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Resting-state networks and neurometabolites in children with ADHD after 10 weeks of treatment with micronutrients: results of a randomised placebo-controlled trial

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ABSTRACT

Children with attention-deficit/hyperactivity disorder (ADHD) show significant abnormalities on MR imaging in network communication and connectivity. The prefrontal-striatal-cerebella circuitry, involved in attention is particularly disrupted. Neurometabolites, the biochemical structures that support neurological structural integrity, particularly in the prefrontal cortex and striatum are associated with symptoms. This study aimed to explore changes in neurometabolite levels through treatment with vitamins and minerals (micronutrients), hypothesising that treatment would impact neural circuitry and correspond to a reduction in symptoms. Twenty-seven non-medicated children ($M = 10.75$ years) with DSM5 diagnosed ADHD were randomised to receive daily micronutrients or placebo for 10 weeks. Main outcome measures included the Clinical Global Impression-Improvement Scale and ADHD-RS-IV Clinician Ratings of ADHD symptoms. Magnetic resonance spectroscopy of the bilateral pre-frontal cortex and bilateral striatum, resting state fMRI and structural images were acquired 1 week pre-treatment, and in the last week of intervention. Results did not show any significant differences in the measured brain metrics and the levels of neurometabolites between treatment and placebo groups after ten weeks of treatment with micronutrients. In the treatment group there was a trend for: decreased choline in the striatum; decreased glutamate in the prefrontal cortex; increased grey matter in the anterior thalamus; increased white matter in the fornix and improved network integrity of the default mode network, dorsal attention network and frontal executive network. The small sample size of the current study limits results, future studies with higher power are warranted to explore any association between micronutrient treatment and neurological changes.

KEYWORDS

ADHD; magnetic resonance spectroscopy; fMRI; micronutrient; vitamin; treatment

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a chronic neurodevelopmental disorder affecting approximately 5% of children.¹ Pharmacological treatments can reduce symptoms, but are often unsatisfactory due to side effects and failure to prevent or alter long-term course.²

ADHD symptom severity has been linked to processed foods, food dyes, and low consumption of fruit and vegetables.³ A recent meta-analysis⁴ and literature review⁵ point toward a role of diet in the expression of ADHD symptoms and benefit of dietary manipulation in improving these symptoms. However, given that diet manipulation can be a challenge for some families, an alternative is to supplement with more nutrients than what might be available through diet alone. As such, dietary supplements in the form of additional

micronutrients offer an alternative to traditional medication approaches and provide another option for children who do not respond to traditional treatment or who experience adverse side effects associated with medications. The evidence for a broad spectrum micronutrient approach is growing with case studies, open label reversal designs and randomised controlled trials all documenting the therapeutic benefit of this approach.⁶ A double-blind, randomised, placebo-controlled trial (RCT) from our own group showed a significant advantage of micronutrients over placebo for general functioning, emotional dysregulation, aggression and inattention. There were no group differences on hyperactivity/impulsivity.⁷

Compared to their age matched counterparts, children with ADHD show significant neurological abnormalities, particularly in the striatum and frontal and

parietal regions and their associated communication networks (see Konrad & Eickhoff⁸ for review). Neurometabolites, compounds that influence metabolic efficiency, energy storage capacity and plasma membrane integrity⁹ are present at abnormal levels in both frontal and striatal regions of the ADHD brain.¹⁰ These compounds are integral to brain health and sensitive to diet and nutritional supplementation¹¹ and can thus serve as biomarkers of dietary intervention.

Cortical networks are categorised by their areas of functional association, that is, all regions involved in the communication of each network are highly correlated. The intrinsic function of these resting state networks is examinable using resting-state fMRI (rs-fMRI). The default mode network (DMN) and attentional cognitive networks have gained particular attention in ADHD. The DMN spans prefrontal, frontal and parietal regions¹² and is most active at rest. The cognitive control networks, such as the dorsal attentional network are inversely associated with DMN and are active when the DMN is at rest. Children with ADHD show reduced activation in the executive function network, and in the total area of the DMN.¹³

To the best of our knowledge, brain changes as a result of micronutrient supplementation have never been examined in ADHD. In the current pilot study, 27 non-medicated children diagnosed with ADHD were randomised to receive a micronutrient formula (ingredients included 13 vitamins, 17 minerals and 4 amino acids) or placebo for 10 weeks as part of the larger study investigating the effects of micronutrients on ADHD symptoms and associated behaviours.⁷ At baseline and at study completion, we performed multimodal MRI, including structural MRI, magnetic resonance spectroscopy, and resting state functional connectivity, to investigate the relationship between micronutrient supplementation and any brain changes.

Methods

Participants in this study were volunteers from a larger clinical trial of 93 medication free children with ADHD who had been assigned to either a micronutrient or placebo treatment for 10 weeks. Methodology of the larger clinical trial is published in detail in Rucklidge et al.⁷ The trial was prospectively registered with the Australia and New Zealand Clinical Trial Registry ACTRN12613000896774.

Participants

Participants were diagnosed with ADHD using the Kiddie Schedule for Affective Disorders and Schizophrenia

Lifetime Version (K-SADS-PL),¹⁴ administered to the participant's parent or guardian by a clinical psychologist or senior graduate clinical psychology student under supervision. Children with other co-occurring disorders (except Autism Spectrum Disorder (ASD)), were purposefully included in order to capture a representative sample of children affected by ADHD, increasing the clinical utility of results.

Eligibility criteria for MRI scanning included: (1) between the ages of 7–12 years; (2) met criteria for ADHD as above and T scores greater than 65 on *both* the hyperactive/impulsive and inattentive indices of the Conners' Parent Rating Scale-Revised: Long Version CRS-R:L,¹⁵ (3) medication-free (psychiatric) for ≥ 4 weeks; (4) able to ingest up to 15 capsules/day with food; and (5) male. Children were screened for history of claustrophobia and ability to complete the imaging sequences. Exclusion criteria were: (1) estimated IQ < 75; (2) ASD; (3) epilepsy; (4) any major psychiatric condition likely to require hospitalisation; (5) any serious medical condition; and (6) allergy to ingredients of the intervention or any known abnormality of mineral metabolism (e.g. Wilson's disease, hemochromatosis).

Thirty boys were recruited for neuroimaging. Three participants were unable to complete the scanning sequences at baseline due to claustrophobia ($n = 1$) or extreme movement that resulted in the termination of image acquisition ($n = 2$), resulting in a final sample of 27 participants who completed two scanning assessments. Ethical approval was obtained from the Human Ethics Committee of the University of Canterbury and the Southern Health and Disability Ethics Committee. Written informed consent was obtained from all of the participants' parents or legal guardians and assent was obtained from all participants.

Procedure

Participants were randomised in a 1:1 ratio to 10 weeks of treatment with either the micronutrients or placebo for 10 weeks of intervention. The sequence allocation was done in blocks of 4 by a research assistant not involved in the study. Participants were instructed to titrate the dose over a week, up to 12 capsules/day, in 3 divided doses, taken with food and water. If there was no clinical response after 4 weeks, families could choose to increase the dose to 15 pills/day. The placebo and micronutrients (Daily Essential Nutrients (DEN): see supporting information in Rucklidge et al.,⁷ for ingredients) were similar in appearance. The placebo included a small amount of riboflavin to mimic the smell and urine colour associated with taking vitamins. DEN were chosen due to research findings indicating greater effects

for combined rather than single nutrient intervention studies (for review see: Popper, Kaplan, & Rucklidge¹⁶). Adherence was measured by collecting unused pills and recordings of the number of doses missed in the previous two weeks. All participants were monitored by a clinical psychologist or psychology graduate student under a psychologist's supervision with face-to-face meetings or phone contact at screening, baseline, and weeks 2, 4, 6, 8 and 10. Image acquisition, processing and statistical analyses were conducted blind to treatment allocation.

Measures

The main clinical outcome measures are detailed in Rucklidge et al.⁷. Primary outcome measures are reported alongside teacher reports and general functioning for this subsample. These included: the Clinical Global Impressions Improvement (CGI-I) scale¹⁷ (completed by the clinician at end of trial identifying improvement from 1 (very much improved) to 7 (very much worse) relative to baseline across global functioning as well as ADHD symptoms); the Children's Global Assessment Scale (CGAS)¹⁸; ADHD Rating Scale IV (ADHD-RS-IV) – clinician version¹⁹ and the DSM-IV ADHD subscales of both the Conners Parent Rating Scale – Revised long version (CPRS-R:L)¹⁵ and the Conners Teacher Rating Scale – Revised long version (CTRS-R:L¹⁵). Other than the CGI-I, all measures were completed at baseline and 10 weeks.

Image acquisition

Magnetic resonance imaging was completed at baseline and 10 weeks. Data were acquired on a 3 T General Electric HDxt scanner (GE Healthcare, Waukesha, USA) with an eight-channel head coil. Imaging protocol included: (1) An axial T1-weighted 3D inversion recovery-prepared fast spoiled gradient echo (BRAVO) sequence (echo time (TE) = 3.8 ms, repetition time (TR) = 9.9 ms, inversion time = 766 ms, flip angle = 15 deg, acquisition matrix = 320 × 320, 222 slices, field of view = 256 mm, slice thickness = 0.8 mm, voxel size = 0.8 × 0.8 × 0.8 mm³); (2) Four single voxel Point Resolved Spectroscopy (PRESS) acquisitions (TE = 30 ms, TR = 1500 ms, voxel size = 20 × 20 × 10 mm³, number of averages = 192 in the right and left striatum and 128 in the right and left prefrontal cortex. Voxels were placed to include a maximum amount of grey matter and minimum amount of cerebrospinal fluid. Pre-scan shimming was performed to achieve full-width half maximum (line width) of ≤ 13 Hz; 3) Resting state functional volumes, acquired using a gradient echo

sequence (TE = 35 ms, TR = 2500 ms, FA = 15 deg, acquisition matrix = 64 × 64, FOV = 240 mm, 36 slices, 160 repetitions, voxel size = 3.75 × 3.75 × 3.8 mm³, scan time = 8:10). During the fMRI acquisition, the participants were asked to lie still and relax while focusing on a fixation cross.

Structural image processing

Data were analysed using Statistical Parametric Mapping Software (SPM 12)²⁰ v7219; the Computational Anatomy Toolbox (CAT12)²¹ v1278, and the CerebroMatic Toolbox.²² First, we created customised tissue probability maps (TPMs) using the matched template approach and a DARTEL template reflective of our sample's age (8.1–13.2 years), sex (all male), and scanner strength (3 T). For each participant, baseline and follow-up T1-weighted images were aligned to a subject-specific mid-point space between the two scans using the longitudinal registration utility in SPM, using default values. The mid-point average image for each individual was then segmented using CAT12 and normalised (using the CerebroMatic-created TPMs and DARTEL template). Grey matter (GM) change images (visit 2 – visit 1) were created by multiplying the native space GM segments (both baseline and follow-up) by the Jacobian rate. GM change images were then warped into MNI-space using the DARTEL deformation fields, modulated, and smoothed (isotropic 8 mm Gaussian kernel).

Magnetic resonance spectroscopy processing

Data were processed using the Magnetic Resonance User Interface (jMURI),²³ v6.0 Beta. Metabolites of interest were N-acetylaspartate (NAA), glutamate (Glu), Creatine (Cr), choline (Cho), and myo-inositol (MI). Spectra were averaged, zero-filled to 5120 points, phase corrected and apodized (1.83 Hz, Lorentzian shape) and frequency-aligned with the residual water signal at 4.7 ppm. The residual water signal was filtered using the Hankel-Lanczos singular value decomposition routine (HLSVD). The metabolite peaks of interest were quantified using the Advanced Method for Accurate, Robust and Efficient Spectral fitting (AMARES). Prior knowledge for peaks was set at the following positions based on previous literature²⁴: NAA, 2.02 parts per million (ppm) and line width (LW) 3.8 Hz; glutamate, 2.35 ppm and 4.8LW; the first creatine peak, 3.01 ppm and 4.8LW; the second creatine peak 3.97 ppm and 4.7LW; choline, 3.2 ppm and 4.8LW, myo-inositol 3.54 ppm and 4.7LW. Line widths were allowed to vary between 2 and 14 Hz. The peak area of each metabolite was normalised by total creatine

peak. Full-width half maximum (line width) of each spectral peak was obtained directly from the raw spectra before pre-processing. ROI's with line width ≥ 10 hz

were excluded ($n = 12$) as this indicates significant abnormalities in the spectral peak, most commonly due to participant movement.

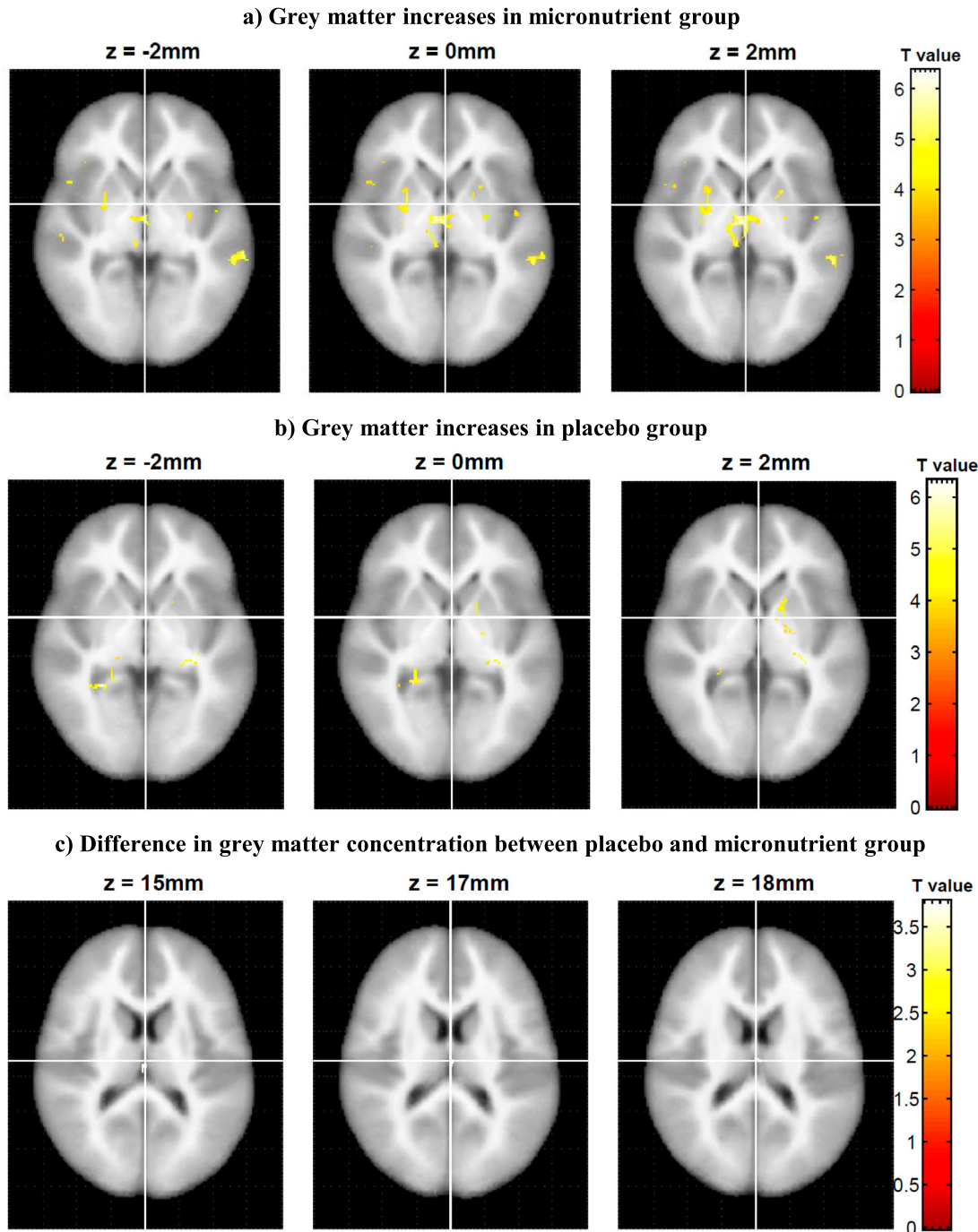


Figure 1. Grey matter atrophy by group (uncorrected, $p < 0.001$) pre and post 10 week intervention. (a) In the micronutrient group, grey matter increases were evident throughout the temporal lobe including within the right thalamus and right parahippocampal gyrus at the conclusion of the intervention period, $T = 3.93$, uncorrected $p < 0.001$. There were no grey matter changes after correcting for multiple comparisons. (b) In the placebo group, grey matter increases were primarily concentrated to the cerebellum, and in small regions of the left amygdala, putamen and hippocampus $T = 3.85$, uncorrected $p < 0.001$. There were no grey matter changes after correcting for multiple comparisons. (c) At the conclusion of the intervention period greater grey matter volume increases were observed in regions of the left and right thalamus and right frontal lobe $T = 3.47$, uncorrected $p < 0.001$. However, there were no grey matter change differences between the two groups after correcting for multiple comparisons. Results are presented on the normalised, smoothed study-specific brain image. Slices displayed are in mm in MNI space.

Resting state fMRI processing

Rs-fMRI data were pre-processed using SPM 12. After excluding the first four volumes, images were realigned and resliced to the first image of the run. We then performed slice timing correction (slice 18/36 was the reference slice). The mean functional image at each timepoint (and all corresponding functional images) was then coregistered to the corresponding time point (baseline or follow-up) structural image. Deformation fields mapping either baseline or follow-up to the mid-point average were combined with the DARTEL deformation fields to normalise all rs-fMRI volumes at each time point, which were also smoothed (8 mm).

Resting state networks (RSNs) were identified using the Group ICA of fMRI Toolbox (GIFT, version 2.e)²¹. Group independent component analysis (GICA) was applied to the aligned, smoothed and normalised data from all participants and all timepoints as a group. The number of components (maps and corresponding time courses) estimated for each participant was set to 20. Maximally independent components were estimated using the infomax algorithm and the data transformed into a linear mixing matrix and 20 ICs. Eleven ICs were identified as meaningful RSNs by evaluating the high to low frequency power ratio in the spectra of components in the RSN time course, and the location of the maxima in the spatial map. The other 9 ICs were discarded from further analysis because they were considered to be related to artefacts, white matter, ventricular, or cerebrospinal fluid maps. Based on the previous literature,²⁵ the 11 ICs were categorised into 10 functional domains of which five were examined; the networks are presented in supplementary Figure 1.

Statistical analysis

All statistical analyses were completed in R (v 3.3.2) or SPM 12. Baseline demographic and clinical group differences were analysed using one way ANOVA or Chi-square as appropriate. Change in clinical outcome variables was examined using a mixed effect general linear model, with group as the between groups factor and participant as the within subjects factor. The baseline measure was entered as a co-variate.

GM and WM change images between baseline and study end were compared between the two groups using a two-sample *t*-test in SPM 12 with intracranial volume as a covariate. The uncorrected threshold was $p < 0.001$. All results were corrected for multiple comparisons using a cluster-wise family wise error rate (FWE, $p < 0.05$).

Bayesian multi-level regression models investigated the relationship between metabolites and treatment

group. Models were fit using the “brms” (v1.10.10) package²⁶ in R. In each model, four chains with 2000 iterations each generated posterior samples. First, group differences were examined separately for each metabolite ratio (NAA; Glu; Cho; Cr; MI), with all four ROIs (right and left prefrontal cortex and striatum) in the same model. For each metabolite ratio, two models were computed. The first modelled the metabolite of interest as a function of age, line width, age-by-ROI and a within-subject session factor (baseline or follow-up). The second modelled the metabolite of interest with the addition of group-by-ROI interaction, time-by-ROI interaction and group-by-time-by-ROI interaction. To determine if the group-by-time-by-ROI predictor was useful at explaining variance in the data (i.e. indicating a treatment effect) the two models were compared using leave-one-out information criterion (LOOIC). A lower LOOIC score, by at least twice the standard error of the estimated difference, indicated that the model minimised out-of-sample individual prediction errors, and was considered as a proxy for a “significant” result. Second, we aimed to predict each clinical variable of interest (e.g. inattention, hyperactivity), as a function of group-by-time-by-ROI. As before, we evaluated the importance of predictors via model comparison by comparing LOOIC scores.

For each of the five resting state networks investigated, a one sample *t*-test was run across all images and participants and thresholded at FWE $p < 0.05$ to create an inclusion mask. Significance testing of resting state networks was executed separately for each network in SPM 12. Maps were compared using a mixed methods ANOVA with time as the within group variable and treatment as the between group variable; the network-specific inclusion masks were used to restrict analyses to voxels present in each network.

Results

Clinical, cognitive and imaging data for at least one MRI sequence at baseline and study end were available for 27 participants. Participants were primarily of NZ European descent with 6 (22%) identifying as NZ Māori. The two groups were well-matched at baseline (Table 1).

There was no statistically significant difference between groups on any demographic or clinical variables and participants in the two groups were similar on their history of psychiatric medication use, adherence rate and average number of pills consumed per day.

Clinical improvement

In line with the clinical changes observed in the larger cohort,⁷ the overall rate of responders (either much or

Table 1. Participant demographics and clinical status at baseline by treatment group ($n = 27$).

	Micronutrient ($n = 13$)	Placebo ($n = 14$)	Statistic
Age, years, mean (sd)	10.75 (1.50)	10.17 (1.36)	$t(1,25) 1.05, p = 0.31$
Male, n (%)	13 (100)	14 (100)	
Ethnicity			
NZ European, n (%)	11 (85)	10 (71)	$\chi^2 = 0.13, df = 1, p = 0.72$
NZ Māori, n (%)	2 (15)	4 (29)	
ADHD Subtype, n (%)			
Inattentive	1 (7.7)	0 (0)	$\chi^2 = 2.33, df = 2, p = 0.31$
Hyperactive Combined	1 (7.7)	0 (0)	
Combined	11 (85)	14 (100)	
Estimated IQ ^a	104.31 (15.65)	100 (16.44)	$F(1,24) = 0.47, p = 0.5$
Co-occurring disorders			
Oppositional defiant disorder	10 (77)	9 (64)	$\chi^2 = 0.09, df = 1, p = 0.77$
Conduct Disorder	2 (15)	1 (7)	$\chi^2 = 0.00, df = 1, p = 0.95$
Learning Disability ^b	3 (23)	2 (14)	$\chi^2 = 0.01, df = 1, p = 0.93$
Tics	2 (15)	2 (14)	$\chi^2 = 5.99^{e-32}, df = 1, p = 1$
Disruptive Mood Dysregulation Disorder	1 (8)	2 (14)	$\chi^2 = 8.23, df = 1, p = 1$
Major Depressive Disorder	0	0 (0)	NA
Generalised Anxiety Disorder	5 (38)	1 (7)	$\chi^2 = 2.23, df = 1, p = 0.14$
Any co-occurring disorder	11 (85)	12 (86)	$\chi^2 = 6.23, df = 1, p = 0.14$
History of past use of psychiatric medications	4 (31)	3 (21)	$\chi^2 = 0.12, df = 1, p = 0.91$
Adherence Rate	95.6% (3.4)	94.7% (6.0)	
Average no of pills per day	12 (1.1)	11.7 (1.1)	

^aAssessed using Block Design and Vocabulary subtests of the WISC-IV.³⁶

^bdefined as having at least one standard score below 100 on either reading or spelling of the WRAT3 or previous assessment confirming an LD.³⁷

very much improved) based on the CGI-I global score for the micronutrient group was 54% versus 29% in the placebo group, comparable to the larger clinical sample (47% and 28% respectively). The micronutrient group also showed greater clinical benefit relative to the placebo group on the CGI-I-ADHD measure. However, given the smaller sample size of the children who underwent neuroimaging, it was not unexpected that other group differences, while all in the same direction of greater benefit for those taking the micronutrients, were not statistically significant (Table 2).

Volume changes

All participants completed structural imaging. The micronutrient group showed a greater increase in grey matter volume between the two time points compared to the placebo group in anterior thalamus (uncorrected $p < 0.001$, Figure 1), and a greater degree of white matter increase, concentrated to a small area of the anterior fornix in the micronutrient group compared to the placebo group (uncorrected $p < 0.001$). Comparisons did not survive correction for multiple comparisons. There were no areas of grey matter increases in the placebo group compared to the micronutrient group and no areas of white matter increases in the placebo group compared to the micronutrient group.

Magnetic resonance spectroscopy changes

Two subjects (both placebo arm) were excluded due to movement. For a further six participants ($n = 4$ micronutrient; $n = 2$ placebo), the following individual regions of interest were excluded due to movement or imaging artefacts: bilateral striatum ($n = 1$); bilateral prefrontal cortex ($n = 2$); left prefrontal cortex ($n = 1$) and right prefrontal cortex ($n = 2$).

Group changes

We found no evidence of interactions between neuro-metabolite ratios and group-by-time-by-ROI, indicating no detectable effect of micronutrient intervention. There was no association between any predictor and the metabolite ratio in any model. Model comparison showed the group-by-time-by-ROI model had a slightly worse fit than the simpler model that did not include a group factor for every metabolite ratio (Table 3), indicating no evidence that group (placebo vs micronutrients) explained any variance in the MRS metabolites. There was a non-significant trend for NAA ratio increase in the left prefrontal cortex in the placebo group during the intervention period (Figure 2(b), Table 3). There was a trend level change in choline ratio in the left and right striatum. Choline increased (worsening) in the placebo group and decreased (improved) in the micronutrient group during the intervention period. Model comparison showed that inclusion of a group-by-time-by-ROI interaction (Table 3) did not improve out of sample prediction, indicating that this was not a significant interaction. There was no association between any metabolite ratios in any region of interest between clinical outcome variables and metabolite ratios.

Table 2. Changes in clinical outcome measures pre to post trial.

	Micronutrient		Placebo Group			<i>p</i>	η^2 (90% CI)*
	Pre	Post	Change	Pre	Post		
Clinician Ratings							
CGI-I							
Overall	NA	2.62		NA	3.21	0.14	0.59 (−0.22, 1.40)*
ADHD	NA	2.54		NA	3.43	0.01	1.03 (0.19, 1.87)*
CGAS	48.31	56.62	8.31	47.43	51.86	4.43	0.08 (0, 0.34)
ADHD-RS-IV							
Inattention	22.92	17.85	−5.08	23.86	21.57	−2.29	0.10 (0, 0.32)
Hyperactivity	22.85	17.00	−5.85	23.57	19.93	−3.64	0.28 (0, 0.24)
Total	45.77	34.85	−10.92	47.43	41.50	−5.93	0.10 (0, 0.33)
Parent Ratings							
CPRS – T Scores							
Inattention	73.77	66.08	−7.69	72.00	69.07	−2.93	0.09 (0, 0.33)
Hyperactivity	85.69	75.31	−10.38	83.93	77.64	−6.29	0.36 (0, 0.22)
Total	81.38	71.77	−9.62	79.86	74.93	−4.93	0.12 (0, 0.31)
Teacher Ratings							
CTRS – T scores							
Inattention	67.67	64.00	−3.67	66.79	68.62	1.83	0.14 (0, 0.33)
Hyperactivity	72.00	67.18	−4.82	71.36	71.31	−0.05	0.25 (0, 0.26)
Total	71.58	67.00	−4.58	70.50	71.69	1.19	0.18 (0, 0.31)

CGI-I: Clinical Global Impression-Improvement, 1 = much improved, 7 = much worse. CGI-I is an improvement score and was thus only measured at study end. **CGAS:** Child Global Assessment Scale, 1–100, 100 = higher functioning. **ADHD-RS-IV:** DSM Clinician Rating of ADHD Symptoms, 0–54, greater scores indicate greater frequency of ADHD behaviours. **CPRS:** Conners' Parent Rating Scale ADHD total subscale and **CTRS:** Conners' Teaching Rating Scale ADHD total subscale. η^2 : Partial eta squared. *Cohen's *d*: used when there was no need to account for pre-trial measures.

Resting state changes

Resting state functional connectivity was not significantly changed for any of the investigated networks (FWE-corrected $p < 0.05$). However, at the uncorrected level, regions within the default mode, dorsal attention and frontal executive showed differences between the groups at the uncorrected level ($p < 0.001$), shown in supplementary Table 1.

Default mode network (uncorrected $p < 0.001$)

Increased connectivity was evident for the micronutrient group within small areas of the default mode network in cerebral frontal, temporal and occipital regions and subcortically in the left thalamus, left caudate nucleus and right parahippocampal gyrus. Compared to the micronutrient group, there were no regions of increased activation in the placebo group within the frontal default mode network, but increased connectivity was evident

in the parietal cortex, regions of the temporal and occipital cortex, cerebellum and, subcortically in the left thalamus (supplementary Table 1).

Dorsal attention (uncorrected $p < 0.001$)

Decreased functional connectivity was evident for the micronutrient group within the dorsal attention network compared to the placebo group. The placebo group showed greater connectivity in the angular gyrus of the parietal lobe and the frontal medial gyrus compared to the micronutrient group. Within the micronutrient group, two very small regions within the orbital gyrus and temporal gyrus showed an increase in functional connectivity (supplementary Table 1).

Frontal executive (uncorrected $p < 0.001$)

Higher resting state connectivity was observed in regions of the frontal gyrus, temporal gyrus, the precentral gyrus in the motor cortex, the parietal cortex and the cerebellum from baseline to study end in the placebo group compared to the micronutrient group (supplementary Table 1).

Frontal parietal (uncorrected $p < 0.001$)

Increased connectivity was observed in the right fusiform gyrus and supplementary motor area of the micronutrient group compared to the placebo group. There was a very small increase in connectivity in the cerebellum of the placebo group compared to the micronutrient group at study end (supplementary Table 1).

Table 3. Model comparisons of metabolite ratios.

Model component	Baseline model	Full model
NAA/tCr	−213.54 ± 19.41	−201.99 ± 19.84
Glu/tCr	−320.01 ± 49.29	−319.60 ± 45.81
Cho/tCr	−439.57 ± 27.30	−434.52 ± 26.91
MI/tCr	−297.13 ± 33.08	−287.14 ± 31.98
tCr	−341.85 ± 28.78	−331.07 ± 26.89

LOOIC +/- SE. A positive difference indicates a better fit than the baseline model and a lower score, a worse fit. When this difference is greater than twice the SE, there is reasonable evidence the better-performing model should be preferred. The baseline model included the regions of interest, line width and age and accounted for the repeated measures component. The full model additionally included the group-by-time interaction effect. In all cases the model fit did not improve with the addition of the group-by-time interaction effect.

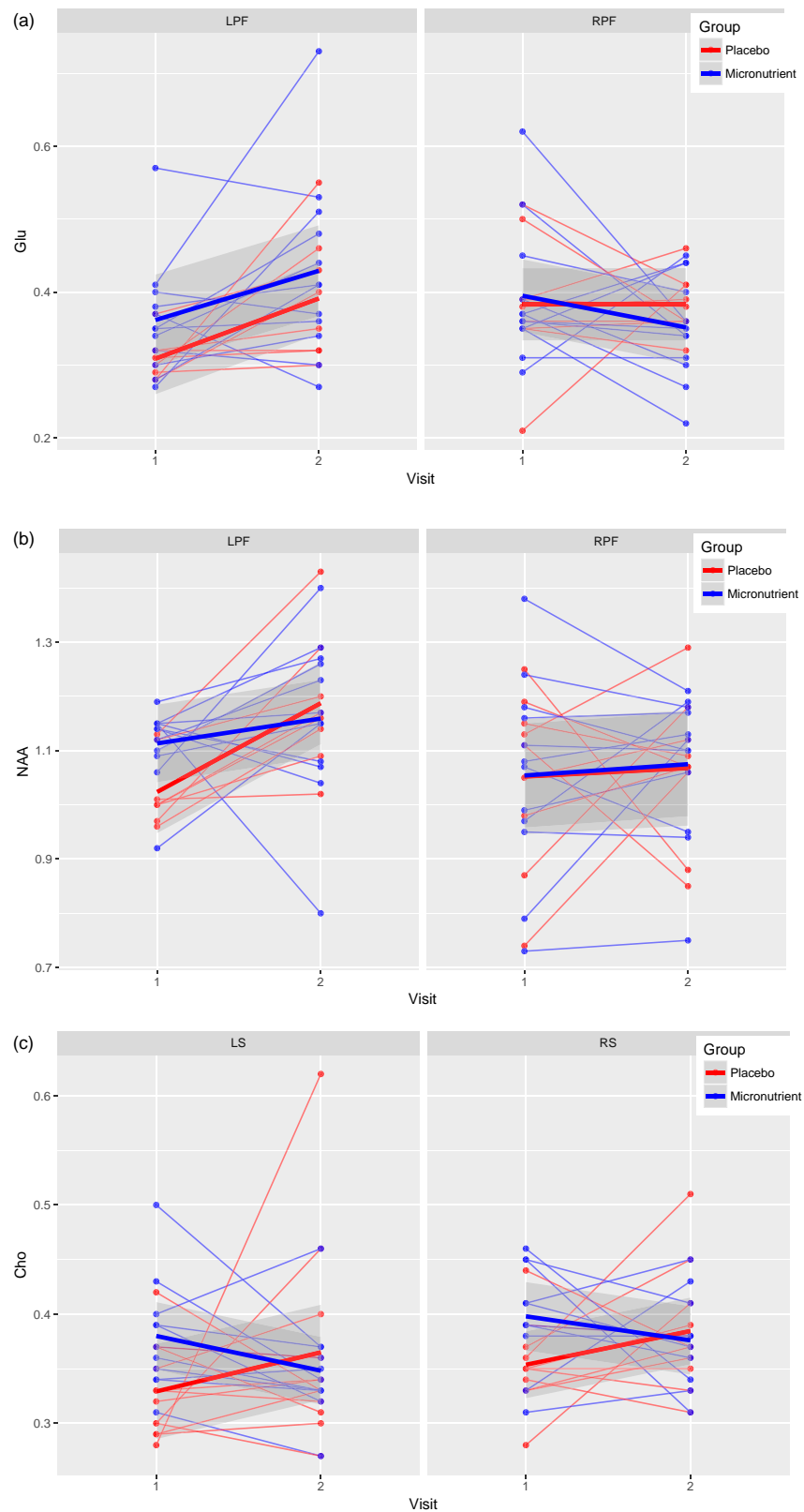


Figure 2. Interactions (non-significant) between treatment group pre-post intervention. **(a)** Non-significant treatment \times time interaction shows glutamate decreases in the right prefrontal cortex of the micronutrient group but not the placebo group during treatment (Interaction effect LOOIC = -319.60 (45.81) vs main effect of time LOOIC = -320.01 (49.29)); **(b)** Non-significant treatment \times time interaction shows N-acetylaspartate increases in the left prefrontal cortex of placebo group more than the micronutrient group (Interaction effect LOOIC = -201.99 (19.84) vs main effect of time LOOIC = -213.54 (19.41)); **(c)** Non-significant treatment \times time interaction shows choline decreases in the striatum of the micronutrient group during treatment (Interaction effect LOOIC = -434.52 (26.91) vs main effect of time LOOIC = -439.57 (27.30)).

Discussion

To our knowledge, this study is the first randomised placebo-controlled trial to use multiple MRI modalities to investigate the impact of a micronutrient treatment intervention for ADHD. Although not statistically significant, there was a greater improvement on all outcome measures in the micronutrient group relative to the placebo group, consistent with the findings reported in the larger clinical sample.⁷ After correcting for multiple comparisons, there was no evidence of difference across multiple brain imaging metrics between the group receiving placebo and the group receiving micronutrient treatment.

Non-significant changes in the expected direction of glutamate ratio and choline ratios in the prefrontal cortex and choline ratio in the striatum were identified. An unexpected finding was an increase of NAA in the left prefrontal cortex of the placebo, rather than micronutrient, group. Our lack of significant findings is largely consistent with those of an earlier drug treatment study²⁷ that examined the left striatum and left pre-frontal cortex in a pre-post design after a 14 week intervention with methylphenidate or similar derivatives (dextroamphetamine, atomoxetine). The participant characteristics were similar to our own and consisted of 14 mixed-sex children aged 7–13 years. There was no significant change in glutamate, choline or NAA in the prefrontal cortex and no change in NAA or choline in the striatum after treatment. They did however report a significant reduction (improvement) in glutamate in the striatum after treatment, in line with our non-significant glutamate reduction in both left and right striatum of the micronutrient group. However, others report no significant change in glutamate in the prefrontal cortex,²⁸ and the prefrontal cortex or striatum²⁹ in children with ADHD after drug treatment. Choline is not sensitive to methylphenidate treatment in children²⁷ with ADHD. NAA appears to be sensitive to methylphenidate; ratios increase in the prefrontal cortex of children with ADHD after 12 weeks of treatment.³⁰ Unfortunately, as the above studies did not include a control group findings may be inflated and the null findings observed in our work may indicate that these reductions are more likely due to an interaction between neurometabolites and the neurodevelopmental processes of ADHD.

Our rs-fMRI findings were in the expected direction, at the end of the treatment period the micronutrient group showed non-significant increased functional connectivity in the default mode network and non-significant reduced connectivity in the dorsal attention and executive function networks compared to the placebo group. Our non-significant findings are in line with Bos et al.³¹ who reported no significant fMRI changes

over 16 weeks in male children after dietary omega-3 fatty acid supplementation. This is in contrast to pharmacology trials where hyperconnectivity in the prefrontal cortex reduced in children and adolescents during a functional task (see Spencer et al.³² for review) after treatment with stimulants.

This study has a number of methodological strengths. The randomised, placebo-controlled study design allows for more robust conclusions regarding the treatment effect on neuroimaging parameters. Several previous reports that have demonstrated a treatment effect on MRS or resting state outcomes have done so using a pre-post design without a well-matched placebo group. This is particularly problematic in a sample of children who undergo complex neurodevelopmental changes in late childhood and early adolescence that vary from their neuro-typical peers.³³ The use of multiple imaging modalities is also advantageous. Abnormalities in NAA levels may be associated with the lack of consolidation during the maturation process observed in ADHD.³⁴

Study limitations include the short-term treatment. Although methylphenidate trials are generally 6–12 weeks, a meta-analysis of omega-3 fatty acid supplementation suggests the optimal duration of intervention is 12–16 weeks.³⁵ It may be that longer intervention is required to detect changes in neural functioning following micronutrient intervention.

The current study was underpowered and therefore can only serve to identify possible regions of change that may be detected using larger samples. The original cohort required 36 participants per group to detect statistically significant between-group effect sizes of at least 0.67 with 80% power.⁷ Due to funding constraints, neuroimaging was limited to 30 participants. A previous fMRI study of Omega-3 supplementation³¹ failed to detect a group effect with a sample size of 40 children with ADHD and 40 controls, each randomised to receive either treatment of placebo.

Conclusion

This study and our previous work⁷ demonstrated that treatment with micronutrients had a beneficial impact on some clinical outcomes for children with ADHD. This exploratory investigation of the neuroimaging component, however, found no evidence of significant brain changes associated with treatment with micronutrients. This is largely in line with previous pharmacological literature. Despite our lack of significant findings, it may be helpful for future research to consider multiple MRI modalities, including fMRI, metabolites, and diffusion imaging to gain a better understanding of the maturation process in ADHD. Other possible mechanism of action of

the micronutrients could also be explored, including the impact on inflammation or microbiome. Larger samples and treatment over a longer duration may be necessary to detect possible neurological changes that may occur.

Declaration of interest

None of the authors have any financial disclosures or competing interests to declare.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

Processed MR data is available at 10.6084/m9.figshare.7144664

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