Dietary reference values of individual micronutrients and nutriomes for genome damage prevention: current status and a road map to the future[1–4]

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ABSTRACT

Damage to the genome is recognized as a fundamental cause of developmental and degenerative diseases. Several micronutrients play an important role in protecting against DNA damage events 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 micronutrient 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 subgroup, or population basis. In this article, the following topics are discussed: *1*) biomarkers used to study genome damage in humans and their validation, *2*) evidence for the association of genome damage with developmental and degenerative disease, *3*) current knowledge of micronutrients required for the maintenance of genome stability in humans, *4*) the effect of nutrient-nutrient and nutrient-genotype interaction on DNA damage, and *5*) strategies to determine dietary reference values of single micronutrients and micronutrient combinations (nutriomes) on the basis of DNA damage prevention. This article also identifies important knowledge gaps and future research directions required to shed light on these issues. The ultimate goal is to match the nutriome to the genome to optimize genome maintenance and to prevent pathologic amounts of DNA damage. *Am J Clin Nutr* doi: 10.3945/ajcn.2010.28674D.

INTRODUCTION

Dietary reference values (DRVs) are intended to provide a guide for the appropriate intake of nutrients for prevention of diseases caused by deficiency (eg, scurvy in the case of vitamin C) or excess (eg, iron-overload disease, which may be fatal) (1). Determining these extremes is important, but the biggest challenge in the prevention of developmental and degenerative disease in populations that are not short of food, fortified food, or supplements is defining the appropriate intakes of micronutrients individually or in combination (nutriomes) to optimize cellular and organism performance on both a personal and a genetic subgroup level at different life stages. Optimization of cellular function ultimately depends on the prevention of damage to the nuclear and mitochondrial genome.

We commence life as a single-cell embryo, which is literally a packet of the human diploid genome primed for replication. This genome has to be replicated with high fidelity millions of times during development to a fetal and adult stage and millions of times thereafter simply to replenish dead cells and cells lost as a result of exfoliation. The capacity to replicate DNA accurately and to produce sufficient daughter cells is limited by the need of cofactors and substrates required for DNA replication and DNA repair as well as accumulated DNA damage that can trigger cell death by apoptosis. 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 recognized as a fundamental underlying cause of developmental defects and accelerated aging as well as of an increased risk of degenerative conditions such as infertility, immune dysfunction, cancer, and 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 article is to consider the proposition that the 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 by using a set of validated and accurate methods for measuring genome damage at both the molecular and the cytogenetic level.

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BIOMARKERS USED TO STUDY GENOME DAMAGE IN HUMANS AND THEIR VALIDATION TO STUDY THE EFFECTS OF NUTRITION

Numerous biomarkers of DNA damage have been developed over the past 30 years, which has enabled the accurate measurement of DNA base damage (eg, hydroxyl radical adducts on nucleotides), microdeletions 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 damage can also be visualized at the cytologic level by using micronucleus cytome assays that were developed for use with cell lines, peripheral blood lymphocytes, erythrocytes, and buccal cells (16–19). Micronuclei originate from acentric chromosome fragments or whole chromosomes that fail to engage the spindle during nuclear division and therefore provide a measure of either chromosome breakage or chromosome loss, respectively. In the cytokinesis-block micronucleus (CBMN) assay, which is commonly used with peripheral blood lymphocytes, once-divided cells that can express this damage are identified as binucleated cells by using a cytokinesis-blocking agent (cytochalasin-B). Within these binucleated cells it is also possible to measure nucleoplasmic bridges (NPBs), which arise from dicentric chromosomes (caused by misrepair of DNA strand breaks or by telomere end fusions) and nuclear buds (NBUDs), biomarkers of gene amplification (**Figure 1** and **Figure 2**).

The presence of micronuclei, NPBs, and/or NBUDs is a strong indicator of chromosomal damage and instability within a cell (17). Micronuclei, NPBs, and NBUDs have been shown to be sensitive to small changes in micronutrient concentration (eg, in folic acid, riboflavin, selenomethionine) within the physiologic range (20–22). It is important to note that these cytogenetic and cytological biomarkers of chromosome damage can detect the genotoxic effects via a multitude of mechanisms and therefore tend to have the advantage of being very sensitive and capable of integrating the effects of multiple interactions and molecular genotoxic events on genome stability. Ideally, these techniques should be combined with other DNA damage diagnostics that can measure specific lesions in the DNA such as oxidized DNA bases, methylation status of DNA at repeat or promoter sequences, and telomere length as well as mitochondrial DNA (mtDNA) deletions (14, 23–30).

A limitation of these more specific techniques is that they reflect only a small portion of the grand total of genomic damage and do not provide information on defects in the functionality of the genome that result from the presence of that lesion. For example, measuring telomere length alone is insufficient to know whether this also results in telomere end fusions, which are the main pathological event that leads to chromosomal instability. However, telomere end fusions could be measured in the nucleoplasmic bridge index within the CBMN cytome assay if combined with telomere labeling (17, 31, 32). For the purpose of in vitro modeling, it is also important that the assays used for in vivo studies can also be used in vitro so that micronutrient combinations and their interactions with endogenous genotoxins (eg, hydrogen peroxide, nitric oxide) and exogenous genotoxins (eg, ultraviolet radiation, dietary carcinogens such as heterocyclic amines in cooked meat) can be efficiently explored.

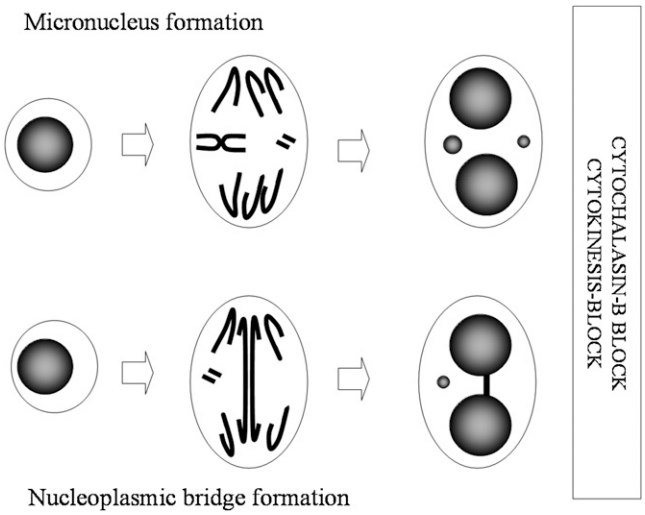

FIGURE 1. Expression of micronuclei and nucleoplasmic bridges (NPBs) during nuclear division. Micronuclei originate either from *1*) lagging whole chromosomes (top) that are unable to engage with the mitotic spindle due to a defect in the spindle or a defect in the centromere/kinetochore complex required to engage with the spindle or from *2*) an acentric chromosome fragment originating from a chromosome break (top and bottom), which lags behind at anaphase because it lacks a centromere/kinetochore complex. Misrepair of 2 chromosome breaks may lead to an asymmetrical chromosome rearrangement, which produces a dicentric (ie, 2 centromeres) chromosome and an acentric fragment (bottom). Frequently, the centromeres of the dicentric chromosome are pulled to opposite poles of the cell at anaphase resulting in the formation of a NPB between the daughter nuclei. NPBs are frequently accompanied by a micronucleus originating from the associated acentric chromosome fragment. NPBs may also originate from dicentric chromosomes caused by telomere end fusions. Because micronuclei and NPBs are expressed only in cells that have completed nuclear division, it is necessary to score these genome instability biomarkers specifically in once-divided cells. This is readily accomplished by blocking cytokinesis using cytochalasin-B. In the cytokinesis-block micronucleus assay, Micronuclei and NPBs are specifically scored in the binucleated cells accumulated in culture by adding cytochalasin-B, the cytokinesis-blocking agent used in the assay. *See* reference 17 for a more detailed explanation.

The DNA damage biomarkers most commonly used in nutrition studies, along with a description of their strengths and weaknesses, are listed in **Table 1**. Most of these techniques can be used in in vitro studies, but others, such as the erythrocyte micronucleus assay, cannot be used for this purpose. Nevertheless, this method, like the others listed in Table 1, is practical for use in in vivo studies also. Although it has been shown to be associated with nutritional status in both cross-sectional and controlled trials and with disease states such as thalassaemia (19, 33, 34), no prospective studies have yet been performed to validate the erythrocyte micronucleus method as a predictor of disease in humans. However, evidence in rodents suggests that it is predictive of cancer risk in animals exposed to carcinogens (33–35). These types of considerations are important when deciding which battery of tests are best suited for determining nutrient reference values for micronutrients to prevent DNA damage.

Ideally, the biomarkers of DNA damage that are of interest would be predictive of human disease risk and 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 developmental and/or degenerative disease risk in humans

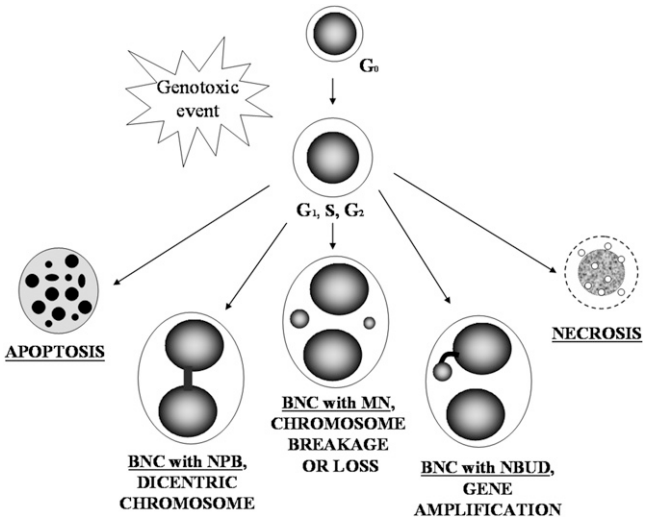

FIGURE 2. The various biomarkers scored in the cytokinesis-block micronucleus cytome assay in lymphocytes. DNA damage biomarkers are scored in binucleated cells (BNC), which accumulate by blocking cytokinesis with cytochalasin-B during ex vivo culture. The DNA damage biomarkers scored are as follows: *1*) micronuclei (MN), biomarkers of chromosome breakage or loss; *2*) nucleoplasmic bridges (NPB), biomarkers of dicentric chromosomes that originate from either misrepair of DNA breaks or telomere end fusions; and *3*) nuclear buds (NBUD), a biomarker of gene amplification. Cell death by necrosis or apoptosis is also measured on the basis of morphologic changes in the nuclei and cytoplasm. The ratio of mononucleated to binucleated cells also provides a measure of mitogenic response and cytostasis. G0, G1, S, and G2 refer to stages in the mitotic cycle. *See* reference 17 for a more detailed explanation.

(22–225). The current status of the 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 micronucleus cytome assay (16), the red blood cell micronucleus assay (33), the comet assay of DNA strand breaks in lymphocytes (36, 37), telomere length (26–28), DNA methylation (24–26), DNA oxidation (38, 39), and mtDNA deletion (29, 30) in leukocytes or lymphocytes—is summarized in **Table 2**. At this point only the micronuclei 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 currently either lack 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. These aspects will be discussed in more detail in the following sections.

EVIDENCE FOR THE ASSOCIATION OF GENOME DAMAGE WITH DEVELOPMENTAL AND DEGENERATIVE DISEASE

Genome damage affects health outcomes at all stages of life. Infertile couples exhibit a higher rate of genome damage than fertile couples when their chromosomal stability is measured in lymphocytes by using the CBMN-Cyt assay (119). Infertility may be due to a reduced production of germ cells because genome damage effectively causes programmed cell death or apoptosis, which is one of the mechanisms by which grossly mutated cells are normally eliminated (226–228). When the latter mechanism fails, reproductive cells with genomic abnormalities may survive, which leads to serious developmental defects (229, 230).

It has also become evident that parental DNA damage level measured by the CBMN or oxidized guanine assays is associated with abnormal pregnancy outcomes such as recurrent pregnancy loss or lower infant birth weight (180, 181, 231). That an elevated rate of chromosomal damage is a cause of cancer has been shown by ongoing prospective cohort studies in European countries, which showed a 2- to 3-fold increased risk of cancer in those whose chromosomal damage rate in lymphocytes was in the highest tertile when measured 10–20 y before cancer incidence was measured (232). It has also been shown that an elevated micronuclei frequency, a robust biomarker of chromosome breakage or loss, in lymphocytes predicts cancer risk (135, 137) and cardiovascular disease mortality in humans (136, 138). Excessive chromosomal damage and micronuclei are also associated with neurodegenerative diseases such as Alzheimer's and Parkinson's disease (126–131). Telomere shortening has been shown to be predictive of risk of certain cancers (207, 211, 217), cardiovascular disease, cognitive decline, and likelihood of mortality (202–206, 212–219). Those individuals with accelerated aging syndromes due to redox imbalances (eg, Down syndrome) and/or suboptimal DNA repair (eg, carriers of deleterious mutations in the ATM or BRCA1 genes) may be particularly susceptible to the genome-damaging effects of suboptimal micronutrient intake. Recent evidence suggests that genome instability in such syndromes might be mitigated by appropriate micronutrient supplementation (233, 234).

CURRENT KNOWLEDGE OF 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, and maintenance methylation of DNA. The role of micronutrients in maintenance of genome stability has been extensively reviewed (2–4, 7–9). Examples of micronutrients involved in various genome stability processes are given in **Table 3**, and some of the various possible mechanisms by which micronutrient deficiency could cause DNA damage, accelerated senescence, and chromosomal instability are illustrated in **Figure 3**.

Both micronutrient deficiency and micronutrient excess can cause genome damage. These effects could be of the same order of magnitude, if not greater, than the genome damage caused by exposure to significant doses of environmental genotoxins such as chemical carcinogens, ultraviolet radiation, and ionizing radiation. An example from our laboratory is the observation that the chromosomal damage in cultured human lymphocytes caused by reducing folate concentration from 120 to 12 nmol/L is equivalent to that induced by an acute exposure to 0.2 Gy of low linear-energy-transfer ionizing radiation (eg, X-rays), a dose of radiation that is $\approx$10 times greater than the annual allowed safety limit of exposure for the general population (239)

TABLE 1
Strengths and weaknesses of best-validated DNA damage assays for nutritional studies in humans[1]

	DNA damage assays							
	CBMN-Cyt	Red blood cell micronucleus	Buccal micronucleus cytome	Comet	DNA oxidation	DNA methylation	Telomere length	mtDNA deletion
DNA damage events measured								
DNA breaks	Yes	Yes	Yes	Yes	No	No	No	Yes
Misrepair of DNA breaks	Yes	Yes	Yes	No	No	No	No	No
Oxidized DNA bases	No	No	No	Yes[2]	Yes	No	No	No
Chromosome malsegregation	Yes[3]	Yes[3]	Yes[3]	No	No	No	No	No
Chromosomal deletions	Yes[3]	Yes[3]	Yes[3]	No	No	No	No	No
Dicentric chromosome or telomere end fusion	Yes[4]	No	No	No	No	No	No	No
Telomere length	No	No	No	No	No	No	Yes	No
Hypo/hypermethylation of DNA	No	No	No	No	No	Yes	No	No
Abasic sites in DNA	No	No	No	Yes[5]	No	No	No	No
mtDNA damage	No	No	No	No	No	No	No	Yes
Other features								
Distinguishes DNA damage in viable cells from cell death[6]	Yes	Yes	Yes	No	No	No	No	No
Suitable for in vitro studies	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Cell type in which assay is performed	PBLs	RBCs	Oral mucosa	Any cell type	Any cell type	Any cell type	Any cell type	Any cell type

[1] CBMN-Cyt, cytokinesis-block micronucleus cytome; mtDNA, mitochondrial DNA; PBLs, peripheral blood lymphocytes; RBCs, red blood cells.
[2] If used in combination with glycosylase enzymes that remove oxidized bases.
[3] By measuring micronuclei with/without centromere staining.
[4] By measuring nucleoplasmic bridges with/without telomere staining.
[5] If alkaline version of the assay is used.
[6] The inability to distinguish between DNA damage from dead or from viable cells may confound DNA damage results.

(**Figure 4**). These results imply that genome damage biomarkers are not only biodosimeters (ie, indicators of the dose experienced by tissues) of exposure to human-made or natural genotoxins but also biodosimeters of the deficiency in micronutrients required *1*) for the prevention of oxidation to DNA (eg, antioxidants), *2*) the prevention of uracil incorporation into DNA (eg, folate), *3*) for the maintenance methylation of CpG in DNA (eg, methionine, choline, folate, vitamin B-12), *4*) as cofactors

TABLE 2
Validation status of DNA damage biomarkers in peripheral blood cells, buccal cells, plasma, or urine with respect to association with nutrition and developmental or degenerative disease or mortality in humans[1]

	Association with nutritional status		Association with developmental or degenerative disease or mortality	
	Cross-sectional studies	Controlled intervention studies	Case-control studies	Prospective cohort studies
Cytokinesis-block micronucleus assay in PBLs	22, 40–46 [H]	40, 41, 46–57 [H]	120–134 [H]	135–138 [M]
Micronucleus assay in buccal cells	58–60 [L]	60–67 [H]	61, 62, 67, 139–152 [H]	NSP
Micronucleus assay in erythrocytes	18, 19, 34, 68–72 [H]	18, 19, 72 [L]	33, 153, 154 [L]	NSP
DNA strand breaks in PBLs: comet assay	73–75 [L]	76–87 [H]	155–176 [H]	NSP
DNA oxidation (8-OHdG in DNA or urine)	88–91 [M]	92–102 [H]	177–192 [H]	NSP
DNA methylation[2]	103–105 [L]	106–109 [M]	193–201 [H]	NSP
Telomere length in PBLs or leukocytes	110–114 [M]	NSP	202–211 [H]	212–219 [H]
Mitochondrial DNA deletion	115–117 [L]	118 [L]	220–225 [M]	NSP

[1] 8-OHdG, 8′hydroxydeoxyguanosine; PBLs, peripheral blood lymphocytes; NSP, no studies yet published. Letters in brackets refer to validation status based on number of published studies: H = high (≥7 published studies), M = medium (4–6 published studies), and L = low (1–3 published studies).
[2] Global or gene-specific methylation.

TABLE 3
Examples of the role and effect of deficiency of specific micronutrients on genomic stability[1]

Micronutrients	Role in genomic stability	Consequence of deficiency
Vitamin C, vitamin E, antioxidant polyphenols (eg, caffeic acid)	Prevention of oxidation to DNA and lipid oxidation.	Increased baseline level of DNA strand breaks, chromosome breaks and oxidative DNA lesions, and lipid peroxide adducts on DNA.
Folate, riboflavin, and vitamins B-6 and B-12	Maintenance methylation of DNA; synthesis of dTMP from dUMP and efficient recycling of folate.	Uracil misincorporation in DNA and increased chromosome breaks and DNA hypomethylation.
Niacin	Required as substrate for PARP, which is involved in cleavage and rejoining of DNA and telomere length maintenance.	Increased number of unrepaired nicks in DNA, increased chromosome breaks and rearrangements, and sensitivity to mutagens.
Zinc	Required as a cofactor for Cu/Zn superoxide dismutase, endonuclease IV, function of p53, Fapy glycosylase, and in zinc-finger proteins such as PARP.	Increased DNA oxidation, DNA breaks, and elevated chromosome damage rate.
Iron	Required as component of ribonucleotide reductase and mitochondrial cytochromes.	Reduced DNA repair capacity and increased propensity for oxidative damage to mitochondrial DNA.
Magnesium	Required as cofactor for a variety of DNA polymerases, in nucleotide excision repair, base excision repair, and mismatch repair. Essential for microtubule polymerization and chromosome segregation.	Reduced fidelity of DNA replication. Reduced DNA repair capacity. Chromosome segregation errors.
Manganese	Required as a component of mitochondrial manganese superoxide dismutase.	Increase susceptibility to superoxide damage to mitochondrial DNA and reduced resistance to radiation-induced damage to nuclear DNA.
Calcium	Required as cofactor for regulation of the mitotic process and chromosome segregation.	Mitotic dysfunction and chromosome segregation errors.
Selenium	Selenoproteins involved in methionine metabolism and antioxidant metabolism (eg, selenomethionine, glutathione peroxidase I).	Increase in DNA strand breaks, DNA oxidation, and telomere shortening.

[1] Data are from references 2–4, 7–9, and 236–245. dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; PARP, poly(ADP-ribose) polymerase.

or as components of DNA repair enzymes (eg, zinc, magnesium), and *5*) for the maintenance of telomere length (eg, niacin, folate) (2–4, 7–9, 236–245). The sensitivity of DNA damage to micronutrient deficiency is underscored by the fact that there are at least 8 human DNA repair glycosylases dedicated to the removal of the type of DNA base damage (eg, 8-hydroxydeoxyguanosine, uracil) that is produced when *1*) antioxidant micronutrients (eg, zinc, vitamin C, and vitamin E) or *2*) methyl donor micronutrients (eg, folate, methionine, and vitamin B-12) are deficient (246, 247).

Results from a recent population study suggest that ≥9 micronutrients affect genome stability in humans in vivo (**Table 4**)

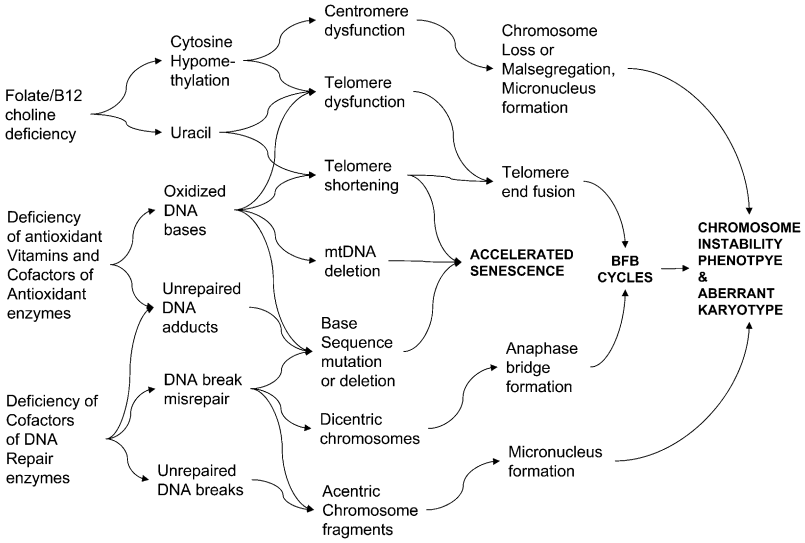

FIGURE 3. Examples of possible mechanisms by which micronutrient deficiency could cause damage to the genome, accelerate senescence, and promote chromosomal instability. mtDNA, mitochondrial DNA; BFB, chromosomal breakage-fusion-bridge cycles.

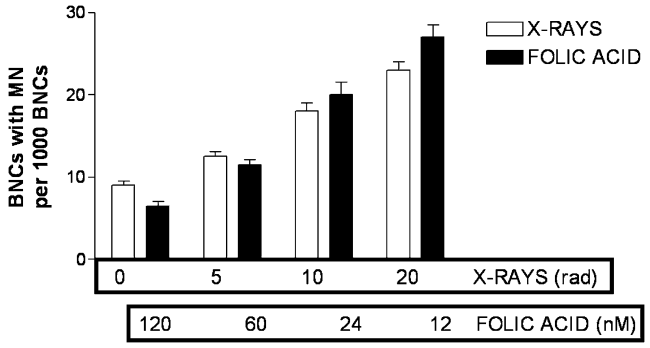

FIGURE 4. Comparison of the dose-response effect on micronucleus induction in cytokinesis-blocked cultured lymphocytes caused by *1*) acute exposure to X-rays up to a maximum dose of 20 rad, which is equivalent to 10 times the annual exposure safety limit for the general public (235), and *2*) folic acid deficiency within the normal physiologic range of 12–120 nM concentration. BNCs, binucleated cells. Results represent the mean ± 1 SEM; $n = 6$ for X-rays and $n = 20$ for folic acid experiments.

(40). This cytogenetic epidemiologic study on 190 healthy individuals (mean age: 47.8 y; 46% males) was designed to determine the association between dietary intake, which was estimated by using a food-frequency questionnaire, and genome damage in lymphocytes, which was measured by using the cytokinesis-block micronucleus assay. Multivariate analysis of baseline data showed that *1*) the highest tertile of intake of vitamin E, retinol, folate, nicotinic acid (preformed), and calcium is associated with significant reductions in micronuclei frequency (ie, −28%, −31%, −33%, −46%, and −49%, respectively; all $P < 0.005$) relative to the lowest tertile of intake and that *2*) the highest tertile of intake of riboflavin, pantothenic acid, and biotin was associated with significant increases in micronuclei frequency [ie, +36% ($P = 0.054$), +51% ($P = 0.021$), and +65% ($P = 0.001$)], respectively, relative to the lowest tertile of intake. Midtertile β-carotene intake was associated with an 18% reduction in micronuclei frequency ($P = 0.038$); however, the highest tertile of intake (>6400 μg/d) resulted in an 18% increment in micronuclei 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 (248–254), and it is probable that in a specific mixed diet, depending on the intake level of an individual, some of the micronutrients may be outside the intake range that is optimal for prevention of genome instability. The results for β-carotene suggest an optimum for genome stability between 4000 and 6000 μg/d with a tendency for marked increase in genome damage at higher or lower intakes, which is consistent with data that suggest an increased cancer risk with deficiency or supplementation above the Recommended Dietary Intake for this vitamin (248, 250, 254).

On the other hand, the apparent genome damage prevention effects associated with vitamin E, retinol, folic acid, preformed nicotinic acid, and calcium were still increasing at the highest

TABLE 4
Association of intake of specific micronutrients with baseline micronuclei frequency in lymphocytes in a South Australian cohort of healthy adults[1]

	Tertiles of intake	Subjects	Variation of micronuclei frequency[2]	95% CI	P^2
		n	%	%	
Calcium (mg/d)	≤927.50	63	0	—	—
	927.51–1249.55	63	−18	(−36, 5)	0.121
	≥1249.56	63	−49	(−63, −30)	<0.001
Nicotinic acid, preformed (mg/d)	≤20.04	63	0	—	—
	20.05–25.72	63	−26	(−40, −9)	0.004
	≥25.73	63	−46	(−58, −30)	0.001
Folate (μg/d)	≤206.64	63	0	—	—
	206.65–256.49	63	−16	(−32, 3)	0.094
	≥256.50	63	−33	(−49, −13)	0.003
Retinol (μg/d)	≤296.37	63	0	—	—
	296.38–457.47	63	−10	(−24, 7)	0.233
	≥457.48	63	−31	(−43, −16)	0.001
Vitamin E (mg/d)	≤7.87	63	0	—	—
	7.88–10.71	64	−15	(−28, 1)	0.066
	≥10.72	62	−28	(−42, −11)	0.003
β-Carotene (μg/d)	≤4161.32	63	0	—	—
	4161.33–6433.12	63	−18	(−32, −1)	0.036
	≥6433.13	63	18	(−6, 48)	0.148
Riboflavin (mg/d)	≤1.84	63	0	—	—
	1.85–2.41	64	41	(11, 78)	0.005
	≥2.42	62	36	(−1, 85)	0.054
Pantothenic acid (mg/d)	≤4.59	63	0	—	—
	4.60–5.64	64	69	(34, 115)	<0.001
	≥5.65	62	51	(6, 114)	0.021
Biotin (μg/d)	≤18.86	63	0	—	—
	18.87–25.49	63	7	(−14, 33)	0.542
	≥25.50	63	65	(22, 123)	0.001

[1] Data are from reference 40.
[2] Percentage variation and *P* values refer to comparison with the lowest tertile of intake.

tertile of intake, which suggests that an optimum could be achieved at even higher intakes or that the maximum beneficial effect is achieved at these intakes. For example, the highest tertile of intake for folate was >256 μg/d, which is consistent with a number of studies showing that developmental defects and cancer, as well as biomarkers for cardiovascular disease risk such as homocysteine, are minimized at folate intakes of ≥400 μg/d (242, 255–258). That both vitamin deficiency and vitamin excess can increase carcinogenesis is supported by several studies (9, 248, 249, 259) and highlights the acute need for better knowledge of dose–response relations between micronutrient intake and genome damage.

We were also interested in investigating the combined effects of calcium or riboflavin with folate consumption because epidemiologic evidence suggests that these dietary factors tend to interact in modifying the risk of cancer (260–262) and that they are also associated with a reduced risk of osteoporosis and hip fracture (263–265). Interactive additive effects, such as the protective effect (−46%) of increased calcium intake and the exacerbating effect (+42%) of higher riboflavin consumption on increased genome damage caused by low folate intake, were observed (**Figure 5**). The results from this study illustrate the strong effect of a wide variety of micronutrients and their interactions on genome health depending on amount of intake. The unexpected effects of these interactions highlight the need to consider not only individual micronutrients but also micronutrient combinations at varying dosages. The term *nutriome* was introduced to define this important aspect of nutritional requirements that needs much attention (266, 267). The ultimate goal is to define for each individual the nutriome that matches their genome to allow optimal genome stability to be achieved. Culturing an individual's cells in an array of multiple micronutrient combinations under physiologic conditions is emerging as the ultimate tool in the genome health nutrigenomics discipline that will allow for the discovery of individualized nutriomes for optimal genome stability tailored to specific genotypes (20, 268–270).

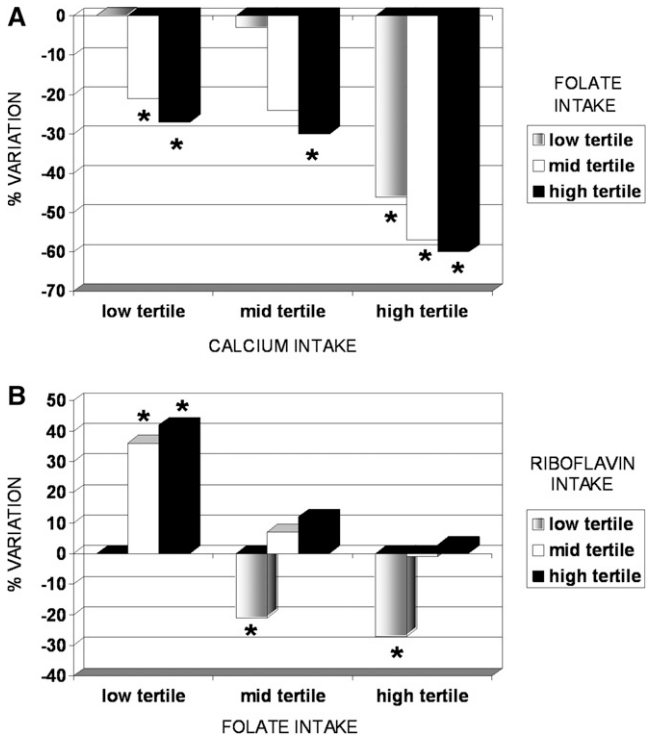

FIGURE 5. The combined effect of (A) calcium and folate intake and (B) riboflavin and folate intake on micronucleus frequency in lymphocytes. Results shown are the percentage variation relative to the combined lowest tertiles of intake in the pair of nutrients examined. *$P < 0.05$ for comparison with the referent value for the combined lowest tertile of intake for the pair of nutrients examined. *See* reference 40 for a more detailed explanation.

The amounts of micronutrients that appear to be protective against genome damage vary greatly between foods (3, 271), and careful choice is needed to design dietary patterns optimized for genome health maintenance. Because dietary choices vary between individuals, due to taste preferences that may be genetically determined (272, 273) or 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 that are rich in micronutrients required for DNA replication and repair and for the prevention of genome-damaging events is essential for individuals to achieve their daily nutrient requirements for genome health maintenance without the intake of excess calories.

An important development is the observation that, although DNA damage measured by both molecular and cytogenetic biomarkers tends to increase with age (274–277) (**Figure 6**), it is possible to attenuate the rate of increase or to reduce the number of these biomarkers by appropriate dietary change or supplementation by specific micronutrient combinations (18, 19, 40, 41, 46–57, 60–67, 72, 76–87, 90–102, 106–109, 114, 118, 278). A limitation of some of these studies is that they are usually performed over brief periods of time (3–6 mo) and limited to single tissues, usually blood cells and single assays of DNA damage. A more robust approach should include the following: *1*) measurements in multiple tissues that can be easily accessed, such as lymphocytes and neutrophils in the hematopoietic system as well as buccal cells as representatives of epithelial cells that compose the bulk of the body, and *2*) a comprehensive set of complementary biomarkers of genome damage to measure both chromosomal instability events that can be readily performed by using micronucleus cytome assays and molecular lesions such as DNA hypo- or hypermethylation, telomere length, DNA oxidation, and mtDNA deletions (Tables 1 and 2).

EFFECT OF NUTRIENT-NUTRIENT AND NUTRIENT-GENOTYPE INTERACTION ON DNA DAMAGE

As indicated above, it is plausible that interactive effects between micronutrients can modify the nutritional requirements for genome maintenance. The detailed study of nutrient–nutrient and nutrient-genotype effects on DNA damage is limited by the resources needed to investigate the numerous possible combinations. However, it has been shown that it is possible to use in vitro modeling to investigate these effects with long-term cultures of peripheral blood lymphocytes (20). The use of lymphocytes for this purpose is ideal because these cells can be studied both in vitro and in vivo, which provides a fully integrated approach by using the same cell system. We tested the hypothesis that the methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism, folic acid deficiency, and riboflavin deficiency, independently or interactively, are important determinants of

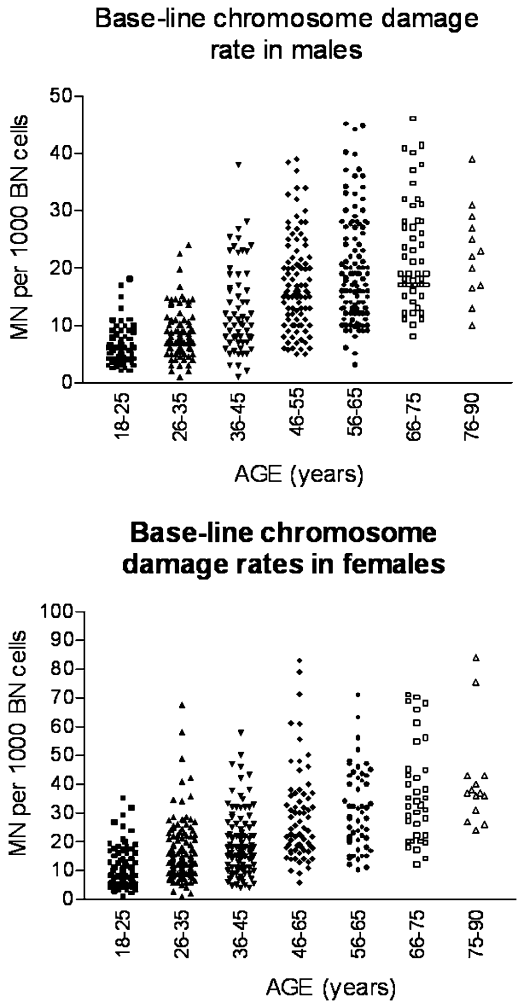

FIGURE 6. Variation in chromosome DNA damage rates of healthy nonsmoking men (n = 495) and women (n = 511) within and between age groups measured by using the cytokinesis-block micronucleus assay. MN, micronuclei; BN, binucleated cells.

genomic stability, cell death, cell proliferation, and homocysteine concentration in 9-d human lymphocyte cultures (20). Lymphocytes of 7 wild-type (CC) and 7 mutant (TT) homozygotes were cultured under the 4 possible combinations of deficiency and sufficiency of riboflavin (0 and 500 nmol/L) and folic acid (20 and 100 nmol/L) at a constant L-methionine concentration of 50 μmol/L. Viable cell growth was 25% greater in TT than in CC cells ($P < 0.05$) and 32% greater at 100 nmol folic acid/L than at 20 nmol folic acid/L ($P = 0.002$). The comprehensive CBMN-Cyt assay (17) was used to measure the DNA damage biomarkers micronucleus, NPB, and NBUD. The micronucleus amounts were 21% higher in TT cells than in CC cells ($P < 0.05$) and 42% lower in the high-folic-acid medium than in the low-folic-acid medium ($P < 0.0001$). The NBUD amounts were 27% lower in TT cells than in CC cells ($P < 0.05$) and 45% lower in the high-folic-acid medium than in the low-folic-acid medium ($P < 0.0001$). High riboflavin concentration (500 nmol/L) increased NBUD amounts by 25% (compared with 0 nmol riboflavin/L) in folate-deficient conditions (20 nmol folic acid medium/L; $P < 0.05$), and there was an interaction between folic acid and riboflavin that affected NBUD amounts ($P = 0.042$). This preliminary investigation suggests that the *MTHFR* C677T polymorphism and riboflavin affect genome instability; however, the effect is relatively small compared with that of folic acid. The fact that the results of this in vitro study are consistent with in vivo observations of elevated homocysteine under low-folate conditions in homozygous carriers (TT) of the *MTHFR* C677T polymorphism (270) and with the apparent increased genomic instability when riboflavin is increased in a low-folate background in vivo (41) suggests that it is feasible to use in vitro approaches to define nutriomes that are optimal for genome stability for individuals and genetic subgroups.

Similar studies have been performed of individuals with *BRCA1* and *BRCA2* mutations on the interaction with folic acid and methionine concentration (268, 269) to investigate the interaction of alcohol and folic acid (279) and define the optimal ratio of seleno:methionine relative to sulfur:methionine at a constant methionine concentration for the prevention of DNA damage and cytotoxicity (21). On the basis of this type of knowledge it is possible to start building plausible mechanistic models of nutrient-nutrient, nutrient-gene interaction, and nutriome-genome interactive effects on genome stability. Two examples of such models are described in **Figure 7** and **Figure 8**. In vitro models are also relevant because we live in an era when cells are taken out of the body and expanded in vitro before being returned to the body (eg, stem cell transplants and immune-system cell transplants). Defining the nutrient composition of the optimal culture medium to prevent DNA damage is critical to keeping oncogenic chromosomal changes from occurring during culture.

STRATEGIES TO DETERMINE DRVs OF SINGLE MICRONUTRIENTS AND MICRONUTRIENT COMBINATIONS (NUTRIOMES) FOR DNA DAMAGE PREVENTION

To determine DRVs of single micronutrients, it is necessary to first perform in vitro dose-response studies on the effect of micronutrient concentration on DNA damage and cytotoxicity and on in vivo cross-sectional investigations of the association of dietary intake with DNA damage biomarkers. Then it is essential to verify this association by appropriate controlled in vivo trials that test both whole foods that are rich in the micronutrient of interest and placebo-controlled trials that use supplements. The various aspects of the best-established assays that have been used successfully in human nutrition studies are summarized in Tables 1 and 2. Ideally, combinations of tests that cover the most important genomic damage pathologies that have been shown to be associated prospectively with adverse health outcomes are used. These include *1*) chromosome deletions or rearrangements that 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 the elevated expression of genes associated with increased DNA damage [eg, *P53*, *WAF1*, and *GADD45* (280, 282)] may be justifiably used as supporting evidence if prospective association with adverse health outcomes is ultimately shown.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.

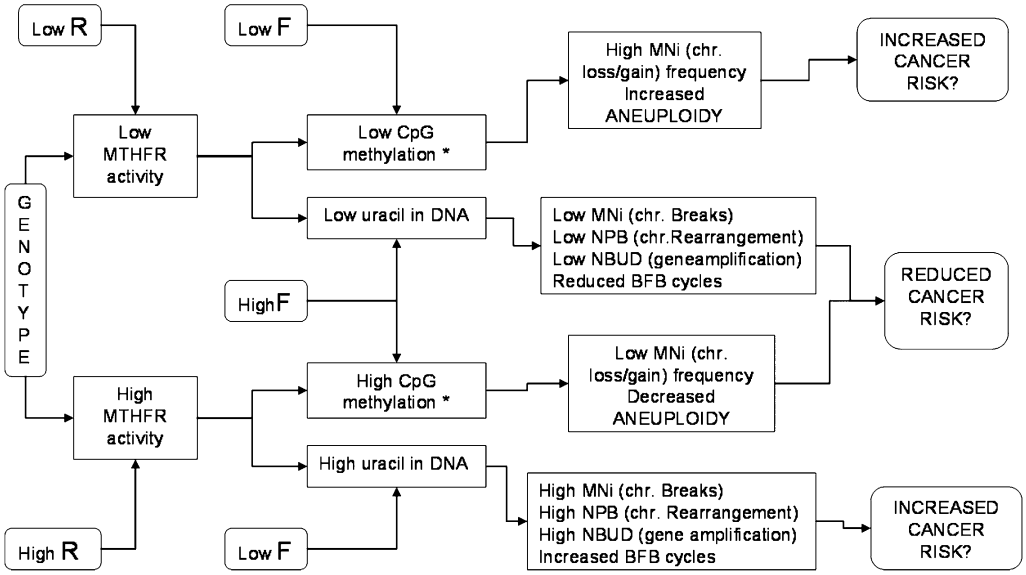

FIGURE 7. Mechanistic framework explaining the interrelation between *MTHFR* genotype, riboflavin (R), and folic acid (F) with respect to the following: *1*) CpG methylation and uracil in DNA; *2*) aneuploidy and micronuclei (MNi) originating from chromosome loss events; *3*) MNi (originating from acentric chromosome fragments), nuclear buds (NBUD), nucleoplasmic bridges (NPB), and breakage-fusion-bridge (BFB) cycles; *4*) initiation of cancer caused by CpG hypomethylation and aneuploidy; and *5*) initiation of cancer caused by increased BFB cycles, MNi (originating from acentric chromosome fragments), NBUDs, and NPBs. *For brevity, other carcinogenic mechanisms induced by altered genome methylation (eg, silencing of tumor suppressor genes and/or activation of oncogenes) are not included in the diagram. chr., chromosome.

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 (eg, leukocytes that 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 hematopoietic and epithelial tissues, it would be preferable to also include measurements in an easily accessible epithelial tissue such as buccal cells in oral mucosa. Using oral mucosa also has the advantage of involving a minimally invasive procedure that can be used for studies in babies, infants, and children. It would also be preferable to miniaturize blood tests so that they can be done on finger-stick blood, which is much easier and less uncomfortable than using venipuncture by syringe. The duration of the intervention studies should also take into consideration the cellular turnover rate of the tissue that is sampled, which can vary greatly from 14 to 21 d in buccal cells to $\geq$6 mo in lymphocytes (16, 17).

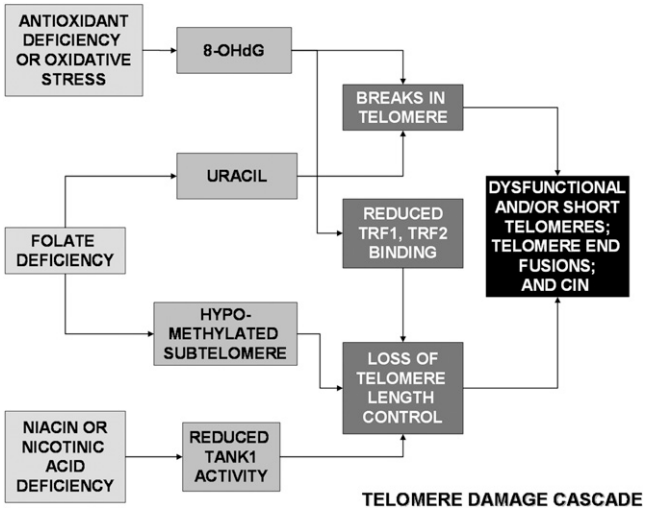

FIGURE 8. Possible mechanisms by which deficiency of folate, niacin (or nicotinic acid), and/or antioxidants may cause dysfunction of telomeres and consequently chromosomal instability (CIN) due to telomere end fusions. 8-OHdG, 8′hydroxydeoxyguanosine.

Given the observed nutrient–nutrient interactive effects and nutrient-genotype effects with respect to genome stability, it will also be necessary to use cross-sectional and intervention study designs that control for these possible interactions by gathering relevant genetic information and by stratifying the analyses according to genotype and the intake or concentration of the interacting nutrient or nutrients. A simple diagram of a possible road map to determine DRVs for genome stability is provided in **Figure 9**.

IMPORTANT TECHNICAL CHALLENGES AND THE NEED FOR HARMONIZATION

For a properly coordinated approach to defining DRVs for DNA damage prevention, it is essential that standard, robust, and transportable protocols for measuring the various DNA damage biomarkers described in Tables 1 and 2 are established. This is already the case for the lymphocyte and buccal micronucleus cytome assays (16, 17), the comet assay (36, 37), some of the DNA oxidation assays (14, 38, 39), DNA methylation (23, 24), telomere length (26–28), and mtDNA deletion assays (29, 30). DNA damage measured by these biomarkers is indicative of residual DNA lesions due to either inefficient or inaccurate DNA repair and/or a level of steady state DNA damage that is excessive relative to normal DNA repair capacity. The effects of micronutrients on the DNA repair process could be analyzed separately from baseline DNA damage by ex vivo/in vitro challenge tests

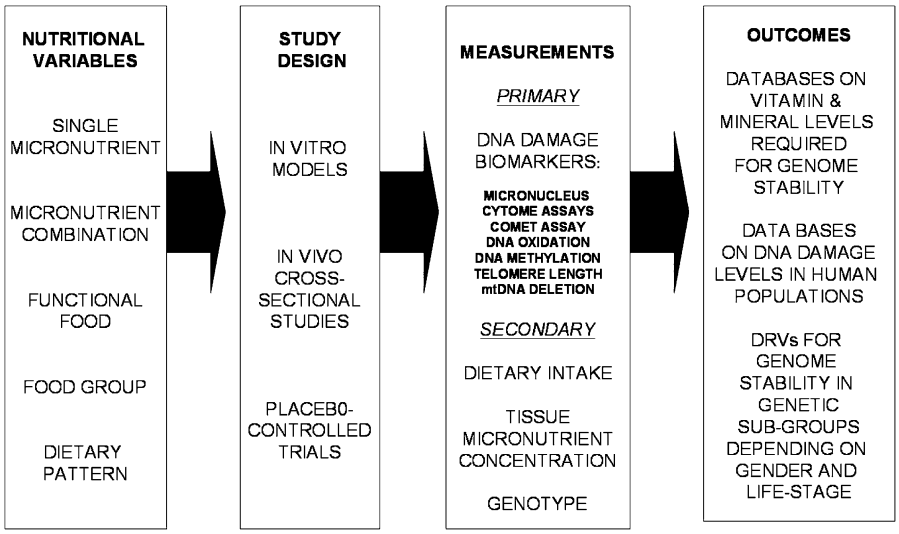

FIGURE 9. A proposed road map for determining dietary reference values (DRVs) of single micronutrients or nutriomes (micronutrient supplement combination, functional food, food group, or dietary pattern) for genome damage prevention by using in vitro and in vivo human models and multiple complementary DNA damage diagnostics. Ideally, this research is also stratified to specific common genetic subgroups, sex, and life stages. mtDNA, mitochondrial DNA.

with a range of genotoxic agents that produce different DNA lesions.

Some of the assays listed above are limited because they provide only arbitrary values (eg, comet assay and quantitative PCR assay for telomere length), but this is being addressed by the use of standards with known amounts of DNA strand breaks or known telomere length or content, respectively. Harmonization is required in both the study designs and the test protocols internationally so that data of cross-sectional and intervention studies from different laboratories and in different countries can be reliably cross-referenced.

Furthermore, given the uncertainties regarding the genotoxic effects of supplementation with supraphysiologic doses of micronutrients, solely or in combination, it is essential to develop in vitro physiologic models such as the peripheral blood lymphocyte culture system, which has been shown to be efficacious in determining the U-shaped relation between micronutrient concentration and genome damage and/or cytotoxicity (20, 21). These tests are essential to define optimal concentration and the safe upper limits of micronutrients and their combinations.

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 sex and that the numbers of these biomarkers that are associated with a substantial elevated risk in adverse health outcomes are determined. These databases 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 establishing and continually refining 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 aging 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.

To date, all studies have been done with adults, and it is now essential to determine the relation between nutrition and DNA damage biomarkers across all life stages, including effects in germ cells, the fetus in utero, premature babies, term babies, infants, children, and teenagers. 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 of the 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 (283–286). This emphasizes the need for reliable and physiologic in vitro systems in this scientific endeavor, given that commonly used culture media (eg, RPMI 1640) are supra-physiologic for key micronutrients such as folate (eg, folic acid concentration in RPMI 1640 is 2000 nM folic but plasma is 20–60 nM folate), which may alter DNA methylation patterns, and that 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 (Table 3).

An important challenge is to devise ways to personalize nutritional requirements for the optimization of genome stability by appropriately matching the nutriome with the genome and its current status of expression (ie, the transcriptome). It has been shown that this is possible by in vitro modeling (20, 268, 270), and by in silico modeling (287–289), but whether these tools can ultimately be translated to predict the in vivo effects of advised personalized nutrition on genome maintenance remains unknown. Until the predictive validity of the in silico and in vitro systems and the resulting expert systems can be proven, we shall have to rely on more robust empirical approaches such as the Genome Health Clinic concept (3, 7, 271), which is based on the diagnosis and nutritional prevention of DNA damage on an individual basis. This approach uses nutritional information that is applicable to the general population but is combined with DNA damage biomarker measurements to verify whether the advice given has actually caused benefit in terms of improved genome stability in the individual. Ideally, both the effect on baseline levels of DNA damage and the effect following ex vivo challenge to moderate genotoxic insult should be measured because challenge tests should provide an indication of the robustness of the homeostatic response to strong perturbations in genome stability (125, 134, 170, 175, 292).

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. These diseases include infertility, pregnancy complications, cancer, cardiovascular disease, and possibly neurodegenerative disease. In silico modeling and in vivo studies suggest that these interventions would have to occur before the disease process caused by excessive DNA damage is initiated (289, 293). 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 (290, 291). Proving this in humans will be more difficult but essential to completely justify nutritional optimization of genome stability for disease prevention.

Research collaborators and participants in research projects conducted at the Commonwealth Scientific and Industrial Research Organisation Food and Nutritional Sciences relating to the field of genome health nutrigenomics, as indicated in the references, are recognized for their important and vital contributions.

The author was solely responsible for designing the manuscript, collecting, reviewing, and interpreting the cited literature as well as for the description of any novel ideas and concepts. The author had no financial or personal interest in any company or organization that sponsored any of the research mentioned in the article, nor did he have an advisory board affiliation with any of these institutions.

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