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Micronutrients and genomic stability: a new paradigm for recommended dietary allowances (RDAs)

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Abstract

Diet as a key factor in determining genomic stability is more important than previously imagined because we now know that it impacts on all relevant pathways, namely exposure to dietary carcinogens, activation/detoxification of carcinogens, DNA repair, DNA synthesis and apoptosis. Current recommended dietary allowances for vitamins and minerals are based largely on the prevention of diseases of deficiency such as scurvy in the case of vitamin C. Because diseases of development, degenerative disease and aging itself are partly caused by damage to DNA it seems logical that we should focus better our attention on defining optimal requirements of key minerals and vitamins for preventing damage to both nuclear and mitochondrial DNA. To date, our knowledge on optimal micronutrient levels for genomic stability is scanty and disorganised. However, there is already sufficient evidence to suggest that marginal deficiencies in folate, vitamin B12, niacin and zinc impact significantly on spontaneous chromosome damage rate. The recent data for folate and vitamin B12 in humans with respect to micronucleus formation in blood and epithelial cells provide compelling evidence of the important role of these micronutrients in maintenance of genome integrity and the need to revise current RDAs for these micronutrients based on minimisation of DNA damage. Appropriately designed in vitro studies and in vivo placebo controlled trials with dose responses using a complementary array of DNA damage biomarkers are required to define recommended dietary allowances for genomic stability. Furthermore these studies would have to be targeted to individuals with common genetic polymorphisms that alter the bioavailability of specific micronutrients and the affinity of specific key enzymes involved in DNA metabolism for their micronutrient co-factor. That there is a need for an international collaborative effort to establish RDAs for genomic stability is self-evident.

Keywords: Recommended dietary allowance; Micronutrients; Genomic stability; DNA damage

1. Introduction

The field of mutation research is founded on the original observations that specific physical and chemical agents, man-made or natural, can produce significant critical alterations to the genome in both somatic and germ cells and that these events are a cause of cancer and developmental defects. In addition, much has been learned about the great complexity of the cells' capacity to repair such DNA lesions (Lindahl and Wood, 1999). Numerous studies have been performed on the effect of in vivo exposure to carcinogens on gene mutation in human populations but it is only very recently that the effect of dietary imbalance has been taken into consideration (Ames, 1998). DNA mutation rates are very high even in the absence of overt exposure to known carcinogens and there is a wide variation in rates of mutation even among individuals of the same age (Fenech, 1998) (Fig. 1). One therefore has to consider whether genetic factors and diet may be the main determinants of variation in background mutation rate.

The focus on diet as a key factor in determining genomic stability is more important than previously imagined because we now know that it impacts on all relevant pathways, namely exposure to dietary carcinogens, activation/detoxification of carcinogens, DNA repair, DNA synthesis and apoptosis (Ames, 1998; Fenech and Ferguson, 2001). This is because all of these critical pathways are dependent not only on enzymes but also on the provision of substrate and co-factors some of which are only available at the right concentration when dietary intake of key minerals and vitamins is adequate. Effectively this means that dietary deficiency in key micronutrients required for DNA maintenance may produce similar effects as inherited genetic disorders that impair activity of enzymes required for genomic stability (Boyonoski et al., 1999;

Abbreviations: dUMP, deoxyuridylic acid; MN, micronucleus; RDA, recommended dietary allowance; TMP, thymidylic acid

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Jacobson et al., 1999; Simulan-Rosenthal et al., 1999, Fenech and Ferguson, 2001) and may damage DNA to similar extents as significant exposure to known carcinogens such as ionising radiation (Ames, 1998).

2. RDAs for genomic stability and disease prevention

Current recommended dietary allowances (RDAs) for vitamins and minerals are based largely on the prevention of diseases of deficiency such as scurvy in the case of vitamin C, anemia in the case of folic acid, and pellagra in the case of niacin. However, these diseases of deficiency are rare in the developed world but degenerative disease and developmental disease are very important. Recently the dietary allowance for folic acid for the prevention of neural tube defects has been revised to more than double the original RDA (Centers for Disease Control, 1992). There is a strong international awareness that it is also necessary to redefine RDAs for the prevention of degenerative disease (such as cancer, cardiovascular disease and Alzheimer's disease) and compression of the morbidity phase during old age. Because diseases of development, degenerative disease and aging itself are partly caused by damage to DNA (Holliday, 1995; Ames, 1998), it seems logical that we should focus better our attention on defining optimal requirements of key minerals and vitamins for preventing damage to both nuclear and mitochondrial DNA. To date, our knowledge on optimal micronutrient levels for genomic stability is scanty and disorganised.

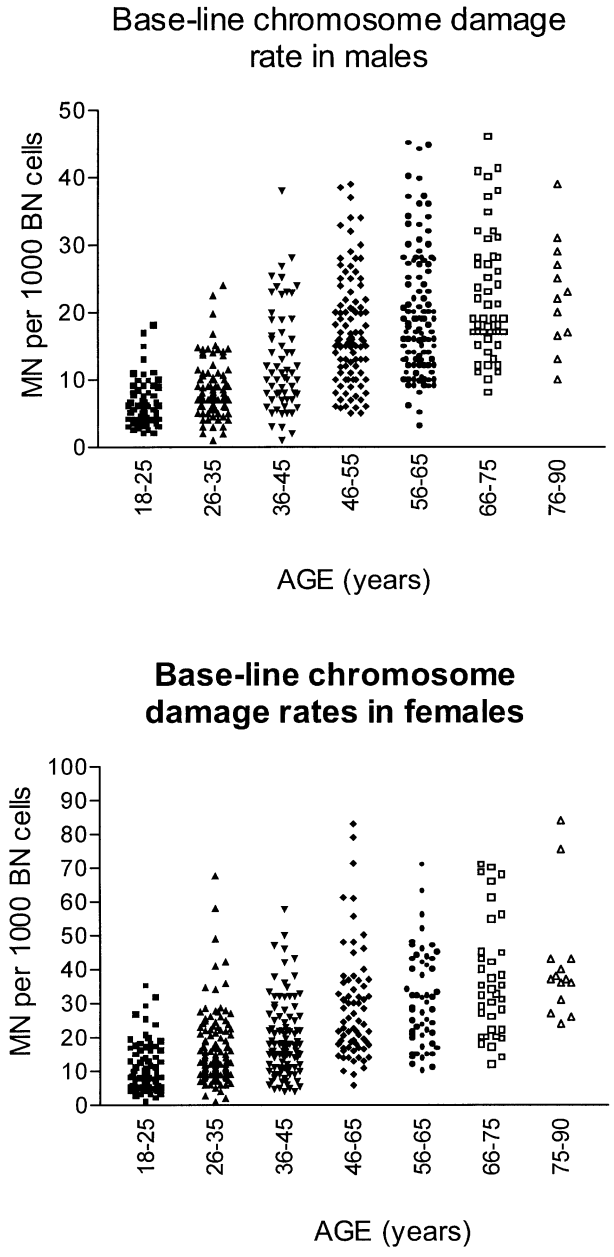

Fig. 1. Variation in chromosome DNA damage rates in peripheral blood lymphocytes of healthy non-smoking males ($N=495$) and females ($N=511$) within and between age groups measured using the micronucleus assay. Each spot represents the micronucleus (MN) frequency in 1000 binucleated (BN) cells of a single and unique individual.

3. Micronutrients required for genomic stability—focus on folate and vitamin B12

Table 1 lists some of the most important minerals and vitamins required for DNA maintenance and prevention of DNA damage and the DNA lesions that could be induced by inadequate intake of these antimutagenic vitamins. Perhaps it is notable that four of eight known human glycosylases are dedicated to the removal of uracil from DNA, the mutation caused by folate deficiency (Lindahl and Wood, 1999).

Both in vitro and in vivo studies with human cells clearly show that folate deficiency, vitamin B12 deficiency and elevated plasma homocysteine are associated with expression of chromosomal fragile sites, chromosome breaks, excessive uracil in DNA, micronucleus formation and DNA hypomethylation (Table 2) (Jacky et al., 1983; Everson et al., 1988; Cravo et al., 1994; Blount and Ames, 1995; Blount et al., 1997; Duthie and Hawdon, 1998; Fenech et al., 1998; Jacob et al., 1998; Titenko-Holland et al., 1998; Crott et al., 2001). In vitro experiments indicate that DNA breaks in human cells are minimised when folic acid concentration in culture medium is greater than 180 nmol/l (80 ng/ml) (Jacky et al., 1983; Duthie and Hawdon, 1998). Recently we have shown that uracil incorporation in human lymphocytes cultured for 8 days is minimised at a folic acid concentration of 120 nmol/l (Crott et al., 2001). Intervention studies in humans taking folate and/or vitamin B12 supplements show that DNA hypomethylation, chromosome breaks, uracil misincorporation and micronucleus formation are minimised when plasma concentration of vitamin B12 is greater than 300 pmol/l, plasma folate concentration is greater than 34 nmol/l,

red cell folate concentration is greater than 700 nmol/l folate and plasma homocysteine is less than 7.5 μmol/l (Everson et al., 1988; Cravo et al., 1994; Blount and Ames, 1995; Blount et al., 1997; Fenech et al., 1998; Jacob et al., 1998; Titenko-Holland et al., 1998). These concentrations are only achievable at intake levels in excess of current RDAs; that is, more than 400 μg folic acid per day and more than 2 μg vitamin B12 per day. Dietary intakes above the current RDA may be particularly important in those with extreme defects in the absorption and metabolism of these vitamins, for which aging is a contributing factor. The above suggests that both controlled in vitro experiments and placebo-controlled in vivo interventions are informative in determining optimal micronutrient intake for prevention of genomic stability.

4. Gene-micronutrient interaction in maintenance of genomic stability

Perhaps, a more useful approach would take into consideration the genotype of individuals with a focus on specific common genetic polymorphisms that alter the bioavailability of specific micronutrients, their metabolism and the affinity of specific key enzymes involved in DNA metabolism for their micronutrient co-factor. Supplementation of diet with appropriate

Table 1
Examples of the role and the effect of deficiency of specific micronutrients on genomic stability

Micronutrient(s)	Role in genomic stability	Consequence of deficiency
Carotenoids, vitamin C, vitamin E	Prevention of oxidation to DNA and lipid oxidation[a,b]	Increased baseline level of DNA strand breaks, chromosome breaks and oxidative DNA lesions and lipid peroxide adducts on DNA[a,b]
Folate and vitamin B12	Maintenance methylation of DNA; synthesis of dTMP from dUMP and efficient recycling of folate[c]	Uracil misincorporation in DNA, increased chromosome breaks and DNA hypomethylation[c]
Niacin	Required as substrate for poly(ADP-ribose) polymerase (PARP) which is involved in cleavage and rejoining of DNA and telomere length maintenance[d]	Increased level of unrepaired nicks in DNA, increased chromosome breaks and sensitivity to mutagens[d]
Zinc	Required as a co-factor for Cu/Zn superoxide dismutase, endonuclease IV, function of p53, Fapy glycosylase and in Zn finger proteins such as PARP[e]	Increased DNA oxidation, DNA breaks and elevated chromosome damage rate[e]

[a] Halliwell (2001).
[b] Claycombe and Meydani (2001).
[c] Fenech (2001).
[d] Hageman and Stierum (2001).
[e] Dreosti (2001).

Table 2
Concentration and dietary intake of folic acid that minimises genomic instability in human tissue

Genomic instability biomarker	Concentration in culture medium—in vitro	Concentration in plasma—in vivo	Concentration in RBCs —in vivo	Daily dietary intake of folic acid via supplementation
SSB/DSB—comet assay	100 ng/ml[c]			
Micronuclei	80 ng/ml[a,d]	15 ng/ml[f] 7.4 ng/ml[g]	600 ng/ml[f] 313 ng/ml[h]	5000 μg/day[f] 228 μg/day[g] 700 μg/day[b,h]
Uracil in DNA	53 ng/ml[e]	53 ng/ml[i,j] 23.7 ng/ml[k]	480 ng/ml[i,j]	5000 μg/day[i,j] 10,000 μg/day[k]
CpG hypomethylation		7.3 ng/ml[l]		516 μg/day[l]

[a] In the presence of thymidine (4.0 mg/l).
[b] Together with 7 μg/day vitamin B12. 1 ng/ml of folic acid = 2.26 nmol/l.
[c] Duthie and Hawdon (1998).
[d] Jacky et al. (1983).
[e] Crott et al. (2001).
[f] Everson et al. (1988).
[g] Titenko-Holland et al. (1998).
[h] Fenech et al. (1998).
[i] Blount and Ames (1995).
[j] Blount et al. (1997).
[k] Cravo et al. (1994).
[l] Jacob et al. (1998).

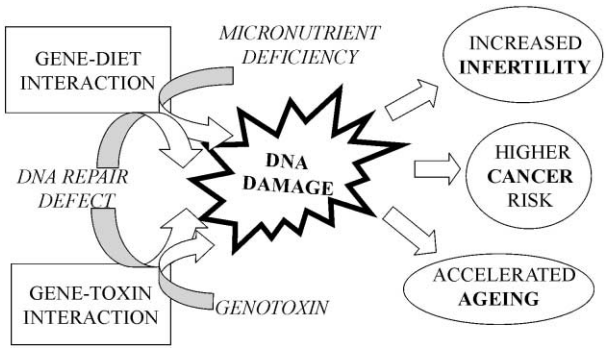

Fig. 2. The concepts of gene–diet and gene–toxin interaction and their impact on various health outcomes.

minerals and vitamins could, in some cases, help overcome inherited metabolic blocks in key DNA maintenance pathways. The interaction between genotype and diet in modulating risk is emerging as an exciting area of research as regards micronutrient effects on DNA. This is illustrated by recent research on the common mutations in the methylene-tetrahydrofolate-reductase (MTHFR) gene. The product of this gene determines the availability of folate for the synthesis of thymidylic acid (TMP) from deoxyuridylic acid (dUMP) and is predicted to minimise uracil misincorporation into DNA while making less methylfolate available for synthesis of *S*-adenosyl methionine, the common methyl donor (Ames, 1999). Epidemiological studies have suggested that individuals with this genotype may be protected against colorectal cancer and acute lymphocytic leukaemia (Chen et al., 1999; Skibola et al., 1999). Other common polymorphisms, such as the manganese superoxide dismutase alanine to valine change in the −9 position, may increase susceptibility to oxidative stress and therefore may necessitate a higher requirement for vitamins C and E (Ambrosone et al., 1999). In the past, considerable attention has been given to gene–environment interaction as it relates to mutagen/carcinogen exposure and genotoxic and cancer risk. Fig. 2 illustrates the concept that perhaps gene–diet interaction as it relates to efficacy of DNA repair/DNA metabolism and micronutrient deficiency may be equally important in determining genomic stability and its consequent impact on fertility, development, cancer risk and the rate of aging (Ames, 1998, 1999; Chen et al., 1999; Skibola et al., 1999; Trkova et al., 2000).

5. Conclusions and the future

Our current stage of knowledge on the role of micronutrients in the maintenance of genomic stability has been recently reviewed in a special issue of *Mutation Research* (Fenech and Ferguson, 2001). These reviews identify the current gaps in our knowledge and provide the basic information for appropriate design of placebo controlled trials that are required to define appropriate RDAs for genomic stability. In the future, clinical trials with a wide array of DNA damage endpoints would be necessary including measures of mitochondrial DNA deletions and point mutations, nuclear microdeletions and point mutations, telomere shortening, balanced chromosomal translocations, chromosome non-disjunction or aneuploidy, micronucleus formation, single and double strand breaks in DNA, DNA adducts and microsatellite instability. It is clear that this objective requires multiple expertise. That there is a need for an international collaborative effort to establish RDAs for genomic stability is evident.

References

Ambrosone, C.B., Freudenheim, J.L., Thompson, P.A., Bowman, E., Vena, J.E., Marshall, J.R., Graham, S., Laughlin, R., Nemoto, T., Shields, P.G., 1999. Managanese superoxide dismutase (MnSOD) genetic polymorphisms, dietary antioxidants and risk of breast cancer. Cancer Research 59, 602–606.

Ames, B.N., 1998. Micronutrients prevent cancer and delay ageing. Toxicology Letters 102103, 5–18.

Ames, B.N., 1999. Cancer prevention and diet: help from single nucleotide polymorphisms. Proceedings of the National Academy of Sciences of the U.S.A 96, 12216–12218.

Blount, B.C., Ames, B.N., 1995. DNA damage in folate deficiency. Bailleres Clinics in Haematology 8, 461–478.

Blount, B.C., Mack, M.M., Wehr, C.M., MacGregor, J.T., Hiatt, R.A., Wang, G., Wickramasinghe, S.N., Everson, R.B., Ames, B.N., 1997. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proceedings of the National Academy of Sciences of the U.S.A. 94, 3290–3295.

Boyonoski, A.C., Gallacher, L.M., Apsimon, M.M., Jacobs, R.M., Shah, G.M., Poirier, G.G., Kirkland, J.B., 1999. Niacin deficiency increases sensitivity of rats to the short and long term effects of ethylnitrosourea treatment. Molecular and Cellular Biochemistry 193, 83–87.

Centers for Disease Control, 1992. Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. MMWR Morbidity and Mortality Weekly Report 41, 1–7.

Chen, J., Giovannucci, E.L., Hunter, D.J., 1999. MTHFR polymorphisms, methyl-replete diets and risk of colorectal carcinoma and adenoma among U. S. men and women: an example of gene-environment interactions in colorectal tumorigenesis. Journal of Nutrition 129, 560S–564S.

Claycombe, K.J., Meydani, S.N., 2001. Vitamin E and genomic stability. Mutation Res. 475, 37–44.

Cravo, M., Fidalgo, P., Pereira, A.D., Gouveia-Oliviera, A., Chaves, P., Selhub, J., Mason, J.B., Mira, F.C., Leitao, C.N., 1994. DNA methylation as an intermediate biomarker in colorectal cancer: modulation by folic acid supplementation. European Journal of Cancer Prevention 3, 473–479.

Crott, J.W., Mashiyama, S.T., Ames, B.N., Fenech, M.F., 2001. MTHFR C677T polymorphism does not alter folic acid deficiency-induced uracil incorporation into primary human lymphocyte DNA in vitro. Carcinogenesis (accelerated paper) 22, 1019–1025.

Duthie, S.J., Hawdon, A., 1998. DNA instability (strand breakage, uracil misincorporation, and defective repair) is increased by folic acid depletion in human lymphocytes in vitro. FASEB Journal 12, 1491–1497.

Dreosti, I.E., 2001. Zinc and the gene. Mutation Research 475, 161–168.
Everson, R.B., Wehr, C.M., Erexson, G.L., MacGregor, J.T., 1988. Association of marginal folate depletion with increased human chromosomal damage in vivo: demonstration by analysis of micronucleated erythrocytes. Journal of the National Cancer Institute 80, 525–529.
Fenech, M., 1998. Chromosomal damage rate, ageing and diet. Annals of the New York Academy of Sciences 854, 23–36.
Fenech, M., 2001. The role of folic acid and vitamin B12 in genomic stability of human cells. Mutation Research 475, 57–68.
Fenech, M., Ferguson, L.R. (Eds.) (2001) Micronutrients and Genomic Stability. Mutation Research 475, 1–6.
Fenech, M., Aitken, C., Rinaldi, J., 1998. Folate, vitamin B12, homocysteine status and DNA damage in young Australian adults. Carcinogenesis 19, 1163–1171.
Hageman, G.J., Stierum, R.H., 2001. Niacin, poly(ADP-ribose) polymerase-1 and genomic stability. Mutation Reearch 475, 45–56.
Halliwell, B., 2001. Vitamin C and genomic stability. Mutation Research 475, 29–35.
Holliday, R., 1995. Understanding Ageing. Cambridge University Press, Cambridge.
Jacky, P.B., Beek, B., Sutherland, G.R., 1983. Fragile sites in chromosomes: possible model for the study of spontaneous chromosome breakage. Science 220, 69–70.
Jacob, R.A., Gretz, D.M., Taylor, P.C., James, S.J., Pogribny, I.P., Miller, B.J., Henning, S.M., Swendseid, M.E., 1998. Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. Journal of Nutrition 128, 1204–1212.
Jacobson, E.L., Shiek, W.M., Huang, A.C., 1999. Mapping the role of NAD metabolism in prevention and treatment of carcinogenesis. Molecular and Cellular Biochemistry 193, 69–74.
Lindahl, T., Wood, R.D., 1999. Quality control by DNA repair. Science 286, 1897–1905.
Simbulan-Rosenthal, C.M., Haddad, B.R., Rosenthal, D.S., Weaver, Z., Coleman, A., Luo, R.B., Young, H.M., Wang, Z.Q., Reid, T., Smulson, E.M., 1999. Chromosomal aberrations in PARP −/−mice: genome stabilisation in immortalised cells by re-introduction of poly(ADP-ribose) polymerase cDNA. Proceedings of the National Academy of Sciences of the U.S.A. 96, 13191–13196.
Skibola, C.F., Smith, M.Y., Kane, E., Roman, E., Rollinson, S., Cartwright, R.A., Morgan, G., 1999. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukaemia in adults. Proceedings of the National Academy of Sciences of the U.S.A. 96, 12810–12815.
Titenko-Holland, N., Jacob, R.A., Shang, N., Balaraman, A., Smith, M.T., 1998. Micronuclei in lymphocytes and exfoliated buccal cells of postmenopausal women with dietary changes in folate. Mutation Research 417, 101–114.
Trkova, M., Kapras, J., Bobkova, K., Stankova, J., Mejsnarova, B., 2000. Increased micronuclei frquencies in couples with reproductive failure. Reproductive Toxicology 14, 331–335.