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Dietary Calcium Intake and HPV Infection Status Among American Women: A Secondary Analysis from National Health and Nutrition Examination Survey (NHANES) Data Set of 2003–2016

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Background: The evidence on the link of dietary calcium (DCa) to human papillomavirus (HPV) infection is limited. Thus, this research was conducted to explore whether DCa is independently associated with HPV infection status in American women with age of 18 to 59 years old.

Material/Methods: We performed a secondary analysis from the National Health and Nutrition Examination Survey (NHANES) data set including 7 cycles from 2003 to 2016. A total of 13 475 selected participants were used for data analysis. The interested independent and the outcome variable were DCa and HPV infection status (HPV infection; HPV subtype). Sociodemographic, dietary, laboratory, questionnaire, and physical examination data were covariates. Weighted binary logistic regression and generalized additive model (GAM) were used for the investigation of both linear and non-linear relationships between DCa and HPV infection status.

Results: Weighted multivariable binary logistic regression indicated DCa was not associated with HPV infection and subtype (OR: 0.93; 95% CI: 0.82–1.05 for HPV infection; OR: 1.09; 95% CI: 0.93–1.28 for HPV subtype). For HPV infection, a non-linear correlation was detected, whose inflection points were 9.78 of log2 DCa. The OR values and the confidence intervals on both sides of inflection point were 0.83 (95% CI: 0.70–0.98) and 1.18 (95% CI: 0.91–1.52), respectively.

Conclusions: At the range of 3.32–9.78 of log2 calcium intake, DCa intake was negatively correlated with HPV infection. After this interval, DCa intake was not associated with the risk of HPV infection.

MeSH Keywords: Americas • Calcium • DNA Probes, HPV

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Background

Human papillomavirus (HPV) infection has always been the focus of public health worldwide. Current data from the United States centers for disease control and prevention (CDC) indicated that nearly 80 million Americans are currently infected with some type of HPV, and about 14 million Americans become infected each year (*https://www.cdc.gov/hpv/*). HPV infection has been confirmed to be closely related to the onset of various tumors, including cervical cancers (91%), vulvar cancers (69%), vaginal cancers (75%), penile cancers (63%), anal cancers in females (93%), and oropharyngeal cancers (63%) in females [1]. Although numerous evidences support that vaccination is an effective measure to control the prevalence of HPV, the HPV vaccination rates are relatively low even in the United States [1–4]. In this context, blocking HPV prevalence must be considered in addition to vaccination and etiological prevention.

Several studies have reported the link of dietary intake of selected nutrients to HPV infection. They have indicated that plant-based foods are negative associate with HPV infection, such as folate, vitamins A, C, and E, and the active Vitamin D [5]. To our best knowledge, literature with respect to the association between dietary calcium (DCa) intake and HPV infection is limited. Results from *in vitro* experiments indicated that extracellular high calcium is associated with HPV persistent infection and progression [6–12]. In addition, findings from epidemiological survey reported that calcium was negatively associated with a risk of CINII/III [13]. Although these studies aimed at different subjects and did not concern with the association between dietary calcium and HPV prevalence, at least the following assumption can be proposed: DCa intake may be associated with HPV infection and DNA types.

Therefore, in the present study, we used existing National Health and Nutrition Examination Survey (NHANES) data to investigate the correlation of dietary calcium intake on HPV infection in American females with age of 18 to 59 years old.

Material and Methods

Data source

The National Health and Nutrition Examination Survey (NHANES), a cross-sectional observational study, was designed to investigate the health and nutritional status in American adults and children. It is a continuous program with about 5,000 people involved each year. NHANES data set include demographic, socioeconomic, dietary, health-related questions, physiological measurements and laboratory tests. The introduction of NHANES can be obtained in detail at official website (*https://www.cdc.Gov/nchs/nhanes/about_nhanes.htm*).

Participants selection and Ethics statement

We performed a secondary analysis based on NHANES data from 2003 to 2016 (7 circles: 03–04, 05–06, 07–08, 09–10, 11–12, 13–14, 15–16). Initially, a total of 71,058 noninstitutionalized civilians during 2003–2016 were involved. Of these 71,058 participants, we excluded participants who did not meet our inclusion criteria: male (n=35,122), age under 18 years (n=14 343, data were unavailable), 59 years old and older (6,752, no records), females without data of HPV testing (n=690), and missing of DCa values (n=676). Finally, a total of 13,475 participants were used for the final data analysis to explore the association of dietary intake and calcium with HPV infection (Figure 1). National Center for Health Statistics (NCHS) approved this observational study, and all participants signed the informed consent form. The Institutional Review Board Approval can be downloaded from CDC official website (*https://www.cdc.gov/nchs/nhanes/about_nhanes.htm*).

Variables involved in the present work

The interested independent variable

In this study, we used the calcium intake in the dietary data as interested independent variable and recorded as a continuous variable. The detailed process of dietary data measurement is described in the NHANES database official website (*https://www.cdc.gov/nchs/nhanes/ContinuousNhanes/Default.aspx*).

The outcome variable

In our present work, we used HPV infection status (dichotomous variable, HPV positive=1; HPV negative=0) and HPV subtype (dichotomous variable, high risk subtype=1; low risk subtype=0) as outcome variables. The test of HPV infection was described in detail in NHANES official website. The subtype of HPV was redefined by the data of HPV DNA typing.

The covariates involved in this secondary analysis

The covariates involved in this study can be summarized into 5 categories, which are: sociodemographic data, dietary data, laboratory data, questionnaire data, and physical examination data. The selection of covariates was mainly based on previous literature, including factors that are known to be associated with HPV infection or DCa intake [5,14–23]. Therefore, following covariates were selected for multivariate model construction:

Continuous variables included: 1) body mass index (BMI, kg/m^2); 2) age (year); 3) frequency of drinking in the past 12 months (time); 4) sex behavior (number of vaginal or anal sex in the past year; number of sexual partners); 5) dietary vitamin B6 intake (mg); 6) folate intake (mcg); 6) vitamin B12 intake (mcg),

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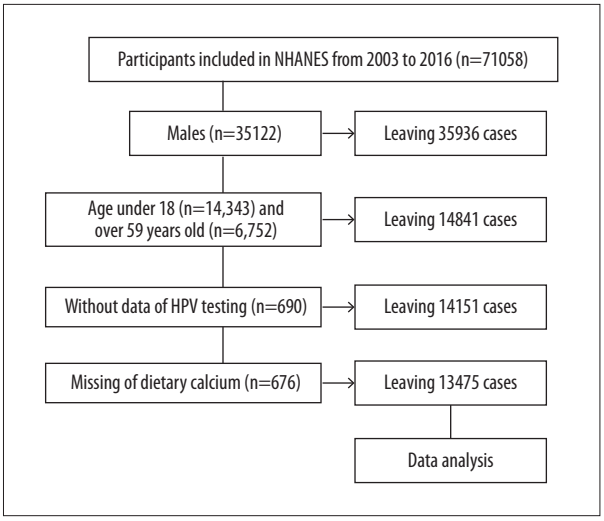

Figure 1. The flow chart is used to illustrate how the object ultimately used for data analysis is selected.

7) vitamin C intake (mg); 8) serum vitamin D level (ng/mL), and 9) albumin-adjusted calcium.

Categorical variables included: 1) history of taking contraceptives (yes/no); 2) female sex hormone user (yes/no); 3) race (Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, Other Race); 4) education level (less than high school diploma, high school, more than high school diploma); 5) marital status (married or living with partner, single); 6) smoking more than 100 cigarettes in life (yes/no); and 7) poverty income ratio (0–4.99/>5).

Statistical analysis

The statistical analyses were guided according to the guideline provided by the CDC official website (*https://www.cdc.gov/nchs/nhanes/tutorials/default.aspx*). Due to the distribution of dietary data were skewed distribution among selected participants, we therefore converted them using the log2 function for subsequent data analysis. To estimate the differences of HPV infection and covariates among different DCa (quartile), weighted chi-square test (categorical variables) or weighted linear regression model (continuous variables) were performed. The entire process of data analyses included 3 aspects with linear association exploration on HPV infection and DCa, nonlinearity addressing and sensitivity analyses. For linear association exploration, weighted binary logistic regression model were used. We built 3 models in this study, they are: non-adjusted model (no covariates were adjusted), minimally-adjusted model (only adjusted sociodemographic data), and fully-adjusted model (all covariates presented in Table 1). Model-based odds ratios (OR) and 95% confidence intervals (CI) were recorded. For nonlinearity addressing, generalized additive model and smooth curve fitting (penalty spline method) were used. If nonlinear relationship between DCa and HPV infection was found, we first used recursive algorithm to get the inflection point, and then established a weighted two-piecewise binary logistic regression model on both sides of inflection point. The purpose of sensitivity analyses in the present work was to ensure the robustness of our findings. Firstly, we converted DCa from continuous variable into categorical variable according to quartile, and calculated *P* for trend. It was aimed to observe whether the trend of nonlinear relationship exists by observation of OR values among different groups. Secondly, in order to ensure that the relationship between all independent variables and the dependent variables in the model is linear. Therefore, we used the generalized additive model (GAM) model to adjust all the continuous variables in the covariate (assuming that these variables are nonlinearly related to HPV infection), and also compared the trends of OR between binary logistic regression and GAM models.

On average, covariates had no record in all covariates (Supplementary Tables 1 and 2). For these missing records of covariates, we used dummy variables to indicate missing covariates values. We assigned missing record for each covariate to 0, and additionally created a dummy variable (0=without missing; 1=missing). Both variables entered in the model at the same time (Y=ax1+b*dummy variable) [24].

All analyses were performed using statistical software R (*http://www.r-project.org*, The R Foundation) and EmpowerStats (*http://www.empower-stats.com*, X&Y Solutions, Inc., Boston, MA, USA). A *P*-value of less than 0.05 (2-sided) was considered statistically significant.

Results

Baseline characteristics of participants

We listed the differences of HPV infection, HPV subtype and covariates among different DCa intake groups (quartile) shown in Table 1. No significant differences were detected on BMI, frequency of sex behavior, alcohol consumption, number of sex partners, and HPV subtype. Compared to the Q1, Q2, and Q3 group participants, the participants in Q4 group was younger, had higher intake of vitamin B6, folate, B12 and C, had higher levels of serum vitamin D and albumin-adjusted calcium, and reported more contraceptive and female hormone users. Most of the participants in the Q4 group were Non-Hispanic White. Besides, fewer single females were observed than those of in the Q1 to Q3 groups. Finally, these participants in the Q4 group showed lower rates of HPV infection than the other groups.

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Table 1. The differences of HPV infection and covariates among different groups of dietary calcium intake.

Quartile	Dietary calcium intake mg/per day log2 transform				*P*-value
	Q1 (3.32–8.91)	Q2 (8.91–9.54)	Q3 (9.54–10.09)	Q4 (10.10–12.64)	
BMI, mean±SD, kg/m^2	28.99±7.46	29.40±7.81	29.06±7.76	29.11±7.85	0.136
Age, mean±SD, year	37.08±12.81	37.34±12.32	37.26±12.19	35.79±11.96	<0.001
Frequency of drinking in the past 12 months, mean±SD, time	3.00±9.13	3.16±9.49	3.30±9.09	3.51±14.66	0.408
Number of vaginal or anal sex in the past year, mean±SD, (times)	1.40±3.14	1.42±4.25	1.32±3.11	1.36±2.42	0.869
Dietary vitamin B6, mean±SD, mg	–0.05±1.01	0.44±0.80	0.67±0.74	1.01±0.71	<0.001
Dietary folate, mean±SD, mcg	6.64±0.99	7.23±0.78	7.48±0.75	7.83±0.71	<0.001
Dietary vitamin B12, mean±SD, mcg	0.60±1.48	1.41±1.06	1.89±0.92	2.44±0.84	<0.001
Dietary vitamin C, mean±SD, mg	4.68±2.07	5.30±1.86	5.65±1.70	6.11±1.68	<0.001
Serum vitamin D level, mean±SD, ng/mL	54.37±26.47	58.02±26.93	61.64±27.05	65.33±26.82	<0.001
Serum albumin-adjusted calcium, mean±SD	9.25±0.33	9.25±0.34	9.25±0.34	9.29±0.35	<0.001
History of taking contraceptives					<0.001
No	32.36%	28.03%	27.61%	27.53%	
Yes	67.64%	71.97%	72.39%	72.47%	
Female sex hormone user					0.041
No	87.45%	88.86%	88.90%	89.86%	
Yes	12.55%	11.14%	11.10%	10.14%	
Race					<0.001
Mexican American	16.69%	18.70%	20.53%	20.46%	
Other Hispanic	8.15%	9.62%	9.18%	8.90%	
Non-Hispanic White	35.47%	36.74%	41.78%	46.86%	
Non-Hispanic Black	30.11%	25.63%	19.61%	16.99%	
Other race/ethnicity	9.58%	9.32%	8.89%	6.79%	
Education					<0.001
<High school	48.28%	44.31%	39.53%	37.32%	
High school	33.77%	33.41%	32.45%	33.88%	
>High school	17.95%	22.28%	28.02%	28.80%	
Marital status					<0.001
Married or living with partner	50.78%	56.80%	59.34%	60.44%	
Single	49.22%	43.20%	40.66%	39.56%	
Smoking more than 100 cigarettes in life					<0.001
No	59.71%	63.76%	65.68%	65.49%	
Yes	40.29%	36.24%	34.32%	34.51%	

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Table 1 continued. The differences of HPV infection and covariates among different groups of dietary calcium intake.

Quartile	Dietary calcium intake mg/per day log2 transform				*P*-value
	Q1 (3.32–8.91)	Q2 (8.91–9.54)	Q3 (9.54–10.09)	Q4 (10.10–12.64)	
Number of vaginal or anal sex in the past year (times)					0.057
1 time	3.67%	2.92%	3.56%	3.46%	
2 times	4.61%	3.50%	4.17%	3.41%	
12–51 times	25.42%	24.32%	21.34%	21.98%	
52–103 times	31.66%	35.59%	34.71%	34.07%	
104–364 times	19.24%	19.36%	20.78%	22.32%	
≥365 times	13.99%	13.31%	14.33%	13.43%	
0 times	1.40%	0.99%	1.12%	1.33%	
Poverty income ratio					<0.001
1	30.18%	26.32%	22.75%	24.94%	
2	25.63%	23.53%	23.23%	25.25%	
3	13.15%	13.67%	14.78%	12.85%	
>3	31.04%	36.47%	39.24%	36.96%	
HPV infection status					<0.001
Negative	51.98%	56.48%	59.52%	58.16%	
Positive	48.02%	43.52%	40.48%	41.84%	
HPV subtype					0.514
Low risk	50.56%	49.38%	49.71%	47.84%	
High risk	49.44%	50.62%	50.29%	52.16%	

BMI – body mass index; HPV – Human papillomavirus. All dietary data were transformed by $\log_2$ function.

Linear association between DCa intake and HPV infection status using univariate and multivariate analyses

Model-based ORs and 95% CIs are listed in Table 2. When no covariates were adjusted, one unit increase of log2 calcium intake was associated with the 11% reductions of risk of HPV infection (OR: 0.89; 95% CI: 0.86–0.92). The same trend was observed on minimally adjusted model (OR: 0.95; 95% CI: 0.91–0.99). However, when we adjusted for all covariates presented in Table 1, the linear associated was not observed (OR: 0.93; 95% CI: 0.82–1.05). For HPV subtype, the DCa intake was not associated with HPV subtype, and supported by all models.

Sensitivity analyses with GAM model and *P* for trend indicated that the findings of fully-adjusted model were robust. Firstly, the OR and confidence interval in the GAM model was almost identical to the fully-adjusted model. Besides, *P* for trend was consistent with DCa when it was used as a continuous variable. However, we also found that the OR values in different calcium intake groups were non-equidistant change (ref in Q1 versus 1.23 in Q2 versus 1.18 in Q3 versus 1.24 in Q4). Therefore, the nonlinearity addressing was needed.

In the GAM model, all covariates which recorded as continuous variables were adjusted as nonlinearity.

Nonlinearity of DCa intake on HPV infection

By generalized additive model and smooth curve fitting (penalty spline method), the saturated effect was found on DCa and HPV infection (Figure 2), the inflection point was 9.78 of log2 calcium intake. At the range of 3.32–9.78 of log2 calcium intake, the log2 calcium intake was negatively associated with HPV infection (OR: 0.83; 95% CI: 0.70–0.98). However, when log2 calcium intake exceed 9.78 (range: 9.78–12.64), the association of them cannot be observed (OR: 1.18; 95% CI:

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Table 2. Linear association of dietary calcium intake and HPV infection by weighted binary logistic regression model.

Exposure	Non-adjusted model OR, 95% CI	Minimally-adjusted model OR, 95% CI	Fully-adjusted model OR, 95% CI	GAM model OR, 95% CI
HPV infection (yes/no)				
Log_2 dietary calcium intake	0.89 (0.86, 0.92)	0.95 (0.91, 0.99)	0.93 (0.82, 1.05)	0.94 (0.83, 1.06)
Q1	Ref	Ref	Ref	Ref
Q2	0.83 (0.76, 0.92)	0.92 (0.83, 1.03)	0.84 (0.65, 1.09)	0.87 (0.67, 1.12)
Q3	0.74 (0.67, 0.81)	0.86 (0.77, 0.96)	0.76 (0.58, 0.99)	0.77 (0.59, 1.01)
Q4	0.78 (0.71, 0.86)	0.89 (0.79, 0.99)	0.88 (0.66, 1.18)	0.88 (0.65, 1.17)
P for trend	<0.0001	0.0186	0.4423	0.3922
HPV subgroup (low and high risk)				
Log_2 dietary calcium intake	1.01 (0.96, 1.06)	1.01 (0.95, 1.07)	1.09 (0.93, 1.28)	1.09 (0.93, 1.28)
Q1	Ref	Ref	Ref	Ref
Q2	1.05 (0.91, 1.21)	1.14 (0.98, 1.34)	1.23 (0.88, 1.72)	1.22 (0.87, 1.71)
Q3	1.03 (0.90, 1.20)	1.11 (0.94, 1.30)	1.18 (0.82, 1.70)	1.18 (0.82, 1.69)
Q4	1.11 (0.97, 1.29)	1.12 (0.95, 1.31)	1.24 (0.84, 1.83)	1.23 (0.84, 1.82)
P for trend	0.1739	0.2478	0.3781	0.3809

Ref – reference; OR – odds ratio; CI – confidence interval. Non-adjusted model – no covariates were adjusted. Minimally adjusted model – only sociodemographic data were adjusted. Fully adjusted model – all covariates were adjusted. GAM model – all covariates which recorded as continuous variables were adjusted as nonlinearity.

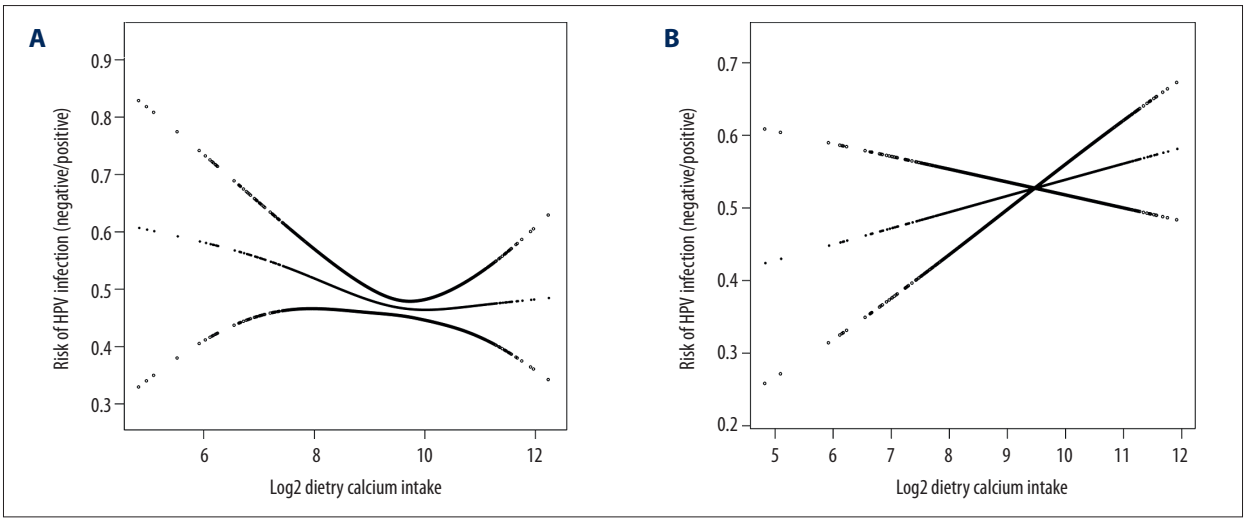

Figure 2. (**A**) The association between dietary calcium intake and human papillomavirus (HPV) infection status is a non-linear relationship. (**B**) There is a non-linear association between dietary calcium intake and HPV infected person type.

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Table 3. Further addressing of nonlinearity between dietary calcium intake and HPV infection status.

Outcome	HPV infection status OR, 95% CI	HPV subtype OR, 95% CI
Fitting by binary logistic regression model	0.93 (0.82, 1.05)	1.09 (0.93, 1.28)
Fitting by weighted two piecewise model		
Inflection point	9.78	10.3
<inflection point	0.83 (0.70, 0.98)	1.07 (0.89, 1.28)
≥inflection point	1.18 (0.91, 1.52)	1.27 (0.70, 2.31)
Log likelihood ratio test	0.037	0.597

HPV – human papillomavirus; OR – odds ratio; CI – confidence interval. The adjusted strategy of covariates was the same as fully-adjusted model.

0.91–1.51). For the result of HPV subtype, we did not find the non-linear linking of DCa and HPV subtype (Table 3).

Discussion

This large-scale and ongoing cross-sectional survey investigated the association of DCa intake with HPV infection status from 2003 to 2016 (7 cycles). Our findings indicated that DCa intake was not independently associated with HPV infection status, including whether HPV duration (negative/positive) or risk type (high or low). However, we found saturated effect on DCa and HPV infection. At the range of 3.32–9.78 of log2 calcium intake, a unit increase of log2 calcium intake was associated with the 17% decrease of HPV infection.

Due to the lack of researches on discovering the association between DCa and HPV infection status, the explanation of our findings from mechanism is needed. Previous studies have indicated that dietary nutrients, especially plant-based food are associated with HPV acquisition and persistence among women, such as folate and vitamins B6 and B12 [5]. These researches attributed the HPV infection-prevented effect of folate, vitamin B6 and B12 to viral integration regulation, DNA synthesis and repair and methylation, antioxidant [5]. Therefore, calcium with the same effect may explain, in part, why calcium is negatively correlated with HPV infection in our results. Michael Fenech, et al. [25] suggested that low DCa intake was associated with higher genome damage rate. Besides, they also pointed out that calcium can restrain cell proliferation, and induces apoptosis and cell differentiation, which were associated with viral integration regulation [25]. The same finding also has been reported by another researcher [26].

Calcium plays an important role in oxidative stress. Previous studies have demonstrated that calcium intake can lead to the modulation of oxidative stress by modulation of calcium transport and signaling lines [27]. Animal experiment with aP2-agouti and wild-type mice show that calcium exerts sustained effects resulting in attenuated adiposity, protection against age-related muscle loss and reduction of oxidative and inflammatory stress in both mouse strains [28]. Another 2 animal studies also suggested that supplemented with calcium can prevent oxidative stress in rats with aldosteronism, and restrict dietary calcium intake can lead to increased oxidative stress and compensatory upregulation of antioxidant enzymes [29,30].

A series of *in vitro* experiments seem to find similar trends: extracellular high calcium can increase HPV virus transcription, which may be one of the factors that cause HPV persistent infection and cancerous (for example: NRIP and calcium/calmodulin activates the phosphatase calcineurin to dephosphorylate E2 and increase E2 protein stability) [6–12]. However, a result from an epidemiological survey is contrary to these *in vitro* studies. Hwang et al. [13] found that calcium (OR, 0.21; 95% CI, 0.08–0.50) was significantly associated with a lower risk of CINII/III. We speculated that the inconsistency between the results of *in vitro* experiments and clinical research may cause by the different research subjects and treatment plans. In addition, these studies focus on the outcome after HPV infection, and we focused on the prevalence of HPV. Therefore, higher-level clinical studies (cohort studies) and mechanism-driven studies are needed to further validate our findings and explore hidden mechanisms in the future.

The clinical value of this study includes: 1) to our best knowledge, it is first time to investigate the association between DCa intake and HPV infection and HPV subtype. This will provide new insights and advice for the development of HPV's etiology, epidemiology prevention, and health policy making, that is, DCa intake is a non-negligible factor for HPV infection. The findings of our present work have identified a novel association between DCa intake and HPV infection. It may

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generate a series of hypotheses that need to be tested systematically by animal models and larger-scale observational studies. These studies should include recommendation of optimal DCa intake, and they make the optimal intake levels for HPV infection prevention can be reliably determined, and the investigation of underlying mechanism.

Our secondary analysis had the following strengths: 1) since the missing data of each covariate is non-randomly missing, we did not use multiple imputation, but used dummy variables to adjust them to minimize the bias caused by missing variables. This method is also used for in a large sample of observational study [24]. 2) The clarification of the nonlinear relationship and the determination of the inflection point have improved the clinical value of our results. In fact, the presence of threshold and saturation effects in most biomedical studies is very common because the human body has its limits and self-regulated instinct. 3) Sensitivity analysis ensures the stability of the results and avoids the contingency in data analysis. 4) Based on a relatively large sample size, we have developed more adequate adjustment strategies, such as folic acid, vitamin B6, B12, and vitamin C, which are recognized as variables associated with HPV infection in previous studies, this adjustment makes our results reliable.

However, the explanation of our findings has some limitations. Firstly, due to NHANES restrictions, we were unable to obtain data on HPV infection in children, so this result could not be used in children. Secondly, this study is limited to American women and therefore it has ethnic and geographical restrictions. Thirdly, the inherent limitations of the cross-sectional study are doomed to the fact that we were unable to obtain an exact causal relationship, resulting in weaker evidence. Fourthly, the variable of "Number of induced abortions" is well-known as a high-risk factor for HPV infection. However, this variable was not included in NHANES database and was not adjusted. Fifthly, the variable of "Number of sex partner" was missing (missing: 7,244, not missing 6,231), but a sensitivity analysis indicated that adjusting or not adjusting this variable has little effect on the association between final dietary calcium and HPV prevalence (see Supplementary Table 3 for details). Finally, in this study, although multiple regression equations were used to adjust for possible potential confounders, adjustments were not possible for those unmeasurable confounders.

Conclusions

In conclusion, we found a saturated effect of DCa intake and HPV infection. In a certain interval (3.32–9.78 of log2 calcium intake), DCa intake was independently and negatively correlated with HPV infection. After this interval, even if the amount of DCa increases, the risk of HPV infection will not decrease further. In addition, DCa intake levels were not associated with HPV subtype of DNA.

Conflict of interest

None.

Supplementary Data

Supplementary Table 1. The description of missing data.

Variables	Without missing*	Missing
Age	13475	0
Race	13475	0
Education level	12758	717
Marital status	13404	71
BMI	13367	108
Poverty income ratio	13190	285
History of takingcontraceptives	12855	620
Female sex hormone user	11546	1929
Smoking more than 100 cigarettes in life	13029	446
Frequency of drinking in the past 12 months	9813	3662
Number of vaginal or anal sex in the past year	7810	5665
Number of sexual partners in the past year	6231	7244
Dietary vitamin B6 (mg) intake	13453	22
Dietary folate intake (mcg)	13452	23
Dietary vitamin B12 intake (mcg)	13413	62
Dietary vitamin C intake (mg)	13398	77
Serum vitamin D level (ng/mL)	11543	1932
Albumin-adjusted calcium	13441	64

* From the description of missing data, the number of missing data were mainly in sex behaviors.

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Supplementary Table 2. Distribution of missing data on HPV infection status of frequency of drinking in past 12 months, number of vaginal or anal sex in past year and number of vaginal or anal sex in past year.

HPV infection	Negative	Positive	P-value
Frequency of drinking in the past 12 months			<0.001
Without missing	5383 (67.43%)	4430 (80.66%)	
Missing record	2600 (32.57%)	1062 (19.34%)	
Number of vaginal or anal sex in the past year			<0.001
1	154 (1.93%)	111 (1.81%)	
2	152 (1.90%)	157 (2.55%)	
12–51	910 (11.40%)	902 (14.67%)	
52–103	1499 (18.78%)	1161 (18.88%)	
104–364	896 (11.22%)	692 (11.26%)	
≥365	558 (6.99%)	525 (8.54%)	
0	43 (0.54%)	50 (0.81%)	
Missing record	3771 (47.24%)	1894 (34.49%)	
Number of sexual partners in the past year (no of sample size)			<0.001
Without missing	3396 (42.54%)	2835 (46.11%)	
Missing record	4587 (57.46%)	2657 (48.38%)	

HPV – human papillomavirus.

Supplementary Table 3. Results of sensitivity analysis with strategy of adjustment.

Outcome	HPV infection status* OR, 95% CI	HPV subtype OR, 95% CI	HPV infection status OR, 95% CI*	HPV subtype OR, 95% CI*
Fitting by binary logistic regression model	0.93 (0.82, 1.05)	1.09 (0.93, 1.28)	0.95 (0.86, 1.05)	1.09 (0.96, 1.24)
Fitting by weighted two piecewise model				
Inflection point	9.78	10.3	10.02	7.9
<Inflection point	0.83 (0.70, 0.98)	1.07 (0.89, 1.28)	0.89 (0.79, 1.00)	0.76 (0.45, 1.29)
≥Inflection point	1.18 (0.91, 1.52)	1.27 (0.70, 2.31)	1.19 (0.93, 1.53)	1.13 (0.99, 1.30)
Log likelihood ratio test	0.037	0.597	0.048	0.155

* Indicated the variable of "number of sexual partners in the past year" was included in model. We can observe that except for HPV genotype changes (because missing data is more obvious), the results of HPV infection status (infection/non-infection) have hardly changed (except for the credibility caused by excessive sample loss beyond widening). HPV – human papillomavirus; OR – odds ratio; CI – confidence interval.

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References:

1. Saslow D, Andrews KS, Manassaram-Baptiste D et al: Human papillomavirus vaccination guideline update: American Cancer Society guideline endorsement. Cancer J Clin, 2016; 66(5): 375–85
2. Rodriguez AM, Do T, Goodman M et al: Human papillomavirus vaccine interventions in the U.S.: A systematic review and meta-analysis. Am J Prev Med, 2019; 56(4): 591–602
3. Das JK, Salam RA, Arshad A et al: Systematic review and meta-analysis of interventions to improve access and coverage of adolescent immunizations. J Adolesc Health, 2016; 59(4S): S40–48
4. Newman PA, Logie CH, Doukas N et al: HPV vaccine acceptability among men: A systematic review and meta-analysis. Sex Transm Infect, 2013; 89(7): 568–74
5. Lopes R, Teixeira JA, Marchioni D et al: Dietary intake of selected nutrients and persistence of HPV infection in men. Int J Cancer, 2017; 141(4): 757–65
6. Tsutsumi K, Iwatake H, Kuwabara D et al: [Effects of calcium on HPV16 gene transcription in cultured laryngeal epithelial cells]. Nihon Jibiinkoka Gakkai Kaiho, 2000; 103(6): 727–33 [in Japanese]
7. Turunen A, Syrjanen S: Extracellular calcium regulates keratinocyte proliferation and HPV 16 E6 RNA expression *in vitro*. APMIS, 2014; 122(9): 781–89
8. Fang NX, Gu W, Ding J et al: Calcium enhances mouse keratinocyte differentiation *in vitro* to differentially regulate expression of papillomavirus authentic and codon modified L1 genes. Virology, 2007; 365(1): 187–97
9. Garrett LR, Coder DM, McDougall JK: Increased intracellular calcium is associated with progression of HPV-18 immortalized human keratinocytes to tumorigenicity. Cell Calcium, 1991; 12(5): 343–49
10. Pei XF, Sherman L, Sun YH et al: HPV-16 E7 protein bypasses keratinocyte growth inhibition by serum and calcium. Carcinogenesis, 1998; 19(8): 1481–86
11. Chang SW, Tsao YP, Lin CY et al: NRIP, a novel calmodulin binding protein, activates calcineurin to dephosphorylate human papillomavirus E2 protein. J Virol, 2011; 85(13): 6750–63
12. Sherman L, Schlegel R: Serum- and calcium-induced differentiation of human keratinocytes is inhibited by the E6 oncoprotein of human papillomavirus type 16. J Virol, 1996; 70(5): 3269–79
13. Hwang JH, Kim MK, Lee JK: Dietary supplements reduce the risk of cervical intraepithelial neoplasia. Int J Gynecol Cancer, 2010; 20(3): 398–403
14. Brouwer AF, Eisenberg MC, Carey TE et al: Multisite HPV infections in the United States (NHANES, 2003–2014): An overview and synthesis. Prev Med, 2019; 123: 288–98
15. Sundaram ME, Mason SM, Basta NE: HPV vaccine uptake among overweight and obese US adolescents: An analysis of the National Health and Nutrition Examination Survey (NHANES) 2009–2014. Vaccine, 2016; 34(22): 2501–6
16. Brouwer AF, Eisenberg MC, Carey TE et al: Trends in HPV cervical and seroprevalence and associations between oral and genital infection and serum antibodies in NHANES, 2003–2012. BMC Infect Dis, 2015; 15: 575
17. Daugherty M, Byler T: Genital wart and HPV prevalence in man in the United States from penile swabs: Results from NHANES. Sex Transm Dis, 2018; 45(6): 412–416
18. Dunne EF, Unger ER, Sternberg M et al: Prevalence of HPV infection among females in the United States. JAMA, 2007; 297(8): 813–19
19. Gillison ML, Broutian T, Pickard RK et al: Prevalence of oral HPV infection in the United States, 2009–2010. JAMA, 2012; 307(7): 693–703
20. D'Souza G, Gross ND, Pai SI et al: Oral human papillomavirus (HPV) infection in HPV-positive patients with oropharyngeal cancer and their partners. J Clin Oncol, 2014; 32(23): 2408–15
21. Kepka D, Coronado G, Rodriguez H et al: Acculturation and HPV infection among Latinas in the United States. Prev Med, 2010; 51(2): 182–84
22. Ortiz AP, Unger ER, Munoz C et al: Cross-sectional study of HPV-16 infection in a population-based subsample of Hispanic adults. BMJ Open, 2014; 4(2): e4203
23. Bhattacharya M, Reiter PL, McRee AL: Nativity status and genital HPV infection among adults in the U.S. Hum Vaccin Immunother, 2019; 15(7–8): 1897–1903
24. Vetter C, Devore EE, Wegrzyn LR et al: Association between rotating night shift work and risk of coronary heart disease among women. JAMA, 2016; 315(16): 1726–34
25. Fenech M, Baghurst P, Luderer W et al: Low intake of calcium, folate, nicotinic acid, vitamin E, retinol, beta-carotene and high intake of pantothenic acid, biotin and riboflavin are significantly associated with increased genome instability – results from a dietary intake and micronucleus index survey in South Australia. Carcinogenesis, 2005; 26(5): 991–99
26. Lamprecht SA, Lipkin M: Chemoprevention of colon cancer by calcium, vitamin D and folate: molecular mechanisms. Nat Rev Cancer, 2003; 3(8): 601–14
27. Ermak G, Davies KJ: Calcium and oxidative stress: From cell signaling to cell death. Mol Immunol, 2002; 38(10): 713–21
28. Bruckbauer A, Zemel MB: Dietary calcium and dairy modulation of oxidative stress and mortality in aP2-agouti and wild-type mice. Nutrients, 2009; 1(1): 50–70
29. Goodwin KD, Sun Y, Weber KT et al: Preventing oxidative stress in rats with aldosteronism by calcitriol and dietary calcium and magnesium supplements. Am J Med Sci, 2006; 332(2): 73–78
30. Itoh M, Oh-Ishi S, Hatao H et al: Effects of dietary calcium restriction and acute exercise on the antioxidant enzyme system and oxidative stress in rat diaphragm. Am J Physiol Regul Integr Comp Physiol, 2004; 287(1): R33–38

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