Damage to the genome is recognised as a fundamental cause of developmental and degenerative diseases. Several micronutrients play an important role in protecting against DNA damage events that are generated through endogenous and exogenous factors by acting as cofactors or substrates for enzymes that detoxify genotoxins as well as enzymes involved in DNA repair, methylation and synthesis. In addition it is evident that either micronutrient deficiency or excess can modify genome stability and that these effects may also depend on nutrient-nutrient and nutrient-gene interaction which is affected by genotype. These observations have led to the emerging science of Genome Health Nutrigenomics which is based on the principle that DNA damage is a fundamental cause of disease that can be diagnosed and nutritionally prevented on an individual, genetic sub-group or population basis. In this review the following topics are discussed: (i) Biomarkers used to study genome damage in humans and their validation; (ii) Current knowledge on micronutrients required for maintenance of genome stability in humans; (iii) Strategies to determine dietary reference values of single micronutrients and micronutrient combinations (nutriomes) based on DNA damage prevention.

Key words: nutrition, dietary reference values, DNA damage, prevention

Introduction

Dietary Reference Values (DRVs) are intended to provide a guide on appropriate intake of nutrients for prevention of diseases caused by deficiency (e.g., scurvy in the case of vitamin C) or excess (e.g., iron-overload disease which may be fatal) (1). Determining these extremes is important but the biggest challenge in prevention of developmental and degenerative disease in populations that are not short of food, fortified food or supplements is defining the appropriate intake levels of micronutrients individually or in combination (nutriomes) to optimise cellular and organism performance on both a personal and genetic sub-group level at different life-stages. Optimisation of cellular function ultimately depends on prevention of damage to the nuclear and mitochondrial genome. The accumulation of mutations at the base sequence or chromosomal level as a result of genotoxic insults due to endogenous and exogenous factors is now recognised as a fundamental underlying cause of developmental defects and accelerated ageing as well as increased risk for degenerative conditions such as infertility, immune dysfunction, cancer, cardiovascular and neurodegenerative diseases (2–6).

This brief review aims to discuss the concept that DRVs need to be focused on defining the optimal intake of micronutrients individually or in combination for prevention of DNA damage because it is becoming increasingly evident that inappropriate nutrition can cause significant harms to the genome that are of a similar magnitude as those induced by environmental genotoxins and carcinogens (2–4,7–9). The central aim of this review is to consider the proposition that prevention of harm to the genome should be a top priority in the setting of nutritional guidelines, in public health strategy and in preventive medicine generally and that this approach is now technically feasible using a set of validated and accurate methods for measuring genome damage at both the molecular and cytogenetic level.

Biomarkers Used to Study Genome Damage in Humans and Their Validation to Study Effects of Nutrition

Numerous biomarkers of DNA damage have been developed over the past thirty years enabling the accurate measurement of DNA base damage (e.g., hydroxyl radical adducts on nucleotides), micro-deletions and amplifications in the DNA sequence, DNA strand breaks, telomere length as well as measurement of DNA damage at the chromosomal level such as acentric chromosome fragments, chromosome rearrangements and loss or gain of whole chromosomes leading to aneuploidy (10–15). Furthermore these biomarkers of chromosome...
### Table 1. Strengths and weaknesses of best validated DNA damage assays for nutritional studies in humans

<table>
<thead>
<tr>
<th>DNA damage events measured</th>
<th>DNA damage assays</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CBMN Cyt</td>
</tr>
<tr>
<td>DNA breaks</td>
<td>YES</td>
</tr>
<tr>
<td>Mis-repair of DNA breaks</td>
<td>YES</td>
</tr>
<tr>
<td>Oxidised DNA bases</td>
<td>NO</td>
</tr>
<tr>
<td>Chromosome malsegregation</td>
<td>YES*</td>
</tr>
<tr>
<td>Chromosomal deletion</td>
<td>YES*</td>
</tr>
<tr>
<td>Dicentric chromosome or telomere end fusion</td>
<td>YES*</td>
</tr>
<tr>
<td>Telomere length</td>
<td>NO</td>
</tr>
<tr>
<td>Hypo/hyper methylation of DNA</td>
<td>NO</td>
</tr>
<tr>
<td>Abasic sites in DNA</td>
<td>NO</td>
</tr>
<tr>
<td>mtDNA damage</td>
<td>NO</td>
</tr>
</tbody>
</table>

**OTHER FEATURES**

- Distinguishes DNA damage in viable cells from cell death
- Suitable for in vitro studies
- Cell type in which assay is performed

<table>
<thead>
<tr>
<th></th>
<th>PBL</th>
<th>RBC</th>
<th>Oral mucosa</th>
<th>Any cell type</th>
<th>Any cell type</th>
<th>Any cell type</th>
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</tr>
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<tbody>
<tr>
<td></td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
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<td>YES</td>
</tr>
</tbody>
</table>

* by measuring micronuclei with/without centromere staining; † bif used in combination with glycosylase enzymes that remove oxidised bases; § if alkaline version of the assay is used; † interpretation of oxidised DNA bases. The inability to distinguish between DNA damage from dead or viable cells may confound DNA damage results. CBMN, cytokinesis-block micronucleus; Cyt, cytoine; PBL, peripheral blood lymphocytes; MN, micronucleus; mtDNA, mitochondrial DNA; RBC, red blood cells.

Damage can also be visualised at the cytological level using micronuclear cytome assays which have been developed for use with cell lines, peripheral blood lymphocytes, erythrocytes and buccal cells (16–19).

Table 1 lists the DNA damage biomarkers most commonly used in nutrition studies describing their strengths and weaknesses. These biomarkers include (i) micronuclear assays in lymphocytes, buccal cells and erythrocytes which provide a measure of chromosome breakage or misrepair and/or loss; (ii) DNA strand break assays such as the comet assay; (iii) DNA oxidation assays that typically measure specific damages such as oxidised guanine; (iv) assays that measure the cytosine methylation status of DNA; (v) telomere length assays which provide a measure of the extent of attrition of the telomere repeat sequence at the end of chromosomes and (vi) global or specific deletions of the mitochondrial genome. Most of these techniques can be used in in vitro studies but others such as the erythrocyte micronuclear assay cannot be used for this purpose. Nevertheless, this method like the others listed in Table 1 is practical to use for in vivo studies also. Although the erythrocyte micronuclear assay has been shown to be associated with nutritional status in both cross-sectional and controlled trials and with disease states such as thalassaemia (19–21) no prospective studies have yet been performed to validate the method as a predictor of disease in humans although evidence in rodents suggest that it is predictive of cancer risk in those animals exposed to carcinogens (20–22). These types of considerations are important when deciding which battery of tests are best suited for determining nutrient reference values for micronutrients based on DNA damage prevention. Although not commonly used, it is also important to note that classical cytogenetic techniques such as metaphase analysis of chromosomes, including chromosome painting methods and sister chromatid exchange assays can also provide more detailed chromosome-specific DNA damage assessments cause by nutritional imbalance.

Ideally the biomarkers of DNA damage that are of interest would be predictive of human disease risk and are modifiable by diet in humans. However, only some of the assays listed in Table 1 have been validated as being responsive to nutritional intervention in placebo-controlled trials as well as being predictive of development and/or degenerative disease risk in humans. The current status of validation of the most commonly used DNA damage biomarkers in nutrition studies which include the cytokinesis-block micronucleus cytome (CBMN-Cyt) assay in lymphocytes (17), the buccal micronuclear cytome (BucMN-Cyt) assay (16), the red blood cell (RBC) micronucleus assay (20,21), the Comet assay of DNA strand breaks in lymphocytes (23,24), telomere length (25–27), DNA methylation (28,29), DNA oxidation (30–32) and mtDNA deletion (33,34) in leukocytes or lymphocytes was recently reviewed (35). At this point in time only the MN frequency index in the CBMN-Cyt assay in human lymphocytes has been substantially validated with respect to its sensitivity to...
changes in nutritional status in both cross-sectional and placebo-controlled trials and its association, via cross-sectional and prospective studies, with developmental and degenerative disease. The other assays are currently either lacking evidence of prospective association with disease outcomes or, as is the case for telomere length, lack evidence from placebo-controlled trials of being modifiable by altered nutrient intake in humans.

Current Knowledge on Micronutrients Required for Maintenance of Genome Stability in Humans

There is overwhelming evidence that a large number of micronutrients (vitamins and minerals) are required as cofactors for enzymes or as part of the structure of proteins (metalloenzymes) involved in DNA synthesis and repair, prevention of oxidative damage to DNA as well as maintenance methylation of DNA. The role of micronutrients in maintenance of genome stability has recently been extensively reviewed (2–4,7–9). Examples of micronutrients involved in various genome stability processes are given in Table 2. All of these micronutrients can also have serious metabolic and health impacts when intake levels are deficient. Notable examples include neurodegenerative disease (vitamin B12 deficiency), cancer (folate deficiency), anaemia (folic and/or iron deficiency), osteoporosis (calcium deficiency), stunted growth and diarrhea in children (zinc deficiency), scurvy (vitamin C deficiency) and immune dysfunction (zinc, folate, selenium deficiency) (1,2,9). The main point is that micronutrient deficiency or excess can also cause genome damage and genome damage caused by micronutrient deficiency could be of the same order of magnitude, if not greater, than the genome damage levels caused by exposure to significant doses of environmental genotoxins such as chemical carcinogens, ultra-violet radiation and ionising radiation. An example from our laboratory is the observation that the chromosomal damage in cultured human lymphocytes caused by reducing folate concentration from 120 nmol/L to 12 nmol/L is equivalent to that induced by an acute exposure to 0.2 Gy of low linear energy transfer (LET) ionising radiation (e.g., X-rays), a dose of radiation which is approximately 10 times greater than the annual allowed safety limit of exposure for the general population (35,36). These results imply that genome damage biomarkers are not only biodosimeters (i.e., indicators of dose experienced by tissues) of exposure to man-made or natural genotoxins but also biodosimeters of deficiency in micronutrients required (a) for the prevention of oxidation to DNA (e.g., antioxidants), (b) prevention of uracil incorporation into DNA (e.g., folate), (c) maintenance methylation of CpG in DNA (e.g., methionine, choline, folate, vitamin B12), (d) as co-factors or as components of DNA repair enzymes (e.g., Zn, Mg) and (e) maintenance of telomere length (e.g., niacin, folate) (2–4,7–9,37–46). The sensitivity of DNA damage to micronutrient deficiency is underscored by the fact that there are at least eight hu-
man DNA repair glycosylases dedicated to the removal of the type of DNA base damage (e.g., 8-hydroxydeoxyguanosine, uracil) that is produced when either antioxidant micronutrients (such as Zn, vitamin C and E) or folate, methionine and vitamin B12 are deficient, respectively (47–49).

Results from a recent population study suggest that at least nine micronutrients affect genome stability in humans in vivo (50). Multivariate analysis of base-line data showed that (a) the highest tertile of intake of vitamin E, retinol, folate, nicotinic acid (preformed) and calcium is associated with significant reductions in micronucleus (MN) frequency, i.e., $-28\%$, $-31\%$, $-33\%$, $-46\%$, and $-49\%$, respectively (all $p < 0.005$), relative to the lowest tertile of intake and (b) the highest tertile of intake of riboflavin, pantothenic acid and biotin was associated with significant increases in MN frequency, i.e., $+36\%$ ($p = 0.054$), $+51\%$ ($p = 0.021$), and $+65\%$ ($p = 0.001$), respectively, relative to the lowest tertile of intake. Mid-tertile β-carotene intake was associated with an 18% reduction in MN frequency ($p = 0.038$), however, the highest tertile of intake (>6400 µg/d) resulted in an 18% increment in MN frequency. In interpreting the data from this study, it is important to note that micronutrients usually exhibit metabolic dose-response effects in which both deficiency and excess can be deleterious (51–56).

The amount of micronutrients that appear to be protective against genome damage vary greatly between foods (57) and careful choice is needed to design dietary patterns optimised for genome health maintenance. Because dietary choices vary between individuals, due to taste preferences which may be genetically determined (58) or due to cultural or religious constraints, several options are required and supplements may be needed to cover gaps in micronutrient requirements. Clearly the development or identification of nutrient dense foods and ingredients which are rich in micronutrients required for DNA replication and repair and prevention of genome damaging events, is essential in making it feasible for individuals to achieve their daily nutrient requirements for genome health maintenance without intake of excess calories.

**Strategies to Determine Dietary Reference Values of Single Micronutrients and Micronutrient Combinations (Nutriomes) Based on DNA Damage Prevention**

To determine DRVs of single micronutrients it is necessary to first perform in vitro dose-response studies on the impact of micronutrient concentration on DNA damage and cytotoxicity and in vivo cross-sectional investigations on the association of dietary intake with DNA damage biomarkers. Secondly, it is essential to verify this association by appropriate controlled in vivo trials testing both whole foods that are rich in the micronutrient of interest as well as placebo-controlled trials using supplements. Table 1 summarises the various aspects of the best established assays that have been used successfully in human nutrition studies. Ideally combinations of tests are used that cover the most important genomic damage pathologies that have been shown to be associated prospectively with adverse health outcomes. These include (1) chromosome deletions or rearrangements which can be measured by the lymphocyte CBMN-Cyt assay; (2) DNA hypomethylation, (3) DNA oxidation, (4) telomere length, and (5) mtDNA deletions. Indirect biomarkers of DNA damage such as elevated expression of genes associated with increased DNA damage (e.g., $p53$, $waf1$, $gadd45$) (59,60) may be justifiably used as supporting evidence and if prospective association with adverse health outcomes is ultimately demonstrated. These DNA damage biomarker studies need to be coupled with robust dietary intake tools and preferably with blood and tissue measures of nutritional status ideally in the target cells in which DNA damage is measured.

To date the intervention studies investigating the effects of micronutrients on DNA damage have been limited by sampling only a single tissue, usually blood cells. Furthermore studies using mixtures of cells (e.g., leukocytes which contain both myeloid and lymphoid cell types) may be confounded by changes in ratios of different cell types if DNA damage rates vary between these subsets. Because of differences in gene expression between haematopoietic and epithelial tissues it would be preferable to also include measurements in an easily accessible epithelial tissue such as buccal cells in oral mucosa. The latter also has the advantage of being a minimally invasive procedure that can be used for studies in babies, infants and children for which it would also be preferable to miniaturise blood tests so that they can be done on a finger-stick blood which is much easier and less discomforting from using venipuncture by syringe. The duration of the intervention studies should also take into consideration the cellular turn-over rate of the tissue that is sampled which can vary greatly from 14–21 days in buccal cells, to 6 months or more in lymphocytes (16,17).

**Knowledge Gaps and Implementation Challenges**

An important knowledge gap is whether DNA damage measurements in lymphocytes and buccal cells would be sufficient to infer DRVs for DNA damage prevention for the whole organism. Furthermore, it is essential that “normal” range values of the validated DNA damage biomarkers are established for each age and gender group and the levels of these biomarkers that are associated with a substantial elevated risk in ad-
verse health outcomes need to be determined. These data bases are available within research laboratories but they should become increasingly accessible and possibly have better quality control if DNA damage tests are also performed routinely within the clinical laboratory setting. If one considers that damage to the genome is the most fundamental pathology or disease it becomes necessary to seriously appreciate the need for the inclusion of the validated DNA damage biomarkers within the clinical setting particularly in this era of preventive and integrative medicine. In other words the adoption of DNA damage biomarkers within mainstream preventive medicine would provide the necessary momentum to establish and continually refine DRVs for prevention of DNA damage. The integration of DNA damage biomarkers in preventive and integrative medicine is starting to occur slowly but infrastructure support, education and training of medical students and integrative medicine practitioners is urgently needed to enable this to happen in a consolidated, sustainable and timely manner given the rapid increase in ageing populations in developed countries. Furthermore, there is an urgent need to translate this knowledge into the design of high nutrient density foods that are appropriate for optimal genome maintenance so that the nutritional requirements of disadvantaged communities and populations are also efficaciously met at the genome level.

Because we live in the era of stem cell technology in which cells grown in culture may be returned to the body it is also increasingly important to define the nutritional requirements in culture medium for prevention of DNA damage because it is known that genomically unstable stem cells have the potential to become progenitor cells for cancer (60–64). The latter emphasises the need of reliable and physiological in vitro systems in this scientific endeavour given that commonly used culture media (e.g., RPMI 1640) are both supra-physiological for key micronutrients such as folate, (e.g., folic acid concentration in RPMI 1640 is 2000 nM folic but plasma is 20–60 nM folate) which may alter DNA methylation patterns, and they are completely deficient for others such as zinc, selenium, iron and manganese all of which are micronutrients required either for genome maintenance and/or prevention of oxidative damage to DNA.

An important challenge is to devise ways to personalise nutritional requirements for optimisation of genome stability by appropriately matching the nutriome with the genome and its current status of expression (i.e., the transcriptome). It was shown that this is possible by in vitro modelling (65–68) and others have shown this to be possible by in silico modelling (68–71) but whether these tools can ultimately be translated to predicting in vivo effects of advised personalised nutrition on genome maintenance remains unknown.

The ultimate and most difficult challenge is to verify that reducing rates of DNA damage in the general population and/or on an individual basis will in fact reduce the incidence and severity of those diseases whose risk has been shown to be prospectively increased if DNA damage is elevated which includes infertility, pregnancy complications, cancer, cardiovascular disease and possibly neurodegenerative disease. In silico modelling and in vivo studies suggest that these interventions would have to occur before the disease process caused by excessive DNA damage is initiated (71,72). Studies in rodents in which DNA damage in peripheral blood and buccal cells was measured together with target tissue disease-specific pathology during dietary intervention support the hypothesis that reducing DNA damage nutritionally is a plausible strategy for prevention of degenerative diseases (73,74). Proving this in humans will be more difficult but essential to completely justify nutritional optimisation of genome stability for disease prevention.

Finally, a possible approach for determining dietary reference values of single micronutrients for genome damage prevention could involve the use of both by in vitro and in vivo human models and measurement of multiple complementary DNA damage biomarkers. However the highest levels of evidence would ultimately be derived from controlled intervention studies covering a wide range of doses but not exceeding the known safe upper limits. The results from such studies for the better established and most commonly used DNA damage biomarkers in nutrition intervention studies (i.e., comet assay and cytokinesis-block micronucleus assay) have been recently reviewed (75,76).

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