Association between Temperament and Empirically Derived Dietary Patterns Among Children Aged 1-2 Years Old

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Abstract

Background: Childhood temperament refers to the innate individual differences in the way a child responds to their environment and regulates their emotions. The current research indicates that a child's temperament can be influenced by a combination of biological and environmental factors, but further investigation is required to identify more potential determinants of temperament development. Recent studies have revealed a correlation between the gut microbiome and temperament, which suggests that dietary intake may impact temperament development.

Objective: The goal of this study is to investigate the associations between empirically derived nutrient-based dietary intake patterns and the development of the three-dimensional temperament traits in toddlers.

Methods: Dietary intake data were collected using a 24-hour recall questionnaire, and a three-dimensional child temperament assessment was obtained from the Revised Infant Behavior Questionnaire (IBQ-R) and Early Childhood Behavior Questionnaire (ECBQ). Nutrients were clustered into dietary patterns using exploratory graph analysis (EGA), and confirmatory factor analysis (CFA) was conducted to obtain a latent factor for each cluster. The association between the latent factors and three temperament traits was estimated utilizing linear mixed effect models.

Results: A significant negative association was identified between the joint effects of EPA and DHA and negative affectivity in temperament, with an effect size of -0.14. These types of polyunsaturated fatty acids are commonly found in fish.

Conclusions: While nutrient-based dietary patterns could influence the development of negativity in early childhood, no significant associations were found between dietary patterns and other aspects of temperament. Further research is needed to investigate the relationship between food-level dietary patterns and more detailed aspects of temperament.

Keywords: early childhood, temperament, dietary intake, nutrient, dietary patterns

1 Introduction

Temperament is a psychobiological concept that refers to an individual's patterns of response to their environment and the regulation of their emotions (Alving-Jessep, Botchway, Wood, Hilton, & Blissett, 2022; M. K. Rothbart, 1981; Sanson, Hemphill, & Smart, 2004). It characterizes the innate differences that can be observed as early as infancy (Alving-Jessep et al., 2022; M. K. Rothbart, 1981; Sanson et al., 2004). Over the years, researchers have proposed various theoretical frameworks to describe and measure temperament (M. K. Rothbart, 1981; Sanson et al., 2004). More recent instruments, such as the Infant Behavior Questionnaire (IBQ) (M. K. Rothbart, 1981), Infant Behavior Questionnaire-Revised (IBQ-R) (Gartstein & Rothbart, 2003), Children's Behavior Questionnaire (CBQ) (M. Rothbart, Ahadi, Hershey, & Fisher, 2001), and the Early Childhood Behavior Questionnaire (ECBQ) (S. P. Putnam, Gartstein, & Rothbart, 2006), have identified three consistent dimensions of temperament: Surgency (or Extraversion), Negative Affectivity, and Regulation (Orienting, or Effortful Control) (Forbes, Rapee, Camberis, & McMahon, 2016; M. Rothbart, 2007; M. Rothbart et al., 2001; S. P. Putnam et al., 2006; Gartstein & Rothbart, 2003; Sanson et al., 2004). These dimensions provide a more comprehensive understanding of the temperament construct and allow for the analysis of the relationship between early childhood characteristics and other developmental outcomes.

Research has shown a growing interest in studying childhood temperament due to its significant and profound relations with various aspects of child development, including mental health (Wiseman, Croft, & Zammit, 2021; Forbes et al., 2016; Rapee, Kennedy, Ingram, Edwards, & Sweeney, 2005; Hirshfeld-Becker et al., 2007), brain connectivity (Zwir et al., 2023; Gilbert et al., 2021), cognitive function (Chong et al., 2019; Lemelin, Tarabulsy, & Provost, 2006), eating habits (Liew, Zhou, Perez, Yoon, & Kim, 2020; Haycraft, Farrow, Meyer, Powell, & Blissett, 2011), and social behavior development (Sanson et al., 2004; M. Rothbart, Ahadi, & Hershey, 1994). The temperament exhibited by a child during early childhood can potentially indicate the emergence of mental illnesses (Sanson et al., 2004; Hirshfeld-Becker et al., 2007; Rapee et al., 2005), predict their social acceptance from early childhood through adolescence (Sanson et al., 2004; Calkins, Dollar, & Wideman, 2019), and forecast their educational achievement at school age (Wright & Jackson, 2022; Chong et al., 2019). Furthermore, some eating behaviors and disorders, such as anorexia nervosa, bulimia nervosa, and binge eating disorder, have been suggested to have a possible foundation in temperament (Liew et al., 2020; Haycraft et al., 2011).

Temperament has traditionally been treated as a moderately stable foundation underlying the development pathways of various physical and personality traits (M. K. Rothbart, 1981; Nigg, 2006; Beekman et al., 2015). However, the stability of temperamental traits over time is still inconclusive, with mixed results from studies (Sanson et al., 2004; Nigg, 2006; Beekman et al., 2015). Some evidence suggests that temperament possesses lower stability before three years old and moderate correlations across time, from toddlers to adolescence (Beekman et al., 2015; M. K. Rothbart & Bates, 2006; Alving-Jessep et al., 2022; Slabach, Morrow, & Wachs, 1991). As a result, many researchers believe that temperament can undergo a considerable amount of changes over time, particularly during significant developmental transitions at early age (S. Putnam, Sanson, & Rothbart, 2002; Sanson et al., 2004). Several environmental and biological factors, including genetics and family environment, can shape a child's temperament (Saudino, 2005). Recent research on the gut microbiome in young children suggests a potential association with early childhood temperament, although the findings are still preliminary (Alving-Jessep et al., 2022; Christian et al., 2015; Kelsey et al., 2021). However, the association between dietary intake patterns and temperament development has not been thoroughly studied.

Nutrition plays a crucial role in supporting healthy brain and body development during infancy, and dietary intake becomes a critical habit to learn as toddlers start to transition from breast milk to solid food (Kolb & Gibb, 2011; Cusick & Georgieff, 2016; Matsuyama et al., 2019). The gut microbial community can be influenced by diet, which creates potential opportunities for dietary interventions to adjust microbial diversity, composition, and stability in the gut, ultimately impacting the development of temperament (Matsuyama et al., 2019; Zhang, 2022; Leeming, Johnson, Spector, & Le Roy, 2019; Alving-Jessep et al., 2022). Therefore, it is meaningful to determine whether particular dietary intake characteristics may have evident associations with the development of certain temperament traits during early childhood. Identifying these connections could provide valuable insight into future clinical trials, studying potential early intervention approaches.

To the best of our knowledge, there has not been research analyzing the relationship of dietary intake as an influential factor with the development of early childhood temperament. Therefore, the aim of this paper is to fill this gap by investigating the associations between empirically derived dietary intake patterns (groups of interacted nutrients derived from sample population) and the development of the three-dimension temperament traits in children aged between 1 and 2 years old.

The following sections will provide an introduction to the background information on temperament and dietary intake analysis. It will cover the measurement instruments of early childhood temperament, explaining the significance of studying temperament, and providing an overview of the current research on temperament development. The method section will describe the data collection and processing procedures, how the dietary patterns were obtained, and the approach taken to analyze the associations between early childhood temperament and dietary patterns. The result section will present the characteristics of the children in the study, the distribution of temperament scales in three dimensions, nutrient-based dietary patterns derived from the data, and the correlation between specific dietary patterns and temperament.

2 Background

2.1 Temperament and Assessment

In theoretical studies, temperament more refers to the differences with a constitutional basis, which means that these differences are innate at birth and can be observed as early as infancy (Alving-Jessep et al., 2022; M. K. Rothbart, 1981; Sanson et al., 2004). The measurement of temperament has been an important topic since 1960s, and various theoretical frameworks have been proposed (M. K. Rothbart, 1981; Sanson et al., 2004). One of the earliest and most influential one was proposed by Thomas and Chess (1963), who identified nine dimensions of temperament, including activity, rhythmicity, approach, adaptability, distractibility, mood, persistence, intensity and threshold (Thomas et al., 1963; M. K. Rothbart, 1981; Sanson et al., 2004). However, this construct was criticized by some researchers for its limited three-point scale, restricted sample and conceptual overlap (M. K. Rothbart, 1981; Sanson et al., 2004). In recent years, researchers have developed more sophisticated and widely-used tools for assessing child temperament, such as the Infant Behavior Questionnaire (IBQ) (M. K. Rothbart, 1981), Infant Behavior Questionnaire-Revised (IBQ-R) (Gartstein & Rothbart, 2003), Children's Behavior Questionnaire (CBQ) (M. Rothbart et al., 2001), and the Early Childhood Behavior Questionnaire (ECBQ) (S. P. Putnam et al., 2006). Three dimensions of temperament, including Surgency (or Extraversion), Negative Affectivity, and Regulation (Orienting, or Effortful Control), can be consistently identified from these instruments and have been widely accepted in literature (Forbes et al., 2016; M. Rothbart, 2007; M. Rothbart et al., 2001; S. P. Putnam et al., 2006; Gartstein & Rothbart, 2003; Sanson et al., 2004).

The IBQ-R and the ECBQ were the two instruments for the assessment of child temperament used in this study. The IBQ-R was originally developed for infants younger than one year old (Gartstein & Rothbart, 2003), while the ECBQ was specifically developed for young children aged between 18 and 36 months (S. P. Putnam et al., 2006). Despite the specified age ranges in the guidelines, the questionnaires can still be effective in evaluating temperament in children who are slightly above or below the recommended ages (Questionnaires, 2023). In a recent study conducted in Vietnam (Takegata et al., 2022), the validity of the Vietnamese version of the Very Short Form of the Infant Behavior Questionnaire-Revised (IBQR-VSF)(S. P. Putnam, Helbig, Gartstein, Rothbart, & Leerkes, 2014) was examined in children aged between 3-18 months, which demonstrated that the IBQR-VSF can be used to assess temperament in children slightly older than one year old. The IBQR-VSF is a short form of IBQ-R with 37 items. Though slightly different items and subscales included in the two instruments, the three factors obtained from IBQ-R and IBQR-VSF have been shown to be consistent (S. P. Putnam et al., 2014), which validates the usage of IBQ-R for children slightly older than 12 months as IBQR-VSF in this study.

Similarly, the three factors identified by IBQ-R and ECBQ were proved to be strongly similar and comparable (S. P. Putnam et al., 2006; Gartstein & Rothbart, 2003). As a result, using IBQ-R and ECBQ together for assessment of temperament in children aged between 1-2 years old in this study is justifiable. IBQ-R's three dimensions are: 1) Negative Affectivity, including Sadness, Discomfort, Anger/Frustration, Fear and negatively loading Reactivity/Soothability; 2) Surgency, with positive loadings on Positive Anticipation, Smiling and Laughter, Impulsivity, High Intensity Pleasure, Activity Level, and negative loading on Shyness Approach; 3) Effortful Control, including Low Intensity Pleasure, Inhibitory Control, Attentional Focusing, and Perceptual Sensitivity (Gartstein & Rothbart, 2003). ECBQ's three dimensions are: 1) Negative Affectivity, comprising Discomfort, Fear, Sadness, Frustration, Soothability, Motor Activation, Perceptual Sensitivity, and Shyness; 2) Surgency/Extraversion, characterized by Impulsivity, Activity Level, High-intensity Pleasure, Sociability, and Positive Anticipation; and 3) Effortful Control, defined by Inhibitory Control, Attention Shifting, Low-intensity Pleasure, Cuddliness, and Attention Focusing (S. P. Putnam et al., 2006).

2.2 Temperament and Early Childhood Development

Temperament can be indicative for the potential emergence of mental diseases (e.g. depression and anxiety), and the social acceptability during early childhood through adolescence. Studies have found that children who exhibit higher levels of negative emotionality are more likely to develop anxiety and depression (Sanson et al., 2004; Hirshfeld-Becker et al., 2007; Rapee et al., 2005), while children with less emotional regulation and higher emotional reaction may find it more challenging to develop and maintain friendships (Sanson et al., 2004; Calkins et al., 2019). In addition, researchers have demonstrated the potential of early childhood temperament from infancy to toddlerhood (0-3 years old) as a predictor of the developmental outcomes in school-age children and even throughout adulthood, especially in the aspects of the cognitive functioning and educational attainment (Wright & Jackson, 2022). Children with high level of persistence, a component of effortful control, at 2-3 years old were found to have achieved higher academic outcomes at ages 6-7 years old (Chong et al., 2019). Moreover, there is a connection between temperament traits and eating behaviors. Early negative affectivity has been found to be associated with increased food avoidance behaviors, such as being selective with food choices and eating at a slower pace (Liew et al., 2020; Haycraft et al., 2011). On the other hand, children with the temperament trait of effortful control have demonstrated greater abilities in regulating their appetite and practicing self-control in relation to food, which shed light on the potential role of temperament traits in the development of obesity and contribute to a greater understanding of the underlying factors of adolescent obesity (Liew et al., 2020; Haycraft et al., 2011). Therefore, it is essential to conduct further research on the development of temperament during early childhood and to identify effective intervention strategies, aiming to enhance early age development outcomes, support children's emotional and physical well-beings, and to promote their positive social and emotional skills.

2.3 Influential Factors in Temperament Development

Although temperament has traditionally been considered moderately stable (M. K. Rothbart, 1981; Nigg, 2006; Beekman et al., 2015), there is evidence suggesting that temperament possesses lower stability before three years old (Beekman et al., 2015; M. K. Rothbart & Bates, 2006; Alving-Jessep et al., 2022; Slabach et al., 1991). It is meaningful to investigate the influential factors of temperament development during early childhood. Based on current research, a child's temperament can be shaped by a combination of environmental and biological factors, including their family environment and genetics (Saudino, 2005). Through literature review, Saudino (2005) estimated that 20-60 percent of the variances in child temperament could be explained by genetics, while environmental factors and the other unexplored biological factors account for the other variances. Non-shared family environment factors, such as differential parenting within the same family, explained more variances on the child temperament compared to the shared family environment factors in multiple twin studies (Saudino, 2005). More studies on the potential factors associated with temperament development are necessary for deeper understanding of the temperament's properties. Recently, early research on the gut microbiome in young children suggests a potential association with early childhood temperament (Alving-Jessep et al., 2022). Study by Christian et al. (2015) found that abundance of butyrate-producing bacteria in the gut of boys is associated with more positive temperament outcomes, while several existing infant studies implied that low gut microbiome diversity are prone to negative temperament outcomes (Kelsey et al., 2021; Alving-Jessep et al., 2022). Although the findings of these studies are still preliminary and are not entirely consistent across studies, they inspire further investigation into the potential factors that can influence or intervene the development of temperament in early childhood.

2.4 Dietary Intake, Microbiome and Temperament

Numerous studies have demonstrated the impact of dietary intake on the physical and mental health of children at all ages. During infancy, nutrition plays a crucial role in supporting healthy brain and body development (Kolb & Gibb, 2011; Cusick & Georgieff, 2016). As toddlers (defined as children aged between 1 and 2 years old) start to transition from breast milk to solid food at 1 year old (Matsuyama et al., 2019), dietary intake, as a major approach to absorb necessary nutrition, becomes a critical habit to learn, and its potential effects on children's development and well-being in every aspect warrant extensive investigation. It has been indicated in research that the gut microbial community can be influenced by diet (Matsuyama et al., 2019; Zhang, 2022; Leeming et al., 2019). In their research, Matsuyama et al. (2019) suggested that a diverse diet rich in fiber and protein may be the primary contributor to the development of alpha-diversity in gut microbiota. This increase in diversity has been found to correlate with higher levels of surgency and pleasure in males, and lower levels of effortful control in females, according to the literature review by Alving-Jessep et al. (2022). Conversely, consuming fewer vegetables and more sugar-sweetened drinks and desserts has been linked to lower gut microbiome diversity and increased colonization of harmful species, which has been studied to be associated with negative temperament outcomes (Alving-Jessep et al., 2022). Therefore, it is reasonable to hypothesize that particular dietary intake characteristics may have evident associations with certain temperament traits.

2.5 Dietary Patterns

Previously, researchers focused on analyzing the roles of a single nutrient on the healthy outcomes. In the past two decades, there has been a growing interest in the analysis of dietary patterns, which provide insights into how groups of nutrients are consumed in combination (Dietary Guidelines Advisory Committee, 2020; Hu, 2002). Dietary pattern analysis is considered more comprehensive for understanding nutrient consumption from an overall perspective, compared to the traditional approach that examines the single nutrients individually (Dietary Guidelines Advisory Committee, 2020; Hu, 2002). Given that people generally consume nutrients in combination, results from dietary pattern analysis is more interpretable and insightful, aligned with the expectations of this study. By considering the overall diet and interactions between nutrients, dietary pattern analysis enables investigators to circumvent the challenges posed by high correlations among nutrients and confounding effects from other nutrients not of interest (Hu, 2002). Moreover, it can aid in the detection of cumulative effects caused by multiple interactive nutrients whose single effects are not sufficiently large to be identified (Hu, 2002). Therefore, dietary pattern analysis was chosen to handle the highly correlated nutrients in this study.

There are two major methods of deriving dietary patterns. One is index-based approach, and the other is data-driven approach. The index-based approach was based on certain dietary guidelines from the prior knowledge and previous research. The data-driven approach is to empirically obtain dietary patterns from sample data using statistical methods, such as principal component analysis (PCA) (Miller et al., 2010). However, PCA has limitations when it comes to labeling and interpreting patterns. Therefore, other methods, such as clustering together with factor analysis, were preferred. In this study, exploratory graph analysis with graphical Lasso and confirmatory factor analysis were conducted to derive the dietary patterns.

3 Methods

The analysis was conducted on the data set collected from a dietary intake analysis study approved by the University of North Carolina at Chapel Hill and the University of Minnesota Institutional Review Boards. The dietary intake data were collected using a 24-hour recall questionnaire, and child temperaments were measured using the widely-used three factors of Surgency (SUR), Negative Affectivity (NEG), and Regulation (REG), derived from the Revised Infant Behavior Questionnaire (IBQ-R) (Gartstein & Rothbart, 2003) and ECBQ (S. P. Putnam et al., 2006). To account for the synergistic effects of nutrients, exploratory graph analysis (EGA) based on graphical lasso algorithm with specified regularization parameter (H. F. Golino & Epskamp, 2017) was conducted to cluster 105 nutrients into several nutrient groups, which were defined as empirically derived dietary patterns. Then, in each nutrient group, confirmatory factor analysis (CFA) (Brown, 2006) was implemented to obtain a latent factor for further analysis. Finally, the association between these latent factors and the three temperament traits was estimated utilizing the linear mixed effect model, taking into account the correlations of multiple visits by the same subject. The hypothesis test results of the estimated effect size of each cluster on the three temperament traits were adjusted for multiple comparison using false discovery rate (FDR) method.

3.1 Study Cohort

Infant-mother dyads were enrolled in this study. The University of North Carolina at Chapel Hill and University of Minnesota Institutional Review Boards approved all study activities. Written informed consent was obtained from the parent for the participation of both themselves and their infants prior to any study activities. Several different recruitment approaches were employed by both institutions, including recruitments from local newborn nurseries, institutional centers with research interest on early brain development, local flyers, and university listservs. The inclusion criteria were: 1) birth at 37-42 weeks of gestational age; 2) appropriate birth weight for gestational age; and 3) no major pregnancy and delivery complications. The exclusion criteria included: 1) adopted child; 2) presence of autism, intellectual disability, schizophrenia, or bipolar disorder related first degree; 3) less than 2 kilograms of birth weight; 4) neonatal hypoxia (10 minute APGAR < 5); 5) having illness requiring more than two days of newborn intensive care unit stay; 6) chromosomal or major congenital abnormality; 7) abnormal magnetic resonance in previous MRI; 8) significant developmental delay or medical illness, or significant genetic or medical conditions impacting growth, development, or cognition (including visual/hearing impairment); 9) contraindication in MRI; and 10) maternal pre-eclampsia, HIV status, placental abruption, and alcohol or illicit drug use during pregnancy.

3.2 Data Collection

The dietary intake data were collected using 24-hour dietary recall questionnaire (24HR), a well-organized interview with the goal of obtaining comprehensive information on all foods and drinks consumed by children within the past 24 hours (National Cancer Institute, 2023). Data collection was via the Nutrition Data System for Research (NDSR), a Windows-based software for collecting and analyzing dietary data. The software collects 24-hour dietary recall data, computes amounts of various nutrients per-ingredient, per-food, per-meal, and per-day, and generates reports in various formats (University of Minnesota, 2023a, 2023b). Due to the age of the children involved in this study, parents completed the questionnaire on behalf of their children regarding their dietary intake.

Demographic information, such as sex, date of birth, race, ethnicity, and parental education level, was collected at the initial visit. Investigators recorded breastfeeding practices throughout the study period, including whether the child was exclusively breastfed, had a mixture of breastmilk and formula, or was exclusively formula-fed. The assessment of temperament was conducted during a separate visit from the dietary intake collection, with a time gap of approximately three months (or 90 days) between the visit for the dietary intake questionnaire and the measurement of temperament.

3.3 Data Preprocessing

To address the possible differences in dietary intake between weekdays and weekends, each child provided at least two dietary intake records within seven days: one from a weekday and one from a weekend. For the convenience of analysis, multiple records from the same subject within seven days were treated as data from the same week, and thus were averaged using different weights to adjust for the difference in the number of weekdays and weekends, with a proportion of 5/7 for weekdays and 2/7 for weekends.

The other data processing procedures involved the following five steps: 1) removing negative values, 2) selecting nutrient variables of interest, 3) removing outliers, 4) adjusting for age effect and 5) matching nutrient consumption data with demographic information and temperament scores. First, three observations containing negative nutrient values were removed. Second, from an initial pool of 338 nutrient variables obtained from questionnaires and NDSR software output, 105 nutrients were selected for analysis. To be specific, nutrients with a proportion of zero values greater than

10% (such as alcohol), as well as summarizing nutrients (nutrients that are calculated or derived from a combination of other nutrients) were excluded from the analysis. Thirdly, outliers were determined using the Median Absolute Deviation (MAD) criterion and Mahalanobis Distance. For each nutrient variable, outliers were identified if the value was five times the MAD away from the median (median ± 5*MAD). If an observation contained more than five outliers among the 105 nutrients, it was removed as an outlier observation. Any observations with extreme outliers (30 times MAD away from the median; median ± 30*MAD) were also excluded. The Mahalanobis Distance was calculated for each observation to identify multivariate outliers that deviated from the distribution of nutrient consumption in the sample population, and two apparent outliers were removed from the dataset. After removing outliers, there were 388 dietary intake observations from 206 children remaining. Fourthly, since most of the nutrients of interest show linear trends across age, the age effect was regressed out using linear regression model. Finally, it was only possible to match temperament scores assessed within a 90-day window to the dietary intake records. There were 226 records that did not have valid temperament assessment results. As a result, the number of observations available to analyze the association between temperament and dietary intake patterns was reduced to 162, obtained from 101 subjects.

3.4 Measurements of Temperament

In this study, temperament in children was assessed using the Revised Infant Behavior Questionnaire (IBQ-R)(Gartstein & Rothbart, 2003) for those aged between 12-17 months, and Early Childhood Behavior Questionnaire (ECBQ) (S. P. Putnam et al., 2006) for those aged between 18-24 months. The IBQ-R comprises 14 scales (Gartstein & Rothbart, 2003), while the ECBQ contains 18 scales (S. P. Putnam et al., 2006). The three factors of temperament obtained from the scales of both instruments were calculated. In analysis, IBQ-R and ECBQ were used together as the assessment of temperament in children aged between 1-2 years old.

For consistency, the three dimensions from both instruments were recorded correspondingly and named Negative Affectivity (NEG), Surgency (SUR), and Regulation (REG) in this paper. NEG measures the tendency of negative mood states, such as fear, anger, and sadness. SUR assesses the inclination towards positive emotions, such as enjoyment of exciting events and willingness to interact with others. REG pertains to the regulation of emotion and attention, including the ability to focus and shift attention, find pleasure in everyday activities, and to calm down after experiencing distress.

3.5 Dietary Patterns

Dietary patterns were empirically derived using exploratory graph analysis with graphical Lasso (EGA) (H. F. Golino & Epskamp, 2017) and confirmatory factor analysis (CFA)(Brown, 2006). EGA is a cutting-edge factor structure model to estimate the number of factors and members within factors, while confirmatory factor analysis (CFA) is a special form of factor analysis, with the indicators of factors predefined to derive latent factors, assuming that the clusters in EGA were formed due to the influences of the underlying latent factors. The EGA was performed using the `EGA` function in the `EGAnet` r package, while CFA was implemented using the `cfa` function in `lavaan` r package.

EGA is a state-of-art method of factor identification through a network, which originally emerged from the field of network psychometrics. It estimates a Gaussian graphical model (GGM) by utilizing the graphical LASSO estimation of inverse covariance matrix (H. F. Golino & Epskamp, 2017). In the estimated network, each element of the inverse covariance matrix represents a connection between two nutrients. The number and members of clusters in the estimated network were determined by the walktrap algorithm, which measures the similarities between nutrients based on random walks (H. F. Golino & Epskamp, 2017). EGA provides a robust and accurate estimation of the number of dimensions in observable variables, particularly in situations where the sample size is small, the factor structure is large (with more than four factors), and there are more items per factor (e.g. 10 items in one factor) and potentially high correlations between factors (e.g. larger than 0.7) (H. F. Golino & Epskamp, 2017; H. Golino et al., 2020), as the case with the dietary data in this study.

The EGA identified 16 clusters that represent the dietary patterns of interest and can be further analyzed. The factors obtained from CFA measure the weighted consumption of the corresponding dietary pattern. To label the dietary patterns, the top 2 or 3 nutrients with the highest loadings on the factors were used as indicators.

3.6 Covariates

Age is a confounding factor that can lead to changes in child temperament, absorbed food energy, and nutrient consumption. To account for the age effects, a piecewise linear regression model was used to regress out age from the temperament scales, while linear regression was used to remove the age effects on food energy and nutrient consumption. The linear mixed effect models examining the association between child temperament and dietary patterns were adjusted for several covariates, including sex (male and female), maternal education level (graduate school or higher, below graduate school), first-four-month breastfeeding practice (exclusively breastfed, more breast milk, formula, not answered), and food energy.

To clarify, graduate school or higher includes those with a graduate degree or enrolled in graduate school, while below graduate school includes those with a college degree (either 4-year or 2-year), with a high school degree, in some college or high school, or not answered (15 subjects). The first-four-month breastfeeding practice categorizes children's feeding practices before four months old, with a focus on whether or not the child was fed with breast milk, since it is of greater interest for most studies in early childhood (Jedrychowski et al., 2012). Thus, those fed with formula, including exclusively formula-fed, more formula-fed (less breast milk fed), and fifty-fifty of formula and breast milk fed, were categorized as "formula". Individuals who were fed a combination of breast milk and formula, with a higher proportion of breast milk, were classified as belonging to the "more breast milk" category.

Additionally, food energy was divided by 1000 to ensure a comparable scale with the other numeric data. These covariates were selected as factors to be controlled for in the regression analysis based on previous research studies, to ensure that the model accounts for potential confounding variables and is as comprehensive and inclusive as possible.

3.7 Statistical Analysis

The characteristics of the children were described in a table, stratified by the index of visit. For numeric variables such as age and food energy, the mean and standard deviation were reported. For categorical numeric variables, the number of subjects and its proportion were reported. The distribution of age-adjusted temperament measures taken from IBQ-R and ECBQ was displayed in a table. A linear mixed effect model was applied to compare the differences between IBQ-R and ECBQ measures separately for each domain, including an intercept and an indicator for ECBQ as the fixed effect, and subject as the random effect. Dietary intake data with 388 observations from 206 children were analyzed using EGA and CFA, and correlations among multiple visits by the same subjects were not considered at this step. The estimated EGA result was visualized in a network graph. The correlation matrices before and after EGA were compared visually using heatmaps. The nutritional categories of the member nutrients in each cluster were analyzed in a bar plot. A linear mixed effect model was estimated for each domain of the temperament measures, adjusting for covariates including sex, maternal education level, first-four-month breastfeeding practice, and food energy. The estimated effect sizes with 95% confidence intervals were reported in a table and visualized in a heatmap. The t test with Satterthwaite's method (Kuznetsova, Brockhoff, & Christensen, 2017) was conducted to determine whether the estimated effect size was significant enough to indicate an association between the cluster of nutrients and temperament. P-values obtained from multiple analysis were adjusted using false discovery rate (FDR) method. All analysis was performed on R (version 4.2.0). The linear mixed effect model was implemented using the `lme4` package (version 1.1-31) and the hypothesis test was implemented using `lmerTest` package (version 3.1-3) (Kuznetsova et al., 2017). A residual diagnosis was conducted to analyze the goodness of fit.

4 Results

4.1 Demographics

Table 1 displays the characteristics of the 101 children with complete information, including age, gender, maternal education level, first-four-month breastfeeding practice, and food energy, stratified by the index of visit. There are 388 observations from 206 children with complete dietary records and demographic information, among which 162 observations from 101 children had matched temperament measurements (within 90 days of a dietary intake record) from either IBQ-R and ECBQ. On average, records without matched temperament measures are 543 days old with a standard deviation of 84 days, which is slightly older than those with matches. The sex distribution for these records is balanced, with only three more males than females, their mothers tend to be well-educated, and majority of them were exclusively breastfed before four months old. Since the distribution of these characteristics is similar to those with matched temperament measures, removing records without a matched temperament measure is not expected to introduce any significant bias in the regression analysis. Most candidates had one or two records, but one candidate had up to seven records. The age ranges for the first and second visit are wide. Overall, the study population is more educated and more exclusively breastfed than the general US population(for Disease Control & (CDC), 2022).

4.2 Temperament

Table 2 presents the distribution of temperament measurements on three dimensions, Negative Affectivity (NEG), Surgency (SUR), and Regulation (REG), obtained from two instruments, IBQ-R and ECBQ. The sample consisted of 92 observations from 77 subjects measured by IBQ-R and 70 observations from 37 subjects measured by ECBQ. As expected, the average age of the observations measured by IBQ-R (403 days, approximately 13.5 months old) was lower than that measured by ECBQ (588 days, around 20 months old). To

Table 1: Demographic characteristics[1], stratified by the index of visits

Demographics	Overall	Visits[2]						
		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
Number of subjects, n(%)[3]	101 (100.0)	101 (100.0)	36 (35.6)	14 (13.9)	5 (5.0)	4 (4.0)	1 (1.0)	1 (1.0)
Number of observations, n(%)[4]	162 (100.0)	101 (62.4)	36 (22.2)	14 (8.6)	5 (3.1)	4 (2.5)	1 (0.6)	1 (0.6)
Age, mean(SD), days	483 (115)	449 (112)	513 (108)	553 (62)	595 (73)	597 (73)	618 (-)[6]	709 (-)[6]
Gender, n(%)								
Male	46 (45.5)	46 (45.5)	16 (44.4)	6 (42.9)	1 (20.0)	1 (25.0)	0 (0.0)	0 (0.0)
Female	55 (54.5)	55 (54.5)	20 (55.6)	8 (57.1)	4 (80.0)	3 (75.0)	1 (100.0)	1 (100.0)
Maternal education level, n(%)								
Graduate school or higher	52 (51.5)	52 (51.5)	17 (47.2)	6 (42.9)	2 (40.0)	1 (25.0)	0 (0.0)	0 (0.0)
Below graduate school	49 (48.5)	49 (48.5)	19 (52.8)	8 (57.1)	3 (60.0)	3 (75.0)	1 (100.0)	1 (100.0)
First-four-month feeding, n(%)								
Exclusively breastfed	76 (75.2)	76 (75.2)	28 (77.8)	10 (71.4)	4 (80.0)	3 (75.0)	1 (100.0)	1 (100.0)
More breast milk	12 (11.9)	12 (11.9)	5 (13.9)	4 (28.6)	1 (20.0)	1 (25.0)	0 (0.0)	0 (0.0)
Formula	10 (9.9)	10 (9.9)	3 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Not answered	3 (3.0)	3 (3.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Food energy, mean(SD), kcal[5]	921.9 (352.8)	893.9 (366.4)	962.1 (329.4)	979.9 (371.0)	927.7 (389.6)	1044.9 (210.3)	807.6 (-)[6]	1082.9 (-)[6]

[1] For the categorical data, the percentage is calculated using the number of subjects in the specific category at certain visit divided by the total number of subjects at that visit.

[2] The age at which each subject visited may vary greatly. Similarly, the time elapsed between visits may differ significantly between subjects. Therefore, their age at each visit may not be directly comparable. Given that there are overlaps in the subjects across visits, it is necessary for the proportions to add up to 100%.

[3] The proportion in this row is calculated using the number of subjects in certain visit divided by the overall number of subjects.

[4] The proportion in this row is calculated using the number of observations in certain visit divided by the overall number of observations. It is expected that the proportions will add up to 100% across all seven visits.

[5] The food energy shown here is in the raw scale. When implementing the linear mixed effect models, food energy is age-adjusted and scaled.

[6] Standard deviation is not available because there is only one subject in the cell.

Table 2: Distribution of temperament obtained from IBQ-R and ECBQ in children aged between 1-2 years old

Dimensions	Overall	Scores (Mean± SD)		
		IBQ-R (n=92)[1]	ECBQ (n=70)[2]	P value[3]
Age, mean(SD), days	483 ± 115	403 ± 33	588 ± 100	-[4]
Surgency (SUR)	-0.09 ± 0.86	-0.03 ± 0.91	-0.17 ± 0.78	0.54
Negative Affectivity (NEG)	-0.01 ± 0.75	0.04 ± 0.81	-0.08 ± 0.66	0.34
Regulation (REG)	0.04 ± 0.95	-0.05 ± 0.97	0.16 ± 0.90	0.59

[1] There are 92 observations from 77 subjects.
[2] There are 70 observations from 37 subjects.
[3] A linear mixed effect model was specified as $y_{ij} = \beta_0 + \beta_1 * I_{ij}(ECBQ) + b_i + \epsilon_{ij}$, where y_{ij} represents the temperament score for the i-th subject at the j-th visit, and ϵ_{ij} and b_i follow the normal distribution. The null hypothesis for this test is $H_0 : \beta_1 = 0$. A t test with Satterthwaite's method was implemented to determine the p-value.
[4] It was expected to have some differences in measurement age for these two instruments. Therefore, it is not of interest to test the difference here.

adjust for age differences, the SUR, NEG, and REG scales were age-adjusted and scaled, as evidenced by the overall mean values of the SUR, NEG, and REG scales around 0 and standard deviations close to 1. The results of the statistical analysis showed that the SUR ($p = 0.54$), NEG ($p = 0.34$), and REG ($p = 0.59$) scales from IBQ-R and ECBQ did not exhibit significant differences, supporting the consistency and similarity of measures obtained from the two instruments.

4.3 Dietary Patterns

To make the best use of the available data, a total of 388 observations from 206 children with complete dietary records and demographic information was used to obtain the dietary patterns. EGA identified 16 clusters, which were labeled depending on the categories of the member nutrients: 1) General carbohydrates, vitamins and minerals group, including Soluble Dietary Fiber, Insoluble Dietary Fiber, Pectins, Natural Folate, Synthetic Folate, Starch, Available Carbohydrate by Added Sugars, Betaine, Vegetable Protein, Thiamin, Niacin, Vitamin B6, Magnesium, Iron, Copper, Potassium, Phytic Acid, Water, Manganese, and Gluten. This cluster includes nutrients commonly found in whole plant-based foods such as fruits, vegetables, whole grains, nuts, and seeds. Examples of foods in this cluster include broccoli, apples, lentils, quinoa, and almonds. 2) Saturated fats and trans fats group, including SFA4:0, SFA6:0, SFA8:0, SFA10:0, SFA12:0, SFA14:0, SFA16:0, SFA17:0, SFA18:0, MUFA14:1, MUFA16:1, MUFA18:1, MUFA20:1, TRANS18:1, TRANS18:2, TRANS16:1, CLA cis9, CLA trans10, and solid fats. This cluster includes foods that are high in saturated and trans fats, such as processed meats, fried foods, and high-fat dairy products. 3) Amino acids and protein group, including Tryptophan, Threonine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Tyrosine, Valine, Histidine, Alanine, Aspartic Acid, Glutamic Acid, Proline, Serine, Animal Protein, and Nitrogen. This cluster includes foods that are high in protein and amino acids, such as meat, fish, poultry, eggs, and legumes. 4) Minerals and electrolytes group, including Galactose, Cystine,

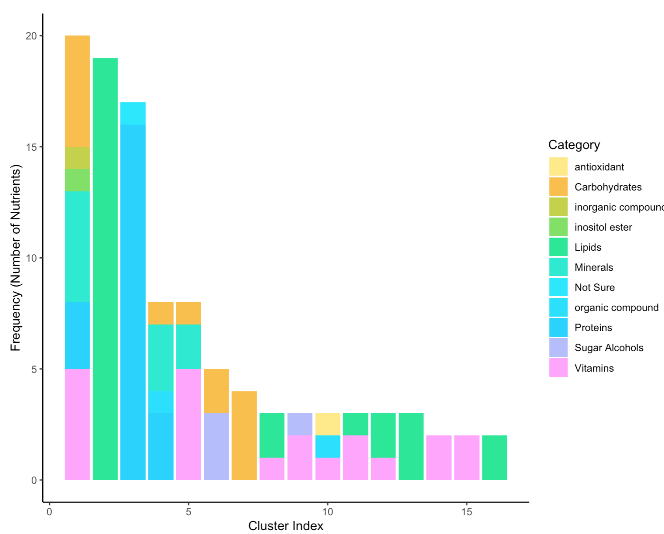

Figure 1: Stacked bar plot showing the nutrient composition of each cluster, stratified by nutritional categories. The x-axis represents the index of clusters, while the y-axis shows the total number of nutrients. The bars are stacked to represent the proportion of each nutritional category within each cluster. The legend displays the correspondence between the colors and the nutritional categories. The height of the bar indicates how many nutrients are included in the cluster.

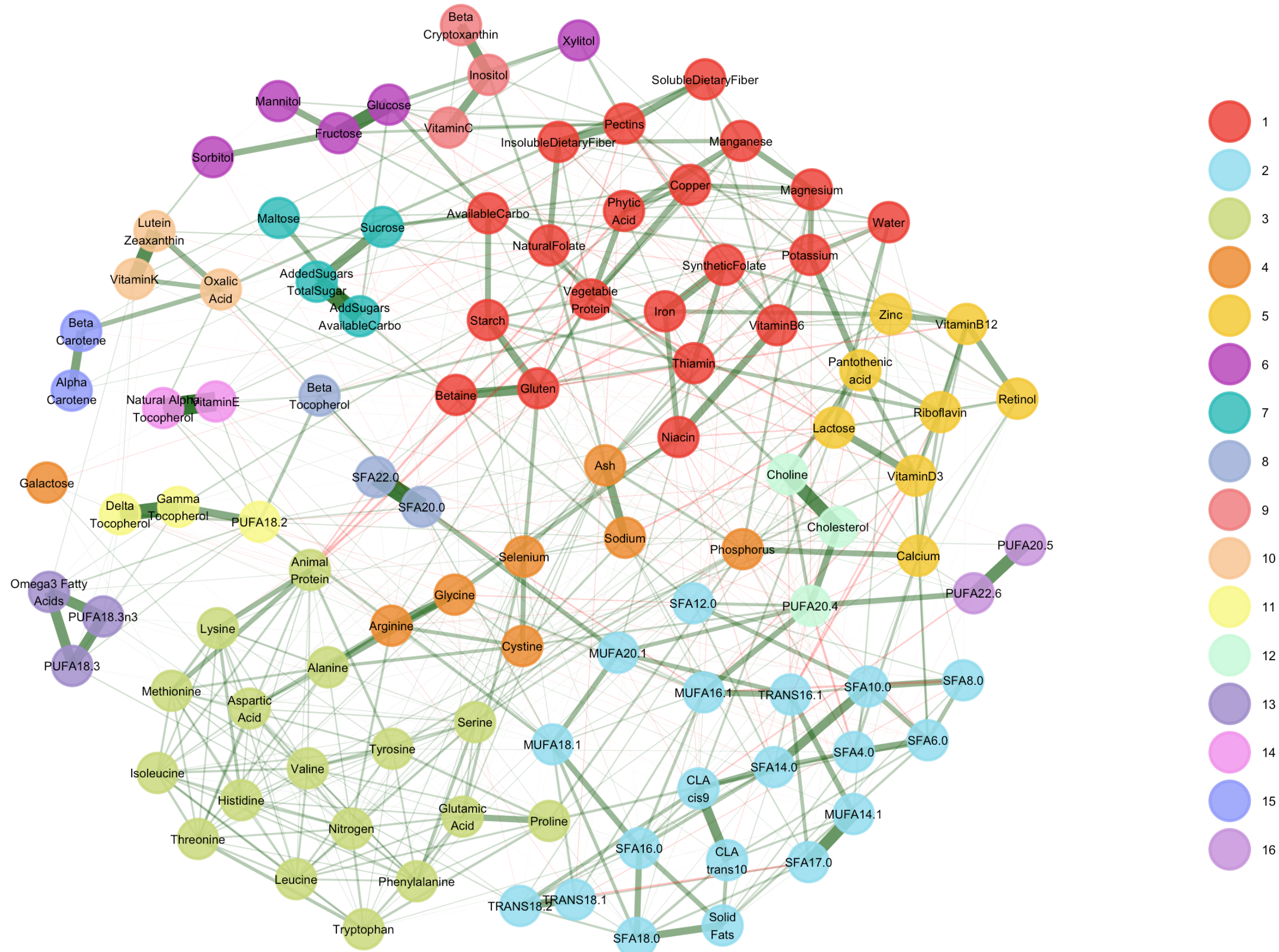

Figure 2: Network graph of EGA clustering results. Each circle represents a nutrient and the color in each circle represents the belonging cluster. The lines connecting circles represent the correlations between the two nutrients. The thicker the line, the higher the correlation is. Red line represents a negative correlation, while the green line represents a positive one.

Arginine, Glycine, Phosphorus, Selenium, Sodium, and Ash. This cluster includes foods that are high in minerals and electrolytes, such as leafy greens, nuts, seeds, and seafood. 5) Vitamins and minerals group, including Lactose, VitaminD3, Retinol, Riboflavin, Pantothenic acid, Vitamin B12, Calcium, Zinc. This cluster includes foods that are high in vitamins and minerals, such as dairy products, fortified cereals, fruits, and vegetables. 6) Sugar alcohols group, including Fructose, Glucose, Mannitol, Sorbitol, and Xylitol. This cluster includes foods that contain sugar alcohols, such as sugar-free gum, candy, and some processed foods. 7) Added sugars group, including Maltose, Sucrose, Add Sugars by Available Carbohydrate, and Added Sugars by Total Sugar. This cluster includes foods that contain added sugars, such as soda, candy, baked goods, and processed snacks. 8) Uncommon saturated fatty acids and vitamin E group, including SFA20:0, SFA22:0, and Beta Tocopherol. This cluster includes foods that are rich in uncommon saturated fatty acids and vitamin E, such as palm oil, beef fat, and sunflower seeds. 9) Carotenoids and vitamin C group, including Beta Cryptoxanthin, Vitamin C, and Inositol. This cluster includes foods that are high in carotenoids and vitamin C, such as carrots, sweet potatoes, peppers, and oranges. 10) Other carotenoids and oxalic acid group, including Lutein Zeaxanthin, Vitamin K, and Oxalic Acid. This cluster includes foods that are high in other carotenoids and oxalic acid, such as spinach, kale, and collard greens. 11) Gamma and Delta tocopherol group, including PUFA18:2 Gamma Tocopherol, and Delta Tocopherol. This cluster includes foods that are high in gamma and delta tocopherol, such as nuts, seeds, and vegetable oils. 12) Omega-6 fatty acids, choline and cholesterol group, including PUFA20:4, Choline, and Cholesterol. This cluster includes foods that are high in omega-6 fatty acids, choline, and cholesterol, such as egg yolks and organ meats. 13) Omega-3 and polyunsaturated

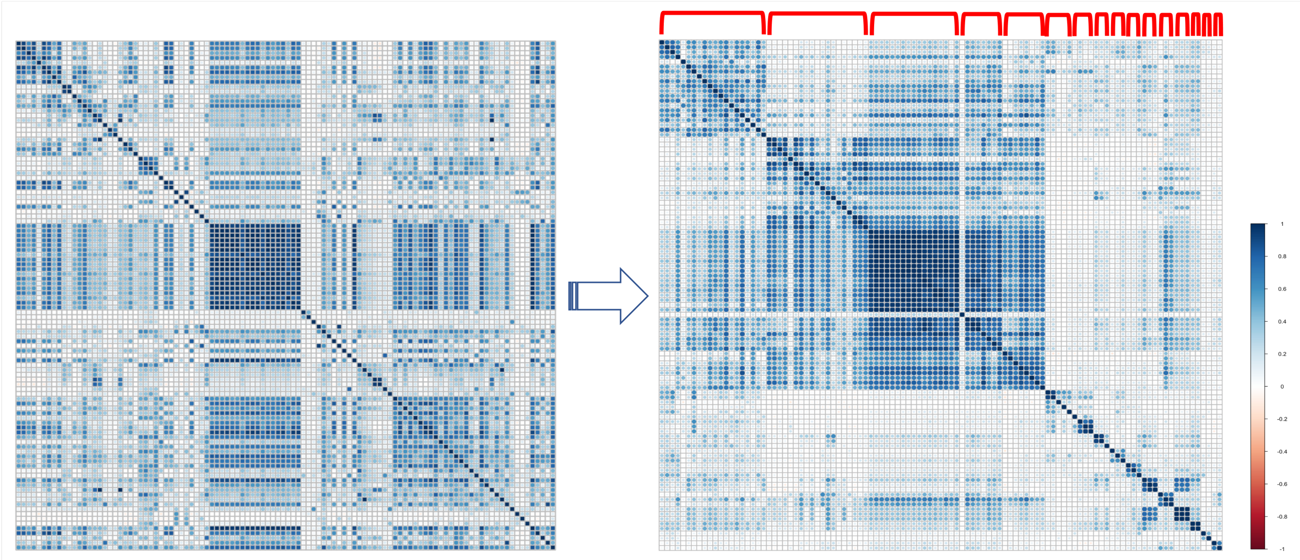

Figure 3: Correlation matrix heatmaps before (left) and after (right) EGA. The blue color showed a positive correlation, while the red color showed a negative correlation. On the right-hand map, the nutrients within the same cluster are enclosed by a red bracket, representing cluster 1 to 16 respectively from left to right.

fatty acids group, including PUFA18:3, PUFA18.3 n3, Omega3 Fatty Acids. This cluster includes foods that are high in omega-3 fatty acids, such as fatty fish, flaxseed, and walnuts. 14) Vitamin E group, including Vitamin E and Natural Alpha Tocopherol. This cluster includes foods that are high in vitamin E, such as almonds, sunflower seeds, and vegetable oils. 15) Carotenoids group, including Beta Carotene and Alpha Carotene. This cluster includes foods that are high in carotenoids, such as tomatoes, papayas, and mangoes. 16) Omega-3 Fatty acids group, including PUFA20:5 and PUFA22.6. This cluster includes foods that are high in omega-3 fatty acids, such as salmon, mackerel, sardines, and herring.

The nutritional categories of the member nutrients in each cluster were visualized in the Figure 1. The clustering results were shown in a network graph (Figure 2). There are 11 categories in total, which are lipids, carbohydrates, proteins, vitamins, minerals, inositol ester, inorganic compound, organic compound, sugar alcohols, and antioxidant. The first cluster is the most diverse and contains the highest amount of nutrients, including carbohydrates and vitamins. It comprises commonly seen and well-known nutrients. The second cluster comprises all lipids but with varying categories, such as fatty acids (saturated, monounsaturated, polyunsaturated, and trans fats). Most proteins are categorized in cluster 3. The fourth group includes minerals, while the fifth group contains mostly vitamins and some carbohydrates. The sixth group consists of sugar alcohols and carbohydrates. All nutrients in the seventh group are carbohydrates. The eighth group comprises lipids and vitamin E, while the ninth group includes sugar alcohols and vitamins. The tenth group is made up of antioxidants, organic compounds, and vitamin K. The remaining groups are dominated by lipids and vitamins. Overall, the grouping appears logical and sensible based on the nutrient categories.

The heatmaps of the nutrients' correlation matrices before and after EGA were shown in Figure 3. The clustering of EGA was based on correlation matrix. As shown in Figure 3, there are apparent blocks on the heatmap after EGA, which indicated that most nutrients with high correlations were grouped into one cluster. These matrices confirmed the groupings identified by EGA, with the majority of highly correlated nutrients forming a single cluster.

The factor loadings that the nutrients weighted in their clusters were shown in Figure 4, which visualized the relative importance of each nutrient in contributing to its clusters. Although the eighth group comprised lipids and vitamin E, SFA 22:00 (behenic acid) and SFA20:00 (arachidic acid) had much higher loadings to this cluster than Beta-Tocopherol, a type of vitamin E. Cluster 4 had a significantly lower loading for galactose compared to all other members in the group, which could be supported from the correlation matrix heatmap on the right of Figure 3, which visualized the correlation matrix after EGA. In the map, galactose is the nutrient with an apparent white line across the square in the middle (the first one within the fourth red bracket, counting from left), which suggested that galactose was not strongly correlated with any of the nutrients of interest in this study. Since the EGA assumed a minimum of two members per cluster, galactose was not placed in a separate group on its

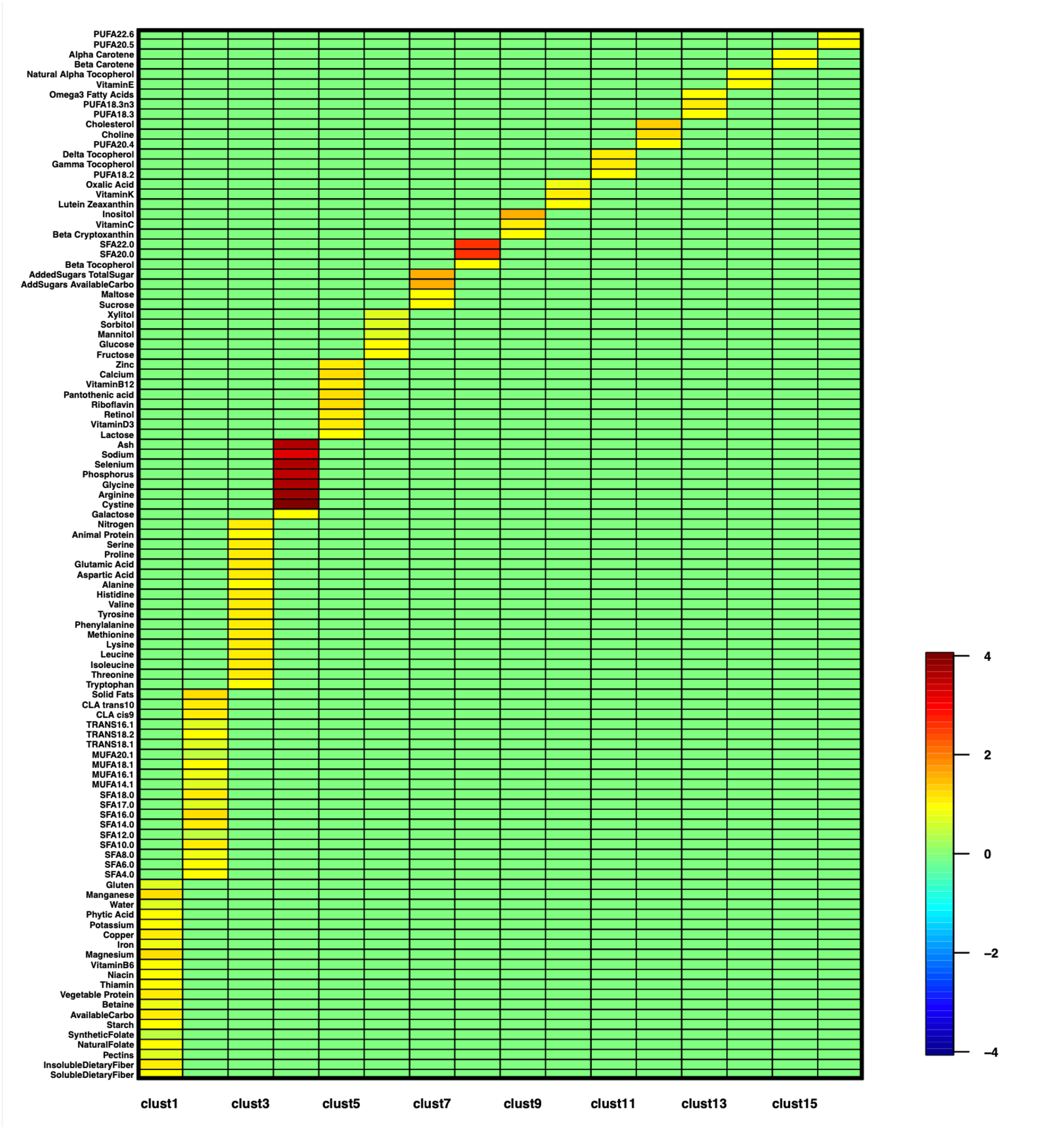

Figure 4: Loading coefficients heatmap of nutrients in the sixteen clusters, estimated by CFA. The x-axis represents the clusters, and the y-axis represents the nutrients. Each cell in the heatmap represents the strength of the relationship between the nutrient and the cluster, which is measured by factor loading score. A color gradient is used to represent such a factor loading score. The darker red color represents a higher positive factor loading score. On the opposite, the darker blue color represents a higher negative factor loading score. The green color means a zero factor loading score. Since CFA was performed on each cluster separately, and the nutrients within each cluster are mutually exclusive, each nutrient had only one non-zero factor loading score in its corresponding cluster.

own. However, CFA identified the low loading of galactose and helped correct this unexpected misgrouping observed in the EGA.

Please see the supplementary material (Appendix A.2) for additional tables (Table 3 and Table 5) with the exact factor loadings for each nutrient within its cluster.

4.4 Dietary Patterns and Temperament

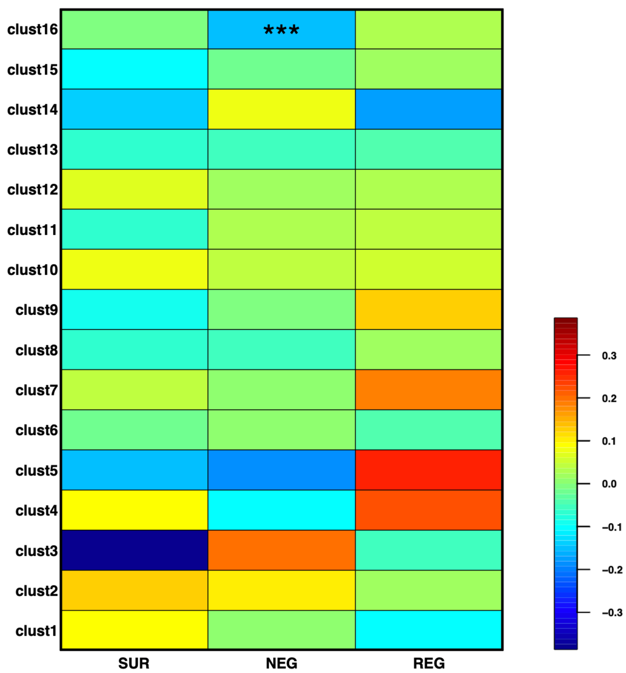

Figure 5: Heatmap showing the effects of the identified nutrient clusters on the early childhood temperament in three dimensions. The x-axis represents the three dimensions of temperament, and y-axis represents the nutrient clusters. Each cell shows an estimated effect size, with warmer colors (red) representing a positive effect and cooler colors (blue) indicating a negative effect. The star symbols show whether the effect was significant based on adjusted p-values, with two stars meaning adjusted $p< 0.05$ and three stars meaning adjusted $p< 0.01$.

The estimated effects of each cluster on the three dimensions of temperament were presented in Figure 5. The darkness of the color in each cell corresponded to the magnitude of the effect. Overall, the effect sizes were not tremendous, with a maximum of 0.26 and a minimum of -0.37. Nonetheless, they were not of moment but still relatively large, considering the overall standard deviation of temperament, which was 0.86, 0.75, and 0.95 for SUR, NEG, and REG respectively. There was a only significantly negative association identified between the Omega-3 fatty acid group, comprised of PUFA 20:05:00 n-3 (eicosapentaenoic acid [EPA]) and PUFA 22:06:00 n-3 (docosahexaenoic acid [DHA]), and the negative affectivity aspect of child temperament (effect size: -0.14; adjusted p-value: <0.01). The two nutrients in the sixteenth cluster are commonly found in fish, such as salmon, mackerel, sardines, and herring. Although not significant, the effect of cluster 3, dominated by proteins, on surgency (effect size: -0.37; adjusted p = 0.52) is the highest among all the estimated effects in this analysis. The corresponding foods for the nutrients in cluster 3 include dairy products (e.g. milk, butter and cheese), poultry, fish and meat. The effect of cluster 5 on NEG is -0.18 (adjusted p=0.16). Cluster 5 includes vitamins, minerals and one carbohydrate. Foods containing the nutrients in cluster 5 are relatively mixed, including milk and dairy products, fish, meat, whole grains, legumes and leafy green vegetables. For REG, cluster 4 and 5 presented high positive effects, with the effect sizes of 0.22 (adjusted p=0.80) and 0.26 (adjusted p=0.16), correspondingly. Cluster 4 nutrients can be found in all kinds of meat, fish, ham and some condiments (e.g. salt). Demographic characteristics, sex, maternal education level, first-four-month breastfeeding practices and age-adjusted energy, did not present any significant effects on the temperament development.

Please see the supplementary material (Appendix A.3) for additional table (Table 6) with the estimated effect size number and 95% confidence interval.

5 Discussion

The major finding from this study is the significant negative association between the Omega-3 fatty acid group, comprised of PUFA20:5 and PUFA22:6, and the negative affectivity aspect of child temperament at early age. PUFA20:5 and PUFA22:6 are mostly included in fish, such as salmon, mackerel, sardines, and herring. This finding is consistent with those of previous research conducted by Wu et al.(2015) in old Chinese adults in Singapore and Yoshikawa, Nishi, Matsuoka, Kim, and Hashimoto (2015) in Japanese company workers, both of which found a significant association between fish consumption and a lower prevalence of depression symptoms. Nevertheless, there are several limitations in this study.

The accuracy of dietary intake records presents a significant concern in this study. The dietary intake data were collected through maternal recall, and some mothers were first-time mothers and not experts in nutrition science. As a result, memory errors and measurement errors may have occurred, leading to a large variance in the data and a considerable number of outliers.

Another limitation of this study is the combined use of IBQ-R and ECBQ as assessments of temperament.

An increase in the sample size can improve the accuracy of model fitting, particularly for EGA and linear mixed effect models. Therefore, this study integrated measurements from IBQ-R and ECBQ on the three broad dimensions of temperament in a single analysis. To enhance the validity of the analysis, it is recommended to separate observations from children aged 1-2 years into two groups based on the instrument used to measure temperament. Generally, children aged 12-18 months and 18-24 months would be the two groups. Collecting more data for both groups would help reduce concerns regarding potential confounding factors resulting from instrument differences.

The differences between the two centers (UNC and UMN) were not considered in the analysis because of the sample size and the unbalanced distribution. UMN had more records collected than UNC initially, but the number of records without temperament measures was similar for both centers. Therefore, after removing those records, UNC had significantly fewer observations remaining compared to UMN (25 vs 137).

6 Conclusion

In conclusion, our study found a significant negative association between the Omega-3 Fatty acids nutrient group, which contains PUFA20:5 and PUFA22:6, and negative affectivity in temperament in children aged 1 to 2 years. This suggests that dietary intake may be a potential factor influencing temperament development in young children. However, we did not find any significant associations between dietary patterns and other aspects of temperament, such as surgency and regulation. Further research is necessary to investigate the relationship between these dietary patterns and the various aspects of temperament in more detail, beyond the three broad dimensions studied here. Moreover, the EGA method in conjunction with CFA, which is a cutting-edge technique from the field of network psychometrics, was utilized to help with the dimension reduction by clustering highly correlated nutrients into groups. The results of this study demonstrated the applicability of this method for future research in this area. Nevertheless, nutrient-based dietary patterns have limitations in terms of interpretation and future insights into the real-world intervention. Future research using food-level dietary data may have the capability to address these limitations and provide more applicable conclusions. Overall, this study highlights the potential for further exploration into the association between dietary intake and temperament, but more researches are necessary and the double blind clinical trials with careful design would give more accurate and insightful conclusions.

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A Supplementary Material

A.1 CFA Implementation

With the nutrients within a cluster assumed as the observed indicators of an unobserved factor, which was called latent factor, an one-factor CFA was fitted to obtain the estimation of the latent factor. Modification indices were used to determine whether the model could be further improved by specifying error covariances for observed variables. This step was implemented using `modificationindices` function in `lavaan` package in r. The model was improved until comparative fit index (CFI) was higher than 0.90 (higher than 0.95 was preferred) and root mean square error of approximation (RMSEA) was lower than 0.08 (preferred lower than 0.05). Both CFI and RMSEA were the assessment of the CFA model's goodness-of-it.

A.2 Factor Loading Table

See Table 3, Table 4 and Table 5

A.3 Coefficient Estimates Table

See Table 6.

Table 3: Factor loadings for nutrients in cluster 1 to cluster 8.

Nutrients	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8
Soluble Dietary Fiber	1.00	-	-	-	-	-	-	-
Insoluble Dietary Fiber	1.06	-	-	-	-	-	-	-
Pectins	0.74	-	-	-	-	-	-	-
Natural Folate	0.95	-	-	-	-	-	-	-
Synthetic Folate	0.48	-	-	-	-	-	-	-
Starch	0.92	-	-	-	-	-	-	-
Available Carbohydrate	1.03	-	-	-	-	-	-	-
Betaine	0.80	-	-	-	-	-	-	-
Vegetable Protein	1.07	-	-	-	-	-	-	-
Thiamin	0.93	-	-	-	-	-	-	-
Niacin	0.94	-	-	-	-	-	-	-
Vitamin B6	0.92	-	-	-	-	-	-	-
Magnesium	1.13	-	-	-	-	-	-	-
Iron	0.80	-	-	-	-	-	-	-
Copper	1.08	-	-	-	-	-	-	-
Potassium	0.94	-	-	-	-	-	-	-
Phytic Acid	0.90	-	-	-	-	-	-	-
Water	0.64	-	-	-	-	-	-	-
Manganese	1.14	-	-	-	-	-	-	-
Gluten	0.72	-	-	-	-	-	-	-
SFA 04:00	-	1.00	-	-	-	-	-	-
SFA 06:00	-	0.99	-	-	-	-	-	-
SFA 08:00	-	0.79	-	-	-	-	-	-
SFA 10:00	-	1.03	-	-	-	-	-	-
SFA 12:00	-	0.45	-	-	-	-	-	-
SFA 14:00	-	1.08	-	-	-	-	-	-
SFA 16:00	-	1.15	-	-	-	-	-	-
SFA 17:00	-	0.74	-	-	-	-	-	-
SFA 18:00	-	1.12	-	-	-	-	-	-
MUFA 14:01	-	0.70	-	-	-	-	-	-
MUFA 16:01	-	0.79	-	-	-	-	-	-
MUFA 18:01	-	0.89	-	-	-	-	-	-
MUFA 20:01	-	0.47	-	-	-	-	-	-
18:01 TRANS	-	0.68	-	-	-	-	-	-
18:02 TRANS	-	0.89	-	-	-	-	-	-
16:01 TRANS	-	0.70	-	-	-	-	-	-
CLA cis-9	-	1.07	-	-	-	-	-	-
CLA trans-10	-	1.02	-	-	-	-	-	-
Solid Fats	-	1.13	-	-	-	-	-	-
Tryptophan	-	-	1.00	-	-	-	-	-
Threonine	-	-	1.04	-	-	-	-	-
Isoleucine	-	-	1.03	-	-	-	-	-
Leucine	-	-	1.04	-	-	-	-	-
Lysine	-	-	1.02	-	-	-	-	-
Methionine	-	-	1.03	-	-	-	-	-
Phenylalanine	-	-	1.04	-	-	-	-	-
Tyrosine	-	-	1.03	-	-	-	-	-
Valine	-	-	1.04	-	-	-	-	-
Histidine	-	-	1.03	-	-	-	-	-
Alanine	-	-	1.00	-	-	-	-	-
Aspartic Acid	-	-	1.02	-	-	-	-	-
Glutamic Acid	-	-	1.02	-	-	-	-	-
Proline	-	-	1.01	-	-	-	-	-
Serine	-	-	1.03	-	-	-	-	-
Animal Protein	-	-	0.99	-	-	-	-	-
Nitrogen	-	-	1.03	-	-	-	-	-

Table 4: Factor loadings for nutrients in cluster 1 to cluster 8 (continued).

Nutrients	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8
Galactose	-	-	-	1.00	-	-	-	-
Cystine	-	-	-	3.90	-	-	-	-
Arginine	-	-	-	3.69	-	-	-	-
Glycine	-	-	-	3.58	-	-	-	-
Phosphorus	-	-	-	3.42	-	-	-	-
Selenium	-	-	-	3.57	-	-	-	-
Sodium	-	-	-	3.20	-	-	-	-
Ash	-	-	-	3.54	-	-	-	-
Lactose	-	-	-	-	1.00	-	-	-
Vitamin D3	-	-	-	-	1.12	-	-	-
Retinol	-	-	-	-	1.05	-	-	-
Riboflavin	-	-	-	-	1.22	-	-	-
Pantothenic Acid	-	-	-	-	1.20	-	-	-
Vitamin B12	-	-	-	-	1.10	-	-	-
Calcium	-	-	-	-	1.18	-	-	-
Zinc	-	-	-	-	1.00	-	-	-
Fructose	-	-	-	-	-	1.00	-	-
Glucose	-	-	-	-	-	1.00	-	-
Mannitol	-	-	-	-	-	0.63	-	-
Sorbitol	-	-	-	-	-	0.63	-	-
Xylitol	-	-	-	-	-	0.63	-	-
Sucrose	-	-	-	-	-	-	1.00	-
Maltose	-	-	-	-	-	-	1.00	-
Add Sugars (by Available Carbo)	-	-	-	-	-	-	1.53	-
Added Sugars (by Total Sugars)	-	-	-	-	-	-	1.53	-
Beta Tocopherol	-	-	-	-	-	-	-	1.00
SFA 20:00	-	-	-	-	-	-	-	2.58
SFA 22:00	-	-	-	-	-	-	-	2.58

Table 5: Factor loadings for nutrients in cluster 9 to cluster 16.

Nutrients	Cluster 9	Cluster 10	Cluster 11	Cluster 12	Cluster 13	Cluster 14	Cluster 15	Cluster 16
Beta Cryptoxanthin	1.00	-	-	-	-	-	-	-
Vitamin C	1.01	-	-	-	-	-	-	-
Inositol	1.55	-	-	-	-	-	-	-
Lutein Zeaxanthin	-	1.00	-	-	-	-	-	-
Vitamin K	-	1.01	-	-	-	-	-	-
Oxalic Acid	-	0.80	-	-	-	-	-	-
PUFA 18:02	-	-	1.00	-	-	-	-	-
Gamma Tocopherol	-	-	1.08	-	-	-	-	-
Delta Tocopherol	-	-	1.08	-	-	-	-	-
PUFA 20:04	-	-	-	1.00	-	-	-	-
Choline	-	-	-	1.21	-	-	-	-
Cholesterol	-	-	-	1.32	-	-	-	-
PUFA 18:03	-	-	-	-	1.00	-	-	-
PUFA 18:03 n-3	-	-	-	-	1.00	-	-	-
Omega3 Fatty Acids	-	-	-	-	1.00	-	-	-
Vitamin E	-	-	-	-	-	1.00	-	-
Natural Alpha Tocopherol	-	-	-	-	-	1.00	-	-
Beta Carotene	-	-	-	-	-	-	1.00	-
Alpha Carotene	-	-	-	-	-	-	1.00	-
PUFA 20:05 n-3	-	-	-	-	-	-	-	1.00
PUFA 22:06 n-3	-	-	-	-	-	-	-	1.00

Table 6: Additional table in the appendix

Cluster	SUR (95% CI)	NEG (95% CI)	REG (95% CI)
Cluster 1	0.09 (-0.24, 0.41)	0.01 (-0.20, 0.22)	-0.10 (-0.42, 0.22)
Cluster 2	0.12 (-0.18, 0.42)	0.10 (-0.09, 0.30)	0.02 (-0.27, 0.32)
Cluster 3	-0.37 (-0.99, 0.25)	0.19 (-0.20, 0.59)	-0.05 (-0.66, 0.55)
Cluster 4	0.09 (-0.60, 0.78)	-0.10 (-0.54, 0.34)	0.22 (-0.46, 0.89)
Cluster 5	-0.14 (-0.46, 0.17)	-0.18 (-0.40, 0.03)	0.26 (-0.06, 0.57)
Cluster 6	-0.01 (-0.24, 0.22)	0.00 (-0.15, 0.15)	-0.04 (-0.27, 0.19)
Cluster 7	0.04 (-0.14, 0.22)	0.01 (-0.12, 0.14)	0.19 (0.00, 0.37)
Cluster 8	-0.06 (-0.21, 0.09)	-0.06 (-0.15, 0.04)	0.01 (-0.13, 0.16)
Cluster 9	-0.09 (-0.25, 0.07)	-0.01 (-0.11, 0.09)	0.12 (-0.03, 0.27)
Cluster 10	0.08 (-0.06, 0.22)	0.04 (-0.06, 0.14)	0.05 (-0.09, 0.19)
Cluster 11	-0.06 (-0.32, 0.19)	0.03 (-0.14, 0.20)	0.04 (-0.21, 0.29)
Cluster 12	0.06 (-0.15, 0.28)	0.02 (-0.12, 0.16)	0.03 (-0.18, 0.24)
Cluster 13	-0.06 (-0.31, 0.18)	-0.06 (-0.22, 0.10)	-0.04 (-0.28, 0.21)
Cluster 14	-0.13 (-0.32, 0.05)	0.08 (-0.04, 0.20)	-0.17 (-0.36, 0.01)
Cluster 15	-0.10 (-0.26, 0.06)	-0.02 (-0.15, 0.11)	0.02 (-0.15, 0.19)
Cluster 16	-0.01 (-0.13, 0.11)	-0.14 (-0.22, -0.07)	0.03 (-0.09, 0.14)