Vitamin C and E Supplementation to Prevent Spontaneous Preterm Birth

A Randomized Controlled Trial

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OBJECTIVE: To estimate whether maternally administered vitamins C and E lower the risk of spontaneous preterm birth.

METHODS: This is a secondary analysis of a randomized, double-masked, placebo-controlled trial in nulliparous women at low-risk administered 1,000 mg vitamin C and 400 international units vitamin E or placebo daily from 9 to 16 weeks of gestation until delivery. Outcomes include preterm birth attributable to premature rupture of membranes (PROM) and total spontaneous preterm births (spontaneous preterm birth attributable to PROM or spontaneous labor).

RESULTS: Of the 10,154 women randomized, outcome data were available for 9,968 (4,992 vitamin group and 4,976 placebo group). A total of 1,038 women (10.4%) delivered preterm, of whom 698 (7.0%) had spontaneous preterm birth. A spontaneous preterm birth occurred in 356 women (7.1%) assigned to daily vitamin C and E supplementation and in 342 (6.9%) assigned to placebo. There were 253 women (2.5%) who delivered after preterm PROM and 445 (4.5%) after a spontaneous preterm labor. In women supplemented with vitamins C and E, births attributed to preterm PROM were similar at less than 37 and 35 weeks of gestation, but births were less frequent before 32 weeks of gestation (0.3% compared with 0.6%, adjusted odds ratio 0.3–0.9). However, total spontaneous preterm births across gestation in women supplemented with vitamins C and E or a placebo were similar.

CONCLUSION: Maternal supplementation with vitamins C and E beginning at 9 to 16 weeks of gestation in nulliparous women at low risk did not reduce spontaneous preterm births.

CLINICAL TRIAL REGISTRATION: ClinicalTrials.gov, www.clinicaltrials.gov, NCT00135707.

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LEVEL OF EVIDENCE: I

** For a list of other members of the NICHD MFMU, see the Appendix online at http://links.lww.com/AOG/A195.*

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Birth before 37 weeks of gestation (preterm) has increased since 1990 and comprised 12.7% of all births in the United States in 2007.[1] There has been consistency among reports that approximately one fourth of preterm births are indicated because of various factors, primarily maternal hypertensive conditions, and three fourths occur spontaneously with or without preterm premature rupture of membranes (PROM).[2,3] In all women with a spontaneous preterm birth, the contribution of premature membrane rupture has been estimated to be up to one third, with two thirds attributable to spontaneous labor.[4] Preterm PROM has been associated with many factors, including ascorbic acid deficiency (vitamin C).[4–7] These observations are of great importance because if vitamin C supplementation reduces the occurrence of preterm PROM, then a deficiency of vitamin C is a modifiable risk factor and supplementation would be a corrective interventional behavior.

Our intent was to assess further the hypothesis that daily maternal antioxidant supplementation with vitamins C and E from early pregnancy would reduce the incidence of spontaneous preterm birth attributable to either spontaneous labor or preterm PROM. To test this hypothesis, we accomplished a secondary analysis of the National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network multicenter, randomized, double-masked trial of nulliparous women at low risk assigned to daily vitamin C and E supplementation or matching placebo to reduce adverse outcomes attributable to pregnancy-associated hypertension.[8]

MATERIALS AND METHODS

The trial was conducted at the 16 clinical centers that were members of the Eunice Kennedy Shriver National Institutes of Child Health and Human Development Maternal-Fetal Medicine Units Network between 2003 and 2008. Full details of the study design and technique of data collection have been previously described.[8] Women with a singleton pregnancy between 9 weeks 0 days and 16 weeks 6 days of gestation were eligible if they had not experienced a previous pregnancy lasting beyond 19 weeks 6 days. Gestational age was determined before randomization by a previously described algorithm[9] using the date of the last menstrual period (if reliable) and results of the earliest ultrasound examination. Women were not eligible if they had elevated systolic (135 mmHg or higher) or diastolic (85 mmHg or higher) blood pressure or proteinuria (at least 300 mg by 24-hour urine collection or 1+ protein by dipstick), were prescribed antihypertensive medication, or were using more than 150 mg vitamin C or more than 75 international units vitamin E daily. Other exclusion criteria were pregestational diabetes, treatment with antiplatelet drugs or nonsteroidal antiinflammatory agents, uterine bleeding within the week before recruitment, uterine malformation, serious medical complication, known fetal anomaly or aneuploidy, in vitro fertilization resulting in the current pregnancy, or illicit drug or alcohol abuse. Eligible and consenting women were randomly assigned to capsules containing a combination of 1,000 mg vitamin C (ascorbic acid) and 400 international units of vitamin E (RRR alpha tocopherol acetate) or matching placebo (mineral oil). Both the vitamin and placebo capsules were manufactured by Strides, which had no role in the design of the study, the analysis or interpretation of the data, the preparation of the manuscript, or the decision to submit the manuscript for publication. The simple urn method, with stratification according to clinical center, was used by the data-coordinating center to create a randomization sequence.[10]

The study was double-masked. Certified research staff collected information on the women's demographic features, medical history, social history at enrollment, and neonatal and maternal outcomes at delivery. The study was approved by the Institutional Review Boards of each clinical site and the data coordinating center. All participants provided written informed consent before enrollment.

The occurrence of spontaneous preterm births (attributable to preterm PROM or to spontaneous preterm labor) in women randomized to vitamin or placebo supplementation was a planned secondary outcome of the original trial. Preterm birth was defined as before 37 weeks of gestation and as an indicated or a spontaneous preterm birth. Spontaneous preterm birth included spontaneous labor with or without subsequent intrapartum oxytocin augmentation. Similar to some previous Maternal-Fetal Medicine Units Network trials, preterm PROM was defined as spontaneous rupture of membranes and one of the following criteria: membrane rupture 60 minutes or more before onset of labor, labor induced for prelabor ruptured membranes, or no labor and onset of rupture 60 minutes or more before delivery.

Data from all women were analyzed according to the group to which they were randomized, regardless of whether they used the study capsules. Continuous variables were compared using the Wilcoxon rank-sum test, and categorical variables were compared using the χ^2 test. Logistic regression was used to calculate odds ratios, and the multivariable analysis included race or ethnic group, smoking, body mass

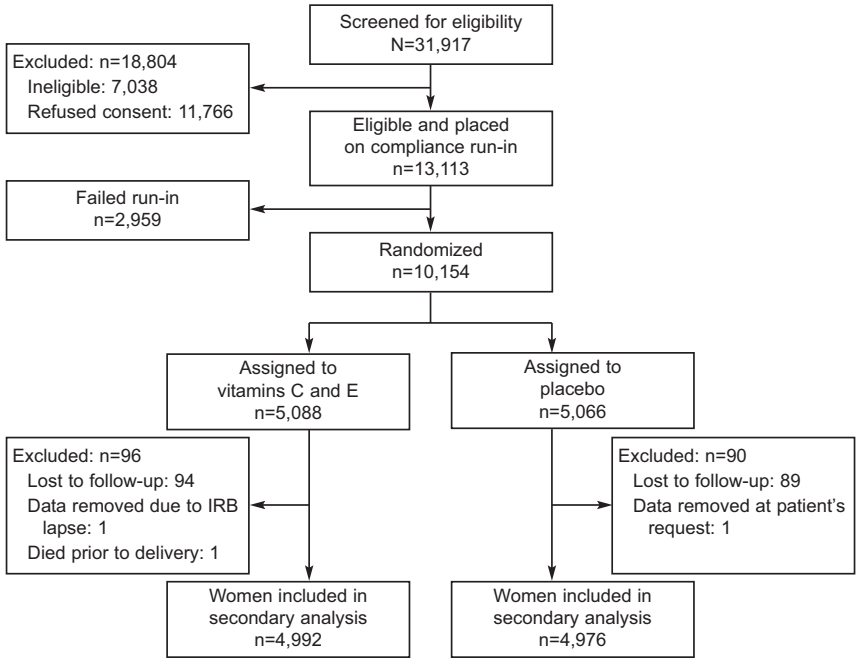

Fig. 1. Enrollment, randomization, and follow-up of participants. IRB, institutional review board.

Hauth. Vitamin C, Vitamin E, and Preterm Birth. Obstet Gynecol 2010.

index at baseline (gestational age 9–16 weeks), and clinical center. Survival analysis, using the Kaplan-Meier method, was performed to estimate whether treatment with vitamins was associated with a longer time to rupture (censoring at term). For each of the outcomes, the Breslow-Day test for homogeneity was used to estimate if there was a difference in the effect among women who started treatment before 13 weeks of gestation compared with those who started on or after 13 weeks of gestation. For all statistical tests, a nominal *P* value less than .05 was considered to indicate statistical significance; no adjustments were made for multiple comparisons. Analyses were performed using SAS software.

RESULTS

Figure 1 details the enrollment, randomization, and follow-up of the participants in this trial. A total of 10,154 women were randomized, and outcome data were available for 9,969 women. One woman who died and never delivered her fetus was excluded, leaving 9,968 women for this analysis (4,992 using vitamins and 4,976 using placebo). Baseline characteristics were similar between the two groups (Table 1). Seventy-seven percent of enrolled participants were using a prenatal vitamin or multivitamin when they were randomized. The median ratio of the number of study capsules used to the number that should have been used between randomization and delivery was 88% in both treatment arms.

A total of 1,038 women (10.4%) delivered preterm, of whom 698 (7.0%) had spontaneous preterm birth. There were 253 women (2.5%) who delivered after preterm PROM and 445 (4.5%) who delivered after a spontaneous preterm labor. Spontaneous preterm birth at less than 37 weeks of gestation occurred in 356 women (7.1%) assigned to daily vitamin C and E supplementation and in 342 (6.9%) assigned a placebo ($P=.61$). These results were similar regarding spontaneous preterm birth before 35 or 32 weeks of gestation (Table 2). Additionally, the survival analysis showed there was no difference in gestational age at membrane rupture (censoring at term) between the two treatment groups ($P=.62$, log-rank test). The frequency of spontaneous preterm birth attributable to preterm PROM before 37 and before 35 weeks of gestation in the vitamin-supplemented and placebo groups were similar (Table 2.) However, spontaneous preterm birth before 32 weeks of gestation and attributable to preterm PROM was less frequent in the vitamin-supplemented group (0.3% compared with 0.6%, adjusted odds ratio 0.3–0.9). Because PROM remote from term often is treated conservatively to prolong pregnancy, we also evaluated the effect of vitamin supplementation based on the gestational age at the time of membrane rupture. Preterm PROM occurring before 32 weeks 0 days of gestation was also less frequent in the women supplemented with vitamins (0.36% compared with 0.64%; $P=.046$). For each of the outcomes, the test for homogeneity was not

Table 1. Baseline Maternal Characteristics

	Vitamins (n=4,992)	Placebo (n=4,976)
Maternal age (y)	23.5±5.2	23.5±5.3
Weeks of gestation at randomization	13.4±2.1	13.4±2.1
Randomization at 12 wk or less	2,181 (43.7)	2,162 (43.4)
Race or ethnic group*		
African American	1,245 (24.9)	1,273 (25.6)
Hispanic	1,559 (31.2)	1,524 (30.6)
Other	2,188 (43.8)	2,179 (43.8)
Body mass index at randomization (kg/m^2)	26.3±6.1	26.3±6.1
Smoker	788 (15.8)	763 (15.3)
Educational level (y)	12.8±2.7	12.8±2.7
Prenatal or multivitamin usage	3,852 (77.2)	3,838 (77.1)
Daily dose of vitamin C (mg)	120 (0–150)	100 (0–300)
Daily dose of vitamin E (international units)	22 (0–100)	22 (0–100)
Previous pregnancy	1,138 (22.8)	1,149 (23.1)

Data are mean±standard deviation, n (%) or median (range).
* Race or ethnic group was self-reported.
$P \geq .05$ for all between-group comparisons.

significant, indicating there was no difference in the effect among women who started treatment before 13 weeks of gestation compared with women who started at or after 13 weeks of gestation.

Neonatal outcomes according to treatment group for the entire cohort previously have been published.[8] Selected neonatal outcomes in the 698 women who had a spontaneous preterm birth before 37 weeks of gestation were similar between those assigned to daily vitamin and placebo supplementation (Table 3).

DISCUSSION

We found a similar occurrence of spontaneous births before 37, 35, and 32 weeks of gestation in women assigned to daily vitamin C and E or to placebo treatment in a planned secondary analysis of a National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network trial. The frequencies of preterm birth before 37 and 35 weeks of gestation attributable to either preterm PROM or preterm labor were also similar between groups. Our analysis does demonstrate less frequent preterm births before 32 weeks of gestation attributable to preterm PROM in women assigned to daily vitamin supplementation (0.30% compared with 0.60%, adjusted odds ratio 0.50, 95% confidence interval 0.27–0.93), and also demonstrates less frequent preterm PROM occurring before 32 weeks of gestation (0.36% compared with 0.64%, adjusted odds ratio 0.56, 95% confidence interval 0.32–1.00). It is possible that this single significant occurrence is attributable to the clinical imprecision of determining the spontaneous preterm birth subcategories of preterm PROM or spontaneous preterm labor. Spontaneous preterm labor and preterm PROM are precisely defined but are less precisely determined because of the difficulties inherent in the "best estimate" of the onset of labor or the timing of membrane rupture in relation to the onset of contractions. Although we limited the diagnosis of PROM to those women with onset of membrane rupture at least 1 hour before the onset of symptomatic contractions, the possibility of incorrect categorization remains and should be considered. Further, the presence of this statistically significant finding for a relatively uncommon finding and the absence of similar trend or pattern for other similar outcomes raise the possibility that the result occurred because of a type I (alpha) error. Most precisely, spontaneous preterm birth includes all of the women who had a preterm birth that was not indicated. We found no difference in spontaneous preterm birth across gestation, whether the women were assigned to daily vitamin supplementation or to a placebo. We also found no benefit of daily maternal vitamin C and E supplementation for any of the prespecified outcomes of the neonates delivered preterm because of spontaneous birth.

Vitamin C deficiency has been associated with the occurrence of spontaneous preterm birth.[4–6,11] Vitamin C is involved in collagen synthesis and may be important to maintain the integrity of the chorioamnion membranes. Woods et al[5] reported that reduced mid-gestational levels of vitamin C are associated with preterm PROM. They noted that reactive oxygen species generated in response to insults such as infection, cigarette smoking, bleeding, or cocaine use can activate collagenolytic enzymes and impair fetal membrane integrity. They hypothesized that an increase in dietary consumption or supplementation of vitamin C and E during pregnancy might reduce the risk of that portion of preterm PROM that may be mediated by oxidative injury to fetal membranes. Plessinger et al[12] report that pretreatment of human amnion-chorion with vitamins C and E prevents hypochlorous acid-induced membrane damage. Casanueva et al[7] tested the hypothesis that maternal supplementation with a relatively low dose of vitamin C (100 mg daily) would prevent spontaneous preterm birth attributable to preterm PROM. In their trial, mean leukocyte vitamin C concentrations decreased in the placebo group and increased in the vitamin C

Table 2. Spontaneous Preterm Birth and Preterm Premature Rupture of Membranes Before 37, 35, and 32 Weeks of Gestation

Outcome	Vitamins (n=4,992)	Placebo (n=4,976)	OR (95% CI)	Adjusted OR* (95% CI)
SPB at less than 37 wk	356 (7.1)	342 (6.9)	1.04 (0.89–1.21)	1.05 (0.90–1.22)
SPB at less than 35 wk	177 (3.6)	185 (3.7)	0.95 (0.77–1.17)	0.96 (0.77–1.18)
SPB at less than 32 wk	109 (2.2)	124 (2.5)	0.87 (0.67–1.13)	0.88 (0.67–1.14)
SPB attributable to preterm PROM at less than 37 wk	124 (2.5)	129 (2.6)	0.96 (0.75–1.23)	0.96 (0.75–1.23)
SPB attributable to preterm PROM at less than 35 wk	56 (1.1)	58 (1.2)	0.96 (0.66–1.39)	0.97 (0.67–1.40)
SPB attributable to preterm PROM at less than 32 wk	15 (0.3)	30 (0.6)	0.50 (0.27–0.92)	0.50 (0.27–0.93)
Preterm PROM at less than 37 wk	130 (2.6)	132 (2.7)	0.98 (0.77–1.25)	0.98 (0.77–1.26)
Preterm PROM at less than 35 wk	58 (1.2)	61 (1.2)	0.95 (0.66–1.36)	0.95 (0.66–1.37)
Preterm PROM at less than 32 wk	18 (0.4)	32 (0.6)	0.56 (0.31–1.00)	0.56 (0.32–1.00)

OR, odds ratio; CI, confidence interval; SPB, total spontaneous preterm births (preterm PROM or preterm labor); PROM, premature rupture of membranes.
Data are n (%) unless otherwise specified.
* Adjusted for race, smoking, baseline body mass index, and center.

group (*P*=.001), and preterm PROM occurred in 14 of 57 (24.6%) women assigned to placebo treatment and in 4 of 52 (7.6%) assigned to vitamin C (relative risk 0.26, 95% confidence interval 0.08–0.83). In a planned secondary analysis of a larger population, Spinnato et al[13] reported that women assigned to 1,000 mg of daily maternal vitamin C and 400 international units of vitamin E or to a placebo had a similar occurrence of spontaneous preterm births. These authors did report an increased frequency of preterm PROM among women randomized to vitamin supplementation (16/349 [4.6%] compared with 6/348 [1.7%]; *P*=.025). In both of these reports and in our analysis, the frequencies of total spontaneous births were not different between groups. The conflicting findings regarding preterm PROM in the Casanueva trial, the Spinnato analysis, and our analysis further raise questions regarding the veracity of our finding that vitamin C and E supplementation reduces the frequency of preterm PROM before 32 weeks of gestation. This is particularly the case for combined vitamin C and E supplementation, because our analysis and the Spinnato analysis had opposite results.

Table 3. Neonatal Outcomes for Spontaneous Preterm Births Ocurring Before 37 Weeks of Gestation

	Vitamins (n=356)	Placebo (n=342)	*P*
Fetal or neonatal death	72 (20.5)	80 (23.5)	.33
Fetal loss at less than 20 wk	45 (12.6)	47 (13.7)	.67
Fetal death at 20 wk or more	8 (2.2)	10 (2.9)	.57
Neonatal death	19 (5.4)	23 (6.8)	.45
SGA less than third percentile*	3 (1.0)	8 (2.8)	.10
LBW less than 2,500 (g)*	157 (52.0)	160 (56.5)	.27
Apgar score 3 or less at 5 min*	14 (4.7)	17 (6.0)	.48
RDS*	56 (18.6)	49 (17.3)	.67
Sepsis*	20 (6.6)	11 (3.9)	.13
Necrotizing enterocolitis*	8 (2.7)	8 (2.8)	.91
IVH (grade III and IV)*	5 (1.7)	7 (2.5)	.49
Retinopathy of prematurity*	16 (5.3)	13 (4.6)	.68
NICU admission*	149 (49.2)	142 (50.0)	.84

SGA, small for gestational age; LBW, low birth weight; RDS, respiratory distress syndrome; IVH, intraventricular hemorrhage; NICU, neonatal intensive care unit.
Data are n (%) unless otherwise specified.
* Live births only (303 in the vitamins group and 285 in the placebo group).

In summary, maternal supplementation with vitamin C and E did not reduce the occurrence of spontaneous preterm birth. Neonatal outcomes were similar in preterm newborns whose mothers were randomized to treatment with vitamin C or E or a placebo. Our results, taken in context with similar trials regarding vitamin C and E supplementation, do not support either the clinical use for prevention of spontaneous preterm birth or its neonatal sequelae or further trials of this treatment in similar populations at low risk.

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