First-trimester plasma tocopherols are associated with risk of miscarriage in rural Bangladesh[1–4]

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ABSTRACT

Background: Tocopherols were discovered for their role in animal reproduction, but little is known about the contribution of deficiencies of vitamin E to human pregnancy loss.

Objective: We sought to determine whether higher first-trimester concentrations of α-tocopherol and γ-tocopherol were associated with reduced odds of miscarriage (pregnancy losses $<$24 wk of gestation) in women in rural Bangladesh.

Design: A case-cohort study in 1605 pregnant Bangladeshi women [median (IQR) gestational age: 10 wk (8–13 wk)] who participated in a placebo-controlled vitamin A– or β-carotene–supplementation trial was done to assess ORs of miscarriage in women with low α-tocopherol ($<$12.0 μmol/L) and γ-tocopherol ($<$0.81 μmol/L; upper tertile cutoff of the γ-tocopherol distribution in women who did not miscarry).

Results: In all women, plasma α- and γ-tocopherol concentrations were low [median (IQR): 10.04 μmol/L (8.07–12.35 μmol/L) and 0.66 μmol/L (0.50–0.95 μmol/L), respectively]. In a logistic regression analysis that was adjusted for cholesterol and the other tocopherol, low α-tocopherol was associated with an OR of 1.83 (95% CI: 1.04, 3.20), whereas a low γ-tocopherol concentration was associated with an OR of 0.62 (95% CI: 0.41, 0.93) for miscarriage. Subgroup analyses revealed that opposing ORs were evident only in women with BMI (in kg/m^2) $\geq$18.5 and serum ferritin concentration $\leq$150 μg/L, although low BMI and elevated ferritin conferred stronger risk of miscarriage.

Conclusions: In pregnant women in rural Bangladesh, low plasma α-tocopherol was associated with increased risk of miscarriage, and low γ-tocopherol was associated with decreased risk of miscarriage. Maternal vitamin E status in the first trimester may influence risk of early pregnancy loss. The JiVitA-1 study, from which data for this report were derived, was registered at clinicaltrials.gov as NCT00198822. *Am J Clin Nutr* 2015;101:294–301.

Keywords alpha-tocopherol, gamma-tocopherol, miscarriage, pregnancy, vitamin E

INTRODUCTION

In 1922, Evans and Bishop (1) discovered that the occurrence of fetal resorption in diet-restricted rats was corrected by the consumption of a lipid extract from various grains. Because of its vital role in fertility, this unknown substance was described as an "antisterility factor" and given the name "tocopherol" from the Greek words tokos, meaning childbirth, and phero, meaning to bring. Since this early discovery, vitamin E's role in fertility has been well established in animal models, including demonstrations of an important role in zygote implantation (2), placental maturation (3), and embryogenesis (4).

In addition to affecting fertility, tocopherols play a significant role in oxidative defense, including mechanisms that protect the maternoplacental fetal units (5). Oxidative stress can arise through the enhanced production of reactive oxygen species or a deficiency of antioxidant defenses resulting from inadequate dietary antioxidant intake, decreased synthesis of antioxidant enzymes, or increased antioxidant use (6). Increased oxidative stress and resulting lipid peroxidation have been linked to pregnancy complications such as preeclampsia (7) and early pregnancy loss (8). For example, elevated plasma lipid peroxide and lower vitamin E status were reported in patients with a recurrent abortion (9).

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[3]Supplemental Table 1 is available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org

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Because of consistent associations between decreased antioxidant defense and pregnancy complications, it was hypothesized that antioxidant supplementation during pregnancy would provide protection against adverse pregnancy outcomes. A recent meta-analysis reported no substantive benefit in the randomized, antenatal use of antioxidant supplements that included vitamin E (α-tocopherol compounds) on risks of miscarriage, preeclampsia, or preterm birth among other outcomes (10). However, the lack of effect may have resulted from the fact that the trials included in the meta-analysis were conducted in developed countries where pregnant women tend to have an adequate vitamin E status. Also, in most trials, antioxidant supplements were started in the second trimester often from ~14–18 wk of gestation, thereby reducing the chance of protecting the pregnancy from miscarriage (11).

Although it has been shown that supplementation of pregnant women with α-tocopherol can also decrease γ-tocopherol status (11), most studies have not included information on associations between the plasma γ-tocopherol concentration and pregnancy outcomes (11), which is a relation about which little is known. Because γ-tocopherol is involved in important biological processes, including presumably anti-inflammatory pathways, a decrease in its plasma concentration could impair a woman's ability to combat the inflammatory response associated with pregnancy, thereby increasing risk of adverse outcomes.

Motivated by these lines of evidence, we undertook an analysis to examine the association between α- and γ-tocopherol status in early pregnancy and risk of miscarriage in women in rural Bangladesh.

SUBJECTS AND METHODS

Data were collected from women early in pregnancy at the outset of their participation in a large, double-masked, cluster-randomized, placebo-controlled vitamin A– and β-carotene–supplementation trial implemented in rural northwest Bangladesh from 2001 to 2007 (i.e., the JiVitA-1 trial; clinicaltrials.gov, NCT00198822) that enrolled over 65,000 pregnancies. Details of the main trial were published elsewhere (12, 13). In brief, women were enrolled in early pregnancy by using urine-based testing triggered by not having reported menstruation in the past 30 d during pregnancy-surveillance home visits conducted by local female staff at 5-weekly intervals. The median (IQR) gestational age at enrollment was 10 wk (9–14 wk) at which time an interview was conducted by trained female interviewers to collect baseline demographic, socioeconomic, dietary, health, and reproductive history data. From the time of enrollment in the study, pregnant women were visited each week by field staff to verify pregnancy status and provide a single, oral capsule that contained a placebo, 7000 μg retinol activity equivalents as preformed vitamin A, or 42 mg β-carotene in soy oil according to a randomized ward assignment (13). Pregnancy losses were recorded each week and classified as miscarriage if not induced and occurring at <24 wk of gestation; thereafter, pregnancy outcomes were classified as live or still births.

Within this setting, a home-based substudy that involved more-intensive assessments of nutritional and health status was initiated in a contiguous area balanced by supplement allocation and representing ~5% of the study site. As part of this activity, nonfasting venous blood samples were collected at early pregnancy before starting women on their weekly dietary supplements. The hemoglobin (g/L) concentration was assessed with a single drop of the venous blood by using a B-Hemoglobin Analyzer (HemoCue Inc.). Remaining venous blood samples were immediately stored in a cool insulation bag for same-day transport and processing at the local field laboratory in Gaibandha. Plasma samples were stored in liquid nitrogen immediately after separation until shipment to laboratories at the Institute of Nutrition, Mahidol University, Bangkok, Thailand, and the Center for Human Nutrition, Johns Hopkins University.

The study was approved by the Bangladesh Medical Research Council and the Johns Hopkins University Institutional Review Board. Verbal informed consent was obtained from all subjects in the presence of a witness and was recorded.

Laboratory analysis

α-Tocopherol and γ-tocopherol were measured simultaneously with retinol and carotenoids by using reverse-phase HPLC at Mahidol University according to the method of Yamini et al. (14). These analyses were completed in ~180 runs from March 2003 through December 2006 by using 3 different quality-control pools over time. Interassay CVs for α-tocopherol were 20.0%, 16.0%, and 12.5%, respectively, by quality-control pool and were 7.5%, 21.0%, and 14.0%, respectively, for γ-tocopherol. Cholesterol was also assessed at Mahidol University by using a commercial colorimetric assay. All other assays were performed at the Johns Hopkins University Center for Human Nutrition. Plasma ferritin and C-reactive protein (CRP) were analyzed by using an Immulite 1000 chemiluminescent immunoassay system (Siemens Diagnostics). α-1 Acid glycoprotein (AGP) was assessed by using a radial immunodiffusion assay (Kent Laboratories). Interassay CVs for all Johns Hopkins–based analyses were ≤5%.

Statistical analysis

All women with α- and γ-tocopherol data available at early pregnancy were included in the analysis if they had their blood drawn in the first 24 wk of gestation and had a subsequent pregnancy outcome other than induced abortion or the birth of multiple infants and had all necessary data available.

Vitamin E deficiency was defined as an α-tocopherol concentration <12.0 μmol/L (15). Because there are no recommended cutoffs for assessing γ-tocopherol status, a tertile distribution was defined in women who did not have a miscarriage against which concentrations in those who later miscarried were classified. The highest one-third of the nonmiscarriage group γ-tocopherol distribution was defined as the referent. This assessment was done after determining that ORs and 95% CIs for miscarriage within the low and middle one-thirds compared with the upper one-third of plasma γ-tocopherol were indistinguishable, with no evidence of a dose response in risk (data not shown).

Age was categorized as <20, 20–29, and ≥30 y. Education was described as no formal education, primary (1–5 y), and secondary or higher (>5 y). Socioeconomic status was defined as low or high by using the median value of a living standard index derived for this population with a principal components analysis (16). General nutritional status was defined by using

BMI (in kg/m^2) categorized as thin (BMI <18.5) and normal (BMI ≥18.5). Regularly chewing tobacco was defined by reported use every day compared with not in the previous 7-d recall period. Iron status was defined as being at risk of iron overload if the plasma ferritin concentration was >150 μg/L (17). Normal iron status (plasma ferritin concentration from 15 to ≤150 μg/L) and depleted iron stores (plasma ferritin concentration ≤15 μg/L) were combined and defined as normal iron status because only 16 of 1605 assessed women had depleted iron stores. The presence of inflammation was defined by an elevated AGP (>1.0 g/L) or CRP (>5.0 mg/L).

Baseline characteristics were summarized and compared by miscarriage status by using the chi-square test. Biochemical data were expressed as medians (IQRs) and compared by outcome status by using the Mann-Whitney *U* test. Simple and multiple logistic regression was used to estimate crude and adjusted ORs, respectively, of miscarriage by plasma tocopherol status by using values above cutoffs for α-tocopherol (≥12.0 μmol/L) and γ-tocopherol (highest tertile, ≥0.81 μmol/L) distribution as referents, with 95% CIs derived from SEs of regression coefficients. ORs from sequential logistic regression models are reported. First, unadjusted ORs are reported, and then, those from logistic regression models adjusted for total cholesterol and the other tocopherol are reported to account for the fact that tocopherols are transported in the lipid fraction of plasma. A subsequent model was further adjusted for potential confounders on the basis of biological plausibility and their significance in a univariate analysis ($P < 0.05$), which resulted in the inclusion of gravidity, maternal age (≥30 compared with <30 y) and gestational age (d) at blood draw. The final model included the previous covariates as well as a dummy variable for an AGP concentration >1.0 g/L, which was intended to examine whether associations were ameliorated when adjusted for the presence of inflammation; if this were the case, it would suggest that associations between tocopherol status and miscarriage were mediated by effects on inflammatory status (18).

We also examined interactions of tocopherols on miscarriage with variables shown or expected to be associated with risk of miscarriage. We considered *P*-interaction < 0.2 as suggestive of an effect modification. Where interactions were shown with one of the tocopherols (i.e., generally α-tocopherol) both tocopherols were presented in stratified analyses to examine a potential effect modification. We present interactions of tocopherols with BMI (≥18.5 or <18.5) and risk of iron overload defined by plasma ferritin (>150 compared with ≤150 μg/L) with the same model-building structure as presented for nonstratified ORs. All analyses were conducted with SAS version 9.3 software (SAS Inc.).

RESULTS

There were 2118 pregnant women enrolled in the biochemical substudy cohort in the trial in whom we excluded those who refused a blood sample collection ($n = 76$), elected to have an abortion ($n = 243$), had a pregnancy outcome before the blood draw ($n = 49$), had twins or triplets ($n = 14$), had a blood collection after 24 wk of gestation ($n = 40$), or had other missing data ($n = 91$). Taken together, subjects excluded for any of these reasons ($n = 513$) were older, of higher gravidity, less educated, and more likely to have inflammation than were those included in the study, which were differences that tended to be greater between participants and nonparticipating women who elected abortions (**Supplemental Table 1**). Of 1605 women (76%) included in the study, 141 women (8.8%) subsequently had a miscarriage, and 1464 women had either a live-born singleton ($n = 1415$, 88.1%) or stillbirth (>24 wk gestational age; $n = 49$; 3.1%). Nearly one-half of studied women were <20 y of age, primigravid, and thin with BMI <18.5 (44.2%) (**Table 1**).

Differences in maternal characteristics by miscarriage status are also shown in Table 1. Women who miscarried were more likely to be older, have had multiple pregnancies, and regularly chewed tobacco. A tendency toward earlier blood draws in women who miscarried was an artifact of miscarriage being an early pregnancy event. The miscarriage occurrence did not differ by years of education or living standard index. In nutritional and inflammatory status variables, miscarriage was associated with elevated AGP, but not CRP, and being at risk of iron overload (plasma ferritin concentration >150 μg/L). The geometric mean ferritin concentration was also higher in women who miscarried than those who did not (92.12 ± 1.85 compared with 79.32 ± 1.84 μg/L; $P = 0.007$). Although not statistically significant, a somewhat higher proportion of women who miscarried had low early pregnancy BMI. Miscarriage rates were not different by study supplementation allocation ($P = 0.167$), and therefore, all results were pooled in subsequent analyses.

Median (IQR) plasma α- and γ-tocopherol concentrations, total cholesterol, and ratios of these are shown in **Table 2** for all subjects and stratified by miscarriage status. α-Tocopherol and total cholesterol concentrations were lower, and ratios of γ-tocopherol to α-tocopherol and γ-tocopherol to total cholesterol were higher in women who progressed to miscarriage than in those who did not (all $P < 0.05$), although γ-tocopherol itself was not associated with subsequent miscarriage (Table 2).

Overall, 72.3% of studied women (1161 of 1605) had vitamin E deficiency by an α-tocopherol concentration <12.0 μmol/L (**Table 3**). When stratified by α-tocopherol status, 5.2% of women with adequate α-tocopherol miscarried compared with 10.2% with an α-tocopherol concentration <12.0 μmol/L, which resulted in an unadjusted OR (95% CI) of 2.07 (1.31, 3.28) ($P = 0.002$) for miscarriage risk in women with vitamin E deficiency. Elevated risk of miscarriage with vitamin E deficiency persisted through subsequent models, although it became weaker after adjustment for inflammation (AGP concentration > 1.0 g/L), which was reflected by an OR (95% CI) of 1.68 (0.95, 2.96) ($P = 0.07$).

For γ-tocopherol, a concentration below the upper tertile (<0.81 μmol/L) was associated with an OR (95% CI) of 0.76 (0.53, 1.09) ($P = 0.13$), which was suggestive of lower risk of miscarriage (Table 3). Adjustment for cholesterol and α-tocopherol (model 1) and maternal age, gravidity, and gestational age at blood draw (model 2) strengthened the association (OR: 0.62; 95% CI: 0.41, 0.93; $P = 0.02$) or an ~40% reduction in the likelihood of miscarriage. However, adjustment for the presence of elevated AGP (model 3) weakened the association (OR: 0.70; 95% CI: 0.46, 1.06; $P = 0.09$). The inclusion of tobacco use had no discernable effect on either tocopherol-associated estimates and was not retained in any models.

Stratification revealed no detectable risk of miscarriage associated with α- or γ-tocopherol status in thin women (BMI <18.5). However, strong, opposing risks of miscarriage in

TABLE 1
Participant characteristics at early pregnancy by miscarriage status in women in rural Bangladesh[1]

Characteristics	All (n = 1605)	Nonmiscarriage (n = 1464)	Miscarriage (n = 141)	P
Age, n (%)				
<20 y	770 (48.0)	705 (48.2)	65 (46.1)	
20–29 y	664 (41.4)	613 (41.9)	51 (36.2)	
≥30 y	170 (10.6)	145 (9.9)	25 (17.7)	0.014
Gravidity, n (%)				
1	767 (47.8)	711 (48.6)	56 (39.7)	
2	337 (21.0)	310 (21.2)	27 (19.2)	
3	239 (14.9)	217 (14.8)	22 (15.6)	
≥4	262 (16.3)	226 (15.4)	36 (25.5)	0.015
Gestational age <12 wk at blood draw, n (%)	1027 (64.0)	903 (61.7)	124 (87.9)	<0.001
Education, n (%)				
None	577 (36.0)	528 (36.1)	49 (34.8)	
Primary (1–5 y)	363 (22.6)	330 (22.6)	33 (23.4)	
Secondary or higher	664 (41.4)	605 (41.4)	59 (41.8)	0.945
LSI[2] less than the median, n (%)	659 (41.1)	604 (41.3)	55 (39.0)	0.604
Chewing tobacco (past 7 d), n (%)				
Less than daily[3]	1462 (91.6)	1345 (92.2)	117 (85.4)	
Daily	134 (8.4)	114 (7.8)	20 (14.6)	0.006
Nutritional and inflammatory status, n (%)				
BMI <18.5 kg/m^2	707 (44.1)	636 (43.6)	71 (50.7)	0.107
Ferritin concentration >150 μg/L	209 (13.5)	183 (12.8)	27 (20.5)	0.01
CRP concentration >5.0 mg/L	128 (8.2)	119 (8.3)	9 (6.8)	0.530
AGP concentration >1.0 g/L	358 (22.8)	311 (21.7)	47 (34.8)	<0.001
Trial allocation				
Placebo	505 (31.5)	463 (31.6)	42 (29.8)	
Vitamin A	518 (32.3)	480 (32.8)	38 (27.0)	
β-carotene	582 (36.3)	521 (35.6)	61 (43.3)	0.167

[1]Miscarriage was defined as pregnancy spontaneously ending at <24 wk of gestational age on the basis of the last menstrual period. Missing values (nonmiscarriage, miscarriage): age (1, 0); education (1, 0); chewing tobacco use (5, 4); BMI (6, 1); ferritin (44, 9); CRP (34, 8); and AGP (30, 6). P values were determined by using the chi-square test. AGP, α-1 acid glycoprotein; CRP, C-reactive protein; LSI, living standard index.

[2]LSI is a composite index reflecting housing material and durable asset ownership with −0.259 representing the median index value for all study households in the placebo group (16).

[3]Only 6 of 1462 women reported chewing tobacco on 1–6 d of the previous 7 d.

women with low plasma tocopherols were observed in women with normal-to-higher BMI reflected by an OR (95% CI) of 4.02 (1.67, 9.71) (P = 0.002) for an α-tocopherol concentration <12.0 μmol/L and 0.46 (0.26, 0.83) (P = 0.009) for a γ-tocopherol concentration <0.81 μmol/L in model 2 (**Table 4**). The strength of the association with miscarriage was attenuated for both α-tocopherol and γ-tocopherol with AGP in the model (model 3) but remained significant for the former. The interaction of BMI with tocopherol status was stronger for α-tocopherol (P-interaction = 0.022) than γ-tocopherol (P-interaction = 0.168).

Similar patterns were observed when stratified by plasma ferritin concentration, which reflected an apparent iron storage (**Table 5**). We observed increased risk of miscarriage associated with low α-tocopherol (<12.0 μmol/L) in women with ferritin concentrations ≤150 μg/L with an OR (95% CI) of 2.13 (1.09, 4.18) (P = 0.03) in model 2 but no discernible change in risk at higher ferritin concentrations. Similarly, in women with normal plasma ferritin, an OR (95% CI) of 0.59 (0.36, 0.94) (P = 0.03) for miscarriage was observed with a γ-tocopherol concentration < 0.81 μmol/L but not in women with higher ferritin. Also,

TABLE 2
Tocopherol and cholesterol status in early pregnancy by miscarriage status in rural Bangladesh[1]

Biochemical indicators	All (n = 1605)	Nonmiscarriage (n = 1464)	Miscarriage (n = 141)	P
α-Tocopherol, μmol/L	10.04 (8.07, 12.35)	10.07 (8.13, 12.48)	9.34 (7.55, 11.19)	0.002
γ-Tocopherol, μmol/L	0.66 (0.50, 0.95)	0.66 (0.50, 0.94)	0.66 (0.51, 1.04)	0.317
γ-Tocopherol:α-tocopherol	0.07 (0.05, 0.10)	0.07 (0.05, 0.09)	0.09 (0.06, 0.11)	<0.001
Total cholesterol, mmol/L	3.30 (2.81, 3.93)	3.32 (2.82, 3.95)	3.18 (2.71, 3.75)	0.026
α-Tocopherol:total cholesterol, μmol/mmol	3.06 (2.53, 3.56)	3.07 (2.54, 3.59)	2.94 (2.44, 3.33)	0.089
γ-Tocopherol:total cholesterol, μmol/mmol	0.21 (0.16, 0.28)	0.21 (0.15, 0.28)	0.23 (0.18, 0.29)	0.015

[1]All values are medians; IQRs in parentheses. Missing values (nonmiscarriage, miscarriage): γ-tocopherol:α-tocopherol (1, 0); total cholesterol, mmol/L (114, 8); α-tocopherol:total cholesterol (114, 8); and γ-tocopherol:total cholesterol (114, 8). P values were determined by using the Mann-Whitney U test.

TABLE 3
Prevalence of miscarriage by tocopherol status and relative odds of miscarriage associated with low plasma α-tocopherol and γ-tocopherol in early pregnancy in women in rural Bangladesh[1]

	α-Tocopherol		γ-Tocopherol	
	≥12 μmol/L (referent; *n* = 444)	<12 μmol/L (*n* = 1161)	≥0.81 μmol/L (referent; *n* = 545)	<0.81 μmol/L (*n* = 1060)
Nonmiscarriage, *n* (%)	421 (94.8)	1043 (89.8)	489 (89.7)	975 (92.0)
Miscarriage, *n* (%)	23 (5.2)	118 (10.2)	56 (10.3)	85 (8.0)
Unadjusted	—	2.07 (1.31, 3.28)[2]	—	0.76 (0.53, 1.09)
Model 1	—	2.12 (1.23, 3.65)	—	0.56 (0.38, 0.82)
Model 2	—	1.83 (1.04, 3.20)	—	0.62 (0.41, 0.93)
Model 3	—	1.68 (0.95, 2.96)	—	0.70 (0.46, 1.06)

[1]Models represent relative odds of miscarriage in the lower compared with higher tocopherol groups on the basis of logistic regression and included the following covariates: model 1 was adjusted for the other tocopherol (μmol/L) and cholesterol (mmol/L); model 2 was adjusted as for model 1 and for maternal age (<30 compared with ≥30 y), gravidity, and gestational age in days at blood draw; and model 3 was adjusted as for model 2 and for α-1 acid glycoprotein (>1.0 g/L). Missing values: model 1, *n* = 122; model 2, *n* = 123; and model 3, *n* = 159.
[2]OR; 95% CI in parentheses (all such values).

the interaction of ferritin with tocopherol status was significant for α-tocopherol (*P*-interaction = 0.054) but not γ-tocopherol (*P*-interaction = 0.538).

DISCUSSION

To our knowledge, this is the first population study of early pregnancy vitamin E nutriture and risk of miscarriage, which was undertaken in a typical, rural, undernourished population in Northern Bangladesh. Motivated by experimental evidence, we tested the hypothesis that low first-trimester vitamin E status by either α- or γ-tocopherol was associated with increased risk of miscarriage. In this setting, ∼10% of all pregnancies ended in miscarriage (13), and vitamin E deficiency, which was defined by a plasma α-tocopherol concentration <12.0 μmol/L (15), was present in >70% of women. The most-important findings were that *1*) women with low α-tocopherol concentrations were ∼1.8 times more likely to miscarry, and *2*) women of lower γ-tocopherol status, which was defined as being below the upper tertile of the nonmiscarrying group, were ∼0.6 times as likely to miscarry than were those with higher concentrations. Stratified analyses revealed that the opposing risk relations of the 2 vitamers were confined to women of normal BMI (i.e., ≥18.5) and ferritin concentrations ≤150 μg/L, which suggested that vitamin E may have been inadequate to benefit women across the entire spectrum of miscarriage risk.

Vitamin E status has been infrequently reported in pregnant women in undernourished societies. The cutoff of a plasma α-tocopherol concentration <12.0 μmol/L was proposed to define vitamin E deficiency in normal, healthy adults (15). To date, there is no consensus on the definition of vitamin E deficiency in pregnant women because α-tocopherol concentrations increase with blood lipids over the course of pregnancy (19, 20). Because tocopherols are transported in the lipid fraction of plasma, we controlled for the total cholesterol concentration and gestational age in our analysis, although we did not control for triglycerides, which are more variable in nonfasting states (21). In this population, α-tocopherol status was similar to that reported in a study in rural Nepal, where the early pregnancy median (IQR) value was 11.44 μmol/L (9.28–14.35 μmol/L) (22). Comparative data on γ-tocopherol are less available, although values observed in these women in Bangladesh were low compared with values reported from most population-based studies in developed countries (23).

Concentrations of α-tocopherol and γ-tocopherol reflect dietary intakes of these vitamers from foods and supplements as well as unique metabolic pathways for each. α-Tocopherol is preferentially bound to the hepatic tocopherol transfer protein, which facilitates the resecretion of α-tocopherol into circulation, thereby promoting a longer half-life (∼60 h) for this form of the

TABLE 4
Prevalence of miscarriage by tocopherol and BMI status and relative odds of miscarriage associated with low plasma α-tocopherol and γ-tocopherol in early pregnancy stratified by BMI in women in rural Bangladesh[1]

	BMI <18.5 kg/m^2	BMI ≥18.5 kg/m^2
α-Tocopherol ≥12 μmol/L, *n*	161	281
Miscarriage, *n* (%)	14 (8.7)	8 (2.8)
α-Tocopherol <12 μmol/L, *n*	546	610
Miscarriage, *n* (%)	57 (10.4)	61 (10.0)
Unadjusted	1.22 (0.66, 2.26)[2]	3.79 (1.79, 8.04)
Model 1	1.14 (0.55, 2.35)	4.66 (1.98, 10.97)
Model 2	0.97 (0.45, 2.07)	4.02 (1.67, 9.71)
Model 3	0.81 (0.37, 1.77)	3.91 (1.60, 9.54)
γ-Tocopherol ≥0.81 μmol/L, n	204	341
Miscarriage, *n* (%)	21 (10.3)	35 (10.3)
γ-Tocopherol <0.81 μmol/L, *n*	503	550
Miscarriage, *n* (%)	50 (9.9)	34 (6.1)
Unadjusted	0.96 (0.56, 1.65)	0.58 (0.35, 0.94)
Model 1	0.75 (0.42, 1.34)	0.40 (0.23, 0.69)
Model 2	0.79 (0.43, 1.45)	0.46 (0.26, 0.83)
Model 3	0.85 (0.45, 1.61)	0.56 (0.31, 1.03)

[1]Models represent relative odds of miscarriage in lower compared with higher tocopherol groups within BMI categories on the basis of logistic regression and included the following covariates: model 1 was adjusted for the other tocopherol (μmol/L) and cholesterol (mmol/L); model 2 was adjusted as for model 1 and for maternal age (<30 compared with ≥30 y), gravidity, and gestational age in days at blood draw; and model 3 was adjusted as for model 2 and for α-1 acid glycoprotein (>1.0 g/L). Missing values for BMI <18.5 kg/m^2: for model 1, *n* = 60; model 2, *n* = 61; and model 3, *n* = 77; missing values for BMI ≥18.5 kg/m^2: model 1, *n* = 62; model 2, *n* = 62; and model 3, *n* = 82. Interaction terms: BMI (<18.5 kg/m^2) × α-tocopherol (<12.0 μmol/L), *P* = 0.02; BMI (<18.5 kg/m^2) × γ-tocopherol (<0.81 μmol/L), *P* = 0.17.
[2]OR; 95% CI in parentheses (all such values).

TABLE 5
Prevalence of miscarriage by tocopherol and ferritin concentration and relative odds of miscarriage associated with low plasma α-tocopherol and γ-tocopherol in early pregnancy stratified by ferritin concentrations in women in rural Bangladesh[1]

	Ferritin ≤150 μg/L	Ferritin >150 μg/L
α-Tocopherol ≥12 μmol/L, *n*	378	49
Miscarriage, *n* (%)	15 (4.0)	7 (14.3)
α-Tocopherol <12 μmol/L, *n*	965	160
Miscarriage, *n* (%)	90 (9.3)	20 (12.5)
Unadjusted	2.49 (1.42, 4.36)[2]	0.86 (0.34, 2.17)
Model 1	2.70 (1.40, 5.21)	0.73 (0.73, 2.26)
Model 2	2.13 (1.09, 4.18)	1.06 (0.30, 3.76)
Model 3	2.11 (1.07, 4.14)	1.06 (0.30, 3.80)
γ-Tocopherol ≥0.81 μmol/L, *n*	462	62
Miscarriage, *n* (%)	43 (9.3)	8 (12.9)
γ-Tocopherol <0.81 μmol/L, *n*	881	147
Miscarriage, *n* (%)	62 (7.0)	19 (12.9)
Unadjusted	0.74 (0.49, 1.11)	1.00 (0.41, 2.43)
Model 1	0.53 (0.34, 0.84)	0.86 (0.33, 2.22)
Model 2	0.59 (0.36, 0.94)	1.08 (0.40, 2.95)
Model 3	0.61 (0.38, 0.98)	1.13 (0.41, 3.10)

[1]Models represent relative odds of miscarriage in the lower compared with higher tocopherol groups within ferritin categories on the basis of logistic regression and included the following covariates: model 1 was adjusted for the other tocopherol (μmol/L) and cholesterol (mmol/L); model 2 was adjusted as for model 1 and for maternal age (<30 compared with ≥30 y), gravidity, and gestational age in days at blood draw; and model 3 was adjusted as for model 2 and for α-1 acid glycoprotein (>1.0 g/L). Missing values for ferritin ≤150 μg/L: model 1, *n* = 102; model 2, *n* = 103; and model 3, *n* = 103; missing values for ferritin >150 μg/L: model 1, *n* = 20; model 2, *n* = 20; and model 3, *n* = 20. Interaction terms: ferritin (≤150 μg/L) × α-tocopherol (<12.0 μmol/L), $P = 0.05$; ferritin (≤150 μg/L) × γ-tocopherol (<0.81 μmol/L), $P = 0.17$.

[2]OR; 95% CI in parentheses (all such values).

vitamin. Conversely, γ-tocopherol is more-rapidly metabolized and excreted with an average half-life of 13 h (24–26). On the basis of a variety of studies, both tocopherols play protective roles against oxidative damage, with some that showed γ-tocopherol to be superior in controlling damage caused by reactive nitrogen oxide species (27, 28) and reducing oxidative DNA damage (29). However, at least one study showed γ-tocopherol to be cytotoxic for macrophage cells, which accumulated γ-tocopherol preferentially to α-tocopherol (30).

On balance, findings that supported a protective role for both tocopherols against oxidative stress motivated our assumption that higher γ-tocopherol would be protective in pregnancy. However, although higher α-tocopherol appeared protective against miscarriage, the lower two-thirds of the γ-tocopherol distribution was associated with benefit. A few studies have shown similar opposing patterns of tocopherols associated with adverse pregnancy outcomes. Eight percent higher risk of preterm birth was observed in Canadian women with highest compared with lowest quartiles of circulating γ-tocopherol, with no association with α-tocopherol observed (31). Scholl et al. (32) showed a positive association of fetal growth with early pregnancy and midpregnancy α-tocopherol but no association with γ-tocopherol. In Zimbabwe, a study that examined both tocopherols in pregnancy showed no clear association of either tocopherol with pre-eclampsia (33), whereas the severity of pre-eclampsia was associated with lower α-tocopherol but not γ-tocopherol in women in New York (34).

Epidemiologic evidence is accumulating to suggest circulating γ-tocopherol may not be advantageous and may occur secondary to a poor diet and oxidative or inflammatory stresses. In this Bangladeshi population, we previously reported that α-tocopherol was positively, and γ-tocopherol negatively, associated with the hemoglobin concentration (35), itself reflective of better micronutrient status. Walter et al. (36) reported that α-tocopherol status was significantly decreased with both thalassemia and sickle cell disease relative to in healthy controls, whereas γ-tocopherol was increased, which suggested that the balance of tocopherols was affected by oxidative stress. The prevalence of thalassemia in women in this region of Bangladesh was shown to be high (28%) (37), implying that a similar mechanism could be at play. Elsewhere, in pregnant women, γ-tocopherol was associated with higher intakes of dietary fat and a lower use of antenatal supplements (11, 32). In an analysis of British adults, α-tocopherol status was positively associated with healthy food choices, whereas the reverse was observed for γ-tocopherol (38). Finally, γ-tocopherol was also higher in adult smokers and those exposed to cigarette smoke (39). These studies suggested that elevated γ-tocopherol may reflect adverse dietary and environmental exposure.

The α-tocopherol transfer protein has been isolated in human placenta, suggesting a pregnancy-specific role for α-tocopherol in fetal development because of its preferential transfer to the fetus (2, 40, 41). However, alleviating maternal oxidative stress and inflammation, both of which may constrain pregnancies in environments such as rural Bangladesh, are distinct and likely pathways through which α-tocopherol may reduce miscarriage apart from mechanisms involved in placental nutrient transfer. A higher prevalence of miscarriage has been reported in women with low BMI in early pregnancy (42, 43), which was an association, albeit not statistically significant, observed in the current study. Higher BMI during pregnancy likely reflects increased adipose storage to support energy demands of pregnancy, especially when dietary intakes are chronically or seasonally inadequate. In women in this study, α-tocopherol may have been insufficient to compensate for nutritional stresses represented by low BMI. Conversely, higher BMI in South Asia may be associated with excess adiposity at lower BMI cutoffs than in other ethnicities as we observed in this population (44). Although adipose tissue may provide a buffer against energy restriction, it is also a source of cytokines that may promote chronic, subclinical inflammation (45). Thus, a protective effect of higher α-tocopherol and lower γ-tocopherol against miscarriage at higher BMI may operate through its role as an anti-inflammatory agent (18). Support for this role for tocopherols comes from our analysis that showed that the benefits of higher α-tocopherol and lower γ-tocopherol on miscarriage were attenuated somewhat after accounting for presence of inflammation.

Plasma ferritin concentrations >150 μg/L may reflect risk of iron overload (17), which may exacerbate oxidative stress (36). Women in this population were at low risk of iron deficiency as a result of routinely consuming iron-rich groundwater (46), with many women entering pregnancy with stores considered high. Elevated ferritin may also be associated with inflammation (17). However, there was little attenuation of the association of either α- or γ-tocopherol with miscarriage risk when accounting for

elevated AGP, which suggests that alleviating oxidative stress at levels below a threshold associated with the highest concentrations of ferritin, rather than reducing inflammation, may have occurred.

In conclusion, this study enrolled a large number of women from whom early pregnancy biospecimens and outcome data were available with minimal loss to follow-up. Nonetheless, limitations included a lack of information on dietary sources of vitamin E as well as more-detailed biochemical data on other forms of vitamin E, markers of oxidative stress, and inflammatory cytokines that could help elucidate specific pathways of the action of vitamin E. Nonetheless, our findings showed an association between adequate α-tocopherol nutriture and reduced risk of miscarriage in human populations, inviting future study of potential beneficial effects of vitamin E status during pregnancy.

The authors' responsibilities were as follows—AAS: conceptualized this analysis and led the writing, editing, and coordination of the manuscript; KS: served as the director of the micronutrient laboratory, oversaw the analysis of nutritional biomarkers, and contributed to the writing and editing the manuscript; RDM, AK, and LW: undertook the data analysis and contributed to the writing of the manuscript; ABL and MR: were lead co-investigators and oversaw the field implementation of the study; HA, SS, and SM: were involved with training of data collectors and managed the collection of biochemical samples; PS and EU: were responsible for laboratory analyses; KPW and PC: oversaw the design and implementation of data collection; KPW: served as principal investigator of the study; was primarily responsible for the design, conduct, and oversight of all aspects of the research; and contributed to the writing and editing the manuscript; and all authors: reviewed, commented on, and approved the manuscript. None of the authors reported a conflict of interest related to the study.

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