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Female dietary antioxidant intake and time to pregnancy among couples treated for unexplained infertility

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Abstract

Objective—To determine whether increased antioxidant intake in women is associated with shorter time to pregnancy (TTP) among a cohort of couples being treated for unexplained infertility.

Design—Secondary data analysis of a randomized controlled trial.

Setting—Academic medical center associated with a private infertility center.

Patients—Females with unexplained infertility.

Interventions—None.

Main Outcome Measure(s)—The time it took to establish a pregnancy that led to a live birth.

Results—Mean nutrient intake exceeded the Estimated Average Requirement (EAR) for vitamins C and E. No differences in mean intake of any of the antioxidants were noted between women who delivered a live-born infant during the study period versus those who did not. In multivariable models, intake of ß-carotene from dietary supplements was associated with shorter TTP among women with BMI ≥ 25 kg/m^2 (HR= 1.29, 95% CI: 1.09, 1.53) and women < 35 y (HR=1.19, 95% CI: 1.01, 1.41). Intake of vitamin C from dietary supplements was associated with shorter TTP among women with BMI < 25 kg/m^2 (HR=1.09, 95% CI: 1.03, 1.15) and women < 35 y (HR=1.10, 95% CI: 1.02, 1.18). Intake of vitamin E from dietary supplements among women ≥ 35 y was also associated with shorter TTP (HR=1.07, 95% CI: 1.01, 1.13).

Conclusions—Shorter TTP was observed among women with BMI < 25 kg/m^2 with increasing vitamin C, women with BMI ≥ 25 kg/m2 with increasing β-carotene, women < 35 y with increasing β-carotene and vitamin C, and women ≥ 35y with increasing vitamin E.

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Conflict of Interest: None to disclose

Keywords

diet; antioxidants; oxidative stress; unexplained infertility; nutritional epidemiology

INTRODUCTION

The role of dietary antioxidant intake in conception and birth outcome is a topic of emerging interest. A growing body of evidence suggests that oxidative stress (OS) and low antioxidant status may be associated with infertility of both known and idiopathic origin (1). Lower total antioxidant status (TAS) is observed in serum of women with polycystic ovarian syndrome (a known risk factor for female infertility) and the peritoneal fluid of women with idiopathic infertility compared to fertile controls (2, 3). *In vivo*, antioxidants scavenge reactive oxygen species (ROS) and other radical species. Oxidative stress occurs when scavenging capacity is exceeded, either because of decreased antioxidant intake or increased antioxidant utilization due to excessive production of ROS. Furthermore, characteristics associated with decreased fertility, such as advanced maternal age and obesity are also associated with increased oxidative stress (4–6).

Other than for folate, specific dietary guidance for women trying to conceive is not included as part of the Institute of Medicine's Dietary Reference Intake (DRI) reports (7). Some, but not all, antioxidants have increased DRIs during pregnancy. For example, the maternal requirement for vitamin C is increased due to hemodilution and active transfer to the fetus (7). Certain populations, such as cigarette smokers and heavy users of alcohol, may have further increased vitamin C requirements during pregnancy as a result of increased lipid peroxidation (7). In contrast, an increased vitamin E requirement during pregnancy is not supported.

The preponderance of lay literature suggesting dietary factors influence female fertility is based on limited scientific research that primarily investigates specific types of infertility, such as ovulatory infertility (8), or the relationship between glucose control and fertility (9, 10), although one recent investigation suggests that women's adherence to national dietary recommendations increases chance of ongoing pregnancy in couples undergoing IVF/ICSI treatment (11). In the present study, we explore antioxidant intake of the female partner in relation to the time it takes a couple to conceive a pregnancy that resulted in a live birth. Given that BMI and age affect fertility and the potential for variation in micronutrient intake among these subgroups (12, 13), we examine diet by age and diet by BMI interactions. We hypothesize that higher intakes of antioxidants β-carotene, vitamin C and vitamin E are associated with a shorter time to conception among a cohort of couples being treated for unexplained infertility.

MATERIALS AND METHODS

Participants

Women participating in The Fast Track and Standard Treatment (FASTT) trial (n=503) who reported reliable dietary information (n=441, defined as those reporting 500 – 5000 kcal / d of intake) and started treatment (n=437, 87%) were included in the present analyses. FASTT was a randomized, controlled clinical trial conducted to evaluate an accelerated treatment strategy for couples with unexplained infertility that consisted of three cycles of clomiphene citrate/intrauterine insemination (CC/IUI) and up to six cycles of IVF compared to a step-wise treatment course of three cycles of CC/IUI, three cycles of gonadotropin/IUI, and up to six cycles of IVF. The study protocol was approved by the Institutional Review Boards at all participating institutions and study participants gave written informed consent. An

independent Data and Safety Monitoring Board (DSMB) met annually. No conflicts of interested exist for the authors of the present investigation. Details of the study are published (14). Briefly, all couples in which the woman was 21–39 years of age who sought care for unexplained infertility at Boston IVF or Harvard Vanguard Medical Associates between September 14, 2001 and August 31, 2005 were screened. Eligibility criteria included 12 months of unsuccessful attempted conception; at least one ovary and ipsilateral patent fallopian tube confirmed by hysterosalpingogram or laparoscopy; no pelvic pathology, ectopic pregnancy, or previous infertility treatment (with the exception of up to three cycles of clomiphene citrate without IUI). Sufficient ovarian reserve, demonstrated by cycle day 3 follicle-stimulating hormone (FSH) and estradiol values of < 15 mIU/mL and < 100 pg/mL, respectively, and a sperm concentration of ≥15 million total motile sperm or ≥5 million total motile sperm from the male partner at reflex IUI preparation were required. Exclusion criteria included the presence of hydrosalpinges, stage III/IV endometriosis, use of donor sperm, or the need for assisted reproductive technology procedures other than IVF. Randomization was performed using permuted blocks of varying sizes, stratified by woman's age (<35 y vs. ≥35 y), laparoscopy within the past year (yes or no), and study site (Boston IVF or Harvard Vanguard Medical Associates). The closing date of the study for delivery of at least one live-born baby was April 30, 2006. Main study results indicated an increased rate of pregnancy in the accelerated arm of the trial, with fewer treatment cycles and at less cost than in the conventional treatment arm (14).

Assessment of dietary intake and supplement use

Participants completed a paper-based, validated 110-item Block Food Frequency Questionnaire (FFQ) at study baseline (15). The FFQ ascertained energy, macro- and micronutrient intake from diet and dietary supplements. Intake of caffeine was not available. Participants were asked to select from 11 options for typical frequency of food and beverage intake, ranging from never to≥6 times/d. In addition, participants selected from five options for frequency of intake of a variety of dietary supplements (<1 day/month, 1–3 days/month, 1–3 days/week, 4–6 days/week, every day) and 6 options for range of dosage, including an option for "I don't know." To reduce extraneous variation in non-energy-bearing nutrient intakes, dietary intake was adjusted for total energy intake with the use of the nutrient residual method (16). In total, we examined three categories of nutrient intake: total nutrient intake (from diet and supplement sources), dietary nutrient intake (diet sources only), and dietary supplements alone.

Assessment of Covariates

Non-diet covariates with known or suspected association with fertility were self-reported as part of the baseline personal health history questionnaire. These included: age, smoking, physical activity, and height and weight. BMI was calculated as kilograms per square meter from the self-reported height and weight.

Assessment of Birth Outcome

The outcome of interest was length of time from the date of randomization to the date of pregnancy resulting in a live birth. Time to pregnancy was determined from the date of randomization to the date of conception of a pregnancy resulting in a live birth and quantified in months (including fractions of a month) for analyses. Analyses were conducted as intention to treat; thus if a couple forewent a treatment cycle they were not excluded from the time to pregnancy calculation. Date of conception was estimated, as follows: the date of the IUI, the oocyte retrieval, or the embryo thaw or, for pregnancies that occurred outside a treatment cycle, the date of coitus, last menstrual period plus 14 days, or 38 weeks prior to the expected delivery date.

All couples were followed until discharge from the hospital of both mother and infant(s), or until one year after completing the treatment protocol. For couples who had not delivered by the closing date of April 30, 2006, time was censored at the date of the ultrasound confirming the pregnancy or at the date of last contact if not pregnant. Hiatus from treatment occurred for medical reasons or personal choice; when couples did not return to treatment within one year they were considered to have completed treatment prior to the break.

Statistical Analysis

Baseline unadjusted descriptive characteristics were assessed using Students *t*-tests for continuous variables and χ^2 tests for categorical variables. Total nutrient intake was reported in three ways: 1.) mean, median and range, 2.) percent of the U.S. Food and Nutrition Board/Institute of Medicine's Estimated Average Requirements (EARs) during pregnancy for women 31–50 years of age, and 3.) percent of participants not meeting the EAR during pregnancy for women 31–50 years of age (7).

Hazard ratios (HR) and 95% confidence intervals (CI) were estimated using Cox proportional hazards regression models with TTP as the underlying time metric. A HR greater than one indicates that the variable was associated with a shorter time to pregnancy. Total nutrient and dietary nutrient intakes were modeled as continuous linear terms and in tertiles, with the lowest tertile as the reference category. For the continuous models, results are presented per unit increase typical for a pre-natal vitamin supplement: β-carotene: 2000 IU, Vitamin C: 60 mg, Vitamin E: 20 mg. Tests for trend were calculated by assigning the median value to each tertile. Due to a large number of tied values for intake from dietary supplements, we examined supplement intake only as a continuous linear variable. Interactions between BMI category (<25.0 vs. ≥25.0 kg/m^2), age (<35 vs. ≥35 y) and antioxidant intake were evaluated by examining the cross-product term for the individual nutrient and category of BMI or age.

The final multivariable model contained only the variables that changed the HR by 10% or more or were *a priori* covariate inclusions. These were: treatment group, total energy intake, BMI (in age stratified models only) and age (in BMI stratified models only). The following potential covariates were examined but did not materially alter the effect of the individual antioxidants and were not included in either the BMI or age stratified models: alcohol intake (g/day), total fat (g/day), saturated fat (g/day), monounsaturated fat (g/day), polyunsaturated fat (g/ day), cigarette use (current, former, never), race (white, black, Asian, other), and physical activity. All analyses were performed using SAS software (version 9.1; SAS Institute Inc., Cary, NC). Statistical significance was defined as $P \leq 0.05$ for all tests and models.

RESULTS

Among the n=437 eligible female participants, there were a total of n=273 pregnancies resulting in a live birth during the study period. The cohort was predominately white with a mean age of 33.1 y. Mean BMI was 23.7 kg/m^2 and 84% of study participants reported taking vitamin supplements regularly. The overwhelming majority of the cohort had no history of cigarette smoking (Table 1). No statistically significant differences were detected between participants who had a live birth during the study period compared to those who did not in age, BMI, smoking status, or regular use of vitamin or mineral supplements. Significant differences in birth outcome by ethnicity were detected, although the number of non-white participants was relatively small. Mean time to conception among the 273 women delivering a live infant(s) was 6.19 months (standard deviation (SD)=5.11), whereas women who did not experience a conception leading to a live birth were censored after a mean time period of 14.74 months (SD=8.99). Mean daily energy, and antioxidant intakes from diet

and dietary supplements are shown in Table 2. The majority of participants exceeded the EAR for pregnancy among women aged 30–51 y. No significant differences were detected in the unadjusted mean intakes for total, dietary, or dietary supplement intake between females who had a live birth during the study period and those who did not. In addition, no significant differences in total energy, intake of antioxidant nutrients, age, or BMI were detected between participants who reported dietary supplements intake versus those who did not (data not shown), and there were no statistically significant associations between with having met the EAR or not and TTP resulting in a live birth among any of the nutrients under investigation.

Significant interactions between BMI category (<25.0 vs. ≥25.0 kg/m^2) and vitamin C (p=0.04, p=0.02, and p=0.05 for dietary supplement, total intake, and dietary sources, respectively) were noted. In addition, significant interactions between age category (<35 vs. ≥35 y) were noted for total vitamin E (p< 0.01), vitamin E from dietary supplements (p<0.001), and β-carotene from dietary supplements (p=0.02). Therefore, we conducted analyses stratified by BMI and age.

Among women with BMI <25 kg/m^2, increased intake of vitamin C from dietary supplements was associated with a shorter TTP (HR= 1.09, 95% CI: 1.03, 1.15). Modeling total vitamin C intake as a continuous variable also indicated a significant association between increased intake and shorter TTP (HR=1.08, 95% CI: 1.03, 1.14). However, no significant associations were detected between tertile of total vitamin C intake (T3 vs. T1, HR: 1.13, 95% CI: 0.80, 1.59), or dietary intake of vitamin C (Table 3). For women with BMI ≥25 kg/m^2, increased intake of ß-carotene from dietary supplements was associated with shorter TTP (HR=1.29, 95% CI: 1.09, 1.53). No significant relationships between intakes of total ß-carotene or dietary ß-carotene were noted (Table 3). Among younger women (<35 y of age), increased ß-carotene from dietary supplements and intake of total ß-carotene (modeled as a continuous variable) were associated with shorter TTP (HR= 1.19, 95% CI: 1.01, 1.41, and HR=1.11, 95% CI: 1.00, 1.23, respectively). However, these relationships were not maintained when total intake was modeled in tertiles, nor was there a significant association between dietary intake of ß-carotene and TTP. Increased supplementary vitamin C and total vitamin C intakes were also associated with shorter TTP (HR=1.10, 95% CI: 1.02, 1.18, and HR=1.07, 95% CI: 1.00, 1.15, respectively) (Table 4), but similar to ß-carotene in the younger women, these relationships were not maintained when total intake was modeled in tertiles, nor was there a significant association between dietary intake of vitamin C and time to pregnancy. Lastly, among older women (≥35 y), increased intake of vitamin E from supplements, as well as total intake of vitamin E modeled as a continuous variable were associated with shorter time to pregnancy (HR= 1.07, 95% CI: 1.01, 1.13, and HR= 1.07, 95% CI: 1.00, 1.13, respectively) (Table 4). These relationships were not maintained when total intake was modeled in tertiles, nor was there a significant association between dietary intake of vitamin E and time to pregnancy. Intakes of total β-carotene and dietary β-carotene in the highest tertiles of consumption were associated with longer time to pregnancy in this age strata (T3 vs. T1 HR= 0.56, 95% CI: 0.34, 0.92, p-trend = 0.02, and HR=0.58, 95% CI: 0.34, 0.96, p-trend=0.03, respectively) (Table 4).

DISCUSSION

In this cohort of women enrolled in a randomized controlled trial to evaluate an accelerated treatment strategy for unexplained infertility, we found evidence that increased intake of certain antioxidants is associated with shorter time to pregnancy, but the relationship varied. Specifically, shortened TTP was observed only among women with BMI < 25 kg/m^2 with increasing vitamin C, women with BMI ≥ 25 kg/m^2 with increasing β-carotene, women < 35y with increasing β-carotene and vitamin C, and women ≥ 35y with increasing vitamin E.

Among women ≥ 35 y, increasing dietary and total intake of β-carotene was associated with increased TTP. Overall, intake of antioxidant nutrients from dietary supplements rather than diet was most relevant. There is no indication in the literature that antioxidants from dietary supplements are biologically superior to those obtained from dietary sources. Rather, it is likely that the use of dietary supplements helped FASTT participants achieve the increased intake necessary to elicit an effect.

It is hypothesized that female antioxidant intake and oxidative stress may influence the timing and maintenance of a viable pregnancy (1), but this relationship has not been evaluated previously. In men, it is known that diet influences oxidative damage to sperm DNA (17) and decreased sperm number, motility, and inhibition of sperm-oocyte fusion are associated with increased reactive oxygen species (18). To the best of our knowledge, the present investigation is the first to investigate female antioxidant intake, conception, and birth outcome.

We stratified our analyses separately by BMI and age for three reasons. Firstly, both high BMI and advanced maternal age are known to adversely affect fertility (19, 20); secondly, U.S. national dietary data suggests that micronutrient intake varies by both age and BMI (13, 21); and lastly, there were significant statistical interactions between BMI, age, and certain antioxidants. Joint stratification by BMI and age at randomization was not feasible due to sample size. Increasing intake of vitamin C from dietary supplements was associated with shorter time to pregnancy among women with BMI <25 kg/m^2 and women < 35 y of age. Throughout the body, vitamin C slows the propagation of the peroxidative process and generation of free radicals and helps recycle oxidized vitamin E and glutathione (22). In addition, animal models suggest a role for vitamin C as an important antioxidant cofactor in the synthesis of collagen in the extracellular matrix of the corpus luteum; which develops from the ovarian follicle and is involved in the production of progestin needed to maintain the endometrium (23). Obesity and advanced maternal age are associated with enhanced lipid peroxidation and overall generation of reactive oxygen species (5, 6, 24). In general, women who are obese experience a higher incidence of delayed conception (4) and obese women, including obese women seeking treatment for infertility, experience an increased risk of miscarriage (25–27). Likewise, advanced maternal age is associated with decreased fertility and increased pregnancy complications. In our study, the mean intakes of total, dietary, and dietary supplement sources of vitamin C were similar among the BMI strata ($p > 0.05$, data not shown). Among the age strata, mean intakes of dietary vitamin C were similar ($p > 0.05$), but mean supplementary vitamin C intakes were higher in the older women relative to the younger women ($p < 0.0001$). However, median intake of supplementary vitamin C was the same in both age strata (60 mg), and closer inspection of the data revealed that the mean value in the older women was skewed by the presence of three women (2% of the older participants) taking ≥ 1000 mg / day, whereas < 0.03% of the younger participants consumed supplementary vitamin C at this level. Thus, intake of vitamin C among the overweight/obese individuals, and most women in the older age strata may not have been high enough to enhance fertility given their inherent oxidative burden.

Increased intake of β-carotene from dietary supplements was associated with shorter TTP in the overweight/obese women and the younger age-group strata. β-carotene has potent antioxidant activity, and lower concentration of β-carotene in follicular fluid is associated with decreased IVF success (28). Bovine models have suggested a role for β-carotene in enhancing the cytoplasmic maturation of oocytes during the second meiotic division (29). Our finding that increased dietary β-carotene intake in the women ≥ 35 years of age was associated with longer TTP was unexpected. Although we were not able to assess plasma carotenes, diet-induced hypercarotenemia is associated with anovulation and amenorrhea (30), possibly due to altered progesterone secretion (31). Unlike dietary supplement intake,

increased dietary consumption of β-carotene is positively associated with intake of the ~600 other carotenoids not included in standard dietary assessment software. If these carotenoids have biologic activity similar to β-carotene, then the dietary effect of β-carotene may be enhanced. This rationale provides one possible explanation why the relationship was not present with dietary supplement intake, which entails only intake of β-carotene. However, we feel this unanticipated finding should be interpreted with caution and it should be emphasized that a delay in TTP with increased dietary β-carotene was not noted among women with BMI < 25 kg/m^2 , BMI ≥ 25 kg/m^2 , or age < 35 y. Lastly, increased intake of vitamin E from dietary supplements and total vitamin E intake were associated with shorter TTP in women ≥ 35y. Although the present study did not assess biologic mechanisms, increased ovarian concentration of vitamin E may be required to help protect the aging ovary during luteolysis and compensate for the decline in the luteal cell ability to quench reactive oxygen species (32). In addition, vitamin E may also prevent damage to the ovarian surface epithelium (33).

The present investigation has several strengths, including a large sample size, prospective, detailed information on TTP, and detailed dietary and supplement data collected via an FFQ at baseline. The FFQ used in present study queried 110 food items and contained 16 questions related to dietary supplement use. Questions related to dietary supplement ascertained use of multiple vitamins, with separate items for regular once-a-day, stress tabs or B-complex type, and antioxidant combination type supplements. Additional items queried single vitamins not part of multiple vitamins, specifically ascertaining data on: Vitamin A, β-carotene, vitamin C, vitamin E, folic acid/ folate, calcium, zinc, iron and selenium. Limitations include reliance on participant recall for diet and supplement intake and a predominately white study population. FFQs do not ascertain all aspects of diet and energy, nor do they query all possible dietary supplements. The generalizability of our study is limited in that subjects were overwhelmingly Caucasian and were seeking treatment for unexplained infertility. It is likely that our subject population engaged in greater dietary supplement use and greater practice of healthy behaviors relative to age-matched females from under-represented minority groups or those without infertility. Furthermore, the present analyses do not consider the male partner's health or diet in the conception and maintenance of a viable pregnancy. FASTT participants were seeking treatment for unexplained infertility, and thus a limitation of our study is that observations may not be generalizable to populations with infertility of known origin or populations without an indication of sub-fertility.

In summary, our results suggest that among the female FASTT participants, increased intakes of β-carotene, vitamin C, and vitamin E were associated with TTP, but the effect of these antioxidant nutrients varies with BMI and age. Specifically, shorter TTP was observed among women with BMI < 25 kg/m^2 with increasing vitamin C, women with BMI ≥ 25 kg/m^2 with increasing β-carotene, women < 35-y with increasing β-carotene and vitamin C, and women ≥ 35 y with increasing vitamin E. These results are consistent with the hypothesis that increased antioxidant intake is positively associated with female fertility. Future work should include prospective pregnancy studies with multiple instances of dietary and supplement intake assessment as well as collection of biological samples to ascertain measures of oxidative stress and to detect early pregnancy loss.

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Table 1

Selected baseline demographic characteristics of female FASTT participants

	Cohort (n=437)	No Live Birth (n=164)	Live Birth (n=273)	P-value[a]
Age, y (mean (SD))	33.09 (3.36)	33.07 (3.26)	33.11 (3.43)	0.92
BMI, kg/m^2 (mean (SD))	23.73 (4.33)	23.92 (4.57)	23.62(4.18)	0.47
Smoking Status, n (%)[b]				
Never	309 (71)	108 (66)	201 (74)	
Former	102 (24)	44 (27)	58 (21)	0.19
Current	23 (5)	11 (7)	12 (4)	
Ethnicity, n (%)				
White	386 (88)	132 (80)	254 (93)	< 0.001
Other /multiracial	51 (12)	31 (20)	19 (7)	
Took vitamin or mineral supplement regularly, n (%)[b,c]				
Yes	368 (85)	135 (83)	233 (86)	0.26
No	66 (15)	28 (17)	37 (14)	

SD= standard deviation, BMI = body mass index

[a] Comparing no live birth to live birth, t-test for continuous variables, $\chi 2$ tests for categorical variables.

[b] data missing for n=1 individual,

[c] As reported in baseline health questionnaire

Table 2

Daily baseline energy and antioxidant intake of female FASTT participants

	Cohort (n=437)						No Live Birth (n=164)						Live Birth (n=273)						
	N	Mean	Median	Range	% EAR[a]	% not meeting EAR[a]	N	Mean	Median	Range	% EAR[a]	% not meeting EAR[a]	N	Mean	Median	Range	% EAR[a]	% not meeting EAR[a]	p-value[b]
Total energy, kcal	437	1532	1511	581 – 4267	--	--	164	1603	1500	581 – 4267	--	--	273	1570	1513	700 – 3435	--	--	0.53
B-carotene, IU[c]																			
Total	437	4072	3239	536 –33056	--	--	164	4100	3344	714 –19345	--	--	273	4056	3239	536 –33056	--	--	0.90
Dietary	437	3151	2452	398 –18145			164	3300	3300	398 – 18145			273	3061	2424	489 – 17233			0.34
Dietary Supplements	312	1290	1200	343 – 26200			119	1100	1200	343 – 4200			193	1407	1200	343 – 26200			0.27
Vitamin C, Mg																			
Total	437	198	198	26 – 1584	283	10	164	189	138	28 – 1148	269	10	273	204	145	26 – 1584	291	10	0.38
Dietary	437	104	104	15 – 358			164	105	94	15 – 352			273	103	95	16 – 358			0.77
Dietary Supplements	322	128	128	17 – 1500			120	114	30	17 – 1060			202	136	60	16 – 1500			0.31
Vitamin E, mg																			
Total	437	37	25	2 – 559	290	30	164	32	25	2 – 559	263	29	273	37	25	2 – 407	308	30	0.34
Dietary	437	7	6	2 – 30			164	7	7	2 – 6			273	7	6	2 – 30			0.49
Dietary Supplements	317	30	16	6 – 556			120	27	16	5 – 445			197	33	16	5 – 322			0.33

EAR = Estimated Average Requirement, defined as the daily nutrient level estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group.

IU = International Unit

[a] EAR during pregnancy, women 30–51 years, EAR is defined as A daily nutrient level estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group.

[b] comparing intakes from female participants without a live birth versus those who had a live birth

[c] No EAR established

Table 3

Hazard Ratios of Time to Pregnancy (TTP) with Live Birth by Antioxidant Intake and BMI

BMI < 25 m^2 / kg (n=319)									
Antioxidant [a,b] **HR (95% CI)**	**Dietary Supplement Intake, Alone**	**Dietary Intake**				**Total Nutrient Intake**[c]			
		T1	T2	T3	p-trend	T1	T2	T3	p-trend
β-carotene	--	1.00	1.09 (0.78, 1.53)	0.92 (0.65, 1.31)	0.57	1.00	0.86 (0.61, 1.20)	0.86 (0.61, 1.20)	0.36
Continuous	1.19 (0.96, 1.48)	1.00 (0.87, 1.15)	--	--		1.03 (0.91, 1.17)	--	--	
Vitamin C	--	1.00	0.93 (0.66, 1.32)	0.95 (0.68, 1.32)	0.77	1.00	1.05 (0.74, 1.50)	1.13 (0.80, 1.59)	0.49
Continuous	1.09 (1.03, 1.15)	0.98 (0.83, 1.17)				1.08 (1.03, 1.14)			
Vitamin E	--	1.00	0.84 (0.60, 1.18)	0.73 (0.52, 1.03)	0.35	1.00	1.01 (0.71, 1.44)	1.01 (0.72, 1.42)	0.94
Continuous	1.00 (0.95, 1.05)	0.71 (0.18, 2.75)	--	--		1.00 (0.95, 1.05)	--	--	
BMI ≥ 25 m^2 / kg (n=115)									
Antioxidant [a,b] **HR (95% CI)**	**Dietary Supplement Intake, Alone**	**Dietary Intake**				**Total Nutrient Intake**[c]			
		T1	T2	T3	p-trend	T1	T2	T3	p-trend
β-carotene	--	1.00	0.76 (0.38, 1.51)	1.21 (0.64, 2.29)	0.34	1.00	1.23 (0.61, 2.46)	1.44 (0.74, 2.79)	0.28
Continuous	1.29 (1.09, 1.53)	1.02 (0.86, 1.21)				1.10 (0.96, 1.26)			
Vitamin C	--	1.00	1.11 (0.57, 2.15)	1.40 (0.73, 2.69)	0.29	1.00	1.05 (0.56, 1.99)	1.14 (0.61, 2.16)	0.67
Continuous	1.00 (0.91, 1.10)	1.02 (0.77, 1.35)				1.00 (0.94, 1.09)			
Vitamin E	--	1.00	0.93 (0.50, 1.73)	1.06 (0.58, 1.95)	0.81	1.00	1.38 (0.72, 2.64)	1.28 (0.68, 2.42)	0.48
Continuous	1.07 (0.97, 1.17)	1.44 (0.28, 7.50)				1.07 (0.97, 1.17)			

[a] adjusted for treatment group, age, and total energy.

[b] for continuous analyses: per unit increase typical for a pre-natal vitamin supplement; β-carotene: 2000 IU, Vitamin C: 60mg, Vitamin E: 20 mg;

[c] ombined dietary and dietary supplement sources.

Table 4

Hazard Ratios of Time to Pregnancy with Live Birth by Antioxidant Intake and Age

Age < 35 y (n=273)									
Antioxidant [a,b] **HR (95% CI)**	**Dietary Supplement Intake, Alone**	**Dietary Intake**				**Total Nutrient Intake**[c]			
		T1	T2	T3	p-trend	T1	T2	T3	p-trend
β-carotene	--	1.00	0.98 (0.67, 1.44)	1.26 (0.86, 1.84)	0.17	1.00	0.96 (0.65, 1.41)	1.18 (0.81, 1.72)	0.38
Continuous	1.19 (1.01, 1.41)	1.08 (0.96, 1.23)	--	--		1.11 (1.00, 1.23)	--	--	
Vitamin C	--	1.00	1.01 (0.69, 1.48)	0.91) (0.62, 1.33)	0.60	1.00	1.00 (0.68, 1.47)	1.19 (0.82, 1.74)	0.36
Continuous	1.10 (1.02, 1.18)	0.94 (0.77, 1.13)				1.07 (1.00, 1.15)			
Vitamin E	--	1.00	0.88 (0.60, 1.29)	0.82 (0.56, 1.20)	0.32	1.00	1.06 (0.72, 1.56)	1.15 (0.79, 1.66)	0.48
Continuous	0.95 (0.87, 1.04)	0.94 (0.28, 3.14)	--	--		0.95 (0.87, 1.04)	--	--	
Age ≥ 35 y (n=154)									
Antioxidant [a,b] **HR (95% CI)**	**Dietary Supplement Intake, Alone**	**Dietary Intake**				**Total Nutrient Intake**[c]			
		T1	T2	T3	p-trend	T1	T2	T3	p-trend
β-carotene	--	1.00	0.87 (0.54, 1.41)	0.58 (0.34, 0.96)	0.03	1.00	0.77 (0.48, 1.25)	0.56 (0.34, 0.92)	0.02
Continuous	1.23 (0.99, 1.51)	0.82 (0.66, 1.00)				0.91 (0.77, 1.08)			
Vitamin C	--	1.00	1.05 (0.63, 1.74)	1.22 (0.75, 1.97)	0.42	1.00	1.06 (0.64, 1.74)	0.86 (0.53, 1.42)	0.46
Continuous	1.03 (0.97, 1.09)	1.11 (0.88, 1.39)				1.03 (0.97, 1.09)			
Vitamin E	--	1.00	0.82 (0.50, 1.36)	0.99 (0.61, 1.61)	0.95	1.00	0.98 (0.60, 1.61)	0.84 (0.52, 1.37)	0.54
Continuous	1.07 (1.01, 1.13)	0.81 (0.10, 6.45)				1.07 (0.97, 1.13)			

[a] adjusted for treatment group, body mass index, and total energy.

[b] for continuous analyses: per unit increase typical for a pre-natal vitamin supplement; β-carotene: 2000 IU, Vitamin C: 60mg, Vitamin E: 20 mg;

[c] combined dietary and dietary supplement sources.