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

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Abstract

Background: Carotenoids and vitamin E play beneficial roles against insulin resistance and β-cell dysfunction.

Objective: To examine the associations of perinatal plasma carotenoids and E vitamers concentrations with glycemia, insulin resistance, gestational and type-2 diabetes mellitus at mid-pregnancy and post-pregnancy in women of the Growing Up in Singapore Towards healthy Outcomes (GUSTO) cohort.

Methods: Plasma concentrations of carotenoids and E vitamers were measured at delivery, and principal component analysis derived patterns of their concentrations. Fasting and 2-hour plasma glucose, and fasting plasma 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). Associations were examined using linear or logistic regressions adjusting for key confounders.

Results: In 678 women, two patterns of carotenoids (CP1: α-, β-carotene, lutein; CP2: zeaxanthin, lycopene, β-cryptoxanthin) and one pattern of E vitamers (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 and post-pregnancy HOMA-IR [gestational: -0.17 (-0.82, 0.01), *P*=0.065; post-pregnancy: -0.11 (-0.15, -0.08) mmol/L]. In contrast, 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], but there were no associations with HOMA-IR.

Conclusions: Higher α-, β-carotene and lutein may lower gestational fasting glycemia, and gestational and post-pregnancy insulin resistance; but higher vitamin E may increase gestational and post-pregnancy glycemia. Further investigation in cohorts with prospective longitudinal measurements of these vitamins are needed.

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 anti-oxidants 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), and their possible roles in insulin resistance and β-cell dysfunction are of interest. However, evidence in pregnant women 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 (11, 12). To the best of our knowledge, no studies have examined other carotenoids 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 (13). 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.

Subjects And Methods

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 (14). Detailed descriptions of the study have been published (14). 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 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 units. 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 (Figure1).

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^{o}C within 4 hours to obtain plasma, stored at -80^{o}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) (15). Method precision was examined using pooled and spiked plasma samples and results were similar to those previously published (15), 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 (16–18) and 2–70 days (19), respectively, thus maternal concentrations around the time of delivery reflect concentrations in the last weeks of gestation.

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 × fasting plasma glucose)/22.5 (20).

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 (21) in use for clinical management at that time. T2DM was defined as a plasma glucose concentration ≥ 7.0 mmol/L fasting and/or ≥ 11.1mmol/L 2-hours post-OGTT, based on the 2019 WHO classification of diabetes (22).

Covariates

Covariates were selected based on previous literature (11, 12, 23, 24) 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 (25) 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 (26). 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.

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 four 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 summed to total tocotrienols before being included in the principal component analysis analysis. 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*).

To enable comparison of effect estimates across exposures, we constructed standard deviation scores [(observed value - mean)/SD] for each carotenoid and E vitamer 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/m^2 according to WHO BMI classification for Asian (27)), 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.

Results

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
Characteristicsa 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)

Pre-pregnancy overweight/obese (BMI ≥ 23.0 kg/m^2), n (%)	**246 (39.6)**
During pregnancy	
Age at delivery, year, mean ± SD	31.4 ± 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

Pre-pregnancy overweight/obese (BMI ≥ 23.0 kg/m^2), n (%)	246 (39.6)
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
Total tocotrienols	0.15 ± 0.10
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])
At 4–6 years' post-pregnancy	
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.

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. Total tocotrienols did not load highly (loading coefficient < 0.30) into any pattern.

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
tocotrienols[a]			
% 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 ([a]absolute values < 0.30 were not listed for simplicity).

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 3). 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* = 0.065) due to small sample size. Individual concentrations of α-, β-carotene and lutein were not significantly associated with gestational HOMA-IR.

No statistical significant associations were observed for individual carotenoids and their combinations with gestational 2-hour glucose and likelihood of GDM.

Table 3
Associations of individual carotenoids and E vitamers, and their patternsa at late-pregnancy with plasma glucose, HOMA-IR during pregnancy as well as GDM in the Growing Up in Singapore Towards healthy Outcomes cohortb,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
Carotenoids[d]								
Individual concentrations								
α-carotene	-0.05 (-0.09, -0.01)	**0.005**	-0.13 (-0.24, 0.01)	0.055	-0.05 (-0.41, 0.32)	0.180	0.89 (0.71, 1.12)	0.332
β-carotene	-0.06 (-0.08, -0.01)	**0.018**	-0.03 (-0.15, 0.10)	0.682	-0.12 (-0.46, 0.01)	0.070	1.11 (0.90, 1.35)	0.325
Lutein	-0.05 (-0.09, -0.01)	**0.019**	0.02 (-0.11, 0.15)	0.764	-0.01 (-0.26, 0.24)	0.946	1.07 (0.85, 1.34)	0.564
CP 1	-0.06 (-0.10, -0.02)	**0.004**	-0.04 (-0.17, 0.08)	0.496	-0.17 (-0.82, 0.01)	0.065	1.05 (0.84, 1.33)	0.649
Individual concentrations								
Zeaxanthin	-0.02 (-0.06, 0.01)	0.218	-0.08 (-0.20, 0.03)	0.169	-0.08 (-0.35, 0.12)	0.100	0.94 (0.74, 1.18)	0.587
Lycopene	-0.02 (-0.05, 0.02)	0.418	-0.05 (-0.16, 0.06)	0.391	0.03 (-0.08, 0.15)	0.576	1.09 (0.89, 1.33)	0.415
β-cryptoxanthin	-0.04 (-0.05, 0.01)	0.050	0.02 (-0.10, 0.13)	0.797	-0.09 (-0.72, 0.01)	0.064	1.01 (0.81, 1.26)	0.911
CP 2	-0.04 (-0.06, 0.01)	0.051	-0.07 (-0.19, 0.05)	0.233	-0.01 (-0.43, 0.20)	0.106	1.02 (0.82, 1.27)	0.856
Vitamin E[e]								
Individual concentrations								

	Fasting glucose (n = 670)		2-hour glucose (n = 670)		HOMA-IR (n = 289)		GDM (n = 130) vs non-GDM (n = 540)	
γ-Tocopherol	0.05 (0.02, 0.09)	**0.004**	0.10 (0.02, 0.17)	**0.010**	0.09 (-0.24, 0.43)	0.575	1.20 (1.00, 1.50)	0.059
δ-Tocopherol	0.05 (0.02, 0.09)	**0.006**	0.07 (-0.01, 0.15)	0.071	0.03 (-0.34, 0.23)	0.111	1.22 (0.99, 1.49)	0.062
α-Tocopherol	-0.001 (-0.04, 0.04)	0.943	0.02 (-0.06, 0.10)	0.554	-0.05 (-0.58, 0.02)	0.073	1.21 (0.99, 1.49)	0.068
VE pattern	0.05 (0.01, 0.08)	**0.015**	0.08 (0.01, 0.16)	**0.033**	0.03 (-0.26, 0.34)	0.796	1.19 (0.99, 1.58)	0.056
Individual concentrations								
Tocotrienols	-0.01 (-0.04, 0.03)	0.703	-0.02 (-0.09, 0.05)	0.584	-0.04 (-0.46, 0.13)	0.095	0.92 (0.73, 1.16)	0.479
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 [d]vitamin A/carotenoids and/or [e]vitamin E.								

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* = 0.064) 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].

Total tocotrienols and α-tocopherol were not associated with gestational fasting and 2-hour glucose. Individual E vitamers and their combinations were not associated with HOMA-IR and likelihood of GDM.

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 4). 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 statistical significant associations were observed for individual α-carotene and lutein with HOMA-IR.

Table 4
Associations of individual carotenoids and E vitamers, and their patternsa 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 cohortb,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
Carotenoids[d]								
Individual concentrations								
α-carotene	-0.02 (-0.09, 0.05)	0.598	-0.03 (-0.21, 0.16)	0.774	-0.06 (-0.13, 0.01)	0.084	0.29 (0.07, 1.20)	0.088
β-carotene	-0.03 (-0.10, 0.04)	0.400	-0.04 (-0.22, 0.14)	0.676	-0.05 (-0.07, -0.04)	**0.001**	0.41 (0.09, 1.78)	0.234
Lutein	-0.01 (-0.10, 0.07)	0.763	0.01 (-0.20, 0.21)	0.943	-0.05 (-0.11, 0.01)	0.108	0.53 (0.17, 1.65)	0.277
CP 1	-0.02 (-0.10, 0.06)	0.638	-0.08 (-0.27, 0.12)	0.425	-0.11 (-0.15, -0.08)	**0.001**	0.32 (0.10, 1.00)	0.051
Individual concentrations								
Zeaxanthin	-0.04 (-0.07, 0.01)	0.063	-0.17 (-0.33, 0.01)	0.057	-0.07 (-0.14, 0.01)	0.065	0.35 (0.19, 1.31)	0.061
Lycopene	-0.04 (-0.12, 0.06)	0.556	0.01 (-0.19, 0.21)	0.915	-0.07 (-0.15, 0.01)	0.096	0.81 (0.38, 1.74)	0.596
β-cryptoxanthin	-0.02 (-0.10, 0.05)	0.553	-0.10 (-0.28, 0.07)	0.248	-0.07 (-0.13, -0.02)	**0.009**	0.36 (0.10, 1.26)	0.111
CP 2	-0.04 (-0.08, 0.01)	0.065	-0.08 (-0.26, 0.11)	0.414	-0.09 (-0.16, 0.02)	0.103	0.42 (0.17, 1.02)	0.059
Vitamin E[e]								

	Fasting glucose (n = 497)		2-hour glucose (n = 497)		HOMA-IR (n = 491)		T2DM (n = 11) vs non-T2DM (n = 486)	
Individual concentrations								
γ-Tocopherol	0.21 (0.09, 0.33)	**0.001**	0.17 (-0.01, 0.34)	0.060	-0.02 (-0.11, 0.06)	0.619	1.16 (0.65, 2.10)	0.617
δ-Tocopherol	0.15 (0.03, 0.27)	**0.015**	0.26 (0.08, 0.44)	**0.006**	0.01 (-0.06, 0.15)	0.339	1.47 (0.85, 2.54)	0.170
α-Tocopherol	0.10 (-0.02, 0.22)	0.104	0.08 (-0.12, 0.27)	0.433	0.05 (-0.04, 0.15)	0.245	0.73 (0.35, 1.50)	0.393
VE pattern	0.19 (0.07, 0.31)	**0.002**	0.24 (0.06, 0.42)	**0.009**	0.10 (-0.03, 0.24)	0.132	1.25 (0.67, 2.34)	0.477
Individual concentrations								
Tocotrienols	-0.02 (-0.14, 0.10)	0.741	0.13 (-0.03, 0.30)	0.117	0.04 (-0.07, 0.15)	0.484	1.10 (0.69, 1.76)	0.702

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 [d]vitamin A/carotenoids and/or [e]vitamin E.

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].

Total tocotrienols and α-tocopherol were not associated with post-pregnancy glucose (Table 3), and there were also no associations of E vitamers and their pattern with HOMA-IR and T2DM.

Discussion

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.

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) (28, 29). 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 (28). When α-, β-carotene and lutein were examined in combination (CP1), the association with fasting glucose during pregnancy was also significant.

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 (23, 28). 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 (30), which are rich sources of β-cryptoxanthin (31)).

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 (32), and thus considered clinically impactful.

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.

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, concentrations 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 (33); 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 (34). A thorough evaluation of the individual cellular actions of γ- or δ-tocopherols, and their impact on glucose metabolism are needed.

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 (35, 36). 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 (37, 38); 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.

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. However, these findings will require confirmation in other similar cohorts with prospective, longitudinal measurements of carotenoids and E vitamers, 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 vitamers and their roles in metabolic health.

Declarations

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Authors' contributions: JSL, JKYC, SYC and MFFC designed the research. JSL performed statistical analysis and wrote the paper. MFFC reviewed and edited the manuscript. JSL and MFFC had primary responsibility for final content. CNO designed the methodology and provided essential reagents for plasma carotenoids and vitamin E assay. KMG, KHT, FY, and YSC led the GUSTO study. All authors critically reviewed the manuscript for scientific content, read and approved the final manuscript.

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Data Availability: The datasets analyzed during the current study are available from the corresponding author on reasonable request, subjected to approval from the executive committee of the GUSTO study.

References

1. Myatt L, Cui X (2004) Oxidative stress in the placenta. Histochem Cell Biol 122(4):369–382
2. Plows JF, Stanley JL, Baker PN, Reynolds CM, Vickers MH (2018) The pathophysiology of gestational diabetes mellitus. Int J Mol Sci 19(11):3342
3. Chen L-W, Soh SE, Tint M-T, Loy SL, Yap F, Tan KH et al (2021) Combined analysis of gestational diabetes and maternal weight status from pre-pregnancy through post-delivery in future development of type 2 diabetes. Sci Rep 11(1):5021
4. Kim C, Newton KM, Knopp RH (2002) Gestational diabetes and the incidence of type 2 diabetes: a systematic review. Diabetes Care 25(10):1862–1868

5. Bellamy L, Casas JP, Hingorani AD, Williams D (2009) Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. Lancet 373(9677):1773–1779
6. Chan JC, Malik V, Jia W, Kadowaki T, Yajnik CS, Yoon KH et al (2009) Diabetes in Asia: epidemiology, risk factors, and pathophysiology. JAMA 301(20):2129–2140
7. International Diabetes Federation. IDF Diabetes Atlas, 9th edn, Brussels B (2019) [Available from: https://www.diabetesatlas.org
8. Phelan S (2016) Windows of opportunity for lifestyle interventions to prevent gestational diabetes mellitus. Am J Perinatol 33(13):1291–1299
9. Bohn T (2019) Carotenoids and markers of oxidative stress in human observational studies and intervention trials: implications for chronic diseases.Antioxidants (Basel). ; 8(6)
10. Winklhofer-Roob BM, Rock E, Ribalta J, Shmerling DH, Roob JM (2003) Effects of vitamin E and carotenoid status on oxidative stress in health and disease. Evidence obtained from human intervention studies. Mol Aspects Med 24(6):391–402
11. Hekmat K, Bagheri R, Abedi P, Tabesh H (2014) The relationship of fat soluble antioxidants with gestational diabetes in Iran: a case-control study. J Matern Fetal Neonatal Med 27(16):1676–1679
12. Parast VM, Paknahad Z (2017) Antioxidant status and risk of gestational diabetes mellitus: a case-control study. Clin Nutr Res 6(2):81–88
13. Jacobs DR Jr, Gross MD, Tapsell LC (2009) Food synergy: an operational concept for understanding nutrition. Am J Clin Nutr 89(5):1543S–8S
14. Soh S-E, Tint MT, Gluckman PD, Godfrey KM, Rifkin-Graboi A, Chan YH et al (2014) Cohort profile: Growing Up in Singapore Towards healthy Outcomes (GUSTO) birth cohort study. Int J Epidemiol 43(5):1401–1409
15. Lee BL, Ong CN (2009) Comprehensive high-performance liquid chromatographic method for the measurements of lipophilic antioxidants in human plasma. J Chromatogr A 1216(15):3131–3137
16. Burri BJ, Neidlinger TR, Clifford AJ (2001) Serum carotenoid depletion follows first-order kinetics in healthy adult women fed naturally low carotenoid diets. J Nutr 131(8):2096–2100
17. Moran NE, Cichon MJ, Riedl KM, Grainger EM, Schwartz SJ, Novotny JA et al (2015) Compartmental and noncompartmental modeling of 13C-lycopene absorption, isomerization, and distribution kinetics in healthy adults. Am J Clin Nutr 102(6):1436–1449
18. Thurmann PA, Schalch W, Aebischer JC, Tenter U, Cohn W (2005) Plasma kinetics of lutein, zeaxanthin, and 3-dehydro-lutein after multiple oral doses of a lutein supplement. Am J Clin Nutr 82(1):88–97
19. Chuang JC, Matel HD, Nambiar KP, Kim S-H, Fadel JG, Holstege DM et al (2011) Quantitation of [5-CH3]-(2R, 4'R, 8'R)-α-Tocopherol in Humans. J Nutr 141(8):1482–1488
20. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28(7):412–419

21. WHO Consultation (1999) Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1: diagnosis and classification of diabetes mellitus. WHO, Geneva
22. World Health Organisation (WHO) (2019) Classification of diabetes mellitus. WHO, Geneva
23. Hozawa A, Jacobs DR Jr, Steffes MW, Gross MD, Steffen LM, Lee DH (2006) Associations of serum carotenoid concentrations with the development of diabetes and with insulin concentration: interaction with smoking: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Am J Epidemiol 163(10):929–937
24. Mayer-Davis EJ, Costacou T, King I, Zaccaro DJ, Bell RA, Insulin Resistance Atherosclerosis Study (IRAS) (2002) Plasma and dietary vitamin E in relation to incidence of type 2 diabetes: The Insulin Resistance and Atherosclerosis Study (IRAS). Diabetes Care 25(12):2172–2177
25. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE et al (2003) International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 35(8):1381–1395
26. Lai JS, Soh SE, Loy SL, Colega M, Kramer MS, Chan JKY et al (2019) Macronutrient composition and food groups associated with gestational weight gain: the GUSTO study. Eur J Nutr 58(3):1081–1094
27. WHO Expert Consultation (2004) Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. The Lancet 363(9403):157–163
28. Coyne T, Ibiebele TI, Baade PD, Dobson A, McClintock C, Dunn S et al (2005) Diabetes mellitus and serum carotenoids: findings of a population-based study in Queensland, Australia. Am J Clin Nutr 82(3):685–693
29. Ylonen K, Alfthan G, Groop L, Saloranta C, Aro A, Virtanen SM (2003) Dietary intakes and plasma concentrations of carotenoids and tocopherols in relation to glucose metabolism in subjects at high risk of type 2 diabetes: the Botnia Dietary Study. Am J Clin Nutr 77(6):1434–1441
30. Alperet DJ, Butler LM, Koh W-P, Yuan J-M, van Dam RM (2017) Influence of temperate, subtropical, and tropical fruit consumption on risk of type 2 diabetes in an Asian population. Am J Clin Nutr 105(3):736–745
31. Burri BJ, La Frano MR, Zhu C (2016) Absorption, metabolism, and functions of β-cryptoxanthin. Nutr Rev 74(2):69–82
32. The HAPO Study Cooperative Research Group (2008) Hyperglycemia and adverse pregnancy outcomes. N Engl J Med 358(19):1991–2002
33. Bates CJ, Mishra GD, Prentice A (2007) γ-Tocopherol as a possible marker for nutrition-related risk: results from four National Diet and Nutrition Surveys in Britain. Br J Nutr 92(1):137–150
34. Lowe LP, Metzger BE, Lowe WL Jr, Dyer AR, McDade TW, McIntyre HD et al (2010) Inflammatory mediators and glucose in pregnancy: results from a subset of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. J Clin Endocrinol Metab 95(12):5427–5434
35. Oostenbrug GS, Mensink RP, Al MD, van Houwelingen AC, Hornstra G (1998) Maternal and neonatal plasma antioxidant levels in normal pregnancy, and the relationship with fatty acid unsaturation. Br

J Nutr 80(1):67–73

36. Herrera E, Ortega H, Alvino G, Giovannini N, Amusquivar E, Cetin I (2004) Relationship between plasma fatty acid profile and antioxidant vitamins during normal pregnancy. Eur J Clin Nutr 58(9):1231–1238
37. Mejía LA, Pineda O, Noriega JF, Benítez J, Falla G (1984) Significance of postprandial blood concentrations of retinol, retinol-binding protein, and carotenoids when assessing the vitamin A status of children. Am J Clin Nutr 39(1):62–65
38. Brown ED, Rose A, Craft N, Seidel KE, Smith JC (1989) Concentrations of carotenoids, retinol, and tocopherol in plasma, in response to ingestion of a meal. Clin Chem 35(2):310–312

Figures

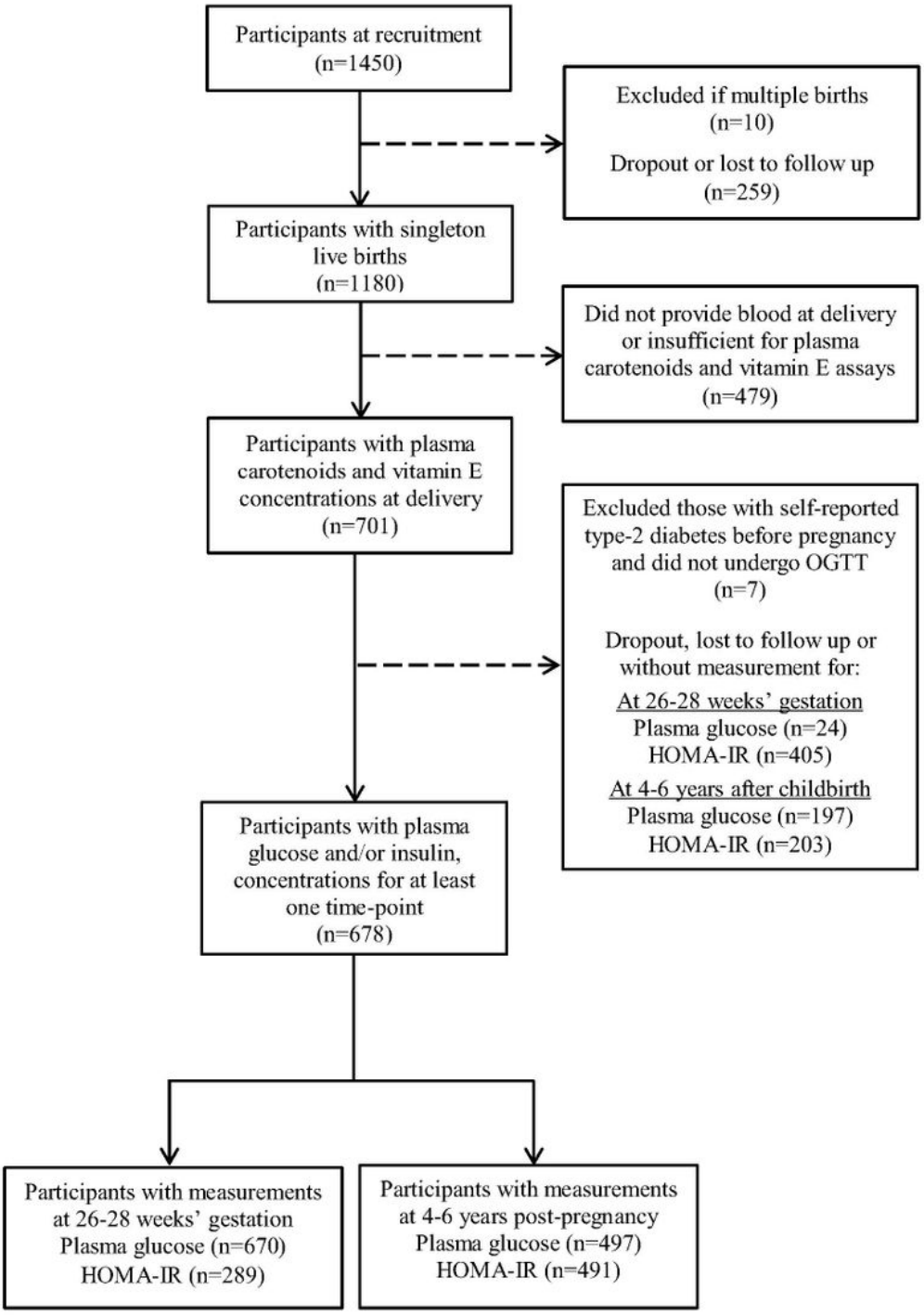

Figure 1

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