Title: Child plasma vitamin B12 concentrations and executive functions at age 7-11 years: the GUSTO study

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**ABSTRACT**

**Purpose:** We examined the associations of plasma vitamin B12 concentrations with executive functions (EFs) in children aged 7-11 years from the Growing Up in Singapore Towards healthy Outcomes (GUSTO) cohort.

**Methods:** Plasma vitamin B12 concentrations were measured at age 8 years. Selected subtests from the Developmental Neuropsychological Assessment (NEPSY-II), Wisconsin Card Sorting Test (WCST-64), Delis-Kaplan Executive Function System (D-KEFS), and the Wechsler Intelligence Scale for Children (WISC-V) were used to assess EFs components – inhibition, working memory and cognitive flexibility at 8.5 and 10.5 years. Brain-network functional connectivity (FC) at 7.5 years were acquired through magnetic resonance imaging. Linear regressions adjusting for key confounders (e.g. socio-demographics, growth and lifestyle factors) examined associations of plasma vitamin B12 concentrations at 8 years with FC at 7.5 years, and with EFs at 8.5 and 10.5 years, respectively.

**Results:** Higher vitamin B12 concentrations at 8 years were associated with better cognitive flexibility at 8.5 years [β (95%CI): 3.23 (0.03, 6.43) for WCST-64 perseverative responses (n=254)], and better working memory at 10.5 years [0.42 (0.04, 0.8) for WISC-V Letter-Number-Sequencing (n=304)]; no associations with NEPSY-II and D-KEFS subtests. Higher vitamin B12 concentrations was associated with lower Dorsal Attention and Frontoparietal networks FC (β -0.125, SE: 0.06381, t-value: -2.03, n=234) linked to better working memory and cognitive flexibility.

**Conclusion:** Higher vitamin B12 concentrations in later childhood may be beneficial for EFs and brain-networks FC but require confirmation in studies using similar EF tests for comparability, and prospective, longitudinal measure of brain-network FC.

**Keywords:** vitamin B12, child, cognition, executive function, functional connectivity

**INTRODUCTION**

It is well established that brain development is most rapid and is most sensitive to environmental factors/insults in the first few years of life [[1](#_ENREF_1)]. Studies have shown that adequate maternal [[2](#_ENREF_2)] and early childhood nutrition [[3](#_ENREF_3)] were associated with better cognitive function in preschool children (aged ≤5 years), but less is known regarding the influence of nutrition on cognitive development in children at later ages. During the “middle childhood” period (commonly defined in literature as ages 6-12 years), there is an increase in synaptic refinement and pruning as well as a peak in the development of the prefrontal cortex which is essential for executive functions (EFs) [[4](#_ENREF_4)].

The core components of EFs are inhibition, cognitive flexibility and working memory which are crucial for regulating attention, thoughts and emotions, planning and organization [[5](#_ENREF_5)]; all of which play important roles in academic performance, social functioning and emotional control [[6](#_ENREF_6),[7](#_ENREF_7)]. As most children would be attending school during the middle-childhood period, it is therefore of great interest to examine the environmental factors influencing EFs; but evidence on the role of nutrition in EFs during the middle-childhood period is limited.

Vitamin B12 is a micronutrient crucial for the development of the brain and central nervous system as it is involved in processes such as myelination of neurons and synaptogenesis [[8](#_ENREF_8)]. Several reviews have identified vitamin B12 as one of the key micronutrients for adequate EFs development alongside iron and omega-3 polyunsaturated fatty acids (PUFAs) [[4](#_ENREF_4),[9](#_ENREF_9),[10](#_ENREF_10)]. While it is clear that iron supplementation [[9](#_ENREF_9)] and higher omega-3 PUFA intake [[10](#_ENREF_10)] improves EFs in school-aged children (aged 6-12 years), limited studies examined the role of vitamin B12 in EFs in school-aged children [[4](#_ENREF_4)]. To our knowledge, only three studies have examined vitamin B12 with EFs, including two intervention trials showing consuming foods rich in or fortified with vitamin B12 resulted in improved short-term memory [[11](#_ENREF_11),[12](#_ENREF_12)]. However, these trials are conducted in populations with a high prevalence of under-nutrition (e.g. stunting, anaemia, and deficiencies in several micronutrients). One study, in contrast, showed higher plasma vitamin B12 concentrations were associated with poorer short-term memory and lower mental processing index scores [[13](#_ENREF_13)] which may also be confounded by the high prevalence of under-nutrition.

In populations with few children classified as vitamin B12 deficient, there remain considerable population variations in circulating vitamin B12 concentrations and intakes [[14](#_ENREF_14)]. It is unclear if these variations contribute to differences in EFs in children without frank vitamin B12 deficiency. With the increasing emphasis on plant-based diets, stimulated in part by efforts to promote planetary health, understanding the potential health effects of following plant-based diets with minimal B12-rich animal products on cognitive functions is important to determine whether such diets should be encouraged in children, even in those not classified as vitamin B12 deficient. To date, only one US study has related serum vitamin B12 to EFs in children aged 6-16 years with few classified as vitamin B12 deficient, reporting no significant associations [[15](#_ENREF_15)]. As the study participants were predominantly European-Americans, the generalisability of study findings, especially to the Asian population, is limited.

Studies examining cognitive abilities in children have largely relied on cognitive test batteries, but they are, to a certain extent, subjective and can be influenced by the participant’s understanding of the cognitive tasks, poor compliance, as well as by interrater reliability [[16](#_ENREF_16)]. As such, complementing cognitive test batteries with an objective tool such as neuroimaging is needed to rigorously assess development of the brain and cognitive abilities [[16](#_ENREF_16)]. A wide network of brain regions are involved in EFs processes and therefore depend on the functional connectivity (FC) between these brain regions [[17](#_ENREF_17)]. The role of nutrition in the brain network FC (using neuroimaging technique) is an emerging area with studies linking a healthy diet to EFs-related FC but mostly in aging brains [[18](#_ENREF_18),[19](#_ENREF_19)]. Only one study examined nutrition and FC in children, finding that higher dietary omega-3 intake correlated with decreased FC between parieto-occipital brain networks involved in EFs [[20](#_ENREF_20)]. No studies have linked vitamin B12 to brain networks FC to support findings with EFs assessed using cognitive test batteries.

This study aims to fill these research gaps by examining the associations between 1) plasma vitamin B12 concentrations and EFs, and 2) plasma vitamin B12 concentrations and FC to explain plausible mechanism underlying the B12-EFs association, in a cohort of Asian children aged 7-11 years.

**METHODS**

Study sample

Data used were obtained from the Growing Up in Singapore Towards healthy Outcomes (GUSTO) study, an ongoing prospective mother-offspring cohort study. Further details regarding the GUSTO study including eligibility and exclusion criteria have been published elsewhere [[21](#_ENREF_21)]. Briefly, between June 2009 and September 2010, 1247 healthy pregnant women aged 18 years and above who were attending their first trimester antenatal clinic at either KK Women’s and Children’s Hospital (KKH) or National University Hospital (NUH) were recruited. A total of 1176 babies were born between 30 November 2009 and 1 May 2011. Follow-up study visits were conducted for both mother and child after pregnancy and throughout childhood. The conduct of the GUSTO study was based on the guidelines in the Declaration of Helsinki and all procedures were approved by the National Healthcare Group Domain Specific Review Board (reference D/2009/021) and the SingHealth Centralised Institutional Review Board (reference 2018/2767). Informed written consent was obtained from all mothers at recruitment as well as written assent from the children at 7 years of age.

Assays of plasma vitamin B12 concentrations

During the year 8 follow-up visit, blood samples were collected from children after an overnight fast, processed within 4 hours and stored at -80 °C until analysis. Plasma concentration of vitamin B12 (pmol/L) was determined at the Bevital AS laboratory using microbiological assay containing a colistin sulphate-resistant strain of *Lactobacillus leichmannii* [[22](#_ENREF_22)]. Participants with plasma vitamin B12 concentration >1200 pmol/L were classified as outliers and excluded from analysis (n=4).

Assessment of executive functions

Due to limited manpower and available test slots, subsets of children were invited to complete tests of EFs at year 8.5 and 10.5 follow-up visits. Priority was given to children who had participated in cognitive assessments prior to 8.5 years. The EFs tests were administered by trained research coordinators with a bachelor’s degree in psychology and trained by GUSTO investigators.

At the year 8.5 visit, the Inhibition (IN) and Word Interference (WI) tasks from the Developmental Neuropsychological Assessment Second Edition (NEPSY-II) were administered to assess three components of EFs: inhibition, switching, and working memory [[23](#_ENREF_23),[24](#_ENREF_24)]. At the same visit, the Wisconsin Card Sorting Test 64-Card Version (WCST-64) was administered to assess cognitive flexibility [[25](#_ENREF_25)]. The WCST-64 yields ‘perseverative responses’, ‘perseverative errors’ and ‘failure-to-maintain-set’ variables. Further details on the test variables have been published elsewhere [[26](#_ENREF_26)]. Standardised scores for ‘perseverative responses’ and ‘perseverative errors’ were used for analysis; ‘failure-to-maintain-set’ was converted to a dichotomous variable for easier analysis (‘0’ for continued one rule to sort cards and ‘1’ for failed to maintain a rule to sort cards).

At the year 10.5 visit, the Colour-Word Interference task from the Delis-Kaplan Executive Function System (D-KEFS) was administered to assess inhibition and cognitive set shifting [[27](#_ENREF_27)]. Additionally, the Letter-Number-Sequencing task from the Wechsler Intelligence Scale for Children Fifth Edition (WISC-V) was administered to evaluate children’s working memory [[28](#_ENREF_28)].

Scaled scores with a mean of 10 and a standard deviation of 3 were used for NEPSY-II, D-KEFS and WISC-V, with higher scores indicating better EFs.

Neuroimaging data acquisition and pre-processing

Children underwent magnetic resonance imaging (MRI) of the brain at age 7.5 years on a 3T scanner (Magnetom Prisma, Siemens, Germany). Resting-state functional MRI (rs-fMRI) images were processed as previously described [[29](#_ENREF_29)], with the default pre-processing pipelines using the CONN Toolbox v20b [[30](#_ENREF_30)]. Detailed methods on neuroimaging data acquisition and pre-processing are in the Supplementary Methods.

Regions of Interests (ROIs) were seven large scale cortical resting state functional networks [Visual Network (VN), Somatomotor Network (SMN), Dorsal Attention Network (DAN), Ventral Attention Network/Salience Network (VAN), Limbic Network (LN), Frontoparietal Network (FPN) and Default Mode Network (DMN)], identified by Yeo et al [[31](#_ENREF_31)]. We computed FC matrices for each subject. Seven ROIs yielded a total of 21 unique between-network rs-FC measures.

Confounders

Confounders for adjustment in statistical models were determined *a priori* based on past literature showing key factors associated with diet or vitamin B12 and cognitive development [[32-34](#_ENREF_32)]. Data on maternal ethnicity was collected at recruitment and maternal highest education level was obtained at the year 5 follow-up visit. By accounting for socio-demographics, we are also accounting for the related family contextual factors influencing the child’s diet and cognitive development. Child sex was retrieved from hospital delivery records. At year 7 visit, dietary intake of children was assessed using a food frequency questionnaire, which was developed specifically to assess dietary intake of GUSTO children (with reference to data from food recalls and diaries) and validated in the same cohort [[35](#_ENREF_35)], and a “Healthy” dietary pattern characterised by higher intakes of fish and seafood, vegetables, nuts and legumes was derived using principal component analysis (PCA). “Healthy” dietary pattern was used to collectively represent diet quality and nutritional status. Few children (n=18, 10.8%) reported consumption of dietary supplements (yes/no) hence was not included. At year 8 visit, the child’s daily duration of moderate-to-vigorous physical activities (MVPA) as reported by the mother was collected, weight was measured with a scale (SECA803, Netherlands), height was measured with a stadiometer (SECA213, Netherlands); age and sex-specific BMI z-scores were derived using WHO reference [[36](#_ENREF_36)], and children with BMI z-scores > 1 SD were classified as overweight or obese. Child BMI was used to reflect growth. For analyses involving rs-fMRI data, mean relative motion over all volumes was included as a co-variate.

Statistical analysis

Plasma vitamin B12 concentration of the child was presented according to maternal and child characteristics. The associations of plasma vitamin B12 concentrations with each EFs tests were examined using linear regressions for normal distributions or inverse Gaussian regressions for positively skewed distributions. Plasma vitamin B12 concentrations were converted to standard deviation (SD) scores for easier comparison of effect sizes to other studies. For the outcome ‘failure-to-maintain-set’, logistic regression was used instead. Results were first presented without adjusting for covariates, followed by results adjusting for maternal ethnicity, maternal educational attainment, child sex, child BMI, child daily MVPA duration, and child “healthy” dietary pattern score. For maternal education, missing data at year 5 follow-up visit were imputed using data collected at recruitment as we hypothesised that maternal education is unlikely to change within 5 years. Multiple imputation with chained equations (20 times) imputed scores for “healthy” dietary pattern due to the larger number of missing data (n=59). All analyses were performed using Stata version 14 (StataCorp LP, College Station, TX, USA). Two-sided P<0.05 was considered statistically significant.All statistical analysis were completed with Stata 17.0 (StataCorp, College Station, TX, USA). Results with two-sided P-values<0.05 were considered statistically significant.

To explore associations between plasma vitamin B12 concentrations and rs-FC, elastic net regression was performed with the caret v6.0-94 and glmnet v4.1-7 packages in R v4.3.0 using 10-fold cross validation and a tuning grid set at α ∈ [0.1, 1], and λ ∈ [0, 1000]. Tuning parameters were selected based on the lowest root mean square error (RMSE) of prediction. Data were scaled and centred before analysis. Predictors included the 21 unique rs-FC and the following confounders/covariates: maternal ethnicity, maternal education level, child sex, child BMI, child daily MVPA duration, child “healthy” dietary pattern score, and rs-fMRI relative mean motion. A final regression model was then performed with the selected predictors from the elastic net model.

**RESULTS**

809 children participated in the year 8 visit (68.8% of the original 1176 infants); those who did not participate were generally due to busy schedules, inability to contact the participants, and dropping out of the GUSTO study. Of whom, 566 provided blood samples for assay of plasma vitamin B12 concentrations; 457 completed MRI at year 7.5, 452 completed both NEPSY-II and WCST-64 at year 8.5, and 433 completed both D-KEFS and WISC-V at year 10.5. Subsets of children with data for vitamin B12 and MRI (n=234), and vitamin B12 and EFs tests at the two respective timepoints (n=356) formed the final analysis sample (**Figure 1**), which were approximately 20-30% of the original 1176 infants and 29-44% of the 809 active participants at year 8. The analysis sample had higher percentages of children of Malay ethnicity, with mothers who attained less than tertiary education, and who were overweight and obese; the MRI subset had higher percentages of children who were less physically active but had higher adherence to the “healthy” dietary pattern (**Online Resource 1**).

A diagram of a patient's dna

Description automatically generated with medium confidence

**Fig. 1** Flow diagram for the analysis of child plasma vitamin B12 concentrations and executive functions in the Growing Up in Singapore Towards healthy Outcomes study. EF, executive function; NEPSY-II, Developmental Neuropsychological Assessment Second Edition; WCST-64, Wisconsin Card Sorting Test 64-Card Version; D-KEFS, Delis-Kaplan Executive Function System; WISC-V, Wechsler Intelligence Scale for Children Fifth Edition; MRI, magnetic resonance imaging; MVPA, moderate-vigorous physical activity; BMI, body mass index

Maternal and child characteristics

Maternal and child characteristics were based on the subset of children with plasma vitamin B12 concentration and completed EFs tests for at least one visit, and who had complete data for covariates (n=356, Figure 1). The mean plasma vitamin B12 concentrations was 521.84 ± 166.80 pmol/L (range: 158.59 - 1096.80 pmol/L); no children were classified as vitamin B12 deficient using a cut-off of <148pmol/L. Children with higher plasma vitamin B12 concentrations tended to be of Chinese ethnicity; their mothers tended to have attained higher education level, and they were less likely to have overweight and obesity (**Table 1**). There were no differences in plasma vitamin B12 concentrations according to child sex, <60 or ≥ 60 min/day MVPA. In a subset of children who provided data on dietary intake (n=297) and consumption of dietary supplement (n=166) at year 7 visit, children with higher adherence to the “Healthy” dietary pattern (defined as PCA z-score >0) had higher plasma vitamin B12 concentrations, but vitamin B12 concentrations did not differ by consumption of dietary supplement containing vitamin B12.

Associations of plasma vitamin B12 concentrations and executive functions

Higher plasma vitamin B12 concentrations at 8 years were associated with higher score in WI-Repetition subtask of the NEPSY-II in the unadjusted model, but the association was attenuated after adjusting for confounders (**Table 2**). Additionally, higher plasma vitamin B12 concentrations at 8 years were associated with higher standard score (i.e., better performance) in perseverative responses and perseverative errors of the WCST-64 at 8.5 years in the unadjusted model. After adjusting for confounders, the association with perseverative responses remained statistically significant (β 3.23, 95% CI: 0.03, 6.43), but the association with perseverative errors was attenuated (β 2.80, 95% CI: -0.34, 5.94).

At 10.5 years, higher plasma vitamin B12 concentrations at 8 years were associated with higher score in Letter-Number-Sequencing task of the WISC-V even after adjusting for covariates (β 0.42, 95% CI: 0.04, 0.80). There were no statistically significant associations between plasma vitamin B12 concentrations and all subtests of D-KEFS.

In a post-hoc analysis examining quartiles of plasma vitamin B12 concentrations, significant associations with WCST-64 perseverative responses at 8.5 years and with WISC-V Letter-Number-Sequencing were only observed for the highest quartile (range of vitamin B12 concentrations: 613.1-1076.6 pmol/L) compared to the lowest quartile (results not shown).

Association of plasma vitamin B12 concentrations and resting state functional connectivity

234 children had both vitamin B12 data and rs-FC data that passed motion exclusion criteria (Figure 1). The results of the elastic net regression were used for variable selection, as predictors that were not selected for would have coefficients reduced to zero. Connectivity between the DAN and FPN (DANxFPN), maternal education, maternal ethnicity, child BMI, and relative mean motion were selected predictors of vitamin B12 concentration. The association between DANxFPN and vitamin B12 concentration was confirmed with regression models without (β -0.119, SE: 0.0652, t-value: -1.83, P=0.069) and with maternal education, maternal ethnicity, child BMI, child “healthy” dietary pattern score, and relative mean motion as co-variate (β -0.125, SE: 0.06381, t-value: -2.03, P=0.045).

**DISCUSSION**

In this group of Asian school-aged children participating in the GUSTO study, we found that higher plasma vitamin B12 concentrations at age 8 years were associated with better cognitive flexibility and working memory as evident from higher scores in perseverative responses of WCST-64 at age 8.5 years and higher scores in the Letter Number Sequencing task of WISC-V at age 10.5 years; albeit without vitamin B12 deficiency. We additionally found plasma vitamin B12 concentrations at age 8 years to be associated with a lower connectivity between the DAN and FPN at age 7.5 years.

Our finding on the association between vitamin B12 concentrations and EFs in children is reminiscent of those by Larnkjær et al. [[37](#_ENREF_37)] reporting higher plasma vitamin B12 concentrations to be associated with higher scores in problem-solving and communication (assessed using the Ages and Stages Questionnaire) in 3 year old children without vitamin B12 deficiency. It is important to note that their children were younger, and the developmental tool used was reported by parents, whereas this study included objective assessments administered in the laboratory. Nonetheless, their findings together with ours suggest higher plasma vitamin B12 concentrations, even in the absence of vitamin B12 deficiency, may still be beneficial to neurocognitive development. In an effort to promote planetary health, there is a recent push for a global shift to plant-based diets; however, our finding suggests that public health recommendation to adopt plant-based diets in children, especially those devoid of B12-rich animal foods, may require careful consideration even in those without vitamin B12 deficiency.

In contrast, the study by Nguyen et al. [[15](#_ENREF_15)] showed no association between serum vitamin B12 concentrations and EFs, assessed by Block Design and Digit Span of WISC-Revised, in healthy children aged 6-16 years. A potential reason for the difference in findings could relate to difference in EFs components measured. Block Design assessed visual-spatial perceptional reasoning while Digit Span assessed a combination of working memory and attention span [[15](#_ENREF_15)]. Our EFs tests assessed cognitive flexibility (perseverative responses) [[25](#_ENREF_25)], and a combination of working memory, processing speed and cognitive flexibility (Letter Number Sequencing) [[28](#_ENREF_28)]. Likewise, we did not find significant association with the NEPSY-II WI task, which also assessed working memory [[23](#_ENREF_23)]. This could be because the NEPSY-II WI task assesses verbal working memory and interference control, which differ from the Letter-Number-Sequencing of the WISC-V.

Additionally, the timing of nutritional influence and development of cognitive functions could also explain the difference in study findings. It is possible that our study captured the period (ages 8-11 years) when the influence of vitamin B12 on the development of brain regions responsible for EFs is strongest; in contrast, the study by Nguyen et al. [[15](#_ENREF_15)] also included children aged 11-16 years. Indeed, during early and middle childhood years, there are significant reshaping and refinement of the prefrontal cortex responsible for EFs; hence, early and mid-childhood have been postulated to be the periods that are most malleable and adaptable to environmental exposures [[4](#_ENREF_4)].

Additionally, we found that vitamin B12 was related to FC between DAN and FPN. Given that DAN is important for working memory [[38](#_ENREF_38)] and FPN is important for cognitive flexibility [[39](#_ENREF_39)], this finding is consistent with our findings using EFs cognitive tests. In general, decreasing between-network FC is expected across childhood due to functional specialization of brain networks, and a lower DAN x FPN FC has been linked to better sustained attention ability (also a component of EFs) [[40](#_ENREF_40)]. In another study examining dietary omega-3 intake (not vitamin B12), higher intake was correlated with a decrease in FC underlying the vigilant attention networks [[20](#_ENREF_20)]. Taken together, a decreasing between-network FC in children may be reflective of better EFs, suggesting a potential beneficial role of vitamin B12 in improving brain networks FC that underlie EFs. However, longitudinal measurements of FC to confirm changes in FC as well as mediation analysis linking B12-FC-EFs will be required to substantiate this finding. We did not find significant associations with the other 20 between-network FC likely because they reflect other aspects of neuro-cognitive development such as visual and motor development, emotion regulation, social behaviour and autobiographical memory which do not regulate EFs [[41](#_ENREF_41)].

While we included other cognitive subtests – NEPSY-II INS task and D-KEFS Colour-Word Interference task, which also assessed cognitive flexibility similar to WCST-64, we did not find significant associations of vitamin B12 with the former two. A possible explanation to the difference in findings could be that the NEPSY-II INS task and D-KEFS Colour-Word Interference task evaluate rule-based flexibility, which also have components of inhibition, naming automaticity, and response time. WCST-64 tests inductive flexibility, which involves adapting to change based on reasoning, and does not test a set of heterogeneous cognitive skills simultaneously unlike NEPSY-II INS and D-KEFS Colour-Word Interference tasks. Our null finding may also be plausible because the domain of inhibition (tested in NEPSY-II INS) may not be an aspect of EFs that is influenced by vitamin B12. In support of our null finding, a previous trial has shown no effect in improving the scores of NEPSY-II INS task at ages 6-9 years after vitamin B12 supplementation at ages 6-30 months [[42](#_ENREF_42)]. A lack of statistical power may also explain the null finding, as a sample size of 254 (mean of 10, standard deviation of 3 based on a US normative sample) only provided 17% power to detect a difference of 0.19 (unadjusted coefficient for NEPSY-II INS) at 5% significance level.

The strengths of our study include examining vitamin B12, FC and EFs which has not been done previously as well as administering a battery of neurocognitive tests to assess EFs components; as there is no one “pure” test specific to each EF component, including a battery could help elucidate the EFs component most influenced by vitamin B12. Several limitations were noted. Selection bias is present; subsets with EFs and FC data differed in ethnic profile, maternal education, and adherence to “healthy” dietary pattern. While this may limit generalizability of study findings to the general population, the exposure-outcome relationships should hold. Although we adjusted for known factors affecting children’s neurocognition in our models, such as maternal education, growth (BMI) and physical activity, we acknowledge that there may be other factors we have not accounted for such as family contextual factors as well as residual confounding. Additionally, we recognised that there are other micronutrients playing critical roles in development of EFs such as iron and omega-3 PUFAs which may have confounded the associations observed; as such, we have adjusted for “healthy” dietary pattern as a surrogate for nutritional status in our analysis. We also acknowledge that the cut off used to define vitamin B12 deficiency (<148 pmol/L) may not be applicable to children of this age especially when studies have found ranges of 245-790 pmol/L for 6-9-year-old; thus, vitamin B12 deficiency may still exist in our cohort. Nevertheless, including vitamin B12 as a continuous variable in our analysis allowed examination of dose-response relationships for the entire range of vitamin B12 concentrations instead of only deficiency versus sufficiency status. The temporality of the B12-FC association is unclear as data on FC was performed before assessment of vitamin B12; the sequence of assessments also precluded performing mediation analysis to confirm that the positive association of vitamin B12 and EF was through improving FC; however, there was a high correlation of vitamin B12 concentrations at age 6 and 8 years (*r* =0.72, n=72) demonstrating stability in plasma vitamin B12 concentrations over time. The timing of FC assessment also means that the association observed may reflect vitamin B12 exposure during the antenatal and early childhood period, but we did not capture FC in early-life (due to limited resources) to differentiate this effect. Lastly, as with all observational studies, our findings only suggest a possible association and not causation.

In conclusion, we found evidence of a beneficial influence of vitamin B12 on cognitive flexibility and working memory in this multi-ethnic Asian cohort of 7–11-year-old children without vitamin B12 deficiency. This suggests that higher intakes of vitamin during the middle-childhood period, even in the absence of vitamin B12 deficiency, may be important for EFs in children. Our findings, however, require confirmation in other studies using similar EFs tests such that findings are directly comparable as well as prospective, longitudinal measurements of functional brain networks before recommendations or interventions can be made.

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Authors’ contributions

JL, EL and JSL designed the research. JL performed statistical analysis and wrote the first draft. SYC contributed to pre-processing of neuroimaging data and analysis of between-network functional connectivity. JSL and EL reviewed and edited the manuscript. JL and JSL had primary responsibility for final content. APT and EL designed the protocols, trained and supervised staffs for executive functions tests and collection of neuroimaging data. FY, YSC, KMG and JGE led the GUSTO study. All authors were involved in data interpretation, critically reviewed the manuscript for intellectual content, read and approved the final manuscript.

Data sharing

Data described in the manuscript, code book, and analytic code will be made available upon request pending approval by lead investigators of the GUSTO study.

Conflicts of interest

FY, KMG and YSC have received reimbursement for speaking at conferences sponsored by companies selling nutritional products. KMG and YSC are part of an academic consortium that has received research funding from Abbott Nutrition, Nestlé and Danone. All other authors declared no conflicts of interest.

Ethics approval

The conduct of the GUSTO study was based on the guidelines in the Declaration of Helsinki and all procedures were approved by the National Healthcare Group Domain Specific Review Board (reference D/2009/021) and the SingHealth Centralised Institutional Review Board (reference 2018/2767).

Consent to participate

Informed written consent was obtained from all GUSTO mothers at recruitment as well as written assent from the children at 7 years of age.

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| --- | --- | --- | --- |
| **Table 1**. Plasma vitamin B12 concentrations of 8 years old children, by maternal and child characteristics, included in the analysis of vitamin B12 and executive functions in the Growing Up Towards healthy Outcomes cohort study (n=356a) | | | |
|  | n (%) | Vitamin B12 (pmol/L) Mean ± SD | P-valueb |
| Maternal Characteristics |  |  |  |
| Ethnicity |  |  |  |
| Chinese | 201 (56.5) | 547.34 ± 171.38a | <0.001 |
| Malay | 107 (30.1) | 500.40 ± 144.09a |
| Indian | 48 (13.5) | 441.46 ± 145.62b |
| Highest education |  |  |  |
| Secondary and below | 105 (29.5) | 473.45 ± 145.45a | <0.001 |
| Post-secondary | 137 (38.5) | 514.42 ± 147.09b |
| Degree and above | 114 (32.0) | 566.32 ± 186.74b |
| Child Characteristics |  |  |  |
| Sex |  |  |  |
| Male | 188 (52.8) | 532.49 ± 176.53 | 0.100 |
| Female | 168 (47.2) | 503.81 ± 148.01 |
| BMI |  |  |  |
| Normal (≤1 SD) | 263 (73.9) | 535.64 ± 173.66 | 0.001 |
| Overweight and Obese (>1 SD) | 93 (26.1) | 471.77 ± 122.36 |
| Daily MVPA |  |  |  |
| < 60 min/day | 126 (35.4) | 514.83 ± 155.58 | 0.726 |
| ≥ 60 min/day | 230 (64.6) | 521.22 ± 168.86 |
| “Healthy” dietary patternc,d |  |  |  |
| High adherence (z-score>0) | 109 (36.7) | 547.9 ± 186.1 | 0.024 |
| Low adherence (z-score≤0) | 188 (63.3) | 502.6 ± 153.4 |
| Intake of supplements containing vitamin B12d |  |  |  |
| Yes | 18 (10.8) | 518.60 ± 196.56 | 0.890 |
| No | 148 (89.2) | 512.78 ± 165.08 |
| BMI, body mass index; MVPA, moderate-to-vigorous physical activity. | | | |
| aChildren with plasma vitamin B12 concentrations, completed executive function tests for at least one visit, and without missing covariates | | | |
| b*P*-values were from t-test or one-way ANOVA with Bonferroni post-hoc test (a,b,c groups with different superscript letter differ) | | | |
| c”Healthy” dietary pattern was characterised by higher intakes of fruit, vegetables, nuts and legumes; fish, seafood, poultry and meat prepared using healthier cooking methods.  dMissing data: n=59 “Healthy” dietary pattern, n=190 intake of supplements containing vitamin B12 | | | |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Table 2.** Associations of plasma vitamin B12 concentrationsa at 8 years with executive functions at 8.5 and 10.5 years in the Growing Up in Singapore Towards healthy Outcomes study | | | | |
|  | **Unadjusted** | | **Adjusted**b | |
| **Tests of Executive Functions** | **β (95% CI)** | **P** | **β (95% CI)** | **P** |
| **Year 8.5** |  |  |  |  |
| NEPSY-II (n=254) |  |  |  |  |
| INI combined scaled score | 0.06 (-0.35, 0.46) | 0.787 | -0.01 (-0.43, 0.41) | 0.964 |
| INS combined scaled score | 0.19 (-0.32, 0.71) | 0.459 | -0.09 (-0.62, 0.44) | 0.743 |
| WI-Repetition scaled score | 0.32 (0.04, 0.61) | 0.026 | 0.19 (-0.11, 0.50) | 0.210 |
| WI-Recall scaled score | 0.19 (-0.24, 0.62) | 0.377 | 0.06 (-0.39, 0.51) | 0.797 |
| WCST-64 (n=254) |  |  |  |  |
| Perseverative responses standard score | 3.70 (0.60, 6.80) | 0.019 | 3.23 (0.03, 6.43) | 0.048 |
| Perseverative errors standard score | 3.28 (0.26, 6.30) | 0.033 | 2.80 (-0.34, 5.94) | 0.080 |
| Failure-to-maintain setc | 0.81 (0.62, 1.06) | 0.121 | 0.80 (0.60, 1.07) | 0.131 |
| **Year 10.5** |  |  |  |  |
| D-KEFS (n=304) |  |  |  |  |
| Inhibition-Switching scaled score | -0.09 (-0.35, 0.16) | 0.456 | -0.21 (-0.48, 0.07) | 0.136 |
| Colour-Naming scaled score | 0.12 (-0.19, 0.43) | 0.436 | -0.06 (-0.39, 0.27) | 0.718 |
| Inhibition scaled score | 0.05 (-0.19, 0.28) | 0.707 | -0.07 (-0.33, 0.18) | 0.581 |
| WISC-V (n=304) |  |  |  |  |
| Letter-Number-Sequencing scaled score | 0.62 (0.26, 0.98) | 0.001 | 0.42 (0.04, 0.80) | 0.033 |

INI, Inhibition Task; INS, Inhibition-Switching Task; NEPSY-II, Developmental Neuropsychological Assessment Second Edition; WCST-64, Wisconsin Card Sorting Test 64-Card Version; D-KEFS, Delis-Kaplan Executive Function System; PSLE, Primary School Leaving Examination; WI, Word Interference Task; WISC-V, Wechsler Intelligence Scale for Children Fifth Edition.

aPlasma vitamin B12 concentrations were converted to per SD increment.

bModels were adjusted for maternal ethnicity, maternal education, child sex, child year 8 BMI z-score, child year 8 moderate-to-vigorous physical activity duration, and “healthy” dietary pattern.

cDichotomous variable; Odds ratio was reported instead.