w)	-													
2'FL (6w)	0.26	-												
2'FL (12w)	0.30*	0.14	-											
3'SL (2w)	0.08	-0.08	-0.17	-										
3'SL (6w)	0.02	0.25	-0.24	-0.30*	-									
3'SL (12w)	0.08	-0.07	0.37**	-0.64***	-0.42**	-								
6'SL (2w)	0.19	-0.12	-0.03	0.08	-0.04	-0.07	-							
6'SL (6w)	-0.13	0.18	-0.13	-0.17	-0.03	0.14	-0.39**	-						
6'SL (12w)	-0.12	-0.09	0.17	-0.10	0.24	-0.03	-0.64***	-0.27*	-					
Fuc HMOs (2w)	0.66***	0.43**	0.34*	0.28*	-0.03	-0.10	0.06	-0.10	0.00	-				
Fuc HMOs (6w)	0.29*	0.85***	0.30*	-0.20	0.42**	-0.08	-0.03	0.20	-0.19	0.33*	-			
Fuc HMOs (12w)	0.34*	0.01	0.73***	-0.21	-0.25	0.40**	-0.01	-0.15	0.15	0.14	0.05	-		
Sial HMOs (2w)	0.25	-0.09	-0.05	0.41**	-0.03	-0.29*	0.71***	-0.30*	-0.51***	0.39**	-0.06	-0.04	-	
Sial HMOs (6w)	-0.37**	0.22	-0.20	-0.29*	0.47***	-0.13	-0.24	0.48***	-0.10	-0.45**	0.41**	-0.38**	-0.43**	-
Sial HMOs (12w)	0.09	-0.11	0.32*	-0.46***	-0.26	0.77***	-0.25	-0.14	0.36**	-0.10	-0.20	0.47***	-0.45***	-0.30*

Note: Correlations are denoted as *r*. Fuc: Fucosylated, Sial: Sialylated, 2w: two weeks, 6w: six weeks, 12w: 12 weeks. HMO concentrations are in grams per litre and adjusted for sample to sample variability (n=63). *p<0.05. **p<0.01. ***p<0.001.

Table 3.5. Correlations between maternal reports on executive functions, inhibitory control composite, and the AUCs of the HMOs (adjusted for sample to sample variability, percentage breastfeeding, and estimated daily intake).

	BRIEF-P by mother	REEF by mother	Inhibitory control composite
AUC of 2'FL	0.00	0.16	0.04
AUC of 3'SL	0.20	-0.12	-0.06
AUC of 6'SL	0.14	-0.16	-0.13
AUC of Fucosylated HMOs	-0.17	0.21	0.14
AUC of Sialylated HMOs	-0.31*	0.03	-0.09

Notes: Correlations are based on imputed data (n=63). HMOs mentioned in this table are the AUCs of the HMOs in grams consumed at 2, 6, and 12 weeks; the BRIEF-P is reverse coded to correspond with the other executive functions and inhibition measures (i.e., higher BRIEF-P scores indicate better executive functions). *$p<0.05$.

Correlations between potential confounding variables and executive functions measures

Potential confounding variables were determined beforehand by the use of the DAG (as mentioned in the confounder section) and subsequently correlated with the outcome variables (see Table 3.6). Only the BRIEF-A correlated significantly with the BRIEF-P (r=0.30) and the inhibitory control composite score (r=0.32). Hence, gestational age and maternal educational level were excluded from the main analysis, and the BRIEF-A was used as confounding factor for the analyses with the BRIEF-P and the inhibitory control composite score.

Main analyses

Analyses with exclusively breastfed infants only

Table 3.7 shows an overview of the multiple regression analyses, as performed in the exclusively breastfed group. Better executive functioning, as measured with the REEF, was associated with more 2'FL (β:5.21, 95%CI:0.84 - 9.57) and grouped fucosylated HMO's (β:3.43, 95%CI:0.30 - 6.56). These results indicate that higher consumption of human milk concentrations of 2'FL and grouped fucosylated HMOs during infancy,

Table 3.6. Correlations between executive function measures and potential confounding variables.

	BRIEF-P Mother	REEF Mother	Inhibitory control composite	Gestational age	Mother educational level	BRIEF-A Mother
BRIEF-P Mother	-					
REEF Mother	0.33**	-				
Inhibitory control composite	0.07	0.34**	-			
Gestational age	-0.01	-0.07	-0.05	-		
Mother educational level	-0.04	0.18	0.17	-0.02	-	
BRIEF-A Mother	0.30*	-0.07	0.32*	0.07	0	-

Note: Correlations are based on imputed data (n=63). The BRIEF-P is reverse coded to correspond with the other executive functions and inhibition measures (i.e., higher BRIEF-P scores indicate better executive functions).*p<0.05, **p<0.01.

are associated with higher executive functions at age three years. No other significant associations were found for the BRIEF-P, the REEF, and inhibitory control. Results were no different with and without winsorizing.

Analyses including partially breastfed infants

The same analyses were also performed including data of partially breastfed infants (see Supplementary Table 7.7). The positive association between 2'FL and the REEF found in the exclusive breastfed group was now marginally significant (p=0.06). Additionally, higher levels of sialylated HMOs were associated with worse executive functions, as measured with the BRIEF-P. No other significant results were found, and results were no different with and without winsorizing.

Table 3.7. Associations between HMOs and executive functions (BRIEF-P and REEF) and inhibitory control (exclusively breastfed infants).

Effect	Estimate (95% CI)	Standard error	*p*-value
BRIEF-P Model 1			
Intercept	147.65 (47.08 - 248.21)**	49.76	0.005
2'FL	-0.34 (-2.07 - 1.40)	0.86	0.70
6'SL	-39.22 (-85.79 - 7.35)	23.04	0.10
3'SL	-29.15 (-118.13 - 59.83)	44.03	0.51
BRIEF-A	0.15 (-0.048 - 0.35)	0.10	0.13
BRIEF-P Model 2			
Intercept	108.24 (22.69 - 193.78)*	42.36	0.015
Fucosylated HMOs	-0.18 (-1.45 - 1.08)	0.63	0.77
Sialylated HMOs	-7.88 (-28.30 - 12.53)	10.11	0.44
BRIEF-A	0.18 (-0.02 - 0.38)	0.10	0.08
REEF Model 1			
Intercept	264.44 (33.40 - 495.48)*	114.40	0.03
2'FL	5.21 (0.84 - 9.57)*	2.16	0.02
6'SL	-14.33 (-131.61 - 102.96)	58.08	0.81
3'SL	-138.79 (-360.91 - 83.34)	109.99	0.21
REEF Model 2			
Intercept	122.34 (-76.57 - 321.26)	98.57	0.22
Fucosylated HMOs	3.43 (0.30 - 6.56)*	1.55	0.03
Sialylated HMOs	-15.75 (-66.45 - 34.95)	25.12	0.53
Inhibitory control Model 1			
Intercept	1.31 (-2.53 - 5.15)	1.90	0.49
2'FL	0.01 (-0.05 - 0.08)	0.03	0.70
6'SL	-0.37 (-2.15 - 1.41)	0.88	0.68
3'SL	0.10 (-3.29 - 3.50)	1.68	0.95
BRIEF-A	-0.01 (-0.02 - -0.002)*	0.004	0.02
Inhibitory control Model 2			
Intercept	2.24 (-0.82 - 5.30)	1.52	0.15
Fucosylated HMOs	0.02 (-0.02 - 0.07)	0.02	0.27
Sialylated HMOs	-0.47 (-1.20 - 0.26)	0.36	0.20
BRIEF-A	-0.01 (-0.02 - -0.002)*	0.004	0.01

Note that analyses were performed on exclusively breastfed infants only, n=45. The REEF models did not include confounders as none of the potential confounders correlated with the REEF. The BRIEF-P is reverse coded to correspond with the other executive functions and inhibition measures (i.e., higher BRIEF-P scores indicate better executive functions). All HMOs and HMO groups mentioned in this table are presented as the Area Under the Curve. *$p<0.05$, **$p<0.01$.

Exploratory analyses

Clinically relevant executive function problems

Multiple logistic regression analyses were performed to check the differences between children with high and low executive functions (Table 3.8). No significant results were found. Results were the same with and without winsorizing. The results including partially breastfed infants were also non-significant (see Supplementary Table 7.8).

Table 3.8. Multiple logistic regression results of the relation between the HMOs and HMO groups, and the BRIEF-P (exclusively breastfed infants).

Effect	Estimate (95% CI)	Standard error	*p*-value
BRIEF-P Model 1			
Intercept	19.90 (-10.91 - 61.23)	17.45	0.27
2'FL	-0.03 (-0.63 - 0.47)	0.26	0.91
6'SL	-10.36 (-26.00 - 1.86)	6.80	0.15
3'SL	-12.84 (-44.72 - 11.80)	13.73	0.36
BRIEF-A	0.03 (-0.02 - 0.10)	0.03	0.29
BRIEF-P Model 2			
Intercept	1.68 (-22.06 –24.82)	11.53	0.89
Fucosylated HMOs	-0.04 (-0.42 - 0.27)	0.16	0.79
Sialylated HMOs	-1.16 (-7.71 - 5.07)	3.14	0.72
BRIEF-A	0.03 (-0.01 - 0.09)	0.02	0.18

Note that the analyses were performed on exclusively breastfed infants only, n=45. All HMOs and HMO groups mentioned in this table are presented as the Area Under the Curve. BRIEF-P coded as: 1, representing the high executive functions group and 0, representing the low executive functions group. Hence, positive values indicate a positive association between higher levels of HMOs and high executive functions.

Individual HMOs and individual time points

All HMOs were added to a random forest model using the data of the children that had been exclusively breastfed during the milk sampling period. All models with the BRIEF-P and REEF yielded a high Mean of Squared Residuals (MSR) (ranging from 163 to 212 for the BRIEF-P and from 1025 to 1371 for the REEF) and a negative % variance explained, also after tuning the models. While the models for the inhibitory

control composite yielded a low MSR, these models also explained negative variance. Because the model fits for all random forest models could not be improved, indicating that the HMOs we selected were unsuitable for predicting our outcomes, the results of the random forest models were not interpreted. Similar results were found after including data from partially breastfed infants.

To still be able to exploratorily inspect the HMOs at separate time points, we ran multiple regression analyses with the separate HMOs predicting the outcome measures, and corrected for multiple testing by dividing the alpha by the number of predictors in the model (Mundfrom et al., 2006). The HMOs at separate time points were not able to significantly predict the outcomes. These results were identical after including the partially breastfed infants.

Discussion

The goal of this study was to investigate links between human milk HMO concentrations during the first 12 postpartum weeks, and executive functions and inhibitory control at three years of age. The analyses performed in the group of exclusive breastfed infants during the 12-week milk sampling period provided evidence that higher milk concentrations of 2'FL and grouped fucosylated HMOs during the first 12 postnatal weeks were associated with better executive functions at age three, as measured with the REEF questionnaire. When partially breastfed infants were added to the analyses, similar results for 2'FL were produced and a negative association between grouped sialylated HMOs and executive functions, as measured with the BRIEF-P questionnaire, appeared. No associations were found with 3'SL, 6'SL, and the inhibitory control composite score. The results from our random forest models with HMOs measured at single time points could not be interpreted due to poor model fits.

We found evidence for an association between higher levels of 2'FL in the first 12 weeks and better executive functions at age three years. This finding seems robust as it

appeared in the analyses with and without including partially breastfed infants. Results of animal studies are also in line with 2'FL leading to better cognition (for a review, see Docq et al. (2020)). Early life administration of 2'FL enhanced long term potentiation (LTP, involved in memory and learning) in rats, improved recognition memory in pigs, and improved performance in operant learning paradigms in mice (Fleming et al., 2020a; Oliveros et al., 2016; Vazquez et al., 2016). Moreover, our results are consistent with two human studies that found an association between 2'FL at one month and better motoric development at six months (Oliveros et al., 2021), and between 2'FL at one month and better cognition at 24 months (Berger et al., 2020b). Interestingly, Berger et al. (2020b) also measured 2'FL concentrations at six months and did not find an association with cognition. Likewise, Jorgensen et al. (2021) also did not find a link between 2'FL at six months and child cognition or executive functions. As such, it might be speculated from these and our findings that early life exposure of 2'FL might be especially important for later cognitive development. It should also be noted that some human studies did not find an association between 2'FL and better cognitive outcomes. Cho et al. (2021) found no evidence for a link between 2'FL concentration (measured at different times for individual infants between ages two to 25 months) and cognition (assessed at ages between two to 25 months). A potential underlying mechanism associating 2'FL with later cognition is the gut microbiota. Indeed, Vázquez et al. (2015) found that 2'FL ingestion in rodents improved learning ability and LTP enhancement, but only when the connection of the vagus nerve was intact (Vazquez et al., 2016). Ingestion of 2'FL may have stimulated production of low molecular components by the gut microbiota, possibly improving executive functions. Reversely, the gut bacteria can alter the integrity of 2'FL (Kuntz et al., 2019), causing 2'FL to reach the brain in a different form. Different forms of 2'FL can exert different effects on LTP in the brain (Vazquez et al., 2016). In addition, fucosyllactose are utilised by *Bifidobacteria* promoting their growth, which may result in positive effects on the brain

(Matsuki et al., 2016; Ojima et al., 2022; Sakanaka et al., 2019). For future studies, it is therefore suggested to include the gut microbiota when investigating the role of HMOs on cognitive outcomes. Also, because HMO levels decrease over time, and both Jorgensen et al. (2021) and Berger et al. (2020b) found no evidence for a relation between future cognition and 2'FL at six months, future sufficiently powered human studies should consider multiple milk samples over a longer period of time to identify sensitive periods for 2'FL concentrations to impact the developing brain.

Our findings also showed that higher concentrations of grouped fucosylated HMOs were present in human milk of children with higher levels of executive functions. Jorgensen et al. (2021) found a positive link between grouped fucosylated HMOs at six months and language, but not executive functions, at age 18 months. Moreover, as more human studies investigating fucosylated HMOs as a group are lacking, and the animal studies on grouped fucosylated HMOs and cognition are scarce, we can only cautiously speculate that grouped fucosylated HMOs may exert positive effects on cognition. Most HMO research to date focused on specific, individual fucosylated HMO's, including 2'FL. For this reason and given our positive findings, future studies may consider also investigating fucosylated HMOs as a group, next to individual HMOs, as the structure of fucosylated HMOs indicate that their physiological functions may be similar (Bode, 2012). More mechanistic studies are also necessary to investigate how grouped fucosylated HMOs might improve cognitive outcomes.

Contrary to our hypothesis, and only in the analyses with the partially breastfed infants included, higher concentrations of grouped sialylated HMOs in mother's milk predicted worse executive functions in 3-year-old children, as measured with the BRIEF-P. Only Jorgensen et al. (2021) investigated grouped sialylated HMOs in humans and found higher levels of grouped sialylated HMOs to be associated with improved language performance at 18 months. Note that our positive associations between 2'FL and grouped fucosylated HMOs and executive functions were obtained with the REEF

questionnaire. Hence, these apparent discrepancies in our results might be explained by the fact that the BRIEF-P assesses child executive functions more in general, while the REEF assesses child behaviour in specific everyday situations. The design of these questionnaires may also explain why paternal BRIEF-P and REEF did not correlate, as in traditional households (like often is the case in the Netherlands (Sociaal en Cultureel Planbureau and Roeters, 2019)), fathers, compared to mothers, spend less time with their children. Fathers may thus have a better view on their child's general executive functions as compared to their child's executive functions in specific daily situations. This could explain why the BRIEF-P was more strongly correlated between parents, compared to the REEF. The BRIEF-P may therefore be a more robust measure of executive functions in general, while the REEF might be more suitable for caregivers who spend more time with their children in different situations. Although the BRIEF-P has been used more often, the use of the newer REEF has been rising.

Our results on grouped sialylated HMOs and worse executive functions was only found when partially breastfed infants were included in the analyses. Because partially breastfed infants by definition consume fewer HMOs than exclusively breastfed infants, we cannot exclude the possibility that these associations between grouped sialylated HMOs and worse executive functions that were only found in the partially breastfed infants may be a chance finding. Additionally, our main analyses were performed on the exclusively breastfed group to correct for potential noise that formula feeding may cause. Some formula feeding includes galactooligosaccharides and fructooligosaccharides (also known as GOS and FOS) which mimic the effects that HMOs have on gut bacteria (Borewicz et al., 2019; Liu et al., 2017), and hence potentially on the brain (Bode, 2015; Totten et al., 2012; Underwood et al., 2014). For this reason, and because the findings differed for the exclusive breastfed versus any breastfed group, we refrain from further interpreting these results.

Furthermore, we found no evidence for a relation between 3'SL concentrations

and executive functions, nor for a relation between 6'SL concentrations and executive functions. Previous results on these HMOs are mixed. Two human studies and one animal study found a positive association with 3'SL and better future cognitive outcomes (Cho et al., 2021; Jorgensen et al., 2021; Pisa et al., 2021), while one human and one animal study found no evidence for an association between 3'SL and future cognition (Berger et al., 2020b; Fleming et al., 2018). Regarding 6'SL, one human study found an association between higher concentrations of 6'SL at one month and better cognition (Oliveros et al., 2021), while another found an association between higher concentrations of 6'SL and a smaller change in infant head circumference between 6-18 months (Jorgensen et al., 2021). Finally, two studies found no evidence for a link between 6'SL and cognition at age 24 months (Berger et al., 2020b; Cho et al., 2021). In piglets, ingestion of 3'SL and 6'SL are related to an increase in sialic acid concentration in the cerebellum and the hippocampus, as well as an expanded hippocampus (Jacobi et al., 2016; Mudd et al., 2017). Whether this mechanism is associated with better executive functions is still unclear. Nonetheless, it is premature to draw conclusions regarding individual sialylated HMOs, as results in human studies are inconsistent, likely due to the different methodology used and ages assessed. Sufficiently powered replication studies are necessary to obtain clarity on if, how, and when sialylated HMOs are associated with child cognition. Curiously, the correlations between all sialylated HMOs (including grouped sialylated HMOs) were negative over time, meaning that higher levels of sialylated HMOs at one time point were correlated with lower levels of sialylated HMOs at another time point. This finding was robust, since removal of outliers did not change these correlations. It is difficult to speculate why these correlations are negative over the first 12 postnatal weeks. How sialylated HMOs develop over time thus requires more research. Future studies in this topic may benefit from adding different time-variant factors, such as maternal diet, or maternal condition and recovery after delivery (Han et al., 2021).

Our study has several strengths. To our knowledge, this study is the first to assess HMO concentrations at three time points early in life and relate these concentrations to cognitive outcomes in toddlerhood. The multiple time points allowed us to investigate HMO concentrations during a critical and sensitive period in life (Martorell, 2017). Second, we used two different types of questionnaires, filled in by mothers and their partners, and several behavioural tasks, to provide a more robust view on child executive functions. A good addition to these measures would be to use eye-tracking (Hodel et al., 2017) or MRI scans (Copeland et al., 2021) for more fine-grained assessments (de Weerth et al., 2022). Our study also has its limitations. The individual milk volume consumption was not measured. This resulted in our estimating HMO exposure based on mean daily intakes known from the literature which is less accurate. Although tedious, future research may benefit from instructing mothers to weigh their infant before and after each feeding to obtain a more precise estimate of their daily milk consumption (Haase et al., 2009; Rankin et al., 2016). Next, the generalizability of our results is limited by our mostly highly educated sample. Lastly, our relatively small sample size reduced our statistical power. However, we preserved our power as much as possible by reducing the number of statistical tests performed, by calculating the AUC of the three HMO measures, by creating a composite score of the observed inhibitory control test scores, and by using partner scores as sensitivity measures.

Despite knowing the beneficial effects of human milk, it is currently one of the most under-investigated biological systems in life sciences (de Weerth et al., 2022). Specifically, human studies investigating HMOs in relation to cognitive outcomes in early childhood are scarce. We found evidence for an association between 2'FL and grouped fucosylated HMOs during the first 12 postnatal weeks and better child executive functions at age three. In the future, larger replication studies should consider collecting multiple mothers' milk samples in early life and extending these findings to later ages as well. Additionally, studies may benefit from including the gut microbiota

in their analyses to be able to investigate the mechanisms underlying HMO associations with child neurodevelopment. Studies should also investigate the effects of HMOs on the development of vulnerable groups who require tailored nutrition but do not always have access to human milk (e.g., preterm born infants). Such studies would aid in the determination of sensitive periods in which HMOs may exert the largest positive effects on cognition and executive functions.

References

Ahlqvist, V. H., Ekström, L. D., Jónsson-Bachmann, E., Tynelius, P., Madley-Dowd, P., Neovius, M., Magnusson, C., and Berglind, D. (2021). Caesarean section and its relationship to offspring general cognitive ability: a registry-based cohort study of half a million young male adults. *Evidence Based Mental Health*, 25(1):7–14.

Anderson, P. J. and Reidy, N. (2012). Assessing executive function in preschoolers. *Neuropsychology Review*, 22(4):345–360.

Ardila, A., Rosselli, M., Matute, E., and Guajardo, S. (2005). The influence of the parents' educational level on the development of executive functions. *Developmental Neuropsychology*, 28(1):539–560.

Bartol, F. F., Wiley, A. A., and Bagnell, C. A. (2008). Epigenetic Programming of Porcine Endometrial Function and the Lactocrine Hypothesis. *Reproduction in Domestic Animals*, 43:273–279.

Bartol, F. F., Wiley, A. A., George, A. F., Miller, D., and Bagnell, C. (2017). PHYSIOLOGY AND ENDOCRINOLOGY SYMPOSIUM: Postnatal reproductive development and the lactocrine hypothesis. *Journal of Animal Science*, 95(5):2200.

Beijers, R., Riksen-Walraven, M., Putnam, S., Jong, M. d., and de Weerth, C. (2013). Early non-parental care and toddler behaviour problems: Links with temperamental negative affectivity and inhibitory control. *Early Childhood Research Quarterly*, 28(4):714–722.

Berger, B., Porta, N., Foata, F., Grathwohl, D., Delley, M., Moine, D., Charpagne, A., Siegwald, L., Descombes, P., Alliet, P., Puccio, G., Steenhout, P., Mercenier, A., and Sprenger, N. (2020a). Linking human milk oligosaccharides, infant fecal community types, and later risk to require antibiotics. *mBio*, 11(2).

Berger, P. K., Plows, J. F., Jones, R. B., Alderete, T. L., Yonemitsu, C., Poulsen, M., Ryoo, J. H., Peterson, B. S., Bode, L., and Goran, M. I. (2020b). Human milk oligosaccharide 2'-fucosyllactose links feedings at 1 month to cognitive development at 24 months in infants of normal and overweight mothers. *PLOS ONE*, 15(2):e0228323.

Bode, L. (2012). Human milk oligosaccharides: Every baby needs a sugar mama. *Glycobiology*, 22(9):1147–1162.

Bode, L. (2015). The functional biology of human milk oligosaccharides. *Early Human Development*, 91(11):619–622.

Bode, L. and Jantscher-Krenn, E. (2012). Structure-function relationships of human milk oligosaccharides. *Advances in Nutrition*, 3(3):383S–391S.

Borewicz, K., Gu, F., Saccenti, E., Hechler, C., Beijers, R., de Weerth, C., van Leeuwen, S. S., Schols, H. A., and Smidt, H. (2020). The association between breastmilk oligosaccharides and faecal microbiota in healthy breastfed infants at two, six, and twelve weeks of age. *Scientific Reports 2020 10:1*, 10(1):1–12.

Borewicz, K., Suarez-Diez, M., Hechler, C., Beijers, R., de Weerth, C., Arts, I., Penders, J., Thijs, C., Nauta, A., Lindner, C., Leusen, E. V., Vaughan, E. E., and Smidt, H. (2019). The effect of prebiotic fortified infant formulas on microbiota composition and dynamics in early life. *Scientific Reports*, 9(1).

Cheng, E. R., Poehlmann-Tynan, J., Mullahy, J., and Witt, W. P. (2014). Cumulative social risk exposure, infant birth weight, and cognitive delay in infancy. *Academic Pediatrics*, 14(6):581–588.

Cho, S., Zhu, Z., Li, T., Baluyot, K., Howell, B. R., Hazlett, H. C., Elison, J. T., Hauser, J., Sprenger, N., Wu, D., and Lin, W. (2021). Human milk 3'-sialyllactose is positively associated with language development during infancy. *The American Journal of Clinical Nutrition*, 114(2):588–597.

Cinelli, C., Forney, A., and Pearl, J. (2022). A crash course in good and bad controls. *Sociological Methods & Research*, page 004912412210995.

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. Hillsdale, NJ: Lawrence Erlbaum Associates, Publishers., second edition.

Cook, F., Giallo, R., Hiscock, H., Mensah, F., Sanchez, K., and Reilly, S. (2019). Infant regulation and child mental health concerns: A longitudinal study. *Pediatrics*, 143(3).

Copeland, A., Silver, E., Korja, R., Lehtola, S. J., Merisaari, H., Saukko, E., Sinisalo, S., Saunavaara, J., Lähdesmäki, T., Parkkola, R., Nolvi, S., Karlsson, L., Karlsson, H., and Tuulari, J. J. (2021). Infant and child MRI: A review of scanning procedures. *Frontiers in Neuroscience*, 15.

Cryan, J. F., O'riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., Codagnone, M. G., Cussotto, S., Fulling, C., Golubeva, A. V., Guzzetta, K. E., Jaggar, M., Long-Smith, C. M., Lyte, J. M., Martin, J. A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., O'connor, R., Cruz-Pereira, J. S., Peterson, V. L., Rea, K., Ritz, N. L., Sherwin, E., Spichak, S., Teichman, E. M., Wouw, M. v. d., Ventura-Silva, A. P., Wallace-Fitzsimons, S. E., Hyland, N., Clarke, G., and Dinan, T. G. (2019). The microbiota-gut-brain axis. *Physiological Reviews*, 99(4):1877–2013.

Dalile, B., Oudenhove, L. V., Vervliet, B., and Verbeke, K. (2019). The role of short-chain fatty acids in microbiota–gut–brain communication. *Nature Reviews Gastroenterology & Hepatology*, 16(8):461–478.

de Weerth, C. (2017). Do bacteria shape our development? Crosstalk between intestinal microbiota and HPA axis. *Neuroscience & Biobehavioral Reviews*, 83:458–471.

de Weerth, C., Aatsinki, A.-K., Azad, M. B., Bartol, F. F., Bode, L., Collado, M. C., Dettmer, A. M., Field, C. J., Guilfoyle, M., Hinde, K., Korosi, A., Lustermans, H., Husna, N., Shukri, M., Moore, S. E., Pundir, S., Rodriguez, J. M., Slupsky, C. M., Turner, S., Goudoever, J. B. V., Ziomkiewicz, A., and Beijers, R. (2022). Human milk: From complex tailored nutrition to bioactive impact on child cognition and behavior. *Critical reviews in food science and nutrition*, pages 1–38.

Docq, S., Spoelder, M., Wang, W., and Homberg, J. R. (2020). The Protective and Long-Lasting Effects of Human Milk Oligosaccharides on Cognition in Mammals. *Nutrients*, 12(11):3572.

Eriksen, B. A. and Eriksen, C. W. (1974). Effects of noise letters upon the identification of a target letter in a nonsearch task. *Perception & Psychophysics*, 16(1):143–149.

Fleming, S., Chichlowski, M., Berg, B., Donovan, S., and Dilger, R. (2018). Dietary sialyllactose does not influence measures of recognition memory or diurnal activity in the young pig. *Nutrients*, 10(4):395.

Fleming, S. A., Mudd, A. T., Hauser, J., Yan, J., Metairon, S., Steiner, P., Donovan, S. M., and Dilger, R. N. (2020a). Dietary oligofructose alone or in combination with 2*r*-fucosyllactose differentially improves recognition memory and hippocampal mRNA expression. *Nutrients*, 12(7):2131.

Fleming, S. A., Mudd, A. T., Hauser, J., Yan, J., Metairon, S., Steiner, P., Donovan, S. M., and Dilger, R. N. (2020b). Human and bovine milk oligosaccharides elicit improved recognition memory concurrent with alterations in regional brain volumes and hippocampal mRNA expression. *Frontiers in Neuroscience*, 14.

Gu, F., ten Kate, G. A., Arts, I. C. W., Penders, J., Thijs, C., Lindner, C., Nauta, A., van Leusen, E., van Leeuwen, S. S., and Schols, H. A. (2021a). Combining HPAEC-PAD, PGC-LC-MS, and 1d ^{1}h NMR to investigate metabolic fates of human milk oligosaccharides in 1-month-old infants: a pilot study. *Journal of Agricultural and Food Chemistry*, 69(23):6495–6509.

Gu, F., Wang, S., Beijers, R., de Weerth, C., and Schols, H. A. (2021b). Structure-specific and individual-dependent metabolization of human milk oligosaccharides in infants: A longitudinal birth cohort study. *Journal of Agricultural and Food Chemistry*, 69(22):6186–6199.

Haase, B., Barreira, J., Murphy, P. K., Mueller, M., and Rhodes, J. (2009). The development of an accurate test weighing technique for preterm and high-risk hospitalized infants. *Breastfeeding Medicine*, 4(3):151–156.

Hack, M., Klein, N. K., and Taylor, H. G. (1995). Long-term developmental outcomes of low birth weight infants. *The Future of Children*, 5(1):176.

Han, S. M., Derraik, J. G. B., Binia, A., Sprenger, N., Vickers, M. H., and Cutfield, W. S. (2021). Maternal and infant factors influencing human milk oligosaccharide composition: Beyond maternal genetics. *The Journal of Nutrition*, 151(6):1383–1393.

Hauser, J., Pisa, E., Vásquez, A. A., Tomasi, F., Traversa, A., Chiodi, V., Martin, F.-P., Sprenger, N., Lukjancenko, O., Zollinger, A., Metairon, S., Schneider, N., Steiner, P., Martire, A., Caputo, V., and Macrì, S. (2021). Sialylated human milk oligosaccharides program cognitive development through a non-genomic transmission mode. *Molecular Psychiatry*, 26(7):2854–2871.

Hechler, C., Beijers, R., Riksen-Walraven, J. M., and de Weerth, C. (2018). Are cortisol concentrations in human breast milk associated with infant crying? *Developmental Psychobiology*, 60(6):639–650.

Hechler, C., Beijers, R., Riksen-Walraven, M., and de Weerth, C. (2019). Prenatal predictors of postnatal quality of caregiving behavior in mothers and fathers. *Parenting*, 19(1-2):101–119.

Hodel, A. S., Senich, K. L., Jokinen, C., Sasson, O., Morris, A. R., and Thomas, K. M. (2017). Early executive function differences in infants born moderate-to-late preterm. *Early Human Development*, 113:23–30.

Hou, L., Li, X., Yan, P., Li, Y., Wu, Y., Yang, Q., Shi, X., Ge, L., and Yang, K. (2021). Impact of the Duration of Breastfeeding on the Intelligence of Children: A Systematic Review with Network Meta-Analysis. *Breastfeeding Medicine*, 16(9):687–696.

Institute of Medicine (US). Subcommittee on Nutrition during Lactation and United States. Health Resources and Services Administration (1991). *Nutrition during lactation*. Henry Holt and Company.

Jacobi, S. K., Yatsunenko, T., Li, D., Dasgupta, S., Yu, R. K., Berg, B. M., Chichlowski, M., and Odle, J. (2016). Dietary isomers of sialyllactose increase ganglioside sialic acid concentrations in the corpus callosum and cerebellum and modulate the colonic microbiota of formula-fed piglets. *The Journal of Nutrition*, 146(2):200–208.

Jorgensen, J. M., Young, R., Ashorn, P., Ashorn, U., Chaima, D., Davis, J. C., Goonatilleke, E., Kumwenda, C., Lebrilla, C. B., Maleta, K., Prado, E. L., Sadalaki, J., Totten, S. M., Wu, L. D., Zivkovic, A. M., and Dewey, K. G. (2021). Associations of human milk oligosaccharides and bioactive proteins with infant growth and development among malawian mother-infant dyads. *The American Journal of Clinical Nutrition*, 113(1):209–220.

Kao, K., Nayak, S., Doan, S. N., and Tarullo, A. R. (2018). Relations between parent EF and child EF: The role of socioeconomic status and parenting on executive functioning in early childhood. *Translational Issues in Psychological Science*, 4(2):122–137.

Kochanska, G., Murray, K., Jacques, T. Y., Koenig, A. L., and Vandegeest, K. A. (1996). Inhibitory control in young children and its role in emerging internalization. *Child Development*, 67(2):490–507.

Krug, M., Wagner, M., Staak, S., and Smalla, K.-H. (1994). Fucose and fucose-containing sugar epitopes enhance hippocampal long-term potentiation in the freely moving rat. *Brain Research*, 643(1-2):130–135.

Kuntz, S., Kunz, C., Borsch, C., Vazquez, E., Buck, R., Reutzel, M., Eckert, G. P., and Rudloff, S. (2019). Metabolic fate and distribution of 2´-fucosyllactose: Direct influence on gut microbial activity but not on brain. *Molecular Nutrition & Food Research*, 63(13).

Liu, F., Li, P., Chen, M., Luo, Y., Prabhakar, M., Zheng, H., He, Y., Qi, Q., Long, H., Zhang, Y., Sheng, H., and Zhou, H. (2017). Fructooligosaccharide (FOS) and galactooligosaccharide (GOS) increase bifidobacterium but reduce butyrate producing bacteria with adverse glycemic metabolism in healthy young population. *Scientific Reports*, 7(1).

Liu, H., Radlowski, E. C., Conrad, M. S., Li, Y., Dilger, R. N., and Johnson, R. W. (2014). Early supplementation of phospholipids and gangliosides affects brain and cognitive development in neonatal piglets. *The Journal of Nutrition*, 144(12):1903–1909.

Martorell, R. (2017). Improved nutrition in the first 1000 days and adult human capital and health. *American Journal of Human Biology*, 29(2):e22952.

Matsuki, T., Yahagi, K., Mori, H., Matsumoto, H., Hara, T., Tajima, S., Ogawa, E., Kodama, H., Yamamoto, K., Yamada, T., Matsumoto, S., and Kurokawa, K. (2016). A key genetic factor for fucosyllactose utilization affects infant gut microbiota development. *Nature Communications*, 7(1).

Matthies, H., Staak, S., and Krug, M. (1996). Fucose and fucosyllactose enhance in-vitro hippocampal long-term potentiation. *Brain Research*, 725(2):276–280.

Montroy, J. J., Bowles, R. P., Skibbe, L. E., McClelland, M. M., and Morrison, F. J. (2016). The development of self-regulation across early childhood. *Developmental Psychology*, 52(11):1744–1762.

Mudd, A., Fleming, S., Labhart, B., Chichlowski, M., Berg, B., Donovan, S., and Dilger, R. (2017). Dietary sialyllactose influences sialic acid concentrations in the prefrontal cortex and magnetic resonance imaging measures in corpus callosum of young pigs. *Nutrients*, 9(12):1297.

Mundfrom, D., Perrett, J., Schaffer, J. R., Piccone, A., and Roozeboom, M. (2006). Bonferroni Adjustments in Tests for Regression Coefficients. *Multiple Linear Regression Viewpoints*.

Nilsen, E. S., Huyder, V., McAuley, T., and Liebermann, D. (2017). Ratings of everyday executive functioning (REEF): A parent-report measure of preschoolers' executive functioning skills. *Psychological Assessment*, 29(1):50–64.

Obelitz-Ryom, K., Bering, S. B., Overgaard, S. H., Eskildsen, S. F., Ringgaard, S., Olesen, J. L., Skovgaard, K., Pankratova, S., Wang, B., Brunse, A., Heckmann, A. B., Rydal, M. P., Sangild, P. T., and Thymann, T. (2019). Bovine milk oligosaccharides with sialyllactose improves cognition in preterm pigs. *Nutrients*, 11(6):1335.

Ojima, M. N., Asao, Y., Nakajima, A., Katoh, T., Kitaoka, M., Gotoh, A., Hirose, J., Urashima, T., Fukiya, S., Yokota, A., Hachem, M. A., Sakanaka, M., and Katayama, T. (2022). Diversification of a fucosyllactose transporter within the genus *Bifidobacterium*. *Applied and Environmental Microbiology*, 88(2).

Oliveros, E., Martín, M. J., Torres-Espínola, F. J., Segura-Moreno, T., Ramírez, M., Santos, A., Buck, R., Rueda, R., Escudero, M., Catena, A., Azaryah, H., and Campoy, C. (2021). Human Milk Levels of 2´-Fucosyllactose and 6´-Sialyllactose are Positively Associated with Infant Neurodevelopment and are Not Impacted by Maternal BMI or Diabetic Status. *Journal of Nutrition & Food Sciences*.

Oliveros, E., Ramirez, M., Vazquez, E., Barranco, A., Gruart, A., Delgado-Garcia, J. M., Buck, R., Rueda, R., and Martin, M. J. (2016). Oral supplementation of 2*r*-fucosyllactose during lactation improves memory and learning in rats. *The Journal of Nutritional Biochemistry*, 31:20–27.

Oliveros, E., Vázquez, E., Barranco, A., Ramírez, M., Gruart, A., Delgado-García, J., Buck, R., Rueda, R., and Martín, M. (2018). Sialic acid and sialylated oligosaccharide supplementation during lactation improves learning and memory in rats. *Nutrients*, 10(10):1519.

Pisa, E., Martire, A., Chiodi, V., Traversa, A., Caputo, V., Hauser, J., and Macrì, S. (2021). Exposure to 3*r*sialyllactose-poor milk during lactation impairs cognitive capabilities in adulthood. *Nutrients*, 13(12):4191.

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., and Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology*, 28(7):916–931.

Rankin, M. W., Jimenez, E. Y., Caraco, M., Collinson, M., Lostetter, L., and DuPont, T. L. (2016). Validation of test weighing protocol to estimate enteral feeding volumes in preterm infants. *The Journal of Pediatrics*, 178:108–112.

Reed, M. A., Pien, D. L., and Rothbart, M. K. (1984). Inhibitory Self-Control in Preschool Children. *Merrill-Palmer Quarterly*, pages 131–147.

Roth, R. M., Isquith, P. K., and Gioia, G. A. (2005). Behavior rating inventory of executive function®–adult version.

Sakanaka, M., Hansen, M. E., Gotoh, A., Katoh, T., Yoshida, K., Odamaki, T., Yachi, H., Sugiyama, Y., Kurihara, S., Hirose, J., Urashima, T., zhong Xiao, J., Kitaoka, M., Fukiya, S., Yokota, A., Leggio, L. L., Hachem, M. A., and Katayama, T. (2019). Evolutionary adaptation in fucosyllactose uptake systems supports bifidobacteria-infant symbiosis. *Science Advances*, 5(8).

Samuel, T. M., Binia, A., de Castro, C. A., Thakkar, S. K., Billeaud, C., Agosti, M., Al-Jashi, I., Costeira, M. J., Marchini, G., Martínez-Costa, C., Picaud, J.-C., Stiris, T., Stoicescu, S.-M., Vanpeé, M., Domellöf, M., Austin, S., and Sprenger, N. (2019). Impact of maternal characteristics on human milk oligosaccharide composition over the first 4 months of lactation in a cohort of healthy european mothers. *Scientific Reports*, 9(1).

Schnaar, R. L., Gerardy-Schahn, R., and Hildebrandt, H. (2014). Sialic acids in the brain: Gangliosides and polysialic acid in nervous system development, stability, disease, and regeneration. *Physiological Reviews*, 94(2):461–518.

Sherman, E. M. S. and Brooks, B. L. (2010). Behavior rating inventory of executive function – preschool version (BRIEF-p): Test review and clinical guidelines for use. *Child Neuropsychology*, 16(5):503–519.

Sociaal en Cultureel Planbureau and Roeters, A. (2019). Looking after the household and family care | Time use in the Netherlands: Edition 2. *Time use in the Netherlands: Edition 2*.

Soyyilmaz, B., Mikš, M. H., Röhrig, C. H., Matwiejuk, M., Meszaros-matwiejuk, A., and Vigsnæs, L. K. (2021). The mean of milk: A review of human milk oligosaccharide concentrations throughout lactation. *Nutrients*, 13(8).

Thum, C., Wall, C. R., Weiss, G. A., Wang, W., Szeto, I. M. Y., and Day, L. (2021). Changes in hmo concentrations throughout lactation: Influencing factors, health effects and opportunities. *Nutrients*, 13(7).

Thurl, S., Munzert, M., Boehm, G., Matthews, C., and Stahl, B. (2017). Systematic review of the concentrations of oligosaccharides in human milk. *Nutrition Reviews*, 75(11):920–933.

Tonon, K. M., Miranda, A., Abrão, A. C. F., de Morais, M. B., and Morais, T. B. (2019). Validation and application of a method for the simultaneous absolute quantification of 16 neutral and acidic human milk oligosaccharides by graphitized carbon liquid chromatography – electrospray ionization – mass spectrometry. *Food Chemistry*, 274:691–697.

Totten, S. M., Zivkovic, A. M., Wu, S., Ngyuen, U., Freeman, S. L., Ruhaak, L. R., Darboe, M. K., German, J. B., Prentice, A. M., and Lebrilla, C. B. (2012). Comprehensive profiles of human milk oligosaccharides yield highly sensitive and specific markers for determining secretor status in lactating mothers. *Journal of Proteome Research*, 11(12):6124–6133.

Underwood, M. A., German, J. B., Lebrilla, C. B., and Mills, D. A. (2014). Bifidobacterium longum subspecies infantis: champion colonizer of the infant gut. *Pediatric Research*, 77(1-2):229–235.

van Buuren, S. and Groothuis-Oudshoorn, K. (2011). mice: Multivariate imputation by chained equations in r. *Journal of statistical software*, 45:1–67.

Vazquez, E., Barranco, A., Ramirez, M., Gruart, A., Delgado-Garcia, J. M., Jimenez, M. L., Buck, R., and Rueda, R. (2016). Dietary 2'-fucosyllactose enhances operant conditioning and long-term potentiation via gut-brain communication through the vagus nerve in rodents. *PLOS ONE*, 11(11):e0166070.

Victora, C. G., Bahl, R., Barros, A. J. D., França, G. V. A., Horton, S., Krasevec, J., Murch, S., Sankar, M. J., Walker, N., Rollins, N. C., and Group, L. B. S. (2016). Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. *The Lancet*, 387(10017):475–90.

Vázquez, E., Barranco, A., Ramírez, M., Gruart, A., Delgado-García, J. M., Martínez-Lara, E., Blanco, S., Martín, M. J., Castanys, E., Buck, R., Prieto, P., and Rueda, R. (2015). Effects of a human milk oligosaccharide, 2'-fucosyllactose, on hippocampal long-term potentiation and learning capabilities in rodents. *The Journal of Nutritional Biochemistry*, 26(5):455–465.

Wang, B. (2009). Sialic acid is an essential nutrient for brain development and cognition. *Annual Review of Nutrition*, 29(1):177–222.

Wang, B. (2012). Molecular mechanism underlying sialic acid as an essential nutrient for brain development and cognition. *Advances in Nutrition*, 3(3):465S–472S.

Wang, B., Yu, B., Karim, M., Hu, H., Sun, Y., McGreevy, P., Petocz, P., Held, S., and Brand-Miller, J. (2007). Dietary sialic acid supplementation improves learning and memory in piglets. *The American Journal of Clinical Nutrition*, 85(2):561–569.

Willemsen, Y., Beijers, R., Vasquez, A. A., and de Weerth, C. (2021). Do breastfeeding history and diet quality predict inhibitory control at preschool age? *Nutrients*, 13(8):2752.

Wright, M. N. and Ziegler, A. (2017). ranger: A fast implementation of random forests for high dimensional data in c++ and r. *Journal of Statistical Software*, 77(1).

Yang, S., Platt, R. W., and Kramer, M. S. (2010). Variation in child cognitive ability by week of gestation among healthy term births. *American Journal of Epidemiology*, 171(4):399–406.

Chapter 4

A Longitudinal Study of the Gut Microbiota during the First Three Years of Life: Links with Problem Behaviour and Executive Functions at Preschool age

Based on: Willemsen, Y.*, Ou, Y.*, Belzer, C., Arias Vásquez, A., Smidt, H., Beijers, R., and de Weerth, C. A longitudinal study of the gut microbiota during the first three years of life: Links with problem behavior and executive functions at preschool age. *Development and Psychopathology*, 2023, 1-17. *Authors contributed equally

Abstract

Early life is a sensitive period when microbiota-gut-brain interactions may have important impact on development. However, evidence from longitudinal studies on low-risk populations is lacking. This study investigated the associations of the gut microbiota in the first three years of life (two, six, and 12 weeks, and one and three years) with problem behaviour and executive functions in n=64 three-year-old children. Higher relative abundance of *Streptococcus* at the age of two weeks, as well as its trajectory over time (including ages two, six, and 12 weeks, and one and three years), was related to worse executive functions. Higher relative abundance of [*Ruminococcus*] *torques* group at the age of three years, as well as its trajectory from one to three years, was associated with less internalizing behaviour. Besides, several robust age-specific associations were identified: higher relative abundance of *Bifidobacterium* (age three years) was associated with more internalizing and externalizing issues; higher relative abundance of *Blautia* (age three years) was linked to less internalizing behaviour; and increased relative abundance of an unidentified *Enterobacteriaceae* genus (age two weeks) was related to more externalizing behaviour. Our findings provide important longitudinal evidence that early life gut microbiota may be linked to behavioural and cognitive development in low-risk children.

Introduction

The human gut harbors a great number of microorganisms, of which bacteria are an essential part. These microorganisms are collectively termed the 'gut microbiota' (Thursby and Juge, 2017). Not only has the gut microbiota been involved in many health outcomes, such as obesity, type 2 diabetes, and irritable bowel syndrome (Vos et al., 2022), it has also been linked to mental health (Cryan et al., 2019). Accumulating evidence from animal and adult human studies has uncovered several key bidirectional communication pathways between the gut microbiota and brain functioning, named the microbiota-gut-brain axis (MGBA) (Cryan et al., 2019). Remarkably, the MGBA is not only functional in adults, but starts playing an equally or even more important role at early ages with regard to child behaviour and cognition (Cryan et al., 2019). Both the gut microbiota and the brain develop at a breathtaking pace during early life, however, only few studies investigated associations between the gut microbiota and behaviour in such sensitive periods. Therefore, this study aimed to investigate the relations of the gut microbiota in the first three years of life with child problem behaviour and executive functions at the age of three years.

The bidirectional interactions of the MGBA occur through intricately innervated and highly adaptable neuronal pathways, and extremely delicate and difficult-to-measure molecular communication systems (Cryan et al., 2019; de Weerth, 2017). For instance, short chain fatty acids (SCFAs), mainly being produced through dietary fiber fermentation by the gut microbiota, likely affect the brain via the vagus nerve, immunity, and the endocrine system (Dalile et al., 2019). Furthermore, specific microbial taxa can generate γ-aminobutyric acid (GABA), which is the main inhibitory neurotransmitter of the central nervous system and regulates many physiological functions (Mazzoli and Pessione, 2016; Silva et al., 2020). The symporter that mediates the uptake of microbiota-derived GABA is present through the gastrointestinal tract, suggesting that

luminal GABA is able to cross the gut barrier and influence extra-gut targets. Although remaining controversial, recent studies suggest the permeability of the blood-brain barrier to GABA, implying its direct impact on the central nervous system (Mazzoli and Pessione, 2016). Besides, GABA receptors are widely expressed in enteric neurons and immune cells, indicating the role of GABA in regulating the gut-to-brain signaling and neuroinflammation (Auteri et al., 2015; Hyland and Cryan, 2010). Such pathways along the MGBA may partially explain how the gut microbiota impacts mental health.

The colonization of the gut by microorganisms mostly commences soon after birth and continues in the following years. The general consensus is that the gut microbiota develops into an adult-like configuration around the age of three years (Derrien et al., 2019), while some studies suggested that this step towards maturation may take longer than previously thought (Ou et al., 2022, 2023a). Gut microbial disturbances during the early dynamic and sensitive colonization period can result in subsequent health problems, such as developing allergies and obesity (Zhuang et al., 2019). This is explained by the early life programming theory, that refers to long lasting changes and disruptions as a consequence of environmental exposures at a young age (Tarry-Adkins and Ozanne, 2011). In early life, the brain experiences numerous quick developments in neuronal proliferation, migration, differentiation, synaptogenesis, myelination, and apoptosis (Rice and Barone, 2000), largely impacting brain functioning, cognition, and behaviour (Erus et al., 2015). Simultaneously, the microbiota is becoming established in the gut of infants and young children (de Weerth, 2017; Wang et al., 2018). Thus, alterations of the gut microbiota in early life may exert considerable effects on the development of the brain. Indeed, there is compelling evidence from animal studies supporting such a hypothesis (Clarke et al., 2014; Leclercq et al., 2017; O'Mahony et al., 2014; Stilling et al., 2015). This marks early life a sensitive time window to obtain and maintain microbiota composition that will promote normal physical and mental development. However, we know little about early-life gut microbiota in asso-

ciation with child behaviour and cognition. Specifically, how the gut microbiota and brain functioning, in particular host behaviour, are interconnected in low-risk community infants and children (i.e., generally healthy and neurotypically developing) is underexplored. Knowledge on these associations, particularly when uncovered by comprehensive longitudinal studies, can provide insight into the typical early development of the gut microbiota in relation to child behaviour and cognition.

First studies have found evidence for associations between the gut microbiota and child behaviour and cognition. Regarding behaviour, Loughman et al. (2020) reported that increased relative abundances of taxa belonging to the genus *Prevotella* at one year of age were associated with less internalizing behaviour at age two (i.e., problem behaviour affecting internal psychological conditions, characterized by withdrawal, anxiety, and emotional problems (Achenbach, 1966)) (Loughman et al., 2020). In our previous study, we found that the rise of *Prevotella* 9 in middle childhood was related to more externalizing behaviour at age ten (i.e., problem behaviour exhibited in the external environment, including features like impulsivity, aggression, and hyperactivity (Achenbach, 1966)) (Ou et al., 2022). Besides, Laue et al. (2022) observed a negative relation between *Streptococcus peroris* and internalizing behaviour in girls before school age, and a positive association between *Lachnospiraceae* species and externalizing behaviour in both genders (Laue et al., 2022). Furthermore, *Lachnospiraceae* species and *Veillonella* were linked to more internalizing behaviour in preschoolers; interestingly, *Veillonella* was positively related to externalizing behaviour as well (van de Wouw et al., 2021). Additionally, increased alpha diversity was observed in preschool children with less internalizing behaviour (Laue et al., 2022; van de Wouw et al., 2021).

Four other studies have found an underlying link between infant gut microbiota and child cognition (Aatsinki et al., 2020; Carlson et al., 2017; Rothenberg et al., 2021; Streit et al., 2021; Tamana et al., 2021). Cognition is fundamental for the development of executive functions, including higher-level cognitive processes like inhibitory control

(Diamond, 2013). Specifically, a cross-sectional study found more *Enterobacteriaceae* species in relation to worse cognition at age 45 months (Streit et al., 2021). Longitudinal research reflected that high relative abundances of *Bacteroides* at age one year were related to better cognition at age two (Carlson et al., 2017; Tamana et al., 2021). Furthermore, *Faecalibacterium* at one year of age was associated with reduced cognitive functions at age two (Carlson et al., 2017). Additionally, a lower relative abundance of *Bifidobacterium* and a higher relative abundance of *Clostridium* at two-and-a-half months were linked to increased attention at eight months (Aatsinki et al., 2020). Moreover, (Rothenberg et al., 2021) found that children with better cognition showed enriched *Faecalibacterium*, *Sutterella*, and *Clostridium* cluster XIVa at age three years. Finally, high alpha diversity at age one year was reported in two-year-old children with worse cognition (Carlson et al., 2017).

To conclude, a number of associations have been observed between the gut microbiota and problem behaviour and cognition in early life, but findings are variable and inconsistent across studies, mainly due to different methodologies used regarding microbiota analyses, genomics, epidemiology, and statistics. Furthermore, most of the previous studies have assessed problem behaviour and cognition by using only one questionnaire of a single reporter. In the current longitudinal study in a community sample of children, we investigated the gut microbiota in relation to problem behaviour (i.e., internalizing and externalizing behaviour) and executive functions (i.e., advanced cognitive abilities, including inhibitory control (Diamond, 2013)) using questionnaires of multiple reporters and behavioural tasks. We had the following two hypotheses: (1) relative abundances and alpha diversity (i.e., Chao1, Shannon, and phylogenetic diversity) of the gut microbiota at age three years are associated with reported problem behaviour and executive functions at the same age; (2) relative abundances and alpha diversity of the gut microbiota at early ages (i.e., two, six, and 12 weeks, and one year) are associated with reported problem behaviour and executive functions at age three.

We investigated these hypotheses in three ways: (1) as the gut microbiota is highly dynamic in early life, its composition at different ages may be differently associated with problem behaviour and executive functions later in life. For this reason, we analyzed the overall gut microbiota composition in relation to preschool-aged cognitive measures in an age-specific manner; (2) for the same reason, relations regarding a single taxon and an alpha diversity index were analyzed in an age-specific manner; (3) based on the age-specific analyses, we explored the trajectories of taxa and alpha diversity parameters in association with mental outcomes over the whole study period. Figure 4.1 shows the workflow of our analyses. Considering that most published findings were at the genus level, we performed our analyses at the same taxonomic level. However, given the paucity of studies on these relations at such early ages, we did not hypothesise specific associations between microbial taxa and mental outcomes.

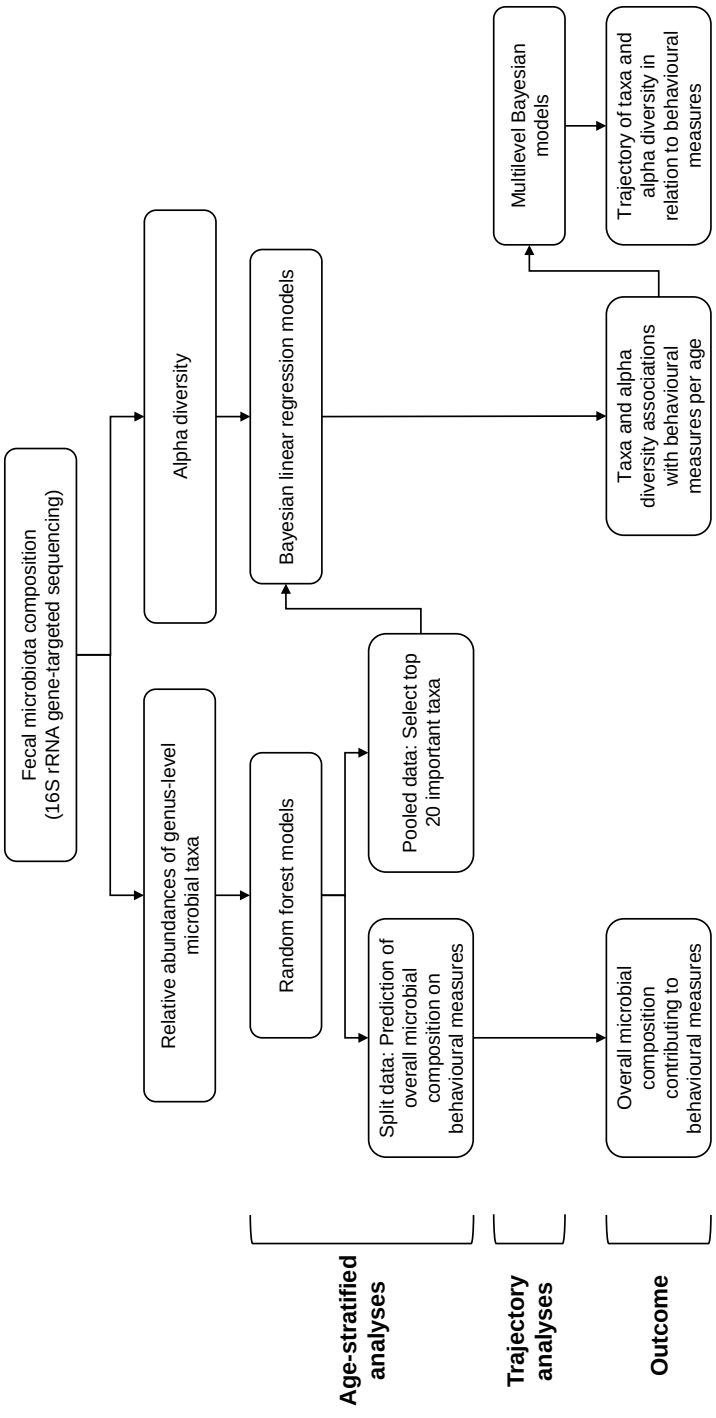


Figure 4.1. Workflow of the analyses.

Materials and methods

Participants

The current study is part of the longitudinal Dutch study named BINGO where early factors associated with child development were investigated. Participants were healthy children and their parents living in the Netherlands. Detailed in- and exclusion criteria are described in a previous publication (Hechler et al., 2018). At baseline, 88 pregnant women were recruited in the Arnhem-Nijmegen region for the BINGO study; 96% were born in the Netherlands. Postnatal exclusion criteria included: complications during pregnancy, gestational age at birth $<$ 37 weeks, birth weight $<$ 2500 g, 5-min Apgar score $<$ 7, and congenital malformations. Seventy-seven mothers were followed up after postnatal exclusion. At three years of child age, 76 families were approached (one drop-out occurred during the previous measurement rounds). Among them, two families could not be contacted, six families did not participate due to time constraints, and one family dropped out due to personal reasons. Parental demographics did not differ significantly between participating and non-participating families. This resulted in a final sample of 67 families. Of them, 64 families participated in home visits when their children reached age three, and the other three families were unable to join home visits but filled out questionnaires in this assessment round. Both parents participated in 54 families (81%, 54/67), and only mothers participated in 13 families (19%, 13/67).

Ethics

The BINGO study was independently reviewed by the Ethics Committee of Social Sciences of Radboud University, and no formal objection to this research was made [ECSW2014–1003–189 and amendment: ECSW–2018–034]. The current study was preregistered on the Open Science Framework: https://osf.io/vwgef with amendment: https://osf.io/nyeb4.

Data collection procedure

Collection of child stool samples was done at the ages of two, six, and 12 weeks, and one and three years. Stool samples were stored in the participant's freezer (-20°C) until they were collected with a portable freezer. The stool samples were stored at -80°C at Radboud University prior to being processed at the Laboratory of Microbiology at Wageningen University & Research. Home visits took place when the child turned three years old. Prior to the home visit, mothers and their partners independently filled in digital questionnaires about their child's problem behaviour and executive functions. During the home visit, the child performed inhibitory control tasks. Tasks were video recorded and afterwards rated by two trained observers. Observers were trained by use of a coding manual specific to each task.

Measures

Gut microbiota composition

Stool samples were collected with a polystyrene 10 mL stool container. Total DNA was extracted from 0.01-0.15 g of stool sample with 300 µL of Stool Transport and Recovery Buffer by double bead-beating steps as previously described (Gu et al., 2018). The variable V4 region of prokaryotic 16S ribosomal RNA (rRNA) genes was then amplified by PCR in duplicate reactions, by using primers 515F-n (5'-GTGCCAGCMGCCGCGGTAA) and 806R-n (5'-GGACTACHVGGGTWTCTAAT) (Gu et al., 2018). The 16S rRNA gene sequencing was completed on the Illumina HiSeq platform by Eurofins Genomics Germany GmbH.

Behavioural measures

Parental questionnaires

Mothers and their partners filled in all questionnaires mentioned below. However, because fewer partners completed the questionnaires, we used partner reports for sensitivity analyses to validate the maternal reports by calculating Kendall correlations between both. The non-parametric Kendall method was chosen due to its better performance in handling non-normally distributed data and tied values (Kendall, 1945). Maternal reports were used as the final measure of reported problem behaviour and executive functions. To assess child problem behaviour, the Child Behavior Checklist (CBCL, 103 items) for ages of one-and-a-half to five years (Achenbach and Ruffle, 2000) and the Strengths and Difficulties Questionnaire (SDQ, 25 items) (Goodman, 1997) were used. The CBCL and the SDQ include internalizing and externalizing subscales, consisting of items scored on a three-point Likert scale. The SDQ can detect problem behaviour as accurately as the CBCL does (Goodman and Scott, 1999). Raw scores for both questionnaires were used as outcome measure in order to compare and possibly aggregate the measures. However, given that the Kendall correlations on the same subscales of the CBCL and the SDQ were lower than 0.5 (Supplementary Table 7.9), we included both instruments separately in the analyses. In both instruments, higher scores on subscales indicate more problem behaviour. To evaluate child executive functions, the Behavior Rating Inventory of Executive Function –preschool version (BRIEF-P, 63 items) questionnaire for preschoolers (Sherman and Brooks, 2010) and the Ratings of Everyday Executive Functions (REEF, 77 items) (Nilsen et al., 2017) were used. The BRIEF-P and the REEF are scored on three- and four-point scales, respectively. A higher score on the BRIEF-P indicates worse executive functions, while a higher score on the REEF indicates better executive functions. The BRIEF-P is a commonly used questionnaire that measures general executive functions and does not

differentiate between different situations. The REEF rates executive functions in different situations (e.g., executive functions around friends, during grocery shopping, or in the community) and determines an average score. Raw scores for both questionnaires were used as outcome measure in order to compare and possibly aggregate the measures. However, Kendall correlations between the BRIEF-P and the REEF were lower than 0.5 (Supplementary Table 7.9), hence both instruments were included in the analyses. Parental questionnaires were considered acceptable and reliable based on their ωtotal (ranged between 0.65-0.94) or Cronbach's α values (ranged between 0.83-0.96) (Supplementary Table 7.10) (Revelle and Condon, 2019).

Inhibitory control tasks

Six different behavioural tasks with good reliability (i.e., Flanker, Whisper, Gift Wrap, Gift Delay, Snack Delay, and Bear Dragon) were performed to measure inhibitory control as previously stated in detail (Willemsen et al., 2021). Observer reliability was determined by the Intraclass Correlation Coefficient (ICC) relying on absolute agreement. The ICC's for the inhibitory control tasks ranged from r=0.84 to r=0.96 (p<0.001). Snack Delay and Bear Dragon were excluded from the analyses due to insufficient variation and low number of children that passed the practice trials, respectively. The other four tasks were included in our study. Higher scores on these tasks indicate better inhibitory control.

Statistical analyses

Pre-processing of sequence data

Sequence data were processed via NG-Tax 2.0 with default settings (Poncheewin et al., 2020; Ramiro-Garcia et al., 2016), with SILVA SSU 16S rRNA gene reference database (version 132) (Quast et al., 2012). The raw amplicon sequence variant (ASV) count data were used to calculate alpha diversity by the 'ape' (Paradis et al., 2020)

and the 'picante' (Kembel, 2020) packages. Then, ASV count data were glommed at the genus level prior to analyses.

Gut microbiota composition and development over the first three years of life

For descriptive purposes, we first delineated gut microbiota composition and development in the first three years after birth (including all samples at the age of two, six, and 12 weeks, and one and three years). We compared differences in alpha diversity indices, including Chao1, Shannon, and phylogenetic diversity, between ages using Wilcoxon rank-sum tests corrected with the False Discovery Rate (FDR) method. Next, we also compared beta diversity between ages by conducting Principal Coordinate Analysis (PCoA) via the 'vegan' package (Oksanen et al., 2020). Considering that PCoA can be applied to different dissimilarity and distance metrics that all differ in specific aspects and corresponding interpretation, we included the Bray-Curtis, weighted Jaccard (formula = 2*Bray-Curtis dissimilarity / (1 + Bray-Curtis dissimilarity)), unweighted UniFrac, weighted UniFrac, and Aitchison (the Euclidean distance based on centered-log-transformed ASV count data) methods, to comprehensively describe the compositional differences. Except for the Aitchison distance, we transformed genus-level count data into relative abundances before calculating other dissimilarity and distance metrices. Significance was determined as a *p*-value lower than 0.05 for non-multiple tests and an FDR-adjusted *p*-value lower than 0.05 for multiple tests. Additionally, we visualized average and individual relative abundances at the genus level over the study period by using a barplot and a heatmap, respectively. To identify differentially abundant microbial taxa at the genus level between ages, we conducted the Linear Discriminant Analysis Effect Size (LEfSe) method by using the 'microbiomeMarker' R package (Segata et al., 2011), with a log-transformed Linear Discriminant Analysis (LDA) score higher than two indicating significance.

Confounding effects

In our original preregistration, we considered child age and diet quality as potential confounders (i.e., variables that influence both the independent variables and the outcome). After reconsideration, both variables were removed as potential confounders due to two major reasons (amendment can be found via https://osf.io/nyeb4): (1) low variation in child age (see Figure 4.2 for notes regarding ages); (2) our previous study using the same cohort found no significant associations of diet quality with behaviour and executive functions (Willemsen et al., 2021). Given these considerations, no confounders were accounted for in the models performed in this study. Note that potential covariates of the independent variables only (i.e., the gut microbiota) were not accounted for in downstream analyses (Cinelli et al., 2020), as they would remove variation in the gut microbiota data, which was not the purpose of this study. These potential confounders and covariates as well as their relations to the gut microbiota and behavioural outcomes are displayed in a directed acyclic graph (DAG) (Supplementary Figure 7.1).

Data imputation and transformation

Missing values (proportion of missing values is shown in Supplementary Table 7.11) in problem behaviour, executive functions, and inhibitory control were imputed ten times together, by using the predictive mean match (PMM) method in the R package - 'mice' (van Buuren and Groothuis-Oudshoorn, 2011). The imputation model was conducted separately at each age. For instance, at the age of three, 64 children provided gut microbiota data, and their missing values in aforementioned behavioural measures were imputed jointly in one model. No auxiliary variables (i.e., variables that are not included in analyses, but are correlated with imputed variables) were considered in the imputation. For both random forest models and the Bayesian linear regression models,

genus-level relative abundance data were used. Numeric variables were standardized (i.e., subtracting the mean and dividing by the standard deviation) for the Bayesian models only, as random forest models rely on decision trees for which standardization is considered unnecessary.

Main analyses

To determine whether gut microbiota composition in the first three years of life (i.e., two, six, and 12 weeks, and one and three years) is associated with problem behaviour (i.e., internalizing and externalizing behaviour) and executive functions (including inhibitory control) at age three, we conducted random forest models and the Bayesian linear regression models (Bürkner, 2017; Kuhn et al., 2020). Random forest is first of all well suited to analyze microbiome data as it is appropriate for high dimensional data, invariant to scaling of inputs, computationally efficient, and able to uncover nonlinear relationships (Belk et al., 2018; Louppe, 2014; Namkung, 2020). The first random forest model was applied to assess the contribution of the total gut microbiota composition on our behavioural outcomes. This was done for the purpose of exploring the gut microbial community as a whole to account for the complex interplay between taxa. The following random forest model was applied as a preselection tool, to select possibly important taxa from high-dimensional data, before passing them on to the Bayesian linear regression model. The Bayesian model was first used to determine age-specific relations (i.e., directions and strengths) of a selected taxon and an alpha diversity index with each outcome measure. By looking at the different time points separately, these analyses can help identify periods of development that are sensitive to certain microbial compositions. Although not preregistered, after reconsideration, we decided to perform an additional analysis to optimize the use of our longitudinal data. Based on the age-specific results, we implemented a multilevel Bayesian model to determine whether trajectories of change in the gut microbiota were associated with

the outcome measures at age three. Figure 4.1 shows the workflow of our analyses.

Age-specific analyses - Determining the contribution of the overall gut microbiota to each behavioural measure through random forest models

Data were imputed ten times: data were randomly split into a training dataset (including 80% participants) and a testing dataset (including 20% participants), leading to ten training datasets and ten corresponding testing datasets. The procedure of data splitting was applied to children who provided gut microbiota information at each age separately. To prevent data leakage, the missing values of behavioural measures were imputed in training and testing datasets separately (ten times) as described earlier. Next, we included genus-level relative abundances of overall gut microbiota as independent variables and one behavioural measure as an outcome. This step was performed on each individual behavioural measure separately. To train the model, a ten-repeated ten-fold cross-validation was conducted on each complete training dataset including imputed values via the 'caret' package (Kuhn et al., 2020). Afterwards, we used the trained model to obtain predicted behavioural outcomes of each corresponding complete testing dataset including imputed values. Similarity between predicted and actual behavioural outcomes of the complete testing dataset was measured by the Pearson correlation with its *p*-value obtained from a permutation test (N=1000). Considering that data splitting and imputation resulted in multiple datasets, we used the median value of the Pearson correlation coefficient from multiple cases to represent the final similarity. The *p*-value corresponding to this median was included. *P*-values were adjusted with FDR methods, with corrected values under 0.05 indicating significance.

Age-specific analyses - Preselecting potentially important gut microbiota contributing to each behavioural measure through random forest models

To identify microbial taxa that contribute to each behavioural outcome, we measured the change in the generalized cross-validation (GCV) value in the random forest model. Larger GCV changes indicate more contribution of the independent variable to the model, in other words, this analysis shows which taxa are potentially more important (Kuhn et al., 2020). Unlike the first random forest model, we did not split the data but used the whole dataset here, because we prioritized the structure of the model and a large sample size can provide more valid information. Missing values of behavioural measures in the whole dataset were imputed as described in the section of data imputation and transformation. Then, relative abundances of all taxa were treated as independent variables with one behavioural measure as an outcome. This procedure was performed on each behavioural measure separately. Next, we carried out a ten-repeated ten-fold cross-validation on each complete dataset containing imputed data and calculated average GCV values of multiple datasets acquired from data imputation. Based on the size of average GCV values, we selected the largest 20 taxa as the top 20 in importance. These 20 taxa were then passed to the Bayesian linear regression models to confirm their actual associations with behavioural measures.

Age-specific analyses - Associating the gut microbiota with behavioural measures by using Bayesian linear regression models

We implemented Bayesian linear regression models to estimate the relations of relative abundances of the selected top 20 microbial taxa with a prevalence value higher than 10% and alpha diversity with the child behavioural measures. Compared to standard linear regression models, the Bayesian linear regression models compute the probability of different effects rather than simply reporting single estimates of the

"true effect" (Bürkner, 2018). We performed the Bayesian models by using the 'brms' R package built based on the programming language Stan (Bürkner, 2017). The brm function within the 'brms' package was used with the Gaussian distribution (mean = 0, std = 1) as the prior distribution for all beta coefficients and the Student's *t*-distribution for error distribution (due to better performance in handling extreme values) (Lange et al., 1989). A list containing multiple complete datasets including imputed data was directly passed to the brm function, which in turn generated a single estimate. Other arguments of the brm function were set as follows: chains = 4, iter = 2000, and warmup = 1000. Under these settings, chains were converged properly with Rhat values lower than 1.01. Regarding the outcomes of the Bayesian models, the less the posterior distribution overlaps with zero, the more likely a relation is positive or negative. In the current study, we defined a relation as positive or negative with confidence when its 95% credible interval (CI) excluded the value zero.

Trajectory analyses - Relating the developmental trajectories of the gut microbiota to behavioural measures through multilevel Bayesian linear regression models

To make maximum use of our longitudinal data, we conducted multilevel models to investigate relations between the developmental trajectories of the gut microbiota and behavioural measures. The multilevel models were performed on microbial taxa and alpha diversity with confident age-specific relations to behavioural measures (i.e., as determined by the Bayesian linear regressions described above). In the multilevel models, microbial and behavioural information as well as the actual age were level 1 variables, and the child was the level 2 variable. Note that missing values in behavioural measures and actual age were not imputed, and that in these analyses we used the same distributions and arguments as described earlier. Before performing a testing model, we first checked the intraclass correlation (ICC) of an intercept-only model. When the 95%

CI of an ICC excluded the value zero in the intercept-only model, multilevel strategies were used. A trajectory relation was considered with confidence when there was no overlap between its 95% CI and zero. With respect to taxa, when their prevalence was higher than 10% at five time points (i.e., two, six, and 12 weeks, and one and three years), multilevel models were performed on the pooled data of all ages. When only the first three time points met the 10% criteria, multilevel models were carried out by pooling samples at these three ages together. When only the prevalences at the last two ages were higher than 10%, multilevel approaches were done in the pooled aged-one-and-three years samples. Rhat values were used to check chain convergence.

Results

Population characteristics and descriptives

Demographic data and descriptives of study variables are shown in Table 4.1. Roughly 50% of the children were girls. Mothers were mostly highly educated (86.2%). Scores on the questionnaires measuring child problem behaviour and executive functions did not differ significantly between mothers and their partners, and they were significantly positively intercorrelated (Supplementary Table 7.9).

Table 4.1. Descriptives of study subjects.

	Characteristics							
Categorical variable	Ratio						Sample size	Completion rate (%)
Child sex	girl:boy = 34:30						64	100
Delivery mode	vaginal: C-section = 54:7						61	95
Educational level (%)	low: middle: high = 0:12.5:87.5						64	100
Numeric variable	Mean ± SD	Minimum	Lower quartile	Median	Upper quartile	Maximum	Sample size	Completion rate (%)
Age at age three in years	3.2 ± 0.1	3.1	3.1	3.2	3.2	3.5	63	99
Gestational age in weeks	39.8 ± 1.5	35.6	38.9	40	40.9	42.1	63	99
Birth weight in grams	3556 ± 426.2	2570	3270	3480	3885	4445	61	95
Total breastfeeding duration in months	9.6 ± 8.1	0	4	8	13.2	36	64	100
Total exclusive breastfeeding duration in months	3.9 ± 1.7	0	3	4	5	7	53	82
Age at solid food introduction in months	4.6 ± 1	3	4	4	5	7	59	92
Average diet quality at age three	4 ± 1.2	2	3.3	3.9	4.8	7.2	64	100
CBCL Internalizing (M)	7.1 ± 5.7	0	3	5.5	10.8	24	62	97
CBCL Internalizing (P)	7.5 ± 5.5	0	4	6	11	22	49	96
CBCL Externalizing (M)	11.8 ± 7.3	0	7	12	15.8	31	62	97
CBCL Externalizing (P)	12.2 ± 5.8	1	8	12	17	24	49	96
SDQ Internalizing (M)	3.6 ± 2.5	0	2	3	5	11	62	97
SDQ Internalizing (P)	3.5 ± 2.4	0	2	3	5	9	44	86
SDQ Externalizing (M)	5.5 ± 3	1	3.2	5	7	14	62	97
SDQ Externalizing (P)	5.4 ± 2.9	0	3	5	7	12	50	98
BRIEF-P Total Score (M)	94.4 ± 15.4	69	83	92	106.5	146	63	99
BRIEF-P Total Score (P)	97.2 ± 17.8	69	86.2	96	109.2	137	50	98
REEF Total Score (M)	151.1 ± 31	74	133.2	153	172.8	215	62	97
REEF Total Score (P)	147 ± 28.9	78	133	148	164	212	49	96
Flanker	1.6 ± 0.3	0.9	1.4	1.7	1.9	2	47	73
Whisper	1.9 ± 0.3	0.9	1.8	2	2	2	60	94
Gift Wrap	2.2 ± 0.9	0	1.5	2.5	3	3	60	94
Gift Delay	3.9 ± 0.2	2.9	3.9	4	4	4	61	95

Notes. In the assessment round at age three, 64 children, 64 mothers, and 51 partners participated in the study. In total, 66, 70, 73, 72, and 64 fecal samples were collected at ages two, six, and 12 weeks, and one and three years, respectively. Completion percentages were based on the number of participating individuals (i.e., completion rates for mothers are based on 64 participating mothers, and completion rates for partners are based on 51 participating partners). P, Partner; M, Mother; CBCL, the Child Behavioral Checklist; SDQ, the Strengths and Difficulties Questionnaire; BRIEF-P, Behavior Rating Inventory of Executive Functions - Preschool; REEF, Ratings of Everyday Executive Functioning. Differences were compared between mother and partner reports by Wilcoxon tests. None of them were significant before or after FDR adjustments.

Gut microbiota composition and development over the first three years of life

We analyzed microbial composition of 345 fecal samples taken at five time points. A total of 42,056,591 high-quality reads were obtained after being processed with NG-Tax 2.0. Within these reads, 220 microbial taxa were identified at the genus level mainly belonging to the phyla *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*, and *Verrucomicrobia*. For descriptive purposes, we compared alpha and beta diversity between ages (Figure 4.2; beta-diversity comparisons between the first three ages and between the last two ages are displayed in Supplementary Figures 7.2 and 7.3) and delineated a general developmental trajectory of the gut microbiota over time (Figure 4.3a). Diversity comparisons reflected profound compositional differences between infancy and toddlerhood. These differences were visualized by the heatmap showing individual relative abundance data (Supplementary Figure 7.4). LEfSe identified a total of 106 differentially abundant microbial taxa between ages (log-transformed LDA scores higher than two; Supplementary Table 7.12). Due to the large number of significant taxa, only the taxa with log-transformed LDA scores higher than four are highlighted and displayed in Figure 4.3b, such as an unidentified genus within *Enterobacteriaceae*, *Lactobacillus*, *Bifidobacterium*, *Faecalibacterium*, and *Blautia*.

Age-specific analyses

Determining the contribution of the overall gut microbiota to each behavioural measure through random forest models

To explore whether the overall microbial composition in the first three years (i.e., at ages two, six, and 12 weeks, and one and three years) contributes to problem behaviour and executive functions at age three, we compared the similarity between the actual and the predicted behavioural results. As shown in Supplementary Table

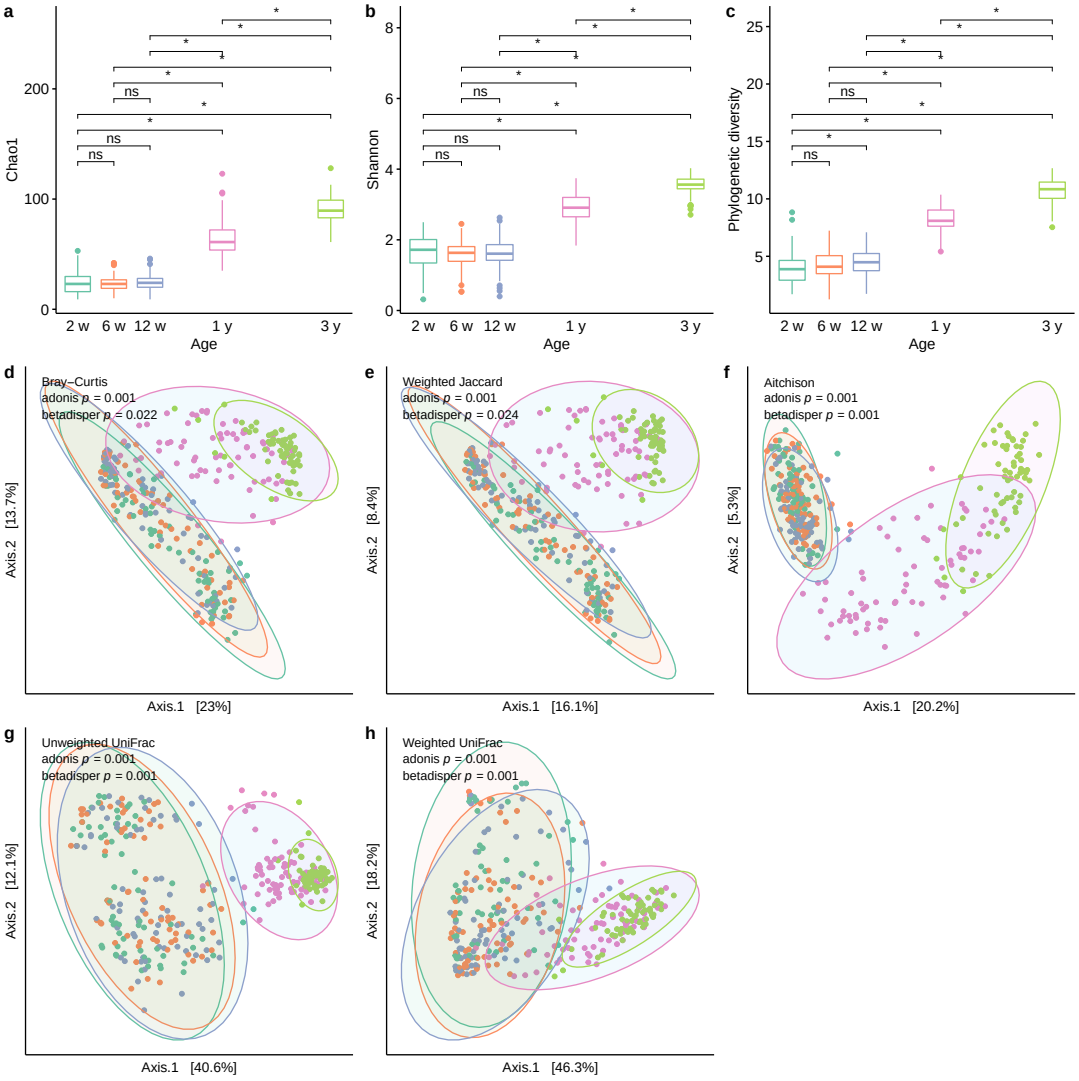


Figure 4.2. Alpha and beta diversity of the gut microbiota in the first three years of life. (a-c) Alpha diversity as measured by Chao1, Shannon, and phylogenetic diversity indices. Wilcoxon rank-sum tests were conducted between ages and corrected with the FDR method (ns, not significant; *, $p<0.01$). Age two weeks: mean ± SD=2.08 ± 0.28. Age six: weeks mean ± SD=6.23 ± 0.55. Age 12 weeks: mean ± SD=12.27 ± 0.42. Age one year: mean ± SD=1.04 ± 0.08. Age three years: mean ± SD=3.18 ± 0.10. (d-h) Principal coordinate plots of beta diversity, based on different pairwise dissimilarity (Bray-Curtis and weighted Jaccard) and distance (UniFrac and Aitchison) matrices, with points and ellipses colored by ages (Lake blue, two weeks; Orange, six weeks; Purple, 12 weeks; Pink, one year; Grass green, three years).

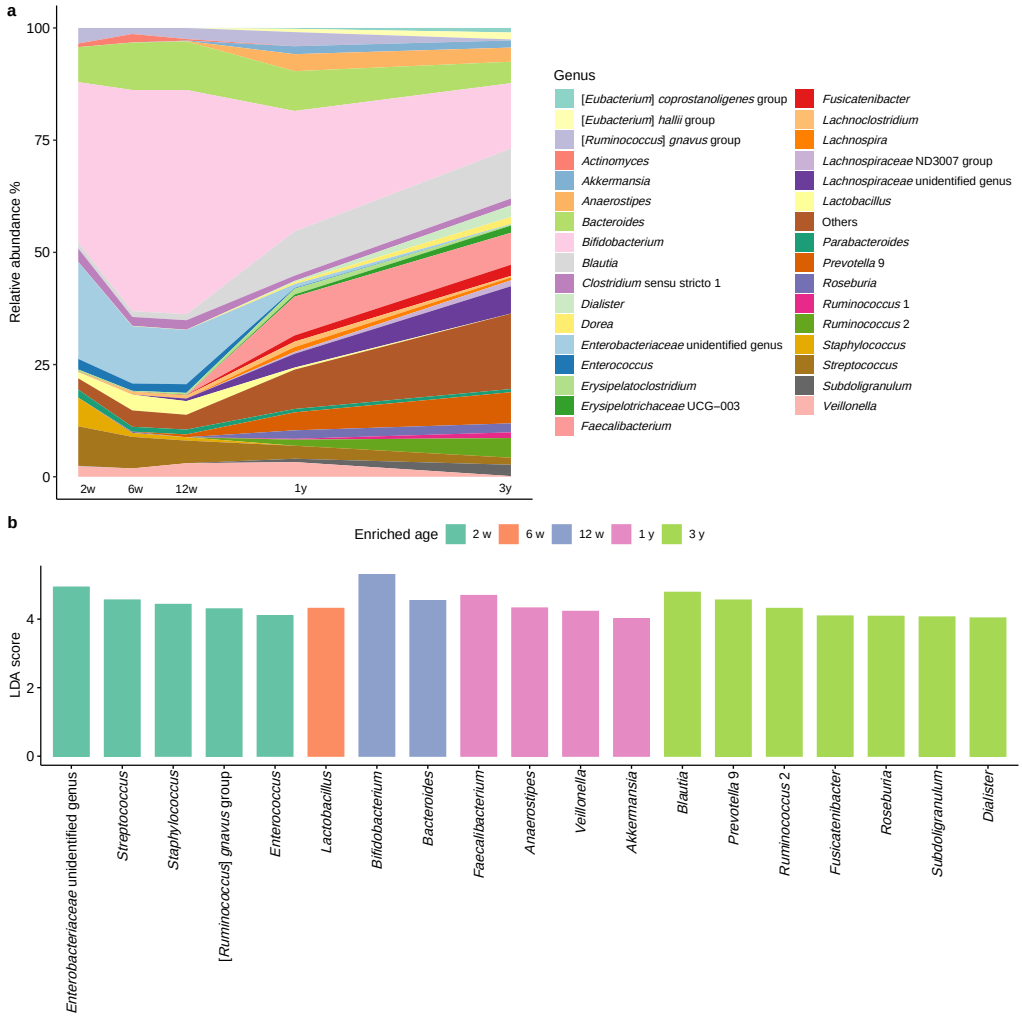


Figure 4.3. Characteristics of the gut microbiota in the first three years of life. (a) Average relative abundances of the gut microbiota at the genus level over time. Others represent genera with relative abundances lower than 1%. (b) Differentially abundant genus-level taxa between ages, identified by Linear Discriminant Analysis Effect Size (LEfSe) with log-transformed Linear Discriminant Analysis (LDA) scores higher than four.

7.13, 92% (46/50) of the models showed insignificant absolute correlation coefficients (i.e., lower than 0.3), indicating a low likelihood that the gut microbiota can contribute to behavioural outcomes. Regarding the 8% (4/50) models with correlation coefficients

higher than 0.3, the similarity remained insignificant between the actual and predicted data, implying the same low likelihood. The random forest models showed that the overall gut microbiota did not contribute to problem behaviour and executive functions in the present study.

Preselecting potentially important gut microbiota contributing to each behavioural measure through random forest models

As planned in our preregistration, we preselected the microbial taxa that may contribute to the behavioural outcomes the most (i.e., top important taxa) based on GCV values, by performing separate random forest models at each age. The top 20 important taxa at the genus level are depicted in Figure 4.4 and Figure 4.5, with the following observations:

1. *Bacteroides* and *Clostridium sensu stricto* 1 were the most frequent contributors to CBCL internalizing behaviour;
2. *Bacteroides* and *Bifidobacterium* were the most frequent contributors to CBCL externalizing behaviour, SDQ internalizing and externalizing behaviour, and BRIEF-P executive functions;
3. *Bacteroides* and *Blautia* were the most frequent contributors to REEF executive functions;
4. Additionally, *Bacteroides* and *Bifidobacterium* were the most frequent contributors to the behavioural measures of inhibitory control (i.e., Flanker, Whisper, Gift Wrap, and Gift Delay) (Figure 4.6).

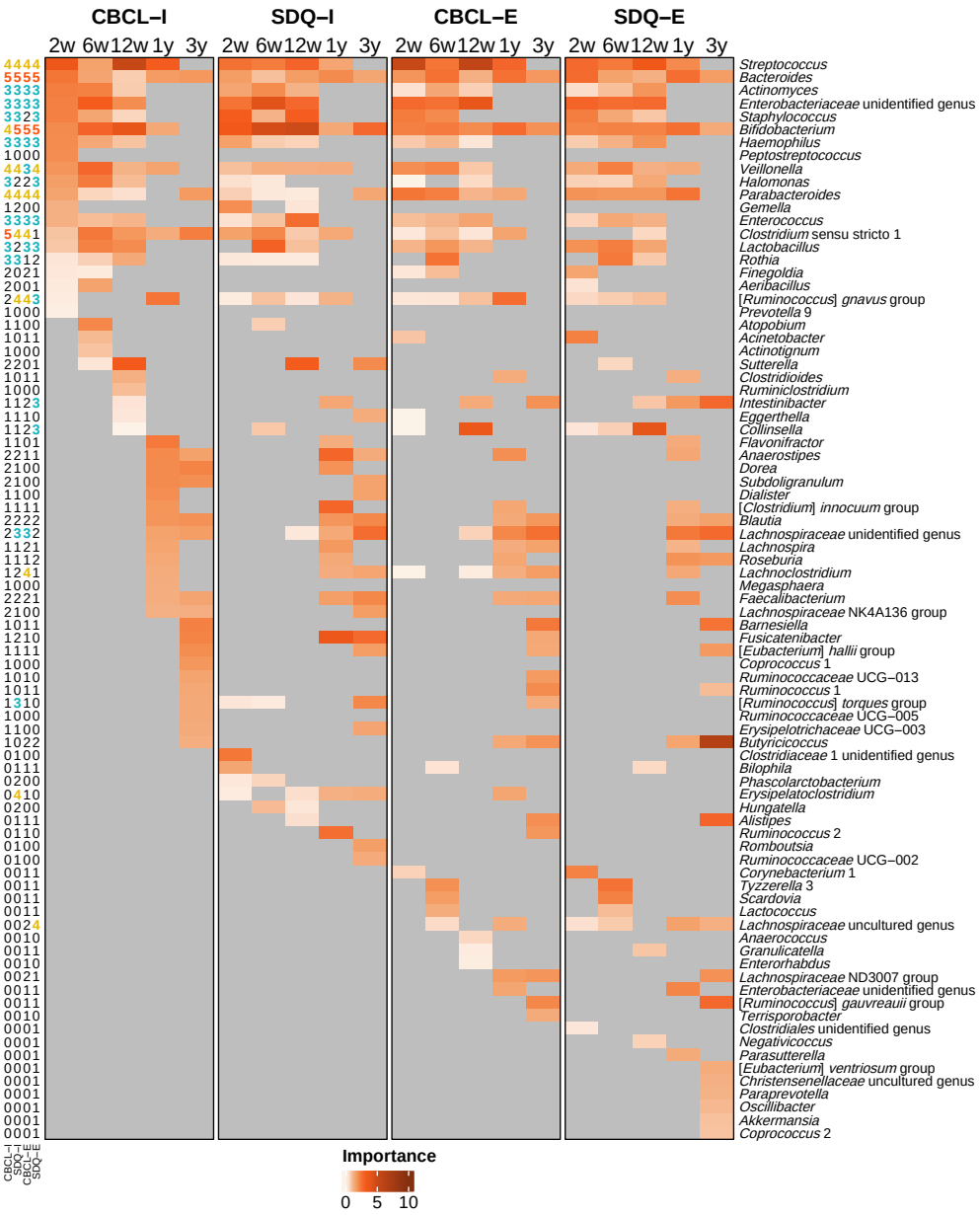


Figure 4.4. Heatmap showing the top 20 important microbial taxa over time and their associations to problem behaviour at age three as reported by the mother. The top 20 important genus-level taxa within each age (i.e., 2w, two weeks; 6w, six weeks; 12w, 12 weeks; 1y, one year; 3y, three years) per behavioural measure are shown on the right side of the figure. Behavioural measures include: CBCL-I, internalizing behaviour measured by the CBCL; SDQ-I, internalizing behaviour measured by the SDQ; CBCL-E, externalizing behaviour measured by the CBCL; SDQ-E, externalizing behaviour measured by the SDQ. The orange scale indicates the importance of the taxa, with darker color referring to increased importance. The importance was determined by the generalized cross-validation value, with a larger value change indicating more contribution of a taxon to the model, i.e., which taxon is more important. As not all taxa appeared in the top 20 list at each time point, these absent taxa are colored in grey. Numbers on the left side of the figure show how many times a taxon appeared to be in the top 20 list of a behavioural measure over time. The frequently appearing taxa are bolded and colored in orange (five times), yellow (four times), or green (three times).

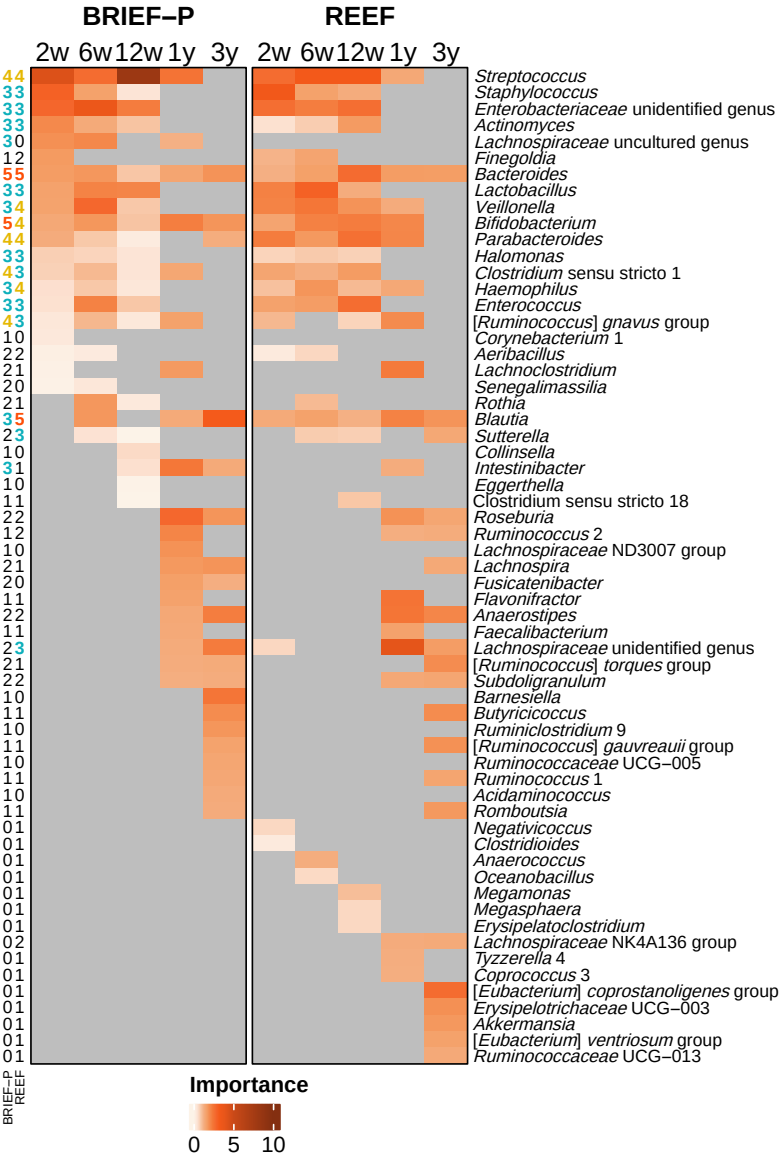


Figure 4.5. Heatmap showing the top 20 important microbial taxa over time and their associations to executive functions at age three as reported by the mother. The top 20 important genus-level taxa within each age (i.e., 2w, two weeks; 6w, six weeks; 12w, 12 weeks; 1y, one year; 3y, three years) per cognitive measure are shown on the right side of the figure. The measures include: BRIEF-P, executive functions measured by the BRIEF-P; REEF, executive functions measured by the REEF. The orange scale indicates the importance of the taxa, with darker color referring to increased importance. The importance was determined by the generalized cross-validation value, with a larger value change indicating more contribution of a taxon to the model, i.e., which taxon is more important. As not all taxa appeared in the top 20 list at each time point, these absent taxa are colored in grey. Numbers on the left side of the figure show how many times a taxon appeared to be in the top 20 list of a measure over time. The frequently appearing taxa are bolded and colored in orange (five times), yellow (four times), or green (three times).

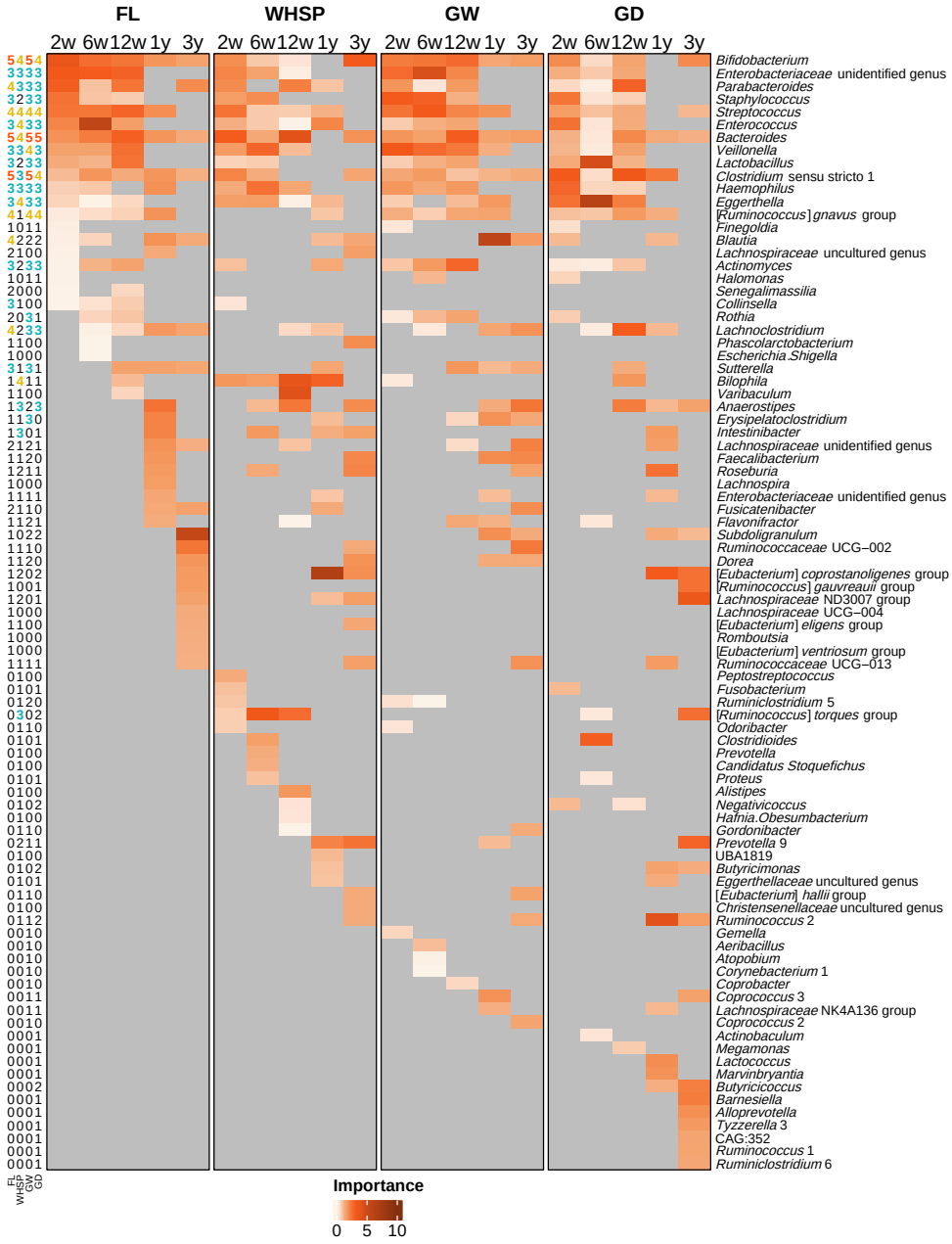


Figure 4.6. Heatmap showing the top 20 important microbial taxa over time and their associations to observed inhibitory control behaviour at age three. The top 20 important genus-level taxa within each age (i.e., 2w, two weeks; 6w, six weeks; 12w, 12 weeks; 1y, one year; 3y, three years) per inhibitory-control task are shown on the right side of the figure. The tasks include: FL, flanker; WHSP, whisper; GW, gift wrap; GD, gift delay. The orange scale indicates the importance of the taxa, with darker color referring to increased importance. The importance was determined by the generalized cross-validation value, with a larger value change indicating more contribution of a taxon to the model, i.e., which taxon is more important. As not all taxa appeared in the top 20 list at each time point, these absent taxa are colored in grey. Numbers on the left side of the figure show how many times a taxon appeared to be in the top 20 list of a task over time. The frequently appearing taxa are bolded and colored in orange (five times), yellow (four times), or green (three times).

Associating the gut microbiota with behavioural measures by using Bayesian linear regression models

To confirm whether the aforementioned top 20 important microbial taxa were associated with problem behaviour and executive functions, we performed the Bayesian linear regression model on each genus-level taxa (relative abundance) and behaviour pair. Table 4.2 shows the strongest observed associations of these pairs (i.e., estimates higher than 0.2 or lower than -0.2). Remarkably, there were several highly present taxa (i.e., prevalent in more than 80% of the samples, relative abundance higher than 10%) in relation to the outcome measures: *Bifidobacterium* at age three years was associated with more internalizing and externalizing behaviour (est.=0.27 for both), *Blautia* at three years was linked to less internalizing behaviour (est.=-0.25), and an unidentified taxa within the *Enterobacteriaceae* family was related to more externalizing behaviour (est.=0.25).

Next, we checked for consensus between the questionnaires assessing the same construct. For internalizing behaviour, there was no consensus between the associations found for the CBCL and the SDQ. For externalizing behaviour, more *Parabacteroides* at two weeks was associated with less externalizing behaviour in both the CBCL (est.=-0.30) and the SDQ (est.=-0.28). An opposite finding was found for *Butyricicoccus* at one year in relation to more externalizing behaviour by the CBCL (est.=0.23), while at three years, it was associated with less externalizing behaviour by the SDQ (est.=-0.35).

Within the CBCL results, *Barnesiella* at age three years was associated with more internalizing (est.=0.31) and externalizing behaviour (est.=0.33). Within the SDQ results, *Bifidobacterium* at age three years was associated with more internalizing and externalizing behaviour (est.=0.27 for both).

Table 4.2. Associations of the gut microbiota in the first three years of life with behavioural measures at age three.

Behaviour at age three	Age of the gut microbiota	Genus	Estimate	Estimate error	95% CI	Prevalence%	Relative abundance %
Mother reported							
CBCL Internalizing	12w	*Intestinibacter*	0.23	0.12	[0.01, 0.47]	16	0.2 ± 0.6
	3y	*Barnesiella*	0.31	0.12	[0.08, 0.55]	50	0.4 ± 0.6
CBCL Externalizing	2w	*Streptococcus*	0.26	0.12	[0.01, 0.5]	94	8.8 ± 10.1
	2w	*Parabacteroides*	-0.3	0.12	[-0.51, -0.06]	35	1.8 ± 4.9
	1y	*Clostridium sensu stricto* 1	0.23	0.11	[0.01, 0.46]	62	1.2 ± 3.5
	1y	*Butyricicoccus*	0.23	0.12	[0.01, 0.48]	56	0.4 ± 0.5
	1y	*Parabacteroides*	-0.22	0.12	[-0.45, -0.01]	44	0.8 ± 1.8
	3y	*Barnesiella*	0.33	0.12	[0.1, 0.56]	50	0.4 ± 0.6
SDQ Internalizing	1y	*Ruminococcus* 2	-0.36	0.11	[-0.58, -0.14]	39	1.4 ± 2.4
	3y	*Bifidobacterium*	0.27	0.13	[0.01, 0.53]	100	14.6 ± 11.6
	3y	*Blautia*	-0.25	0.12	[-0.48, 0]	100	11.1 ± 4.5
	3y	[*Ruminococcus*] *torques* group	-0.25	0.13	[-0.51, -0.01]	84	0.8 ± 0.7
	3y	*Sutterella*	0.25	0.13	[0.01, 0.5]	61	0.3 ± 0.3
SDQ Externalizing	2w	*Enterobacteriaceae* unidentified genus	0.25	0.12	[0.01, 0.5]	89	21.6 ± 24.1
	2w	*Parabacteroides*	-0.28	0.12	[-0.51, -0.05]	35	1.8 ± 4.9
	6w	*Halomonas*	0.28	0.11	[0.06, 0.5]	11	0.1 ± 0.2
	3y	*Butyricicoccus*	-0.35	0.12	[-0.57, -0.11]	89	0.4 ± 0.3
	3y	*Bifidobacterium*	0.27	0.13	[0.01, 0.52]	100	14.6 ± 11.6
	3y	*Oscillibacter*	0.28	0.12	[0.04, 0.51]	22	0 ± 0.1
BRIEF-P	2w	*Streptococcus*	0.4	0.12	[0.15, 0.64]	94	8.8 ± 10.1
	6w	*Halomonas*	0.24	0.12	[0.01, 0.49]	11	0.1 ± 0.2
	12w	*Streptococcus*	0.31	0.12	[0.07, 0.55]	88	5.1 ± 10.4

Table 4.2 (continued).

	12w	*Intestinibacter*	0.3	0.11	[0.08, 0.53]	16	0.2 ± 0.6
	1y	*Ruminococcus* 2	-0.3	0.12	[-0.54, -0.08]	39	1.4 ± 2.4
	1y	*Clostridium sensu stricto* 1	0.27	0.12	[0.03, 0.5]	62	1.2 ± 3.5
	3y	*Blautia*	-0.3	0.13	[-0.57, -0.05]	100	11.1 ± 4.5
REEF	2w	*Parabacteroides*	0.25	0.12	[0, 0.47]	35	1.8 ± 4.9
	6w	*Halomonas*	-0.24	0.12	[-0.48, -0.01]	11	0.1 ± 0.2
	1y	*Lachnospiraceae* unidentified genus	0.28	0.11	[0.06, 0.5]	78	3.1 ±4.1
	3y	[*Ruminococcus*] *torques* group	-0.24	0.13	[-0.49, -0.01]	84	0.8 ±0.7
Child tasks							
	6w	*Bacteroides*	0.28	0.12	[0.05, 0.51]	59	10.6 ± 16.3
	1y	*Anaerostipes*	-0.29	0.12	[-0.51, -0.07]	96	3.8 ± 3.6
Flanker	1y	*Sutterella*	-0.24	0.12	[-0.48, -0.01]	46	0.3 ± 0.6
	3y	*Subdoligranulum*	-0.25	0.13	[-0.5, 0]	95	2.5 ± 1.8
	3y	*Ruminococcaceae* UCG-013	0.26	0.12	[0.03, 0.51]	73	0.2 ± 0.2
	1y	*Subdoligranulum*	-0.31	0.12	[-0.54, -0.08]	31	0.7 ± 1.4
	1y	*Coprococcus* 3	-0.26	0.12	[-0.48, -0.02]	14	0.1 ± 0.3
Gift Wrap	1y	*Veillonella*	0.24	0.12	[0.01, 0.47]	71	3.3 ± 5
	1y	*Lachnospiraceae* NK4A136 group	-0.3	0.12	[-0.55, -0.08]	38	0.4 ± 0.7

Notes. Associations of estimates with 95% credible intervals (CIs) excluding 0 are presented. Behavioural measures include: CBCL, Child Behavioral Checklist; SDQ, Strengths and Difficulties Questionnaire; BRIEF-P, Behavior Rating Inventory of Executive Functions - Preschool; REEF, Ratings of Everyday Executive Functioning.

Regarding executive functions, *Ruminococcus* 2 at one year and [*Ruminococcus*] *torques* group at age three years, were associated with better executive functions as measured by the BRIEF-P (est.=-0.30, note that higher scores on the BRIEF-P indicate worse executive functions) and worse executive functions measured by the REEF (est.=-0.24), respectively. Lastly, *Halomonas* at six weeks was associated with worse executive functions as measured by the BRIEF-P (est.=0.24) and the REEF (est.=-0.24).

Different associations were found for the Flanker and the Gift Delay tasks. For the Flanker, relations were identified at the age of six weeks, and one and three years, while for the Gift Wrap, associations were observed at age one year only. This may be due to a highly dynamic gut microbiota ecosystem in early life, of which composition at different ages may be variously linked to executive functions.

There were some overlapping associations between the questionnaires of problem behaviour and executive functions. *Parabacteroides* at two weeks was associated with better executive functions (REEF, est.=0.25), and less externalizing behaviour (CBCL and SDQ, est.=-0.30 and -0.28, respectively). Another consistent result was *Streptococcus* at two weeks in relation to worse executive functions (BRIEF-P, est.=0.40) and more externalizing behaviour (CBCL, est.=0.26).

We also measured behavioural relations to alpha diversity, including Chao1, Shannon, and phylogenetic diversity by using the Bayesian linear regression models (strongest results are displayed in Table 4.3). Interestingly, relations were only observed for alpha diversity at age two weeks. Higher Chao1 values were associated with less internalizing behaviour (CBCL) (est.=-0.28). Furthermore, Chao1 values were in positive relation to better executive function performance (REEF and Gift Wrap, est.=0.31 and 0.43, respectively). Lastly, higher phylogenetic diversity at age two weeks was also linked to better inhibitory control during the Gift Wrap task (est.=0.32).

Table 4.3. Associations of alpha diversity in the first three years of life with problem behaviour, executive functioning, and inhibitory controls at age three.

Behaviour at age three	Age of the gut microbiota	Alpha diversity	Estimate	Estimate error	95% CI
Mother reported					
CBCL Internalizing	2w	Chao1	-0.28	0.12	[-0.51, -0.04]
REEF	2w	Chao1	0.31	0.13	[0.07, 0.57]
Child tasks					
Gift Wrap	2w	Chao1	0.43	0.12	[0.19, 0.64]
	2w	PD	0.32	0.12	[0.08, 0.56]

Notes. Associations of estimates with 95% credible intervals (CIs) excluding 0 are presented. CBCL, the Child Behavioral Checklist; REEF, Ratings of Everyday Executive Functioning.

Trajectory analyses - Relating the developmental trajectories of the gut microbiota to behavioural measures through multilevel Bayesian linear regression models

Based on the results of age-specific Bayesian models and the 10% prevalence rule applied to microbial taxa (Supplementary Table 7.14), we identified 16 pairs (including 12 pairs of taxa and behavioural measures, and four pairs of alpha diversity and behavioural measures) available at all five ages (i.e., two, six, and 12 weeks, and one and three years), three at the first three ages, and 12 at the last two ages (Supplementary Table 7.15). Higher relative abundances of *Streptococcus* over the first three years of life were weakly related to worse executive functions reported by the BRIEF-P (est.=0.05; higher scores on the BRIEF-P indicating worse performance), conforming to earlier age-specific findings. We also found that the trajectory of [*Ruminococcus*] *torques* group from age one to three was negatively related to internalizing behaviour (SDQ, est.=-0.22), implying that higher relative abundances were associated with fewer internalizing difficulties during this period. No enduring associations were observed with confidence regarding alpha diversity.

Discussion

In this longitudinal study, we investigated associations of the gut microbiota during early life with problem behaviour and executive functions, including inhibitory control, at three years of age. Several associations with behaviour and cognition were found for relative abundances of microbial taxa and alpha diversity throughout the first three years of life, in concordance with the early life programming theory (Tarry-Adkins and Ozanne, 2011). Supplementary Table 7.16 provides an overview of the different microbiota taxa and microbial diversity index at different ages relative to the developmental findings. In addition, Supplementary Table 7.16 shows the existing literature relative to our findings. Based on this table, we discuss the most prominent findings below.

We found evidence that increased relative abundance of *Streptococcus*, specifically at the age of two weeks and over the first three years after birth, was associated with worse executive functions at the age of three years. This result indicates that *Streptococcus* might affect cognitive development throughout early life. Relations between early-life relative abundances of *Streptococcus* and behaviour and cognition in typically developing children have not been observed in previous literature. For comparative purposes, we therefore examined studies on microbiota composition in children diagnosed with neurodevelopmental disorders as they mostly have comorbid behavioural and cognitive issues (Schoemaker et al., 2014). According to a systematic review, children with autism spectrum disorder (ASD) frequently show an overgrowth of *Streptococcus* (Bundgaard-Nielsen et al., 2020). Although gut microbiota dysbiosis in ASD was seemingly partially attributed to an altered dietary pattern (Li et al., 2022; Welberg, 2022), diet was not correlated to the mental outcomes of our community samples. In addition to *Streptococcus*, both age-specific and trajectory relations were discerned for the [*Ruminococcus*] *torques* group: higher relative abundances at the age of three years and throughout the period from one to three years of age were associated with fewer

child internalizing problems. Previous research showed excessive absolute abundances of fecal [*Ruminococcus*] *torques* group in children with ASD (Wang et al., 2013) and this taxon was strikingly increased in patients with inflammatory bowel disease (Png et al., 2010).

With respect to microbial taxa that only showed age-specific associations, we observed higher relative abundances of *Bifidobacterium* at age three years related to more internalizing and externalizing behaviour at the same age. Interestingly, a systematic review showed decreased *Bifidobacterium* in ASD children compared to neurotypically developing controls (Xu et al., 2019). Besides, supplementing ASD children with a prebiotic galacto-oligosaccharide increased *Bifidobacterium* populations in the gut and alleviated autistic symptoms (Grimaldi et al., 2017). However, opposite roles of *Bifidobacterium* have been described in major depressive disorder (MDD) (Cheung et al., 2019; Knudsen et al., 2021). Such inconsistency also takes place within ADHD studies. Two studies reported that *Bifidobacterium longum* mitigated ADHD (Finegold et al., 2010; Pärtty et al., 2015), while another study found overgrowing *Bifidobacterium* species in ADHD subjects (Aarts et al., 2017). In addition, in the current study, a higher relative abundance of *Blautia* at the age of three years was related to fewer internalizing difficulties (as well as to better executive functions at the same age). *Blautia* is suggested to play an important role in nutrient absorption and digestion (Eren et al., 2015), and in child gut microbiota development towards a normal adult-like configuration (Hsiao et al., 2014). In line with our findings, depleted *Blautia* was seen in ASD populations aged from two to 18 years old by several studies as concluded in a systematic review (Liu et al., 2019). However, elevated levels of *Blautia* were reported in relation to MDD in adults (Cheung et al., 2019) and ADHD in three-year-old children (Laue et al., 2022), indicating that different mechanisms may be involved depending on the psychopathology and chronological age. Lastly, we observed a positive relation between one unidentified *Enterobacteriaceae* genus at the age of two weeks

and externalizing problems at the age of three years. Of interest, more *Enterobacteriaceae* species were cross-sectionally related to decreased cognitive functioning at the age of 45 months (Streit et al., 2021).

Another of our findings was that higher alpha diversity at age two weeks was linked to fewer internalizing problems and better executive functions at age three years. In accordance with our internalizing behaviour result, Laue et al. (2022) observed that higher alpha diversity in the first two months of life was related to less internalizing behaviour in three-year-old boys. Furthermore, van de Wouw et al. (2021) found lower alpha diversity in three-to-five year old children with clinically relevant CBCL cut-off scores for internalizing behaviour. Besides, Eckermann et al. (2022) observed higher Shannon alpha diversity at the age of one, three, and four months in relation with better cognitive ability as measured by Digit Span forwards test at the age of ten years, although not for Shannon alpha diversity at the age of six and ten years. Despite the generally weak or absent relations found between child gut microbiota and executive functions, the above-mentioned studies may indicate that higher alpha diversity in the first years of life is related to improved subsequent mental outcomes at later ages. On the contrary, Carlson et al. (2017) found higher alpha diversity at age one year related to worse cognition at age two years in typically developing toddlers. Additionally, a recent paper from van de Wouw et al. (2023) found evidence for a weak to modest cross sectional relation between higher Shannon diversity and worse verbal comprehension in three- to four-year-old children. Together, these findings illustrate the fact that we are yet to understand the potential impact of alpha diversity levels at different developmental stages. In general, alpha diversity levels of newborns start increasing immediately after birth due to colonization of microorganisms. With time, breastfed-infants tend to form a *Bifidobacterium*-predominated configuration which is often less diverse than formula-fed infants. After the introduction of solid food, child alpha diversity starts to increase, gradually reaching a steady state resembling gut microbiota composition of

adults. Given these apparent normative fluctuations in levels of alpha diversity in the first months and years of life, having a comparatively high (or low) alpha diversity will potentially impact a child's development differently depending on the child's specific age.

Our study contributes to the growing body of literature on the gut microbiota, problem behaviour, and executive functions. A strength of our study is the longitudinal design, which covered the period from birth to age three years and allowed for the assessment of multiple developmental stages of the gut microbiota. Another advantage was that questionnaires were filled in by both mother and partner. Partner reports were used for sensitivity analyses and because they showed positive correlations with maternal reports, they enhanced the reliability of our study measures. Furthermore, problem behaviour and executive functions were assessed with multiple questionnaires (i.e., CBCL and SDQ for problem behaviour, and BRIEF-P and REEF for executive functions), allowing us to determine conformity and consistency between various questionnaires. Finally, we used standardized behavioural tasks as a tool to objectively determine child executive functions.

A limitation of our study is the possible overreliance on the compositional features of the gut microbiota using relative abundances instead of absolute abundances. This approach may increase the chance of spurious associations as relative abundances are dependent on each other. Besides, 16S rRNA gene sequence data are limited at species-level resolution and profiling precise gene functions (Durazzi et al., 2021). Hence, although multiple associations were identified, it is worth noting that these relations do not indicate causality. The results await follow-up studies, preferably preclinical experimental studies, to determine if individual microbes (e.g., aforementioned *Streptococcus* and *Bifidobacterium*) and microbial communities as a whole influence behaviour and cognition, e.g., by generating neurotransmitters (e.g., GABA and serotonin) and their precursors (e.g., tryptophan and phenylalanine) in the gut (Altaib et al., 2021; Baran-

douzi et al., 2022; Biederman and Spencer, 1999; Gizer et al., 2009; Kandel et al., 2000; Kanehisa et al., 2022; Staller and Faraone, 2007). Further research including such as quantitative PCR, whole-genome shotgun metagenomic sequencing, targeted fecal metabolomics, and experimental studies in animal models, would improve the understanding of current correlational results and provide insight into microbial functions and even causality. An analytic limitation of our study is LEfSe, which was used to identify differentially abundant taxa in this study, and has recently been pointed out to have higher sensitivity to false positive rates compared to other microbial composition analyses, such as ALDEx2, ANCOM-II, and DESeq2 (Nearing et al., 2021). Due to such methodological limitations, LEfSe-based significant findings should be carefully validated in future studies. Another limitation of our study is the relatively small sample size and mostly highly educated study population, limiting the generalizability of the findings. The restricted sample size may also hamper deep inference with respect to taxa with low prevalence rates to some degree. Our findings on microbial relations to the mental outcomes need to be confirmed in a larger, more representative cohort. Also, the participants were highly educated, which may hamper translating our findings to individuals with a lower socioeconomic status. Lastly, recent developments in longitudinal analytical approaches (Kodikara et al., 2022), such as zero-inflated beta regression models, block bootstrap methods, and SplinectomeR, will better facilitate the identification of differentially abundant microbial taxa between groups (e.g., below and above clinical cut-offs) over time.

To conclude, our results provide tentative evidence supporting the idea that in a child's first years of life the gut microbiota might play a vital role in the development of the brain, in line with the early life programming theory (Tarry-Adkins and Ozanne, 2011). Potential mechanisms are likely related to microbiota-derived metabolites (Ahmed et al., 2022). As the nature of this study was exploratory and the body of similar research needs to grow to a large extent, it is still premature to translate our cor-

relational findings into clinical implications. Replications in other longitudinal studies on healthy community children are necessary to confirm our findings. Ideally, to avoid inconsistent results caused by different methods used, replication studies should apply the same methodology regarding microbiology, genomics, epidemiology, and statistics (Ou et al., 2023b). This will shed more light on key microbial taxa and latent pathways of associations between early gut microbiota and child behaviour and cognition.

References

Aarts, E., Ederveen, T. H. A., Naaijen, J., Zwiers, M. P., Boekhorst, J., Timmerman, H. M., Smeekens, S. P., Netea, M. G., Buitelaar, J. K., Franke, B., Hijum, S. A. F. T. v., and Vasquez, A. A. (2017). Gut microbiome in ADHD and its relation to neural reward anticipation. *PloS one*, 12(9):e0183509–e0183509.

Aatsinki, A.-K., Kataja, E.-L., Munukka, E., Lahti, L., Keskitalo, A., Korja, R., Nolvi, S., Häikiö, T., Tarro, S., Karlsson, H., and Karlsson, L. (2020). Infant fecal microbiota composition and attention to emotional faces. *Emotion*, 22(6):1159.

Achenbach, T. M. (1966). The classification of children's psychiatric symptoms: a factor-analytic study. *Psychological monographs*, 80(7):1–37.

Achenbach, T. M. and Ruffle, T. M. (2000). The child behavior checklist and related forms for assessing behavioral/emotional problems and competencies. *Pediatrics in Review*, 21(8):265–271.

Ahmed, H., Leyrolle, Q., Koistinen, V., Kärkkäinen, O., Layé, S., Delzenne, N., and Hanhineva, K. (2022). Microbiota-derived metabolites as drivers of gut-brain communication. *Gut microbes*, 14(1):2102878.

Altaib, H., Nakamura, K., Abe, M., Badr, Y., Yanase, E., Nomura, I., and Suzuki, T. (2021). Differences in the Concentration of the Fecal Neurotransmitters GABA and Glutamate Are Associated with Microbial Composition among Healthy Human Subjects. *Microorganisms*, 9(2):378.

Auteri, M., Zizzo, M. G., and Serio, R. (2015). GABA and GABA receptors in the gastrointestinal tract: from motility to inflammation. *Pharmacological Research*, 93:11–21.

Barandouzi, Z. A., Lee, J., Rosas, M. D. C., Chen, J., Henderson, W. A., Starkweather, A. R., and Cong, X. S. (2022). Associations of neurotransmitters and the gut microbiome with emotional distress in mixed type of irritable bowel syndrome. *Scientific reports*, 12(1):1648.

Belk, A., Xu, Z. Z., Carter, D. O., Lynne, A., Bucheli, S., Knight, R., and Metcalf, J. L. (2018). Microbiome Data Accurately Predicts the Postmortem Interval Using Random Forest Regression Models. *Genes*, 9(2):104.

Biederman, J. and Spencer, T. (1999). Attention-deficit/hyperactivity disorder (adhd) as a noradrenergic disorder. *Biological Psychiatry*, 46(9):1234–1242.

Bundgaard-Nielsen, C., Knudsen, J., Leutscher, P. D. C., Lauritsen, M. B., Nyegaard, M., Hagstrøm, S., and Sørensen, S. (2020). Gut microbiota profiles of autism spectrum disorder and attention deficit/hyperactivity disorder: A systematic literature review. *Gut microbes*, 11(5):1172–1187.

Bürkner, P. C. (2017). brms: An R package for Bayesian multilevel models using Stan. *Journal of Statistical Software*, 80(1).

Bürkner, P. C. (2018). Advanced Bayesian multilevel modeling with the R package brms. *R Journal*, 10(1):395–411.

Carlson, A. L., Xia, K., Azcarate-Peril, M. A., Goldman, B. D., Ahn, M., Styner, M. A., Thompson, A. L., Geng, X., Gilmore, J. H., and Knickmeyer, R. C. (2017). Infant gut microbiome associated with cognitive development. *Biological Psychiatry*, 83(2):148–159.

Cheung, S. G., Goldenthal, A. R., Uhlemann, A.-C., Mann, J. J., Miller, J. M., and Sublette, M. E. (2019). Systematic Review of Gut Microbiota and Major Depression. *Frontiers in psychiatry*, 10:34.

Cinelli, C., Forney, A., and Pearl, J. (2020). A Crash Course in Good and Bad Controls. *SSRN Electronic Journal*, pages 1–10.

Clarke, G., O'Mahony, S. M., Dinan, T. G., and Cryan, J. F. (2014). Priming for health: gut microbiota acquired in early life regulates physiology, brain and behaviour. *Acta Paediatrica*, 103(8):812–819.

Cryan, J. F., O'riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., Codagnone, M. G., Cussotto, S., Fulling, C., Golubeva, A. V., Guzzetta, K. E., Jaggar, M., Long-Smith, C. M., Lyte, J. M., Martin, J. A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., O'connor, R., Cruz-Pereira, J. S., Peterson, V. L., Rea, K., Ritz, N. L., Sherwin, E., Spichak, S., Teichman, E. M., Wouw, M. v. d., Ventura-Silva, A. P., Wallace-Fitzsimons, S. E., Hyland, N., Clarke, G., and Dinan, T. G. (2019). The microbiota-gut-brain axis. *Physiological Reviews*, 99(4):1877–2013.

Dalile, B., Oudenhove, L. V., Vervliet, B., and Verbeke, K. (2019). The role of short-chain fatty acids in microbiota–gut–brain communication. *Nature Reviews Gastroenterology & Hepatology*, 16(8):461–478.

de Weerth, C. (2017). Do bacteria shape our development? Crosstalk between intestinal microbiota and HPA axis. *Neuroscience & Biobehavioral Reviews*, 83:458–471.

Derrien, M., Alvarez, A.-S., and Vos, W. M. d. (2019). The Gut Microbiota in the First Decade of Life. *Trends in Microbiology*, 27(12):997–1010.

Diamond, A. (2013). Executive functions. *Annual review of psychology*, 64:135–68.

Durazzi, F., Sala, C., Castellani, G., Manfreda, G., Remondini, D., and Cesare, A. D. (2021). Comparison between 16S rRNA and shotgun sequencing data for the taxonomic characterization of the gut microbiota. *Scientific Reports*, 11(1):3030.

Eckermann, H. A., Ou, Y., Lahti, L., and de Weerth, C. (2022). Can gut microbiota throughout the first 10 years of life predict executive functioning in childhood? *Developmental Psychobiology*, 64(3):1–14.

Eren, A. M., Sogin, M. L., Morrison, H. G., Vineis, J. H., Fisher, J. C., Newton, R. J., and McLellan, S. L. (2015). A single genus in the gut microbiome reflects host preference and specificity. *The ISME journal*, 9(1):90–100.

Erus, G., Battapady, H., Satterthwaite, T. D., Hakonarson, H., Gur, R. E., Davatzikos, C., and Gur, R. C. (2015). Imaging patterns of brain development and their relationship to cognition. *Cerebral cortex (New York, N.Y. : 1991)*, 25(6):1676–1684.

Finegold, S. M., Dowd, S. E., Gontcharova, V., Liu, C., Henley, K. E., Wolcott, R. D., Youn, E., Summanen, P. H., Granpeesheh, D., Dixon, D., Liu, M., Molitoris, D. R., and Green, J. A. (2010). Pyrosequencing study of fecal microflora of autistic and control children. *Anaerobe*, 16(4):444–453.

Gizer, I. R., Ficks, C., and Waldman, I. D. (2009). Candidate gene studies of ADHD: a meta-analytic review. *Human Genetics*, 126(1):51–90.

Goodman, R. (1997). The Strengths and Difficulties Questionnaire: A Research Note. *Journal of Child Psychology and Psychiatry*, 38(5):581–586.

Goodman, R. and Scott, S. (1999). Comparing the strengths and difficulties questionnaire and the child behavior checklist: Is small beautiful? *Journal of Abnormal Child Psychology*, 27(1):17–24.

Grimaldi, R., Cela, D., Swann, J. R., Vulevic, J., Gibson, G. R., Tzortzis, G., and Costabile, A. (2017). In vitro fermentation of B-GOS: impact on faecal bacterial populations and metabolic activity in autistic and non-autistic children. *FEMS microbiology ecology*, 93(2):fiw233.

Gu, F., Borewicz, K., Richter, B., Zaal, P. H. v. d., Smidt, H., Buwalda, P. L., and Schols, H. A. (2018). In Vitro Fermentation Behavior of Isomalto/Malto-Polysaccharides Using Human Fecal Inoculum Indicates Prebiotic Potential. *Molecular Nutrition & Food Research*, 62(12):1800232.

Hechler, C., Beijers, R., Riksen-Walraven, J. M., and de Weerth, C. (2018). Are cortisol concentrations in human breast milk associated with infant crying? *Developmental Psychobiology*, 60(6):639–650.

Hsiao, A., Ahmed, A. M. S., Subramanian, S., Griffin, N. W., Drewry, L. L., Jr, W. A. P., Haque, R., Ahmed, T., and Gordon, J. I. (2014). Members of the human gut microbiota involved in recovery from Vibrio cholerae infection. *Nature*, 515(7527):423–426.

Hyland, N. P. and Cryan, J. F. (2010). A Gut Feeling about GABA: Focus on GABA(B) Receptors. *Frontiers in pharmacology*, 1:124.

Kandel, E. R., Schwartz, J. H., Jessell, T. M., Siegelbaum, S., Hudspeth, A. J., Mack, S., et al. (2000). *Principles of neural science*, volume 4. McGraw-hill New York.

Kanehisa, M., Sato, Y., and Kawashima, M. (2022). KEGG mapping tools for uncovering hidden features in biological data. *Protein science : a publication of the Protein Society*, 31(1):47–53.

Kembel, S. W. (2020). Picante: Integrating phylogenies and ecology.

Kendall, M. G. (1945). The Treatment of Ties in Ranking Problems. *Biometrika*, 33(3):239–251.

Knudsen, J. K., Bundgaard-Nielsen, C., Hjerrild, S., Nielsen, R. E., Leutscher, P., and Sørensen, S. (2021). Gut microbiota variations in patients diagnosed with major depressive disorder-A systematic review. *Brain and behavior*, 11(7):e02177–e02177.

Kodikara, S., Ellul, S., and Cao, K.-A. L. (2022). Statistical challenges in longitudinal microbiome data analysis. *Briefings in bioinformatics*, 23(4):bbac273.

Kuhn, M., Wing, J., Weston, S., Williams, A., Keefer, C., Engelhardt, A., Cooper, T., Mayer, Z., Kenkel, B., Benesty, M., Lescarbeau, R., Ziem, A., Scrucca, L., Tang, Y., Candan, C., Hunt, T., and Team, R. C. (2020). caret: a classification and regression training.

Lange, K. L., Little, R. J. A., and Taylor, J. M. G. (1989). Robust Statistical Modeling Using the t Distribution. *Journal of the American Statistical Association*, 84(408):881.

Laue, H. E., Karagas, M. R., Coker, M. O., Bellinger, D. C., Baker, E. R., Korrick, S. A., and Madan, J. C. (2022). Sex-specific relationships of the infant microbiome and early-childhood behavioral outcomes. *Pediatric research*, 92(2):580–591.

Leclercq, S., Mian, F. M., Stanisz, A. M., Bindels, L. B., Cambier, E., Ben-Amram, H., Koren, O., Forsythe, P., and Bienenstock, J. (2017). Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior. *Nature communications*, 8:15062.

Li, G., Song, B., Wang, C., Tang, D., Li, K., He, X., and Cao, Y. (2022). Diet, microbe, and autism: Cause or consequence? *Cell Host & Microbe*, 30(1):5–7.

Liu, F., Li, J., Wu, F., Zheng, H., Peng, Q., and Zhou, H. (2019). Altered composition and function of intestinal microbiota in autism spectrum disorders: a systematic review. *Translational psychiatry*, 9(1):43.

Loughman, A., Ponsonby, A.-L., O'Hely, M., Symeonides, C., Collier, F., Tang, M. L. K., Carlin, J., Ranganathan, S., Allen, K., Pezic, A., Saffery, R., Jacka, F., Harrison, L. C., Sly, P. D., Vuillermin, P., and Group, B. I. S. I. (2020). Gut microbiota composition during infancy and subsequent behavioural outcomes. *EBioMedicine*, 52:102640.

Louppe, G. (2014). Understanding random forests: From theory to practice. *arXiv preprint arXiv:1407.7502*.

Mazzoli, R. and Pessione, E. (2016). The Neuro-endocrinological Role of Microbial Glutamate and GABA Signaling. *Frontiers in microbiology*, 7:1934.

Namkung, J. (2020). Machine learning methods for microbiome studies. *Journal of Microbiology*, 58(3):206–216.

Nearing, J. T., Douglas, G. M., Hayes, M., MacDonald, J., Desai, D., Allward, N., Jones, C. M. A., Wright, R., Dhanani, A., Comeau, A. M., and Langille, M. G. I. (2021). Microbiome differential abundance methods produce disturbingly different results across 38 datasets. *Nature Communications*, 13(1):1–16.

Nilsen, E. S., Huyder, V., McAuley, T., and Liebermann, D. (2017). Ratings of everyday executive functioning (REEF): A parent-report measure of preschoolers' executive functioning skills. *Psychological Assessment*, 29(1):50–64.

Oksanen, J., Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P., O'Hara, R., Simpson, G., Solymos, P., et al. (2020). Vegan community ecology package version 2.5-7 november 2020. *R Project for Statistical Computing: Vienna, Austria*.

O'Mahony, S. M., Felice, V. D., Nally, K., Savignac, H. M., Claesson, M. J., Scully, P., Woznicki, J., Hyland, N. P., Shanahan, F., Quigley, E. M., Marchesi, J. R., O'Toole, P. W., Dinan, T. G., and Cryan, J. F. (2014). Disturbance of the gut microbiota in early-life selectively affects visceral pain in adulthood without impacting cognitive or anxiety-related behaviors in male rats. *Neuroscience*, 277:885–901.

Ou, Y., Belzer, C., Smidt, H., and de Weerth, C. (2022). Development of the gut microbiota in healthy children in the first ten years of life: associations with internalizing and externalizing behavior. *Gut Microbes*, 14(1).

Ou, Y., Belzer, C., Smidt, H., and de Weerth, C. (2023a). Development of the gut microbiota in the first 14 years of life and its relations to internalizing and externalizing difficulties and social anxiety during puberty. *European Child & Adolescent Psychiatry*, pages 1–14.

Ou, Y., Belzer, C., Smidt, H., and de Weerth, C. (2023b). Methodological recommendations for human microbiota-gut-brain axis research. *Microbiome Research Reports*.

Paradis, E., Bolker, B., Claude, J., Sien Cuong, H., Desper, R., Durand, B., Dutheil, J., Gascuel, O., Heibl, C., Lawson, D., Lefort, V., Legendre, P., Lemon, J., Noel, Y., Nylander, J., Opgen-Rhein, R., Popescu, A.-A., Schliep, K., Strimmer, K., and de Vienne, D. (2020). APE: Analyses of Phylogenetics and Evolution in R language.

Png, C. W., Lindén, S. K., Gilshenan, K. S., Zoetendal, E. G., McSweeney, C. S., Sly, L. I., McGuckin, M. A., and Florin, T. H. J. (2010). Mucolytic bacteria with increased prevalence in IBD mucosa augment in vitro utilization of mucin by other bacteria. *The American journal of gastroenterology*, 105(11):2420–2428.

Poncheewin, W., Hermes, G. D. A., Dam, J. C. J. v., Koehorst, J. J., Smidt, H., and Schaap, P. J. (2020). NG-Tax 2.0: A Semantic Framework for High-Throughput Amplicon Analysis. *Frontiers in Genetics*, 10(January):1–12.

Pärtty, A., Kalliomäki, M., Wacklin, P., Salminen, S., and Isolauri, E. (2015). A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: a randomized trial. *Pediatric research*, 77(6):823–828.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research*, 41(Database issue):D590–D596.

Ramiro-Garcia, J., Hermes, G. D. A., Giatsis, C., Sipkema, D., Zoetendal, E. G., Schaap, P. J., and Smidt, H. (2016). NG-Tax, a highly accurate and validated pipeline for analysis of 16S rRNA amplicons from complex biomes. *F1000Research*, 5:1791.

Revelle, W. and Condon, D. M. (2019). Reliability from α to ω: A tutorial. *Psychological Assessment*, 31(12):1395–1411.

Rice, D. and Barone, S. (2000). Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environmental health perspectives*, 108(suppl 3):511–533.

Rothenberg, S. E., Chen, Q., Shen, J., Nong, Y., Nong, H., Trinh, E. P., Biasini, F. J., Liu, J., Zeng, X., Zou, Y., Ouyang, F., and Korrick, S. A. (2021). Neurodevelopment correlates with gut microbiota in a cross-sectional analysis of children at 3 years of age in rural China. *Scientific Reports*, 11(1):1–11.

Schoemaker, K., Bunte, T., Espy, K. A., Deković, M., and Matthys, W. (2014). Executive functions in preschool children with ADHD and DBD: an 18-month longitudinal study. *Developmental neuropsychology*, 39(4):302–315.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., and Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology*, 12(6).

Sherman, E. M. S. and Brooks, B. L. (2010). Behavior rating inventory of executive function – preschool version (BRIEF-p): Test review and clinical guidelines for use. *Child Neuropsychology*, 16(5):503–519.

Silva, Y. P., Bernardi, A., and Frozza, R. L. (2020). The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. *Frontiers in endocrinology*, 11:25.

Staller, J. A. and Faraone, S. V. (2007). Targeting the dopamine system in the treatment of attention-deficit/hyperactivity disorder. *Expert Review of Neurotherapeutics*, 7(4):351–362.

Stilling, R. M., Ryan, F. J., Hoban, A. E., Shanahan, F., Clarke, G., Claesson, M. J., Dinan, T. G., and Cryan, J. F. (2015). Microbes & neurodevelopment –Absence of microbiota during early life increases activity-related transcriptional pathways in the amygdala. *Brain, Behavior, and Immunity*, 50:209–220.

Streit, F., Prandovszky, E., Send, T., Zillich, L., Frank, J., Sabunciyan, S., Foo, J., Sirignano, L., Lange, B., Bardtke, S., Hatfield, G., Witt, S. H., Gilles, M., Rietschel, M., Deuschle, M., and Yolken, R. (2021). Microbiome profiles are associated with cognitive functioning in 45-month-old children. *Brain, behavior, and immunity*, 98:151–160.

Tamana, S. K., Tun, H. M., Konya, T., Chari, R. S., Field, C. J., Guttman, D. S., Becker, A. B., Moraes, T. J., Turvey, S. E., Subbarao, P., Sears, M. R., Pei, J., Scott, J. A., Mandhane, P. J., and Kozyrskyj, A. L. (2021). Bacteroides-dominant gut microbiome of late infancy is associated with enhanced neurodevelopment. *Gut Microbes*, 13(1):1–17.

Tarry-Adkins, J. L. and Ozanne, S. E. (2011). Mechanisms of early life programming: current knowledge and future directions. *The American Journal of Clinical Nutrition*, 94:S1765–S1771.

Thursby, E. and Juge, N. (2017). Introduction to the human gut microbiota. *Biochemical Journal*, 474(11):1823.

van Buuren, S. and Groothuis-Oudshoorn, K. (2011). mice: Multivariate imputation by chained equations in r. *Journal of statistical software*, 45:1–67.

van de Wouw, M., Rojas, L., Vaghef-Mehrabani, E., Wang, Y., Fichter, C., Workentine, M. L., Dewey, D., Arrieta, M.-C., Reimer, R. A., Tomfohr-Madsen, L., et al. (2023). Exploring associations between the gut microbiota and full-scale intelligence in preschool children. *Neuroscience Letters*, page 137357.

van de Wouw, M., Wang, Y., Workentine, M. L., Vaghef-Mehrabany, E., Dewey, D., Reimer, R. A., Tomfohr-Madsen, L., and Giesbrecht, G. F. (2021). Associations Between the Gut Microbiota and Internalizing Behaviors in Preschool Children. *Psychosomatic medicine*, 84(2):159–169.

Vos, W. M. d., Tilg, H., Hul, M. V., and Cani, P. D. (2022). Gut microbiome and health: mechanistic insights. *Gut*, 71(5):1020–1032.

Wang, L., Christophersen, C. T., Sorich, M. J., Gerber, J. P., Angley, M. T., and Conlon, M. A. (2013). Increased abundance of Sutterella spp. and Ruminococcus torques in feces of children with autism spectrum disorder. *Molecular autism*, 4(1):42.

Wang, S., Harvey, L., Martin, R., Beek, E. M. v. d., Knol, J., Cryan, J. F., and Renes, I. B. (2018). Targeting the gut microbiota to influence brain development and function in early life. *Neuroscience & Biobehavioral Reviews*, 95:191–201.

Welberg, L. (2022). Diet drives altered microbiota in autism. *Nature Neuroscience*, 25(1):2.

Willemsen, Y., Beijers, R., Vasquez, A. A., and de Weerth, C. (2021). Do breastfeeding history and diet quality predict inhibitory control at preschool age? *Nutrients*, 13(8):2752.

Xu, M., Xu, X., Li, J., and Li, F. (2019). Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. *Frontiers in psychiatry*, 10:473.

Zhuang, L., Chen, H., Zhang, S., Zhuang, J., Li, Q., and Feng, Z. (2019). Intestinal Microbiota in Early Life and Its Implications on Childhood Health. *Genomics, proteomics & bioinformatics*, 17(1):13–25.

Chapter 5

Are Adolescent Diet Quality and Emotional Eating Predicted by History of Maternal Caregiving Quality and Concurrent Inhibitory Control?

Based on: Willemsen, Y., Vacaru, S.V., Beijers, R., and de Weerth, C. Are Adolescent Diet Quality and Emotional Eating Predicted by History of Maternal Caregiving Quality and Concurrent Inhibitory Control? *Appetite*, 2023, page 107020.

Abstract

The risk for unhealthy eating behaviour, including poor diet quality and emotional eating, is heightened in adolescence and could result in profound and long-lasting psychological and physical implications. Caregiving quality and adolescents' regulatory skills, such as inhibitory control, may play an essential role in the development of adolescent eating behaviour. This preregistered study investigated whether maternal caregiving throughout the first 14 years of life predicts adolescent diet quality and emotional eating and whether potential associations are mediated by adolescents' inhibitory control. In this low-risk community cohort, maternal caregiving quality was observed at child ages five weeks, 12 months, 2.5, 10, and 14 years. At age 14, diet quality and emotional eating were assessed through self-report. Adolescent inhibitory control was assessed with three behavioural tasks and a maternal report. Mediation analyses were performed with structural equation modelling in R. No evidence was found for links between maternal caregiving quality, and adolescent diet quality and emotional eating. Higher levels of adolescent inhibitory control predicted better adolescent diet quality. Longitudinal and experimental studies are needed to investigate directionality, and replication studies are needed in more representative samples (e.g., including high-risk families). Such studies will shed further light on potential links between the history of caregiving behaviour and adolescent regulatory and eating behaviour.

Introduction

Adolescence is known for its heightened risk for unhealthy eating behaviours, including low diet quality and emotional eating (Limbers et al., 2021; World Health Organization, 2005). Diet quality refers to the healthiness of the dietary intake, while emotional eating is the urge to consume food in response to negative emotions instead of feelings of hunger (van Strien et al., 2016). The magnitude of eating behavioural problems during adolescence is widely recognized, with profound and long-lasting psychological and physical implications (Movassagh et al., 2017). However, little is known about the origins and underlying mechanisms that explain variation in diet quality and emotional eating (abbreviated as DQ/EE) between adolescents. This pre-registered study investigated whether the history of maternal caregiving quality throughout the first 14 years of life contributes to adolescent DQ/EE, and whether adolescent inhibitory control plays a potential mediating role.

High caregiving quality is characterized by sensitivity, responsiveness, and consistency in meeting the child's needs (Layzer and Goodson, 2006). Moreover, showing respect for child autonomy and being cooperative are also categorised as high quality caregiving (Layzer and Goodson, 2006). High quality of caregiving contributes to a range of child well-being and developmental outcomes (Bechtel-Kuehne et al., 2016). For example, high caregiving quality in early life has been shown to predict educational performance, psychobiological and psychosocial development in childhood, adolescence and even adulthood (Guyon-Harris et al., 2021; Raby et al., 2015; Scherer et al., 2019; Sroufe et al., 2010). Though early life is suggested to be a window of opportunity where caregiving quality may be an important contributor for future child development, there is also evidence that high quality caregiving at later ages is associated with beneficial child psychosocial and cognitive outcomes (Casale et al., 2015).

With respect to diet, several cross sectional, as well as some longitudinal, studies

have shown that high caregiving quality is related to healthier dietary intake of toddlers and children (Gubbels et al. (2011); Romanos-Nanclares et al. (2018), for reviews see Hughes and Papaioannou (2018); Sleddens et al. (2011)). Studies with adolescents indicate more contradictory results. Specifically, higher caregiving quality at age 12 years was associated with higher diet quality at age 15 years (Zietz et al., 2022). Furthermore, high caregiving quality was associated with higher adolescent intake of fruits, vegetables (Kremers et al., 2003; Lytle et al., 2003; Pearson et al., 2010), dietary fibres (Kim, 2007; Kim et al., 2008), and lower caloric intake (Kim, 2007; Kim et al., 2008), while higher maternal control was associated with lower snacking frequency in adolescents (Kim, 2007; Kim et al., 2008). In contrast, other studies found no relation between caregiving quality and adolescent dietary behaviours, including snacking (Kim, 2007; Kim et al., 2008), and soft drinks intake (Vereecken et al., 2009). Though less research focused on emotional eating as outcome, such studies found lower caregiving quality to be associated with more child (Schuetzmann et al., 2008; Topham et al., 2011) and adolescent emotional eating (Snoek et al., 2007; van Strien et al., 2019b). Notably, van Strien et al. (2019b) found this relation in a longitudinal study, with lower early life caregiving quality predicting more adolescent emotional eating. This relation was mediated by emotion regulation, indicating that adolescents who experienced lower quality of maternal caregiving had poorer control over their emotions, and in turn, showed more emotional eating.

One way through which high caregiving quality could contribute to healthy eating behaviours in adolescence is through its contribution to the development of adolescent inhibitory control (IC). IC can be defined as the ability to suppress impulses and consider the consequences of our actions (Diamond, 2013). IC is considered an important ability that is a core component of many psychosocial and cognitive outcomes (Anzman-Frasca et al., 2015; Diamond, 2013; Nigg, 2017). A major contributing pathway for IC development is parental coregulation. Coregulation is characterised by sensitive and

responsive behaviour towards the child's needs, hereby building the child's internal regulatory capacities (Bechtel-Kuehne et al., 2016; Bernier et al., 2012; Merz et al., 2016). Parental coregulation thus gradually shapes the child's capacity to regulate attention and emotions, thereby helping the child to inhibit impulses and achieve their goals (Belsky, 2002; Bernier et al., 2010, 2012; Cassidy, 1994; Gärtner et al., 2018; Merz et al., 2016; Schore, 2001; von Suchodoletz et al., 2011). Indeed, maternal sensitivity (Frick et al., 2019a,b; Geeraerts et al., 2021; Jennings et al., 2008; Spinrad et al., 2012; Vrijhof et al., 2020; Wu et al., 2022), responsiveness (Brophy-Herb et al., 2012; Kochanska et al., 2000; von Suchodoletz et al., 2011), supportive presence (Bosquet Enlow et al., 2019; Bravo et al., 2023; Brophy-Herb et al., 2012; Kok et al., 2013), and autonomy support (Bernier et al., 2010), all predicted better toddler and child IC. Whether this prediction holds for adolescence is still under investigation, as only one study investigated and found maternal sensitivity at age seven years to be negatively associated to 14-year-olds' over-inhibited, withdrawn, and anxious-depressed behaviour (van der Voort et al., 2014); behaviours found to be related to poorer child's self-regulation and inhibitory control capacities (Buffie and Nangle, 2022; Diamond, 2013; Valikhani et al., 2021).

In turn, adolescent IC may relate to adolescents' eating behaviours through how IC helps suppress automatic behaviours, emotions, and thoughts (Bari and Robbins, 2013). Adolescents with low IC may find it difficult to resist the temptation to consume high-calorie, low-nutrient-dense foods as it satisfies their immediate reward system (Casey et al., 2008). Consumption of these foods decreases the diet quality. However, the evidence regarding IC and adolescent DQ/EE is mixed. IC has been associated with better adolescent diet quality (i.e., lower sugar-sweetened beverages, snacks, and higher whole grain consumption) (Ames et al., 2014) and lower total energy intake during a lab-based food task (Byrne et al., 2021a). However, another study found no associations between adolescent IC, diet quality, and beverages consumed during a

lab-based food task (Ames et al., 2016). Hence, there is only one study on adolescent IC and overall diet quality (Ames et al., 2014), which needs replication studies to confirm their results. With respect to emotional eating, IC could relate to emotional eating via the diminished ability to regulate stress and emotions (Bari and Robbins, 2013). Adolescents with lower levels of IC could therefore be more inclined to engage in emotional eating as a coping mechanism (Shriver et al., 2020). However, research regarding emotional eating and adolescents' IC is scarce as only three studies were conducted and no associations were found in participants aged 8-17 years (Byrne et al., 2021b; Mayer et al., 2022; Nelson et al., 2020).

In sum, high caregiving quality may relate to adolescent diet quality and emotional eating. A potential underlying mechanism might be adolescent IC, as higher caregiving quality is suggested to support the development of IC, which in turn is suggested to be associated with healthier DQ/EE. We investigated whether the history of maternal caregiving quality throughout the first 14 years of life (observed at age five weeks, 12 months, 2.5, 10, and 14 years) contributed to DQ/EE, namely diet quality and emotional eating at 14 years of age, hypothesizing that earlier and concurrent higher maternal caregiving quality leads to higher diet quality and less emotional eating during adolescence. Moreover, we investigated whether IC mediated the expected associations, hypothesizing that higher maternal caregiving quality relates to better IC and, in turn, better DQ/EE. Next to questionnaires, we incorporated behavioural tasks of often-investigated IC subdomains in adolescents, namely: delayed discounting (the tendency to devalue delayed rewards), interference control (the ability to suppress or disregard irrelevant information), and response inhibition (the ability to inhibit or stop a pre-potent response) (Ames et al., 2014; Byrne et al., 2021a; Mayer et al., 2022; Nelson et al., 2020; van der Bij et al., 2020). This study was preregistered on Open Science Framework (https://doi.org/10.17605/OSF.IO/7K9NX, registered on November 24th, 2022).

Materials and methods

Participants

This study is a part of the ongoing longitudinal BIBO (Dutch acronym for Basal influences on Child Development) study that follows women and their children from late pregnancy through childhood and adolescence (see Beijers et al. (2011) for a description of the study). Mothers in the Netherlands were recruited on a voluntary basis via flyers distributed at midwife practices in the cities of Nijmegen, Arnhem, and surrounding areas. The inclusion criteria were an uncomplicated singleton pregnancy, no current physical or mental health problems, no drugs and/or alcohol use during pregnancy, gestational age ≥37 weeks, and a children's 5-min APGAR score of ≥7. From 220 women recruited, eight were excluded due to medical reasons (e.g., preterm birth), and 19 dropped out within the first three postpartum months due to personal circumstances, leading to a sample of 193 mother-infant dyads (see Figure 5.1 for the flowchart). Between the child age of three months and 14 years of age, 34 participants dropped out (18%) for various reasons. As a result, 159 mother-adolescent dyads were approached to participate in the measurement round at child age 14. From this group, 150 dyads participated. The reasons for non-participation were personal (n=8) and COVID-related reasons (n=1). No significant differences in maternal age, educational level, and child sex were found between the dyads who participated (n=150), not participated (n=9), and dropped out (n=34). The BIBO study was reviewed by the Ethics Committee of the Faculty of Social Sciences (ECSW) of the Radboud University Nijmegen, The Netherlands, and no formal objection to this research was made (SW2017-1303-497, SW2017-1303-498, and ECSW-2018-067).

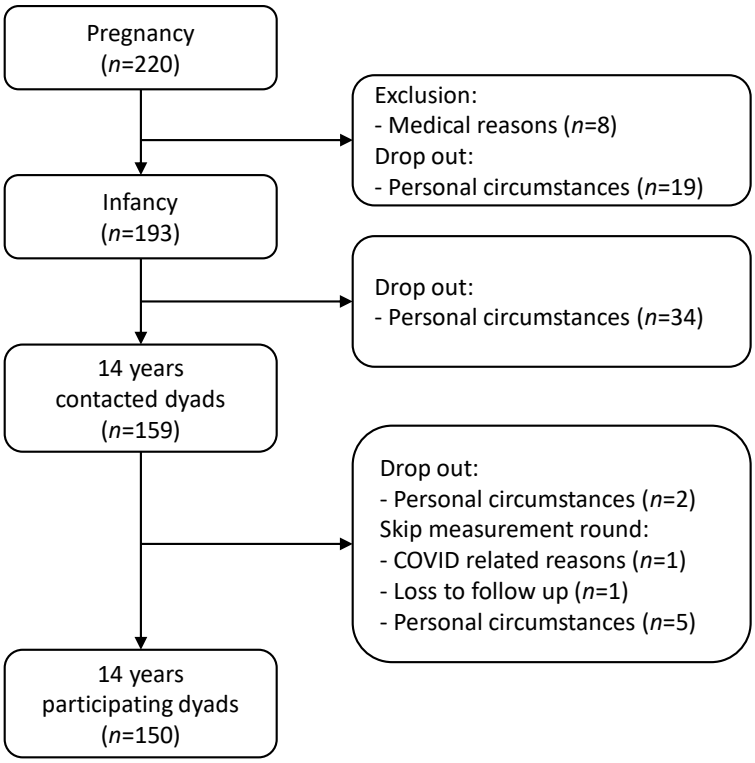


Figure 5.1. Flowchart of study participants.

Procedure

The BIBO study entailed home visits (aged five weeks, 12 months, 2.5 years, 10 years, and 14 years) in which mothers and children were observed during naturalistic interactions. At five weeks of age (Mean child age=33.5 days, SD=4.9), mothers and their infants were filmed at home during a bathing session: the infant was undressed, bathed, and dressed again. At 12 months of age (Mean child age=53 weeks and 6 days, SD=19 days), mothers and their infants visited the lab and were instructed to play together with four toys (i.e., hand puppets, books, and two types of puzzles) for 12 minutes in total (three minutes per toy). At age 2.5 years (Mean child age=30 months and 5 days, SD=19 days), mothers and infants were filmed at home while playing with

three toys (i.e., puzzles, blocks, and a fishing game) for 12 minutes in total (4 minutes per toy). During a home visit at child age 10 years (Mean child age=10 years and 1 month, SD=2 months), mother and child were asked to discuss two different emotions for three minutes each and to play a Tangram game for six minutes. During a home visit at age 14 years (Mean child age=14 years and 5 months, SD=2 months), mother and adolescent were asked to discuss two topics for three minutes each for the first interaction task. The topics were determined based on the 44-item issues checklist (Robin and Foster, 1989), which includes common discussion topics between parents and adolescents (e.g., low grades at school, how to spend money, and helping around the house). At the start of the home visit, the parent indicated per topic whether they had discussed it within the past four weeks 'yes' or 'no'. If they scored 'yes', they rated on a 1-5 Likert scale how calm (1) or angry (5) the discussion about the topic made them feel. The two highest-scoring topics were used for the interaction task. For the second interaction task, mother-adolescent dyads were asked to organize and write down details of an event for the child's classmates, for seven minutes. Furthermore, during the home visit at age 14, adolescent IC was assessed with three behavioural tasks (each lasting less than five minutes) and via a mother-report online questionnaire. Details on the behavioural tasks are mentioned in the 'Behavioural tasks' section under the 'Inhibitory control' paragraph. Adolescent diet quality and emotional eating were assessed with online and paper self-report questionnaires, respectively.

Reliability of coding

Parent-child interactions were videotaped during home visits and rated afterward by at least two independent observers using the sensitivity scales mentioned below. An experienced senior coder (the third author of this paper) trained the independent observers using gold standard training videos. The independent observers, unaware of the goals of the current study, independently practiced until they reached adequate

reliability (intraclass correlation coefficient (ICC) ≥0.80) with gold standard training videos. After, the independent observers scored the study videos, with regular observer team meetings and checks to detect and prevent observer drift. At least 30 percent of the data was doubly entered or scored to check for reliability (ICC, two-way mixed effects, relying on absolute agreement; Koo and Li (2016)). Reliability was good when ICC≥0.80 (Koo and Li, 2016). If there was a large discrepancy between two observer scores, a third observer (e.g., the experienced senior observer) was included and independently observed the video again. For each questionnaire and recording that needed to be entered or scored, a codebook was made to set the coding rules.

Measures

Caregiving quality

To rate maternal behaviour at infant age five weeks, the 9-point Ainsworth scale was used (Ainsworth et al., 2015). Interactions were rated for sensitivity, defined as the extent to which the mother responds to the infant's needs and signals in a timely and sensitive manner, and cooperation, defined as the extent to which the mother adjusts her behaviour to the infant and does not interfere with the infant's ongoing activity. As the two subscales were highly correlated (r=0.86, p<0.01), the scores of sensitivity and cooperation were averaged. Higher scores represent higher caregiving quality. Interrater reliability for this scale was 0.81 in previous literature (Stiles, 2004), and it showed correlations with a different sensitivity questionnaire (Maternal Sensitivity Q-Sort), supporting construct validity (Stiles, 2004). The interobserver reliability in this study was good, exceeding an ICC of 0.90 for both constructs.

To rate maternal behaviour at child age 12 months, 2.5 years, 10 years, and 14 years, the 7-point Erickson scale were used (Erickson et al., 1985). Interactions were rated for supportive presence, defined as the extent to which the parent provides emotional support and confidence in the child, and respect of child autonomy, defined as

the extent to which the parent respects the validity of the child's individuality, motives, and perspectives. Scores on supportive presence and respect of child autonomy correlated significantly at 12 months (r=0.62), 2.5 years (r=0.46), 10 years (r=0.60), and 14 years (r=0.75), and were therefore averaged. Higher scores represent higher caregiving quality. The intraclass coefficients were good for supportive presence at 12 months (ICC=0.95), 2.5 years (ICC=0.91), 10 years (ICC=0.97), and 14 years (ICC=0.81). The intraclass coefficients were also good for respect of child autonomy at age 12 months (ICC=0.70), 2.5 years (ICC=0.70), 10 years (ICC=0.93), and 14 years (ICC=0.77).

Inhibitory control (14 years)

Behavioural tasks

Interference control, STROOP task: The STROOP measures verbal IC (Stroop, 1935). Adolescents were presented with three cards. The first card was a word card (W) and displayed four Dutch words (i.e., green, blue, red, and yellow), 100 times (i.e., 10 lines with 10 words), printed in black. The adolescent was asked to read all 100 words of the first card as fast as possible from left to right. The second card was a colour card (C), on which four different colours (i.e., green, blue, red, and yellow rectangular shapes) were printed 100 times (i.e., 10 lines with 10 colours). The adolescent was asked to name all 100 colours as fast as possible from left to right. The third card was a combination of colours and words (CW) and presented 100 words: the four different words in four different colours, congruent and incongruent with their words (e.g., the word 'yellow' was written in yellow font, and the word 'blue' was written in yellow font). The adolescent was asked to name the colour of the word and inhibit naming the written word. Each card was timed by the experimenter, and afterwards the number of mistakes were counted per card by checking the recordings. Reliability between raters was high (ICC: 0.95). IC was measured with Golden's method (Golden et al., 1978), which is the

most used method in literature (Scarpina and Tagini, 2017). The number of correctly named items within 45 seconds in all conditions was calculated. A predicted score (i.e., the score if the task was performed with perfect IC, taken into account the normal reading speed) of the third card (CW) was calculated with the following formula: (W x C) / (W + C). Then the inference was calculated by subtracting the predicted score from the actual number of items correctly named in the third card (CW). A high score indicates better IC.

Response inhibition. Go/No-Go task: The Go/No-Go task measures the motoric IC of the adolescent (Murphy et al., 1999). The task contained two parts of 60 trials each and was programmed in Psychopy. For each part, 25% of the stimuli were 'no-go' stimuli. Adolescents were asked to press a button when a 'go' stimulus was shown; however, pressing should be inhibited when the 'no go' stimulus was shown. Each image was shown for 400 milliseconds with an interstimulus time of 500 milliseconds. After the first 30 trials, there was a break until the adolescent was ready to continue (breaks took no more than one minute). For the first 60 trials, 'go' stimuli were a yellow square, blue triangle, and blue square, and the 'no go' stimulus was a yellow triangle. For the second part (next 60 trials), 'go' stimuli were the letters 'P', 'D', and 'B', and the 'no go' stimulus was the letter 'R'. See Figure 5.2 for a visualization of the Go/No-go task. IC was determined by the number of errors made in the No-Go trials. A higher number indicates worse IC.

Delayed discounting. Monetary choice task: The Monetary choice task is a 27-item questionnaire where the adolescent is asked to choose between receiving a smaller amount of money immediately, or a larger amount of money with delay (e.g., "Would you prefer €19 today, or €25 in 53 days?") (Kirby and Maraković, 1996). Adolescents filled in this questionnaire digitally. Answers were entered in the automated scoring system of the Monetary choice task (Kaplan et al., 2016). The Cronbach's alpha was 0.90, and the overall consistency was 0.96. The log Geomean of the k was used as a

measure of delayed discounting. A higher k corresponds with a greater proportion of choices for the smaller immediate rewards, indicating worse IC.

Correlations between the tasks were non-significant and ranged from r=-0.15 to r=-0.04. Regardless of these correlation coefficients, we preferred forming a composite score over keeping the tasks separate because we assume that the tasks measure different forms of the same overarching construct (Epstein, 1983). Consequently, and following our preregistration (https://doi.org/10.17605/OSF.IO/7K9NX), the three IC tasks scores were standardized and subsequently aggregated into a composite score to represent adolescent behavioural IC. The Go/No-Go task and the Monetary choice task were reverse coded before aggregation. Subsequently, a higher IC composite score indicates better IC.

Questionnaire

The Behavior Rating Inventory of Executive Function 5-18 (BRIEF 5-18; Roth et al. (2013)) is a 75-item caregiver-report questionnaire used to assess executive functions of children between 5-18 years. It contains a 10-item IC subscale, scored on a 3-point Likert scale (1=never, 2=sometimes, 3=often). The parent completed the entire questionnaire online, however, only the IC subscale was used for this study. Because higher sum scores on this subscale indicate worse IC, the outcome of the BRIEF was reverse-coded in the analyses to align with our other IC composite score in which a higher score also presents better IC. Internal consistency analyses yielded Cronbach's alpha of 0.83 for the IC subscale. The internal consistency in previous literature ranges from α=0.80-0.98, and test-retest reliability was r=0.82 for parents (Dodzik, 2017).

The BRIEF IC subscale was positively correlated with the number of mistakes made in the Go/No-Go task (r=0.22), indicating that low IC is associated with worse performance on the Go/No-Go. The BRIEF did not correlate with the STROOP and monetary choice task.

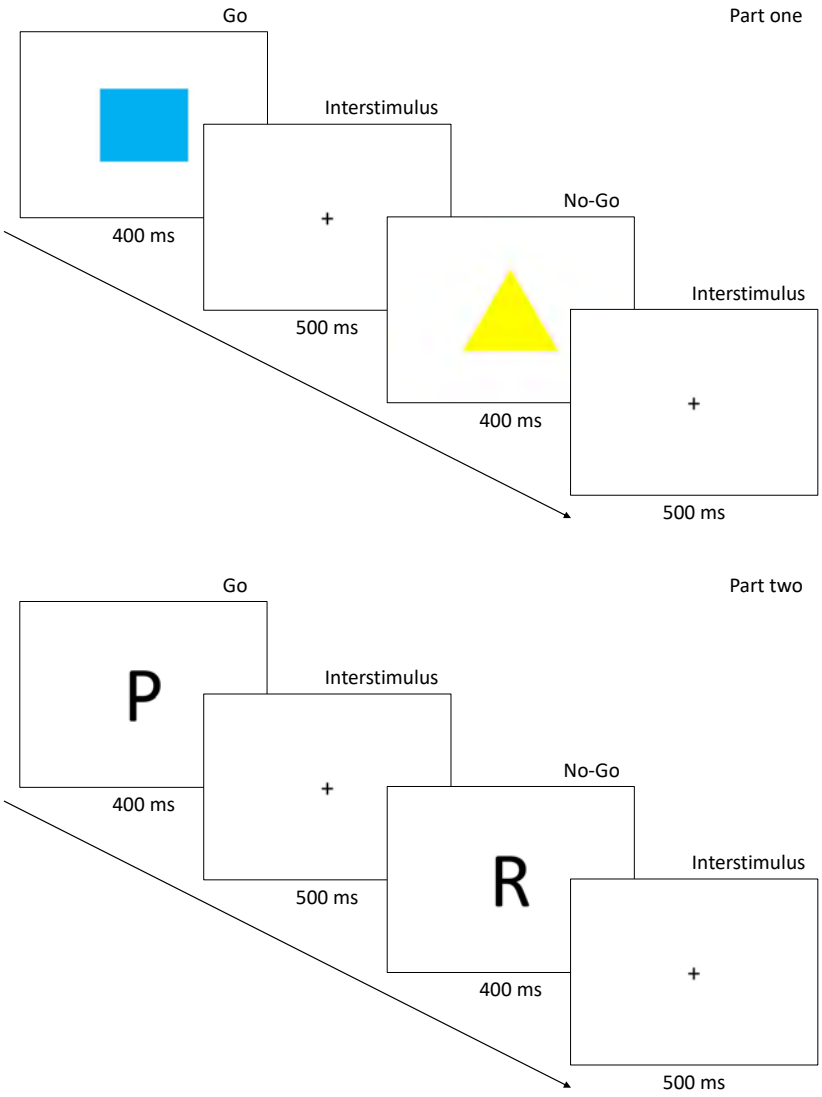


Figure 5.2. Go/No-Go task stimuli. In part one, the stimuli were coloured figures, shown for 400 ms. In part two, the stimuli were black letters, shown for 400 ms. Stimuli were always followed by an interstimulus which was a fixation cross, shown for 500 ms.

Diet quality

The 'Eetscore' (in English: Diet score) is a validated online food frequency questionnaire (FFQ) that assesses diet quality (Rijk et al., 2021; Lee et al., 2015), and was filled in by the adolescents. The Eetscore assesses intake of 16 food categories, based on the food categories of the Dutch Healthy Diet index 2015 (Looman et al., 2017): vegetables, fruit, whole grain products, legumes, nuts, dairy, fish, tea, coffee, spreading and cooking fats, red meat, processed meat, sugary beverages, alcohol, salt, and unhealthy snacks. The number of items in the Eetscore ranges from 40-76. A score between 0 and 10 was given for each food category. The ratio of the intake was calculated according to the cut off, e.g., fruit intake of 100 grams per day was assigned a score of 5 (100/200 g/d *10). This was reverse scored for the unhealthy food categories (Looman et al., 2017): red meat, processed meat, sugary drinks, alcohol, sodium, and unhealthy snacks. All scores were summed to obtain the total diet quality score (ranges between 0 and 160). A higher score represents better diet quality. See Table 5.1 for the scoring per food category.

Emotional eating

The Dutch Eating Behavior Questionnaire (DEBQ) is a 33-item self-report questionnaire that assesses eating behaviour and contains a 13-item subscale to assess emotional eating, on a 5-point Likert scale (1=never, 5=very often) (van Strien et al., 1986). Only the emotional eating subscale was assessed and filled in by the adolescent on paper. The scores on this subscale were summed. Higher scores indicate more emotional eating. In a nonclinical sample, the emotional eating subscale has good internal reliability with coefficient alphas ranging from 0.96 to 0.97 (Bohrer et al., 2015). The Cronbach alpha was good: α =0.88. The ICC for this questionnaire was >0.99. This scale has not been validated in adolescents yet, and current results on the validity of

this subscale are still divergent (Domoff et al., 2013).

Table 5.1. Scores for food categories in the Eetscore.

	Minimum score (=0)	Maximum score (=10)
Vegetables	0 g	≥ 200 g
Fruits	0 g	≥ 200 g
Whole grain products	0 g or ratio of whole grain products/refined grain products ≤0.7	≥ 90 g No consumption of refined grain products or ratio of whole grain/ refined grain products ≥ 11 g
Legumes	0 g	≥10 g
Nuts	0 g	≥15 g
Dairy	0 g or ≥750 g	300-450 g
Fish	0 g	≥15 g
Tea	0 mL	≥ 450 mL
Spreading and cooking fats	0 g of soft margarines, liquid cooking fats, and plant-based oils or ≤0.6 g ratio of liquid cooking fat/ hard cooking fat	No consumption of butter, hard margarines, and hard cooking fats or ratio or of liquid fats/ hard fats ≥13
Coffee	Unfiltered coffee	Consumption of only filtered coffee or no coffee consumption
Red meat	100 g	≤ 45 g
Processed meat	≥ 50 g	0 g
Sugary drinks	≥ 250 g	0 g
Alcohol	Female ≥20 g Male ≥30 g	Female ≤10 g Male ≤10 g
Sodium	≥3.8 g	<1.9 g
Unhealthy snacks	>7 unhealthy snacks/week	≤3 unhealthy snacks/week

Note: The minimum score represents scores of a low quality diet, and the maximum score represents scores of a high quality diet.

Confounders

Confounding variables were determined based on previous literature and plotted as directed acyclic graphs (DAGs), which show the contribution of these potential confounding variables to the model (Cinelli et al., 2020). See Figure 5.3 for a visualization of the DAGs. The following potential confounding variables were considered for the models with diet quality as well as emotional eating as dependent variable: child sex (Deslippe et al., 2022; Özcan et al., 2020), and maternal educational level (Assari, 2020; Davis-Kean et al., 2021; Drywień et al., 2021; Gouveia et al., 2018; Özcan et al.,

2020). Maternal educational level was assessed on an 8-point Likert scale (ranging from 1=primary education to 8=university education). These potential confounding variables were subsequently correlated with the dependent variables. When the associations were significant, the variables were added to the statistical models to correct for confounding.

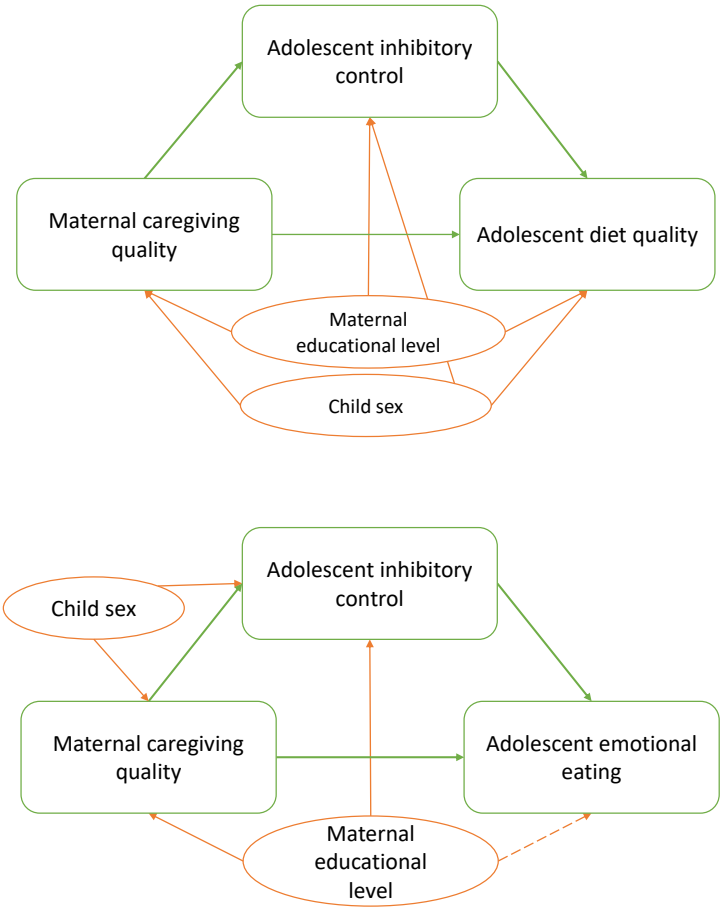


Figure 5.3. Directed acyclic graphs for determining potential confounders for adolescent diet quality and adolescent emotional eating. Green coloured arrows indicate the links between predictor, mediator and outcome variables. Orange coloured arrows indicate the links between potential confounding variables and predictor, mediator, and outcome variables. The dotted line indicates unclear relation between the two measures.

Missing data

Out of 150 mother-adolescent dyads, seven were excluded from the final analyses as they did not fill in the questionnaires on dietary intake and emotional eating. From the remaining 143, the following data were missing: Eetscore (n=11), DEBQ (n=1), maternal supportive presence/respect for autonomy at age 12 months (n=4), age 2.5 years (n=1), age 10 years (n=4), age 14 years (n=5), Go/No-Go (n=6), STROOP (n=6), Delayed Discounting (n=6), and BRIEF (n=1). No data was missing at five weeks of age. The LittleMCAR test indicated that data were missing completely at random (χ^2=208.376, p=0.136). In total, 2.1% of the data were missing. From the 143 participants, a sample size of n=132 was used for the dietary intake analyses (because 11 Eetscore FFQs were missing) and n=142 for the emotional eating analyses (because one DEBQ was missing). We did not impute missing data for our dependent variables, and imputed data in our independent variables by means of Expectation Maximization, which predicts the missing data based on existing data (Dempster et al., 1977).

Statistics

Preliminary analyses

Preliminary analyses were performed in R. Statistical significance was considered at a p-value of $<$0.05, and when the 95% confidence interval did not include 0. Continuous variables were checked for normality by visual inspection and the Shapiro-Wilk test, and the following variables were non-normally distributed: sensitivity and cooperation at five weeks, supportive presence and respect for child autonomy at 12 months, 2.5 years, 10 years, and 14 years, maternal educational level, and the BRIEF. The BRIEF was log-transformed to obtain a normal distribution for the main analyses. Transforming the caregiving quality measures and maternal educational level did not improve the distribution, hence these were left untransformed. Wilcoxon tests were performed

to check for significant differences between boys and girls for non-normally distributed data. Variables were furthermore checked for outliers with the Grubbs test. Diet quality contained one outlier that was winsorized. All analyses were performed with and without winsorized data. Pearson and Spearman correlations were performed to measure correlations between normally and non-normally distributed variables, respectively (see Supplementary Table 7.17 for correlations between all measured variables).

Due to the longitudinal nature of our study, the sample size could not be adjusted. However, the sample size provided enough statistical power for the structural equation models, according to the most commonly used rule of thumb of ten cases per parameter for structural equation modelling (Schreiber et al., 2006). Sample sizes of n=132 and n=142 would be sufficient for the eleven parameters used in the models.

Main analyses

Mediation analysis was performed in R with the 'Lavaan' package (Rosseel, 2012). Two models were run to explore if maternal caregiving quality at different ages independently predicted diet quality, and whether this was mediated by IC as assessed by the questionnaire (model 1) and the behavioural tasks (model 2). Another two models were run to explore if maternal caregiving quality at different ages independently predicted emotional eating, and whether this was mediated by IC as assessed by the questionnaire (model 3) and the behavioural tasks (model 4). Potential confounding variables were only added to the model as extra variables if they correlated significantly with the outcome measure. Maternal educational level correlated with diet quality (r=0.26), emotional eating (r=0.21), and the IC composite score (r=0.18), and was hence added as a confounder in the analyses including these measures. Furthermore, on average, girls had significantly higher diet quality than boys, hence we corrected for child sex in the main analyses including diet quality. There were no significant differences between boys' and girls' scores on the IC subscale of the BRIEF and on the DEBQ.

Exploratory analyses

Exploratorily, we investigated the mediating effects of the three IC tasks separately, yielding an extra six models (three models per outcome variable). The reason for exploring these tasks separately is because these IC tasks each measure different types of IC. We furthermore created a single score of all the caregiving quality measures by converting them to z-scores and calculating the average. The sensitivity composite score was then used as a predictor variable, i.e., caregiving quality, in the models for diet quality and emotional eating. For this purpose, four extra models were run.

Results

Preliminary analyses

Table 5.2 shows the descriptive statistics of the study population and the primary measures of this study. In two cases, fathers performed the interaction task with their child. No difference in the results was found after excluding these two father-child dyads from the analyses.

Table 5.3 shows the correlations between the variables used for the final analyses and potential confounding variables. Higher caregiving quality at 10 years correlated significantly with higher caregiving quality at 2.5 years (r=0.25) and 14 years (r=0.24). Furthermore, a higher IC composite score correlated significantly with higher diet quality (r=0.26). Diet quality and emotional eating did not correlate significantly.

Main analyses

Mediation analyses were run to investigate the associations between caregiving quality and diet quality as mediated by IC (BRIEF) (model 1) and the IC composite (model 2). Similarly, mediation analyses were run to investigate the association between caregiving quality and emotional eating as mediated by IC (BRIEF) (model 3), and the

IC composite (model 4). The results are visualized in Figure 5.4 (model 1 and 2) and Figure 5.5 (model 3 and 4). Model 2 shows that the IC composite score was positively associated with diet quality (β=5.22, 95%CI=0.64 - 9.80), indicating that better IC is related to higher diet quality. Additionally, the covariate maternal educational level was associated with better diet quality, better observed IC, and more emotional eating (as seen in model 2 and 3). No other significant results were found. Supplementary Table 7.18 shows the estimates of models 1 and 2, and Supplementary Table 7.19 shows the estimates of models 3 and 4. Results were no different with and without winsorizing diet quality.

Table 5.2. Descriptive statistics of participant characteristics and measures.

	n(%)	**Min - max**	
Characteristics			
Child sex			
Female	68 (47.6)		
Male	75 (52.4)		
Maternal educational level		2 - 8	
Low	0 (0)		
Middle	19 (13.3)		
High	124 (86.7)		
	Mean age (±SD)	**Min - max**	***n***
Child age (years ±SD)	14.4 (0.2)	169 - 181	143
Maternal age (years ±SD)	46.5 (3.9)	35 - 57	143
Maternal caregiving quality scores	**Mean score (±SD)**	**Min - max**	***n***
5 weeks	5.6 (2.2)	1 - 9	143
12 months	4.3 (1.3)	1 - 7	139
2.5 years	5.3 (0.7)	3 - 7	142
10 years	5.1 (1.0)	2 - 7	139
14 years	4.6 (1.4)	1 - 7	138
Inhibitory control	**Mean score (±SD)**	**Min - max**	***n***
Questionnaire			
BRIEF IC	3.8 (3.4)	0 - 14	142
Tasks			
STROOP	9.7 (7.0)	-3.95 - 26.85	137
Go/No-Go	13.5 (5.2)	3 - 25	137
Monetary choice	-2.4 (0.62)	-3.8 - -0.9	137
DQ/EE	**Mean score (±SD)**	**Min - max**	***n***
Diet quality	87.1 (16.8)	31 - 132	132
Emotional eating	31.6 (8.9)	13 - 60	142

Notes. SD: Standard deviation, Min: minimum, Max: maximum, BRIEF: Behavior Rating Inventory of Executive Function, IC: Inhibitory control, DQ/EE: Diet quality and Emotional eating. Higher scores on the STROOP indicates better inhibitory control. Higher scores on the Go/No-Go and Monetary choice task indicate worse inhibitory control. Educational level: 1=primary education to 8=university education; low=1-2, medium=3-4, and high=5-8. Combined maternal caregiving scores are the average of the two subscales.

Table 5.3. Correlation coefficients between potential confounding variables, predictors, and outcome variables.

	Maternal educational level	Child (1=boy/ 2=girl)	CQ 5w	CQ 12m	CQ 2.5y	CQ 10y	CQ 14y	IC questionnaire	IC tasks	Diet Quality	Emotional eating
Maternal educational level	-										
Child sex (1=boy/2=girl)	0.11	-									
CQ 5w	0.04	-0.03	-								
CQ 12m	-0.03	0.05	0.12	-							
CQ 2.5y	-0.01	0.09	0.00	-0.02	-						
CQ 10y	0.04	0.00	0.08	0.07	**0.25****	-					
CQ 14y	0.09	-0.07	0.14	0.09	0.05	**0.24****	-				
IC questionnaire	-0.03	0.12	0.08	0.10	0.06	0.14	0.13	-			
IC tasks	**0.18***	0.16	-0.13	-0.13	0.00	-0.03	-0.02	0.14	-		
Diet Quality	**0.26****	**0.18***	-0.13	-0.02	-0.03	-0.05	-0.02	0.02	**0.26****	-	
Emotional eating	**0.21***	0.09	0.05	-0.06	0.07	0.02	-0.08	-0.06	0.13	-0.03	-

Note: CQ, Caregiving quality. IC questionnaire: Score on the Behavior Rating Inventory of Executive Functions. IC tasks: Aggregated score of the inhibitory control tasks. The BRIEF indicates higher scores as worse inhibitory control. 5w: five weeks, 12m: 12 months, 2.5y: 2.5 years, 10y: 10 years, 14y: 14 years, *$p<0.05$, **$p<0.01$.

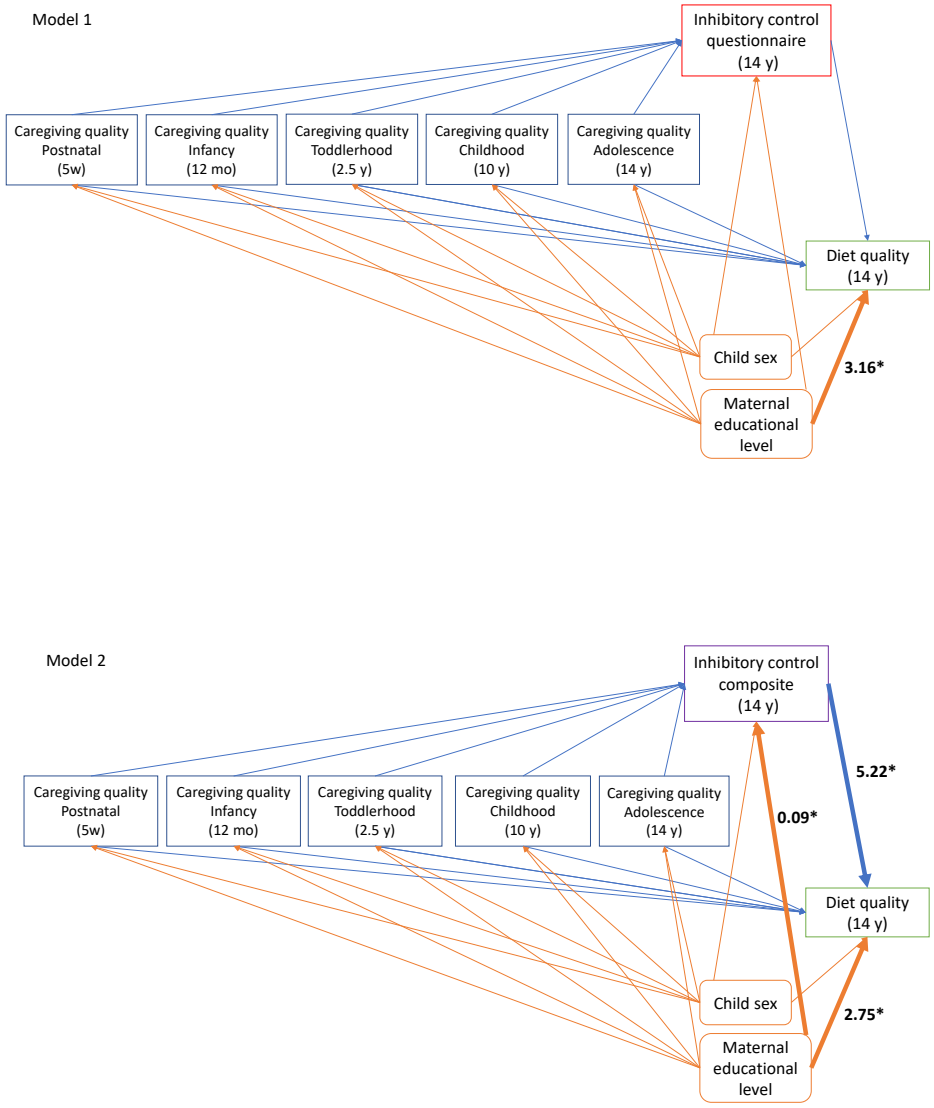


Figure 5.4. Mediation model results of diet quality as outcome. Only the significant estimates (betas) are shown. Blue arrows indicate links between predictor, mediator and outcome variables, and orange arrows indicate links between confounding variables and predictor, mediator, and outcome variables.

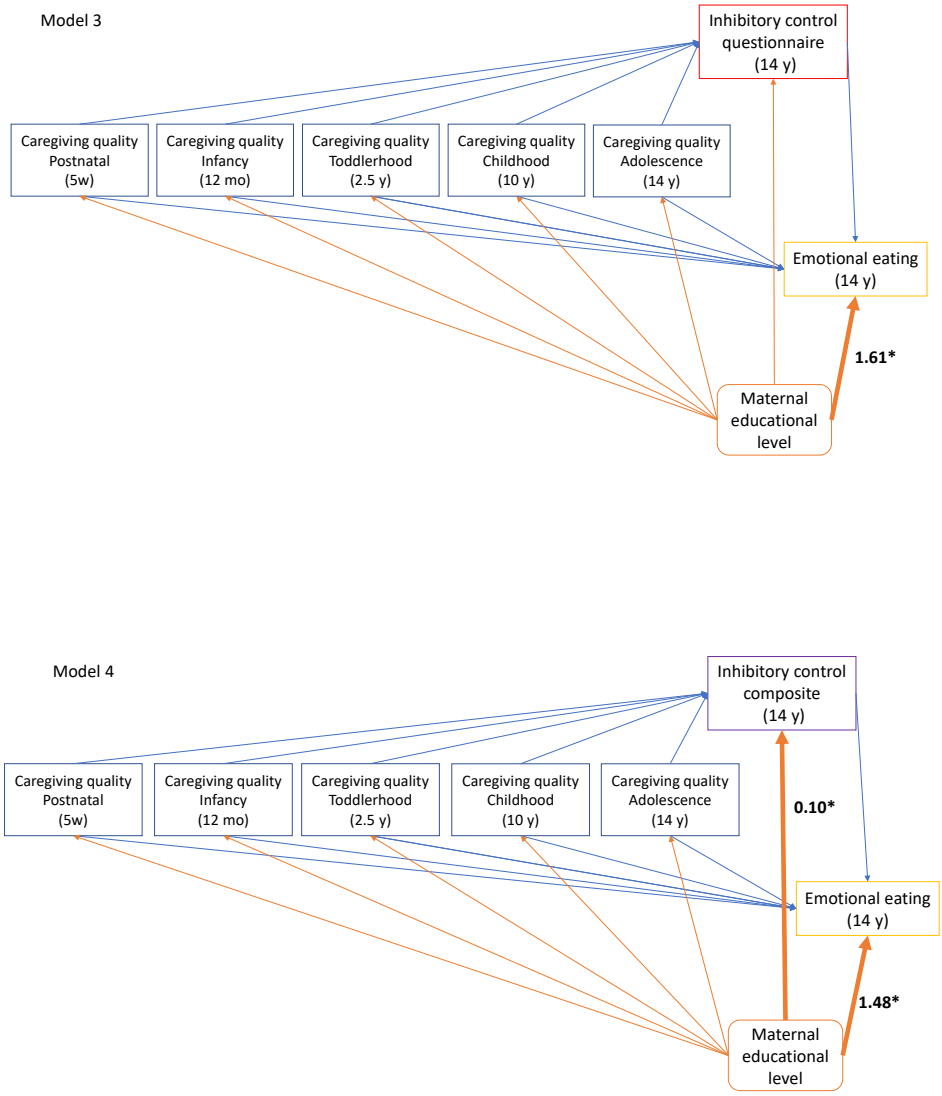


Figure 5.5. Mediation model results of emotional eating as outcome. Only the significant estimates (betas) are shown. Blue arrows indicate links between predictor, mediator and outcome variables, and orange arrows indicate links between confounding variables and predictor, mediator, and outcome variables.

Exploratory analyses

We explored whether the different behavioural tasks (i.e., STROOP, Go/No-Go, and Monetary choice) independently mediated the associations between caregiving quality and adolescent DQ/EE. Results showed that higher maternal caregiving quality at five weeks was associated with more mistakes in the Go/No-Go task (β=0.43, CI=0.03 - 0.84) and higher maternal caregiving quality at 12 months with worse performance on the STROOP task (β=-1.21, CI=-2.13 - -0.29). Furthermore, there was a negative association between delayed discounting and diet quality (β=-5.51, CI=-10.01 - -1.01). As high levels of delayed discounting indicate low IC, this indicates that better IC is associated with better diet quality. See Supplementary Tables 7.20 and 7.21 for an overview of all the estimates.

Additionally, we combined all caregiving quality measures over the ages into one composite score and investigated the relation between caregiving quality over the whole 14-year period and adolescent DQ/EE mediated by IC (i.e., BRIEF and IC composite score). No significant results were found (see Supplementary Table 7.22 for an overview of all the estimates).

Discussion

This study investigated whether the history of maternal caregiving quality from birth until 14 years of age predicts adolescent diet quality and emotional eating. Maternal caregiving quality was investigated at five weeks, 12 months, 2.5 years, 10 years, and 14 years. Moreover, inhibitory control (IC) was investigated as a potential mediator in these associations. We found that better adolescent IC was associated with better adolescent diet quality. There was no evidence for a relation between maternal caregiving quality at any age and adolescent diet quality or emotional eating. Lastly, we found no evidence for a relation between maternal caregiving quality at all ages and

IC, nor for mediation effects.

The better the IC of an adolescent, as assessed with several behavioural tasks, the higher the diet quality, as reported by the adolescent. This finding is in line with the studies of Ames et al. (2014) and Byrne et al. (2021a), who found that better IC, also assessed with behavioural tasks, was associated with fewer sugar-sweetened beverages and fewer snacks (Ames et al., 2014), and lower total energy intake (but not snack intake) (Byrne et al., 2021a). The focus of previous studies was on response inhibition, using a (food) Go/No-go task (Ames et al., 2014; Byrne et al., 2021a). We broadened our measure of the adolescents' IC by collecting multiple IC measures, including response inhibition, interference control, and delayed discounting (Epstein, 1983). The fact that our aggregated IC score related to better adolescent diet quality, indicates that different aspects of inhibitory control may impact dietary intake. Indeed, for example, nutritional intake can be affected by delaying a food reward for later (delayed discounting) (Barlow et al., 2016; Ortega et al., 2023), by choosing a healthier snack over other present food temptations (interference control) (Zuniga et al., 2015), as well as by controlling direct impulsivity to fulfil a momentarily food desire (response inhibition) (Ames et al., 2014; Byrne et al., 2021a). Our exploratory analyses revealed that especially IC behaviour measured with the monetary choice task, measuring delayed gratification, was associated with higher diet quality. Although this is an exploratory finding, it is congruent with previous research that found delayed gratification to be associated with lower child weight and less eating in the absence of hunger (Giuliani and Kelly, 2021). Note, however, that while in this study delayed discounting was used as a measure for inhibitory control, and it has indeed been related to various maladaptive behaviours such as substance abuse and gambling (Amlung et al., 2017), delayed discounting is not necessarily an indicator of executive functioning (see e.g., Yeh et al. (2021)).

Nonetheless, future research should investigate different constructs of IC to confirm

our exploratory finding. Additionally, our choice for assessing total diet quality was based on the fact that individuals do not consume foods in isolation, and nutrients of individual food groups interact with each other (Tapsell et al., 2016). Research on overall diet instead of separate food groups and health outcomes has therefore been growing (Panagiotakos, 2008; Wirfält et al., 2013). Thus, future research aimed at replicating and extending these findings would benefit from investigating the entire dietary pattern in addition to separate food groups.

It is important to note that our evidence for a relation between IC and diet quality was cross-sectional. As such, we cannot establish directionality. We have to acknowledge the possibility that better diet quality predicts better adolescent IC. Indeed, studies found that diet can affect cognitive ability and behaviour in children and adolescents (see reviews by Bellisle (2004) and Martin et al. (2018)). However, it is still unclear whether dietary manipulations could affect behaviour on long term. One potential underlying mechanism in such relation is the microbiota-gut-brain axis, the bi-directional biological communication route between the brain and microbiota in the gut (Cryan et al., 2019). Microbiota in the gut are able to produce metabolites that travel to the brain through different pathways, influencing brain metabolism and function (Cryan et al., 2019). Because dietary intake affects gut microbiota composition, subsequently, cognition and behaviour might also be impacted (Liang et al., 2022; Vernocchi et al., 2020). To unravel directionality in the adolescent IC-diet association, future longitudinal, experimental and intervention studies (e.g., improving adolescent diet quality or IC) are warranted.

Our result on higher adolescent diet quality and better IC was found when IC was assessed by behavioural tasks, and not the questionnaire. Furthermore, our executive functions questionnaire was filled in by the mother, not the adolescent. Many studies have previously assessed adolescent executive functions, of which IC is part, via parent reports (see review by Nyongesa et al. (2019)). Inter-informant reliability of

executive functions between parents and adolescents is moderately positive (Hughes et al., 2009; Wilson et al., 2011). Nonetheless, there are some discrepancies between the two raters, making it valuable to assess executive functions and IC with multiple informants and measures. We investigated adolescent IC with several behavioural tasks, but the composite of these tasks did not correlate with the maternal report of adolescent IC. Exploratory results revealed, when decomposing the composite, that only the better performance on the Go/No-go task correlated with better IC assessed with the BRIEF in our study. This result is exploratory. Nevertheless, similar patterns are seen in previous literature on adolescent IC that included behavioural tasks and the BRIEF IC subscale (filled in by parents). Those studies show divergent results with non-significant (Toplak et al., 2008) or significant (Hummer et al., 2010) intercorrelations between behavioural tasks and questionnaires. As the adolescent becomes more independent and desires more privacy when he grows older (Sanders, 2013), it is possible that the view the mother has on her adolescent behaviour becomes less clear. Note that behavioural tasks have some disadvantages (i.e., being a momentary assessment of IC, subject to time-variant factors, such as time of day, how the adolescent slept (Goldstein et al., 2007; Jankowski et al., 2023), or attention span (Markant and Amso, 2014)). However, self-report questionnaires can also be flawed as they are subject to potential biases, such as socially desirable answers (De Vriendt et al., 2009; Stanton et al., 1996). Hence, future research should ideally include both behavioural tasks and (self-report) questionnaires of IC.

Contrary to our hypothesis, we found no evidence for a relation for caregiving quality at any age on IC, and adolescent diet quality. These results align to some extent with previous literature that found no link between caregiving quality and adolescent dietary intake (snacks and soft drinks intake, specifically) (Kim, 2007; Kim et al., 2008; Vereecken et al., 2009). Conversely, studies are showing higher caregiving quality to be associated with adolescent (over-)inhibition (van der Voort et al., 2014) and higher

adolescent diet quality (Kim, 2007; Kim et al., 2008; Kremers et al., 2003; Lytle et al., 2003; Pearson et al., 2010; Zietz et al., 2022). Similar to the current study, Zietz et al. (2022) investigated general diet quality, while the other studies investigated specific food groups. The discrepancy between our and Zietz et al.'s (2022) results could be because caregiving quality was assessed via adolescent report (i.e., study of Zietz et al. (2022)) instead of objective observations of mother-child interactions (i.e., our study).

Next, we found no evidence for relations of our study variables with adolescent emotional eating. While this is seemingly different from van van Strien et al. (2019b), who found a longitudinal relation between lower caregiving quality in early life and more emotional eating during adolescence, they only found evidence for an indirect effect through emotion suppression and alexithymia (the difficulty to identify one's own emotions) (van Strien et al., 2019b). Notably, one study found a cross-sectional link between child emotional eating and parental caregiving quality, only when caregiving quality was reported by the adolescent, but not the parent (Snoek et al., 2007). In line with our results, three studies found no link between emotional eating and IC (Byrne et al., 2021b; Mayer et al., 2022; Nelson et al., 2020). Nonetheless, literature regarding caregiving and IC in relation to emotional eating is scarce. Possibly, adolescent awareness of their own behaviour is an important factor for self-reporting emotional eating behaviour, though no research has been published on this. Moreover, the emotional eating questionnaire has been validated in adults (Bailly et al., 2012; Cebolla et al., 2014), but not yet in adolescents. Though the DEBQ has been used before in adolescents (van Strien et al., 2019b,a) producing reliable results (Cronbach α=0.94, similar to ours α=0.88) and adequate variation in the data. Nonetheless, validation of this questionnaire in adolescents is recommended. Notably, all studies, thus far, on parental caregiving quality and adolescent DQ/EE have investigated caregiving quality using adolescent (Kim, 2007; Kim et al., 2008; Kremers et al., 2003; Lytle et al., 2003; Pearson et al., 2010; Snoek et al., 2007; Zietz et al., 2022) or parent (Vereecken

et al., 2009) reports. Adolescent reports and observations of parenting behaviour have been found to correlate moderately for positive parenting behaviours, but not for negative parenting behaviours (Arney, 2004; Parent et al., 2014). Moreover, self-reports have been observed to be more valid and reliable for assessing harsh and overreactive parenting, while observations appear to be more valid and reliable for assessing permissive and inconsistent parenting (Arney, 2004). We therefore recommend future research to employ both reports and observations on parenting behaviour to obtain a comprehensive assessment of parental caregiving quality.

Although we find no link between maternal caregiving quality and adolescent IC, the exploratory results revealed longitudinal associations (age five weeks and 12 months) between caregiving quality and individual behavioural tasks. Curiously, the associations were opposite of what we expected, namely, higher maternal caregiving quality was associated with worse performance on the IC tasks. As these findings are the result from exploratory analyses, they may suffer from type II errors (Akobeng, 2016). Hence, we refrain from further interpreting these results. Future studies should investigate maternal caregiving quality in direct relation to different measures of IC. Ideally, potential moderators and/or mediators (e.g., alexithymia) should be included to determine the possible mechanism behind expected relations.

Notably, we found that maternal educational level was a predictor of better adolescent diet quality, more emotional eating, and better IC (measured by the behavioural tasks). Higher educated mothers possibly have more knowledge about nutrition, and a higher income to provide healthy nutrition for the child (Pearson et al., 2009). Additionally, higher parental educational level was previously also shown to predict IC in adolescents (Assari, 2020), likely due to the fact that mothers with higher educational levels have increased access to healthcare, nutrition, and better child education, which could enhance the development of self-regulation (Noble et al., 2007). So far, the role of maternal educational level on emotional eating is still unclear, as one study

found that low parental education is associated with more emotional eating in primary school children (Umoke et al., 2020), while three studies found no relations between child/adolescent emotional eating behaviour and parental educational level (Gouveia et al., 2019; Stone et al., 2022; van Strien et al., 2019b). Note that educational level correlates highly with socioeconomic status (SES) (Aikens and Barbarin, 2008; Morgan et al., 2009). For this reason, we are unable, as we did not measure other indicators of SES such as income, to disentangle potential effects of maternal education from those due to SES. Nonetheless, maternal educational level seems to play a significant role in adolescent DQ/EE and IC, making it an important aspect to include in future research.

Our study has several strengths and limitations. This study leveraged maternal caregiving data across the first 14 years of children's age, which allowed us to investigate the importance of early life as well as later life caregiving for adolescent IC and DQ/EE. We performed multiple behavioural tasks and used maternal reports to obtain a comprehensive measure of adolescent IC. Adolescent diet quality was assessed with a food frequency questionnaire which was validated for reproducibility and relative validity, allowing us to compare the scores within our study population (Rijk et al., 2021; Lee et al., 2015). Validation studies in Dutch adolescents would benefit the use of this FFQ. However, our mostly highly educated sample limits the generalizability of our results. Future (replication) studies including lower educated parents may shed light on the relations between history of caregiving quality, adolescent IC, and adolescent DQ/EE.

Overall, this study contributes to the growing body of literature confirming the relations between adolescent IC and diet quality. To unravel directionality, longitudinal and experimental study designs are needed. Such studies can inform targeted interventions to improve IC and diet quality during adolescence. Additionally, inclusion of lower educated families may shed light on the role of maternal caregiving quality

on adolescent DQ/EE and IC, as this group may provide more variation in caregiving quality (Davis-Kean et al., 2021). Including more lower educated families is also important as maternal educational level was already found to be a predictor of better adolescent diet quality, more emotional eating, and better IC within the limited educational range of our study. Of relevance, variables other than IC may also play a role in their diet quality, including adolescent nutritional knowledge, self-efficacy, mental health, and peer influence (Chung et al., 2017; O'Neil et al., 2014; Story et al., 2002). To explore what predicts adolescent diet quality the best, future studies may therefore want to include more adolescent variables. In sum, high diet quality and IC skills are both of major importance during adolescence, as many developmental changes occur wherein they both play significant roles (Norris et al., 2022). Hence, future research aimed at investigating the (early) predictors of IC and dietary behaviours, as well as the directionality of their associations with diet quality, are warranted.

References

Aikens, N. L. and Barbarin, O. (2008). Socioeconomic Differences in Reading Trajectories: The Contribution of Family, Neighborhood, and School Contexts. *Journal of Educational Psychology*, 100(2):235–251.

Ainsworth, M. D. S., Blehar, M. C., Waters, E., and Wall, S. N. (2015). *Patterns of attachment: A psychological study of the strange situation*. Psychology Press.

Akobeng, A. K. (2016). Understanding type I and type II errors, statistical power and sample size. *Acta Paediatrica*, 105(6):605–609.

Ames, S. L., Kisbu-Sakarya, Y., Reynolds, K. D., Boyle, S., Cappelli, C., Cox, M. G., Dust, M., Grenard, J. L., Mackinnon, D. P., and Stacy, A. W. (2014). Inhibitory control effects in adolescent binge eating and consumption of sugar-sweetened beverages and snacks. *Appetite*, 81:180–192.

Ames, S. L., Wurpts, I. C., Pike, J. R., MacKinnon, D. P., Reynolds, K. R., and Stacy, A. W. (2016). Self-regulation interventions to reduce consumption of sugar-sweetened beverages in adolescents. *Appetite*, 105:652–662.

Amlung, M., Vedelago, L., Acker, J., Balodis, I., and MacKillop, J. (2017). Steep delay discounting and addictive behavior: a meta-analysis of continuous associations. *Addiction*, 112(1):51–62.

Anzman-Frasca, S., Francis, L. A., and Birch, L. L. (2015). Inhibitory Control is Associated with Psychosocial, Cognitive, and Weight Outcomes in a Longitudinal Sample of Girls. *Translational issues in psychological science*, 1(3):203–216.

Arney, F. M. (2004). *A comparison of direct observation and self-report measures of parenting behaviour*. PhD thesis, Adelaide University.

Assari, S. (2020). Parental Education on Youth Inhibitory Control in the Adolescent Brain Cognitive Development (ABCD) Study: Blacks' Diminished Returns. *Brain sciences*, 10(5).

Bailly, N., Maitre, I., Amanda, M., Hervé, C., and Alaphilippe, D. (2012). The Dutch Eating Behaviour Questionnaire (DEBQ). Assessment of eating behaviour in an aging French population. *Appetite*, 59(3):853–858.

Bari, A. and Robbins, T. W. (2013). Inhibition and impulsivity: Behavioral and neural basis of response control. *Progress in Neurobiology*, 108:44–79.

Barlow, P., Reeves, A., McKee, M., Galea, G., and Stuckler, D. (2016). Unhealthy diets, obesity and time discounting: a systematic literature review and network analysis. *Obesity Reviews*, 17(9):810–819.

Bechtel-Kuehne, S., Strodthoff, C. A., and Pauen, S. (2016). Co-and self-regulation in the caregiver-child dyad: Parental expectations, children's compliance, and parental practices during early years. *Journal of Self-regulation and Regulation*, 2:33–56.

Beijers, R., Jansen, J., Riksen-Walraven, M., and de Weerth, C. (2011). Attachment and infant night waking: A longitudinal study from birth through the first year of life. *Journal of Developmental and Behavioral Pediatrics*, 32(9):635–643.

Bellisle, F. (2004). Effects of diet on behaviour and cognition in children. *The British journal of nutrition*, 92 Suppl 2(S2):S227–S232.

Belsky, J. (2002). Developmental origins of attachment styles. *Attachment & Human Development*, 4(2):166–170.

Bernier, A., Carlson, S. M., Deschênes, M., and Matte-Gagné, C. (2012). Social factors in the development of early executive functioning: A closer look at the caregiving environment. *Developmental science*, 15(1):12–24.

Bernier, A., Carlson, S. M., and Whipple, N. (2010). From external regulation to self-regulation: early parenting precursors of young children's executive functioning. *Child Development*, 81(1):326–339.

Bohrer, B. K., Forbush, K. T., and Hunt, T. K. (2015). Are common measures of dietary restraint and disinhibited eating reliable and valid in obese persons? *Appetite*, 87:344–351.

Bosquet Enlow, M., Petty, C. R., Svelnys, C., Gusman, M., Huezo, M., Malin, A., and Wright, R. J. (2019). Differential Effects of Stress Exposures, Caregiving Quality, and Temperament in Early Life on Working Memory versus Inhibitory Control in Preschool-Aged Children. *Developmental Neuropsychology*, 44(4):339–356.

Bravo, P., Cárcamo, R., Santos, S., Ringoot, A., IJzendoorn, M. H. v., Hillegers, M. H. J., and Jansen, P. W. (2023). Maternal sensitivity in early childhood and body mass index in adolescence: A population-based study on the role of self-regulation as a mediator. *Appetite*, 182:106418.

Brophy-Herb, H. E., Stansbury, K., Bocknek, E., and Horodynski, M. A. (2012). Modeling maternal emotion-related socialization behaviors in a low-income sample: Relations with toddlers' self-regulation. *Early Childhood Research Quarterly*, 27(3):352–364.

Buffie, M. L. and Nangle, D. W. (2022). The Interaction Between Self-Regulation Deficits and Negative Problem Orientation Exacerbates Depressive, but not ADHD Symptoms. *Journal of Rational - Emotive and Cognitive - Behavior Therapy*, 40(3):647–662.

Byrne, M. E., Shank, L. M., Altman, D. R., Swanson, T. N., Ramirez, E., Moore, N. A., Rubin, S. G., LeMay-Russell, S., Parker, M. N., Kaufman, R. E., Yang, S. B., Torres, S. L., Brady, S. M., Kelly, N. R., Tanofsky-Kraff, M., and Yanovski, J. A. (2021a). Inhibitory control and negative affect in relation to food intake among youth. *Appetite*, 156:104858.

Byrne, M. E., Tanofsky-Kraff, M., Lavender, J. M., Parker, M. N., Shank, L. M., Swanson, T. N., Ramirez, E., LeMay-Russell, S., Yang, S. B., Brady, S. M., Zenno, A., Chivukula, K. K., Kelly, N. R., and Yanovski, J. A. (2021b). Bridging Executive Dysfunction and Disinhibited Eating among Youth: A Network Analysis. *The International journal of eating disorders*, 54(5):721.

Casale, M., Cluver, L., Crankshaw, T., Kuo, C., Lachman, J. M., and Wild, L. G. (2015). Direct and Indirect Effects of Caregiver Social Support on Adolescent Psychological Outcomes in Two South African AIDS-Affected Communities. *American journal of community psychology*, 55(3-4):336–346.

Casey, B. J., Getz, S., and Galvan, A. (2008). The adolescent brain. *Developmental review*, 28(1):62–77.

Cassidy, J. (1994). Emotion Regulation: Influences of Attachment Relationships. *Monographs of the Society for Research in Child Development*, 59(2-3):228–249.

Cebolla, A., Barrada, J. R., van Strien, T., Oliver, E., and Baños, R. (2014). Validation of the Dutch Eating Behavior Questionnaire (DEBQ) in a sample of Spanish women. *Appetite*, 73:58–64.

Chung, S. J., Ersig, A. L., and McCarthy, A. M. (2017). The Influence of Peers on Diet and Exercise Among Adolescents: A Systematic Review. *Journal of pediatric nursing*, 36:44–56.

Cinelli, C., Forney, A., and Pearl, J. (2020). A Crash Course in Good and Bad Controls. *SSRN Electronic Journal*, pages 1–10.

Cryan, J. F., O'riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., Codagnone, M. G., Cussotto, S., Fulling, C., Golubeva, A. V., Guzzetta, K. E., Jaggar, M., Long-Smith, C. M., Lyte, J. M., Martin, J. A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., O'connor, R., Cruz-Pereira, J. S., Peterson, V. L., Rea, K., Ritz, N. L., Sherwin, E., Spichak, S., Teichman, E. M., Wouw, M. v. d., Ventura-Silva, A. P., Wallace-Fitzsimons, S. E., Hyland, N., Clarke, G., and Dinan, T. G. (2019). The microbiota-gut-brain axis. *Physiological Reviews*, 99(4):1877–2013.

Davis-Kean, P. E., Tighe, L. A., and Waters, N. E. (2021). The Role of Parent Educational Attainment in Parenting and Children's Development. *Current Directions in Psychological Science*, 30(2):186–192.

De Vriendt, T., Huybrechts, I., Ottevaere, C., Van Trimpont, I., and De Henauw, S. (2009). Validity of self-reported weight and height of adolescents, its impact on classification into bmi-categories and the association with weighing behaviour. *International journal of environmental research and public health*, 6(10):2696–2711.

Dempster, A. P., Laird, N. M., and Rubin, D. B. (1977). Maximum Likelihood from Incomplete Data Via the*EM*Algorithm. *Journal of the Royal Statistical Society: Series B (Methodological)*, 39(1):1–22.

Deslippe, A. L., O'Connor, T. M., Brussoni, M., and Mâsse, L. C. (2022). The association of food parenting practices with adolescents' dietary behaviors differ by youth gender, but not by parent gender. *Appetite*, 169:105846.

Diamond, A. (2013). Executive functions. *Annual review of psychology*, 64:135–68.

Dodzik, P. (2017). Behavior Rating Inventory of Executive Function, Second Edition Gerard A. Gioia, Peter K. Isquith, Steven C. Guy, and Lauren Kenworthy. *Journal of Pediatric Neuropsychology*, 3(3-4):227–231.

Domoff, S. E., Meers, M. R., Koball, A. M., and Musher-Eizenman, D. R. (2013). The validity of the Dutch Eating Behavior Questionnaire: some critical remarks. *Eating and Weight Disorders - Studies on Anorexia, Bulimia and Obesity*, 19(2):137–144.

Drywień, M., Górnicka, M., Kulik, S., and Górnicki, K. (2021). Patterns of Avoiding Nutrition Mistakes in Metropolitan Adolescents Are Associated with Sex, Nutrition Knowledge, Physical Activity, and Family Environment. *Nutrients*, 13(2):433.

Epstein, S. (1983). Aggregation and beyond: Some basic issues on the prediction of behavior. *Journal of Personality*, 51(3):360–392.

Erickson, M. F., LA, S., and Egeland, B. (1985). The relationship between quality of attachment and behavior problems in preschool in a high-risk sample. *Monographs of the Society for Research in Child Development*, 50(1-2):147–166.

Frick, M. A., Forslund, T., and Brocki, K. C. (2019a). Can reactivity and regulation in infancy predict inattentive and hyperactive/impulsive behavior in 3-year-olds? *Development and Psychopathology*, 31(2):619–629.

Frick, M. A., Forslund, T., and Brocki, K. C. (2019b). Does child verbal ability mediate the relationship between maternal sensitivity and later self-regulation? A longitudinal study from infancy to 4 years. *Scandinavian journal of psychology*, 60(2):97–105.

Geeraerts, S. B., Endendijk, J. J., Deković, M., Huijding, J., Deater-Deckard, K., and Mesman, J. (2021). Inhibitory Control Across the Preschool Years: Developmental Changes and Associations with Parenting. *Child Development*, 92(1):335.

Giuliani, N. R. and Kelly, N. R. (2021). Delay of Gratification Predicts Eating in the Absence of Hunger in Preschool-Aged Children. *Frontiers in psychology*, 12:650046.

Golden, C., Freshwater, S. M., and Golden, Z. (1978). Stroop color and word test. *APA PsycTests*.

Goldstein, D., Hahn, C. S., Hasher, L., Wiprzycka, U. J., and Zelazo, P. D. (2007). Time of day, Intellectual Performance, and Behavioral Problems in Morning Versus Evening type Adolescents: Is there a Synchrony Effect? *Personality and individual differences*, 42(3):431–440.

Gouveia, M. J., Canavarro, M. C., and Moreira, H. (2018). Is Mindful Parenting Associated With Adolescents' Emotional Eating? The Mediating Role of Adolescents' Self-Compassion and Body Shame. *Frontiers in Psychology*, 9(OCT).

Gouveia, M. J., Canavarro, M. C., and Moreira, H. (2019). How can mindful parenting be related to emotional eating and overeating in childhood and adolescence? The mediating role of parenting stress and parental child-feeding practices. *Appetite*, 138:102–114.

Gubbels, J. S., Kremers, S. P. J., Stafleu, A., de Vries, S. I., Goldbohm, R. A., Dagnelie, P. C., de Vries, N. K., van Buuren, S., and Thijs, C. (2011). Association between parenting practices and children's dietary intake, activity behavior and development of body mass index: the KOALA Birth Cohort Study. *The international journal of behavioral nutrition and physical activity*, 8:18.

Guyon-Harris, K. L., Humphreys, K. L., Miron, D., Tibu, F., Fox, N. A., Nelson, C. A., and Zeanah, C. H. (2021). Early caregiving quality predicts consistency of competent functioning from middle childhood to adolescence following early psychosocial deprivation. *Development and psychopathology*, 33(1):18–28.

Gärtner, K. A., Vetter, V. C., Schäferling, M., Reuner, G., and Hertel, S. (2018). Inhibitory control in toddlerhood –the role of parental co-regulation and self-efficacy beliefs. *Metacognition and Learning 2018 13:3*, 13(3):241–264.

Hughes, D., Turkstra, L., and Wulfeck, B. (2009). Parent and self-ratings of executive function in adolescents with specific language impairment. *International Journal of Language & Communication Disorders*, 44(6):901–916.

Hughes, S. O. and Papaioannou, M. A. (2018). Maternal Predictors of Child Dietary Behaviors and Weight Status. *Current nutrition reports*, 7(4):268–273.

Hummer, T. A., Kronenberger, W. G., Wang, Y., Dunn, D. W., Mosier, K. M., Kalnin, A. J., and Mathews, V. P. (2010). Executive Functioning Characteristics Associated with ADHD Comorbidity in Adolescents with Disruptive Behavior Disorders. *Journal of Abnormal Child Psychology*, 39(1):11–19.

Jankowski, K. S., Díaz-Morales, J. F., and Vollmer, C. (2023). Chronotype, Time of Day, and Performance on Intelligence Tests in the School Setting. *Journal of Intelligence 2023, Vol. 11, Page 13*, 11(1):13.

Jennings, K. D., Sandberg, I., Kelley, S. A., Valdes, L., Yaggi, K., Abrew, A., and Macey-Kalcevic, M. (2008). Understanding of self and maternal warmth predict later self-regulation in toddlers. *International Journal of Behavioral Development*, 32(2):108–118.

Kaplan, B. A., Amlung, M., Reed, D. D., Jarmolowicz, D. P., McKerchar, T. L., and Lemley, S. M. (2016). Automating Scoring of Delay Discounting for the 21- and 27-Item Monetary Choice Questionnaires. *The Behavior analyst*, 39(2):293–304.

Kim, M. J. (2007). *Parenting style and older children's and young adolescents' dietary intake and nutritional status*. PhD thesis, Texas A&M University.

Kim, M. J., McIntosh, W. A., Anding, J., Kubena, K. S., Reed, D. B., and Moon, G. S. (2008). Perceived parenting behaviours predict young adolescents' nutritional intake and body fatness. *Maternal & child nutrition*, 4(4):287–303.

Kirby, K. N. and Maraković, N. N. (1996). Delay-discounting probabilistic rewards: Rates decrease as amounts increase. *Psychonomic Bulletin & Review 1996 3:1*, 3(1):100–104.

Kochanska, G., Murray, K. T., and Harlan, E. T. (2000). Effortful control in early childhood: continuity and change, antecedents, and implications for social development. *Developmental psychology*, 36(2):220–232.

Kok, R., Lucassen, N., Bakermans-Kranenburg, M. J., Ijzendoorn, M. H. V., Ghassabian, A., Roza, S. J., Govaert, P., Jaddoe, V. W., Hofman, A., Verhulst, F. C., and Tiemeier, H. (2013). Parenting, corpus callosum, and executive function in preschool children. *Child neuropsychology : a journal on normal and abnormal development in childhood and adolescence*, 20(5):583–606.

Koo, T. K. and Li, M. Y. (2016). A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *Journal of Chiropractic Medicine*, 15(2):155.

Kremers, S. P. J., Brug, J., Vries, H. D., and Engels, R. C. M. E. (2003). Parenting style and adolescent fruit consumption. *Appetite*, 41(1):43–50.

Layzer, J. I. and Goodson, B. D. (2006). The "Quality " of Early Care and Education Settings. *Evaluation review*, 30(5):556–576.

Lee, L. v., Feskens, E. J. M., Meijboom, S., Huysduynen, E. J. C. H. v., van't Veer, P., Vries, J. H. M. d., and Geelen, A. (2015). Evaluation of a screener to assess diet quality in the Netherlands. *British Journal of Nutrition*, 115(3):517–526.

Liang, X., Fu, Y., Cao, W.-T., Wang, Z., Zhang, K., Jiang, Z., Jia, X., Liu, C.-Y., Lin, H.-R., Zhong, H., Miao, Z., Gou, W., Shuai, M., Huang, Y., Chen, S., Zhang, B., Chen, Y.-M., and Zheng, J.-S. (2022). Gut microbiome, cognitive function and brain structure: a multi-omics integration analysis. *Translational neurodegeneration*, 11(1):49.

Limbers, C. A., Summers, E., Smith, K., and Lavender, J. (2021). Emotional Eating and Weight Status in Adolescents: A Systematic Review. *International Journal of Environmental Research and Public Health*, 18(3):991.

Looman, M., Feskens, E. J., Rijk, M. d., Meijboom, S., Biesbroek, S., Temme, E. H., Vries, J. d., and Geelen, A. (2017). Development and evaluation of the Dutch Healthy Diet index 2015. *Public health nutrition*, 20(13):2289–2299.

Lytle, L. A., Varnell, S., Murray, D. M., Story, M., Perry, C., Birnbaum, A. S., and Kubik, M. Y. (2003). Predicting adolescents' intake of fruits and vegetables. *Journal of nutrition education and behavior*, 35(4):170–178.

Markant, J. and Amso, D. (2014). Leveling the playing field: attention mitigates the effects of intelligence on memory. *Cognition*, 131(2):195–204.

Martin, A., Booth, J. N., Laird, Y., Sproule, J., Reilly, J. J., and Saunders, D. H. (2018). Physical activity, diet and other behavioural interventions for improving cognition and school achievement in children and adolescents with obesity or overweight. *The Cochrane database of systematic reviews*, 1(1).

Mayer, M. A., Catalani, F., Fraire, J., Deltetto, N., Martín, L., Beneitez, A., Fischman, D., and Orden, Á. B. (2022). Inhibitory control and obesity in adolescents: A prospective cohort study. *Appetite*, 171:105910.

Merz, E. C., Landry, S. H., Zucker, T. A., Barnes, M. A., Assel, M., Taylor, H. B., Lonigan, C. J., Phillips, B. M., Clancy-Menchetti, J., Eisenberg, N., Spinrad, T. L., Valiente, C., and Villiers, J. d. (2016). Parenting Predictors of Delay Inhibition in Socioeconomically Disadvantaged Preschoolers. *Infant and Child Development*, 25(5):371–390.

Morgan, P. L., Farkas, G., Hillemeier, M. M., and MacZuga, S. (2009). Risk factors for learning-related behavior problems at 24 months of age: Population-based estimates. *Journal of Abnormal Child Psychology*, 37(3):401–413.

Movassagh, E. Z., Baxter-Jones, A. D. G., Kontulainen, S., Whiting, S. J., and Vatanparast, H. (2017). Tracking Dietary Patterns over 20 Years from Childhood through Adolescence into Young Adulthood: The Saskatchewan Pediatric Bone Mineral Accrual Study. *Nutrients*, 9(9):1–14.

Murphy, F. C., Sahakian, B. J., Rubinsztein, J. S., Michael, A., Rogers, R. D., Robbins, T. W., and Paykel, E. S. (1999). Emotional bias and inhibitory control processes in mania and depression. *Psychological medicine*, 29(6):1307–1321.

Nelson, T. D., James, T. D., Nelson, J. M., Johnson, A. B., Mason, W. A., Yaroch, A. L., and Espy, K. A. (2020). Associations between specific components of executive control and eating behaviors in adolescence: A study using objective and subjective measures. *Appetite*, 154:104784.

Nigg, J. T. (2017). Annual Research Review: On the relations among self-regulation, self-control, executive functioning, effortful control, cognitive control, impulsivity, risk-taking, and inhibition for developmental psychopathology. *Journal of child psychology and psychiatry, and allied disciplines*, 58(4):361.

Noble, K. G., McCandliss, B. D., and Farah, M. J. (2007). Socioeconomic gradients predict individual differences in neurocognitive abilities. *Developmental Science*, 10(4):464–480.

Norris, S. A., Frongillo, E. A., Black, M. M., Dong, Y., Fall, C., Lampl, M., Liese, A. D., Naguib, M., Prentice, A., Rochat, T., Stephensen, C. B., Tinago, C. B., Ward, K. A., Wrottesley, S. V., and Patton, G. C. (2022). Nutrition in adolescent growth and development. *The Lancet*, 399(10320):172–184.

Nyongesa, M. K., Ssewanyana, D., Mutua, A. M., Chongwo, E., Scerif, G., Newton, C. R. J. C., and Abubakar, A. (2019). Assessing Executive Function in Adolescence: A Scoping Review of Existing Measures and Their Psychometric Robustness. *Frontiers in Psychology*, 10:311.

O'Neil, A., Quirk, S. E., Housden, S., Brennan, S. L., Williams, L. J., Pasco, J. A., Berk, M., and Jacka, F. N. (2014). Relationship between diet and mental health in children and adolescents: a systematic review. *American journal of public health*, 104(10):e31–e42.

Ortega, A., Bejarano, C. M., Hesse, D. R., Reed, D., and Cushing, C. C. (2023). Temporal discounting modifies the effect of microtemporal hedonic hunger on food consumption: An ecological momentary assessment study. *Eating Behaviors*, 48:101697.

Özcan, B. A., Yesilkay, B., Yaldız, N., and Pehliva, M. (2020). Factors affecting diet quality in adolescents: the effect of sociodemographic characteristics and meal consumption. *Progress in Nutrition*.

Panagiotakos, D. (2008). α-priori versus α-posterior methods in dietary pattern analysis: a review in nutrition epidemiology. *Nutrition Bulletin*, 33(4):311–315.

Parent, J., Forehand, R., Dunbar, J. P., Watson, K. H., Reising, M. M., Seehuus, M., and Compas, B. E. (2014). Parent and Adolescent Reports of Parenting When a Parent Has a History of Depression: Associations with Observations of Parenting. *Journal of abnormal child psychology*, 42:173–183.

Pearson, N., Atkin, A. J., Biddle, S. J. H., Gorely, T., and Edwardson, C. (2010). Parenting styles, family structure and adolescent dietary behaviour. *Public health nutrition*, 13(8):1245–1253.

Pearson, N., Biddle, S. J. H., and Gorely, T. (2009). Family correlates of fruit and vegetable consumption in children and adolescents: a systematic review. *Public Health Nutrition*, 12(2):267–283.

Raby, K. L., Roisman, G. I., Fraley, R. C., and Simpson, J. A. (2015). The Enduring Predictive Significance of Early Maternal Sensitivity: Social and Academic Competence Through Age 32 Years. *Child Development*, 86(3):695–708.

Rijk, M. G. d., Slotegraaf, A. I., Brouwer-Brolsma, E. M., Perenboom, C. W. M., Feskens, E. J. M., and Vries, J. H. M. d. (2021). Development and evaluation of a diet quality screener to assess adherence to the Dutch food-based dietary guidelines. *The British journal of nutrition*, 128(8):1–11.

Robin, A. L. and Foster, S. L. (1989). *Negotiating parent–adolescent conflict: A behavioral–family systems approach. - PsycNET*. Guilford Press.

Romanos-Nanclares, A., Zazpe, I., Santiago, S., Marín, L., Rico-Campà, A., and Martín-Calvo, N. (2018). Influence of Parental Healthy-Eating Attitudes and Nutritional Knowledge on Nutritional Adequacy and Diet Quality among Preschoolers: The SENDO Project. *Nutrients*, 10(12):1875.

Rosseel, Y. (2012). lavaan: An r package for structural equation modeling. *Journal of Statistical Software*, 48(2).

Roth, R. M., Isquith, P. K., and Gioia, G. A. (2013). Assessment of executive functioning using the behavior rating inventory of executive function (brief). In *Handbook of executive functioning*, pages 301–331. Springer.

Sanders, R. A. (2013). Adolescent psychosocial, social, and cognitive development. *Pediatrics in review*, 34(8):354–359.

Scarpina, F. and Tagini, S. (2017). The Stroop Color and Word Test. *Frontiers in Psychology*, 8(APR):557.

Scherer, E., Hagaman, A., Chung, E., Rahman, A., O'Donnell, K., and Maselko, J. (2019). The relationship between responsive caregiving and child outcomes: evidence from direct observations of mother-child dyads in Pakistan. *BMC public health*, 19(1).

Schore, A. N. (2001). Effects of a secure attachment relationship on right brain development, affect regulation, and infant mental health. *Infant Ment. Health J*, 22:7–66.

Schreiber, J. B., Stage, F. K., King, J., Nora, A., and Barlow, E. A. (2006). Reporting Structural Equation Modeling and Confirmatory Factor Analysis Results: A Review. *The Journal of Educational Research*, 99(6):323–338.

Schuetzmann, M., Richter-Appelt, H., Schulte-Markwort, M., and Schimmelmann, B. G. (2008). Associations Among the Perceived Parent–Child Relationship, Eating Behavior, and Body Weight in Preadolescents: Results from a Community-based Sample. *Journal of Pediatric Psychology*, 33(7):772–782.

Shriver, L. H., Dollar, J. M., Calkins, S. D., Keane, S. P., Shanahan, L., and Wideman, L. (2020). Emotional Eating in Adolescence: Effects of Emotion Regulation, Weight Status and Negative Body Image. *Nutrients*, 13(1):79.

Sleddens, E. F. C., Gerards, S. M. P. L., Thijs, C., Vries, N. K. D., and Kremers, S. P. J. (2011). General parenting, childhood overweight and obesity-inducing behaviors: a review. *International journal of pediatric obesity*, 6(sup3):e12–27.

Snoek, H. M., Engels, R. C. M. E., Janssens, J. M. A. M., and van Strien, T. (2007). Parental behaviour and adolescents' emotional eating. *Appetite*, 49(1):223–230.

Spinrad, T. L., Eisenberg, N., Silva, K. M., Eggum, N. D., Reiser, M., Edwards, A., Iyer, R., Kupfer, A. S., Hofer, C., Smith, C. L., Hayashi, A., and Gaertner, B. M. (2012). Longitudinal Relations Among Maternal Behaviors, Effortful Control and Young Children's Committed Compliance. *Developmental Psychology*, 48(2):552.

Sroufe, L. A., Coffino, B., and Carlson, E. A. (2010). Conceptualizing the Role of Early Experience: Lessons from the Minnesota Longitudinal Study. *Developmental review*, 30(1):36.

Stanton, W. R., McClelland, M., Elwood, C., Ferry, D., and Silva, P. A. (1996). Prevalence, reliability and bias of adolescents' reports of smoking and quitting. *Addiction*, 91(11):1705–1714.

Stiles, A. S. (2004). Measuring maternal sensitivity in teen mothers: reliability and feasibility of two instruments. *Journal of nursing measurement*, 12(3):195–214.

Stone, R. A., Blissett, J., Haycraft, E., and Farrow, C. (2022). Predicting preschool children's emotional eating: The role of parents' emotional eating, feeding practices and child temperament. *Maternal & Child Nutrition*, 18(3).

Story, M., Neumark-Sztainer, D., and French, S. (2002). Individual and environmental influences on adolescent eating behaviors. *Journal of the American Dietetic association*, 102(3):S40–S51.

Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of Experimental Psychology*, 18(6):643–662.

Tapsell, L. C., Neale, E. P., Satija, A., and Hu, F. B. (2016). Foods, Nutrients, and Dietary Patterns: Interconnections and Implications for Dietary Guidelines. *Advances in nutrition (Bethesda, Md.)*, 7(3):445–454.

Topham, G. L., Hubbs-Tait, L., Rutledge, J. M., Page, M. C., Kennedy, T. S., Shriver, L. H., and Harrist, A. W. (2011). Parenting styles, parental response to child emotion, and family emotional responsiveness are related to child emotional eating. *Appetite*, 56(2):261–264.

Toplak, M. E., Bucciarelli, S. M., Jain, U., and Tannock, R. (2008). Executive Functions: Performance-Based Measures and the Behavior Rating Inventory of Executive Function (BRIEF) in Adolescents with Attention Deficit/Hyperactivity Disorder (ADHD). *Child Neuropsychology*, 15(1):53–72.

Umoke, M. J., Umoke, P. C. I., Onyeke, N. G., Victor-Aigbodion, V., Eseadi, C., Ebizie, E. N., Obiweluozo, P. E., Uzodinma, U. E., Chukwuone, C. A., Dimelu, I. N., Uwakwe, R. C., Uba, M. B. I., Elom, C. O., and Folorunsho, R. O. B. (2020). Influence of parental education levels on eating habits of pupils in Nigerian primary schools. *Medicine*, 99(43):e22953.

Valikhani, A., Abbasi, Z., Radman, E., Goodarzi, M. A., and Moustafa, A. A. (2021). Insecure Attachment and Subclinical Depression, Anxiety, and Stress: A Three-Dimensional Model of Personality Self-Regulation As a Mediator. *Mental Health and Psychopathology*, pages 88–112.

van der Bij, J., Kelder, R. O. d., Montagne, B., and Hagenaars, M. A. (2020). Inhibitory control in trauma-exposed youth: A systematic review. *Neuroscience & Biobehavioral Reviews*, 118:451–462.

van der Voort, A., Linting, M., Juffer, F., Bakermans-Kranenburg, M. J., Schoenmaker, C., and IJzendoorn, M. H. v. (2014). The development of adolescents' internalizing behavior: longitudinal effects of maternal sensitivity and child inhibition. *Journal of youth and adolescence*, 43(4):528–540.

van Strien, T., Beijers, R., Smeekens, S., and Winkens, L. H. (2019a). Duration of breastfeeding is associated with emotional eating through its effect on alexithymia in boys, but not girls. *Appetite*, 132:97–105.

van Strien, T., Beijers, R., Smeekens, S., Winkens, L. H. H., and Konttinen, H. (2019b). Parenting quality in infancy and emotional eating in adolescence: Mediation through emotion suppression and alexithymia. *Appetite*, 141:104339.

van Strien, T., Donker, M. H., and Ouwens, M. A. (2016). Is desire to eat in response to positive emotions an 'obese' eating style: Is Kummerspeck for some people a misnomer? *Appetite*, 100:225–235.

van Strien, T., Frijters, J. E. R., Bergers, G. P. A., and Defares, P. B. (1986). The Dutch Eating Behavior Questionnaire (DEBQ) for assessment of restrained, emotional, and external eating behavior. *International Journal of Eating Disorders*, 5(2):295–315.

Vereecken, C., Legtest, E., Bourdeaudhuij, I. D., and Maes, L. (2009). Associations between General Parenting Styles and Specific Food-Related Parenting Practices and Children's Food Consumption. *American Journal of Health Promotion*, 23(4):233–240.

Vernocchi, P., Chierico, F. D., and Putignani, L. (2020). Gut microbiota metabolism and interaction with food components. *International Journal of Molecular Sciences*, 21(10):3688.

von Suchodoletz, A., Trommsdorff, G., and Heikamp, T. (2011). Linking Maternal Warmth and Responsiveness to Children's Self-regulation. *Social Development*, 20(3):486–503.

Vrijhof, C. I., Euser, S., Bakermans-Kranenburg, M. J., Bulk, B. G. v. d., Bosdriesz, J. R., Linting, M., Wijk, I. C. v., Visser, I. d., and IJzendoorn, M. H. v. (2020). Effects of parental sensitivity in different contexts on children's hot and cool effortful control. *Journal of family psychology*, 34(4).

Wilson, K. R., Donders, J., and Nguyen, L. (2011). Self and Parent Ratings of Executive Functioning After Adolescent Traumatic Brain Injury. *Rehabilitation Psychology*, 56(2):100–106.

Wirfält, E., Drake, I., and Wallström, P. (2013). What do review papers conclude about food and dietary patterns? *SNF Swedish Nutrition Foundation*, 57.

World Health Organization (2005). Nutrition in adolescence: issues and challenges for the health sector: issues in adolescent health and development.

Wu, Q., Jalapa, K., Han, S. J., Tawfiq, D., and Cui, M. (2022). A dynamic systems perspective towards executive function development: Susceptibility at both ends for inhibitory control. *Development and Psychopathology*, pages 1–9.

Yeh, Y.-H., Myerson, J., and Green, L. (2021). Delay discounting, cognitive ability, and personality: What matters? *Psychonomic bulletin & review*, 28(2):686–694.

Zietz, S., Cheng, E., Lansford, J. E., Deater-Deckard, K., Giunta, L. D., Dodge, K. A., Gurdal, S., Liu, Q., Long, Q., Oburu, P., Pastorelli, C., Skinner, A. T., Sorbring, E., Steinberg, L., Tapanya, S., Tirado, L. M. U., Yotanyamaneewong, S., Alampay, L. P., Al-Hassan, S. M., Bacchini, D., Chang, L., and Bornstein, M. H. (2022). Positive parenting, adolescent adjustment, and quality of adolescent diet in nine countries. *Journal of adolescence*, 94(8):1130–1141.

Zuniga, K. E., Mackenzie, M. J., Roberts, S. A., Raine, L. B., Hillman, C. H., Kramer, A. F., and McAuley, E. (2015). Relationship between fruit and vegetable intake and interference control in breast cancer survivors. *European Journal of Nutrition*, 55(4):1555–1562.

Chapter 6

General Discussion

Executive functions (EF) and inhibitory control (IC) are essential skills that are important for executing goal-directed behaviours (Diamond, 2013). Early life nutrition is suggested to have a pivotal role in explaining inter-individual differences in these behaviours (Costello et al., 2020). The general goal of this thesis was to uncover unknown links between nutrition and behaviour in early life. We investigated this by means of three aims. The **first aim** was to investigate the relations between early life nutrition-related predictors and future cognitive and behavioural outcomes. The **second aim** was to investigate potential mechanisms underlying the relations between early life nutrition and cognitive and behavioural outcomes. Because of the important role of the microbiota-gut-brain axis on behaviour (Chakrabarti et al., 2022; Cryan et al., 2019; Morais et al., 2020), we focused on the gut bacteria in early as well as later life (two weeks to three years of age). Lastly, as nutrition is important for the development of many physiological, and likely psychological systems (Norris et al., 2022), it is important to study predictors of healthy nutritional behaviours, especially in phases of life where risk for unhealthy nutritional behaviours is heightened, such as adolescence. The **third aim** was therefore to investigate the role of maternal caregiving behaviour on adolescent nutritional behaviours.

In light of **aim 1**, we investigated whether the duration of breastfeeding predicted better toddler EF and IC, and if this was mediated by toddler diet quality (**Chapter 2**), and whether the human milk oligosaccharide (HMO) composition in breast milk at two, six, and 12 weeks predicted better toddler EF and IC (**Chapter 3**). We found that duration of breastfeeding, and toddler diet quality did not predict toddler IC and EF (**Chapter 2**). However, we did find a relation between longer breastfeeding duration and better toddler diet quality (**Chapter 2**). Furthermore, in **Chapter 3**, we found that higher concentrations of 2'FL and grouped fucosylated HMOs in mother milk predicted better toddler EF in exclusively breastfed toddlers. These relations were not found for 3'SL, 6'SL, and grouped sialylated HMOs.

For **aim 2**, gut microbiota composition at ages two, six, and 12 weeks, and one and three years, were assessed in relation to EF, IC, and problem behaviour in toddlerhood (**Chapter 4**). One of the most important findings was the association between higher relative abundances of *Streptococcus* throughout the first three years of life and worse EF at age three years.

Lastly, for **aim 3**, we investigated the relation between maternal caregiving quality, measured from the early postnatal period until adolescence, and adolescent diet quality and emotional eating behaviour, and whether adolescent IC mediated these potential relations (**Chapter 5**). We found that higher diet quality was associated with better IC in adolescence. However, adolescent emotional eating behaviour was not related to adolescent IC. Finally, we did not find support for a relation between maternal caregiving quality (at all ages) and adolescent inhibitory control or diet quality and emotional eating behaviour.

Figure 6.1 shows a summary of the results found. The following paragraphs discuss how these findings contribute to research on early life and development, as well as their limitations and directions for future research.

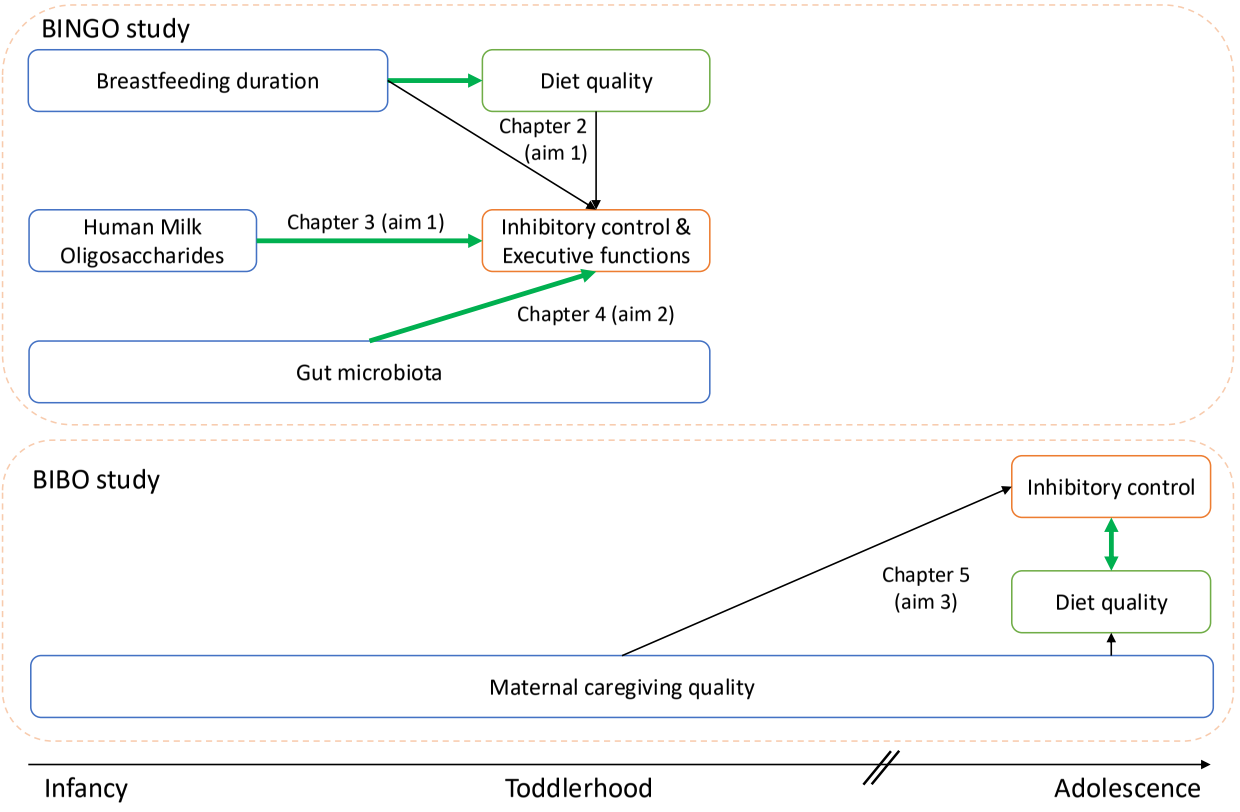


Figure 6.1. Overview of the research topics per chapter. Blue boxes indicate the expected predictors. The orange and green boxes indicate the outcome measures. Light orange dotted lines indicate the cohorts used. The black arrows indicate the expected direction of the association, as causality could not be determined in the present data. The bold green arrows indicate the associations found.

The impact of early life nutrition on future behaviour

Early life has been shown to be an extremely important phase predicting future development (Robertson et al., 2019). In line with this, many studies investigating early life factors, such as early life stress and early life nutrition, have found impactful health consequences on children's future physiological and psychological wellbeing (Connor, 2019; Smith and Pollak, 2020). In the current studies, we also found that early life nutrition predicted future child health outcomes. First, we found that longer exclusive breastfeeding duration predicted higher diet quality in toddlers (**Chapter 2**). This is in line with the results of many studies that found longer (exclusive and total) breastfeeding duration to be related to higher intake of vegetables and fruits in toddlers, contributing to a higher toddler diet quality (see reviews by Ventura (2017) and Ventura et al. (2021)). A potential mechanism underlying these findings could be that the mother shapes healthier food preferences in the child through breastfeeding (Ventura, 2017; Ventura et al., 2021). Mother milk is the first exposure to flavours for most infants. The flavour of mother milk changes depending on the maternal diet. Specifically, compounds of garlic, carrot, anise, mint, eucalyptus, alcohol, and molecular structures of fruit and vegetables are transferred to human milk (Spahn et al., 2019). An infant responds to this flavour change by increasing or decreasing sucking time and number of sucks (Mennella and Beauchamp, 1991). Long-term studies show that during solid food consumption, young children show greater preferences (defined as greater duration of mouthing behaviour) for the flavours they have been exposed to through breast milk, with lasting effects until at least ten years of age (e.g., maternal diet with higher vegetable intake is associated with greater child preferences for, and intake of vegetables) (Mennella et al., 2001; Sausenthaler et al., 2010; Ventura et al., 2021). This explanation falls under the Lactocrine Programming hypothesis, which states that milk constituents can have long term effects on an infant's development

(Bartol et al. (2008); Hinde et al. (2013), for a review, see de Weerth et al. (2022)). In this case, the effect would be a programming of the child's food preferences. Note that this proposed explanation is plausible under the assumption that mothers who breastfeed longer have healthier diets than mothers who breastfeed for shorter times (Amir and Donath, 2012; Beckerman et al., 2020). An alternative and most probable complementary explanation for our findings is that mothers who breastfeed longer also provide and feed their children healthier foods in toddlerhood.

A clear result in this thesis that is supported by the Lactocrine Programming hypothesis is the evidence we found for higher levels of fucosylated HMOs in mother milk predicting better toddler EF (**Chapter 3**). This is in line with rodent studies that confirmed a causal relation between early life HMOs and better cognition (Docq et al., 2020). The review by Docq et al. (2020) described rodent studies that administered HMOs in early life and assessed their behaviour in adulthood. In addition, several human studies also found relations between HMOs and better cognitive outcomes (Berger et al., 2020; Cho et al., 2021; Jorgensen et al., 2021). When comparing the results of **Chapters 2** and **3**, they suggest that breastfeeding duration independently does not predict EF or IC, but that the constituents of mother's milk, i.e., HMOs, may be important for predicting cognitive outcomes. As there are still unclear relations between breastfeeding duration and child and toddler EF (Belfort et al., 2016; Julvez et al., 2007; Lopez et al., 2021), both breastfeeding duration and milk-borne bioactive factors should be considered in future research to obtain a clear view on how Lactocrine Programming predicts toddler behaviour. Note, however, that causality cannot be established with observational longitudinal studies. Animal studies that include early life manipulations in experimental trials can unveil causal relations between early life nutrition and cognition, though results are still difficult to translate to humans (Mudd et al., 2017). Hence, a combination of these methods (i.e., longitudinal observational human studies, experimental animal studies, randomised controlled trials), as well as

replication studies to confirm previous results (to allow for performing meta-analyses), are necessary to determine whether and how early life nutrition causally affects future cognitive and behavioural outcomes in human children.

The microbiota-gut-brain axis: mechanism underlying the relations between nutrition and behaviour?

We found that early life fucosylated HMOs predicted better toddler EF (**Chapter 3**). Interestingly, the main functions of HMOs are to serve as nutrition for the gut microbiota (Bode, 2015; Totten et al., 2012; Underwood et al., 2014). Indeed, in the same BINGO cohort, HMO levels were positively related to higher levels of specific gut microbiota (e.g., high levels of 2'FL were related to higher levels of *Bifidobacteria*) (Borewicz et al., 2020). In addition, animal studies as well as human studies have observed an interplay between the gut microbiota and brain functioning (Bundgaard-Nielsen et al., 2020; Cheung et al., 2019; Cryan et al., 2019; Jiang et al., 2018; Sukmajaya et al., 2021). Because of the potential regulatory function of the gut microbiota on the brain (e.g., through production of short chain fatty acids that affect the central nervous system (Dong and Cui, 2022)), the microbiota-gut-brain axis is a strong candidate for explaining the relations found between early life nutrition and cognition. In line with previous findings (Carlson et al., 2017; Guzzardi et al., 2022; Tamana et al., 2021), we found that high levels of *Veillonella* and *Bacteroides* in early life were related to better toddler IC (**Chapter 4**). In addition, findings came forth that were not seen in previous literature, e.g., we found that higher levels of gut *Streptococcus* in early life as well as throughout the first three years of life, predicted worse toddler EF (**Chapter 4**). Although we speculated about potential pathways through which these gut microbiota may regulate behaviour, we were unable to confirm these pathways in the current thesis. To discover the causal role of gut microbiota in

the relation between behaviour and cognition, future research should aim at identifying the function of bacteria, involving a multi-omics approach (Ou, 2023), as explained below.

Metagenomics, including marker gene analyses (16S rRNA in bacteria) and shotgun sequencing, have allowed us to reveal the composition of bacteria in a stool sample (Almeida et al., 2019). Many studies identify the relative abundance of bacteria and infer on the relations found with the health outcome, leading to insightful discoveries, such as that bacterial composition between depressed, and anxious individuals differ significantly from healthy controls (Pinart et al., 2021). Note, however, that interpretation of relative abundance data could lead to false discovery rates (Gloor et al., 2017), and correlation biases (Tsilimigras and Fodor, 2016). This is due to the fact that an increase in abundance of one taxa is equal to a decrease in all other taxa, meaning that relative abundance of one taxa is dependent on the abundance of the remaining taxa (Barlow et al., 2020). Identifying absolute bacterial abundances (measured by e.g., quantitative PCR or flow cytometry) may aid in the interpretation of biological mechanisms regarding the microbiota-gut-brain axis. Importantly, as gut microbiota composition is subject to day-to-day, and within-person variation (Vandeputte et al., 2021), it is advisable to include repeated measures of stool sampling. To increase the reliability of earlier findings, replication studies are necessary (Peels and Bouter, 2021). When consistency in results is found over multiple studies, the next step is to explore the mechanism behind the relations.

Nonetheless, assessing relative or absolute abundance of bacteria does not reveal their transcriptional activities (Abu-Ali et al., 2018; Granata et al., 2020; Jia et al., 2019). Metatranscriptomics, which is the identification of microbial mRNA, allows for identification of bacterial metabolic activities. Interestingly, this technique has helped uncover different functions of the same bacterial species. For example, in patients with Crohn's disease, *Ruminococcus gnavus* is able to produce inflammatory glucorhamnan

that induces production of inflammatory cytokines in the gut (Henke et al., 2019). This same bacterium was also found to modulate mucin production which fortifies gut barrier functions (Graziani et al., 2016), subsequently preventing gut inflammation (Luissint et al., 2016). This phenomenon is likely explained by interactions between different bacterial taxa, through communication, cooperation, and competition (Coyte et al., 2015; Coyte and Rakoff-Nahoum, 2019; Zhang et al., 2022). This means that gut bacteria exert different effects depending on which other bacteria are present in the gut. Different functions of the same bacterial species could also be explained by the fact that bacteria exert different activities based on their host's conditions, also known as phenotypic switching (Sousa et al., 2012; Tadrowski et al., 2018). Interestingly, some mRNAs have weak ribosome binding sites and are therefore poorly translated, while those with strong binding sites are easier to translate (Liang et al., 2000). This means that not all microbial mRNA detected by metatranscriptomics may be involved in the expected metabolic processes, indicating that the interpretation of the results of these techniques must be done with caution.

Identifying microbial proteins (metaproteomics) and metabolites (metabolomics) might give a clearer picture of the role of gut bacteria in behaviour. Metaproteomics identifies levels of expressed proteins. The identification of these proteins is reliant on pipelines that process these data and match the peptides with online metagenomic databases to discover the most probable bacteria that might have expressed them (Mesuere et al., 2016). The reliance on these metagenomic databases is also a flaw, as they are dependent on the previously detected proteins. Hence, newly found peptides may not be in the database yet. Metabolomics is the study of metabolites in a biological sample, and allows for identification of key metabolites of specific pathways linked to a disease (Vignoli et al., 2019). However, as some metabolites are produced by different bacterial strains (Venegas et al., 2019), it is difficult to disentangle which bacteria are related to the identified metabolites. Note that including metaproteomics, and

metabolomics result in more variables and therefore more potential interactions to be investigated. Hence, it is extremely important that this type of research is properly powered.

All of the above supports the notion that identification of relative abundance is just a first step to understanding the role of gut bacteria in behaviour. Although all omics-techniques may have flaws, these techniques can get us closer to identifying causality in the microbiota-gut brain axis. A multi-omics approach could therefore lead to a better understanding on how gut microbiota may play a causal role in the relation between nutrition and behaviour (Daliri et al., 2021).

Before validating the potential molecular mechanisms of bacteria, it is important to first isolate, culture, and characterise them. Although approximately only 20% of the human gut bacteria have been cultured so far (Eckburg et al., 2005), it might be possible to culture hundreds of gut bacterial strains in a short timespan in the near future, due to rapid developments in high-throughput cultivation approaches (Clavel et al., 2022). Furthermore, besides bacteria, the gut is also colonised by fungi, archaea, and viruses. Fungi and archaea have different functions and have been associated with host phenotype, such as gut-related diseases and gut motility, respectively (Borrel et al., 2020; Richard and Sokol, 2019). These microorganisms also interact with bacteria and their derived products (Borrel et al., 2020; Richard and Sokol, 2019). Viruses present in the gut interact and coexist with gut bacteria through lysogeny (i.e., integration of the virus' nucleic acid into the bacterial genome or formation of a viral replicon in the bacterial cytoplasm). This way, viruses directly impact gut microbiota composition and the immune system, possibly modulating drivers of health and disease (Kirsch et al., 2021). Identifying the function of individual micro-organisms may be challenging. However, considering the complex interactions between the host, bacteria, fungi, and archaea, it is extremely valuable to obtain a detailed view of these dynamic interactions before validating these pathways.

Lastly, in vivo and in vitro validation studies can help confirm the expected pathways that are likely involved in the microbiota-gut-brain axis (Morais et al., 2020). In vitro studies are studies outside of a (animal) body. For example, organoids, three-dimensional tissues cultures grown from stem cells, have been used more and more to model gut and microbial interactions (Moysidou and Owens, 2021). In these models, microbes or their derived metabolites are injected into gut organoids to determine interactions between the gut and the microbiota (Moysidou and Owens, 2021). In vivo studies model the potential causes of health outcomes, such as depression and anxiety, in animal models. These studies grant evidence for causal relations (Nagpal and Cryan, 2021; Nestler and Hyman, 2010). For example, after transplanting microbiota of mice with high-anxiety into mice with low-anxiety, the behaviour of these recipient mice changed according to the donor's behavioural profile (Bercik et al., 2011). Furthermore, transplantation of gut microbiota from humans with autism spectrum disorder into germ-free mice resulted in the induction of autistic behaviours in the mice (Sharon et al., 2019). However, interpretation of these human microbiota-associated rodent studies must be done with caution. Overall, the majority of these studies do not attempt to gain insight in the mechanisms (e.g., which genes are up-, or down-regulated after the stool transplant), and they use a small number of human donors (Walter et al., 2020). In addition, studies with null-results or negative outcomes are rarely published (Nissen et al., 2016), and causal claims are overstated, also due to the fact that rodents are proxies for human diseases that do not occur naturally in rodents (e.g., what exactly are 'autistic behaviours' or other behaviours in rodents?) (Freudenberg et al., 2017; Kazdoba et al., 2016). Improving the experiments, and changing mindset and policies would aid in discovering true causality between gut microbiota composition and behaviour (Walter et al., 2020). All in all, the steps for turning correlational relations into causal relations are many and complex. Nonetheless, it is clear that gut microbiota research would benefit from applying repeated measures, identifying

absolute gut bacterial abundances, applying a multi-omics approach, and validating the pathways in in vitro and in vivo studies, to confirm causal relations between the gut microbiota and the brain. This approach, in combination with more specific hypothesis formation, larger sample sizes, and rigorous data collection, storage, and processing, will create evidence that can become substantial enough to result in future clinical implications.

Maternal educational level: Key player contributing to child health

Maternal educational level was found to be important when investigating early life nutrition and later child behavioural outcomes (**Chapter 2** and **Chapter 5**). Higher maternal educational level correlated with later breastfeeding cessation age (**Chapter 2**), better adolescent inhibitory control, higher adolescent diet quality, and more adolescent emotional eating behaviour (**Chapter 5**). A vast majority of previous literature, related to this thesis' topic, included educational level in their analyses, and found it to impact their results (Belfort et al., 2016; Julvez et al., 2007; Kim et al., 2008; Kremers et al., 2003; Lopez et al., 2021; Lytle et al., 2003; Snoek et al., 2007; van Strien et al., 2019; Vereecken et al., 2009). Despite educational level being determined differently in each country and culture, study results regarding relations between educational level and child health are consistent. Higher parental educational level is consistently positively related to child physical and mental health (Fakhrunnisak and Patria, 2022; Quesnel-Vallée and Taylor, 2012; Wu and Qi, 2021). There are some potential suggested mechanisms behind this relation. Parental education was shown to relate to improved parenting abilities and marital quality (Oreopoulos and Salvanes, 2011), as well as improved maternal ability to manage finances, choose the qualitatively best child educational programs, and control family health (Samarakoon and Parinduri,

2015). Furthermore, higher maternal educational level is usually accompanied by a higher income, allowing for provision of expensive healthier nutrition (Rippin et al., 2020). One study also found that parental educational level affects child health through their own parental health and family living conditions (Wu and Qi, 2021). As such, maternal educational level appears to impact child health in different ways, making it a key variable to include in future research.

In the current thesis, we observed potential effects of maternal educational level within a generally highly educated sample. Since lower educated and higher educated families differ in their dietary intake (i.e., lower educated families have less healthy diets) (Fard et al., 2021; Guerra-Carrillo et al., 2017), it is imperative to include and retain lower educated families in future studies. As achieving this goal is unfortunately a common challenge for scientific studies, previous research has reported on the barriers and strategies that improve the inclusion and retention rates of lower educated families (Barnett et al., 2012; Brannon et al., 2013; Nicholson et al., 2011; Teague et al., 2018). Generally, strategies to reduce participant burden (e.g., travel time, and flexible data collection methods) are most effective in retaining large sample sizes (Teague et al., 2018). To reduce the burden in lower educated families, a higher budget may be necessary (e.g., in the form of a higher incentive, or time and labor of the staff to allow for stepped-interventions and rapport-building between staff and participants) (Barnett et al., 2012; Brannon et al., 2013; Nicholson et al., 2011). Summarizing, future research on nutrition and behaviour should include lower educated families. By applying the abovementioned strategies, future studies can discover and obtain consistent results from a more diverse sample (e.g., inclusion of a wider variety of educational levels), allowing for generalization of the results.

Assessment of child behaviour: challenges and future directions

The assessment of behaviour is reliant on behavioural tests, (semi-)naturalistic observations of behaviour, and questionnaires, all of which have been applied in all our studies. At different ages, different types of measures may be more suitable than others for characterizing behaviour. Indeed, we found changing relations depending on the measure used, and age assessed (in **Chapters 3**, and **4**, we found results for toddler behaviour assessed with parental questionnaires by the primary caregiver, and in **Chapter 5**, we found results for adolescent behaviour assessed with behavioural tasks). This could be due to the fact that toddlers spend a large part of their time together with parents. Furthermore, toddler behaviour during a behavioural task could be variable as it may be dependent on their hunger levels, sleep quality, and the examiner performing the tasks (e.g., sex, examiner-toddler interaction) (Srinath et al., 2019). Adolescent behaviour is less affected by these factors (Srinath et al., 2019). However, as children become adolescents, they become more independent and desire more privacy (Sanders, 2013), resulting in parents having a less clear view on their adolescent's behaviour. Adolescent self-report on behaviour had not been assessed in our studies due to time and burden constraints. However, it could be very valuable to include adolescent self-reports next to behavioural tasks, as, commonly, literature finds equivocal correlations between behavioural tasks and questionnaires (Toplak et al., 2008). Both methods are valuable but also have their biases, with questionnaires being prone to socially desirable answers (De Vriendt et al., 2009; Stanton et al., 1996), and behavioural tasks being a momentary assessment dependent on other factors, such as attention span (Markant and Amso, 2014). Hence, future research on toddler and adolescent behaviour ideally should include both behavioural tasks as well as (self-report) questionnaires. In case of time and/or budget restrictions, it may be advisable to assess toddler behaviour with

parental questionnaires, and adolescent behaviour with behavioural tasks.

Assessment of child dietary intake: challenges and future directions

Diet quality was determined by assessing the dietary intake with parental reports (**Chapter 2**, **3** and **4**) and self-reports (**Chapter 5**). The most commonly used methods for dietary assessment are 24-hour recalls, food frequency questionnaires, and food records (Amoutzopoulos et al., 2018). Although keeping a food record is currently the most valid method for assessing total dietary intake (i.e., 'gold-standard'), the fact that the most commonly used dietary assessment methods are reliant on self-reports and parent reports, means that they are prone to memory bias as well as social desirability bias (Hebert et al., 1995; Subar et al., 2015). This makes the assessment of dietary intake in a general population one of the largest challenges in nutritional sciences. Additionally, assessing dietary intake in specific age-groups within a general population has its own specific challenges. Toddlers cannot report their own dietary intake, hence, we are dependent on parental reports. It is a logical choice, since toddlers spend most of their time with their parents, and parents provide the toddler with nutrition, allowing them to know exactly what their child consumes. However, this also makes parent report highly subject to socially desirable answers, as it is the parent who is responsible for the toddler's dietary intake. In addition, there are moments when the parents do not know the dietary intake of their child, such as when it goes to kindergarten or is cared for by babysitters (which is often the case in the Netherlands (Jeugdinstituut, 2022)). This makes parental report still challenging. Regarding adolescent dietary intake, as children become more independent (Sanders, 2013), the parent's view on the dietary intake of their adolescent child becomes less clear. This means that self-reports are more representative and reliable for assessing adolescent dietary intake. However, the multiple

24-hour recalls needed to obtain a reliable picture of diet quality cause a relatively high participant burden. As recruitment and retention of adolescents in studies is already challenging (Jong et al., 2023), a food frequency questionnaire, which is less burdensome than three unannounced 24-hour recalls, is more appropriate for assessing habitual dietary intake in adolescents. Note that self-reported nutritional questionnaires are also prone to socially desirable answers, recall bias, and misreporting in adolescents (Jones et al., 2021). Fortunately, developments regarding the assessment of nutritional intake have been rising. Technology-based assessments, including image- and sensor-based technologies, are being developed and show promising results (Ho et al., 2020; Kouvari et al., 2020; Zhao et al., 2020). Specifically, image-based technologies might be interesting for assessing dietary intake in the general population, as participants can simply take a picture of their food before and after the meal with a smartphone. This method reduces subject bias, burden, and provides a more accurate view on portion size and type of food, compared to 24-hour recalls, food frequency questionnaires, and food records. The largest challenge with this technology is for algorithms to accurately identify the nutritional value of the food on the image (Dalakleidi et al., 2022). More references, and thus, more time are necessary to improve this algorithm. All in all, dietary assessment methods are advancing, and technology-based assessments could aid in reducing the burden and improving the accuracy of reporting dietary intake. As the utilisation and development of technological image-based dietary assessment started roughly 10 years ago (Forster et al., 2014; Kristal et al., 2014), it is likely that these technologies will be applied either as a stand-alone method, or in combination with traditional dietary assessment methods, in the foreseeable future. For the time being, the best way to assess dietary intake in toddlers is through parental reports. Specifically, multiple unannounced 24-hour dietary recall reports, although tedious, provide the most reliable data. Furthermore, adolescent dietary intake is most reliably assessed with a self-reported food frequency questionnaire.

Conclusion and future perspectives

As reflected in this thesis, early life nutrition is an important life phase for future health and behaviour. We found evidence that supports the Lactocrine Programming hypothesis: fucosylated HMOs are related to better EF in our study. Furthermore, the microbiota-gut-brain axis may be an important mediator between nutrition and behaviour as we found certain bacteria in early life and throughout the first three years of life to be related to better EF and IC in toddlerhood. To identify the likely causal role of gut microbiota on behaviour, recommendations for future research are: performing repeated measures, identifying absolute gut bacterial abundances, applying a multi-omics approach, validating the pathways in in vitro and in vivo studies, and replicating studies. Additionally, including lower educational levels is imperative as the role of maternal educational level was already shown to be important in our generally highly educated sample. A higher budget is necessary to aid the inclusion and retention of this target group. Lastly, the methods for assessing nutritional intake have their flaws. However, it is likely that technological image-based assessment methods will be applied more commonly in the foreseeable future. This will allow for accurate assessment of dietary intake in the general population, resulting in more accurate inferences on the relation between dietary intake and health outcomes.

The development of EF skills is important for many future outcomes, such as academic performance, socioemotional competence, and general health (Ayduk et al., 2000; Duckworth et al., 2013; Eisenberg et al., 2007; Munakata and Michaelson, 2021; Cortés Pascual et al., 2019; Robson et al., 2020; Shoda et al., 1990). This thesis contributes to understanding the role of early life breastfeeding duration and HMO exposure on toddler EF, and the possible regulating role of gut microbiota on toddler EF. Replicating this thesis' results will help confirm the roles of early life nutritional predictors for future child health and behaviour. These studies would add to the body

of literature that shows the importance of early life predictors for future healthy diet and behaviour. In turn, this would provide input for policy makers and health care institutions aiming to improve an infant's early life exposures.

References

Abu-Ali, G. S., Mehta, R. S., Lloyd-Price, J., Mallick, H., Branck, T., Ivey, K. L., Drew, D. A., DuLong, C., Rimm, E., Izard, J., Chan, A. T., and Huttenhower, C. (2018). Metatranscriptome of human faecal microbial communities in a cohort of adult men. *Nature microbiology*, 3(3):356–366.

Almeida, A., Mitchell, A. L., Boland, M., Forster, S. C., Gloor, G. B., Tarkowska, A., Lawley, T. D., and Finn, R. D. (2019). A new genomic blueprint of the human gut microbiota. *Nature*, 568(7753):499–504.

Amir, L. H. and Donath, S. M. (2012). Maternal diet and breastfeeding: A case for rethinking physiological explanations for breastfeeding determinants. *Early Human Development*, 88(7):467–471.

Amoutzopoulos, B., Steer, T., Roberts, C., Cade, J. E., Boushey, C. J., Collins, C. E., Trolle, E., Boer, E. J. d., Ziauddeen, N., Rossum, C. v., Buurma, E., Coyle, D., and Page, P. (2018). Traditional methods v. new technologies - dilemmas for dietary assessment in large-scale nutrition surveys and studies: a report following an international panel discussion at the 9th International Conference on Diet and Activity Methods (ICDAM9), Brisban. *Journal of nutritional science*, 7:e11–e11.

Ayduk, O., Mendoza-Denton, R., Mischel, W., Downey, G., Peake, P. K., and Rodriguez, M. (2000). Regulating the interpersonal self: Strategic self-regulation for coping with rejection sensitivity. *Journal of Personality and Social Psychology*, 79(5):776–792.

Barlow, J. T., Bogatyrev, S. R., and Ismagilov, R. F. (2020). A quantitative sequencing framework for absolute abundance measurements of mucosal and lumenal microbial communities. *Nature communications*, 11(1):2590.

Barnett, J., Aguilar, S., Brittner, M., and Bonuck, K. (2012). Recruiting and retaining low-income, multi-ethnic women into randomized controlled trials: successful strategies and staffing. *Contemporary clinical trials*, 33(5):925–932.

Bartol, F. F., Wiley, A. A., and Bagnell, C. A. (2008). Epigenetic Programming of Porcine Endometrial Function and the Lactocrine Hypothesis. *Reproduction in Domestic Animals*, 43:273–279.

Beckerman, J. P., Slade, E., and Ventura, A. K. (2020). Maternal diet during lactation and breastfeeding practices have synergistic association with child diet at 6 years. *Public Health Nutrition*, 23(2):286–294.

Belfort, M. B., Rifas-Shiman, S. L., Kleinman, K. P., Bellinger, D. C., Harris, M. H., Taveras, E. M., Gillman, M. W., and Oken, E. (2016). Infant Breastfeeding Duration and Mid-Childhood Executive Function, Behavior, and Social-Emotional Development. *Journal of developmental and behavioral pediatrics*, 37(1):43–52.

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., Deng, Y., Blennerhassett, P., Macri, J., McCoy, K. D., Verdu, E. F., and Collins, S. M. (2011). The Intestinal Microbiota Affect Central Levels of Brain-Derived Neurotropic Factor and Behavior in Mice. *Gastroenterology*, 141(2):599–609.e3.

Berger, P. K., Plows, J. F., Jones, R. B., Alderete, T. L., Yonemitsu, C., Poulsen, M., Ryoo, J. H., Peterson, B. S., Bode, L., and Goran, M. I. (2020). Human milk oligosaccharide 2'-fucosyllactose links feedings at 1 month to cognitive development at 24 months in infants of normal and overweight mothers. *PLOS ONE*, 15(2):e0228323.

Bode, L. (2015). The functional biology of human milk oligosaccharides. *Early Human Development*, 91(11):619–622.

Borewicz, K., Gu, F., Saccenti, E., Hechler, C., Beijers, R., de Weerth, C., van Leeuwen, S. S., Schols, H. A., and Smidt, H. (2020). The association between breastmilk oligosaccharides and faecal microbiota in healthy breastfed infants at two, six, and twelve weeks of age. *Scientific Reports 2020 10:1*, 10(1):1–12.

Borrel, G., Brugère, J.-F., Gribaldo, S., Schmitz, R. A., and Moissl-Eichinger, C. (2020). The host-associated archaeome. *Nature Reviews Microbiology*, 18(11):622–636.

Brannon, E. E., Kuhl, E. S., Boles, R. E., Aylward, B. S., Ratcliff, M. B., Valenzuela, J. M., Johnson, S. L., and Powers, S. W. (2013). Strategies for Recruitment and Retention of Families from Low-Income, Ethnic Minority Backgrounds in a Longitudinal Study of Caregiver Feeding and Child Weight. *Children's health care : journal of the Association for the Care of Children's Health*, 42(3):198.

Bundgaard-Nielsen, C., Knudsen, J., Leutscher, P. D. C., Lauritsen, M. B., Nyegaard, M., Hagstrøm, S., and Sørensen, S. (2020). Gut microbiota profiles of autism spectrum disorder and attention deficit/hyperactivity disorder: A systematic literature review. *Gut microbes*, 11(5):1172–1187.

Carlson, A. L., Xia, K., Azcarate-Peril, M. A., Goldman, B. D., Ahn, M., Styner, M. A., Thompson, A. L., Geng, X., Gilmore, J. H., and Knickmeyer, R. C. (2017). Infant gut microbiome associated with cognitive development. *Biological Psychiatry*, 83(2):148–159.

Chakrabarti, A., Geurts, L., Hoyles, L., Iozzo, P., Kraneveld, A. D., Fata, G. L., Miani, M., Patterson, E., Pot, B., Shortt, C., and Vauzour, D. (2022). The microbiota-gut-brain axis: pathways to better brain health. Perspectives on what we know, what we need to investigate and how to put knowledge into practice. *Cellular and molecular life sciences : CMLS*, 79(2):80.

Cheung, S. G., Goldenthal, A. R., Uhlemann, A.-C., Mann, J. J., Miller, J. M., and Sublette, M. E. (2019). Systematic Review of Gut Microbiota and Major Depression. *Frontiers in psychiatry*, 10:34.

Cho, S., Zhu, Z., Li, T., Baluyot, K., Howell, B. R., Hazlett, H. C., Elison, J. T., Hauser, J., Sprenger, N., Wu, D., and Lin, W. (2021). Human milk 3'-sialyllactose is positively associated with language development during infancy. *The American Journal of Clinical Nutrition*, 114(2):588–597.

Clavel, T., Horz, H. P., Segata, N., and Vehreschild, M. (2022). Next steps after 15 stimulating years of human gut microbiome research. *Microbial Biotechnology*, 15(1):164–175.

Connor, K. (2019). *Early Life Nutrition and Future Health*. MDPI.

Cortés Pascual, A., Moyano, N., and Robres, A. Q. (2019). The relationship between executive functions and academic performance in primary education: Review and meta-analysis. *Frontiers in psychology*, 10:1582.

Costello, S. E., Geiser, E., and Schneider, N. (2020). Nutrients for executive function development and related brain connectivity in school-aged children. *Nutrition Reviews*, 79(12):1293–1306.

Coyte, K. Z. and Rakoff-Nahoum, S. (2019). Understanding Competition and Cooperation within the Mammalian Gut Microbiome. *Current biology : CB*, 29(11):R538–R544.

Coyte, K. Z., Schluter, J., and Foster, K. R. (2015). The ecology of the microbiome: Networks, competition, and stability. *Science*, 350(6261):663–666.

Cryan, J. F., O'riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., Codagnone, M. G., Cussotto, S., Fulling, C., Golubeva, A. V., Guzzetta, K. E., Jaggar, M., Long-Smith, C. M., Lyte, J. M., Martin, J. A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., O'connor, R., Cruz-Pereira, J. S., Peterson, V. L., Rea, K., Ritz, N. L., Sherwin, E., Spichak, S., Teichman, E. M., Wouw, M. v. d., Ventura-Silva, A. P., Wallace-Fitzsimons, S. E., Hyland, N., Clarke, G., and Dinan, T. G. (2019). The microbiota-gut-brain axis. *Physiological Reviews*, 99(4):1877–2013.

Dalakleidi, K. V., Papadelli, M., Kapolos, I., and Papadimitriou, K. (2022). Applying Image-Based Food-Recognition Systems on Dietary Assessment: A Systematic Review. *Advances in Nutrition*, 13(6):2590.

Daliri, E. B.-M., Ofosu, F. K., Chelliah, R., Lee, B. H., and Oh, D.-H. (2021). Challenges and Perspective in Integrated Multi-Omics in Gut Microbiota Studies. *Biomolecules*, 11(2):300.

De Vriendt, T., Huybrechts, I., Ottevaere, C., Van Trimpont, I., and De Henauw, S. (2009). Validity of self-reported weight and height of adolescents, its impact on classification into bmi-categories and the association with weighing behaviour. *International journal of environmental research and public health*, 6(10):2696–2711.

de Weerth, C., Aatsinki, A.-K., Azad, M. B., Bartol, F. F., Bode, L., Collado, M. C., Dettmer, A. M., Field, C. J., Guilfoyle, M., Hinde, K., Korosi, A., Lustermans, H., Husna, N., Shukri, M., Moore, S. E., Pundir, S., Rodriguez, J. M., Slupsky, C. M., Turner, S., Goudoever, J. B. V., Ziomkiewicz, A., and Beijers, R. (2022). Human milk: From complex tailored nutrition to bioactive impact on child cognition and behavior. *Critical reviews in food science and nutrition*, pages 1–38.

Diamond, A. (2013). Executive functions. *Annual review of psychology*, 64:135–68.

Docq, S., Spoelder, M., Wang, W., and Homberg, J. R. (2020). The Protective and Long-Lasting Effects of Human Milk Oligosaccharides on Cognition in Mammals. *Nutrients*, 12(11):3572.

Dong, Y. and Cui, C. (2022). The role of short-chain fatty acids in central nervous system diseases. *Molecular and Cellular Biochemistry*, 477(11):2595–2607.

Duckworth, A. L., Tsukayama, E., and Kirby, T. A. (2013). Is It Really Self-Control? Examining the Predictive Power of the Delay of Gratification Task. *Personality and Social Psychology Bulletin*, 39(7):843–855.

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S. R., Nelson, K. E., and Relman, D. A. (2005). Diversity of the human intestinal microbial flora. *Science (New York, N.Y.)*, 308(5728):1635–1638.

Eisenberg, N., Hofer, C., and Vaughan, J. (2007). Effortful control and its socioemotional consequences. *Handbook of emotion regulation*, 2:287–288.

Fakhrunnisak, D. and Patria, B. (2022). The positive effects of parents' education level on children's mental health in Indonesia: a result of longitudinal survey. *BMC public health*, 22(1):949.

Fard, N. v., Morales, G. D. F., Mejova, Y., and Schifanella, R. (2021). On the interplay between educational attainment and nutrition: a spatially-aware perspective. *EPJ Data Science*, 10(1).

Forster, H., Fallaize, R., Gallagher, C., O'Donovan, C. B., Woolhead, C., Walsh, M. C., Macready, A. L., Lovegrove, J. A., Mathers, J. C., Gibney, M. J., Brennan, L., and Gibney, E. R. (2014). Online dietary intake estimation: the Food4Me food frequency questionnaire. *Journal of medical Internet research*, 16(6):e150–e150.

Freudenberg, F., O'Leary, A., Aguiar, D. C., and Slattery, D. A. (2017). Challenges with modelling anxiety disorders: a possible hindrance for drug discovery. *Expert Opinion on Drug Discovery*, 13(4):279–281.

Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., and Egozcue, J. J. (2017). Microbiome Datasets Are Compositional: And This Is Not Optional. *Frontiers in microbiology*, 8:2224.

Granata, I., Nardelli, C., D'Argenio, V., Tramontano, S., Compare, D., Guarracino, M. R., Nardone, G., Pilone, V., and Sacchetti, L. (2020). Duodenal Metatranscriptomics to Define Human and Microbial Functional Alterations Associated with Severe Obesity: A Pilot Study. *Microorganisms*, 8(11):1811.

Graziani, F., Pujol, A., Nicoletti, C., Dou, S., Maresca, M., Giardina, T., Fons, M., and Perrier, J. (2016). *Ruminococcus gnavus*E1 modulates mucin expression and intestinal glycosylation. *Journal of Applied Microbiology*, 120(5):1403–1417.

Guerra-Carrillo, B., Katovich, K., and Bunge, S. A. (2017). Does higher education hone cognitive functioning and learning efficacy? Findings from a large and diverse sample. *PloS one*, 12(8):e0182276–e0182276.

Guzzardi, M. A., Ederveen, T. H. A., Rizzo, F., Weisz, A., Collado, M. C., Muratori, F., Gross, G., Alkema, W., and Iozzo, P. (2022). Maternal pre-pregnancy overweight and neonatal gut bacterial colonization are associated with cognitive development and gut microbiota composition in preschool-age offspring. *Brain, Behavior, and Immunity*, 100:311–320.

Hebert, J. R., Clemow, L., Pbert, L., Ockene, I. S., and Ockene, J. K. (1995). Social Desirability Bias in Dietary Self-Report May Compromise the Validity of Dietary Intake Measures. *International Journal of Epidemiology*, 24(2):389–398.

Henke, M. T., Kenny, D. J., Cassilly, C. D., Vlamakis, H., Xavier, R. J., and Clardy, J. (2019). Ruminococcus gnavus, a member of the human gut microbiome associated with Crohn's disease, produces an inflammatory polysaccharide. *Proceedings of the National Academy of Sciences of the United States of America*, 116(26):12672–12677.

Hinde, K., Foster, A. B., Landis, L. M., Rendina, D., Oftedal, O. T., and Power, M. L. (2013). Daughter dearest: Sex-biased calcium in mother's milk among rhesus macaques. *American Journal of Physical Anthropology*, 151(1):144–150.

Ho, D. K. N., Tseng, S.-H., Wu, M.-C., Shih, C.-K., Atika, A. P., Chen, Y.-C., and Chang, J.-S. (2020). Validity of image-based dietary assessment methods: A systematic review and meta-analysis. *Clinical Nutrition*, 39(10):2945–2959.

Jeugdinstituut, N. (2022). Cijfers over kinderopvang.

Jia, Y., Leung, M. H. Y., Tong, X., Wilkins, D., and Lee, P. K. H. (2019). Rare Taxa Exhibit Disproportionate Cell-Level Metabolic Activity in Enriched Anaerobic Digestion Microbial Communities. *mSystems*, 4(1):e00208–18.

Jiang, H.-y., Zhou, Y.-y., Zhou, G.-l., Li, Y.-c., Yuan, J., Li, X.-h., and Ruan, B. (2018). Gut microbiota profiles in treatment-naïve children with attention deficit hyperactivity disorder. *Behavioural Brain Research*, 347:408–413.

Jones, L., Ness, A., and Emmett, P. (2021). Misreporting of Energy Intake From Food Records Completed by Adolescents: Associations With Sex, Body Image, Nutrient, and Food Group Intake. *Frontiers in nutrition*, 8:749007.

Jong, S. T., Stevenson, R., Winpenny, E. M., Corder, K., and Sluijs, E. M. F. v. (2023). Recruitment and retention into longitudinal health research from an adolescent perspective: a qualitative study. *BMC medical research methodology*, 23(1):16.

Jorgensen, J. M., Young, R., Ashorn, P., Ashorn, U., Chaima, D., Davis, J. C., Goonatilleke, E., Kumwenda, C., Lebrilla, C. B., Maleta, K., Prado, E. L., Sadalaki, J., Totten, S. M., Wu, L. D., Zivkovic, A. M., and Dewey, K. G. (2021). Associations of human milk oligosaccharides and bioactive proteins with infant growth and development among malawian mother-infant dyads. *The American Journal of Clinical Nutrition*, 113(1):209–220.

Julvez, J., Ribas-Fitó, N., Forns, M., Garcia-Esteban, R., Torrent, M., and Sunyer, J. (2007). Attention behaviour and hyperactivity at age 4 and duration of breast-feeding. *Acta Paediatrica*, 96(6):842–847.

Kazdoba, T. M., Leach, P. T., Yang, M., Silverman, J. L., Solomon, M., and Crawley, J. N. (2016). Translational Mouse Models of Autism: Advancing Toward Pharmacological Therapeutics. *Current topics in behavioral neurosciences*, 28:1–52.

Kim, M. J., McIntosh, W. A., Anding, J., Kubena, K. S., Reed, D. B., and Moon, G. S. (2008). Perceived parenting behaviours predict young adolescents' nutritional intake and body fatness. *Maternal & child nutrition*, 4(4):287–303.

Kirsch, J. M., Brzozowski, R. S., Faith, D., Round, J. L., Secor, P. R., and Duerkop, B. A. (2021). Bacteriophage-Bacteria Interactions in the Gut: From Invertebrates to Mammals. *Annual review of virology*, 8(1):95–113.

Kouvari, M., Mamalaki, E., Bathrellou, E., Poulimeneas, D., Yannakoulia, M., and Panagiotakos, D. B. (2020). The validity of technology-based dietary assessment methods in childhood and adolescence: a systematic review. *Critical Reviews in Food Science and Nutrition*, 61(7):1065–1080.

Kremers, S. P. J., Brug, J., Vries, H. D., and Engels, R. C. M. E. (2003). Parenting style and adolescent fruit consumption. *Appetite*, 41(1):43–50.

Kristal, A. R., Kolar, A. S., Fisher, J. L., Plascak, J. J., Stumbo, P. J., Weiss, R., and Paskett, E. D. (2014). Evaluation of web-based, self-administered, graphical food frequency questionnaire. *Journal of the Academy of Nutrition and Dietetics*, 114(4):613–621.

Liang, S. T., Xu, Y. C., Dennis, P., and Bremer, H. (2000). mRNA composition and control of bacterial gene expression. *Journal of Bacteriology*, 182(11):3037–3044.

Lopez, D. A., Foxe, J. J., Mao, Y., Thompson, W. K., Martin, H. J., and Freedman, E. G. (2021). Breastfeeding Duration Is Associated With Domain-Specific Improvements in Cognitive Performance in 9–10-Year-Old Children. *Frontiers in Public Health*, 9:657422.

Luissint, A.-C., Parkos, C. A., and Nusrat, A. (2016). Inflammation and the Intestinal Barrier: Leukocyte-Epithelial Cell Interactions, Cell Junction Remodeling, and Mucosal Repair. *Gastroenterology*, 151(4):616–632.

Lytle, L. A., Varnell, S., Murray, D. M., Story, M., Perry, C., Birnbaum, A. S., and Kubik, M. Y. (2003). Predicting adolescents' intake of fruits and vegetables. *Journal of nutrition education and behavior*, 35(4):170–178.

Markant, J. and Amso, D. (2014). Leveling the playing field: attention mitigates the effects of intelligence on memory. *Cognition*, 131(2):195–204.

Mennella, J. A. and Beauchamp, G. K. (1991). The Transfer of Alcohol to Human Milk. *New England Journal of Medicine*, 325(14):981–985.

Mennella, J. A., Jagnow, C. P., and Beauchamp, G. K. (2001). Prenatal and postnatal flavor learning by human infants. *Pediatrics*, 107(6):E88–E88.

Mesuere, B., Willems, T., Jeugt, F. V. d., Devreese, B., Vandamme, P., and Dawyndt, P. (2016). Unipept web services for metaproteomics analysis. *Bioinformatics*, 32(11):1746–1748.

Morais, L. H., Schreiber, H. L., and Mazmanian, S. K. (2020). The gut microbiota–brain axis in behaviour and brain disorders. *Nature Reviews Microbiology*, 19(4):241–255.

Moysidou, C.-M. and Owens, R. M. (2021). Advances in modelling the human microbiome-gut-brain axis in vitro. *Biochemical Society transactions*, 49(1):187–201.

Mudd, A., Fleming, S., Labhart, B., Chichlowski, M., Berg, B., Donovan, S., and Dilger, R. (2017). Dietary sialyllactose influences sialic acid concentrations in the prefrontal cortex and magnetic resonance imaging measures in corpus callosum of young pigs. *Nutrients*, 9(12):1297.

Munakata, Y. and Michaelson, L. E. (2021). Executive Functions in Social Context: Implications for Conceptualizing, Measuring, and Supporting Developmental Trajectories. *Developmental Psychology*, 3(1):139–163.

Nagpal, J. and Cryan, J. F. (2021). Microbiota-brain interactions: Moving toward mechanisms in model organisms. *Neuron*, 109(24):3930–3953.

Nestler, E. J. and Hyman, S. E. (2010). Animal models of neuropsychiatric disorders. *Nature neuroscience*, 13(10):1161–1169.

Nicholson, L. M., Schwirian, P. M., Klein, E. G., Skybo, T., Murray-Johnson, L., Eneli, I., Boettner, B., French, G. M., and Groner, J. A. (2011). Recruitment and retention strategies in longitudinal clinical studies with low-income populations. *Contemporary clinical trials*, 32(3):353–362.

Nissen, S. B., Magidson, T., Gross, K., and Bergstrom, C. T. (2016). Publication bias and the canonization of false facts. *eLife*, 5:e21451.

Norris, S. A., Frongillo, E. A., Black, M. M., Dong, Y., Fall, C., Lampl, M., Liese, A. D., Naguib, M., Prentice, A., Rochat, T., Stephensen, C. B., Tinago, C. B., Ward, K. A., Wrottesley, S. V., and Patton, G. C. (2022). Nutrition in adolescent growth and development. *The Lancet*, 399(10320):172–184.

Oreopoulos, P. and Salvanes, K. G. (2011). Priceless: The Nonpecuniary Benefits of Schooling. *Journal of Economic Perspectives*, 25(1):159–184.

Ou, Y. (2023). *The microbiota-gut-brain axis: the development of the gut microbiota and mental health over the first 14 years of life*. PhD thesis, Wageningen University and Research.

Peels, R. and Bouter, L. (2021). Replication and trustworthiness. *Replication and trustworthiness, Accountability in Research*, 30(2):77–87.

Pinart, M., Dötsch, A., Schlicht, K., Laudes, M., Bouwman, J., Forslund, S. K., Pischon, T., and Nimptsch, K. (2021). Gut Microbiome Composition in Obese and Non-Obese Persons: A Systematic Review and Meta-Analysis. *Nutrients*, 14(1):12.

Quesnel-Vallée, A. and Taylor, M. (2012). Socioeconomic pathways to depressive symptoms in adulthood: evidence from the National Longitudinal Survey of Youth 1979. *Social science & medicine (1982)*, 74(5):734–743.

Richard, M. L. and Sokol, H. (2019). The gut mycobiota: insights into analysis, environmental interactions and role in gastrointestinal diseases. *Nature Reviews Gastroenterology & Hepatology*.

Rippin, H. L., Hutchinson, J., Greenwood, D. C., Jewell, J., Breda, J. J., Martin, A., Rippin, D. M., Schindler, K., Rust, P., Fagt, S., Matthiessen, J., Nurk, E., Nelis, K., Kukk, M., Tapanainen, H., Valsta, L., Heuer, T., Sarkadi-Nagy, E., Bakacs, M., Tazhibayev, S., Sharmanov, T., Spiroski, I., Beukers, M., Rossum, C. v., Ocke, M., Lindroos, A. K., Lemming, E. W., and Cade, J. E. (2020). Inequalities in education and national income are associated with poorer diet: Pooled analysis of individual participant data across 12 European countries. *PloS one*, 15(5):e0232447–e0232447.

Robertson, R. C., Manges, A. R., Finlay, B. B., and Prendergast, A. J. (2019). The Human Microbiome and Child Growth - First 1000 Days and Beyond. *Trends in microbiology*, 27(2):131–147.

Robson, D. A., Allen, M. S., and Howard, S. J. (2020). Self-Regulation in Childhood as a Predictor of Future Outcomes: A Meta-Analytic Review. *Psychological Bulletin*, 146(4).

Samarakoon, S. and Parinduri, R. A. (2015). Does Education Empower Women? Evidence from Indonesia. *World Development*, 66:428–442.

Sanders, R. A. (2013). Adolescent psychosocial, social, and cognitive development. *Pediatrics in review*, 34(8):354–359.

Sausenthaler, S., Koletzko, S., Koletzko, B., Reinhardt, D., Krämer, U., Berg, A. v., Berdel, D., Bauer, C. P., Grübl, A., Wichmann, H.-E., and Heinrich, J. (2010). Effect of hydrolysed formula feeding on taste preferences at 10 years. Data from the German Infant Nutritional Intervention Program Plus Study. *Clinical Nutrition*, 29(3):304–306.

Sharon, G., Cruz, N. J., Kang, D.-W., Gandal, M. J., Wang, B., Kim, Y.-M., Zink, E. M., Casey, C. P., Taylor, B. C., Lane, C. J., Bramer, L. M., Isern, N. G., Hoyt, D. W., Noecker, C., Sweredoski, M. J., Moradian, A., Borenstein, E., Jansson, J. K., Knight, R., Metz, T. O., Lois, C., Geschwind, D. H., Krajmalnik-Brown, R., and Mazmanian, S. K. (2019). Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice. *Cell*, 177(6):1600–1618.e17.

Shoda, Y., Mischel, W., and Peake, P. K. (1990). Predicting Adolescent Cognitive and Self-Regulatory Competencies From Preschool Delay of Gratification: Identifying Diagnostic Conditions. *Developmental Psychology*, 26(6):978–986.

Smith, K. E. and Pollak, S. D. (2020). Early life stress and development: potential mechanisms for adverse outcomes. *Journal of neurodevelopmental disorders*, 12(1):34.

Snoek, H. M., Engels, R. C. M. E., Janssens, J. M. A. M., and van Strien, T. (2007). Parental behaviour and adolescents' emotional eating. *Appetite*, 49(1):223–230.

Sousa, A. M., Machado, I., and Pereira, M. O. (2012). Phenotypic switching: an opportunity to bacteria thrive. *Science against microbial pathogens: communicating current research and technological advances*.

Spahn, J. M., Callahan, E. H., Spill, M. K., Wong, Y. P., Benjamin-Neelon, S. E., Birch, L., Black, M. M., Cook, J. T., Faith, M. S., Mennella, J. A., and Casavale, K. O. (2019). Influence of maternal diet on flavor transfer to amniotic fluid and breast milk and children's responses: a systematic review. *The American Journal of Clinical Nutrition*, 109:1003S–1026S.

Srinath, S., Jacob, P., Sharma, E., and Gautam, A. (2019). Clinical Practice Guidelines for Assessment of Children and Adolescents. *Indian Journal of Psychiatry*, 61(Suppl 2):158.

Stanton, W. R., McClelland, M., Elwood, C., Ferry, D., and Silva, P. A. (1996). Prevalence, reliability and bias of adolescents' reports of smoking and quitting. *Addiction*, 91(11):1705–1714.

Subar, A. F., Freedman, L. S., Tooze, J. A., Kirkpatrick, S. I., Boushey, C., Neuhouser, M. L., Thompson, F. E., Potischman, N., Guenther, P. M., Tarasuk, V., Reedy, J., and Krebs-Smith, S. M. (2015). Addressing Current Criticism Regarding the Value of Self-Report Dietary Data. *The Journal of nutrition*, 145(12):2639–2645.

Sukmajaya, A. C., Lusida, M. I., Soetjipto, and Setiawati, Y. (2021). Systematic review of gut microbiota and attention-deficit hyperactivity disorder (ADHD). *Annals of general psychiatry*, 20(1):12.

Tadrowski, A. C., Evans, M. R., and Waclaw, B. (2018). Phenotypic Switching Can Speed up Microbial Evolution. *Scientific reports*, 8(1):8941.

Tamana, S. K., Tun, H. M., Konya, T., Chari, R. S., Field, C. J., Guttman, D. S., Becker, A. B., Moraes, T. J., Turvey, S. E., Subbarao, P., Sears, M. R., Pei, J., Scott, J. A., Mandhane, P. J., and Kozyrskyj, A. L. (2021). Bacteroides-dominant gut microbiome of late infancy is associated with enhanced neurodevelopment. *Gut Microbes*, 13(1):1–17.

Teague, S., Youssef, G. J., Macdonald, J. A., Sciberras, E., Shatte, A., Fuller-Tyszkiewicz, M., Greenwood, C., McIntosh, J., Olsson, C. A., Hutchinson, D., and Theme, S. L. S. (2018). Retention strategies in longitudinal cohort studies: a systematic review and meta-analysis. *BMC medical research methodology*, 18(1):151.

Toplak, M. E., Bucciarelli, S. M., Jain, U., and Tannock, R. (2008). Executive Functions: Performance-Based Measures and the Behavior Rating Inventory of Executive Function (BRIEF) in Adolescents with Attention Deficit/Hyperactivity Disorder (ADHD). *Child Neuropsychology*, 15(1):53–72.

Totten, S. M., Zivkovic, A. M., Wu, S., Ngyuen, U., Freeman, S. L., Ruhaak, L. R., Darboe, M. K., German, J. B., Prentice, A. M., and Lebrilla, C. B. (2012). Comprehensive profiles of human milk oligosaccharides yield highly sensitive and specific markers for determining secretor status in lactating mothers. *Journal of Proteome Research*, 11(12):6124–6133.

Tsilimigras, M. C. B. and Fodor, A. A. (2016). Compositional data analysis of the microbiome: fundamentals, tools, and challenges. *Annals of Epidemiology*, 26(5):330–335.

Underwood, M. A., German, J. B., Lebrilla, C. B., and Mills, D. A. (2014). Bifidobacterium longum subspecies infantis: champion colonizer of the infant gut. *Pediatric Research*, 77(1-2):229–235.

van Strien, T., Beijers, R., Smeekens, S., Winkens, L. H. H., and Konttinen, H. (2019). Parenting quality in infancy and emotional eating in adolescence: Mediation through emotion suppression and alexithymia. *Appetite*, 141:104339.

Vandeputte, D., Commer, L. D., Tito, R. Y., Kathagen, G., Sabino, J., Vermeire, S., Faust, K., and Raes, J. (2021). Temporal variability in quantitative human gut microbiome profiles and implications for clinical research. *Nature Communications 2021 12:1*, 12(1):1–13.

Venegas, D. P., Fuente, M. K. D. I., Landskron, G., González, M. J., Quera, R., Dijkstra, G., Harmsen, H. J. M., Faber, K. N., and Hermoso, M. A. (2019). Corrigendum: Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. *Frontiers in immunology*, 10:1486.

Ventura, A. K. (2017). Does Breastfeeding Shape Food Preferences? Links to Obesity. *Annals of Nutrition and Metabolism*, 70(3):8–15.

Ventura, A. K., Phelan, S., and Garcia, K. S. (2021). Maternal Diet During Pregnancy and Lactation and Child Food Preferences, Dietary Patterns, and Weight Outcomes: a Review of Recent Research. *Current Nutrition Reports*, 10(4):413–426.

Vereecken, C., Legtest, E., Bourdeaudhuij, I. D., and Maes, L. (2009). Associations between General Parenting Styles and Specific Food-Related Parenting Practices and Children's Food Consumption. *American Journal of Health Promotion*, 23(4):233–240.

Vignoli, A., Ghini, V., Meoni, G., Licari, C., Takis, P. G., Tenori, L., Turano, P., and Luchinat, C. (2019). High-Throughput Metabolomics by 1D NMR. *Angewandte Chemie International Edition*, 58(4):968–994.

Walter, J., Armet, A. M., Finlay, B. B., and Shanahan, F. (2020). Establishing or Exaggerating Causality for the Gut Microbiome: Lessons from Human Microbiota-Associated Rodents. *Cell*, 180(2):221–232.

Wu, Y. and Qi, D. (2021). The Effects of Family Income and Parents' Educational Status on Child Health Status: Examining the Mediation Effects of Material Deprivation and Parents' Health. *Journal of Family Issues*, 43(9):2255–2274.

Zhang, Y., Ma, N., Tan, P., and Ma, X. (2022). Quorum sensing mediates gut bacterial communication and host-microbiota interaction. *Critical Reviews in Food Science and Nutrition*, pages 1–13.

Zhao, X., Xu, X., Li, X., He, X., Yang, Y., and Zhu, S. (2020). Emerging trends of technology-based dietary assessment: a perspective study. *European Journal of Clinical Nutrition*, 75(4):582–587.

Chapter 7

Supplementary Materials

Supplementary materials of chapter 2

Supplementary Table 7.1. Correlations between mother and partner reports.

	1. BRIEF-P inh (M)	2. BRIEF-P inh (P)	3. BRIEF-P total (M)	4. BRIEF-P total (P)	5. REEF (M)	6. REEF (P)	7. BRIEF-A (M)	8. BRIEF-A (P)
1	-							
2	0.415**	-						
3	0.800**	0.539**	-					
4	0.248	0.857**	0.535**	-				
5	-0.426**	-0.279*	-0.461**	-0.259	-			
6	-0.182	-0.182	-0.181	-0.147	0.407**	-		
7	0.359*	0.366*	0.354**	0.318*	0.033	-0.067	-	
8	0.256	0.428**	0.324*	0.518*	-0.178	-0.049	0.385**	-

Correlations are denoted as *r*. BRIEF-P inh: Score of the inhibitory control scale of the BRIEF-P; BRIEF-P total: Total score of the BRIEF-P; (M): Questionnaire filled in by mother; (P): Questionnaire filled in by partner. * indicates a *p*-value lower than 0.05, ** indicates a *p*-value lower than 0.01.

Supplementary Table 7.2. Correlations between potential confounding variables and independent and outcome variables.

	Maternal educational level	BRIEF-A-comp	Child Gender
Breastfeeding data			
Exclusive breastfeeding	0.191	0.126	-0.026
Breastfeeding cessation age	0.290*	-0.042	0.098
Behavioural tasks			
Flanker	0.106	-0.006	-0.289*
Whisper	-0.002	0.102	-0.124
Gift Wrap	0.063	-0.179	0.396**
Gift Delay	0.433***	-0.270*	0.140
Inhibitory control composite	0.217	-0.250*	0.132
Questionnaires			
BRIEF-P-inh	0.008	0.351**	0.157
BRIEF-P	-0.084	0.437***	-0.012
REEF	0.080	-0.019	0.021

Correlations are denoted as *r*. BRIEF-P-inh: Score of the inhibitory control scale of the BRIEF-P. BRIEF-A-comp: Composite score of the BRIEF-A filled in by mother and partner. Child gender: 1=boy, 2=girl. *$p<0.05$. **$p<0.01$. ***$p<0.001$.

Supplementary Table 7.3. Parameter Estimates and bootstrapped Confidence Intervals for models 7 and 8.

	B	SE	Lower CI	Upper CI
	Model 7: Exclusive breastfeeding duration → diet quality score → reported executive functioning (BRIEF-P)			
Regression paths				
Reported Executive functioning (BRIEF-P)				
Exclusive breastfeeding duration	-0.242	1.198	-2.590	2.107
Diet quality score	1.007	1.484	-1.902	3.917
Parental executive functioning	0.763***	0.056	0.653	0.873
Diet quality score				
Exclusive breastfeeding duration	0.173*	0.070	0.035	0.310
Mediation effect	0.174	0.260	-0.336	0.684
Total effect	0.695	1.083	-1.429	2.818
	Model 8: Breastfeeding cessation age → diet quality score → reported executive functioning (BRIEF-P)			
Regression paths				
Reported Executive functioning (BRIEF-P)				
Breastfeeding cessation age	0.338	0.204	-0.061	0.738
Diet quality score	1.143	1.364	-1.531	3.817
Parental executive functioning	0.801***	0.081	0.642	0.960
Maternal educational level	-1.272	1.257	-3.736	1.191
Diet quality score				
Breastfeeding cessation age	0.154	0.131	-0.102	0.411
Mediation effect	0.177	0.223	-0.260	0.613
Total effect	0.044	1.123	-2.158	2.245

MLR estimator used to calculate parameter estimates, bootstrapping used to calculate bias-corrected confidence intervals. Model 7: $\chi^2(3)=1.043$, $p=0.307$; CFI=0.998, RMSEA=0.024, SRMR=0.032, $n=67$. Model 8: $\chi^2(4)=1.817$, $p=0.611$, RMSEA=0.000, SRMR=0.046, $n=67$. *$p<0.05$. ***$p<0.001$.

Supplementary Table 7.4. Parameter Estimates and bootstrapped Confidence Intervals for Exploratory Models 1, 2, 5, 6, 7, and 8.†

	B	SE	Lower CI	Upper CI
	Exploratory model 1: Exclusive breastfeeding duration → Diet quality score → Flanker			
Regression paths				
Flanker				
Exclusive breastfeeding duration	0.059	0.048	-0.035	0.153
Diet quality score	-0.065	0.098	-0.257	0.128
Gender	-0.313	0.211	-0.726	0.099
Diet quality score				
Exclusive breastfeeding duration	0.223**	0.072	0.081	0.365
Mediation effect	-0.014	0.021	-0.056	0.028
Total effect	-0.269	0.225	-0.710	0.173
	Exploratory model 2: Breastfeeding cessation age → Diet quality score → Flanker			
Regression paths				
Flanker				
Breastfeeding cessation age	0.026*	0.012	0.003	0.048
Diet quality score	-0.062	0.087	-0.234	0.109
Parental executive functioning	-0.406*	0.188	-0.775	-0.036
Maternal educational level	0.046	0.054	-0.059	0.151
Diet quality score				
Breastfeeding cessation age	0.029	0.018	-0.007	0.065
Mediation effect	-0.002	0.003	-0.007	0.003
Total effect	-0.336	0.190	-0.709	0.036
	Exploratory model 5: Exclusive breastfeeding duration → Diet quality score → Gift Wrap task			
Regression paths				
Gift Wrap task				
Exclusive breastfeeding duration	-0.093	0.048	-0.188	0.001
Diet quality score	0.168	0.092	-0.013	0.349
Gender	0.683**	0.204	0.283	1.082
Diet quality score				
Exclusive breastfeeding duration	0.155*	0.071	0.016	0.293
Mediation effect	0.026	0.019	-0.011	0.063
Total effect	-0.067	0.044	-0.153	0.018
	Exploratory model 6: Breastfeeding cessation age → Diet quality score → Gift Wrap task			
Regression paths				
Gift Wrap task				
Breastfeeding cessation age	-0.008	0.011	-0.030	0.015
Diet quality score	0.114	0.092	-0.066	0.295
Parental executive functioning	0.727***	0.196	0.343	1.112

Supplementary Table 7.4 (continued).

Maternal educational level	0.091	0.059	-0.026	0.207
Diet quality score				
Breastfeeding cessation age	0.156	0.121	-0.082	0.393
Mediation effect	0.018	0.019	-0.020	0.056
Total effect	0.828***	0.174	0.487	1.169
	Exploratory model 7: Exclusive breastfeeding duration → Diet quality score → Gift Delay task			
Regression paths				
Gift Delay task				
Exclusive breastfeeding duration	1.260	1.414	-1.511	4.032
Diet quality score	-1.790	2.187	-6.076	2.497
Parental executive functioning	-0.441*	0.176	-0.788	-0.096
Maternal educational level	6.195**	2.325	1.639	10.752
Diet quality score				
Exclusive breastfeeding duration	0.160*	0.068	0.027	0.294
Mediation effect	-0.287	0.399	-1.069	0.495
Total effect	6.726**	2.496	1.834	11.619
	Exploratory model 8: Breastfeeding cessation age → Diet quality score → Gift Delay task			
Regression paths				
Gift Delay task				
Breastfeeding cessation age	0.603	0.333	-0.049	1.256
Diet quality score	-1.781	2.118	-5.933	2.371
Parental executive functioning	-0.423*	0.167	-0.751	-0.096
Maternal educational level	5.826*	2.255	1.406	10.247
Diet quality score				
Breastfeeding cessation age	0.153	0.125	-0.093	0.398
Mediation effect	-0.272	0.392	-1.040	0.497
Total effect	0.332	0.443	-0.537	1.200

MLR estimator used to calculate parameter estimates, bootstrapping used to calculate bias-corrected confidence intervals. Exploratory model 1: $\chi^2(3)=0.696$, $p=0.404$, CFI=1.000, RMSEA=0.000, SRMR=0.035, $n=52$. Exploratory model 2: $\chi^2(4)=1.592$, $p=0.661$; CFI=1.000, RMSEA=0.000, SRMR=0.047, $n=52$. Exploratory model 5: $\chi^2(2)=0.980$, $p=0.613$; CFI=1.000, RMSEA=0.000, SRMR=0.034, $n=65$. Exploratory model 6: $\chi^2(3)=2.079$, $p=0.721$; CFI=1.000, RMSEA=0.000, SRMR=0.047, $n=65$. Exploratory model 7: $\chi^2(4)=1.881$, $p=0.598$; CFI=1.000, RMSEA=0.000, SRMR=0.045, $n=66$. Exploratory model 8: $\chi^2(4)=1.765$, $p=0.623$, CFI=1.000, RMSEA=0.000, SRMR=0.044, $n=66$. *$p<0.05$. **$p<0.01$. ***$p<0.001$.

†Model 3 (Exclusive breastfeeding duration → Diet quality score → Whisper) and Model 4 (Breastfeeding cessation age → Diet quality score → Whisper) could not be fitted; therefore no parameter estimates could be produced.

Supplementary Table 7.5. Parameter Estimates and bootstrapped Confidence Intervals for Exploratory Models 9a, 10a, 11a, 12a, and 14a.†

	B	SE	Lower CI	Upper CI
	Exploratory model 9a: Exclusive breastfeeding duration → Vegetable intake → Executive functioning (BRIEF-P)			
Regression paths				
Executive functioning (BRIEF-P)				
Exclusive breastfeeding duration	0.647	0.872	-1.062	2.356
Vegetable intake	-0.391	0.239	-0.860	0.077
Parental executive functioning	0.363***	0.088	0.190	0.535
Vegetable intake				
Exclusive breastfeeding duration	0.092	0.461	-0.812	0.996
Mediation effect	-0.036	0.185	-0.400	0.327
Total effect	0.974	0.892	-0.775	2.723
	Exploratory model 10a: Breastfeeding cessation age → Vegetable intake → Executive functioning (BRIEF-P)			
Regression paths				
Executive functioning (BRIEF-P)				
Breastfeeding cessation age	0.341	0.197	-0.044	0.726
Vegetable intake	-0.367	0.232	-0.822	0.089
Parental executive functioning	0.378***	0.088	0.205	0.550
Maternal educational level	-1.004	1.141	-3.247	1.239
Vegetable intake				
Breastfeeding cessation age	-0.033	0.108	-0.246	0.179
Mediation effect	0.012	0.040	-0.066	0.090
Total effect	-0.274	1.129	-2.486	1.939
	Exploratory model 11a: Exclusive breastfeeding duration → Fruit intake → Executive functioning (BRIEF-P)			
Regression paths				
Executive functioning (BRIEF-P)				
Exclusive breastfeeding duration	0.452	0.932	-1.374	2.278
Fruit intake	0.150	0.222	-0.285	0.584
Parental executive functioning	0.352***	0.087	0.182	0.522
Fruit intake				
Exclusive breastfeeding duration	1.215*	0.538	0.160	2.270
Mediation effect	0.178	0.291	-0.392	0.748
Total effect	0.981	0.889	-0.761	2.724
	Exploratory model 12a: Breastfeeding cessation age → Fruit intake → Executive functioning (BRIEF-P)			
Regression paths				
Executive functioning (BRIEF-P)				
Breastfeeding cessation age	0.338	0.201	-0.055	0.731
Fruit intake	0.132	0.202	-0.264	0.528
Parental executive functioning	0.362***	0.090	0.185	0.538

Supplementary Table 7.5. (continued).

Maternal educational level	-0.940	1.284	-3.457	1.576
Fruit intake				
Breastfeeding cessation age	0.107	0.110	-0.108	0.322
Mediation effect	0.014	0.029	-0.043	0.071
Total effect	-0.227	1.271	-2.718	2.265
	Exploratory model 14a: Breastfeeding cessation age → Snack and Candy intake → Executive functioning (BRIEF-P)			
Regression paths				
Executive functioning (BRIEF-P)				
Breastfeeding cessation age	0.347	0.199	-0.043	0.736
Snack and Candy intake	-0.076	0.546	-1.146	0.994
Parental executive functioning	0.358***	0.096	0.170	0.545
Maternal educational level	-1.048	1.274	-3.546	1.449
Snack and Candy intake				
Breastfeeding cessation age	-0.075	0.043	-0.159	0.009
Mediation effect	0.006	0.042	-0.076	0.087
Total effect	-0.338	1.260	-2.808	2.132

MLR estimator used to calculate parameter estimates, bootstrapping used to calculate bias-corrected confidence intervals. Exploratory model 9a: $\chi^2(3)=0.932$, $p=0.334$; CFI=1.000, RMSEA=0.000, SRMR=0.033. Results based on $n=67$. Exploratory model 10a: $\chi^2(3)=1.292$, $p=0.863$; CFI=1.000, RMSEA=0.000, SRMR=0.038. Results based on $n=67$. Exploratory model 11a: $\chi^2(3)=0.684$, $p=0.408$; CFI=1.000, RMSEA=0.000, SRMR=0.025. Results based on $n=67$. Exploratory model 12a: $\chi^2(3)=2.060$, $p=0.725$; CFI=1.000, RMSEA=0.000, SRMR=0.049. Results based on $n=67$. Results based on $n=66$. Exploratory model 14a: $\chi^2(3)=1.939$, $p=0.747$; CFI=1.000, RMSEA=0.000, SRMR=0.049. Results based on $n=67$. *$p<0.05$. ***$p<0.001$.

†Model 13a (Exclusive breastfeeding duration → Snack and Candy intake → Executive functioning (BRIEF-P)) could not be fitted; therefore no parameter estimates could be produced.

Supplementary Table 7.6. Parameter Estimates and bootstrapped Confidence Intervals for Exploratory Models 9b, 10b, 11b, 13b, and 14b.†

	B	SE	Lower CI	Upper CI
	Exploratory model 9b: Exclusive breastfeeding duration → Vegetable intake → Executive functioning (REEF)			
Regression paths				
Executive functioning (REEF)				
Exclusive breastfeeding duration	-1.959	1.530	-4.958	1.041
Vegetable intake	0.379	0.586	-0.769	1.528
Maternal educational level	3.239	2.258	-1.186	7.665
Vegetable intake				
Exclusive breastfeeding duration	0.077	0.461	-0.826	0.981
Mediation effect	0.029	0.173	-0.310	0.368
Total effect	1.310	2.446	-3.484	6.103
	Exploratory model 10b: Breastfeeding cessation age → Vegetable intake → Executive functioning (REEF)			
Regression paths				
Executive functioning (REEF)				
Breastfeeding cessation age	-0.202	0.341	-0.870	0.466
Vegetable intake	0.369	0.596	-0.799	1.538
Maternal educational level	3.087	2.215	-1.255	7.429
Vegetable intake				
Breastfeeding cessation age	-0.006	0.108	-0.218	0.206
Mediation effect	-0.014	0.047	-0.107	0.079
Total effect	2.870	2.157	-1.358	7.099
	Exploratory model 11b: Exclusive breastfeeding duration → Fruit intake → Executive functioning (REEF)			
Regression paths				
Executive functioning (REEF)				
Exclusive breastfeeding duration	-2.547	1.713	-5.905	0.812
Fruit intake	0.443	0.381	-0.304	1.191
Maternal educational level	3.732	2.257	-0.692	8.156
Fruit intake				
Exclusive breastfeeding duration	1.263**	0.470	0.341	2.185
Mediation effect	0.560	0.588	-0.592	1.712
Total effect	1.746	2.383	-2.925	6.417
	Exploratory model 13b: Exclusive breastfeeding duration → Snacks and candy intake → Executive functioning (REEF)			
Regression paths				
Executive functioning (REEF)				
Exclusive breastfeeding duration	-1.943	1.599	-5.077	1.192
Snacks and candy intake	0.163	0.805	-1.415	1.740
Maternal educational level	3.296	2.199	-1.015	7.606
Fruit intake				

Supplementary Table 7.6. (continued).

Exclusive breastfeeding duration	-0.310	0.209	-0.720	0.100
Mediation effect	-0.050	0.251	-0.543	0.442
Total effect	1.303	2.367	-3.336	5.941
	Exploratory model 14b: Breastfeeding cessation age → Snack and Candy intake → Executive functioning (REEF)			
Regression paths				
Executive functioning (REEF)				
Breastfeeding cessation age	-0.230	0.367	-0.949	0.488
Snack and Candy intake	0.274	0.784	-1.263	1.811
Maternal educational level	3.176	2.169	-1.075	7.426
Snack and candy intake				
Breastfeeding cessation age	-0.076	0.049	-0.172	0.020
Mediation effect	-0.021	0.062	-0.142	0.100
Total effect	2.924	2.097	-1.186	7.035

MLR estimator used to calculate parameter estimates, bootstrapping used to calculate bias-corrected confidence intervals. Exploratory model 9b: $\chi^2(2)=0.859$, $p=0.651$; CFI=1.000, RMSEA=0.000, SRMR=0.034. Results based on $n=67$. Exploratory model 10b: $\chi^2(3)=0.204$, $p=0.651$; CFI=1.000, RMSEA=0.000, SRMR=0.016. Results based on $n=67$. Exploratory model 11b: $\chi^2(3)=0.791$, $p=0.374$; CFI=1.000, RMSEA=0.000, SRMR=0.034. Results based on $n=67$. Exploratory model 13b: $\chi^2(2)=0.720$, $p=0.698$; CFI=1.000, RMSEA=0.000, SRMR=0.032. Results based on $n=67$. Exploratory model 14b: $\chi^2(3)=0.019$, $p=0.889$; CFI=1.000, RMSEA=0.000, SRMR=0.005. Results based on $n=67$. $^{**}p<0.01$.
†Model 12b (Breastfeeding cessation age → Fruit intake → Executive functioning (REEF)) could not be fitted; therefore no parameter estimates were produced.

Supplementary materials of chapter 3

Supplementary Table 7.7. Associations between HMOs and HMO groups and measures of executive functioning including partially breastfed infants.

		Estimate (95% CI)	Standard error	*p*-value
BRIEF-P				
Model 1	Intercept	65.07 (40.01 - 90.12)***	12.52	0.00
	2'FL	-0.78 (-2.66 - 1.10)	0.94	0.41
	6'SL	-22.34 (-63.77 - 19.08)	20.69	0.28
	3'SL	34.57 (-11.013 - 80.15)	22.77	0.13
	BRIEF-A	0.20 (0.006 - 0.40)*	0.10	0.04
BRIEF-P				
Model 2	Intercept	139.06 (73.73 - 204.38)***	32.64	0.00
	Fucosylated HMOs	-0.62 (-1.78 - 0.54)	0.58	0.29
	Sialylated HMOs	-14.24 (-28.45 - -0.02)*	7.10	0.05
	BRIEF-A	0.19 (0.002 - 0.38)*	0.09	0.05
REEF				
Model 1	Intercept	166.97 (132.28 - 201.66)	17.34	0.00
	2'FL	3.87 (-0.19 - 7.93)†	2.03	0.06
	6'SL	-40.99 (-130.72 - 48.74)	44.84	0.36
	3'SL	1.18 (-97.01 - 99.36)	49.07	0.98
REEF				
Model 2	Intercept	88.35 (-39.60 - 216.30)	63.97	0.17
	Fucosylated HMOs	2.19 (-0.44 - 4.82)	1.31	0.10
	Sialylated HMOs	1.94 (-29.79 - 33.67)	15.86	0.90
Inhibitory control				
Model 1	Intercept	1.01 (0.22 - 1.80)	0.40	0.01
	2'FL	0.01 (-0.05 - 0.07)	0.03	0.75
	6'SL	-0.94 (-2.25 - 0.37)	0.65	0.16
	3'SL	0.88 (-0.55 - 2.32)	0.72	0.22
	BRIEF-A	-0.01 (-0.01 - -0.002)*	0.003	0.01
Inhibitory control				
Model 2	Intercept	1.54 (-0.57 - 3.65)	1.05	0.15
	Fucosylated HMOs	0.02 (-0.02 - 0.06)	0.02	0.29
	Sialylated HMOs	-0.27 (-0.73 - 0.19)	0.23	0.24
	BRIEF-A	-0.01 (-0.01 - -0.002)*	0.003	0.01

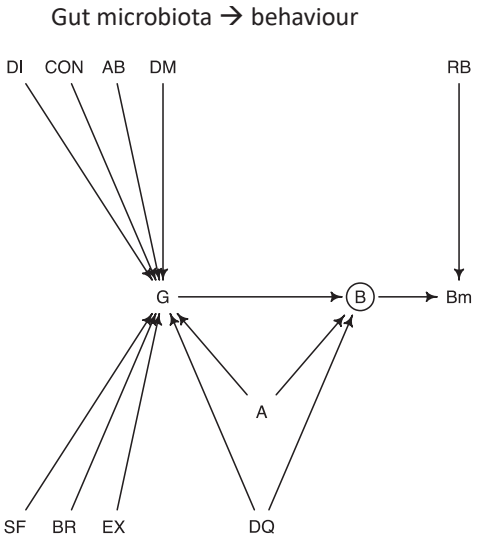
Note that the analyses were performed on data including partially breastfed infants, n=63. The REEF models did not include confounders as none of the potential confounders correlated with the REEF. The BRIEF-P is reverse coded to correspond with the other executive functions and inhibition measures (i.e., higher BRIEF-P scores indicate better executive functions). All HMOs and HMO groups mentioned in this table are presented as the Area Under the Curve. †$p<0.1$, *$p<0.05$, ***$p<0.001$.

Supplementary Table 7.8. Multiple logistic regression results between the HMOs and HMO groups and the BRIEF-P including partially breastfed infants.

		Estimate (95% CI)	Standard error	*p*-value
BRIEF-P				
Model 1	Intercept	-2.79 (-7.51 –1.51)	2.25	0.23
	2'FL	- 0.03 (-0.63 -0.47)	0.26	0.27
	6'SL	-10.36 (-26.00 - 1.86)	6.80	0.65
	3'SL	-12.84 (-44.72 - 11.80)	13.73	0.54
	BRIEF-A	0.03 (-0.02 - 0.10)	0.03	0.14
BRIEF-P				
Model 2	Intercept	3.31 (-10.17 –17.88)	6.97	0.64
	Fucosylated HMOs	-0.04 (-0.42 - 0.27)	0.16	0.27
	Sialylated HMOs	-1.16 (-7.71 - 5.07)	3.14	0.61
	BRIEF-A	0.03 (-0.01 - 0.09)	0.02	0.17

Note that the analyses were performed on data including partially breastfed infants, n=63. All HMOs and HMO groups mentioned in this table are presented as the Area Under the Curve. BRIEF-P coded as: 1, representing the high executive functions group and 0, representing the low executive functions group. Hence, positive values indicate a positive association between higher levels of HMOs and high executive functions.

Supplementary materials of chapter 4



G: relative abundance of the gut microbiota

B: behavioural score of problem behaviour, cognition and inhibitory control at age three (with missing values)

Bm: behavioural score of problem behaviour, cognition and inhibitory control at age three (without missing values)

RB: reasons for missingness

A: child age

DQ: dietary quality at age three

DI: diarrhea in the past one year

CON: constipation in the past one year

AB: antibiotic treatment in the past one year

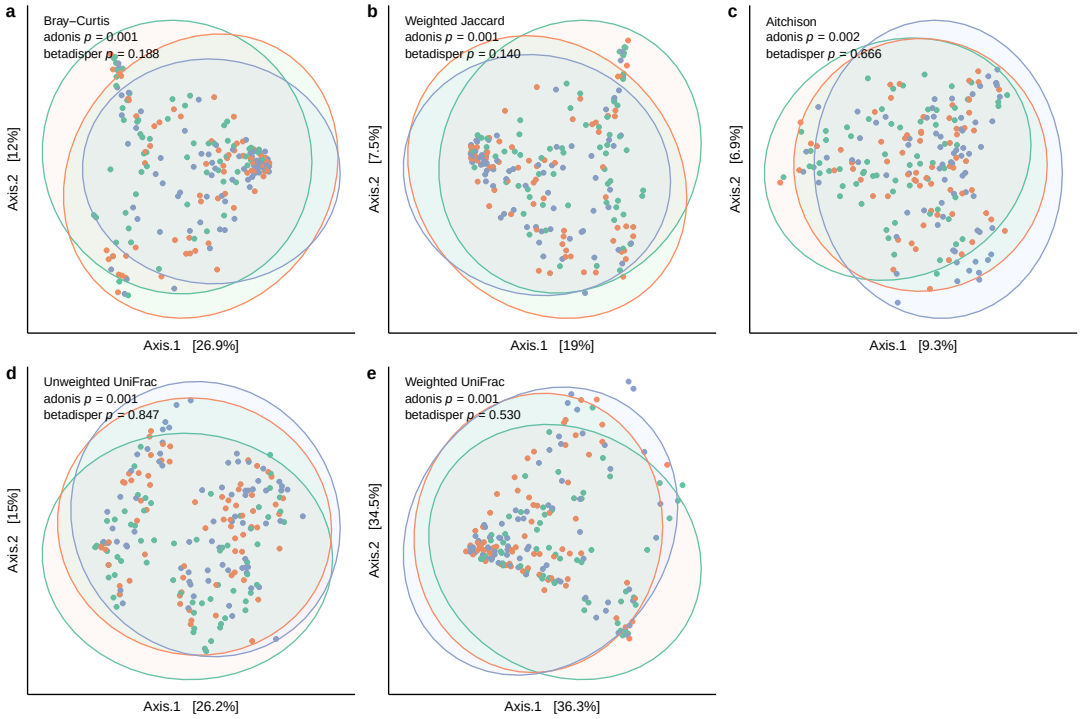
DM: delivery mode

SF: the first time when solid food was introduced

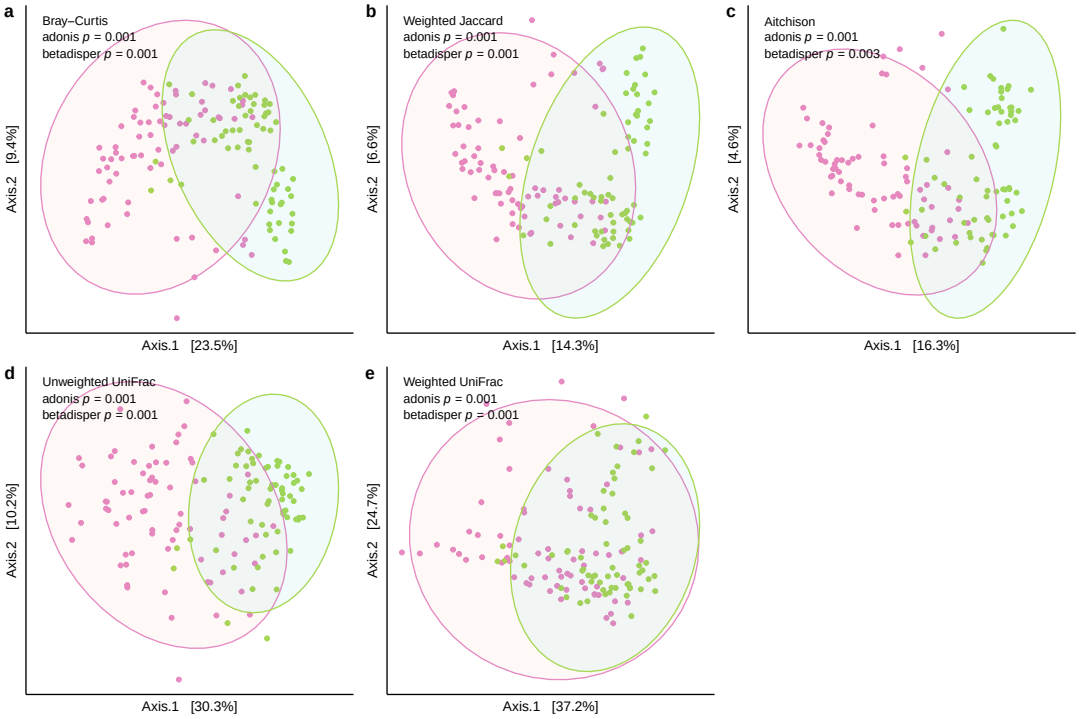
BR: breastfeeding duration

EX: exclusive breastfeeding duration

Supplementary Figure 7.1. Directed Acyclic Graph for determining confounders. Colors: black, predictors (G) and outcomes (B); grey, variables (Bm) and (RB) related to missingness; green, potential confounders that influence (G) and (B), including (A) and (DQ); orange, potential covariates of (G), including (DI), (CON), (AB), (DM), (SF), (BR), and (EX).



Supplementary Figure 7.2. Beta diversity of the gut microbiota at the age of two, six, and 12 weeks. (a-e) Principal coordinate plots of beta diversity, based on different pairwise dissimilarity (Bray-Curtis and weighted Jaccard) and distance (UniFrac and Aitchison) matrices, with points and ellipses colored by ages (Lake blue, two weeks; Orange, six weeks; Purple, 12 weeks).



Supplementary Figure 7.3. Beta diversity of the gut microbiota at the age of one and three years. (a-e) Principal coordinate plots of beta diversity, based on different pairwise dissimilarity (Bray-Curtis and weighted Jaccard) and distance (UniFrac and Aitchison) matrices, with points and ellipses colored by ages (Pink, one year; Grass green, three years).

Supplementary Figure 7.4. Heatmap showing relative abundances of the gut microbiota at the genus level over time. Bacteria with average relative abundances lower than 1% across the first three years, were assigned to 'Others'. Rows of bacteria were clustered based on Euclidean distance.

Supplementary Table 7.9. Kendall correlations between behavioural measures.

	CBCL (M) Internalizing	CBCL (M) Externalizing)	CBCL (P) Internalizing	CBCL (P) Externalizing	SDQ (M) Internalizing	SDQ (M) Externalizing	SDQ (P) Internalizing	SDQ (P) Externalizing	BRIEF-P (M) Total Score	BRIEF-P (P) Total Score	REEF (M) Total Score	REEF (P) Total Score	Flanker	Gift Wrap	Gift Delay
(M) CBCL Externalizing	0.49*	-													
(P) CBCL Internalizing	0.4*	0.21	-												
(P) CBCL Externalizing	0.32*	0.29*	0.54*	-											
(M) SDQ Internalizing	0.4*	0.12	0.21	0.14	-										
(M) SDQ Externalizing	0.14	0.39*	-0.02	0.22	0	-									
(P) SDQ Internalizing	0.3*	0.17	0.34*	0.24	0.31*	-0.06	-								
(P) SDQ Externalizing	0.11	0.26	0.16	0.35*	0.15	0.32*	0.18	-							
(M) BRIEF-P Total Score	0.44*	0.53*	0.27	0.24	0.2	0.29*	0.18	0.22	-						
(P) BRIEF-P Total Score	0.32*	0.21	0.38*	0.37*	0.27	0.21	0.38*	0.23	0.39*	-					
(M) REEF Total Score	-0.19	-0.27*	-0.21	-0.18	-0.1	-0.16	-0.09	0.03	-0.34*	-0.21	-				
(P) REEF Total Score	0.02	-0.19	-0.03	-0.23	-0.01	-0.34*	-0.09	-0.25	-0.13	-0.13	0.24	-			
Flanker	-0.01	0.02	-0.18	-0.02	0.08	0.01	0.04	0.09	0.01	-0.16	0.07	0.04	-		
Gift Wrap	-0.07	-0.03	0.06	0.05	0	-0.09	-0.09	0.17	0.02	0.01	0.15	0.1	0.05	-	
Gift Delay	0.06	0.02	0.15	0.29*	0.14	-0.17	0.22	0.2	0.01	0	0.18	0.07	0.19	0.11	-
Whisper	-0.06	-0.04	-0.1	-0.09	-0.01	-0.1	-0.21	-0.21	-0.14	-0.23	0.21	0.19	0.11	-0.04	0.25*

M, Mother; P, Partner; CBCL, the Child Behavioral Checklist; SDQ, the Strengths and Difficulties Questionnaire; BRIEF-P, Behavior Rating Inventory of Executive Functions - Preschool; REEF, Ratings of Everyday Executive Functioning. Note that increased internalizing and externalizing scores refer to more corresponding behavioural problems. A higher score on the BRIEF-P indicates worse executive functions while a higher score on the REEF indicates better executive functions. Higher scores on the four behavioural tasks mean better performances in inhibitory control. * indicates a *p*-value lower than 0.05.

Supplementary Table 7.10. Reliability of parental questionnaires.

Filler	Questionnaire	Behaviour	ωtotal	Cronbach's α
Mother	CBCL	Internalizing	IC	0.83
		Externalizing	0.92	-
	SDQ	Internalizing	0.65	-
		Externalizing	0.74	-
	BRIEF-P	Executive functions	0.94	-
	REEF	Executive functions	IC	0.96
Partner	CBCL	Internalizing	IC	0.83
		Externalizing	0.84	-
	SDQ	Internalizing	0.65	-
		Externalizing	0.72	-
	BRIEF-P	Executive functions	IC	0.95
	REEF	Executive functions	IC	0.95

IC indicates the estimate was incalculable. Due to better ability at assessing reliability, ωtotal values were used as the first important estimates in determining reliability. For those subscales and questionnaires with incalculable ωtotal, Cronbach's α values were computed as alternatives. All estimates were above 0.65, of which most of estimates were higher than 0.7, indicating good reliability of the scales.

Supplementary Table 7.11. Proportion of missing values in problem behaviour and executive functions.

	Proportion of missing values (%)				
	2w	6w	12w	1y	3y
CBCL Internalizing (M)	16.7	14.3	13.7	12.5	3.1
CBCL Externalizing (M)	16.7	14.3	13.7	12.5	3.1
SDQ Internalizing (M)	16.7	14.3	13.7	12.5	3.1
SDQ Externalizing (M)	16.7	14.3	13.7	12.5	3.1
BRIEF-P Total Score (M)	15.2	12.9	12.3	11.1	1.6
REEF Total Score (M)	16.7	14.3	13.7	12.5	3.1
Flanker	40.9	35.7	37	34.7	26.6
Whisper	19.7	17.1	17.8	15.3	6.2
Gift Wrap	21.2	20	19.2	16.7	6.2
Gift Delay	19.7	18.6	17.8	15.3	4.7

Notes. M, Mother; CBCL, the Child Behavioral Checklist; SDQ, the Strengths and Difficulties Questionnaire; BRIEF-P, Behavior Rating Inventory of Executive Functions - Preschool; REEF, Ratings of Everyday Executive Functioning.

Supplementary Table 7.12. Differentially abundant microbial taxa at the genus level over time with linear discriminant analysis (LDA) scores higher than 2.

Enriched age	Genus	LDA score
2 w	*Enterobacteriaceae* unidentified genus	4.93
2 w	*Streptococcus*	4.56
2 w	*Staphylococcus*	4.43
2 w	[*Ruminococcus*] *gnavus* group	4.3
2 w	*Enterococcus*	4.1
2 w	*Clostridium sensu stricto* 1	3.99
2 w	*Parabacteroides*	3.84
2 w	*Finegoldia*	2.49
2 w	*Negativicoccus*	2.3
6 w	*Lactobacillus*	4.31
6 w	*Actinomyces*	3.99
6 w	*Hungatella*	3.42
6 w	*Megasphaera*	3.4
6 w	*Candidatus Stoquefichus*	2.5
6 w	*Halomonas*	2.49
6 w	*Aeribacillus*	2.12
12 w	*Bifidobacterium*	5.3
12 w	*Bacteroides*	4.54
12 w	*Rothia*	2.85
12 w	*Varibaculum*	2.85
12 w	*Ruminiclostridium*	2.15
1 y	*Faecalibacterium*	4.69
1 y	*Anaerostipes*	4.32
1 y	*Veillonella*	4.23
1 y	*Akkermansia*	4.01
1 y	*Lachnoclostridium*	3.79
1 y	*Erysipelatoclostridium*	3.77
1 y	*Lachnospira*	3.75
1 y	[*Eubacterium*] *eligens* group	3.55
1 y	*Prevotella* 2	3.38
1 y	[*Clostridium*] *innocuum* group	3.38
1 y	*Flavonifractor*	3.2
1 y	*Sutterella*	3.16
1 y	*Tyzzerella* 4	3.09
1 y	*Lachnospiraceae* UCG-004	3.08
1 y	*Eggerthella*	2.98
1 y	*Parasutterella*	2.97
1 y	*Clostridioides*	2.67
1 y	*Tyzzerella* 3	2.6
1 y	CAG:352	2.54
1 y	*Lactococcus*	2.48

Supplementary Table 7.12 (continued).

3 y	*Blautia*	4.78
3 y	*Prevotella* 9	4.56
3 y	*Ruminococcus* 2	4.31
3 y	*Fusicatenibacter*	4.09
3 y	*Roseburia*	4.08
3 y	*Subdoligranulum*	4.06
3 y	*Dialister*	4.03
3 y	[*Eubacterium*] *hallii* group	3.88
3 y	*Erysipelotrichaceae* UCG-003	3.87
3 y	*Dorea*	3.85
3 y	*Lachnospiraceae* ND3007 group	3.79
3 y	*Ruminococcus* 1	3.74
3 y	*Lachnospiraceae* NK4A136 group	3.65
3 y	*Intestinibacter*	3.65
3 y	[*Eubacterium*] *coprostanoligenes* group	3.65
3 y	[*Ruminococcus*] *torques* group	3.58
3 y	*Ruminococcaceae* UCG-002	3.53
3 y	*Alistipes*	3.5
3 y	uncultured genus	3.49
3 y	*Christensenellaceae* R-7 group	3.45
3 y	*Romboutsia*	3.42
3 y	*Phascolarctobacterium*	3.41
3 y	*Butyricicoccus*	3.4
3 y	*Coprococcus* 2	3.4
3 y	uncultured bacterium	3.35
3 y	[*Ruminococcus*] *gauvreauii* group	3.33
3 y	*Barnesiella*	3.26
3 y	*Coprococcus* 3	3.25
3 y	*Prevotella* 7	3.2
3 y	*Senegalimassilia*	3.14
3 y	*Coprococcus* 1	3.06
3 y	*Ruminococcaceae* UCG-013	3.02
3 y	*Holdemanella*	2.99
3 y	*Paraprevotella*	2.99
3 y	*Terrisporobacter*	2.98
3 y	[*Eubacterium*] *ventriosum* group	2.98
3 y	*Ruminococcaceae* NK4A214 group	2.94
3 y	*Sarcina*	2.93
3 y	*Ruminococcaceae* UCG-005	2.93
3 y	*Sellimonas*	2.9
3 y	[*Eubacterium*] *ruminantium* group	2.81
3 y	*Lachnospiraceae* UCG-001	2.81
3 y	*Ruminiclostridium* 6	2.8

Supplementary Table 7.12 (continued).

3 y	*Lachnospiraceae* FCS020 group	2.78
3 y	*Ruminococcaceae* UCG-014	2.76
3 y	*Ruminococcaceae* UCG-004	2.75
3 y	*Adlercreutzia*	2.67
3 y	*Lachnospiraceae* UCG-003	2.64
3 y	CAG:56	2.63
3 y	[*Eubacterium*] *xylanophilum* group	2.63
3 y	*Alloprevotella*	2.62
3 y	*Gordonibacter*	2.54
3 y	*Ruminiclostridium* 5	2.53
3 y	*Ruminococcaceae* UCG-003	2.52
3 y	*Turicibacter*	2.51
3 y	*Butyrivibrio*	2.5
3 y	Family XIII AD3011 group	2.45
3 y	*Odoribacter*	2.4
3 y	*Mollicutes* RF39 uncultured bacterium	2.4
3 y	*Oscillibacter*	2.36
3 y	*Anaeroplasma*	2.23
3 y	*Methanobrevibacter*	2.22
3 y	*Marvinbryantia*	2.15
3 y	*Butyricimonas*	2.1
3 y	*Ruminiclostridium* 9	2.09

Supplementary Table 7.13. Pearson correlations between actual and predicted results from random forest models.

Behaviour at age three	Age of the gut microbiota	Median of Pearson correlation coefficient	Permutation *p*-value	Adjusted permutation *p*-value
CBCL Internalizing (M)	2w	0.04	0.91	1.00
	6w	0.05	0.87	1.00
	12w	0.02	0.96	1.00
	1y	-0.09	0.80	1.00
	3y	-0.16	0.59	1.00
CBCL Externalizing (M)	2w	0.00	0.99	1.00
	6w	-0.33	0.32	1.00
	12w	0.13	0.71	1.00
	1y	0.09	0.78	1.00
	3y	-0.11	0.73	1.00
SDQ Internalizing (M)	2w	0.05	0.88	1.00
	6w	-0.03	0.91	1.00
	12w	0.28	0.37	1.00
	1y	-0.16	0.67	1.00
	3y	0.06	0.85	1.00
SDQ Externalizing (M)	2w	0.05	0.88	1.00
	6w	-0.29	0.37	1.00
	12w	0.10	0.78	1.00
	1y	0.02	0.94	1.00
	3y	0.14	0.67	1.00
BRIEF-P Total Score (M)	2w	0.12	0.70	1.00
	6w	0.07	0.85	1.00
	12w	0.10	0.77	1.00
	1y	0.06	0.82	1.00
	3y	-0.09	0.76	1.00
REEF Total Score (M)	2w	-0.15	0.65	1.00
	6w	-0.07	0.84	1.00
	12w	-0.15	0.66	1.00
	1y	-0.01	0.99	1.00
	3y	-0.11	0.74	1.00
Flanker	2w	-0.07	0.83	1.00
	6w	0.32	0.32	1.00
	12w	0.07	0.84	1.00
	1y	-0.17	0.63	1.00
	3y	0.07	0.83	1.00
Whisper	2w	0.09	0.82	1.00
	6w	-0.06	0.88	1.00
	12w	0.23	0.52	1.00
	1y	0.28	0.37	1.00
	3y	-0.03	0.93	1.00
Gift Wrap	2w	0.02	0.94	1.00
	6w	0.25	0.39	1.00
	12w	0.14	0.67	1.00

Supplementary Table 7.13 (continued).

	1y	-0.02	0.93	1.00
	3y	-0.37	0.23	1.00
	2w	-0.15	0.56	1.00
	6w	-0.09	0.89	1.00
Gift Delay	12w	0.00	1.00	1.00
	1y	0.32	0.32	1.00
	3y	0.04	0.91	1.00

N=1000 permutation tests were performed. FDR adjustments were conducted to the *p*-values.

Supplementary Table 7.14. Microbial taxa and alpha diversity with confident age-stratified relations to behavioural measures and taxa prevalence over time.

Type	Behaviour at age three	Taxa or alpha diversity	Prevalence at 2w	Prevalence at 6w	Prevalence at 12w	Prevalence at 1y	Prevalence at 3y	Prevalence >10% at all ages	Prevalence >10% only at the first three ages	Prevalence >10% only at the last two ages
genus	CBCL Internalizing	*Barnesiella*	0	1	0	7	50	no	no	no
genus	CBCL Internalizing	*Intestinibacter*	0	9	16	78	88	no	no	no
genus	CBCL Externalizing	*Barnesiella*	0	1	0	7	50	no	no	no
genus	CBCL Externalizing	*Butyricicoccus*	0	0	3	56	89	no	no	yes
genus	CBCL Externalizing	*Clostridium sensu stricto* 1	33	50	44	62	83	yes	no	no
genus	CBCL Externalizing	*Parabacteroides*	35	34	36	44	83	yes	no	no
genus	CBCL Externalizing	Streptococcus	94	93	88	90	88	yes	no	no
genus	SDQ Internalizing	[*Ruminococcus*] *torques* group	5	6	4	32	84	no	no	yes
genus	SDQ Internalizing	*Bifidobacterium*	79	87	93	99	100	yes	no	no
genus	SDQ Internalizing	*Blautia*	6	11	14	90	100	no	no	no
genus	SDQ Internalizing	*Ruminococcus* 2	0	0	0	39	94	no	no	yes
genus	SDQ Internalizing	*Sutterella*	8	10	12	46	61	no	no	no
genus	SDQ Externalizing	*Bifidobacterium*	79	87	93	99	100	yes	no	no
genus	SDQ Externalizing	*Butyricicoccus*	0	0	3	56	89	no	no	yes
genus	SDQ Externalizing	*Enterobacteriaceae* unidentified genus	89	93	97	68	31	yes	no	no
genus	SDQ Externalizing	*Halomonas*	12	11	12	0	0	no	yes	no
genus	SDQ Externalizing	*Oscillibacter*	0	0	0	4	22	no	no	no
genus	SDQ Externalizing	*Parabacteroides*	35	34	36	44	83	yes	no	no
genus	BRIEF-P	*Blautia*	6	11	14	90	100	no	no	no
genus	BRIEF-P	*Clostridium sensu stricto* 1	33	50	44	62	83	yes	no	no
genus	BRIEF-P	*Halomonas*	12	11	12	0	0	no	yes	no
genus	BRIEF-P	*Intestinibacter*	0	9	16	78	88	no	no	no
genus	BRIEF-P	*Ruminococcus* 2	0	0	0	39	94	no	no	yes
genus	BRIEF-P	*Streptococcus* [*Ruminococcus*]	94	93	88	90	88	yes	no	no
genus	REEF		5	6	4	32	84	no	no	yes

Supplementary Table 7.14. (continued).

genus	REEF	*torques* group *Halomonas*	12	11	12	0	0	no	yes	no
genus	REEF	*Lachnospiraceae* unidentified genus	5	4	15	78	100	no	no	no
genus	REEF	*Parabacteroides*	35	34	36	44	83	yes	no	no
genus	Flanker	*Anaerostipes*	0	1	3	96	100	no	no	yes
genus	Flanker	*Bacteroides*	56	59	66	86	97	yes	no	no
genus	Flanker	*Ruminococcaceae* UCG-013	0	0	0	33	73	no	no	yes
genus	Flanker	*Subdoligranulum*	2	0	0	31	95	no	no	yes
genus	Flanker	*Sutterella*	8	10	12	46	61	no	no	no
genus	Gift Wrap	*Coprococcus* 3	0	0	0	14	66	no	no	yes
genus	Gift Wrap	*Lachnospiraceae* NK4A136 group	0	0	0	38	89	no	no	yes
genus	Gift Wrap	*Subdoligranulum*	2	0	0	31	95	no	no	yes
genus	Gift Wrap	*Veillonella*	62	64	62	71	17	yes	no	no
alpha diversity	CBCL Internalizing	Chao1	-	-	-	-	-	-	-	-
alpha diversity	REEF	Chao1	-	-	-	-	-	-	-	-
alpha diversity	Gift Wrap	Chao1	-	-	-	-	-	-	-	-
alpha diversity	Gift Wrap	PD	-	-	-	-	-	-	-	-

Supplementary Table 7.15. The multilevel Bayesian results of selected genera and alpha diversity with behavioural measures.

Behaviour at age three	Taxa or alpha diversity	Age of the gut microbiota	Rhat <1.01	Estimate	Estimate error	95% CI	95% CI excluding 0
CBCL Externalizing	*Clostridium sensu stricto* 1	2w, 6w, 12w, 1y, 3y	yes	0	0	[-0.01, 0.01]	no
CBCL Externalizing	*Parabacteroides*	2w, 6w, 12w, 1y, 3y	no	-	-	-	-
CBCL Externalizing	*Streptococcus*	2w, 6w, 12w, 1y, 3y	yes	0.03	0.02	[0, 0.07]	no
SDQ Internalizing	*Bifidobacterium*	2w, 6w, 12w, 1y, 3y	yes	0.09	0.07	[-0.04, 0.22]	no
SDQ Externalizing	*Bifidobacterium*	2w, 6w, 12w, 1y, 3y	yes	-0.04	0.07	[-0.17, 0.09]	no
SDQ Externalizing	*Enterobacteriaceae* unidentified genus	2w, 6w, 12w, 1y, 3y	yes	0.01	0.01	[-0.01, 0.03]	no
SDQ Externalizing	*Parabacteroides*	2w, 6w, 12w, 1y, 3y	no	-	-	-	-
BRIEF-P	*Clostridiumsensu stricto* 1	2w, 6w, 12w, 1y, 3y	yes	0	0	[0, 0.01]	no
BRIEF-P	*Streptococcus*	2w, 6w, 12w, 1y, 3y	yes	0.05	0.02	[0.02, 0.09]	yes
REEF	*Parabacteroides*	2w, 6w, 12w, 1y, 3y	no	-	-	-	-
Flanker	*Bacteroides*	2w, 6w, 12w, 1y, 3y	no	-	-	-	-
Gift Wrap	*Veillonella*	2w, 6w, 12w, 1y, 3y	yes	0.01	0	[0, 0.02]	no
CBCL Internalizing	Chao1	2w, 6w, 12w, 1y, 3y	yes	-0.01	0.02	[-0.06, 0.04]	no
REEF	Chao1	2w, 6w, 12w, 1y, 3y	yes	0.04	0.03	[-0.01, 0.09]	no
Gift Wrap	Chao1	2w, 6w, 12w, 1y, 3y	yes	0.02	0.03	[-0.03, 0.07]	no
Gift Wrap	PD	2w, 6w, 12w, 1y, 3y	yes	0.05	0.03	[-0.01, 0.12]	no
CBCL Externalizing	*Parabacteroides*	2w, 6w, 12w	no	-	-	-	-
SDQ Externalizing	*Halomonas*	2w, 6w, 12w	no	-	-	-	-
SDQ Externalizing	*Parabacteroides*	2w, 6w, 12w	no	-	-	-	-
BRIEF-P	*Halomonas*	2w, 6w, 12w	no	-	-	-	-
REEF	*Halomonas*	2w, 6w, 12w	no	-	-	-	-
REEF	*Parabacteroides*	2w, 6w, 12w	no	-	-	-	-
Flanker	*Bacteroides*	2w, 6w, 12w	yes	0.01	0.01	[-0.01, 0.03]	no
CBCL Externalizing	*Butyricicoccus*	1y, 3y	yes	-0.01	0.08	[-0.17, 0.15]	no
CBCL Externalizing	*Parabacteroides*	1y, 3y	no	-	-	-	-
SDQ Internalizing	[*Ruminococcus*] *torques* group	1y, 3y	yes	-0.22	0.07	[-0.35, -0.07]	yes
SDQ Internalizing	*Ruminococcus* 2	1y, 3y	yes	-0.1	0.08	[-0.26, 0.05]	no
SDQ Externalizing	*Butyricicoccus*	1y, 3y	yes	-0.09	0.09	[-0.25, 0.08]	no
SDQ Externalizing	*Parabacteroides*	1y, 3y	yes	-0.04	0.03	[-0.09, 0.02]	no
BRIEF-P	*Ruminococcus* 2	1y, 3y	yes	-0.11	0.08	[-0.25, 0.04]	no
REEF	[*Ruminococcus*] *torques* group	1y, 3y	yes	-0.05	0.08	[-0.21, 0.09]	no
REEF	*Parabacteroides*	1y, 3y	yes	-0.01	0.03	[-0.06, 0.05]	no
Flanker	*Anaerostipes*	1y, 3y	yes	-0.05	0.09	[-0.23, 0.11]	no

Supplementary Table 7.15. (continued).

Flanker	*Bacteroides*	1y, 3y	yes	-0.07	0.08	[-0.23, 0.08]	no
Flanker	*Ruminococcaceae* UCG-013	1y, 3y	yes	0.1	0.1	[-0.09, 0.29]	no
Flanker	*Subdoligranulum*	1y, 3y	no	-	-	-	-
Gift Wrap	*Coprococcus* 3	1y, 3y	no	-	-	-	-
Gift Wrap	*Lachnospiraceae* NK4A136 group	1y, 3y	yes	-0.07	0.05	[-0.17, 0.01]	no
Gift Wrap	*Subdoligranulum*	1y, 3y	yes	-0.03	0.06	[-0.16, 0.06]	no

Notes. Multilevel Bayesian linear regression models were performed on taxa and alpha diversity over time. Relations in grey rows are confident with 95% CI excluding zero. Chains were regarded converged when Rhat values lower than 1.01. Models that did not meet the Rhat criteria under current settings were not considered in the present study. Given that *Parabacteroides* and *Bacteroides* did not meet the Rhat requirement in the pooled data of all five ages, we did extra trajectory analyses for them after splitting the data into two periods (2w, 6w, and 12w; 1y and 3y; grey coloured text).

Supplementary Table 7.16. Overview of the associations in our study in comparison with findings reported in literature.

Microbial taxa and alpha diversity	In the present study	Literature about problem behaviour or executive functions and inhibitory control	Other literature with similar findings	Other literature with divergent findings	Potential mechanisms
↑ *Parabacteroides* (2w)	↓ Externalizing behaviour	NF	↓ *Parabacteroides* in children with ASD (Strati et al., 2017) (Averina et al., 2020) ↓ *Parabacteroides* in ADHD (Prehn-Kristensen et al., 2018)	↑ *Parabacteroides* in children with ASD (Inoue et al., 2016)	GABA
	↑ Executive functions	NF			
↑ *Parabacteroides* (1y)	↓ Externalizing behaviour	NF			
↑ *Ruminococcus* 2 (1y)	↓ Internalizing behaviour	NF	↓ *Ruminococcus* 2 in MDD patients (Cheung et al., 2019) (Jiang et al., 2015)		Tryptophan/ serotonin
	↑ Executive functions	NF		↑ *Ruminococcaceae* in ADHD patients,and inattention (Szopinska-Tokov et al., 2020)	
↑ [*Ruminococcus*] *Torques* group (3y)	↓ Executive functions	NF			
↑ *Barnesiella* (3y)	↑ Internalizing and externalizing behaviour	NF	↑ *Barnesiella* in (constipated) ASD (Liu et al., 2019a) (Zhao et al., 2019)	↓ *Barnesiella* in ASD (Averina et al., 2020)	GABA
↑ *Butyricicoccus* (1y)	↓ Externalizing behaviour	NF	↑ *Butyricicoccus* in constipated ASD vs non-constipated ASD (Dan et al., 2020)		Butyrate
↑ *Butyricicoccus* (3y)	↑ Externalizing behaviour	NF	↓ *Butyricicoccus* in ASD (Liu et al., 2019a)		
↑ *Streptococcus* (2w)	↑ Externalizing behaviour	NF	↑ *Streptococcus* in ASD (Bundgaard-Nielsen et al., 2020)		GABA and tryptophan
	↓ Executive	NF	↑ *Streptococcus* in Bipolar disorder		

Supplementary Table 7.16. (continued).

	functions		(Järbrink-Sehgal and Andreasson, 2020)		
↑ *Streptococcus* (12w)	↓ Executive functions	NF			
↑ *Clostridium sensu stricto* 1 (1y)	↑ Externalizing behaviour		↑*Clostridium* in ASD (De Angelis et al., 2013); (Kandeel et al., 2020)		Neurotoxins
	↓ Executive functions	↑ *Clostridium* at 2.5 months with higher attention (Aatsinki et al., 2020)			
↑ *Intestinibacter* (12w)	↑ Internalizing behaviour	NF	↑ *Intestinibacter bartlettii* in children with neurodevelopmental disorders (Bojović et al., 2020)		Neurotoxins
	↓ Executive functions	NF			
↑ *Bifidobacterium* (3y)	↑ Internalizing behaviour	NF	↑ *Bifidobacterium* in MDD patients (Knudsen et al., 2021)	↓ *Bifidobacterium* in ASD (Xu et al., 2019) ↑ *Bifidobacterium* less ASD symptoms (Grimaldi et al., 2017) ↓ *Bifidobacterium* in MDD patients (Cheung et al., 2019)	GABA, dopamine, and noradrenaline
	↑ Externalizing behaviour	NF	↑ *Bifidobacterium* in ADHD patients (Aarts et al., 2017)	↑ *Bifidobacterium longus* positive on ADHD (Finegold et al., 2010) (Pärtty et al., 2015)	
↑ *Blautia* (3y)	↓ Internalizing behaviour	NF	↓ *Blautia* in ASD patients	↑ *Blautia* in MDD	NF
	↑ Executive functions	NF	(Liu et al., 2019b)	(Cheung et al., 2019) ↑ *Blautia* with worse ADHD symptoms (Laue et al., 2022)	
↑ *Halomonas* (6w)	↑ Externalizing behaviour	NF			GABA and tryptophan
	↓ Executive functions	NF	↑ *Halomonas* in Alzheimers (Wu et al., 2021)		

Supplementary Table 7.16. (continued).

↑ *Bacteroides* (6w)	↑ Inhibitory control	↑ *Bacteroides* with better cognition at 2 years (Carlson et al., 2017) (Tamana et al., 2021)			GABA
↑ *Subdoligranulum* (1y and 3y)	↓ Inhibitory control	NF		↓ *Subdoligranulum* in patients with anxiety (Chen et al., 2019)	NF
↑ *Anaerostipes* (1y)	↓ Inhibitory control	NF	↓ *Anaerostipes* in children with autism (Iglesias-Vázquez et al., 2020)		Butyrate
↑ *Lachnospiraceae* NK4A136 (1y)	↓ Inhibitory control	NF			NF
↑ *Ruminococcaceae* UCG-013 (1y)	↑ Inhibitory control	NF			NF
↑ *Sutterella* (1y)	↓ Inhibitory control	↑ *Sutterella* with better cognition at age three years (Rothenberg et al., 2021)	↑ *Sutterella* in children with autism (Wang et al., 2013) (Williams et al., 2012)		NF
↑*Coprococcus* 3 (1y)	↓ Inhibitory control	NF		↑ *Coprococcus* 3 in healthy patients compared to patients with anxiety disorder (Chen et al., 2019)	Tryptophan
↑ *Veillonella* (1y)	↑ Inhibitory control	↑ *Veillonella* with better cognition at five years (Guzzardi et al., 2022)			Immune system, interleukin pathways
↑ Alpha diversity (2w)	↓ Internalizing behaviour	↑ Alpha diversity with less internalizing behaviour in boys (Laue et al., 2022) ↓ Alpha diversity in children above the clinical threshold for internalizing behaviour	↓ alpha diversity in ASD children (Kang et al., 2018) (Liu et al., 2019a; Ma et al., 2019)	No difference in alpha diversity between ASD patients and healthy controls (Li et al., 2021)	GABA and norepinephrine

Supplementary Table 7.16. (continued).

	(van de Wouw et al., 2021)		
↑ Executive functions	↑ alpha diversity and worse cognition (Carlson et al., 2017)	↓ alpha diversity in ADHD (Prehn-Kristensen et al., 2018)	No differences in alpha diversity between ADHD patients and healthy controls (Jiang et al., 2018) (Szopinska-Tokov et al., 2020) (Wan et al., 2020) (Richarte et al., 2021)

Notes. NF, Not Found (i.e., no comparable findings in the literature for behavioural problems or executive functions).

Supplementary materials of chapter 5

Supplementary Table 7.17. Raw correlation coefficients between all measured variables.

	Sens 5w	Coop 5w	SP 12m	RA 12m	SP 2.5y	RA 2.5y	SP 10y	RA 10y	SP 14y	RA 14y	DQ	EE	BRIEF IC	GNG	STROOP
Sens 5w	-														
Coop 5w	0.86***	-													
SP 12m	0.17	0.10	-												
RA 12m	0.14	0.12	0.62***												
SP 2.5y	0.03	-0.01	0.06	-0.12	-										
RA 2.5y	0.05	-0.05	-0.04	-0.03	0.46***	-									
SP 10y	0.11	0.12	0.16	-0.01	0.15	0.14	-								
RA 10y	0.04	0.03	0.07	0.07	0.17*	0.19*	0.60***	-							
SP 14y	0.12	0.13	0.01	0.06	0.08	0.01	0.22*	0.20*	-						
RA 14y	0.15	0.14	0.08	0.19*	0.05	0.07	0.21*	0.18*	0.75***	-					
DQ	-0.05	-0.17*	-0.06	0.06	0.00	-0.02	-0.08	0.00	-0.04	-0.02	-				
EE	0.05	0.05	-0.05	-0.07	0.09	0.11	0.04	0.03	-0.07	-0.05	-0.03	-			
BRIEF IC	-0.07	-0.09	-0.08	-0.1	-0.11	0.02	-0.13	-0.11	-0.15	-0.09	-0.02	0.06	-		
GNG	0.21*	0.11	0.09	0.01	0.01	0.01	-0.03	-0.01	-0.01	0.06	-0.07	0.01	0.22**	-	
STROOP	-0.06	0.06	-0.20*	-0.14	0.05	0.00	-0.08	0.01	-0.08	-0.08	0.15	0.18*	-0.10	-0.15	-
Monetary Choice	0.04	0.09	-0.02	-0.13	0.00	0.06	0.06	0.03	-0.09	-0.15	-0.21*	0.00	-0.04	-0.04	-0.07

Sens = sensitivity, Coop = cooperation, SP = supportive presence, RA = respect for child autonomy, DQ = diet quality, EE = Emotional eating, BRIEF = Behavior Rating Inventory of Executive Functions, IC = inhibitory control, GNG = Go/No-Go. *p<0.05, **p<0.01, ***p<0.001.

Supplementary Table 7.18. Mediation analyses estimates of model 1 and model 2 with diet quality as dependent variable.

Model description	Effect (beta)	SE	95% CI LL	95% CI UL
Model 1 Caregiving quality, IC BRIEF, Diet quality				
Direct effect				
Caregiving quality 5w → Diet quality	-0.97	0.64	-2.22	0.29
Caregiving quality 12m → Diet quality	-0.20	1.15	-2.45	2.05
Caregiving quality 2.5y → Diet quality	-0.61	2.08	-4.68	3.46
Caregiving quality 10y → Diet quality	-0.97	1.40	-3.72	1.79
Caregiving quality 14y → Diet quality	-0.15	1.00	-2.10	1.81
IC BRIEF → Diet quality	-1.17	3.15	-7.34	4.99
Caregiving quality 5w → IC BRIEF	0.03	0.02	0.00	0.07
Caregiving quality 12m → IC BRIEF	0.00	0.03	-0.06	0.06
Caregiving quality 2.5y → IC BRIEF	0.03	0.06	-0.09	0.14
Caregiving quality 10y → IC BRIEF	-0.01	0.04	-0.08	0.07
Caregiving quality 14y → IC BRIEF	0.00	0.03	-0.05	0.06
Partial mediation				
Caregiving quality 5w → IC BRIEF → Diet quality	-0.04	0.10	-0.23	0.16
Caregiving quality 12m → IC BRIEF → Diet quality	0.00	0.04	-0.07	0.07
Caregiving quality 2.5y → IC BRIEF → Diet quality	-0.03	0.11	-0.24	0.18
Caregiving quality 10y → IC BRIEF → Diet quality	0.01	0.05	-0.09	0.11
Caregiving quality 14y → IC BRIEF → Diet quality	0.00	0.03	-0.07	0.06
Full mediation				
Caregiving quality 5w → IC BRIEF → Diet quality ← Caregiving quality 5w	-1.00	0.63	-2.24	0.24
Caregiving quality 12m → IC BRIEF → Diet quality ← Caregiving quality 12m	-0.20	1.15	-2.45	2.05
Caregiving quality 2.5y → IC BRIEF → Diet quality ← Caregiving quality 2.5y	-0.64	2.08	-4.70	3.43
Caregiving quality 10y → IC BRIEF → Diet quality ← Caregiving quality 10y	-0.96	1.40	-3.71	1.80
Caregiving quality 14y → IC BRIEF → Diet quality ← Caregiving quality 14y	-0.15	1.00	-2.11	1.80
Model 2 Caregiving quality, IC composite, Diet quality				
Direct effect				
Caregiving quality 5w → Diet quality	-0.88	0.58	-2.02	0.25
Caregiving quality 12m → Diet quality	-0.17	1.20	-2.52	2.18
Caregiving quality 2.5y → Diet quality	-1.18	1.94	-4.97	2.62
Caregiving quality 10y → Diet quality	-1.17	1.23	-3.58	1.23
Caregiving quality 14y → Diet quality	-0.51	0.86	-2.19	1.18
IC composite → Diet quality	5.22*	2.34	0.64	9.80
Caregiving quality 5w → IC composite	-0.04	0.02	-0.08	0.01
Caregiving quality 12m → IC composite	-0.06	0.04	-0.14	0.02
Caregiving quality 2.5y → IC composite	0.03	0.08	-0.12	0.18
Caregiving quality 10y → IC composite	-0.04	0.05	-0.13	0.06
Caregiving quality 14y → IC composite	0.00	0.04	-0.07	0.08
Partial mediation				

Supplementary Table 7.18. (continued).

Caregiving quality 5w → IC composite → Diet quality	-0.19	0.16	-0.50	0.11
Caregiving quality 12m → IC composite → Diet quality	-0.30	0.24	-0.78	0.18
Caregiving quality 2.5y → IC composite → Diet quality	0.15	0.39	-0.62	0.91
Caregiving quality 10y → IC composite → Diet quality	-0.20	0.26	-0.70	0.31
Caregiving quality 14y → IC composite → Diet quality	0.03	0.19	-0.35	0.40
Full mediation				
Caregiving quality 5w → IC composite → Diet quality ← Caregiving quality 5w	-1.08	0.59	-2.24	0.08
Caregiving quality 12m → IC composite → Diet quality ← Caregiving quality 12m	-0.47	1.21	-2.84	1.90
Caregiving quality 2.5y → IC composite → Diet quality ← Caregiving quality 2.5y	-1.03	1.97	-4.90	2.84
Caregiving quality 10y → IC composite → Diet quality ← Caregiving quality 10y	-1.37	1.22	-3.76	1.01
Caregiving quality 14y → IC composite → Diet quality ← Caregiving quality 14y	-0.48	0.89	-2.23	1.27

Note that the BRIEF is reverse scored to align with our inhibitory control measures. Hence higher scores on the BRIEF indicate better inhibitory control. SE: Standard Effect, CI: Confidence interval, LL: Lower limit, UL: Upper limit, IC: Inhibitory control, BRIEF: Behavior Rating Inventory of Executive Functions, 5w: five weeks, 12m: 12 months, 2.5y: 2.5 years, 10y: 10 years, 14y: 14 years, *$p<0.05$.

Supplementary Table 7.19. Mediation analyses estimates of model 3 and model 4 with emotional eating as dependent variable.

Model description	Effect (beta)	SE	95% CI LL	95% CI UL
Model 3 Caregiving quality, IC BRIEF, Emotional Eating				
Direct effect				
Caregiving quality 5w → Emotional Eating	0.25	0.33	-0.41	0.90
Caregiving quality 12m → Emotional Eating	-0.36	0.58	-1.49	0.77
Caregiving quality 2.5y → Emotional Eating	1.03	1.07	-1.07	3.13
Caregiving quality 10y → Emotional Eating	0.00	0.74	-1.45	1.46
Caregiving quality 14y → Emotional Eating	-0.78	0.53	-1.82	0.25
IC BRIEF → Emotional Eating	-0.17	1.68	-3.46	3.13
Caregiving quality 5w → IC BRIEF	0.03	0.02	0.00	0.06
Caregiving quality 12m → IC BRIEF	-0.01	0.03	-0.06	0.05
Caregiving quality 2.5y → IC BRIEF	0.02	0.05	-0.09	0.12
Caregiving quality 10y → IC BRIEF	-0.02	0.04	-0.09	0.05
Caregiving quality 14y → IC BRIEF	0.00	0.03	-0.05	0.05
Partial mediation				
Caregiving quality 5w → IC BRIEF → Emotional Eating	0.00	0.05	-0.10	0.09
Caregiving quality 12m → IC BRIEF → Emotional Eating	0.00	0.01	-0.02	0.03
Caregiving quality 2.5y → IC BRIEF → Emotional Eating	0.00	0.03	-0.06	0.05
Caregiving quality 10y → IC BRIEF → Emotional Eating	0.00	0.03	-0.06	0.06
Caregiving quality 14y → IC BRIEF → Emotional Eating	0.00	0.01	-0.01	0.01
Full mediation				
Caregiving quality 5w → IC BRIEF → Emotional Eating ← Caregiving quality 5w	0.24	0.33	-0.41	0.89
Caregiving quality 12m → IC BRIEF → Emotional Eating ← Caregiving quality 12m	-0.36	0.58	-1.49	0.77
Caregiving quality 2.5y → IC BRIEF → Emotional Eating ← Caregiving quality 2.5y	1.03	1.07	-1.07	3.13
Caregiving quality 10y → IC BRIEF → Emotional Eating ← Caregiving quality 10y	0.01	0.74	-1.44	1.46
Caregiving quality 14y → IC BRIEF → Emotional Eating ← Caregiving quality 14y	-0.78	0.53	-1.82	0.25
Model 4 Caregiving quality, IC composite, Emotional Eating				
Direct effect				
Caregiving quality 5w → Emotional Eating	0.29	0.33	-0.36	0.94
Caregiving quality 12m → Emotional Eating	-0.30	0.58	-1.43	0.83
Caregiving quality 2.5y → Emotional Eating	1.01	1.07	-1.08	3.10
Caregiving quality 10y → Emotional Eating	0.05	0.74	-1.40	1.50
Caregiving quality 14y → Emotional Eating	-0.79	0.53	-1.82	0.24
IC composite → Emotional Eating	1.33	1.29	-1.20	3.85
Caregiving quality 5w → IC composite	-0.04	0.02	-0.08	0.01

Supplementary Table 7.19. (continued).

Caregiving quality 12m → IC composite	-0.05	0.04	-0.12	0.03
Caregiving quality 2.5y → IC composite	0.02	0.07	-0.12	0.15
Caregiving quality 10y → IC composite	-0.03	0.05	-0.12	0.06
Caregiving quality 14y → IC composite	0.00	0.03	-0.06	0.07
Partial mediation				
Caregiving quality 5w → IC composite → Emotional Eating	-0.05	0.06	-0.16	0.06
Caregiving quality 12m → IC composite → Emotional Eating	-0.06	0.08	-0.22	0.09
Caregiving quality 2.5y → IC composite → Emotional Eating	0.02	0.09	-0.16	0.21
Caregiving quality 10y → IC composite → Emotional Eating	-0.04	0.08	-0.19	0.11
Caregiving quality 14y → IC composite → Emotional Eating	0.01	0.05	-0.08	0.10
Full mediation				
Caregiving quality 5w → IC composite → Emotional Eating ← Caregivingquality 5w	0.24	0.33	-0.41	0.89
Caregiving quality 12m → IC composite → Emotional Eating ← Caregiving quality 12m	-0.36	0.58	-1.49	0.77
Caregiving quality 2.5y → IC composite → Emotional Eating ← Caregiving quality 2.5y	1.03	1.07	-1.07	3.13
Caregiving quality 10y → IC composite → Emotional Eating ← Caregiving quality 10y	0.01	0.74	-1.44	1.46
Caregiving quality 14y → IC composite → Emotional Eating ← Caregiving quality 14y	-0.78	0.53	-1.82	0.25

Note that the BRIEF is reverse scored to align with our inhibitory control measures. Hence higher scores on the BRIEF indicate better inhibitory control. CI: Confidence interval, LL: Lower limit, UL: Upper limit, IC: Inhibitory control, BRIEF: Behavior Rating Inventory of Executive Functions, 5w: five weeks, 12m: 12 months, 2.5y: 2.5 years, 10y: 10 years, 14y: 14 years.

Supplementary Table 7.20. Mediation analyses estimates of Exploratory model 1, 2 and 3 with diet quality as dependent variable.

Model description	Effect (beta)	SE	95% CI LL	95% CI UL
Exploratory model 1 Caregiving quality, STROOP, Diet quality				
Direct effect				
Caregiving quality 5w → Diet quality	-1.10	0.64	-2.36	0.1
Caregiving quality 12m → Diet quality	0.33	1.19	-2.00	2.66
Caregiving quality 2.5y → Diet quality	-0.73	2.11	-4.86	3.4
Caregiving quality 10y → Diet quality	-0.79	1.42	-3.58	2.0
Caregiving quality 14y → Diet quality	-0.31	1.01	-2.29	1.6
STROOP → Diet quality	0.28	0.22	-0.14	0.70
Caregiving quality 5w → STROOP	0.08	0.26	-0.43	0.59
Caregiving quality 12m → STROOP	-1.21*	0.47	-2.13	-0.29
Caregiving quality 2.5y → STROOP	0.61	0.85	-1.05	2.28
Caregiving quality 10y → STROOP	-0.03	0.58	-1.16	1.10
Caregiving quality 14y → STROOP	-0.22	0.41	-1.02	0.58
Partial mediation				
Caregiving quality 5w → STROOP → Diet quality	0.02	0.07	-0.12	0.17
Caregiving quality 12m → STROOP → Diet quality	-0.34	0.29	-0.91	0.23
Caregiving quality 2.5y → STROOP → Diet quality	0.17	0.27	-0.36	0.70
Caregiving quality 10y → STROOP → Diet quality	-0.01	0.16	-0.32	0.31
Caregiving quality 14y → STROOP → Diet quality	-0.06	0.12	-0.30	0.18
Full mediation				
Caregiving quality 5w → STROOP → Diet quality ← Caregiving quality 5w	-1.08	0.65	-2.35	0.19
Caregiving quality 12m → STROOP → Diet quality ← Caregiving quality 12m	-0.01	1.17	-2.30	2.28
Caregiving quality 2.5y → STROOP → Diet quality ← Caregiving quality 2.5y	-0.56	2.11	-4.70	3.59
Caregiving quality 10y → STROOP → Diet quality ← Caregiving quality 10y	-0.80	1.43	-3.60	2.01
Caregiving quality 14y → STROOP → Diet quality ← Caregiving quality 14y	-0.37	1.01	-2.36	1.62
Exploratory Model 2 Caregiving quality, Go/No-Go, Diet quality				
Direct effect				
Caregiving quality 5w → Diet quality	-1.01	0.66	-2.30	0.28
Caregiving quality 12m → Diet quality	0.00	1.17	-2.28	2.29
Caregiving quality 2.5y → Diet quality	-0.53	2.11	-4.67	3.61
Caregiving quality 10y → Diet quality	-0.81	1.43	-3.61	1.99
Caregiving quality 14y → Diet quality	-0.35	1.01	-2.33	1.64
Go/No-Go → Diet quality	-0.16	0.27	-0.70	0.37
Caregiving quality 5w → Go/No-Go	0.43*	0.21	0.03	0.84
Caregiving quality 12m → Go/No-Go	0.07	0.37	-0.66	0.80
Caregiving quality 2.5y → Go/No-Go	0.16	0.68	-1.17	1.48
Caregiving quality 10y → Go/No-Go	-0.07	0.46	-0.96	0.83
Caregiving quality 14y → Go/No-Go	0.16	0.32	-0.48	0.80
Partial mediation				

Supplementary Table 7.20. (continued).

Caregiving quality 5w → Go/No-Go → Diet quality	-0.07	0.12	-0.31	0.17
Caregiving quality 12m → Go/No-Go → Diet quality	-0.01	0.06	-0.14	0.11
Caregiving quality 2.5y → Go/No-Go → Diet quality	-0.03	0.12	-0.26	0.21
Caregiving quality 10y → Go/No-Go → Diet quality	0.01	0.08	-0.14	0.16
Caregiving quality 14y → Go/No-Go → Diet quality	-0.03	0.07	-0.16	0.11
Full mediation				
Caregiving quality 5w → Go/No-Go → Diet quality ← Caregiving quality 5w	-1.08	0.65	-2.35	0.19
Caregiving quality 12m → Go/No-Go →Diet quality ← Caregiving quality 12m	-0.01	1.17	-2.30	2.28
Caregiving quality 2.5y → Go/No-Go → Diet quality ← Caregiving quality 2.5y	-0.56	2.11	-4.70	3.59
Caregiving quality 10y → Go/No-Go → Diet quality ← Caregiving quality 10y	-0.80	1.43	-3.60	2.01
Caregiving quality 14y → Go/No-Go → Diet quality ← Caregiving quality 14y	-0.37	1.01	-2.36	1.62
Exploratory model 3 Caregiving quality, Monetary choice, Diet quality				
Direct effect				
Caregiving quality 5w → Diet quality	-0.97	0.63	-2.21	0.28
Caregiving quality 12m → Diet quality	-0.24	1.15	-2.49	2.01
Caregiving quality 2.5y → Diet quality	-0.79	2.07	-4.85	3.27
Caregiving quality 10y → Diet quality	-0.43	1.41	-3.19	2.33
Caregiving quality 14y → Diet quality	-0.72	1.00	-2.69	1.25
Monetary choice → Diet quality	-5.51*	2.29	-10.01	-1.01
Caregiving quality 5w → Monetary choice	0.02	0.02	-0.03	0.07
Caregiving quality 12m → Monetary choice	-0.04	0.04	-0.13	0.04
Caregiving quality 2.5y → Monetary choice	-0.04	0.08	-0.20	0.11
Caregiving quality 10y → Monetary choice	0.07	0.05	-0.04	0.17
Caregiving quality 14y → Monetary choice	-0.06	0.04	-0.14	0.01
Partial mediation				
Caregiving quality 5w → Monetary choice → Diet quality	-0.12	0.14	-0.39	0.16
Caregiving quality 12m → Monetary choice → Diet quality	0.23	0.26	-0.27	0.74
Caregiving quality 2.5y → Monetary choice → Diet quality	0.23	0.44	-0.64	1.10
Caregiving quality 10y → Monetary choice → Diet quality	-0.37	0.33	-1.02	0.28
Caregiving quality 14y → Monetary choice → Diet quality	0.35	0.25	-0.15	0.85
Full mediation				
Caregiving quality 5w → Monetary choice → Diet quality ← Caregiving quality 5w	-1.08	0.65	-2.35	0.19
Caregiving quality 12m → Monetary choice →Diet quality ← Caregiving quality 12m	-0.01	1.17	-2.30	2.28
Caregiving quality 2.5y → Monetary choice → Diet quality ← Caregiving quality 2.5y	-0.56	2.11	-4.70	3.59

Supplementary Table 7.20. (continued).

Caregiving quality 10y → Monetary choice → Diet quality ← Caregiving quality 10y	-0.80	1.43	-3.60	2.01
Caregiving quality 14y → Monetary choice → Diet quality ← Caregiving quality 14y	-0.37	1.01	-2.36	1.62

SE: Standard Effect, CI: Confidence interval, LL: Lower limit, UL: Upper limit, 5w: five weeks, 12m: 12 months, 2.5y: 2.5 years, 10y: 10 years, 14y: 14 years, *$p<0.05$.

Supplementary Table 7.21. Mediation analyses estimates of Exploratory model 4, 5 and 6 with emotional eating as dependent variable.

Model description	Effect (beta)	SE	95% CI LL	95% CI UL
Exploratory model 4 Caregiving quality, STROOP, Emotional eating				
Direct effect				
Caregiving quality 5w → Emotional eating	0.22	0.33	-0.42	0.87
Caregiving quality 12m → Emotional eating	-0.15	0.59	-1.30	1.00
Caregiving quality 2.5y → Emotional eating	0.97	1.06	-1.12	3.05
Caregiving quality 10y → Emotional eating	0.04	0.73	-1.40	1.48
Caregiving quality 14y → Emotional eating	-0.73	0.52	-1.75	0.30
STROOP → Emotional eating	0.16	0.11	-0.05	0.38
Caregiving quality 5w → STROOP	0.13	0.26	-0.37	0.63
Caregiving quality 12m → STROOP	-1.33*	0.45	-2.20	-0.46
Caregiving quality 2.5y → STROOP	0.32	0.83	-1.30	1.95
Caregiving quality 10y → STROOP	-0.11	0.57	-1.23	1.01
Caregiving quality 14y → STROOP	-0.26	0.40	-1.06	0.53
Partial mediation				
Caregiving quality 5w → STROOP → Emotional eating	0.02	0.04	-0.07	0.11
Caregiving quality 12m → STROOP → Emotional eating	-0.22	0.16	-0.53	0.10
Caregiving quality 2.5y → STROOP → Emotional eating	0.05	0.14	-0.22	0.33
Caregiving quality 10y → STROOP → Emotional eating	-0.02	0.09	-0.20	0.17
Caregiving quality 14y → STROOP → Emotional eating	-0.04	0.07	-0.18	0.10
Full mediation				
Caregiving quality 5w → STROOP → Emotional eating ← Caregiving quality 5w	0.25	0.33	-0.40	0.89
Caregiving quality 12m → STROOP → Emotional eating ← Caregiving quality 12m	-0.37	0.58	-1.50	0.76
Caregiving quality 2.5y → STROOP → Emotional eating ← Caregiving quality 2.5y	1.02	1.07	-1.08	3.12
Caregiving quality 10y → STROOP → Emotional eating ← Caregiving quality 10y	0.02	0.74	-1.43	1.48
Caregiving quality 14y → STROOP → Emotional eating ← Caregiving quality 14y	-0.77	0.53	-1.80	0.26
Exploratory Model 5 Caregiving quality, Go/No-Go, Emotional eating				
Direct effect				
Caregiving quality 5w → Emotional eating	0.24	0.34	-0.42	0.90
Caregiving quality 12m → Emotional eating	-0.36	0.58	-1.49	0.77
Caregiving quality 2.5y → Emotional eating	1.03	1.07	-1.07	3.13
Caregiving quality 10y → Emotional eating	0.01	0.74	-1.44	1.46
Caregiving quality 14y → Emotional eating	-0.79	0.53	-1.82	0.25
Go/No-Go → Emotional eating	0.01	0.14	-0.27	0.29
Caregiving quality 5w → Go/No-Go	0.39*	0.19	0.01	0.77

Supplementary Table 7.21. (continued).

Caregiving quality 12m → Go/No-Go	0.04	0.34	-0.62	0.70
Caregiving quality 2.5y → Go/No-Go	0.13	0.63	-1.10	1.37
Caregiving quality 10y → Go/No-Go	-0.19	0.43	-1.04	0.67
Caregiving quality 14y → Go/No-Go	0.09	0.31	-0.52	0.69
Partial mediation				
Caregiving quality 5w → Go/No-Go → Emotional eating	0.00	0.06	-0.11	0.11
Caregiving quality 12m → Go/No-Go → Emotional eating	0.00	0.01	-0.01	0.01
Caregiving quality 2.5y → Go/No-Go → Emotional eating	0.00	0.02	-0.04	0.04
Caregiving quality 10y → Go/No-Go → Emotional eating	0.00	0.03	-0.05	0.05
Caregiving quality 14y → Go/No-Go → Emotional eating	0.00	0.01	-0.02	0.02
Full mediation				
Caregiving quality 5w → Go/No-Go → Emotional eating ← Caregiving quality 5w	0.24	0.33	-0.41	0.89
Caregiving quality 12m → Go/No-Go →Emotional eating ← Caregiving quality 12m	-0.36	0.58	-1.49	0.77
Caregiving quality 2.5y → Go/No-Go → Emotional eating ← Caregiving quality 2.5y	1.03	1.07	-1.07	3.13
Caregiving quality 10y → Go/No-Go → Emotional eating ← Caregiving quality 10y	0.01	0.74	-1.45	1.46
Caregiving quality 14y → Go/No-Go → Emotional eating ← Caregiving quality 14y	-0.78	0.53	-1.82	0.25
Exploratory model 6 Caregiving quality, Monetary choice, Emotional eating				
Direct effect				
Caregiving quality 5w → Emotional eating	0.25	0.33	-0.40	0.90
Caregiving quality 12m → Emotional eating	-0.37	0.58	-1.50	0.76
Caregiving quality 2.5y → Emotional eating	1.03	1.07	-1.07	3.12
Caregiving quality 10y → Emotional eating	0.03	0.74	-1.42	1.49
Caregiving quality 14y → Emotional eating	-0.81	0.53	-1.85	0.24
Monetary choice → Emotional eating	-0.42	1.19	-2.74	1.91
Caregiving quality 5w → Monetary choice	0.03	0.02	-0.02	0.07
Caregiving quality 12m → Monetary choice	-0.02	0.04	-0.10	0.06
Caregiving quality 2.5y → Monetary choice	0.00	0.08	-0.15	0.15
Caregiving quality 10y → Monetary choice	0.05	0.05	-0.05	0.15
Caregiving quality 14y → Monetary choice	-0.06	0.04	-0.13	0.01
Partial mediation				
Caregiving quality 5w → Monetary choice → Emotional eating	-0.01	0.03	-0.08	0.05
Caregiving quality 12m → Monetary choice → Emotional eating	0.01	0.03	-0.05	0.07
Caregiving quality 2.5y → Monetary choice → Emotional eating	0.00	0.03	-0.06	0.06
Caregiving quality 10y → Monetary choice	-0.02	0.06	-0.14	0.10

Supplementary Table 7.21. (continued).

→ Emotional eating				
Caregiving quality 14y → Monetary choice	0.03	0.07	-0.12	0.17
→ Emotional eating				
Full mediation				
Caregiving quality 5w → Monetary choice	0.24	0.33	-0.41	0.89
→ Emotional eating ← Caregiving quality 5w				
Caregiving quality 12m → Monetary choice	-0.36	0.58	-1.49	0.77
→ Emotional eating ← Caregiving quality 12m				
Caregiving quality 2.5y → Monetary choice l	1.03	1.07	-1.07	3.12
→ Emotional eating ← Caregiving quality 2.5y				
Caregiving quality 10y → Monetary choice	0.01	0.74	-1.44	1.46
→ Emotional eating ← Caregiving quality 10y				
Caregiving quality 14y → Monetary choice	-0.78	0.53	-1.81	0.25
→ Emotional eating ← Caregiving quality 14y				

SE: Standard Effect, CI: Confidence interval, LL: Lower limit, UL: Upper limit, 5w: five weeks, 12m: 12 months, 2.5y: 2.5 years, 10y: 10 years, 14y: 14 years, *$p<0.05$.

Supplementary Table 7.22. Exploratory mediation models with the caregiving scores combined.

Model description	Effect (beta)	SE	95% CI LL	95% CI UL
Exploratory model 7 Caregiving quality composite, IC BRIEF, Diet quality				
Direct effect				
Caregiving quality composite → Diet quality	-3.99	2.66	-9.20	1.21
IC BRIEF → Diet quality	-1.54	3.13	-7.68	4.60
Caregiving quality composite → IC BRIEF	0.08	0.07	-0.07	0.22
Partial mediation				
Caregiving quality composite → IC BRIEF → Diet quality	-0.12	0.26	-0.64	0.40
Full mediation				
Caregiving quality composite → IC BRIEF → Diet quality ← Caregiving quality composite	-4.11	2.65	-9.30	1.08
Exploratory model 8 Caregiving quality composite, IC composite, Diet quality				
Direct effect				
Caregiving quality composite → Diet quality	-3.46	2.41	-8.18	1.26
IC composite → Diet quality	5.46*	2.33	0.91	10.02
Caregiving quality composite → IC composite	-0.12	0.10	-0.31	0.08
Partial mediation				
Caregiving quality composite → IC composite → Diet quality	-0.65	0.63	-1.89	0.60
Full mediation				
Caregiving quality composite → IC composite → Diet quality ← Caregiving quality composite	-4.11	2.44	-8.89	0.67
Exploratory model 9 Caregiving quality composite, IC BRIEF, Emotional eating				
Direct effect				
Caregiving quality composite → Emotional eating	-0.41	1.39	-3.13	2.31
IC BRIEF → Emotional eating	0.07	1.69	-3.24	3.37
Caregiving quality composite → IC BRIEF	0.04	0.07	-0.10	0.17
Partial mediation				
Caregiving quality composite → IC BRIEF → Emotional eating	0.00	0.06	-0.12	0.13
Full mediation				
Caregiving quality composite → IC BRIEF → Emotional eating ← Caregiving quality composite	-0.41	1.39	-3.12	2.31
Exploratory model 10 Caregiving quality composite, IC composite, Emotional eating				
Direct effect				
Caregiving quality composite → Emotional eating	-0.22	1.40	-2.95	2.52
IC composite → Emotional eating	1.27	1.30	-1.28	3.81
Caregiving quality composite → IC composite	-0.15	0.09	-0.32	0.03
Partial mediation				
Caregiving quality composite → IC composite →	-0.19	0.22	-0.63	0.25

Supplementary Table 7.22. (continued).

Emotional eating				
Full mediation				
Caregiving quality composite → IC composite → Emotional eating ← Caregiving quality composite	-0.50	1.38	-3.20	2.21

Note that the BRIEF is reverse scored to align with our inhibitory control measures. Hence higher scores on the BRIEF indicate better inhibitory control. *$p<0.05$, CI: Confidence interval, LL: Lower limit, UL: Upper limit, IC: Inhibitory control, BRIEF: Behavior Rating Inventory of Executive Functions.

References

Aarts, E., Ederveen, T. H. A., Naaijen, J., Zwiers, M. P., Boekhorst, J., Timmerman, H. M., Smeekens, S. P., Netea, M. G., Buitelaar, J. K., Franke, B., Hijum, S. A. F. T. v., and Vasquez, A. A. (2017). Gut microbiome in ADHD and its relation to neural reward anticipation. *PloS one*, 12(9):e0183509–e0183509.

Aatsinki, A.-K., Kataja, E.-L., Munukka, E., Lahti, L., Keskitalo, A., Korja, R., Nolvi, S., Häikiö, T., Tarro, S., Karlsson, H., and Karlsson, L. (2020). Infant fecal microbiota composition and attention to emotional faces. *Emotion*, 22(6):1159.

Averina, O. V., Kovtun, A. S., Polyakova, S. I., Savilova, A. M., Rebrikov, D. V., and Danilenko, V. N. (2020). The bacterial neurometabolic signature of the gut microbiota of young children with autism spectrum disorders. *Journal of medical microbiology*, 69(4):558–571.

Bojović, K., Ignjatović, Đ.-d. i., Soković Bajić, S., Vojnović Milutinović, D., Tomić, M., Golić, N., and Tolinački, M. (2020). Gut microbiota dysbiosis associated with altered production of short chain fatty acids in children with neurodevelopmental disorders. *Frontiers in Cellular and Infection Microbiology*, 10:223.

Bundgaard-Nielsen, C., Knudsen, J., Leutscher, P. D. C., Lauritsen, M. B., Nyegaard, M., Hagstrøm, S., and Sørensen, S. (2020). Gut microbiota profiles of autism spectrum disorder and attention deficit/hyperactivity disorder: A systematic literature review. *Gut microbes*, 11(5):1172–1187.

Carlson, A. L., Xia, K., Azcarate-Peril, M. A., Goldman, B. D., Ahn, M., Styner, M. A., Thompson, A. L., Geng, X., Gilmore, J. H., and Knickmeyer, R. C. (2017). Infant gut microbiome associated with cognitive development. *Biological Psychiatry*, 83(2):148–159.

Chen, Y.-h., Bai, J., Wu, D., Yu, S.-f., Qiang, X.-l., Bai, H., Wang, H.-n., and Peng, Z.-w. (2019). Association between fecal microbiota and generalized anxiety disorder: Severity and early treatment response. *Journal of Affective Disorders*, 259:56–66.

Cheung, S. G., Goldenthal, A. R., Uhlemann, A.-C., Mann, J. J., Miller, J. M., and Sublette, M. E. (2019). Systematic Review of Gut Microbiota and Major Depression. *Frontiers in psychiatry*, 10:34.

Dan, Z., Mao, X., Liu, Q., Guo, M., Zhuang, Y., Liu, Z., Chen, K., Chen, J., Xu, R., Tang, J., et al. (2020). Altered gut microbial profile is associated with abnormal metabolism activity of autism spectrum disorder. *Gut microbes*, 11(5):1246–1267.

De Angelis, M., Piccolo, M., Vannini, L., Siragusa, S., De Giacomo, A., Serrazzanetti, D. I., Cristofori, F., Guerzoni, M. E., Gobbetti, M., and Francavilla, R. (2013). Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. *PloS one*, 8(10):e76993.

Finegold, S. M., Dowd, S. E., Gontcharova, V., Liu, C., Henley, K. E., Wolcott, R. D., Youn, E., Summanen, P. H., Granpeesheh, D., Dixon, D., Liu, M., Molitoris, D. R., and Green, J. A. (2010). Pyrosequencing study of fecal microflora of autistic and control children. *Anaerobe*, 16(4):444–453.

Grimaldi, R., Cela, D., Swann, J. R., Vulevic, J., Gibson, G. R., Tzortzis, G., and Costabile, A. (2017). In vitro fermentation of B-GOS: impact on faecal bacterial populations and metabolic activity in autistic and non-autistic children. *FEMS microbiology ecology*, 93(2):fiw233.

Guzzardi, M. A., Ederveen, T. H. A., Rizzo, F., Weisz, A., Collado, M. C., Muratori, F., Gross, G., Alkema, W., and Iozzo, P. (2022). Maternal pre-pregnancy overweight and neonatal gut bacterial colonization are associated with cognitive development and gut microbiota composition in pre-school-age offspring. *Brain, Behavior, and Immunity*, 100:311–320.

Iglesias-Vázquez, L., Van Ginkel Riba, G., Arija, V., and Canals, J. (2020). Composition of gut microbiota in children with autism spectrum disorder: a systematic review and meta-analysis. *Nutrients*, 12(3):792.

Inoue, R., Sakaue, Y., Sawai, C., Sawai, T., Ozeki, M., Romero-Pérez, G. A., and Tsukahara, T. (2016). A preliminary investigation on the relationship between gut microbiota and gene expressions in peripheral mononuclear cells of infants with autism spectrum disorders. *Bioscience, biotechnology, and biochemistry*, 80(12):2450–2458.

Järbrink-Sehgal, E. and Andreasson, A. (2020). The gut microbiota and mental health in adults. *Current opinion in neurobiology*, 62:102–114.

Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., Wang, W., Tang, W., Tan, Z., Shi, J., et al. (2015). Altered fecal microbiota composition in patients with major depressive disorder. *Brain, behavior, and immunity*, 48:186–194.

Jiang, H.-y., Zhou, Y.-y., Zhou, G.-l., Li, Y.-c., Yuan, J., Li, X.-h., and Ruan, B. (2018). Gut microbiota profiles in treatment-naïve children with attention deficit hyperactivity disorder. *Behavioural Brain Research*, 347:408–413.

Kandeel, W. A., Meguid, N. A., Bjørklund, G., Eid, E. M., Farid, M., Mohamed, S. K., Wakeel, K. E., Chirumbolo, S., Elsaeid, A., and Hammad, D. Y. (2020). Impact of clostridium bacteria in children with autism spectrum disorder and their anthropometric measurements. *Journal of Molecular Neuroscience*, 70:897–907.

Kang, D.-W., Ilhan, Z. E., Isern, N. G., Hoyt, D. W., Howsmon, D. P., Shaffer, M., Lozupone, C. A., Hahn, J., Adams, J. B., and Krajmalnik-Brown, R. (2018). Differences in fecal microbial metabolites and microbiota of children with autism spectrum disorders. *Anaerobe*, 49:121–131.

Knudsen, J. K., Bundgaard-Nielsen, C., Hjerrild, S., Nielsen, R. E., Leutscher, P., and Sørensen, S. (2021). Gut microbiota variations in patients diagnosed with major depressive disorder-A systematic review. *Brain and behavior*, 11(7):e02177–e02177.

Laue, H. E., Karagas, M. R., Coker, M. O., Bellinger, D. C., Baker, E. R., Korrick, S. A., and Madan, J. C. (2022). Sex-specific relationships of the infant microbiome and early-childhood behavioral outcomes. *Pediatric research*, 92(2):580–591.

Li, N., Chen, H., Cheng, Y., Xu, F., Ruan, G., Ying, S., Tang, W., Chen, L., Chen, M., Lv, L., et al. (2021). Fecal microbiota transplantation relieves gastrointestinal and autism symptoms by improving the gut microbiota in an open-label study. *Frontiers in cellular and infection microbiology*, 11:948.

Liu, F., Li, J., Wu, F., Zheng, H., Peng, Q., and Zhou, H. (2019a). Altered composition and function of intestinal microbiota in autism spectrum disorders: a systematic review. *Translational psychiatry*, 9(1):43.

Liu, S., Li, E., Sun, Z., Fu, D., Duan, G., Jiang, M., Yu, Y., Mei, L., Yang, P., Tang, Y., et al. (2019b). Altered gut microbiota and short chain fatty acids in chinese children with autism spectrum disorder. *Scientific reports*, 9(1):287.

Ma, B., Liang, J., Dai, M., Wang, J., Luo, J., Zhang, Z., and Jing, J. (2019). Altered gut microbiota in chinese children with autism spectrum disorders. *Frontiers in cellular and infection microbiology*, 9:40.

Prehn-Kristensen, A., Zimmermann, A., Tittmann, L., Lieb, W., Schreiber, S., Baving, L., and Fischer, A. (2018). Reduced microbiome alpha diversity in young patients with adhd. *PloS one*, 13(7):e0200728.

Pärtty, A., Kalliomäki, M., Wacklin, P., Salminen, S., and Isolauri, E. (2015). A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: a randomized trial. *Pediatric research*, 77(6):823–828.

Richarte, V., Sánchez-Mora, C., Corrales, M., Fadeuilhe, C., Vilar-Ribó, L., Arribas, L., Garcia, E., Rosales-Ortiz, S. K., Arias-Vasquez, A., Soler-Artigas, M., et al. (2021). Gut microbiota signature in treatment-naïve attention-deficit/hyperactivity disorder. *Translational psychiatry*, 11(1):382.

Rothenberg, S. E., Chen, Q., Shen, J., Nong, Y., Nong, H., Trinh, E. P., Biasini, F. J., Liu, J., Zeng, X., Zou, Y., Ouyang, F., and Korrick, S. A. (2021). Neurodevelopment correlates with gut microbiota in a cross-sectional analysis of children at 3 years of age in rural China. *Scientific Reports*, 11(1):1–11.

Strati, F., Cavalieri, D., Albanese, D., De Felice, C., Donati, C., Hayek, J., Jousson, O., Leoncini, S., Renzi, D., Calabrò, A., et al. (2017). New evidences on the altered gut microbiota in autism spectrum disorders. *Microbiome*, 5:1–11.

Szopinska-Tokov, J., Dam, S., Naaijen, J., Konstanti, P., Rommelse, N., Belzer, C., Buitelaar, J., Franke, B., Bloemendaal, M., Aarts, E., and Vasquez, A. A. (2020). Investigating the gut microbiota composition of individuals with attention-deficit/hyperactivity disorder and association with symptoms. *Microorganisms*, 8(3):406.

Tamana, S. K., Tun, H. M., Konya, T., Chari, R. S., Field, C. J., Guttman, D. S., Becker, A. B., Moraes, T. J., Turvey, S. E., Subbarao, P., Sears, M. R., Pei, J., Scott, J. A., Mandhane, P. J., and Kozyrskyj, A. L. (2021). Bacteroides-dominant gut microbiome of late infancy is associated with enhanced neurodevelopment. *Gut Microbes*, 13(1):1–17.

van de Wouw, M., Wang, Y., Workentine, M. L., Vaghef-Mehrabany, E., Dewey, D., Reimer, R. A., Tomfohr-Madsen, L., and Giesbrecht, G. F. (2021). Associations Between the Gut Microbiota and Internalizing Behaviors in Preschool Children. *Psychosomatic medicine*, 84(2):159–169.

Wan, L., Ge, W.-R., Zhang, S., Sun, Y.-L., Wang, B., and Yang, G. (2020). Case-control study of the effects of gut microbiota composition on neurotransmitter metabolic pathways in children with attention deficit hyperactivity disorder. *Frontiers in Neuroscience*, 14:127.

Wang, L., Christophersen, C., Sorich, M., Gerber, J., and Angley, M. (2013). A conlon, m. increased abundance of sutterella spp. and ruminococcus torques in feces of children with autism spectrum disorder. mol. *Autism*, 4:42.

Williams, B. L., Hornig, M., Parekh, T., and Lipkin, W. I. (2012). Application of novel pcr-based methods for detection, quantitation, and phylogenetic characterization of sutterella species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. *MBio*, 3(1):10–1128.

Wu, S., Liu, X., Jiang, R., Yan, X., and Ling, Z. (2021). Roles and mechanisms of gut microbiota in patients with alzheimer's disease. *Frontiers in Aging Neuroscience*, 13:650047.

Xu, M., Xu, X., Li, J., and Li, F. (2019). Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. *Frontiers in psychiatry*, 10:473.

Zhao, R.-H., Zheng, P.-Y., Liu, S.-M., Tang, Y.-C., Li, E.-Y., Sun, Z.-Y., and Jiang, M.-M. (2019). Correlation between gut microbiota and behavior symptoms in children with autism spectrum disorder. *Zhongguo Dang dai er ke za zhi= Chinese Journal of Contemporary Pediatrics*, 21(7):663–669.

Chapter 8

Appendices

Nederlandse samenvatting

Research data management statement

Acknowledgements

Curriculum Vitae

Donders Graduate School

Nederlandse samenvatting

Dit proefschrift bevat cross-sectionele en longitudinale onderzoeken die verbanden tussen voeding en gedrag onderzochten. Voor gedrag is er onder andere specifiek gekeken naar executieve functies en inhibitievermogen. Executieve functies zijn belangrijke processen die plaatsvinden in het brein om doelgerichte handelingen zo efficiënt mogelijk uit te voeren. Hierbij kan men bijvoorbeeld denken aan het bakken van een taart: wanneer de oven wordt aangezet voordat het beslag wordt gemaakt, dan is de oven al op temperatuur wanneer het beslag klaar is. Inhibitie, ofwel het vermogen om impulsen te beheersen, speelt een belangrijke rol in deze executieve functies.

De onderzochte verbanden in dit proefschrift reiken van het vroege leven tot de adolescentie. De darmbacterie-brein as, ofwel de communicatieroute tussen de darmbacteriën en het brein, is een belangrijk mechanisme dat de verwachte verbanden zou kunnen verklaren. Het eerste doel van dit proefschrift was om de relaties te onderzoeken tussen borstvoedingsfactoren en gedrag bij peuters. Het tweede doel was om de relaties te onderzoeken tussen darmbacteriën en gedrag bij peuters. Het derde doel was om de rol van de kwaliteit van de zorg van de moeder, meerdere keren gemeten tussen geboorte van het kind en de adolescentie, in het voedingsgedrag van adolescenten te onderzoeken.

In **hoofdstuk 2** is onderzocht of de lengte van de borstvoedingsperiode, ofwel borstvoedingsduur, het inhibitievermogen van een peuter voorspelt. Vervolgens is onderzocht of de dieetkwaliteit van een peuter een tussenrol speelt in deze voorspelling. In de eerste drie jaren na de bevalling, hebben moeders de borstvoedingsduur bijgehouden. Op driejarige leeftijd is het inhibitievermogen van het kind in kaart gebracht met behulp van vier verschillende gedragstaken. Ook zijn er vragenlijsten bij beide ouders afgenomen om het gedrag van het kind in kaart te brengen. De voedingsvragenlijst over de voedingsinname van de peuters is ingevuld door één van de ouders. Er is geen bewijs

gevonden voor een verband tussen borstvoedingsduur en inhibitievermogen. Resultaten van voorgaand onderzoek op dit onderwerp liepen al uiteen, waarschijnlijk onder meer door het gebruik van verschillende meetmethoden. Net als voorgaand onderzoek wees ons onderzoek wel uit dat langere borstvoedingsduur een betere dieetkwaliteit van de peuters voorspelt. Het is echter niet duidelijk wat het mechanisme achter dit verband is. Hoe belangrijk moeders gezonde voeding vinden is mogelijk een belangrijke speler in dit verband.

In **hoofdstuk 3** is er onderzocht of humane melk oligosachariden (HMOs), de executieve functies en het inhibitievermogen van peuters voorspelt. HMOs zijn complexe suikers die aanwezig zijn in moedermelk. Moeders hebben hiervoor op twee, zes en 12 weken een kleine hoeveelheid melk verzameld. Van deze melk is de HMO-samenstelling geanalyseerd. Dezelfde meetmethoden benoemd in hoofdstuk 2 zijn gebruikt om executieve functies en inhibitie bij de peuters te meten. Resultaten lieten zien dat hoge niveaus van gefucosyleerde HMOs gerelateerd zijn aan betere executieve functies bij peuters. Er is geen bewijs gevonden voor een verband tussen gesialyleerde HMOs en executieve functies bij peuters. Onze resultaten wat betreft gefucosyleerde HMOs komen overeen met voorgaand soortgelijk onderzoek in dieren en mensen. Onze resultaten over gesialyleerde HMOs komen gedeeltelijk overeen met soortgelijke studies. Dit komt omdat er uiteenlopende resultaten zijn gevonden in voorgaand onderzoek. Deze verschillen zijn te verklaren door het gebruik van verschillende onderzoeksmethoden en de leeftijden waarop het gedrag van de kinderen is gemeten. Dit is het eerste onderzoek bij mensen dat HMOs heeft gemeten op drie tijdspunten binnen de eerste drie maanden. Replicatie van dit onderzoek is daarom belangrijk.

In **hoofdstuk 4** zijn de relaties tussen darmbacteriën en executieve functies (inclusief inhibitievermogen) van peuters onderzocht. Dezelfde meetmethoden benoemd in hoofdstuk 2 zijn gebruikt om de executieve functies en inhibitie te meten. Op twee, zes en 12 weken en op één en drie jaar, is de compositie van de darmbacteriën van

het kind geanalyseerd. Er zijn verbanden gevonden tussen hogere niveaus van *Streptococcus*, [*Ruminococcus*] *Torques* groep, *Clostridium sensu stricto* 1, *Intestinibacter*, en *Halomonas* en verminderde executieve functies bij peuters. Een hoger niveau van *Bacteroides*, *Parabacteroides*, *Ruminococcus* 2, en *Blautia* en een hogere diversiteit aan verschillende soorten darmbacteriën bleken gerelateerd aan betere executieve functies. Hogere niveaus van *Bacteroides*, *Ruminococcaceae* UCG-013 en *Veillonella* voorspelden betere inhibitievermogen. Hogere niveaus van *Subdoligranulum*, *Lachnospiraceae* NK4A136, *Anaerostipes*, *Sutterella* en *Coprococcus* 3 voorspelden verminderde inhibitievermogen in peuters. Resultaten van voorgaande onderzoeken overlappen gedeeltelijk met de bevindingen van dit proefschrift. Zo heeft eerder onderzoek ook een verband gevonden tussen *Bacteroides* en beter inhibitievermogen. Er zijn echter ook verbanden gevonden die niet in voorgaand onderzoek naar voren kwamen. Verschillen tussen de resultaten van ons onderzoek en eerder onderzoek komt mede doordat er sprake is van verschillende leeftijden en gedragsmaten die zijn onderzocht. Meer (replicatie) onderzoek is nodig om de gevonden relaties te bevestigen en de bewijskracht te versterken.

In **hoofdstuk 5** zijn de voorspellers van de kwaliteit van voeding van adolescenten onderzocht. Kwaliteit van zorg van de moeder is onderzocht op de kinderleeftijden van vijf weken, 12 maanden, twee-en-een-half jaar, 10 jaar en 14 jaar. Dit is onderzocht aan de hand van video's van interacties tussen moeder en kind die door onafhankelijke beoordelaars scores hebben gekregen. Voedingsinname en emotie-eten van adolescenten is middels zelfrapportage verzameld. Verder is het inhibitievermogen van de adolescent gemeten met behulp van drie gedragstaken en een vragenlijst die is ingevuld door de moeder. Er is geen bewijs gevonden voor maternale zorgkwaliteit en inhibitievermogen of dieetkwaliteit. Wel is er bewijs gevonden voor een verband tussen betere inhibitievermogen en betere dieetkwaliteit bij adolescenten. Toekomstig onderzoek zou (zelf)rapportage en objectieve observaties moeten combineren om een beter beeld te

krijgen over kwaliteit van de zorg van moeders (en partners) en hoe dat relateert aan het gedrag van adolescenten (voedingsinname en inhibitievermogen). Langlopende en experimentele onderzoeken zijn nodig om de richting van de verbanden te achterhalen.

Samenvattend, specifieke suikers in moedermelk en bepaalde darmbacteriën voorspellen betere executieve functies bij peuters. Tevens is er een verband gevonden tussen het inhibitievermogen en de dieetkwaliteit van een adolescent. Ondanks dat deze bevindingen geen oorzakelijke verbanden kunnen aantonen, wijzen de resultaten erop dat voeding in het vroege leven en de darmbacteriën mogelijk een rol spelen in de executieve functies en het inhibitievermogen van peuters. Daarnaast suggereren de resultaten dat er samenspel is tussen inhibitievermogen en voedingsinname tijdens adolescentie.

Belangrijkste bevindingen van dit proefschrift:

- Langere borstvoedingsduur voorspelt niet het inhibitievermogen van een peuter, maar wel betere dieetkwaliteit van de peuter op de leeftijd van drie jaar.
- Hogere concentraties van gefucosyleerde HMOs in moedermelk voorspellen betere executieve functies op peuterleeftijd.
- We vonden geen bewijs voor een verband tussen gesialyleerde HMOs in moedermelk en executieve functies op peuterleeftijd.
- Hogere niveaus van de darmbacterieën *Streptococcus*, [*Ruminococcus*] *Torques* groep, *Clostridium sensu stricto* 1, *Intestinibacter*, en *Halomonas* zijn gerelateerd aan verminderde executieve functies in peuters.
- Hogere niveaus van de darmbacterieën *Bacteroides*, *Parabacteroides*, *Ruminococcus* 2 en *Blautia* en hogere diversiteit aan verschillende soorten darmbacteriën zijn gerelateerd aan betere executieve functies in peuters.
- Hogere niveaus van de darmbacterieën *Bacteroides*, *Ruminococcaceae* UCG-013

en *Veillonella* voorspellen beter inhibitievermogen in peuters.

- Hogere niveaus van de darmbacterieën *Subdoligranulum*, *Lachnospiraceae* NK4A136, *Anaerostipes*, *Sutterella* en *Coprococcus* 3 voorspellen verminderd inhibitievermogen in peuters.
- Beter inhibitievermogen van adolescenten is gerelateerd aan betere dieetkwaliteit.
- Er is geen bewijs gevonden dat de zorgkwaliteit van de moeder tijdens de kindertijd de inhibitie en dieetkwaliteit van adolescenten voorspelt.

Research data management statement

Ethics

The BINGO and BIBO study were conducted in accordance with the 1964 Declaration of Helsinki and its later amendments. No formal objections were made against the study protocol of the BINGO study (ECSW2014-1003-189, and ECSW-2018-034) and the BIBO study (SW2017-1303-497, SW2017-1303-498, and ECSW-2018-067) by the ethics committee of the Social Science faculty of the Radboud University, Nijmegen, the Netherlands. A written informed consent was obtained from all participants regarding the use and storage of their data. Participants were informed that they may decide to discontinue the study at any moment, without giving a reason. This research was supported by the European Union's Horizon 2020 Eat2beNice grant (728018 to C. de Weerth and A. Arias Vásquez), a Jacobs Foundation Advanced Research Fellowship (to C. de Weerth), and a Netherlands Organization for Scientific Research VENI grant (016.195.197 to R. Beijers), VIDI grant (575-25-009 to C. de Weerth), and VICI grant (016.Vici.185.038 to C. de Weerth).

FAIR principles

1. Findable

All data from the BINGO and BIBO study, including the raw, cleaned, and master data are stored at the secure network drive of the Donders Institute for Brain, Cognition, and Behavior. The pseudonymization key is included in a subfolder of the BINGO and BIBO folder. Physical data, indicating paper data, are stored in the locked archive of the department of Cognitive Neuroscience at the Donders Institute for Brain, Cognition, and Behavior.

2. Accessible

The data and research documentation are only accessible to researchers involved in the BINGO and/or BIBO project, and to the Developmental Psychobiology (DPB) lab

manager. The data are not freely available since we did not ask participants for consent to store their data in a public online depository. Researchers may ask for permission for the re-use of the data with a methodologically sound proposal. The proposal to access the BINGO and/or BIBO should be directed to carolina.deweerth@radboudumc.nl. If approved, data requestors need to sign a data transfer agreement or research collaboration agreement, depending on the level of collaboration. The anonymous processed data will be shared with the collaborator, and researchers are asked to analyze the data and/or publish the results within two years.

3. Interoperable

Documentation on the set-up of the BINGO and BIBO study can be found in the BINGO and BIBO folder and published papers. Results are reproducible and interpretable as the data, documentation files, and R scripts for the papers described in the current thesis are stored at the secure network drive of the Donders Institute for Brain, Cognition, and Behavior.

4. Reusable

All data will be stored for at least 15 years from moment of data collection and can be reused within this period, as stated in the informed consent forms.

Privacy

The privacy of the BINGO and BIBO study participants was guaranteed by pseudonymization. The pseudonymization key that linked the code to personal data was stored on the secure network drive of the Donders Institute for Brain, Cognition, and Behavior in separate folders for the BINGO and BIBO studies. This key is only accessible to the DPB lab manager, and researchers that are involved in the project depending on their role. To contact participants for new measurement waves, the pseudonymization key was not destroyed since the BINGO study is halted, and may continue, and the BIBO study is currently ongoing. If data is shared, no personal data (e.g., addresses, videos with participant faces) will be shared.

Acknowledgements

It is difficult to describe how I feel about everyone who has been with me in this journey towards getting my doctorate degree. However, I will do my best to put my feelings into words!

First and foremost, thank you **Carolina** for giving me the opportunity to perform research in your lab! Although you might be notoriously famous for your "RBF" ;), I felt that it did not represent how you are as a promotor and a person. Your compassionate guidance and commitment to the improvement of your students have shaped me into the scientist that I am today. Your eye for detail, scientific knowledge, and critical thinking showed me that I have had a professional and experienced promotor on my side. I am fortunate to have had you as my mentor, or rather, a science mom. :)

Roseriet, as my co-promotor, you have been a gigantic help throughout this entire journey. Details on projects were easy for you to remember, which made me feel at ease knowing that you were extremely involved in all phases of my PhD track. Your meticulous mind and pragmatic thinking were a great combination of skills that helped me make important decisions. Thank you! Both you and Carolina are hard workers, and you both have also taught me the value of work-life balance. I think I have grown the most in my life during the years that I was under both your supervision, on a professional as well as personal level.

Alejandro, as my co-promotor, you've been supportive of me since the start of my PhD track. Although our contact was limited, your support came at the right times when situations seemed challenging, or even gloomy. I am grateful for the opportunities you facilitated for me to engage with colleagues both within and beyond the Radboud community. The experience in Rome was unforgettable.

Major gratitude goes to **every single participant of the BINGO and BIBO study**! The completion of this dissertation would have been impossible without your commitment, excitement, and dedication. BINGO was such a wonderful first experience with human data collection! Every single toddler was a pleasure to work with. During the COVID pandemic, my life took a somber turn as I missed the human interactions.

I therefore felt truly fortunate to have the opportunity to visit all BIBO participants, with each one of you adding an extra touch of sunshine to my life.

Thanks to the other co-authors of my papers, **Henk Schols**, **Fangjie Gu**, **Clara Belzer**, and **Hauke Smidt**, whose expertise and input were extremely valuable in guiding our research and in understanding what our data was showing us and how it could be interpreted. Special thanks go to **Jeanne de Vries** and **Corine Perenboom** for their advice and help regarding the assessment of dietary intake.

My PhD journey started at the Developmental Psychology department at the Radboud University in the first 2 years. I have had warm interactions with many who are already continuing their professional journey. **Christine**, your initiation of the BINGO study set the stage for an incredible journey. Thank you so much! **Bonnie**, your quirkiness was refreshing. You showed me that being yourself is the strongest asset one can express. **Pamela**, you are a kind-hearted colleague whose calmness and knowledge were inspirational. **Kelly**, as you always provided a listening ear, your kindness never went unnoticed. It was always good to see you. **Maartje**, of the few interactions that we've had, you struck me as a kind and gentle person. Thanks for handing BIBO over to me :) **Anouk**, thank you for your advice and knowledge about executive functions, and the shared stories. I would also like to thank **Yvonne van den Berg**, **Toon Cillessen**, **Bill Burk**, **Tessa Lansu**, **Remco**, **Sterre**, **Nicole**, **Jesse**, **Ilja**, **Nina**, **Lisan**, and **Geert** for making me feel welcome at the department at the Radboud University (and thanks to the latter 3 for being my brief tennis partners!). I also want to thank **Brianna**, **Daria**, and **Esther** for including me in their activities during the first year of my PhD (I still remember the tasty pumpkin bread!). Special thanks to **Julian** who helped me create a behavioural task in Psychopy for the BIBO study. **Peiying**, I always felt so comfortable with you talking about anything, and I had a lot of fun playing tennis with you! Hope you're doing well! **Kris**, thank you for your major support during this PhD journey!

My PhD journey continued at the Radboudumc. As our lab expanded, I had the pleasure of encountering many kind and intelligent people. **Irene**, my Paranymph, you were the first person that I felt connected to in our new-forming lab. Your presence, your humour, and your accent (:P) made me smile! I am delighted that we could share

our hardships and learnings in the past years. :) **Hellen**, my Paranymph, radiates a contagious brightness. I loved our conversations about science, sustainability, and more personal topics. I admire you and have learned invaluable lessons from our chats. :) **Yang**, my precious colleague and co-author, collaborating on our microbiota paper was a pleasure (despite the challenges we encountered!). Your admirable strength and kindness shine through, and I cherish the conversations we shared. Wishing you all the best in China! **Kathi**, it was so nice to have you as a colleague because of your bright and kind personality. I was happy to hear that you are pursuing something you truly enjoy. **Nicole**, you are the gentlest and kindest person I know. I very much enjoyed our random dinners together, and your BIBO knowledge tests, haha! I wish you all the best for your PhD! **Henrik**, you are intelligent, with a sense of humour and a down-to-earth nature. I am very thankful that I could always ask you microbiota-related questions and receive the most well-constructed answers. **Stefania**, you are such an intelligent and passionate person, and that makes you shine! It was so nice collaborating with you on our caregiving paper :) **Nina**, I can always count on you being at the office ;) I enjoyed our chats (and rants :P) about our PhD lives during lunch. **Evie**, I admire your strong, righteous, and gentle personality. It was always a pleasure to chat with you :) **Ana**, though we were situated in different buildings, it was always good to see you in our lab meetings and at other meet ups :) **Marjorie** and **Dafna**, we have not seen each other that much, but it was always fun and pleasant to chat with you!

Other colleagues, students, and research assistants have played a major role in my PhD trajectory! Special thanks to **Jana**, **Rosa**, and **Ilse** who spent a large amount of time helping me with the home visits, and data coding for BINGO and BIBO! You made my data collection time so much smoother, and much more fun! Thank you **Anna**, for your help setting up the BIBO 12.5 years round. It was a reassurance knowing that we had a brain expert by our side :). **Timothy**, thanks for your help with programming BIBO's curiosity task! Thank you **Els**, **Lisanne**, **Eefje**, **Annefleur**, **Ismay**, **Noortje**, **Hilde**, **Jasmijn**, **Danique**, and **Lonneke** for your help with picking up samples, processing samples, preparing materials and/or entering and coding data for BINGO and BIBO, and the great conversations! Thanks to **Sofia** for helping with

the creation of the BIBOok ;D It was such a comforting feeling to have you all by my side!

Other people walking around at the Donders were a major part of how I experienced my work life. **Vab**, **Casper** (/former Nijmegen tennis partner), **Carolina** (/moving partner), **Alejandro**, **Mariana**, **Sevgi**, **Lea**, **James**, **Emma** (/coffee break partner), **Michael**, **Claire**, **Angelos**, and **Shery** you have all been such great mood-makers during my time at the CNS department. Thank you all for the cosy lunches, shared food, and good/random/silly conversations. :) **Jo** and **Kübra**, two of my former 'Best Office Ever' mates, together with Irene, were such pleasant office mates to be around! Thanks for tolerating my phone calls to participants ;) I really enjoyed our dinners together. Let's do that again sometime! Of course, I want to thank **Erna**, **Inge**, **Annemarie**, and **Ellen** at the secretary! You have always been there to help me out. And thanks to **Nino** for your help with my administrative things (e.g., reminding me to fill in my hours hehe). Thanks to **Andrea** and **Max** from Betty and Mora for the tasty fresh Italian lunches I could always count on!

I would like to thank everyone outside the Radboud who has supported me during my PhD journey. To **Nard**, the first to believe in this PhD adventure, thank you for your unwavering belief and support! **Olga**, our conversations have helped me get through my darker days, thank you! **Dewi**, our annual life updates feel like a comforting tradition. I'm grateful to have you capturing my major life event. **Marjolein**, I loved our catch-ups every so often. Your empathy always made me feel comfortable to talk to you about everything. **Ivy Mok**, I enjoyed our chats and rants about life! I hope you are doing well.

I would like to thank **my colleagues at Danone Nutricia Research**, as the end my PhD journey partly overlapped with my start at this company within this amazing team. From the first moment, I felt very welcomed by everyone. All of your interest in my PhD research has made me feel recognised and extra proud. So thank you all, and especially **Raka**, **Nana**, **Elly**, **Loret**, and **Evelien**.

My most heartfelt gratitude goes to the closest people in my life, people who know best how this entire PhD journey impacted me. **Aaron**, we met quite close to the start

of my PhD and our conversations have been a constant source of comfort. **Ivy**, from elementary school age to adulthood, our occasional contact is a cherished comfort I can always count on. **Liselotte**, since the age of 12, you have always been by my side. I think we both have grown a lot. Thanks for hearing out about my PhD (and other) struggles and joys these past 6 years. **Maga**, throughout the challenges during this PhD, you have been my rock. Thank you for always being there. Of course, I want to thank **my parents** for being patient with me, and hearing out all my experiences during the past 6 years (and my entire life!). I'm excited to bring the first Doctor's title into the family. **Nishant**, you've been there with me at the end of my PhD journey, and have helped me tremendously in ways I cannot describe. Thank you for your unwavering support and love. I love you all!

Thank you all! Dank jullie wel! 謝謝大家!

Curriculum Vitae

Yvonne Willemsen was born in Wageningen on December 6th, 1992. She obtained her Bachelor's degree of Nutrition and Dietetics, at the Hogeschool Arnhem en Nijmegen in Nijmegen, minoring in infant and child nutrition. To understand the science behind what was taught during her Bachelor's studies, she continued studying Nutrition and Health at Wageningen University & Research, where she completed two specializations: Molecular Nutrition & Toxicology and Nutritional Physiology & Health Status. For both these specializations, her theses focused on toddler, and child nutrition. Within this Master's track, she also did her internship at TNO where she wrote a review on gut microbiota, antibiotics use in early life, and its relations with obesity development. All these previous experiences contributed to her growing interest in the interactions between gut microbiota, nutrition, and health. She was granted an ideal fitting PhD position at the Radboud University (RU) and the Radboudumc (RUMC) in Nijmegen. Under the supervision of Prof. dr. Carolina de Weerth (RUMC), Dr. Roseriet Beijers (RU) and Dr. Alejandro Arias Vásquez (RUMC), she conducted research on (early life) nutrition, gut microbiota, and behaviour as described in this thesis.

Donders Graduate School for Cognitive Neuroscience

For a successful research Institute, it is vital to train the next generation of young scientists. To achieve this goal, the Donders Institute for Brain, Cognition and Behaviour established the Donders Graduate School for Cognitive Neuroscience (DGCN), which was officially recognised as a national graduate school in 2009. The Graduate School covers training at both Master's and PhD level and provides an excellent educational context fully aligned with the research programme of the Donders Institute.

The school successfully attracts highly talented national and international students in biology, physics, psycholinguistics, psychology, behavioral science, medicine and related disciplines. Selective admission and assessment centers guarantee the enrolment of the best and most motivated students.

The DGCN tracks the career of PhD graduates carefully. More than 50% of PhD alumni show a continuation in academia with postdoc positions at top institutes worldwide, e.g., Stanford University, University of Oxford, University of Cambridge, UCL London, MPI Leipzig, Hanyang University in South Korea, NTNU Norway, University of Illinois, North Western University, Northeastern University in Boston, ETH Zürich, University of Vienna etc. Positions outside academia spread among the following sectors: specialists in a medical environment, mainly in genetics, geriatrics, psychiatry and neurology. Specialists in a psychological environment, e.g., as specialist in neuropsychology, psychological diagnostics or therapy. Positions in higher education as coordinators or lecturers. A smaller percentage enters business as research consultants, analysts or head of research and development. Fewer graduates stay in a research environment as lab coordinators, technical support or policy advisors. Upcoming possibilities are positions in the IT sector and management position in pharmaceutical industry. In general, the PhDs graduates almost invariably continue with high-quality positions that play an important role in our knowledge economy.

For more information on the DGCN as well as past and upcoming defenses please visit: http://www.ru.nl/donders/graduate-school/phd/

9 789493 296374

DONDERS INSTITUTE

Radboud University

In Dei Nomine Feliciter

Radboudumc