Human Papillomavirus Persistence and Nutrients Involved in the Methylation Pathway among a Cohort of Young Women[1]

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Abstract

Persistent oncogenic human papillomavirus (HPV) infection is associated with cervical dysplasia. Cofactors, such as nutrient status, may be required for the progression of HPV infection to neoplasia. HPV DNA methylation patterns *in vitro* have been shown to be associated with viral transcriptional activity. Folate, vitamin B_{12}, vitamin B_6, and methionine may function to prevent cervical cancer through their role in DNA methylation. This study was conducted to examine the relationship of dietary intake of folate, vitamin B_{12}, vitamin B_6, and methionine, as well as circulating levels of folate and vitamin B_{12} to HPV persistence. Oncogenic HPV status was determined at baseline and at ~3 and 9 months postbaseline. Multivariate logistic regression analysis was used to determine the adjusted odds ratios for persistent HPV infection associated with each tertile of individual nutrient among 201 women with a persistent or intermittent HPV infection. Circulating vitamin B_{12} levels were inversely associated with HPV persistence (*P* for trend, 0.037) after adjusting for age, age at first intercourse, marital status, cigarette smoking status, race, and body mass index. In addition, women with circulating levels in the highest tertile (>493.2 pg/ml) of vitamin B_{12} were less likely to have a persistent infection (adjusted odds ratio = 0.4; 95% confidence interval = 0.17–0.96). No significant associations were observed between HPV persistence and dietary intake of folate, vitamin B_{12}, vitamin B_6, or methionine from food alone or from food and supplements combined or from circulating folate. These data suggest a role for circulating vitamin B_{12} in early cervical carcinogenesis.

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Introduction

Cervical cancer is the third leading cause of cancer in women, comprising 9.8% of new cancer cases worldwide (1). Infection with oncogenic HPVs[3] has been shown to be the primary etiological factor for cervical cancer (2–4), as well as its precursor lesion, cervical intraepithelial neoplasia (5–7). HPV infections are often transient (8, 9), but persistent infections, especially with oncogenic HPV types, can further increase the risk of cervical dysplasia (5, 6, 10, 11). Although HPV infection is essential for cervical cancer, not all women infected with the virus progress to invasive cervical cancer. Nutritional factors may contribute to the progression of HPV infection to neoplasia (12).

In vitro evidence indicates that HPV DNA methylation patterns are associated with viral transcriptional activity (13). Methylation of the upstream regulatory region of HPV has been shown to prevent transcription *in vitro*, suggesting that methylation can decrease viral proliferation (14). Folate, as well as vitamins B_{12} and B_6, may function to prevent cervical cancer through their role in DNA methylation as they contribute to the synthesis of S-adenosylmethionine (15, 16), the primary donor for the methylation of DNA and RNA (17).

Despite compelling biochemical evidence for a role of folate and vitamin B_{12}, the epidemiological literature has not supported an association with cervical neoplasia. Case control studies assessing dietary folate intake have not shown a protective effect against cervical dyplasia (18–21), carcinoma *in situ* (22, 23), or invasive cervical cancer (24–26).

Furthermore, of the five case control studies that assessed the association between cervical dysplasia and circulating folate concentrations (18, 20, 27–29), only two reported a protective effect (18, 27). Similarly, higher circulating concentrations of folate were not protective against invasive cervical cancer (30–32). Two investigations assessed circulating vitamin B_{12} levels, but neither observed an association between cervical dysplasia (28) and cancer (30). Finally, three clinic trials of folic acid supplementation were conducted among women with cervical dysplasia (33–35). Only one of these trials resulted in cytological improvement (33).

As the majority of epidemiological studies that assessed the association between methyl donor nutrients and risk of cervical neoplasia did not include measures of HPV status, these studies must be interpreted with caution. No previous investigation has evaluated the association between folate, vitamin B_{12}, vitamin B_6, and methionine and the early carcinogenic event of HPV persistence. Therefore, this study was conducted to test whether increased dietary intake of folate, vitamin B_{12}, vitamin B_6, or methionine, or circulating levels of

[3] The abbreviations used are: HPV, human papillomavirus; RLU, relative light unit; AFFQ, Arizona Food Frequency Questionnaire; USDA, United States Dietary Association; BMI, body mass index; CI, confidence interval; OR, odds ratio.

folate or vitamin B_{12}, are associated with decreased risk of HPV persistence.

Materials and Methods

The Young Women's Health Study was a prospective cohort study designed to investigate the natural history of HPV infections among healthy women. Study visits occurred at baseline and ~3- and 9-months postbaseline. The University of Arizona's Human Subjects Committee approved this study protocol. Before study entry, all participants signed an informed consent form.

Subjects. From September 1996 to August 1999, all healthy women receiving routine gynecological care at a reproductive health care clinic were approached by a study interviewer for participation in the Young Women's Health Study. Of the 1950 women approached, 1541 women met the eligibility criteria, and 1342 women were enrolled in the baseline visit. Eligibility criteria included being aged 18–35 years, currently sexually active or seeking birth control, resident of the Tucson metropolitan area, no treatment for cervical intraepithelial neoplasia within the last 18 months, no abnormal Pap smear in the last 18 months, no history of chronic illness, not currently pregnant and >2 months postpartum, still having menstrual periods (*i.e.*, no hysterectomy), and no relocation plans over the next 12 months. At the baseline visit, each participant completed an extensive self-administered questionnaire regarding socio-demographics, cigarette smoking history, sexual behaviors, and reproductive history. During each participant's routine gynecological examination, exfoliated cells for HPV analysis were collected after Pap smear sample collection.

A group of 822 women (61%) who completed the baseline visit and had a baseline Pap smear of negative or atypical squamous cells of undetermined significance were invited to participate in the follow-up visits. Women, who were positive for any one of 13 oncogenic HPV types or HPV negative at baseline, were recruited to the follow-up visits. Women who were HPV negative were included in the study as they were at risk for acquiring an HPV infection over the study period. Groups of 346 and 206 women completed scheduled 3- and 9-month study visits, respectively. Of the 346 women who completed at least one follow-up visit, 187 (54%) were positive for an oncogenic HPV infection at baseline, and the remaining were HPV negative. Cytology and HPV testing were performed as part of each follow-up gynecological examination. Participants were monetarily compensated ($50) for participation in each of the follow-up visits. Women completing follow-up visits were more educated ($P = 0.05$) and less likely to be current smokers ($P < 0.01$) than women completing the baseline visit only; however, there were no significant differences in age, race, marital status, age at first intercourse, number of sexual partners, parity, oral contraceptive use, and ever having a Pap smear (data not shown).

HPV Analysis. HPV status at the baseline and follow-up visits was determined using Hybrid Capture II (Digene Corp, Beltsville, MD) to detect oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Positive samples were defined as a ratio of greater than 1 RLU of sample divided by RLUs of a positive control. Repeat testing was conducted on samples with equivocal readings (range 1–2 RLUs) with results of ≥1 RLU classified as positive. Samples with repeated values <1 RLU were analyzed a third time and classified based on the results of the two concordant results. Approximately, 1 pg of viral DNA/ml sample can be detected by this method (36).

Because the objectives of this investigation were to test the association of nutrients involved in methylation with HPV persistence, only participants who tested positive for an HPV infection and had at least one follow-up visit were included in this analysis. HPV persistence was defined as women who were HPV positive for any oncogenic HPV infection at two or more consecutive time points ($n = 131$), whereas women who were positive for any oncogenic type infection at one or more nonconsecutive time points were classified as having intermittent infections ($n = 70$). Women who tested negative for HPV until the last clinic visit, and then tested positive ($n = 24$), and women who were consistently HPV negative ($n = 121$) were excluded from these analyses.

Dietary Intake Analysis. Trained interviewers administered the AFFQ at the 3-month visit. The AFFQ is a modification of the dietary portion of the Block National Cancer Institute Health Habits and History Questionnaire (37) and includes foods common to the Southwestern United States (*i.e.*, salsa and burritos), as well as low-fat food items. Vitamin supplement duration, dosage, and brand are included. This optically scannable, semiquantitative questionnaire measured participants' usual intake by asking questions regarding the frequency and portion size of 153 food items over the previous 3 months. The validity and reliability of this questionnaire have been investigated through comparisons with four 24-h food recalls, as well as repeated administration after 1 year of both AFFQ and four 24-h recalls (38) or four dietary records (39) in two different prospective studies.

Nutrient values were calculated from the participants' reported dietary and supplement intake using a database derived from the 1994 USDA Food Composition Database, the USDA Continuing Survey of Food Intake of Individuals 1994–1996 and 1998, and the Nutrient Database for Standard Reference (version 11–13). To capture the additional folate intake because of fortification of enriched grain products, mandated by the United States Food and Drug Administration as of January 1, 1998 (40), two databases were used. Questionnaires completed on or after January 1, 1998 were analyzed from the database, which included fortified grain products, whereas questionnaires completed before this date were analyzed without the updated fortification values. A group of 140 participants completed the AFFQ before January 1, 1998, and 206 participants completed the AFFQ after this date. All dietary calculations used age-specific portion sizes for women. From these databases, semiquantitative nutrient intake values were obtained for folate, vitamin B_{12}, vitamin B_6, and methionine.

Circulating Folate and Vitamin B_{12} Measures. At the 3- and 9-month clinic visits, fasting blood samples were collected by venipuncture using Monoject evacuated tubes containing EDTA (Sherwood Medical, St. Louis, MO) for quantitative assessment of circulating nutrient values. All samples were separated, and the resulting plasma aliquots were stored at −80°C until analyses were conducted.

Plasma samples were obtained from 285 participants, 82% who completed the 3-month study visit, and 180 participants, 87% who completed the 9-month study visit. Twenty women completed both the 3- and 9-month visits, but blood was obtained only at the 9-month visit. The most common reasons for not obtaining blood ($n = 41$) were that the phlebotomist was unable to find a vein ($n = 29$), only a small amount of blood was obtained ($n = 8$), or the participant refused ($n = 4$). Participants with and without samples were comparable on age, race, education, marital status, cigarette smoking, and BMI.

Plasma folate and vitamin B_{12} were quantified in duplicate using the Quantaphase II B_{12} and Folate Radioassay (Bio-Rad

Table 1 Unadjusted mean[a] values of dietary intake and circulating nutrient levels by case control status

	Intermittent HPV infection		Persistent HPV infection		*P*
	Mean	(SD)	Mean	(SD)	
Dietary Intake of Micronutrients					
Foods Only[b]					
Folate (μg)	453.3	(330.7)	396.3	(220.4)	0.51
Vitamin B_{12} (μg)	4.9	(3.2)	4.6	(2.7)	0.85
Vitamin B_6 (mg)	2.4	(1.4)	2.1	(1.1)	0.48
Methionine (gm)	1.7	(1.1)	1.7	(0.9)	0.81
Food and Supplements Combined[c]					
Folate (μg)	626.6	(420.3)	578.6	(346.7)	0.54
Vitamin B_{12} (μg)	12.0	(16.1)	15.8	(52.5)	0.68
Vitamin B_6 (mg)	5.4	(7.5)	5.6	(9.1)	0.60
Plasma Levels					
Folate (ng/ml)[d]	7.9	(3.3)	8.8	(3.7)	0.16
Vitamin B_{12} (pg/ml)[e]	439.8	(159.5)	405.5	(176.8)	0.10

[a] Means are presented as untransformed values but were calculated from log-transformed values.
[b] Intermittent = 70, Persistent = 131.
[c] Intermittent = 70 with 34 consuming supplements, Persistent = 131 with 66 consuming supplements.
[d] Intermittent = 60, Persistent = 109.
[e] Intermittent = 60, Persistent = 110.

Laboratories, Inc., Hercules, CA). To minimize interference because of EDTA, plasma samples were filtered using serum filter columns (Image Molding, Denver, CO) before analysis. On the basis of repeated measurements of a pooled plasma sample, the interassay coefficient of variation was 13% for plasma folate and 0.41% for plasma vitamin B_{12}.

The average of two plasma values was used unless only one sample was available, in which case, the single value was used. Overall, 60 (86%) participants with intermittent infections and 109 (83%) participants with persistent infections had available plasma samples for analysis. The Pearson correlation coefficients of log-transformed 3- and 9-month nutrient levels were significant at $r = 0.45$ ($P < 0.01$) for folate and $r = 0.42$ ($P < 0.01$) for vitamin B_{12} among participants with both measures. Although at least one plasma sample was available for 298 participants, only participants who had an intermittent or persistent HPV infection were included in the statistical analyses ($n = 170$). Because of a single inadequate sample, 169 participants had samples available for folate analysis.

Statistical Analysis. To test whether increased dietary intake levels of nutrients involved in the methylation pathway were associated with decreased HPV persistence, two groups were compared: women with persistent infections ($n = 131$) and women with intermittent infections ($n = 70$). Nutrient intake and plasma levels were categorized into tertiles based on the distribution of the intermittent group. Bivariate analyses were conducted for age, race, education, marital status, cigarette smoking history, age at first intercourse, lifetime number of sexual partners, parity, number of pregnancies, oral contraceptive use, Depo Provera use, and Pap smear history, as well as variables that would account for under and over-reporting of dietary intake, such as BMI and total energy intake. χ^2 analyses for categorical variables and two sample *t* tests or ANOVAs for continuous variables were used. Non-nutrient risk factors associated with both HPV persistence and any dietary or plasma nutrient at $P \leq 0.2$ were assessed in multivariate logistic regression models. To maximize statistical power, only those variables associated with both nutrient status and HPV persistence were retained as covariates using multivariate analysis. Backwards stepwise elimination was performed, and only significant variables at $P < 0.05$ (age at first intercourse, marital status, race, and BMI) were retained in the final model. Age and cigarette smoking ($P = 0.052$) were also included in the model to be consistent with the literature. Because of the high intercorrelation of the nutrients, risk of HPV persistence was estimated individually for each nutrient. Multivariate logistic regression modeling of HPV persistence was performed to obtain an estimate of the association (adjusted OR) and 95% CIs. Treating categorical nutrient variables as continuous variables in multivariate logistic regression allowed for the assessment of linear trends. Pearson correlation coefficients were estimated between circulating and dietary nutrient levels using the log-transformed values as these data were highly skewed. All statistical tests performed were two sided with an α level of 0.05 and were performed using Intercooled STATA (2001 Stata Statistical Software: Release 7.0; StataCorp., College Station, TX).

Results

The unadjusted mean values of dietary intake and circulating nutrient levels for participants with intermittent and persistent HPV infections are reported in Table 1. No significant differences were observed in dietary intake from food alone or food and supplements combined for folate, vitamin B_{12}, vitamin B_6, and methionine among participants with persistent HPV infections and participants with intermittent HPV infections. Circulating folate and vitamin B_{12} levels were equivalent when participants with persistent and intermittent HPV infections were compared.

Dietary folate, vitamin B_{12}, vitamin B_6, and methionine intakes from food sources were highly intercorrelated, *e.g.*, a correlation coefficient of $r = 0.92$ ($P < 0.01$) was observed between dietary folate and vitamin B_6. The high correlation coefficients were likely because of fortification of these nutrients simultaneously in certain foods, such as fortified breakfast cereals, which were reported frequently by study participants. In this cohort, the correlations between dietary nutrients from foods and circulating folate and vitamin B_{12} concentrations were low; $r = 0.10$ ($P = 0.21$) for folate and $r = 0.15$ ($P = 0.18$) for vitamin B_{12}. Eighty-three (41%) women consumed vitamin supplements of folate, vitamin B_6, and B_{12}. The correlations between dietary nutrients and their plasma concentrations remained low when nutrients from supplements were

Table 2 Distribution of socio-demographic and cervical neoplasia risk factor characteristics by tertiles of dietary intake and circulating measures among study participants

Tertiles	Dietary food intake						Plasma levels					
	Folate (μg/day)			Vitamin B_{12} (μg/day)			Folate (ng/ml)			Vitamin B_{12} (pg/ml)		
	1	2	3	1	2	3	1	2	3	1	2	3
No.	77	66	58	65	78	58	50	52	67	74	51	45
Age (yr), mean (SD)[a]	24 (4)	25 (4)	23 (4)	24 (4)	24 (4)	23 (4)	23 (4)	25 (5)	24 (5)	25 (4)	24 (5)	23 (5)
Race (%)[b]												
Caucasian	85.7	84.9	65.5	86.2	79.5	72.4	72.0	84.6	80.6	87.8	68.6	77.8
Hispanic	9.1	10.6	29.3	7.7	16.7	22.4	22.0	11.5	16.4	9.5	23.5	20.0
Other	5.2	4.6	5.1[c]	6.2	3.9	5.2	6.0	3.9	3.0	2.7	7.8	2.2[c]
Education (% ≥some college)[b]	70.1	68.2	77.6	73.9	71.8	69.0	66.0	67.3	77.6	74.3	29.4	33.3
Marital status (%)[b]												
Single	64.9	72.7	74.1	64.6	74.4	70.7	60.0	67.3	73.1	66.2	70.6	66.7
Married	11.7	7.6	5.2	10.8	11.5	1.7	14.0	7.7	4.5	10.8	5.9	6.7
Cohabiting	7.8	12.1	15.5	10.8	7.7	17.2	14.0	13.5	11.9	14.9	13.7	8.9
Divorced/separated	15.6	7.6	5.2	13.9	6.4	10.3	12.0	11.5	10.5	8.1	9.8	17.8
BMI, mean (SD)[a]	23.8 (5.5)	23.6 (5.2)	22.8 (4.5)	23.5 (5.2)	23.6 (5.1)	23.1 (5.3)	24.3 (6.1)	23.3 (4.2)	22.9 (5.6)	23.3 (5.1)	24.0 (6.4)	23.1 (4.5)
Total energy (kcal), mean (SD)[a]	1221 (531)	2127 (717)	3095[c] (1278)	1248 (624)	1933 (704)	3138[c] (1257)	2322 (1312)	2091 (1401)	1879 (856)	2041 (956)	1959 (983)	224 (1668)
Cigarette smoking (% ever)[b]	48.1	50.0	55.2	52.3	43.6	58.6	48.0	55.8	53.7	54.1	51.0	53.3
Age @ 1st intercourse (yr), mean (SD)[a]	17 (3)	17 (3)	17 (2)	17 (3)	17 (3)	16 (2)	17 (2)	17 (4)	16 (2)	17 (3)	17 (4)	16 (2)
Lifetime # sexual partners, mean (SD)[a]	9 (8)	10 (10)	9 (7)	10 (9)	9 (8)	10 (9)	8 (9)	11 (11)	10 (7)	11 (9)	9 (11)	9 (7)
Parity (% ever)[b]	27.3	21.2	13.8	30.7	15.4	19.0	28.0	30.8	15.0	23.0	23.5	24.4
Oral contraceptive use (%)[b]												
Never	14.3	10.6	19.0	16.9	6.4	22.4	22.0	11.5	7.5	9.5	13.7	17.8
Current	47.8	36.4	39.7	47.7	41.0	34.5	42.0	46.2	41.8	56.8	43.1	20.0
Past	39.0	53.0	41.4	35.3	52.6	43.1	36.0	42.3	50.8	33.8	43.1	62.2[c]

[a] Analysis of covariance.
[b] Pearson λ^2 test of association.
[c] $P < 0.05$.

Table 3 Association between specific dietary micronutrient intake and HPV persistence, $n = 201$

	Intermittent No.	Persistent No.	Crude OR	Adjusted OR (95% CI)
Food only				
Folate (μg)				
Low (<301.6)	24	53	1.00	1.00
Medium (301.6–481.6)	23	43	0.85	0.63 (0.29–1.38)
High (>481.6)	23	35	0.69	0.52 (0.23–1.18)
P for trend				0.109
Vitamin B_{12} (μg)				
Low (<2.9)	24	41	1.00	1.00
Medium (2.9–6.0)	23	55	1.40	1.47 (0.68–3.19)
High (>6.0)	23	35	0.89	0.68 (0.30–1.54)
P for trend				0.439
Vitamin B_6 (mg)				
Low (<1.6)	24	44	1.00	1.00
Medium (1.6–2.7)	23	53	1.26	0.92 (0.43–2.00)
High (>2.7)	23	34	0.81	0.61 (0.27–1.39)
P for trend				0.254
Methionine (grams)				
Low (<1.2)	24	34	1.00	1.00
Medium (1.2–1.9)	23	55	1.69	1.29 (0.58–2.86)
High (>1.9)	23	42	1.29	1.25 (0.56–2.80)
P for trend				0.590
Food + supplements combined				
Folate (μg)				
Low (<301.6)	24	49	1.00	1.00
Medium (301.6–481.6)	23	33	0.70	0.80 (0.38–1.69)
High (>481.6)	23	49	1.05	1.16 (0.56–2.39)
P for trend				0.686
Vitamin B_{12} (μg)				
Low (<2.9)	24	48	1.00	1.00
Medium (2.9–6.0)	23	46	1.00	1.11 (0.53–2.31)
High (>6.0)	23	37	0.80	0.96 (0.46–2.03)
P for trend				0.933
Vitamin B_6 (mg)				
Low (<2.32)	24	51	1.00	1.00
Medium (2.32–4.18)	23	45	0.92	1.03 (0.50–2.13)
High (>4.18)	23	35	0.72	0.83 (0.39–1.79)
P for trend				0.647

[a] Adjusted for age, age at first intercourse, marital status, cigarette smoking status, race, and BMI.

combined with food values; $r = 0.23$ ($P < 0.01$) for folate and $r = 0.26$ ($P < 0.01$) for vitamin B_{12}.

The distributions of socio-demographic and cervical neoplasia risk factor characteristics by tertiles of dietary food intake and circulating measures are reported in Table 2. Race was significantly associated with dietary folate intake from food and circulating vitamin B_{12} concentrations, and energy was associated with both food folate and vitamin B_{12} intake. The oral contraceptive used was significantly associated with circulating vitamin B_{12} concentrations.

Table 3 presents the association between specific dietary nutrients and HPV persistence. No significant associations were observed between HPV persistence and dietary folate, vitamin B_{12}, vitamin B_6, and methionine. Furthermore, no association was observed with HPV persistence when combined measures of food and supplement intake for folate, vitamin B_6, or vitamin B_{12} were examined. Although not statistically significant, an inverse association was observed between dietary folate from food alone and HPV persistence. Among women consuming the highest level of folate from food sources, risk of HPV persistence was 0.52 (95% CI = 0.23–1.18).

Table 4 presents the association between circulating nutrients and HPV persistence. No association was observed between HPV persistence and circulating folate levels. After adjustment, circulating vitamin B_{12} concentrations remained inversely associated with HPV persistence (*P* for trend, 0.037). Women with circulating levels in the highest tertile (>493.2 pg/ml) of vitamin B_{12} were significantly less likely to have a persistent infection (OR = 0.4; 95% CI = 0.17–0.96).

Table 4 Association between circulating nutrients and HPV persistence

	Intermittent No.	Persistent No.	Crude OR	Adjusted OR ([a]95%CI)
Folate (μg/ml)[b]				
Low (<6.48)	20	30	1.00	1.00
Medium (6.48–8.80)	20	32	1.07	0.95 (0.39–2.29)
High (>8.80)	20	47	1.57	1.20 (0.51–2.78)
P for trend				0.662
Vitamin B_{12} (pg/ml)[c]				
Low (<357.7)	20	54	1.00	1.00
Medium (357.7–493.2)	20	31	0.57	0.57 (0.24–1.32)
High (>493.2)	20	25	0.46	0.40 (0.17–0.96)
P for trend				0.037

[a] Adjusted for age, age at first intercourse, marital status, cigarette smoking status, race, and BMI.
[b] $n = 169$.
[c] $n = 170$.

Discussion

Infection with persistent oncogenic HPV infection is the major risk factor for the development of cervical dysplasia (5, 6, 10, 11). In previous studies, folate, carotenoids, vitamin C, vitamin E, and retinols have been investigated as potential modifiers of cervical dysplasia and cancer risk (12). The current study, however, is the first conducted to examine the association of nutrients involved in DNA methylation (*i.e.*, folate, vitamin B_{12}, vitamin B_6, and methionine) and risk of HPV persistence. The findings from this investigation suggest that circulating vitamin B_{12} concentrations may be protective against HPV persistence. However, no significant reduction in HPV persistence risk was observed for dietary intake of folate, vitamin B_{12}, vitamin B_6, methionine from food alone, and food and supplements combined or for circulating concentrations of folate.

Most investigations of diet and cervical neoplasia have used a retrospective design and, therefore, were unable to measure HPV persistence. To our knowledge, no study has previously addressed the association between vitamin B_{12} and HPV persistence, although two previous studies have assessed circulating vitamin B_{12} concentrations in the prevention of cervical dysplasia (28) or cancer (30). Goodman *et al.* (28) reported no association between circulating vitamin B_{12} concentrations and atypical squamous cells of undetermined significance and low-grade or high-grade squamous intraepithelial lesions. Differences in study findings may be because of dissimilarities in the racial distributions of cohorts, a factor known to influence food choices. In a nested case control study, no association was observed between serum vitamin B_{12} concentrations and carcinoma *in situ* or invasive cervical cancer (28). However, this study assessed HPV-16 antibody status at baseline only and, therefore, could not assess other oncogenic HPV types.

In our population, high circulating vitamin B_{12} concentrations were protective against HPV persistence. One hypothesized mechanism by which vitamin B_{12} may protect against cervical carcinogenesis is through viral DNA methylation.

DNA methylation plays a significant role in gene expression, DNA structural stability, and DNA damage and repair mechanisms (16, 41), all of which are believed to be important in preventing carcinogenesis. A randomized trial examining the effect of supplemental folate (42), a methyl donor nutrient, among participants with colon adenomas reported that folate supplementation increased genomic methylation, suggesting that higher dietary consumption of certain nutrients may be protective through their influence on gene methylation status. In support of this hypothesis, a decrease in *in vitro* HPV transcription has been shown when the regulatory upstream region was methylated (13, 14), suggesting a role for methylation in preventing the maintenance of HPV infection.

It is also possible that circulating vitamin B_{12} is a surrogate marker for some other unmeasured factor that is protective against HPV persistence. In our study, circulating vitamin B_{12} concentrations and dietary intake measures were poorly correlated, which is consistent with a previous report from a similar population (43). These poor correlations suggest that diet and circulating concentrations measure different exposures and may help to explain why a relationship between circulating vitamin B_{12} concentrations, but not dietary vitamin B_{12} intake, was observed in this study.

There are several strengths to consider when interpreting the results of this study. HPV status was determined with multiple measures of HPV infection over a 9-month period. This study measured both dietary intake and circulating measures of folate and vitamin B_{12}, within the same woman. The database used to calculate folate nutrient values from the AFFQ was established to account for the addition of fortified grain products to the American diet, as determined by the completion date of the AFFQ.

As well, potential limitations need to be considered when interpreting the results from this study. Only 346 of women invited to the follow-up visits participated, thus limiting the power to detect statistically significant differences between groups. Because this study was conducted at only one clinic site, the results may not be generalizable to other populations. Although we were not able to assess type-specific oncogenic infections because of the molecular method used to detect HPV, the risk of cervical dysplasia has been shown to increase with either the same or different type oncogenic HPV persistence (6). Therefore, HPV persistence with an oncogenic type, as determined in our study, provides a useful measure of subsequent cervical neoplasia risk. As with any study, assessing the dietary intake of folate is problematic as the dietary intake database is limited because of the difficulties of analytically quantifying food folate content (44). Errors in the USDA nutrient database for folate (45) may have resulted in misclassification of dietary folate status leading to bias toward the null value. Such bias may explain why a protective association between dietary folate intake from food sources and HPV persistence was not observed. Similarly, RBC folate is a more precise measure of long-term circulating folate levels, whereas serum and plasma are more variable based on recent dietary intake (46). Although RBC folate was not assessed, the influence of participants' most recent meal on circulating folate concentrations was minimized by collecting blood samples from fasting participants.

In conclusion, results from this study indicate that elevated circulating levels of vitamin B_{12} reduce the risk of HPV persistence. Although these findings suggest a role for vitamin B_{12} in preventing cervical carcinogenic events, larger studies using multiple measures over time need to be conducted to further investigate this relationship.

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