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Diet and premalignant lesions of the cervix: evidence of a protective role for folate, riboflavin, thiamin, and vitamin B_{12}

Brenda Y. Hernandez[1,*], Katharine McDuffie[1], Lynne R. Wilkens[1], Lori Kamemoto[2] & Marc T. Goodman[1]
[1]*Cancer Research Center of Hawaii, University of Hawaii, 1236 Lauhala Street, Honolulu, Hawaii 96813, USA;* [2]*John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii, USA*

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Abstract

Objective: A case–control study was conducted among a population of multiethnic women identified from clinics on Oahu, Hawaii between 1992 and 1996 to explore the relationship between diet and cervical dysplasia.
Methods: Two-hundred and fourteen women with biopsy-confirmed high and low grade squamous intraepithelial lesions of the cervix (SIL) and 271 controls were identified. Exfoliated cervical cells were collected for HPV DNA testing. Surveys were administered to assess non-dietary risk factors and intake of nutrients from over 250 specific food items as well as nutritional supplements.
Results: Riboflavin and thiamin from food sources, vitamin B_{12} supplements, and total (food and supplements) folate displayed inverse, dose-responsive associations with high-grade SIL (HSIL). Riboflavin from food sources and total folate also demonstrated inverse, dose-responsive associations with low-grade SIL (LSIL). The odds ratios for LSIL and HSIL were reduced by 50–90% for the highest compared to the lowest levels of intake of these nutrients. A number of major food sources of these vitamins, including all types of breads, bran cereal, and fruit juice, also demonstrated inverse associations with HSIL. There was some evidence that the increased risk of HSIL associated with low nutrient intake was most pronounced among drinkers and smokers.
Conclusions: This investigation provides evidence that thiamin, riboflavin, folate, and vitamin B_{12} may play a protective role in cervical carcinogenesis.

Introduction

Human papillomavirus (HPV) has been clearly established as the major causal agent in the etiology of cervical cancer and its precursor lesions [1, 2]. Nevertheless, most HPV infections do not result in cervical malignancies. Cofactors acting independently or with HPV to influence carcinogenesis appear to be important but remain largely unidentified. Protective effects have been observed for specific dietary and plasma nutrients including tocopherols [3, 4] carotenoids [4, 5], vitamin C [5], and folate [6, 7]. Overall, however, epidemiologic studies have been inconsistent with respect to the relationship between specific foods and nutrients and cervical neoplasia. We previously explored the relationship between plasma micronutrients and premalignant lesions of the cervix [4]. The present study explores the relationship between diet and cervical dysplasia while adjusting for potentially confounding dietary and non-dietary factors.

Materials and methods

Recruitment of cases and controls

We conducted a case–control study among women attending three hospital-based clinics on the island of Oahu, Hawaii for cervical cytological screening between

* Address correspondence to: Brenda Y. Hernandez, PhD, Caner Research Center of Hawaii, University of Hawaii, 1236 Lauhala Street, Honolulu, Hawaii 96813, USA. Ph.: +1-808-586-2992; Fax: +1-808-586-2982, E-mail: brenda@crch.hawaii.edu

June, 1992 and December, 1996. This study was approved by the Committee on Human Subjects of the University of Hawaii and individual hospital institutional review boards. Written, informed consent was obtained from all study subjects. Eligible cases and controls included women 18 and older who were residents of Oahu. Women who had a prior hysterectomy, had been pregnant or lactating within the past 6 months, or had a previous diagnosis of cervical dysplasia within the past 3 years were ineligible to participate.

Cases were identified at participating clinics and initially included all women with a cytological classification of low-grade and high-grade squamous intraepithelial lesions (LSIL and HSIL, respectively) of the cervix. These women, who had been scheduled to return to the clinic for follow-up examination, were contacted by letter and telephone for permission to participate in the study. At the follow-up visit, written consent was obtained and colposcopy and biopsy were obtained. All smears and biopsies were read by study pathologists and classified according to the Bethesda classification system [8]. Of the 303 women with biopsy-confirmed dysplasia, 214 (71%) consented to participate. The 137 and 77 women with biopsy-confirmed LSIL and HSIL, respectively, are the subjects of the present investigation.

Controls were women with negative cytological smears attending the same clinics as cases. Eligible women were selected from the admission logs of the participating clinics on a randomly selected day of the month. Potential controls were met at the clinics by one of the interviewers who explained the purpose of the study. Of the 533 potential controls initially identified, 389 consented to participate (73%). Of these, 118 had a diagnosis of atypical squamous cells of undetermined significance and were excluded, leaving 271 cytologically normal controls.

Specimen collection and HPV DNA detection

All procedures including Pap smears, colposcopy, biopsy, and HPV specimen collection were done at the clinic sites. HPV specimens were collected using Dacron swabs and placed in 1 ml of specimen transport media (Digene Corporation, Gaithersburg, MD, USA). Specimens were transported to the nearby research lab where they were temporarily stored at −20 °C.

Batched frozen cell specimens (1 ml) were then shipped on dry ice to the HPV laboratory of Harborview Medical Center in Seattle, Washington. There they were prepared for HPV DNA detection by polymerase chain reaction (PCR) and dot blot hybridization. The PCR amplifies the highly conserved L1 region of the viral genome using degenerate primers (MY09 and MY11) [9]. The primers amplify over 45 types of HPV, including the most common oncogenic types found in the cervix [10]. PCR products were assessed through dot blot hybridization using biotin-labeled oligonucleotide DNA probes for HPV types 6/11, 16, 18, 31/33/35/39, and 45, as well as a generic probe, which detects a broad spectrum of HPV types.

Dietary and non-dietary survey

Following the clinical visit, participants were scheduled for a personal interview at their homes or another convenient location. Two separate standardized questionnaires were used. The first was a survey on demographic and background information, tobacco use, and sexual, reproductive, and medical histories. The second was a dietary survey querying the frequency of consumption and portion sizes of over 250 specific food items or food categories. The survey also covered use of nutritional supplements including single and multivitamins. Subjects were queried about the frequency and quantity of vitamin intake as well as the brand of supplements. This survey had been previously validated in our population [11]. The food items in the survey are the minimum set that contributes 85% or more of the major dietary components for the ethnic groups included in this study. The food sources of these dietary components and common serving sizes for each were derived from measured food records among a random sample of the major ethnic groups in Hawaii. The survey also included questions about added fats and oils as well as supplements. For each food item, the usual frequency of consumption per day, week, or month was assessed. For seasonal items such as certain fruits, yearly frequencies were queried. Color photographs illustrating the most representative serving sizes were used to assist subjects in estimating serving sizes. The quantity of each food item consumed on a daily basis was calculated as the product of the frequency and serving size.

Specific nutrient intakes for each person were compiled using a food composition database, which contained over 3000 foods and 95 components. The food composition database was compiled from the United States Department of Agriculture [12] data and other sources [13, 14] and was customized for the diverse diets of Hawaii's multiethnic population. The vitamin supplement database included information on about 2700 vitamins by brand name and this was used to determine the quantity of each type of supplement included in single and multivitamins. The dietary reference period was the year prior to diagnosis for cases and the year prior to study enrollment for controls. The median time

between diagnosis/enrollment and interview was 4 months.

Alcohol intake was assessed in both the nondietary and dietary surveys. The nondietary survey queried lifetime alcohol use including past and current frequency of intake by alcohol type. The dietary survey queried the quantity of consumption for specific types of alcohol in the previous 12 months. Alcohol quantity was calculated by converting the volume of drinks and frequency of intake into grams for all types of drinks (beer, wine, hard liquor).

Statistical analysis

Analysis of covariance [15] was used to compare the means of log-transformed intakes of nutrients and foods between cases and controls while adjusting for age, ethnicity, and energy (kilocalorie) intake. Partial Pearson correlations (r_p) of log-transformed values of dietary items were also calculated (adjusting for age and ethnicity) in order to evaluate colinearity among both cases and controls.

The analysis focused on the association of specific foods and nutrients with the risk of LSIL and HSIL. For specific micronutrients such as vitamins and minerals, estimates were based on intake from foods, supplements, and both food and supplements (total intake). Exposures elicited through the non-dietary survey, such as cigarette smoking and sexual history, were analyzed as potential confounders. Unconditional logistic regression [16] modeling case–control status was used to evaluate the risk associated with levels of intake of dietary items. Odds ratios (OR) and 95% confidence intervals (CI) were computed by exponentiating the coefficients and 95% CI for the indicator variables representing tertiles of the food or nutrient of interest. The tertile cut points were based on the distributions of intake among controls. The final model was selected based on a previous analysis of a subset of this study population [4] in addition to analysis of nondietary risk factors in the current population.

Because HPV has been established as a necessary cause of cervical cancer [2], direct adjustment for viral status may obscure the relationship of other risk factors. For this reason, models were included with and without adjustment for HPV DNA infection (positive/negative). Other adjustment variables included energy intake (continuous variable using log-transformed kilocalories), age (continuous variable), race/ethnicity (white, Japanese, Hawaiian, other), cigarette smoking (ever/never daily for 6 months or more), alcohol drinking (ever/never at least weekly for 6 months or more), and number of sexual partners prior to age 20 (continuous variable). A test for linear trend in the logit of risk was performed by comparing twice the difference in log-likelihoods for models with and without a trend variable, based on a χ^2 distribution with one degree of freedom. The trend variable was assigned the median for the appropriate tertile.

The joint associations of certain nutrients with alcohol and smoking, respectively, on the risk of high-grade dysplasia were evaluated. The likelihood ratio test with one degree of freedom was used to compare a no-interaction model containing main effect terms with a fully parameterized model containing all possible interaction terms for the variables of interest. In these models, dummy variables were created to represent the interaction terms of total nutrient intake (dichotomized at the median intake level of controls) with cigarette smoking (ever/never daily for 6 months or more) and alcohol drinking (ever/never at least weekly for 6 months or more), respectively.

Results

Table 1 compares the characteristics of SIL cases (HSIL and LSIL combined) and controls. Over 70% of cases were positive for HPV DNA compared to 10% of controls. HPV was more strongly associated with HSIL than LSIL (OR = 39.4, 95% CI = 18.7–82.8 and OR = 16.9, 95% CI = 9.7–39.3, respectively, adjusted for age and race/ethnicity). Compared to controls, SIL cases were younger and more likely to be of non-white race/ethnicity. Cases also had more sexual partners prior to age 20 and were more likely to have a history of smoking and drinking.

We examined the relationship of consumption of individual nutrients and food items with the risk of LSIL and HSIL. No association was observed for major macronutrients including kilocalories, proteins, carbohydrates, or fats (data not shown).

Table 2 shows the relationship of selected nutrients with LSIL and HSIL risk. Estimates were made with and without adjustment for HPV DNA infection (positive/negative) as well as age, race/ethnicity, smoking, alcohol, (log-transformed) kilocalories, and number of sexual partners prior to age 20. There was a general pattern of decreasing HSIL risk with increasing nutrient levels for thiamin, riboflavin, and vitamin B_{12} (food, supplements, and total sources), and folate (supplement and total sources). When adjustment was made for HPV, the inverse associations for thiamin and riboflavin from food, total folate, and supplemental B_{12} were significant and dose-responsive (p for trend < 0.05).

Table 1. Background characteristics of women with SIL of the cervix and normal controls in Hawaii

	Cases (n = 214)	Controls (n = 271)	HPV-adjusted[a] OR (95% CI)	Non HPV-adjusted[b] OR (95% CI)
Any HPV DNA[c]				
Negative	59 (28%)	244 (90%)	–	1.0[d]
Positive	155 (72%)	27 (10%)	N/A	22.6 (13.5–38.1)
HPV 16	62	4		
HPV 6/11	7	2		
HPV 18	9	1		
HPV 31/33/35/39	36	3		
HPV 45	5	0		
Other HPV	48	19		
Age	32 (mean)	39 (mean)		
<30	111 (52%)	83 (31%)[e]	1.0[d]	1.0[d]
30–39	58 (27%)	72 (27%)	1.0 (0.6–1.8)	0.6 (0.4–1.0)
≥40	45 (21%)	116 (43%)	0.4 (0.2–0.8)	0.3 (0.2–0.5)
			p trend = 0.004	p trend < 0.0001
Race/ethnicity				
White	75 (35%)	121 (45%)[e]	1.0[d]	1.0[d]
Japanese	26 (12%)	42 (16%)	1.7 (0.8–3.6)	1.0 (0.6–1.8)
Hawaiian	38 (18%)	39 (15%)	1.4 (0.7–2.7)	1.2 (0.7–2.0)
Other	75 (35%)	69 (25%)	1.8 (1.0–3.2)	1.6 (1.0–2.5)
Household income				
< $25,000	66 (31%)	61 (23%)	1.0[d]	1.0[d]
$25,000– < 50,000	78 (36%)	101 (37%)	1.1 (0.6–2.1)	0.9 (0.6–1.5)
≥$50,000	70 (33%)	109 (40%)	1.3 (0.7–2.5)	0.8 (0.5–1.4)
Marital status				
Single (never married)	58 (27%)	49 (18%)[e]	1.0[d]	1.0[d]
Married/living as married	103 (48%)	159 (59%)	1.6 (0.8–3.2)	1.0 (0.6–1.6)
Divorced/separated	52 (24%)	53 (20%)	2.0 (0.9–4.4)	1.7 (0.9–3.2)
Widowed	1 (1%)	10 (4%)	0.2 (0.01–2.4)	0.2 (0.02–1.9)
Number of sexual partners prior to age 20[f]				
0	43 (20%)	98 (37%)	1.0[d]	1.0[d]
1	51 (24%)	72 (27%)	1.7 (0.8–3.3)	1.4 (0.8–2.4)
2	40 (19%)	40 (15%)	2.4 (1.1–5.1)	1.7 (0.9–3.1)
3	78 (37%)	56 (21%)	2.0 (1.0–4.1)	1.9 (1.1–3.3)
			p trend = 0.06	p trend = 0.02
Cigarette smoking[g]				
Never	120 (56%)	172 (63%)	1.0[d]	1.0[d]
Ever	94 (44%)	99 (37%)	1.8 (1.1–2.9)	1.6 (1.1–2.4)
Alcohol drinking[h]				
Never	95 (44%)	149 (55%)	1.0[d]	1.0[d]
Ever	119 (56%)	122 (45%)	1.8 (1.1–3.0)	1.8 (1.2–2.8)

[a] Adjusted for age, race/ethnicity, and HPV DNA.
[b] Adjusted for age and race/ethnicity.
[c] Positive for one or more types of HPV DNA detected by MY09/MY11 PCR and dot blot hybridization.
[d] Referent group.
[e] Percentages do not total 100% due to rounding.
[f] Excludes cases and controls under age 20.
[g] Cigarette smoking daily for 6 months or more.
[h] Alcohol drinking at least weekly for 6 months or more.

Without HPV adjustment, these associations were neither significant nor dose-responsive.

For LSIL, there was also a general pattern of decreasing risk with increasing nutrient levels for thiamin and riboflavin (food, supplements, and total sources) and for folate and vitamin B_{12} (supplements and total sources). When adjustment was made for HPV, these associations were neither significant nor dose-

Table 2. Association of selected nutrients with low- and high-grade SIL of the cervix in Hawaii

Nutrient (average daily intake)	Controls (n = 271)	LSIL (n = 137)	HSIL (n = 77)	LSIL odds ratio (95% CI)		HSIL odds ratio (95% CI)	
				HPV-adjusted[a]	Non HPV-adjusted[b]	HPV-adjusted[a]	Non HPV-adjusted[b]
Thiamin (food sources), mg							
<1.2	81	57	23	1.0[c]	1.0[c]	1.0[c]	1.0[c]
1.2–1.8	98	38	27	0.5 (0.3–1.2)	0.6 (0.3–1.1)	0.4 (0.1–1.2)	0.7 (0.3–1.5)
>1.8	92	42	27	0.7 (0.2–1.9)	0.5 (0.2–1.1)	0.1 (0.03–0.7)	0.4 (0.1–1.1)
				p trend = 0.47	*p* trend = 0.08	*p* trend = 0.01	*p* trend = 0.08
Thiamin (supplements), mg							
0	161	91	51	1.0[c]	1.0[c]	1.0[c]	1.0[c]
0.1–1.7	52	24	16	1.0 (0.4–2.0)	0.9 (0.5–1.6)	0.7 (0.3–1.9)	0.9 (0.5–1.9)
>1.7	58	22	10	0.8 (0.4–1.7)	0.7 (0.4–1.2)	0.5 (0.2–1.3)	0.5 (0.2–1.1)
				p trend = 0.57	*p* trend = 0.21	*p* trend = 0.15	*p* trend = 0.11
Thiamin (total), mg							
<1.5	83	52	26	1.0[c]	1.0[c]	1.0[c]	1.0[c]
1.5–2.9	92	50	21	1.0 (0.5–2.1)	0.9 (0.5–1.6)	0.4 (0.1–1.0)	0.5 (0.2–1.1)
>2.9	96	35	30	0.9 (0.4–1.9)	0.6 (0.3–1.2)	0.6 (0.2–1.6)	0.7 (0.3–1.5)
				p trend = 0.65	*p* trend = 0.11	*p* trend = 0.63	*p* trend = 0.61
Riboflavin (food sources), mg							
<1.5	78	55	29	1.0[c]	1.0[c]	1.0[c]	1.0[c]
1.5–2.1	102	43	17	0.7 (0.3–1.5)	0.5 (0.3–1.0)	0.2 (0.1–0.7)	0.3 (0.2–0.7)
>2.1	91	39	31	0.6 (0.2–1.6)	0.4 (0.2–0.9)	0.2 (0.1–0.9)	0.4 (0.2–1.1)
				p trend = 0.32	*p* trend = 0.03	*p* trend = 0.03	*p* trend = 0.07
Riboflavin (supplements), mg							
0	163	91	51	1.0[c]	1.0[c]	1.0[c]	1.0[c]
0.1–1.8	51	23	16	0.9 (0.4–2.0)	0.8 (0.4–1.5)	0.8 (0.4–2.4)	1.0 (0.5–2.0)
>1.8	57	23	10	0.9 (0.4–1.8)	0.8 (0.4–1.4)	0.5 (0.2–1.3)	0.5 (0.2–1.1)
				p trend = 0.72	*p* trend = 0.22	*p* trend = 0.15	*p* trend = 0.12
Riboflavin (total), mg							
<1.7	86	49	27	1.0[c]	1.0[c]	1.0[c]	1.0[c]
1.7–3.3	87	52	22	1.1 (0.5–2.1)	0.9 (0.5–1.6)	0.4 (0.2–1.1)	0.5 (0.2–1.0)
>3.3	98	36	28	0.9 (0.4–1.9)	0.6 (0.3–1.1)	0.5 (0.2–1.4)	0.5 (0.2–1.1)
				p trend = 0.65	*p* trend = 0.08	*p* trend = 0.35	*p* trend = 0.16
Niacin (food sources), mg							
<16	88	54	20	1.0[c]	1.0[c]	1.0[c]	1.0[c]
16–23	87	46	29	1.3 (0.6–2.9)	1.0 (0.5–1.9)	1.3 (0.4–3.7)	1.3 (0.6–2.8)
>23	96	37	28	1.0 (0.4–2.7)	0.6 (0.3–1.5)	0.5 (0.1–2.0)	0.8 (0.3–2.3)
				p trend = 0.97	*p* trend = 0.30	*p* trend = 0.30	*p* trend = 0.71
Pantothenic acid (food sources), mg							
<3.9	86	54	21	1.0[c]	1.0[c]	1.0[c]	1.0[c]
3.9–5.6	101	38	24	1.0 (0.4–2.1)	0.7 (0.4–1.4)	1.3 (0.4–3.8)	1.0 (0.4–2.3)
>5.6	84	45	32	1.2 (0.4–3.7)	0.9 (0.4–2.2)	1.7 (0.4–7.6)	1.3 (0.4–3.9)
				p trend = 0.70	*p* trend = 0.87	*p* trend = 0.46	*p* trend = 0.64
Vitamin B_6 (food sources), mg							
<1.6	83	55	23	1.0[c]	1.0[c]	1.0[c]	1.0[c]
1.6–2.2	91	45	26	1.0 (0.5–2.1)	0.8 (0.4–1.4)	0.8 (0.3–2.2)	0.9 (0.4–1.9)
>2.2	97	37	28	0.8 (0.3–2.2)	0.5 (0.2–1.2)	0.4 (0.1–1.4)	0.6 (0.2–1.7)
				p trend = 0.69	*p* trend = 0.11	*p* trend = 0.14	*p* trend = 0.38

Table 2. (Continued)

Nutrient (average daily intake)	Controls (n = 271)	LSIL (n = 137)	HSIL (n = 77)	LSIL odds ratio (95% CI)		HSIL odds ratio (95% CI)	
				HPV-adjusted[a]	Non HPV-adjusted[b]	HPV-adjusted[a]	Non HPV-adjusted[b]
Vitamin B_6 (supplements), mg							
0	163	90	49	1.0[c]	1.0[c]	1.0[c]	1.0[c]
0.1–2	52	26	18	1.2 (0.6–2.4)	1.0 (0.5–1.8)	1.0 (0.4–2.5)	1.1 (0.6–2.2)
>2	56	21	10	0.6 (0.3–1.4)	0.7 (0.4–1.2)	0.5 (0.2–1.3)	0.6 (0.3–1.3)
				p trend = 0.40	*p* trend = 0.26	*p* trend = 0.20	*p* trend = 0.30
Vitamin B_6 (total), mg							
1.9	85	47	29	1.0[c]	1.0[c]	1.0[c]	1.0[c]
1.9–3.7	82	59	21	1.5 (0.7–3.0)	1.5 (0.9–2.7)	0.3 (0.1–0.9)	0.5 (0.3–1.1)
>3.7	104	31	27	0.7 (0.3–1.6)	0.7 (0.3–1.3)	0.4 (0.2–1.1)	0.6 (0.3–1.2)
				p trend = 0.29	*p* trend = 0.09	*p* trend = 0.17	*p* trend = 0.25
Folate (food sources), mcg							
<244	86	55	21	1.0[c]	1.0[c]	1.0[c]	1.0[c]
244–368	96	42	23	1.1 (0.5–2.2)	0.8 (0.4–1.3)	0.8 (0.3–2.2)	1.0 (0.5–2.2)
>368	89	40	33	1.5 (0.6–3.6)	0.9 (0.4–1.8)	1.3 (0.4–4.1)	1.6 (0.7–3.7)
				p trend = 0.37	*p* trend = 0.67	*p* trend = 0.65	*p* trend = 0.31
Folate (supplements), mcg							
0	165	95	53	1.0[c]	1.0[c]	1.0[c]	1.0[c]
0.1–400	87	35	21	0.7 (0.4–1.3)	0.7 (0.4–1.2)	0.5 (0.2–1.2)	0.7 (0.4–1.3)
>400	19	7	3	0.4 (0.1–1.5)	0.4 (0.2–1.2)	0.3 (0.1–1.5)	0.4 (0.1–1.4)
				p trend = 0.13	*p* trend = 0.08	*p* trend = 0.07	*p* trend = 0.13
Folate (total), mcg							
<294	81	54	26	1.0[c]	1.0[c]	1.0[c]	1.0[c]
294–534	89	48	25	1.0 (0.5–1.9)	1.0 (0.6–1.7)	0.6 (0.2–1.5)	0.8 (0.4–1.6)
>534	101	35	26	0.6 (0.3–1.2)	0.5 (0.3–1.0)	0.3 (0.1–0.9)	0.6 (0.3–1.2)
				p trend = 0.15	*p* trend = 0.04	*p* trend = 0.04	*p* trend = 0.13
Vitamin B_{12} (foods), mcg							
<3.3	91	49	22	1.0[c]	1.0[c]	1.0[c]	1.0[c]
3.3–5.5	91	46	25	0.9 (0.4–1.9)	0.8 (0.4–1.4)	0.6 (0.2–1.7)	0.9 (0.4–1.9)
>5.5	89	42	30	1.0 (0.4–2.4)	0.7 (0.3–1.4)	0.7 (0.2–2.2)	0.8 (0.3–2.0)
				p trend = 0.91	*p* trend = 0.30	*p* trend = 0.51	*p* trend = 0.65
Vitamin B_{12} (supplements), mcg							
0	161	90	52	1.0[c]	1.0[c]	1.0[c]	1.0[c]
0.1–6	54	28	15	1.2 (0.6–2.5)	0.9 (0.5–1.7)	0.7 (0.3–1.8)	0.8 (0.4–1.6)
>6	56	19	10	0.6 (0.3–1.2)	0.6 (0.3–1.1)	0.3 (0.1–1.0)	0.5 (0.2–1.1)
				p trend = 0.35	*p* trend = 0.18	*p* trend = 0.05	*p* trend = 0.10
Vitamin B_{12} (total), mcg							
<4.4	91	47	24	1.0[c]	1.0[c]	1.0[c]	1.0[c]
4.4–10	82	55	24	1.3 (0.7–2.7)	1.0 (0.6–1.8)	0.6 (0.2–1.7)	1.0 (0.4–1.5)
>10	98	35	29	0.6 (0.3–1.4)	0.6 (0.3–1.1)	0.5 (0.2–1.2)	0.8 (0.4–1.6)
				p trend = 0.24	*p* trend = 0.08	*p* trend = 0.12	*p* trend = 0.49

[a] Adjusted for age, race/ethnicity, smoking, alcohol, (log-transformed) kilocalories, lifetime number of sexual partners, and HPV.
[b] Adjusted for age, race/ethnicity, smoking, alcohol, (log-transformed) kilocalories, and lifetime number of sexual partners.
[c] Referent group.

responsive. Without HPV adjustment, however, the inverse associations for riboflavin from food and total folate were significant and dose-responsive (p for trend <0.05).

Fortified cereal, bran cereal, and other dry cereals were among the major food sources of thiamin, riboflavin, folate, B_6 and B_{12} in this study population. Other major food sources included white rice and orange juice for thiamin, milk (non-fat, low-fat, and whole milk) for riboflavin, orange juice and broccoli for folate, white rice and bananas for B_6, and cream soups and liver for B_{12}. There was no association of any individual food item with LSIL risk. A number of associations were observed for HSIL risk.

Consumption of bread (all types), bran cereal, and fruit juice (all types, predominantly orange juice) was inversely associated with HSIL risk and these relationships were dose-responsive for bread and bran cereal (OR = 0.2, 95% CI 0.1–0.6, p for trend = 0.01; OR = 0.3, 95% CI 0.1–0.8, p for trend = 0.04; and OR = 0.3, 95% CI 0.2–0.8, p for trend = 0.01, respectively, for the highest relative to the lowest intake, adjusted for age, race/ethnicity, smoking, alcohol drinking, sexual partners prior to age 20, and HPV). None of these associations were significant or dose-responsive when HPV was excluded as a covariate (OR = 0.5, 95% CI 0.2–1.1, p for trend = 0.37; OR = 0.6, 95% CI 0.3–1.1, p for trend = 0.34; and OR = 0.5, 95% CI 0.2–1.1, p for trend = 0.18, for bread, bran cereal, and fruit juice, respectively).

Beer and wine showed an increasing risk of HSIL with higher levels of consumption although the relationships were not statistically significant (data not shown). When all alcoholic drinks were considered together, however, there was a positive, dose-responsive association between total alcohol intake and HSIL (OR = 3.5, 95% CI 1.1–11.0, p for trend = 0.04) for the highest relative to the lowest tertile of intake. This increased risk of HSIL was also significant albeit attenuated when HPV was not included in the model (OR = 2.6, 95% CI 1.1–5.8, p for trend = 0.03) for the highest relative to the lowest tertile of intake.

Lutein and iodine from food sources each displayed a dose-responsive, increased risk of HSIL for the highest relative to the lowest level of intake (OR = 3.2, 95% CI 1.1–10.1, p for trend = 0.02 and OR = 4.3, 95% CI 1.2–15.4, p for trend = 0.02, respectively, for the highest relative to the lowest intake, adjusted for age, race/ethnicity, smoking, alcohol drinking, sexual partners prior to age 20, and HPV. There was no association of iodine with HSIL when HPV was excluded as a covariate (OR = 1.8, 95% CI 0.7–4.6, p for trend = 0.21). The positive association of lutein with HSIL remained significant but was not dose-responsive when HPV was excluded (OR = 2.2, 95% CI 1.0–5.1, p for trend 0.06 for the highest relative to the lowest level of intake).

Spinach, dark green lettuce, and broccoli were the major sources of lutein and white rice, dried seaweed, and low-fat milk the major sources of iodine and in this study population. A positive association was observed between spinach intake and HSIL (OR = 2.7, 95% CI 1.0–7.1, p for trend = 0.04) for the highest relative to the lowest intake, adjusted for age, race/ethnicity, smoking, alcohol drinking, sexual partners prior to age 20, and HPV. This relationship was attenuated when HPV was excluded in the model (OR = 2.0, 95% CI 1.0–4.1, p for trend = 0.50).

Selected nutrients were evaluated for joint association with alcohol and smoking, with HSIL risk (Tables 3 and 4, respectively). ORs were calculated using statistical models with and without HPV status as a covariate. Compared to non-drinkers with high intake, there was a statistically significant increased risk of HSIL among alcohol drinkers with low total intakes of thiamin, riboflavin, folate, vitamin B_{12} and vitamin B_6. This was observed both with and without HPV-adjustment although the magnitude of risk was consistently higher with HPV adjustment. Statistical interaction (departure from additivity in the coefficients) between nutrient levels and alcohol use was not significant for any of the vitamins.

Compared to non-smokers with high intake, a statistically significant increased risk of HSIL was observed among smokers with low intakes of thiamin, riboflavin, folate, vitamin B_6, and vitamin B_{12}. Unlike joint associations with alcohol, however, these relationships were only observed with HPV adjustment. Statistical interaction between nutrient levels and alcohol use was not significant for any of the vitamins.

Discussion

To date, diet has not been consistently observed to play a role in the etiology of cervical cancer and its precursors. The present investigation yields evidence that dietary thiamin, riboflavin, folate, and vitamin B_{12} may protect against premalignant cervical lesions, particularly high-grade SIL, which, compared to low-grade dysplasia, are the more immediate precursors of cervical cancer [17]. Intake of these vitamins reduced the OR for HSIL by 50–90% for the highest compared to the lowest levels of consumption. The consistency of the relationships for individual vitamins and a few of their major food sources, including bread, bran cereal, and fruit juice, makes our study results even more compelling.

The very strong association of HPV with HSIL, which was twice the magnitude as that of HPV with LSIL, supports the direct role of HPV in cervical carcinogenesis.

Table 3. Joint association of alcohol consumption and intake of selected nutrients with the risk of HSIL of the cervix in Hawaii

Nutrient (average daily intake[b])	Alcohol consumption[a]: Never		Alcohol consumption[a]: Ever		p[d]	
	Cases/Controls	OR[c] (95% CI)	Cases/Controls	OR[c] (95% CI)		
Thiamin (mg)						
>1.9	18/74	1.0[e]	23/76	1.6 (0.6–4.7)		
≤1.9	14/75	1.9 (0.6–6.3)	22/46	5.7 (1.7–19.2)	0.55	HPV-adjusted
		1.0[e]		1.5 (0.7–3.2)		
		1.0 (0.4–2.5)		3.3 (1.4–7.8)	0.18	Non HPV-adj.
Riboflavin (mg)						
>2.2	16/71	1.0[e]	25/76	1.7 (0.6–5.1)		
≤2.2	16/78	1.6 (0.5–2.7)	20/46	4.3 (1.3–14.3)	0.87	HPV-adjusted
		1.0[e]		1.7 (0.8–3.8)		
		1.1 (0.5–2.7)		3.0 (1.2–7.1)	0.46	Non HPV-adj.
Folate (mcg)						
>422	15/63	1.0[e]	21/72	1.2 (0.4–3.5)		
≤422	17/86	1.0 (0.3–3.2)	24/50	4.0 (1.2–13.2)	0.10	HPV-adjusted
		1.0[e]		1.4 (0.6–3.1)		
		0.9 (0.4–2.2)		2.9 (1.2–7.1)	0.13	Non HPV-adj.
Vitamin B_6 (mg)						
>2.5	15/69	1.0[e]	25/76	1.7 (0.6–4.8)		
≤2.5	17/80	1.5 (0.5–4.8)	20/46	4.6 (1.4–15.5)	0.42	HPV-adjusted
		1.0[e]		1.8 (0.8–4.0)		
		1.1 (0.5–2.7)		2.9 (1.2–7.1)	0.52	Non HPV-adj.
Vitamin B_{12} (mcg)						
>6.1	15/67	1.0[e]	26/73	2.4 (0.8–6.9)		
≤6.1	17/82	2.1 (0.6–6.8)	19/49	4.3 (1.3–14.6)	0.84	HPV-adjusted
		1.0[e]		2.1 (0.9–4.6)		
		1.3 (0.5–3.0)		2.7 (1.1–6.4)	0.97	Non HPV-adj.

[a] Consumption of alcohol at least once a week for 6 months or more.
[b] Total intake from foods and supplements.
[c] Adjusted for age, race/ethnicity, smoking, (log-transformed) kilocalories, and number of sexual partners prior to age 20; estimates provided with and without additional adjustment for HPV.
[d] Based on the likelihood ratio test comparing models with and without an interaction term (1 degree of freedom).
[e] Referent group.

HPV adjustment resulted in stronger and more precise risk estimates of the association of the various nutrients and foods with HSIL. This indicates that HPV is a strong confounder in the relationship between specific nutrients and HSIL. That is, HPV is associated with thiamin, riboflavin, folate, and vitamin B_{12} – as well as HSIL. Furthermore, this may indicate that women with lower intakes of these vitamins are more susceptible to HPV-infection than those with higher intakes.

By contrast to HSIL, HPV adjustment did not substantially alter risk estimates for LSIL and yielded risk estimates that were less precise. Two nutrients – riboflavin from food and total folate – displayed a significant, inverse association with LSIL only when HPV was not taken into account. The addition of HPV as a covariate attenuated these associations. This indicates that HPV is not a strong confounder in the relationship between these nutrients and LSIL risk.

In addition to its availability from natural sources, a number of nutrients have been made more widely available in the American diet through fortification of commonly eaten foods. In addition to the United States, a number of other countries in Africa, South and Central America, and Asia have adopted some form of mandatory or voluntary practices for the fortification of foods with nutrients including thiamin, riboflavin, niacin, and/or folate. Most European countries, however, have not adopted mandatory fortification policies. Thiamin and riboflavin has been added to refined grains, including flour and prepared cereals, for a number of decades in the United States. Folate fortification, by contrast, was not done on a widespread basis until after 1996 – after this investigation was concluded. In addition to fortification, intake of specific nutrients has been enhanced through its availability as supplements. In the early 1990s, the US government supported recommendations for daily supplemental use of folic acid among women of childbearing age in order to prevent fetal neural tube defects [18, 19]. Accordingly, the intake of folic acid as well as B_6 and B_{12} – the latter

Table 4. Joint association of cigarette smoking and intake of specific nutrients with the risk of HSIL of the cervix in Hawaii

Nutrient (average daily intake[b])	Cigarette smoking[a]: Never		Cigarette smoking[a]: Ever		p^d	
	Cases/Controls	OR[c] (95% CI)	Cases/Controls	OR[c] (95% CI)		
Thiamin (mg)						
>1.9	23/96	1.0[e]	18/54	2.1 (0.7–5.9)		
≤1.9	21/75	3.0 (1.1–8.8)	15/46	4.7 (1.4–15.5)	0.69	HPV-adjusted
		1.0		1.4 (0.7–3.0)		
		1.6 (0.8–3.4)		2.2 (0.9–5.1)	0.96	Non HPV-adj.
Riboflavin (mg)						
>2.2	22/93	1.0[e]	19/54	2.0 (0.7–5.5)		
≤2.2	22/78	2.2 (0.8–6.0)	14/46	3.7 (1.1–12.0)	0.83	HPV-adjusted
		1.0		1.5 (0.7–3.2)		
		1.6 (0.7–3.3)		1.9 (0.8–4.5)	0.70	Non HPV-adj.
Folate (mcg)						
>422	20/83	1.0[e]	16/52	1.4 (0.5–4.1)		
≤422	24/88	1.5 (0.6–4.2)	17/48	4.2 (1.3–13.9)	0.39	HPV-adjusted
		1.0		1.3 (0.6–3.0)		
		1.4 (0.7–3.0)		2.1 (0.9–4.8)	0.81	Non HPV-adj.
Vitamin B_6 (mg)						
>2.5	23/92	1.0[e]	17/53	1.4 (0.5–3.9)		
≤2.5	21/79	1.7 (0.6–4.6)	16/47	4.2 (1.3–13.4)	0.42	HPV-adjusted
		1.0		1.3 (0.6–2.8)		
		1.3 (0.6–2.8)		1.9 (0.9–4.4)	0.81	Non HPV-adj.
Vitamin B_{12} (mcg)						
>6.1	22/88	1.0[e]	19/52	2.2 (0.8–6.1)		
≤6.1	22/83	2.2 (0.8–6.1)	14/48	3.5 (1.1–11.7)	0.66	HPV-adjusted
		1.0		1.6 (0.7–3.3)		
		1.4 (0.8–6.1)		1.7 (0.7–4.0)	0.65	Non HPV-adj.

[a] Cigarette smoking daily for 6 months or more.
[b] Total intake from food and supplements.
[c] Adjusted for age, race/ethnicity, alcohol, (log-transformed) kilocalories, and number of sexual partners prior to age 20; estimates provided with and without additional adjustment for HPV.
[d] Based on the likelihood ratio test comparing models with and without an interaction term (1 degree of freedom).
[e] Reference category.

two which were commonly included with folic acid in multivitamin form – increased among women [18, 19].

Our study findings are consistent with those of other case–control investigations which have observed inverse associations with cervical neoplasia risk for dietary folate [6, 7], riboflavin [20], and fruit juices [5, 21, 22]. Other studies have not supported our findings. In our previous investigation, which included a subset of women in the present study, we observed no association between circulating levels of folate or vitamin B_{12} and cervical dysplasia [23]. This incongruence may be explained by the different periods of exposure measured by blood and dietary intake levels of the vitamins, respectively. While circulating levels of the water-soluble B vitamins likely reflect relatively recent exposure, the dietary reference period was the 12 months prior to SIL diagnosis. Other studies have observed a poor correlation between circulating and dietary levels of B vitamins [24]. Clinical trials of folic acid supplementation in women with cervical dysplasia showed no evidence that folate promotes regression or otherwise favorably alters the course of disease [25]. Intervention studies have not, however, examined the effects of supplementation on the development of cervical dysplasia among cytologically normal women.

B vitamins are a group of heterogeneous vitamins with distinct and diverse functions. Specific B vitamins can potentially influence carcinogenesis through its effect on DNA synthesis or methylation. Folate, vitamin B_{12}, vitamin B_6, and riboflavin are involved in metabolic pathways resulting in either DNA synthesis or methylation of DNA [26, 27]. Key to these metabolic pathways is the enzyme 5-,10-methylenetetrahydrofolate reductase (MTHFR). MTHFR converts folate, in the form of 5,10-methylenetetrahydrofolate, to 5-methyltetrahydrofolate. 5,10-methylenetetrahydrofolate is required for the biosynthesis of the thymidylate [27]. Low levels of folate and subsequent reduced levels of 5, 10-methylenetetrahydrofolate can result in misincorporation of uracil into DNA and a large number of

chromosomal breaks [27]. Riboflavin is also necessary for MTHFR activity as it is the precursor to flavin adenine dinucleotide (FAD), which is a coenzyme to MTHFR [26]. Low riboflavin levels can impede MTHFR activity [28] and may therefore also contribute to DNA instability.

Vitamin B_{12} is required for the activity of another enzyme, methionine synthase, which synthesizes methionine, an essential amino acid and precursor to the universal methyl donor, S-adenosylmethionine (SAM) [29]. A by-product of the conversion of methionine to SAM is homocysteine [30]. Homocysteine is subsequently removed by one of two metabolic pathways requiring different B vitamins: remethylation and transulfuration.

In the remethylation pathway, folate and vitamin B_{12} serve as substrate and cofactor, respectively, whereby 5-methyltetrahydrofolate provides the methyl group to remethylate homocysteine into methionine [30]. Lower levels of folate and vitamin B_{12} in the diet may therefore result in reduced levels of DNA methylation [31] as well as elevated levels of homocysteine [32].

Vitamin B_6 is required in the alternative pathway for homocysteine metabolism, the transulfuration pathway, through which homocysteine is degraded to cystathionine [30]. Vitamin B_6 deficiency disrupts this metabolic pathway and results in excessive homocysteine levels.

Folate, vitamin B_{12}, vitamin B_6, and riboflavin all are essential to these metabolic pathways involved in DNA synthesis and methylation such that low intake of these nutrients could potentially disrupt these pathways and result in chromosomal instability or altered DNA methylation – which can result in cancer. Indeed it has been shown that low levels of B vitamins can result in reduced levels of DNA methylation [31], which, in turn, may be related to increasing severity of cervical dysplasia and cancer [33]. Folate levels in cervical tissue have been correlated with DNA methylation level in the tissue [34]. It has also been suggested that low folate levels resulting in hypomethylation of DNA and DNA strand breaks may enhance the integration of HPV DNA into human DNA [35, 36].

As previously outlined, deficiency of folate, vitamin B_{12}, vitamin B_6, and/or riboflavin and resultant disruption of the various metabolic pathways can also lead to excessive levels of homocysteine [30, 32]. Elevated homocysteine levels are an established risk factor for cardiovascular disease [37, 38]. Several studies have observed an association between elevated homocysteine levels and cervical disease including cervical dysplasia [39] and cervical cancer [40]. In a multiethnic case–control study in five geographic areas, high homocysteine levels were associated with a 2- to 3-fold increased risk for invasive cervical cancer [41].

A fairly common polymorphism of MTHF involves a cytosine to thymine substitution at nucleotide 677 [42]. Individuals who are homozygous for the TT genotype have lower MTHFR enzyme activity and consequent elevated homocysteine levels [42, 43]. In our own previous investigation, we observed a weakly positive association between plasma homocysteine levels and risk of any SIL [23]. However, among individuals with the variant T allele (CT or TT) and plasma folate levels below the median, there was a 5-fold risk of cervical SIL compared to women with CC genotype and folate intakes above the median [23].

In the present investigation, there was a positive, dose-responsive relationship between alcohol consumption and HSIL risk and this has been supported by other studies [44]. Smoking was also positively associated with HSIL risk and this is also consistent with previous studies [45]. We observed some evidence of interaction of B vitamins with both alcohol intake and smoking, although none of the relationships reached significance. Interactions between folate and alcohol have been observed in previous studies whereby low levels of folate combined with excessive alcohol intake was associated with increased risk of colorectal [46] and breast cancer [47].

The relationship between alcohol and HSIL may be mediated by homocysteine as alcohol consumption has been shown to increase homocysteine levels [48]. Alcohol can also contribute to elevated homocysteine levels by impeding the absorption of B vitamins levels such as folate [49]. The inverse association between folate intake and homocysteine levels has been observed to be strongest among alcohol drinkers [48].

The results of the present investigation should be considered preliminary as they are limited by the relatively small sample size as well as the retrospective study design. The dietary assessment tool was a major strength of the study as it had been previously validated in our population and included a wide range of foods and food groups, including ethnic-specific foods. The retrospective design of this study may have resulted in some degree of misclassification in the ascertainment of dietary intake. In general, however, there is not widespread public awareness that diet can alter cervical cancer risk. Consequently, any dietary misclassification was likely to have been non-differential.

Although the strong association between HPV and cervical neoplasia was confirmed in this Hawaii population, there were limitations to the evaluation of HPV status. HPV is a transient infection in the majority of women while a minority develop persistent infections, which in turn, increases the risk of cervical carcinogenesis [50]. Because assessment of HPV infection was

limited to one rather than multiple time periods, we were not able to consider the relative duration of infection. Although only 10% of controls were HPV positive, it is possible that this group included previously infected women who had since cleared their infection. We were, however, able to control for a number of other potential confounders including age and sexual history.

Our study provides compelling evidence that certain B vitamins and their food sources may reduce the risk of cervical disease. The results are consistent with a number of previous epidemiologic investigations. Additionally, there is some evidence that alcohol use and cigarette smoking influence the effects of specific nutrients. These observations warrant further exploration as these findings provide evidence that risk of cervical disease may be modifiable through the diet.

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