THE ASSOCIATION BETWEEN DIETARY FOLATE AND VITAMIN B12 INTAKE AND GENITAL HUMAN PAPILLOMAVIRUS (HPV) INFECTION IN MEN

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAIʻI AT MĀNOA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

EPIDEMIOLOGY

APRIL 2019

By

Somsook Santibenchakul

Dissertation Committee:

Alan R. Katz, Chairperson

Eric L. Hurwitz

Brenda Y. Hernandez

Yan Yan Wu

Carol J. Boushey

Yurii B. Shvetsov

Dedication

To my fiancé, Assistant Professor Pitch Sutheerawatthana, PhD, thank you for your love, understanding, and patience during these past years. Your keenness has guided me throughout this journey.

To my mom and dad for your unconditional love. You have inspired me to believe that education will change my entire life.

To the Cole family – Patcharin, John, and Tui – thank you for all your love and support starting from my MPH and all the way through the end of my PhD. A million thanks to you for being my best Hawai‘i family.

To my brothers, Dr. Somtop Santibenchakul, PhD; and Dr. Somruk Santibenchakul, MD, your little sister has followed in your footsteps.

To Dr. Tagayasu Anzai, MD, thank you for being a great friend, from MPH through the end of my PhD. During this long journey, you inspired me to believe in the power of hard work.

To Assistant Professor Thomas Lee, PhD; and Professor Jonna Estudillo, PhD, thank you for sharing your exciting PhD journey and inspiring me to pursue my own.

To Ms. Tani Loo and Mr. Alexander Casey at the Writing Center, Department of English, University of Hawai‘i at Mānoa, thank you so much for your kindness and helping me working on this dissertation. Thanks a million.

To Dr. Stephen Kūhiō Vogeler, PhD, my East-West Center program coordinator, the success I have had today is due in no small part to your support. Big mahalo for all your kind support during my past five years at the East-West Center.

To Professor Khunying Kobchitt Limpaphayom, MD; Associate Professor Unnop Jaisamrarn, MD; and Professor Nimit Techakraichana, MD, thank you for all your kind support and encouragement and for believing in me.

To Associate Professor Damrong Tresukosol, MD, you helped me when I needed help the most. I am forever grateful for your support.

Acknowledgments

I want to express the biggest thanks to my committee members. Thank you for your constant guidance and support. To Professor Katz, MD, thank you for all your kindness and encouragement. During the past decades of being a student, you are the best advisor I have ever known. To Professor Hurwitz, PhD, from my master's degree advisor to my PhD graduate chair in epidemiology, thank you for all your patience, kindness, and support during these past five years. I am more than appreciative. To Assistant Professor Wu, PhD, you are the first Biostatistics teacher who helped me understand the subject – from a medical student who got a D in a statistics class to become a master and doctoral student who got an A for all biostatistics classes. Words can neither qualify nor quantify how helpful your Biostatistics classes have been. I am forever grateful. To Assistant Professor Shvetsov, PhD, thank you for your guidance, support, and encouragement. Without you, I am not able to complete all the sophisticated analyses in this dissertation. All the accomplishments I have achieved, I owe to you. To Associate Professor Hernandez, PhD; and Associate Professor Boushey, PhD. I am grateful for being given the opportunity to work with you and for your professional guidance**.**

To the East-West Center, for awarding me the Graduate Degree Fellowship, the East-West Center Alumni Scholarship, the Royal Sala Thai Scholarship, the Trudy and Al Wong `Ohana Scholarship, and field research funding, you have lightened my financial burden, which allowed me to focus more on my studies. As I promised, I would work hard and try my best to shorten this journey. To the Office of Public Health Studies, for awarding me research funding, I am grateful. To King Chulalongkorn Memorial Hospital Funding, thank you for investment in a student like me. I am grateful. Finally, to the National Institutes of Health grant number

(P20GM103516) for the funding source for the Hawai‘i HPV Male Longitudinal Study. Mahalo for all your support.

Abstract

Human papillomavirus (HPV) infection, which is an established cause of venereal warts and anogenital carcinoma, is one of the most prevalent sexually transmitted infections worldwide. Most HPV infections are asymptomatic, suggesting that other co-factors might play a role in the progression from infections to HPV-related diseases. Nutritional status is known to influence the immune system and alter host defense mechanisms, so it may be a determinant factor of the body's cellular and biological response toward HPV infection. This dissertation presents an analysis which aims to understand the role of dietary intake – as a proxy of nutritional status – and the natural history of HPV infection in men. The first research question focuses on the association between dietary folate and vitamin B12 intake and persistent genotype specific HPV infections. The acquisition and clearance of genotype specific HPV infections and dietary folate and vitamin B12 intake are investigated in the second and third research question. All the analyses accounted for potential confounders such as energy intake, age, and risky sexual behaviors. This dissertation utilized data from the Hawai‘i HPV male longitudinal study, which followed 445 adult men between 2004-2006. Compared to those with the highest intake level, our analyses did not yield strong evidence to support our hypothesis that men who consumed less dietary folate and vitamin B12 were at a higher risk of genotype specific HPV infections. However, we did find that men whose dietary folate and vitamin B12 intake were in the lowest and middle tertiles had higher odds of persistent genotype specific HPVs. Men whose dietary vitamin B12 intake was in the middle tertile had a higher risk of acquiring genotype specific HPVs. During the persistent stage of infection (> 130 days), men whose dietary folate intake was in the lowest tertile

had a lower rate of any risk and oncogenic HPV clearance. Men whose dietary vitamin B12 intake was in the middle tertile had a lower rate of oncogenic HPV clearance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

List of Abbreviations:

95% CI	95% confidence interval
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CMI	Cell-mediated immunity
DFE	Dietary folate equivalents
DNA	Deoxyribonucleic acid
E region	Early region
Kcal	Kilocalorie
HR	Hazard ratio
HBV	Hepatitis B virus
HC II	Hybrid capture II
HCV	Hepatitis C virus
HIM	HPV in Men
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HR-HPV	High-risk human papillomavirus
LCR	Long control region
L region	Late region
LR-HPV	Low-risk human papillomavirus
Min	Minimum
Max	Maximum
MTHFR	Methylenetetrahydrofolate reductase
NCHS	National Health and Nutrition Examination Survey
OR	Odds ratio
ORFs	Open-reading frames
PCR	Polymerase chain reaction
QFFQ	Quantitative food frequency questionnaire
RBC	Red blood cell
SAM	S-adenosylmethionine
SD	Standard deviation
STIs	Sexually transmitted infections
U.S.	United States
WLW	Wei, Lin, and Weissfeld approach
WHO	World Health Organization

Chapter 1

Introduction

Human papillomavirus (HPV) is a non-enveloped, icosahedral capsid, double strand deoxyribonucleic acid (DNA) virus of the Papillomaviridae family [1]. At present, scientists have identified more than 100 HPV genotypes, about 40 of which cause anogenital diseases and subsets of head and neck squamous cell carcinoma [2, 3]. Based on molecular biological data, HPV is further classified into high-risk (HR-HPV), or oncogenic HPV; and low-risk (LR-HPV), or non-oncogenic HPV. Oncogenic HPV infection is an established cause of premalignant lesions, carcinoma of anogenital tracts, and subsets of oropharyngeal and oral cancers, while a non-oncogenic HPV infection is associated with anogenital warts. The following HPV genotypes, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68, are considered high-risk HPVs based on oncogenic potential [1, 3]. All other genotypes are considered non-oncogenic or undetermined risk genotypes. The double stranded circular DNA genome of HPVs encodes about eight open-reading frames (ORFs), which can be divided into three functional parts [1]. First, the early (E) region encodes E1 to E7 proteins, which are required for viral replication. The E6 and E7 proteins of oncogenic HPV genotypes are the most significant HPV proteins in the pathogenesis of HPV-related malignancy. The binding of these proteins with host intracellular proteins, p53 and retinoblastoma proteins, respectively, immortalize epithelial cells and lead to malignant transformation of anogenital cells [4] Second, the late (L) region encodes L1 and L2 proteins, which are structural proteins necessary for virion assembly. Third, the long control region (LCR) contains *cis* elements that are essential for the viral DNA replication and transcription.

According to the World Health Organization (WHO), HPV infection is one of the most common sexually transmitted infections (STIs) globally [1]. In the United States (U.S.), HPV

infection is the most common STI among sexually active adults [5]. The prevalence of the HPV infection in males is higher than females [6, 7]. As demonstrated by the National Health and Nutrition Examination Survey (NHANES) during 2013-2014, the prevalence of genital HPV infection among adult males and females aged 18-59 were 45.2% and 39.9%, respectively [7]. Among this population, the prevalence of oncogenic HPV were 25.1% and 20.4% for adult males and females, respectively [7]. Despite the higher prevalence of oncogenic HPV infection in males, the impact of oncogenic HPV as a causal factor in cervical cancer in females has received greater attention historically. Globally, HPV infection causes 528,000 cases of cervical cancer, which is the fourth most common cancer in women, and there are 266,000 cervical cancer deaths each year [8]. Worldwide, the incidence of penile cancer varies according to geographic regions. Compared to Africa, Southeast Asia, and Latin America, penile cancer incidence is lower in the U.S., Europe, and other Western countries. Cancer of the penis accounts for less than 1% of male cancer in the U.S. while it accounts for 10% of male cancer in certain geographic areas [9, 10]. Compared to cervical carcinoma, incidence rates of anal and penile cancers are very low [11]. HPV is also a causal agent in subsets of oropharyngeal and oral cancers, primarily involving the tonsils and base of tongue [12]. In the U.S. and Europe, HPV accounts for 70%-80% of oropharyngeal cancers, and the incidence is rising in the U.S. [13, 14]. While this dissertation focuses mainly on HPV-related anogenital diseases, our results might have an implication on future studies on HPV-related oropharyngeal and oral cancers.

Most HPV infections are asymptomatic and become undetectable over time. Within the first year, 70% of incident infections are resolved, and 90% of incident infections are resolved in the second year [15]. Although the majority of HPV infections resolve spontaneously, persistent infections are associated with detrimental diseases. Persistent infection with oncogenic HPV is the

established cause of anogenital carcinoma. Infections with oncogenic HPVs account for 85% and 50% of anal and penile carcinoma in males [11]. HPVs are detected in 87% of cases of penile high-grade squamous intraepithelial lesions [11]. Worldwide, approximately 45% of penile carcinoma is associated with HPVs [16]. The prevalence of HPVs among invasive penile cancer cases is as high as 63% in the U.S. [17]. Different sensitivity of HPV detecting tests is one possible explanation why the prevalence in U.S. is higher than other countries. HPV types 16 and 18 account for 35% to 40% of overall penile carcinoma and account for 70% to 80% of all HPV positive penile carcinoma [18]. The latter group occurs at a younger age and is associated with sexual risk habits [19]. In some case series, HPV positive penile carcinoma is associated with a better survival rate [20, 21]. Non-oncogenic HPV infection, primarily HPV 6 and 11, can cause highly contagious anogenital warts, wherein about 65% of patients' sexual partners become anogenital wart cases within three weeks to eight months [22, 23]. Anogenital warts are also associated with a very high recurrence rate. Despite receiving appropriate treatment, most warts will recur within the first three months of infection [24]. Venereal warts increase psychosocial concerns for both patients and their sexual partners; however, these anogenital lesions rarely have malignant transformation.

Most genital HPV infections are asymptomatic and do not require treatment. Removal of anogenital warts and amelioration of symptoms are recommended [25]. A number of treatment modalities are available for premalignant penile lesions and penile cancers depending on type, site, and stage of disease [10, 26]. To prevent HPV infection, sexual abstinence is considered the single most effective strategy. Condoms can lower the risk of contracting a genital HPV infection. However, HPV is transmitted by skin to skin contact, so the uncovered genital area may still be prone to infection [5]. The Centers for Disease Control and Prevention (CDC) recommends routine

HPV vaccine for all girls and women ages 9-11 years through age 26 years; meanwhile, it is recommended for boys and men ages 9-11 years through age 21 years, and men who have sex with men through age 26 years [27]. To maximize the benefits of HPV immunization, the HPV vaccine should be administered prior to becoming sexually active. Three different vaccines, which target varying HPV genotypes, are currently available in different geographic locations. Bivalent vaccine targets HPV types 16 and 18; quadrivalent HPV vaccine targets HPV types 6, 11, 16, and 18; 9-valent vaccine targets HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58. Since 2017, only the 9-valent vaccine is available in the U.S. [28]. Though excellent seroconversion rates have been reported following immunization with HPV vaccine, HPV vaccination can protect against only vaccine covered HPV types [29].

To further lower the burden of HPV-related diseases, it is necessary to provide additional effective prevention strategies focusing on modifying cofactors in the natural history of HPV infection. Most infections with HPV are asymptomatic, which suggest that particular cofactors might play an important role in the progression from infections to HPV-related diseases. Two significant co-factors associated with the pathogenesis of HPV-related diseases have been identified. Viral determinants including HPV genotypes, viral load, and its genetic variants are the first proposed determinant in the pathogenesis of HPV-related conditions [30]. Individual factors that affect the human immune system are the second proposed determinant as immunosuppressive individuals are at greater risk of having anogenital warts and HPV-related malignancies [31]. Nutritional status, a known fundamental determinant of human immune systems, might be a critical variable that is often overlooked by researchers regarding its role in modifying the risk of HPV infection. Due to the established link between HPV infections and cervical carcinoma, most efforts regarding the understanding of the natural history of HPV infections have focused on

females, though males play a major role in HPV infections of females, as HPV is a sexually transmitted infection. HPV transmission was evaluated in a study of 25 monogamous heterosexual couples, which included males from the current cohort. During 7.5 months of follow-up, the overall rate of HPV transmissions from the penis to the cervix was 4.9/100 person-months, which was lower than the rate of cervix-to-penile transmission [32].

Scant epidemiologic studies on the natural history of HPV infection have been conducted to investigate the role of specific dietary intake in HPV infection [33-35]. Some of these published studies demonstrated the protective role of consuming certain kinds of dietary micronutrients, such as folate and vitamin B12 [34, 35]. The following putative mechanisms, with regards to the role of folate and vitamin B12 in modifying body immune response toward HPV infection, have been proposed. Folate and vitamin B12 can alter the risks of HPV infection through several metabolic pathways involving either DNA synthesis or methylation of DNA [36, 37]. The enzyme 5-,10-methylenetetrahydrofolate reductase (MTHFR) plays a major role in these biochemical pathways. This key enzyme converts folate in the form of 5-,10- methylenetetrahydrofolate to 5-methytetrahydrofolate. The 5-,10- methylenetetrahydrofolate is essential in the biosynthesis of the thymidylate, a nucleotide needed for DNA synthesis [36]. A low level of folate, resulting in a reduced level of the 5-,10- methylenetetrahydrofolate, leads to the misincorporation of uracil into DNA and the breaking of chromosomes [36]. These common chromosome fragility sites correspond with the site that HPV types 16 and 18 integrate into host DNA [38, 39]. Folate and vitamin B12 are also involved in the methionine synthesis pathway which is another significant pathway associated with DNA methylation [40]. Vitamin B12 is required in the synthesis of methionine, a precursor of a universal methyl donor, namely S-adenosylmethionine (SAM) [40]. Homocysteine, a by-product of the methionine synthesis pathway, is subsequently eliminated by

remethylation and transulfuration [41]. The remethylation process requires both folate and vitamin B12 as a substrate and a cofactor, respectively [41]. The remethylation pathway also requires 5-methyltetrahydrofolate to serve the methyl group to change homocysteine into methionine [41]. Therefore, a low level of folate and vitamin B12 may affect HPV infection by either involving DNA methylation or increasing homocysteine levels which have been shown to be associated with persistent HPV infections in clinical studies [42, 43].

Research questions

The aims of this dissertation are: (1) To estimate the association (odds ratio) of dietary folate and vitamin B12 intake with persistent genotype specific HPV infections; (2) To estimate the association (hazard ratio) of dietary folate and vitamin B12 intake with incident genotype specific HPV infections; and (3) To estimate the association (hazard ratio) of dietary folate and vitamin B12 intake with clearance of incident genotype specific HPV infections.

Hypotheses

Paper 1: Men with lower levels of dietary folate and vitamin B12 intake will have higher odds of persistent genotype specific HPV infections.

Paper 2: Men with lower levels of dietary folate and vitamin B12 intake will be at a higher risk of incident genotype specific HPV infections.

Paper 3: Men with lower levels of dietary folate and vitamin B12 intake will have a lower rate of clearance of incident genotype specific HPV infections.

Database

All of the analyses in this dissertation used data derived from the Hawai‘i HPV male longitudinal study, which was approved by the Committee on Human Studies of the University of

Hawai‘i (Appendix, IRB Approval) [32, 44-46]. Research subjects were primarily recruited from a university-based population in Hawai‘i through campus flyers and newspaper advertisements. The invitations were also sent to the emails of enrolled undergraduate and graduate male students. Eligible subjects were English speaking men whose age was at least 18 years and who had no history of bleeding disorders. This criterion was required because blood was collected for serologic evaluation, which is not a part of the current analyses. All study subjects provided written informed consent (Appendix, Informed Consent). Between July 2004 and December 2006, 445 adult men were recruited, and the follow-up visits were conducted at intervals of approximately 2 months for up to 20 visits. Study visits were conducted at the University Health Services of the University of Hawai‘i and the Cancer Research Center of Hawai‘i. At enrollment, a trained interviewer conducted a structured survey of study participants covering sociodemographic, medical, sexual, and reproductive histories. During each subsequent visit, information was updated regarding smoking, alcohol use, medical, sexual and reproductive histories during the intervening period between study visits.

HPV specimen collection

Prior to initiation of the study, university-based clinicians were specifically trained to collect genital HPV specimens. A training video was used throughout the study period to ensure the standardization and uniformity of specimen collections. At each study visit, exfoliated cell samples for HPV DNA detection were obtained by clinicians using textured paper and a saline moistened swab. Separate specimens were collected from the glans penis/corona sulcus, penile shaft, and scrotum. Among uncircumcised men, an additional specimen was collected from the inner foreskin. Between each sampling site, clinicians' disposable gloves were changed to avoid

the risk of contamination between sites. Visible warts and lesions were physically avoided in sampling the genitals.

HPV DNA testing and genotyping

DNA was extracted from specimens by using commercial reagents (QIAGEN, Valencia, CA, USA). The polymerase chain reaction (PCR) used PGMY09/PGMY11 primers to amplify a 450-bp region of the L1 HPV genome [47]. HPV positive specimens were subsequently genotyped using a reverse line blot detection method for 37 different HPV types [47]. HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 were considered oncogenic genotypes [1]. All other genotypes were considered non-oncogenic/undetermined risk genotypes. HPV positive specimens that were subsequently found to be negative in the genotyping assay were considered to be unclassified HPV positive specimens. This PCR-based assay has demonstrated a high degree of sensitivity and reproducibility [48-50]. As an internal control, all specimens were tested for sample sufficiency by using GH20 and PC04 primers to amplify a 268-bp region of the human-globin gene, from which insufficient specimens were excluded from analyses.

Dietary intake assessment using a food frequency questionnaire

Dietary intake was determined by a structured interview using a quantitative food frequency questionnaire (QFFQ), which was designed to assess typical food intake of populations in Hawai‘i. The questionnaire included eight frequency categories which ranged from “never or hardly ever eat” to “two or more times a day.” Three choices of portion size (with a few food items listed to have four choices of portion size) were provided in the questionnaire. Pictures of selected foods representing portion sizes were added at the top of several pages of the questionnaire to aid the participants in choosing their portion size. For alcohol and other beverages, the questionnaire

included nine frequency categories which ranged from “never to hardly ever drink” to “drinking four or more times a day.” For vitamin and mineral supplements, the questionnaire included six frequency categories which ranged from “never use” to “three or more times a day.” Three choices of duration were provided which ranged from “one year or less” to “five years or more,” and dose per tablet of each supplement was inquired.

The development of the quantitative food frequency questionnaire used in this study

The QFFQ was primarily developed as a dietary assessment tool for use in the Multiethnic cohort study in Hawai‘i and Los Angeles [51]. Briefly, sixty men and women aged between 40-75 years from five principle ethnic groups, including African-American, Japanese-American, Latino, Native Hawaiian, and White, provided 3-day measured food records. These records were used to identify food items included in the questionnaire. The contribution of each food item to the total intake of the major nutrients of interest — such as fat, dietary fiber, vitamin A, carotenoids, and vitamin C — was computed for each ethnic group. For the other nutrients, food items were ordered from highest to lowest dietary contributions. The minimum set of food items that made up at least 85% of the intake was chosen. Specific food items of traditional diets, such as tofu and salted fish consumed by Japanese-Americans, were also identified from food records and included in the questionnaire regardless of contribution to the intake. Similar foods with comparable nutrient composition were grouped into food items. The food items listed in the final questionnaire accounted for more than 85% of the intake of major nutrients. The QFFQ’s performance was assessed in a calibration study that compared diet reported from the QFFQ with three 24-hour dietary recalls. Estimates of the correlation between the QFFQ and 24-hour recalls after energy adjustment ranged from 0.57–0.74 for nutrient densities and from 0.55–0.74 for energy-adjusted nutrients [52].

Chapter 2

Examining the Relationship between Dietary Folate and Vitamin B12 Intake and Persistent Genotype Specific HPV Infections

Introduction

Persistence of HPV infection is an established cause of both anogenital warts and malignancies. In the United States, 2.2% of men reported a history of genital warts [53]. Each year, in the U.S., the estimated number of HPV-related penile cancer cases is 800, and the estimated number of HPV-related anal cancer cases in men is 1,900 [54]. Worldwide, the annual incidence of anogenital warts is 137 per 100,000 men, while the annual incidence of recurrent anogenital warts is as high as 163 per 100,000 men [55]. Among men, the overall prevalence of anogenital warts ranges from 0.16% to 0.20%. Considering prospectively collected physician reports, the prevalence of anogenital wart is as high as 4.1% to 5.1% [55]. Globally, the annual incident cancer cases attributable to HPV among men are 66,000 of which 13,000 are penile cancer and 17,000 are anal cancer [56]. Theoretically, as high as 70% to 90% of all HPV attributable anogenital diseases may be prevented by universal HPV vaccination. However, achieving high coverage of HPV vaccination is not easily afforded in resource-limited settings. To deal with the burden of HPV-related diseases, the role of nutrition as a risk reduction strategy to prevent persistent HPV infection could be another interesting approach.

Nutritional status, a known elemental determinant of the immune system, might be a significant co-factor in the pathogenesis of HPV-related diseases. Theoretically, folate and vitamin B12 function in a key role of one-carbon metabolism which is crucial in DNA methylation and gene expression [57]. DNA hypomethylation is an important epigenetic event in cervical

carcinogenesis with which an increasing degree of DNA hypomethylation is associated with higher grade of cervical intraepithelial neoplasm [58]. The degree of DNA hypomethylation in biopsied cervical tissue is significantly related to folate concentrations in both serum and cervical tissue [59]. To date, there is limited evidence regarding the role of dietary folate and vitamin B12 intake on persistence of HPV infections. To the best of our knowledge, only three articles focusing on the role of dietary intake and persistent genital HPV infection have been published since 2002. Regarding the association between dietary folate and vitamin B12 intake and persistent genital HPV infections, these articles demonstrated inconsistent results. The details of each article were summarized in the appendices (Appendix, Literature Review Chapter 2, Table 1).

Methods

Assessment of HPV status

HPV positive was defined by a positive HPV test for at least one of the following genital specimens: glans penis, coronal sulcus, foreskin, shaft, or scrotum. Men with persistent genotype specific HPV infections were defined as men who were HPV positive for at least two consecutive visits with the same classified HPV genotype. Men with transient HPV infection were defined as men who were HPV positive in only one of the evaluations or non-consecutively positive for the same classified HPV genotype.

Eligible participants (n=218) were classified into one of the following groups: (1) case group or persistent genotype specific HPV infection group (n=142) or (2) control group or transient HPV infection group (n=76). Men who had a persistent genotype specific HPV infection, despite concurrently having had a transient HPV infection of another HPV genotype were still classified

into the persistent group. Figure 2.2 summarizes the flow diagram classifying men into either case or control status.

Assessment of dietary intakes

Using the validated QFFQ, Dietary folate [μg dietary folate equivalents (DFE) per day], vitamin B12 (μg per day), and energy [kilocalorie (kcal) per day] intakes derived from the baseline interview were used in this analysis. All dietary intakes were presented as nutrient densities, which are the measures of dietary composition computed by dividing nutrient values by total energy intake and expressed as intake per 1,000 kcal [60]. Dietary folate (μg DFE per 1,000 kcal per day) and vitamin B12 (μg per 1,000 kcal per day) intakes were categorized according to the tertile cut points of men in the control group – transient HPV infection group [34].

For all the analyses in this dissertation, the intake of folate was estimated as DFE. Most nutrition labels do not use DFE, which takes into account the different bioavailabilities of folate sources. One DFE is equivalent to 1 μg of food folate, or 0.6 μg of folic acid from fortified food or as a supplement. The DFE reported in this analysis did not account for the supplement being taken with food or on an empty stomach as this level of detail was not collected.

Measure of potential confounders

Selected covariates derived from the baseline interview regarding sociodemographic, reproductive, medical, and sexual history were evaluated and used as potential confounders in the multivariable analysis. These potential confounders, which were determined by the earlier published studies of this cohort and the literature regarding HPV infection, include age, race/ethnicity, birthplace, marital status, education attainment, income level, smoking habits, alcohol drinking habits, age at first sexual intercourse with a female, total number of lifetime

female sexual partners, sexual preferences, condom use, a history of genital warts, a history of STIs and circumcision status [11, 32, 44, 46, 61-64]. Number of follow-up visits was included as a potential confounder in multivariable analysis, as this covariate might contribute to the assessment of persistent genotype specific HPV infection status. Smoking habits were self-reported and were categorized as either "ever smoke" or "never smoke." "Ever smoker" was defined as "ever smoked cigarettes daily for at least 6 months or more." The remaining participants were defined as "never smoker." Alcohol use was self-reported and separated by current alcohol drinking status as "yes" or "no." Current alcohol drinking was defined as having drank alcohol at least once a week during the past 4 months.

Statistical analysis

Statistical analysis was conducted using SAS software version 9.4 (SAS Institute, Cary, NC). For this study, individual participant was used as a unit of analysis in a nested case-control study. Men with persistent genotype specific HPV infections were defined as the case group, while men with transient HPV infection were defined as the control group. Baseline sociodemographic, reproductive, medical, and sexual history were explored according to HPV status. Proportion, Chi-square, and Fisher Exact statistics were used to compare the differences between groups. The distribution of median level of dietary folate and vitamin B12 intake according to HPV status were evaluated. To test the hypothesis of whether men with lower levels of dietary folate and vitamin B12 intake have higher odds of persistent genotype specific HPV infections, we conducted two separate multivariable logistic regression models, as dietary folate and vitamin B12 intake are highly correlated. Selected potential confounders, which were determined by the earlier published studies of this cohort and the literature review regarding HPV infection, were evaluated. The purposeful selection algorithm was used for variable selection [65]. The p-value cut-off point of

0.25 was defined as the significance level to include the variable as a candidate for multivariable analysis. In the process of variable selection of multivariable analysis, the covariates were removed from the model if they were non-significant or were ascertained not to be a confounder. The p-value cut-off point of 0.1 was defined as the significance level, and confounders were defined as variables that made a change in any remaining parameter estimate greater than 15% as compared to the full model. At this point, the model contained significant covariates and confounders. Any variable not selected for the original multivariable model was then added back into the multivariable model one at a time. Any variables that were significant (p-value < 0.1) were put in the model, and the model was iteratively reduced, as before. This iterative process involved only the variables that were additionally added.

Results

This first study focused on persistent genotype specific HPV infections. Among 445 adult men in the Hawai‘i HPV male cohort, we included only men who had at least one HPV infection during the study period. Fifty-four men whose HPV tests were negative for all study visits were excluded. To assess the persistence of HPV infection status, we included only men who had at least two follow-up visits. Seventy-six men were excluded because they had only one visit. Seven men who did not complete the QFFQ were excluded. Twelve men who both had only one visit and did not complete the QFFQ were excluded. Two men who reported consuming more than 10,000 kcal of energy intake per day were excluded. Due to the strong association of human immunodeficiency virus infection (HIV) with HPV infection and HPV-related neoplasia, thirty men with self-reported HIV infection status were excluded [66]. We further excluded forty-six men who did not have classified HPV genotype but who had only two consecutive positive visits for unclassified HPV (referred to as “XX”), as this group of men could not be defined as either

case or control for this analysis. A total of 218 men were eligible for this first research question. Figure 2.1 summarizes the flow diagram of included and excluded participants.

Of the 218 men who were included in this study, a total of 142 men were classified into persistent genotype specific HPVs group (cases), while 76 men were classified into transient or non-persistent genotype specific HPVs group (controls). The basic demographics and lifestyle variables of the study population by HPV status are shown in Table 2.1. Men in the case group were older than those in the control group with a mean age (standard deviation, [SD]) of 29.1 (11.9) years and 26.5 (10.0) years, respectively. The number of visits among the cases was greater than the controls with a median (range) of 7 (2-19) and 5 (2-19), respectively. Men in the case group had a greater number of sexual partners compared to men in the control group of with a median (range) of 10 (1-1,000) and 6 (1-300), respectively. Fig 2.3 and 2.4 show the distribution of dietary folate and vitamin B12 intakes by HPV status. The median intake of dietary folate equivalents (µg DFE per 1,000 kcal per day) among the cases was marginally lower than the controls, of which the median intakes (min-max) were 270.58 (101.63-944.82) and 272.24 (133.10-594.72), respectively. The median intake of dietary vitamin B12 (µg per 1,000 kcal per day) among the cases was marginally lower than the controls with the median intakes (min-max) of 2.26 (0.39-6.12) and 2.34 (0.74-6.27), respectively. Table 2.2 shows the distribution of study participants according to their tertile values of dietary folate and vitamin B12 intakes by HPV status. Among cases, the lowest proportion of men (28.9%) had dietary folate intake in the lowest tertile compared to the middle (35.9%) and highest (35.2%) tertiles. The lowest proportion of men (30.3%) had dietary vitamin B12 intake in the highest tertile compared to the middle (35.9%) and lowest (33.8%) tertiles. However, the Chi-square tests show that neither dietary folate (p-value <

0.72) nor vitamin B12 (p-value < 0.77) intakes are associated with persistent genotype specific HPV infections.

Table 2.3 displays the results of logistic regression analysis for nutrients variables associated with persistent genotype specific HPV infections which include men who had the highest tertile of nutrients intakes as a reference group. Univariate analysis shows that men whose dietary folate intake were in the lowest tertile had lower odds of persistent genotype specific HPV infections: the odds ratio (OR) = 0.79 (95% confidence interval [CI] 0.45-1.57). Men whose dietary folate intake were in the middle tertile had higher odds of persistent genotype specific HPV infections: the OR = 1.02 (95% CI 0.52-2.01). Regarding dietary vitamin B12 intake, univariate analysis shows that men in the lowest and middle tertiles had higher odds of persistent genotype specific HPV infections: the OR = 1.12 (95% CI 0.57-2.21) for the lowest tertile and the OR = 1.29 (95% CI 0.65-2.56) for the middle tertile, respectively. Multivariable analysis, adjusted for age, number of visits, education, and number of lifetime female sexual partners, shows that men whose dietary folate and vitamin B12 intakes were in the lowest and middle tertiles had higher odds of persistent genotype specific HPV infections: the OR = 1.21 (95% CI 0.54-2.68) for men whose dietary folate intake were in the lowest tertile, the OR = 1.55 (95% CI 0.70-3.42) for men whose dietary folate intake were in the middle tertile, the OR = 1.01 (95% CI 0.46-2.18) for men whose dietary vitamin B12 intake were in the lowest tertile, and the OR = 1.40 (95% CI 0.64-3.06) for men whose dietary vitamin B12 intake were in the middle tertile.

Table 2.4 shows the results of logistic regression analysis for potential confounders initially identified as associated with persistent genotype specific HPV infections. Table 2.5 and 2.6 show final logistic regression models of the association between dietary folate and vitamin B12 intake and persistent genotype specific HPV infection. The number of visits, education, and number of

lifetime female sexual partners reached statistically significant differences in univariate analysis and remained significant in the multivariable analysis. Table 2.6 shows that compared to men who had less than five follow-up visits, men who had at least five follow-up visits had higher odds of persistent genotype specific HPV infections: the OR = 2.95 (95% CI 1.51-5.79). Compared to men who had graduate or professional degree, those who had no or some college degree had higher odds of persistent genotype specific HPV infections: the OR = 6.84 (95% CI 2.38-19.64). Men who had college degree had higher odds of persistent genotype specific HPV infections: the OR = 16.29 (95% CI 4.38-60.61). Compared to men who had zero to one partner, men who had two to ten partners had higher odds of persistent genotype specific HPV infection: the OR = 3.91 (95% CI 1.34-11.46). Men who had more than ten partners had higher odds of persistent genotype specific HPV infection: the OR = 5.48 (95% CI 1.75-17.19).

Discussion

The median intakes of dietary folate and vitamin B12 among men with persistent genotype specific HPV infections (cases) were marginally lower than men with transient HPV infection (controls). The univariate analysis of the association between dietary folate intake and persistent genotype specific HPV infections does not support our hypothesis that men with lower levels of dietary folate intake are at higher odds of persistent genotype specific HPV infections. After adjustment for potential confounders, men whose dietary folate intake was in the middle and lowest tertiles had higher odds of persistent genotype specific HPV infections. The number of visits, education, and number of lifetime female sexual partners were independently associated in multivariable analysis. Among these three variables, number of lifetime female sexual partners was the most significant confounder. Compared to the crude ORs, the adjusted OR of men in the

lowest tertile changed 21%, and the adjusted OR of men in the middle tertile changed 34.6% after adjustment for number of female lifetime sexual partners.

Our results are somewhat consistent with the recent publication by Lopez et al. in 2017 [34]. This study found an inverse association between dietary vitamin B12 intake and persistent genotype specific HPV infections. Compared to the lowest quartile, the adjusted ORs of the other quartiles were lower than one, which suggested that men whose dietary vitamin B12 intake was higher had lower odds of persistent genotype specific HPV infections: regarding oncogenic HPVs, for the second quartile: the OR = 0.94 (95% CI 0.65-1.37), for the third quartile: the OR = 0.72 (95% CI 0.47-1.09), and for the highest quartile: the OR = 0.98 (95% CI 0.61-1.58); regarding non-oncogenic HPV infections, for the second quartile: the OR = 0.77 (95% CI 0.54-1.10), for the third quartile the OR = 0.70 (95% CI 0.49-1.02), and for the highest quartile: the OR = 0.55 (95% CI 0.38-0.81). The association between dietary folate intake and persistent genotype specific HPV infections in this study was inconsistent among each quartile: regarding oncogenic HPV infections, for the second quartile: the OR = 0.96 (95% CI0.64-1.43), for the third quartile: the OR = 1.24 (95% CI 0.80-1.92), and for the highest quartile: the OR = 0.95 (95% CI 0.54-1.69). The multivariable analysis of this study adjusted for the following confounders: age, number of lifetime female sexual partners, smoking habits, and marital status. We did additional analysis by using the same set of potential confounders as this study did and found consistent results. The study published by Sedjo et al. in 2002 found that women whose dietary folate was in the middle and highest tertiles had lower odds of persistent oncogenic HPV infections: the OR = 0.63 (95% CI0.29-1.38) for the middle tertile and the OR = 0.52 (95% CI 0.23-1.18) for the highest tertile [35]. These results are consistent with our findings. Regarding dietary vitamin B12, the results of Sedjo et al.'s study were inconsistent among each tertile of dietary vitamin B12 intake: the OR =

1.47 (95% CI0.68-3.19) for the middle tertile and the OR = 0.68 95% CI (0.30-1.54) for the highest tertile. These results are inconsistent with our findings. None of the results in Sedjo et al.'s study reached a statistically significant difference. The methodology of this study is quite different from ours, as this study does not focus on genotype specific HPVs, which might introduce misclassification bias in detecting persistent HPV infections.

Regarding other potential confounders, number of visits, education, and number of lifetime female sexual partners were associated with persistent genotype specific HPVs. The greater number of visits the participant had, the more likely that he would test positively for HPVs. This may be explained by the greater sensitivity of HPV tests when tested frequently. Another possible explanation is that men who had a first positive HPV test might have been more likely to return for another HPV test. Our results show that graduate or professional degree is associated with the lowest risk of persistent genotype specific HPVs. This association existed even after adjusted for risky sexual behaviors – number of lifetime female sexual partners, sexual preference, age at first sexual intercourse with female, and condom use within the past four months. We did not see the effect of risky behaviors on the association between education and persistent genotype specific HPV infections. This might be explained by the changing of risky sexual behaviors over time in which our analyses did not account for. A greater number of lifetime female sexual partners was associated with persistent genotype specific HPV infections in our study. This finding was consistent with a publication by Kjaer et al. in 2011 that the number of lifetime female sexual partners is a significant predictor of HPV infections among men [67].

The strength of this study is that we focus on persistent genotype specific HPV infections. This approach mitigates misclassification bias of defining persistent HPV infection by implementing genotype specific information. We did the nested case-control design which

provided us with less information bias of defining both exposures (dietary intakes) and outcome (persistent genotype specific HPV infections). The following limitations of this study should be considered when interpreting the results. First, we have a limited sample size, and this precludes additional analysis focused on individual HPV genotypes. Additionally, all the analyses do not provide enough evidence to support our hypothesis, as all 95% CIs covered one. Second, we decided to define two positive consecutive visits for the same HPV genotypes as persistent genotype specific HPV infections. In other words, the duration of persistent HPV infection was defined as approximately two months. This might not represent the true duration of a persistent infection for particular HPV genotypes such as HPV 16, for which the median duration of infection is as long as twelve months [61]. Third, our results do not demonstrate a dose response relationship [68]. The ORs of men whose dietary intakes were in the middle tertile were higher than the ORs of men whose dietary intakes were in the lowest tertile.

Conclusion

The analysis of our first research question did not provide enough evidence to support our hypothesis that men with lower dietary folate and vitamin B12 are at lower odds of persistent genotype specific HPV infections.

Figure 2. 1 Study flow chart of included and excluded participants

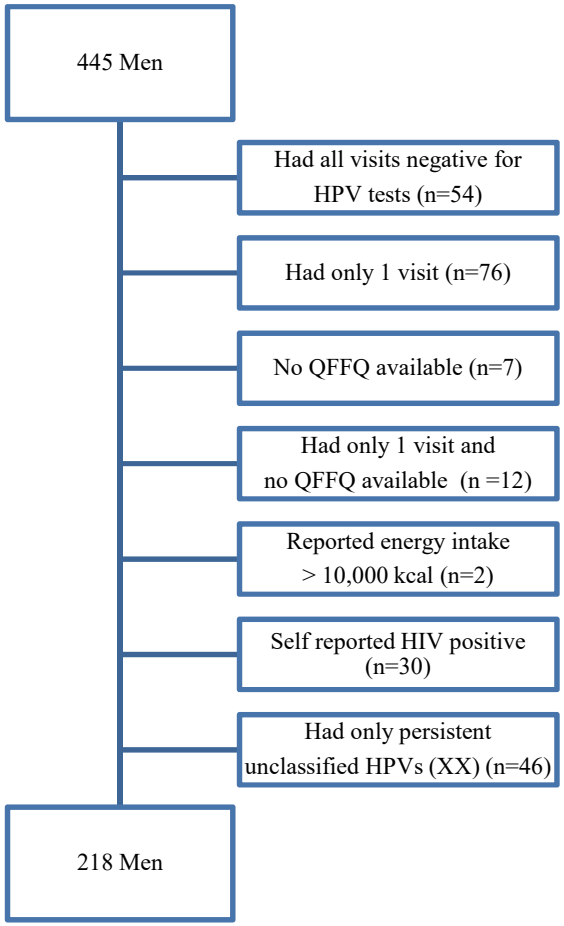


Figure 2. 2 Flow diagram classifying men into either case or control status

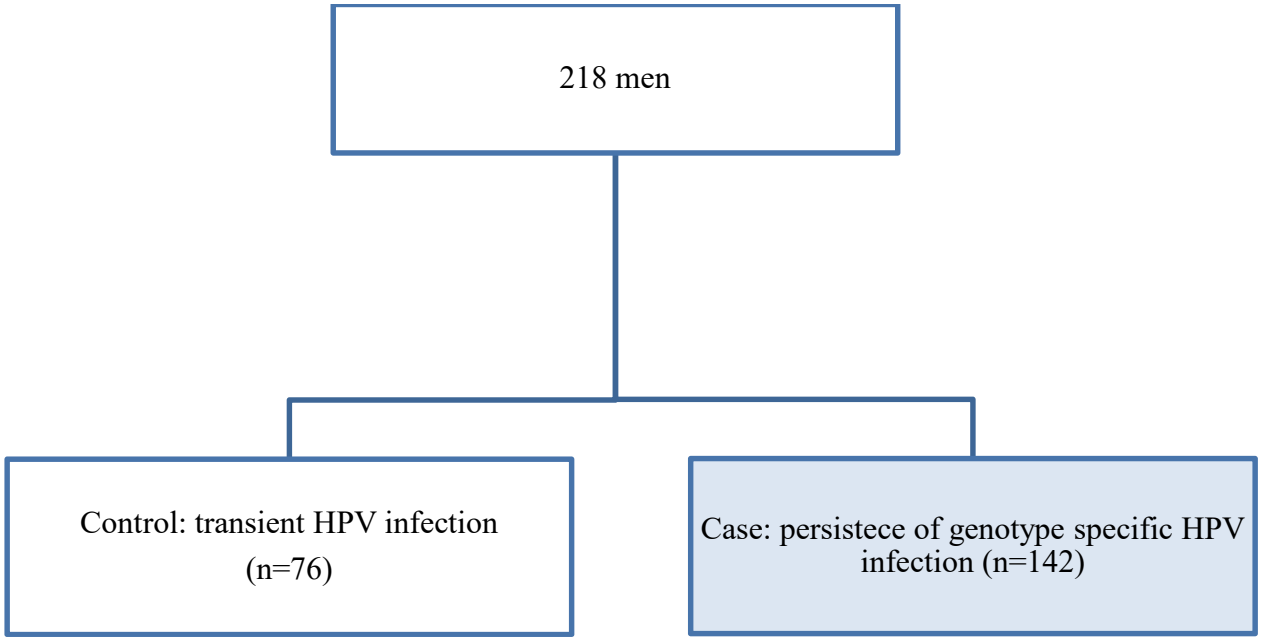
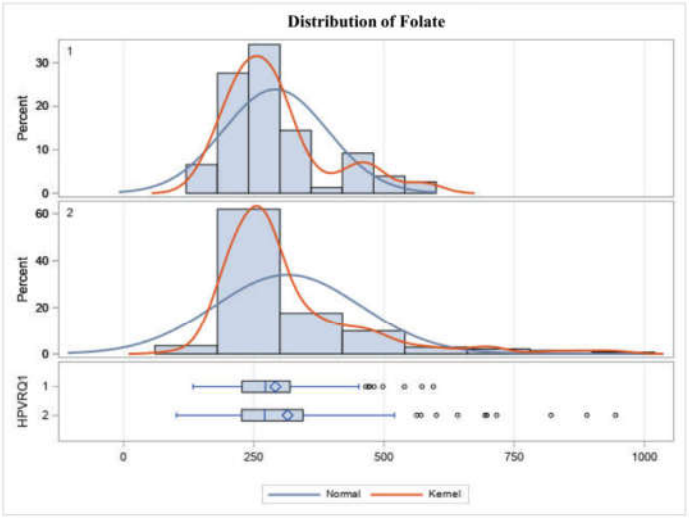


Figure 2. 3 The distribution of dietary folate intake by HPV status

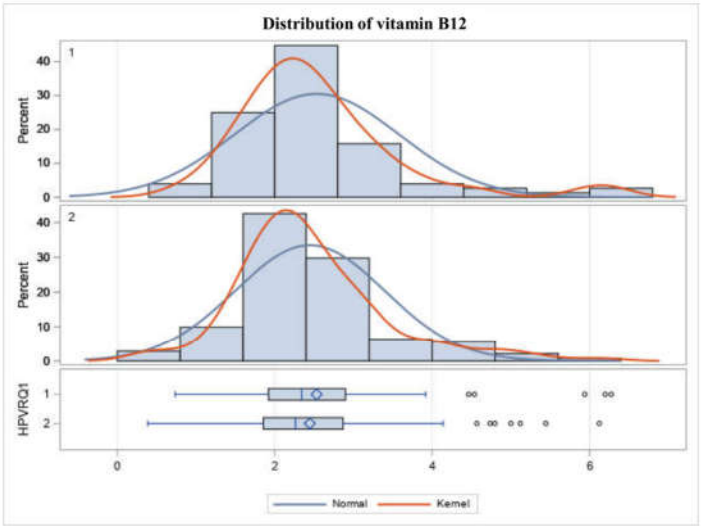


1. **Transient HPV (Controls)**
2. **Persistent HPV (Cases)**

Folate post fortification (ug DFE/1,000kcal/day)	Transient HPV infection (n=76) - Controls	Persistent genotype specific HPV infection (n=142) - Cases
median (min-max)	272.24 (133.10-594.72)	270.58 (101.63-944.82)
mean (SD)	291.32 (100.20)	314.73 (140.80)

Figure 2.4 The distribution of dietary vitamin B12 intake by HPV status

1. **Transient HPV (Controls)**
2. **Persistent HPV (Cases)**



Vitamin B12 (ug/1,000 kcal/day)	Transient genotype specific HPV infection (n=76) - Controls	Persistent genotype specific HPV infection (n=142) - Cases
median (min-max)	2.34 (0.74-6.27)	2.26 (0.39-6.12)
mean (SD)	2.53 (1.05)	2.45 (0.95)

Table 2. 1 Demographic and lifestyle variables by HPV status (n=218)

Variable	Non-persistent genotype specific HPV infection group (76 men) n (%)	Persistent genotype specific HPV infection group (142 men) n (%)	All men (218 men) n	P-value[a]
Age				
< 24 years	43 (41.0)	62 (59.1)	105	0.07
>= 24 years	33 (29.2)	80 (70.8)	113	
Number of visits				
< 5 visits	36 (45.0)	44 (55.0)	80	0.02
>= 5 visits	40 (28.9)	98 (71.1)	138	
Race				
Non-White	31 (37.8)	51 (62.2)	82	0.48
White	45 (33.1)	91 (66.9)	136	
Birthplace				
Non-USA	15 (37.5)	25 (62.5)	40	0.70
USA	61 (34.3)	117 (65.7)	178	
Marital status				

Variable	Non-persistent genotype specific HPV infection group (76 men) n (%)	Persistent genotype specific HPV infection group (142 men) n (%)	All men (218 men) n	P-value[a]
Single/Divorced/Separated	64 (34.8)	120 (65.2)	184	0.95
Married/Cohabiting	12 (35.3)	22 (64.7)	34	
Income				
< $1000	22 (30.6)	50 (69.4)	72	0.31
>= $1000 to < $1500	26 (32.5)	54 (67.5)	80	
> $1500	28 (42.4)	38 (57.6)	66	
Education				
High school graduate	1 (33.3)	2 (66.7)	3	0.0002
Vocational/ technical/trade school or business college	0	5 (100)	5	
Some college or university	52 (36.4)	91 (63.6)	143	
Bachelor's degree	5 (13.2)	33 (86.8)	38	
Graduate or professional degree	18 (62.1)	11 (37.9)	29	
Number of lifetime female sexual partners				
0 to 1	14 (66.7)	7 (33.3)	21	0.002

Variable	Non-persistent genotype specific HPV infection group (76 men) n (%)	Persistent genotype specific HPV infection group (142 men) n (%)	All men (218 men) n	P-value[a]
2 to 10	38 (35.5)	69 (64.5)	107	
> 10	22 (26.2)	62 (73.8)	84	
Sexual preference				
No sex	1 (50)	1 (50)	2	0.87
Sex with women only	61 (34.1)	118 (65.9)	179	
Sex with men only	1 (33.3)	2 (66.7)	3	
Sex with women and men	13 (40.6)	19 (59.4)	32	
Age at 1st SI with female, years				
< 16	13 (28.3)	33 (71.7)	46	0.47
16 to 18	44 (38.3)	71 (61.7)	115	
> 18	17 (33.3)	34 (66.7)	51	
Smoking habits				
Current	14 (38.9)	22 (61.1)	36	0.13
Ever[b]	9 (21.4)	33 (78.6)	42	

Variable	Non-persistent genotype specific HPV infection group (76 men) n (%)	Persistent genotype specific HPV infection group (142 men) n (%)	All men (218 men) n	P-value[a]
Never	53 (37.9)	87 (62.1)	140	
Alcohol drinking habits				
Ever[c]	42 (32.3)	88 (67.7)	130	0.36
Never	33 (38.4)	53 (61.6)	86	
Condom use within past 4 months				
No	22 (36.7)	38 (63.3)	60	0.32
Half and less than half the time	12 (26.1)	34 (73.9)	46	
More than half the time	28 (39.4)	43 (60.6)	71	
Circumcised				
Yes	65 (36.7)	112 (63.3)	177	0.23
No	11 (26.8)	30 (73.2)	41	
History of genital warts				
Current	5 (31.3)	11 (68.7)	16	0.23
Ever	2 (14.3)	12 (85.7)	14	

Variable	Non-persistent genotype specific HPV infection group (76 men) n (%)	Persistent genotype specific HPV infection group (142 men) n (%)	All men (218 men) n	P-value[a]
Never	69 (36.7)	119 (63.3)	188	
History of chlamydia infection				
Ever	4 (25)	12 (75)	16	0.39
Never	72 (35.6)	130 (64.4)	202	
History of gonorrhea infection				
Ever	5 (55.6)	4 (44.4)	9	0.18
Never	71 (34.0)	138 (66.0)	209	
History of hepatitis B infection				
Current	1 (50.0)	1 (50.0)	2	0.69
Ever	0	1 (50.0)	1	
Never	75 (34.9)	140 (65.1)	215	
History of hepatitis C infection				
Current	0	1 (100.0)	1	0.36
Ever	0	2 (100.0)	2	

Variable	Non-persistent genotype specific HPV infection group (76 men) n (%)	Persistent genotype specific HPV infection group (142 men) n (%)	All men (218 men) n	P-value[a]
Never	76 (35.5)	138 (64.5)	214	
History of genital herpes infection				
Current	1 (12.5)	7 (87.5)	8	0.36
Ever	1 (25.0)	3 (75.0)	4	
Never	74 (35.9)	132 (64.1)	206	
History of non-gonococcal urethritis				
Ever	1 (25)	3 (75)	4	0.68
Never	75 (35.01)	139 (65.0)	214	
History of syphilis infection				
Ever	0	0	0	N/A
Never	76 (34.9)	142 (65.1)	218	
History of yeast infection				
Ever	2 (40)	3 (60)	5	0.81
Never	74 (34.7)	139 (65.3)	213	

Variable	Non-persistent genotype specific HPV infection group (76 men) n (%)	Persistent genotype specific HPV infection group (142 men) n (%)	All men (218 men) n	P-value[a]
a: Chi-square/ Fisher's exact test b: ever smoked cigarettes daily for at least 6 months or more c: ever drink alcohol at least once a week for 6 months or more				

Table 2.2 The distribution of study participants according to their tertiles of dietary folate and vitamin B12 intakes by HPV status (n=218)

Variable	Non-persistent genotype specific HPV infection group (76 men) - controls n (%)	Persistent genotype specific HPV infection group (142 men) - cases n (%)	All men (218 men) n	P-value[a]
Folate, ug DFE per 1,000 kcal/ day				
=< 237.62	26 (34.2)	41 (28.9)	67	0.72
237.62 to =< 296.31	25 (32.9)	51 (35.9)	76	
> 296.31	25 (32.9)	50 (35.2)	75	
B12, ug per 1,000 kcal/ day				
=< 2.06	26 (34.2)	48 (33.8)	74	0.77
2.06 to =< 2.65	24 (31.6)	51 (35.9)	75	
> 2.65	26 (34.2)	43 (30.3)	69	
a: Chi-square/ Fisher's exact test				

Table 2.3 Odds ratios and 95% confidence intervals for persistent genotype specific HPV infection by levels of dietary folate and vitamin B12 intake (n=218)

Outcome	Variable	Number of participants	Univariate analysis		Multivariable analysis	
			Odds Ratio (95% CI)	P-value	Odds Ratio[a] (95% CI)	P-value
Any risk HPVs	**Folate, ug DFE per 1,000 kcal/ day**					
	=< 237.62	67	0.79 (0.40-1.57)	0.50	1.21 (0.54-2.68)	0.65
	237.62 to =< 296.31	76	1.02 (0.52-2.01)	0.95	1.55 (0.70-3.42)	0.28
	> 296.31	75	reference	(0.72)[b]	reference	(0.56)[b]
	B12, ug per 1,000 kcal/ day					
	=< 2.06	74	1.12 (0.57-2.21)	0.75	1.01 (0.46-2.18)	0.99
	2.06 to =< 2.65	75	1.29 (0.65-2.56)	0.47	1.40 (0.64-3.06)	0.40
	> 2.65	69	reference	(0.77)[b]	reference	(0.63)[b]

a: adjusted for age, number of visits, education, and number of lifetime female sexual partners
b: type 3 tests with sandwich variance estimate

Table 2.4 Odds ratios and 95% confidence intervals for persistent genotype specific HPV infection by potential confounders (n=218)

Variable	Numbers of participants in Uni/Bivariate analysis	Uni/Bivariate analysis	P-value
Age			
< 24 years	105	0.6 (0.34-1.04)	0.07
>= 24 years	113	reference	
Number of visits			
< 5 visits	80	reference	
>= 5 visits	138	2.01 (1.13-3.56)	0.02
Race			
Non-White	82	0.81 (0.46-1.44)	0.48
White	136	reference	
Birthplace			
Non-USA	40	0.87 (0.43-1.77)	0.70
USA	178	reference	
Marital status			
Single/Divorced/Separated	184	1.02 (0.48-2.20)	0.95
Married/Cohabiting	34	reference	
Education			

Variable	Numbers of participants in Uni/Bivariate analysis	Uni/Bivariate analysis	P-value
No college/ Some college	151	3.03 (1.33-6.88)	0.008
College	38	10.8 (3.24-35.95)	0.0001
Graduate/Professional degree	29	reference	(0.0005)[a]
Income			
< $1000	72	1.68 (0.83-3.37)	0.15
>= $1000 to < $1500	80	1.53 (0.78-3.01)	0.22
> $1500	66	reference	(0.30)[a]
Number of lifetime female sexual partners			
0 to 1	21	reference	(0.004)[a]
2 to 10	107	3.63 (1.35-9.77)	0.01
> 10	84	5.64 (2.01-15.78)	0.001
Sexual preference			
No sex	2	reference	(0.82)[a]
Sex with women only	179	1.93 (0.12-31.46)	0.64
Sex with men only/Sex with women and men	35	1.64 (0.10-28.41)	0.73
Age at 1st sexual intercourse with female, years			
< 16	46	1.27 (0.53-3.02)	0.59
16 to 18	115	0.81 (0.40-1.61)	0.54

Variable	Numbers of participants in Uni/Bivariate analysis	Uni/Bivariate analysis	P-value
> 18	51	reference	(0.47)[a]
Smoking habits			
Current/Ever[b]	78	1.46 (0.80-2.64)	0.21
Never	140	reference	
Alcohol drinking habits			
Ever[c]	130	1.31 (0.74-2.31)	0.36
Never	86	reference	
Condom use within past 4 months			
No	60	1.13 (0.55-2.29)	0.75
Half and less than half the time	46	1.85 (0.82-4.16)	0.14
More than half the time	71	reference	(0.32)[a]
Circumcised			
Yes	177	reference	
No	41	1.58 (0.74-3.37)	0.23
History of genital warts			
Current/Ever	30	1.91 (0.78-4.67)	0.16
Never	188	reference	
History of chlamydia infection			

Variable	Numbers of participants in Uni/Bivariate analysis	Uni/Bivariate analysis	P-value
Ever	16	1.66 (0.52-5.34)	0.39
Never	202	reference	
History of gonorrhea infection			
Ever	9	0.41 (0.11-1.58)	0.20
Never	209	reference	
History of hepatitis B infection			
Current/Ever	3	1.07 (0.10-12.00)	0.96
Never	215	reference	
History of hepatitis C infection			
Current/Ever	3	N/A	N/A
Never	214	reference	
History of genital herpes infection			
Current/Ever	12	1.91 (0.78-4.67)	0.16
Never	206	reference	
History of non-gonococcal urethritis			
Ever	4	1.62 (0.17-15.83)	0.68
Never	214	reference	
History of yeast infection			

Variable	Numbers of participants in Uni/Bivariate analysis	Uni/Bivariate analysis	P-value
Ever	5	0.80 (0.13-4.89)	0.81
Never	213	Reference	

a: type 3 tests with sandwich variance estimate
b: ever smoked cigarettes daily for at least 6 months or more
c: ever drink alcohol at least once a week for 6 months or more

Table 2.5 Final logistic regression model of the association between dietary folate intake and persistent genotype specific HPV infections

Outcome	Variable	Univariate analysis		Multivariable analysis	
		Odds Ratio (95% CI)	**P-value**	**Odds Ratio[a] (95% CI)**	**P-value**
Any risk HPVs	**Folate, ug DFE per 1,000 kcal/ day**				
	=< 237.62	0.79 (0.40-1.57)	0.50	1.21 (0.54-2.68)	0.65
	237.62 to =< 296.31	1.02 (0.52-2.01)	0.95	1.55 (0.70-3.42)	0.28
	> 296.31	Reference	(0.72)[b]	reference	(0.56)[b]
	Age				
	< 24 years	0.6 (0.34-1.04)	0.07	0.46 (0.21-1.03)	0.06
	>= 24 years	Reference		reference	
	Number of visits				
	< 5 visits	Reference		reference	
	>= 5 visits	2.01 (1.13-3.56)	0.02	2.93 (1.49-5.74)	0.002
	Education				
	No college/ Some college	3.03 (1.33-6.88)	0.008	6.65 (2.31-19.16)	0.0005
	College	10.8 (3.24-35.95)	0.0001	16.60 (4.44-62.06)	< 0.0001
	Graduate/Professional degree	Reference	(0.0005)[b]	reference	(< 0.0001)[b]
	Number of lifetime female sexual partners				
	0 to 1	Reference	(0.004)[b]	reference	(0.01)[b]

	2 to 10	3.63 (1.35-9.77)	0.01	4.08 (1.37-12.09)	0.01
	> 10	5.64 (2.01-15.78)	0.001	5.62 (1.78-17.78)	0.003
a: adjusted for age, number of visits, education, and number of sexual partners b: type 3 tests with sandwich variance estimate					

Table 2.6 Final logistic regression model of the association between dietary vitamin B12 intake and persistent genotype specific HPV infections

Outcome	Variable	Univariate analysis		Multivariable analysis	
		Odds Ratio (95% CI)	**P-value**	**Odds Ratio [a] (95% CI)**	**P-value**
Any risk HPVs	**B12, ug per 1,000 kcal/ day**				
	=< 2.06	1.12 (0.57-2.21)	0.75	1.01 (0.46-2.18)	0.99
	2.06 to =< 2.65	1.29 (0.65-2.56)	0.47	1.40 (0.64-3.06)	0.40
	> 2.65	Reference	(0.77)[b]	reference	(0.63)[b]
	Age				
	< 24 years	0.6 (0.34-1.04)	0.07	0.49 (0.22-1.08)	0.08
	>= 24 years	Reference		reference	
	Number of visits				
	< 5 visits	Reference		reference	
	>= 5 visits	2.01 (1.13-3.56)	0.02	2.95 (1.51-5.79)	0.002
	Education				
	No college/ Some college	3.03 (1.33-6.88)	0.008	6.84 (2.38-19.64)	0.0004
	College	10.8 (3.24-35.95)	0.0001	16.29 (4.38-60.61)	< 0.0001
	Graduate/Professional degree	Reference	(0.0005)[b]	reference	(< 0.0001)[b]
	No. lifetime sexual partners				
	0 to 1	Reference	(0.004)[b]	reference	(0.01)[b]

	2 to 10	3.63 (1.35-9.77)	0.01	3.91 (1.34-11.46)	0.01
	> 10	5.64 (2.01-15.78)	0.001	5.48 (1.75-17.19)	0.004
a: adjusted for age, number of visits, education, and number of lifetime female sexual partners b: type 3 tests with sandwich variance estimate					

Chapter 3

Examining the Relationship between Dietary Folate and Vitamin B12 Intake and the Acquisition of Genotype Specific Genital HPV Infections

Introduction

Worldwide, the most common STI is HPV infection. As most infections are asymptomatic and the anogenital tract can be infected with more than 40 HPV genotypes, many experts believe that almost all sexually active adults have been infected with at least one HPV genotype at least once in their lifetime [1]. In the U.S., the estimated lifetime probability of acquiring HPV in sexually active adults is 90% in men and 85% in women [69]. Among men, the incidence of genital HPV is 38.4 per 1,000 person-months and the annual cumulative incidence is 39.3% [61]. At the 24-month follow-up, the cumulative incidence of HPV infection is increased to 62% [70]. Among women, the annual cumulative incidence of first HPV infection is 28.5%. By 36 months, the cumulative incidence of genital HPV infection is increased to almost 50% [71]. Though the rate of HPV acquisition in men is higher, the burden of HPV-related diseases is more dominant in women, as the number of cervical cancer cases is far higher than the number of penile cancer cases [54].

The most consistent predictors of acquiring genital HPV infection are sexual behaviors, as the incidence of genital HPV among virgin women is far lower than sexually active women [72, 73]. Increasing number of lifetime sexual partners is a significant predictor of acquiring genital HPV infection [61]. Furthermore, the number of a partner's additional sexual partners is also significantly associated with the acquisition of HPV in women [71]. Evidence regarding the concordance of HPV genotype in a sexual couple supports the importance of HPV transmission

through sexual activities [32]. The transmission of HPV infection from men to women plays an important role in the risk of cervical cancer, so in addition to receiving an HPV vaccination, practicing safe sex behaviors is the primary strategy to deal with HPV-related diseases. Additionally, to effectively prevent HPV-related diseases globally, vaccine coverage must be high, but only developed countries may be able to afford a national vaccination program.

A nutrient-mediated program, which can modify a human's immune system and is a significant co-factor in the pathogenesis of HPV-related disease, is another interesting strategy to deal with the burden of HPV-related diseases. Nutrient deficiencies can cause DNA damage leading to genetic alteration and compromising the body's immune system [36, 37]. Subsequently, this puts the body at risk for HPV-related diseases. The deficiency of vitamin B12 and folate might affect the risk of HPV acquisition, as folate and vitamin B12 play a vital role in DNA synthesis and repair of breaking chromosome. This breaking point is the site that HPV incorporates into human chromosome [38, 39]. To the best of our knowledge, only two studies, which focused on the role of nutrition and incident HPV infection, have been published [43, 64]. The summaries of both articles are included in the appendix (Appendix, Literature review, chapter 3). Until present, no published studies focus on dietary folate and vitamin B12 intake and the acquisition of genotype specific HPV infection.

Methods

Assessment of HPV status

HPV positive was defined by a positive HPV test for at least one of the following genital specimens: glans penis, coronal sulcus, foreskin, shaft, or scrotum. To prevent bias due to left censoring, we did not include the HPV genotypes that were found at the baseline examination

because we did not know when participants acquired those particular HPV genotypes. In other words, we defined the acquisition of genotype specific HPV infections as the acquisition of HPV genotypes that were found after at least one preceding negative visit for those particular genotypes. Participants could be infected with more than one HPV genotypes at the same time, and each HPV infection was used as a separate unit of analysis. For participants who had re-infection with the same HPV genotypes, we considered only the first HPV infection, as this approach mitigated the misclassification bias from detecting re-activation of that particular genotype.

Assessment of dietary intakes

Using the validated QFFQ, Dietary folate (µg DFE per day), vitamin B12 (µg per day), and energy (kcal per day) intakes derived from the baseline interview were used in this analysis. All dietary intakes were presented as nutrient densities. Dietary folate (µg DFE per 1,000 kcal per day) and vitamin B12 (µg per 1,000 kcal per day) intakes were categorized according to the tertile cut points of all participants (n=318) [64].

Measure of potential confounders

As we did in the analysis of our first research question, selected covariates derived from the baseline interview regarding sociodemographic, reproductive, medical, and sexual history were evaluated and used as potential confounders in the multivariable analysis.

Statistical analysis

Statistical analysis was conducted using SAS software version 9.4 (SAS Institute, Cary, NC). Sociodemographic characteristics and lifestyle variables of included men were summarized by using frequency statistics, and the results are shown in Table 3.1. For this study, each genotype

specific HPV infection was used as a separate unit of analysis. We excluded infections with unclassified HPV genotypes and clinic visits with missing or inadequate specimens. To test the hypothesis of whether dietary folate and vitamin B12 intake were associated with the acquisition of genotype specific HPV infections, recurrent events survival analysis was conducted. The Wei, Lin, and Weissfeld (WLW) approach, which accounts for the concurrent acquisition of more than one genotype specific HPV infections, was used [74]. Days since participant entered into the study until he acquired genotype specific HPV infections were used as the time metric. To account for the correlation within subjects, we used the robust sandwich variance estimate to test the hypotheses and confidence intervals of model parameters [75]. We conducted the Schoenfeld residual analysis to test the proportional hazard assumption regarding each category of dietary folate and vitamin B12 intake and the hazard of acquiring genotype specific HPV infections. Univariate analysis was conducted by using each baseline sociodemographic and lifestyle variable as an independent variable and the acquisition of genotype specific HPV infection as a dependent variable. Two separate multivariable analyses were conducted using either dietary folate or vitamin B12 intakes as the independent variable. The purposeful selection algorithm was used for variable selection to select potential confounders to include in the multivariable models [65]. Further conducted analyses were with classified genotype specific incident HPV infection according to the oncogenic or non-oncogenic genotypes.

Results

This study focused on the acquisition of HPVs. Among 445 adult men in the Hawai‘i HPV male cohort, we included only men who had at least two follow-up visits so that we could assess the acquisition of genotype specific HPV infections. Seventy-six men were excluded because they had only one visit. Seven men who did not complete the QFFQ were excluded. Twelve men who

both had only one visit and did not complete the QFFQ were excluded. Two men who reported consuming more than 10,000 kcal of energy intake per day were excluded. Due to a high correlation between HIV and HPV infection, thirty men with self-reported HIV infection status were excluded [76]. A total of 318 men were eligible for this second research question. Figure 3.1 summarizes the flow diagram of included and excluded participants.

The basic demographics and lifestyle variables of the study population are shown in Table 3.1. The mean (SD) age of participants was 27.7 (11.0) years. The median (range) number of visits was 6 (2-19) visits. The majority of participants (80.8%, 257/318) were U.S. born and (59.1%, 188/318) were White. The highest proportion (65.7%, 209/318) of participants had some college education. The majority of participants (60.1%, 191/318) had monthly income lower than $1,000. The majority of participant (81.7%, 258/316) were heterosexual. The majority of participants (53.6%, 162/302) had their first-time sexual intercourse with a female when they were 16-18 years old. The median (range) number of lifetime female sexual partners was 7 (1-100). During the past four months, 69% (167/242) of participants used condoms when they had sexual intercourse with a female. Among these, 62% (105/167) used condoms more than half of the time. Few participants (11.1%, 35/318) had a history of genital warts, of which about 50% currently had genital warts. Very few participants (0.9%, 3/317) had a history of hepatitis C virus (HCV) infection. Approximately 70% (226/318) of participants were defined as “a never smoker.”

There are 174 (54.7%, 174/318) men who acquired at least one incident genotype specific HPV infection during the study period. Among these men, 125 (39.3%, 125/318) men had at least one oncogenic HPV genotype, while 143 (45.0%, 143/318) men had at least one non-oncogenic HPV genotype. Among all men, 566 incident genotype specific HPVs were acquired during the study period. Among these, 45.8% (259/566) were oncogenic HPVs, while 54.2% (318/566) were

non-oncogenic HPVs. The distribution of incident genotype specific HPV infection by dietary folate and vitamin B12 intake status is summarized in Tables 3.2 and 3.3.

Figures 3.2 and 3.3 show Schoenfeld residual plots between the tertiles of dietary intake and follow-up time. Neither dietary folate nor vitamin B12 intake violated the proportional hazards assumption. Tables 3.2 and 3.3 show the results of survival analysis for nutrients variables associated with incident genotype specific HPV infection, which included men who had the highest tertile of nutrients intakes as a reference group. Univariate analysis shows that men whose dietary folate intake were in the lowest tertile were at lower risk of incident genotype specific HPV infections. These associations were consistent among any risk, oncogenic, and non-oncogenic HPV infections: the hazard ratio [HR] = 0.76 (95% CI 0.52-1.10), the HR = 0.75 (95% CI 0.46-1.21), and the HR = 0.76 (95% CI 0.51-1.13), respectively. Among men whose dietary folate intake were in the middle tertile: the HR = 1.00 (95% CI 0.71-1.41) for any risk HPV infections, the HR = 1.00 (95% CI 0.67-1.51) for oncogenic HPV infections, and the HR = 0.99 (95% CI 0.69-1.45) for non-oncogenic HPV infections. After adjustment for potential confounders, men whose dietary folate intake were in the lowest tertile: the HR = 0.74 (95% CI 0.52-1.05) for any risk HPV infections, the HR = 0.93 (95% CI 0.58-1.47) for oncogenic HPV infections, and 0.77 (0.52-1.33) for non-oncogenic HPV infections. Men whose dietary folate intake were in the middle tertile: the HR = 0.97 (95% CI 0.71-1.32) for any risk HPV infections, the HR = 1.16 (95% CI 0.80-1.69) for oncogenic HPV infections, and the HR = 0.94 (95% CI 0.66-1.33) for non-oncogenic HPV infections. Regarding dietary vitamin B12 intake, univariate analysis shows that men whose dietary vitamin B12 intake was in the lowest tertile were at lower risk of incident genotype specific HPV infections. These associations were consistent among any risk, oncogenic, and non-oncogenic HPV infections: the HR = 0.75 (95% CI 0.52-1.10), the HR = 0.67 (95% CI 0.43-1.05),

and the HR = 0.83 (95% CI 0.56-1.22), respectively. Among men whose dietary vitamin B12 intake was in the middle tertile: the HR = 1.02 (95% CI 0.73-1.44) for any risk HPV infections, the HR = 0.94 (95% CI 0.62-1.44) for oncogenic HPV infections, and the HR = 1.10 (95% CI 0.76-1.60) for non-oncogenic HPV infections. After adjustment for potential confounders, men whose dietary vitamin B12 intake were in the lowest tertile: the HR = 1.01 (95% CI 0.71-1.45) for any risk HPV infections, the HR = 0.81 (95% CI 0.53-1.26) for oncogenic HPV infections, and the HR = 1.10 (95% CI 0.75-1.60) for non-oncogenic HPV infections. Men whose dietary vitamin B12 intake were in the middle tertile: the HR = 1.42 (95% CI 1.04-1.95) for any risk HPV infections, the HR = 1.03 (95% CI 0.70-1.52) for oncogenic HPV infections, and the HR = 1.47 (95% CI 1.01-2.12) for non-oncogenic HPV infections.

Table 3.4 shows the results of survival analysis for potential confounders initially identified as associated with incident genotype specific HPV infections. Race, number of lifetime female sexual partners, alcohol drinking habits, a history of genital warts, and a history of HCV infection reached statistically significant differences in univariate analysis of any risk, oncogenic, and non-oncogenic HPV infections. Income, age at first sexual intercourse with a female, smoking habits reached statistically significant differences in univariate analysis of any risk and non-oncogenic HPV infections. A history of genital herpes infection reached statistically significant differences in univariate analysis of oncogenic HPV infections. A history of non-gonococcal urethritis reached statistically significant differences in univariate analysis of oncogenic and non-oncogenic HPV infections. However, the direction of HRs is different.

Tables 3.5-3.10 show final Cox proportional hazard model of the association between dietary folate and vitamin B12 intake and the acquisition of HPVs – any risk, oncogenic, and non-oncogenic HPVs. Tables 3.5 and 3.6 show multivariable analysis of any risk HPV infections, of

which number of lifetime female sexual partners and a history of HCV infection were independently associated with incident genotype specific HPV infections. Tables 3.7 and 3.8 show multivariable analysis of oncogenic HPV infections, of which number of lifetime female sexual partners, a history of genital wart, and a history of HCV infection were independently significantly associated with incident genotype specific HPV infections. Regarding non-oncogenic HPV infections, multivariable analysis shows that age, number of lifetime female sexual partners, and a history of HCV infection were independently associated with incident genotype specific HPV infections. These results are shown in Tables 3.9 and 3.10.

Discussion

Among all acquired genotype specific HPV infections, the proportion of oncogenic HPVs (45.8%) was slightly lower than non-oncogenic HPVs (54.2%). This is consistent with the previous publication by Giuliano et al. in 2011 that the incidence of oncogenic HPVs was slightly lower than non-oncogenic HPVs. Giuliano et al. reported that the incidence per 1000 month of oncogenic HPVs was 22.2 (19.8-24.9), while the incidence per 1000 month of non-oncogenic HPVs was 27.8 (24.8-31.0) [61].

The univariate analysis of the association between dietary folate intake and the acquisition of genotype specific HPV infections does not support our hypothesis that men with lower levels of dietary folate intake will be at higher risk of acquiring HPVs. After adjustment for potential confounders, men whose dietary folate intake was in the middle tertile had higher risk of acquiring oncogenic HPVs. None of these associations reached a statistically significant difference. Regarding dietary vitamin B12 intake, men whose dietary vitamin B12 intake was in the middle tertile had higher risk of acquiring any risk and non-oncogenic HPVs. After adjustment for

potential confounders, most of the associations between dietary vitamin B12 intake and the acquisition of genotype specific HPV infections support our hypothesis. Men whose dietary vitamin B12 intake was in the middle tertile was independently significantly associated with acquiring any risk and non-oncogenic HPVs. The biological plausibility of this association is that vitamin B12 plays an important role in modifying our body immune system through several metabolic pathways involving either DNA synthesis or methylation of DNA, so that men with lower levels of dietary vitamin B12 intake might have weaker immune to protect against HPVs [36, 37].

Our results are inconsistent with the publication by Piyathilake et al. in 2004 [43]. This study found that women with higher plasma and red blood cell (RBC) folate had lower odds of acquiring oncogenic HPVs: the OR = 0.27 (95% CI 0.08-0.91). One possible explanation why we did not see this association is that the methodology of this study is quite different from ours. While our exposure was dietary folate intake, the combination of plasma and RBC folate was used in Piyathilake et al.'s study. Additionally, our analyses accounted for all possible incident genotype specific HPVs, Piyathilake et al.'s study did not use the genotype specific information as an outcome.

Regarding other potential confounders, number of lifetime female sexual partners and a history of HCV infection were independently associated in all multivariable analyses – any risk, oncogenic, and non-oncogenic HPV infections. Our results are consistent with previous publications that the number of lifetime sexual partners is a strong risk of acquiring HPVs [61, 63]. We have very few participants (0.9%, 3/317) with a history of HCV infection. Injection drug use is the primary risk of acquiring HCV infection among young adults in the U.S. [77]. However, all these three participants in our study were in their late 40s. In other words, they were born in the

baby boomer (1945-1965) period, when the majority of HCV transmission occurred through medical procedures or illicit drug use [78]. The acquiring of HPV might not be related to this etiology. One possible explanation is that these participants might have unreported risky sexual behaviors. Participants with a history of non-gonococcal urethritis had a higher risk of acquiring non-oncogenic HPV infections, as this STD shares similar etiology with genital HPVs. However, this association was inverse in the analysis of oncogenic HPVs. We do not have an explanation to support the plausibility of this association.

The strength of this study is that, to the best of our knowledge, is the first that investigates the association between dietary intake and incident genotype specific HPV infections among men. Second, we did the recurrent survival analysis to capture all possible genotype specific HPV infections. The robust sandwich variance estimate was used to account for the correlation within subjects. Third, our study conducted HPV testing frequently, giving the method a high sensitivity to detect the acquisition of HPVs. The limitation of this study is that the HRs (and 95% CIs) are more apparent among men in the middle tertile, so causal relationships must be interpreted with caution. In other words, a biological gradient or a dose-response relationship does not exist among these associations [68]. Additionally, even though folate and vitamin B12 work closely in the DNA synthesis pathways, the association between dietary vitamin B12 intake and acquiring HPVs is more noticeable.

Conclusion

Men with lower levels of dietary vitamin B12 intake might have a higher risk of acquiring any risk and non-oncogenic HPV infections. These associations are more noticeable among men whose dietary vitamin B12 intake was in the middle, not the lowest tertile. Men with

lower levels of dietary folate intake were not associated with a higher risk of acquiring genotype specific HPV infections.

Figure 3.1 Study flow chart of included and excluded participants

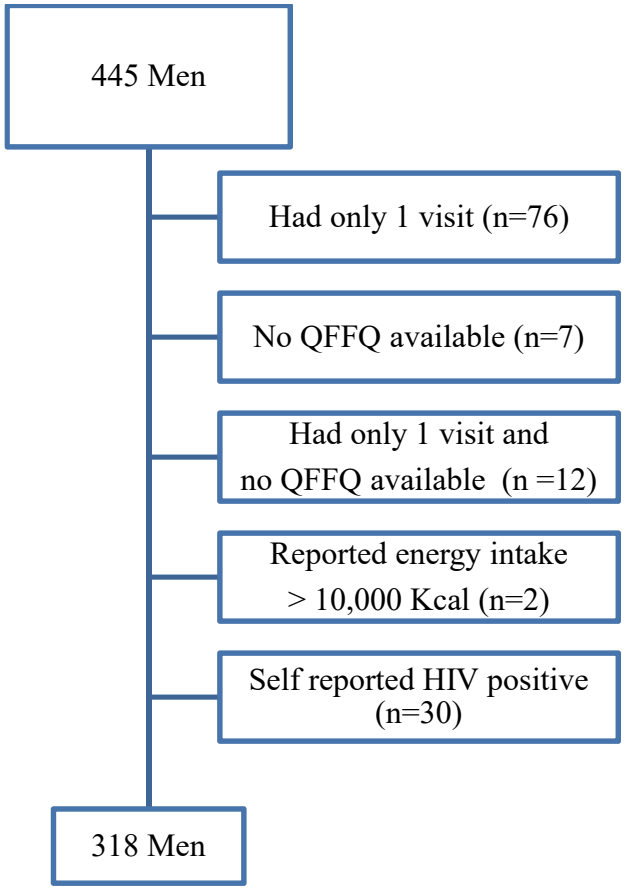


Figure 3.2 Scaled Schoenfeld residual for dietary folate intake

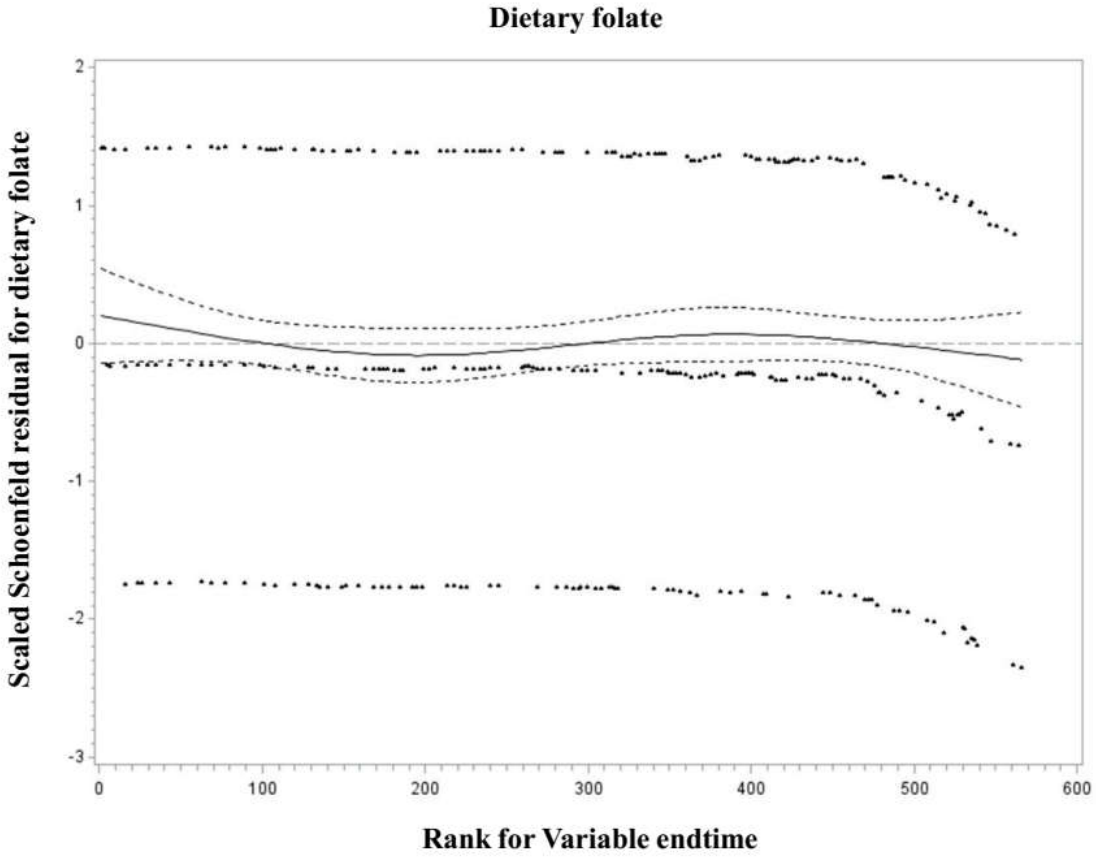


Figure 3.3 Scaled Schoenfeld residual for dietary vitamin B12 intake

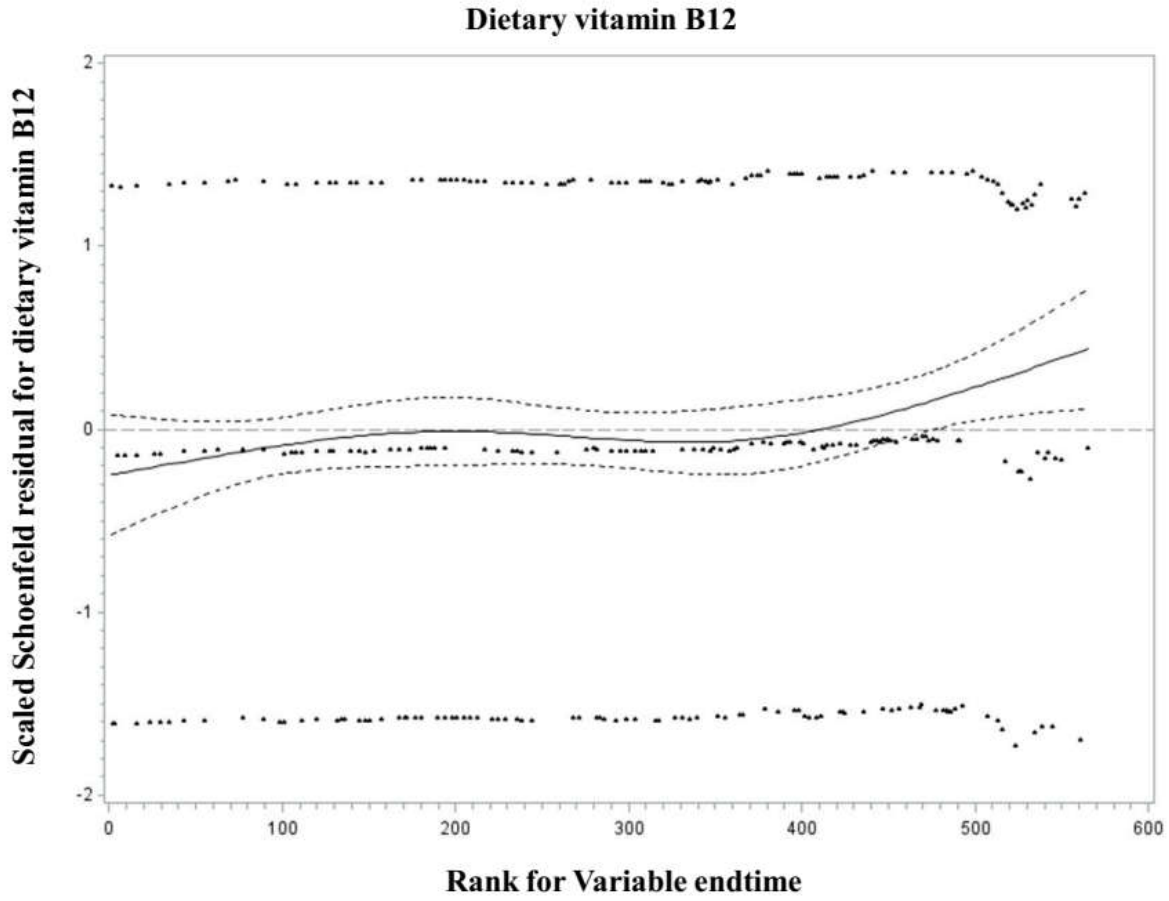


Table 3.1 Demographic and lifestyle variables by HPV status (n=318)

Variable	All men (318 men) n (%)
Age	
< 24 years	159 (50.0)
>= 24 years	159 (50.0)
Race	
Non-White	130 (40.9)
White	188 (59.1)
Birthplace	
Non-USA	61 (19.2)
USA	257 (80.8)
Marital status	
Single/Divorced/Separated	273 (85.9)
Married/Cohabiting	45 (14.2)
Income	
< $1000	191 (60.1)

Variable	All men (318 men) n (%)
>= $1000 to < $1500	62 (19.5)
> $1500	65 (20.4)
Education	
High school graduate	3 (0.9)
Vocational/ technical/trade school or business college	6 (1.9)
Some college or university	209 (65.7)
Bachelor's degree	55 (17.3)
Graduate or professional degree	45 (14.2)
Number of lifetime female sexual partners	
0 to 1	37 (12.3)
2 to 10	167 (55.3)
> 10	98 (32.5)
Sexual preference	
No sex	9 (2.9)
Sex with women only	258 (81.7)

Variable	All men (318 men) n (%)
Sex with men only	6 (1.9)
Sex with women and men	43 (13.6)
Age at 1st SI with female, years	
< 16	60 (19.9)
16 to 18	162 (53.6)
> 18	98 (32.5)
Smoking habits	
Current	40 (12.6)
Ever[a]	52 (16.3)
Never	226 (71.1)
Alcohol drinking habits	
Ever[b]	174 (56.3)
Never	135 (43.7)
Condom use within past 4 months	
No	75 (31.0)

Variable	All men (318 men) n (%)
Half and less than half the time	62 (25.6)
More than half the time	105 (43.4)
Circumcised	
Yes	261 (82.1)
No	57 (17.9)
History of genital warts	
Current	17 (5.4)
Ever	18 (5.7)
Never	283 (89.0)
History of chlamydia infection	
Ever	18 (5.7)
Never	300 (94.3)
History of gonorrhea infection	
Ever	11 (3.5)
Never	307 (96.5)

Variable	All men (318 men) n (%)
History of hepatitis B infection	
Current	3 (0.9)
Ever	2 (0.6)
Never	313 (98.4)
History of hepatitis C infection	
Current	1 (0.3)
Ever	2 (0.6)
Never	314 (99.1)
History of genital herpes infection	
Current	10 (3.1)
Ever	5 (1.6)
Never	303 (95.3)
History of non-gonococcal urethritis	
Ever	5 (1.6)
Never	313 (98.4)

Variable	All men (318 men) n (%)
History of syphilis infection	
Ever	0
Never	318 (100)
History of yeast infection	
Ever	7 (2.2)
Never	311 (97.8)
a: ever smoked cigarettes daily for at least 6 months or more b: ever drink alcohol at least once a week for 6 months or more	

Table 3.2 Hazard ratios and 95% confidence intervals for acquisition of HPV infections by levels of dietary folate intake (n=318)

Outcome	Variable	Number of participants/ Number of infections	Univariate analysis		Multivariable analysis	
			Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
Any risk HPVs	**Folate, ug DFE per 1,000 kcal/ day**					
	=< 243.40	104/135	0.76 (0.52-1.10)	0.14	0.74 (0.52-1.05)[a]	0.09
	> 243.40 to =< 301.42	105/207	1.00 (0.71-1.41)	0.99	0.97 (0.71-1.32)[a]	0.85
	> 301.42	109/224	reference	(0.24)[b]	reference	(0.20)[b]
Oncogenic HPVs	**Folate, ug DFE per 1,000 kcal/ day**					
	=< 243.40	104/61	0.75 (0.46-1.21)	0.24	0.93 (0.58-1.47)[c]	0.75
	> 243.40 to =< 301.42	105/95	1.00 (0.67-1.51)	0.98	1.16 (0.80-1.69)[c]	0.43
	> 301.42	109/103	reference	(0.42)[b]	reference	(0.58)[b]
Non-Oncogenic HPVs	**Folate, ug DFE per 1,000 kcal/ day**					
	=< 243.40	104/74	0.76 (0.51-1.13)	0.17	0.77 (0.52-1.13)[d]	0.18
	> 243.40 to =< 301.42	105/112	0.99 (0.69-1.45)	0.99	0.94 (0.66-1.33)[d]	0.71
	> 301.42	109/121	reference	(0.29)[b]	reference	(0.40)[b]

a: adjusted for age, education, number of lifetime female sexual partners, sexual preference, condoms use in the past 4 months, and history of HCV infection
b: type 3 tests with sandwich variance estimate
c: adjusted for age, number of lifetime female sexual partners, sexual preference, history of genital warts, and history of HCV infection

d: adjusted for age, number of lifetime female sexual partners, condoms use in the past 4 months, and history of HCV infection

Table 3.3 Hazard ratios and 95% confidence intervals for acquisition of HPV infections by levels of dietary B12 intake (n=317)

Outcome	Variable	Number of participants/ Number of infections	Univariate analysis		Multivariable analysis	
			Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
Any risk HPVs	**B12, ug per 1,000 kcal/ day**					
	=< 1.94	105/162	0.75 (0.52-1.10)	0.14	1.01 (0.70-1.45)[a]	0.97
	> 1.94 to =< 2.57	105/197	1.02 (0.73-1.44)	0.89	1.42 (1.04-1.95)[a]	0.03
	> 2.57	107/206	reference	(0.20)[b]	reference	(0.03)[b]
Oncogenic HPVs	**B12, ug per 1,000 kcal/ day**					
	=< 1.94	105/70	0.67 (0.43-1.05)	0.08	0.81 (0.53-1.26)[c]	0.35
	> 1.94 to =< 2.57	105/88	0.94 (0.62-1.44)	0.78	1.03 (0.70-1.52)[c]	0.86
	> 2.57	107/100	reference	(0.16)[b]	reference	(0.46)[b]
Non-Oncogenic HPVs	**B12, ug per 1,000 kcal/ day**					
	=< 1.94	105/92	0.83 (0.56-1.22)	0.34	1.10 (0.75-1.60)[d]	0.63
	> 1.94 to =< 2.57	105/109	1.10 (0.76-1.60)	0.61	1.47 (1.01-2.12)[d]	0.04
	> 2.57	107/106	reference	(0.38)[b]	Reference	(0.10)[b]

a: adjusted for age, education, number of lifetime female sexual partners, sexual preference, condoms use in the past 4 months, and history of HCV infection
b: type 3 tests with sandwich variance estimate
c: adjusted for age, number of lifetime female sexual partners, sexual preference, history of genital warts, and history of HCV infection

d: adjusted for age, number of lifetime female sexual partners, condoms use in the past 4 months, and history of HCV infection

Table 3.4 Hazard ratios and 95% confidence intervals for acquisition of HPV infection by potential confounders (n=318)

Variable	Numbers of participants	Any risk HPV		Oncogenic HPV		Non-oncogenic HPV	
		Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value
Age, years							
< 24 years	159	0.89 (0.66-1.19)	0.43	0.81 (0.57-1.16)	0.25	0.96 (0.69-1.32)	0.79
>= 24 years	159	reference		reference		reference	
Race							
Non-White	130	0.64 (0.46-0.87)	0.01	0.68 (0.46-0.995)	0.05	0.60 (0.43-0.86)	0.005
White	188	reference		reference		reference	
Birthplace							
Non-USA	61	0.84 (0.57-1.24)	0.37	0.87 (0.55-1.38)	0.56	0.81 (0.53-1.23)	0.32
USA	257	reference		reference		reference	
Marital status							
Single/Divorced/Separated	273	1.11 (0.76-1.63)	0.59	1.06 (0.66-1.73)	0.80	1.16 (0.74-1.81)	0.52
Married/Cohabiting	45	reference		reference		reference	
Education							

Variable	Numbers of participants	Any risk HPV		Oncogenic HPV		Non-oncogenic HPV	
		Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value
No college/some college	218	reference	(0.25)[a]	reference	(0.56)[a]	reference	(0.14)[a]
Bachelor's degree	55	0.98 (0.69-1.38)	0.89	0.89 (0.57-1.38)	0.59	1.05 (0.73-1.52)	0.79
Graduate/professional degree	45	0.67 (0.42-1.07)	0.10	0.76 (0.45-1.29)	0.31	0.60 (0.35-1.02)	0.06
Income							
< $1000	191	reference	(0.01)[a]	reference	(0.05)[a]	reference	(0.03)[a]
>= $1000 to < $1500	62	1.03 (0.74-1.44)	0.86	1.09 (0.72-1.65)	0.69	0.99 (0.69-1.40)	0.93
> $1500	65	0.61 (0.42-0.89)	0.01	0.64 (0.40-1.01)	0.06	0.59 (0.39-0.89)	0.01
Number of lifetime female sexual partners							
0 to 1	37	reference	(< 0.0001)[a]	reference	(<0.0001)[a]	reference	(<.0001)[a]
2 to 10	167	2.85 (1.29-6.31)	0.01	1.87 (0.80-4.42)	0.15	4.81 (2.18-10.62)	0.0001
> 10	98	6.30 (2.89-13.76)	< 0.0001	4.45 (1.90-10.43)	0.001	10.02 (4.60-21.82)	<.0001
Sexual preference							
Sex with women only	258	reference		reference		reference	
Sex with men only/Sex with women and men	49	0.78 (0.53-1.15)	0.21	0.69 (0.45-1.07)	0.10	0.86 (0.54-1.36)	0.52

Variable	Numbers of participants	Any risk HPV		Oncogenic HPV		Non-oncogenic HPV	
		Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value
Age at 1st SI with female, years							
< 16	60	1.80 (1.22-2.67)	0.003	1.72 (1.09-2.73)	0.02	1.88 (1.20-2.94)	0.01
16 to 18	162	1.23 (0.85-1.77)	0.27	1.24 (0.80-1.92)	0.34	1.22 (0.81-1.83)	0.34
> 18	98	reference	(0.01)[a]	reference	(0.06)[a]	reference	(0.01)[a]
Smoking habits							
Current/Ever[b]	92	1.46 (1.10-1.95)	0.01	1.38 (0.95-1.98)	0.09	1.54 (1.13-2.10)	0.01
Never	226	reference		reference		reference	
Alcohol drinking habits							
Ever[c]	174	1.50 (1.12-2.02)	0.01	1.58 (1.10-2.26)	0.01	1.44 (1.03-2.02)	0.03
Never	135	reference		reference		reference	
Condom use within past 4 months							
No	75	1.15 (0.80-1.65)	0.46	1.28 (0.81-2.03)	0.29	1.04 (0.70-1.56)	0.84
Half and less than half the time	62	1.37 (0.94-2.01)	0.10	1.49 (0.92-2.40)	0.11	1.29 (0.85-1.95)	0.23
More than half the time	105	reference	(0.26)[a]	reference	(0.25)[a]	reference	(0.45)[a]

Variable	Numbers of participants	Any risk HPV		Oncogenic HPV		Non-oncogenic HPV	
		Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value
Circumcised							
Yes	261	1.12 (0.77-1.62)	0.57	1.15 (0.73-1.82)	0.55	1.08 (0.74-1.59)	0.68
No	57	reference		reference		reference	
History of genital warts							
Current/Ever	35	1.98 (1.39-2.81)	0.0001	2.10 (1.37-3.23)	0.001	1.87 (1.27-2.77)	0.002
Never	283	reference		reference		reference	
History of chlamydia infection							
Ever	18	1.32 (0.75-2.30)	0.34	1.26 (0.63-2.51)	0.51	1.36 (0.80-2.31)	0.25
Never	300	reference		reference		reference	
History of gonorrhea infection							
Ever	11	0.45 (0.14-1.49)	0.19	0.66 (0.19-2.36)	0.52	0.27 (0.06-1.17)	0.08
Never	307	reference		reference		reference	
History of hepatitis B infection							
Current/Ever	5	0.53 (0.11-2.44)	0.41	0.45 (0.10-2.05)	0.30	0.60 (0.13-2.80)	0.51

Variable	Numbers of participants	Any risk HPV		Oncogenic HPV		Non-oncogenic HPV	
		Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value
Never	313	reference		reference		reference	
History of hepatitis C infection							
Current/Ever	3	3.01 (1.85-4.91)	< 0.001	2.84 (1.21-6.66)	0.02	3.15 (2.00-4.96)	<0.0001
Never	314	reference		reference		reference	
History of genital herpes infection							
Current/Ever	15	1.39 (0.84-2.31)	0.20	1.99 (1.09-3.61)	0.02	0.94 (0.49-1.80)	0.94
Never	303	reference		reference		reference	
History of non-gonococcal urethritis							
Ever	5	1.11 (0.77-1.59)	0.59	0.19 (0.04-0.82)	0.03	1.96 (1.27-3.01)	0.002
Never	313	reference		reference		reference	
History of yeast infection							
Ever	7	0.54 (0.23-1.28)	0.16	0.68 (0.23-2.03)	0.49	0.42 (0.19-0.96)	0.04
Never	311	reference		reference		reference	

a: type 3 tests with sandwich variance estimate
b: ever smoked cigarettes daily for at least 6 months or more

Variable	Numbers of participants	Any risk HPV		Oncogenic HPV		Non-oncogenic HPV	
		Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value
c: ever drink alcohol at least once a week for 6 months or more							

Table 3.5 Final Cox proportional hazard model of the association between dietary folate intake and the acquisition of any risk HPV infections

Outcome	Variable	Univariate analysis		Multivariable analysis	
		Hazard Ratio (95% CI)	**P-value**	**Hazard Ratio[a] (95% CI)**	**P-value**
Any risk HPVs	**Folate, ug DFE per 1,000 kcal/ day**				
	=< 243.40	0.76 (0.52-1.10)	0.14	0.74 (0.52-1.05)	0.09
	> 243.40 to =< 301.42	1.00 (0.71-1.41)	0.99	0.97 (0.71-1.32)	0.85
	> 301.42	reference	(0.24)[b]	reference	(0.20)[b]
	Age, years				
	< 24 years	0.89 (0.66-1.19)	0.43	1.10 (0.75-1.60)	0.68
	>= 24 years	reference		reference	
	Education				
	No college/some college	1.48 (0.93-2.36)	0.10	1.68 (1.01-2.81)	0.05
	Bachelor's degree	1.45 (0.86-2.45)	0.17	1.17 (0.67-2.03)	0.59
	Graduate/professional degree	reference	(0.25)[b]	reference	(0.07)[b]
	Number of lifetime female sexual partners				
	0 to 1	reference	(< 0.0001)[b]	reference	(< 0.0001)[b]

	2 to 10	2.85 (1.29-6.31)	0.01	5.10 (1.89-13.74)	0.001
	> 10	6.30 (2.89-13.76)	< 0.0001	10.78 (4.06-28.64)	< 0.0001
	Sexual preference				
	Sex with women only	reference		reference	
	Sex with men only/Sex with women and men	0.78 (0.53-1.15)	0.21	0.75 (0.46-1.22)	0.25
	Condom use within past 4 months				
	No	1.15 (0.80-1.65)	0.46	1.05 (0.76-1.46)	0.76
	Half and less than half the time	1.37 (0.94-2.01)	0.10	1.24 (0.89-1.73)	0.20
	More than half the time	reference	(0.26)[b]	reference	(0.43)[b]
	History of hepatitis C infection				
	Current/Ever	3.01 (1.85-4.91)	< 0.001	2.93 (1.97-4.36)	< 0.0001
	Never	reference		reference	

a: adjusted for age, education, number of lifetime female sexual partners, sexual preference, condoms use in the past 4 months, and history of HCV infection
b: type 3 tests with sandwich variance estimate

Table 3.6 Final Cox proportional hazard model of the association between dietary vitamin B12 intake and the acquisition of any risk HPV infections

Outcome	Variable	Univariate analysis		Multivariable analysis	
		Hazard Ratio (95% CI)	**P-value**	**Hazard Ratio[a] (95% CI)**	**P-value**
Any risk HPVs	**B12, ug per 1,000 kcal/ day**				
	=< 1.94	0.75 (0.52-1.10)	0.14	1.01 (0.70-1.45)	0.97
	> 1.94 to =< 2.57	1.02 (0.73-1.44)	0.89	1.42 (1.04-1.95)	0.03
	> 2.57	reference	(0.20)[b]	reference	(0.03)[b]
	Age, years				
	< 24 years	0.89 (0.66-1.19)	0.43	1.10 (0.75-1.60)	0.63
	>= 24 years	reference		reference	
	Education				
	No college/some college	1.48 (0.93-2.36)	0.10	1.79 (1.03-3.09)	0.04
	Bachelor's degree	1.45 (0.86-2.45)	0.17	1.31 (0.75-2.30)	0.35
	Graduate/professional degree	reference	(0.25)[b]	reference	(0.08)[b]
	Number of lifetime female sexual partners				
	0 to 1	reference	(< 0.0001)[b]	reference	(< 0.0001)[b]

	2 to 10	2.85 (1.29-6.31)	0.01	5.22 (1.95-13.97)	0.001
	> 10	6.30 (2.89-13.76)	< 0.0001	11.13 (4.22-29.36)	(< 0.0001)
	Sexual preference				
	Sex with women only	reference		reference	
	Sex with men only/Sex with women and men	0.78 (0.53-1.15)	0.21	0.75 (0.46-1.21)	0.24
	Condom use within past 4 months				
	No	1.15 (0.80-1.65)	0.46	1.15 (0.83-1.59)	0.40
	Half and less than half the time	1.37 (0.94-2.01)	0.10	1.32 (0.94-1.84)	0.10
	More than half the time	reference	(0.26)[b]	reference	(0.27)[b]
	History of hepatitis C infection				
	Current/Ever	3.01 (1.85-4.91)	< 0.001	2.93 (1.82-4.72)	< 0.001
	Never	reference		reference	

a: adjusted for age, education, number of lifetime female sexual partners, sexual preference, condoms use in the past 4 months, and history of HCV infection
b: type 3 tests with sandwich variance estimate

Table 3.7 Final Cox proportional hazard model of the association between dietary folate intake and the acquisition of oncogenic HPV infections

Outcome	Variable	Univariate analysis		Multivariable analysis	
		Hazard Ratio (95% CI)	**P-value**	**Hazard Ratio[a] (95% CI)**	**P-value**
oncogenic risk HPVs	**Folate, ug DFE per 1,000 kcal/ day**				
	=< 243.40	0.75 (0.46-1.21)	0.24	0.93 (0.58-1.47)	0.75
	> 243.40 to =< 301.42	1.00 (0.67-1.51)	0.98	1.16 (0.80-1.69)	0.43
	> 301.42	reference	(0.42)[b]	reference	(0.58)[b]
	Age, years				
	< 24 years	0.81 (0.57-1.16)	0.25	1.10 (0.75-1.60)	0.63
	>= 24 years	reference		reference	
	Number of lifetime female sexual partners				
	0 to 1	reference	(<0.0001)[b]	reference	(<0.0001)[b]
	2 to 10	1.87 (0.80-4.42)	0.15	1.78 (0.74-4.31)	0.20
	> 10	4.45 (1.90-10.43)	0.001	3.91 (1.59-9.60)	0.003
	Sexual preference				
	Sex with women only	reference		reference	

	Sex with men only/Sex with women and men	0.69 (0.45-1.07)	0.10	0.65 (0.40-1.07)	0.09
	History of genital warts				
	Current/Ever	2.10 (1.37-3.23)	0.001	1.84 (1.21-2.80)	0.004
	Never	reference			
	History of hepatitis C infection				
	Current/Ever	2.84 (1.21-6.66)	0.02	2.97 (1.33-6.59)	0.008
	Never	reference		reference	
a: adjusted for age, number of lifetime female sexual partners, sexual preference, history of genital warts, and history of HCV infection b: type 3 tests with sandwich variance estimate					

Table 3.8 Final Cox proportional hazard model of the association between dietary vitamin B12 intake and the acquisition of oncogenic HPV infections

Outcome	Variable	Univariate analysis		Multivariable analysis	
		Hazard Ratio (95% CI)	P-value	Hazard Ratio[a] (95% CI)	P-value
oncogenic risk HPVs	**B12, ug per 1,000 kcal/ day**				
	=< 1.94	0.67 (0.43-1.05)	0.08	0.81 (0.53-1.26)	0.35
	> 1.94 to =< 2.57	0.94 (0.62-1.44)	0.78	1.03 (0.70-1.52)	0.86
	> 2.57	reference	(0.16)[b]	reference	(0.46)[b]
	Age, years				
	< 24 years	0.81 (0.57-1.16)	0.25	1.12 (0.77-1.63)	0.56
	>= 24 years	reference		reference	
	Number of lifetime female sexual partners				
	0 to 1	reference	(<0.0001)[b]	reference	(<0.0001)[b]
	2 to 10	1.87 (0.80-4.42)	0.15	1.80 (0.75-4.32)	0.19
	> 10	4.45 (1.90-10.43)	0.001	3.90 (1.60-9.51)	0.003
	Sexual preference				
	Sex with women only	reference		reference	

	Sex with men only/Sex with women and men	0.69 (0.45-1.07)	0.10	0.68 (0.42-1.10)	0.11
	History of genital warts				
	Current/Ever	2.10 (1.37-3.23)	0.001	1.88 (1.24-2.85)	0.0003
	Never	reference		reference	
	History of hepatitis C infection				
	Current/Ever	2.84 (1.21-6.66)	0.02	2.91 (1.32-6.41)	0.008
	Never	reference		reference	
a: adjusted for age, number of lifetime female sexual partners, sexual preference, history of genital warts, and history of HCV infection b: type 3 tests with sandwich variance estimate					

Table 3.9 Final Cox proportional hazard model of the association between dietary folate intake and the acquisition of non-oncogenic HPV infections

Outcome	Variable	Univariate analysis		Multivariable analysis	
		Hazard Ratio (95% CI)	P-value	Hazard Ratio[a] (95% CI)	P-value
Non-oncogenic HPVs	**Folate, ug DFE per 1,000 kcal/ day**				
	=< 243.40	0.77 (0.53-1.11)	0.17	0.77 (0.52-1.13)	0.18
	> 243.40 to =< 301.42	0.91 (0.66-1.27)	0.99	0.94 (0.66-1.33)	0.71
	> 301.42	reference	(0.29)[b]	reference	(0.40)[b]
	Age, years				
	< 24 years	0.96 (0.69-1.32)	0.79	1.51 (1.08-2.11)	0.02
	>= 24 years	reference		reference	
	Number of lifetime female sexual partners				
	0 to 1	reference	(<.0001)[b]	reference	(<.0001)[b]
	2 to 10	4.81 (2.18-10.62)	0.0001	6.42 (2.25-18.32)	0.001
	> 10	10.02 (4.60-21.82)	<.0001	13.01 (4.60-36.79)	<.0001
	Condom use within past 4 months				
	No	1.04 (0.70-1.56)	0.84	0.95 (0.66-1.37)	0.79

	Half and less than half the time	1.29 (0.85-1.95)	0.23	1.20 (0.83-1.75)	0.34
	More than half the time	reference	(0.45)[b]	reference	(0.50)[b]
	History of hepatitis C infection				
	Current/Ever	3.15 (2.00-4.96)	<0.0001	2.75 (1.84-4.10)	<0.0001
	Never	reference		reference	

a: adjusted for age, number of lifetime female sexual partners, condoms use in the past 4 months, and history of HCV infection
b: type 3 tests with sandwich variance estimate

Table 3.10 Final Cox proportional hazard model of the association between dietary vitamin B12 intake and the acquisition of non-oncogenic HPV infections

Outcome	Variable	Univariate analysis		Multivariable analysis	
		Hazard Ratio (95% CI)	**P-value**	**Hazard Ratio[a] (95% CI)**	**P-value**
Non-oncogenic HPVs	**B12, ug per 1,000 kcal/ day**				
	=< 1.94	0.83 (0.56-1.22)	0.34	1.10 (0.75-1.60)	0.63
	> 1.94 to =< 2.57	1.10 (0.76-1.60)	0.61	1.47 (1.01-2.12)	0.04
	> 2.57	reference	(0.38)[b]	Reference	(0.10)[b]
	Age, years				
	< 24 years	0.96 (0.69-1.32)	0.79	1.53 (1.08-2.16)	0.02
	>= 24 years	reference		reference	
	Number of lifetime female sexual partners				
	0 to 1	reference	(<.0001)[b]	reference	(<.0001)[b]
	2 to 10	4.81 (2.18-10.62)	0.0001	6.50 (2.25-18.78)	0.001
	> 10	10.02 (4.60-21.82)	<.0001	13.57 (4.73-38.94)	<.0001
	Condom use within past 4 months				

	No	1.04 (0.70-1.56)	0.84	1.04 (0.72-1.49)	0.85
	Half and less than half the time	1.29 (0.85-1.95)	0.23	1.30 (0.90-1.88)	0.16
	More than half the time	reference	(0.45)[b]	reference	(0.35)[b]
	History of hepatitis C infection				
	Current/Ever	3.15 (2.00-4.96)	<0.0001	2.64 (1.81-3.85)	<0.0001
	Never	reference		reference	
a: adjusted for age, number of lifetime female sexual partners, condoms use in the past 4 months, and history of HCV infection b: type 3 tests with sandwich variance estimate					

Chapter 4

Examining the Relationship between Dietary Folate and Vitamin B12 Intake and Clearance of Genital Genotype Specific Incident HPV Infections

Introduction

Globally, genital HPV infection is one of the most common STIs. The estimated lifetime probability of acquiring HPV in sexually active adults is up to 90% [1]. Due to the body's cell-mediated immunity (CMI) response toward the clearing of HPV, most infections are transient and asymptomatic, while persistent infection increases the likelihood of anogenital warts and cancers [2-3]. Worldwide, almost 5% of all cancers are attributable to HPV, of which 630,000 new cancer cases occur each year [79]. Among these, the majority are cervical cancer cases, while the incidence of penile cancer is very low. Even though the majority of HPV attributable cancer affects women, men play an important role in the infection of women [32]. At present, a detailed understanding about the natural history of HPV in men is still lacking. These HPV related cancer cases present unique opportunities for prevention. To reduce the burden of HPV-related diseases, the modification of significant cofactors that affect the progression from asymptomatic HPV infection to persistent infection and HPV-related diseases are crucial.

Significant determinants in the natural history of HPV infection, including viral and individual attributes, play an important role in the clearance of genital HPV in men. The median duration of HPV infection differs according to HPV specific genotypes. HPV 16 has the longest median duration of infection (12.19 months) compared with any other HPV genotypes (7.52 months, on average) [61]. Increasing age and fewer risky sexual preference contribute to greater probability of clearing genital HPV infection [63]. Circumcision status affects the clearance of

genital HPV, as the median duration of HPV infection at the glans/coronal sulcus is longer in uncircumcised men [46]. Additional interesting co-factors that are often overlooked by researchers are individuals' nutritional status, as it plays a significant role in modifying host immunity which might affect the clearance of HPV. Nutritional status might affect the clearance of HPV by promoting the apoptosis of HPV infected cells [80]. At present, few epidemiologic studies demonstrated that particular plasma/ serum micronutrients such as folate, carotene, and tocopherol might affect the clearance of genital HPV. Individuals with higher concentrations of these micronutrients had the shorter duration of anogenital HPV infection [64]. Due to the limited numbers of publications, the role of nutrition on the clearance of genital HPV is still inconclusive. To our knowledge, at present, only four published articles focus on the role of nutrition and the clearance of HPV infection [43, 62, 64, 81]. The summaries of these studies are included in the appendix. No published studies focus on the dietary folate and vitamin B12 intakes and the clearance of genotype specific HPV infections.

Methods

Assessment of HPV status

HPV positive was defined by a positive HPV test for at least one of the following genital specimens: glans penis, coronal sulcus, foreskin, shaft, or scrotum. To prevent bias in clearance, we focused mainly on incident HPV infections – HPV infections first detected at the second or subsequent visit. The time to clearance was defined as the time from HPV detection until the time that participant had his first negative result for that particular genotype.

Assessment of dietary intakes

Using the validated QFFQ, Dietary folate (µg DFE per day), vitamin B12 (µg per day), and energy (kcal per day) intake derived from the baseline interview were used in this analysis. All dietary intakes were presented as nutrient densities. Dietary folate (µg DFE per 1,000 kcal per day) and vitamin B12 (µg per 1,000 kcal per day) intake were categorized according to the tertile cut points, which were the same cut points used in our second research question [64].

Measure of potential confounders

As we did in the analysis of our first research question, selected covariates derived from the baseline interview regarding sociodemographic, reproductive, medical, and sexual history were evaluated and used as potential confounders in the multivariable analysis.

Statistical analysis

Statistical analysis was conducted using SAS software version 9.4 (SAS Institute, Cary, NC). Sociodemographic characteristics and lifestyle variables of included men were summarized by using frequency statistics, and the results are given in Table 4.1. For this study, each genotype specific HPV infections was used as a separate unit of analysis. We excluded infections with unclassified HPV genotypes (XX) and clinic visits with missing or inadequate specimens. To test the hypothesis of whether dietary folate and vitamin B12 intake were associated with clearance of genotype specific incident HPV infections, recurrent events survival analysis was conducted. We used the WLW approach, which accounts for the concurrent clearance of more than one genotype specific HPV infections [74]. For the time metric, we utilized days since participant acquired genotype specific incident HPV infection until he cleared that particular HPV genotype. To account for the correlation within subjects, we used the robust sandwich variance estimate to test the hypotheses and confidence intervals of model parameters [75]. We conducted the

Schoenfeld residual analysis to test the proportional hazard assumption regarding each category of dietary folate and vitamin B12 intake and the hazard of clearance of HPV infection. Univariate analysis was conducted by using each baseline sociodemographic and lifestyle variable as an independent variable and used clearance of incident genotype specific HPV as a dependent variable. Two separate multivariable analyses were conducted with dietary folate or vitamin B12 intake as independent variables. The purposeful selection algorithm was used for variable selection to select potential confounders to include in the multivariable models [65]. Further conducted analyses were classified genotype specific clearance of the HPV infection according to the oncogenic or non-oncogenic genotypes.

Results

This third study focused on clearance of genotype specific HPV infections. Among 445 adult men in the Hawai‘i HPV male cohort, we included only men who had at least one genotype specific incident HPV infection and at least one follow-up visit after acquiring that particular HPV genotype, so that we could assess the clearance status. Men who completed the QFFQ, reported consuming less than 10,000 kcal of energy intake per day, and self-reported negative HIV infection status were included in this analysis. A total of 149 men were eligible for this third research question. Figure 4.1 summarizes the flow diagram of included and excluded participants.

The basic demographics and lifestyle variables of the study population are shown in Table 4.1. The mean (SD) age of participants was 29.3 (12.2) years. The majority of participant (82.4%, 122/148) were heterosexual. Half of participants (50%, 72/144) had their first sexual intercourse with a female when they were 16-18 years old. The median (range) number of lifetime female sexual partners was 10 (1-1,000). During the past four months, 65.3% (77/118) of participants used

condoms when they had sexual intercourse with a female. Among these, 58.4% (45/77) used condoms more than half of the time. Approximately 35% (52/149) of participants reported ever having smoked cigarettes daily for at least six months. Among these, 44% (23/52) were current smokers. Approximately 60% (92/149) of participants reported ever having drunk alcohol at least once a week for at least six months.

There were 149 men who acquired at least one incident genotype specific HPV infection and had at least one visit after acquiring infection. A total of 407 incident genotype specific HPV infections were cleared during the study period. Among these, 46.8% (191/408) genotypes were oncogenic HPVs, while 53.2 (217/408) genotypes were non-oncogenic HPVs. The distribution of incident genotype specific HPV infections that were cleared by dietary folate and vitamin B12 intakes status is summarized are Tables 4.2 and 4.3. The distributions of total follow-up time (person-month) by dietary folate and vitamin B12 intakes status are summarized in the same table.

Figures 4.2 and 4.3 show Schoenfeld residual plots between the tertiles of dietary intakes and the follow-up time to HPV clearance. The plot of three tertiles of dietary vitamin B12 intakes did not violate the proportional hazards assumption. However, the plot of dietary folate intake violated the proportional hazard assumption, of which an inflection located between 100 -160 days. We selected 130 days as the change point for the clearance analysis, as this was the median time interval between the second and the third clinic visits. The Cox model was fit separately for the transient stage of HPV infection (first 130 days after acquisition) and persistent stage (from 131 days until clearance or censoring) for the clearance analysis of dietary folate intake.

Tables 4.2-4.4 shown the results of survival analysis for nutrients variables associated with clearance of incident genotype specific HPV infections, which include men who had the highest

tertile of nutrients intakes as a reference group. Regarding transient stage (< 130 days) of acquiring HPV infections, univariate analysis shows that men whose dietary folate intake was in the lowest tertile had a higher rate of clearance of incident genotype specific HPV infections for any risk and non-oncogenic HPV infections. The HR = 1.16 (95% CI 0.79-1.71) for any risk HPV infection and the HR = 1.40 (95% CI 0.86-2.28) for non-oncogenic HPV infection. Regarding oncogenic HPV infection, men whose dietary folate intake was in the lowest tertile had a lower rate of clearance of incident genotype specific HPV infections: the HR = 0.92 (95% CI 0.56-1.52). Men whose dietary folate intake was in the middle tertile had a higher rate of clearance of incident genotype specific HPV infections for any risk, oncogenic, and non-oncogenic HPV infections. The HR = 1.18 (95% CI 0.81-1.71) for any risk HPV infections, the HR = 1.09 (95% CI 0.69-1.75) for oncogenic HPV infections, and the HR = 1.28 (95% CI 0.81-2.01) for non-oncogenic HPV infections. After adjustment for potential confounders, the effect estimates slightly changed. Men whose dietary folate intake was in the lowest tertile: the HR = 1.28 (95% CI 0.87-1.88) for any risk HPV infections, the HR = 1.18 (95% CI 0.74-1.88) for oncogenic HPV infections, and the HR = 1.42 (95% CI 0.85-2.36) for non-oncogenic HPV infections. Men whose dietary folate intake was in the middle tertile: the HR = 1.19 (95% CI 0.83-1.70) for any risk HPV infections, the HR = 1.15 (95% CI 0.72-1.83) for oncogenic HPV infections, and the HR = 1.25 (95% CI 0.82-1.90) for non-oncogenic HPV infections.

Regarding persistent stage (> 130 days) of acquiring HPV infections, univariate analysis shows that men whose dietary folate intake was in the lowest and middle tertiles had a higher rate of clearance of incident genotype specific HPV infections for any risk and non-oncogenic HPV infections. Regarding any risk HPV infections, men whose dietary intake was in the lowest and middle tertiles: the HR = 1.00 (95% CI 0.54-1.18) and the HR = 1.51 (95% CI 0.68-1.95),

respectively. Regarding non-oncogenic HPV infections, men whose dietary intake was in the lowest and middle tertiles: the HR = 1.34 (95% CI 0.59-3.05) and the HR = 1.53 (95% CI 0.78-2.99), respectively. Regarding oncogenic HPV infection, men whose dietary folate intake was in the lowest and middle tertiles had a lower rate of clearance of incident genotype specific HPV infections: the HR = 0.70 (95% CI 0.34-1.46) and the HR = 0.82 (95% CI 0.42-1.62), respectively. After adjustment for potential confounders, almost all the HRs (and 95% CIs) attenuated toward the null. Regarding any risk HPV infections, men whose dietary intake was in the lowest and middle tertiles: the HR = 0.81 (95% CI 0.46-1.43) and the HR = 1.31 (95% CI 0.78-2.20), respectively. Regarding oncogenic HPV infections, men whose dietary intake was in the lowest and middle tertiles: the HR = 0.47 (95% CI 0.22-0.99) and the HR = 0.65 (95% CI 0.32-1.36), respectively. Regarding non-oncogenic HPV infection, men whose dietary intake was in the lowest and middle tertiles: the HR = 1.16 (95% CI 0.52-2.60) and the HR = 2.33 (95% CI 0.93-5.82), respectively.

Regarding dietary vitamin B12 intake, univariate analysis shows that men whose dietary vitamin B12 intake was in the lowest tertile had a lower rate of clearance of incident genotype specific HPV infections for any risk and oncogenic HPV infections. The HR = 0.91 (95% CI 0.67-1.25) for any risk HPV infections, and the HR = 0.77 (95% CI 0.52-1.15) for oncogenic HPV infections. Regarding non-oncogenic HPV infection, men whose dietary vitamin B12 intake was in the lowest tertile had a higher rate of clearance of incident genotype specific HPV infections: the HR 1.08 (95% CI 0.73-1.58). Among men whose dietary vitamin B12 intake was in the middle tertile: the HR = 0.86 (95% CI 0.64-1.16) for any risk HPV infections, the HR = 0.73 (95% CI 0.50-1.04) for oncogenic HPV infections, and the HR= 1.003 (95% CI 0.69-1.46) for non-oncogenic HPV infections. After adjustment for potential confounders, men whose dietary vitamin

B12 intake was in the lowest tertile: the HR = 0.94 (95% CI 0.73-1.23) for any risk HPV infections, the HR = 0.77 (95% CI 0.53-1.13) for oncogenic HPV infections, and the HR = 1.02 (95% CI 0.72-1.44) for non-oncogenic HPV infections. Men whose dietary vitamin B12 intake was in the middle tertile: the HR = 0.85 (95% CI 0.65-1.13) for any risk HPV infections, the HR = 0.69 (95% CI 0.48-0.99) for oncogenic HPV infections, and the HR = 0.97 (95% CI 0.67-1.39) for non-oncogenic HPV infections.

Table 4.5 shows the results of survival analysis for potential confounders initially identified as associated with clearance of incident genotype specific HPV infections. Age, education, and a history of gonorrhea infection reached statistically significant differences in univariate analysis of any risk, oncogenic, and non-oncogenic HPV infections. Sexual preference, number of lifetime female sexual partners, and a history of hepatitis B virus (HBV) infection reached statistically significant differences in univariate analysis of any risk and non-oncogenic HPV infections. A history of HCV infection reached a statistically significant difference in univariate analysis of any risk HPV infections. A history of non-gonococcal urethritis reached a statistically significant difference in univariate analysis of oncogenic HPV infections. Income reached a statistically significant difference in univariate analysis of non-oncogenic HPV infections. Tables 4.6-4.14 show final Cox proportional hazard models of the association between dietary folate and vitamin B12 intake and clearance of incident HPV infections – any risk, oncogenic, and non-oncogenic HPVs. Number of lifetime female sexual partners were independently associated with clearance of genotype specific HPV infections in most of the analyses.

Discussion

Among all participants (n=149), there were 408 HPV genotypes that cleared during the study period. The proportion of oncogenic HPVs (46.8%, 191/408) were slightly lower than non-oncogenic HPVs (53.2%, 217/408). Since, the proportion of non-oncogenic incident genotype specific HPVs is higher than the oncogenic HPVs among our study population, this might affect the different proportion of HPVs cleared during the study period.

The univariate analysis of the association between dietary folate intake and the clearance of incident genotype specific HPV infections during the transient stage does not support our hypothesis that men with lower levels of dietary folate intake will have a lower rate of HPV clearance. Even after adjustment for potential confounders, all the results still do not support our hypothesis Regarding the clearance during the persistent stage, only the clearance of oncogenic HPVs supports our hypothesis in both univariate and multivariable analysis. In multivariable analysis, men whose dietary folate intake was in the lowest tertile had 53% lower rate of oncogenic HPV clearance and this association reached a statistically significant difference. Men whose dietary folate intake was in the middle tertile had 35% lower rate of oncogenic HPV clearance. These results are somewhat consistent with previous publications [43, 81]. The study, by Piyathilake et al. in 2004, found that women with higher plasma and red blood cell (RBC) folate had higher odds of oncogenic HPV clearance, of which the OR (and 95% CI) were 2.50 (1.18-5.30) [43]. Another study, by Sedjo et al. in 2003, found that women with higher plasma folate had higher odds of oncogenic HPV clearance, of which the ORs (and 95% CIS) were 1.24 (0.56-2.76) for the middle tertile, and 1.73 (0.80-3.75) for the highest tertile [81]. However, as we stated in the discussion of our second study, the methodology of these studies is quite different from ours.

Regarding dietary vitamin B12 intake, the associations among any risk and oncogenic HPVs support our hypothesis. After adjustment for potential confounders, almost all the associations support our hypothesis throughout all analyses – any risk, oncogenic, and non-oncogenic HPV infections. Men whose dietary vitamin B12 intake was in the lowest tertile had 23% lower rate of HPV clearance in the oncogenic HPV analysis. Men whose dietary folate intake was in the middle tertile had 31% lower rate of oncogenic HPV clearance and this association reached a statistically significant difference. In 2003, Sedjo et al. investigated the association between plasma vitamin B12 and oncogenic HPV clearance. This study found inconsistent results, of which the ORs (and 95% CIs) were 1.24 (0.56-2.75) for women whose serum vitamin B12 was in the middle tertile, and 0.93 (0.40-2.14) for women whose serum vitamin B12 was in the lowest tertile [81].

Our study found that men with lower levels of dietary folate and vitamin B12 intakes might have a lower rate of oncogenic HPV clearance. One possible explanation is that both folate and vitamin B12 act in several metabolic pathways of DNA synthesis [36, 37]. They play a crucial role in modifying our body's immune system against the infection.

Regarding other confounders, younger men had a significantly lower rate of HPV clearance compared to the older group. This association reached a statistically significant difference in multivariable analysis. One possible explanation is that older men had a higher prevalence of HPV antibodies [6]. The greater number of lifetime sexual partners were independently associated with a slower rate of HPV clearance in almost most multivariable analysis. This finding is consistent in the clearance of genital HPVs among both genders [61, 63].

The strength of this third study is that, to our knowledge, is the first that investigates the association between dietary intakes and the clearance of incident genotype specific HPV infections among men. Second, our analytic method – recurrent survival analysis – allows us to capture all possible HPV clearance. Also, we did the robust sandwich variance estimate to account for the correlation within subjects. Third, our study conducted HPV testing frequently, giving the method a high sensitivity to detect the clearance of genotype specific HPVs. The limitation of this study is that we included only the clearance of incident genotype specific HPV infections, so the precision of estimation is limited. However, this approach prevents us from defining a re-activation of HPV as a recurrent infection, which can introduce misclassification bias in a study of the clearance of genotype specific HPV infections [82]. Another limitation is that the duration of the follow-up time was limited: the median duration of the longest follow-up time among each participant was 130 days. Some HPV genotypes, such as HPV-16, have long durations of infection. The median duration of infection for HPV-16 has been documented at over 12 months [61].

Conclusion

Men with lower levels of dietary folate and vitamin B12 intakes might have a lower rate of oncogenic HPV clearance. However, this association of dietary folate intake existed only during the persistent stage of acquiring incident genotype specific HPV infections.

Figure 4.1 Study flow chart of included and excluded participants

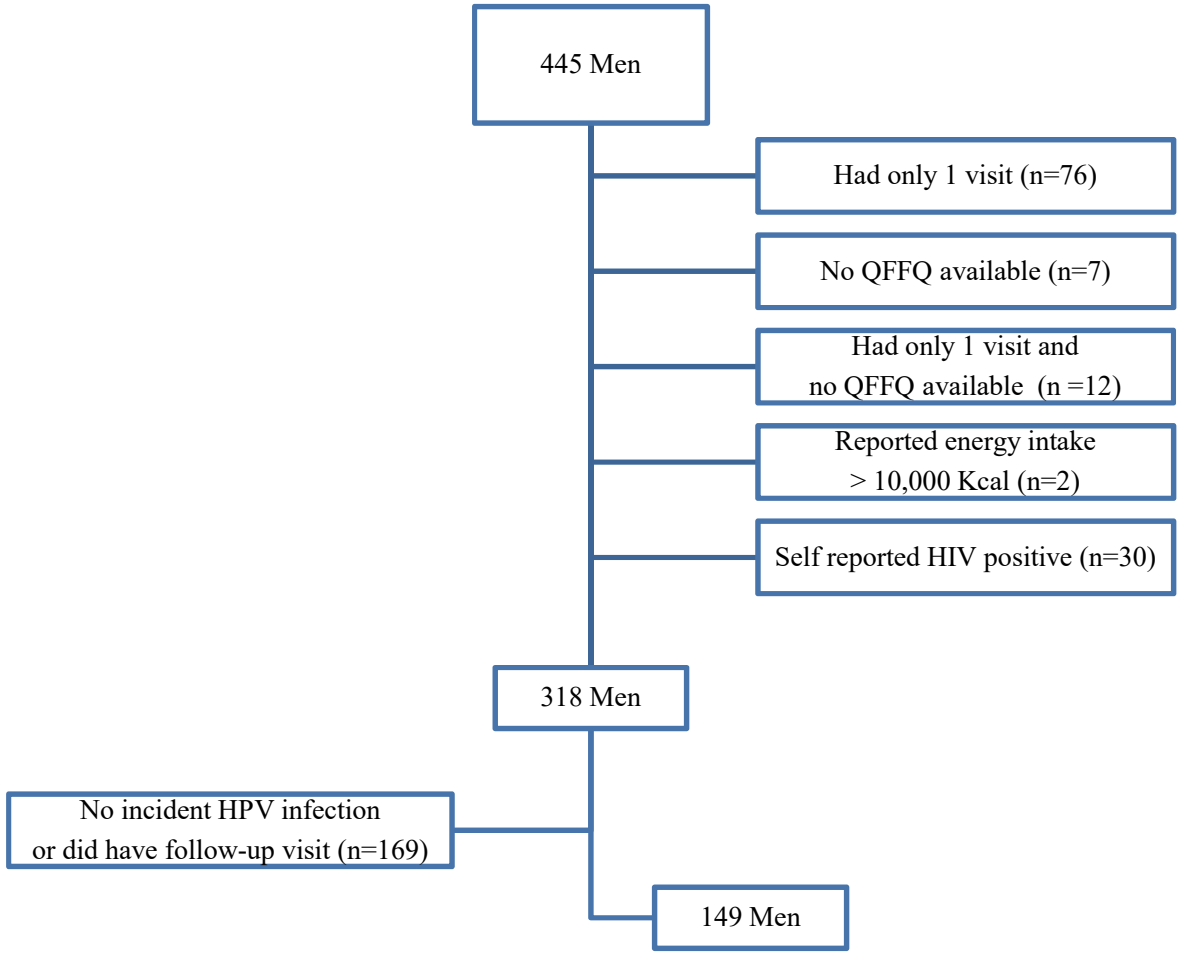


Figure 4.2 Scaled Schoenfeld residual for dietary folate intake

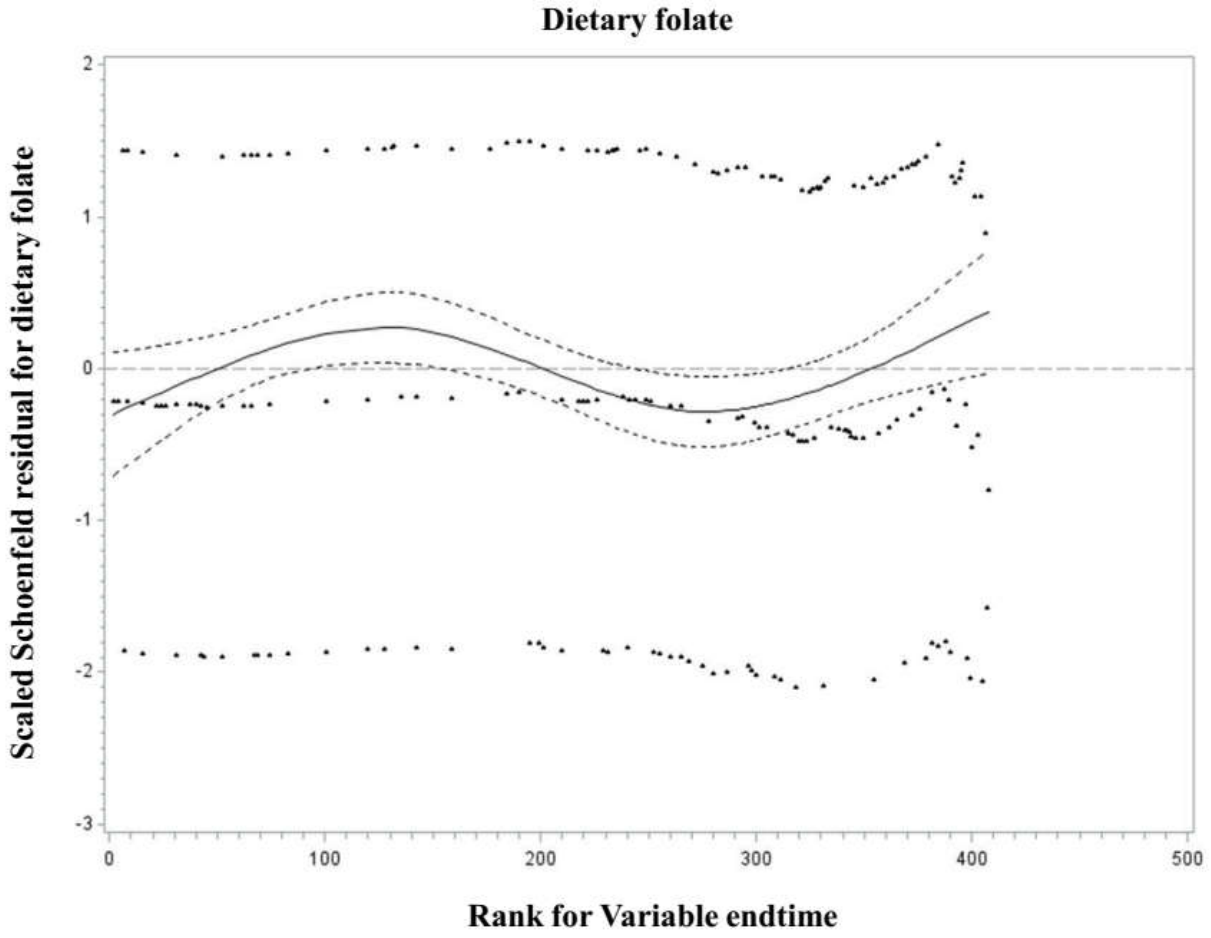


Figure 4.3 Scaled Schoenfeld residual for dietary vitamin B12 intake

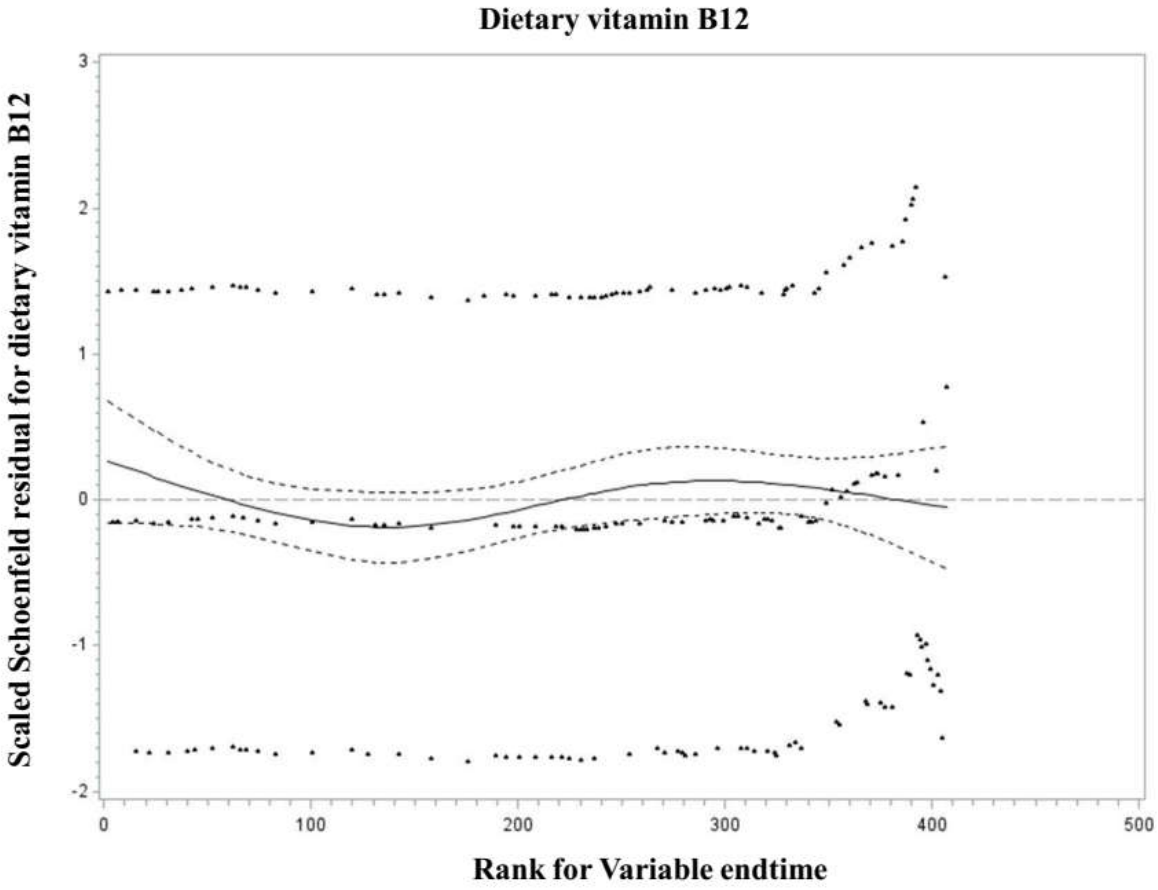


Table 4.1 Demographic and lifestyle variables (n=149)

Variable	All men (149 men) n (%)
Age	
< 24 years	68 (45.6)
>= 24 years	81 (54.4)
Race	
Non-White	55 (36.9)
White	94 (63.1)
Birthplace	
Non-USA	26 (17.5)
USA	123 (82.6)
Marital status	
Single/Divorced/Separated	128 (85.9)
Married/Cohabiting	21 (14.1)
Income	
< $1000	52 (34.9)

Variable	All men (149 men) n (%)
>= $1000 to < $1500	53 (35.6)
> $1500	44 (29.5)
Education	
High school graduate	2 (1.3)
Vocational/ technical/trade school or business college	4 (2.7)
Some college or university	90 (60.4)
Bachelor's degree	33 (22.2)
Graduate or professional degree	20 (13.4)
Number of female lifetime sexual partners	
0 to 1	8 (335.6)
2 to 10	70 (48.6)
> 10	66 (45.8)
Sexual preference	
No sex	1 (0.7)
Sex with women only	122 (82.4)

Variable	All men (149 men) n (%)
Sex with men only	4 (2.7)
Sex with women and men	21 (14.2)
Age at 1st SI with female, years	
< 16	33 (22.9)
16 to 18	72 (50.0)
> 18	39 (27.1)
Smoking habits	
Current	23 (15.0)
Ever[a]	29 (19.0)
Never	97 (65.0)
Alcohol drinking habits	
Ever[b]	92 (61.7)
Never	57 (38.3)
Condom use within past 4 months	
No	41 (34.8)

Variable	All men (149 men) n (%)
Half and less than half the time	32 (27.1)
More than half the time	45 (38.1)
Circumcised	
Yes	123 (82.6)
No	26 (17.4)
History of genital warts	
Current	11 (7.4)
Ever	14 (9.4)
Never	124 (83.2)
History of chlamydia infection	
Ever	12 (8.1)
Never	137 (91.9)
History of gonorrhea infection	
Ever	2 (1.3)
Never	147 (98.7)

Variable	All men (149 men) n (%)
History of hepatitis B infection	
Ever	2 (1.3)
Never	147 (98.7)
History of hepatitis C infection	
Ever	2 (1.4)
Never	146 (98.7)
History of genital herpes infection	
Current	6 (4.0)
Ever	4 (2.7)
Never	139 (93.3)
History of non-gonococcal urethritis	
Ever	3 (2.0)
Never	146 (98.0)
History of syphilis infection	
Ever	0

Variable	All men (149 men) n (%)
Never	149 (100)
History of yeast infection	
Ever	4 (2.7)
Never	145 (97.3)
a: ever smoked cigarettes daily for at least 6 months or more b: ever drink alcohol at least once a week for 6 months or more	

Table 4.2 Hazard ratios and 95% confidence intervals for clearance of incident HPV infections by levels of dietary folate intake (Transient infection, < 130 days)

Outcome	Variable	No. cleared/ Follow-up (person-months)	Univariate analysis		Multivariable analysis	
			Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
Any risk HPVs	**Folate, ug DFE per 1,000 kcal/ day**					
	=< 243.40	72/320.4	1.16 (0.79-1.71)	0.45	1.28 (0.87-1.88)[a]	0.20
	> 243.40 to =< 301.42	117/524.4	1.18 (0.81-1.71)	0.39	1.19 (0.83-1.70)[a]	0.34
	> 301.42	110/572.1	reference	(0.66)[b]	reference	(0.44)[b]
Oncogenic HPVs	**Folate, ug DFE per 1,000 kcal/ day**					
	=< 243.40	30/153.9	0.92 (0.56-1.52)	0.75	1.18 (0.74-1.88)[c]	0.48
	> 243.40 to =< 301.42	54/243.7	1.09 (0.69-1.75)	0.71	1.15 (0.72-1.83)[c]	0.56
	> 301.42	55/268.9	reference	(0.80)[b]	Reference	(0.72)[b]
Non-Oncogenic HPVs	**Folate, ug DFE per 1,000 kcal/ day**					
	=< 243.40	42/166.5	1.40 (0.86-2.28)	0.18	1.42 (0.85-2.36)[a]	0.19
	> 243.40 to =< 301.42	63/280.7	1.28 (0.81-2.01)	0.29	1.25 (0.82-1.90)[a]	0.30
	> 301.42	55/303.2	reference	(0.40)[b]	reference	(0.39)[b]

a: adjusted for age, number of lifetime female sexual partners, and circumcision
b: type 3 test with sandwich variance estimates
c: adjusted for age, education, number of lifetime female sexual partners, and circumcision

Table 4.3 Hazard ratios and 95% confidence intervals for clearance of incident HPV infections by levels of dietary folate intake (Persistent infection, > 130 days)

Outcome	Variable	No. cleared/ Follow-up (person-months)	Univariate analysis		Multivariable analysis	
			Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
Any risk HPVs	**Folate, ug DFE per 1,000 kcal/ day**					
	=< 243.40	20/255.7	1.00 (0.54-1.18)	0.99	0.81 (0.46-1.43)[a]	0.47
	> 243.40 to =< 301.42	41/488.2	1.51 (0.68-1.95)	0.60	1.31 (0.78-2.20)[a]	0.30
	> 301.42	48/612.0	reference	(0.85)[b]	reference	(0.36)[b]
Oncogenic HPVs	**Folate, ug DFE per 1,000 kcal/ day**					
	=< 243.40	11/152.4	0.70 (0.34-1.46)	0.35	0.47 (0.22-0.99)[c]	0.04
	> 243.40 to =< 301.42	18/229.6	0.82 (0.42-1.62)	0.57	0.65 (0.32-1.36)[c]	0.26
	> 301.42	23/235.5	reference	(0.61)[b]	Reference	(0.12)[b]
Non-Oncogenic HPVs	**Folate, ug DFE per 1,000 kcal/ day**					
	=< 243.40	9/103.3	1.34 (0.59-3.05)	0.49	1.16 (0.52-2.60)[a]	0.71
	> 243.40 to =< 301.42	23/258.6	1.53 (0.78-2.99)	0.22	2.33 (0.93-5.82)[a]	0.07
	> 301.42	25/376.4	reference	(0.46)[b]	reference	(0.19)[b]

a: adjusted for age, number of lifetime female sexual partners, and circumcision
b: type 3 test with sandwich variance estimates
c: adjusted for age, education, number of lifetime female sexual partners, and circumcision

Table 4.4 Hazard ratios and 95% confidence intervals for clearance of incident HPV infection by levels of dietary vitamin B12 intake (n=148)

Outcome	Variable	No. cleared/ Follow-up (person-months)	Univariate analysis		Multivariable analysis	
			Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
Any risk HPVs	**B12, ug per 1,000 kcal/ day**					
	=< 1.94	123/657.8	0.91 (0.67-1.25)	0.56	0.94 (0.73-1.23)[a]	0.66
	> 1.94 to =< 2.57	135/752.10	0.86 (0.64-1.16)	0.33	0.85 (0.65-1.13)[a]	0.27
	> 2.57	149/693.4	reference	(0.60)[b]	reference	(0.54)[b]
Oncogenic HPVs	**B12, ug per 1,000 kcal/ day**					
	=< 1.94	51/282.8	0.77 (0.52-1.15)	0.21	0.77 (0.53-1.13)[c]	0.18
	> 1.94 to =< 2.57	62/356.7	0.73 (0.50-1.04)	0.08	0.69 (0.48-0.99)[c]	0.04
	> 2.57	77/334.9	reference	(0.18)[b]	reference	(0.11)[b]
Non-Oncogenic HPVs	**B12, ug per 1,000 kcal/ day**					
	=< 1.94	72/375	1.08 (0.73-1.58)	0.70	1.02 (0.72-1.44)[a]	0.91
	> 1.94 to =< 2.57	73/395.4	1.003 (0.69-1.46)	0.99	0.97 (0.67-1.39)[a]	0.85
	> 2.57	72/358.5	reference	(0.92)[b]	reference	(0.96)[b]

a: adjusted for age, number of lifetime sexual partners, and circumcision
b: type 3 test with sandwich variance estimates
c: adjusted for age, education, number of lifetime sexual partners, and circumcision

Table 4.5 Hazard ratios and 95% confidence intervals for clearance of incident HPV infections by potential confounders (n=149)

Variable	Numbers of participants	Any risk HPV		Oncogenic HPV		Non-oncogenic HPV	
		Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value
Age, years							
< 24 years	68	0.67 (0.51-0.86)	0.002	0.64 (0.47-0.87)	0.005	0.70 (0.50-0.97)	0.03
>= 24 years	81	reference		reference		reference	
Race							
Non-White	55	0.94 (0.70-1.24)	0.64	1.14 (0.78-1.67)	0.51	0.79 (0.56-1.10)	0.16
White	94	reference		reference		reference	
Birthplace							
Non-USA	26	1.04 (0.71-1.53)	0.84	1.25 (0.80-1.94)	0.33	0.93 (0.60-1.42)	0.72
USA	123	reference		reference		reference	
Marital status							
Single/Divorced/Separated	128	1.00 (0.71-1.42)	1.00	0.91 (0.61-1.36)	0.65	1.08 (0.68-1.72)	0.74
Married/Cohabiting	21	reference		reference		reference	
Education							

Variable	Numbers of participants	Any risk HPV		Oncogenic HPV		Non-oncogenic HPV	
		Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value
No college/some college	96	0.42 (0.31-0.56)	< 0.001	0.47 (0.31-0.72)	0.0005	0.35 (0.25-0.49)	< 0.0001
Bachelor's degree	33	0.64 (0.47-0.88)	0.01	0.87 (0.55-1.39)	0.57	0.47 (0.32-0.70)	0.0002
Graduate/professional degree	20	reference	(< 0.001)[a]	reference	(0.0002)[a]	reference	(< 0.0001)[a]
Income							
< $1000	52	reference	(0.25)[a]	reference	(0.32)[a]	reference	(0.02)[a]
>= $1000 to < $1500	53	1.27 (0.95-1.71)	0.11	1.12 (0.77-1.64)	0.55	0.70 (0.50-1.00)	0.05
> $1500	44	1.23 (0.87-1.72)	0.24	0.81 (0.53-1.22)	0.31	1.24 (0.83-1.86)	0.29
Number of lifetime female sexual partners							
0 to 1	8	reference	(0.004)[a]	reference	(0.10)[a]	reference	(0.007)[a]
2 to 10	70	0.53 (0.34-0.83)	0.01	0.59 (0.35-1.01)	0.06	0.32 (0.12-0.84)	0.02
> 10	66	0.78 (0.52-1.18)	0.24	0.83 (0.52-1.32)	0.44	0.50 (0.20-1.25)	0.14
Sexual preference							
Sex with women only	**122**	reference		reference		reference	
Sex with men only/Sex with women and men	25	1.58 (1.20-2.09)	0.001	1.32 (0.87-2.00)	0.19	1.79 (1.27-2.51)	0.001

Variable	Numbers of participants	Any risk HPV		Oncogenic HPV		Non-oncogenic HPV	
		Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value
Age at 1st SI with female, years							
< 16	33	0.75 (0.53-1.07)	0.12	0.66 (0.42-1.05)	0.08	0.83 (0.54-1.27)	0.40
16 to 18	72	0.87 (0.62-1.22)	0.41	0.77 (0.49-1.20)	0.25	0.93 (0.63-1.37)	0.70
> 18	39	reference	(0.28)[a]	reference	(0.21)[a]	reference	(0.69)[a]
Smoking habits							
Current/Ever[b]	52	0.98 (0.74-1.30)	0.88	0.84 (0.60-1.16)	0.29	1.09 (0.77-1.53)	0.64
Never	97	reference		reference		reference	
Alcohol drinking habits							
Ever[c]	92	1.18 (0.88-1.59)	0.26	1.08 (0.76-1.52)	0.68	1.28 (0.90-1.81)	0.17
Never	57	reference		reference		reference	
Condom use within past 4 months							
No	41	1.08 (0.77-1.53)	0.65	1.06 (0.68-1.65)	0.81	1.14 (0.73-1.77)	0.57
Half and less than half the time	32	0.87 (0.61-1.25)	0.46	0.99 (0.64-1.53)	0.95	0.79 (0.52-1.19)	0.26
More than half the time	45	reference	(0.50)[a]	reference	(0.94)[a]	reference	(0.24)[a]

Variable	Numbers of participants	Any risk HPV		Oncogenic HPV		Non-oncogenic HPV	
		Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value
Circumcised							
Yes	123	reference		reference		reference	
No	26	0.99 (0.68-1.45)	0.96	1.12 (0.73-1.72)	0.61	0.91 (0.60-1.40)	0.67
History of genital warts							
Current/Ever	25	1.09 (0.83-1.42)	0.54	1.22 (0.87-1.72)	0.25	0.998 (0.69-1.44)	0.99
Never	124	reference		reference		reference	
History of chlamydia infection							
Ever	12	0.88 (0.64-1.22)	0.44	0.67 (0.34-1.32)	0.25	1.05 (0.72-1.53)	0.80
Never	137	reference		reference		reference	
History of gonorrhea infection							
Ever	2	0.47 (0.39-0.58)	< 0.0001	0.42 (0.31-0.56)	< 0.0001	0.53 (0.42-0.65)	< 0.0001
Never	147	reference		reference		reference	
History of hepatitis B infection							
Current/Ever	1	1.34 (1.16-1.54)	< 0.0001	0.93 (0.76-1.15)	0.50	1.84 (1.51-2.25)	< 0.0001

Variable	Numbers of participants	Any risk HPV		Oncogenic HPV		Non-oncogenic HPV	
		Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value
Never	148	reference		reference		reference	
History of hepatitis C infection							
Current/Ever	2	1.63 (1.08-2.44)	0.02	1.90 (0.82-4.39)	0.13	1.43 (0.95-2.18)	0.09
Never	146	reference		reference		reference	
History of genital herpes infection							
Current/Ever	10	1.19 (0.90-1.58)	0.21	1.29 (0.86-1.94)	0.22	1.11 (0.56-2.22)	0.76
Never	139	reference		reference		reference	
History of non-gonococcal urethritis							
Ever	3	1.12 (0.55-2.26)	0.76	2.65 (2.01-3.48)	< 0.0001	1.04 (0.55-1.97)	0.90
Never	146	reference		reference		reference	
History of yeast infection							
Ever	4	1.43 (0.94-2.19)	0.10	1.30 (0.56-3.04)	0.54	1.72 (1.07-2.74)	0.02
Never	145	reference		reference		reference	

a: type 3 test with sandwich variance estimates
b: ever smoked cigarettes daily for at least 6 months or more

Variable	Numbers of participants	Any risk HPV		Oncogenic HPV		Non-oncogenic HPV	
		Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value	Uni/Bivariate analysis	P-value
b: ever drink alcohol at least once a week for 6 months or more							

Table 4.6 Final Cox proportional hazard model of the association between dietary folate intake and clearance of incident HPV infections (Any risk, Transient infection, < 130 days)

Outcome	Variable	Univariate analysis		Multivariable analysis	
		Hazard Ratio (95% CI)	**P-value**	**Hazard Ratio[a] (95% CI)**	**P-value**
Any risk HPVs	**Folate, ug DFE per 1,000 kcal/ day**				
	=< 243.40	1.16 (0.79-1.71)	0.45	1.28 (0.87-1.88)	0.20
	> 243.40 to =< 301.42	1.18 (0.81-1.71)	0.39	1.19 (0.83-1.70)	0.34
	> 301.42	reference	(0.66)[b]	reference	(0.44)[b]
	Age, years				
	< 24 years	0.67 (0.49-0.92)	0.01	0.70 (0.50-0.98)	0.04
	>= 24 years	reference		reference	
	Number of lifetime female sexual partners				
	0 to 1	reference	(0.07)[b]	reference	(0.33)[b]
	2 to 10	0.69 (0.36-1.32)	0.26	0.84 (0.44-1.59)	0.59
	> 10	1.02 (0.56-1.86)	0.95	1.10 (0.61-1.99)	0.75
	Circumcised				

	Yes	1.09 (0.75-1.59)	0.65	1.01 (0.69-1.48)	0.95
	No	reference		reference	

a: adjusted for age, number of lifetime sexual partners, and circumcision
b: type 3 test with sandwich variance estimates

Table 4.7 Final Cox proportional hazard model of the association between dietary folate intake and clearance of incident HPV infections (oncogenic HPVs, Transient infection, < 130 days)

Outcome	Variable	Univariate analysis		Multivariable analysis	
		Hazard Ratio (95% CI)	**P-value**	**Hazard Ratio[a] (95% CI)**	**P-value**
Oncogenic HPVs	**Folate, ug DFE per 1,000 kcal/ day**				
	=< 243.40	0.92 (0.56-1.52)	0.75	1.18 (0.74-1.88)	0.48
	> 243.40 to =< 301.42	1.09 (0.69-1.75)	0.71	1.15 (0.72-1.83)	0.56
	> 301.42	reference	(0.80)[b]	Reference	(0.74)[b]
	Age, years				
	< 24 years	0.57 (0.38-0.86)	0.007	0.70 (0.43-1.13)	0.14
	>= 24 years	reference		reference	
	Education				
	No college/some college	0.48 (0.30-0.78)	0.003	1.46 (0.85-2.49)	0.17
	Bachelor's degree	0.78 (0.44-1.39)	0.40	1.78 (0.94-3.37)	0.08
	Graduate/professional degree	reference	(0.007)[b]	reference	(0.16)[b]
	No. lifetime sexual partners				

	0 to 1	reference	(0.46)[b]	reference	(0.58)[b]
	2 to 10	0.97 (0.37-2.57)	0.95	1.46 (0.85-2.49)	0.17
	> 10	1.28 (0.51-3.21)	0.61	1.78 (0.94-3.37)	0.08
	Circumcised				
	Yes	reference	0.57	0.94 (0.54-1.62)	0.81
	No	1.15 (0.72-1.84)		reference	
a: adjusted for age, education, number of lifetime sexual partners, and circumcision b: type 3 test with sandwich variance estimates					

Table 4.8 Final Cox proportional hazard model of the association between dietary folate intake and clearance of incident HPV infections (Non-oncogenic HPVs, Transient infection, < 130 days)

Outcome	Variable	Univariate analysis		Multivariable analysis	
		Hazard Ratio (95% CI)	P-value	Hazard Ratio[a] (95% CI)	P-value
Non-oncogenic HPVs	**Folate, ug DFE per 1,000 kcal/ day**				
	=< 243.40	1.40 (0.86-2.28)	0.18	1.42 (0.85-2.36)	0.19
	> 243.40 to =< 301.42	1.28 (0.81-2.01)	0.29	1.25 (0.82-1.90)	0.30
	> 301.42	reference	(0.40)[b]	reference	(0.39)[b]
	Age, years				
	< 24 years	0.76 (0.51-1.12)	0.17	0.83 (0.54-1.27)	0.40
	>= 24 years	reference		reference	
	Number of lifetime female sexual partners				
	0 to 1	reference	(0.02)[b]	reference	(0.04)[b]
	2 to 10	0.31 (0.12-0.83)	0.02	0.35 (0.13-0.92)	0.03
	> 10	0.50 (0.20-1.27)	0.15	0.52 (0.20-1.38)	0.19
	Circumcised				

	Yes	reference		reference	
	No	1.04 (0.66-1.65)	0.85	1.04 (0.67-1.62)	0.85
a: adjusted for age, number of lifetime sexual partners, and circumcision b: type 3 test with sandwich variance estimates					

Table 4.9 Final Cox proportional hazard model of the association between dietary folate intake and clearance of incident HPV infections (Any risk, Persistent infection, > 130 days)

Outcome	Variable	Univariate analysis		Multivariable analysis	
		Hazard Ratio (95% CI)	P-value	Hazard Ratio[a] (95% CI)	P-value
Any risk HPVs	**Folate, ug DFE per 1,000 kcal/ day**				
	=< 243.40	1.00 (0.54-1.86)	0.99	0.81 (0.46-1.43)	0.47
	> 243.40 to =< 301.42	1.51 (0.68-1.95)	0.60	1.31 (0.78-2.20)	0.30
	> 301.42	reference	(0.85)[b]	reference	(0.36)[b]
	Age, years				
	< 24 years	0.66 (0.42-1.04)	0.08	0.63 (0.38-1.05)	0.07
	>= 24 years	reference		reference	
	Number of lifetime female sexual partners				
	0 to 1	reference	(0.007)[b]	reference	(0.001)[b]
	2 to 10	0.18 (0.06-0.53)	0.002	0.12 (0.04-0.39)	0.001
	> 10	0.27 (0.09-0.76)	0.01	0.17 (0.05-0.52)	0.002
	Circumcised				

	Yes	reference		reference	
	No	0.71 (0.31-1.60)	0.40	0.48 (0.18-1.31)	0.15

a: adjusted for age, number of lifetime sexual partners, and circumcision
b: type 3 test with sandwich variance estimates

Table 4.10 Final Cox proportional hazard model of the association between dietary folate intake and clearance of incident HPV infections (oncogenic HPVs, Persistent infection, > 130 days)

Outcome	Variable	Univariate analysis		Multivariable analysis	
		Hazard Ratio (95% CI)	**P-value**	**Hazard Ratio[a] (95% CI)**	**P-value**
Oncogenic HPVs	**Folate, ug DFE per 1,000 kcal/ day**				
	=< 243.40	0.70 (0.34-1.46)	0.35	0.47 (0.22-0.99)	0.04
	> 243.40 to =< 301.42	0.82 (0.42-1.62)	0.57	0.66 (0.32-1.36)	0.26
	> 301.42	reference	(0.61)[b]	Reference	(0.12)[b]
	Age, years				
	< 24 years	0.85 (0.48-1.50)	0.58	1.37 (0.59-3.18)	0.47
	>= 24 years	reference		reference	
	Education				
	No college/some college	0.31 (0.14-0.67)	0.003	0.25 (0.08-0.81)	0.02
	Bachelor's degree	0.67 (0.16-2.72)	0.57	0.95 (0.27-3.35)	0.94
	Graduate/professional degree	reference	(0.009)[b]	reference	(0.004)[b]
	Number of lifetime female sexual partners				

	0 to 1	reference	(0.004)[b]	reference	(0.001)[b]
	2 to 10	0.12 (0.03-0.41)	0.001	0.07 (0.02-0.28)	0.0001
	> 10	0.19 (0.06-0.62)	0.006	0.10 (0.03-0.38)	0.0006
	Circumcised				
	Yes	reference		reference	
	No	0.98 (0.28-3.37)	0.97	0.44 (0.12-1.55)	0.20

a: adjusted for age, education, number of lifetime sexual partners, and circumcision
b: type 3 test with sandwich variance estimates

Table 4.11 Final Cox proportional hazard model of the association between dietary folate intake and clearance of incident HPV infections (Non-oncogenic HPVs, Persistent infection, > 130 days)

Outcome	Variable	Univariate analysis		Multivariable analysis	
		Hazard Ratio (95% CI)	P-value	Hazard Ratio[a] (95% CI)	P-value
Non-oncogenic HPVs	**Folate, ug DFE per 1,000 kcal/ day**				
	=< 243.40	1.34 (0.59-3.05)	0.49	1.16 (0.52-2.60)	0.71
	> 243.40 to =< 301.42	1.53 (0.78-2.99)	0.22	2.33 (0.93-5.82)	0.07
	> 301.42	reference	(0.46)[b]	reference	(0.39)[b]
	Age, years				
	< 24 years	0.56 (0.31-1.03)	0.06	0.37 (0.15-0.92)	0.03
	>= 24 years	reference		reference	
	No. lifetime sexual partners				
	0 to 1				
	2 to 10	reference		reference	
	> 10	1.36 (0.74-2.51)	0.33	0.87 (0.50-1.52)	0.63
	Circumcised				

	Yes	reference		reference	
	No	0.62 (0.24-1.62)	0.33	0.37 (0.08-1.65)	0.19

a: adjusted for age, number of lifetime sexual partners, and circumcision
b: type 3 test with sandwich variance estimates

Table 4.12 Final Cox proportional hazard model of the association between dietary vitamin B12 intake and clearance of incident HPV infections (Any risk HPVs)

Outcome	Variable	Univariate analysis		Multivariable analysis	
		Hazard Ratio (95% CI)	P-value	Hazard Ratio[a] (95% CI)	P-value
Any risk HPVs	**B12, ug per 1,000 kcal/ day**				
	=< 1.94	0.91 (0.67-1.25)	0.56	0.94 (0.73-1.23)	0.66
	> 1.94 to =< 2.57	0.86 (0.64-1.16)	0.33	0.85 (0.65-1.13)	0.27
	> 2.57	reference	(0.60)[b]	reference	(0.54)[b]
	Age, years				
	< 24 years	0.67 (0.51-0.86)	0.002	0.73 (0.57-0.95)	0.02
	>= 24 years	reference		reference	
	No. lifetime sexual partners				
	0 to 1	reference	(0.004)[b]	reference	(0.02)[b]
	2 to 10	0.53 (0.34-0.83)	0.01	0.52 (0.32-0.85)	0.01
	> 10	0.78 (0.52-1.18)	0.24	0.69 (0.44-1.08)	0.11
	Circumcised				

	Yes	0.99 (0.68-1.45)	0.96	0.88 (0.60-1.29)	0.52
	No	reference		reference	

a: adjusted for age, number of lifetime sexual partners, and circumcision
b: type 3 test with sandwich variance estimates

Table 4.13 Final Cox proportional hazard model of the association between dietary vitamin B12 intake and clearance of incident HPV infections (Oncogenic HPVs)

Outcome	Variable	Univariate analysis		Multivariable analysis	
		Hazard Ratio (95% CI)	**P-value**	**Hazard Ratio[a] (95% CI)**	**P-value**
Oncogenic HPVs	**B12, ug per 1,000 kcal/ day**				
	=< 1.94	0.77 (0.52-1.15)	0.21	0.80 (0.54-1.18)	0.27
	> 1.94 to =< 2.57	0.73 (0.50-1.04)	0.08	0.69 (0.48-0.98)	0.04
	> 2.57	reference	(0.18)[b]	reference	(0.11)[b]
	Age, years				
	< 24 years	0.64 (0.47-0.87)	0.005	0.91 (0.64-1.31)	0.62
	>= 24 years	reference		reference	
	Education				
	No college/some college	0.47 (0.31-0.72)	0.00001	0.48 (0.29-0.79)	0.004
	Bachelor's degree	0.87 (0.55-1.39)	0.57	0.84 (0.50-1.43)	0.53
	Graduate/professional degree	reference	(0.0002)[b]	reference	(0.001)[b]
	No. lifetime sexual partners				

	0 to 1	reference	(0.10)[b]	reference	(0.35)[b]
	2 to 10	0.59 (0.35-1.01)	0.06	0.65 (0.36-1.17)	0.15
	> 10	0.83 (0.52-1.32)	0.44	0.76 (0.46-1.27)	0.29
	Circumcised				
	Yes	reference		reference	
	No	1.12 (0.73-1.72)	0.61	0.75 (0.46-1.21)	0.24
a: adjusted for age, education, number of lifetime sexual partners, and circumcision b: type 3 test with sandwich variance estimates					

Table 4.14 Final Cox proportional hazard model of the association between dietary vitamin B12 intake and clearance of incident HPV infections (Non-oncogenic HPVs)

Outcome	Variable	Univariate analysis		Multivariable analysis	
		Hazard Ratio (95% CI)	P-value	Hazard Ratio[a] (95% CI)	P-value
Non-oncogenic HPVs	**B12, ug per 1,000 kcal/ day**				
	=< 1.94	1.08 (0.73-1.58)	0.70	1.02 (0.72-1.44)	0.91
	> 1.94 to =< 2.57	1.003 (0.69-1.46)	0.99	0.97 (0.67-1.39)	0.85
	> 2.57	reference	(0.92)[b]	reference	(0.96)[b]
	Age, years				
	< 24 years	0.70 (0.50-0.97)	0.03	0.77 (0.55-1.08)	0.14
	>= 24 years	reference		reference	
	No. lifetime sexual partners				
	0 to 1	reference	(0.007)[b]	reference	(0.02)[b]
	2 to 10	0.32 (0.12-0.84)	0.02	0.31 (0.12-0.78)	0.01
	> 10	0.50 (0.20-1.25)	0.14	0.43 (0.17-1.07)	0.07
	Circumcised				

	Yes	reference		reference	
	No	0.91 (0.60-1.40)	0.67	0.87 (0.55-1.36)	0.54

a: adjusted for age, number of lifetime sexual partners, and circumcision
b: type 3 test with sandwich variance estimates

Chapter 5

Discussions

The final chapter of this dissertation will discuss the innovation, strengths and limitations, and implication of the results as well as possible next steps. In order to aid our readers, we have provided a brief summary of the objectives of each research question and findings.

Summary of research questions

Research question 1: Do dietary folate and vitamin B12 intakes have an association with persistent genotype specific HPV infections?

Hypothesis: Men with lower levels of dietary folate and vitamin B12 intakes will have higher odds of persistent genotype specific HPV infections.

Conclusion: Our analyses do not provide enough evidence to support our hypothesis that men with lower dietary folate and vitamin B12 will be at lower odds of persistent genotype specific HPV infections.

Research question 2: Do dietary folate and vitamin B12 intakes have an association with acquisition of incident genotype specific HPV infections?

Hypothesis: Men with lower levels of dietary folate and vitamin B12 intakes will be at higher risk of acquiring incident genotype specific HPV infections.

Conclusion: Our analyses demonstrate that men with lower levels of dietary vitamin B12 intake might have a higher risk of acquiring any risk and non-oncogenic HPV infections. However, these associations do not exist among men with lower levels of dietary folate intake.

Research question 3: Do dietary folate and vitamin B12 intakes have an association with clearance of incident genotype specific HPV infections.

Hypothesis: Men with lower levels of dietary folate and vitamin B12 intakes will have a lower rate of clearance of incident genotype specific HPV infections.

Conclusion: Our analyses demonstrate that men with lower dietary folate intake might have a lower rate of oncogenic HPV clearance during the persistent stage (>131 days) of acquiring HPVs. Meanwhile, men with lower dietary vitamin B12 intake might have a lower rate of oncogenic HPV clearance.

Although we use three different approaches – persistent, acquisition, and clearance of HPVs – to study the role of dietary intake and the natural history of HPV infections among men, these three research questions shared the following innovation, strengths and limitations.

Innovation

To our knowledge, very few studies have investigated the association between dietary intakes and persistent genital HPV infection [33-35]. Only one study was conducted among men, and the authors focused on persistent genotype specific HPV infections, as we did in our first research question [34]. Compared to the only published study, our method of assessing participants' HPV status was similar, but participants were tested frequently to yield more sensitivity in capturing genotype specific persistent HPV infections. Our method of assessing participants' dietary intakes was structured with different detail variation to account for cultural dietary differences. Regarding the second and third research questions – acquisition and clearance of incident genotype specific HPV infections – until present, none of the published studies have been focused on the role of dietary intakes nor on the acquisition and clearance of incident

genotype specific HPV infections. The second and third studies in this dissertation were the first to assess these associations. The frequent HPV testing and the use of a highly sensitive method to detect the acquisition and clearance of incident genotype specific HPV infections did contribute to reduce measurement bias.

Strengths and limitations

The three research studies in this dissertation use strong methodology. First, a validated QFFQ was used to assess study participant's dietary intakes, which acted as a proxy for nutrition status. This questionnaire was primarily developed to use in a multi-ethnic cohort study in Hawai‘i and southern California; thus, it provides nutritional assessment in this population with reduced bias. Second, the intake of folate was estimated as dietary folate equivalents (DFE), which takes into account the different bioavailabilities of folate sources, as mandatory fortification of enriched cereal grain products with folic acid was fully implemented in the United States since 1998 [83]. This procedure reduces information bias in studying dietary folate intake. Third, HPV status was assessed by a PCR-based technique, which provides high sensitivity and specificity [84, 85]. Specimens testing positive were genotyped for 37 different HPV types. This genotype specific information mitigates bias in studying the persistence and clearance of genotype specific HPV infections. Fourth, HPV was assessed frequently to capture persistence, acquisition, and clearance of genotype specific HPV infections. Fifth, HPV was sampled from multiple sampling sites of study participants' external genitalia because this technique provides higher sensitivity in detecting genital HPV [85]. Sixth, we implement the purposeful selection of variables as our variable selection algorithm. Our multivariable model had the capacity to retain important confounding variables to yield an unbiased association between dietary intakes and genital HPV infections [65].

The major limitations of this study are first, that nutritional status is determined by dietary assessment using QFFQ. Even though this QFFQ was validated and the procedure showed medium to high correlation between dietary intake and the 24-hour dietary recall method, this method introduces recall bias, unlike biomarkers of nutritional exposure [52]. However, subjective dietary intake assessment represents a reasonable estimation of an individual's dietary intake over time and might be more informative to modify an individual's dietary habits by providing dietary recommendations. Second, only the baseline dietary information is available, so we cannot examine the relation of change in nutritional status over time with the persistence, acquisition, and clearance of HPV infection. Third, consumed dietary folate and vitamin B12 among this population is quite high. In other words, we have too few participants to study this association at low levels of dietary intake. Fourth, we did not have the information regarding body mass index (BMI) and physical activity which might significantly contribute to the association between dietary intake and HPV infection. In other words, there is potential confounding due to variables not included in the data set. Fifth, the study participants knew their HPV status, so they may have modified their risky behaviors toward acquiring HPV infection. This could have introduced misclassification bias of the observed association. Sixth, this database has a limited sample size. This precludes a stratified analysis focusing specifically on individual HPV genotypes and contributes to lack of precision in the estimates. Seventh, most of our study population are college students who might have different dietary, sociodemographic, and sexual behaviors compared to the general population. So, the generalizability of our results to other populations is limited. Reported income of students might not truly reflect the socioeconomic status of a student study population [86]. To deal with this issue, all our multivariable analyses did not include income as an adjusted covariate.

Implication of the results and Possible next steps

All the analyses in this dissertation investigate the association between dietary folate and vitamin B12 intake and the natural history of genital HPV infections among men. Since there are very few published articles of this association, this dissertation provides exploratory evidence about the role of dietary intakes and genital HPVs among men. Consuming a diet rich in vitamin B12 might be associated with a lower risk of acquiring and enhanced clearance of incident genotype specific HPVs.

To provide stronger evidence about this association, further research with the following characteristics are needed. First, a larger sample size is needed to provide greater power in statistical analysis contributing to the more precise estimates of effect. Second, serial dietary information along the study period is needed to capture the changing consuming behaviors. Third, serum micronutrient information is needed to provide a more valid assessment of nutritional status. Fourth, participants with a variety of consuming behaviors are needed to capture the association among different levels of dietary intake and genital HPVs.

In conclusion, the results from these three studies should spur future studies to confirm whether dietary folate and vitamin B12 intake are associated with genital HPV incidence, persistence, or clearance among men. As HPV-related diseases/cancers are major public health issues, especially in resource limited countries, further studies on the role of dietary intake, could identify public health interventions to reduce the burden of HPV-related diseases.

Appendix

Literature review, Chapter 2

To our knowledge, since 2002, only three articles focusing on the role of dietary folate and vitamin B12 intake and persistent genital HPV infections have been published. In 2017, Lopes et al. conducted a study to investigate the role of dietary intake and persistence of genotype specific HPV infections among 1,078 men living in southern Florida-USA, Sao Paulo-Brazil or Cuernavaca-Mexico [34]. This longitudinal cohort, which was a part of the HPV in Men (HIM) study, followed participants every six months for a median of four years of follow-up. A validated QFFQ for the Brazilian population was used to assess the nutritional status of men. Genital specimens, including penile and scrotal samples, were obtained at each visit approximately six months apart for up to four years. The PCR was used to amplify a fragment of the HPV L1 gene. HPV genotyping was conducted with the linear array method. Persistence of genotype specific HPV infections was defined as having at least two consecutive positive tests for the same HPV genotype. This study showed that participants with persistence of genotype specific oncogenic HPV infections had lower median values of both dietary folate and vitamin B12 intakes compared to participants with transient HPV infections. Participants with persistence of genotype specific non-oncogenic HPV infections also had lower median values of dietary vitamin B12 intake compared to participants with transient HPV infections. Multivariable logistic regression analysis showed that participants with higher quartile of dietary vitamin B12 intakes were associated with lower odds of persistence of both oncogenic and non-oncogenic genotype specific HPV infections. However, only the association between dietary vitamin B12 and non-oncogenic HPV infections reached statistical significance. Participants with a higher quartile of dietary folate intake were not associated with lower odds of persistence of genotype specific oncogenic HPV infections. The article did not show the result regarding dietary folate and persistence of genotype specific non-

oncogenic HPV infections. In sum, the authors concluded that no association was observed between dietary intakes and persistence of genotype specific oncogenic HPV infections; however, dietary vitamin B12 intake was inversely associated with persistence of genotype specific non-oncogenic HPV infections.

Apart from the first and only article that was conducted among men, the other two articles were conducted among women and focused on cervical HPV infections. In 2003, Giuliano et al. conducted a study to investigate the role of dietary intake and persistence of genotype specific HPV infections among 433 women attending a comprehensive maternal and child health maintenance program catering to low-income families, in Sao Paulo-Brazil [33]. A validated QFFQ for this population was used to assess the nutritional status of all participants. The PCR method was used to detect HPV DNA. HPV status was based on HPV evaluations at the baseline, and additional three follow-up visits were conducted every four months. Persistence of genotype specific HPV infections was defined as women who tested positive for the same HPV genotype on two or more consecutive visits. This study demonstrated that the mean values of dietary folate and vitamin B12 intake among participants with persistence of genotype specific HPV infections were lower than participants with transient HPV infections. However, none of these associations reached statistical significance. This study did not demonstrate these associations in multivariable analysis.

The last study was conducted in 2002 by Sedjo et al. The authors examined the association between dietary intake and persistence of oncogenic HPV infections among 201 healthy women in Arizona-USA [35]. A validated QFFQ for this population was used to assess the nutritional status of participants. HPV tests were conducted at baseline, and the follow-up visits were conducted at approximately three and nine months after enrolling into the study. Hybrid capture II

(HC II) (Digene Corp, Belts-ville, MD) was used to detect up to thirteen oncogenic HPV genotypes. Persistence of oncogenic HPV infections was defined as testing positive for HPV on at least two consecutive follow-up visits. This study showed that the mean values of both dietary folate and vitamin B12 among women with persistent oncogenic HPV infections were lower than women with transient oncogenic HPV infections. Multivariable analysis showed that women with higher tertile of dietary folate were associated with lower odds of persistence of oncogenic HPV infections. However, this association did not reach a statistical significance. Women with higher tertile of dietary vitamin B12 intake were not associated with lower odds of persistence of oncogenic HPV infections. The results of all three studies are summarized in Table 1.

Table 1 A literature review summarizing three articles which studied the association between dietary folate and vitamin B12 intake and persistent HPV infection[a] [33-35]

Authors/ Year/ Place(s)	Participants	Dietary assessment	HPV testing	Results of crude analysis	Results of multivariable logistic regression analysis
Lopes et al. 2017 Southern Florida US/ Sao Paolo Brazil/ Cuernavaca Mexico	1,248 men 18-70 years	QFFQ	PCR method Repeated testing for every 6 months for 4 years	(1) Persistent genotype specific oncogenic HPV (n=458) VS. No[b] (n=790) Folate (ug DFE), Median 707.02 VS. 720.49 Vitamin B12 (ug), Median 5.65 VS. 5.74 (2) Persistent genotype specific non-oncogenic HPV (n=636) VS. No[c] (n=612) Folate (ug DFE), Median 718.12 VS. 713.00 Vitamin B12 (ug), Median 5.68 VS. 5.73	(1) Persistent genotype specific oncogenic HPV (n=458) VS. Transient HPV[d] (n=620) Folate (DFE) (MCG) Quartile | Adjusted OR (95% CI) 1 | 1.00 2 | 0.96 (0.64-1.43) 3 | 1.24 (0.80-1.92) 4 | 0.95 (0.54-1.69) Vitamin B12 (MCG) Quartile | Adjusted OR (95% CI) 1 | 1.00 2 | 0.94 (0.65-1.37) 3 | 0.72 (0.47-1.09) 4 | 0.98 (0.61-1.58) (2) Persistent genotype specific non-oncogenic HPV (n=636) VS. Transient[d] HPV (n=442) Folate (DFE) (MCG) No reported results Vitamin B12 (MCG) Quartile | Adjusted OR (95% CI) 1 | 1.00 2 | 0.77 (0.54-1.10) 3 | 0.70 (0.49-1.02) 4 | 0.55 (0.38-0.81)
Giuliano et al.	433 women	QFFQ included vitamin and	PCR method	Persistent genotype specific HPV (n=185) VS. Transient HPV (n=248)	No reported results

<table>
<tr>
<td>2003

Sao Paulo Brazil</td>
<td>Mean age 31 years</td>
<td>mineral supplements</td>
<td>Repeated testing for every 4 months for 1 year</td>
<td>Folate (ug), Mean (SD)
107.33 (56.24) VS. 105.26 (53.25)

Vitamin B12 (ug), Mean (SD)
5.07 (5.11) VS. 4.80 (4.87)</td>
<td></td>
</tr>
<tr>
<td>Sedjo et al.

2002</td>
<td>201 women

Mean age (SD)
23-25 (4)</td>
<td>QFFQ included vitamin and mineral supplements</td>
<td>Hybrid capture II

Repeated testing at 3 and 9 months after the enrollment</td>
<td>(1) Food only:
Persistent oncogenic HPV (n=131)
VS.
Intermittent oncogenic HPV (n=70)

Folate (ug DFE), Mean (SD)
396.3 (220.4) VS. 453.3 (330.7)

Vitamin B12 (ug), Mean (SD)
4.6 (2.7) VS. 4.9 (3.2)

(2) Food and supplement combined:
Persistent oncogenic HPV (n=131) with consuming supplements (n=66)
VS.
Intermittent oncogenic HPV (n=70) with consuming supplements (n=34)

Folate (ug DFE), Mean (SD)
578.6 (346.7) VS. 626.6 (420.3)

Vitamin B12 (ug), Mean (SD)
15.8 (52.5) VS. 12.0 (16.1)</td>
<td>(1) Food only:
Persistent oncogenic HPV (n=131)
VS.
Intermittent oncogenic HPV (n=70)

Folate (MCG)
<table>
<tr><td>Tertile</td><td>Adjusted OR (95% CI)</td></tr>
<tr><td>Low</td><td>1.00</td></tr>
<tr><td>Medium</td><td>0.63 (0.29-1.38)</td></tr>
<tr><td>High</td><td>0.52 (0.23-1.18)</td></tr>
</table>
Vitamin B12 (MCG)
<table>
<tr><td>Tertile</td><td>Adjusted OR (95% CI)</td></tr>
<tr><td>Low</td><td>1.00</td></tr>
<tr><td>Medium</td><td>1.47 (0.68-3.19)</td></tr>
<tr><td>High</td><td>0.68 (0.30-1.54)</td></tr>
</table>
(2) Food and supplement combined:
Persistent oncogenic HPV (n=131) with (n=66) consuming supplements
VS.
Intermittent oncogenic HPV (n=70) with (n=34) consuming supplements

Folate (MCG)
<table>
<tr><td>Tertile</td><td>Adjusted OR (95% CI)</td></tr>
<tr><td>Low</td><td>1.00</td></tr>
<tr><td>Medium</td><td>0.80 (0.38-1.69)</td></tr>
<tr><td>High</td><td>1.16 (0.56-2.39)</td></tr>
</table>
Vitamin B12 (MCG)
<table>
<tr><td>Tertile</td><td>Adjusted OR (95% CI)</td></tr>
</table></td>
</tr>
</table>

					Low	1.00
					Medium	1.11 (0.53-2.31)
					High	0.96 (0.46-2.03)

a: all these published articles defined persistent HPV infection as at least two consecutive visits positive for HPV.
b: includes individuals with transitory and no infection for oncogenic HPV, but at least one positive result for other HPV types.
c: includes individuals with transitory and no infection for non-oncogenic HPV, but at least one positive result for other HPV types.
d: includes individuals with at least one HPV infection during four years of follow-up visits and only individuals with classified HPV infection.

Literature review, Chapter 3

To our knowledge, only two studies which focused on the role of nutrition and incident HPV infections have been published. In 2004, Piyathilake et al. published a longitudinal study aimed at investigating the role of plasma micronutrients in the natural history of HPV infections [43]. This was a 24-month prospective study which followed study participants and tested for cervical HPV status every 6 months. HC II assay was used to classify individuals as positive or negative for oncogenic HPV infections. At baseline, serum micronutrients, including combined plasma and RBC folate, vitamin B12, vitamin C, vitamin A, vitamin E, and total carotene, were assessed. The analysis was based on a nested case control design. Women who had at least three consecutive visits during the study period were included and were classified according to their HPV status. Women were classified as having incident HPV infections if they had at least one negative HPV test preceding a positive HPV test and then continued to test positive until the end of the study. Women with incident HPV infections were defined as cases, while women with all visits negative for HPV were defined as controls. Logistic regression analysis was conducted to test the association between plasma micronutrients and incident HPV infections. The multivariable regression analysis, adjusted for age, number of lifetime partners, smoking status, and the other micronutrients concentration, showed that women with higher concentration of plasma folate (>= 20.4 nmol/L) and RBC folate (>= 847.5 nmol/L) had lower odds of acquiring HPV infections: the adjusted OR = 0.27 (95% CI 0.08-0.91). However, women with higher concentration of plasma vitamin B12 (> 341.5 pmol/L) did not have lower odds of acquiring HPV: the adjusted OR = 1.46 (95% CI 0.51-4.18). The authors of this study concluded that higher concentration of plasma and RBC folate showed protective effects toward acquiring oncogenic HPV infections after controlling for known risk factors and other micronutrients.

Another study investigating the role of nutrition on the acquisition of HPV infections was published by Shvetsov et al. in 2010 [64]. This study focused on the association between plasma micronutrient concentrations and anal HPV infections. A cohort of 279 women were followed at 4-month intervals for a mean duration of 16 months. During each visit, plasma micronutrient concentrations were measured and anal HPV testing using PCR methods followed by genotyping using a reverse line blot detection method were conducted. The Cox proportional hazard model was used to determine the association between micronutrient concentrations and the acquisition of anal HPV infections. Women with higher circulating levels of plasma carotenoids were associated with a reduced risk of acquiring anal HPV infections, of which the highest quartile of plasma trans-zeaxanthin (>82.6-281.1 ng/ml) and plasma total beta carotene (>364.8-1762.1) showed 43-50% significant risk reduction. However, individuals with higher concentration of either plasma retinol or plasma tocopherol did not show a significant trend toward reduced risk of acquiring anal HPV infections. The authors of this study concluded that several carotenoids can reduce the risk of acquiring anal HPV infections.

Literature review, Chapter 4

To our knowledge, four longitudinal studies which focused on the role of nutrition and the clearance of HPV infections have been published since 1997 [43, 62, 64, 81]. Among these studies, two were aimed at the clearance of HPV infections, and the other two were focused on the clearance of incident HPV infections. In 2003, Sedjo et al. published a study to investigate the effect of plasma micronutrients on the clearance of oncogenic HPV infections [81]. The results of this study showed that higher concentrations of trans- and cis- lycopene reduced time to clearance of oncogenic HPV infections. For the highest tertiles of trans- and cis- lycopene: the HR = 2.79 (95% CI 1.17-6.66) and the HR = 2.92 (95% CI 1.28-6.63). Higher concentration of folate showed reduced time to clearance of oncogenic HPV infections. For the highest tertile: the adjusted HR = 1.73 (0.80-3.75). However, higher concentration of vitamin B12 was not associated with the reduced clearance time of oncogenic HPV infections in this study.

In 2004, Piyathilake et al. published a study aimed at investigating the role of folate on the clearance of incident oncogenic HPV infections, in which the details were described earlier in the literature review section of the first research question [43]. The authors compared all women who were HPV positive initially and then tested and remained HPV negative during the follow-up visit to women who remained HPV positive during the follow-up period. The multivariable logistic regression analysis adjusted for age, number of lifetime partners, smoking status, and micronutrient concentration, and showed that higher concentration of either combined plasma folate and RBC folate or vitamin B12 was positively associated with becoming HPV negative during the follow-up visit. Compared to women who remained HPV positive during the follow-up visit, becoming HPV negative for the higher concentrations of combined plasma folate and RBC

folate and vitamin B12: the OR = 2.50 (95% CI 1.18-5.30) and the HR = 1.14 (95% CI 0.53-2.46), respectively.

In 2007, Goodman et al. published a longitudinal study aimed at investigating the association between serum micronutrient concentration of retinol, carotenoid, and tocopherol and the clearance of incident oncogenic HPV [62]. Women were followed at an interval of every four months with a median follow up time of 15.3 months. The interview, cervical, and blood specimen collection were done for each woman with a median of six visits. A multivariable Cox proportional hazard model adjusted for age, current smoking status, number of lifetime sexual partners, age at first sex, blood frozen over one-year indicator variable, and co-infection status was used to study the association between serum micronutrients and the clearance of 189 genotype specific incident oncogenic HPV infections of 122 women. During the early stage of infections, which was defined as an infection that lasted for less than 120 days, higher circulating levels of several carotenoids were associated with a significant decrease in the clearance time of genotype specific HPV infections. Clearance of persistent HPV infections, which was defined as an infection lasting for more than 120 days, was not significantly associated with circulating levels of carotenoids or tocopherols.

A recent study to investigate the role of plasma micronutrients on the clearance of HPV infections was published by Shvetsov in 2010 [64]. The authors examined the clearance of 189 incident anal HPV infections of which 113 infections cleared during the study period. The details of this study, which were described previously in the literature review section of the second research question, used a multivariable Cox proportional hazard model adjusted for age, current smoking status, current practice of anal sex, history of pregnancy over a lifetime, period of time the blood was kept frozen from blood draw until laboratory analysis, coinfection in the anus with

other HPV genotypes, and cervical infections with the index HPV type. Clearance of persistent incident anal HPV infections, which was defined as an infection that lasted for less than 150 days, was associated with higher concentrations of beta- plus gamma- tocopherol: the adjusted HR = 3.67 (95% CI 1.82-7.39). However, few associations of circulating micronutrient levels with clearance of transient incident anal HPV infections were observed.

IRB Approval

UNIVERSITY of HAWAI'I SYSTEM

Office of Research Compliance
Human Studies Program

TO:	Hernandez, Brenda, MPH/PhD, University of Hawaii at Manoa, University of Hawaii Cancer Center Okiyama, Eugene, BS/MPH, University of Hawaii at Manoa, University of Hawaii Cancer Center, Santibenchakul, Somsook, MS/MD, University of Hawaii at Manoa, University of Hawaii Cancer Center
FROM:	Rivera, Victoria, Interim Dir, Ofc of Rsch Compliance, Biomedical IRB
PROTOCOL TITLE:	The Association between Dietary Folate and Vitamin B12 Intake and Genital Human Papillomavirus (HPV) Infection in Men
FUNDING SOURCE:	
PROTOCOL NUMBER:	2018-00071 Approval Date: February 26, 2018 Expiration Date: December 31, 2999

NOTICE OF APPROVAL FOR HUMAN RESEARCH

This letter is your record of the Human Studies Program approval of this study as exempt.

On February 26, 2018, the University of Hawaii (UH) Human Studies Program approved this study as exempt from federal regulations pertaining to the protection of human research participants. The authority for the exemption applicable to your study is documented in the Code of Federal Regulations at 45 CFR 46.101(b) 4.

Exempt studies are subject to the ethical principles articulated in The Belmont Report, found at the OHRP Website www.hhs.gov/ohrp/humansubjects/guidance/belmont.html.

Exempt studies do not require regular continuing review by the Human Studies Program. However, if you propose to modify your study, you must receive approval from the Human Studies Program prior to implementing any changes. You can submit your proposed changes via email at uhirb@hawaii.edu. (The subject line should read: Exempt Study Modification.) The Human Studies Program may review the exempt status at that time and request an application for approval as non-exempt research.

In order to protect the confidentiality of research participants, we encourage you to destroy private information which can be linked to the identities of individuals as soon as it is reasonable to do so. Signed consent forms, as applicable to your study, should be maintained for at least the duration of your project.

This approval does not expire. However, please notify the Human Studies Program when your study is complete. Upon notification, we will close our files pertaining to your study.

If you have any questions relating to the protection of human research participants, please contact the Human Studies Program by phone at 956-5007 or email uhirb@hawaii.edu. We wish you success in carrying out your research project.

1960 East-West Road
Biomedical Sciences Building B104
Honolulu, Hawai'i 96822
Telephone: (808) 956-5007
Fax: (808) 956-8683

Informed Consent

UNIVERSITY OF HAWAI'I AT MĀNOA

Cancer Research Center of Hawai'i
Cancer Epidemiology Program

CONSENT TO PARTICIPATE IN AN INVESTIGATION OF HUMAN PAPILLOMAVIRUS (HPV) IN MEN

TITLE OF STUDY: Molecular Epidemiology and Natural History of Human Papillomavirus (HPV) Infection in Men

SPONSOR: National Institutes of Health

PRINCIPAL INVESTIGATOR: Brenda Y. Hernandez, M.P.H., Ph.D., Cancer Research Center of Hawaii, University of Hawaii, 1236 Lauhala Street, Honolulu, Hawaii, 96813; telephone (808) 586-2992.

INFORMED CONSENT: Before you decide whether or not to participate in this research study, it is important that you understand the purpose of the study, how it may help you, what you will be asked to do, possible risks, and your rights as a research subject. This process is called informed consent. This consent form gives you information about the study, and research staff will discuss it with you and answer your questions. Once you understand the study, and if you agree to take part, you will be asked to sign this consent form. A copy will be given to you to keep. Taking part in the study is completely voluntary (of your own free will) and you can choose not to take part in the study at any time.

STUDY PURPOSE: Human Papillomavirus (HPV) is a virus that is transmitted through sexual intercourse. The virus is common in men and women, but most people with HPV have no signs of infection. Some types of HPV can cause warts. HPV is also the principal cause of cervical cancer in women. Most studies of HPV have been done with women, and little is known about it in men. This study is being conducted by the University of Hawaii Cancer Research Center to learn more about the occurrence and course of HPV infection in men, to examine the factors associated with HPV infection in men, and to better understand the factors influencing transmission of HPV between men and women. You will be one of more than 600 men who will take part in this study.

STUDY PROCEDURES: If you decide to take part in the study you will be asked to attend this visit and follow-up visits to the clinic every 2 to 6 months for up to 4 years. Each visit will take about 60-90 minutes. You will be asked to do the following:

1. At each visit, you will be asked to complete a survey with a research assistant. You will be asked questions about your sexual and medical history as well as other things about your personal habits and background. All of these questions will take between 10-30 minutes. You can refuse to answer any questions and all of your answers will be kept completely confidential.

2. At each visit, trained clinic staff will draw 30 cc (about 1½ tablespoons) of blood from a vein in your arm using a sterile, one-time use needle. The blood will be tested for HPV antibodies.

3. At each visit, you will be asked to give a urine sample of about ¾ cup. The urine will be tested for HPV.

4. At each visit, a clinician will take samples, using emery paper and a wet swab, of the outside surface of your genitals, including the penile glans and coronal sulcus (head of penis), the penile shaft, and the scrotum. If you are uncircumcised, a swab of the foreskin on your penis will also be obtained. If visible lesions or warts are present in the anal/genital region, the clinician will take samples using a wet swab. A sample of anal cells will be collected using a wet swab. The clinician will also obtain samples of cells inside your mouth using a soft brush and cells on your hands (palms, fingertips, and fingernails) using a wet swab. All samples will be tested for HPV.

5. At each visit, you will be asked to provide a self-collected semen sample. At the first visit, you will be given a kit with instructions for collection of the semen sample, which should be returned to the clinic within 24-48 hours of your visit. At follow-up visits, you will be asked to bring the semen sample with you to the clinic at the time of your visit.

1236 Lauhala Street, Honolulu, Hawai'i 96813
Telephone: (808) 586-2985, Facsimile: (808) 586-2982
An Equal Opportunity/Affirmative Action Institution

References

1. World Health Organization International Agency for Research on Cancer, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Human Papillomaviruses. 2007, the International Agency for Research on Cancer: 150 cours Albert Thomas, 69372 Lyon Cedex 08, France.
2. Doorbar, J., et al., Human papillomavirus molecular biology and disease association. Rev Med Virol, 2015. 25 Suppl 1: p. 2-23.
3. Dayyani, F., et al., Meta-analysis of the impact of human papillomavirus (HPV) on cancer risk and overall survival in head and neck squamous cell carcinomas (HNSCC). Head Neck Oncol, 2010. 2: p. 15.
4. Munger, K., et al., The E6 and E7 genes of the human papillomavirus type 16 together are necessary and sufficient for transformation of primary human keratinocytes. J Virol, 1989. 63(10): p. 4417-21.
5. Fact sheet: Sexually transmitted infections (STIs). August 2016 [cited 2017 7/25]; Available from: http://www.who.int/mediacentre/factsheets/fs110/en/.
6. Markowitz, L.E., et al., Seroprevalence of human papillomavirus types 6, 11, 16, and 18 in the United States: National Health and Nutrition Examination Survey 2003-2004. J Infect Dis, 2009. 200(7): p. 1059-67.
7. McQuillan G, K.-M.D. and U.E. Markowitz LE, Paulose-Ram R. Prevalence of HPV in adults aged 18–69: United States, 2011–2014. NCHS data brief, no 280, 2017.
8. GLOBOCAN 2012 (IARC) , S.o.C.S. Cervical Cancer: Estimated Incidence, Mortality and Prevalence Worldwide in 2012. [cited 2017 7/25]; Available from: http://globocan.iarc.fr/old/FactSheets/cancers/cervix-new.asp.
9. Siegel, R.L., K.D. Miller, and A. Jemal, Cancer statistics, 2018. CA Cancer J Clin, 2018. 68(1): p.7-30.
10. Richter, S., et al., Management of carcinoma of the penis: Consensus statement from the Canadian Association of Genitourinary Medical Oncologists (CAGMO). Can Urol Assoc J, 2013. 7(11-12): p. E797-811.
11. Giuliano, A.R., et al., Epidemiology of human papillomavirus infection in men, cancers other than cervical and benign conditions. Vaccine, 2008. 26 Suppl 10: p. K17-28.
12. Haeggblom, L., et al., Time to change perspectives on HPV in oropharyngeal cancer. A systematic review of HPV prevalence per oropharyngeal sub-site the last 3 years. Papillomavirus Res, 2017. 4: p. 1-11.
13. Chaturvedi, A.K., et al., Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol, 2011. 29(32): p. 4294-301.
14. O'Sullivan, B., et al., Development and validation of a staging system for HPV-related oropharyngeal cancer by the International Collaboration on Oropharyngeal cancer Network for Staging (ICON-S): a multicentre cohort study. Lancet Oncol, 2016. 17(4): p. 440-451.
15. Lee, S.L. and A.M. Tameru, A Mathematical Model of Human Papillomavirus (HPV) in the United States and its Impact on Cervical Cancer. J Cancer, 2012. 3: p. 262-8.
16. Miralles-Guri, C., et al., Human papillomavirus prevalence and type distribution in penile carcinoma. J Clin Pathol, 2009. 62(10): p. 870-8.
17. Hernandez, B.Y., et al., Human papillomavirus genotype prevalence in invasive penile cancers from a registry-based United States population. Front Oncol, 2014. 4: p. 9.

18. Backes, D.M., et al., Systematic review of human papillomavirus prevalence in invasive penile cancer. Cancer Causes Control, 2009. 20(4): p. 449-57.
19. Chaux, A. and A.L. Cubilla, The role of human papillomavirus infection in the pathogenesis of penile squamous cell carcinomas. Semin Diagn Pathol, 2012. 29(2): p. 67-71.
20. Djajadiningrat, R.S., et al., Human papillomavirus prevalence in invasive penile cancer and association with clinical outcome. J Urol, 2015. 193(2): p. 526-31.
21. Gunia, S., et al., p16(INK4a) is a marker of good prognosis for primary invasive penile squamous cell carcinoma: a multi-institutional study. J Urol, 2012. 187(3): p. 899-907.
22. Garland, S.M., et al., Natural history of genital warts: analysis of the placebo arm of 2 randomized phase III trials of a quadrivalent human papillomavirus (types 6, 11, 16, and 18) vaccine. J Infect Dis, 2009. 199(6): p. 805-14.
23. Oriel, J.D., Natural history of genital warts. Br J Vener Dis, 1971. 47(1): p. 1-13.
24. Yanofsky, V.R., R.V. Patel, and G. Goldenberg, Genital warts: a comprehensive review. J Clin Aesthet Dermatol, 2012. 5(6): p. 25-36.
25. Division of STD Prevention, N.C.f.H.A., Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention. Anogenital warts. 2015 Sexually Transmitted Diseases Treatment Guidelines [cited 2019 04/28]; Available from: https://www.cdc.gov/std/tg2015/warts.htm.
26. Shabbir, M., S. Minhas, and A. Muneer, Diagnosis and management of premalignant penile lesions. Ther Adv Urol, 2011. 3(3): p. 151-8.
27. HPV Vaccine Recommendations. Vaccines and Preventable Diseases 2016 December 15, 2016 [cited 2019 March 18,]; Available from: https://www.cdc.gov/vaccines/vpd/hpv/hcp/recommendations.html.
28. HPV Vaccines. July 13, 2016 October 8, 2018 [cited 2018 October 31]; Available from: https://www.cancer.org/cancer/cancer-causes/infectious-agents/hpv/hpv-vaccines.html.
29. Reisinger, K.S., et al., Safety and persistent immunogenicity of a quadrivalent human papillomavirus types 6, 11, 16, 18 L1 virus-like particle vaccine in preadolescents and adolescents: a randomized controlled trial. Pediatr Infect Dis J, 2007. 26(3): p. 201-9.
30. Xavier Castellsagué , L.B., Laia Alemany , Mireia Diaz, Silvia de Sanjosé , and F. Xavier Bosch, The Epidemiology of Cervical Cancer, in HPV and Cervical Cancer, F. Borruto, De Ridder, Marc (Eds.), Editor. 2012, Springer Science+Business Media, LLC: 233 Spring Street, New York, NY 10013, USA. p. 63-84.
31. Wieland, U., A. Kreuter, and H. Pfister, Human papillomavirus and immunosuppression. Curr Probl Dermatol, 2014. 45: p. 154-65.
32. Hernandez, B.Y., et al., Transmission of human papillomavirus in heterosexual couples. Emerg Infect Dis, 2008. 14(6): p. 888-94.
33. Giuliano, A.R., et al., Dietary intake and risk of persistent human papillomavirus (HPV) infection: the Ludwig-McGill HPV Natural History Study. J Infect Dis, 2003. 188(10): p. 1508-16.
34. Lopes, R., et al., Dietary intake of selected nutrients and persistence of HPV infection in men. Int J Cancer, 2017. 141(4): p. 757-765.
35. Sedjo, R.L., et al., Human papillomavirus persistence and nutrients involved in the methylation pathway among a cohort of young women. Cancer Epidemiol Biomarkers Prev, 2002. 11(4): p. 353-9.

36. Blount, B.C., et al., Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A, 1997. 94(7): p. 3290-5.
37. Duthie, S.J., et al., Impact of folate deficiency on DNA stability. J Nutr, 2002. 132(8 Suppl): p. 2444S-2449S.
38. Wilke, C.M., et al., FRA3B extends over a broad region and contains a spontaneous HPV16 integration site: direct evidence for the coincidence of viral integration sites and fragile sites. Hum Mol Genet, 1996. 5(2): p. 187-95.
39. Popescu, N.C., J.A. DiPaolo, and S.C. Amsbaugh, Integration sites of human papillomavirus 18 DNA sequences on HeLa cell chromosomes. Cytogenet Cell Genet, 1987. 44(1): p. 58-62.
40. Banerjee, R.V. and R.G. Matthews, Cobalamin-dependent methionine synthase. FASEB J, 1990. 4(5): p. 1450-9.
41. Ubbink, J.B., et al., Vitamin B-12, vitamin B-6, and folate nutritional status in men with hyperhomocysteinemia. Am J Clin Nutr, 1993. 57(1): p. 47-53.
42. Abike, F., et al., Human papilloma virus persistence and neopterin, folate and homocysteine levels in cervical dysplasias. Arch Gynecol Obstet, 2011. 284(1): p. 209-14.
43. Piyathilake, C.J., et al., Folate is associated with the natural history of high-risk human papillomaviruses. Cancer Res, 2004. 64(23): p. 8788-93.
44. Hernandez, B.Y., et al., Reduced clearance of penile human papillomavirus infection in uncircumcised men. J Infect Dis, 2010. 201(9): p. 1340-3.
45. Hernandez, B.Y., et al., Acceptability of prophylactic human papillomavirus vaccination among adult men. Hum Vaccin, 2010. 6(6): p. 467-75.
46. Hernandez, B.Y., et al., Circumcision and human papillomavirus infection in men: a site-specific comparison. J Infect Dis, 2008. 197(6): p. 787-94.
47. Gravitt, P.E., et al., Improved amplification of genital human papillomaviruses. J Clin Microbiol, 2000. 38(1): p. 357-61.
48. Coutlee, F., et al., Enhanced detection and typing of human papillomavirus (HPV) DNA in anogenital samples with PGMY primers and the Linear array HPV genotyping test. J Clin Microbiol, 2006. 44(6): p. 1998-2006.
49. Van Hamont, D., et al., Evaluation of the SPF10-INNO LiPA human papillomavirus (HPV) genotyping test and the roche linear array HPV genotyping test. J Clin Microbiol, 2006. 44(9): p. 3122-9.
50. Giuliani, L., et al., Comparison of DNA sequencing and Roche Linear array in human papillomavirus (HPV) genotyping. Anticancer Res, 2006. 26(5B): p. 3939-41.
51. Kolonel, L.N., et al., A multiethnic cohort in Hawaii and Los Angeles: baseline characteristics. Am J Epidemiol, 2000. 151(4): p. 346-57.
52. Stram, D.O., et al., Calibration of the dietary questionnaire for a multiethnic cohort in Hawaii and Los Angeles. Am J Epidemiol, 2000. 151(4): p. 358-70.
53. Daugherty, M. and T. Byler, Genital Wart and HPV Prevalence in Males in the United States from Penile Swabs: Results from NHANES. Sex Transm Dis, 2017.
54. How Many Cancers Are Linked with HPV Each Year? HPV and Cancer 2018 August 22, 2018 [cited 2018 November 10]; Available from: https://www.cdc.gov/cancer/hpv/statistics/cases.htm.

55. Patel, H., et al., Systematic review of the incidence and prevalence of genital warts. BMC Infect Dis, 2013. 13: p. 39.
56. De Martel, C., et al., Worldwide burden of cancer attributable to HPV by site, country and HPV type. Int J Cancer, 2017. 141(4): p. 664-670.
57. Kim, Y.I., et al., Folate deficiency in rats induces DNA strand breaks and hypomethylation within the p53 tumor suppressor gene. Am J Clin Nutr, 1997. 65(1): p. 46-52.
58. Kim, Y.I., et al., Global DNA hypomethylation increases progressively in cervical dysplasia and carcinoma. Cancer, 1994. 74(3): p. 893-9.
59. Fowler, B.M., et al., Hypomethylation in cervical tissue: is there a correlation with folate status? Cancer Epidemiol Biomarkers Prev, 1998. 7(10): p. 901-6.
60. Walter, W., Implications of total energy intake for epidemiologic analyses, in Monographs in epidemiology and biostatistics: Nutritional epidemiology. 2012, Oxford university press: New York, NY. p. 271.
61. Giuliano, A.R., et al., Incidence and clearance of genital human papillomavirus infection in men (HIM): a cohort study. Lancet, 2011. 377(9769): p. 932-40.
62. Goodman, M.T., et al., Hawaii cohort study of serum micronutrient concentrations and clearance of incident oncogenic human papillomavirus infection of the cervix. Cancer Res, 2007. 67(12): p. 5987-96.
63. Goodman, M.T., et al., Prevalence, acquisition, and clearance of cervical human papillomavirus infection among women with normal cytology: Hawaii Human Papillomavirus Cohort Study. Cancer Res, 2008. 68(21): p. 8813-24.
64. Shvetsov, Y.B., et al., Plasma micronutrients and the acquisition and clearance of anal human papillomavirus infection: the Hawaii HPV cohort study. Cancer Res, 2010. 70(23): p. 9787-97.
65. Bursac, Z., et al., Purposeful selection of variables in logistic regression. Source Code Biol Med, 2008. 3: p. 17.
66. Houlihan, C.F., et al., Human papillomavirus infection and increased risk of HIV acquisition. A systematic review and meta-analysis. AIDS, 2012. 26(17): p. 2211-22.
67. Kjaer, S.K., et al., Acquisition and persistence of human papillomavirus infection in younger men: a prospective follow-up study among Danish soldiers. Cancer Epidemiol Biomarkers Prev, 2005. 14(6): p. 1528-33.
68. Hill, A.B., The Environment and Disease: Association or Causation? Proc R Soc Med, 1965. 58: p. 295-300.
69. Chesson, H.W., et al., The estimated lifetime probability of acquiring human papillomavirus in the United States. Sex Transm Dis, 2014. 41(11): p. 660-4.
70. Partridge, J.M., et al., Genital human papillomavirus infection in men: incidence and risk factors in a cohort of university students. J Infect Dis, 2007. 196(8): p. 1128-36.
71. Winer, R.L., et al., Risk of female human papillomavirus acquisition associated with first male sex partner. J Infect Dis, 2008. 197(2): p. 279-82.
72. Ventimiglia, E., et al., Human Papillomavirus Infection and Vaccination in Males. Eur Urol Focus, 2016. 2(4): p. 355-362.
73. Karlsson, R., et al., Lifetime number of partners as the only independent risk factor for human papillomavirus infection: a population-based study. Sex Transm Dis, 1995. 22(2): p. 119-27.

74. L. J. Wei, D.Y.L., L. Weissfeld., Regression Analysis of Multivariate Incomplete Failure Time Data by Modeling Marginal Distributions. Journal of the American Statistical Association, 1989. 84(408): p. 1065-1073.
75. Wei, D.Y.L.a.L.J., The Robust Inference for the Cox Proportional Hazards Model. Journal of the American Statistical Association, 1989. 84(408): p. 1074-1078.
76. Akogbe, G.O., et al., Race and prevalence of human papillomavirus infection among men residing in Brazil, Mexico and the United States. Int J Cancer, 2012. 131(3): p. E282-91.
77. Zibbell, J.E., et al., Increases in hepatitis C virus infection related to injection drug use among persons aged </=30 years - Kentucky, Tennessee, Virginia, and West Virginia, 2006-2012. MMWR Morb Mortal Wkly Rep, 2015. 64(17): p. 453-8.
78. Services, U.S.D.o.H.a.H. Hepatitis C Why People Born from 1945–1965 Should Get Tested. 2016.
79. Plummer, M., et al., Global burden of cancers attributable to infections in 2012: a synthetic analysis. Lancet Glob Health, 2016. 4(9): p. e609-16.
80. Ruffin, M.T.R., Cheryl L., Human Papillomavirus Infection in Men and Women: The Impact of Nutrition on Cervical Cancer, in Principles of Gender-Specific Medicine. . 2004, Elsevier Inc. p. 796-812.
81. Sedjo, R.L., et al., Effect of plasma micronutrients on clearance of oncogenic human papillomavirus (HPV) infection (United States). Cancer Causes Control, 2003. 14(4): p. 319-26.
82. Gravitt, P.E. and R.L. Winer, Natural History of HPV Infection across the Lifespan: Role of Viral Latency. Viruses, 2017. 9(10).
83. Administration., U.S.F.a.D., Food Standards: Amendment of Standards of Identity for Enriched Grain Products to Require Addition of Folic Acid: Final Rule. 1996a, Federal Register. p. 8781–97.
84. Abreu, A.L., et al., A review of methods for detect human Papillomavirus infection. Virol J, 2012. 9: p. 262.
85. Giuliano, A.R., et al., The optimal anatomic sites for sampling heterosexual men for human papillomavirus (HPV) detection: the HPV detection in men study. J Infect Dis, 2007. 196(8): p. 1146-52.
86. JS, K., Social Epidemiology, in Modern Epidemiology, G.S. Rothman KJ, Lash TL, Editor. 2008, Wolters Kluwer: Philadelphia. p. 532-48.