Serum Carotenoids and Fat-Soluble Vitamins in Women With Type 1 Diabetes and Preeclampsia

A longitudinal study

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OBJECTIVE—Increased oxidative stress and immune dysfunction are implicated in preeclampsia (PE) and may contribute to the two- to fourfold increase in PE prevalence among women with type 1 diabetes. Prospective measures of fat-soluble vitamins in diabetic pregnancy are therefore of interest.

RESEARCH DESIGN AND METHODS—Maternal serum carotenoids (α- and β-carotene, lycopene, and lutein) and vitamins A, D, and E (α- and γ-tocopherols) were measured at first (12.2 ± 1.9 weeks [mean $\pm$ SD], visit 1), second (21.6 ± 1.5 weeks, visit 2), and third (31.5 ± 1.7 weeks, visit 3) trimesters of pregnancy in 23 women with type 1 diabetes who subsequently developed PE (DM PE+) and 24 women with type 1 diabetes, matched for age, diabetes duration, HbA$_{1c}$, and parity, who did not develop PE (DM PE−). Data were analyzed without and with adjustment for baseline differences in BMI, HDL cholesterol, and prandial status.

RESULTS—In unadjusted analysis, in DM PE+ versus DM PE−, α-carotene and β-carotene were 45 and 53% lower, respectively, at visit 3 ($P < 0.05$), before PE onset. In adjusted analyses, the difference in β-carotene at visit 3 remained significant. Most participants were vitamin D deficient (<20 ng/mL), and vitamin D levels were lower in DM PE+ versus DM PE− throughout the pregnancy, although this did not reach statistical significance.

CONCLUSIONS—In pregnant women with type 1 diabetes, low serum α- and β-carotene were associated with subsequent development of PE, and vitamin D deficiency may also be implicated.

Preeclampsia (PE) is defined as new-onset hypertension and proteinuria during pregnancy. PE is a major cause of maternal and infant morbidity and mortality, and its incidence is increased approximately fourfold by the presence of maternal type 1 diabetes (1,2). Placental oxidative stress is implicated in the pathogenesis of PE (3,4), and compared with the general population, women with type 1 diabetes may be particularly prone to and affected by impaired antioxidant status. Any alterations in maternal antioxidant status in pregnancy are therefore of interest. Risk for PE has also been associated with vitamin D deficiency, perhaps mediated through sequelae such as immune dysfunction, inflammation, and hypertension (5,6).

Several cross-sectional PE studies have been performed, mostly in nondiabetic women. Altered levels of carotenoids (α- and β-carotene, lycopene, and lutein) have been reported (7). Mikhail et al. (8) found decreased maternal β-carotene levels in PE, but other studies were not confirmatory (9,10). Vitamin A, or retinol, is an essential fat-soluble vitamin with physiologic functions in mammalian reproduction (11). Cross-sectional studies in nondiabetic women with and without PE report both lower (9) and higher (10) vitamin A levels. Vitamin E or α-tocopherol, which has antioxidant functions, has been reported to be lower (8,12), higher (9,10), or similar (13) between PE and normotensive nondiabetic pregnancies.

There are some longitudinal PE studies, although none in type 1 diabetes. Bodnar et al. (5) found that severe vitamin D deficiency [25(OH)D <15 ng/mL] in early pregnancy was associated with a fivefold increased risk of PE. Halhali et al. (14) showed that serum 1, 25(OH)$_2$D was not altered in pregnant women who subsequently developed PE. In a longitudinal study of nondiabetic women with a risk of high PE, Chappell et al. (15) found no significant differences in α-tocopherol concentrations.

Thus, longitudinal studies are few, and cross-sectional studies are not informative on the temporal relationship between antioxidant status and subsequent PE. Furthermore, there are no prospective data relating serum carotenoids, fat-soluble vitamins, and PE in diabetes. We report a multicenter prospective study examining differences in

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Table 1—*Clinical profiles of 47 type 1 diabetic and 20 nondiabetic participants*

	Nondiabetic	P value: DM− vs. DM PE−	Diabetic		
			No PE (PE−)	P value: DM PE− vs. DM PE+	PE (PE+)
N	20		24		23
Age (years)	31.7 ± 4.6	0.17	29.9 ± 3.8	0.31	28.5 ± 5.6
BMI (kg/m^2)	23.6 ± 3.7	0.41	24.6 ± 4.1	**0.025**	28.0 ± 5.8
Alcohol use (%)					
None	11*		25		18
Stopped during pregnancy	68	0.55	58	0.39	68
Smoking (%)					
No	100*		88		91
Quit because of pregnancy	0	0.55	4	0.69	5
First pregnancy (%)	55	0.57	75	0.26	76
Gravida (*n*)	1.7 ± 1.0	0.24	1.3 ± 0.7	0.99	1.3 ± 0.7
Para (*n*)	0.5 ± 0.9	0.18	0.2 ± 0.5	0.91	0.2 ± 0.5
Abortus (*n*)	0.2 ± 0.4	0.86	0.1 ± 0.3	0.91	0.1 ± 0.4
Age at diabetes onset (years)	—		15 ± 8	0.065	12 ± 6
Duration of diabetes (years)	—		15 ± 7	0.32	17 ± 7
HbA$_{1c}$ (%)	5.3 ± 0.3	**<0.0001**	6.7 ± 1.0	0.13	7.3 ± 1.2
Blood pressure (mmHg)					
Systolic	112 ± 9	0.35	109 ± 10	0.27	113 ± 12
Diastolic	67 ± 8	0.24	64 ± 8	0.27	67 ± 9
Microalbumin (mg/dL)	0.47 ± 0.17	0.58	0.43 ± 0.20	0.12	1.03 ± 1.78
Total cholesterol (mg/dL)	187 ± 26	0.22	176 ± 34	0.54	182 ± 28
HDL cholesterol (mg/dL)	81 ± 22	0.63	84 ± 18	**0.035**	74 ± 14
LDL cholesterol (mg/dL)	88 ± 30	0.22	77 ± 28	0.10	91 ± 28
Triglycerides (mg/dL)	94 ± 34	0.076	75 ± 31	0.17	87 ± 24
Gestational age (weeks)					
Visit 1	12.6 ± 1.7	0.56	12.3 ± 1.7	0.98	12.3 ± 2.1
Visit 2	21.5 ± 1.2	0.97	21.4 ± 1.2	0.15	22.1 ± 1.7
Visit 3	31.2 ± 1.1	0.80	31.3 ± 1.5	0.39	31.7 ± 1.7
Term	39.2 ± 1.5	**0.013**	38.0 ± 1.4	**0.036**	37.2 ± 1.2

Values are means ± SD. Measurements refer to visit 1 unless otherwise indicated. *P* values < 0.05 are denoted in boldface. *For these data, *P* value refers to combined percentage (i.e., "none" and "stopped during pregnancy," or "no" and "quit because of pregnancy").

serum carotenoids, vitamins A (retinol), D [25(OH)D], and E (tocopherols) between women with type 1 diabetes with and without subsequent PE. Levels were measured in each trimester of pregnancy and before PE onset. Our study evaluated the temporal association of vitamin and antioxidant provitamin levels with PE in type 1 diabetes.

Because our major goal was to compare pregnant women with type 1 diabetes who subsequently developed PE with those who did not, our primary comparison is between these two groups. We also included healthy, normotensive, nondiabetic pregnant women to obtain reference values during pregnancy, and for illustrative purposes we report some secondary analyses using their data.

RESEARCH DESIGN AND METHODS—This is a substudy of a previously described prospective cohort of 151 non-Hispanic white women with type 1 diabetes and 24 nondiabetic subjects enrolled during the first trimester of pregnancy and followed until delivery (16). Clinical data and specimens were collected at each trimester (visit 1: 12.2 ± 1.9 weeks; visit 2: 21.6 ± 1.5 weeks; and visit 3: 31.5 ± 1.7 weeks of gestation [mean ± SD]; no overlap) and at term (37.6 ± 2.0 weeks). Visit 3 was before PE onset. Subjects were requested to fast overnight, actual prandial status was recorded, and serum and urine were obtained before any exogenous insulin administration. The study was approved by the institutional review boards of participating centers in Norway, Australia, and the U.S. Exclusion criteria were renal impairment (including microalbuminuria), cardiovascular disease, hypertension, or other significant medical problems prepregnancy or at visit 1. PE was defined as new-onset hypertension (>140/90 mmHg) after 20 weeks' gestation in a previously normotensive woman, accompanied by proteinuria (>300 mg/24 h). Of the 26 diabetic PE (DM PE+) cases in the larger cohort (16), samples from 23 were available for this substudy because of sample attrition. Of 26 DM PE− cases matched on the basis of age, diabetes duration, HbA$_{1c}$, and parity, samples from 24 were available for this study (sample attrition). For reference values, 20 of 24 pregnant, nondiabetic, non-PE women (DM−) were also studied (3 were previously excluded as described [16]; 1 additional exclusion due to sample attrition).

Laboratory analyses
Procedures have been detailed (16). Serum carotenoids, retinol, and tocopherols were measured by high-performance liquid chromatography (HPLC) using a modified combined version of methods of Lee et al. (17) and Karppi et al. (18).

Serum vitamin D [25(OH)D] was measured by HPLC/tandem mass spectrometry through Quest Diagnostics Co. (19).

Briefly, 200 μL serum was deproteinized with 200 μL ethanol-butylated hydroxytoluene containing 50 μL internal standard cocktail, vortexed, extracted (1,000 μL *n*-hexane, 60 s), dried (nitrogen, 10 min), and reconstituted in 200 μL ethanol-butylated hydroxytoluene solution. Then, 50 μL was injected onto a 4.6-mm C-18 Ultrasphere ODS HPLC column (Beckman Coulter, Inc., Danvers, MA) and eluted (flow rate 0.8 mL/min) with an isocratic solvent (methanol, 60%; acetonitrile, 20%; and dichloromethane, 20%). The HPLC system included a 515 pump, 2996 photodiode array detector, and a Rheodyne 7725i manual injector (Waters, Milford, MA). Data acquisition was via Waters Empower data software. Interassay coefficient of variation for pooled quality samples was ≤10%. All assays were performed by operators masked to sample clinical status.

Statistics

The primary analyses compared DM PE+ with DM PE−. A secondary analysis evaluated differences between DM PE− and nondiabetic non-PE control subjects (DM−). Carotene and tocopherol levels were analyzed both with and without correction for total lipids (cholesterol + triglyceride). Minimum differences between DM PE+ and DM PE− (*n* ≥20/group) could be detected with 80% power as follows: lutein: 0.4 μmol/L; α-tocopherol: 5.4 μmol/L; vitamin A: 0.18 μmol/L; vitamin D: 3.08 μg/L. Results are reported (Table 1) as means (± SD) or, for non-normally distributed measures (per Shapiro-Wilks test), were transformed logarithmically and reported as geometric means (95% CI) (all carotenoids [except lutein], γ-tocopherol, and α- or γ-tocopherol:lipid ratios). Differences between groups were analyzed by Student *t* test for continuous measures and χ^2 test for categoric measures. Analyses were performed with and without inclusion of parameters that differed between DM PE+ and DM PE− (BMI, HDL cholesterol), and prandial status, as covariates. Microsoft Excel 2007 (Microsoft Corp., Redmond, WA) and SPSS for Windows 15.0 (SPSS Inc., Chicago, IL) were used.

RESULTS—As shown in Table 1, at baseline, the diabetic groups were comparable except for higher BMI and lower HDL cholesterol in DM PE+ versus DM PE−.

Serum β-carotene was 37% lower at visit 1 (*P* < 0.05), and α- and β-carotene were 45 and 53% lower, respectively, at visit 3, in DM PE+ versus DM PE− (*P* < 0.05) (Fig. 1*A* and *B*), although after adjustment for covariates, only β-carotene at visit 3 remained significant. Serum lutein levels were similar in DM PE+ versus DM PE− at all visits (Fig. 1*C*); lycopene was 52% higher in DM PE+ versus DM PE− at visit 2 (*P* < 0.05) (Fig. 1*D*).

Serum lutein was lower at visits 1 and 2 in DM PE− versus DM− (Fig. 1*C*; *P* < 0.05). Differences in lutein and lycopene were not affected by the covariate analysis.

Serum vitamin A levels did not differ significantly between the diabetic groups at any visit (Fig. 2*A*), but were lower in DM PE− versus DM− at visits 2 and 3 (Fig. 2*A*; *P* < 0.05). Consideration of covariates had no effect.
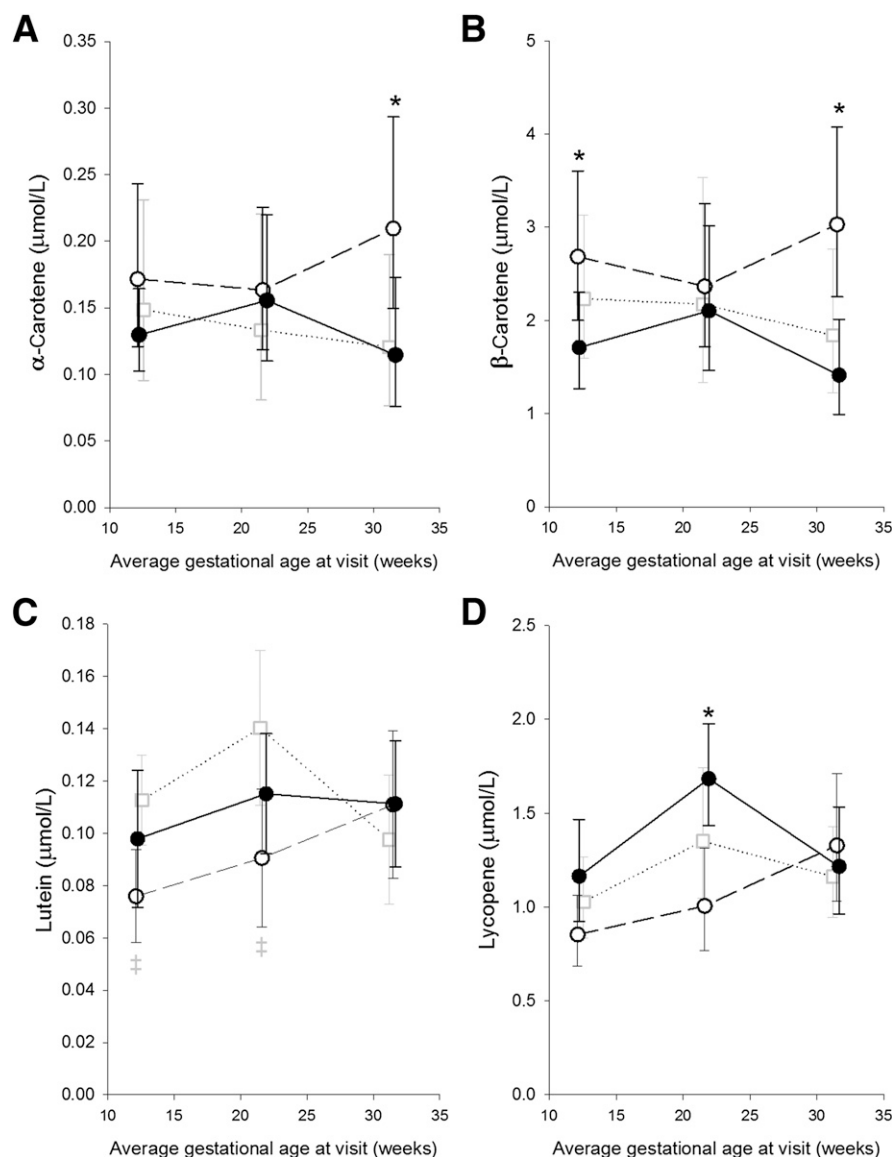
Figure 1—*Longitudinal changes in* (A) *α-carotene,* (B) *β-carotene,* (C) *lutein, and* (D) *lycopene during gestation. Values are geometric means for α-carotene, β-carotene, and lycopene, and arithmetic means for lutein. Error bars show the 95% CI for the mean. Three groups of participants are shown; white circle, dashed line: DM PE− (n = 24); black circle, solid line: DM PE+ (n = 23); white square, dotted lines: DM− (nondiabetic control subjects, n = 20). Except lutein, all values were log-transformed before testing for significant differences. Values significantly (P < 0.05) different between DM PE− and DM PE+ groups are indicated with an asterisk (*) and between DM PE− and DM− groups are indicated with a double dagger (‡). After adjustment for covariates (BMI, HDL cholesterol, and prandial status), differences in α-carotene at visit 3 and β-carotene at visit 1 were no longer significant (P = 0.09 and 0.12, respectively).*

Vitamin D [25(OH)D] insufficiency, deficiency, and severe deficiency were defined as <30, <20, and <15 ng/mL, respectively (20,21). Mean 25(OH)D levels did not differ significantly between DM PE+ and DM PE− groups, although there was a slight trend toward lower levels in DM PE+ throughout pregnancy (Fig. 2*B*). Also, women with type 1 diabetes in general, and those who developed PE in particular, were more likely to be vitamin D deficient or severely deficient than the nondiabetic group, and the relative risk of PE associated with vitamin D deficiency was increased, although not significantly at any visit (Table 2). Of

relevance to vitamin D, there were no differences between groups in the seasons of sample collection (data not shown), and all enrolled participants were Caucasian.

Unadjusted α-tocopherol levels were ~18% higher in DM PE+ versus DM PE− at visit 2 (Fig. 2*C*; $P < 0.05$), whereas γ-tocopherol was similar in all groups at all visits (Fig. 2*D*). Mean serum α-tocopherol was lower in DM PE− versus DM− only at visit 2 (Fig. 2*C*; $P < 0.05$). No differences were noted for γ-tocopherol at any visit (Fig. 2*D*). Covariate analysis did not affect significance.

Because carotenes and tocopherols circulate primarily within lipoproteins,

levels were adjusted for serum total lipids (Fig. 3). Lipid-adjusted carotenes were lower in DM PE+ than DM PE− at visit 1 (β-carotene only) and visit 3 (both carotenes) (Fig. 3*A* and *B*; $P < 0.05$). Again, after inclusion of covariates, only β-carotene at visit 3 remained significant. Adjusted lycopene and lutein levels showed no differences between groups at any visit (data not shown). By comparing DM PE− with DM−, adjusted α- and β-carotenoids were not significantly different, but they tended to decline with gestational age in both groups. Lipid-adjusted α- and γ-tocopherols did not differ between DM PE+ and DM PE−, or between DM− and DM PE−, although α-tocopherol trended downward in all groups during pregnancy (Fig. 3*C*). Differences in tocopherol: lipid ratios were not affected by covariate analysis.

CONCLUSIONS—In a prospective study of pregnant women with type 1 diabetes, we demonstrated lower serum carotenoids, especially β-carotene, in affected versus unaffected women before PE onset. We also observed a high prevalence of vitamin D insufficiency, most frequently in women who developed PE. Relative to early pregnancy, there was also a downward trend in lipid-adjusted carotenoids and tocopherols in the third trimester, perhaps representing antioxidant reserve depletion. This decline occurred because total cholesterol and triglycerides increased by 1.5- and twofold, respectively, between visits 1 and 3 (not shown) in all groups. We have previously shown that pregnant women with type 1 diabetes are already "primed" for PE by having elevated levels of endoglin (16), and in this setting, antioxidant depletion may trigger PE.

We identified significantly lower serum β-carotene, and by some analyses, lower α-carotene, in pregnant women with type 1 diabetes who subsequently developed PE versus those who did not. Our longitudinal data, particularly between-group differences in serum carotenoids (DM PE+ vs. DM PE−) in the third trimester, concur with existing cross-sectional studies, performed later in pregnancy in nondiabetic women, that show lower circulating carotenoids in PE (8,10). β-Carotene also possesses pro-vitamin A activity (22), and we postulate that the low β-carotene in the third trimester could be explained by increased endogenous antioxidant consumption due to elevated oxidative stress or
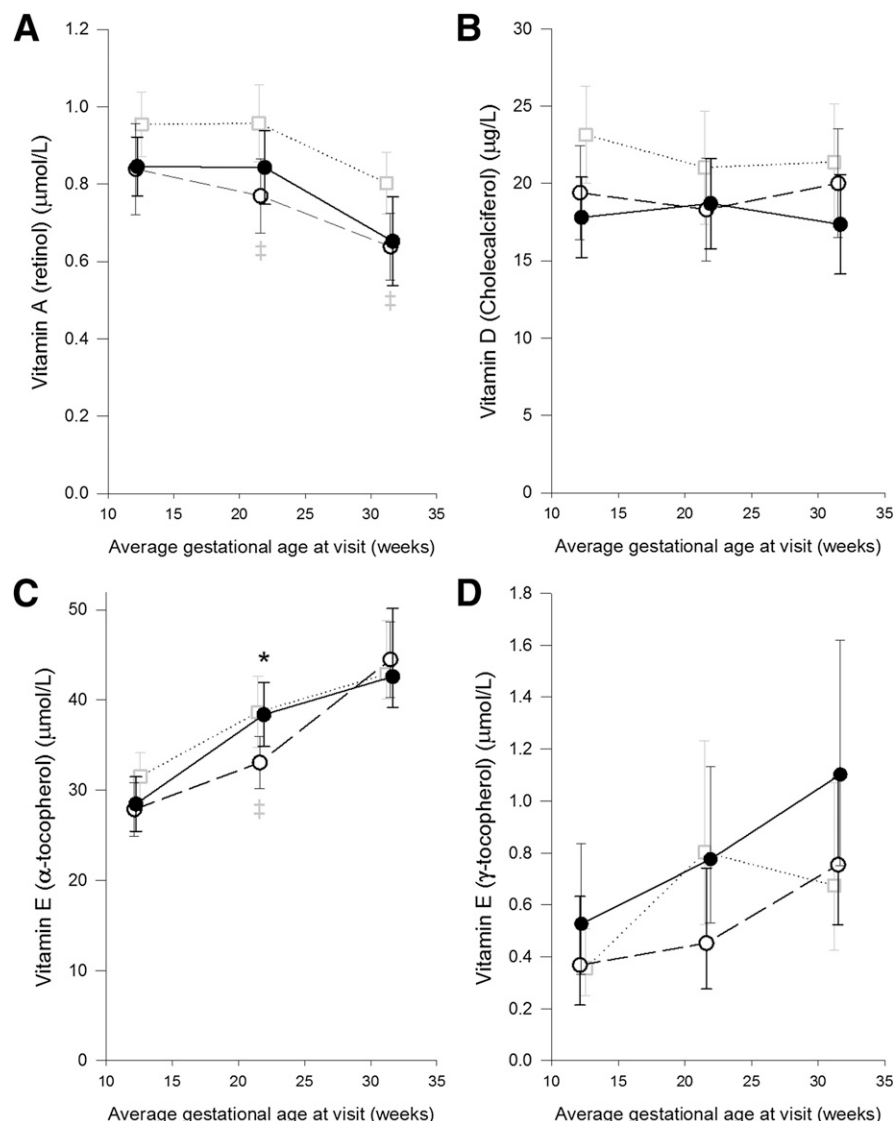
Figure 2—*Longitudinal changes in* (A) *retinol,* (B) *vitamin D* (*cholecalciferol*), (C) *α-tocopherol, and* (D) *γ-tocopherol* (*log transformed*) *during gestation. Values are arithmetic means for retinol, cholecalciferol, and α-tocopherol, and geometric means (95% CI) for γ-tocopherol. Group and statistical symbols are as in Fig. 1. Adjustment for covariates had no effect on significance. Statistically significant differences between visits were seen for* (C) *α-tocopherol in DM PE+ between gestational age 12 and 22 weeks, and in DM PE− between gestational age 22 and 32 weeks* (P < 0.05).

Table 2—Percentage of participants who are vitamin D [25(OH)D] insufficient (<30 ng/mL), deficient (<20 ng/mL), or severely deficient (<15 ng/mL), and relative risk of PE associated with low vitamin D levels (<30, <20, and <15 ng/mL) in diabetic participants

Vitamin D level (ng/mL)	Visit 1				Visit 2				Visit 3			
	DM PE+ (%)	DM PE− (%)	DM− (%)	RR (95% CI)	DM PE+ (%)	DM PE− (%)	DM− (%)	RR (95% CI)	DM PE+ (%)	DM PE− (%)	DM− (%)	RR (95% CI)
<30	96	87	84	2.1 (0.4–11.8)	95	90	79	1.4 (0.3–7.9)	96	91	75	1.5 (0.3–7.7)
<20	78	65	37	2.2 (0.4–12.3)	60	70	53	1.8 (0.3–10.8)	61	52	45	1.9 (0.3–11.0)
<15	39	22	0	2.5 (0.4–14.2)	30	45	26	1.8 (0.3–11.2)	39	30	30	2.1 (0.4–12.4)

PE+: PE (n = 23); PE−: no PE (n = 24); DM−: nondiabetic, non-PE pregnant control subjects (n = 20). Reference group for relative risk in all cases comprises women in whom vitamin D level is sufficient (>30 ng/mL). RR, relative risk.
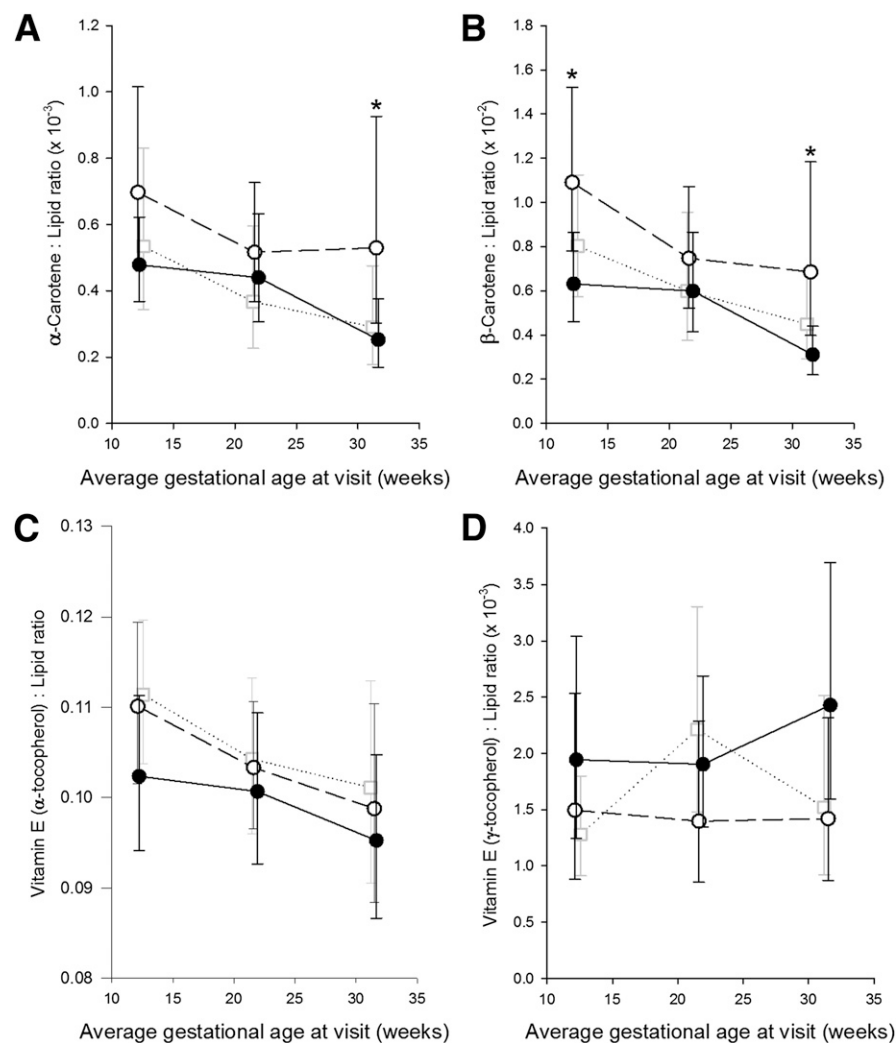
Figure 3—*Longitudinal changes in ratios* (A) α-*carotene: total lipids,* (B) β-*carotene: total lipids,* (C) α-*tocopherol: total lipids, and* (D) γ-*tocopherol: total lipids, during gestation. Values are geometric means (95% CI). Ratios are expressed as serum tocopherol and carotenoid molarity (μmol/L) to total lipid mass (serum total cholesterol and triglycerides) (mg/dL). Group and statistical symbols are as in Fig. 1. After adjustment for covariates (BMI, HDL cholesterol, and prandial status), differences in α-carotene at visit 3 and β-carotene at visit 1 were no longer significant* (P *= 0.08 and 0.07, respectively).*

increased conversion to vitamin A to compensate for low vitamin A levels. In our study, low vitamin A levels in women with type 1 diabetes in the first trimester are comparable to those previously reported (9,10) and to levels in U.S. adult women (23). Thus, although not statistically significant, decreasing vitamin A levels between the second and third trimesters in women with type 1 diabetes could promote PE.

Vitamin D deficiency, an increasingly prevalent condition in women of childbearing age (20), has been associated with PE in a large prospective study (5). We found that the majority of our participants, regardless of geographic location (which included sunny regions such as Oklahoma, South Carolina, and Australia), were vitamin D deficient. Levels did not differ between sites. We lack information on dietary intake or ultraviolet light exposure. The women with type 1 diabetes as a whole had lower levels than our nondiabetic reference group (data not shown). The reason is unclear. Occult celiac disease, which may cause vitamin D malabsorption, is prevalent (5–10%) in type 1 diabetes (24) and could be contributory.

Links between vitamin D deficiency and PE are unclear. Hyppönen (6) proposed an immune hypothesis, whereby local disruption of vitamin D status promotes loss of maternal–fetal immune tolerance and immune maladaptation.

Although this is intriguing, it has yet to be established as a cause for PE in humans. Another possibility is diminished placental 1α-hydroxylase activity, which occurs in PE (25) and lowers local and circulating $1,25(OH)_2D$.

In agreement with Williams et al. (10), our study does not support a relationship between serum α- and γ-tocopherols and PE, although the decline in adjusted α-tocopherol levels throughout the pregnancy might have a permissive effect in women already predisposed. The mean first trimester serum α-tocopherol values (25–30 μmol/L) in the women with type 1 diabetes in our study were comparable to normal values in U.S. adults and above the threshold indicating vitamin E deficiency (<11.6 μmol/L) (23).

Our study has several limitations. The findings merit confirmation in larger prospective studies involving diabetic and nondiabetic women. Furthermore, confounding due to certain group differences in this small cohort, although nonsignificant, cannot be excluded. More data regarding maternal diet, vitamin supplement use, and ultraviolet exposure would have been helpful to dissect cause and effect. Measures of other antioxidant vitamins and enzymes would be of interest. Fasting overnight is challenging for pregnant women with type 1 diabetes, and at each visit, a similar proportion from each diabetic group (range 2–6 of 23 or 24) attended nonfasting. However, our conclusions were not substantially affected by statistical adjustment for prandial status (as presented) or exclusion of nonfasting visits (data not shown).

We believe this is the first longitudinal study of the relationship between PE in pregnant women with type 1 diabetes and antecedent serum levels of carotenoids and vitamins A, D, and E. We found that women with type 1 diabetes who developed PE had lower antecedent serum α- and β-carotene concentrations than those who did not. Vitamin D insufficiency affected most women with type 1 diabetes, and levels tended to be lower in those who developed PE. Lipid-adjusted levels of many of the fat-soluble antioxidants declined during pregnancy. Our study sample, although small, represented women with type 1 diabetes from different geographic locations. Further studies are needed to define whether optimizing fat-soluble antioxidant and vitamin status throughout pregnancy can reduce the high incidence of PE in those with type 1 diabetes.

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