REVIEW

Effect of dietary intervention on human micronucleus frequency in lymphocytes and buccal cells

Philip Thomas*, Jing Wu, Varinderpal Dhillon and Michael Fenech

CSIRO Division of Food and Nutritional Sciences, PO Box 10041, 13 Kintore Avenue, Adelaide BC, Adelaide, South Australia 5000, Australia

*To whom correspondence should be addressed. CSIRO Division of Food and Nutritional Sciences, PO Box 10041, 13 Kintore Avenue, Adelaide BC, Adelaide, South Australia 5000, Australia. Tel: +08 83038897; Fax: +08 83038899; Email: philip.thomas@csiro.au

Received on June 17, 2010; revised on August 4, 2010; accepted on August 27, 2010

Successful maintenance of accurate DNA replication and repair is critical to human health. Once this homeostatic balance is impaired, genomic instability events occur, compromising the integrity of the genome, which may initiate fundamental events leading to human diseases. Biomarkers of DNA damage, such as the micronucleus (MN) index, are elevated both in developmental and degenerative diseases and have been shown to be predictive of increased cancer risk and cardiovascular disease mortality. Several micronutrients have been identified as being effective in reducing and/or protecting against DNA damage. Micronutrients act as co-factors for enzymes required in DNA repair or maintenance of methylation patterns essential for optimal gene expression. In this review, published human intervention studies are examined with respect to the efficacy of micronutrient supplementation in reducing MN frequency in both lymphocytes and buccal cells. Important knowledge gaps and future research directions are also explored. The outcomes of these studies suggest that supplementation with antioxidant vitamins and certain B vitamins may cause a substantial reduction in MN frequency.

Introduction

Genome damage including DNA strand breakage, chromosome rearrangement, aneuploidy or alterations in methylation patterns and subsequent alterations in gene dosage and gene expression have been identified as being fundamental to the development of human diseases, such as cancer (1,2).

Biomarkers of chromosome damage need to be sensitive enough to reflect changes within the genome as a result of exposure to exogenous and endogenous agents. One such established biomarker is the micronucleus (MN) index, which is reflective of whole chromosome loss or breakage (3). MN frequency has been extensively used as a biomarker to gauge rates of chromosomal damage within human populations investigating exposure to genotoxic agents (4), micronutrient deficiency or excess (5,6) or differences in genotypic profiles (7). An elevated MN frequency in lymphocytes is predictive of increased cancer risk as well as cardiovascular mortality and has been found to be elevated in both Alzheimer’s and Parkinson’s disease (8–11).

Micronutrient status plays an important role in the protection against genome damage by providing co-factors required for the efficient function of enzymes involved in DNA repair, detoxification or maintenance of methylation of the genome (12,13). Micronutrient deficiency or excess can have modifying effects on genomic integrity that may involve nutrient–nutrient or nutrient–gene interactions and may depend on an individual’s genetic constitution (14).

It is important to identify candidate dietary factors that minimise genomic instability events including MN frequency with the eventual goal of potentially reducing future disease risk. This review aims to explore the relationship between micronutrient status and the effect on MN frequency in lymphocytes and buccal cells in light of human intervention studies. Intervention studies performed over the last two decades have mainly been placebo controlled. This review will focus on supplementation studies involving antioxidant vitamins (both single and multiple) and certain B vitamins and their effects on MN frequency.

Lymphocytes

The results of studies in lymphocytes are summarised in Table I.

Folate and vitamin B12

Folate and vitamin B12 perform an important function supplying methyl groups essential for DNA metabolism and maintenance (1,5). Folate is required for the synthesis of deoxythymidine monophosphate (dTMP) from deoxyuridine monophosphate (dUMP) and plays a key role as a methyl donor within the folate–methionine and DNA methylation maintenance pathways (Figure 1) (29). It has been shown that both micronutrient deficiency and/or excess can have detrimental effects in terms of genome damage (30).

Under conditions of folate deficiency, dUMP accumulates resulting in uracil being incorporated into DNA instead of thymine (31). Excessive incorporation of uracil not only leads to point mutations but also results in single- and double-strand DNA breaks, chromosome breakage and MN formation (32,33). Vitamin B12 deficiency similarly causes high uracil incorporation by restricting synthesis of the form of folate required for dTMP synthesis (i.e. 5,10 methylentetrahydrofolate), resulting in increased chromosome breakage (1,34). Folate and vitamin B12 are required for the synthesis of methionine through the remethylation of homocysteine (Hcy) and the synthesis of S-adenosylmethionine (SAM), the common methyl donor required for the maintenance of methylation patterns involving cytosine that determines gene expression and DNA conformation (34,35). Under low
### Table I. Human dietary intervention studies investigating micronutrient status in relation to MN frequency in lymphocytes

<table>
<thead>
<tr>
<th>Micronutrient tested</th>
<th>Nutrient supplement or dietary change tested</th>
<th>Type and duration of intervention</th>
<th>No. of subjects and age</th>
<th>Effect on MN frequency</th>
<th>Study author (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B vitamins</strong></td>
<td>Folic acid (0.7 mg/day for 2 months, 2.0 mg/day for 2 months)</td>
<td>Placebo-controlled over 4 months</td>
<td>N = 64 (all males), age 50–70 years</td>
<td>No significant difference</td>
<td>Fenech et al. (15)</td>
</tr>
<tr>
<td></td>
<td>Folic acid and vitamin B12 [700 μg FA, 7 μg B12/40 g cereal (3.5 × RDI), for 3 months, 2000 μg FA, 20 μg B12 (10 × RDI) for 3 months]</td>
<td>Randomised double-blind placebo-controlled over 6 months</td>
<td>N = 63 (32 females, 31 males), age 18–32 years</td>
<td>Significant 15.3% reduction (P &lt; 0.03) for the treated group and 25.4% reduction (P &lt; 0.01) for those in the treated group with above average MN frequency at baseline</td>
<td>Fenech et al. (6)</td>
</tr>
<tr>
<td><strong>Single antioxidants</strong></td>
<td>Folic acid (15 mg thrice weekly) and vitamin B12 (1000 μg/week)</td>
<td>Randomised controlled prospective study over 20 weeks</td>
<td>N = 27 (8 females, 19 males), age 52–84 years</td>
<td>Significant 59% reduction (P = 0.001)</td>
<td>Stopper et al. (16)</td>
</tr>
<tr>
<td></td>
<td>Vitamin C, 2 g supplement</td>
<td>Double-blind placebo-controlled intervention with a crossover. Samples collected 2 and 4 h post-supplementation</td>
<td>N = 11 (all males), age 21–44 years</td>
<td>No significant effect</td>
<td>Crott et al. (17)</td>
</tr>
<tr>
<td></td>
<td>Beta-carotene (30 mg/day), vitamin C (300 mg/day)</td>
<td>Placebo-controlled 7-day intervention</td>
<td>N = 17 (all females), age 20–21 years</td>
<td>Significant inverse correlation with plasma beta-carotene (P &lt; 0.001)*</td>
<td>Umegaki et al. (18)</td>
</tr>
<tr>
<td></td>
<td>Vitamin E 1. 50 mg for 8 weeks 2. 500 IU for 8 weeks</td>
<td>Placebo-controlled double-blind intervention over 16 weeks</td>
<td>N = 60 (all males), age 50–70 years</td>
<td>No significant reduction</td>
<td>Fenech et al. (19)</td>
</tr>
<tr>
<td></td>
<td>Selenium: 75, 150, 225 μg daily (8 weeks at each concentration)</td>
<td>Placebo-controlled double-blind intervention over 24 weeks</td>
<td>N = 81 (all males), age 40–70 years</td>
<td>No significant reduction</td>
<td>Wu et al. (20)</td>
</tr>
<tr>
<td></td>
<td>Coenzyme Q10 (100 mg/day)</td>
<td>Intervention over 2 weeks</td>
<td>N = 10 (4 females, 6 males), age 29–74 years</td>
<td>Significant 38% reduction (P &lt; 0.05)</td>
<td>Migliore et al. (21)</td>
</tr>
<tr>
<td><strong>Dual antioxidants</strong></td>
<td>Vitamin C (1000 mg/day)—7 days, Vitamin C (1000 mg/day) and Vitamin E (335 mg/day)—7 days</td>
<td>Controlled 14-day intervention</td>
<td>N = 24 (12 females, 12 males), age 19–33 years</td>
<td>Significant 49% reduction (P &lt; 0.05)</td>
<td>Schneider et al. (22)</td>
</tr>
<tr>
<td></td>
<td>Alpha-tocopherol (100 mg/day), beta-carotene (6 mg/day), vitamin C (100 mg/day), selenium (50 μg/day)</td>
<td>Randomised placebo-controlled intervention over 12 weeks</td>
<td>N = 106 (all males), age 39–51 years</td>
<td>Significant 39% reduction (P = 0.015)</td>
<td>Smolkova et al. (23)</td>
</tr>
<tr>
<td></td>
<td>Beta-carotene (18 mg/day), ascorbic acid (900 mg/day), L-alpha-tocopherol succinate (250 mg/day), zinc (12 mg/day)</td>
<td>Controlled intervention over 6 months</td>
<td>N = 178 (101 females, 77 males), age 32–62 years</td>
<td>Significant 25% reduction (P &lt; 0.05)</td>
<td>Fenech et al. (24)</td>
</tr>
<tr>
<td></td>
<td>Beta-carotene (15 mg/day), rutin (75 mg/day), retinol acetate (3 mg/day), alpha-tocopherol (30 mg/day) ascorbic acid (150 mg/day), folic acid (0.2 mg/day)</td>
<td>Controlled intervention over 4 months</td>
<td>N = 60 (30 females, 30 males), age 23–82 years</td>
<td>Significant 24.7% reduction (P &lt; 0.05)*</td>
<td>Gaziev et al. (25)</td>
</tr>
<tr>
<td><strong>Wine</strong></td>
<td>Polyphenols (300 ml of wine)</td>
<td>Crossover intervention samples collected 1, 3, 8 and 24 h after consumption</td>
<td>N = 4 (all males), age 20–45 years</td>
<td>Significant 76.7% reduction (P &lt; 0.01)*</td>
<td>Fenech et al. (26)</td>
</tr>
<tr>
<td></td>
<td>Polyphenols (300 ml of wine)</td>
<td>Crossover intervention samples collected 0.5, 1 and 2.0 h after consumption</td>
<td>N = 6 (all males), age 21–26 years</td>
<td>Significant 20% reduction (P = 0.0002)*</td>
<td>Greenrod et al. (27)</td>
</tr>
</tbody>
</table>

Recommended daily intake: folic acid (400 μg), vitamin B12 (6 μg), vitamin C (75 mg), vitamin E/tocopherol (10 mg), selenium (35 μg), coenzyme Q10 (30–200 mg), beta-carotene (10 mg), zinc (15 mg) and rutin (1000–3000 mg).

*Effect on radiation-induced MN frequency.
concentrations of vitamin B12 and methionine, both SAM synthesis and DNA methylation are reduced; furthermore, 5-methyltetrahydrofolate cannot be converted to 5,10-methylenetetrahydrofolate which is required as a methyl donor for dTMP synthesis, thus favouring an increase in the dUMP pool and uracil incorporation into DNA (5).

In a study of older men aged between 50 and 70 years, the MN index was significantly elevated in individuals with non-optimal values for serum folate (<6.8 nmol/l), B12 (<150 pmol/l) and HCy (>10 μmol/l) compared to individuals with higher levels (P = 0.02) (15). Individuals who had HCy levels above 10 μmol/l but had normal folate and B12 ranges had a significantly elevated MN index (P = 0.05) compared to those with normal folate and B12 and a HCy level of <10 μmol/l. This suggests that elevated HCy concentrations even in the presence of normal B vitamin status or low levels of folate and B12 is an important factor for increased chromosome damage (15). A placebo-controlled trial in this cohort showed that supplementing daily for 2 months with 0.7 mg folic acid followed by another 2 months with 2.0 mg folic acid only produced an 11% reduction in HCy with no significant change in MN frequency (15).

A cross-sectional study in young adults aged 18–32 years showed a positive correlation between MN frequency and plasma HCy in males (R = 0.293, P < 0.05) and a negative correlation with serum B12 in females (R = −0.36, P < 0.05) (6). A follow on placebo-controlled dietary intervention study employing folate and B12 at 3.5 times, the recommended dietary intake (RDI) for 12 weeks followed by a tablet supplementation regimen of 10 times the RDI for a further 12 weeks, found that the MN frequency was significantly reduced by 15.3% (P < 0.03) in the treated cohort and by 25% (P < 0.01) in those individuals with a baseline MN frequency within the high 50th percentile. The MN frequency positively correlated with plasma HCy (R = 0.39, P < 0.006) and negatively correlated with serum B12 (R = −0.49, P < 0.0005). The main result showed that the MN frequency was significantly reduced when plasma HCy was <7.5 μmol/l and serum B12 was in excess of 300 pmol/l. It was shown that intake of 700 μg of folic acid and 7 μg B12 was sufficient to minimise both plasma HCy and the MN frequency (6,36).

Folic acid and B12 supplementation reduced the MN frequency in individuals suffering end-stage renal failure (16). Both cancer incidence and MN frequency are significantly elevated in renal patients (37). Patients were supplemented with either 15 mg folic acid three times a week following dialysis or given folic acid together with 1000 μg of hydroxycobalamin (vitamin B12) once a week over a 17-week period. The MN index was reduced by 28% in the folic acid group and 41% in the combined folic acid and B12 cohort. Plasma HCy levels were also significantly reduced by 32% in the folic acid group and by 47% in the combined folic acid and B12 group (16).

Single and multiple antioxidants
Free radical generation results from normal metabolic processes that are maintained within normal homeostatic boundaries by an elaborate endogenous antioxidant system (38). When levels of free radicals exceed the normal clearing capacity of the cell, oxidative stress follows resulting in cellular and genome damage.

Single antioxidant supplements
Vitamin C acts as both an antioxidant and a pro-oxidant, which in this latter role may involve the reduction of DNA-bound anions, such as copper and iron that have the capacity to reduce hydrogen peroxide to form the highly reactive hydroxyl radical. Interaction of these hydroxyl radicals with the DNA backbone can lead to single- or double-strand breaks leading to MN formation (39). Vitamin C in vitro has been shown to increase DNA damage in a dose-dependent manner and at higher doses to enhance the cytotoxicity of hydrogen peroxide to human lymphocytes (40). The anti-oxidant capacity of vitamin C stems from the poor reactivity of the semi-hydroxascorbate radical produced upon reaction with reactive oxygen metabolites (41). Its ability to quench free radicals efficiently in vivo may reduce damage to proto-oxidant and tumour suppressor genes, thereby lowering the risk of cancer. Epidemiological evidence shows that a high intake of vitamin C-rich foods reduces the risk of certain cancers by up to 50% (42).
A double-blind placebo-controlled intervention consisting of 11 male subjects determined whether high plasma vitamin C promotes or protects against genetic damage and cell death ex vivo. Venous blood was collected before and after an antioxidant-poor diet, which reduced plasma vitamin C by 15% \( (P < 0.05) \), and was followed with a 2-g vitamin C supplement, which raised plasma concentrations by 115 and 125% \( (0.12 \text{ mM}) \) after 2 and 4 h, respectively \( (P < 0.05) \). Plasma collected post-vitamin C ingestion did not alter MN expression or apoptosis in control or hydrogen peroxide-treated lymphocytes but moderately increased necrosis \( (P < 0.08) \). Analysis of combined data showed that necrotic cell frequency correlated positively with micronucleated cell frequency \( (R = 0.66, P < 0.0001) \) and negatively with apoptotic cell frequency \( (R = -0.81, P < 0.0001) \). Overall, vitamin C supplementation did not appear to cause DNA damage under normal physiological conditions nor did it protect cells against hydrogen peroxide-induced toxicity \( (17) \).

The potential protective effects of beta-carotene and ascorbic acid on the spontaneous and x-ray-induced MN frequency have been investigated \( (18) \). Three groups of volunteers having been on a 12-day beta-carotene-deficient diet were given beta-carotene \( (30 \text{ mg}) \), ascorbic acid \( (300 \text{ mg}) \) or a placebo. It was shown that the x-ray-induced MN frequency was less in the beta-carotene-supplemented group showing a significant inverse correlation with plasma beta-carotene \( (P < 0.001) \), suggesting a protective effect from exogenously induced genetic damage \( (18) \).

Vitamin E prevents the production of reactive oxygen species forming when fat undergoes oxidation. Results of a placebo-controlled double-blind intervention study were inconclusive on whether vitamin E intake exceeding the RDI \( (10 \text{ mg/day}) \) could afford protection against DNA damage in 60 male volunteers \( (19) \). The intervention was split into two phases of 8 weeks each with supplementation of vitamin E at 5 \( \times \) RDI during Phase 1 followed by 10 \( \times \) RDI of vitamin E during Phase 2. The results showed a significant 32% decrease \( (P < 0.007) \) in the MN frequency for both control and treated groups and further did not mitigate against the effects of endogenous hydrogen peroxide. It was unclear whether the observed reduction was due to the soya bean oil carrier used in the placebo and vitamin E capsules \( (19) \). Consequently in future studies, associations of reduced MN in vitamin E interventions need to consider that the lipid carrier itself maybe genome protective.

Selenium is an essential co-factor in antioxidant defense enzymes, such as glutathione peroxide and certain forms of thioredoxin reductase \( (43) \). It has been suggested that inadequate levels of selenium intake may cause an increased risk for some cancers particularly prostate cancer \( (44,45) \). A placebo-controlled double-blind study investigated the effect of increased selenium intake over a 24-week period on the effect on DNA damage biomarkers in a cohort of healthy elderly South Australian men. The consumption of wheat biscuits biofortified with selenium (three/day) increased plasma selenium from a baseline level of 122–192 \( \mu \text{g/ml} \). Improvement of selenium status had no modifying effect on MN frequency leading to the conclusion that this cohort was selenium replete at levels sufficient to minimise baseline DNA damage \( (20) \). In fact, plasma selenium concentration of 120 \( \mu \text{g/ml} \) is considered optimal for prostate cancer prevention \( (46) \).

Endogenous oxidative stress has been shown to play a key role in the pathogenesis of mitochondrial disease. These disorders are characterised by increased free radical production resulting from mitochondrial dysfunction affecting both nuclear and mitochondrial integrity \( (21) \). The objective of this intervention study was to supplement 13 mitochondrial disease patients with ubiquinone \( (100 \text{ mg/day}) \), a coenzyme Q10 analogue, and measure the effect on MN frequency. Following 2-week supplementation, a significant 38% reduction \( (P < 0.05) \) in MN frequency was evident in this cohort when compared to baseline values \( (21) \).

**Dual antioxidants**

An intervention study involving cohorts of smokers and non-smokers were supplemented with vitamin C and E to investigate the impact on MN frequency. Baseline concentrations of both vitamins were lower in the smokers, who also had higher MN frequency compared to the non-smoker cohort. Both cohorts were supplemented with 1000 mg vitamin C daily for 7 days and then for a further 7 days with both 1000 mg vitamin C and 335 mg vitamin E. The MN frequency was significantly reduced in both cohorts \( (P < 0.05) \) but was more pronounced in the smoker cohort \( (22) \).

**Multiple antioxidants and wine**

A placebo-controlled intervention investigated the effect of alpha-tocopherol \( (100 \text{ mg/day}) \), beta-carotene \( (6 \text{ mg/day}) \), vitamin C \( (100 \text{ mg/day}) \) and selenium \( (50 \mu \text{g/day}) \) on oxidative stress and MN incidence and the influence of the methylenetetrahydrofolate reductase gene \( (MTHFR) \). The study comprised of two groups of middle-aged men differing in cardiovascular disease risk factors. The intervention was performed over a 12-week period and MN frequencies following the intervention were measured in relation to MTHFR genotype, HCy and folate levels. The MN frequencies and plasma folate levels did not vary in relation to genotype status. MN frequency showed a significant 39% \( (P = 0.015) \) decrease specifically in individuals with folate levels within the normal physiological range. In individuals with low folate levels, a significant correlation existed between folate and HCy following supplementation. This is suggestive that folate deficiency further increases the effects of potential risk factors such as Hcy levels in relation to DNA damage \( (23) \).

A randomised controlled intervention, performed in 190 healthy individuals \( (\text{mean age 47.8 years, 46% males}) \), examined whether supplementation for 6 months with beta-carotene, vitamins C, E and zinc \( (ACEZn) \) improved genome stability \( (24) \). Half the volunteers were randomly selected to take a daily dose, as a tablet, of an antioxidant mixture \( (ACEZn) \), supplying 18 mg beta-carotene, 900 mg ascorbic acid, 250 mg \( \alpha \)-alpha-tocopheryl succinate and 12 mg Zn. The rest of the volunteers were given no supplement and served as controls. The duration of the intervention was 6 months with volunteers providing a blood sample at the end of the intervention period. A subset of volunteers \( (12 \text{ males and 11 females in the control group and 10 males and 12 females in the ACEZn group}) \) agreed to donate an additional blood sample 3 months into the intervention to assess changes in plasma ascorbic acid, alpha-tocopherol, beta-carotene and zinc. The efficacy of the ACEZn intervention was tested by measuring changes in these plasma micronutrients in a subset of the control group \( (n = 23) \) and the supplemented group \( (n = 22) \) after 3 months of supplementation. There was no change in these micronutrients in the plasma of the control group. In contrast, the plasma ascorbic acid, alpha-tocopherol and
beta-carotene in the supplemented group increased by 27% ($P < 0.05$), 55% ($P < 0.01$) and 500% ($P < 0.001$), respectively, but there was no change in plasma zinc. A significant 25% decrease in MN frequency ($P < 0.05$) occurred in the supplemented group at the end of the study relative to baseline values. The final net effect of treatment, obtained by comparing the supplemented group and control group, after adjusting for individual characteristics, dietary habit and baseline values of MN frequency, was a 13% significant reduction in MN frequency (95% confidence interval = −1%, −24%, $P = 0.038$) (24).

A vitamin antioxidant combination containing the vitamins A, C, E as well as beta-carotene, folic acid and rutin, when taken daily for 4 months, reduced spontaneous and gamma-radiation-induced MN frequency significantly ($P < 0.01$) in both young and older subjects. This is suggestive that antioxidant micronutrient combinations may be effective in reducing DNA damage, resulting from both exogenous and endogenous insults (25).

Epidemiological evidence suggests that a diet-containing phenolic compounds may decrease genomic instability by protecting DNA from oxidative damage (47,48). In an intervention study, where individuals were placed on a low-polyphenol diet for 48 h prior to the consumption of 300 ml of red or white wine, it was shown that plasma collected at time points up to 3 h following wine consumption produced a significant 70% reduction in hydrogen peroxide-induced MN frequency (26). This suggests that consumption of wine may bring about changes within blood plasma that may have a protective effect on DNA damage levels, resulting from exposure to exogenous and/or endogenous sources of reactive oxygen metabolites.

A crossover intervention study investigated both the alcoholic and non-alcoholic fraction of wine in relation to the potential protective effects against DNA damage induced by oxidative stress (27). Similarly, individuals were placed on a low-polyphenol diet for 48 h prior to the consumption of 300 ml of complete red wine, de-alcoholised red wine or ethanol on three separate occasions 1 week apart. The de-alcoholised wine significantly reduced radiation-induced MN frequency at 1 and 2 h post-consumption by 20%. Interestingly, the ethanol fraction increased radiation-induced DNA damage, whereas the complete wine was more effective in reducing MN frequency relative to the ethanol fraction but was not as effective as the de-alcoholised wine (27).

**Buccal mucosa cells**

Other candidate human tissues have been investigated as potential models to reflect genomic instability status. The buccal mucosa is a stratified squamous epithelial layer that allows a minimally invasive approach towards cellular collection. In light of the fact that >90% of cancers are epithelial in origin (49), buccal cell utilisation has great epidemiological potential both as a non-invasive means for genotoxic assessment and in identifying potential biomarkers for future disease assessment. The results of dietary supplement intervention on MN frequency in buccal cells are summarised in Table II.

**Folate**

Titenko-Holland et al. (50) showed in a study consisting of nine healthy postmenopausal women that controlled changes in folate intake could alter MN frequency. The participants were subjected to a baseline week with a folate intake of 195 µg/day, 5-week depletion at 56 µg/day and gradual repletion including 4 weeks at 111 µg/day, 11 days at 286 µg/day and 9 days at 516 µg/day. The results showed that the MN frequency in exfoliated buccal cells was successfully decreased following a period of dietary supplementation with 516 µg of folate per day (50).

A controlled intervention study determined the MN frequency within the buccal mucosa of patients diagnosed with diabetes mellitus (DM) compared to healthy controls (51). One hundred and fifty-nine individuals were subdivided into three subgroups: Group 1 were healthy individuals ($n = 81$); Group 2 were patients with DM ($n = 48$) and Group 3 were DM patients ($n = 30$) who were supplemented with 5 mg folic acid three times a day for 30 days. It was shown that the MN frequency in patients with DM (prior to supplementation) was 2-fold higher than in healthy subjects ($P < 0.001$). The 30 DM patients who took folic acid showed a significant reduction ($P < 0.001$) in the MN frequency after 30 days supplementation ($0.67 ± 0.55$) compared to their baseline frequency ($2.47 ± 1.43$) (51).

**Single and multiple antioxidants**

**Single antioxidants**. An earlier study showed that the MN frequency was reduced following a trial of vitamin E for chemoprevention of oral leukoplakia. Following 24 weeks of supplementation with 400 U of vitamin E twice daily, the MN frequency was significantly reduced from sites of visible lesions ($P < 0.01$) and normal looking mucosa ($P < 0.01$) (52). This reduction in MN frequency may be the result of modulation of the DNA repair system resulting in efficient removal of damaged DNA in vitamin E treated cells (58), although no evidence was provided in the study by Benner et al. (52).

Stich et al. performed a study utilising MN frequency to determine the potential protective effects of vitamin A, beta-carotene and canthaxanthine on the buccal mucosa of betel nut/tobacco chewers. Following 9-week supplementation with either vitamin A (150 000 IU/week) or beta-carotene (180 mg/week), the frequency of micronucleated cells was significantly reduced compared to baseline frequency ($P < 0.001$) (53). Stich et al. (53) suggest that vitamin A and beta-carotene may have an inhibitory effect on MN formation utilising mechanisms not involving free radical scavenging. This is proposed as canthaxanthine was ineffective in reducing MN formation but has been shown to be effective in its role as a free radical scavenger (53).

Individuals with oral lichen planus (OLP), a chronic inflammatory disease resulting in striations and plaques on the buccal mucosa, have been reported to have an elevated frequency of micronucleated exfoliated cells (59). An open trial containing 20 patients were supplemented with 15 mg of beta-carotene four times daily for 3 months. Buccal MN frequency was measured in both lesions and adjacent normal tissue both pre- and post-supplementation. A significant reduction in the MN frequency ($P < 0.01$) was reported in the OLP lesions but no significant reduction occurred within the normal mucosa (54).

An intervention trial in an Inuit cohort involved the supplementation of beta-carotene (180 mg/week, given twice weekly in six capsules of 30 mg each) to cohorts of smokeless tobacco and non-tobacco users to determine the effect on MN
frequency. Prior to beta-carotene administration, the MN index was $1.87 \pm 0.92\%$ at the gingival groove, where tobacco was usually held in the mouth. A significant decrease ($P < 0.001$) in the MN frequency ($0.74 \pm 0.42\%$) was evident following 10-week beta-carotene supplementation (55).

**Dual antioxidants.** A short-term intervention trial determined the effects of beta-carotene alone and in combination with vitamin A on leukoplakia development (precancerous patches of oral keratosis) and MN frequency in Indian tobacco/betel quid chewers (56). The three groups under investigation were supplemented as follows: Group 1, beta-carotene only (180 mg/week); Group 2 beta-carotene (180 mg/week) plus vitamin A (100 000 IU/week) and Group 3 received a placebo. All supplementation capsules were administered twice weekly for 6 months. After 3 months, the MN frequency was significantly reduced in Group 1 (4.09–1.1% in areas of leukoplakia and 4.1–1.0% within the normal mucosa). Following 6 months of treatment, significant remission of leukoplakias occurred in Group 1 (14.8%) and Group 2 (27.5%) compared to the placebo group, suggesting potential protective effects against both leukoplakia and MN formation (56).

**Multiple antioxidants.** A randomised double-blind intervention study determined whether weekly intake of retinol (15 mg), riboflavin (200 mg) and zinc (50 mg) was effective in reducing both precancerous lesions in oesophageal tissue and MN incidence in a population with a high incidence of oesophageal cancer. Following 1 year of treatment, there were no statistically significant differences in the buccal mucosa MN frequency before and after supplementation (57).

**Conclusions and future directions**

This review highlights significant findings from a small number of human intervention studies showing that micronutrient supplementation in some instances significantly reduces MN frequency. The data derived from these intervention studies suggest that multiple micronutrient combinations appear to be more efficacious under conditions of normal folate and B12 status in the reduction of MN frequency compared to studies investigating the effects of individual micronutrients. In lymphocytes, the mean reduction in baseline MN for multiple micronutrients was 33.4% (mean reduction for vitamin A (P < 0.001), significant 58% reduction for vitamin A (P < 0.001)) compared to studies investigating the effects of individual micronutrients. In lymphocytes, the mean reduction in baseline MN for multiple micronutrients was 33.4% (mean ± SD), while for single micronutrients, the effect size was 7.6 ± 16.9% (P < 0.04). For single micronutrients, the following studies were examined for effect size (refs 15, 17, 19–21 in Table I), whereas for multiple micronutrients, studies (refs 6, 16, 22–25 in Table I) were considered. All micronutrient doses, length of supplementation and sample sizes are available in Table I.

The presence of MN is a strong indicator of chromosomal damage resulting from either whole chromosome loss or breakage. As genome damage is considered the most fundamental of all disease pathologies, it is essential to determine which micronutrient supplementation is necessary to maintain optimal genome health and who is likely to benefit. In negative outcome studies, where no additional health benefits are conferred upon individuals with adequate baseline levels, future studies need to address whether specific

---

**Table II. Human dietary intervention studies investigating micronutrient status in relation to MN frequency in buccal mucosa**

<table>
<thead>
<tr>
<th>Micronutrient tested</th>
<th>Nutrient supplement or dietary change tested</th>
<th>Type and duration of intervention</th>
<th>No. of subjects and age</th>
<th>Effect on MN frequency</th>
<th>Study author (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B vitamins</td>
<td>Controlled changes in folate intake—56, 111, 286, 516 μg/day 5 mg folic acid, 3 times daily</td>
<td>Depletion, repletion intervention over 13 weeks Controlled intervention over 30 days</td>
<td>N = 9 (all females), age 49–63 years</td>
<td>Significant 57% reduction ($P = 0.010$)</td>
<td>Titenko-Holland et al. (50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N = 159 (88 females, 71 males), age 35.16 ± 12.8 years</td>
<td>Significant 73% reduction ($P &lt; 0.001$)</td>
<td>Zunigá-Gonzalez et al. (51)</td>
</tr>
<tr>
<td>Single antioxidant</td>
<td>Alpha-tocopherol, 400 U twice daily</td>
<td>Intervention over 24 weeks</td>
<td>N = 24 (11 females, 13 males), age 29–83 years</td>
<td>Significant 64%, reduction ($P &lt; 0.01$)</td>
<td>Benner et al. (52)</td>
</tr>
<tr>
<td></td>
<td>Beta-carotene (180 mg/week), vitamin A (150 000 IU/week), canthaxanthine (180 mg/week)</td>
<td>Placebo-controlled intervention over 9 weeks</td>
<td>N = 89, gender and age not stated</td>
<td>Significant 61% reduction for beta-carotene ($P &lt; 0.001$), significant 58% reduction for vitamin A ($P &lt; 0.001$)</td>
<td>Stich et al. (53)</td>
</tr>
<tr>
<td></td>
<td>Beta-carotene (15 mg/4 times daily)</td>
<td>Intervention over 3 months</td>
<td>N = 20 (19 females, 1 males), age 31–61 years</td>
<td>Significant 78% reduction ($P &lt; 0.001$)</td>
<td>Buajeeb et al. (54)</td>
</tr>
<tr>
<td></td>
<td>Beta-carotene (180 mg/week)</td>
<td>Placebo-controlled intervention over 10 weeks</td>
<td>N = 23</td>
<td>Significant reduction ($P &lt; 0.001$)</td>
<td>Stich et al. (55)</td>
</tr>
<tr>
<td>Dual antioxidants</td>
<td>1. Beta-carotene (180 mg/week) 2. Beta-carotene (180 mg/week) and vitamin A (100 000 IU/week)</td>
<td>Placebo-controlled intervention over 6 months</td>
<td>N = 130, age 48.8 ± 12.9 years, gender not stated</td>
<td>Significant 14.8% reduction for beta-carotene ($P &lt; 0.001$), significant 27.5% reduction for beta-carotene and vitamin A ($P &lt; 0.001$)</td>
<td>Stich et al. (56)</td>
</tr>
<tr>
<td>Multiple antioxidants</td>
<td>Retinol (15 mg), riboflavin (200 mg), zinc (50 mg/week)</td>
<td>Randomised double-blind intervention over 1 year</td>
<td>N = 170</td>
<td>No significant difference</td>
<td>Munoz et al. (57)</td>
</tr>
</tbody>
</table>

Recommended daily intake: folic acid (400 μg), vitamin E/tocopherol (10 mg), beta-carotene (10 mg), vitamin A (600 μg), retinol (600 μg), riboflavin (1.6 mg) and zinc (15 mg).
genotypes or higher susceptibility groups, such as cancer cohorts, are more likely to benefit or be at risk of toxic effects from increased micronutrient supplementation. One of the current challenges in the design of future dietary intervention trials is the potential impact of baseline variability upon MN frequency. Age and gender are the most important demographic variables impacting on the MN index with MN frequencies in females being greater than those in males by a factor of 1.2–1.6 depending on the age group. For both sexes, MN frequency was significantly and positively correlated with age ($R = 0.62$ in males and $R = 0.65$ in females). The main dietary factors influencing the MN index in subjects, who are not folate deficient, are plasma B12 ($R = -0.315$, $P = 0.0127$) and plasma HCY ($R = 0.415$, $P = 0.0086$) (60). There is a need to consider the development of personalised micronutrient combinations (nutrimes), consisting of those dietary components that based on current evidence can maximise effective genome maintenance depending on genetic background, lifestyle choices and disease state. An important knowledge gap is to determine whether the relationship between nutrient profiles and DNA damage events is substantially different at various stages of the human lifecycle. Perhaps, the most challenging of all knowledge gaps is whether reducing genomic instability events, such as the MN frequency by micronutrient supplementation, results in a minimisation of individual risk to future diseases for which the probability of occurring is increased when DNA damage or MN frequency rates are elevated (8,9,11). Further interventions should be undertaken to determine MN frequencies, where data are currently unavailable involving micronutrients, such as B6, which is thought to be protective against disease states, such as colorectal cancer to investigate potential levels of DNA damage, cytostasis and cytotoxicity resulting from micronutrient supplementation.

Acknowledgements

Conflict of interest statement: None declared.

References

24. Fenech, M., Baghurst, P., Luderer, W., Turner, J., Record, S., Ceppi, M. and Bonassi, S. (2005) Low intake of calcium, folate, nicotinic acid, vitamin A, retinol, vitamin E and riboflavin are significantly associated with increased genome instability—results from a dietary intake and micronucleus index survey in South Australia. Carcinogenesis, 26, 991–999.

Diet and MN frequency