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Original Contribution

Shorter Time to Pregnancy With Increasing Preconception Carotene Concentrations Among Women With 1–2 Previous Pregnancy Losses

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Although maternal nutrition may affect fecundity, associations between preconception micronutrient levels and time to pregnancy (TTP) have not been examined. We assessed the relationship between preconception fat-soluble micronutrient concentrations and TTP among women with 1–2 prior pregnancy losses. This was a prospective cohort study of 1,228 women set within the Effects of Aspirin in Gestation and Reproduction (EAGeR) Trial (United States, 2007–2011), which assessed the association of preconception-initiated daily low-dose aspirin with reproductive outcomes. We measured preconception levels of zeaxanthin, cryptoxanthin, lycopene, α- and β-carotene, and α- and γ-tocopherol in serum. We used discrete Cox regression models, accounting for left-truncation and right-censoring, to calculate fecundability odds ratios and 95% confidence intervals. The models adjusted for age, body mass index, race, smoking, alcohol, physical activity, income, vitamin use, cholesterol, treatment arm, and study site. Serum α-carotene levels (per log unit (μg/dL) increase, fecundability odds ratio (FOR) = 1.17, 95% confidence interval (CI): 1.00, 1.36) and serum α-carotene concentrations at or above the US average (2.92 μg/dL) versus below the average (FOR = 1.21, 95% CI: 1.02, 1.44) were associated with shorter TTP. Compared with levels below the US average (187 μg/dL), γ-tocopherol concentrations at or above the average were associated with longer TTP (FOR = 0.83, 95% CI: 0.69, 1.00). The potential for these nutrients to influence fecundability deserves further exploration.

antioxidants; carotenes; fecundability; lipophilic micronutrients; time to pregnancy; tocopherols

Abbreviations: BMI, body mass index; CI, confidence interval; FOR, fecundability odds ratio; hCG, human chorionic gonadotropin; TTP, time to pregnancy.

Time to pregnancy (TTP) is a widely accepted measure of couple fecundity and is of great interest for couples seeking pregnancy. Factors affecting couple fecundity include the ages of both partners, body mass index (BMI), and various environmental exposures (1–4). Modifiable lifestyle risk factors, such as cigarette smoking and consumption of alcohol and caffeinated beverages, have also been suggested as potential factors influencing fecundity (5, 6). However, though the overall nutritional quality of the diet has been associated with female fertility (7), relationships between specific dietary components and couple fecundity are less understood.

Fat-soluble micronutrients, including carotenoids, retinol, and tocopherols, are obtained exclusively via the diet, as they are not synthesized in the body system. The most common dietary sources of carotenoids are yellow- and orange-colored fruits and vegetables and green leafy vegetables, whereas tocopherols are abundant in nuts, seeds, and nut and seed oils. These micronutrients are transported by lipoproteins in the body system and have antioxidant properties, as they scavenge reactive oxygen species (8). They are critical to normal cellular metabolism (9–11) and have been shown to be important for reproductive hormonal function (12). Antioxidant status (assessed by concentrations of carotenoids, retinol, and tocopherols in blood (13) and follicular fluid (13–15)) in women undergoing in vitro fertilization has been associated with embryo quality, which supports a potential role for antioxidants in the early stages of human reproduction.

Despite their potential importance to human fertility, only 1 study has evaluated the associations between intake of vitamins via supplements and diet and TTP among women with unexplained infertility (16). To our knowledge, no studies to date have investigated the association of preconception fat-soluble micronutrients measured in blood with fecundability. Therefore, we evaluated the relationship between preconception fat-soluble micronutrient concentrations and fecundability among women with proven fecundity and no history of infertility.

METHODS

Study design

We used data from the Effects of Aspirin in Gestation and Reproduction (EAGeR) Trial, which enrolled 1,228 women at 4 university medical centers in the United States (2007–2011). The study design of the original trial is described elsewhere (17). Briefly, participants were 18–40 years of age, had 1 or 2 documented prior pregnancy losses, had up to 2 prior live births, had regular menstrual cycles (i.e., 21–42 days), and were attempting pregnancy without the use of infertility treatment. Women with a known history of infertility treatment or the presence of major medical disorders were excluded.

At baseline, participants completed questionnaires on demographic factors, lifestyle, medical and reproductive history, and family medical history (17). Height and weight were measured, and BMI was calculated (weight (kg)/height $(m)^2$). Participants were followed for 6 menstrual cycles while attempting pregnancy or throughout pregnancy for those who became pregnant. We provided fertility monitors (Clearblue Easy Fertility Monitor; Inverness Medical Innovations, Waltham, Massachusetts) to assist couples with the timing of intercourse and timing of study visits.

The institutional review board at each study site approved the trial protocol. All participants provided written informed consent. The trial was registered on ClinicalTrials.gov (trial NCT00467363).

Analysis of fat-soluble micronutrients

Blood specimens were collected at baseline, processed, and stored at −80°C prior to analysis. Preconception levels of serum fat-soluble micronutrients, including zeaxanthin, cryptoxanthin, lycopene, α-carotene, β-carotene, α-tocopherol, and γ-tocopherol, were measured at the University of Minnesota (Minneapolis, Minnesota) using high-performance liquid chromatography (18, 19) in 1,207 women (21 women did not have sufficient sample volume). The mobile phase used for tocopherol was 100% methanol (19). For carotenoids, acetonitrile/methylene chloride/methanol (70:20:10 by volume) was used as the mobile phase (19), with modification by adding 0.015% diisopropylethylamine to aid in analyte recovery. The interassay laboratory coefficients of variation ranged from 5.9% for γ-tocopherol to 13.3% for α-tocopherol. Total cholesterol concentration was measured using a Roche COBAS 6000 chemistry analyzer (Roche Diagnostics, Indianapolis, Indiana), with coefficients of variation of 2.1% and 2.2% at mean concentrations of 178.6 mg/dL and 258.9 mg/dL, respectively.

Outcome assessment

The primary outcome was TTP or the number of menstrual cycles needed to achieve pregnancy over 6 consecutive months of follow-up. Pregnancy was determined by positivity for urinary human chorionic gonadotropin (hCG) and ultrasonography (20). In brief, urine hCG tests (Quidel Quickvue; Quidel Corporation, San Diego, California), which were sensitive to 25 mIU/mL hCG, were conducted at home or at the clinic when participants reported missing menses at the end of each cycle during follow-up. For a more sensitive detection of early pregnancy, we also measured free β-hCG levels in daily first-morning urine samples collected on the last 10 days of each woman's first and second cycles of study participation and in spot urine samples collected at clinic visits that took place at the end of each cycle. Among women with positive urinary hCG, a 6- to 7-week ultrasound examination was performed to confirm pregnancy.

Statistical analysis

We determined demographic and reproductive history characteristics by tertiles of selected micronutrients and compared them (using Fisher's exact test or the χ^2 test as appropriate) among 1,207 women with available micronutrient measurements. Concentrations of fat-soluble micronutrients were log-transformed for normality. Geometric mean values and standard deviations of micronutrients were calculated overall, by number of previous losses, by number of previous live births, and by pregnancy status and were compared using Student's *t* test or analysis of variance, as appropriate. Pearson correlation coefficients were obtained for log-transformed fat-soluble micronutrient concentrations to assess correlations. Distributions of measured micronutrient concentrations were compared with the US average levels reported for women aged 20–39 years in the 2005–2006 National Health and Nutrition Examination Survey. Reported geometric mean values for each micronutrient in the National Health and Nutrition Examination Survey were: 13.8 μg/dL (95% confidence interval (CI): 13.2, 14.5) for zeaxanthin; 8.0 μg/dL (95% CI: 7.5, 8.6) for cryptoxanthin; 41.3 μg/dL (95% CI: 40.2, 42.4) for lycopene; 2.9 μg/dL (95% CI: 2.5, 3.4) for α-carotene; 12.2 μg/dL (95% CI: 10.7, 13.9) for β-carotene; 1,010 μg/dL (95% CI: 981, 1,040) for α-tocopherol; and 187 μg/dL (95% CI: 173, 202) for γ-tocopherol (21).

We used Cox proportional hazards regression models for discrete survival time, accounting for left-truncation and right-censoring, to estimate fecundability odds ratios and 95% confidence intervals for relationships between fat-soluble micronutrients and TTP. A fecundability odds ratio greater than 1 is interpreted as indicating increased fecundability. We imputed missing values for fat-soluble micronutrient concentrations (n = 21; 1.7%) and covariates (BMI, 1.6%; alcohol drinking, 1.3%; physical activity, 0.1%; income, 0.1%; vitamin use, 1.5%; total cholesterol concentration, 1.7%; number of cycles of attempting pregnancy before study entry, 7.9%) using a multiple imputation model with the fully conditional specification method and 5 imputations (22), with PROC MIANALYZE being used to combine the results. In addition to the aforementioned variables, the imputation models included age, race, cigarette smoking, treatment arm, and study site. The models adjusted for age, BMI, race, cigarette smoking, alcohol drinking, physical activity, income, vitamin use, treatment arm, study site, and serum cholesterol level

(17). Serum cholesterol was included because lipids are one of the major factors that could affect the bioavailability of fat-soluble micronutrients (23) and are also associated with TTP (24). We investigated treatment arm and BMI as potential effect-measure modifiers.

Because 128 women did not have complete outcome information due to early withdrawal from the study and because lower levels of micronutrients were observed in those women (see Web Figure 1, available at https://academic.oup.com/aje), we performed several sensitivity analyses to investigate potential bias. We compared complete-case results ($n = 1{,}100$) with those from an analysis where the potential pregnancy outcomes of women who withdrew were imputed using 3 strategies: 1) the survival probability of no pregnancy achieved derived from the complete cases using Kaplan-Meier multiple imputation (1,000 imputations), 2) achievement of pregnancy in 1 cycle after withdrawal, and 3) no pregnancy achieved after 6 cycles. The Kaplan-Meier–based imputation is a missing-at-random–like principled approach that is plausible and considers covariates (25), while the latter 2 methods are extreme possibilities of the influence of potential unobserved outcomes.

We also considered several sensitivity analyses to evaluate the assumptions of our underlying causal framework. In this setting we were interested in investigating the association between fat-soluble micronutrient concentrations and TTP among women with 1–2 prior losses, without generalizing our findings to all reproductive-age women. If, however, one were interested in answering this question for the broader population, there may be potential concerns regarding selection bias, as our participants were restricted to women with a specific reproductive history, and because past micronutrient levels may be associated with reproductive history (Web Figure 2). Under this framework, we considered 3 possible sources of confounding. First, we evaluated the potential impact of an unmeasured dietary factor (U_1) that is associated with past and current micronutrient concentrations across a range of correlations from −0.8 to 0.8. Second, we evaluated the impact of adjustment for previous fecundability (TTP_0) to block the pathway using a proxy for previous infertility (i.e., attempting pregnancy for longer than 1 year). Third, we assessed an unmeasured confounder of the relationship between reproductive history (S) and current TTP, U_2, as this may introduce selection bias if it is strongly correlated with S and TTP (26). We simulated a variable which could represent genetic factors (27, 28) across a range of correlations between U_2 and S (odds ratios of 1.7 and 2.7) and U_2 and TTP (fecundability odds ratios of 0.5 and 0.7). SAS, version 9.4 (SAS Institute, Inc., Cary, North Carolina), was used for all statistical analysis.

RESULTS

Women of normal weight (BMI <25), nonsmoking women, women with more than a high school education, and women in higher income categories were more likely to be in the middle or upper tertile of serum α-carotene concentrations than in the first tertile (Table 1). Women whose last pregnancy loss had occurred less than 4 months previously or who achieved pregnancy after randomization also tended to be in the middle and upper tertiles of α-carotene concentration compared with the first. Though we did not detect differences in tertiles of α-carotene by treatment arm, differences by study site were detected ($P = 0.001$). For γ-tocopherol, we observed an opposite trend in α-carotene levels for most demographic characteristics. Women whose last pregnancy loss had occurred less than 8 months previously were more likely to be in the middle or upper tertile of γ-tocopherol concentrations than in the lower tertile. Unlike the case for α-carotene, women who achieved pregnancy after randomization were more likely to be in the lower tertile of γ-tocopherol compared with the higher tertiles. Most women reported having taken vitamin supplements (92.4%). Subsequent to inception of the study, 22.0%, 16.2%, 10.8%, 7.3%, 5.1%, and 3.8% of women achieved pregnancy during menstrual cycles 1–6, respectively; 34.8% of women did not achieve pregnancy within 6 menstrual cycles of study follow-up or withdrew from the study. Levels of all measured fat-soluble micronutrients were positively correlated (ranging from $r = 0.11$ for zeaxanthin and γ-tocopherol to $r = 0.68$ for α-carotene and β-carotene), except for correlations between α-carotene and γ-tocopherol ($r = -0.08$) and between β-carotene and γ-tocopherol ($r = -0.20$).

Serum concentrations of fat-soluble micronutrients did not differ significantly by number of previous pregnancy losses (Table 2). Mean cryptoxanthin concentrations were higher in nulliparous women (8.1 μg/dL) than in parous women (7.4 μg/dL in women who had 1 previous live birth and 7.5 μg/dL in women who had 2 previous live births; $P < 0.01$), whereas no significant differences across parity were detected for the other micronutrients.

Mean concentrations of fat-soluble micronutrients differed by pregnancy status, except for lycopene (Web Figure 1). Except for γ-tocopherol, higher concentrations were detected for all measured micronutrients in pregnant women relative to nonpregnant women and women who withdrew early from the study. Compared with the US average level reported for women aged 20–39 years (21), levels of zeaxanthin, α-carotene, and β-carotene were higher among the women in our study, whereas mean concentrations of cryptoxanthin, lycopene, α-tocopherol, and γ-tocopherol were lower than the US average.

A log unit increase in α-carotene (μg/dL) was associated with a shorter TTP (fecundability odds ratio (FOR) = 1.17, 95% CI: 1.00, 1.36) after adjustment for potential confounders (Table 3). For α-carotene, concentrations at or above the US average (≥2.9 μg/dL) were associated with a shorter TTP (FOR = 1.21, 95% CI: 1.02, 1.44) than concentrations below the US average. Similarly, higher lycopene and β-carotene concentrations were associated with a shorter TTP, although the association was imprecise or attenuated after adjusting for the covariates. A log unit increase in γ-tocopherol concentration (μg/dL) was associated with a longer TTP (FOR = 0.66, 95% CI: 0.52, 0.84), and a consistent yet attenuated association was detected for concentrations at or above the US average (≥187 μg/dL) (FOR = 0.73, 95% CI: 0.62, 0.87), compared with those below the US average (<187 μg/dL). After adjustment for covariates, however, a log unit increase in γ-tocopherol was not associated with TTP, whereas γ-tocopherol concentrations at or above the US average remained associated with a longer TTP (FOR = 0.83, 95% CI: 0.69, 1.00) relative to those below the US average. No other significant associations were identified for other measured fat-soluble micronutrients and TTP. We did not detect any effect-measure modification by treatment arm or BMI in our data.

For our sensitivity analysis, we used 3 different methods to impute pregnancy during our 6 cycles of follow-up. By design, all

Table 1. Demographic Characteristics of 1,207 Study Participants With Fat-Soluble Micronutrient Measurements Taken at Baseline, by Tertiles (μg/dL) of α-Carotene and γ-Tocopherol, Effects of Aspirin in Gestation and Reproduction Trial, 2007–2011

Demographic Characteristic	α-Carotene[a]							γ-Tocopherol[b]						
	Tertile 1 (n = 403)		Tertile 2 (n = 402)		Tertile 3 (n = 402)		*P* Value	Tertile 1 (n = 404)		Tertile 2 (n = 404)		Tertile 3 (n = 399)		*P* Value
	No.	%	No.	%	No.	%		No.	%	No.	%	No.	%	
Age, years							0.71							0.69
<35	359	89.1	356	88.6	351	87.3		361	89.4	353	87.4	352	88.2	
≥35	44	10.9	46	11.4	51	12.7		43	10.6	51	12.6	47	11.8	
Body mass index[c]							<0.0001							<0.0001
<25	145	36.6	210	53.2	268	67.7		266	67.0	212	53.3	145	37.0	
≥25	251	63.4	185	46.8	128	32.3		131	33.0	186	46.7	247	63.0	
Race							<0.01							0.42
White	368	91.3	387	96.3	388	96.5		385	95.3	385	95.3	373	93.5	
Nonwhite	35	8.7	15	3.7	14	3.5		19	4.7	19	4.7	26	6.5	
Frequency of cigarette smoking							<0.0001							<0.01
Never smoker	319	79.4	358	90.4	372	93.2		361	89.6	364	90.6	324	82.7	
Fewer (<6 times/week)	40	10.0	24	6.1	21	5.3		25	6.2	25	6.2	35	8.9	
Daily	43	10.7	14	3.5	6	1.5		17	4.2	13	3.2	33	8.4	
Frequency of alcohol drinking							0.36							<0.0001
Never drinker	261	65.1	264	66.7	267	67.6		297	74.1	266	66.3	229	58.7	
Sometimes (up to 2–3 times/week)	135	33.7	123	31.1	116	29.4		100	24.9	123	30.7	151	38.7	
Often (4–6 times/week or more)	5	1.3	9	2.3	12	3.0		4	1.0	12	3.0	10	2.6	
Physical activity level[d]							0.02							0.17
High	145	36.0	120	29.9	133	33.1		147	36.4	122	30.2	129	32.3	
Moderate	141	35.0	177	44.0	179	44.5		165	40.8	177	43.8	155	38.9	
Low	117	29.0	105	26.1	90	22.4		92	22.8	105	26.0	115	28.8	
Educational level							<0.0001							0.02
More than high school	305	75.9	363	90.3	374	93.0		365	90.4	346	85.6	331	83.2	
High school	84	20.9	32	8.0	24	6.0		34	8.4	52	12.9	54	13.6	
Less than high school	13	3.2	7	1.7	4	1.0		5	1.2	6	1.5	13	3.3	
Annual income, dollars							<0.0001							0.61
<20,000	41	10.2	22	5.5	29	7.2		30	7.4	28	6.9	34	8.5	
20,000–39,999	143	35.5	89	22.2	77	19.2		94	23.3	105	26.0	110	27.6	
40,000–74,999	53	13.2	60	15.0	63	15.7		69	17.1	51	12.6	56	14.0	
75,000–99,999	24	6.0	60	15.0	64	15.9		49	12.2	55	13.6	44	11.0	
≥100,000	142	35.2	170	42.4	169	42.0		161	40.0	165	40.8	155	38.9	
Employment							0.44							0.03
Yes	285	74.6	301	78.0	296	74.6		285	73.1	290	73.8	307	80.4	
No	97	25.4	85	22.0	101	25.4		105	26.9	103	26.2	75	19.6	
Time since last pregnancy loss, months							0.02							0.04
≤4	187	46.8	226	57.4	226	57.4		210	52.4	218	55.3	211	53.7	
5–8	79	19.8	72	18.3	66	16.8		71	17.7	72	18.3	74	18.8	
9–12	42	10.5	30	7.6	26	6.6		39	9.7	40	10.2	19	4.8	
>12	92	23.0	66	16.8	76	19.3		81	20.2	64	16.2	89	22.7	
No. of pregnancies, not including losses							0.14							0.55
0	167	41.4	168	41.8	180	44.8		157	38.9	183	45.3	175	43.9	
1	162	40.2	139	34.6	126	31.3		152	37.6	138	34.2	137	34.3	
2	70	17.4	86	21.4	88	21.9		89	22.0	77	19.1	78	19.6	
3	4	1.0	9	2.2	8	2.0		6	1.5	6	1.5	9	2.3	

Table continues

Table 1. Continued

Demographic Characteristic	α-Carotene[a]							γ-Tocopherol[b]						
	Tertile 1 (*n* = 403)		Tertile 2 (*n* = 402)		Tertile 3 (*n* = 402)		*P* Value	Tertile 1 (*n* = 404)		Tertile 2 (*n* = 404)		Tertile 3 (*n* = 399)		*P* Value
	No.	%	No.	%	No.	%		No.	%	No.	%	No.	%	
No. of previous live births							0.36							0.28
0	186	46.2	183	45.5	190	47.3		170	42.1	193	47.8	196	49.1	
1	156	38.7	148	36.8	133	33.1		160	39.6	144	35.6	133	33.3	
2	61	15.1	71	17.7	79	19.7		74	18.3	67	16.6	70	17.5	
No. of previous pregnancy losses							0.46							0.12
1	261	64.8	277	68.9	269	66.9		258	63.9	285	70.5	264	66.2	
2	142	35.2	125	31.1	133	33.1		146	36.1	119	29.5	135	33.8	
Treatment arm							0.24							0.14
Low-dose aspirin	201	49.9	190	47.3	214	53.2		218	54.0	199	49.3	188	47.1	
Placebo	202	50.1	212	52.7	188	46.8		186	46.0	205	50.7	211	52.9	
Study site							0.001							0.57
Utah	311	77.2	325	80.9	348	86.6		340	84.2	325	80.5	319	80.0	
New York	39	9.7	24	6.0	13	3.2		20	5.0	26	6.4	30	7.5	
Colorado	20	5.0	27	6.7	25	6.2		21	5.2	29	7.2	22	5.5	
Pennsylvania	33	8.2	26	6.5	16	4.0		23	5.7	24	5.9	28	7.0	
Pregnancy after randomization							<0.0001							0.001
Yes	215	53.4	283	70.4	289	71.9		283	70.1	272	67.3	232	58.2	
No	188	46.7	119	29.6	113	28.1		121	30.0	132	32.7	167	41.9	

Abbreviation: IPAC, International Physical Activity Questionnaire.
[a] Range (minimum–maximum) of α-carotene levels: tertile 1, 0.21–2.22 μg/dL; tertile 2, 2.23–4.31 μg/dL; tertile 3, 4.33–46.95 μg/dL.
[b] Range (minimum–maximum) of γ-tocopherol levels: tertile 1, 65.0–143.0 μg/dL; tertile 2, 144.0–192.0 μg/dL; tertile 3, 193.0–476.0 μg/dL.
[c] Weight (kg)/height (m)^2.
[d] Physical activity, assessed by means of the IPAQ–Short Form, was categorized on the basis of standard IPAQ cutoffs (42).

3 methods resulted in dissimilar levels of censoring of women at 6 months of follow-up. The results of the Kaplan-Meier–based imputation were similar to the complete-case results for all fat-soluble micronutrients, implying that plausible outcomes of withdrawals were unlikely to have substantively changed our results. Moreover, the 2 extreme cases for potential outcomes of withdrawals also resulted in largely similar results, further supporting the robustness of our findings (Web Table 1).

To investigate the potential for unmeasured confounders to introduce selection bias, we performed additional sensitivity

Table 2. Distributions of Preconception Serum Concentrations (μg/dL) of Fat-Soluble Micronutrients (*n* = 1,207), Effects of Aspirin in Gestation and Reproduction Trial, 2007–2011[a]

Micronutrient	Total	No. of Previous Pregnancy Losses			No. of Previous Live Births			
		1 (*n* = 807)	2 (*n* = 400)	*P* Value	0 (*n* = 559)	1 (*n* = 437)	2 (*n* = 211)	*P* Value[b]
Zeaxanthin	15.7 (1.5)	15.8 (1.5)	15.5 (1.5)	0.50	15.9 (1.5)	15.3 (1.5)	16.0 (1.4)	0.31
Cryptoxanthin	7.7 (1.6)	7.7 (1.6)	7.6 (1.6)	0.59	8.1 (1.7)[c]	7.4 (1.6)[c]	7.5 (1.6)	0.01
Lycopene	26.7 (1.6)	27.0 (1.7)	26.2 (1.6)	0.31	26.8 (1.6)	26.0 (1.7)	27.9 (1.6)	0.21
α-Carotene	4.3 (1.8)	4.3 (1.8)	4.3 (1.8)	0.84	4.3 (1.7)	4.1 (1.8)[c]	4.6 (1.8)[c]	0.11
β-Carotene	15.4 (2.1)	15.5 (2.1)	15.4 (2.1)	0.95	15.9 (2.0)	15.1 (2.1)	15.0 (2.1)	0.48
α-Tocopherol	912.6 (1.3)	911. (1.3)	914.2 (1.3)	0.88	925.3 (1.3)[c]	892.9 (1.3)[c]	920.8 (1.3)	0.09
γ-Tocopherol	167.6 (1.4)	167.2 (1.4)	168.3 (1.4)	0.74	170.8 (1.4)[c]	163.9 (1.4)[c]	166.7 (1.4)	0.14

[a] Values are presented as geometric mean (geometric standard deviation).
[b] Overall group difference calculated using analysis of variance.
[c] $P < 0.05$ for difference between groups.

Table 3. Associations Between Log-Transformed Levels of Fat-Soluble Micronutrients and Time to Pregnancy (n = 1,228),[a] Effects of Aspirin in Gestation and Reproduction Trial, 2007–2011

Micronutrient	Unadjusted		Adjusted[b]	
	FOR	95% CI	FOR	95% CI
Continuous variable, μg/dL				
Zeaxanthin	1.19	0.98, 1.45	1.12	0.89, 1.39
Cryptoxanthin	1.12	0.95, 1.32	1.06	0.88, 1.28
Lycopene	1.15	0.98, 1.35	1.19	0.99, 1.43
α-Carotene	1.23	1.08, 1.41	1.17	1.00, 1.36
β-Carotene	1.24	1.11, 1.39	1.12	0.98, 1.28
α-Tocopherol	1.15	0.85, 1.55	1.23	0.85, 1.78
γ-Tocopherol	0.66	0.52, 0.84	0.83	0.62, 1.11
Dichotomous variable (≥US average)[c]				
Zeaxanthin	1.10	0.94, 1.29	1.04	0.87, 1.24
Cryptoxanthin	1.09	0.93, 1.29	1.04	0.87, 1.24
Lycopene	1.03	0.81, 1.30	1.02	0.79, 1.32
α-Carotene	1.29	1.10, 1.52	1.21	1.02, 1.44
β-Carotene	1.26	1.07, 1.48	1.11	0.92, 1.33
α-Tocopherol	1.04	0.88, 1.23	1.04	0.87, 1.25
γ-Tocopherol	0.73	0.62, 0.87	0.83	0.69, 1.00

Abbreviations: CI, confidence interval; FOR, fecundability odds ratio.

[a] Imputed data for missing micronutrient concentrations and covariates were used in the analyses.

[b] Models adjusted for age (years), body mass index (weight (kg)/height (m)^2), race (white or nonwhite), frequency of cigarette smoking (never smoker, fewer (<6 times/week), or daily), frequency of alcohol drinking (never drinker, sometimes (up to 2–3 times/week), or often (4–6 times/week or more)), physical activity level (high, moderate, or low; see Table 1), annual income (<$20,000, $20,000–$39,999, $40,000–$74,999, $75,000–$99,999, or ≥$100,000), vitamin use (yes or no), total cholesterol concentration (mg/dL), treatment arm (low dose aspirin or placebo), and study site.

[c] A micronutrient level less than the US average (<138 μg/dL for zeaxanthin, <8 μg/dL for cryptoxanthin, <41.3 μg/dL for lycopene, 2.9 μg/dL for α-carotene, <12.2 μg/dL for β-carotene, <1,010 μg/dL for α-tocopherol, and <187 μg/dL for γ-tocopherol) was used as the referent.

analyses. Adjustment for an unmeasured dietary factor (U_1), past TTP (TTP_0), or an unmeasured genetic factor (U_2), either individually (Web Figures 3A and 3B) or together (Web Figure 3C), would block the backdoor pathways and did not alter our results.

DISCUSSION

Overall, we found that increasing preconception serum carotenoid concentrations were associated with improved fecundability and a shorter TTP among women with proven fecundity and no history of infertility. On the other hand, our data also suggested that serum γ-tocopherol levels at or above the US average were associated with a longer TTP. TTP is a widely used measure of couple fecundity, and our data support a possible role of fat-soluble micronutrients, which are easily obtained through diet, in fecundability.

A positive association between carotenoids and shortened TTP was also reported in a recent study of women diagnosed with unexplained infertility (16). In that study, intake of β-carotene from dietary supplements was associated with a shorter TTP in women with BMI ≥25 or age <35 years. Although β-carotene was only marginally associated with a shorter TTP in our study, we observed that α-carotene was significantly associated with a 17% shorter TTP. Although we did not observe any effect-measure modification by BMI or age, taken together these data support a beneficial role of carotenoids in TTP and necessitate further exploration of a potential role of carotenoids in fertility.

Carotenoids, which are mostly obtained from fruits and vegetables, have been shown to inhibit free radical reactions in body systems and to protect cellular membranes from lipid peroxidation (11). Specific biological mechanisms to account for the association between antioxidant micronutrients and TTP are uncertain. However, antioxidants have been shown to be associated with reproductive hormone concentrations in healthy women (12), suggesting potentially complex hormonal interactions, which may subsequently lead to improved reproductive function. Our finding highlights a potential role of preconception carotenoids in fecundability, although the clinical implications of these results for women attempting pregnancy in the general population warrant further investigation.

In our data, serum γ-tocopherol concentrations at or above the US average and increased γ-tocopherol concentrations were associated with a longer TTP. Our results differed from the

results of other studies, which observed positive associations between tocopherols and reproductive outcomes. In particular, Browne et al. (13) identified a positive association between γ-tocopherol measured in follicular fluid and better embryo quality in women undergoing in vitro fertilization, suggesting a potential beneficial role of γ-tocopherol in embryo-level outcomes. Differences between serum and follicular fluid measures might explain the discrepant findings. In a study of women with unexplained infertility, vitamin E supplementation was associated with a shorter TTP in women aged ≥35 years (16); however, serum γ-tocopherol levels were not measured.

Though the differences might also be related to differences in study design, primary outcomes, and assessment of micronutrient status, most importantly, the women in our study have demonstrated fecundity rather than infertility. Interestingly, serum γ-tocopherol concentrations measured in our women at baseline did not vary by reproductive history characteristics, such as number of previous losses or live births; however, all women had a history of prior losses and no history of infertility treatment. Although mean concentrations differed by pregnancy status, with lower levels being observed in pregnant women (163.4 μg/dL) than in nonpregnant women (171.3 μg/dL) and women who withdrew from the study (186.1 μg/dL), our sensitivity analyses indicated a minimal influence of the difference in γ-tocopherol concentrations by pregnancy and withdrawal status on our results. Given potentially differential impacts of γ-tocopherol on fertility outcomes, further investigation is necessary to elucidate its potential role in fecundability.

The differences we observed between carotene and tocopherol levels and TTP in our study may be explained by several factors. Serum carotene concentrations measured in our study were inversely correlated with γ-tocopherol concentrations, a result consistent with that of a study carried out among postmenopausal women in the Women's Health Initiative (29). Competition for micellar solubilization before absorption was noted in an animal study in which simultaneous administration of large doses of α-tocopherol reduced the utilization of β-carotene (30). In a clinical trial conducted among 59 adults, Willett et al. (31) reported a reduction in plasma carotenoid levels after 16 weeks of daily α-tocopherol administration (800 IU) as well, suggesting competitive absorption and subsequent interactive bioactivity of carotenes and tocopherols. Almost all demographic factors, including BMI, cigarette smoking, alcohol drinking, physical activity, education, and income, when characterized by tertiles of γ-tocopherol and α-carotene, showed opposing trends in our study. Thus, it appears that numerous lifestyle variables as well as dietary habits could affect bioavailability of carotenes and tocopherols (32).

Our study had multiple strengths. Measurement of TTP was prospective, which minimized the misclassification that can occur in many retrospective observational studies (33). The prospective study design particularly strengthens our findings, as selected micronutrients were measured in women while they were trying to conceive. However, there were several limitations in our study. Although TTP is a measure of couple fecundity, data on the micronutrient status of male partners were not available in our study. Therefore, future investigation of a potential contribution of male partners' macro- and micronutrient status to couples' TTP is of interest. Further, intake of vitamin supplements tends to increase plasma levels of micronutrients and may affect the bioavailability of other micronutrients (31). Most study participants had taken vitamin supplements prior to enrollment and during the trial, which may have affected our study results. Although we included use of vitamin supplements during the multivariable regression analysis, we did not have data regarding the dose and specific type of vitamin supplements taken. Data on dietary intake, which influences levels of micronutrients (34), were not available. However, our fat-soluble micronutrient concentrations measured in serum are useful markers of intakes of foods rich in those micronutrients, particularly fruits and vegetables, though correlations vary by specific micronutrient (35, 36). Given the short half-lives of fat-soluble micronutrients (37–39), our micronutrient concentrations measured at baseline may not have adequately reflected micronutrient status at the time of conception. Our follow-up duration of 6 months was comparable to that of other studies that investigated TTP (40, 41) and allowed us to investigate factors associated with subfertility. However, our study was limited in that we may have been able to identify infertility had we been able to follow the women for 12 months, rather than 6 months.

Information on pregnancy status and timing of pregnancy was not available for approximately 10% of our participants who withdrew early, and their preconception levels of fat-soluble micronutrients were different from those who were retained in the study. We performed sensitivity analyses based on different statistical approaches to address potential bias due to such missingness. Overall, our sensitivity analyses demonstrated consistency of results across several different imputation methods, confirming the robustness of our results to the impact of participant withdrawal. Because of the original clinical trial enrollment criteria, the generalizability of our findings is limited to women with 1–2 prior losses. Although we are not attempting to generalize our findings to all reproductive-age women, we investigated the potential for selection bias due to such restriction. Reassuringly, the results were not different from our observed findings, even after accounting for strong potential unmeasured confounders.

To our knowledge, this was the first study assessing associations between preconception fat-soluble micronutrients measured in blood—reflecting an individual's antioxidant status—and couple fecundity. Our data support a beneficial role for carotenes in TTP among women who have demonstrated fecundity and are attempting pregnancy. However, because the effect sizes tended to be small overall, additional research is needed to better describe the clinical significance and relevance of these findings. Fat-soluble micronutrients are abundant in fruits, vegetables, and seed oils and are easily obtainable through a healthy diet in the general population. Given the positive associations between micronutrients and TTP and the fact that diet is a modifiable lifestyle factor, further exploration of the roles of fat-soluble micronutrients and fecundability in the general population of reproductive-age women is warranted.

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REFERENCES

1. Menken J, Trussell J, Larsen U. Age and infertility. *Science*. 1986;233(4771):1389–1394.
2. Dunson DB, Colombo B, Baird DD. Changes with age in the level and duration of fertility in the menstrual cycle. *Hum Reprod*. 2002;17(5):1399–1403.
3. Sharpe RM, Franks S. Environment, lifestyle and infertility—an inter-generational issue. *Nat Cell Biol*. 2002;4(suppl):s33–s40.
4. Hauser R. The environment and male fertility: recent research on emerging chemicals and semen quality. *Semin Reprod Med*. 2006;24(3):156–167.
5. Augood C, Duckitt K, Templeton AA. Smoking and female infertility: a systematic review and meta-analysis. *Hum Reprod*. 1998;13(6):1532–1539.
6. Hassan MA, Killick SR. Negative lifestyle is associated with a significant reduction in fecundity. *Fertil Steril*. 2004;81(2): 384–392.
7. Chavarro JE, Rich-Edwards JW, Rosner BA, et al. Diet and lifestyle in the prevention of ovulatory disorder infertility. *Obstet Gynecol*. 2007;110(5):1050–1058.
8. Aust O, Sies H, Stahl W, et al. Analysis of lipophilic antioxidants in human serum and tissues: tocopherols and carotenoids. *J Chromatogr A*. 2001;936(1–2):83–93.
9. Esterbauer H, Striegl G, Puhl H, et al. The role of vitamin E and carotenoids in preventing oxidation of low density lipoproteins. *Ann N Y Acad Sci*. 1989;570:254–267.
10. Esterbauer H, Dieber-Rotheneder M, Striegl G, et al. Role of vitamin E in preventing the oxidation of low-density lipoprotein. *Am J Clin Nutr*. 1991;53(1 suppl):314S–321S.
11. Sies H, Stahl W. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. *Am J Clin Nutr*. 1995;62(6 suppl): 1315S–1321S.
12. Mumford SL, Browne RW, Schliep KC, et al. Serum antioxidants are associated with serum reproductive hormones and ovulation among healthy women. *J Nutr*. 2016;146(1): 98–106.
13. Browne RW, Bloom MS, Shelly WB, et al. Follicular fluid high density lipoprotein-associated micronutrient levels are associated with embryo fragmentation during IVF. *J Assist Reprod Genet*. 2009;26(11–12):557–560.
14. Schweigert FJ, Steinhagen B, Raila J, et al. Concentrations of carotenoids, retinol and alpha-tocopherol in plasma and follicular fluid of women undergoing IVF. *Hum Reprod*. 2003; 18(6):1259–1264.
15. Pauli SA, Session DR, Shang W, et al. Analysis of follicular fluid retinoids in women undergoing in vitro fertilization: retinoic acid influences embryo quality and is reduced in women with endometriosis. *Reprod Sci*. 2013;20(9): 1116–1124.
16. Ruder EH, Hartman TJ, Reindollar RH, et al. Female dietary antioxidant intake and time to pregnancy among couples treated for unexplained infertility. *Fertil Steril*. 2014;101(3): 759–766.
17. Schisterman EF, Silver RM, Perkins NJ, et al. A randomised trial to evaluate the effects of low-dose aspirin in gestation and reproduction: design and baseline characteristics. *Paediatr Perinat Epidemiol*. 2013;27(6):598–609.
18. Bieri JG, Brown ED, Smith JC. Determination of individual carotenoids in human plasma by high performance liquid chromatography. *J Liquid Chromatogr*. 1985;8(3): 473–484.
19. Craft NE, Brown ED, Smith JC Jr. Effects of storage and handling conditions on concentrations of individual carotenoids, retinol, and tocopherol in plasma. *Clin Chem*. 1988;34(1):44–48.
20. Schisterman EF, Mumford SL, Schliep KC, et al. Preconception low dose aspirin and time to pregnancy: findings from the Effects of Aspirin in Gestation and Reproduction randomized trial. *J Clin Endocrinol Metab*. 2015;100(5):1785–1791.
21. Centers for Disease Control and Prevention. *Second National Report on Biochemical Indicators of Diet and Nutrition in the US Population*. Atlanta, GA: Centers for Disease Control and Prevention; 2012. https://www.cdc.gov/nutritionreport/. Accessed April 18, 2018.
22. van Buuren S. Multiple imputation of discrete and continuous data by fully conditional specification. *Stat Methods Med Res*. 2007;16(3):219–242.
23. Roodenburg AJ, Leenen R, van het Hof KH, et al. Amount of fat in the diet affects bioavailability of lutein esters but not of alpha-carotene, beta-carotene, and vitamin E in humans. *Am J Clin Nutr*. 2000;71(5):1187–1193.
24. Schisterman EF, Mumford SL, Browne RW, et al. Lipid concentrations and couple fecundity: the LIFE Study. *J Clin Endocrinol Metab*. 2014;99(8):2786–2794.
25. Zhao Y, Herring AH, Zhou H, et al. A multiple imputation method for sensitivity analyses of time-to-event data with possibly informative censoring. *J Biopharm Stat*. 2014;24(2): 229–253.
26. Whitcomb BW, Schisterman EF, Perkins NJ, et al. Quantification of collider-stratification bias and the birthweight paradox. *Paediatr Perinat Epidemiol*. 2009; 23(5):394–402.
27. Baxter N, Sumiya M, Cheng S, et al. Recurrent miscarriage and variant alleles of mannose binding lectin, tumour necrosis factor and lymphotoxin alpha genes. *Clin Exp Immunol*. 2001; 126(3):529–534.

28. Sierra S, Stephenson M. Genetics of recurrent pregnancy loss. *Semin Reprod Med*. 2006;24(1):17–24.
29. White E, Kristal AR, Shikany JM, et al. Correlates of serum alpha- and gamma-tocopherol in the Women's Health Initiative. *Ann Epidemiol*. 2001;11(2):136–144.
30. Johnson RM, Baumann CA. The effect of alpha-tocopherol on the utilization of carotene by the rat. *J Biol Chem*. 1948;175(2): 811–816.
31. Willett WC, Stampfer MJ, Underwood BA. Vitamins A, E, and carotene: effects of supplementation on their plasma levels. *Am J Clin Nutr*. 1983;38(4):559–566.
32. van Het Hof KH, West CE, Weststrate JA, et al. Dietary factors that affect the bioavailability of carotenoids. *J Nutr*. 2000; 130(3):503–506.
33. Cooney MA, Buck Louis GM, Sundaram R, et al. Validity of self-reported time to pregnancy. *Epidemiology*. 2009;20(1): 56–59.
34. Al-Delaimy WK, Ferrari P, Slimani N, et al. Plasma carotenoids as biomarkers of intake of fruits and vegetables: individual-level correlations in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Eur J Clin Nutr*. 2005;59(12):1387–1396.
35. Campbell DR, Gross MD, Martini MC, et al. Plasma carotenoids as biomarkers of vegetable and fruit intake. *Cancer Epidemiol Biomarkers Prev*. 1994;3(6):493–500.
36. El-Sohemy A, Baylin A, Ascherio A, et al. Population-based study of alpha- and gamma-tocopherol in plasma and adipose tissue as biomarkers of intake in Costa Rican adults. *Am J Clin Nutr*. 2001;74(3):356–363.
37. Burri BJ, Neidlinger TR, Clifford AJ. Serum carotenoid depletion follows first-order kinetics in healthy adult women fed naturally low carotenoid diets. *J Nutr*. 2001;131(8):2096–2100.
38. Leonard SW, Paterson E, Atkinson JK, et al. Studies in humans using deuterium-labeled alpha- and gamma-tocopherols demonstrate faster plasma gamma-tocopherol disappearance and greater gamma-metabolite production. *Free Radic Biol Med*. 2005;38(7):857–866.
39. Wise JA, Kaats GR, Preuss HG, et al. Beta-carotene and alpha-tocopherol in healthy overweight adults; depletion kinetics are correlated with adiposity. *Int J Food Sci Nutr*. 2009;60(suppl 3): 65–75.
40. Wise LA, Rothman KJ, Mikkelsen EM, et al. An Internet-based prospective study of body size and time-to-pregnancy. *Hum Reprod*. 2010;25(1):253–264.
41. Buck Louis GM, Sundaram R, Schisterman EF, et al. Heavy metals and couple fecundity, the LIFE Study. *Chemosphere*. 2012;87(11):1201–1207.
42. Craig CL, Marshall AL, Sjöström M, et al. International Physical Activity Questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc*. 2003;35(8):1381–1395.