

Perinatal plasma carotenoids and vitamin E concentrations with glycemia and insulin resistance in women during and after pregnancy

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Abstract: We examined the associations of perinatal plasma carotenoids and E vitamers concentrations with glycemia, insulin resistance, gestational and type-2 diabetes mellitus at pregnancy and post-pregnancy in GUSTO women. Plasma carotenoids and E vitamers concentrations were measured at delivery, and principal component analysis derived patterns of their concentrations. Fasting and 2-hour glucose, and fasting insulin were measured at 26-28 weeks' gestation and 4-6 years' post-pregnancy, with derivation of homeostatic model assessment for insulin resistance (HOMA-IR). In 678 women, two carotenoids patterns (CP1: α -, β -carotene, lutein; CP2: zeaxanthin, lycopene, β -cryptoxanthin) and one E vitamers pattern (VE: γ -, δ -, α -tocopherols) were derived. A higher CP1 score (1-SD) was associated with lower gestational fasting glucose [β (95%CI): -0.06 (-0.10, -0.02) mmol/L], and lower gestational [-0.17 (-0.82, 0.01) mmol/L, $P=0.06$] and post-pregnancy HOMA-IR [-0.11 (-0.15, -0.08) mmol/L]. A higher VE score (1-SD) was associated with higher gestational and post-pregnancy fasting and 2-hour glucose [gestational: 0.05 (0.01, 0.08) and 0.08 (0.01, 0.16); post-pregnancy: 0.19 (0.07, 0.31) and 0.24 (0.06, 0.42) mmol/L]. Higher α -, β -carotene and lutein may be beneficial for gestational fasting glycemia, but higher vitamin E may increase gestational and post-pregnancy glycemia, although requires confirmation in cohorts with prospective longitudinal measurements of these vitamins.

Keywords: carotenoids; vitamin E; glycemia; insulin resistance; pregnancy; post-pregnancy

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1. Introduction

During pregnancy, high amounts of circulating reactive oxygen species is generated by the placenta for optimal maternal adaptation to pregnancy and for the normal

development of the fetus [1]. These oxidative processes are counter-balanced by antioxidants to protect against oxidative damage. However, an imbalance between these oxidative processes and anti-oxidant capacity can lead to oxidative stress, which has adverse effects on pregnancy and fetal development [1]. Emerging evidence suggests that increased oxidative stress may be involved in the pathogenesis of gestational diabetes mellitus (GDM) [2].

Women who experienced GDM are at higher risk of developing insulin resistance and T2DM later in life [3]; an estimated 15–25% of women with prior GDM develop T2DM within 1–2 years after pregnancy, and 35%–70% develop T2DM by 10–15 years after pregnancy [4,5]. Asian populations are at a disproportionately higher risk of T2DM [6], and the prevalence of GDM (23.5%) in Singapore is among the highest in the world [7]. As such, potential interventions to prevent GDM and its progression to T2DM, including improving diet during the antenatal period, might have utility in reducing the life-time risk of T2DM [8].

Dietary antioxidants such as carotenoids and vitamin E (comprising tocopherol and tocotrienols vitamers) are known to reduce oxidative stress [9,10]. Carotenoids can reduce reactive oxygen species such as singlet oxygen and peroxy radicals as well as transcription factors such as nuclear factor κ B and nuclear factor erythroid 2-related factor 2 [9], which are responsible for insulin resistance and β -cell dysfunction [11]. Similarly, vitamin E especially α -tocopherol has been shown to reduce lipid peroxidation [10], which produces superoxide that damages the structural and functional components of β -cells crucial for maintaining normal glucose concentrations [11].

However, evidence relating carotenoids and vitamin E to glycemia and insulin resistance in pregnant women or to GDM is scarce, with two case-control studies reporting no differences in dietary β -carotene and vitamin E intakes, as well as no difference in serum α -tocopherol concentrations, between women with and without GDM [12,13]. To the best of our knowledge, no studies have examined other carotenoids such as lutein and zeaxanthin, and E vitamers in relation to glycemia and insulin resistance during pregnancy or investigated whether gestational plasma concentrations relate to women's glycemia and insulin resistance post-pregnancy.

There is increasing recognition that nutrients and dietary compounds have synergistic effects on health [14]. Thus, assessing combinations of dietary compounds using pattern analysis may be more appropriate for assessing the influence of highly correlated dietary compounds on glycemia. We aimed to examine the associations of individual plasma carotenoids and E vitamers concentrations at delivery and their combination, with gestational glycemia, insulin resistance and GDM, as well as glycemia, insulin resistance and risk of T2DM at 4–6 years' post-pregnancy.

2. Materials and Methods

2.1. Study sample

We analyzed data from the Growing Up in Singapore Towards healthy Outcomes (GUSTO) study, which is a prospective mother-offspring cohort in Singapore [15]. Detailed descriptions of the study have been published [15]. In brief, pregnant women (≥ 18 years) of Chinese, Malay or Indian ethnicity with homogenous parental ethnic background were recruited during their first trimester (< 14 weeks) in June 2009–September 2010 from two major maternity hospitals (National University Hospital and KK Women's and Children's Hospital) in Singapore. All procedures of GUSTO were conducted according to the guidelines in the Declaration of Helsinki and have received ethics approval from the Institutional Review Board governing the two maternity hospitals. Written informed consent was obtained from all participants at recruitment.

A total of 1450 pregnant women were recruited at baseline, and 1098 had singleton live births. The present analysis included all GUSTO women who provided sufficient blood for plasma carotenoids and E vitamers assays at delivery, and had information on

plasma glucose and/or insulin during pregnancy, as well as plasma glucose and/or insulin at 4-6 years' post-pregnancy (**Figure 1**).

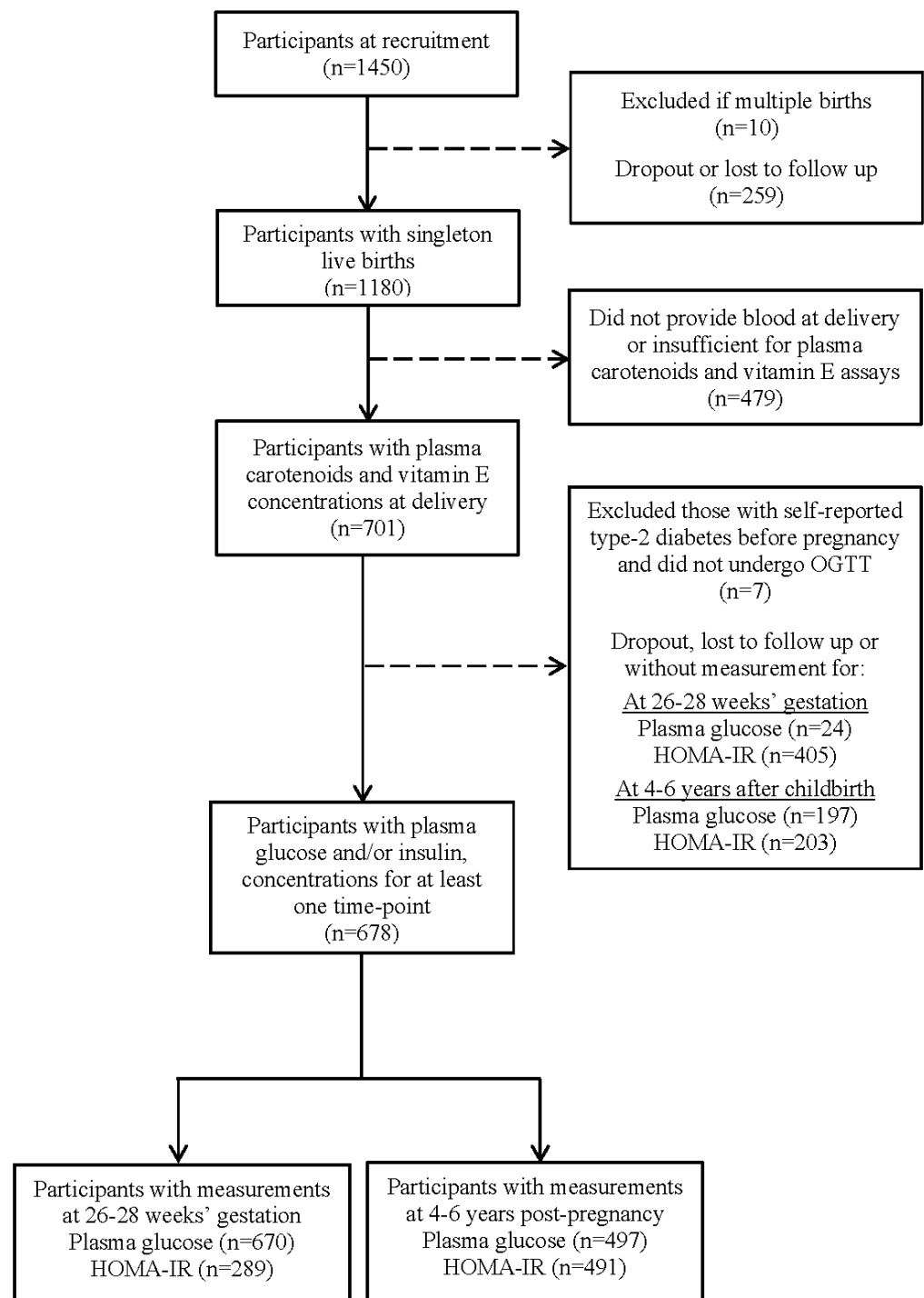


Figure 1. Participants included in the analysis of plasma carotenoids and vitamin E concentrations with glycemia and insulin during and post-pregnancy in the Growing Up in Singapore Towards healthy Outcomes cohort. HOMA-IR, homeostatic model assessment for insulin resistance; OGTT, oral glucose tolerance test.

2.2. Plasma concentrations of carotenoids and E vitamers

Non-fasting blood samples were collected from pregnant women (median gestation: 39 weeks, interquartile range: 38-40 weeks') around the time of delivery (up to 2 weeks

prior or within 17 hours after) by standard venipuncture. The blood samples were collected in EDTA tubes, centrifuged at 1600g for 10 minutes at 4°C within 4 hours to obtain plasma, stored at -80°C and thawed prior to analysis. Ultra High Performance Liquid Chromatography with Photo-Diode Array detection was used to determine plasma concentrations of carotenoids (α -carotene, β -carotene, β -cryptoxanthin, lutein and zeaxanthin) and E vitamers (α -, γ -, δ -tocopherols and tocotrienols) [16]. Method precision was examined using pooled and spiked plasma samples and results were similar to those previously published [16], with the relative standard deviations of within day assays and between-day assays generally <10% and <15%, respectively. The half-lives of circulating carotenoids and E vitamers are 5-45 days [17-19] and 2-70 days [20], respectively, thus maternal concentrations around the time of delivery reflect concentrations in the last weeks of gestation.

2.3. Plasma glucose and insulin concentrations, GDM and T2DM

At 26-28 weeks' gestation, we measured plasma glucose after an overnight fast and 2 hours following a 75g load in an oral glucose tolerance test (OGTT). Women with self-reported pre-existing T2DM before pregnancy were excluded from OGTT. Fasting plasma insulin concentrations were measured in a subset of women with available fasting blood sample. Similarly, at 4-6 years' post-pregnancy, fasting and 2-hour plasma glucose following OGTT and fasting plasma insulin were measured. Insulin and glucose concentrations were quantified using the colorimetry method (Advia 2400 Chemistry system, Siemens Medical Solutions Diagnostics; and Beckman LX20 Pro analyzer, Beckman Coulter). HOMA-IR was calculated as (fasting plasma insulin \times fasting plasma glucose)/22.5 [21].

GDM was defined as a plasma glucose concentration ≥ 7.0 mmol/L fasting and/or ≥ 7.8 mmol/L 2-hours post-OGTT, based on the 1999 World Health Organization (WHO) standard criteria [22] in use for clinical management at that time. T2DM at 4-6 years' post-pregnancy was defined as a plasma glucose concentration ≥ 7.0 mmol/L fasting and/or ≥ 11.1 mmol/L 2-hours post-OGTT, based on the 2019 WHO classification of diabetes [23]; or self-reported to be diagnosed with T2DM after GUSTO index pregnancy (n=1).

2.4. Covariates

Covariates were selected based on previous literature [12,13,24,25] and a directed acyclic graph. Information on women's age, ethnicity, highest education attained, self-reported existing T2DM and family history of T2DM were collected at recruitment. Women's pre-pregnancy body mass index (BMI) was calculated as weight divided by height squared (kg/m^2), based on self-reported pre-pregnancy weight, and height measured with a stadiometer (SECA model 213) at 26-28 weeks' gestation. Parity was retrieved from hospital delivery records. At 26-28 weeks' gestation, self-reported cigarette smoking and alcohol intake during pregnancy were ascertained; moderate and vigorous physical activity in the past 7 days were self-reported using the International Physical Activity Questionnaire [26] and categorized as follows: never, <150 and ≥ 150 min/week; food and dietary supplement intakes were assessed using a single 24-hour recall administered by trained research staff. Total fat intake was estimated using nutrient analysis software (Dietplan, Forestfield Software, UK) based on a food composition database containing local foods [27]. The use of dietary supplements (yes/no) containing any amounts of preformed vitamin A (retinol or retinyl esters), carotenoids, vitamin E or its vitamers were considered.

2.5. Statistical analysis

Descriptive statistics were presented for demographic, nutritional and clinical measures for those included in the present analysis.

To examine carotenoids and E vitamers in combination, we constructed patterns from six carotenoids and three E vitamers using principal component analysis with the

use of varimax rotation. As a high percentage of participants had concentrations below the detection limit for each form of tocotrienols, all forms of tocotrienols were removed from subsequent analyses. The number of patterns chosen for retention was determined by the break point of the Scree plot and eigenvalue of >1.0 (determined *a priori*). Differences in intakes of fruit, vegetables, total fat and dietary supplements between tertiles of carotenoids and E vitamers patterns were compared using chi-squared or one-way ANOVA tests.

To enable comparison of effect estimates across exposures, we constructed standard deviation scores [(observed value - mean)/SD] for each carotenoid and E vitamers as well as the scores for their patterns. Associations of individual carotenoids and E vitamers, and their patterns: 1) with continuous measures of plasma glucose and HOMA-IR were examined using linear regressions for normal distributions or inverse Gaussian regressions for positively skewed distributions, 2) with categorical outcomes – GDM and T2DM, were examined using logistic regression. All models were adjusted for women’s age at delivery, ethnicity, education, pre-pregnancy overweight or obesity (BMI ≥ 23 kg/m² according to WHO BMI classification for Asian [28]), family history of diabetes mellitus, parity, smoking, alcohol intake, moderate-strenuous physical activity, total fat and dietary supplement intakes.

Missing data for covariates were imputed using multiple imputation with chained equations (20 times) for the following confounding variables: highest education attained (n=5), pre-pregnancy BMI (n=56), n=10 family history of T2DM, n=5 smoking, n=19 alcohol, n=4 physical activity, n=10 total fat intake, and n=47 dietary supplement intakes. All analyses were performed using Stata version 14 (StataCorp LP, College Station, TX, USA). Two-sided $P < 0.05$ was considered statistically significant.

3. Results

3.1. Study sample characteristics

Table 1 presents the demographic, lifestyle and clinical characteristics along with the average concentrations of individual carotenoids and E vitamers for the 678 women included. The women were on average 31 years old at delivery. The majority was of Chinese ethnicity (59%), had attained tertiary education (37.4%), and was multiparous (56.2%) at recruitment, and did not engage in moderate-vigorous physical activity (70%). Approximately 39.6% women were with overweight or obesity before pregnancy, 2% smoked and drank alcohol during pregnancy, and 73.5% and 26.8% of women were taking dietary supplements containing vitamin A/carotenoids and vitamin E at mid-late pregnancy, respectively. A total of 204 (30.5%) women reported a family history of T2DM, 130 (19.4%) were classified as having GDM and 11 (2.2%) as having T2DM at 4-6 years’ post-pregnancy.

Table 1. Characteristics^a of participants for the associations of plasma carotenoids and vitamin E concentrations with glycemia and insulin resistance during and post-pregnancy in the Growing Up in Singapore Towards healthy Outcomes cohort (n=678).

Characteristics	n (%)
Pre-pregnancy overweight/obese (BMI ≥ 23.0 kg/m ²), n (%)	246 (39.6)
<u>During pregnancy</u>	
Age at delivery, year, mean \pm SD	31.4 \pm 5.0
Ethnicity, n (%)	
Chinese	400 (59.0)
Malay	158 (23.3)
Indian	120 (17.7)
Highest education, n (%)	

≤Secondary	203 (30.2)
Post-secondary	218 (32.4)
Tertiary	252 (37.4)
Parity, n (%)	
Nulliparous	297 (43.8)
Primi- / Multiparous	381 (56.2)
Smoking, n (%)	13 (1.9)
Alcohol intake, n (%)	15 (2.3)
Moderate-vigorous physical activity, n (%)	
Never	472 (70.0)
<150 min/week	135 (20.0)
≥150 min/week	67 (9.9)
Total fat intake, g/day, mean ± SD	69.6 ± 29.1
Intake of supplements containing, n (%)	
Vitamin A/carotenoids	462 (73.5)
Vitamin E	169 (26.8)
Plasma carotenoids concentrations, μmol/L, mean ± SD	
α-carotene	0.12 ± 0.09
β-carotene	0.45 ± 0.36
β-cryptoxanthin	0.45 ± 0.33
Lutein	0.46 ± 0.26
Zeaxanthin	0.30 ± 0.12
Lycopene	0.23 ± 0.13
Plasma vitamin E concentrations, μmol/L, mean ± SD	
α-tocopherol	52.45 ± 13.09
γ-tocopherol	1.47 ± 0.77
δ-tocopherol	0.47 ± 0.29
Family history of diabetes mellitus, n (%)	204 (30.5)
Fasting plasma glucose, mmol/L, mean ± SD	4.35 ± 0.49
2-hour plasma glucose, mmol/L, mean ± SD	6.56 ± 1.54
HOMA-IR, median (IQR)	1.18 (0.80, 1.70)
Gestational diabetes, n (%)	130 (19.4 ^b)
<u>At 4-6 years' post-pregnancy</u>	
Fasting plasma glucose, mmol/L, mean ± SD	4.94 ± 0.73
2-hour plasma glucose, mmol/L, mean ± SD	6.36 ± 1.89
HOMA-IR, median (IQR)	1.22 (0.81, 1.97)
Type-2 diabetes, n (%)	11 (2.2 ^c)

^a Characteristics were based on data obtained during pregnancy or at 4-6 years' post-pregnancy unless otherwise specified. Missing data: n=5 highest education attained, n=56 pre-pregnancy BMI, n=10 family history of T2DM, n=5 smoking, n=19 alcohol, n=4 physical activity, n=10 total fat intake, and n=47 dietary supplements intakes. ^b Percentage calculated based on 670 women with plasma glucose concentrations at 26-28 weeks' gestation ^c Percentage calculated based on 497 women with plasma glucose concentrations at 4-6 years' post-pregnancy.

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3.2. Patterns of carotenoid and E vitamers

Three patterns were derived (Table 2). Carotenoid pattern 1 (CP1) was represented by α -carotene, β -carotene and lutein; vitamin E (VE) pattern consisted of all forms of tocopherols (γ -, δ - and α -tocopherols); and carotenoid pattern 2 (CP2) comprised zeaxanthin, lycopene and β -cryptoxanthin.

Table 2. Carotenoid and E vitamers patterns construction: Pattern structure and variance explained.

Carotenoid/E vitamers	Carotenoid pattern 1 (CP1)	Vitamin E (VE) pattern	Carotenoid pattern 2 (CP2)
α -carotene	0.56		
β -carotene	0.51		
lutein	0.48		
γ -tocopherol		0.61	
δ -tocopherol		0.60	
α -tocopherol		0.42	
zeaxanthin			0.59
lycopene			0.55
β -cryptoxanthin			0.46
% variance explained by each pattern	21.9	20.5	17.1
Cumulative % of variance explained	21.9	42.4	39

Values are loading coefficients derived from principal component analysis (^aabsolute values <0.30 were not listed for simplicity).

We found that women who were in the highest tertile of CP1 scores had significantly higher fruit and vegetables intakes (Table 3). Only total fat intake was significantly different according to tertiles of VE scores, whilst only vegetables intake was significantly different according to tertiles of CP2 scores (higher intakes for those in highest tertiles). There were no significant differences in the proportion of women consuming dietary supplements containing vitamin A and carotenoids or containing vitamin E according to the patterns scores.

Table 3. Intakes of fruit, vegetables, total fat, and dietary supplements according to tertiles of carotenoid and E vitamers patterns scores

	Carotenoid pattern 1 (CP1)			Vitamin E (VE) pattern			Carotenoid pattern 2 (CP2)		
	Tertile 1	Tertile 3	<i>p</i>	Tertile 1	Tertile 3	<i>p</i>	Tertile 1	Tertile 3	<i>p</i>
Total fruit intake, g/day, mean \pm SD	63.7 \pm 119.4	163.1 \pm 189.7	<0.001	122.1 \pm 171.1	104.2 \pm 165.4	0.09	99.0 \pm 144.6	127.6 \pm 185.9	0.37
Total vegetables intake, g/day, mean \pm SD	56.0 \pm 64.2	80.6 \pm 74.9	0.001	66.7 \pm 69.5	67.6 \pm 70.6	0.09	59.0 \pm 67.8	73.7 \pm 69.7	0.02
Total fat intake, g/day, mean \pm SD	67.3 \pm 29.3	72.5 \pm 28.2	0.15	68.3 \pm 30.6	73.8 \pm 28.9	0.02	68.1 \pm 26.7	71.4 \pm 30.5	0.57

Supplements containing Vitamin A, n (%)	167 (75.9)	146 (70.9)	0.49 9	164 (76.3)	150 (69.4)	0.23 3	161 (75.2)	156 (74.3)	0.59 9
Supplements containing Vitamin E, n (%)	49 (22.3)	65 (31.6)	0.09 7	63 (29.3)	47 (21.8)	0.10 7	61 (28.5)	52 (24.8)	0.66 9

^a P-values were obtained from chi-squared or one-way ANOVA tests.

3.3. Carotenoid and E vitamers with gestational plasma glucose, HOMA-IR and GDM

Higher α -, β -carotene and lutein concentrations (per SD increment), examined individually, were significantly associated with 0.05 mmol/L (95% CI: -0.09, -0.01), 0.06 mmol/L (95% CI: -0.08, -0.01) and 0.05 mmol/L (95% CI: -0.09, -0.01) lower gestational fasting glucose, respectively (Table 4). Likewise, the combination of α -, β -carotene and lutein was inversely associated with gestational fasting glucose, as reflected by a higher CP1 score (per SD increment) significantly associating with a 0.06 mmol/L (95% CI: -0.10, -0.02; $P=0.004$) lower gestational fasting glucose. There was a trend towards higher CP1 score (combination of α -, β -carotene and lutein) associating with lower gestational HOMA-IR (β -0.17, 95% CI: -0.82, 0.01), but the association was borderline significant ($P=0.06$) due to small sample size. Individual concentrations of α -, β -carotene and lutein were not significantly associated with gestational HOMA-IR. No statistically significant associations were observed for individual carotenoids and their combinations with gestational 2-hour glucose and likelihood of GDM.

Additionally, there was a trend towards higher β -cryptoxanthin concentrations associating with lower gestational HOMA-IR (β -0.09, 95% CI: -0.72, 0.01 per SD increment in concentrations), but the association was borderline significant ($P=0.06$) due to small sample size. The combination of zeaxanthin, lycopene, and β -cryptoxanthin was not associated with gestational HOMA-IR. Overall, zeaxanthin, lycopene, and β -cryptoxanthin, whether individually or in combination (CP2), were not associated with gestational fasting and 2-hour glucose or likelihood of GDM.

Individually, higher γ -tocopherols concentrations (per SD increment) were associated with higher gestational glucose concentrations [fasting 0.05 mmol/L (95% CI: 0.02, 0.09), 2-hour 0.10 mmol/L (95% CI: 0.02, 0.17)]. Additionally, higher δ -tocopherol (per SD increment) were associated with a 0.05 mmol/L (95% CI: 0.02, 0.09) higher gestational fasting glucose but not significantly associated with 2-hour glucose. The combination of γ -, δ - and α -tocopherols (VE pattern) was associated with higher gestational glucose concentrations [fasting 0.05 mmol/L (95% CI: 0.01, 0.08), 2-hour 0.08 mmol/L (95% CI: 0.01, 0.16) per SD score increment].

No statistically significant association was observed for α -tocopherol with gestational fasting and 2-hour glucose. Individual E vitamers and their combinations were not associated with HOMA-IR and likelihood of GDM.

Table 4. Associations of individual carotenoids and E vitamers, and their patterns^a at late-pregnancy with plasma glucose, HOMA-IR during pregnancy as well as GDM in the Growing Up in Singapore Towards healthy Outcomes cohort^{b,c}

	Fasting glucose (n=670)		2-hour glucose (n=670)		HOMA-IR (n=289)		GDM (n=130) vs non-GDM (n=540)	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	OR (95% CI)	P
<u>Carotenoids^d</u>								
Individual concentrations								
α -carotene	-0.05 (-0.09, -0.01)	0.005	-0.13 (-0.24, 0.01)	0.05	-0.05 (-0.41, 0.32)	0.18	0.89 (0.71, 1.12)	0.33
β -carotene	-0.06 (-0.08, -0.01)	0.018	-0.03 (-0.15, 0.10)	0.68	-0.12 (-0.46, 0.01)	0.07	1.11 (0.90, 1.35)	0.32
Lutein	-0.05 (-0.09, -0.01)	0.019	0.02 (-0.11, 0.15)	0.76	-0.01 (-0.26, 0.24)	0.95	1.07 (0.85, 1.34)	0.56
CP 1	-0.06 (-0.10, -0.02)	0.004	-0.04 (-0.17, 0.08)	0.50	-0.17 (-0.82, 0.01)	0.06	1.05 (0.84, 1.33)	0.65
Individual concentrations								
Zeaxanthin	-0.02 (-0.06, 0.01)	0.22	-0.08 (-0.20, 0.03)	0.17	-0.08 (-0.35, 0.12)	0.10	0.94 (0.74, 1.18)	0.59
Lycopene	-0.02 (-0.05, 0.02)	0.42	-0.05 (-0.16, 0.06)	0.39	0.03 (-0.08, 0.15)	0.58	1.09 (0.89, 1.33)	0.41
β -cryptoxanthin	-0.04 (-0.05, 0.01)	0.05	0.02 (-0.10, 0.13)	0.80	-0.09 (-0.72, 0.01)	0.06	1.01 (0.81, 1.26)	0.91
CP 2	-0.04 (-0.06, 0.01)	0.05	-0.07 (-0.19, 0.05)	0.23	-0.01 (-0.43, 0.20)	0.11	1.02 (0.82, 1.27)	0.86
<u>Vitamin E^e</u>								
Individual concentrations								
γ -Tocopherol	0.05 (0.02, 0.09)	0.004	0.10 (0.02, 0.17)	0.010	0.09 (-0.24, 0.43)	0.57	1.20 (1.00, 1.50)	0.06
δ -Tocopherol	0.05 (0.02, 0.09)	0.006	0.07 (-0.01, 0.15)	0.07	0.03 (-0.34, 0.23)	0.11	1.22 (0.99, 1.49)	0.06
α -Tocopherol	-0.001 (-0.04, 0.04)	0.94	0.02 (-0.06, 0.10)	0.55	-0.05 (-0.58, 0.02)	0.07	1.21 (0.99, 1.49)	0.07
VE pattern	0.05 (0.01, 0.08)	0.015	0.08 (0.01, 0.16)	0.033	0.03 (-0.26, 0.34)	0.80	1.19 (0.99, 1.58)	0.06

GDM, gestational diabetes mellitus; HOMA-IR, homeostatic model assessment for insulin resistance

^a CP 1: α -, β -carotene and lutein; CP 2: zeaxanthin, lycopene and β -cryptoxanthin; VE pattern: γ -, δ -, α -tocopherols

^b Effect estimates are per SD increment in pattern scores or individual carotenoids and vitamin E concentrations (P<0.05 in bold)

^c All models adjusted for age, ethnicity, education, pre-pregnancy overweight and obese status, parity at recruitment, family history of T2DM, and the following at mid-late pregnancy: smoking status, alcohol intake, moderate-strenuous physical activity, total fat intake and intake of any supplement containing ^dvitamin A/carotenoids and/or ^evitamin E.

3.4. Carotenoid and E vitamers with post-pregnancy plasma glucose, HOMA-IR and T2DM

Higher β -carotene concentrations (per SD increment) were associated with 0.05 (95% CI: -0.07, -0.04) lower HOMA-IR (Table 5). Higher score in the combination of α -, β -carotene and lutein (per SD increment in CP1 score), was also associated with 0.11 (95% CI: -0.15, -0.08) lower HOMA-IR. No statistically significant associations were observed for individual α -carotene and lutein with HOMA-IR.

Additionally, higher β -cryptoxanthin concentrations (per SD increment) were associated with 0.07 (95% CI: -0.13, -0.02) lower HOMA-IR. However, individual zeaxanthin and lycopene, as well as the combination of zeaxanthin, lycopene and β -cryptoxanthin (CP2) were not associated with HOMA-IR.

Individual carotenoids and their combinations were not associated with post-pregnancy fasting and 2-hour glucose, nor with risk of T2DM.

Individually, higher δ -tocopherol concentrations (per SD increment) were associated with higher glucose concentrations [fasting 0.15 mmol/L (95% CI: 0.03, 0.27) and 2-hour 0.26 mmol/L (95% CI: 0.08, 0.44)]. Additionally, higher γ -tocopherol concentrations (per SD increment) were associated with 0.21 mmol/L (95% CI: 0.09, 0.33) higher fasting glucose, but not significantly associated with 2-hour glucose. The combination of γ -, δ - and α -tocopherols (VE pattern) was associated with higher glucose concentrations [(fasting 0.19 mmol/L (95% CI: 0.07, 0.31) and 2-hour 0.24 mmol/L (95% CI: 0.06, 0.42) per SD increment in VE pattern score].

No statistically significant associations were observed for α -tocopherol with post-pregnancy glucose, and there were also no associations of E vitamers and their pattern with HOMA-IR and T2DM.

Table 5. Associations of individual carotenoids and E vitamins, and their patterns^a at late-pregnancy with plasma glucose and HOMA-IR as well as T2DM at 4–6 years' post-pregnancy in the Growing Up in Singapore Towards healthy Outcomes cohort^{b,c}

	Fasting glucose (n=497)		2-hour glucose (n=497)		HOMA-IR (n=491)		T2DM (n=11) vs non-T2DM (n=486)	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	OR (95% CI)	P
<u>Carotenoids^d</u>								
Individual concentrations								
α -carotene	-0.02 (-0.09, 0.05)	0.60	-0.03 (-0.21, 0.16)	0.77	-0.06 (-0.13, 0.01)	0.08	0.29 (0.07, 1.20)	0.09
β -carotene	-0.03 (-0.10, 0.04)	0.40	-0.04 (-0.22, 0.14)	0.68	-0.05 (-0.07, -0.04)	0.001	0.41 (0.09, 1.78)	0.23
Lutein	-0.01 (-0.10, 0.07)	0.76	0.01 (-0.20, 0.21)	0.94	-0.05 (-0.11, 0.01)	0.11	0.53 (0.17, 1.65)	0.28
CP 1	-0.02 (-0.10, 0.06)	0.64	-0.08 (-0.27, 0.12)	0.42	-0.11 (-0.15, -0.08)	0.001	0.32 (0.10, 1.00)	0.05
Individual concentrations								
Zeaxanthin	-0.04 (-0.07, 0.01)	0.06	-0.17 (-0.33, 0.01)	0.06	-0.07 (-0.14, 0.01)	0.06	0.35 (0.19, 1.31)	0.06
Lycopene	-0.04 (-0.12, 0.06)	0.56	0.01 (-0.19, 0.21)	0.91	-0.07 (-0.15, 0.01)	0.10	0.81 (0.38, 1.74)	0.60
β -cryptoxanthin	-0.02 (-0.10, 0.05)	0.55	-0.10 (-0.28, 0.07)	0.25	-0.07 (-0.13, -0.02)	0.009	0.36 (0.10, 1.26)	0.11
CP 2	-0.04 (-0.08, 0.01)	0.06	-0.08 (-0.26, 0.11)	0.41	-0.09 (-0.16, 0.02)	0.10	0.42 (0.17, 1.02)	0.06
<u>Vitamin E^e</u>								
Individual concentrations								
γ -Tocopherol	0.21 (0.09, 0.33)	0.001	0.17 (-0.01, 0.34)	0.06	-0.02 (-0.11, 0.06)	0.62	1.16 (0.65, 2.10)	0.62
δ -Tocopherol	0.15 (0.03, 0.27)	0.015	0.26 (0.08, 0.44)	0.006	0.01 (-0.06, 0.15)	0.34	1.47 (0.85, 2.54)	0.17
α -Tocopherol	0.10 (-0.02, 0.22)	0.10	0.08 (-0.12, 0.27)	0.43	0.05 (-0.04, 0.15)	0.24	0.73 (0.35, 1.50)	0.39
VE pattern	0.19 (0.07, 0.31)	0.002	0.24 (0.06, 0.42)	0.009	0.10 (-0.03, 0.24)	0.13	1.25 (0.67, 2.34)	0.48

GDM, gestational diabetes mellitus; HOMA-IR, homeostatic model assessment for insulin resistance; T2DM, type-2 diabetes mellitus.

^a CP 1: α -, β -carotene and lutein; CP 2: zeaxanthin, lycopene and β -cryptoxanthin; VE pattern: γ -, δ -, α -tocopherols

^b Effect estimates are per SD increment in pattern scores or individual carotenoids and vitamin E concentrations (P<0.05 in bold)

^c All models adjusted for age, ethnicity, education, pre-pregnancy overweight and obese status, parity at recruitment, family history of T2DM, and the following at mid-late pregnancy: smoking status, alcohol intake, moderate-strenuous physical activity, total fat intake and intake of any supplement containing ^dvitamin A/carotenoids and/or ^evitamin E.

4. Discussion

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This study found associations of higher late-pregnancy concentrations of α -, β -carotene and lutein (in combination) with lower fasting glucose during pregnancy, as well as lower HOMA-IR during pregnancy and at 4-6 years' post-pregnancy. Additionally, a higher late-pregnancy β -cryptoxanthin concentration was individually associated with lower HOMA-IR during pregnancy and at 4-6 years' post-pregnancy. In contrast, higher concentrations of γ -, δ - and α -tocopherols in combination were associated with higher fasting and 2-hour glucose during pregnancy and post-pregnancy, but not with HOMA-IR.

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The associations we observed for α - or β -carotene (when examined individually) with plasma glucose during pregnancy are reminiscent of studies in non-pregnant populations. Overall, the effect size of the lowered fasting glucose during pregnancy we found for a 1-SD increment in α - or β -carotene concentrations (0.05-0.06 mmol/L) approximated to studies in non-pregnant populations (0.05-0.25 mmol/L) [29,30]. However, our findings of a higher lutein concentration associating with lower fasting glucose but not 2-hour glucose during pregnancy is in direct contrast to a study in a non-pregnant population which observed significant associations with lower 2-hour glucose but not fasting glucose [29]. When α -, β -carotene and lutein were examined in combination (CP1), the association with fasting glucose during pregnancy was also significant.

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We additionally found higher β -cryptoxanthin concentrations to associate with lower HOMA-IR during pregnancy (albeit a trending association due to small sample size) and 4-6 years' post-pregnancy, which contrasted studies showing no significant association between serum β -cryptoxanthin concentrations and HOMA-IR in non-pregnant populations [24,29]. The difference in findings may be due to variations in carotenoid concentrations influenced by the pregnancy-related hyperlipidemic state, placental transfer, or consumption of foods rich in β -cryptoxanthin (e.g. a greater consumption of tropical fruits in Singapore [31], which are rich sources of β -cryptoxanthin [32]).

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The magnitude of the above associations may appear modest (0.06 mmol/L lower fasting glucose), but such an effect size has been associated with an appreciable reduction in odds of delivery by cesarean section, delivery of a neonate with birth weight >90th percentile, neonatal hypoglycemia, and fetal hyperinsulinemia in the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study [33], and thus considered clinically impactful.

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Our findings support the beneficial role of combined α -, β -carotene and lutein (CP1) in insulin resistance (HOMA-IR) during pregnancy (albeit borderline significance due to small sample size) and post-pregnancy. The significant association with post-pregnancy HOMA-IR could be mediated through a lowered gestational HOMA-IR, changes to the physiological function of tissues influencing insulin resistance that persist beyond pregnancy, or consistent adherence to a diet high in these carotenoids post-pregnancy. Further studies specifically designed to address this will be needed. Despite observing associations with HOMA-IR at both time points, the beneficial associations of these carotenoids with gestational fasting glucose did not persist beyond pregnancy. One possible explanation could be a heightened state of physiological insulin resistance during pregnancy compared to a milder state of insulin resistance post-pregnancy. The heightened state of insulin resistance during pregnancy returns to pre-pregnancy state after delivery, as such, these women may not be sufficiently resistant to insulin at 4-6 years' post-pregnancy to observe an appreciable change in glycemia. In observing consistent associations between CP1 and HOMA-IR during pregnancy as well as post-pregnancy, but less consistent associations when these carotenoids were examined individually, further support the value of examining combinations of carotenoids to account for their synergistic activities.

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We observed differences in fruit and vegetables intakes according to tertiles of CPI scores, but no significant differences in the proportion of individuals consuming vitamin A/carotenoids supplements. Additionally, in the same cohort, we previously found women with higher concentrations of β -cryptoxanthin to have higher intakes of fruit and vegetables during pregnancy [34], but the concentrations did not differ significantly by intake of vitamin A/carotenoids supplements [35]. These women were also found to have lower mean concentrations of α -carotene, β -cryptoxanthin, and lutein compared to other pregnant cohorts even with approximately 70% of them consuming vitamin A/carotenoids-containing supplements, possibly due to lower fruit and vegetables intake within the cohort or the dietary supplements consumed did not contain the full range of carotenoids [35]. Taken together, our findings suggest greater consumption of fruit and vegetables, but not dietary supplements containing vitamin A/carotenoids, to be beneficial for lowering glycemia and insulin resistance during and/or after pregnancy. Fruit and vegetables, apart from their antioxidative properties, are also higher in fibre and lower in glycaemic index, which aligned with existing dietary approaches for glycaemic control in non-pregnant populations [36]. Continued health promotion efforts encouraging higher fruits and vegetables consumption in pregnant women are needed for better glycemic outcomes during and after pregnancy.

Our analysis may be underpowered to detect statistically significant differences in 2-hour glucose concentrations (due to a much wider variation in concentrations compared to fasting glucose), as well as in odds of GDM and T2DM (categorical variables with small number of cases). Further investigations in studies with larger sample sizes are needed.

Contrasting the associations for carotenoids, γ -, δ -, and α -tocopherols in combination were associated with higher fasting and 2-hour glucose during pregnancy. This association is likely driven by γ - and δ -tocopherols, considering their higher loadings in this pattern as well as their individual associations with plasma glucose. However, the literature is sparse on mechanism of action linking γ - and δ -tocopherols to glucose metabolism. There is some evidence suggesting that γ -tocopherol may be pro-inflammatory [37]; as such the positive associations with plasma glucose and GDM may be a result of increased inflammation – one of the underlying mechanisms of hyperglycemia during pregnancy [38]. Alternatively, higher concentrations of γ - and δ -tocopherols may reflect the widespread use of refined blended plant oils around the world which have very high content of γ -tocopherol [39]. One study in Chinese adults found higher intakes of refined blended plant oils to be associated with increased risk of T2DM, likely because frying is the predominant cooking method adopted for this type of oil [40], or due to its higher content of n-6 fatty acids which play conflicting roles in glucose metabolism [41]. While our study also observed those who are in the highest tertile of CP2 scores to have significantly higher total fat intake, a thorough evaluation of γ - or δ -tocopherols concentrations and their food sources, as well as comprehensive understanding of their individual cellular actions and impact on oxidative stress, are needed to elucidate the impact of the different E vitamers on glucose metabolism.

Strengths of this study include being the first to relate maternal plasma concentrations of individual carotenoids and E vitamers to glycemic measures during pregnancy and a few years' post-pregnancy, and the derivation of carotenoid and E vitamers combinations to capture their synergistic activities. Several limitations are noted. Blood samples (around the time of delivery) used for plasma carotenoid and vitamin E assays were taken after the measurement of plasma glucose during pregnancy (26–28 weeks' gestation), as such reverse causation cannot be ruled out. However, studies have shown stability in carotenoids and α -, γ -tocopherols concentrations from second trimester to delivery [42,43], as well as stability in dietary patterns throughout pregnancy [44,45]. The use of non-fasting plasma samples may have introduced systematic bias, but studies have shown non-significant differences in carotenoid concentrations pre- and post-meal [46,47]; the effect of fasting compared with non-fasting is less clear for plasma E vitamers due to limited literature. Findings with gestational HOMA-IR were also limited by the smaller sample

size as more than half of the cohort did not provide sufficient fasting blood samples for insulin measurements. While the aim of the study is to examine the influences of carotenoids and vitamin E during the perinatal period, our study could benefit from having measurements of carotenoid and vitamin E concentrations at 4-6 years' post-pregnancy.

5. Conclusions

Our study showed that higher maternal concentrations of α -, β -carotene and lutein are associated with lower fasting glucose and HOMA-IR during pregnancy as well as lower HOMA-IR at 4-6 years' post-pregnancy. These findings alluded to the importance of consuming greater quantity and variety of fruit and vegetables during pregnancy to ensure sufficient concentrations of a range of carotenoids (α -, β -carotene, lutein and β -cryptoxanthin); however, these findings will require confirmation in other similar cohorts with prospective, longitudinal measurements of carotenoids and E vitamins, plasma glucose and insulin during and after pregnancy. Replication of findings in populations of different ethnic characteristics with different dietary patterns will also be required before recommendations can be made. Our findings of higher concentrations of γ - and δ -tocopherols associating with higher plasma glucose during pregnancy, highlight the need to further investigate specific E vitamins and their roles in metabolic health.

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