**TITLE PAGE**

**Title:** Higher maternal plasma β-cryptoxanthin concentration is associated with better cognitive and motor development in offspring at 2 years of age.

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**ABSTRACT**

**Purpose:** Current literature on the roles of α-, β-carotene and β-cryptoxanthin in neurocognitive function has largely focused on preventing cognitive decline in older people, and less on neuro-development in children. We examined the relations of maternal plasma carotenoids concentrations with offspring cognitive development up to age 4.5 years in the Growing Up in Singapore Towards healthy Outcomes mother-offspring cohort study.

**Methods:** Maternal plasma α-, β-carotene and β-cryptoxanthin concentrations at delivery were determined by ultra-performance liquid chromatography. Children’s cognition was assessed at ages 2 (Bayley Scales of Infant and Toddler Development) and 4.5 (Kaufman Brief Intelligence Test) years. Associations were examined in 419 mother-offspring pairs using linear regressions adjusting for key confounders.

**Results:** Median and interquartile range of maternal plasma concentrations (mg/L) were: α-carotene 0.052 (0.032, 0.081), β-carotene 0.189 (0.134, 0.286), and β-cryptoxanthin 0.199 (0.123, 0.304). In 2 years old children, higher maternal carotenoids [per standard deviation (SD) log-concentration] were positively associated with neurocognitive functions: β-cryptoxanthin with higher scores in cognitive [β=0.18, (0.08, 0.28) SD], receptive language [β=0.17 (0.07, 0.27) SD], fine motor [β=0.16 (0.05, 0.26) SD], and gross motor [β=0.16 (0.06, 0.27) SD] scales; β-carotene with higher cognitive score [β=0.17 (0.05, 0.29) SD]. No significant associations were observed with neurocognitive functions at age 4.5 years.

**Conclusion:** Our study provides novel data suggesting a potential role of prenatal carotenoids, particularly β-cryptoxanthin, on early offspring cognitive and motor development. Whether the prenatal influences sustain beyond early childhood requires further investigation in longer term studies.

**Keywords:** carotenoids, pregnancy, cognition, motor, children

**TEXT**

**Introduction**

Optimizing brain development and cognitive function in early ages including the *in utero* period have important economic and public health implications. Children who develop strong cognitive skills in younger ages perform better in school [1], subsequently contributing to attainment of higher education [2] – a key determinant of better health behaviors and greater contribution to economic growth [3,4]. Thus, strategies aiming to optimize early cognitive development through improving maternal nutrition during pregnancy are therefore warranted.

Carotenoids are proposed to be important for cognitive health [5,6]. Growing evidence supports the role of lutein and zeaxanthin in early neurocognitive development [7,8]. This includes detection of high lutein and zeaxanthin concentrations across a number of infant brain regions [9], as well as associations of a higher macular lutein and zeaxanthin density in children or higher lutein concentrations in breastmilk, with better neurocognitive functioning in infants/children [10-12].

Separately, pro-vitamin A carotenoids such as α-carotene, β-carotene and β-cryptoxanthin may also be important for neurocognitive development due to their ability to convert to vitamin A [13]. This is supported by evidence showing higher maternal plasma vitamin A concentrations (above median value) to be associated with better offspring cognitive performance [14-16]. The role of pro-vitamin-A carotenoids on cognition thus differs to that of lutein and zeaxanthin which do not have vitamin A activity [17,18]. In addition, their role in early neurocognitive development could be attributable to their own biological activity [13]. These carotenoids have anti-oxidative properties which can protect the developing brain from increased oxidative and inflammatory stress [19], and their involvement in activation of gene expressions associated with neuronal gap junctions communication [20] may be important for fetal brain development.

The rationale to look into the role of pro-vitamin-A carotenoids on neurocognitive outcomes is further substantiated by evidence showing not only the presence of lutein and zeaxanthin, but also cryptoxanthin and β-carotene in brain regions of infants associated with memory, executive function, vision, and hearing [9]. However, little is known about the influences of pro-vitamin-A carotenoids in early neurocognitive functions, with only one study showing higher β-carotene in breastmilk to be associated with better infant motor development [21].

Currently, majority of studies examined pro-vitamin-A carotenoids with cognitive decline amongst the elderly. These studies showed higher β-cryptoxanthin concentrations to associate with higher intelligence [22], or higher β-carotene concentrations to associate with lower cognitive decline [22,23] as well as β-carotene supplementation to improve cognitive performance [24]. Furthermore, those with Alzheimer’s disease were found to have lower concentrations of α-carotene and β-cryptoxanthin [25].

Although there is evidence to suggest accelerated accumulation of carotenoids in fetal brain during the last trimester[9], no studies have examined whether exposures to varying concentrations of pro-vitamin-A carotenoids *in utero* influences neurocognitive performance in early childhood. While studies demonstrated higher fruit and vegetable intake in mothers during pregnancy to associate with better cognitive performance in children [26,27], the association may not be attributable to the effects of pro-vitamin-A carotenoids. Therefore, this study aimed to examine the relations of maternal α-carotene, β-carotene and β-cryptoxanthin concentrations during pregnancy with cognitive development of the offspring at ages 2 and 4.5 years.

**Subjects and Methods**

Study population

Data for the present analysis were from the Growing Up in Singapore Towards healthy Outcomes (GUSTO) study – a prospective mother-offspring cohort in Singapore [28]. Detailed descriptions of the GUSTO study have been published [28]. In brief, pregnant women (≥18 years) in their first trimester (<14 weeks) were recruited from the National University Hospital (NUH) and KK Women’s and Children’s Hospital (KKH) between June 2009 and September 2010. To be eligible for the study, the pregnant women had to: 1) be Singapore citizens or permanent residents of Chinese, Malay or Indian ethnicity with homogenous parental ethnic background; 2) have intention to deliver in the two hospitals and to reside in Singapore for the next five years; and 3) consent to donate birth tissues at delivery. Women receiving chemotherapy, psychotropic drugs or diagnosed with type-1 diabetes were not eligible to participate. The GUSTO study received ethics approval from the Institutional Review Board of NUH and KKH, and all procedures were conducted according to the guidelines laid down in the Declaration of Helsinki. Written informed consent was obtained from all participants at each study visit. The GUSTO study was registered at www.clinicaltrials.gov as NCT01174875.

Maternal plasma pro-vitamin-A carotenoid concentrations

Non-fasting bloods samples were obtained from mothers upon arrival at the hospitals for delivery by standard venipuncture technique. The blood samples were collected in EDTA tubes, processed within 4 hours (centrifuged at 1600g for 10 minutes at 4oC) to obtain the plasma, stored at -80oC and thawed prior to analysis. Ultra High Performance Liquid Chromatography with Photo-Diode Array detection was used to determine plasma concentrations of α-carotene, β-carotene and β-cryptoxanthin. The precision of the method was examined using pooled and spiked plasma samples and the results were similar as published earlier, with the relative standard deviations (n = 6) of within day assays and between-day assays generally <10% and <15%, respectively [29]. A detailed description of the methodology is provided in the **Online Resource 1: Supplementary Methods**.

Cognitive outcomes in young children

The Bayley Scales of Infant and Toddler Development, 3rd edition (BSID-III) [30], was administered to children at age 2 years (± 1 month), in their homes when they were alert and awake. The test is usually performed in a quiet space (e.g. television off, away from traffic/construction work) and when there were fewer people at home to minimize distractions. The BSID-III is a standardized test that assesses development of children 1-42 months of age in the following domains: cognitive, receptive and expressive language, and fine and gross motor [30].

At age 4.5 years (± 1 month), the child’s cognitive functioning was assessed using the Kaufman Brief Intelligence Test, 2nd edition (KBIT-2) in KKH clinic. The KBIT-2 is an assessment of verbal and non-verbal intelligence for individuals aged 4-90 years [31]. The verbal scale assesses knowledge of words and their meanings, whereas the non-verbal scale assesses the ability to solve new problems by perceiving relationships and completing analogies.

English is the main language in Singapore, thus majority of the assessments were conducted in English (n=229/361 for BSID-III and n=252/322 for KBIT-2). For children with very limited exposure to English, the BSID-III and KBIT-2 were informally adapted into Chinese and Malay or Tamil equivalents. Only a total of 67, 44 and 21 BSID-III were administered in Chinese, Malay and Tamil languages respectively, while a total of 50 and 20 KBIT-2 were administered in Chinese or Malay languages respectively. Tamil dominant speaking children (n=10) were administered the English version of KBIT-2, as all of them were bilingual in Tamil and English at age 4.5 years. The language adaptations were according to common practice by Singapore’s clinical psychologists, and scored as follows: a correct score is given for responses in a dominant language, a mix of dominant or non-dominant languages, or entirely in a non-dominant language [32]. Furthermore, administration and scoring was performed by research coordinators of the same ethnicity to the child. Previous study has shown minimal influence of cultural or language bias on test performance [32].

The research coordinators have been trained in accordance to the manual by the head psychologist from KKH (for BSID-III) or GUSTO investigators with a background in psychiatry (for KBIT-2) [33,34], and were also experienced in administering a range of neurocognitive assessments in GUSTO [33,34]. Actual administration only began after the trainers deemed them as adequately trained (i.e. following closely to protocols). Cleaning of test scores followed strict protocols set by the trainers.

The BSID-III and KBIT-2 were performed in a subset of children. Priority in scheduling to complete the BSID-III and KBIT-2 (test batteries measuring global cognition) was given to those who had participated in neurodevelopmental assessments examining specific cognitive domains (e.g. memory, attention and executive function) prior to 2 or 4.5 years [33,34]. The principal reasons for non-participation were busy schedules, lack of interest, inability to contact the participants, or drop out from the GUSTO study. None of the children in GUSTO were reported to have any illnesses or in poor health condition. Raw test scores were used as age-specific norms were not available for our population.

Covariates

Covariates were selected based on previous literature [14,16,35]. Information on maternal age, ethnicity, highest education attained, and monthly household income were collected during recruitment visit (<14 weeks’ gestation). At the clinic visit at 26-28 weeks’ gestation, antenatal mental well-being was assessed with the Edinburgh Postnatal Depression Scale (EPDS) [36] and the State-Trait Anxiety Inventory (STAI) [37]. Maternal pre-pregnancy BMI was calculated as weight divided by height squared (kg/m2), based on self-reported pre-pregnancy weight and height measured with a stadiometer (SECA model 213) at 26-28 weeks’ gestation. Maternal dietary intake as well as use of any dietary supplements during pregnancy was assessed also at 26–28 weeks’ gestation using a single 24-hour recall by trained clinical staff. Overall diet quality was assessed with the Healthy Eating Index for pregnant women in Singapore (HEI-SGP) [38]. The use of dietary supplements containing any amounts of preformed vitamin A (retinol or retinyl esters) and/or pro-vitamin-A carotenoids were considered. Maternal parity and delivery complications (as a result of pre-eclampsia and gestational diabetes); infant gestational age (determined by a dating ultrasound scan in the first trimester), birth weight (measured by midwives within 72 hours of delivery), and Apgar scores were retrieved from hospital delivery records. Small-for-Gestational Age (SGA) was defined as birth weight <10th percentile for gestational age and Large-for-Gestational Age (LGA) as birth weight >90th percentile for gestational age based on the centile charts generated previously for GUSTO neonates [39].

Statistical analysis

Maternal plasma carotenoid concentrations were summarized according to maternal and child characteristics for the 419 mother-offspring pairs with data for at least one cognitive test. Differences in concentrations between groups were compared using non-parametric analyses – Mann-Whitney U and Kruskal-Wallis tests, as data were not normally distributed. Bonferroni post hoc analysis was performed to identify groups which differed if the Kruskal Wallis test was significant. For these analyses, *P*<0.05 was considered statistically significant.

The values for individual maternal plasma carotenoids were log-transformed, then converted to SD scores for easier interpretation. The BSID-III and KBIT-2 raw scores were also converted to SD scores to facilitate comparison across the cognitive tests and domain scales.

Associations of each maternal plasma carotenoid with each BSID-III or KBIT-2 scales in the children were examined using linear regressions. Several statistical models were employed: Model 1 – basic model with adjustment for child’s exact age at cognitive testing; Model 2 – additional adjustment for maternal age, ethnicity, education, household income, maternal pre-pregnancy BMI, parity, diet quality and antenatal depressive symptoms and anxiety levels (potential confounders). If attenuation was observed after adjusting for confounders, stepwise regression was performed to identify the main attenuating factor (i.e. the covariate that resulted in greatest attenuation of effect estimates). The selection of covariates for adjustment in statistical models were based on a theoretical model in the form of a directed acyclic graph constructed *a priori* (**Online Resource 1: Supplementary Fig. 1**). We did not adjust for: 1) maternal fruit and vegetables intakes as plasma carotenoids concentrations acted as proxies to dietary carotenoids, and; 2) infant gestational age, birth weight and breastfeeding as these factors may be on the causal pathway between maternal carotenoids concentrations and offspring cognitive development, and 3) maternal postpartum factors or early childhood factors (e.g. child’s preschool attendance and early infancy/childhood diet) as these are not confounders. For these analyses, *P*<0.01 was considered statistically significant to account for multiple testing.

Missing data for covariates were imputed using multiple imputation with chained equations (20 times) for the following confounding variables: n=3 maternal education, n=21 household income, n=7 EPDS, n=21 STAI, n=40 maternal pre-pregnancy BMI, and n=32 diet quality. All analyses were performed using Stata version 14 (StataCorp LP, College Station, TX, USA).

**Results**

A total of 1247 pregnant women participated at baseline, to whom 1176 babies were born. The present analysis included all GUSTO mothers who provided sufficient blood for plasma carotenoids assays (n=701), and whose offspring completed cognitive assessments at ages 2 (n=361) and 4.5 (n=332) years (**Fig. 1**). A total of 419 children completed at least one cognitive assessment, with 274 of them completing cognitive assessments at both time-points. The characteristics of the 757 mother-offspring pairs without data on maternal carotenoids and/or their offspring cognitive assessments at 2 and 4.5 years were comparable to those included in the analysis (**Online Resource 1: Supplementary Table 1**).

Characteristics of mother-offspring pairs

Pregnant women of Chinese ethnicity tended to have the highest concentrations of α-and β-carotene, while women of Malay and Indian ethnicity were more likely to have the lowest concentrations of α-carotene and β-cryptoxanthin respectively (**Table 1**). Those with lower concentrations of α- and β-carotene tended to have attained a lower educational level, and were more likely to be obese and to have antenatal depressive symptoms and anxiety. Additionally, women with lower concentrations of α-carotene tended to be younger and those with lower concentrations of β-cryptoxanthin were more likely to have probable anxiety. Those from households with the highest monthly income tended to have the highest concentrations of all three carotenoids. Not surprising, women with better diet quality, and higher intakes of fruit and vegetables have higher concentrations of α- and β-carotene, but significant difference in β-cryptoxanthin concentrations was only observed for fruit intake (higher concentration with higher intake) and not according to vegetables intake and diet quality. No significant differences in plasma carotenoids concentrations were observed comparing children of mothers who consume or did not consumed supplements containing vitamin A, <37 or ≥37 weeks’ gestation, different status of birth-weight-for-gestational age, and low or normal birth weight. All included children had an Apgar score ≥9, and their mothers did not experience delivery complications as a result of pre-eclampsia or gestational diabetes.

Maternal carotenoids and offspring BSID-III outcomes at age 2 years

The associations of maternal plasma carotenoid concentrations with scores for each BSID-III scale in children at 2 years are presented in **Table 2**.

Higher maternal β-carotene concentrations (per SD increment in log-transformed concentrations) was associated with a 0.17 SD (95% CI: 0.05, 0.29) higher cognitive scores, after adjusting for key confounders (Model 2). Associations between higher maternal β-carotene concentrations and higher scores in receptive and expressive language, and fine and gross motor were attenuated after adjusting for confounders (Model 2). Stepwise adjustment revealed maternal education and/or household income as strong attenuating factor(s) (data not shown).

Higher maternal β-cryptoxanthin (per SD increment in log-transformed concentrations) was associated with 0.18 SD (95% CI: 0.08, 0.28 SD) higher cognitive scores, 0.17 SD (95% CI: 0.07, 0.27) higher receptive language scores, 0.16 SD (95% CI: 0.05, 0.26) higher fine motor scores, and 0.16 SD (95% CI: 0.06, 0.27) higher gross motor scores after adjustment for potential confounders (Model 2).

No significant associations were observed for maternal α-carotene concentrations in relation to BSID-III subscales in children.

Maternal carotenoids and KBIT-2 outcomes in children at 4.5 years

The associations of maternal plasma carotenoid concentrations with scores of each KBIT-2 scale in children at 4.5 years are presented in **Table 3**. Higher maternal α- and β-carotene concentrations were associated with higher scores in verbal score, but these were attenuated after adjustment for confounders (Model 2). Maternal education was a strong attenuating factor when stepwise adjustment was performed (data not shown). There was a positive trend between maternal β-cryptoxanthin and children’s verbal score but this was also attenuated after adjusting for confounders.

**Discussion**

In this prospective mother-offspring cohort study, we observed associations of higher maternal plasma β-cryptoxanthin concentrations with better offspring cognitive, receptive language, fine and gross motor development at age 2 years. Additionally, higher maternal plasma β-carotene concentrations were associated with better offspring cognitive development at 2 years of age. However, we did not observe long term relationships of maternal β-cryptoxanthin and β-carotene with offspring cognition at 4.5 years of age. There were no significant relationships between maternal plasma α-carotene and offspring cognitive development.

The positive associations observed for β-cryptoxanthin and β-carotene were in line with earlier study demonstrating presence of cryptoxanthins and β-carotene in several brain regions of infants associated with ‘higher-level’ cognitive functions [9]. As detailed in Introduction, their antioxidant activity [19], their role in promoting formation of gap junctions between cells [20], as well as their ability to convert to vitamin A (also important for neurodevelopment), ensures optimal growth and development of the brain and central nervous system during the *in utero* period, which may subsequently impact on cognitive development in the long term [40]. The associations were likely not acting through improved pregnancy or birth outcomes, which have been previously described as mechanisms linking improved maternal nutrition to offspring cognitive performance [41], as we found no correlations of maternal pro-vitamin A carotenoids concentration with gestational age and birth weight; all children had normal Apgar score and their mothers did not experience delivery complications due to pre-eclampsia and gestational diabetes.

Higher maternal β-cryptoxanthin concentrations were most consistently associated with better neurocognitive development in children at 2 years, as we observed significant associations with all BSID-III scales. Furthermore, the associations remained significant after controlling for important contributors to infant/child’s cognition such as maternal education [42] and maternal mental health [43]. These findings are akin to one study showing higher serum β-cryptoxanthin concentrations correlated with better concept formation in people aged >80 years [22], and another reporting higher plasma β-cryptoxanthin concentrations in healthy subjects versus those with Alzheimer’s disease [25].

A possible explanation to why we observed most consistent association between maternal β-cryptoxanthin and offspring neurocognitive development could be due to a greater antioxidant activity and higher bioaccessibility and bioavailability from its food sources, compared to other pro-vitamin A carotenoids [44]. Furthermore, β-cryptoxanthin is more abundant and has greater bioavailability in tropical fruits [45,46], suggesting that the role of β-cryptoxanthin in cognition may be more prominent in populations where the consumption of tropical fruits is high. This is supported by our observation in GUSTO that differences in fruit intake contributed to the differences in β-cryptoxanthin concentrations, rather than differences in vegetables intake. Our findings thus have important practical implications for populations from tropical countries with differing fruits and vegetables consumption patterns to non-tropical countries. However, owing to the lack of studies in pregnant populations in general, comparison of β-cryptoxanthin concentrations/findings observed in our cohort to others was not possible.

Contrary to the study showing higher β-carotene concentrations in breastmilk to be associated with better infant motor development [21], we only found higher maternal β-carotene concentrations during pregnancy to be associated with their offspring performing better in the BSID-III cognitive scale but not for fine and gross motor scales. One possible explanation could be the timing of the cognitive assessments – the previous study assessed neurocognitive functions at 6 months of age whilst our assessments were conducted at 2 years of age. As different brain regions have different neurodevelopmental trajectories that span and peak at different times [47]; whether the influences of prenatal β-carotene on cognitive functions can be detected may depend on the maturation stage of the brain and the timing of assessment.

This may also explain why we observed no significant associations with verbal and non-verbal intelligence at 4.5 years. The prefrontal cortex (involved in ‘higher-level’ cognitive control) develops in growth spurts during the first two years of life, then again at ages 7-9 years and 15 years. Thus, to observe longer term effects of maternal pro-vitamin-A carotenoids on cognitive development throughout childhood and adolescence period may require assessments during those specific time points. Alternatively, the cognitive testing batteries used at ages 2 (psychomotor and mental development) and 4.5 years (global intelligence) may differ in the cognitive aspects they measure, resulting in inconsistent findings. Longer term studies using comparable cognitive assessment instruments across follow-up time points up until the adolescence period are needed to determine whether the associations sustain beyond age 2 years.

We also noted that maternal education was a strong attenuating factor for the associations between maternal pro-vitamin A carotenoids and offspring intelligence at 4.5 years. This finding indicates that the offspring environmental influences (e.g. parental education and socio-economic status) and/or heritability of intelligence (using maternal education as a proxy) [48,49] may be stronger determinants of offspring intelligence later in life compared to the effects of prenatal nutrition.

Our study is novel in several ways. It is the first study to examine associations between maternal pro-vitamin A carotenoids during pregnancy and neurocognitive development in the offspring, while most studies have focused on the elderly or postnatal carotenoids exposures. We found that higher concentrations of maternal β-cryptoxanthin and β-carotene may be important for offspring early cognitive development. Furthermore, our study is the first to demonstrate a potential role of β-cryptoxanthin in cognitive functions – a carotenoid less known for its health benefits, while the majority of literature concentrated on β-carotene. The time point (late pregnancy) selected to measure maternal carotenoids may have been more sensitive to brain development (e.g. myelination and hippocampus growth increases from 32 weeks’ gestation [50]), which may explain why we observed more significant associations.

Several limitations are noted. Due to limited manpower and resources, the BSID-III and KBIT-2 were performed in a subset of children which may have led to selection bias; however, comparison of participant characteristics showed that non-participants were similar in profile for a number of key determinants. We recognized that our analyses have not accounted for dietary intakes; however, plasma carotenoids strongly reflect intakes of fruit and vegetables [51] and adjusting for them may remove the dietary effects we aimed to observe. We have adjusted for overall diet quality instead. A number of confounders were not measured and accounted for such as maternal intelligence (but maternal education was used as a proxy [52]) and home environment which may have biased the associations observed. The use of cognitive assessments developed in the United States of America may introduce cultural and language bias, but much effort was undertaken to minimize these biases by: 1) accepting all correct responses regardless of whether participants were given the dominant or non-dominant languages or a mix of both, 2) by assigning research coordinators of same ethnic background to the child to administer the cognitive assessments [32].

In conclusion, higher maternal plasma β-cryptoxanthin and β-carotene concentrations during pregnancy were associated with better neurocognitive development in offspring at age 2 years. Majority of previous pro-vitamin-A carotenoids and cognition literature has focused on preventing or delaying cognitive decline or Alzheimer’s disease in the elderly, which differ to the neurocognitive developmental pathways that we have studied. Our study provides novel data suggesting potential *in utero* influences of pro-vitamin-A carotenoids, such as β-cryptoxanthin and β-carotene, in early neurocognitive development. The present study alluded to the importance of consuming orange- and yellow-coloured fruits which are high in β-cryptoxanthin during pregnancy, and adds new support to current recommendations encouraging consumption of sufficient quantity and variety of fruits and vegetables, although further studies are needed to confirm the effects of these maternal carotenoids on offspring cognitive development.

**Ethical Standards**

The GUSTO study received ethics approval from the Institutional Review Board of NUH and KKH, and all procedures were conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all participants at each study visit.

**Conflict of Interest**

FY, KMG, PDG and YSC have received reimbursement for speaking at conferences sponsored by companies selling nutritional products. KMG, PDG and YSC are part of an academic consortium that has received research funding from Abbott Nutrition, Nestlé and Danone. All other authors (JSL, SC, BLL, LPS, KHT, CNO, MJM, AR-G, BFPB, MFFC) declared that they have no competing interests.

**Authors contributions**

JSL, CNO, BFPB, AR-G and MFFC designed the research. JSL performed statistical analysis and wrote the manuscript. MFFC, BFPB and AR-G reviewed and edited the manuscript. JSL and MFFC had primary responsibility for final content. BLL and CNO designed the methodology and provided essential reagents for plasma carotenoids assay; SC, MJM, AR-G and BFPB designed the protocol and provided essential materials for the cognitive assessments. LPS, FY, KHT, PDG, YSC and KMG led the GUSTO study. All authors critically reviewed the manuscript for scientific content, read and approved the final manuscript.

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| **Table 1**: Concentrations of maternal plasma carotenoids according to maternal and child characteristics in the Growing Up in Singapore Towards healthy Outcomes cohorta |
|  | *N*b | α-carotene, mg/L | *P*c | β-carotene, mg/L | *P* | β-cryptoxanthin, mg/L | *P* |
| All mother-child pairs | 419 | 0.052 (0.032, 0.081) |  | 0.189 (0.134, 0.286) |  | 0.199 (0.123, 0.304) |  |
|  |  |  |  |  |  |  |  |
| **Maternal Characteristics** |  |  |  |  |  |  |  |
| Age, year |  |  |  |  |  |  |  |
| <35 | 311 | 0.048 (0.031, 0.080) | 0.013\* | 0.176 (0.130, 0.284) | 0.052 | 0.190 (0.119, 0.292) | 0.093 |
| ≥35 | 108 | 0.058 (0.041, 0.085) |  | 0.206 (0.146, 0.309) |  | 0.213 (0.158, 0.320) |  |
| Ethnicity |  |  |  |  |  |  |  |
| Chinese | 241 | 0.061 (0.042, 0.090) | <0.001\* | 0.230 (0.158, 0.333) | <0.001\* | 0.211 (0.121, 0.344)1 | <0.001\* |
| Malay | 99 | 0.036 (0.026, 0.052) |  | 0.153 (0.111, 0.200)1 |  | 0.207 (0.152, 0.292)1 |  |
| Indian | 79 | 0.044 (0.029, 0.073) |  | 0.144 (0.105, 0.218)1 |  | 0.150(0.102, 0.225) |  |
| Highest education |  |  |  |  |  |  |  |
| ≤Secondary | 122 | 0.043 (0.029, 0.069)1 | <0.001\* | 0.172 (0.125, 0.244)1 | <0.001\* | 0.200 (0.128, 0.280) | 0.602 |
| Post-secondary | 145 | 0.048 (0.030, 0.069)1 |  | 0.185 (0.127, 0.291)1 |  | 0.189 (0.127, 0.292) |  |
| University  | 149 | 0.067 (0.044, 0.095) |  | 0.219 (0.146, 0.329) |  | 0.211 (0.119, 0.329) |  |
| Monthly Household income, SGD |  |  |  |  |  |  |  |
| <2000 | 60 | 0.038 (0.027, 0.055) | 0.001\* | 0.167 (0.110, 0.226)1 | 0.001\* | 0.184 (0.128, 0.275)1 | 0.004\* |
| 2000-5999 | 218 | 0.048 (0.031, 0.070) |  | 0.175 (0.124, 0.254)1 |  | 0.194 (0.127, 0.294)1 |  |
| ≥6000 | 120 | 0.067 (0.044, 0.100) |  | 0.247 (0.164, 0.363) |  | 0.240 (0.142, 0.375) |  |
| Pre-pregnancy BMI, kg/m2 |  |  |  |  |  |  |  |
| Under- (<18.5) | 39 | 0.052 (0.032, 0.076)1,2 | <0.001\* | 0.203 (0.149, 0.289)1 | <0.001\* | 0.196 (0.129, 0.275) | 0.109 |
| Normal (18.5-22.9) | 177 | 0.063 (0.039, 0.099)1 |  | 0.225 (0.157, 0.333)1 |  | 0.219 (0.122, 0.338) |  |
| Over- (23-27.4) | 90 | 0.049 (0.031, 0.074)2 |  | 0.164 (0.120, 0.256)1 |  | 0.198 (0.128, 0.293) |  |
| Obese (≥27.5) | 73 | 0.035 (0.026, 0.054) |  | 0.138 (0.101, 0.186) |  | 0.170 (0.114, 0.249) |  |
| Antenatal EPDS score |  |  |  |  |  |  |  |
| Normal (<15) | 373 | 0.053 (0.034, 0.083) | 0.016\* | 0.195 (0.137, 0.298) | 0.036\* | 0.201 (0.125, 0.305) | 0.192 |
| Probable depressive symptoms (≥15) | 39 | 0.035 (0.024, 0.071) |  | 0.160 (0.110, 0.234) |  | 0.158 (0.109, 0.294) |  |
| Antenatal STAI state-score |  |  |  |  |  |  |  |
| Normal (<40) | 286 | 0.054 (0.034, 0.084) | 0.001\* | 0.197 (0.137, 0.310) | 0.009\* | 0.207 (0.125, 0.325) | 0.014\* |
| Probable anxiety (≥40) | 112 | 0.044 (0.029, 0.068) |  | 0.172 (0.117, 0.238) |  | 0.169 (0.111, 0.252) |  |
| Parity |  |  |  |  |  |  |  |
| Nulliparous | 183 | 0.055 (0.034, 0.085) | 0.322 | 0.194 (0.137, 0.311) | 0.218 | 0.201 (0.127, 0.332) | 0.337 |
| Primiparous | 140 | 0.048 (0.030, 0.080) |  | 0.180 (0.124, 0.267) |  | 0.193 (0.113, 0.285) |  |
| Multiparous | 96 | 0.052 (0.032, 0.075) |  | 0.195 (0.139, 0.250) |  | 0.201 (0.146, 0.292) |  |
| Diet quality (HEI-SGP) |  |  |  |  |  |  |  |
| <75th percentile (<62.4) | 291 | 0.047 (0.029, 0.072) | <0.001\* | 0.174 (0.125, 0.256) | 0.003\* | 0.186 (0.117, 0.291) | 0.050 |
| ≥ 75th percentile (≥62.4) | 96 | 0.068 (0.043, 0.097) |  | 0.212 (0.146, 0.316) |  | 0.222 (0.124, 0.338) |  |
| Fruit intake |  |  |  |  |  |  |  |
| <75th percentile (<151g) | 314 | 0.046 (0.030, 0.077) | <0.001\* | 0.173 (0.121, 0.276) | <0.001\* | 0.182 (0.114, 0.279) | 0.001\* |
| ≥ 75th percentile (≥151g) | 100 | 0.064 (0.048, 0.085) |  | 0.233 (0.175, 0.309) |  | 0.245 (0.173, 0.352) |  |
| Vegetables intake |  |  |  |  |  |  |  |
| <75th percentile (<173g) | 314 | 0.048 (0.030, 0.076) | <0.001\* | 0.181 (0.130, 0.266) | 0.027\* | 0.198 (0.119, 0.291) | 0.130 |
| ≥ 75th percentile (≥173g) | 100 | 0.063 (0.043, 0.095) |  | 0.229 (0.149, 0.325) |  | 0.213 (0.135, 0.346) |  |
| Supplements containing Vitamin A |  |  |  |  |  |  |  |
| Yes | 294 | 0.050 (0.031, 0.078) | 0.514 | 0.185 (0.134, 0.276) | 0.872 | 0.199 (0.121, 0.297) | 0.831 |
| No | 98 | 0.055 (0.031, 0.084) |  | 0.180 (0.127, 0.284) |  | 0.196 (0.123, 0.279) |  |
|  |  |  |  |  |  |  |  |
| **Child Characteristics** |  |  |  |  |  |  |  |
| Gestational Age |  |  |  |  |  |  |  |
| <37 weeks | 29 | 0.048 (0.031, 0.075) | 0.721 | 0.185 (0.137, 0.253) | 0.715 | 0.202 (0.129, 0.253) | 0.651 |
| ≥37 weeks | 390 | 0.052 (0.032, 0.081) |  | 0.192 (0.134, 0.289) |  | 0.199 (0.123, 0.308) |  |
| Birth Weight for Gestational Age |  |  |  |  |  |  |  |
| SGA | 48 | 0.056 (0.040, 0.084) | 0.207 | 0.210 (0.144, 0.313) | 0.445 | 0.188 (0.106, 0.264) | 0.504 |
| AGA | 329 | 0.050 (0.031, 0.081) |  | 0.187 (0.131, 0.284) |  | 0.200 (0.126, 0.306) |  |
| LGA | 42 | 0.055 (0.028, 0.071) |  | 0.194 (0.137, 0.316) |  | 0.209 (0.121, 0.343) |  |
| Low Birth Weight |  |  |  |  |  |  |  |
| < 2.5kg | 30 | 0.052 (0.032, 0.080) | 0.852 | 0.196 (0.147, 0.302) | 0.728 | 0.199 (0.119, 0.233) | 0.249 |
| ≥ 2.5kg | 389 | 0.052 (0.032, 0.081) |  | 0.189 (0.133, 0.286) |  | 0.200 (0.125, 0.307) |  |

a Values are presented as median (IQR) unless otherwise specified.BMI, body mass index; EPDS, Edinburgh Postnatal Depression Scale; HEI-SGP, Healthy Eating Index for Pregnant women in Singapore; STAI, State-Trait Anxiety Inventory; SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age.

b Missing data: n=3 maternal education, n=21 monthly household income, n=40 maternal pre-pregnancy BMI, n=7 EPDS, n=32 HEI-SGP, n=21 STAI, n=5 fruit intake, n=5 vegetables intake, n=27 Vitamin A supplements.

c *P*-values (\**P*<0.05) were obtained from Mann-Whitney U and Kruskal-Wallis tests with Bonferroni post hoc analysis. 1, 2 groups with the same superscript number in a column indicate no significant difference.

**Table 2:** Associations of maternal plasma carotenoids concentrations with scores of Bayley Scale of Infant and Toddler Development-III at 2 years of age in the Growing Up in Singapore Towards healthy Outcomes study (n=361)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Cognitive | Receptive language | Expressive language | Fine motor | Gross motor |
|  | β (95% CI)a | *P* | R2 | β (95% CI) | *P* | R2 | β (95% CI) | *P* | R2 | β (95% CI) | *P* | R2 | β (95% CI) | *P* | R2 |
| α-carotene |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Model 1b | 0.06 (-0.03, 0.16) | 0.20 | 2.3 | 0.09 (-0.01, 0.19) | 0.08 | 2.4 | 0.10 (0.01, 0.20) | 0.04 | 2.1 | 0.08 (-0.01, 0.18) | 0.09 | 4.7 | 0.08 (-0.02, 0.18) | 0.11 | 0.8 |
| Model 2c | -0.01 (-0.12, 0.10) | 0.86 | 10.0 | -0.003 (-0.11, 0.10) | 0.95 | 12.3 | 0.05 (-0.05, 0.16) | 0.29 | 11.7 | 0.06 (-0.05, 0.16) | 0.32 | 6.4 | 0.07 (-0.04, 0.18) | 0.22 | 5.7 |
| β-carotene |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Model 1 | 0.22 (0.11, 0.32) | 0.001 | 6.2 | 0.23 (0.12, 0.33) | 0.001 | 6.4 | 0.18 (0.08, 0.29) | 0.001 | 4.1 | 0.14 (0.04, 0.25) | 0.008 | 5.8 | 0.15 (0.04, 0.26) | 0.006 | 2.1 |
| Model 2 | 0.17 (0.05, 0.29) | 0.006 | 11.9 | 0.15 (0.04, 0.27) | 0.01 | 14.0 | 0.14 (0.03, 0.26) | 0.01 | 13.0 | 0.12 (0.002, 0.24) | 0.05 | 7.3 | 0.15 (0.03, 0.27) | 0.02 | 6.7 |
| β-cryptoxanthin |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Model 1 | 0.21 (0.11, 0.31) | 0.001 | 6.2 | 0.21 (0.10, 0.31) | 0.001 | 5.7 | 0.15 (0.05, 0.25) | 0.004 | 3.2 | 0.17 (0.07, 0.27) | 0.001 | 6.9 | 0.17 (0.06, 0.27) | 0.001 | 2.9 |
| Model 2 | 0.18 (0.08, 0.28) | 0.001 | 12.9 | 0.17 (0.07, 0.27) | 0.001 | 15.0 | 0.12 (0.02, 0.22) | 0.02 | 12.8 | 0.16 (0.05, 0.26) | 0.004 | 8.5 | 0.16 (0.06, 0.27) | 0.003 | 7.6 |

a Effect estimates are per SD increment in log-transformed maternal plasma carotenoids concentrations, and per SD BSID-III score.

b Model 1 was adjusted for child’s age at cognitive testing

c Model 2 was adjusted as for Model 1 and maternal age, ethnicity, education, household income, pre-pregnancy BMI, parity, diet quality, antenatal depressive symptoms and anxiety levels.

|  |
| --- |
| **Table 3:** Associations of maternal plasma carotenoids concentrations with scores of Kaufman Brief Intelligence Test-2 at 54 months of age in the Growing Up in Singapore Towards healthy Outcomes study (n=332) |
|  | Verbal | Non-verbal |
|  | β (95% CI)a | P | R2 | β (95% CI) | P | R2 |
| α-carotene |  |  |  |  |  |  |
| Model 1b | 0.14 (0.04, 0.25) | 0.007 | 2.2 | 0.10 (-0.0005, 0.21) | 0.05 | 1.2 |
| Model 2c | 0.02 (-0.09, 0.13) | 0.70 | 15.4 | 0.03 (-0.08, 0.14) | 0.60 | 6.2 |
| β-carotene |  |  |  |  |  |  |
| Model 1 | 0.18 (0.07, 0.28) | 0.001 | 3.1 | 0.06 (-0.04, 0.17) | 0.24 | 0.4 |
| Model 2 | 0.05 (-0.06, 0.16) | 0.36 | 15.6 | -0.03 (-0.14, 0.09) | 0.66 | 6.1 |
| β-cryptoxanthin |  |  |  |  |  |  |
| Model 1 | 0.13 (0.02, 0.24) | 0.02 | 1.6 | 0.09 (-0.02, 0.20) | 0.10 | 0.8 |
| Model 2 | 0.06 (-0.04, 0.16) | 0.25 | 15.7 | 0.04 (-0.06, 0.15) | 0.42 | 6.3 |
| a Effect estimates are per SD increment in log-transformed maternal plasma carotenoids concentrations, and per SD KBIT-2 score.b Model 1 was adjusted for child’s age at cognitive testing c Model 2 was adjusted as for Model 1 and maternal age, ethnicity, education, household income, pre-pregnancy BMI, parity, diet quality, antenatal depressive symptoms and anxiety levels.  |

**FIGURE CAPTIONS**

**Fig. 1** Participant flow diagram for the analysis of associations of maternal plasma carotenoids with offspring neurocognitive outcomes in the Growing Up in Singapore Towards healthy Outcomes study. BSID-III, Bayley Scale of Infant and Toddler Development 3rd edition; KBIT-2, Kaufman Brief Intelligence Test 2nd edition