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The role of diet and nutrition in cervical carcinogenesis: A review of recent evidence

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Our objective was to provide an update on recent epidemiologic evidence about the role of diet and nutrition on the risk of human papillomavirus (HPV) persistence and cervical neoplasia, taking HPV into account. We conducted a systematic review and qualitative classification of all observational studies controlling for HPV infection published between March 1995 and November 2003 and of all randomized clinical trials published between January 1991 and November 2003. Scientific evidence was classified as convincing, probable, possible or insufficient, as used in a previous study on diet and cancer. Thirty-three studies were eligible for this review (10 clinical trials, 8 observational prospective studies and 15 case-control studies). The few studies on HPV persistence showed a possible protective effect of fruits, vegetables, vitamins C and E, beta- and alpha-carotene, lycopene, luterin/zeaxanthin and cryptoxanthin. Evidence for a protective effect of cervical neoplasia was probable for folate, retinol and vitamin E and possible for vegetables, vitamins C and B12, alpha-carotene, beta-carotene, lycopene, lutein/zeaxanthin and cryptoxanthin. Evidence for an increased risk of cervical neoplasia associated with high blood homocysteine was probable. Results did not differ between studies looking at preneoplastic and invasive lesions or between retrospective and prospective studies. The available evidence for an association between diet and nutritional status and cervical carcinogenesis taking HPV infection into account is not yet convincing. Large cohort studies are needed to adequately assess the role of foods and nutrients in cervical HPV carcinogenesis.

Key words: nutrition; human papillomavirus; cervical cancer; epidemiology; review

Although incidence and mortality from invasive cervical cancer declined substantially from the 1950s onward in developed countries, cancer of the cervix remains the second most common cancer among women in the world, accounting for about 10% of all new cancer cases.[1] Approximately 471,000 cases are newly diagnosed and 233,000 women die each year from cervical cancer, most of them in less-developed countries.[1]

Cervical squamous carcinoma results from a continuous process, starting from normal cervical epithelium that after human papillomavirus (HPV) infection and persistence progresses to intraepithelial lesions, carcinoma *in situ* (CIS) and finally, invasive squamous carcinoma (ISC). However, up to 60% of these preinvasive intraepithelial lesions experience spontaneous regression, with progression to ISC a relatively uncommon event.[2]

Persistent infection by certain HPV genotypes has thus been recognized as a necessary step for the development, maintenance and progression of cervical intraepithelial neoplasia (CIN) and cervical cancer.[3] Despite this, HPV infection is most likely not a sufficient cause of cervical cancer, since prospective studies consistently show that only a small fraction of infected women do eventually develop the disease.[4] Cervical cancer is believed to have a cofactorial etiology in which HPV interacts with other cofactors, including nutritional ones,[5] that influence the risk of HPV persistence and progression to CIN. Established factors that influence the probability of HPV acquisition are having had an early age at first intercourse, a history of multiple sexual partners and contact with promiscuous partners.[5] Cofactors that influence the risk of progression from HPV infection to HPV persistence and development of squamous intraepithelial lesions (SIL) include environmental and lifestyle cofactors such as cigarette smoking, diet, long-term oral contraceptive (OC) use, high parity and coinfection with other sexually transmitted infections, host cofactors and viral cofactors such as viral load, integration, genotype and variants.[5–7]

There are no published studies exploring the possible role of diet and nutritional status on the risk of HPV infection acquisition and few on the risk of HPV persistence. Concerning cervical cancer and diet, early epidemiologic research on diet and cervical cancer focused mainly on invasive disease. Available evidence was reviewed in the 1990s.[8–10] In the most comprehensive review, Potishman and Brinton concluded that there was fairly consistent evidence that the risk of cervical cancer and its precursors may be related to low intake of carotenoids and especially vitamin C.[8] They found a less consistent association with low intake of vitamin E and folate. On the other hand, an international expert panel concluded that there was no convincing evidence that any dietary factor increases the risk of cervical cancer but that diets high in vegetables and fruits, carotenoids, vitamin C and vitamin E were possibly protective and that folate and retinol possibly had no relationship with ISC.[9] Finally, another expert committee concluded that the evidence was strongly consistent for a protective effect of fruits and vegetables, moderately consistent for vitamin C and folate and weakly consistent for retinol and/or carotenoids.[10]

Overall, authors of these reviews acknowledged that firm associations between nutritional factors and SIL or ISC could not be established and that the most important limitation of the reviewed studies was lack of control for HPV infection. Since the key role of HPV has been established and reliable techniques for its detection have been suitable for epidemiologic research, most recent nutritional studies on cervical cancer have taken HPV infection into account. These studies have tried to ascertain if previously suspected dietary factors act as independent risk factors, as cofactors of HPV or are merely confounders of HPV and HPV-related factors.

The aim of our study is to provide an update on recent epidemiologic evidence about the role of diet and nutritional status on the risk of HPV persistence as well as on the risk of SIL and ISC, taking HPV into account.

Material and methods

Sources of data were all published peer-reviewed observational studies available in the Medline and Cancerlit databases from March 1995 to November 2003 and all clinical trials published from January 1991 to November 2003. The searching was completed with references included in retrieved papers that were not previously identified through electronic search. To be eligible for this review, studies had to fulfill the following inclusion criteria:

(i) Study design: observational or intervention epidemiologic studies that included a control group.

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TABLE I – CLASSIFICATION OF THE SCIENTIFIC EVIDENCE FOR ASSOCIATIONS OF DIET AND NUTRITION WITH HPV PERSISTENCE AND CERVICAL NEOPLASIA AND CANCER

Dietary and nutritional variables	HPV persistence			Cervical neoplasia/cancer		
	No. of studies (no. protective) [no. significant][1]	No. of cases/ controls	Scientific evidence	No. of studies (no. protective) [no. significant][1]	No. of cases/ controls	Scientific evidence
Folate	2 (0) [0]	150/96	INS	11 (7) [2]	1,874/3,171	PRB
Vitamin B6	1 (1) [0]	131/70	INS	1 (1) [1]	150/179	INS
Vitamin B12	2 (2) [1]	150/96	INS	2 (2) [0]	521/441	PSB
Homocysteine[2]	1 (0) [0]	19/26	INS	5 (5) [2]	898/1,021	PRB
Betacarotene	3 (3) [0]	418/383	PSB	7 (5) [0]	1,445/2,466	PSB
Alphacarotene	3 (0) [0]	418/383	INS	4 (3) [2]	412/616	PSB
Lycopene	3 (1) [1]	233/160	PSB	4 (4) [1]	412/616	PSB
Lutein/zeaxanthin	4 (2) [1]	349/408	PSB	4 (4) [1]	412/616	PSB
Cryptoxanthin	3 (1) [1]	349/408	PSB	4 (2) [1]	412/616	PSB
Retinol	1 (0) [0]	200/70	INS	7 (4) [2]	1,585/2,628	PRB
Vitamin C	2 (2) [1]	218/338	PSB	7 (3) [0]	1,493/2,574	PSB
Vitamin E	1 (1) [1]	102/90	PSB	7 (6) [3]	1,446/2,463	PRB
Fruits	2 (2) [1]	316/318	PSB	0	-	-
Vegetables	2 (2) [1]	316/318	PSB	1 (1) [0]	184/509	INS

[1]$p = \leq 0.05$. –[2]Risk increased. – PRB, probable; PSB, possible; INS, insufficient.

(ii) Exposure variables: usual food or nutrient intake or nutrient blood levels (serum, plasma or red cells).

(iii) Outcome variables: HPV persistence, occurrence of cervical squamous neoplasia, CIS and ISC. Classification systems used by different authors were respected since they are not fully interchangeable. The Bethesda Classification System classifies preinvasive disease in low-grade and high-grade squamous intraepithelial lesions (LSIL and HSIL, respectively). LSIL include condylomatous atypia and grade 1 of the CIN classification system, and HSIL encompasses CIN 2 and CIN 3/CIS.[5] Studies looking only at atypical squamous cell of undetermined significance (ASCUS) were excluded.

(iv) Confounding variables: only observational studies that controlled for infection by HPV measured by PCR with blot hybridization, Hybrid Capture System, Southern blot and HPV serology were included in our review. Randomized clinical trials, including those that did not measure HPV infection, were also eligible.

After an initial selection of all eligible papers, a systematic review and qualitative classification of each study was undertaken. For each study, potential methodologic limitations regarding statistical power, bias and control for confounding were evaluated. Odds ratios and 95% CIs for highest *vs.* lowest categories of food/nutrient intake or nutrient blood concentration are presented graphically, only for those food groups or nutrients assessed in at least 3 studies. The most fully adjusted ORs were used from each included article. Odds ratios for each study were plotted as diamonds whose size is proportional to the study size.

To classify scientific evidence, the widely accepted methods and terminology adopted by the panel of the World Cancer Research Fund & American Institute for Cancer Research (WCRF & AICR) in their study on diet and cancer were used.[9] In brief, evidence was classified as convincing, probable, possible or insufficient. Evidence was convincing when epidemiologic studies showed consistent associations, with little or no evidence to the contrary. Also, there should be a substantial number of acceptable studies (more than 20), preferably including prospective designs, conducted in different population groups and controlling for possible confounding factors. Associations should be biologically plausible, and laboratory evidence had to be usually supportive or strongly supportive. Evidence was classified as probable when epidemiologic studies showing associations were either not too consistent, with a number and/or proportion of studies not supporting the association, or the number or type of studies was not extensive enough to make a more definite judgement. Also, mechanistic and laboratory evidence had to be usually supportive or strongly supportive. Evidence was considered as possible when epidemiologic studies were generally supportive but limited in quantity, quality or consistency. There may or may not be supportive mechanistic or laboratory evidence. Alternatively, few or no epidemiologic data were available, but there was strongly supportive evidence from other disciplines. Finally, evidence was considered insufficient when there were only a few studies, which were generally consistent but do no more than hint at a possible relationship. If a significant portion of the data was inconsistent, amounting to possible or insufficient evidence of a causal relationship, the inconsistent evidence was noted as such. In some cases, the data were extremely limited and/or inconsistent; for such relationships, no judgement could be made.

Results

Description of studies

Overall, 33 studies were eligible for our review: 10 clinical trials, 8 prospective studies (3 of them nested case-control) and 15 case-control studies. All but 2 studies were carried out in developed countries, mainly in the U.S. Approximately 50% of studies enrolled women of multiethnic origin, 25% only nonwhite and 25% only white or not specified. Studies that presented their results in different publications were reported once. Among the 23 observational studies, 14 assessed nutrient circulating levels, 3 assessed nutrient intake, 4 assessed both blood levels and nutrient intake, 1 assessed only food intake, and 1 looked at both food and nutrient intake. The most frequently assessed nutrients were folate, retinol, beta-carotene and other specific carotenoids, vitamin E and vitamin C. Almost half of the reviewed studies analyzed preneoplastic lesions without separate analyses for different preinvasive lesions. Among studies doing separate analyses, most of them looked at CIN2-3/HSIL. Classification of the scientific evidence for each studied food or nutrient and stratified by studies on HPV persistence and studies on cervical neoplasia is shown in Table I.

Folate, vitamin B6, vitamin B12, homocysteine, methionine and cysteine

The 2 studies looking at folate and HPV persistence showed no association (Fig. 1).[11,12] Among the 5 reviewed studies on dietary folate and cervical neoplasia,[13–17] 4 presented inverse associations,[13–16] but only 1 achieved statistical significance.[15] One had a low statistical power,[14] and the other was a relatively large nested study that suggested a protective effect of dietary intake among HPV-positive women but not among HPV-negative women. Five[14,18–21] of 7 studies[14,18–23] looking at blood folate concentration and cervical neoplasia showed a protective effect for high serum or RBC folate, but only 1 was statistically significant, showing a strong protection of plasma folate for CIN 1-3 HPV-

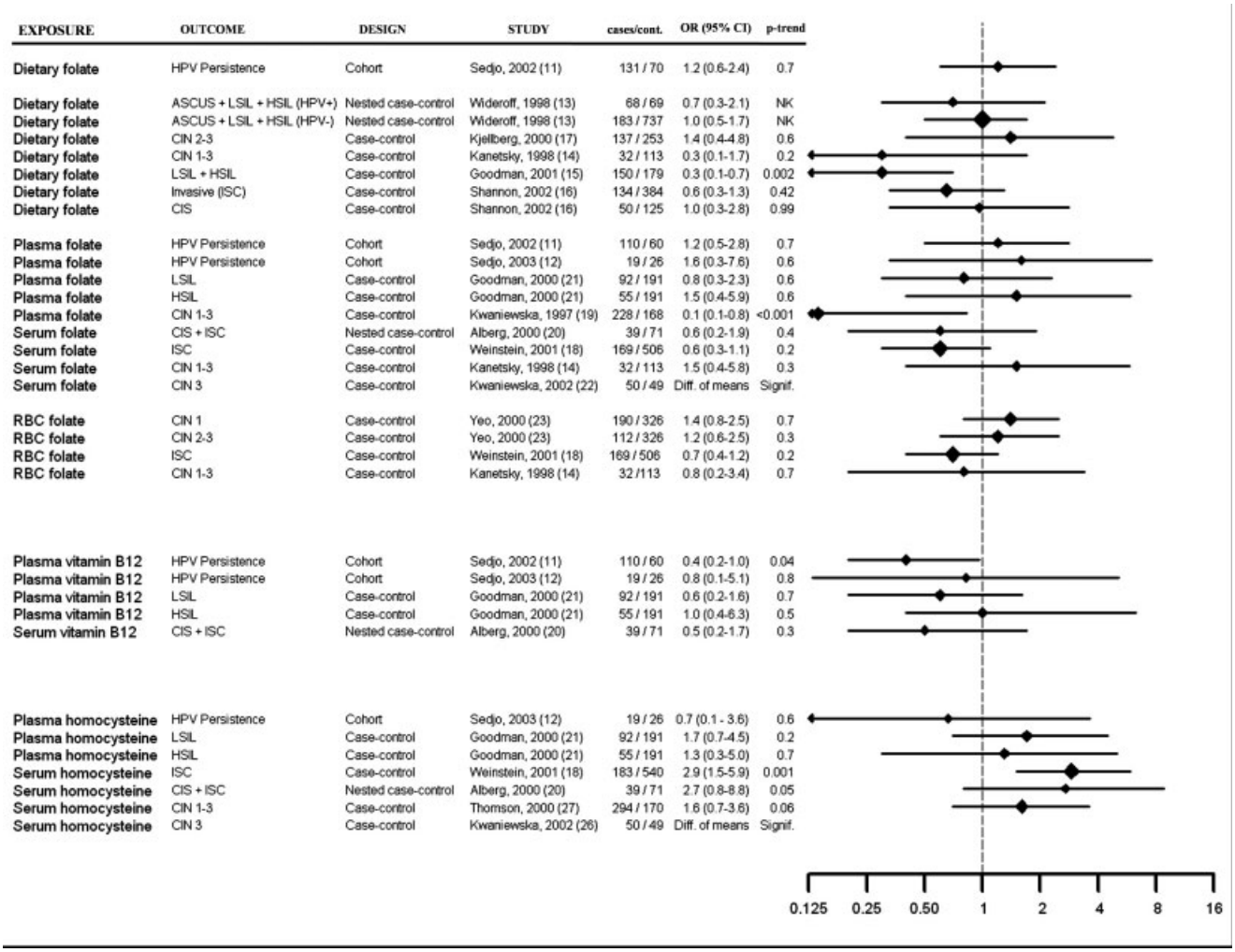

FIGURE 1 – Selected case-control and cohort studies (1995–2003) on folate, vitamin B12 and homocysteine and cervical carcinogenesis. Odds ratio for highest vs. lowest category of exposure.

positive women compared to normal HPV-negative women.[19] Of the 2 studies observing statistical negative association with folate,[15,19] adjustment for all relevant confounding factors was done in 1 of them,[19] and adjustment for all confounding factors except oral contraceptives was done in the other.[15] Both studies were hospital based and, as such, they were subject to potential selection bias. Two chemoprevention trials[24,25] assessing the effect of oral supplementation with oral folic acid in CIN 1-2 women concluded that folic acid does not enhance the regression of early epithelial lesions, although they do not address the potential effect in preventing this type of lesion.

Among the 2 studies looking at vitamin B6, 1 showed a nonsignificant inverse association,[11] whereas the other showed a strong significant protection for SIL.[15] Two studies[11,12] found a nonsignificant inverse association of plasma vitamin B12 with HPV persistence, whereas 2 studies found inverse but not significant associations with SIL[21] and CIS+ISC.[20]

Serum/plasma homocysteine, which is inversely associated with folate, vitamin B12 and vitamin B6 intake, increased the risk of SIL in 5 of 5 studies,[20–22,26,27] but only 1 was significant.[26] This was a population-based case-control study adjusted for all relevant confounding factors.

Overall, insufficient evidence for a protective effect of folic acid, vitamin B6 and vitamin B12 on HPV persistence was found. In relation to cervical cancer, there is probably a protective effect of folate and a risk effect of homocysteine, whereas there is possibly a protective effect of vitamin B12 (Table I). Ziegler *et al*. suggested that elevated homocysteine could be a more accurate measure of inadequate folate nutritional status than direct measurement of circulating folate, and a biomarker of disruption of 1-carbon metabolism, which may be related to cervical carcinogenesis.[28]

Carotenoids

A weak and nonsignificant protective effect of dietary intake or low serum concentration of beta-carotene was observed for HPV persistence in 3[29–31] of 4 prospective studies (Fig. 2).[29–32] Five[13,16,33,35,36] of 7 observational studies[13,14,16,33–36] suggested a protective effect of beta-carotene with SIL, although none of them was significant.

The 2 biggest studies—one of them a prospective study adjusting for all relevant confounding factors—showed no association.

In a randomized clinical trial investigating the association of beta-carotene with early preinvasive lesions,[37] no favorable influence of oral administration of beta-carotene on ASCUS-CIN1 regression rates was observed after 2 years of follow-up. Other clinical trials of oral beta-carotene supplementation could not demonstrate enhanced regression of CIN 2-3 lesions,[38] CIN1-2[39] or CIN 1-3,[40,41] although small sample sizes or short treatment duration in most of these trials limited their conclusions.

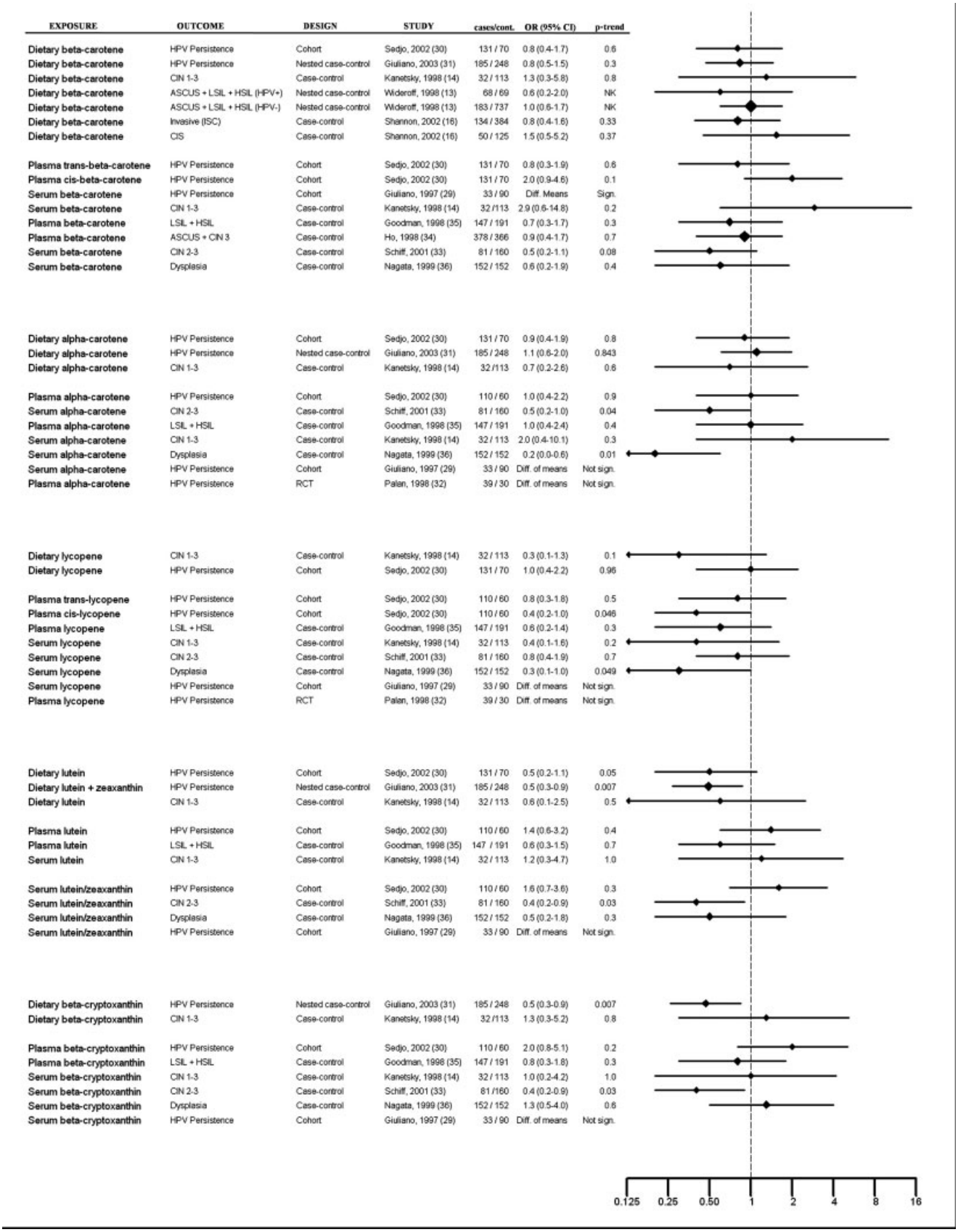

FIGURE 2 – Selected case-control and cohort studies (1995–2003) on carotenoids and cervical carcinogenesis. Odds ratio for highest vs. lowest category of exposure.

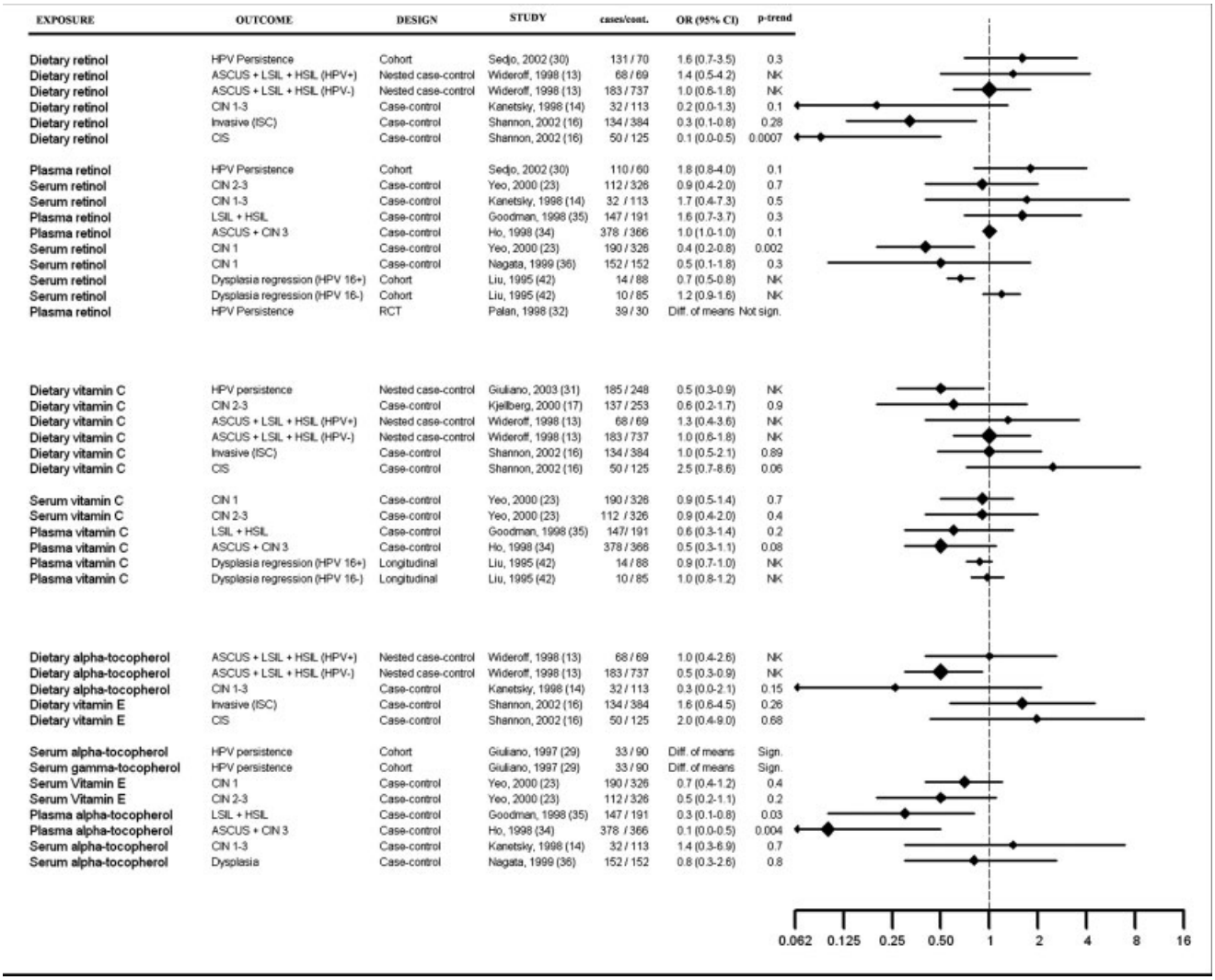

FIGURE 3 – Selected case-control and cohort studies (1995–2003) on retinol, vitamin C and tocopherol and cervical carcinogenesis. Odds ratio for highest vs. lowest category of exposure.

None of 4 studies looking at alpha-carotene showed a significant protective effect for HPV persistence.[29–32] With respect to cervical neoplasia, 3[14,33,36] of 4[14,33,35,36] studies showed inverse associations with alpha-carotene, being significant in 2 of them.[33,36]

Only 1[30] of 3[29,30,32] studies on lycopene showed a protective effect for HPV persistence, whereas the 4 studies looking at lycopene and cervical neoplasia[14,33,35,36] showed a protective effect, although only 1 was significant.[35]

Lutein or lutein/zeaxanthin protected from HPV persistence in 2 studies looking at diet[30,31] but not in 2 studies looking at serum levels.[29,32] Lutein or lutein/zeaxanthin were protective for cervical neoplasia in the 4 studies looking at it.[14,33,35,36]

Beta-cryptoxanthin presented an inverse association in 1[31] of 3 studies[29–31] looking at HPV persistence and in 2 of 4 studies looking at cervical neoplasia.[14,33,35,36]

In conclusion, a protective effect of beta-carotene and other carotenoids in both HPV persistence and cervical cancer is possible, although clinical trials showed no beneficial effect of oral supplementation with beta-carotene on regression of preneoplastic lesions.

Retinoids

None of 2 studies looking at HPV persistence[30,32] showed a protective effect of serum/plasma retinol concentration (Fig. 3). Two[14,16] of 3 observational studies[13,14,16] showed inverse associations of retinol intake with cervical neoplasia. One of them showed a strong significant protective effect of high-retinol foods on CIS.[16] Two limitations of this study have to be stressed. First, a high percentage of cases completed the food frequency questionnaire 1 year after initial diagnosis, allowing for potential recall bias and/or for influence of the disease process on food intake. The second limitation was that selected controls for CIS were replaced if they refused to participate until 2 controls per case were recruited, allowing for potential selection bias. On the other hand, a strong marginally significant protective effect of dietary retinol on CIN1-3 was shown in a case-control study conducted in the U.S.[14] With respect to serum/plasma retinol and the risk of cervical neoplasia, a protective effect was shown in 3[23,36,42] of 6 observational studies.[14,23,34–36,42] An interaction between HPV-16 seropositivity and low serum retinol was found in a cohort study.[43] In relation to intervention studies, a randomized placebo-controlled trial evaluating the efficacy of topical application of aliretinoin (9-cis-retinoic acid) on CIN2-3[44] found that regression rates of CIN2 but not CIN3 lesions in the treated patients were nonsignificantly higher than in the placebo ones, especially in the high-dosage group (CIN2: 50%, 33% and 20% in the high-dosage, low-dosage and placebo groups, respectively; CIN3: 32%, 31% and 41%); however, a high percentage of toxic effects

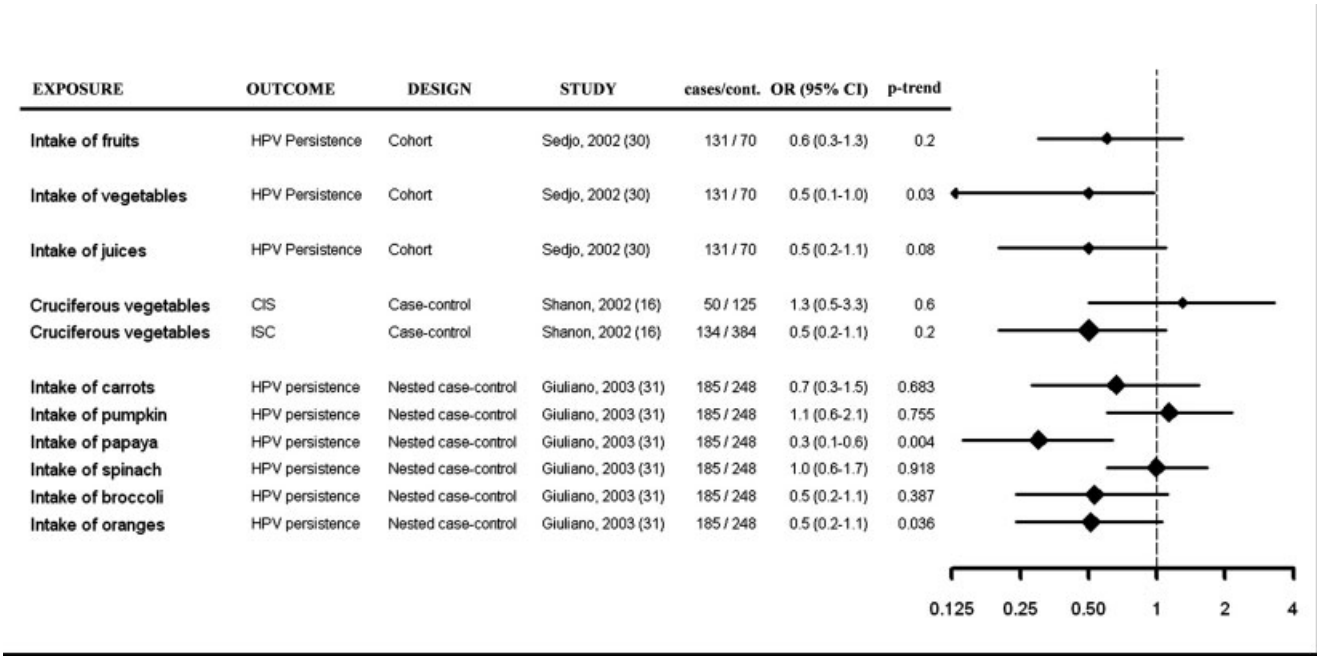

FIGURE 4 – Selected case-control and cohort studies (1995–2003) on fruit and vegetables intake and cervical carcinogenesis. Odds ratio for highest vs. lowest category of exposure.

were observed in the high-dosage group. Results of this trial are consistent with a previous and more powerful trial evaluating the effect of all-trans-retinoic acid, showing an increase in CIN2 but not CIN3 regression rate in the intervention group.[45] Short follow-up (12 weeks) and small sample size (38 subjects in each arm) were the main drawbacks of the 9-cis-retinoic acid trial. In another randomized trial assessing the efficacy of an oral retinol derivative in CIN 2-3 regression rate, a poorer outcome for the treated patients than for the placebo ones was shown.[46]

Overall, evidence for a protective effect of retinol was insufficient for HPV persistence and probable for cervical neoplasia. Clinical trials evaluating the effect of topically applied retinoic acid[44,45] showed an increase in the regression rates of CIN2 but not CIN3, supporting a role in early lesions.

Vitamin C

Vitamin C presented inverse associations with HPV persistence in 2 of 2 studies[29,31] and inverse associations with SIL in 4[17,23,34,35] of 7 observational studies (Fig. 3).[13,16,17,23,34,35,42] Important limitations of 1 of these studies[16] were stated before. Plasma vitamin C was not related to dysplasia progression in a longitudinal analysis,[42] but the number of cases in this study was too low. The only available clinical trial assessing the effect of vitamin C oral supplementation on regression of early lesions showed no benefit.[37] In this trial, a nonsignificant unfavorable effect of vitamin C in decreasing regression rates and increasing progression rates of CIN1 was observed, either administered alone or in combination with beta-carotene.[35]

Overall, evidence for a protective effect of vitamin C was possible for HPV persistence and cervical neoplasia, although the only available clinical trial showed no benefit of oral supplementation on early lesion regression.

Vitamin E

Serum tocopherol concentration was related to HPV persistence in 1[29] of 2 prospective studies.[29,32] Two[13,14] of 3 studies[13,14,16] looking at dietary vitamin E found inverse associations with SIL. Four[23,34–36] of 5[14,23,34–36] observational studies also showed inverse relationships of serum/plasma vitamin E with SIL. The observed protective effect of vitamin E was significant in 3 studies.[13,34,35] The only study not showing a protective effect had several limitations that were previously specified. In summary, evidence for a protective effect of vitamin E was possible for HPV persistence and probable for cervical neoplasia.

Foods

Surprisingly, only 3 studies[16,30,31] that fulfilled the inclusion criteria for this review looked at food intake (Fig. 4). Low intake of vegetables and, marginally, low intake of fruits and juices were associated with HPV persistence in a cohort study.[30] In a cohort of women with low levels of fruit and vegetable intake, increased dietary consumption of papaya, orange and broccoli conferred strong protection to HPV persistence.[31] Finally, a hospital-based case-control study found a marginally significant reduced risk of ISC for high cruciferous vegetable intake.[16]

In summary, although data were limited, a protective effect of fruits and vegetables was possible for HPV persistence. No studies were available on the association of fruit intake with cervical neoplasia, and evidence from the only study looking at vegetable intake and cervical neoplasia was insufficient.

Discussion

Although there is some epidemiologic support for a role of diet and nutritional status on cervical carcinogenesis, the available evidence taking HPV infection into account is not yet convincing. One of the most relevant new findings according to 2 recent prospective studies is a possible and promising protective effect of fruits and vegetables on HPV persistence. In relation to nutrients, evidence for a protective effect on HPV persistence is possible for some specific carotenoids, vitamin C and vitamin E. Evidence for a protective effect of cervical neoplasia was probable for folate, retinol and vitamin E and possible for vegetables, vitamin C, vitamin B12, alpha-carotene, beta-carotene, lycopene, lutein/zeaxanthin and cryptoxanthin. Evidence for an increased risk of cervical neoplasia associated with high blood homocysteine was probable. Overall, conclusions for nutrients from studies taking HPV infec-

tion into account do not differ substantially from those previous studies that did not control for it.[8–10]

Results did not differ between studies looking at preneoplastic and invasive lesions or between retrospective and prospective studies.

Finally, the consistent protective effects found in studies using HPV persistence as an endpoint are especially relevant because a possible protective effect of diet and nutrition on the cancer process is more plausible for early lesions[47] than for more advanced phases of the disease process.

Limitations of studies

Evidence from strong study designs such as chemoprevention trials and prospective studies is discouraging, although in a critical review of all chemoprevention trials available by 2001, it was pointed out that none of them had enough power to detect clinical significant differences in response rates.[48] Furthermore, most of them have been carried out without adequate information about the phase in which it could be more effective and about duration of treatment and the length of follow-up needed to demonstrate an effect.[49]

Most observational studies included in our review were case-control studies. Conclusions from case-control studies have to be cautious because potential for selection and recall bias needs to be considered. Also, the possible influence of the disease process on nutrient blood concentration has to be evaluated.[50]

Only 4 studies looked at invasive cervical cancer. Thus, the conclusions from our review are mostly relevant for preinvasive cervical neoplasia. Half of reviewed studies looked at the effect of nutrients on SIL without separating LSIL and HSIL. Dietary factors that act in early phases of the natural history of the disease could be different than those acting in more advanced phases. Folate and homocysteine were the only nutrients assessed in different studies with respect to both invasive and preinvasive disease. However, given the small number of studies in each stage group and the inconsistent results, no conclusions could be drawn on possible differences between low- and high-grade SIL.

Another limitation of available studies that could partly explain inconsistent results is potential misclassification of nutritional or dietary exposures and HPV infection.[51] With respect to HPV assessment, misclassification due to differences in assay sensitivity is possible, leading potentially to residual confounding.

High correlation among specific nutrients or between nutrients and other cofactors such as tobacco, OC use, multiparity or socioeconomic status could hamper the assessment of individual effects. Particularly important is the possible confounding effect of smoking and of oral contraceptive use on the assessment of the effect of beta-carotene, folate and vitamin C.[49]

Finally, insufficient power to detect statistically significant differences between groups is a limitation of some of the available studies. Seven of 23 studies had fewer than 100 cases and 6 studies had 100–200 cases. Those numbers are not sufficient to detect differences when stratifying by different outcomes.

Biologic plausibility

There are plausible biologic mechanisms by which dietary factors may protect from progression of transient to persistent HPV infection, SIL and ISC. Vitamins A and E regulate cell differentiation and proliferation.[52] On the other hand, vitamin C, vitamin E, carotenoids and other dietary constituents could act as efficient scavengers of free radicals and oxidants. These substances, which are produced during normal metabolism and the inflammation process and which are also contained in high amounts in tobacco smoke, could lead to extensive damage of DNA, proteins and lipids if not counteracted by antioxidant molecules.[53] Products arising from lipid peroxidation and protein modification can interact in turn with cigarette smoke products, creating additional toxic products. These products are thought to activate inflammatory immune responses, which may play a relevant role in oxidative tissue damage.[53] Vitamins C and E could protect from HPV persistence and inhibit cervical carcinogenesis by enhancing immunologic functions and by modulating inflammatory response to infection.[52] Also, vitamin C, vitamin E and other dietary constituents could inhibit DNA adduct formation, which is induced by tobacco products and other chemicals.[52,54,55] On the other hand, antioxidant nutrients could modulate immune response and decrease viral replication and gene expression.[49] It is worth noting that smoking and OC use, both established cofactors for cervical cancer, could decrease circulating levels of carotenoids, vitamin C and folate independently of intake.[49,56]

HPV, a necessary cause for cervical cancer, is integrated into the host DNA, especially at fragile sites, and activation of specific oncogenes appears to be a prerequisite for cervical carcinogenesis.[49] Low tissue folate levels increase the frequency of fragile sites on DNA, increase the risk of DNA damage by carcinogens and viruses and increase the potential for chromosomal damage and oncogene expression.[49] Folate and vitamins B6 and B12 are involved in DNA synthesis and repair and in DNA/RNA methylation, which may play a role in virus integration and gene stability.[8,9,46,49] Global hypomethylation is an early epigenetic event in cervical carcinogenesis and *in vitro* methylation selectively downregulates HPV 18 transcription.[49] Elevated plasma homocysteine concentration may be involved in cervical carcinogenesis since it is a biomarker of disruption of one-carbon metabolism.[28] Plasma homocysteine increases when intake of folate, vitamin B12, vitamin B6 and/or B2 are insufficient or by genetic variation in critical pathway enzymes, such MTHFR.[28]

Future directions

At present, there are no published cohort studies on SIL and few on HPV persistence that comprehensively assess suspected nutritional or dietary factors controlling for HPV status. Thus, further studies are needed, in particular prospective studies with long follow-up periods and enough power to detect small effects. It would be important to perform prospective studies in populations with a wide range of food intake. The following study designs would be valuable: *(i)* prospective cohort studies using HSIL and ISC as an endpoint; *(ii)* prospective studies on HPV persistence, with multiple HPV measures at different points in time; *(iii)* clinical trials looking at the effect of fruit and vegetable intake on HPV persistence and SIL. These trials would be preferably conducted in populations with low fruit and vegetable intake. Given the scarce number of studies on ISC taking HPV infection into account, case-control studies on diet and ISC would also be useful.

The use of serologic markers of HPV infection as a means of HPV adjustment may prove valid with the introduction of newer assays and the detection of antibodies against the most frequent genotypes.

Control for all known or suspected cofactors such as tobacco, parity and OC use would also be necessary, and investigation of possible interactions of dietary factors with genetic polymorphisms found to be biologically relevant could be of interest when looking at certain nutrients. Finally, further studies should assess both usual food, mainly fruits and vegetables, and usual nutrient intake through detailed questionnaires and, simultaneously, measure biochemical markers of nutrient intake.

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