Maternal Serum Antioxidants in Mid Pregnancy and Risk of Preterm Delivery and Small for Gestational Age Birth: Results from a Prospective Pregnancy Cohort

Jean M. Kerver, PhD, MSc, RD,[1] Claudia B. Holzman, PhD, DVM,[1] Yan Tian, PhD,[2]
Bertha L. Bullen, PhD,[1] Rhobert W. Evans, PhD,[3] and Jamil B. Scott, PhD[4]

Abstract

Background: Preterm delivery (PTD) and poor fetal growth are major contributors to neonatal mortality and morbidity that can extend from birth onward. Although overt maternal nutrient deficiencies are associated with adverse pregnancy outcomes, such deficiencies are rare in developed countries. However, some evidence suggests that even within the normal range, higher levels of antioxidant nutrients are protective against adverse pregnancy outcomes.

Materials and Methods: Using data from the prospective Pregnancy Outcomes and Community Health (POUCH) Study ($n = 301$ preterm; $n = 246$ term), we examined associations between maternal blood levels of selected antioxidants and pregnancy outcomes. Serum collected at 16–27 weeks' gestation was analyzed for carotenoids, retinol, and α- and γ-tocopherol. Using weighted polytomous regression, these nutrient concentrations were assessed in relation to (1) PTD (<37 weeks gestation) overall and grouped as spontaneous or medically indicated; and (2) small for gestational age (SGA) defined as birthweight-for-gestational age <10th percentile of a national reference population.

Results: Women with total serum carotenoids in the upper quartile (Q4) had significantly lower odds of medically indicated PTD compared with women in the lower quartiles (Q1–Q3) even after adjustment for maternal characteristics (aOR = 0.4; 95% CI: 0.2–0.9). Odds ratios for SGA were consistently ≤0.5 among women with any of the serum nutrients in Q4 as compared with Q1–Q3, but final models did not reach statistical significance.

Conclusion: Results support the possibility that high maternal serum antioxidants and/or the larger dietary or lifestyle pattern they represent may play a protective role in preventing adverse pregnancy outcomes.

Keywords: pregnancy, antioxidant vitamins, carotenoids, preterm, birthweight

Introduction

IN THE UNITED STATES, PRETERM DELIVERY (PTD) (<37 weeks completed gestation) and poor fetal growth (as estimated by small for gestational age, SGA) occur in ∼9.9% and 8.2% of live births, respectively.[1,2] Both PTD and SGA, which can be overlapping, represent strong predictors of neonatal mortality and morbidity.[3,4] Globally, risk factors for both PTD and SGA include numerous maternal micronutrient deficiencies,[5,6] although such deficiencies are not common in developed countries. In the United States, risk factors for these adverse birth outcomes include African American race/ethnicity, maternal smoking, nulliparity, and lean maternal body mass index.[7]

While the biological causes of PTD and SGA are not well understood, nutrient status potentially affects several of the putative mechanisms, including infection, uterine muscle contractility, inflammation, and oxidative stress.[8] The demands of pregnancy impose substantial oxidative and inflammatory stress on the maternal system.[9] Antioxidants can partially

[1]Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, Michigan, USA.
[2]Michigan Department of Health and Human Services, Lansing, Michigan, USA.
[3]Department of Epidemiology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.
[4]Division of Public Health, Michigan State University, Flint, Michigan, USA.

mitigate damage from oxidative reactions originating from both physiological (*e.g.,* pregnancy) and pathological (*e.g.,* smoking) pathways.[10] Additionally, antioxidants can enhance immune responses and decrease inflammatory responses. Because of these properties, antioxidants have been hypothesized to play a part in the prevention of adverse pregnancy conditions such as preeclampsia[11] and pregnancy outcomes, including PTD[12,13] and SGA.[14–17]

In this study, we focus on serum carotenoids and vitamins A and E, which are all fat-soluble compounds with antioxidant properties. Vitamin A is required for cell differentiation and growth, and its deficiency (overt or subclinical) has been associated with an increased risk of PTD and maternal anemia.[18] Vitamin E is essential for normal reproductive physiology and its deficiency is linked to PTD.[19] Vitamins A and E are essential dietary micronutrients, and some carotenoids have provitamin A activity (*i.e.,* biologic conversion to vitamin A).[20,21]

Serum levels of carotenoids and other antioxidants reflect dietary intake and individual host factors (*e.g.,* absorption, transport, and metabolism).[22–25] Serum carotenoids are notable because they are more reflective of dietary intake than other nutrients that are homeostatically controlled. The best food sources of carotenoids are yellow/orange and dark green vegetables,[26] whereas vitamin A is derived mainly from animal products (meat and dairy), and vitamin E from vegetable oils and nuts.[27] To the extent that serum levels of antioxidant nutrients represent dietary intake, they represent a modifiable factor in gravid health.

This study assesses the relations among selected fat-soluble serum antioxidant vitamins and two pregnancy outcomes, PTD and SGA (used in this study as a proxy for poor fetal growth). Data are from a prospective pregnancy cohort—the Pregnancy Outcomes and Community Health (POUCH) Study. We hypothesized that serum concentrations of selected antioxidants (*i.e.,* carotenoids, vitamin A [measured as retinol], and vitamin E [measured as α- and γ-tocopherol]) would be inversely associated with risk of PTD (overall and grouped by spontaneous or medically indicated) and SGA (defined as birthweight-for-gestational age <10th percentile of a national reference population).

Materials and Methods

Study design: POUCH study cohort and subcohort

The POUCH Study is a prospective pregnancy cohort designed to elucidate pathways to adverse pregnancy outcomes. The study received Institutional Review Board approval at Michigan State University, the Michigan Department of Community Health (now known as the Michigan Department of Health and Human Services), and nine community hospitals. Women were provided initial information about the POUCH Study through their prenatal care providers. Eligibility criteria included: 16th–27th week of pregnancy, maternal serum alpha-fetoprotein (MSAFP) screening, prenatal care at one of 52 clinics in 5 Michigan communities between 1998 and 2004, singleton pregnancy with no known chromosomal abnormality or birth defect, maternal age ≥15 years, no prepregnancy diabetes mellitus, and ability to complete study interviews in English.

Women who met the eligibility criteria and expressed interest in the study constituted the sampling frame. A stratified random sample was used to oversample African American women and those with unexplained high MSAFP levels (≥ 2 multiples of the median), as these groups are known to be at increased risk for adverse pregnancy outcomes.[28] Of the 3,038 women recruited into the cohort, 3,019 (99%) were followed through delivery. A stratified comparison of maternal characteristics between POUCH Study participants and all mothers delivering within the five study communities during the same period (data derived from birth certificates) showed that the study sample was predominantly reflective of women with live births in the communities at large.

To conserve resources, some costly data elements (*e.g.,* placental examinations, medical record abstraction, assays of stored biologic samples) were obtained from only a subsample of POUCH Study participants, referred to as the subcohort. The subcohort of 1,371 women includes all PTD, all pregnancies with high MSAFP and a stratified random sample of term deliveries with oversampling of African American women as described in detail elsewhere.[29] To account for the cohort and subcohort sampling strategy, inverse-probability sampling weights are used in all POUCH Study analyses. For example, African American women, women with high MSAFP, and women with PTD who were oversampled into the subcohort are assigned a 'smaller weight' so that they represent their proportion in the eligible population agreeing to participate in the original POUCH Study.

Data collection and variable specification

At enrollment (16–27 weeks' gestation), participants met with a study nurse who obtained consent, conducted interviews, measured body weight, and collected biologic specimens, including nonfasting blood. Participants also completed self-administered questionnaires. Interviews and questionnaires provided the covariate data used in this study, that is, maternal age, self-identified race/ethnicity, medical insurance (Medicaid yes/no), maternal education level, parity, and smoking history. The number of women whose race/ethnicity was neither non-Hispanic White nor African American were too few to constitute additional groups. Their other maternal characteristics and pregnancy outcomes most closely paralleled that of non-Hispanic Whites in the POUCH Study and were therefore combined with this group in the race/ethnicity variable.

Pregnancy outcomes were determined from labor and delivery records by trained study staff. PTD was defined as birth before 37 completed weeks' gestation. Gestational week at delivery was calculated using date of last menstrual period (LMP). Alternatively, in instances where the gestational age estimate based on the LMP differed from the ultrasound estimate by more than 2 weeks, the ultrasound estimate was used. Early ultrasound (≤ 25 weeks) data were available for 93% of the participants.

PTD was categorized into subtypes based on clinical presentations noted in the medical record review: (1) spontaneous labor (defined as cervix dilated ≥2 cm and regular contractions) or preterm premature rupture of the membranes (PPROM—defined as rupture of membranes before or simultaneous with the onset of labor); and (2) medically indicated, defined as delivery that began by induction or Cesarean section in the absence of spontaneous labor or rupture of

membranes as an initiating event. Using a national birth-weight reference population that is sex specific for singleton births,[30] birthweight for gestational age was modeled as SGA at <10%tile, appropriate for gestational age (AGA), and large for gestational age (LGA) at >90%tile.

Laboratory analyses

Nonfasting blood samples were drawn at enrollment (range 16–27 weeks' gestation), centrifuged within 45 minutes of collection, aliquoted (1 mL), and stored at −80°C. Subcohort stored serum specimens were shipped to the Heinz Nutrition Laboratory at the University of Pittsburgh for multiple assays, including lipid analyses described elsewhere.[31] All serum specimens were transported on dry ice and protected from light. Procedures conducted in the laboratory were performed under subdued lighting in amber tubes. The final assays completed were of the antioxidant nutrients presented in this study, which were performed on specimens with sufficient remaining serum after two freeze/thaw cycles: all preterm (n = 301) and a random sample of term (n = 246). The sample size for term births was dictated by available resources (Fig. 1).

Antioxidants were measured using an isocratic high-performance liquid chromatography (HPLC) procedure with UV detection developed by Chromsystems Diagnostics (Munich, Germany). The HPLC consists of Waters (Milford, MA) modules: two 515 pumps, a 717 Plus autosampler, and a 2,996 photodiode array detector. The tocopherols, retinol, and carotenoids were detected at 290, 325, and 453 nm, respectively. Calibrators and controls were run with each set of samples and the intra-assay coefficients of variation ranged from 3.1% to 8.0%.

Statistical analyses

As noted above, the POUCH Study cohort and subcohort were constructed by probability sampling from the original sampling frame. Sampling weights were used in all analyses to produce results that are generalizable to the sampling frame and maintain correct standard errors for hypothesis testing and confidence intervals. Statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

Descriptive statistics for maternal characteristics and serum individual and total carotenoids, retinol, and α- and γ-tocopherol concentrations were calculated using mean and 95% confidence intervals (CI) for continuous variables and percentages for categorical variables. We assessed correlations between individual carotenoids and summed individual carotenoid concentrations to obtain total carotenoids. Distributions of the serum nutrient concentrations were skewed; therefore, all analyses used least squares (LS) geometric mean of log-transformed serum nutrient concentrations (SAS SurveyReg procedure, incorporating sampling weights). For presentation, results are expressed on the original scale after back transforming the LS geometric mean (± 95% CI).

We applied weighted polytomous regression (Proc Surveylogistic) to examine the odds of pregnancy outcomes associated with maternal serum nutrient concentrations. Our gestational age range (16–27 weeks) was limited to mid pregnancy, but because serum antioxidant status has been shown to vary over the course of pregnancy,[32] we included gestational age at serum collection in all models. Additionally, maternal race/ethnicity was included in all models to account for potential confounding. Initial models included other covariates for which the mean nutrient concentrations were significantly different among maternal characteristic groups, that is, age, education, Medicaid enrollment, parity, season of enrollment, smoking status at enrollment, and BMI at enrollment. Final adjusted models included only covariates that met the following prespecified criteria: (1) remained significant in the multivariable model (along with gestational age at serum collection and race/ethnicity); and (2) removal did not alter the other main effect estimates by >10%. Serum nutrient concentrations were analyzed in quartiles using values from women with normal MSAFP and term deliveries to determine quartile cutpoints (<25th percentile = Q1; 26th – 49.9th percentile = Q2; 50th– 74.9th percentile = Q3; ≥ 75th percentile = Q4). After finding a protective effect in the highest quartile indicative of a threshold effect, we dichotomized the distribution of each nutrient (Q4 vs. Q1–Q3 combined).

In a second series of analyses, final adjusted models for carotenoids and tocopherols incorporated an additional covariate, serum total cholesterol, to account for potential
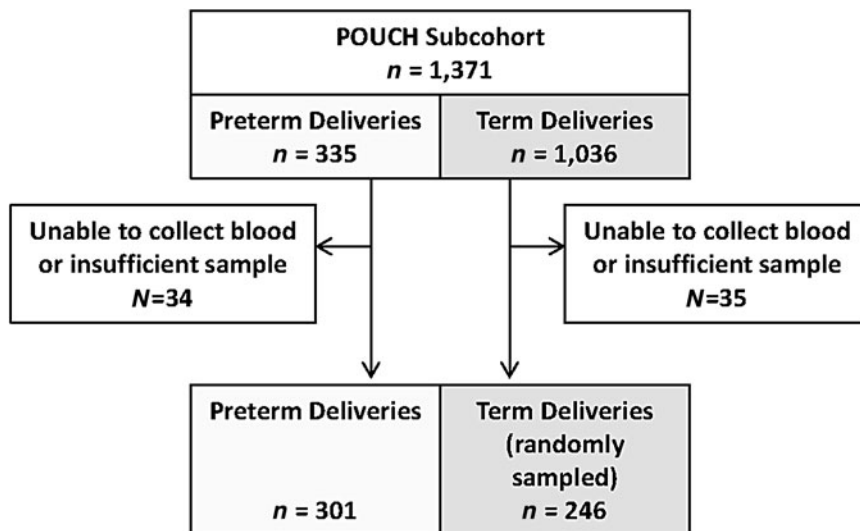
FIG. 1. Flow Diagram of POUCH Study Participants Eligible for Maternal Serum Antioxidant Analyses. POUCH, pregnancy outcomes and community health.

confounding by blood lipid concentrations. The rationale for adjusting for serum cholesterol is to avoid under- or over-estimating concentrations of the lipophilic compounds, carotenoids, and tocopherols, which circulate in blood primarily transported by lipoproteins.[32–35] For example, tocopherols have no specific plasma transport protein, but they are transported in plasma lipoproteins and their distribution parallels that of total lipids.[35] Plasma vitamin E levels have been shown to increase during pregnancy in response to the physiologic hyperlipidemia of pregnancy, such that the ratio of tocopherol to β-lipoprotein remains unchanged, supporting a role of vitamin E as a protective molecule inhibiting lipoprotein oxidation.[33]

Results

Maternal characteristics reflected the source population in this community-based Michigan cohort with 43% using Medicaid insurance (Table 1). Most participants (56%) were between 20 and 29 years at enrollment, with 16% under 20 years and 28% age 30 years or older. Race/ethnicity distribution was 25% African American and 75% White/Other. Slightly over half (52%) had more than a high school education, whereas 26% had a high school degree or equivalent and 22% had an education level of less than 12th grade.

Most (68%) were enrolled between 20 and 24 weeks gestation and 47% were nulliparous. The reasons specified for

TABLE 1. MEAN SERUM ANTIOXIDANT BIOMARKER CONCENTRATIONS BY MATERNAL CHARACTERISTICS IN THE POUCH STUDY (N = 547)[1]

		Total carotenoids (ng/mL)		Retinol (µg/mL)		α-Tocopherol (µg/mL)		γ-Tocopherol (µg/mL)	
	n (%)[2]	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Maternal age (y)									
<20[3]	98 (15.5)	942	(843–1053)	0.28	(0.25–0.31)	8.56	(7.99–9.16)	1.49	(1.31–1.70)
20–29	301 (56.2)	849	(782–922)	0.31	(0.29–0.33)	8.86	(8.42–9.31)	1.63	(1.51–1.76)
≥30	148 (28.3)	892	(782–1017)	**0.33[4]**	**(0.30–0.35)**	9.35	(8.69–10.1)	1.47	(1.30–1.66)
Race/ethnicity									
White/Others[3]	342 (75.4)	875	(809–947)	0.33	(0.31–0.34)	9.27	(8.85–9.70)	1.54	(1.44–1.66)
African American	205 (24.6)	874	(810–943)	**0.26[4]**	**(0.25–0.27)**	**8.03[4]**	**(7.69–8.39)**	1.62	(1.50–1.75)
Maternal education (y)									
<12[3]	135 (21.7)	851	(756–959)	0.29	(0.28–0.32)	8.40	(7.92–8.92)	1.63	(1.45–1.84)
12	160 (26.1)	786	(702–879)	0.31	(0.29–0.33)	8.56	(8.01–9.14)	1.69	(1.51–1.88)
>12	252 (52.2)	934	(853–1022)	0.32	(0.30–0.33)	**9.39[4]**	**(8.88–9.92)**	1.48	(1.36–1.60)
Medicaid[6]									
No[3]	250 (56.6)	905	(829–988)	0.32	(0.30–0.34)	9.30	(8.83–9.80)	1.51	(1.39–1.64)
Yes	296 (43.4)	837	(768–912)	**0.29[5]**	**(0.28–0.31)**	**8.51[4]**	**(8.10–8.93)**	1.63	(1.51–1.76)
Gestational age at enrollment (wks)									
<20[3]	103 (16.1)	823	(737–920)	0.33	(0.31–0.36)	8.35	(7.70–9.04)	1.74	(1.55–1.95)
20–24	371 (67.9)	870	(806–940)	**0.31[5]**	**(0.29–0.32)**	8.93	(8.58–9.30)	**1.50[5]**	**(1.40–1.62)**
25–27	73 (16.0)	951	(801–1129)	0.29	(0.26–0.33)	**9.67[5]**	**(8.57–10.90)**	1.65	(1.44–1.90)
Parity[6]									
None[3]	242 (46.6)	988	(907–1076)	0.31	(0.29–0.33)	9.29	(8.81–9.80)	1.41	(1.31–1.52)
≥ 1	304 (53.4)	**786[4]**	**(724–854)**	0.31	(0.29–0.33)	8.66	(8.25–9.09)	**1.71[4]**	**(1.57–1.86)**
Enrollment date									
Spring[3]	139 (25.2)	906	(813–1009)	0.33	(0.30–0.35)	9.04	(8.45–9.67)	1.57	(1.48–1.67)
Summer	150 (27.3)	915	(816–1026)	0.30	(0.28–0.32)	9.14	(8.47–9.86)	1.54	(1.24–1.83)
Fall	125 (22.7)	848	(745–965)	0.31	(0.29–0.34)	8.73	(8.26–9.23)	1.59	(1.43–1.76)
Winter	133 (24.9)	828	(718–954)	0.30	(0.28–0.33)	8.85	(8.13–9.63)	1.56	(1.37–1.77)
Smoking at enrollment									
No[3]	454 (88.9)	886	(827–948)	0.31	(0.30–0.32)	9.00	(8.65–9.37)	1.57	(1.48–1.67)
Yes	93 (11.1)	792	(707–888)	0.28	(0.25–0.32)	8.51	(7.88–9.19)	1.51	(1.24–1.83)
BMI (kg/m²) at enrollment									
Quartile 1[3]	137 (24.2)	1030	(900–1178)	0.29	(0.27–0.32)	8.76	(8.14–9.42)	1.27	(1.13–1.43)
Quartile 2	136 (27.9)	980	(889–1080)	0.31	(0.29–0.33)	9.56	(9.00–10.15)	**1.52[5]**	**(1.37–1.69)**
Quartile 3	137 (26.0)	910	(839–986)	0.33	(0.30–0.35)	9.60	(8.87–10.38)	**1.75[4]**	**(1.57–1.95)**
Quartile 4	137 (21.9)	**604[4]**	**(532–687)**	0.31	(0.29–0.33)	**7.75[4]**	**(7.32–8.21)**	**1.78[4]**	**(1.59–1.99)**

[1]Least square geometric mean ± 95% confidence intervals (CI) of the log values, which were retransformed back to non-log values for presentation here.

[2]Weighted for the cohort and subcohort sampling design; weighted percentages reflect prevalence in sampled population.

[3]Referent group.

[4]$p \leq 0.01$ for mean difference from the referent group, bold text used for clarity.

[5]$p \leq 0.05$ for mean difference from the referent group, bold text used for clarity.

[6]Missing data: Medicaid (n = 1); Parity (n = 1).

medically indicated PTD were determined by study obstetricians who reviewed prenatal and labor and delivery records. Many women had more than one specified reason for medically indicated PTD but we created a hierarchical list of primary reasons as follows: 37% maternal hypertension, 10% intrauterine growth restriction, 8% oligohydramnios, 3% abruption, 24% grouped as other maternal indications, and 18% grouped as other fetal indications.

Mean (*i.e.,* LS geometric mean) serum nutrient concentrations are presented by maternal characteristics in Table 1. While there was wide variation in total carotenoid concentrations (overall mean ± SD = 956 ± 441, range 230–3,432 ng/mL), mean subgroup values did not vary significantly by most maternal characteristics assessed. However, mean serum carotenoids were highest in primiparous women and women with lower BMI ($p < 0.01$ for both). There was also wide variation in retinol and tocopherol concentrations (overall mean ± SD were: 0.32 ± 0.10, range 0.09–0.79 µg/mL for retinol; 9.22 ± 2.78, range 3.49–23.22 µg/mL for α-tocopherol; and 1.73 ± 0.80, range 0.35–5.44 µg/mL for γ-tocopherol). Mean serum retinol concentrations were highest in women ≥30 years and in women whose blood was sampled earlier in gestation ($p < 0.05$). Both retinol and α-tocopherol concentrations were highest in women in the White/Other race/ethnicity category, and in those with private insurance ($p < 0.01$ except where noted). Additionally, mean serum α-tocopherol concentrations were higher in women with more than a high school education ($p < 0.01$) as well as in women whose blood was sampled later in gestation ($p < 0.05$). Mean serum γ-tocopherol concentrations were higher in multiparous women and higher in those with higher BMIs ($p < 0.01$). None of the serum antioxidant nutrient concentrations varied significantly by smoking status or season of enrollment.

Correlations among retinol, α- and γ-tocopherol, the individual carotenoids, and total cholesterol are shown in Table 2. The highest correlation between individual carotenoids was 0.55 between β-cryptoxanthin and its immediate precursor β-carotene and, the lowest was 0.30 between β-carotene and lycopene. Correlations of each individual carotenoid with total carotenoids was high, ranging from 0.61 for α-cryptoxanthin to 0.81 for β-carotene.

Mean serum carotenoid concentrations are shown in Table 3 by pregnancy outcome. It is apparent across all pregnancy outcomes that α- and β-cryptoxanthin had the lowest concentrations, whereas lutein/zeaxanthin and lycopene had the highest. Within pregnancy outcome categories, lower mean (95%CI) concentrations of β-cryptoxanthin were significantly associated with medically indicated PTD [65.9 ng/mL (58.0–74.9)] compared with term delivery [77.8 ng/mL (71.3–84.9)] and with SGA [60.0 ng/mL (51.2–70.3)] compared with AGA [7.8 ng/mL (70.3–83.9)]. Lower mean (95%CI) concentrations of lutein/zeaxanthin were also significantly associated with SGA [173.2 ng/mL (145.1–206.8)] compared with AGA [215.9 ng/mL (204.2–228.3)]. In contrast, higher mean (95%CI) concentrations of β-carotene and α-cryptoxanthin were significantly associated with LGA [205.8 ng/mL (152.9–277.0) and 47.0 ng/mL (38.7–57.1), respectively] compared with AGA [136.6 ng/mL (123.3–151.4) and 32.5 ng/mL (30.4–34.6), respectively].

Because individual carotenoids were highly correlated with total carotenoids, subsequent analyses focus on total

TABLE 2. PEARSON CORRELATIONS BETWEEN SERUM ANTIOXIDANT BIOMARKERS AND CHOLESTEROL IN THE POUCH STUDY (N = 547)[1,2]

	Retinol (µg/mL)	α-Tocopherol (µg/mL)	γ-Tocopherol (µg/mL)	β-Carotene (ng/mL)	α-Cryptoxanthin (ng/mL)	β-Cryptoxanthin (ng/mL)	Lycopene (ng/mL)	Lutein/Zeaxanthin (ng/mL)	Total carotenoid (ng/mL)	Total cholesterol (mg/dL)
Retinol	1.00									
α-tocopherol	0.38[1]	1.00								
γ-tocopherol	0.08	0.29[1]	1.00							
β-carotene	0.11[1]	0.34[1]	-0.18[1]	1.00						
α-Cryptoxanthin	0.13[1]	0.31[1]	0.01	0.35[1]	1.00					
β-Cryptoxanthin	0.09[1]	0.20[1]	-0.06	0.55[1]	0.39[1]	1.00				
Lycopene	0.08	0.32[1]	0.03	0.30[1]	0.49[1]	0.32[1]	1.00			
Lutein/zeaxanthin	0.09[1]	0.44[1]	0.05	0.41[1]	0.47[1]	0.45[1]	0.39[1]	1.00		
Total carotenoid	0.14[1]	0.45[1]	-0.07	0.81[1]	0.61[1]	0.69[1]	0.69[1]	0.72[1]	1.00	
Total cholesterol	0.06	0.46[1]	0.31[1]	0.23[1]	0.27[1]	0.19[1]	0.34[1]	0.36[1]	0.37	1.00

[1]$p \le 0.05$ for correlation (*r*), bold text used for clarity.
[2]Weighted for the cohort and subcohort sampling design; weighted percentages reflect prevalence in sampled population.

TABLE 3. MEAN SERUM INDIVIDUAL CAROTENOID CONCENTRATIONS BY PREGNANCY OUTCOME IN THE POUCH STUDY (N=547)[1,2]

	n (%)[3]	β-Carotene (ng/mL)		α-Cryptoxanthin (ng/mL)		β-Cryptoxanthin (ng/mL)		Lycopene (ng/mL)		Lutein/Zeaxanthin (ng/mL)	
		Mean	(95% CI)	Mean	(95% CI)	Mean	(95% CI)	Mean	(95% CI)	Mean	(95% CI)
Pregnancy outcome											
Preterm[4]	301 (10.7)	127.2	(115.1– 140.4)	33.0	(31.2– 34.9)	69.4	(64.4– 74.8)	223.5	(207.3– 241.0)	215.0	(203.8– 226.8)
Term[4]	246 (89.3)	142.6	(128.8– 157.8)	33.1	(31.0– 35.3)	77.8	(71.4– 84.9)	231.3	(213.0– 251.1)	212.5	(201.6– 224.1)
Pregnancy outcome subtype											
Spontaneous	122 (4.4)	133.9	(114.7– 156.3)	33.7	(31.1– 36.5)	69.1	(61.4– 77.8)	224.7	(201.1– 251.2)	223.5	(205.5– 243.1)
PPROM	82 (2.9)	126.5	(107.0– 149.7)	33.2	(29.8– 37.0)	74.1	(65.5– 83.8)	225.5	(201.2– 252.8)	215.2	(195.7– 236.6)
Medically indicated[4]	97 (3.4)	119.6	(100.6– 142.1)	31.9	(29.0– 35.1)	65.9	(58.0– 74.9)[6]	220.1	(196.1– 247.1)	204.4	(186.7– 223.8)
Term[4]	246 (89.3)	142.6	(128.8– 157.8)	33.1	(31.0– 35.3)	77.8	(71.3– 84.9)	231.3	(213.0–251.1)	212.5	(201.5– 224.1)
Size for gestational age[5]											
AGA[4]	458 (81.8)	136.6	(123.3– 151.4)	32.5	(30.4– 34.6)	76.8	(70.3– 83.9)	226.3	(208.1– 246.1)	215.9	(204.2– 228.3)
SGA (<10th %tile)	58 (7.8)	131.1	(102.3– 167.8)	29.0	(25.1– 33.6)	60.0	(51.2– 70.3)[6]	232.7	(190.9– 283.7)	173.2	(145.1– 206.8)[6]
LGA (>90th %tile)	30 (10.4)	205.8	(152.9– 277.0)[6]	47.0	(38.7– 57.1)[7]	106.9	(76.4– 149.6)	263.5	(220.0– 315.7)	244.6	(210.1– 284.7)

[1]Least square geometric means ±95% confidence intervals (CI) of log values, retransformed back to non-log values for presentation.
[2]Adjusted for: maternal race (White/Other vs. African American), parity (≥1 vs. none), gestational age, and BMI at the time of enrollment.
[3]Weighted percentages reflect oversampling of African Americans and preterm into the subcohort.
[4]Referent group.
[5]Birthweight missing ($n=1$).
[6]$p<0.05$; bold text used for clarity.
[7]$p<0.01$; bold text used for clarity.
PPROM, preterm premature rupture of membranes; AGA, appropriate for gestational age; SGA, small for gestational age; LGA, large for gestational age.

TABLE 4. ODDS RATIOS OF ADVERSE PREGNANCY OUTCOMES BY MATERNAL SERUM CONCENTRATIONS OF VITAMIN A COMPOUNDS IN THE POUCH STUDY (N = 547)[1,2]

	Total carotenoids (ng/mL)				Retinol (µg/mL)			
	Q4 vs.	Q1–3			Q4 vs.	Q1–Q3		
	(≥1,206)	(<1,206)	Unadjusted	Adjusted	(≥0.37)	(<0.37)	Unadjusted	Adjusted
Pregnancy outcome	N	n	OR (95% CI)	OR[2] (95% CI)	n	n	OR (95% CI)	OR[2] (95% CI)
Term (referent)	63	183	1.0	1.0	66	180	1.0	1.0
Preterm (<37 weeks)	61	240	0.7 (0.4–1.1)	0.7 (0.4–1.1)	94	207	1.0 (0.7–1.5)	1.1 (0.7–1.6)
Medically Indicated	10	87	**0.4 (0.2–0.7)**	**0.4 (0.2–0.9)**	34	63	1.2 (0.7–2.0)	1.3 (0.7–2.3)
Spontaneous or PPROM	51	153	0.9 (0.5–1.4)	0.8 (0.5–1.3)	60	144	0.9 (0.6–1.4)	1.0 (0.6–1.6)
Size for gestational age[3]								
AGA (referent)	109	349	1.0	1.0	138	320	1.0	1.0
SGA (<10th percentile)	7	51	0.5 (0.2–1.3)	**0.2 (0.1–0.8)**	13	45	**0.3 (0.1–0.8)**	0.4 (0.1–1.2)
LGA (>90th percentile)	8	22	1.2 (0.4–3.3)	1.9 (0.5–6.5)	9	21	1.7 (0.7–4.5)	1.5 (0.5–4.1)

95% CI does not include 1; bold text used for clarity.
[1]All analyses were weighted for the cohort and subcohort sampling design; Q1–Q3 combined (<75th %tile) was used as the referent category when calculating odds ratios.
[2]Adjusted for: maternal race (White/Other vs. African American); parity (≥1 vs. none); gestational age and BMI at the time of enrollment.
[3]Birthweight missing (n = 1).
OR, odds ratio.

carotenoids. As seen in Table 4, high serum carotenoids (≥ 75th percentile or Q4) were associated with lower odds of medically indicated PTD. While the number of women in Q4 carotenoids with medically indicated PTD was not large (n = 10), the strength of this association was substantial, an estimated 60% lower odds, that is, adjusted odds ratio (aOR) = 0.4; 95% CI: 0.2–0.9). Upon further adjustment for serum cholesterol (not shown in table), the association was slightly stronger (aOR = 0.3; 95% CI: 0.1–0.9). The primary reason listed in the medical record for medically indicated PTD in our sample was: hypertension (n = 36); intrauterine growth retardation (n = 11); oligohydramnios (n = 8); abruption (n = 3); other maternal in-

dication (n = 23); and other fetal indication (n = 16). In sensitivity analyses that removed women with preeclampsia (n = 31), results were similar (data not shown). Odds ratios for the relation between high serum carotenoids and SGA were ≤0.5 in all models but confidence intervals in two of the three models included 1.0 (i.e., the unadjusted model and the adjusted model that included serum cholesterol).

Serum retinol levels exhibited no significant association with PTD (Table 4). For SGA, odds ratios were <0.5 in all models among women in Q4 versus Q1–3, but the confidence interval for the adjusted model included 1.0. Similarly, results shown in Table 5 indicate no significant association

TABLE 5. ODDS RATIOS OF ADVERSE PREGNANCY OUTCOMES BY MATERNAL SERUM CONCENTRATIONS OF VITAMIN E COMPOUNDS IN THE POUCH STUDY (N = 547)[1,2]

	α-Tocopherol (µg/mL)				γ-Tocopherol (µg/mL)			
	Q4 vs.	Q1–3			Q4 vs.	Q1–3		
	(≥<10.44)	(<10.44)	Unadjusted	Adjusted	(≥2.14)	(<2.14)	Unadjusted	Adjusted
Pregnancy outcome	N	N	OR (95% CI)	OR[2] (95% CI)	n	n	OR (95% CI)	OR[2] (95% CI)
Term (referent)	64	182	1.0	1.0	60	186	1.0	1.0
Preterm (<37 weeks)	85	216	1.0 (0.6–1.4)	1.1 (0.7–1.6)	73	228	1.0 (0.7–1.6)	1.1 (0.7–1.7)
Medically indicated	18	79	0.6 (0.3–1.1)	0.6 (0.3–1.2)	21	76	0.9 (0.5–1.6)	0.8 (0.4–1.5)
Spontaneous or PPROM	67	137	1.2 (0.7–1.8)	1.3 (0.8–2.0)	52	152	1.1 (0.7–1.8)	1.2 (0.8–2.0)
Size for gestational age[3]								
AGA (referent)	132	326	1.0	1.0	113	345	1.0	1.0
SGA (<10th percentile)	8	50	**0.2 (0.1–0.7)**	**0.3 (0.1–0.8)**	11	47	**0.3 (0.1–0.7)**	**0.3 (0.1–0.8)**
LGA (>90th percentile)	9	21	1.6 (0.6–4.3)	1.9 (0.7–5.2)	9	21	1.3 (0.5–3.6)	1.0 (0.3–2.9)

95% CI does not include 1; bold text used for clarity.
[1]All analyses were weighted for the cohort and subcohort sampling design; Q1–Q3 combined (<75th %tile) was used as the referent category when calculating odds ratios.
[2]Adjusted for: maternal race (White/Other vs. African American); parity (≥1 vs. none); gestational age and BMI at the time of enrollment.
[3]Birthweight missing (n = 1).

between serum α-tocopherol or γ-tocopherol levels and PTD. However, again among women in Q4 versus Q1–3 for both α-tocopherol and γ-tocopherol, odds ratios for SGA were <0.5 in all models, but the confidence intervals included 1.0 after serum cholesterol was added to the models (aORs = 0.5; 95% CI: 0.2–1.6 for α-tocopherol and 0.3; 95% CI: 0.1–1.5 for γ-tocopherol).

Discussion

In this prospective study of pregnancy outcomes, high concentrations of serum carotenoids measured in mid pregnancy were significantly associated with a lower risk for medically indicated PTD. This relation was not observed for the other serum antioxidant nutrients examined (*i.e.,* retinol, α- and γ-tocopherol). Protective associations also were observed among high serum concentrations of total carotenoids, retinol, α- and γ-tocopherol, and the risk of SGA, although these associations were not consistent among individual carotenoids and did not remain statistically significant in adjusted models.

Our results are largely consistent with existing literature assessing the relations among antioxidants and PTD and/or SGA. We are aware of only one other prospective pregnancy cohort[12,14] in a developed country (*i.e.,* where few women have overt micronutrient deficiencies) that assessed associations among serum retinol, tocopherols, and carotenoids measured at mid pregnancy and the birth outcomes PTD and SGA. Results from that Canadian pregnancy cohort demonstrated a link between high concentrations of plasma carotenoids, but not retinol or tocopherols, and lower risk of spontaneous PTD; women with medically indicated PTD were not included in the cohort.[12] In the same cohort, high concentrations of plasma carotenoids were also associated with lower risk of SGA, whereas high concentrations of retinol were associated with higher risk of SGA.[14]

A 1994 case/control study of 80 pregnant women reported significantly lower serum β-carotene levels measured after birth, but no differences in retinol or α-tocopherol, among women who experienced preterm premature rupture of membranes (PPROM) in comparison to serum levels in the control women.[36] An important difference to note between the aforementioned study and the results presented here is the timing of blood collection. In the present prospective cohort study as well as the other described above,[12,14] the blood was collected mid pregnancy, whereas in the case/control study that collected blood after birth,[36] the lower serum β-carotene could be indicative of a depletion of β-carotene due to potentially increased antioxidant needs associated with PPROM.

In studies of nonpregnant adults, body composition has been shown to be inversely related to serum carotenoid concentrations,[37,38] which is consistent with the POUCH Study results described in this study. The decreased risk for medically indicated PTD with high serum carotenoid concentrations in the POUCH Study remained significant after controlling for BMI.

Much of the focus in research on nutrient status during pregnancy includes vitamin and/or mineral supplementation, which may or may not be applicable to dietary intake of whole foods. Carotenoids are naturally occurring lipid-soluble plant pigments that provide the red, orange, and yellow colors in fruits and vegetables. Serum carotenoids

have been shown to be a good marker of the relative consumption of fruit and vegetable intake within populations[39,40] and have been shown to increase significantly in response to fruit/vegetable behavioral interventions.[41,42] Fruit and vegetable intake is often associated with other healthy dietary behaviors, and accordingly, serum carotenoids have been shown to be related to multiple summary measures of dietary intake indicating they are a good marker for healthy dietary patterns.[43] Therefore, protective associations with high serum carotenoids may be related to direct effects or because healthy dietary patterns are part of a larger, potentially synergistic effect.

The mechanism by which carotenoids or other antioxidants may be directly associated with pregnancy outcomes is unclear; however, some fraction of PTD and SGA may arise from insufficient placental perfusion, either due to problems with placentation or later-onset vascular events.[44] Thus, our results support the possibility that this pathway may be protected by a threshold of maternal serum carotenoids, similar to mechanistic hypotheses posed to describe cardioprotective effects of carotenoids in nonpregnant adults.[45] Alternatively, the placental perfusion pathway may be protected by other unmeasured correlates of high serum carotenoids.

Major strengths of this study are the carefully collected prospective data, the expert adjudication of pregnancy outcomes using medical records, high participant retention through delivery, and low rates of missing data.[28] Women were sampled from community prenatal clinics and represent a diverse socioeconomic spectrum. Additionally, antioxidants and lipids were assessed using state-of-the-art laboratory methods.

The single serum measurement at mid pregnancy to characterize a woman's nutrient status may be considered a limitation of this study, although serum carotenoids are more representative of habitual dietary intake than many nutrients.[46] Because we did not assess dietary intake or collect detailed dosage information about vitamin/mineral supplementation in the present study, we were unable to determine the extent to which the serum carotenoids or other antioxidant nutrients are reflective of usual dietary intake in our sample. Furthermore, with only one serum measure at mid pregnancy, we could not know whether we captured the critical time period during pregnancy that may most influence the pregnancy outcomes studied here. Maternal lipid levels have been shown to vary by fetal sex,[47] suggesting that stratification by fetal sex may be important, however, given our sample size, this was not feasible. Similarly, there may be additional confounding factors that we did not capture, and we were limited by small sample sizes in certain cells, most notably cells with women in the highest quartile of serum antioxidant nutrients and medically indicated PTD or SGA infant (sample sizes ranged from 7 to 13 women). Although our odds ratios were consistently between 0.2 and 0.5 for all the serum antioxidant nutrients in relation to SGA, 95% confidence intervals were wide.

Conclusion

While there are well-known adverse effects of overt micronutrient deficiency during pregnancy, less is known about the potential benefits of above average intakes of micronutrients and other associated phytochemicals found in foods. Maternal serum antioxidant nutrients and/or the larger

dietary and/or lifestyle pattern they represent may be partially protective against pathology leading to adverse pregnancy outcomes. Serum carotenoids are reflective of dietary intake and are therefore potentially modifiable. Dietary guidelines for pregnant women already include recommendations for intakes of food groups high in carotenoids (*e.g.,* vegetables and fruits) and our results support the importance of efforts to assist women in adhering to these guidelines.

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Address correspondence to:
Jean M. Kerver, PhD, MSc, RD
Department of Epidemiology and Biostatistics
Michigan State University
909 Fee Road
East Lansing, MI 48824
USA

E-mail: kerverje@msu.edu