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Title: Perinatal plasma carotenoid and vitamin E concentrations with maternal blood pressure during and after pregnancy

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Abstract

Background and Aims: Few studies examined influence of carotenoids and vitamin E on blood pressure or hypertension during and after pregnancy. We related perinatal plasma concentrations of individual carotenoids and forms of vitamin E, and their combination, to blood pressure and hypertension at late-pregnancy and 4 years post-pregnancy.

Methods and Results: In 684 women of the Growing Up in Singapore Towards healthy Outcomes cohort, we quantified plasma carotenoids and vitamin E concentrations at delivery. Systolic and diastolic blood pressure (SBP, DBP) around 37-39 weeks' gestation were extracted from obstetric records, and measured at 4 years post-pregnancy. Principal component analysis derived patterns of carotenoids (CP) and vitamin E. Associations were examined using linear or logistic regressions adjusting for confounders. Two carotenoid (CP1: α-, β-carotene, lutein; CP2: zeaxanthin, lycopene, β-cryptoxanthin) and one vitamin E (γ-, δ-, α-tocopherols) patterns were derived. CP1 (1SD score increment) was associated with lower SBP and DBP [β (95% CI): -2.36 (-3.47, -1.26) and -1.37 (-2.21, -0.53) mmHg] at late-pregnancy, and 4 years post-pregnancy [-1.45 (-2.72, -0.18) and -0.99 (-1.98, -0.01) mmHg]. Higher β-cryptoxanthin concentrations were associated with lower SBP and DBP [-1.50 (-2.49, -0.51) and -1.20 (-1.95, -0.46) mmHg] at late-pregnancy. Individual vitamin E and their pattern were not associated with blood pressure or hypertension.

Conclusion: Higher perinatal α-, β-carotene and lutein concentrations are associated with lower blood pressure in women at late-pregnancy and post-pregnancy. Foods rich in these carotenoids such as red-, orange- and dark-green-colored vegetables might be beneficial for blood pressure during and after pregnancy.

Keywords: carotenoids, vitamin E, blood pressure, pregnancy, post-pregnancy

Main Text

Introduction

Oxidative stress has been shown to associate with several metabolic and cardiovascular diseases including hypertension in non-pregnant populations [1]. Increased oxidative stress enhanced production of endothelium-derived contractile factors, and reduced bioavailability of nitric oxide (vasodilator), leading to endothelial dysfunction and impaired vascular relaxation in hypertension [2].

Pregnancy is a state of increased oxidative stress, as a pro-oxidant environment plays an important role in the normal development of the placenta [3]. However, when oxidative stress surpasses the antioxidant defense in the placenta, the oxidative damage could lead to development of pregnancy complications [3]. Emerging evidence suggest increased oxidative stress to have implications in the pathology of pregnancy-induced hypertension (PIH) [4, 5].

Furthermore, there is accumulating evidence linking poor metabolic health during pregnancy with worse metabolic health later in life. Women who experienced PIH are at a higher risk of developing hypertension later in life [6, 7]. Pregnancy, a period of perturbed metabolic conditions, could benefit from early interventions to delay or prevent future metabolic diseases.

Carotenoids and vitamin E are postulated to have anti-oxidative properties, as such, play important roles in lowering blood pressure via reducing oxidative stress [8, 9]. Carotenoids are the yellow, orange, and red pigments synthesized by plants, which makes them reliable markers of fruit and vegetables intake [9]. Vitamin E comprised of tocopherols and tocotrienols (α, β, γ, and δ), from amongst which α-tocopherol is most biologically active and abundant in human tissues, and γ-tocopherol is the most common form found in diet [10]. Tocopherols and tocotrienols are found in plants with high lipid content, as such, plant-based edible oils are rich

sources of these E vitamers. Studies in non-pregnant populations have demonstrated the beneficial roles of carotenoids and vitamin E in blood pressure and hypertension. For example, observational studies found higher serum concentrations of α- and β-carotene, β-cryptoxanthin and total vitamin E to associate with lower blood pressure or incident hypertension [11-13], while intervention trials have demonstrated a beneficial effect of supplementation with lycopene on blood pressure [14].

In pregnant populations, intervention trials showed no beneficial effects of vitamin E supplementation in preventing pre-eclampsia [15], and one case-control study reported no association between higher total vitamin E intake and gestational hypertension [16]. These studies, however, did not examine individual forms of vitamin E. A meta-analysis of observational studies relating individual carotenoids with risk of preeclampsia reported potential beneficial associations [17], but these studies did not consider gestational hypertension which has implications on pregnancy and fetal outcomes as well as future metabolic risk similar to preeclampsia [7]. To the best of our knowledge, no studies have investigated individual carotenoids and forms of vitamin E with women's blood pressure levels during pregnancy and post-pregnancy, as well as risks of PIH and long-term hypertension.

There is increasing recognition that nutrients or dietary compounds do not act alone, but often in synergy with each other by enhancing or balancing each other's effects on health [18]. Deriving patterns of multiple dietary compounds has been suggested as a viable method to assess synergy among nutrients or dietary compounds [19, 20]. Therefore, we aimed to: 1) examine the associations of perinatal plasma carotenoids (α-, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene) and forms of vitamin E (α-, γ-, δ-tocopherols, and α-, γ-, δ-tocotrienols) concentrations at late-pregnancy, and 2) their combination using pattern analysis, with blood pressure and PIH at late-pregnancy and 4 years post-pregnancy.

Methods

Data for the present study were drawn from the Growing Up in Singapore Towards healthy Outcomes (GUSTO) study, which is a prospective mother-offspring cohort in Singapore aimed to examine influences of environmental factors during pregnancy on health and wellbeing of mothers and offspring later in life [21]. The GUSTO study research staffs recruited pregnant women (≥18 years) who were attending their first trimester (<14 weeks) antenatal dating ultrasound scan at two major public maternity units in Singapore (KK Women's and Children's Hospital and National University Hospital) during June 2009-September 2010. To be eligible, women needed to be Singapore citizens or permanent residents of Chinese, Malay or Indian ethnicity with homogenous parental ethnic background. Further details on the GUSTO study including the eligibility criteria for participation have been published [21]. The GUSTO study has received ethics approval from the Institutional Review Board of the two maternity units, 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 present study followed the group of women with data for plasma carotenoids and vitamin E at late-pregnancy, as well as blood pressure measurements at late-pregnancy and/or at 4 years post-pregnancy. Women self-reported to have existing hypertension prior to pregnancy were excluded from analysis.

Assays of plasma carotenoids and vitamin E concentrations

We used plasma samples collected around the time of delivery to reflect concentrations of carotenoids and vitamin E at late-pregnancy as the half-lives of carotenoids and vitamin E are 26-76 days [22] and 2-70 days [23] respectively. Non-fasting bloods samples were obtained from pregnant women (median gestation: 39 weeks', inter-quartile range: 38-40 weeks') at the hospitals by standard venipuncture technique up to 2 weeks before delivery or within 17 hours

after delivery. 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 [24] was used to determine plasma concentrations of carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin and lycopene) and vitamin E (α-, γ-, δ-tocopherols and tocotrienols). The precision of the method was examined using pooled and spiked plasma samples and the results were similar as published earlier [24], with the relative standard deviations (n = 6) of within day assays and between-day assays generally <10% and <15%, respectively.

Blood pressure measurements and PIH at late-pregnancy

To closely match the timing of carotenoids and vitamin E measurements as well as to account for PIH that developed near the end of pregnancy [25], peripheral systolic and diastolic blood pressures (SBP and DBP) of pregnant women measured during the last antenatal visit (median: 38 weeks' gestation, inter-quartile range: 37-39 weeks' gestation) were extracted from clinical obstetric records. Pregnancy-induced hypertension was defined as: 1) hypertension that appears *de novo* after 20 weeks' gestation without proteinuria (SBP ≥130mm Hg or DBP ≥90mm Hg according to the American College of Cardiology and American Heart Association to better identify women at risk of adverse events [26]), 2) identified from hospital delivery records to have a diagnosis of PIH, pre-eclampsia or eclampsia. Chronic hypertension (SBP ≥130mm Hg or DBP ≥90mm Hg at ≤20 weeks' gestation or identified from hospital delivery records to have a diagnosis of chronic hypertension) was not considered.

Blood pressure measurements and hypertension at 4 years post-pregnancy

Blood pressure of GUSTO women was measured again at 4 years post-pregnancy as several studies showed that the risk of hypertension following PIH was greatest within the 5 years post-

pregnancy [27, 28]. Peripheral SBP and DBP were measured twice from the right upper arm using a Dinamap CARESCAPE V100 (GE Healthcare, Milwaukee, WI) by trained research staffs. An average of both BP readings was calculated if the difference between readings was less than 10 mmHg; otherwise, a third reading was taken and the average of the three readings used instead. Hypertension was defined as having SBP ≥130 mm Hg or DBP ≥90 mm Hg according to the ACC and AHA [29] or self-reported to being diagnosed with hypertension post-pregnancy.

Covariates

Covariates were selected based on previous literature [12, 13, 16]. Information on women's age, ethnicity, highest education attained, and self-reported hypertension before pregnancy were collected during recruitment visit (<14 weeks' gestation). 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. Pre-pregnancy overweight or obesity was defined as BMI ≥23 kg/m^2 according to WHO Asian BMI classification [30]. Parity was retrieved from hospital delivery records. At the 26-28 weeks' gestation follow-up visit, moderate and vigorous physical activity in the past 7 days were self-reported using the International Physical Activity Questionnaire [31] and categorized as follows: never, <150 and ≥150 min/week; food and dietary supplements intakes were assessed using a single 24-hour recall by trained research staffs with the use of the 5-stage, multiple-pass interviewing technique [32, 33]. Total fat intake was estimated using nutrient analysis software (Dietplan, Forestfield Software, UK) based on a food composition database containing local foods [34]. The use of dietary supplements (yes/no) containing any amounts of preformed vitamin A (retinol or retinyl esters), carotenoids, vitamin E and its forms were considered.

143 Carotenoids and vitamin E patterns

144 To examine the influence of carotenoids and vitamin E in combination, we constructed patterns
145 from six carotenoids and four forms of vitamin E plasma biomarkers using principal component
146 (PC) analysis with varimax rotation. All forms of tocotrienols were summed to total
147 tocotrienols before being included in the PC analysis, as a high percentage of participants had
148 concentrations below the detection limit for each form of tocotrienols. The number of patterns
149 (or PCs) chosen to retain was determined by the break point of the Scree plot and eigenvalue
150 of >1.0 (determined *a priori*). The pattern score (or PC score) was calculated by summing the
151 standardized concentrations of biomarkers weighted by their PC loadings [35]. Each participant
152 received a pattern score for each derived pattern, with a higher score indicating greater
153 adherence to the derived pattern.

154 Statistical analysis

155 To enable comparison of effect estimates across exposures, we constructed standard deviation
156 scores [(observed value - mean)/SD] for concentrations of each form of carotenoids and
157 vitamin E as well as the scores of each pattern. We did not standardize blood pressure to
158 facilitate comparison of effect sizes with other studies.

159 Associations of individual carotenoids and vitamin E, and their patterns: 1) with continuous
160 measures of blood pressure were examined using linear regression, 2) with PIH or hypertension
161 at 4 years post-pregnancy examined using logistic regression. All models adjusted for the
162 following 8 covariates: women's age at delivery, ethnicity, education, pre-pregnancy
163 overweight and obesity, parity at recruitment, moderate-strenuous physical activity as well as
164 total dietary fat and dietary supplements intakes at mid-late-pregnancy.

The main analyses above were performed using all available data at each time point. Several sensitivity analyses were performed to determine the robustness of the associations observed: 1) restricted to participants with blood pressure data at both late-pregnancy and post-pregnancy, 2) restricted to participants with blood samples collected before delivery to ≤1 hour after delivery, and within ±1 week of blood pressure measurement.

Missing data for covariates were imputed using multiple imputation with chained equations (20 times) for the following confounding variables: n=5 highest education attained, n=58 pre-pregnancy overweight and obesity status, n=2 moderate-strenuous physical activity, and n=43 total fat and dietary supplements intakes. All analyses were performed using Stata version 14 (StataCorp LP, College Station, TX, USA). We considered two-sided $P<0.05$ to be statistically significant; adjusting for multiple testing is not relevant as the current study involves exploratory data analysis of observational data [36].

Results

A total of 1450 pregnant women participated at baseline, of which 1180 of them remained in the study until delivery and had singleton live births. The present analysis included women who provided sufficient blood for plasma carotenoids and vitamin E assays, as well as had blood pressure measurements for at least one time-point (n=684; n=676 last antenatal visit or n=473 at 4 years post-pregnancy) (**Figure 1**). Compared to the 684 women included in analysis, those who were excluded (n=496) were more likely to have attained lower educational levels, and less likely to engage in moderate-strenuous physical activity and to consume vitamin E supplements (**Supplementary Table 1**).

Sample characteristics

Table 1 presents the demographic and clinical characteristics of the 684 women with plasma carotenoids and vitamin E, and blood pressure data at either late-pregnancy or post-pregnancy, along with the average concentrations of each carotenoid and vitamin E. The women were on average 31.4 ± 5.0 years old at delivery. Majority of women were of Chinese ethnicity (58.5%), attained tertiary education (37.3%), and were primi- or multiparous (57%) at recruitment, and did not engage in moderate-strenuous physical activity (70%) at mid-late-pregnancy. Approximately 26.6% were overweight or obese before pregnancy. The average total fat intake was 70.0 ± 31.0g/day, and 73.6% of women were taking dietary supplements containing vitamin A/carotenoids whilst 27% were taking dietary supplements containing vitamin E at mid-late-pregnancy. A total of 244 (39%) reported to have a family history of high blood pressure, 118 (17.5%) were classified as having PIH and 31 (6.5%) were classified as having hypertension at 4 years post-pregnancy.

Carotenoid and vitamin E patterns

Three patterns were extracted (**Table 2**): Carotenoid pattern 1 (CP1) was characterized by higher concentrations of α-carotene, β-carotene and lutein; Vitamin E (VE) pattern comprised of all forms of tocopherols (γ-, δ- and α-tocopherols); and Carotenoid pattern 2 (CP2) was represented by higher concentrations of zeaxanthin, lycopene and β-cryptoxanthin. Total tocotrienols did not load highly (loading coefficient <0.30) into any pattern.

Associations of carotenoids and vitamin E with blood pressure and hypertension at late-pregnancy

The associations of individual plasma carotenoids and forms of vitamin E, and their patterns, with blood pressure and hypertension at late-pregnancy are presented in **Table 3**. Results were similar with or without adjustment for covariates (**Supplementary Table 2**); the following description reports associations adjusted for covariates.

When examined individually, higher β-carotene concentrations (per SD increment) were associated with 2.46 mmHg lower SBP (*P*=0.001) and 1.45 mmHg lower DBP (*P*=0.001) at late-pregnancy. Higher α-carotene and lutein concentrations were associated with 1.27 mmHg (*P*=0.015) and 1.45 mmHg (*P*=0.012) lower SBP at late-pregnancy, respectively; but both carotenoids were not individually associated with DBP at late-pregnancy.

The combination of α-, β-carotene and lutein showed inverse association with blood pressure at late-pregnancy, as reflected by higher scores (per SD increment) in CP1 associating with 2.36 mmHg lower SBP (*P*=0.001) and 1.37 mmHg lower DBP (*P*=0.001).

Additionally, 1-SD increment in β-cryptoxanthin concentrations were individually associated with 1.50 mmHg lower SBP (*P*=0.003) and 1.20 mmHg lower DBP (*P*=0.002) at late-pregnancy. No significant associations were observed for zeaxanthin and lycopene with blood pressure and hypertension at late-pregnancy, when examined individually.

Trending inverse associations were observed for the combination of zeaxanthin, lycopene, β-cryptoxanthin (CP2) with SBP (*P*=0.051) and DBP (*P*=0.069) at late-pregnancy.

With the exception of higher β-carotene concentrations individually associating with 35% lower odds of PIH (*P*=0.013), all other carotenoids as well as the carotenoid patterns were not significantly associated with PIH.

There were no significant associations for forms of Vitamin E, whether individually or in combination (VE pattern), with blood pressure and hypertension at late-pregnancy.

<u>Associations of carotenoids and vitamin E with blood pressure and hypertension at 4 years post-pregnancy</u>

The associations of individual plasma carotenoids and forms of vitamin E, and their patterns, with blood pressure and hypertension at 4 years post-pregnancy are also presented in Table 3 (unadjusted associations can be found in Supplementary Table 2).

When examined individually, higher β-carotene and lutein concentrations (per SD increment) were associated with 1.40 mmHg (*P*=0.015) and 1.56 mmHg (*P*=0.018) lower SBP as well as 0.90 mmHg (*P*=0.042) and 1.19 mmHg (*P*=0.019) lower DBP at 4 years post-pregnancy respectively. No significant associations were observed between α-carotene and SBP or DBP at 4 years post-pregnancy.

Higher scores (per SD increment) in CP1 (combination of α-, β-carotene and lutein) were associated with 1.45 mmHg lower SBP (*P*=0.025) and 0.99 mmHg lower DBP (*P*=0.049) at 4 years post-pregnancy.

Additionally, a 1-SD increment in zeaxanthin concentrations was individually associated with 1.29 mmHg lower SBP (*P*=0.024) at 4 years post-pregnancy, but not with DBP. There were no significant associations observed for lycopene and β-cryptoxanthin, when examined individually or in combination with zeaxanthin (CP2), with SBP and DBP at 4 years post-pregnancy.

All carotenoids and their patterns were not significantly associated with hypertension at 4 years post-pregnancy.

There were no significant associations for forms of Vitamin E, whether individually or in combination (VE pattern), with blood pressure and hypertension at 4 years post-pregnancy.

Sensitivity analysis

Effect estimates were in the same direction with similar magnitude as those observed in the main analyses when analyses were limited to the subset with 1) blood pressure at both late-

pregnancy and 4 years post-pregnancy (**Supplementary Table 3**), and 2) blood samples collected before delivery to ≤1 hour after delivery, and within ±1 week of blood pressure measurement (Supplementary Table 4).

Discussion

This study found that higher perinatal plasma concentrations of α-, β-carotene and lutein in combination were associated with lower maternal blood pressure at late-pregnancy and at 4 years post-pregnancy. When examined individually, we additionally found associations of higher β-carotene and lutein concentrations with lower blood pressure at both late-pregnancy and post-pregnancy, as well as associations between higher β-cryptoxanthin concentrations and lower blood pressure at late-pregnancy. Individual forms of vitamin E and their patterns were not associated with blood pressure or hypertension.

The associations observed when examining carotenoids individually with blood pressure aligned with evidence in non-pregnant populations. The effect estimates were also comparable. It is likely that the beneficial associations observed in pregnant and non-pregnant populationss share similar mechanisms; the anti-oxidative properties of carotenoids reduce excessive oxidative stress involved in the development of metabolic disorders [1]. We found higher concentrations of β-carotene and lutein to be associated with 1-2 mmHg lower SBP and/or DBP at late-pregnancy and 4 years post-pregnancy. Similarly, studies in non-pregnant populations consistently showed that higher β-carotene concentrations were associated with 1-3 mmHg lower SBP and DBP [12, 37, 38], and higher lutein concentrations were associated with, or supplementation with lutein resulted in, 1-3 mmHg lower DBP [37, 39]. We additionally found evidence supporting a beneficial association between lutein and SBP. On the other hand, the association between α-carotene and blood pressure was less consistent, with higher concentrations associating with lower SBP at late-pregnancy only. Evidence for α-

279 carotene in studies of non-pregnant populations is mixed, with two studies observing higher α-
280 carotene concentrations to associate with both lower SBP and DBP [13, 37], one study finding
281 an association with lower SBP but not DBP [12] and another observing an association with
282 lower DBP but not SBP [40].

283 Importantly, when the above carotenoids were examined in combination (CP1), we observed
284 consistent significant inverse associations with both SBP and DBP at both time points. This
285 observation supports the value of examining carotenoids in combination, as their synergistic
286 activities may influence blood pressure more consistently. Studies have shown a 1-2 mmHg
287 population-wide reduction in SBP or DBP to be associated with lower risks or fewer cases of
288 cardiovascular diseases such as 6% and 15% lower risks of coronary heart disease and stroke
289 respectively [41], or 13-20 fewer heart failure events per 100 000 person-years [42]; shedding
290 some light on the significance of the effect estimates observed for CP1 with blood pressure.

291 Of note, our study raises the possibility that adhering to a dietary pattern high in α-, β-carotene
292 and lutein during pregnancy may be beneficial not only on blood pressure during pregnancy
293 but also in the longer term post-pregnancy. Possible mechanisms underlying the association
294 observed with blood pressure post-pregnancy could be through a lowered blood pressure during
295 pregnancy [43], or a result of continued adherence to a dietary pattern high in these carotenoids
296 post-pregnancy [44]. Further studies specifically designed to address this will be needed.

297 Additionally, higher β-cryptoxanthin concentrations, when examined individually, were
298 associated with SBP and DBP at late-pregnancy; but no associations were observed with blood
299 pressure at 4 years post-pregnancy. We found higher zeaxanthin concentrations to associate
300 with lower SBP post-pregnancy, but several studies in non-pregnant populations did not
301 observe associations between zeaxanthin and blood pressure [13, 37, 40]. Results may differ
302 because we measured zeaxanthin concentrations in the perinatal period. On the contrary, we

303 did not observe significant associations between lycopene and blood pressure at late-pregnancy
304 and post-pregnancy, despite evidence showing lycopene supplementation to reduce SBP in
305 non-pregnant populations [14, 45]. However, positive findings from intervention trials may
306 have resulted from having highly motivated individuals and consumption of a higher dosage
307 of lycopene; which does not reflect the level and variation in free-living populations [46].

308 When zeaxanthin, lycopene and β-cryptoxanthin were examined in combination, there were
309 weak associations with lower SBP and DBP at late-pregnancy, likely driven by β-
310 cryptoxanthin. As carotenoids with higher loadings in this pattern (zeaxanthin and lycopene)
311 did not demonstrate individual significant associations with blood pressure at late-pregnancy,
312 this has likely attenuated the associations observed for β-cryptoxanthin when combined.

313 Carotenoids are mainly found in fruit and vegetables [47]. Likewise, we have shown in a
314 previous study that women with higher concentrations of carotenoids around the time of
315 delivery have higher intakes of fruit and vegetables at 26-28 weeks' gestation [48].
316 Furthermore, plasma carotenoids concentrations did not differ significantly by intake of
317 vitamin A/carotenoids supplements (**Supplementary Table 4**), although this finding is limited
318 by a lack of details on dosage and frequency of intake. Our findings aligned with
319 recommendations in non-pregnant populations which encourage a diet high in fruit and
320 vegetables for lower blood pressure such as the DASH (Dietary Approaches to Stop
321 Hypertension) diet [49], suggesting that similar dietary recommendations can be adopted
322 during pregnancy for lower blood pressure. Additionally, when considered together with
323 existing evidence showing beneficial roles of carotenoids in other health outcomes (e.g.
324 advanced macular degeneration, cardiometabolic diseases and cancer) [50], suggest the need
325 to propose carotenoid-specific intake recommendations beyond recommending more fruits and
326 vegetables intakes. Nevertheless, replication of our findings in other cohorts is required before
327 recommendations to increase carotenoids and vitamin E during pregnancy can be made. We

328 observed GUSTO pregnant women to have lower mean concentrations of α-carotene (0.12 vs
329 0.22 μmol/L), β-cryptoxanthin (0.45 vs 0.47 μmol/L), and lutein (0.46 vs 0.61-0.65 μmol/L)
330 compared to other pregnant cohorts [51, 52], which could be due to less than half of GUSTO
331 women meeting the daily recommendations for fruit and vegetables intake [53]; even with 74%
332 of women consuming carotenoids-containing supplements, not all supplements contain the full
333 range of carotenoids and the dosages required to achieve the desired concentrations. The
334 concentration of β-carotene is similar, however, to other pregnant cohorts, likely due to most
335 women taking dietary supplements which is a significant source of β-carotene.

336 The individual forms of Vitamin E and their combination were not associated with blood
337 pressure and hypertension at late-pregnancy and post-pregnancy. This is similar to findings
338 from Wang *et al.* [16] who reported no association between dietary vitamin E intake
339 (preconception and across pregnancy trimesters) and PIH. A study in non-pregnant populations
340 also did not find significant associations between serum α-tocopherol concentrations and blood
341 pressure among women [12]. The null association between α-tocopherol and blood pressure
342 may be due to lack of variation in α-tocopherol concentrations as majority of GUSTO pregnant
343 women (98%) had α-tocopherol concentrations above 30 μmol/L – a level proposed to be
344 beneficial for human health [54]. No studies have examined γ- and δ-tocopherols or
345 tocotrienols and we could not compare our results.

346 To the best of our knowledge, this is the first study to relate perinatal plasma concentrations of
347 individual carotenoids and forms of vitamin E as well as their combinations to blood pressure
348 during pregnancy and post-pregnancy. Identifying biomarker patterns is advantageous as they
349 capture the interactive effect of dietary compounds in combination. Unlike intervention trials,
350 the observational nature of this study depicts concentrations of carotenoids and vitamin E in
351 free-living populations.

Several limitations of our study must be noted. Blood pressure measurements at late-pregnancy were taken slightly before the blood samples collection for plasma carotenoids assays (mean ± SD time difference = 6 ± 8 days), as such the temporality of the associations cannot be established. Our study could benefit from having repeated measures of carotenoid and vitamin E concentrations at early-mid pregnancy and post-pregnancy to confirm the robustness and temporality of the associations. The use of non-fasting plasma samples may have introduced systematic bias but studies have shown non-significant differences in carotenoids concentrations pre- and post-meal [55, 56]. In a study relating plasma carotenoids to cognition, effect estimates remained similar whether including or excluding non-fasting samples [57]. Differences in blood pressure measurement protocols at late-pregnancy (from obstetric records) and at 4 years post-pregnancy (measured at GUSTO clinic visit) may contribute to differences in findings between time points. While our analysis adjusted for dietary (total fat) and supplements intakes, the use of a 24-hour recall reflects only a single day's intake rather than usual diet, while supplements lack details on the combinations of vitamins/carotenoids included as well as dosage and frequency of intake. We acknowledged the presence of retention bias as those included in the analysis attained higher educational levels and have a healthier lifestyle (e.g. a higher percentage of participants engaged in moderate-strenuous physical activity and consumed dietary supplements), but this will likely result in an underestimation of associations [58]. Misclassification of hypertension cases before pregnancy is possible because it is self-reported. As with any observational study, residual confounding is likely present.

In conclusion, our study showed higher perinatal maternal concentrations of α-, β-carotene and lutein in combination, are associated with lower maternal blood pressure at late-pregnancy and post-pregnancy. These carotenoids are abundant in red-, orange- and dark-green-colored vegetables, suggesting a potential benefit of encouraging greater consumption of these types

376 of vegetables in pregnant women. Further investigations are required to explore the temporal
377 relationship of β-cryptoxanthin with blood pressure.

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Authors' contributions: JSL, SES, JKYC, SYC and MFFC designed the research. JSL performed statistical analysis and wrote the manuscript. 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; WLY and YSL designed the protocol and supervised collection of blood pressure data. KHT,

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Table 1: Characteristics[a] of participants for the associations of plasma carotenoids and vitamin E concentrations with blood pressure in the Growing Up in Singapore Towards healthy Outcomes study (n=684[b])

Age at delivery, year, mean ± SD	31.4 ± 5.0
Ethnicity, n (%)	
Chinese	400 (58.5)
Malay	158 (23.1)
Indian	126 (18.4)
Highest education, n (%)	
≤Secondary	208 (30.6)
Post-secondary	218 (32.1)
University	253 (37.3)
Parity, n (%)	
Nulliparous	294 (43.0)
Primi- / Multiparous	390 (57.0)
Pre-pregnancy overweight/obese (BMI≥23.0 kg/m^2), n (%)	170 (26.6)
Moderate-strenuous physical activity, n (%)	
Never	475 (70.0)
<150 min/week	138 (20.3)
≥150 min/week	66 (9.7)
Total fat intake, g/day, mean ± SD	70.0 ± 31.0
Intake of supplements containing, n (%)	
Vitamin A/carotenoids	472 (73.6)
Vitamin E	173 (27.0)
Family history of high blood pressure, n (%)	244 (39.0)
Systolic blood pressure at last antennal visit, mean ± SD	119 ± 13
Diastolic blood pressure at last antennal visit, mean ± SD	71 ± 10
Pregnancy-induced hypertension, n (%)	118 (17.5[c])
Pre-eclampsia, n (%)	15 (2.2[c])
Systolic blood pressure at 4 years post-pregnancy, mean ± SD	110 ± 13
Diastolic blood pressure at 4 years post-pregnancy, mean ± SD	66 ± 9
Hypertension at 4 years post-pregnancy, n (%)	31 (6.5[d])
Plasma carotenoid 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
Total tocotrienols (α-, γ-, δ-)	0.15 ± 0.10

[a] Characteristics were based on data obtained during pregnancy unless otherwise specified.

[b] Missing data: n=5 highest education, n=58 pre-pregnancy overweight/obese status, n=9 family history, n=2 moderate-strenuous physical activity, n=43 total fat intake and dietary supplements intake
[c] Based on 676 women with blood pressure measurements at last antenatal visit.
[d] Based on 473 women with blood pressure measurements at 4 years post-pregnancy

Table 2: Carotenoids and vitamin E biomarker patterns construction: Pattern structure and variance explained[a]

Carotenoids/Vitamin E	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
total tocotrienols			
% variance explained by each pattern	21.9	20.5	17.1
Cumulative % of variance explained	21.9	42.4	59.5

[a] Values are loading coefficients derived from principal component analysis. Absolute values <0.30 were not listed for simplicity.

Table 3: Associations of individual carotenoids and forms of vitamin E, and their patterns with blood pressure and hypertension at late-pregnancy and 4 years post-pregnancy in the Growing Up in Singapore Towards healthy Outcomes study[a,b,c]

	Late-pregnancy (n=676)				4 years post-pregnancy (n=473)				Pregnancy-induced hypertension (n=118)		Hypertension post-pregnancy (n=31)	
	Systolic BP		Diastolic BP		Systolic BP		Diastolic BP					
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Carotenoids[d]												
Individual concentrations												
α-carotene	-1.27 (-2.30, -0.25)	0.015	-0.54 (-1.32, 0.23)	0.168	-0.74 (-1.90, 0.42)	0.212	-0.40 (-1.30, 0.50)	0.376	0.99 (0.79, 1.23)	0.922	0.98 (0.59, 1.64)	0.951
β-carotene	-2.46 (-3.48, -1.43)	0.001	-1.45 (-2.22, -0.67)	0.001	-1.40 (-2.53, -0.27)	0.015	-0.90 (-1.80, -0.02)	0.042	0.65 (0.46, 0.91)	0.013	0.59 (0.25, 1.39)	0.239
Lutein	-1.45 (-2.57, -0.33)	0.012	-0.70 (-1.54, 0.15)	0.107	-1.56 (-2.85, -0.27)	0.018	-1.19 (-2.19, -0.20)	0.019	0.85 (0.66, 1.10)	0.235	0.87 (0.47, 1.57)	0.632
Carotenoid pattern 1	-2.36 (-3.47, -1.26)	0.001	-1.37 (-2.21, -0.53)	0.001	-1.45 (-2.72, -0.18)	0.025	-0.99 (-1.98, -0.01)	0.049	0.84 (0.65, 1.09)	0.187	0.88 (0.48, 1.60)	0.688
Individual concentrations												
Zeaxanthin	-0.86 (-1.87, 0.15)	0.095	-0.66 (-1.42, 0.10)	0.090	-1.29 (-2.41, -0.17)	0.024	-0.68 (-1.57, 0.21)	0.118	0.98 (0.80, 1.21)	0.860	1.09 (0.74, 1.62)	0.663
Lycopene	-0.16 (-1.15, 0.84)	0.754	0.18 (-0.57, 0.93)	0.635	-0.53 (-1.65, 0.59)	0.353	-0.56 (-1.41, 0.30)	0.194	1.07 (0.89, 1.30)	0.480	1.22 (0.88, 1.70)	0.247
β-cryptoxanthin	-1.50 (-2.49, -0.51)	0.003	-1.20 (-1.95, -0.46)	0.002	-0.77 (-1.84, 0.28)	0.154	-0.51 (-1.32, 0.31)	0.221	0.91 (0.73, 1.14)	0.420	1.13 (0.73, 1.73)	0.594
Carotenoid pattern 2	-1.10 (-2.00, 0.001)	0.051	-0.70 (-1.45, 0.06)	0.069	-0.95 (-2.06, 0.15)	0.090	-0.65 (-1.50, 0.20)	0.134	0.95 (0.77, 1.18)	0.651	1.20 (0.81, 1.77)	0.355
Vitamin E[e]												
Individual concentrations												
γ-tocopherol	0.11 (-0.89, 1.11)	0.839	0.32 (-0.44, 1.06)	0.414	-0.40 (-1.52, 0.71)	0.488	-0.47 (-1.35, 0.42)	0.305	1.05 (0.80, 1.38)	0.743	1.04 (0.68, 1.59)	0.861
δ-tocopherol	0.39 (-0.63, 1.41)	0.452	0.69 (-0.10, 1.43)	0.096	0.14 (-1.02, 1.30)	0.813	0.08 (-0.83, 0.99)	0.861	1.16 (0.90, 1.50)	0.257	0.99 (0.62, 1.58)	0.969
α-tocopherol	-0.62 (-1.62, 0.37)	0.228	-0.12 (-0.88, 0.62)	0.755	0.87 (-0.27, 2.01)	0.147	0.52 (-0.36, 1.40)	0.259	0.86 (0.64, 1.15)	0.304	0.96 (0.62, 1.49)	0.852
Vitamin E pattern	0.11 (-0.89, 1.10)	0.833	0.44 (-0.30, 1.19)	0.241	0.06 (-1.09, 1.22)	0.924	-0.12 (-1.01, 0.77)	0.792	1.05 (0.80, 1.37)	0.746	1.00 (0.64, 1.58)	0.988
Individual concentrations												
Total tocotrienols	0.25 (-0.73, 1.22)	0.627	0.15 (-0.58, 0.89)	0.689	-0.08 (-1.14, 0.97)	0.886	-0.22 (-1.02, 0.59)	0.598	1.05 (0.82, 1.35)	0.685	1.09 (0.75, 1.59)	0.653

[a] BP, blood pressure; Carotenoid pattern 1, α-, β-carotene and lutein; Carotenoid pattern 2, zeaxanthin, lycopene and β-cryptoxanthin; Vitamin E pattern, γ-, δ-, α-tocopherols

[b] Effect estimates are per SD increment in pattern scores or individual carotenoids and vitamin E concentrations

[c] All models adjusted for age, ethnicity, education, pre-pregnancy overweight and obesity, parity at recruitment, and the following at mid-late-pregnancy: moderate-strenuous physical activity, total fat intake, and intake of any supplement containing [d]vitamin A/carotenoids, [e]vitamin E.

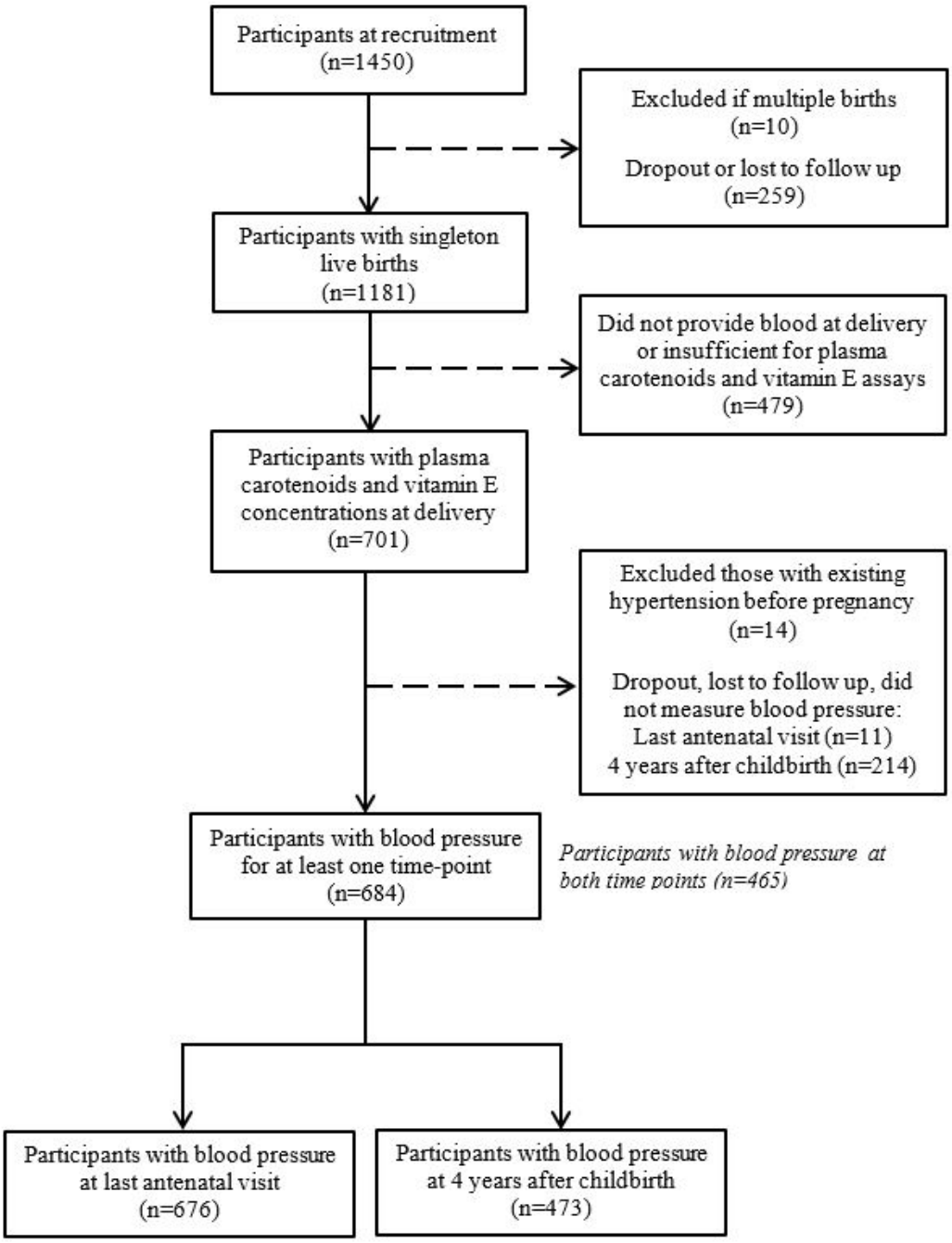

Fig. 1 Flowchart of participants included in the analysis of plasma carotenoids and vitamin E concentrations with blood pressure at late-pregnancy and 4 years post-pregnancy