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ORIGINAL ARTICLE

Dietary Factors Impact Developmental Trajectories in Young Autistic Children

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Abstract

Purpose The purpose of this research was to investigate the impact of dietary factors on developmental trajectories in young autistic children.

Methods A gluten-free and casein-free diets, as well as six types of food (meat and eggs, vegetables, uncooked vegetables, sweets, bread, and "white soft bread that never molds") were investigated observationally for up to three years in 5,553 children 2 to 5 years of age via parent-report measures completed within a mobile application. Children had a parent-reported diagnosis of Autism Spectrum Disorder (ASD); 78% were males; the majority of participants resided in the USA. Outcome was monitored on five orthogonal subscales: Language Comprehension, Expressive Language, Sociability, Sensory Awareness, and Health, assessed by the Autism Treatment Evaluation Checklist (ATEC) (Rimland & Edelson, 1999) and Mental Synthesis Evaluation Checklist (MSEC) (Arnold & Vyshedskiy, 2022; Braverman et al., 2018).

Results Consumption of fast-acting carbohydrates – sweets, bread, and "white soft bread that never molds" – was associated with a significant and a consistent Health subscale score decline. On the contrary, a gluten-free diet, as well as consumption of meat, eggs, and vegetables were associated with a significant and consistent improvement in the Language Comprehension score. Consumption of meat and eggs was also associated with a significant and consistent improvement in the Sensory Awareness score.

Conclusion The results of this study demonstrate a strong correlation between a diet and developmental trajectories and suggest possible dietary interventions for young autistic children.

Keywords diet · ASD · Autism · Dietary intervention · Language comprehension · Complex-language

Introduction

Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental condition that includes life-affecting social communication issues as well as restricted or repetitive patterns of behavior (CDC, 2020). ASD is estimated to occur in around 2.8% of the 8-year-old children in the USA (Maenner et al., 2023) and is generally considered to most frequently result from genetic factors (Thapar & Rutter, 2021). However, recent research has shown that environmental factors can increase the likelihood of autism's development and exacerbate its symptoms (Chaste & Leboyer, 2012; Ratajczak, 2011).

One environmental factor that affects all children's development is diet (Whiteley et al., 2013). Autistic children have been shown to be especially susceptible to imbalances and abnormalities in gastrointestinal function (Adams et al., 2018; Peretti et al., 2019). Imbalances in gut function heavily influence the central nervous system through the brain-gut axis, and dysfunction of this broad neural circuitry can significantly impact the development of the higher-order cognitive functions that autistic children struggle with (Carabotti et al., 2015; Jones et al., 2022). Thus, many recent studies have attempted to elucidate the correlation between autistic children's behaviors and their diet (Adams et al., 2018; González-Domenech et al., 2020; Jones et al.,

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2022; Keller et al., 2021; Klaveness et al., 2013; Peretti et al., 2019; Piwowarczyk et al., 2018).

Various fundamental factors of a diet, especially those that are known to specifically affect or reshape the gut microbiome, have been explored, such as gluten-free and casein-free. Gluten is a protein found in wheat, barley, and rye that has been the focus of much nutrition research due to the role it plays in celiac disease (Catassi et al., 2022). Casein is a protein found in milk and dairy products that has been a recent focus of autism research due to its effect on the gut microbiome (Zhao et al., 2022). Past studies attempting to link gluten-free and casein-free diets to an increase in overall health of autistic children have struggled to create conclusive evidence, with some finding a significant correlation (Quan et al., 2022), a weak correlation (González-Domenech et al., 2020; Piwowarczyk et al., 2018), or no correlation at all (Keller et al., 2021; Sausmikat & Smollich, 2016).

In 2015 we developed a language therapy app and made it available gratis at all major app stores (Dunn, Elgart, Lokshina, Faisman, Khokhlovich et al., 2017a, 2017b; Dunn, Elgart, Lokshina, Faisman, Waslick et al., 2017; Vyshedskiy et al., 2020; Vyshedskiy and Dunn, 2015). In return for access to structured language exercises inside the app, parents are asked to complete a 133-question survey every three months. As a result, we accumulated more than 100,000 longitudinal assessments. The app is primarily popular with autistic children and therefore the majority of our participants are young children diagnosed with ASD.

The 133-question parent-report assesses children's development on five orthogonal axes: language comprehension using the Mental Synthesis Evaluation Checklist (MSEC) (Arnold & Vyshedskiy, 2022; Braverman et al., 2018), as well as expressive language, sociability, sensory awareness, and health, assessed by the Autism Treatment Evaluation Checklist (ATEC) (Rimland & Edelson, 1999). In 2019 we added questions polling parents on the children's diet (gluten-free and casein-free) and consumption of six types of food (meat and eggs, vegetables, uncooked vegetables, sweets, bread, and "white soft bread that never molds"). This study reports associations between the diet and children's developmental trajectories.

Table 1 Approximate relationship between ATEC total score, age, and ASD severity as described in (Jagadeesan et al., 2022). At any age, a greater ATEC score indicates greater ASD severity

Severity	Age (years)		
	2–3	3–4	4–5
Mild	< 69	< 65	< 61
Moderate	69–81	65–79	61–76
Severe	81–179	79–179	76–179

Methods

Participants

Participants were users of a language therapy app that was made available gratis at all major app stores in September 2015. Once the app was downloaded, caregivers were asked to register and to provide demographic details, including the child's diagnosis and age. Caregivers consented to anonymized data analysis and completed the Autism Treatment Evaluation Checklist (ATEC) (Rimland & Edelson, 1999), an evaluation of the language comprehension using the Mental Synthesis Evaluation Checklist (MSEC) (Arnold & Vyshedskiy, 2022; Braverman et al., 2018), as well as the diet assessment. The first evaluation was administered approximately one month after the download. The subsequent evaluations were administered at approximately three-month intervals for up to three years. To enforce regular evaluations, the app became unusable at the end of each three-month interval and parents were required to complete an evaluation to regain its functionality.

Inclusion Criteria

Inclusion criteria were identical to our previous studies of this population (Forman et al., 2022; Fridberg et al., 2021; Levin et al., 2022; Mahapatra et al., 2018; Vyshedskiy et al., 2020; Vyshedskiy & Khokhlovich, 2023a, b). Specifically, we selected participants based on the following criteria:
1) Consistency: Participants' caregivers must have filled out at least three ATEC evaluations, and the interval between the first and the last evaluation was six months or longer (N = 10,891). Among the selected participants, the average number of evaluations was 5.7 ± 4.1. The average number of days between the 1st and the last evaluation was 667 ± 1399.
2) Diagnosis: Parent-reported ASD diagnosis at the end of the study. Children without ASD diagnosis were excluded from the study (N = 1517). A good reliability of parent-reported diagnosis was previously demonstrated (Jagadeesan et al., 2022). The parent-reported ASD diagnosis was supported by ATEC scores. Average initial ATEC total score was 68.4 ± 24.1 which corresponds to moderate ASD as delineated earlier (Jagadeesan et al., 2022) and Table 1.

Exclusion Criteria

1) Maximum age: Participants older than five years of age at the time of their first evaluation were excluded from this study (N = 3479). Note that children who started at 5 years of age potentially remained in the study until 8 years of age.

2) Minimum age: Participants younger than two years of age at the time of their first evaluation were excluded from this study (N = 342).

After excluding participants that did not meet these criteria, there were 5,553 total participants. Children's age at baseline was 3.5 ± 0.8. 78% were males.

Evaluations

A caregiver-completed Autism Treatment Evaluation Checklist (ATEC) (Rimland & Edelson, 1999) and Mental Synthesis Evaluation Checklist (MSEC) (Braverman et al., 2018) were used to track children's development. The ATEC questionnaire is comprised of four subscales: (1) Speech/Language/Communication, (2) Sociability, (3) Sensory/Cognitive Awareness, and (4) Physical/Health/Behavior. The first subscale, Speech/Language/Communication, contains 14 items and its score ranges from 0 to 28 points. The Sociability subscale contains 20 items and its score ranges from 0 to 40 points. The third subscale, referred here as the Sensory Awareness subscale, has 18 items and score range from 0 to 36 points. The fourth subscale, referred here as the Health subscale, contains 25 items and score range from 0 to 75 points. The scores from each subscale are combined in order to calculate a Total Score, which ranges from 0 to 179 points. A lower score indicates lower severity of ASD symptoms and a higher score indicates more severe symptoms of ASD. While ATEC is not a diagnostic checklist [16], there is strong correlation between the total ATEC score and ASD severity. Table 1 lists approximate ATEC total score as related to ASD severity and age as described in (Jagadeesan et al., 2022).

ATEC was selected as a tool since it is one of the few measures validated to evaluate treatment effectiveness. In contrast, another popular ASD assessment tool, Autism Diagnostic Observation Schedule or ADOS, (Lord et al., 2000) has only been validated as a diagnostic tool. Various studies confirmed the validity and reliability of ATEC. For example, Geier et al. reported a strong correlation between the CARS score and the total ATEC score ($r = 0.71$) in 56 ASD children (2–16 years of age) (Geier et al., 2013). Similarly, Freire et al. reported a strong correlation between the CARS score and the total ATEC score ($r = 0.8$) in 42 ASD children (2–6 years of age) (Freire et al., 2018). Furthermore, several trials confirmed ATEC's ability to longitudinally measure changes in participant performance. One trial conducted by Magiati et al. utilized ATEC to monitor the progress of 22 schoolchildren over a five-year period. ATEC score was compared to age-specific cognitive, language, and behavioral metrics such as the Wechsler Preschool and Primary Scale of Intelligence. The researchers noted ATEC's high level of internal consistency as well as a high correlation with other standardized assessments used to measure the same capacities in children with ASD (Magiati et al., 2011). Charman et al. utilized ATEC amongst other measures to test the feasibility of tracking the longitudinal changes in children using caregiver-administered questionnaires and noted differential effects across subscales of ATEC, possibly driven by development-focused vs. symptom-focused subscales that are conflated in the ATEC total score (Charman et al., 2004). Another study assessing the ability of dietary intervention to affect ASD symptoms also utilized ATEC as a primary measure (Klaveness et al., 2013), concluding that it has "high general reliability," coupled with an ease of access. These studies support the effectiveness of ATEC as a tool for longitudinal tracking of symptoms and assessing changes in ASD severity.

Expressive Language Subscale

The ATEC Speech/Language/Communication subscale includes the following questions: (1) Knows own name, (2) Responds to 'No' or 'Stop', (3) Can follow some commands, (4) Can use one word at a time (No!, Eat, Water, etc.), (5) Can use 2 words at a time (Don't want, Go home), (6) Can use 3 words at a time (Want more milk), (7) Knows 10 or more words, (8) Can use sentences with 4 or more words, (9) Explains what he/she wants, (10) Asks meaningful questions, 11) Speech tends to be meaningful/relevant, 12) Often uses several successive sentences, 13) Carries on fairly good conversation, and 14) Has normal ability to communicate for his/her age. The answers choices were: not true, somewhat true, very true. With the exception of the first three items, all items in the ATEC subscale 1 primarily measure expressive language. Accordingly, the ATEC subscale 1 is herein referred to as the Expressive Language subscale to distinguish it from the Language Comprehension subscale tested by the MSEC evaluation.

Sociability Subscale

The ATEC Sociability subscale includes the following questions: (1) Seems to be in a shell – you cannot reach him/her; (2) Ignores other people; (3) Pays little or no attention when addressed; (4) Uncooperative and resistant; (5) No eye contact; (6) Prefers to be left alone; (7) Shows no affection; (8) Fails to greet parents; (9) Avoids contact with others; (10) Does not imitate; 11) Dislikes being held/cuddled; 12) Does not share or show; 13) Does not wave 'bye bye'; 14) Disagreeable/not compliant; 15) Temper tantrums; 16) Lacks friends/companions; 17) Rarely smiles; 18) Insensitive to other's feelings; 19) Indifferent to being liked; 20) Indifferent if parent(s) leave. The answers choices were: not descriptive, somewhat descriptive, very descriptive.

Sensory Awareness Subscale

The ATEC Sensory/Cognitive awareness subscale includes the following questions: (1) Responds to own name; (2) Responds to praise; (3) Looks at people and animals; (4) Looks at pictures (and T.V.); (5) Does drawing, coloring, art; (6) Plays with toys appropriately; (7) Appropriate facial expression; (8) Understands stories on T.V.; (9) Understands explanations; (10) Aware of environment; 11) Aware of danger; 12) Shows imagination; 13) Initiates activities; 14) Dresses self; 15) Curious, interested; 16) Venturesome – explores; 17) "Tuned in" — Not spacey; 18) Looks where others are looking. The answers choices were: not descriptive, somewhat descriptive, very descriptive.

Health Subscale

The ATEC Physical/Health/Behavior subscale includes the following questions: (1) Bed-wetting; (2) Wets pants/diapers; (3) Soils pants/diapers; (4) Diarrhea; (5) Constipation; (6) Sleep problems; (7) Eats too much/too little; (8) Extremely limited diet; (9) Hyperactive; (10) Lethargic; 11) Hits or injures self; 12) Hits or injures others; 13) Destructive; 14) Sound-sensitive; 15) Anxious/fearful; 16) Unhappy/crying; 17) Seizures; 18) Obsessive speech; 19) Rigid routines; 20) Shouts or screams; 21) Demands sameness; 22) Often agitated; 23) Not sensitive to pain; 24) "Hooked" or fixated on certain objects/topics; 25) Repetitive movements (stimming, rocking, etc.). The answers choices were: not a problem, minor problem, moderate problem, and serious problem.

Language Comprehension Subscale

The MSEC evaluation was designed to be complementary to ATEC in measuring complex-language comprehension. Out of 20 MSEC items, those that directly assess language comprehension are the following: 1) Understands simple stories that are read aloud; 2) Understands elaborate fairy tales that are read aloud (i.e., stories describing FANTASY creatures); 3) Understands some simple modifiers (i.e., green apple vs. red apple or big apple vs. small apple); 4) Understands several modifiers in a sentence (i.e., small green apple); 5) Understands size (can select the largest/smallest object out of a collection of objects); 6) Understands possessive pronouns (i.e. your apple vs. her apple); 7) Understands spatial prepositions (i.e., put the apple ON TOP of the box vs. INSIDE the box vs. BEHIND the box); 8) Understands verb tenses (i.e., I will eat an apple vs. I ate an apple); 9) Understands the change in meaning when the order of words is changed (i.e., understands the difference between 'a cat ate a mouse' vs. 'a mouse ate a cat'); 10) Understands explanations about people, objects or situations beyond the immediate surroundings (e.g., "Mom is walking the dog," "The snow has turned to water"). The answers choices were: not true, somewhat true, very true. MSEC consists of 20 questions within a score range of 0 to 40 points; similarly to ATEC, a lower MSEC score indicates a better developed language comprehension.

The psychometric quality of MSEC was tested with 3,715 parents of ASD children (Braverman et al., 2018). Internal reliability of MSEC was good (Cronbach's alpha > 0.9). MSEC exhibited adequate test–retest reliability, good construct validity, and good known group validity as reflected by the difference in MSEC scores for children of different ASD severity levels. MSEC norms have been reported earlier (Arnold & Vyshedskiy, 2022).

To simplify interpretation of figure labels, the subscale 1 of the ATEC evaluation is herein referred to as the Expressive Language subscale and the MSEC scale is referred to as the Language Comprehension subscale.

Diet Questionnaire

Parents of participants were asked: (1) Is your child following a gluten-free diet? (gluten is a protein found in many grains; this diet eliminates such grains); (2) Is your child following a casein-free diet? (this diet eliminates milk and all by-products of milk). The answer-options were: "I do not know this diet", "No", "Sometimes", "Usually", "Always". To study the effect of gluten-free and casein-free diets, we compared two groups of participants: those who followed the diet "usually", or "nearly always", or "always" (dieting group) and those who did not follow the diet or were not familiar with this diet (control group).

Food Questionnaire

Parents were also asked about consumption of six types of food: (1) Meat from animals (one serving = one egg or slice of meat); (2) Vegetables (cooked or uncooked): carrots, broccoli, cauliflower, salad, apples, etc.; (3) Uncooked vegetables only: carrots, broccoli, cauliflower, salad, apples, etc. (one serving = one apple or one carrot or one plate of salad); (4) Sweets (one serving = one candy or one teaspoon of sugar); (5) Bread (one serving = one slice of bread); (6) White soft bread that never molds (one serving = one slice of bread). The answer-options were: "Never", "Half serving a day", "One serving a day", "Two servings a day", "Three servings a day", "Four or more servings a day." To study the effect of a food type, we compared two groups of participants: those who on average consumed two or more servings per day (food type-eating group) and those who consumed half a serving or less (control group).

Statistical Analysis

The framework for the evaluation of score changes over time has been earlier explained in detail (Forman et al., 2022; Fridberg et al., 2021; Levin et al., 2022; Mahapatra et al., 2018; Vyshedskiy et al., 2020; Vyshedskiy & Khokhlovich, 2023a). In short, the concept of a "Visit" was developed by dividing the three-year-long observation interval into 3-month periods. All evaluations were mapped into 3-month-long bins with the first evaluation placed in the first bin. When more than one evaluation was completed within a bin, their results were averaged to calculate a single number representing this 3-month interval. Thus, we had 12 quarterly evaluations.

It was then hypothesized that there was a two-way interaction between a participant-group and Visit. Statistically, this hypothesis was modeled by applying the Linear Mixed Effect Model with Repeated Measures (MMRM), where a two-way interaction term was introduced to test the hypothesis. The model (Endpoint ~ Baseline + Gender + Age + Severity + Participant-Group * Visit) was fit using the R Bioconductor library of statistical packages, specifically the "nlme" package. The subscale score at baseline, as well as gender and severity were used as covariates. Severity was adjudicated as defined in Table 1.

Conceptually, the model fits a plane into n-dimensional space. This plane considers a complex variability structure across multiple visits, including baseline differences. Once such a plane is fit, the model calculates Least Squares Means (LS Means) for each subscale and group at each visit. The model also calculates LS Mean differences between the groups at each visit. Missing data was handled by the MMRM without imputation, as suggested by the National Research Council in its report "Prevention & Treatment of Missing Data in Clinical Trials" (National Research Council, 2010). The MMRM approach to analysis makes use of all available data, including subjects with partial data in order to arrive at an estimate of the mean treatment effect without filling in the missing data.

In preparation for statistical analysis, participants in the control group were matched to those in the diet group using a propensity score (Schneider et al., 2007) based on age, gender, expressive language, language comprehension, sociability, sensory awareness, and health at the 1st evaluation (baseline).

Results

Gluten-free Diet

Participants who followed the gluten-free diet "usually", or "nearly always", or "always" (gluten-free group) were matched to those who did not follow the gluten-free diet (control group) using a propensity score (Schneider et al., 2007) based on age, gender, language comprehension, expressive language, sociability, sensory awareness, and health score at the 1st evaluation (baseline). Participants who followed the diet "sometimes" were not included in the analysis. The number of matched participants was 526 out of 526 in the gluten-free group and 526 out of 3,664 in the control group. We then analyzed trajectories of children development on five orthogonal subscales: Language Comprehension, Expressive Language, Sociability, Sensory Awareness, and Health.

On the Language Comprehension subscale, the average improvement in the gluten-free group over 36 months was 8.77 points ($SE = 0.52$, $p < 0.0001$) compared to 5.84 points ($SE = 0.61$, $p < 0.0001$) in the control group, Fig. 1, Table S41, Table S1. The difference in the gluten-free relative to the control at Month 36 was statistically significant: -2.97 points ($SE = 0.75$, $p < 0.0001$). The negative difference indicates that the control group had greater scores at Month 36 and, therefore, more severe symptoms. On an annualized basis, the participants from the gluten-free group improved their language comprehension 1.5-times faster than those in the control group (gluten-free = 2.9 points/year; control = 1.9 points/year).

On the Expressive Language subscale, the participants in the gluten-free group improved over the 36-month period by 6.03 points ($SE = 0.39$, $p < 0.0001$) compared to 5.74 points ($SE = 0.46$, $p < 0.0001$) improvement in the control group, Table S2. The difference between the groups at Month 36 was not statistically significant: -0.3 points ($SE = 0.56$, $p = 0.5918$).

On the Sociability subscale, the subjects in the gluten-free group improved over the 36-month period by 1.78 points ($SE = 0.53$, $p = 0.0008$) compared to 1.70 points ($SE = 0.62$, $p = 0.0065$) improvement in those in the control group, Table S3. The difference between the groups at Month 36 was not statistically significant: 0.02 points ($SE = 0.76$, $p = 0.9746$).

On the Sensory Awareness subscale, the subjects in the gluten-free group improved over the 36-month period by 3.07 points ($SE = 0.44$, $p < 0.0001$) compared to 2.15 points ($SE = 0.55$, $p < 0.0001$) improvement in the control group, Table S4. The difference between the groups at Month 36 was not statistically significant: -0.93 points ($SE = 0.64$, $p = 0.1513$).

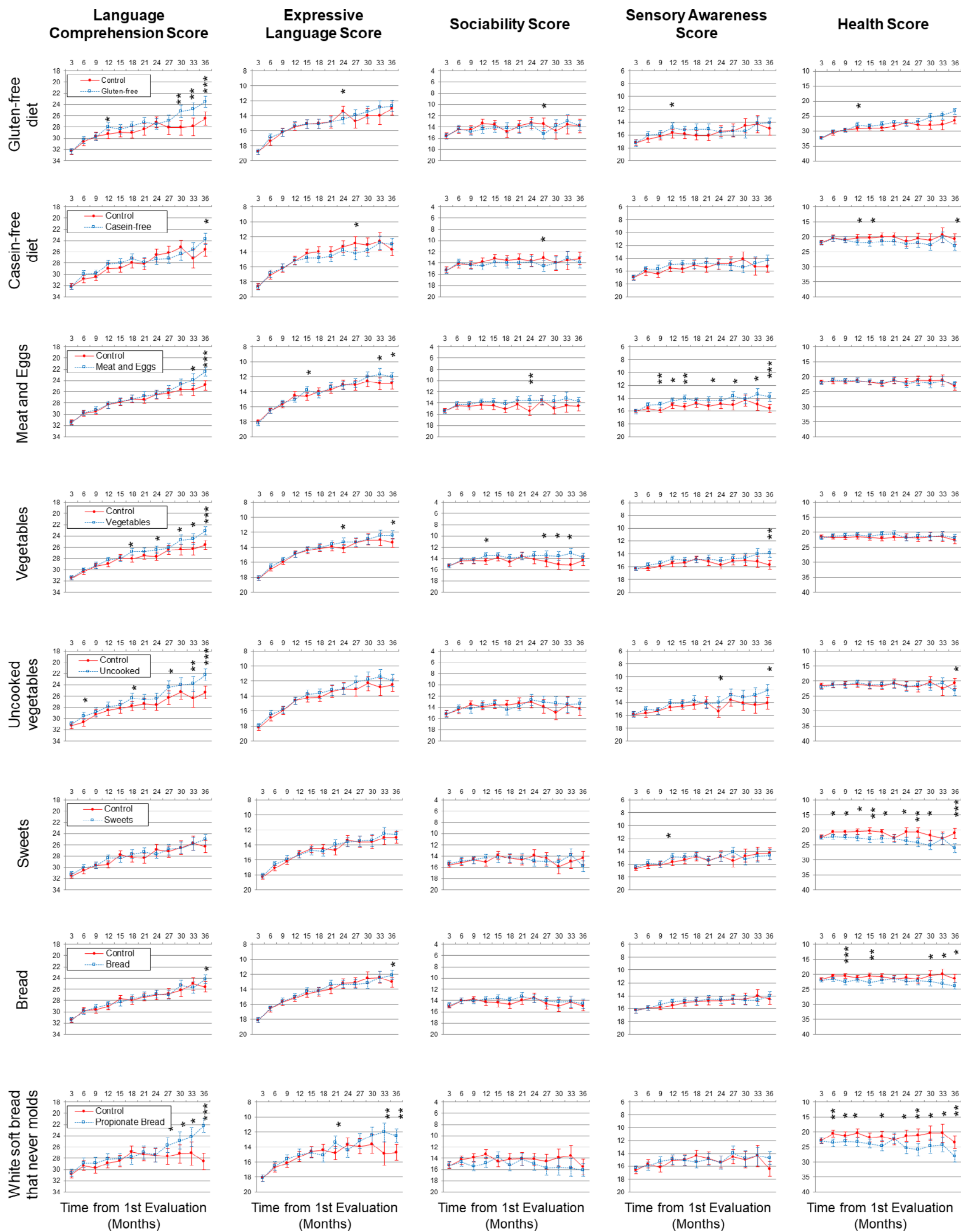

Fig. 1 Longitudinal plots of subscale scores LS Means from the regression model. Horizontal axis shows months from the 1st evaluation (0 to 36 months). Error bars show the 95% confidence interval. To facilitate comparison between subscales, all vertical axes' ranges have been normalized to show 40% of their corresponding subscale's maximum available score. A lower score (the upward direction on the graph) indicates symptom improvement. P-value indicates statistical significance of the difference between the groups: ***<0.0001; **<0.001; *<0.05

On the Health subscale, the subjects in the gluten-free group improved over the 36-month period by 0.89 points (SE = 0.81, p = 0.2741) compared to 0.76 points (SE = 0.95, p = 0.4281) in the control group, Table S5. The difference between the groups at Month 36 was not statistically significant: 0.1 points (SE = 1.17, p = 0.9339).

Casein-free Diet

Participants who followed the casein-free diet "usually", or "nearly always", or "always" (casein-free group) were matched to those who did not follow the casein-free diet (control group) using a propensity score based on age, gender, language comprehension, expressive language, sociability, sensory awareness, and health score at the 1st evaluation (baseline). Participants who followed the diet "sometimes" were not included in the analysis. The number of matched participants was 581 out of 581 in the casein-free group and 581 out of 3,759 in the control group. We then analyzed trajectories of children development on five orthogonal subscales: Language Comprehension, Expressive Language, Sociability, Sensory Awareness, and Health.

On the Language Comprehension subscale, the average improvement in the casein-free group over 36 months was 8.48 points (SE = 0.52, p < 0.0001) compared to 6.49 points (SE = 0.58, p < 0.0001) in the control group, Fig. 1, Table S42, Table S6. The difference in the casein-free relative to the control at Month 36 was statistically significant: -1.93 points (SE = 0.73, p < 0.0087). The negative difference indicates that the control group had greater scores at Month 36 and, therefore, more severe symptoms. On an annualized basis, the participants from the casein-free group improved their language comprehension 1.3-times faster than those in the control group (casein-free = 2.8 points/year; control = 2.2 points/year).

On the Expressive Language subscale, the participants in the casein-free group improved over the 36-month period by 5.62 points (SE = 0.39, p < 0.0001) compared to 4.91 points (SE = 0.44, p < 0.0001) improvement in the control group, Table S7. The difference between the groups at Month 36 was not statistically significant: -0.66 points (SE = 0.55, p = 0.2334).

On the Sociability subscale, the subjects in the casein-free group improved over the 36-month period by 1.37 points (SE = 0.52, p = 0.0089) compared to 1.96 points (SE = 0.59, p = 0.0008) improvement in those in the control group, Table S8. The difference between the groups at Month 36 was not statistically significant: 0.74 points (SE = 0.73, p = 0.3095).

On the Sensory Awareness subscale, the subjects in the casein-free group improved over the 36-month period by 2.68 points (SE = 0.44, p < 0.0001) compared to 1.7 points (SE = 0.49, p = 0.0006) improvement in the control group, Table S9. The difference between the groups at Month 36 was not statistically significant: -0.1 points (SE = 0.62, p = 0.1053).

On the Health subscale, the subjects in the casein-free group declined over the 36-month period by 1.07 points (SE = 0.79, p = 0.1779) compared to an improvement of 0.96 points (SE = 0.89, p = 0.282) in the control group, Table S10. The difference between the groups at Month 36 was statistically significant: 2.27 points (SE = 1.11, p = 0.0414).

Animal Meat- and Eggs-eating

Participants who consumed two or more servings of "animal meat" per day (one serving = one egg or slice of meat; meat-eating group) were matched to those who consumed half a serving or less (control group) using a propensity score based on age, gender, language comprehension, expressive language, sociability, sensory awareness, and health score at the 1st evaluation (baseline). Participants who consumed one serving a day were not included in the analysis. The number of matched participants was 1,067 out of 1,071 in the meat-eating group and 1,067 out of 1,265 in the control group. We then analyzed trajectories of children development on five orthogonal subscales: Language Comprehension, Expressive Language, Sociability, Sensory Awareness, and Health.

On the Language Comprehension subscale, the average improvement in the meat-eating group over 36 months was 9.04 points (SE = 0.42, p < 0.0001) compared to 5.84 points (SE = 0.58, p < 0.0001) in the control group, Fig. 1, Table S43, Table S11. The difference in the meat-eating relative to the control at Month 36 was statistically significant: -2.4 points (SE = 0.56, p < 0.0001). The negative difference indicates that the control group had greater scores at Month 36 and, therefore, more severe symptoms. On an annualized basis, the participants from the meat-eating group improved their language comprehension 1.6-times faster than those in the control group (meat-eating = 3 points/year; control = 1.9 points/year).

On the Expressive Language subscale, the participants in the meat-eating group improved over the 36-month period by 6.2 points (SE = 0.31, p < 0.0001) compared to 5.06 points (SE = 0.32, p < 0.0001) improvement in the control group, Table S12. The difference between the groups at Month 36 was statistically significant: -0.92 points (SE = 0.42, p = 0.0279). On an annualized basis, the participants from the meat-eating group improved their expressive language 1.1-times faster than those in the control group (meat-eating = 2.1 points/year; control = 1.7 points/year).

On the Sociability subscale, the subjects in the meat-eating group improved over the 36-month period by 1.77 points (SE = 0.42, p < 0.0001) compared to 1.74 points

(SE = 0.43, p = 0.0902) improvement in those in the control group, Table S13. The difference between the groups at Month 36 was not statistically significant: -0.93 points (SE = 0.55, p = 0.0944).

On the Sensory Awareness subscale, the subjects in the meat-eating group improved over the 36-month period by 2.21 points (SE = 0.35, p < 0.0001) compared to 0.35 points (SE = 0.36, p = 0.3344) improvement in the control group, Table S14. The difference between the groups at Month 36 was statistically significant: -1.89 points (SE = 0.46, p < 0.0001).

On the Health subscale, the subjects in the meat-eating group declined over the 36-month period by 0.45 points (SE = 0.63, p = 0.4725) compared to a decline of 1.53 points (SE = 0.65, p = 0.0186) in the control group, Table S15. The difference between the groups at Month 36 was not statistically significant: -0.78 points (SE = 0.83, p = 0.3467).

Vegetable-eating

Participants who consumed two or more servings of "cooked or uncooked vegetables (carrots, broccoli, cauliflower, salad, apples, etc.; one serving = one apple or one carrot or one plate of salad)" per day (vegetable-eating group) were matched to those who consumed half a serving or less (control group) using a propensity score based on age, gender, language comprehension, expressive language, sociability, sensory awareness, and health score at the 1st evaluation (baseline). Participants who consumed one serving a day were not included in the analysis. The number of matched participants was 1,232 out of 1,232 in the vegetable-eating group and 1,232 out of 1,581 in the control group. We then analyzed trajectories of children development on five orthogonal subscales: Language Comprehension, Expressive Language, Sociability, Sensory Awareness, and Health.

On the Language Comprehension subscale, the average improvement in the vegetable-eating group over 36 months was 8.27 points (SE = 0.38, p < 0.0001) compared to 5.82 points (SE = 0.42, p < 0.0001) in the control group, Fig. 1, Table S44, Table S16. The difference in the vegetable-eating group relative to the control group at Month 36 was statistically significant: -2.49 points (SE = 0.53, p < 0.0001). The negative difference indicates that the control group had greater scores at Month 36 and, therefore, more severe symptoms. On an annualized basis, the participants from the vegetable-eating group improved their language comprehension 1.5-times faster than those in the control group (vegetable-eating = 2.8 points/year; control = 1.9 points/year).

On the Expressive Language subscale, the participants in the vegetable-eating group improved over the 36-month period by 5.65 points (SE = 0.29, p < 0.0001) compared to 4.66 points (SE = 0.32, p < 0.0001) improvement in the control group, Table S17. The difference between the groups at Month 36 was statistically significant: -0.99 points (SE = 0.40, p = 0.0137). On an annualized basis, the participants from the vegetable-eating group improved their expressive language 1.2-times faster than those in the control group (vegetable-eating = 1.9 points/year; control = 1.6 points/year).

On the Sociability subscale, the subjects in the vegetable-eating group improved over the 36-month period by 1.55 points (SE = 0.39, p < 0.0001) compared to 0.87 points (SE = 0.42, p = 0.0389) improvement in those in the control group, Table S18. The difference between the groups at Month 36 was not statistically significant: -0.60 points (SE = 0.53, p = 0.2537).

On the Sensory Awareness subscale, the subjects in the vegetable-eating group improved over the 36-month period by 2.34 points (SE = 0.33, p < 0.0001) compared to 0.64 points (SE = 0.36, p = 0.077) improvement in the control group, Table S19. The difference between the groups at Month 36 was statistically significant: -1.74 points (SE = 0.45, p = 0.0001).

On the Health subscale, the subjects in the vegetable-eating group declined over the 36-month period by 0.09 points (SE = 0.58, p = 0.8749) compared to a decline of 1.14 points (SE = 0.63, p = 0.0715) in the control group, Table S20. The difference between the groups at Month 36 was not statistically significant: -0.73 points (SE = 0.79, p = 0.3538).

Uncooked-vegetables Only

Participants who consumed two or more servings of "uncooked vegetables (carrots, broccoli, cauliflower, salad, apples, etc.; one serving = one apple or one carrot or one plate of salad)" per day (uncooked-vegetable-eating group) were matched to those who consumed half a serving or less (control group) using a propensity score based on age, gender, language comprehension, expressive language, sociability, sensory awareness, and health score at the 1st evaluation (baseline). Participants who consumed one serving a day were not included in the analysis. The number of matched participants was 616 out of 617 in the uncooked-vegetable-eating group and 616 out of 2,559 in the control group. We then analyzed trajectories of children development on five orthogonal subscales: Language Comprehension, Expressive Language, Sociability, Sensory Awareness, and Health.

On the Language Comprehension subscale, the average improvement in the uncooked-vegetable-eating group over 36 months was 8.76 points (SE = 0.57, p < 0.0001) compared to 5.87 points (SE = 0.61, p < 0.0001) in the control group, Fig. 1, Table S45, Table S21. The difference in the

uncooked-vegetable-eating group relative to the control group at Month 36 was statistically significant: -3.11 points (SE = 0.74, $p < 0.0001$). The negative difference indicates that the control group had greater scores at Month 36 and, therefore, more severe symptoms. On an annualized basis, the participants from the uncooked-vegetable-eating group improved their language comprehension 1.5-times faster than those in the control group (uncooked-vegetable-eating = 2.9 points/year; control = 2.0 points/year).

On the Expressive Language subscale, the participants in the uncooked-vegetable-eating group improved over the 36-month period by 6.16 points (SE = 0.43, $p < 0.0001$) compared to 5.64 points (SE = 0.46, $p < 0.0001$) improvement in the control group, Table S22. The difference between the groups at Month 36 was not statistically significant: -0.66 points (SE = 0.55, p = 0.2307).

On the Sociability subscale, the subjects in the uncooked-vegetable-eating group improved over the 36-month period by 1.8 points (SE = 0.57, p = 0.0016) compared to 0.86 points (SE = 0.61, p = 0.1576) improvement in those in the control group, Table S23. The difference between the groups at Month 36 was not statistically significant: -0.90 points (SE = 0.73, p = 0.2165).

On the Sensory Awareness subscale, the subjects in the uncooked-vegetable-eating group improved over the 36-month period by 3.61 points (SE = 0.48, $p < 0.0001$) compared to 1.67 points (SE = 0.51, p = 0.011) improvement in the control group, Table S24. The difference between the groups at Month 36 was statistically significant: -1.97 points (SE = 0.62, p = 0.0014).

On the Health subscale, the subjects in the uncooked-vegetable-eating group declined over the 36-month period by 1.21 points (SE = 0.84, p = 0.1467) compared to an improvement of 0.74 points (SE = 0.89, p = 0.4091) in the control group, Table S25. The difference between the groups at Month 36 was statistically significant: 2.46 points (SE = 1.08, p = 0.0222).

Sweets

Participants who consumed two or more servings of "Sweets (one serving = one candy or one teaspoon of sugar)" per day (sweets-eating group) were matched to those who consumed half a serving or less (control group) using a propensity score based on age, gender, language comprehension, expressive language, sociability, sensory awareness, and health score at the 1st evaluation (baseline). Participants who consumed one serving a day were not included in the analysis. The number of matched participants was 732 out of 732 in the sweets-eating group and 732 out of 2,197 in the control group. We then analyzed trajectories of children development on five orthogonal subscales: Language Comprehension, Expressive Language, Sociability, Sensory Awareness, and Health.

On the Language Comprehension subscale, the average improvement in the sweets-eating group over 36 months was 6.23 points (SE = 0.52, $p < 0.0001$) compared to 5.19 points (SE = 0.59, $p < 0.0001$) in the control group, Fig. 1, Table S46, Table S26. The difference in the sweets-eating group relative to the control group at Month 36 was not statistically significant: -1.27 points (SE = 0.67, $p < 0.0565$). The negative difference indicates that the control group had greater scores at Month 36 and, therefore, more severe symptoms.

On the Expressive Language subscale, the participants in the sweets-eating group improved over the 36-month period by 5.55 points (SE = 0.38, $p < 0.0001$) compared to 5.25 points (SE = 0.44, $p < 0.0001$) improvement in the control group, Table S27. The difference between the groups at Month 36 was not statistically significant: -0.48 points (SE = 0.49, p = 0.3227).

On the Sociability subscale, the subjects in the sweets-eating group declined over the 36-month period by 0.34 points (SE = 0.53, p = 0.5253) compared to improvement of 1.16 points (SE = 0.61, p = 0.0552) in the control group, Table S28. The difference between the groups at Month 36 was not statistically significant: 1.31 points (SE = 0.68, p = 0.0535).

On the Sensory Awareness subscale, the subjects in the sweets-eating group improved over the 36-month period by 1.79 points (SE = 0.44, $p < 0.0001$) compared to 2.32 points (SE = 0.50, $p < 0.0001$) improvement in the control group, Table S29. The difference between the groups at Month 36 was not statistically significant: -0.31 points (SE = 0.56, p = 0.5806).

On the Health subscale, the subjects in the sweets-eating group declined over the 36-month period by 3.64 points (SE = 0.78, $p < 0.0001$) compared to an improvement of 1.35 points (SE = 0.89, p = 0.1312) in the control group, Table S30. The difference between the groups at Month 36 was statistically significant: 5.11 points (SE = 1.0, $p < 0.0001$). The positive difference indicates that the sweets group had greater scores at Month 36 and, therefore, more severe symptoms.

Bread-eating

Participants who consumed two or more servings of bread "(one serving = one slice of bread)" per day (bread-eating group) were matched to those who consumed half a serving or less (control group) using a propensity score based on age, gender, language comprehension, expressive language, sociability, sensory awareness, and health score at the 1st evaluation (baseline). Participants who consumed one

serving a day were not included in the analysis. The number of matched participants was 1036 out of 1038 in the bread-eating group and 1,036 out of 1,608 in the control group. We then analyzed trajectories of children development on five orthogonal subscales: Language Comprehension, Expressive Language, Sociability, Sensory Awareness, and Health.

On the Language Comprehension subscale, the average improvement in the bread-eating group over 36 months was 7.17 points (SE = 0.43, $p < 0.0001$) compared to 5.95 points (SE = 0.49, $p < 0.0001$) in the control group, Fig. 1, Table S47, Table S31. The difference in the bread-eating group relative to the control group at Month 36 was statistically significant: -1.39 points (SE = 0.57, p = 0.0153). The negative difference indicates that the control group had greater scores at Month 36 and, therefore, more severe symptoms. On an annualized basis, the participants from the bread-eating group improved their language comprehension 1.2-times faster than those in the control group (bread-eating = 2.4 points/year; control = 2.0 points/year).

On the Expressive Language subscale, the participants in the bread-eating group improved over the 36-month period by 5.93 points (SE = 0.32, $p < 0.0001$) compared to 5.10 points (SE = 0.37, $p < 0.0001$) improvement in the control group, Table S32. The difference between the groups at Month 36 was statistically significant: -0.91 points (SE = 0.42, p = 0.0308).

On the Sociability subscale, the subjects in the bread-eating group declined over the 36-month period by 0.21 points (SE = 0.43, p = 0.633) compared to 0.05 points (SE = 0.5, p = 0. 9221) decline in the control group, Table S33. The difference between the groups at Month 36 was not statistically significant: -0.29 points (SE = 0.57, p = 0.6066).

On the Sensory Awareness subscale, the subjects in the bread-eating group improved over the 36-month period by 2.22 points (SE = 0.35, $p < 0.0001$) compared to 1.82 points (SE = 0.41, p = 0.0011) improvement in the control group, Table S34. The difference between the groups at Month 36 was not statistically significant: -0.48 points (SE = 0.47, p = 0. 3091).

On the Health subscale, the subjects in the bread-eating group declined over the 36-month period by 1.74 points (SE = 0.64, p = 0.0066) compared to an improvement of 0.25 points (SE = 0.74, p = 0. 7373) in the control group, Table S35. The difference between the groups at Month 36 was statistically significant: -2.34 points (SE = 0.84, p = 0.0056).

White Soft Bread that Never Molds

Participants who consumed two or more servings of “White soft bread that never molds (one serving = one slice of bread)” per day (never-mold-bread-eating group) were matched to those who consumed half a serving or less (control group) using a propensity score based on age, gender, language comprehension, expressive language, sociability, sensory awareness, and health score at the 1st evaluation (baseline). Participants who consumed one serving a day were not included in the analysis. The number of matched participants was 407 out of 407 in the never-mold-bread-eating group and 407 out of 3,106 in the control group. We then analyzed trajectories of children development on five orthogonal subscales: Language Comprehension, Expressive Language, Sociability, Sensory Awareness, and Health.

On the Language Comprehension subscale, the average improvement in the never-mold-bread-eating group over 36 months was 8.39 points (SE = 0.69, $p < 0.0001$) compared to 2.27 points (SE = 0.79, p = 0.0041) in the control group, Fig. 1, Table S48, Table S36. The difference in the never-mold-bread-eating group relative to the control group at Month 36 was statistically significant: -6.32 points (SE = 0.91, $p < 0.0001$). The negative difference indicates that the control group had greater scores at Month 36 and, therefore, more severe symptoms. On an annualized basis, the participants from the never-mold-bread-eating group improved their language comprehension 3.7-times faster than those in the control group (never-mold-bread-eating = 2.8 points/year; control = 0.76 points/year).

On the Expressive Language subscale, the participants in the never-mold-bread-eating group improved over the 36-month period by 5.59 points (SE = 0.51, $p < 0.0001$) compared to 3.46 points (SE = 0.59, $p < 0.0001$) improvement in the control group, Table S37. The difference between the groups at Month 36 was statistically significant: -2.21 points (SE = 0.67, p = 0.001). On an annualized basis, the participants from the never-mold-bread-eating group improved their language comprehension 1.7-times faster than those in the control group (never-mold-bread-eating = 1.9 points/year; control = 1.2 points/year).

On the Sociability subscale, the subjects in the never-mold-bread-eating group declined over the 36-month period by 0.87 points (SE = 0.64, p = 0.1711) compared to 0.29 points (SE = 0.74, p = 0.6969) decline in the control group, Table S38. The difference between the groups at Month 36 was not statistically significant: 0.58 points (SE = 0.84, p = 0.4885).

On the Sensory Awareness subscale, the subjects in the never-mold-bread-eating group improved over the 36-month period by 1.54 points (SE = 0.55, p = 0.0054) compared to 0.2 points (SE = 0.64, p = 0.7489) improvement in the control group, Table S39. The difference between the groups at Month 36 was statistically significant: -1.64 points (SE = 0.73, p = 0.0250).

On the Health subscale, the subjects in the never-mold-bread-eating group declined over the 36-month period by

5.21 points (SE = 1.02, $p < 0.0001$) compared to a decline of 0.58 points (SE = 1.17, p = 0.6195) in the control group, Table S40. The difference between the groups at Month 36 was statistically significant: 4.76 points (SE = 1.35, p = 0.0004). The positive difference indicates that the never-mold-bread group had greater scores at Month 36 and, therefore, more severe symptoms.

Discussion

Effects of Diet on Outcome Measures

This is the largest and longest study to date demonstrating a strong association between diets and children's developmental trajectories. Over the period of three years, parents assessed the development of 5,553 young children with ASD quarterly. Two diets (gluten-free and casein-free) and six types of foods (meat/eggs, vegetables, uncooked vegetables, sweets, bread, and "white soft bread that never molds") were investigated. To study the effect of a diet, participants were divided into two groups: those who followed the diet "usually", "nearly always", or "always" were compared to those who did not follow the diet (those who followed the diet "sometimes" were not included in either group). Similarly, to study an effect of a food type consumption, participants were divided into two groups: those who consumed two or more servings per day of a food type were compared to those who consumed half a serving or less (those who consumed one serving a day were not included in either group). In order to compare the two groups, participants were matched using a propensity score (Schneider et al., 2007) based on age, gender, expressive language, language comprehension, sociability, sensory awareness, and health at the 1st evaluation (baseline).

Different diets resulted in wide ranges of effects: some had diffuse beneficial effects on various outcome measures, some had diffuse adverse, and others had both beneficial and adverse effects. The most diffusely beneficial diets included consumption of meat and eggs, vegetables, and uncooked vegetables. These three diets are correlated with significant and consistent improvements in complex-language comprehension (Fig. 1, the blue dashed line raises toward a lower score which corresponds to improved language comprehension). The consumption of meat and eggs also demonstrated a significant and consistent correlation with improvement in sensory awareness, while consumption of vegetables was also linked to some improvement in expressive language and sociability.

The gluten-free diet was another diet that only had beneficial effects on outcome measures. A gluten-free diet had significant and consistent effects on complex-language comprehension and small positive nonsignificant effect in other subscales. The casein-free diet did not have significant and consistent effects on evaluation scores. Future studies shall investigate combinations of diets, which may have a synergistic effect.

Consumption of "fast" carbohydrates – sweets, bread, and "white soft bread that never molds"– was associated with a significant and consistent health decline over the three years of observation (Fig. 1, the blue dashed line declines toward a greater health score, which corresponds to increased symptoms severity). Other studies have similarly found that regular consumption of simple carbohydrates can deteriorate health in various ways, including putting individuals at risk for metabolic disease (Clemente-Suárez et al., 2022; Plaza-Diaz et al., 2021).

Surprisingly, while consumption of "white soft bread that never molds" (most frequently treated with the preservative calcium propionate) was associated with a significant and consistent decline of health, it was also associated with a greater improvement of complex-language comprehension and expressive language. Usually, a decline in the health score is associated with a comparable decline in the language score. For example, we have previously reported that sleep problems in young autistic children were associated with a significant and consistent health decline and also with a lower language development (Levin et al., 2022). Similarly, the presence of seizures in young autistic children was associated with a health decline and with a lower expressive language development (Forman et al., 2022). Likewise, increased video- and television-watching was associated with a health decline and also with a lower complex-language comprehension development (Fridberg et al., 2021). This is the first time in our research when we observed a dissociation between the directions of the health and the language developmental trajectories. The mechanism of such a dissociation is unclear, but it may result from an interaction between simple carbohydrates of the bread with the preservative propionate.

Possible Mechanisms of Correlation Between Diet, Health, and Language Development

High levels of both simple carbohydrates and propionate are known to affect metabolic health and homeostasis. Children's gut microbiome and their metabolic health are more susceptible to rapid changes than that of adults (Peretti et al., 2019; van De Sande et al., 2014). Simple carbohydrates, like those found in sweets and refined grains like white bread, provide fast energy. Simple carbohydrates do not require diverse microorganisms to break down the long carbohydrate molecules in the large intestine and are instead quickly absorbed by the small intestine. Conversely,

complex dietary fibers are broken by microorganisms into smaller substrates, like the short chain fatty acids (SCFAs). Propionate itself is in fact a SCFA that is produced by gut microorganisms along with other SCFAs butyrate and acetate (Clemente-Suárez et al., 2022).

SCFAs work together to help regulate metabolism through many pathways, such as gluconeogenesis, satiety signaling, lipogenesis, cell differentiation, and cholesterol metabolism (Lin et al., 2012; Louis & Flint, 2017). Of all the SCFAs, propionate plays the largest role in gluconeogenesis, which means it promotes the generation of glucose in the liver from non-carbohydrate substrates, such as amino acids and complex proteins (Høgh et al., 2020; Wang et al., 2018). The other SCFAs work to downregulate gluconeogenesis and upregulate production of other molecules in order to maintain glucose secretion at stable levels (Wong et al., 2006). However, this stability may be disrupted when propionate is provided directly by food, as it is in the digestion of white preserved bread, rather than being a byproduct of metabolism of complex dietary fibers. Such presence of propionate may disrupt homeostatic regulation of metabolites (Clemente-Suárez et al., 2022).

Both simple carbohydrates and propionate increase glucose levels and speed up metabolism, providing a boost of energy to the brain and quickly increasing alertness, wakefulness, and cognitive performance (Clemente-Suárez et al., 2022). This has been confirmed experimentally in studies where children's cognitive performance is compared immediately after consumption of easily digestible food (e.g., French fries) versus slower-digestible food (e.g., roasted potatoes) (Lee et al., 2019). However, this quick metabolism comes at a cost. Consumption of these easily digestible high energy substances lead to mood swings between alertness and tiredness. Long-term consumption of easily digestible high energy substances like simple carbohydrates and propionate can lead to an overproduction of insulin and subsequent insulin resistance, the foundation of many metabolic diseases, such as obesity and diabetes (Freeman & Pennings, 2022). In the gut microbiome, overprovision of these substances leads to a lack of microbial diversity (Plaza-Diaz et al., 2021) and reduces the ability to digest complex metabolites. It also leaves the gut susceptible to damage, infection, and inflammation (Plaza-Díaz et al., 2022).

Recent experiments of propionate application to cell colonies and animal models have found similarly concerning effects. Rats administered propionate-heavy diets displayed stereotypical behaviors of autism, such as aggression, sensory sensitivity, and a lack of the ability to inhibit reflexes (Choi et al., 2018; Foley et al., 2015). Human stem cell colonies treated with propionic acid led to an over-proliferation of glial cells and decreased proportion of neurons (Abdelli et al., 2019).

While possible mechanisms linking long-term propionate consumption to disrupted metabolic health are abundant, the association of propionate with long-term language improvement remains unclear. If the mechanism is based on short-term increase in alertness, wakefulness, and cognitive performance, then we would expect to observe a similar effect in all "fast" carbohydrates, including sweets and bread, which was not observed in this study (Fig. 1). Accordingly, a propionate mechanism of action may be completely separate from that of "fast" carbohydrates. One interesting possibility is an antidepressant effect induced in rats administered low-dose propionate (Hao et al., 2020). Another possibility is propionate regulation of fat metabolism and its effect on reduction of obesity (Chambers et al., 2015; Li et al., 2021; Psichas et al., 2015). Investigation of the propionate role in autism remains a promising area of research.

Limitations

The observational design of this study cannot definitively prove causality since unknown confounders may influence the study results. The golden standard of testing therapeutic interventions is a randomized controlled trial (RCT). RCTs, however, are usually limited to 6-months or less, which may not be enough to detect an effect of a dietary intervention on a developmental trajectory. Unlike RCTs, epidemiological studies can run for many years. In this study, dissociation between the groups in terms of their language abilities is not detected until the third year of the investigation (Fig. 1; gluten-free diet, meat-eating, vegetable-eating, uncooked-vegetable-eating, and never-mold-bread-eating). Furthermore, this type of study involves real-time coding of dietary habits and, therefore, minimizes guessing and telescoping effects associated with a single survey.

Food consumption questionnaires inevitably contain items such as "usually" and "always," which are open to different interpretations among different groups of people. In this study we combined the participants who followed a diet "usually," "nearly always," or "always" into one dieting group and those who did not follow a diet or were not familiar with a diet into the control group. Participants who followed a diet "sometimes" were excluded from the study. Such an approach should have reduced the variability associated with various questionnaire interpretations.

Another limitation is that there was no checking for gluten-free and casein-free diets purity. Exclusion diets can sometimes be contaminated by other foods and, therefore, checking for this cross-contamination (e.g., using direct

questions about the exemption of food items) can improve data quality.

Other limitations include relying on parent-reports for diagnosis and assessments. Participants of this study had their diagnosis identified by caregivers as ASD at their last evaluation. Diagnosis misidentification should not have been a significant problem. The app is popular primarily in the ASD community. Most app users (82%) reported ASD diagnosis by their last evaluation. If ASD participants were mislabeled as a different condition, they would have been excluded from this study. Second, if participants without ASD diagnosis were misidentified by their caregivers as ASD, the caregivers must have also misrepresented participants' ATEC score (calculated based on 77 questions) as the average initial ATEC total score was 68.4 ± 24.1, which corresponds to moderate ASD as delineated earlier (Jagadeesan et al., 2022) and shown in Table 1. It is highly unlikely that caregivers went through such an effort to consciously misrepresent participants' condition since there was no benefit in doing so, although random events of this kind cannot be completely excluded.

We have also previously explored the relationships between different levels of ASD severity reported by parents and the reported scores. We hypothesized that if parents clearly understood and honestly communicated their child's diagnosis, the reported ASD severity level would have a consistent relationship with assessment subscales. Wherein, greater ASD severity would correspond to worse assessment scores and vice versa. Conversely, if parents misreported their children's diagnosis, no difference in the average assessment score would be expected between the groups. This cross-sectional analysis of 9,573 children has demonstrated statistically significant differences between mild and moderate ASD diagnosis as well as between moderate and severe ASD diagnosis in each subscale and in every age group in children 3 years of age and older (Jagadeesan et al., 2022). These findings contribute to support good reliability of parents' reports of children's diagnosis.

In terms of evaluation scores, two biases are possible. Parents may yield to wishful thinking and overestimate their children's abilities on a single assessment (Scattone et al., 2011). This, however, is not a problem for a longitudinal study as the pattern of changes generated by measuring the score dynamics over multiple assessments provides meaningful data on a child's developmental trajectory even if wishful thinking was inflating each individual assessment. A second possible bias concerns parents who may wish to exaggerate their child's improvement by purposefully entering a score that is better than their previous assessment. If this was the case, one would surmise that parents who have invested more time and energy into the app were also more likely to rate their child as improving. We have previously addressed this possibility analytically by calculating the correlation coefficient between the app usage measured in days/week with child's improvement in language comprehension ($r = -0.01$), expressive language ($r = -0.06$), sociability ($r = -0.04$), cognitive awareness ($r = -0.01$), and health ($r=0.01$) (Vyshedskiy et al., 2020). Low absolute values of correlation coefficients, as well as the variability of the direction of correlation (positive correlation for improvement of health and negative correlation for other subscales) were not consistent with the hypothesis that parents who invested more time into working with the app were also more likely to rate their child as improving. Additionally, even if parents wanted to score their children as improving (consciously or unconsciously), this would have been very difficult as parents were blinded to their answers at all the previous evaluations and it is near-impossible to recall one's answers to 133 questions (with each question coming with 3 to 6 options) for three months (time between evaluations). We conclude that it is unlikely that evaluation biases influenced the results of this study.

Another confounder could be a parent educational level. Parent education information was collected by our survey, and we repeated analysis with parent education level entered as a covariate. Adjusting for parent education level did not alter the results. The presented results are based on analyses without covarying parent education level.

Still another confounder could be a duration of speech and language therapy. We repeated analysis with duration of speech and language therapy entered as a covariate. Adjusting for duration of speech and language therapy did not alter the results. The presented results are based on analyses without covarying duration of speech and language therapy.

We did not have any information about participants' celiac disease, allergies, or intolerances, and, therefore, could not investigate the association of these conditions with diets and symptom improvement.

Conclusions

Diet can be a powerful determinant of a child's developmental trajectory. The results of this study demonstrate strong correlation between consumption of "fast" carbohydrates (sweets and bread) and a significant and a consistent health decline. On the contrary, a gluten-free diet, meat-, eggs-, and vegetable-eating were associated with a significant and consistent improvement of complex-language comprehension. Obviously, these conclusions are drawn from averaged results and diet choices must always be governed by an individual's needs.

Importantly, the complex-language comprehension scale MSEC was the only outcome measure sensitive to

the positive effect of most food types, confirming the value of including a complex-language subscale into the battery of outcome measures. Furthermore, this effect was only detected during the third year of observation, suggesting the need for long-term studies.

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Data Availability De-identified raw data from this manuscript are available from the corresponding author upon reasonable request.

Code Availability Statement Code is available from the corresponding author upon reasonable request.

Declarations

Informed Consent Caregivers have consented to anonymized data analysis and publication of the results. The study was conducted in compliance with the Declaration of Helsinki (World Medical Association, 2013).

Compliance with Ethical Standards Using the Department of Health and Human Services regulations found at 45 CFR 46.101(b)(4), the Biomedical Research Alliance of New York LLC Institutional Review Board (IRB) determined that this research project is exempt from IRB oversight.

Competing Interests Authors declare no competing interests.

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