# Eat2beNICE

**Effects of Nutrition and Lifestyle on Impulsive, Compulsive, and Externalizing Behaviours**

**H2020 - 728018**

# D5.1: Manuscript: Manuscript on the effect of early diet (birth to 2.5y) on microbiome composition and its relation with impulsive/compulsive behaviour

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## Author list


| Organisation | Name | Contact information |
|---|---|---|
| RUMC | Yvonne Willemsen | Yvonne.willemsen1@radboudumc.nl |
| RU | Roseriet Beijers | r.beijers@psych.ru.nl |
| RUMC | Alejandro Arias Vasquez | Alejandro.AriasVasquez@radboudumc.nl |
| RUMC | Carolina de Weerth | Carolina.deweerth@radboudumc.nl |


# Introduction

Inhibitory control is important for controlling impulsive behavior (American Psychological Association, 2018), and is an integral part of higher-order executive functioning (EF) (Diamond, 2013). Studies have associated poor inhibitory control with psychopathology, such as attention deficit hyperactivity disorder (ADHD), and child internalizing and externalizing problems (Gagne, Chang, Fang, Spann, & Kwok, 2019; Gagne, Saudino, & Asherson, 2011; Lipszyc & Schachar, 2010). Several factors have been associated with higher levels of inhibitory control, such as high socioeconomic status, high parenting quality, and genetic factors (Cheng, Lu, Archer, & Wang, 2018; Engelhardt, Briley, Mann, Harden, & Tucker-Drob, 2015; St. John, Kibbe, & Tarullo, 2019). Nutrition has also been associated with inhibitory control, though its exact role remains unclear (Egbert, Creber, Loren, & Bohnert, 2019). 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 an infant. As such, breastfeeding plays an important role in health and early development (Duijts, Ramadhani, & Moll, 2009; Victora et al., 2016). For example, children who were breastfed longer than six months have better cognitive outcomes compared to children who were breastfed for shorter times (Bar, Milanaik, & Adesman, 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). Additionally, while one study found that preschoolers EF was higher when they were breastfed longer (Julvez et al., 2007), another study found no relation between breastfeeding and EF in 6- to 7-year-old children (Belfort et al., 2016). To our knowledge, no study has specifically examined associations between breastfeeding history and inhibitory control in preschool children.

The association between habitual diet and child EF, including inhibitory control, is equivocal (for a review, see Egbert et al., 2019). For example, the two studies performed in preschool-aged children did not find a relation between inhibitory control and calories or protein consumed (Levitan et al., 2015; Pieper & Laugero, 2013), though Levitan et al. (2015) did find that higher intake of sugars was associated with worse inhibitory control in preschoolers. As the development of problem behavior typically occurs around preschool age, and behavior 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, to our knowledge, the role of breastfeeding has only been investigated in relation to ADHD and EF. 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. The main aim of this study was to investigate whether breastfeeding history (i.e exclusive breastfeeding duration and breastfeeding cessation age) is predictive of inhibitory control behavior at age 3 years, and if this association is partially mediated by habitual diet quality. As most previous studies investigated EF (Belfort et al., 2016; Hayatbakhsh, O'Callaghan, Bor, Williams, & Najman, 2012; Julvez et al., 2007), for generalization purposes, this study investigated EF as a secondary outcome.

## Abbreviations

**ADHD** Attention-Deficit/Hyperactivity Disorder
**EF** Executive functioning
**24-h** 24 hour
**BRIEF** Behavior Rating Inventory of Executive Function
**REEF** Ratings of Everyday Executive Functioning

## 1. Deliverable report

Note: publishers' policies prevent sharing of detailed results prior to publication in a peer-reviewed journal. Detailed results, tables and uncompromised figures will be made available upon acceptance and/or as soon as the publisher's embargo has been lifted.

### Methods

#### Participants

This study is part of the ongoing longitudinal *BINGO* study investigating early predictors of child development. Participants are healthy children living in the Netherlands, whose parents were recruited during pregnancy. Detailed information about the in- and exclusion criteria are mentioned in the paper by Hechler, Beijers, Riksen-Walraven, & de Weerth (2018). At the 36-month measurement round, 76 families were contacted. Six families did not participate due to lack of time, 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 nonparticipating families. In total, 67 families participated. For 54 families (81%), both parents participated, and for 13 families (19%), only mothers participated. Three families were too busy to participate in a home visit and only filled in the questionnaires. The *BINGO* study was approved by the Ethical Committee of the faculty of Social Sciences of the Radboud University [ECSW2014-1003-189 and amendment: ECSW-2018-034]. The current study was preregistered at the Open Science Framework: https://osf.io/5mgnf and amendment: https://osf.io/35tg6.

#### Procedure

Information on breastfeeding cessation age (age when child stopped receiving breastfeeding) and exclusive breastfeeding duration (no additional solids or formula feeding next to breastfeeding) was collected via maternal questionnaires at two, six and 12 weeks, and at 12 and 36 months of age. At 36 months, six inhibitory control tasks were performed during a home visit. The tasks were video recorded, and afterwards rated by two independent, 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 (24h) recalls before the home visit to assess their child's habitual nutritional intake. If parents had missed a 24h-recall, a recall was performed during the home visit, or was sent online after the home visit.

### Measures

#### *Breastfeeding history*

At age two, six and 12 weeks, mothers were asked if they exclusively breastfed the child. At age 12 months, mothers reported the child's feeding history (i.e. 1) are you breastfeeding? (yes or no; when 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, if applicable. To determine breastfeeding history as accurately as possible, the breastfeeding data from the 36-months measurement round was compared to that of the 12-months, 12-weeks, 6-weeks and 2-weeks rounds. If data between the time points was conflicting, data with the shortest recall time was used. Breastfeeding cessation age correlated significantly with exclusive breastfeeding duration ($r$=0.59,

$p$<0.001). Because the significant correlation was lower than 0.7, the main analyses were ran for both variables separately.

*Diet quality*

Three online 24h food intake recalls, using *Compl-eat*™ (Meijboom *et al.*, 2017), were used to measure children's nutritional intake. Parents were asked to report the dietary intake of their child from the previous day on two random weekdays and one random weekend day. Scores were given to each 24h-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 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. For example, vegetable intake of 80 grams per day was assigned a score of 0.8 (80/100 g/d). The score was truncated at 1. This was reverse-scored for intake of candy and snacks, and sugar-sweetened beverages. The scores for the 10 food groups were summed up to a total diet quality score for each 24-hour recall. The average of the three diet scores was used as the overall diet quality score. A higher score corresponds with a higher diet quality.

*Inhibitory control tasks*

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

Snack Delay (Beijers, Riksen-Walraven, Putnam, de Jong, & de Weerth, 2013; Kochanska, Murray, Jacques, Koenig, & Vandegeest, 1996)**.** To measure delay of gratification, the Snack Delay task 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 refrain from taking the snack until 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. The child's waiting behavior 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). Due to insufficient variation (no child ate the snack or touched the cup), this task was excluded from analyses.

Whisper (Beijers *et al.*, 2013; Kochanska *et al.*, 1996)**.** To measure verbal inhibition, the Whisper task 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). Non-responding was coded as missing. Mean scores were used to determine inhibitory control.

Bear Dragon (Kochanska *et al.*, 1996; Reed, Pien, & Rothbart, 1984)**.** To measure go/no-go, the Bear Dragon task 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). The children were told to obey the commands of the bear and ignore the commands of the dragon. After a maximum of three practice trials, 10 test trials followed. Child behavior 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 summed. Due to the low number of children (*n*=31) that passed the practice trials, this task was excluded from the analyses. This low number is similar to a study by Kloo & Sodian (2017), where more than 50% of the preschoolers failed the practice trials.

Flanker (Eriksen & Eriksen, 1974). The Flanker task was used to measure motoric 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 (other fish oriented in the same or opposite directions, flanking the target fish). 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 subsequently averaged.

Gift Wrap (Beijers *et al.*, 2013; Kochanska *et al.*, 1996). To measure motoric inhibition, the Gift Wrap task 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. Gift-wrapping lasted one minute. The child's waiting behavior 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). The scores were summed up.

Gift Delay (Kochanska *et al.*, 1996). To measure impulse control, the Gift Delay task 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 minute. The child's latency (measured in seconds) towards touching the present was used as a measure of impulse control. A higher score indicates better inhibitory control.

*Reliability of coding*

All recordings were observed by two observers independently. To determine inter-rater reliability, 30 out of 65 tapes 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).

*Parental questionnaires on inhibitory control and executive functioning*

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

BRIEF-P (Sherman & 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 inhibitory subscale, scored on a 3-point scale. As higher scores indicate worse EF and inhibitory control, and to align with our other inhibitory control 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 alpha's were good for the inhibitory control subscale and the total EF score (mothers: $\alpha$=0.89, and $\alpha$=0.94, respectively, and partners: $\alpha$=0.84, and $\alpha$=0.96, respectively).

REEF (Nilsen, Huyder, McAuley, & Liebermann, 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 inhibitory 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 variables*

The following confounding variables were taken into account: maternal educational level (ranging from 1= Primary education, to 8=University education), child gender (1=boy, 2=girl), and parental inhibitory control. For this last confounder, parental inhibitory control was assessed with the Behavior Rating Inventory of Executive Function-Adult (BRIEF-A). The BRIEF-A is a 75-item self-report questionnaire of EF in adults, and contains an 8-item inhibitory 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 alpha's for the subscale inhibitory control were insufficient for both mothers ($\alpha$=0.57) and partners ($\alpha$=0.54). Therefore, the parental total score of the BRIEF-A was used as a confounder (Cronbach's alpha was 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, Zoccali, MacLeod, & Dekker, 2008).

## Statistical analysis

First, descriptive analyses and normality analyses were computed. As not all variables were normally distributed, the main analyses accounted for non-normally distributed data by using robust estimators. Moreover, the data was inspected for outliers. Two variables contained one outlier each (breastfeeding cessation age, and the maternal BRIEF-P), and were subsequently winsorized (Blaine, 2018). Results were similar with and without winsorinzing. Pearson and Spearman correlations were performed to correlate (non-normally distributed) variables. Due to the longitudinal nature of our study, the sample size could not be adjusted for the data collection. According to Fritz & MacKinnon (2007), with a power of 0.8, our study was able to detect medium to large effect sizes ($\beta \geq 0.39$ and $\beta \geq 0.59$, respectively). Robust estimators were used to account for small sample size (Tabachnick & Fidell, 2014).

*Missing Data*

The following 24h-recall data was missing: day one (*n*=1), day two (*n*=6) and day three (*n*=12). Missing 24h-recall data were imputed by means of expected maximization to allow for calculation of the average diet quality score. The following behavioral data was missing: Whisper (*n*=4), Flanker (*n*=18), Gift Wrap (*n*=5), and Gift Delay (*n*=4). The following questionnaires data was missing: REEF of the mother (*n*=1), and the REEF (*n*=16), BRIEF-P (*n*=15) and BRIEF-A (*n*=21) of the partner. The LittleMCAR test from the BaylorEdPsych package indicated that data was missing completely at random ($X^2$=272.547, $p$=0.445). Missing data were accounted for by means of Full Information Maximum Likelihood (FIML) in the analyses. In addition, the Yuan-Bentler scaled Chi-square estimator with Huber-White covariance adjustment to the standard errors of each parameter estimate was used.

*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 & 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). The models were adjusted based on the 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 of CFI >0.95, RMSEA <0.05, SRMR <0.05 and a non-significant $X^2$ ($p$ >0.05) (Tabachnick & Fidell, 2014). Bias-corrected confidence intervals were obtained by use of bootstrapping.

To test whether breastfeeding history was predictive of inhibitory control, and if this association was mediated by diet quality at age 3, four SEM models were run. 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 behavioral tests. In model 3 and 4, reported inhibitory control (BRIEF-P inhibitory control scale, mother and partner combined) was used as the outcome measure.

*Generalization analysis*
For generalization purposes, we investigated whether breastfeeding duration (model 5) and breastfeeding cessation age (model 6) were related to EF at age 3, 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 breastfeeding cessation age (model 8) were predictive of reported EF determined by the composite score of the BRIEF-P, and whether exclusive breastfeeding duration (model 9) and breastfeeding cessation age (model 10) were predictive of reported EF determined by the composite score of the REEF. In model 5 to 10, the mediating role of habitual diet quality at age 3 was also assessed.

*Exploratory analysis*
Exploratorily, we tested if exclusive breastfeeding duration and breastfeeding cessation age were predictive of individual inhibitory control behavior tasks, and whether this was mediated by diet quality at age 3. 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. Because of low variation of fish intake in the current study population, fish intake was not investigated.

## Results

### Preliminary analyses

*Descriptives*
Descriptive statistics of the study population are shown in Table 2. The study population is mostly highly educated. Scores on questionnaires differed significantly between mothers and partners for the BRIEF-P and the REEF. Average diet quality score was a 3.9. Mean diet quality score for a previous study in Dutch children between 12-28 months=4.1 (Voortman *et al.*, 2015). Table 3 shows the correlations between the study variables. Longer duration of exclusive breastfeeding is significantly correlated with cessation of breastfeeding ($r$=0.638, $p$<0.001), and with better diet quality at age 3 ($r$=0.321, $p$=0.009). There were no significant correlations between all behavioral tasks ($r$ between -0.117 and 0.211).

*Latent variable and composite score creation*

Main analyses. Because the behavioral tasks and questionnaires did not correlate highly ($r$=<0.7), assumption testing for latent variable creation was performed (see Statistical Analyses). First, assumption testing was performed for the behavioral tasks: Flanker, Whisper, Gift Wrap, and Gift Delay. KMO analysis values ranged between 0.41 and 0.55. Bartlett's test did not reach significance, and determination testing showed a value of 0.934. Because KMO values and Bartlett's test did not meet the cut off values, a latent variable for the behavioral tasks was not created. Instead, a composite score was made by averaging z-scores of the inhibitory control tasks. An average score was calculated if a maximum of one test score was missing. In total, four children had more than one behavioral task score missing. Next, we performed assumption testing for creating latent variables from the parental reports on inhibitory control. We first tested assumptions for the BRIEF-P inhibitory control subscale filled in by mother and partner. KMO analysis showed a value of 0.5 for maternal and partner reports. Bartlett's test reached significance, and determinant testing yielded results $p$>0.0001. However, because the 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.

Generalization analyses. To define reported EF for the generalization analyses, assumption testing for latent variable creation was performed from the four EF questionnaires (BRIEF-P and the REEF, both filled in by mother and partner). KMO measures 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 BRIEF-P filled in by mother 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, an adequate model fit could not be reached for the models with the latent variable for EF (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. KMO analysis showed a value of 0.5 for all questionnaires, Bartlett's test reached significance for all analyses, and data was 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 the models utilizing the composite score of the BRIEF-P as the outcome variable (models 7 and 8), but not for models utilizing a composite score of the REEF as the 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 inhibitory control subscale ($r$=0.351, $p$=0.004) and with 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 inhibitory control subscale, BRIEF-P total questionnaire, and observed inhibitory control were used as outcome variable. A detailed overview of all correlations with potential confounding variables, including correlations with exploratory outcome measures (Flanker, Whisper, Gift Wrap, and Gift Delay), is shown in the supplementary materials in **Fehler! Verweisquelle konnte nicht gefunden werden.**.

## Main analyses

Goodness of fit measures showed an adequate fit of final models 1 through 4. The parameter estimates, and bootstrapped confidence intervals per model are shown in Table 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 ($\beta$=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. In addition, no significant mediation effect of diet quality score was found for model 1 to 4. Furthermore, worse parental EF is associated with high levels of observed child inhibitory control at age three ($\beta$=-0.009, 95% CI [-0.017, -0.001]). Contrarily, high levels of parental EF is associated with high levels of child inhibitory control reported by both parents at age three ($\beta$=0.091, 95% CI [0.034, 0.148]).

### Generalization 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-). Neither did diet quality have a mediating effect in both models. Parameter estimates and their respective confidence intervals are shown in supplementary materials in **Fehler! Verweisquelle konnte nicht gefunden werden.**.

### Exploratory analyses

Separate analyses per task and food group were performed for exploratory purposes. We investigated whether breastfeeding history was predictive for the outcome of the separate behavioral tasks, with diet quality score as mediator. Parameter estimates, and bootstrapped confidence intervals are shown in **Fehler! Verweisquelle konnte nicht gefunden werden.** of the supplementary materials. No significant relations were found between breastfeeding history and the separate behavioral tasks.

We also investigated whether separate food groups (vegetable intake, fruit intake, and snacks and candy intake) would mediate the association between breastfeeding history and EF. Because all exploratory models could not be fitted using the latent variable for EF, the composite score for the BRIEF-P and the composite score for the REEF were used as outcome variables in separate models. No significant mediation effects for food groups were found (see supplementary materials **Fehler! Verweisquelle konnte nicht gefunden werden.** and **Fehler! Verweisquelle konnte nicht gefunden werden.** for the parameter estimates).

## Discussion

This study did not provide evidence that breastfeeding history (i.e. exclusive breastfeeding duration and breastfeeding cessation age) are associated with inhibitory control and EF in 3-year-old children. Furthermore, diet quality at age 3 was not associated with inhibitory control or EF in 3-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 3.

Contrary to our hypotheses, breastfeeding history did not predict inhibitory control or EF in 3-year-olds. These results are in line with the results from Belfort *et al.* (2016), who also did not find a relation between breastfeeding and EF of 6 to 7-year-old children. Contrarily, Julvez *et al.* (2007) found that breastfeeding was associated with high levels of EF in a sample of 500 4-year-old children. The observational tasks used in this last study focused more broadly 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, more specific aspects of EF.

Another explanation for our null-results with breastfeeding history might be that small amounts of breastfeeding are already beneficial for toddler 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 longer period, and less variation in mothers breastfeeding for a shorter period (*n*=7 breastfed <12 weeks) or non-breastfeeding mothers (*n*=5), future studies should investigate a sample with more variation in breastfeeding duration.

Furthermore, 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, Quinte, Cruz, Grellert, & Santos, 2019; Egbert *et al.*, 2019). However, the two studies that assessed nutritional intake in relation to inhibitory control in preschool-aged children indicate mixed results, as one study found that higher intake of sugars was associated with worse inhibitory control (Levitan *et al.*, 2015), while the other study found no association between nutritional intake and inhibitory control (Pieper & Laugero, 2013). While Levitan *et al.* (2015) and Pieper & Laugero (2013) assessed spontaneous food choices, our study is the first to study habitual diet and toddler's inhibitory control. Apparently, the relation between diet quality and inhibitory control and EF is still unclear, especially in preschool-aged children. As such, replication studies are needed, preferably using the same measurement methods to assess dietary intake, inhibitory control and EF.

It might also be possible that the effects of breastfeeding history and diet quality may not be the same for all children, but instead might be moderated by individual differences. One such factor that could moderate an association between diet and child outcomes, that has recently been receiving attention, are the gut microbiota. Gut microbiota are a collection of bacteria, archaea and eukarya colonizing the gastrointestinal tract of the human (Thursby & 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 & Hasan Mohajeri, 2019; Szopinska-Tokov *et al.*, 2020). Since gut microbiota play a major role in the processing of nutrients (Vernocchi, Chierico, & Putignani, 2020), it is possible that the gut microbiota moderate the effects of diet on the brain (Cryan *et al.*, 2019).

Lastly, our results indicated that longer exclusive breastfeeding duration is predictive of better diet quality in toddlerhood. This is consistent with previous studies (Ventura, 2017; Weinfield, Borger, & Gola, 2020). Ventura's (2017) review included studies investigating food groups and showed that longer breastfeeding duration is associated with higher intake of vegetables and fruits in young children, contributing to better diet quality. Furthermore, Weinfield, Borger & Gola (2020) found a positive relation between longer breastfeeding duration and diet quality of preschool-aged children in low-income populations. Because worse diet quality is also associated with different negative child outcomes (Marshall, Burrows, & Collins, 2014; Wu *et al.*, 2019), we recommend future studies to consider the role of diet quality when studying relations between breastfeeding and child outcomes.

Our study has several strengths including the longitudinal nature, -covering the period from birth until age 3 years, and allowing for frequent maternal reports on breastfeeding status-, the assessment of inhibitory control using multiple behavioral tasks, and reports by both mother and partner, and the assessment of habitual diet with three 24h-recalls, which are considered the least biased self-report instrument (Thompson & Subar, 2017). The current study also has limitations to note: the generalizability of our findings is limited by our mostly highly educated sample, and the relatively small sample size reduced our statistical power. We preserved power by using composite scores for outcome variables and performing exploratory analyses for individual scores, but replications in larger and more varied study populations are highly recommended.

To conclude, no relation was found between breastfeeding history and inhibitory control or EF, and no mediating effect of diet quality was found. Nonetheless, we found that longer exclusive breastfeeding duration predicted better diet quality at age 3, which corresponds with previous literature. It is possible that the relation between breastfeeding history, habitual diet and inhibitory control/EF exists, but was not found in our study due to small sample size and mostly highly educated families. Due to the importance of inhibitory control in the occurrence of psychopathology and child behavior problems, we recommend future studies to accurately investigate inhibitory control and its predictors in a bigger and more generalizable sample of toddlers.

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## 2. Tables and other supporting documents where applicable and necessary

Table 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 |

g/d: grams per day.

Table 2. Characteristics of the study population.

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

SD: Standard deviation

[a]Partner educational level was assessed in the prenatal measurement round.

****p* < 0.001

Table 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 | BRIEF-P inh-P |
|---|---|---|---|---|---|---|---|---|---|
| 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.

*$p < 0.05$. **$p < 0.01$.

Table 4. Parameter Estimates and bootstrapped Confidence Intervals for Model 1, 2, 3, and 4.

| | B | SE | Lower CI | Upper CI |
|---|---|---|---|---|
| **Regression Paths** | Model 1: Exclusive breastfeeding duration → diet quality score → Observed inhibitory control | | | |
| 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 |
| **Regression Paths** | Model 2: Breastfeeding cessation age → Diet quality score → Observed inhibitory control | | | |
| 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 |
| **Regression Paths** | Model 3: Exclusive breastfeeding duration → diet quality score → reported inhibitory control | | | |

| | | | | |
|---|---|---|---|---|
| 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 |
| **Regression Paths** | Model 4: Breastfeeding cessation age → diet quality score → reported inhibitory control | | | |
| 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 |
| 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** | -0.003 | 0.011 | -0.025 | 0.019 |
| **Total model** | 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: $X^2$ (3)= 0.919, *p*=0.338; CFI = 1.000, RMSEA = 0.000, SRMR = 0.029, *n*=66. Model 2: $X^2$ (4)= 1.804, p= 0.614; CFI = 1.000, RMSEA = 0.000, SRMR = 0.045, *n*=66. Model 3: $X^2$ (3)= 0.996, *p*=0.307; CFI = 0.996, RMSEA = 0.026, SRMR = 0.031, *n*=67. Model 4: $X^2$ (4)= 1.779, *p*= 0.619; CFI = 1.000, RMSEA = 0.000, SRMR = 0.044, *n*=67.

*$p < 0.05$. **$p < 0.01$.

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