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Effects of prenatal vitamin-D, multivitamin and folic acid supplementation on brain development in children with ADHD and ASD traits

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ABSTRACT

This study aims to examine the associations between vitamin suppletion (including folic acid vitamin D and multivitamin supplements), taken general food patterns into account as factor, and differences in structure of the offspring's brain, offspring risk of neurodevelopmental disorders (ASD and ADHD), as reflected in symptom scores on questionnaires for ASD and ADHD, and if so, test whether differences in the brain mediate association with neurodevelopmental disorders.

A total of 3937 children (age 9-11) from the Generation-R project were included in the current study. Maternal Vitamin-D and Folic-acid serum levels, multivitamin supplement use and overall dietary quality as assessed by the Food Frequency Questionaire (FFQ) during pregnancy were used as predictors. Brain volumes of children were decomposed into four independent components and used as mediators. ADHD and ASD symptoms as measured by CBCL and SRS respectively were used as outcome variables.

Our results show 1) significant association between Maternal vitamin-D, Multivitamin use and diet quality and ADHD or ASD symptoms. 2) Vitamin-D and diet quality are associated with childhood brain volume components. 3) brain volume components are associated with ADHD and ASD symptoms scores, and these can be dissociated by component and 4) part of the association between dietary factors in pregnancy on ADHD and ASD symptoms is mediated through the brain volume components of the children.

INTRODUCTION AND RATIONALE

Preconception and prenatal nutrition are critical for fetal brain development and deficiencies in certain nutrients have been linked to higher risk of neurodevelopmental disorders particularly in low and middle income countries. However, associations of use of multivitamin / dietary supplements (including, among others, folic acid, fatty acids and vitamin D, in combination or alone) with offspring neurodevelopmental disorders are not well understood and results across studies are not fully consistent. A recent systematic review and meta-analysis of studies in mostly European populations found an overall inverse association between maternal folic acid or multivitamin supplementation and children's risk of Autism Spectrum Disorders (ASD). This meta-analysis synthesized findings of six prospective cohort studies and estimated a RR of ASD of 0.64 (95% CI: 0.46, 0.90) (Li et al., 2019). Just to highlight two studies that were part of this meta-analysis, Levine at al. (2018) report an association between maternal use of folic acid and multivitamin supplements in the periods before and during pregnancy with a 50% or larger reduction of risk for autism in a cohort of more than 45,000 children at age 10 year. Another study included in the meta-analysis, (DeVilbiss et al., 2017) found an about 40% lower risk for autism with intellectual disability (but not for autism per se, thus without intellectual disability) in more than 270,000 mother-child pairs from population registers for mothers who used multivitamin supplements during pregnancy. Yet, there was no evidence that either iron or folic acid supplement use were associated with ASD prevalence (DeVilbiss et al., 2017). Also, among very young children at high familial risk for ASD, those with a mother who used multivitamins before and/or in the first period of pregnancy were less likely to develop full ASD, compared to those whose mother did not use multivitamins (Schmidt et al. 2019).

Findings of studies on the associations between dietary factors and/or vitamin use during pregnancy and risk for ADHD in the offspring were inconsistent (Li et al., 2019).

In summary, data on associations of multivitamin supplements (either combinations of vitamin or single vitamins) and risk for later ASD, and in particular later ADHD and related outcomes overall are inconclusive and warrant future investigation. It is unclear which food components are more critical than others, what the role is of the overall food pattern, and it is also unclear to which extent early nutrition is associated with differences in brain structure and function of the offspring and whether differences in brain structure and function mediate offspring's risk for neurodevelopmental disorders.

Aim

This study aims to examine the associations between vitamin suppletion (including folic acid vitamin D and multivitamin supplements), taken general food patterns into account as factor, and differences in structure of the offspring's brain, offspring risk of neurodevelopmental disorders (ASD and ADHD), as reflected in symptom scores on questionnaires for ASD and ADHD, and if so, test whether differences in the brain mediate association with neurodevelopmental disorders.

METHODS

We used the data of the Generation R study. This is a population-based prospective cohort study of about 10,000 children from fetal life until adulthood (Jaddoe et al., 2006). The study is designed to identify early environmental and genetic causes and causal pathways leading to normal and abnormal growth, development and health from fetal life, over childhood and into young adulthood. Details about study characteristics, data acquisition and preprocessing can be found in the primary design paper as well as subsequent updates on longitudinal data acquisition (Jaddoe et al., 2006; 2007; 2012). Population & recruitment

Sample characteristics

The main predictors used for this study are the maternal use of dietary vitamin-D, folic acid and multivitamin supplements during pregnancy. Information on use of maternal multivitamin supplements is based on a self-report questionnaire on dietary habits. Folic acid and vitamin-D use are based on blood biosamples during pregnancy. Overall patterns of material dietary quality were additionally derived from the self-report Food Frequency Questionnaire (FFQ) (Nguyen et al., 2017). The main outcome for our models is severity of ADHD and ASD symptoms at age 9. ADHD symptoms are measured by parental questionnaire (CBCL) at age 9. ASD symptoms are measured by parental SRS questionnaire at age 9.

Mediator variables are the cortical and subcortical brain volume of children at age 9. These data are based on standard freesurfer segmentation, and averaged over both hemispheres, leading to a total of 43 included brain segmentations per subject (see Table 3).

A total of 3737 subjects in the Generation-R datasets had brain imaging data available at age 9 and were included in the current analyses.

N (males/females)	1951	1986
Age (mean/SD)	10.12	0.59
Folic acid serum levels mother during pregnancy (available)	2571	
Vitamin D serum levels mother during pregnancy (available)	3072	
Multivitamin use mother during pregnancy (available)	2930	
Diet quality score mother during pregnancy (available)	2709	

Table 1. Participant characteristics and data availability

Data analyses

Given our aim and hypotheses to test a mediating role for brain structure in regulating the association between the dietary supplements and ASD/ADHD symptoms, our data analyses will take the form of 5 sequential models:

1) Effects of dietary supplements during pregnancy on ADHD and ASD symptoms in children will be analysed using regression analyses with the 3 supplements (vitamin-d serum levels, folic acid serum levels and recent multivitamin use self-report (yes/no)) as predictors and either total ADHD symptoms reported by parental CBCL score or total ASD symptoms as reported by parental SRS score as outcome measures.
2) Effects of dietary supplements during pregnancy on brain volumes will be analyzed using a set of regression analyses with the 3 supplements (vitamin-d serum levels, folic acid serum levels and recent multivitamin use self-report (yes/no)) as predictors each brain volume segment as outcome measure. These 43 models will be corrected for multiple comparisons using false discovery rate (fdr) correction.
   Given the large number of brain volumes, ICA decomposition of these areas into several underlying independent factors will be performed as a data-reduction step. This allows for the

use of the resulting independent components (ICs) as subsequent predictors and mediators. The analyses from step 3 will be repeated using these ICs instead of segmented brain volumes.

3) Association between ADHD and ASD symptoms and brain volumes in children will be analysed using a set of regression models using the symptom scores as outcome variable and each brain volume segment as predictor. These resulting 2 x 43 models will be corrected for multiple comparisons using fdr corrections.
4) The final mediation models will use the 3 supplements (vitamin-d serum levels, folic acid serum levels and recent multivitamin use self-report (yes/no)) as predictors, either total ADHD symptoms reported by parental CBCL score or total ASD symptoms as reported by parental SRS score as outcome measures, and the brain volume ICs as mediators. One model will be run for each IC, separately for ADHD and ASD symptoms.

RESULTS

Effects of dietary supplements during pregnancy on ADHD and ASD symptoms in children

The regression models in Table2 indicate a significant association between maternal multivitamin use, overall dietary quality, and ADHD (t=-202.79, p<0.04; t=-361.05, p<0.001 respectively). The association reflects that use of multivitamins and better dietary quality is associated with lower scores for ADHD symptoms. Additionally, a significant association between vitamin-d use and multivitamin use and ASD is observed as well (t=-29.73, p<0.001; t=-202.26, p<0.04), again reflecting that higher levels of vitamin D and better dietary quality is associated with lower scores for ASD symptoms.

	ADHD				**ASD**			
	Estimate	Std. Error	t value	Pr(>|t|)	Estimate	Std. Error	t value	Pr(>|t|)
Vitamin D	-0.01	0.00	-119.88	0.23	**-0.01**	**0.00**	**-29.73**	**0.00**
Folic Acid	-0.02	0.01	-107.75	0.28	-0.01	0.01	-0.50	0.62
Multivitamin	**-0.54**	**0.26**	**-202.79**	**0.04**	**-0.44**	**0.22**	**-202.26**	**0.04**
Diet score pregnancy	**-0.30**	**0.08**	**-361.05**	**0.00**	-0.11	0.07	-160.92	0.11

Table 2. regression outcomes of dietary supplementation during pregnancy on ADHD and ASD symptoms in children.

Effects of dietary supplement during pregnancy on brain volumes in children

The regression models in Table 3 Indicate that both maternal vitamin-D and overall diet score are significantly associated with many brain volumes. Vitamin-D is associated mainly with brain volumes in the frontal and temporal areas, whilst diet score is associated with most brain volumes over the whole brain. Multivitamin use and folic acid use do not show any significant associations with brain volumes after FDR correction.

The regression models in Table 4. indicate a significant association between maternal vitamin-D and brain volumes in the frontal-temporal/parietal IC as well as the frontal-occipital IC (t=-2.2022 p<0.027; t=-3.56, p<0.001 respectively.). Additionally, a significant association between overall maternal dietary quality and brain volume in the subcortical IC, frontal-temporal/parietal IC and frontal-occipital IC was observed (t=2.036, p<0.04; t=-2.383, p<0.017; t=3.629, p<0.001 respectively).

	Vitamin D				Folic Acid				Dietscore				Multivitamin			
	estimate	std.error	tvalue	p-corrected	estimate	std.error	tvalue	p-correcte	estimate	std.error	tvalue	p-corrected	estimate	std.error	tvalue	p-correcte
supramarginal_vol	0.003	0.001	306.07	0.010	0.004	0.003	145.11	0.574	0.074	0.016	460.76	0.000	0.042	0.052	0.8007	0.628
parsorbitalis_vol	0.002	0.001	299.87	0.010	0.001	0.003	0.4348	0.897	0.074	0.016	45.146	0.000	0.082	0.053	155.14	0.372
postcentral_vol	0.002	0.001	288.19	0.011	0.004	0.003	145.65	0.574	0.071	0.016	438.04	0.000	0.024	0.052	0.4653	0.786
lateralorbitofrontal_vol	0.003	0.001	319.69	0.010	0.003	0.003	0.9338	0.887	0.068	0.016	418.17	0.000	0.064	0.052	121.37	0.461
paracentral_vol	0.001	0.001	0.9525	0.358	0.002	0.003	0.772	0.887	0.066	0.016	403.9	0.000	0.075	0.053	141.93	0.419
Caudate_vol	0.002	0.001	225.97	0.052	0.001	0.003	0.4282	0.897	0.063	0.016	385.34	0.001	0.037	0.053	0.6999	0.671
medialorbitofrontal_vol	0.002	0.001	222.32	0.054	0.002	0.003	0.597	0.887	0.063	0.016	385.83	0.001	0.096	0.053	181.16	0.331
bankssts_vol	0.001	0.001	172.72	0.113	0.002	0.003	0.8294	0.887	0.063	0.016	389.5	0.001	0.018	0.052	0.3491	0.823
parsopercularis_vol	0.001	0.001	157.73	0.141	-0.002	0.003	-0.622	0.887	0.061	0.016	376.4	0.001	0.070	0.053	131.31	0.452
precentral_vol	0.003	0.001	312.07	0.010	0.006	0.003	205.46	0.516	0.060	0.016	37.313	0.001	0.042	0.052	0.8003	0.628
precuneus_vol	0.002	0.001	310.17	0.010	0.005	0.003	171.41	0.516	0.058	0.016	36.907	0.001	0.023	0.051	0.4427	0.786
isthmuscingulate_vol	0.003	0.001	339.84	0.007	-0.001	0.003	-0.218	0.961	0.060	0.017	36.444	0.001	0.006	0.054	0.1154	0.912
lingual_vol	0.002	0.001	214.69	0.060	-0.002	0.003	-0.686	0.887	0.058	0.016	356.08	0.001	0.056	0.053	106.53	0.529
superiortemporal_vol	0.002	0.001	264.04	0.022	0.000	0.003	-0.053	0.970	0.058	0.016	352.94	0.001	0.115	0.053	215.4	0.301
CSF_vol	-0.001	0.001	-0.624	0.545	0.001	0.003	0.2863	0.954	0.057	0.017	337.76	0.002	0.093	0.055	168.43	0.331
insula_vol	0.003	0.001	348.63	0.007	0.000	0.003	0.0757	0.970	0.054	0.016	328.49	0.003	0.048	0.053	0.8956	0.628
superiorparietal_vol	0.002	0.001	251.83	0.028	0.006	0.003	218.56	0.516	0.052	0.016	329.49	0.003	0.020	0.051	0.3829	0.816
inferiorparietal_vol	0.001	0.001	162.73	0.131	0.005	0.003	166.58	0.516	0.050	0.016	317.61	0.004	0.063	0.051	122.85	0.461
middletemporal_vol	0.003	0.001	314.61	0.010	0.005	0.003	177.19	0.516	0.048	0.016	303.89	0.005	0.009	0.051	0.1772	0.901
pericalcarine_vol	0.002	0.001	194.93	0.088	-0.001	0.003	-0.4	0.897	0.049	0.016	305.19	0.005	0.014	0.052	0.2749	0.842
caudalmiddlefrontal_vol	0.002	0.001	18.864	0.095	0.005	0.003	157.22	0.555	0.049	0.016	302.3	0.005	0.072	0.053	135.64	0.443
lateraloccipital_vol	0.003	0.001	41.97	0.001	0.000	0.003	-0.097	0.970	0.047	0.016	294.3	0.006	0.104	0.052	200.7	0.301
fusiform_vol	0.002	0.001	240.58	0.037	0.002	0.003	0.6929	0.887	0.047	0.016	293.72	0.006	0.038	0.052	0.7175	0.671
cuneus_vol	0.004	0.001	451.83	0.000	-0.001	0.003	-0.474	0.897	0.047	0.016	286.53	0.007	0.113	0.053	213.54	0.301
parstriangularis_vol	0.002	0.001	184.63	0.098	-0.003	0.003	-117.1	0.790	0.046	0.016	286.22	0.007	0.100	0.052	191.68	0.301
superiorfrontal_vol	0.002	0.001	295.36	0.011	0.002	0.003	0.62	0.887	0.044	0.016	266.96	0.012	0.102	0.053	191.21	0.301
Hippocampus_vol	0.002	0.001	214.8	0.060	0.000	0.003	-0.038	0.970	0.043	0.016	266.72	0.012	0.074	0.052	142.42	0.419
Amygdala_vol	0.002	0.001	190.82	0.093	-0.003	0.003	-103.5	0.807	0.043	0.016	2.647.	0.012	0.056	0.053	104.65	0.529
rostralanteriorcingulate_vol	0.001	0.001	119.64	0.255	0.001	0.003	0.2545	0.954	0.043	0.016	264.88	0.012	0.083	0.053	155.81	0.372
rostralmiddlefrontal_vol	0.002	0.001	309.43	0.010	0.001	0.003	0.402	0.897	0.041	0.016	260.8	0.013	0.164	0.052	317.48	0.066
inferiortemporal_vol	0.002	0.001	301.99	0.010	0.002	0.003	0.5565	0.887	0.041	0.016	256.23	0.015	0.016	0.052	0.3028	0.840
Thalamus_Proper_vol	0.002	0.001	199.56	0.083	0.003	0.003	110.12	0.790	0.038	0.016	237.31	0.024	0.066	0.052	127	0.461
posteriorcingulate_vol	0.002	0.001	260.03	0.024	0.000	0.003	0.0826	0.970	0.036	0.016	21.917	0.037	0.062	0.053	115.91	0.482
caudalanteriorcingulate_vol	0.000	0.001	0.1964	0.844	0.002	0.003	0.7225	0.887	0.031	0.016	189	0.075	0.089	0.053	169.97	0.331
Brain_Stem_vol	0.001	0.001	173.67	0.113	0.003	0.003	110.38	0.790	0.029	0.016	18.443	0.080	0.006	0.052	0.1109	0.912
Accumbens_area_vol	0.002	0.001	177.39	0.109	0.003	0.003	109.05	0.790	0.031	0.017	180.06	0.086	0.049	0.055	0.876	0.628
transversetemporal_vol	0.002	0.001	293.07	0.011	0.000	0.003	0.1259	0.970	0.026	0.017	155.61	0.139	0.130	0.054	240.12	0.301
parahippocampal_vol	0.001	0.001	167.83	0.122	-0.005	0.003	-182	0.516	0.019	0.017	117.15	0.273	0.109	0.054	201.44	0.301
Putamen_vol	0.001	0.001	128.92	0.230	0.003	0.003	0.883	0.887	0.018	0.017	105.4	0.322	0.026	0.054	0.4825	0.786
Pallidum_vol	0.001	0.001	0.9824	0.350	0.006	0.003	191.54	0.516	0.015	0.016	0.9125	0.389	-0.030	0.053	-0.565	0.768
frontalpole_vol	0.001	0.001	149.91	0.160	0.007	0.003	213.6	0.516	0.012	0.017	0.7204	0.494	0.044	0.055	0.8147	0.628
entorhinal_vol	0.001	0.001	121.67	0.253	0.002	0.003	0.5768	0.887	0.009	0.017	0.5302	0.610	0.094	0.054	17.498	0.331
temporalpole_vol	0.002	0.001	184	0.098	0.001	0.003	0.2562	0.954	0.000	0.017	0.0117	0.991	0.025	0.054	0.4609	0.786

Table 3. regression outcomes of dietary supplementation during pregnancy on brain volumes in children.

	IC1: subcortical				**IC2:** frontal-temporal/parietal				**IC3:** frontal-occipital				**IC4:** hippocampal			
	Est	std	t	p-fdr	Est	std	t	p-fdr	Est	std	t	p-fdr	Est	std	t	p-fdr
vitamin-d	0.001	0.001	0.787	0.431	**0.002**	**0.001**	**2.220**	**0.027**	**-0.003**	**0.001**	**-3.563**	**0.000**	-0.001	0.001	-1.312	0.190
folic-acid	0.005	0.003	1.698	0.090	0.001	0.003	0.242	0.809	0.002	0.003	0.655	0.512	0.005	0.003	1.786	0.074
dietscore_pregnancy	**0.033**	**0.016**	**2.036**	**0.042**	**0.038**	**0.016**	**2.383**	**0.017**	**-0.059**	**0.016**	**-3.629**	**0.000**	0.027	0.017	1.624	0.105
multivitamin	0.002	0.052	0.039	0.969	0.083	0.052	1.598	0.110	-0.083	0.052	-1.592	0.112	-0.021	0.054	-0.383	0.702

Table 4. regression outcomes of dietary supplementation during pregnancy on brain independent components in children.

Associations between brain volumes and ADHD and ASD symptoms in children

The regression models in Table 5. show significant associations between ADHD and ASD symptoms and volumes of the brain regions. ADHD in particular is significantly associated with lower brain volume on a large portion of the areas of the brain, whilst ASD is negatively associated mainly with volumes in the frontal and temporal areas.

In Table 6. the regression models utilizing the brain IC's show that ADHD symptoms are negatively associated with brain volumes in the subcortical and frontal-occipital IC (t=-1.958, p<0.05; t=-4.498, p<0.001 respectively). ASD symptoms are negatively associated with frontal- temporal/parietal volumes (t=-3.101, p<0.002).

	ADHD				ASD			
	estimate	std.error	tvalue	p-correcte	estimate	std.error	tvalue	p-correcte
caudalmiddlefrontal_vol	-0.438	0.088	-49.848	2,80E+09	-0.264	0.070	-377.790	0.007
lateralorbitofrontal_vol	-0.382	0.089	-429.820	0.000	-0.242	0.070	-345.710	0.008
middletemporal_vol	-0.361	0.087	-412.860	0.001	-0.245	0.070	-351.110	0.008
postcentral_vol	-0.355	0.089	-40.003	0.001	-0.219	0.070	-314.250	0.012
superiortemporal_vol	-0.335	0.088	-381.500	0.001	-0.220	0.069	-3.174	0.012
supramarginal_vol	-0.330	0.088	-373.800	0.001	-0.225	0.069	-324.160	0.012
inferiortemporal_vol	-0.329	0.087	-378.680	0.001	-0.214	0.070	-30.457	0.014
bankssts_vol	-0.329	0.088	-373.960	0.001	-0.188	0.069	-27.129	0.018
caudalanteriorcingulate_vol	-0.339	0.088	-384.430	0.001	-0.189	0.070	-271.570	0.018
parstriangularis_vol	-0.305	0.087	-349.390	0.002	-0.189	0.070	-270.660	0.018
precentral_vol	-0.289	0.088	-32.868	0.003	-0.190	0.070	-272.620	0.018
precuneus_vol	-0.292	0.088	-332.000	0.003	-0.192	0.070	-276.280	0.018
rostralanteriorcingulate_vol	-0.293	0.089	-330.850	0.003	-0.193	0.069	-279.040	0.018
superiorparietal_vol	-0.292	0.088	-329.710	0.003	-0.195	0.070	-279.460	0.018
transversetemporal_vol	-0.298	0.089	-335.930	0.003	-0.193	0.070	-276.480	0.018
insula_vol	-0.296	0.088	-337.040	0.003	-0.198	0.070	-283.750	0.018
superiorfrontal_vol	-0.283	0.087	-325.670	0.003	-0.173	0.070	-2.483	0.033
Thalamus_Proper_vol	-0.281	0.088	-320.450	0.003	-0.172	0.070	-244.410	0.035
Caudate_vol	-0.275	0.088	-313.790	0.004	-0.168	0.069	-242.620	0.035
parsorbitalis_vol	-0.271	0.087	-310.630	0.004	-0.158	0.070	-227.040	0.050
CSF_vol	-0.270	0.087	-309.300	0.004	0.150	0.069	218.276	0.060
parsopercularis_vol	-0.268	0.088	-306.560	0.004	-0.147	0.070	-211.930	0.064
rostralmiddlefrontal_vol	-0.268	0.089	-301.470	0.005	-0.149	0.070	-212.800	0.064
paracentral_vol	-0.266	0.088	-301.950	0.005	-0.141	0.070	-202.040	0.078
cuneus_vol	-0.260	0.087	-298.730	0.005	-0.138	0.070	-198.290	0.082
fusiform_vol	-0.259	0.088	-293.840	0.005	-0.128	0.070	-184.490	0.104
Accumbens_area_vol	-0.243	0.088	-276.850	0.009	-0.129	0.070	-18.469	0.104
posteriorcingulate_vol	-0.240	0.088	-272.370	0.010	-0.125	0.070	-179.290	0.112
lateraloccipital_vol	-0.234	0.088	-266.210	0.012	-0.107	0.070	-152.330	0.189
isthmuscingulate_vol	-0.220	0.088	-249.560	0.018	-0.096	0.069	-139.790	0.233
parahippocampal_vol	-0.214	0.089	-241.330	0.022	-0.088	0.069	-126.770	0.284
inferiorparietal_vol	-0.195	0.088	-221.700	0.036	-0.076	0.070	-108.650	0.373
medialorbitofrontal_vol	-0.190	0.088	-216.360	0.040	-0.063	0.069	-0.903	0.477
temporalpole_vol	-0.189	0.089	-212.910	0.042	-0.061	0.070	-0.878	0.481
lingual_vol	-0.173	0.088	-196.250	0.061	-0.050	0.069	-0.731	0.571
pericalcarine_vol	0.157	0.088	178.549	0.089	-0.042	0.069	-0.600	0.629
Hippocampus_vol	-0.150	0.088	-171.050	0.101	-0.041	0.070	-0.589	0.629
Brain_Stem_vol	-0.146	0.088	-165.830	0.110	0.043	0.070	0.607	0.629
Putamen_vol	-0.122	0.088	-138.230	0.184	-0.038	0.071	-0.534	0.654
Pallidum_vol	-0.085	0.088	-0.971	0.356	-0.021	0.071	-0.293	0.827
frontalpole_vol	-0.082	0.089	-0.923	0.374	0.019	0.070	0.266	0.828
entorhinal_vol	0.073	0.087	0.836	0.413	0.013	0.069	0.189	0.870
Amygdala_vol	-0.033	0.088	-0.375	0.708	-0.010	0.071	-0.139	0.889

Table 5. regression outcomes associations between brain volumes and ADHD and ASD symptoms in children

Associations between brain ICA components and ADHD and ASD symptoms in children

	ADHD				ASD			
	Estimate	Std. Error	t value	Pr(>|t|)	Estimate	Std. Error	t value	Pr(>|t|)
IC1	**-0.172**	**0.088**	**-1.958**	**0.050**	-0.045	0.070	-0.640	0.522
IC2	-0.151	0.088	-1.712	0.087	**-0.217**	**0.070**	**-3.101**	**0.002**
IC3	**-0.393**	**0.087**	**4.498**	**0.0001**	0.117	0.069	1.697	0.090
IC4	-0.108	0.087	-1.234	0.217	-0.111	0.069	-1.595	0.111

Table 6. regression outcomes associations between brain independent components and ADHD and ASD symptoms in children

Mediation of dietary supplement association with ADHD and ASD by brain ICA components

The mediation models depicted in Table 7. indicate that the indirect effect from Vitamin-D to ADHD symptoms via the frontal-occipital IC is significant (-2.668, p<0.008). The indirect effect from multivitamin use to ADHD symptoms via the frontal-occipital IC is significant (t=-2.18; p<0.029). The indirect effect from maternal dietary score to ADHD symptoms via the frontal-occipital IC is significant (t=-2.506, p<0.012).

The mediation models in Table 8. indicate that the indirect effect from multivitamin use to ASD symptoms via the frontal-temporal/parietal IC is significant (t=-2.003, p<0.045).

Mediation model 1

ADHD + VIT-D	effect	Est	StE	z-value	P(>|z|)
IC 1: subcortical	indirect1	0	0	-1.263	0.207
IC2: frontal-temporal/parietal	indirect2	0	0	-1.307	0.191
IC3: frontal-occipital	**indirect3**	**-0.001**	**0**	**-2.668**	**0.008**
IC4: hippocampal	indirect4	0	0	-0.431	0.666
	total	-0.006	0.003	-1.661	0.097

Mediation model 2

ADHD + multivit	effect	Est	StE	z-value	P(>|z|)
IC 1: subcortical	indirect1	-0.01	0.011	-0.96	0.337
IC2: frontal-temporal/parietal	indirect2	-0.02	0.015	-1.379	0.168
IC3: frontal-occipital	**indirect3**	**-0.044**	**0.02**	**-2.18**	**0.029**
IC4: hippocampal	indirect4	-0.004	0.008	-0.442	0.659
	total	0.053	0.21	0.255	0.799

Mediation model 3:

ADHD + dietscore	effect	Est	StE	z-value	P(>|z|)
IC 1: subcortical	indirect1	-0.006	0.004	-1.526	0.127
IC2: frontal-temporal/parietal	indirect2	-0.008	0.006	-1.501	0.133
IC3: frontal-occipital	**indirect3**	**-0.015**	**0.006**	**-2.506**	**0.012**
IC4: hippocampal	indirect4	-0.001	0.003	-0.457	0.648
	total	**-0.257**	**0.063**	**-4.105**	**0**

Table 7. mediation models indicating the direct and indirect effects (mediated through brain IC's) of dietary factors on ADHD

Mediation model 4

ASD + VIT-D	effect	Est	StE	z-value	P(>|z|)
IC 1: subcortical	indirect1	0	0	-0.209	0.835
IC2: frontal-temporal/parietal	indirect2	0	0	-1.865	0.062
IC3: frontal-occipital	indirect3	0	0	-0.11	0.913
IC4: hippocampal	indirect4	0	0	-0.283	0.778
	total	**-0.011**	**0.002**	**-4.767**	**0**

Mediation mode 5

ASD + multivit	effect	Est	StE	z-value	P(>|z|)
IC 1: subcortical	indirect1	-0.001	0.003	-0.278	0.781
IC2: frontal-temporal/parietal	**indirect2**	**-0.03**	**0.015**	**-2.003**	**0.045**
IC3: frontal-occipital	indirect3	-0.018	0.014	-1.259	0.208
IC4: hippocampal	indirect4	0	0.004	-0.066	0.948
	total	0.093	0.199	0.467	0.64

Mediation model 6

ASD + dietscore	effect	Est	StE	z-value	P(>|z|)
IC 1: subcortical	indirect1	0	0.003	0.017	0.986
IC2: frontal-temporal/parietal	indirect2	-0.008	0.005	-1.566	0.117
IC3: frontal-occipital	indirect3	-0.004	0.004	-1.056	0.291
IC4: hippocampal	indirect4	-0.001	0.002	-0.564	0.573
	total	**-0.196**	**0.051**	**-3.875**	**0**

Table 8. mediation models indicating the direct and indirect effects (mediated through brain IC's) of dietary factors on ADHD

DISCUSSION

Our first set of analyses indicate that there is a significant association between maternal dietary factors and ADHD or ASD symptoms. In particular, we show that multivitamin supplementation and better overall diet quality of the mother are associated with lower ADHD scores in children, whilst vitamin-D serum levels are associated with lower ASD scores.

Our second set of models show that these dietary factors are associated with brain volumes in children. Vitamin-D levels during pregnancy are associated with higher frontal-occipital and frontal-temporal/parietal volumes in children. Overall diet quality of the mothers is associated with higher subcortical, frontal-occipital and frontal-temporal/parietal volumes in children.

Our third set of analyses show that there is a significant association between brain volume and ADHD/ASD in children. Specifically, we show that ADHD symptoms are associated with lower subcortical and frontal-occipital volumes, whilst ASD symptoms are associated with lower frontal-temporal/parietal volumes.

Lastly, our fourth set of models show that part of the maternal vitamin-D level, multivitamin supplementation and dietary quality effects on ADHD are mediated through frontal-occipital volumes in children, and maternal multivitamin supplementation effects on ASD are partly mediated through frontal-temporal/parietal effects in children.

Taken together, this research shows several important effects. Firstly, we show that the maternal dietary factors seem to influence the brain and behavior of their children many years later. Second, we also show that we can dissociate which dietary factors and which brain IC's are associated with different neurodevelopmental disorders. So both dietary factors and brain volume patterns are specific for either ADHD or ASD. Lastly, we show that through these mediation models, it is likely that some of the influence of diet is mediated through structural brain development, indicating a potential causal pathway through which the influence of vitamin and other dietary factors may influence behavioral outcomes.

An important caveat of the current research is the difficulty of dissociating covarying environmental factors during development. In particular, our models will need to be expanded to investigate effects of other prenatal factors such as maternal mental health, the child's birthweight, duration of pregnancy and smoking/alcohol use during pregnancy. We also need to take into account the wider environment in which the child subsequently developed. This includes factors like parental socio-economic status, parental educational attainment, child dietary quality, ethnicity, and the presence of internalizing mental disorders in children. Finally, part of these effects may be mediated by or driven by genetic factors in the mother and the offspring.

We conclude that maternal dietary practices and choices likely influence both the brain development and neurodevelopmental symptoms of their offspring, but acknowledge that causal inferences are challenging to make within a complex system of covarying environmental influences.

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