

Review
The role of folic acid and Vitamin B12 in genomic
stability of human cells

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Received 24 August 2000; received in revised form 17 October 2000; accepted 24 October 2000

Abstract

Folic acid plays a critical role in the prevention of chromosome breakage and hypomethylation of DNA. This activity is compromised when Vitamin B12 (B12) concentration is low because methionine synthase activity is reduced, lowering the concentration of S-adenosyl methionine (SAM) which in turn may diminish DNA methylation and cause folate to become unavailable for the conversion of dUMP to dTMP. The most plausible explanation for the chromosome-breaking effect of low folate is excessive uracil misincorporation into DNA, a mutagenic lesion that leads to strand breaks in DNA during repair. Both in vitro and in vivo studies with human cells clearly show that folate deficiency causes expression of chromosomal fragile sites, chromosome breaks, excessive uracil in DNA, micronucleus formation and DNA hypomethylation. In vivo studies show that Vitamin B12 deficiency and elevated plasma homocysteine are significantly correlated with increased micronucleus formation. In vitro experiments indicate that genomic instability in human cells is minimised when folic acid concentration in culture medium is >227 nmol/l. Intervention studies in humans show: (a) that DNA hypomethylation, chromosome breaks, uracil misincorporation and micronucleus formation are minimised when red cell folate concentration is >700 nmol/l folate; and (b) micronucleus formation is minimised when plasma concentration of Vitamin B12 is >300 pmol/l and plasma homocysteine is <7.5 μ mol/l. These concentrations are achievable at intake levels in excess of current RDIs i.e. more than 200–400 μ g folic acid per day and more than 2 μ g Vitamin B12 per day. A placebo-controlled study with a dose–response suggests that based on the micronucleus index in lymphocytes, an RDI level of 700 μ g/day for folic acid and 7 μ g/day for Vitamin B12 would be appropriate for genomic stability in young adults. Dietary intakes above the current RDI may be particularly important in those with extreme defects in the absorption and metabolism of these Vitamins, for which ageing is a contributing factor. © 2001 Elsevier Science B.V. All rights reserved.

Key to unit conversion: 1 ng/ml folic acid, 2.26 nmol/l folic acid; 1 pg/ml Vitamin B12, 0.74 pmol/l Vitamin B12

Keywords: Genomic stability; Chromosome aberrations; Micronuclei; DNA methylation; Recommended daily allowance; Vitamin B12; Folic acid; Homocysteine

Abbreviations: HC: homocysteine; MN: micronucleus; Mned: micronucleated; RDI: recommended dietary intake; SSB: single-stranded break; DSB: double-stranded break; RBCs: red blood cells

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PII: S0027-5107(01)00079-3

1. Introduction

Folic acid and Vitamin B12 (B12) play an important role in DNA metabolism [1] (Fig. 1). Folic acid is required for the synthesis of dTMP from dUMP. Under conditions of folic acid deficiency, dUMP accumulates and as a result uracil is incorporated into DNA instead of thymine [2]. There is good evidence suggesting that excessive incorporation of uracil in DNA not only leads to point mutation but may also result in the generation of single- and double-stranded DNA breaks, chromosome breakage and micronucleus formation [3,4]. The mutagenic effects of uracil are underscored by the observation that of eight known human glycosylases, four (UNG, TDG, hSMUG1, MBD4) are dedicated to the removal of uracil [5]. Folic acid and Vitamin B12 are also required for the synthesis of methionine and S-adenosyl methionine (SAM), the common methyl donor required for the maintenance of methylation patterns in DNA that determine gene expression and DNA conformation [6]. When the concentration of Vitamin B12 and methionine is low, SAM synthesis is reduced, methylation of DNA is reduced, inhibition by SAM of methylenetetrahydrofolate reductase (MTHFR) is minimised resulting in the irreversible conversion of 5,10-methylenetetrahydrofolate to

5-methyltetrahydrofolate, thus, favouring an increase in the dUMP pool and uracil incorporation into DNA. Deficiencies in folic acid and Vitamin B12, therefore, can lead to: (a) elevated DNA damage rate and altered methylation of DNA, both of which are important risk factors for cancer [3–5]; and (b) an increased level in homocysteine status, an important risk factor for cardiovascular disease [7]. These same defects may also play an important role in developmental and neurological abnormalities [3,4].

The blood levels of folate and Vitamin B12 required to prevent anaemia and hyperhomocysteinemia are properly defined, however, it is still uncertain whether such accepted levels of sufficiency are in fact adequate to minimise chromosome damage rates and optimise DNA methylation status. In this paper evidence is provided from in vitro studies with human cells and in vivo cross-sectional and intervention studies in humans to identify the concentration or intake level at which potential genotoxic effects of low folate and Vitamin B12 status may be prevented. In addition, the potential impact of genetic polymorphisms in key transport molecules and enzymes required for the metabolism and of folic acid and Vitamin B12 are discussed as factors that should be considered when determining recommended dietary intakes (RDI) of these Vitamins based on genomic stability.

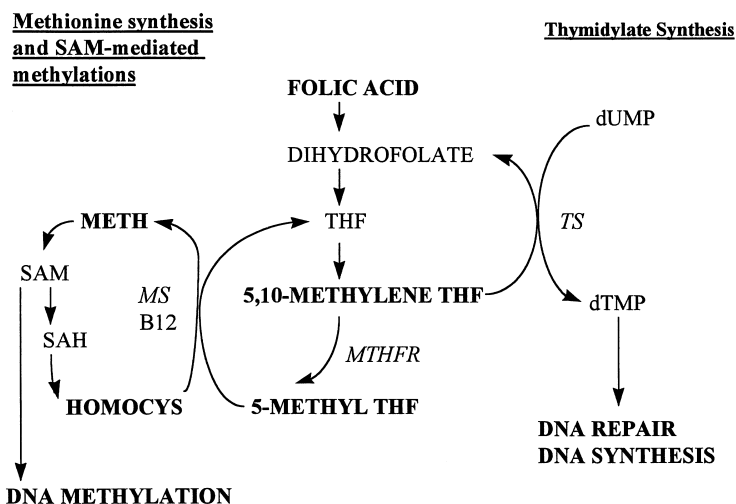


Fig. 1. The main metabolic pathways in folate and homocysteine (homocys) metabolism. B12: Vitamin B12; meth: methionine; THF: tetrahydrofolate; TS: thymidylate synthase; MS: methionine synthase; MTHFR: methylenetetrahydrofolate reductase; SAM: S-adenosyl methionine; SAH: S-adenosyl homocysteine.

2. Evidence from in vitro cultures with human cells

It has been shown that fragile sites in chromosomes are expressed when human lymphocytes are cultured in the absence of folic acid and thymidine in culture medium [8,9]. Furthermore, under these conditions chromosome breakage and micronucleus (MN) expression are increased simultaneously suggesting a similar mechanism underlying the expression of fragile sites and chromosome breakage [8–10]. Reidy's experiments showed that lymphocytes cultured in folic acid deficient medium exhibit increased levels of excision repair during G₂ because the cytosine arabinoside-induced chromosome aberration level (which is indicative of excision repair activity) was more than doubled by this treatment and further enhanced by addition of deoxyuridine [11,12]. Treatment of human lymphoid cells in culture with methotrexate results in a large increase in the dUTP/dTTP ratio and a much increased incorporation rate of uracil in DNA [13]. The connection between uracil incorporation and the generation of DNA strand breaks was confirmed in more recent studies using the single cell gel electrophoresis method, in this method, the extent of uracil incorporation was measured by treating the nuclei with uracil DNA glycosylase followed by measurement of resulting DNA breaks [14,15].

It appears that no studies have yet been published showing a link between Vitamin B12 deficiency in vitro and increased genomic instability in human cells. Conclusive experiments to answer this question are yet to be performed.

3. Evidence from in vivo studies with humans

Results from studies in rodents suggest that extreme folate deficiency (i.e. on diets without folic acid that also include succinyl sulphathiazole, an antibiotic that eradicates folate producing bacteria in the gut) causes DNA strand breaks, hypomethylation of DNA, increased uracil and apurinic sites in DNA [16–18] and caffeine synergistically increased folate-deficiency-induced micronucleus frequencies in peripheral blood erythrocytes [19]. However, marginal folic acid deficiency (400 nmol/kg diet) did not increase micronucleus levels in maternal or foetal

reticulocytes but it did cause a significant three-fold increase in foetal developmental abnormalities [20]. The data from these rodent studies suggest that folic acid intake levels are critical for genomic stability in vivo and indicate that optimal thresholds for prevention of genomic stability and prevention of developmental defects may be different. Studies on the effects of Vitamin B12 deficiency on genomic instability in rodents have not been published.

The early evidence of chromosome damage in human cells in vivo from folate and Vitamin B12 deficiency was first obtained from studies linking the expression of Howell–Jolly bodies in erythrocytes with megaloblastic anaemias [21–23]. Howell–Jolly bodies are whole chromosomes or chromosome fragments that lag behind at anaphase during production and maturation of the red blood cell and in fact they are the same as micronuclei, the alternative and most commonly used term for this chromosome damage biomarker. Micronucleated erythrocytes in humans are most readily observed in splenectomised subjects because the spleen actively filters micronucleated erythrocytes from the blood [24,25].

A case study of a 30-year-old male with Crohn's disease with a very high level of micronuclei in erythrocytes (67/1000 cells) showed that this was associated with a low serum folate (1.9 ng/ml; normal range > 2.5 ng/ml) and a low red cell folate (70 ng/ml; normal range > 225 ng/ml). Micronucleus frequency was reduced to 12/1000 cells, serum folate increased to >20 ng/ml, red cell folate increased to 1089 ng/ml after 25 days with daily oral dose of 25 mg folic acid [24]. One of the main observations of this study was that minimum spontaneous MN frequencies were observed only when serum folate levels exceeded 15–20 ng/ml which was higher than the values accepted as normal by clinicians (i.e. 6–15 ng/ml).

A cross-sectional study of smokers ($N = 30$) and non-smokers ($N = 30$) showed a significant inverse relationship between chromosome aberrations and blood folate status and that smoking and blood folate status are interrelated in their association with chromosome fragility. In this study of ex vivo expressed DNA damage the cells were cultured in low folate medium and the results may, therefore, reflect the expression of fragile sites within chromosomes [26].

Another small ($N = 22$) cross-sectional study on the influence of blood micronutrients on micronucleus

frequency in erythrocytes of splenectomised subjects pre-selected because they had amongst the highest or lowest micronucleus frequencies from a larger population ($N = 122$) showed that the elevated micronucleus index was only strongly associated with low levels of serum folate (<4 ng/ml) and low levels of plasma B12 (<200 pg/ml). Vitamins C, E and beta-carotene did not show a strong inverse correlation with the micronucleus index [27].

Blood samples from the same cohort of individuals analysed in the studies of splenectomised individuals [24,26] were also analysed for uracil content [3,4]. The results showed that uracil level in DNA was 70-fold higher in individuals with serum folate <4 ng/ml relative to individuals with serum folate >4 ng/ml. Uracil levels in DNA were rapidly reduced (within 3 days) after daily supplementation with 5 mg folic acid in both the deficient (<4 ng/ml serum folate) and non-deficient groups (>4 ng/ml serum folate). These changes were accompanied by corresponding reductions in erythrocyte micronucleus frequency but over a longer time-frame [3,4].

A folic acid depletion/repletion study (base-line: 195 ug/day; depletion: 5 weeks 65 ug/day; repletion: 4 weeks 111 ug/day followed by 20 days >280 ug/day) of nine post-menopausal women in a metabolic unit showed a significant increase in micronucleus frequency in lymphocytes following depletion and a decrease following repletion; micronucleus frequency in buccal cells decreased after the repletion phase [28]. The depletion phase in this study also resulted in increased DNA hypomethylation, increased dUTP/dTTP ratio and lowered NAD levels in lymphocytes [29].

A cross-sectional study on buccal mucosal folate and Vitamin B12 and its relation to micronucleus frequency in buccal cells revealed that buccal mucosal folate and Vitamin B12 are significantly lower in current smokers than in non-current smokers [30]. Although current smokers in this study were three times more likely to have micronucleated buccal cells, this chromosome damage index was not associated with localised folate and Vitamin B12 deficiencies. However, an elevated salivary Vitamin B12 was associated with a reduced micronucleus frequency in the buccal cells. This was the first study investigating the hypothesis that epithelial cancers such as those of the cervix, lung, bladder and oropharyngeal region could be due to localised deficiencies in folic acid and

Vitamin B12 which was suggested from the observation that megaloblastic changes in such tissues can be corrected by folate/Vitamin B12 supplementation [31].

A small number of case studies link Vitamin B12 deficiency with increased levels of chromosome aberrations [32,33]. Of the 10 patients with pernicious anaemia (which is a manifestation of Vitamin B12 deficiency), three had elevated chromosome aberrations, and eight had increased levels of micronucleus frequency in bone marrow preparations [34]. A female infant with transcobalamin II (the transporter for Vitamin B12 in plasma) deficiency showed elevated levels of aneuploidy (hypodiploidy in approximately 30% of cells) and increased chromosomal breakage in the bone marrow with reduction in hypodiploidy to 10% of cells after 5 months treatment with folate and Vitamin B12 supplements [35]. Combined deficiency in folic acid and Vitamin B12 was associated: (a) with transient 7q- in one patient [36]; and (b) in a series of patients produced a persistent abnormal deoxyuridine suppression test result (which is indicative of inadequate capacity to generate dTMP) and increased frequency of chromosomes showing despiralisation and chromosomal breaks [37]. The latter studies showed that it took up to 84 days after supplementation with folic acid and Vitamin B12 before the deoxyuridine suppression and the chromosomal morphology tests returned to normal. With regard to the question of chromosome despiralisation, it may be important to note that the DNA methylation inhibitor, 5-azacytidine, induces distinct undercondensation of the heterochromatin regions of chromosomes 1, 9, 15, 16 and Y, and the specific loss of these chromosomes as micronuclei in human lymphocytes *in vitro* [38]. Similarly the ICF immunodeficiency syndrome, which is caused by mutation in the DNA methyl transferase gene, is characterised by despiralisation of heterochromatin of chromosomes 1, 9 and 16 and loss of this chromatin into micronuclei and nuclear blebs [39].

A cross-sectional study in Japan involving 18 college students aged 19–23 years and 15 laboratory workers aged 24–69 year performed to investigate the age-adjusted micronucleus index in cytokinesis-blocked lymphocytes with serum Vitamins found a protective effect of increased folic acid which was marginally significant (multiple regression beta value -4.00 , $P = 0.06$) but no apparent protective effect associated with elevated Vitamin B12 concentration;

none of the subjects were Vitamin B12 deficient (<200 pg/ml) or folic acid deficient (<3.5 ng/ml) and mean values were 544 pg/ml and 7 ng/ml, respectively [40].

We have performed a series of studies to investigate the interrelationship between DNA damage in somatic cells and blood status for folate, Vitamin B12 and homocysteine. As a marker of chromosome damage, we have used the cytokinesis-block micronucleus method in lymphocytes which has been shown in numerous studies to be a reliable and sensitive biomarker of chromosome breakage and chromosome loss that occurs spontaneously [41] or as a result of elevated exposure to genotoxins [42].

Our preliminary studies comparing DNA damage rate and micronutrient status in vegetarians and non-vegetarians had indicated that there was a significant negative correlation between the micronucleus frequency in lymphocytes and plasma Vitamin B12 status in young men [43]. Therefore, we investigated the prevalence of folate deficiency, Vitamin B12 deficiency and hyperhomocysteinemia in 64 healthy men aged between 50 and 70 years and determined the relationship of these micronutrients with the micronucleus frequency in cytokinesis-blocked lymphocytes [44]. In total, 23% of the men had serum folate concentration <6.8 nmol/l, 16% had red blood cell folate concentration <317 nmol/l, 4.7% were Vitamin B12 deficient (<150 pmol/l) and 37% had plasma homocysteine levels >10 μ mol/l. In total, 56% of the apparently healthy men had non-optimal values for folate, Vitamin B12 or homocysteine. The micronucleus index of these men (19.2 ± 1.1 , $N = 34$) was significantly elevated ($P = 0.02$) when compared to that of men who had higher concentrations of folate and Vitamin B12 and lower plasma homocysteine (16.3 ± 1.3 , $N = 30$). Interestingly, the micronucleus index in men with normal concentrations of folate and Vitamin B12 but homocysteine levels > 10 μ mol/l (19.4 ± 1.7 , $N = 15$) was also significantly higher ($P = 0.05$) when compared to those with normal folate, Vitamin B12 and homocysteine <10 μ mol/l. Micronucleus index and plasma homocysteine were also significantly ($P = 0.0086$) and positively correlated ($r = 0.415$) in those subjects who were not deficient in folate or Vitamin B12. The micronucleus index was not significantly correlated with folate indices but there was a significant ($P = 0.013$) negative

correlation with serum Vitamin B12 ($r = -0.315$). It was apparent that elevated homocysteine status, in the absence of Vitamin deficiency, and low, but not deficient, Vitamin B12 status are important risk factors for increased chromosome damage in lymphocytes.

Subsequently, we performed a cross-sectional study ($N = 49$ males, 57 females) and a randomised double-blind placebo-controlled dietary intervention study ($N = 31$, 32 per group) to determine the effect of folate and B12 on DNA damage (micronucleus formation and DNA methylation) and plasma homocysteine (HC) in young Australian adults aged 18–32 years [45]. None of the volunteers were folate deficient (i.e. red cell folate <136 nmol/l) and only 4.4% (all females) were Vitamin B12 deficient (i.e. serum B12 <150 pmol/l). The cross-sectional study showed that (a) the frequency of micronucleated (MNed) cells was positively correlated with plasma HC in males ($r = 0.293$, $P < 0.05$); and (b) in females MNed cell frequency was negatively correlated with serum B12 ($r = -0.359$, $P < 0.01$); but (c) there was no significant correlation between micronucleus index and red cell folate status. The results also showed that the level of unmethylated CpG (DNA) (measured using the Sss1 methylase method), was not significantly related to Vitamin B12 or folate status. The dietary intervention involved supplementation with 700 μ g folic acid and 7 μ g Vitamin B12 in wheat bran cereal for 3 months followed by 2000 μ g folic acid and 20 μ g Vitamin B12 via tablets for a further 3 months. In the supplemented group, MNed cell frequency was significantly reduced during the intervention by 25.4% in those subjects with initial MNed cell frequency in the high 50th percentile but there was no change in those subjects in the low 50th percentile for initial MNed cell frequency. The reduction in MNed cell frequency was significantly correlated with serum B12 ($r = -0.49$, $P < 0.0005$) and plasma HC ($r = 0.39$, $P < 0.006$), but was not significantly related to red cell folate. DNA methylation status was not altered in the supplemented group. The greatest decrease in plasma HC (by 37%) during the intervention was observed in those subjects in the supplemented group with initial plasma HC in the high 50th percentile, and correlated significantly with increases in red cell folate ($r = -0.64$, $P < 0.0001$) but not with serum B12. The results from this study suggest that (a) MNed cell frequency is minimised when plasma HC is

below 7.5 $\mu\text{mol/l}$ and serum B12 is above 300 pmol/l ; and (b) dietary supplement intake of 700 μg folic acid and 7 μg Vitamin B12 is sufficient to minimise MNed cell frequency and plasma homocysteine in young adults. Thus, it appears that elevated plasma HC, a risk factor for cardiovascular disease, may also be a risk factor for chromosome damage.

Other studies have shown that global DNA methylation in lymphocytes or colonic tissue is influenced by the extent of folate intake. The depletion–repletion study performed by Jacob et al. [29] with post-menopausal women in a metabolic unit showed more than 100% increase in DNA hypomethylation after 9 weeks on low folate (56–111 $\mu\text{g/day}$) and a subsequent increase in DNA methylation after a further 3 weeks on a high folate diet (286–516 $\mu\text{g/day}$). Fowler et al. [46] and Cravo and colleague [47] showed using the Sss1 methylase assay that cervical and gastric/colonic/rectal epithelium DNA methylation is significantly correlated to serum and tissue folate concentration, respectively. Furthermore, it was shown that intrinsic methylation of DNA was lower in the normal colorectal mucosa of adenoma and carcinoma patients, however, supplementation with 10 mg folic acid/day for 6 months increased methylation 15-fold ($P < 0.0002$) and 3 months after cessation of therapy methylation decreased four-fold [47].

Studies on breast cancer patients at the time of disease presentation and before chemotherapy showed that elevated mutant frequencies in the HPRT gene occurred in those individuals with serum folate in the deficient range and serum Vitamin B12 levels were correlated negatively with sister chromatid exchange levels [48]. The extent of increase in HPRT mutant frequency in lymphocytes of breast cancer patients after chemotherapy tended to correlate negatively with serum folate level [49]. In this respect, it is important to note that murine cells deficient in DNA methyl transferase exhibit elevated point mutation rates due mainly to gene deletions caused by mitotic recombination or chromosome loss [50].

Another important possibility of prevention of genomic instability could be the prevention of integration of oncogenic virus DNA. Prevention of hypomethylation may enable a better surveillance of foreign DNA integration into human DNA because DNA methylation appears to have evolved partly for this purpose [51]. It is interesting to note in this regard

that HPV virus tends to integrate in fragile sites that may be folate-dependent [52] which raises the hypothesis that viral integration into DNA in vivo may be facilitated when folate status is low enough to cause fragile site expression. It is also important to note that transcription of retroviral or parasitic DNA sequences integrated into mammalian DNA is inhibited by cytosine methylation, and conversely demethylation may activate transcription of endogenous retroviruses, the significance of these observations is underscored by the fact that the large majority of 5-methylcytosine in the genome actually lies within parasitic, retroviral or transposon DNA [53,54]. Whether folate deficiency can activate transcription of retroviral DNA remains untested. Vitamin B12 may also play a direct role in the prevention of integration of oncogenic viruses because it has been shown that cobalamin inhibits HIV integrase and the integration of HIV-DNA into nuclear DNA [55]. On the basis of these results, combination treatment with folic acid and Vitamin B12 supplements has been used in the treatment of AIDS patients with apparent success [56].

4. Environmental and genetic factors that determine the bioavailability of folic acid and Vitamin B12

Alcoholism is associated with significantly reduced levels of tissue folate, Vitamin B12 and B6 in humans; at intakes $>3.0 \text{ g/kg/day}$ there was a doubling in the level of DNA hypomethylation of lymphocytes [57]. The reduced folate level in alcoholics may be due to reduced absorption or sub-optimal dietary intake. However, if results in the rat model reflect the situation in humans, then there is a good probability that the microbial metabolism of alcohol can result in exceedingly high levels of acetaldehyde which destroys folate in the intestine — this has been shown to be associated with localised folate deficiency in the colonic mucosa [58].

Reduced absorption of protein-bound B12 due to atrophic gastritis caused by autoantibodies to gastric parietal cells and reduced absorption due to autoantibodies to intrinsic factor are two of the main causes of Vitamin B12 deficiency which may affect between 10–40% of the elderly (>60 year) [59]. An increasingly important cause of cobalamin deficiency is exposure

to nitrous oxide either due to abuse [60], exposure during anesthesia [61] or occupational exposure of hospital personnel during surgery procedures [62]. Nitrous oxide inactivates cobalamin rendering exposed individuals effectively B12 deficient. It is interesting to note that hospital personnel exposed to nitrous oxide had four times the MN frequency of matched controls [62] which could be explained in part by the genotoxic effect of functional B12 deficiency.

The conversion of dietary folate and Vitamin B12 to intracellular active co-enzyme requires many physiological and biochemical processes including stomach release of bound Vitamin, intestinal uptake, blood transport proteins, cell uptake receptors, and enzymatic conversion to the active co-enzyme [63]. In the case of Vitamin B12 at least five different peptides (R binder, intrinsic factor, ileal receptors, transcobalamin I, transcobalamin II) are required to deliver Vitamin B12 from the gut to the tissues and a further four enzymes (cblF, cblC/D, microsomal reductase, cblE/G) are necessary to convert Vitamin B12 to the appropriate reduced state for function as a co-enzyme with methionine synthase. In the case of folate, a conjugate enzyme is required to deconjugate polyglutamated folate in the small intestine, receptors are required for active uptake into the intestinal brushborder epithelium, carried to the liver by the hepato-portal circulation where monoglutamated folate (i.e. folic acid) is reduced and methylated to form 5-methyltetrahydrofolate which is exported into the blood, it is then taken up by receptor/pinocytosis mechanism where it is subsequently stored in cells in the polyglutamated form by the activity of folyl-gamma-polyglutamate synthetase. The capacity of 5-methyltetrahydrofolate to donate its methyl group for the regeneration of methionine from homocysteine is dependent on the activity of methionine synthase. On the other hand, the activity of thymidylate synthase and methylenetetrahydrofolate reductase determine the probability of 5,10-methylenetetrahydrofolate donating its methyl group for the conversion of dUMP to dTMP. All of the above indicates that genetic defects in one or more of the key enzymes and uptake proteins can limit the bioavailability of folate and Vitamin B12. It may, therefore, be necessary for above RDI intake of these Vitamins to overcome defects relating to uptake or reduced activity of enzymes as in fact

has been shown in subjects defective in intrinsic factor or cobalamin reductase in the case of Vitamin B12 and subjects defective in MTHFR in the case of folate [63–65]. It is also interesting to note in the case of MTHFR that polymorphisms reducing its activity, such as the C677T mutation may on the one hand protect against cancer while on the other hand increase the risk for developmental defects such as Down syndrome and neural tube defects [66–71]. The most plausible explanation is that the MTHFR mutation minimises incorporation of uracil into DNA and, therefore, chromosome breakage and rearrangement while only having a relatively minor impact on DNA methylation — this implies that chromosome breakage/rearrangement may be more critical than hypomethylation for carcinogenesis, although this emphasis may change depending on folate intake and the extent to which hypomethylation of DNA causes aneuploidy, a potential carcinogenic event [66,67,72]. However, the impact of the MTHFR mutation on DNA methylation status may be important enough during the finely tuned development process when the concerted and timely expression of genes is critical and possibly more susceptible to appropriate DNA methylation status. Either way the observations with the C677T and A1298C MTHFR polymorphism [69,70] show the potential importance of using folate supplements to overcome metabolic limitations and the expectation is that the same will apply to Vitamin B12 for defects of other key enzymes such as methionine synthase and methionine synthase reductase [63,73].

5. Recommended dietary intakes (RDIs) for folate and Vitamin B12 based on genomic stability

There is now increasing interest to redefine RDIs of minerals and Vitamins not only to prevent diseases of extreme deficiency but also to prevent developmental abnormalities and degenerative diseases of old age as well as optimising cognition [74]. Prevention of chromosome breakage and aneuploidy is an important parameter for the definition of new RDIs for micronutrients [75], such as folic acid and Vitamin B12 because increased rates of DNA damage have been shown to be associated with increased cancer risk [76–78] and accelerated ageing [79]. Table 1

Table 1
Concentration and dietary intake of folic acid that minimises genomic instability in human tissue^a

Genomic instability biomarker	Concentration in culture medium in vitro (ng/ml)	Concentration in plasma in vivo (ng/ml)	Concentration in RBCs in vivo (ng/ml)	Daily dietary intake (ug/day)
SSB/DSB — comet assay	100 [15]			
Micronuclei	80 [8] ^b	15 [24] 7.4 [28]	600 [24]	5000 [24] 228 [28]
Uracil in DNA		53 [3,4]	313 [45] 480 [3,4]	700 [45] ^c 5000 [3,4]
CpG hypomethylation		23.7 [47] 7.3 [29]		10000 [47] 516 [29]

^a 1 ng/ml of folic acid = 2.26 nmol/l.

^b In the presence of thymidine (4.0 mg/l).

^c Together with 7 ug/day Vitamin B12.

summarises the information from in vitro and in vivo controlled experiments in human cells and human subjects with a view to defining, based on current knowledge, the optimal concentration and dietary intake of folic acid for minimising genomic instability. The results from a variety of DNA damage biomarkers suggest that above RDI levels of folic acid intake are required to minimise DNA damage, furthermore, the current sufficiency levels of folate in plasma (i.e. 2.2 ng/ml or 4.9 nmol/l) and red blood cells (i.e. 132 ng/ml or 298 nmol/l), which are based on prevention of anaemia [80], are much lower than the averaged concentration levels at which DNA damage is minimised i.e. 21 ng/ml (range 7.3–53.0 ng/ml) for plasma and 464 ng/ml (range 313–600 ng/ml) for red blood cells (results from Table 1). In this regard, it is interesting to note that the red cell folate concentration that corresponds to minimisation of risk of neural tube defects in the unborn child is approximately 400 ng/ml [81,82] which is of a similar magnitude to that which appears to be required for minimising genomic instability. The data from our intervention studies [44,45] also suggest that the current sufficiency level of Vitamin B12 in plasma for prevention of anaemia (i.e. 150 pmol/l or 203 pg/ml) is almost a half of the level at which the micronucleus index in lymphocytes is observed to be minimised (i.e. 300 pmol/l or 406 pg/ml) — intakes of three and half times the current Australian RDI were required to achieve this level in plasma. Only with careful choice and sufficient intake of folate-rich foods such as aleurone flour, certain fruits and vegetables, and Vitamin B12-rich foods such as liver is it

possible to achieve the required above RDI intake of folate and Vitamin B12 [82,83]. The use of fortified foods or tablet supplements may be a more practical proposition for achieving these levels of intake [84]. Combining folic acid with Vitamin B12 in supplements or in a fortification programme not only has the benefit of maximising the impact of these Vitamins because Vitamin B12 makes folate more bioavailable both for synthesis of dTMP and methionine, but it also enhances the homocysteine-lowering capacity of folate [45] and prevents the possibility of masking neuropathies (associated with pernicious anaemia) when folate supplements are taken on their own in individuals who have an underlying Vitamin B12 deficiency [85].

6. Conclusion

The accumulated evidence to date suggests that folate and Vitamin B12 play an important role in genomic stability. Above RDI intakes of these Vitamins may be required for a large proportion of humans because of the increasing evidence for common single nucleotide polymorphisms that alter significantly the activity of proteins required for the absorption, transport and metabolism of these Vitamins to their active forms. Current evidence from prospective studies suggest a reduced risk of cancer in those with lower chromosomal damage rates regardless of exposure to man-made carcinogens. It is, therefore, anticipated that increased genomic stability resulting from adequate intake of folate and Vitamin B12 may result

in reduced cancer risk and some epidemiological evidence for this view is already emerging [66,67,86,87].

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