Eat2beNICE

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

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ABSTRACT

The current report describes the results of the TRACE-MRI study, that is an add-on to the TRACE-RCT protocol. The TRACE-RCT (part of WP2 of Eat2beNICE) is a randomized clinical trial with the goal to investigate and compare the short-term (5 weeks) and long-term (12 months) effects of the Elimination Diet (ED) and Healthy Diet (HD) on ADHD and emotion dysregulation symptoms. In the current study, we assess the effect of these diets on functional and structural brain measures assessed by MRI scanning and on accompanying cognitive measures, and explore whether changes in behaviour are mediated by changes in brain structure and/or function.

The conduct of this TRACE-MRI study was severely negatively affected by the Covid-19 pandemic, since the brain imaging centre had been closed for a long-time, just when a large sample of participants had been enrolled in the clinical RCT.

A total of 26 subjects were enrolled in the imaging part of this experiment. The sample included approximately equal numbers of subjects that showed positive effects of the dietary intervention (responders) as well as subjects that showed no such effects (non-responders).

We observed small longitudinal effects on one structural brain measure, and this did not differentiate between dietary conditions.

Due to unforseen circumstances, the sample size and thereby statistical power of this data set was very limited. Our main recommendation is that further research employing larger sample sizes is initiated to explore subtle effects of the different dietary interventions that the current study might have been underpowered to detect.

INTRODUCTION AND RATIONALE

Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder defined by symptoms of developmentally inappropriate inattention, impulsivity and hyperactivity with an estimated prevalence of 5-6% [1,2]. About 50% of all patients still show ADHD symptoms and related impairments in their adult life [3]. This places them at high risk of social and economic disadvantage in the course of their life, and creates a considerable demand for social, educational and healthcare services. Furthermore, patients with ADHD often have comorbid psychiatric problems, such as substance abuse, aggression and depression [4]. Taken together this makes ADHD a costly societal problem.

Food seems to trigger ADHD symptoms in some children and an individually constructed elimination diet (ED) might be an effective treatment for ADHD [5]. ED involves a temporary (2-5 weeks) total change of diet, in which the patient is only allowed to eat a few different hypo-allergenic foods (for instance rice, turkey, lettuce, pears, and water). Thereafter, a 12 month reintroduction phase is needed to find out which products trigger ADHD symptoms. The rationale for this diet is that a patient may show adverse reactions to any type of food and that it is important to determine the individual susceptibility to the specific foods that causes adverse reactions. The role of ED as ADHD intervention has been investigated [6-14]. A recent meta-analysis [26] concluded that about 42% of the children with ADHD show an excellent (≥30% symptom reduction) response. However, these studies have only focused on the short-term effects of ED. There are no studies reporting on the long-term effectiveness and cost-effectiveness of ED. Recently, an independent committee has accredited ED as an intervention with strong evidence for effectiveness and as such it is included in the Database Effective Youth Interventions (see PVG-dieet at www.NJI.nl). Currently, four private ADHD research centers in the Netherlands offer ED. ED is not included in guidelines for the treatment of ADHD or reimbursed for by public health insurance. This qualifies ED as a Type 2A intervention.

There is a growing awareness that food may play a role in our psychological well-being [15,16]. However, the exact mechanisms underlying the ED effect in ADHD are unknown and whether the dietary change is the underlying determinant of the supposed effect of ED is topic of scientific [12] and public debate (De Volkskrant, December 29, 2012). It has been suggested that the gut-microbiome-brain connection may play a significant role [17], by altered microbial colonization and activity of the gut [18]. Therefore, further research into the relationship between diet and ADHD symptoms has been recommended by the National Institutes of Health in the USA [19] as well as emphasized by the Dutch Patient Organization 'Balans' [www.balans.nl].

In a broader context, there is growing societal discussion and concern about year by year increasing prescription rates of medication (mostly psychostimulants) to children with ADHD (www.sfk.nl). Between 2005 and 2009, the number of prescriptions of methylphenidate among children aged 0 to 10 years has increased yearly with 16% [20]. The costs for ADHD medication have increased from 12 million euros in 2007 to 22 million euros in 2011 [21]. The Dutch government has setup an official policy to reduce medicalization of children. Development and testing of effective and cost-effective alternatives to medication treatment of children with ADHD has thus enormous societal relevance and fits perfectly in this policy. In addition, medication can have side-effects and only suppresses ADHD symptoms. There is a demand for alternative treatments for ADHD, in particular for ED and cognitive training. This is illustrated by the fact that on average children wait 6 months before they can start with an ED diagnostic trajectory at one of the private ADHD research centers, despite the fact that it is not reimbursed at present.

To support the further development and integration of ED and other dietary interventions in ADHD, it is important to explore the neurobiological underpinnings of the behavioral effect of these interventions.

Previous research shows that ADHD is associated with a 3-5% smaller total brain size compared to controls due to a reduction of gray matter [22], and with smaller volumes of the accumbens, amygdala, caudate, hippocampus, and putamen. Case-control differences were larger for children and adolescents than for adults with ADHD [23]. ADHD is also associated with delayed maturation of cerebral cortex [24].

Functional magnetic resonance imaging (fMRI) studies using inhibitory control, working memory, and attentional tasks have documented under-activation of frontostriatal, frontoparietal and ventral attention networks [25]. The frontoparietal network supports goal-directed executive processes while the ventral attention network facilitates attentional reorienting to salient and behaviorally relevant external stimuli. In reward processing paradigms, most studies report lower activation of the ventral striatum in ADHD compared to controls in anticipation of reward [26]. ADHD is also associated with hyperactivation in somatomotor and visual systems [24], which possibly compensates for impaired functioning of the prefrontal and anterior cingulate cortices [26]. Resting-state MRI studies report that ADHD is associated with reduced or absent anti-correlations between the default mode network (DMN) and the cognitive control network, lower connectivity within the DMN itself, and lower connectivity within the cognitive and motivational loops of the fronto-striatal circuits [27].

Given the important role of the frontal-striatal circuits in the underlying neurobiology of ADHD, we hypothesize that these structures may also mediate the effects of dietary inventions on ADHD behavior.

Objectives

The main aim of the current study is to explore whether an ED, compared to healthy diet, affects the fronto-striatal and fronto-amygdalar brain systems, and whether effects on the brain mediate effects on behaviour (i.e. symptoms of inattention and impulsivity and emotional dysregulation).

METHODS

A-priori power analysis

Based on previous intervention studies, we expect cluster level effect sizes of d=0.6 in the primary fMRI outcome measures [31]. Extrapolating from this, our power estimation indicates minimum group sizes of 28 subjects per group to detect the main intervention effect. With an allowance for 10% subject dropout due to excessive motion in the scanner, this means we planned to include 30 subjects per dietary group for the TRACE-MRI study.

Population & recruitment

As described above, the recruitment for TRACE-MRI was linked to the TRACE-RCT, which was initiated significantly earlier than TRACE-MRI. When recruitment for TRACE-MRI was started up, only a limited number of subjects could be tested in the MRI paradigm before the lab space was shut down due to COVID-19 restrictions. TRACE-RCT suffered no such restrictions, and was able to continue testing and recruitment, leading to a large number of subjects being unable to attend the MRI part of this study. When COVID-19 restrictions were subsequently lifted, almost all residual subjects recruited for TRACE-RCT also attended the TRACE-MRI study, but given the small number of subjects still needed for the RCT as well as natural dropout and the strict exclusion criteria for TRACE-MRI, we were able to include less then half the planned subjects.

Hence, for the current study, we fully included 26 subjects that were enrolled in the TRACE RCT [28]. Children were all under treatment at Karakter Nijmegen, and were aged between 6 and 12 years. All children were enrolled in the dietary intervention tract of TRACE, and randomized across the Elimination Diet (ED) and Healthy Diet (HD) conditions.

All subjects should have been conferred a clinical ADHD diagnosis according to the DSM-5 (any subtype). A clinical ADHD diagnosis has to be confirmed by a structured psychiatric interview with the parents (K-SADS) [30] and the Strengths and Weaknesses of ADHD-symptoms and Normal-behaviors (SWAN) rating scale [31] which teachers fill out. Comorbidities were allowed (except for eating disorders).

Exclusion criteria for the RCT entailed:

- Use of any psychotropic medication or adhering to another elimination diet.
- Children who have received treatment (e.g., medication, behavioral therapy, diet) for ADHD in the past two months.
- Children diagnosed with diabetes mellitus.
- Children diagnosed with eating disorder.
- Children and/or parents with inadequate mastery of the Dutch language.
- High a priori risk of not being able to adhere to the diet (lack of motivation and/or significant parent-child relationship problems requiring family therapy).
- Children who refuse to have meat or animal food products in general in their diet or children who are already following an elimination diet.

Exclusion criteria for MRI scanning:

- The presence of metal objects in or around the body.
- Claustrophobia.

Dietary interventions

Elimination diet

The first part of the ED trajectory consists of a 5-week elimination phase, where children follow a standardized ED, supervised by a dietician and a health care psychologist. All food that contain major allergens and gluten, and that are high in histamine activating substances, will be eliminated in the elimination phase. Weekly face-to-face or telephone contacts with the dietician are scheduled to keep the patient and family motivated and respond to any questions they may have about the diet. Also, researchers contact the family after two weeks to evaluate the experiences with the diet so far and to answer questions parents may have. After these 5 weeks, it is determined if the child responds to the diet with a clinical significant reduction of the ADHD symptoms (≥30%) according to the parent and/or teacher ratings. Responders are invited to continue with the second phase of the diet. Non-responders are switched to care-as-usual (CAU).

The second phase (reintroduction phase) may last up to 12 months and includes children who respond to the first phase. The basis is the ED followed during the first phase, but every week a new food is introduced, according to a standardized scheme, in a sufficient amount as to be able to trigger ADHD symptoms. If the reintroduction of a food does not trigger recurrence of ADHD symptoms, this food is added to the diet and can be eaten ad libitum. If a food does seem to trigger recurrence of ADHD symptoms according to parental ratings, the food is listed in the category 'to be avoided'. In the next week, no new food introduction takes place, to allow the ADHD symptoms to decrease again to baseline. When ADHD symptoms have returned to baseline in this period, another new food is introduced in the week thereafter. During this phase, parents have contact with a dietician and a health care psychologist every month to identify foods that trigger ADHD symptoms in their child, to give mental support and to answer questions about the diet. In addition, according to the parents' needs, the researchers provide mental support and advice to the parents via telephone. Eventually this phase leads to a consolidated dietary advice about the specific foods (on average 3-5) to be avoided, while maintaining an otherwise normal diet. Nutritional adequacy of the overall diet is continuously monitored by the dietician during the whole study and if necessary dietary supplements are recommended to the children. Patients who drop-out at any time, are switched to CAU.

Healthy diet

The healthy diet is based on the strict guidelines of the World Health Organization. Some foods are allowed in unlimited quantities and frequencies, others in restricted quantities and frequencies and some in very restricted quantities and frequencies. This healthy diet is prescribed in a strict and structured manner making the diet comparable to ED regarding impact to the family, structure and attention towards the child. Contacts with a dietician, health care psychologist and researchers are at the same frequency as with ED. The healthy diet does not aim to affect the allergen content of the diet or at losing or gaining weight. After these 5 weeks, it will be determined if the child responds to the diet with a clinical significant reduction of the ADHD symptoms (≥30%) according to the parent and/or teacher ratings. Responders are invited to continue with the second phase of the diet. Non-responders are switched to CAU. The second phase of the healthy diet consists of two-monthly supervising by a dietician, health care psychologist and researcher. During these supervisions, adhering to the guidelines and the ADHD symptoms are evaluated.

MRI acquisition and analysis

Participants were asked to remove all metal objects before entering the scanner room. Special care was taken to make the child/adolescent comfortable on the scanner bed. Participants were be provided with earplugs to protect against scanner noise. The participants were able to communicate with the experimenter over an intercom and can press an alarm button in case of emergency. The MRI

session consists of different types of scans that range between 5 to 15 minutes, with short breaks in between, and takes at maximum 50 minutes.

We collected the following MRI measures: Anatomical MRI scan (sMRI); Resting state MRI scan (rsMRI); Task-based functional MRI scans (fMRI).

For the first and second-level analyses of structural and functional MRI data, brain imaging analysis with conventional software packages (e.g., SPM12, FSL, FreeSurfer) were used to (1) derive neuroanatomical features (e.g. measures of cortical surface, cortical thickness, grey/ white matter volumes), and to (2) carry out structural region of interest analyses of the MRI data. To maximize consistency and comparability between individual research projects, for each data type (e.g., structural T1-weighted ADNI, task-related fMRI, resting-state MRI) "standard" pre-processed data (e.g., motion-corrected, normalized, smoothed images) and first-level contrast maps will be made available to all partners via the central database.

The primary longitudinal between-group analyses will be implemented as general linear model using each brain metric at T0 as well as the change during intervention (T1-T0) as dependent variable, including intervention group as predictor and age, gender and IQ as covariate. The following metrics will be used as dependent variable:

fMRI:

- Whole-brain neural activation during Stop-task (inhibition) performance
- Whole-brain neural connectivity during Stop-task (inhibition) performance
- Whole-brain neural activation during Hariri-task (emotional reactivity) performance
- Whole-brain neural connectivity during Hariri-task (emotional reactivity) performance

Rs-MRI

- Whole-brain neural connectivity

Correction for multiple comparisons

All separate imaging modalities were statistically assessed as separate independent analyses. The structural imaging analyses were corrected for multiple comparisons using Bonferroni correction based on the total number of Regions of Interest (ROIs) assessed. Each of the functional imaging metrics is whole brain voxel-level corrected for multiple comparisons during the group-level analyses. Follow up connectivity analyses will be further corrected based on the number of observed clusters which serve as input for the connectivity matrix.

Mediation

As a secondary step, all neural metrics which show a positive correlation with the behavioural change in ADHD symptoms during intervention will be used to test for mediation, as depicted in Figure 1.

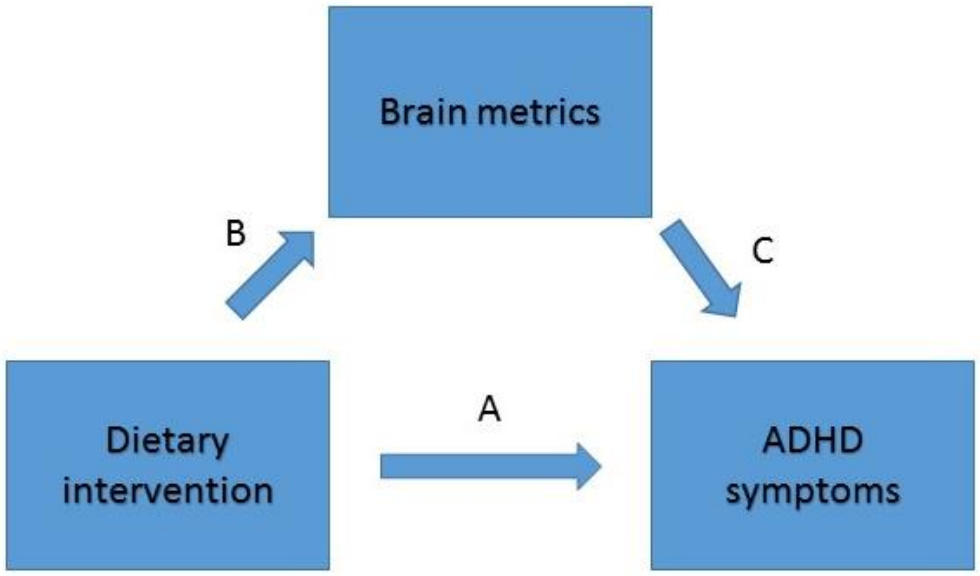

Figure 1. schematic overview of the mediation analysis structure.

In the mediation analysis, we will first establish if association A (intervention to behaviour) is significant, and subsequently if association B (intervention to brain) is as well. If both these conditions are true, we will run a third model for each brain metric using ADHD symptoms as dependent variable, Dietary Intervention group as the main predictor and brain metric as covariate. This will allow us to dissociate whether the effect of dietary intervention on ADHD is indeed mediated by our observed brain changes or not.

RESULTS

Out of the 26 participants that completed at least one element of the longitudinal data acquisition, the respondership to the diet is presented in Table 1. A responder indicates a significant improvement in ADHD symptoms between T0 and T1. A full responder indicates this improvement is observed by both parent and teacher ratings. Partial responders show improvement to one of these observers only, and mixed responders show improvement in one observer and deterioration in the other. Non-responders show no effect in either observer and deterioration indicated increased ADHD symptoms as reported by both observers.

Our results indicate a relative equal distribution of respondership status over these groups, with no difference in respondership status observed as a function of dietary intervention type (ED vs HD).

	N
1. Full responder	7
2. Partial responder	7
3. Mixed responder	8
4. Non-responder	3
5. Deterioration	1
Total N (longitudinal)	26

Table 1. Distribution of respondership status across the sample.

Effects of dietary intervention on Structural Brain Imaging

Structural brain images were automatically segmented using Freesurfer into 9 subcortical volumes and 51 cortical surface area and 51 cortical thickness regions of interest (ROIs).

Of these, only a single region of interest showed a significant effect of the dietary intervention (difference between T0 and T1) as a function of respondership, namely the Right Paracentral Gyrus ($p<0.01$, uncorrected), which showed a decrease in cortical thickness at T1 as compared to T0 for non-responders (m= and deteriorated subjects. No main effect of diet type were observed, neither any interactions between diet*respondership.

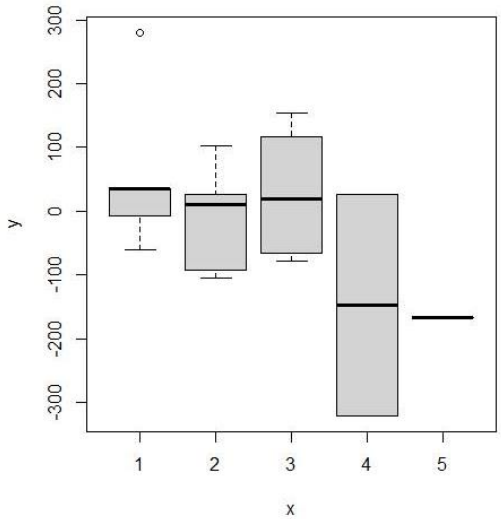

Figure 1. Change in the right-paracentral gyrus surface area as function of responder status. The y-axis indicates change in estimated means in the longitudinal model comparing thickness at T0 and T1. The x-axis indicates respondership status (1=full responder, 2=partial responder, 3=mixed responder, 4=non-responder, 5=deterioration).

Effects of dietary intervention on Functional Brain Imaging

In Table 2, the numbers of subjects which successfully complete the Hariri task, Stop-task and Resting State (RS) sequence are presented. These numbers indicate that we had significant dropout, especially on the longitudinal scan, which further decreased the statistical power in our GLM models.

Treatment	Respondership_simple	T1_struct	T1_Hariri	T1_Stoptask	T1_RS	T2_struct	T2_Hariri	T2_Stoptask	T2_RS
ED	responder	7	7	5	5	7	6	4	4
ED	non-responder	7	6	4	5	7	6	5	4
HD	responder	7	7	4	6	6	6	3	5
HD	non-responder	5	5	1	3	5	5	3	3

Table 2. number of subjects which completed each imaging measure from the Elimination Diet (ED) and Healthy Diet (HD) intervention conditions, subdivided by responder vs non-responder status.

We did not observe any significant effects at T0, T1 or between T0 and T1, on any of the functional imaging measures as a function of dietary condition, nor of respondership status, nor as a function of the interaction between diet and respondership.

Given the absence of any significant association in these models, the statistical assumptions underlying our planned mediation models were not met and we did not continue with these secondary analyses.

DISCUSSION

During the course of the current study, severe COVID-19 restriction on lab-space use lead to a significant drop in the numbers of subjects which could be tested. According to our a-priori power analyses, this study was underpowerd to detect small and medium effects of the intervention on brain structure and activation.

With the limited size of this MRI subsample no differences were found in respondership rates between the ED and the ED. We further demonstrate that on none of our cognitive or imaging measures, the ED and HD groups show any a-priori differences at T0.

When looking at longitudinal effect over the intervention, we only observe a difference in the structural brain measures. Across all subjects in the two dietary conditions, the cortical thickness of a single brain region, the right paracentral gyrus varies as a function of responder ship status. We observe a decrease in cortical thickness over time for the non-responder and deterioration conditions. This effect however is slightly puzzling, as a decrease in thickness was not expected at such short interval.

Given that respondership status was based on the observed ADHD symptoms, our results might be explained by potential measurement error of cortical thickness associated with increased fidgeting inside the scanner. We corrected for movement effects during our analyses, and in the functional imaging datasets we observed no residual effect of movement as a function of respondership, but nevertheless the subtle influence of small scale movements during the structural imaging remains a potential confounder that we cannot rule out at this point, with this limited sample size. It must also be noted that this longitudinal effect did not differ between dietary conditions, as there was no diet*respondership interaction. So for all our effects, no distinction between the two diets could be made.

The different functional imaging measures did not show differences over time. As shown in the results section, the numbers of subjects which successfully completed each of the functional measurements are very low, which gives us very limited power to observe longitudinal and/or differential treatment effects. This means that any small or even moderate effects on these metrics may be obscured due to random noise and lack of statistical power. In short, for these measures we cannot conclude any effects of the intervention, but neither can we really robustly exclude the presence of these effects. We were unable to test the hypothesized mediation models, given that the initial statistical assumptions of significant associations between our predictors and brain outcome variables was not met. Given these limitations, we recommend that future research would be primarily aimed at increasing the numbers of subjects in imaging metrics of dietary interventions.

To conclude, our hypotheses of a mediating role for the frontal-striatal brain networks underlying the effect of dietary interventions in ADHD could not be verified. Some small effects on the cortical thickness in the right paracentral gyrus are observed, but further studies are needed to accurately determine the veracity and causal role of this potential effect. Based on these initial findings, we primarily argue for follow up studies with increased sample sizes to better understand the neurobiological changes underlying dietary intervention in ADHD.

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