Eat2beNICE

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

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D5.2: Manuscript on the effect of dietary interventions on microbiome composition and its relation with impulsive/compulsive behaviour relationship

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1. DELIVERABLE REPORT

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INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental condition [1]. Symptoms of hyperactivity/impulsivity and inattention persist into adulthood in more than half of the affected individuals [2]. Up to 70 % of adults with persistent ADHD are further affected by emotion regulation problems, such as reactive aggression [3]. Aggressive behavior is a frequent catalyst for diagnostic consultation [4] and has a large impact on social and functional impairment, such as dysfunctional relationships, peer rejection, impairments in school/occupation, and higher risk of engaging in criminal behavior or suicidal attempts [5-8] Little is known about potential mechanisms underlying the co-occurrence of ADHD with reactive aggression. Alterations of immune response, inflammatory processes affecting brain development and altered neurotransmission and brain functioning in ADHD might play a role for the development of aggressive behavior. In fact, people with ADHD exhibited altered brain development in regions of emotion regulation [9, 10] and altered brain functioning during emotion processing in relation to elevated reactive aggression [11]. Next to genetic predisposition, these processes are likely influenced by environmental factors [12-14] Recently, diet and the gut-microbiome have received attention with regard to alterations in ADHD and emotional behavior, representing potential targets for prevention and treatment support [15-18].

Diet and ADHD

Multiple studies have reported different eating behaviour in children and adolescents with ADHD compared to neurotypical peers (for a review, see [19]). For example, Western diet, high in fats, proteins and sugars, as well as low consumption of fruits, vegetables, and foods that are rich in polyunsaturated fatty acids (PUFAs) and minerals, were associated with an increased risk for ADHD (for a review, see [16]). Furthermore, some dietary interventions could partially ameliorate the symptoms of ADHD by either restricting sugar consumption, imposing additive and preservative free, or hypoallergenic diets (for a review see [20]) or adding supplements such as omega 3 PUFAs, minerals such as zinc and iron, and multivitamins [19].

Gut Microbiota and ADHD

The gut microbiota can influence brain functioning, development and behavior relevant for ADHD by modulating the synthesis and bioavailability of key neurotransmitters such as dopamine and serotonin [21] or neuroinflammatory processes, which have been associated with ADHD and related behaviour.

In this study we aim to investigate the *direct* associations between diet and behavior and the *mediator* role of the gut-microbiota in diet - behavior relationships.

METHODS

Participants

A total of 83 adults with and 79 without ADHD participated in the IMpACT2-NL study. We excluded participants with missing fecal samples, irritable bowel syndrome (IBS), > 30% missing answers in the relevant online questionnaires and unclear ADHD status (control subjects with >6 symptoms in one DIVA subscale or cases with < 6 symptoms over both subscales), resulting in 77 participants with and 77 without ADHD. Both groups had comparable distributions of age, sex and BMI (**See Table 1**). All participants provided written informed consent before participating in the study and received monetary compensation. Among a battery of neuropsychological tests and questionnaires, participants completed a short semiquantitative food questionnaire and the Reactive-Proactive

Aggression Questionnaire (RPQ). Participants were instructed to collect their fecal samples at home using a validated protocol by OMNIgene•GUT kit (DNAGenotek, Ottawa, CA) and send them back to our laboratory for gut-microbiota analyses.

Measures

Dietary patterns

Dietary patterns were investigated using an Exploratory Factor Analysis (EFA) (*psych* package [22]) on the weekly quantities of the food items from the semiquantitative food frequency questionnaire. We used the heterogeneous correlation matrix to assess the factor loadings, accounting for mixed ordinal categorical and continuous input from the questionnaire [23, 24].

Analyses

Diet-Behavior associations

We performed logistic regression to investigate the associations of ADHD diagnosis and rank-based regression (*Rfit* package [25]) to investigate associations of ADHD and begavior with all of the resulting diet factors.

Microbiota-Behavior associations

We investigated associations of Shannon diversity (alpha diversity) and Aitchison distance (beta diversity) on amplicon-sequence-variant (ASV) level with reactive aggression and ADHD diagnosis using rank-based regression and permanova respectively. Bacterial abundance was transformed applying the center-log-ratio (CLR) transformation. We further applied randomized Lasso feature selection (*monaLisa* package, 5% selection probability [26]) to select genera to test associations with each behavior. The association between CLR transformed abundances and both behaviors was done applying rank-based- and logistic regression respectively.

Mediation Analysis of the gut-microbiota / Diet

We first selected genera for the relevant diet factor using randomized Lasso stability selection (≥ 5% selection probability). We then tested which of these genera (CLR-transformed abundance) were potential mediators (using the *mma* package [27], default $p < .1$ for correlations with diet and behavior) using nonparametric mediation analysis of the diet-behavior relationship (*mediation* package [28], see Figure 1).

RESULTS

Diet

Parallel analysis suggested to apply the EFA on 3 factors, (see scree plots, Figure 3). The three resulting dietary patterns were characterized as follows:

- Factor1 was characterized by high consumption of alcohol and meat and low consumption of sweetened beverages and chocolate – we describe this factor as alcohol-rich/savory diet;
- Factor2 was defined by a high consumption of sweetened beverages, milk and meat and low consumption of vegetables – resembling an “unhealthy” diet similar to a western diet;
- Factor3 showed high consumption of legumes, fruits and vegetables, low consumption of meat, milk and chocolate – describing a “healthy”/ vegetarian diet.

Figure 4 shows the loadings of each food item on the three factors.

Diet and ADHD diagnosis

Dietary factors, age, sex and BMI were not associated with ADHD diagnosis, smoking status showed a positive association with ADHD diagnosis, see Table 2.

Microbiota-Behavior associations

Alpha and beta diversity were not significantly associated with ADHD diagnosis but with age and sex (and alpha diversity also with BMI), see figures 4 & 5. Differential abundance analysis showed significant associations of 14 out of 15 selected genera for ADHD and 8 out of 14 selected genera for reactive aggression with the respective behavior, see table 5.

The gut-microbiota as a mediator of diet and behavior

We performed mediation analyses of the gut-microbiota on the significant association between the ***"unhealthy"*** diet factor and reactive aggression scores. Two of these genera, *Eubacterium_nodatum_group* and *Lachnospiraceae_UCG_010,* were identified as potential mediators.

Eubacterium_nodatum_group was significantly associated with the "unhealthy" diet (t = -1.99, p = 5.00 E^{-2}) The partial mediation effect of *Eubacterium nodatum group* on the diet-behavior relationship did not reach significance (6.6 E^{-2}), see Table T4.

DISCUSSION

In the current study we showed that diet and the gut-microbiota play a role in reactive aggression in adults with and without ADHD, answering the research questions.

1. Are dietary patterns related to ADHD and reactive aggression and is there an overlap between dietary patterns relevant for ADHD and reactive aggression that might be related to the co-occurrence? -While an "unhealthier" high-energy diet was associated with reactive aggression, associations between diet and ADHD diagnosis were not shown in our adult population.
2. Are alterations in diversity and composition of the gut microbiota related to ADHD and reactive aggression and is there an overlap of microbial alterations between both? -Gut microbial diversity was not associated with either outcome. The composition showed associations with both behaviors, the abundance of 14 genera was significantly altered in adults with ADHD and 8 genera were associated with reactive aggression scores. However, compositional alterations related to ADHD and reactive aggression did not overlap.
3. Is diet functioning as a mediator between associations of behavioral outcomes and gut-microbiome signatures? While a diet rich in alcohol might mediate the association between Caulobacter and ADHD diagnosis, this result did not survive multiple testing correction.

These results show that diet and the microbiota affect reactive aggression and ADHD individually. If replicated, these results could help identify targets for nutritional or pre-/probiotic interventions as treatment support for reactive aggression in the context of adult ADHD. While inflammatory processes might play a role for both, reactive aggression and ADHD, the interplay of diet, the gut-microbiota and these behaviors as well as potential mechanism have to be investigated further. To do so, large, functional studies with deep dietary phenotyping are needed to robustly identify dietary and microbial signatures.

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2. Tables and other supporting documents where applicable and necessary

Table 1. Demographic description of the sample

	ADHD	Controls	*p*-value
N	77	77	
Age (SD)	34.09 (10.37)	34.47 (13.10)	7.37 E^{-1}
Sex, % male	42.86 %	48.05 %	6.27 E^{-1}
BMI (SD)	24.90 (4.51)	24.93 (4.24)	9.93 E^{-1}
Smoking,% current non-smokers	70.13 %	90.91 %	2.27 E^{-3}
Stimulant medication, % current users	57.14 %	0 %	1.72 E^{-14}
Reactive aggression scores (SD)	8.23 (4.04)	5.64 (3.15)	3.33 E^{-5}
Number of inattentive symptoms DIVA (SD)	7.34 (1.90)	0.84 (1.29)	2.20 E^{-16}
Number of hyperactive/impulsive symptoms DIVA (SD)	5.62 (2.22)	0.87 (1.26)	2.20 E^{-16}
Scores Diet1 (SD)	-0.15 (1.06)	0.14 (1.44)	3.42 E^{-1}
Scores Diet2 (SD)	0.02 (1.46)	-0.05 (1.38)	8.28 E^{-1}
Scores Diet3 (SD)	-0.01 (1.06)	-0.07 (1.30)	3.66 E^{-1}

Table T1. Demographic description of the sample including mean and standard deviation of age, BMI, mean centered diet scores and reactive aggression scores as well as percentage of current stimulant users, current non-smokers and male participants. Antibiotic and probiotic usage was assessed in the scale of often, sometimes, rarely or never: no participant used antibiotics frequently, 7 participants used probiotics frequently. Statistical testing was performed using the Mann Whitney test as well as the Chi-squared test for distribution free comparisons of independent samples, significant results are highlighted.

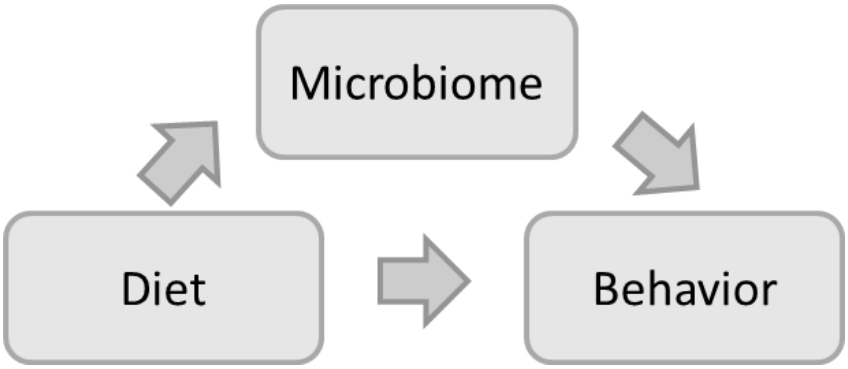

Figure 1. Diagram of potential mediating effects of the gut-microbiota on the relationship between the diet factors and behavioral outcome measures such as ADHD diagnosis and reactive aggression.

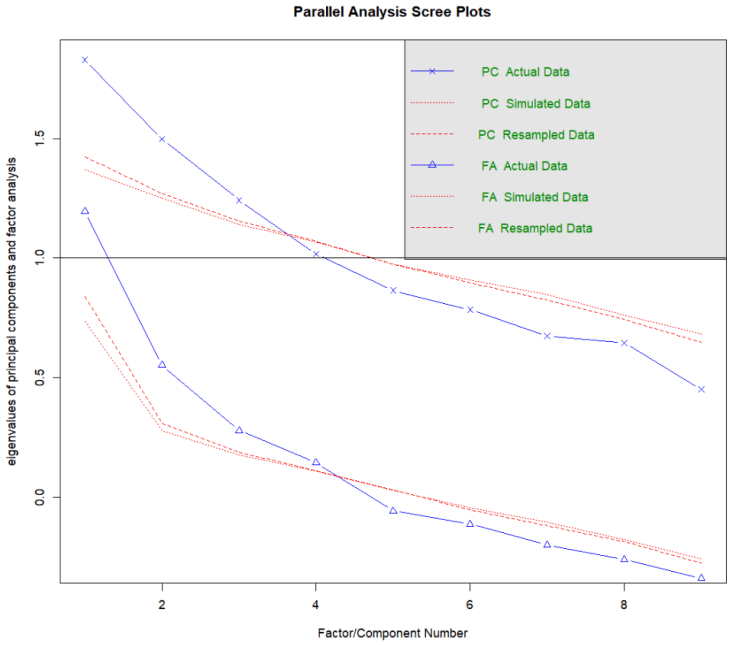

Figure 2. Scree plot, results of the parallel analysis. Actual, simulated and resampled data bend between 2 and 4 factors suggest a 3 factor solution.

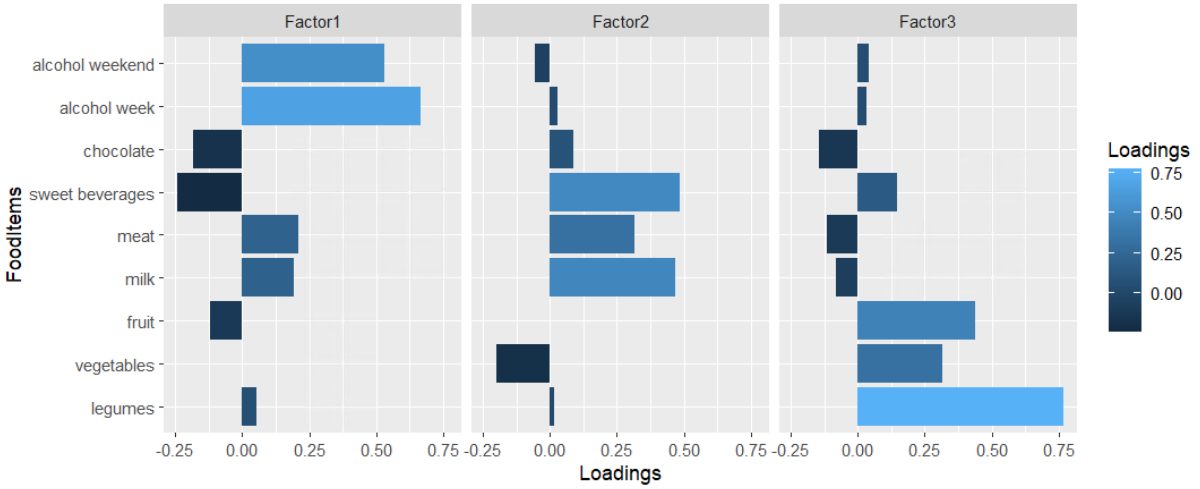

Figure 3. Factor loadings of the food items for the three diet factors suggested by EFA. Factor 1, alcohol-rich/savory diet (left); Factor 2, "unhealthy" diet (middle) and Factor 3, "healthy"/vegetarian diet (right).

Table 2. Diet-behavior associations

	Standard Estimate	Estimate	Std. Error	z-value/ t-value	*p*-value	*Fdr*-corrected
ADHD Diagnosis						
Factor1	-0.26	-0.20	0.19	-1.37	1.71 E^{-1}	8.58 E^{-1}
Factor2	-0.01	-0.01	0.18	-0.06	9.53 E^{-1}	1.00
Factor3	0.02	-0.02	0.17	0.14	8.85 E^{-1}	1.00
Age	0.04	0.00	0.17	-0.23	8.82 E^{-1}	-
Sex	0.08	0.16	0.17	0.49	6.27 E^{-1}	-
BMI	0.07	-0.02	0.17	-0.41	6.79 E^{-1}	-
Smoke	0.59	1.48	0.19	3.10	1.94 E^{-3}	-

Table 2. Results from the logistic regression analysis of dietary factors with ADHD diagnosis corrected for age, sex, BMI, smoking and ADHD medication, showing standardized regression estimates, estimates, standard errors, and p-values. Fdr correction was applied to p-values of associations of the dietary factors with ADHD diagnosis.

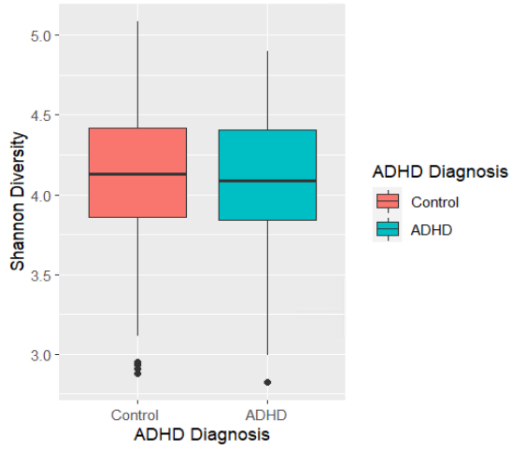

Figure 4. Boxplot visualizing the Shannon index in participants with and without ADHD. Individuals without ADHD are marked red. Individuals with ADHD are marked in blue.

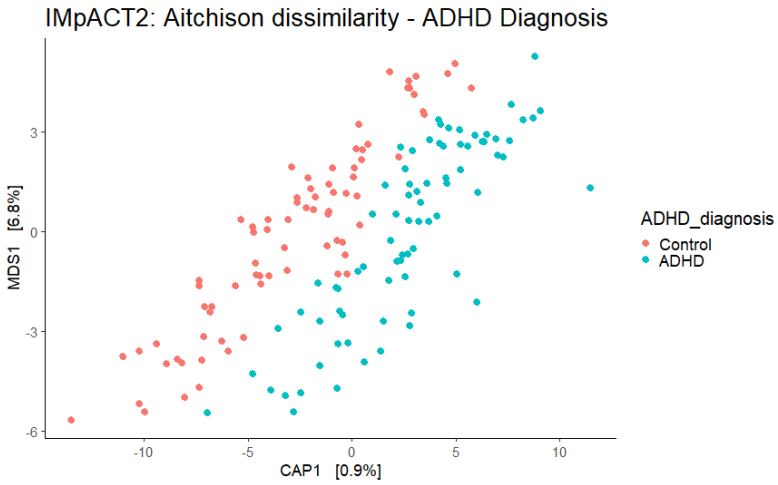

Figure 5. CAP plot supervised for ADHD diagnosis. Individuals without ADHD are marked red. Individuals with ADHD are marked in blue.

Table 3. Feature selection and differential abundance analysis

Genus	Selection probability	Standard Error	Estimate	Z value	*p*-value	Fdr-corrected
ADHD Diagnosis						
Tyzzerella	0.59	0.06	0.21	3.82	1.31 E^{-4}	3.80 E^{-3}
RF39	0.25	0.06	-0.19	-3.39	7.11 E^{-4}	1.03 E^{-2}
Sutterella	0.19	0.08	-0.24	-2.85	4.34 E^{-3}	2.31 E^{-2}
Sanguibacteroides	0.16	0.15	-0.40	-2.58	9.78 E^{-3}	2.40 E^{-2}
Uncultured.6	0.16	0.06	-0.18	-2.78	5.39 E^{-3}	2.31 E^{-2}
Ruminoclostridium	0.15	0.11	0.30	2.62	8.66 E^{-3}	2.40 E^{-2}
Eisenbergiella	0.12	0.09	0.25	2.77	5.57 E^{-3}	2.31 E^{-2}
Caulobacter	0.11	0.16	0.43	2.64	8.31 E^{-3}	2.40 E^{-2}
Eubacterium fissicatena group	0.11	0.11	0.28	2.55	1.07 E^{-2}	2.40 E^{-2}
Eubacterium ruminantium group	0.09	0.05	-0.12	-2.45	1.41 E^{-2}	2.69 E^{-2}
CHKCI002	0.09	0.18	0.45	2.55	1.07 E^{-2}	2.39 E^{-2}
Uncultured.10	0.08	0.08	-0.19	-2.43	1.48 E^{-2}	2.69 E^{-2}
Pseudomonas	0.07	0.13	-0.26	-1.98	4.79 E^{-2}	6.05 E^{-2}
Paraprevotella	0.06	0.05	-0.09	-2.08	3.73 E^{-2}	4.92 E^{-2}
Bifidobacterium	0.05	0.07	-0.15	-2.14	3.13 E^{-2}	4.36 E^{-2}

Table T3. Results from the feature selection and differential abundance analysis. Logistic regression of ADHD with 15 selected genera. Associations that were not significant after correction in grey text.

Table 4. Mediation Analysis

Mediation	Estimate	95% CI lower	95% CI upper	*p*-value
Eubacterium_nodatum_group				
ACME	0.07	-0.01	0.15	6.6 E^{-2}
ADE	0.74	0.25	1.15	4.0 E^{-3}
Total effect	0.81	0.34	1.15	4.0 E^{-3}
Proportion Mediated	0.08	-0.02	0.30	NA

Table T4. Results of the mediation analyses of Unhealthy Diet on the association of Eubacterium_nodatum_group with ADHD diagnosis, Estimates, p values, lower and upper boundaries of the confidence intervals for the Average Causal Mediated Effect (ACME), Average Direct Effect (ADE), total effect and the proportion of the effect that was mediated are presented.

3. Acknowledgement and Disclaimer

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