Micronutrient Deficiencies

A Major Cause of DNA Damage

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ABSTRACT: Deficiencies of the vitamins B_{12}, B_6, C, E, folate, or niacin, or of iron or zinc mimic radiation in damaging DNA by causing single- and double-strand breaks, oxidative lesions, or both. The percentage of the population of the United States that has a low intake (<50% of the RDA) for each of these eight micronutrients ranges from 2% to 20+ percent. A level of folate deficiency causing chromosome breaks occurred in approximately 10% of the population of the United States, and in a much higher percentage of the poor. Folate deficiency causes extensive incorporation of uracil into human DNA (4 million/cell), leading to chromosomal breaks. This mechanism is the likely cause of the increased colon cancer risk associated with low folate intake. Some evidence, and mechanistic considerations, suggest that vitamin B_{12} and B_6 deficiencies also cause high uracil and chromosome breaks. Micronutrient deficiency may explain, in good part, why the quarter of the population that eats the fewest fruits and vegetables (five portions a day is advised) has about double the cancer rate for most types of cancer when compared to the quarter with the highest intake. Eighty percent of American children and adolescents and 68% of adults do not eat five portions a day. Common micronutrient deficiencies are likely to damage DNA by the same mechanism as radiation and many chemicals, appear to be orders of magnitude more important, and should be compared for perspective. Remedying micronutrient deficiencies is likely to lead to a major improvement in health and an increase in longevity at low cost.

INTRODUCTION

Approximately 40 micronutrients (the vitamins, essential minerals, and other compounds required in small amounts for normal metabolism) are required in the human diet.[1] For each micronutrient, metabolic harmony requires an optimal intake (i.e., to give maximal life span); deficiency distorts metabolism in numerous and complicated ways, many of which may lead to DNA damage. The recommended dietary allowance (RDA)[2–4] of a micronutrient is mainly based on information on acute effects, because the optimum amount for long-term health is generally not known. For many micronutrients, a sizable percentage of the population is deficient relative to the current RDA.[5] Remedying these deficiencies, which can be done at low cost, is likely to lead to a major improvement in health and an increase in longevity. The optimum intake of a micronutrient can vary with age and genetic constitution and be influenced by other aspects of diet. Determining these optima, and

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remedying deficiencies, and in some cases excesses, will be a major public health project for the coming decades. Long-term health is also influenced by many other aspects of diet.

Micronutrient deficiency can mimic radiation (or chemicals) in damaging DNA by causing single- and double-strand breaks, or oxidative lesions, or both. Those micronutrients whose deficiency mimics radiation are folic acid, B_{12}, B_6, niacin, C, E, iron, and zinc, with the laboratory evidence ranging from likely to compelling. The percentage of the population that is deficient for each of these eight micronutrients ranges from 2% to 20+%, and may constitute *in toto* a considerable percentage of the population of the United States. Micronutrient deficiency is a plausible explanation for the strong epidemiological evidence that shows an association between low consumption of fruits and vegetables and cancer at most sites.

DIETARY FRUITS AND VEGETABLES AND CANCER PREVENTION

Greater consumption of fruits and vegetables is associated with a lower risk of degenerative diseases, including cancer, cardiovascular disease, cataracts, and brain dysfunction.[6] More than 200 studies in the epidemiological literature have been reviewed and show, with great consistency, an association between low consumption of fruits and vegetables and the incidence of cancer.[7–9] The quarter of the population with the lowest dietary intake of fruits and vegetables has roughly twice the cancer rate for most types of cancer (lung, larynx, oral cavity, esophagus, stomach, colon and rectum, bladder, pancreas, cervix, and ovary[7]) when compared to the quarter with the highest intake. In a different survey the lowest quartile of adults consumed 2.7 portions or less and the highest quartile 5.6 portions or more (Krebs-Smith, personal communication). These observations are consistent with data on the Seventh Day Adventists, who are nonsmokers and mostly vegetarians, and have about half the cancer mortality rate and a longer life span, than the average American.[10] Eighty percent of American children and adolescents[11] and 68% of adults[12] did not meet the intake recommended by the National Cancer Institute and the National Research Council: five servings of fruits and vegetables per day. Publicity about hundreds of minor hypothetical risks, such as that from pesticide residues in the diet,[13] has contributed to a lack of perspective on disease prevention. Half of the American population does not list fruit and vegetable consumption as a protective factor against cancer,[14] and two-thirds think that for good health only two servings per day need to be consumed.[15] Fruit and vegetable consumption is lowest among the poor, particularly African-Americans.[12,16]

Many components of fruits and vegetables may be responsible for their protective effect, such as micronutrients, plant phenolics, and fiber. This paper argues that inadequate intake of many micronutrients, such as folic acid and vitamins C and B_6 contributes to DNA damage, cancer, and degenerative disease. A major part of the protective effect of fruits and vegetables may be due to their micronutrient content. In addition, dietary deficiencies of micronutrients whose sources are not primarily fruits and vegetables, such as zinc, iron, niacin, vitamin E, and vitamin B_{12}, also appear to contribute to DNA damage and are also common in the United States. Other micronutrients are likely to be added to this list in the coming years.

Table 1. Micronutrient deficiency and DNA Damage

Micronutrient	Percent[a] U.S. Deficient	DNA Damage	Health Effects
Folic acid	10%	Chromosome breaks (Radiation mimic)	Cancer: colon Heart disease Brain dysfunction Birth defects
Vitamin B_{12}	4% (<half RDA)	Chromosome breaks?	(see Folic acid) Neuronal damage
Vitamin B_6	10% (<half RDA)	Chromosome breaks?	(see Folic acid)
Vitamin C	15% (<half RDA)	Radiation mimic (DNA oxidation)	Cataract 4× Cancer Heart disease
Vitamin E	20% (<half RDA) (RDA may be too low)	Radiation mimic (DNA oxidation)	Cancer: Colon 2× Heart disease 1.5× Immune dysfunction
Iron	7% (<half RDA) (19% women 12–50 yr)	DNA breaks Radiation mimic	Brain dysfunction Immune dysfunction Cancer
Zinc	18% (<half RDA)	Chromosome breaks Radiation mimic	Brain dysfunction Immune dysfunction Cancer
Niacin	2% (<half RDA)	Disables DNA repair (poly-ADP-ribose)	Neurological symptoms Memory loss
Selenium		Radiation mimic (DNA oxidation)	Cancer: Prostate

[a]1% = 2.7 million people

FOLIC ACID

Folate deficiency, a common vitamin deficiency in people who eat few fruits and vegetables, causes chromosome breaks in human genes.[17] Approximately 10% of the U.S. population[18,19] are deficient at the level causing chromosome breaks. In two small studies of low-income (mainly African-American) elderly[20] and adolescents[21] done nearly 20 years ago, about half had a folate deficiency at this level, though the issue should be reexamined. The mechanism of chromosome breaks has now been shown to be deficient methylation of uracil to thymine, and subsequent incorporation of uracil into human DNA (4 million/cell).[17] Uracil in DNA is excised by a repair glycosylase with the formation of a transient single-strand break in the DNA; two opposing single-strand breaks cause a double-strand chromosome break, which is difficult to repair. Both high DNA uracil levels and chromosome breaks in humans are reversed by folate administration.[17] Folate supplementation above the RDA minimized chromosome breakage.[22] Folate deficiency has been associated with increased risk of colon cancer,[23,24] and the 15-year use of a multivitamin supplement containing folate lowered colon cancer risk by about 75 percent.[25] Folate and B_{12}

deficiencies are associated with cognitive defects in humans,[17] and neurotoxicity in children is caused by methotrexate, which lowers folate pools if folate is not replenished.[26] Chromosome breaks could contribute to the increased risk of cancer, and possibly cognitive defects, associated with folate deficiency in humans.[17] Folate deficiency causes increased homocysteine accumulation, which has been associated with neural tube defects in the fetus and an estimated 10% of heart disease in the United States, both of which could be eliminated by folate supplements, food fortification, or better diets.[27–32] Homocysteine damages endothelial cells in culture and is a risk factor for arterial endothelial dysfunction in humans.[33]

A polymorphism (a common, alternate, form of a gene) in the gene for methylene-tetrahydrofolate (THF) reductase, the enzyme responsible for reducing methylene-THF to methyl-THF, results in homozygotes having a decreased activity and a twofold increase in plasma homocysteine. Homozygotes, 5–25% of individuals, depending on the ethnicity,[34,35] have an increased risk of heart disease,[30] stroke, [28,36] and neural tube defects.[35,37] This polymorphism increases the methylene-THF pool at the expense of the methyl-THF pool, resulting in decreased DNA uracil levels and increased serum homocysteine. The potential role in human carcinogenesis of uracil misincorporation is supported by two studies that show a two- to fourfold lower risk of colon cancer for individuals who are homozygous for the mutant alleles of methylene-THF reductase compared to controls.[32,38]

VITAMIN B_{12}

The main dietary source of B_{12} is meat. About 4% of the U.S. population consumes below half of the RDA of vitamin B_{12}.[5] About 14% of elderly Americans and about 24% of elderly Dutch have mild B_{12} deficiency, in part accountable by the Americans taking more vitamin supplements.[39] Vitamin B_{12} would be expected to cause chromosome breaks by the same mechanism as folate deficiency. Both B_{12} and methyl-THF are required for the methylation of homocysteine to methionine. If either folate or B_{12} is deficient, then homocysteine, a major risk factor for heart disease,[28,29] accumulates. When B_{12} is deficient, then tetrahydrofolate is trapped as methyl-THF; the methylene-THF pool, which is required for methylation of dUMP to dTMP, is consequently diminished. Therefore, B_{12} deficiency, like folate deficiency, should cause uracil to accumulate in DNA, and there is accumulating evidence for this [Ingersoll *et al*., unpublished and ref. 40]. The two deficiencies may act synergistically. In a study of healthy elderly men,[22] or young adults,[41] increased chromosome breakage was associated with either a deficiency in folate, or B_{12}, or with elevated levels of homocysteine. B_{12} supplementation above the RDA was necessary to minimize chromosome breakage.[41] B_{12} deficiency is known to cause neuropathy due to demyelination and loss of peripheral neurons (reviewed in ref. 17).

VITAMIN B_6

About 10% of the U.S. population consumes less than half of the RDA (1.6 mg/day) of vitamin B_6.[5] Vitamin B_6 deficiency causes a decrease in the enzyme activity

of serine hydroxymethyl transferase, which supplies the methylene group for methylene-THF.[42] If the methylene-THF pool is decreased in B_6 deficiency, then uracil incorporation, with associated chromosome breaks, would be expected. Evidence for this has been found in women at a level of 32 nmol/L of vitamin B_6 in blood (0.5 mg/day intake) (Ingersoll, Shultz, and Ames, unpublished). In a case-control study of diet and cancer, vitamin B_6 intake was inversely associated with prostate cancer.[43] Vitamin B_6 deficiency appears to contribute to heart disease and supplementation reduces risk;[44] levels above the RDA may be necessary to minimize risk.[31] A level of vitamin B_6 in blood below 23 nmol/L is a risk factor for stroke and atherosclerosis.[45] Diets low in vitamin B_6 are associated with brain dysfunction in children and adults.[46] Good sources of vitamin B_6 are whole grain bread and cereal, liver, bananas, and green beans. A major source in the United States is fortified breakfast cereal.

ANTIOXIDANTS

The beneficial effects of fruits and vegetables may be due, in part, to antioxidants and other micronutrients.[6,7,47–51] Oxidant by-products of normal energy metabolism—superoxide, hydrogen peroxide, and hydroxyl radical—are the same mutagens produced by radiation. Ingesting inadequate amounts of dietary antioxidants, such as vitamins C and E, mimics radiation exposure.[6,52–54] Oxidative damage to DNA and other macromolecules appears to have a major role in aging and degenerative diseases associated with aging, such as cancer.[6,55–57] Oxidative lesions accumulate with age in DNA[58] and protein.[59] A young rat has about 24,000 oxidative DNA lesions per cell and an old rat has about 66,000.[58] DNA is oxidized in normal metabolism because antioxidant defenses, though numerous, are not perfect. When lipid is oxidized, aldehydes are produced,[60,61] some of which are mutagenic.[62–64] Aldehydes, such as malondialdehyde, bind to protein and accumulate with age.[65]

White cells and other phagocytic cells of the immune system combat bacteria, parasites, and virus-infected cells by destroying them with the mutagenic oxidizing agents NO, HOCl, and H_2O_2.[66,67] The burst of oxidants, and consequent inflammation, from phagocytic cells is a major source of NOx (a mixture of reactive nitrogen oxides) and contributes to both cancer and heart disease.[66] These oxidants protect humans from immediate death from infection, but they also cause oxidative damage to DNA, chronic cell killing with compensatory cell division, and mutation,[68,69] thus contributing to the carcinogenic process and perhaps to aging.[70] Chronic infections cause about 21% of new cancer cases in developing countries and 9% in developed countries.[71]

Antioxidants may explain much of the protective effect of fruit and vegetable intake against the stomach and lung cancer caused by chronic inflammation.[7,8] NOx is the main oxidative mutagen in cigarette smoke; it depletes the antioxidants ascorbate and α-tocopherol in smokers. Smokers in the bottom quartile of fruit and vegetable intake have about double the risk of lung cancer compared to smokers in the top quartile of intake,[7,8] probably because of decreased antioxidant intake.[72] The risk of bladder cancer is increased by smoking and decreased by supplementation with multivitamins or ascorbate.[73] Supplementation of the diet with a mixture of the antioxidant vitamins C, E, and β-carotene significantly lowered oxidative DNA

damage in lymphocytes of both smokers and nonsmokers, as measured by the comet assay.[74] In a randomized trial in China in a poorly nourished population, cancer mortality was significantly decreased by a supplement of vitamin E, selenium, and β-carotene.[75] Antioxidant defenses against oxidative damage involve ascorbate (vitamin C), α-tocopherol (vitamin E), such carotenoids as β-carotene and lycopene, glutathione, lipoic acid, and selenium, zinc, and copper.

VITAMIN E

Vitamin E**,** the major fat-soluble antioxidant, is consumed primarily from dietary vegetable oils and nuts: 20% of the population consumes less than half of the RDA.[5] Evidence is accumulating that the optimum intake may be higher, as discussed below. Studies on vitamin E supplementation have all been done with α-tocopherol, but γ-tocopherol, the main form in the U.S. diet, has a different function than α-tocopherol, and the two complement each other.[76] γ-Tocopherol is a powerful nucleophile and thus traps electrophilic mutagens that reach the membrane. In the soluble part of the cell, glutathione acts as both an antioxidant and a nucleophile. In the membrane α-tocopherol is the antioxidant and γ-tocopherol (or lycopene) can act as a nucleophile. An important electrophilic mutagen destroyed by γ-tocopherol is NOx. γ-Tocopherol reacts with NOx to form nitro-γ-tocopherol, thus protecting lipids, DNA, and protein.[76–78]

People taking vitamin E supplements (200 IU/day) for 10 years reduced their risk of colon cancer by about half,[79] and evidence suggests a marked protective effect of a supplement (50 IU/day) on prostate cancer.[80,81] Vitamin E appears to protect against brain dysfunction,[82,83] and deficiency leads to various neuropathologies.[84]

Vitamin E supplements (100 IU–400 IU) also reduced the risk of coronary heart disease by about 40%,[85–90] as well as mortality from all causes.[87] The role of oxidants and the protective role of antioxidants in heart disease have recently been reviewed.[49,50] In a study of a population with low levels of vitamin C and E, doses of vitamin E from 70 to 560 IU lowered lipid peroxidation, whereas a dose of 1050 IU increased it,[91] emphasizing that information on the toxic level, as well as the optimum level, of each micronutrient is desirable.

Both vitamin E and selenium enhance the immune system in animals,[92] and vitamin E supplementation (200–400 units/day) enhances human immunity.[93] Vitamin E[94] or vitamin C[95] reduced oxidative stress and malformations in offspring of diabetic rats.

VITAMIN C

Fifteen percent of the U.S. population consumes less than half the RDA (60 mg/day) of ascorbate,[5] which comes from dietary fruits and vegetables.

There is a large literature on supplementation studies with vitamin C in humans using biomarkers of oxidative damage to DNA, lipids (lipid oxidation releases mutagenic aldehydes), and protein. Though there are positive and negative studies, if the fact that the blood cell saturation occurs at about 100 mg/day[96,97] is taken into

consideration, then the evidence suggests that this level minimizes DNA damage.[74,98,99]

Cataracts appear to be due to oxidation of lens protein, and such antioxidants as vitamins C and E, and carotenoids appear to protect against cataracts and macular degeneration of the eye in rodents and humans.[100–102] The use of vitamin C supplements for 10 years or more reduced lens opacities by about 80 percent.[103]

Spontaneous oxidative damage in the DNA of an old rat is about 66,000 adducts per diploid cell[58] and, unlike uracil misincorporation, is likely to be equally frequent on both strands. Glycosylase repair of oxidative adducts also results in transient single-strand breaks in DNA. Therefore, increased oxidative damage, together with elevated levels of uracil in DNA, would be expected to lead to more double-strand (chromosome) breaks in individuals who are deficient in both folate and antioxidants. There is some evidence for this synergy,[104,105] which may be important, because 10–15% of men in the United States had serum ascorbate levels (< 0.3 mg/dL) close to the scurvy threshold.[5,106]

Some studies suggest that vitamin C protects against cancer, which would be plausible based on the mechanistic data, though other studies show no effect. The variable of tissue saturation again is critical. A significant protective effect was observed for renal cancer in nonsmokers, though not in smokers.[107] In a review of nutrition and pancreatic cancer, fruit and vegetable intake and vitamin C were protective, though it is difficult to rule out that vitamin C is a surrogate for some other compounds in fruits and vegetables.[108] Both experimental and epidemiological data suggest that vitamin C protects against stomach cancer,[109] a result that is plausible because of the role of oxidative damage from inflammation by *Helicobacter pylori* infection, which is the main risk factor for stomach cancer. The role of vitamin C in inhibiting oral cancer has recently been reviewed.[110] Vitamin C improves endothelial dysfunction, an early stage of atherosclerosis, in heavy smokers.[111] Vitamin C supplementation was associated with a reduction in overall mortality, and in cardiovascular disease, in a follow up of the NHANES I study.[112]

Men with low consumption of antioxidants, or who smoke, oxidize the DNA of their sperm as well as their somatic DNA. When the level of dietary vitamin C is insufficient to maintain seminal fluid vitamin C, the oxidative lesions in sperm DNA are more than doubled.[98,113] Oxidative lesions in sperm DNA are higher in smokers than nonsmokers.[53] Smoking is a severe oxidative stress, and the NOx in cigarette smoke depletes both vitamin C and vitamin E.[114] Thus, smokers must ingest two to three times more vitamin C than nonsmokers to achieve the same level in blood. They rarely do, however. Inadequate vitamin C levels are more common among the poor and smokers. Smokers also have more chromosomal abnormalities in their sperm than nonsmokers.[115]

Germ line mutations, and their associated cancer and genetic abnormalities, are predominately of paternal origin.[116] Smoking by fathers, therefore, may plausibly increase the risk of childhood cancer and birth defects, a thesis supported by epidemiological evidence.[113,114] The evidence on smoking fathers' offspring having an increased rate of childhood cancer is becoming more persuasive.[117–120] A new epidemiological study from China makes the case stronger; acute lymphocytic leukemia, lymphoma, and brain cancer are each increased three- to fourfold in offspring of male smokers.[117] The studies on paternal smoking and childhood cancer did not

examine the effect of diet. It seems likely, given the above evidence, that the cancer risk to offspring of male smokers would be higher when dietary antioxidant intake is low. Maternal use of multivitamins lowers the risk of childhood cancer in offspring.[121] In one study the maternal use of vitamins throughout the pregnancy lowered the risk of brain tumors in the offspring by about half.[122] In a study of children with childhood cancer, serum levels of β-carotene, vitamin E, and zinc were significantly lower than controls.[123] Thus a multivitamin supplement (or a better diet) for both parents might markedly lower childhood cancer. In addition, several studies suggest an increased rate of birth defects in offspring of smoking fathers (reviewed in refs. 113 and 114).

Diets deficient in fruits and vegetables are commonly low in folate, antioxidants, (e.g., vitamin C) and many other micronutrients, and it seems plausible that the higher cancer rates associated with consuming deficient diets are due, in good part, to increased DNA damage.[7,17,66]

SELENIUM

Selenium is important in enzymatic defenses against oxidants, and deficiency would be expected to lead to oxidative DNA damage.[124] An RDA of 70 μg/day of selenium and an upper limit of 350 μg/day has been proposed.[125] The average intake in the United States is about 100 μg/day, though different areas of the country have different selenium levels in the soil, and the bioavailability depends on the selenium form in foods.[124]

A growing body of evidence suggests that selenium plays an important role in the prevention of cancer in a variety of organs and species.[126,127] Prostate cancer incidence was reduced by two-thirds in the selenium-supplemented group (200 g/day) compared to the placebo group in a randomized, double-blind, cancer-prevention trial; total cancer mortality, lung, and colorectal cancer were also significantly reduced.[128,129] In a cohort study,[130] men in the highest selenium quintile of intake had only one-half the odds ratio of prostate cancer as men in the lowest quintile. In a nested, case-control prospective study on ovarian cancer, serum selenium was associated with decreased risk.[131] In a study of postmenopausal breast cancer patients, a strong inverse relationship was observed between triiodothyronine (T3) levels and cancer (OR=0.17; CI95%=0.08–0.36) between the highest and lowest tertiles.[132] Toenail selenium was positively associated with T3 levels in both cases and controls; the selenoenzyme, iodothyronine deiodinase, synthesizes T3. Prostate and breast cancer cells were about 25 times more sensitive than normal cells to selenomethionine, a major form of selenium in cells.[133] In a study of selenium intake and colorectal cancer that adjusted for possible confounders, the individuals in the lowest quartile of plasma selenium had four times the risk of colorectal adenomas compared to those in the highest quartile.[134] Selenium and glutathione peroxidase levels were found to be lowered in patients with uterine cervical carcinoma.[135] In a Chinese study, cervical cancer mortality was inversely associated with several factors, including serum selenium levels.[136]

Several hypotheses have been proposed to explain the protection against carcinogenesis by supplemental selenium.[124] One of these is its protection against oxidative

damage involving selenium as an essential component of the antioxidant enzyme glutathione peroxidase,[137] or selenoprotein-P.[138–140]

Excess selenium intake appears to cause oxidative damage and cancer in rodents.[141] The case for selenium supplementation is becoming stronger, though the toxicity of high selenium levels must be taken into account.

IRON

A major dietary source of iron is meat. The United Nations Food and Agriculture Organization has estimated that the world has about 2 billion people at risk for iron deficiency, mainly women and children. In the United States about 7% of the population and about 19% of women, aged 12–50, ingest below 50% of the RDA;[5] about nine million people have been estimated to be clinically deficient.[142] Iron deficiency appears to lead to oxidative DNA damage.[143] Iron deficiency in children is associated with cognitive dysfunction.[144,145] Low iron intake results in anemia, immune dysfunction, and adverse pregnancy outcomes, such as prematurity.[145]

Excess iron appears to also lead to oxidative DNA damage in rats that is reversed by vitamin E.[146] Increased risk of human cancer[145,147] and heart disease[148,149] has been associated with excess iron.

ZINC

Major sources of zinc are meat, eggs, nuts, and whole grains. Zinc deficiency causes a variety of health effects that have been reviewed in depth.[150] Eighteen percent of the U.S. population consumes less than half the RDA for zinc (12 mg women, 15 mg men).[5] Mean daily intakes reported for poor children (5 mg), middle income children (6.3 mg), and vegetarians (6.4 mg) in the United States appear insufficient.[150] Zinc is a component of over 300 proteins, over 100 DNA-binding proteins with zinc fingers, Cu/Zn superoxide dismutase, the estrogen receptor, and the synaptic transmission protein.[150] Functioning of p53, a zinc protein that is mutated in half of human tumors, is disrupted on loss of zinc.[151] P53 prevents mutation by inhibiting cell division and inducing apoptosis in response to DNA lesions.[152]

Chromosome breaks in rats have been reported with a zinc-deficient diet.[153] The offspring of zinc-deficient rhesus monkeys also have increased chromosome breaks.[154] The chromosome breaks might be due to increased oxidative damage,[154,155] perhaps due to loss of activity of Cu/Zn superoxide dismutase or the zinc-containing DNA-repair enzyme, Fapy glycosylase, which repairs oxidized guanine.[156] Zinc deficiency has been suggested as a contributor to esophageal cancer in humans and has been shown to cause esophageal tumors in rats in conjunction with a single low dose of a nitrosamine.[157–159] Severe zinc deficiency by itself can cause esophageal tumors in rats.[159]

Zinc is known to be an essential trace element for testicular development and spermatogenesis.[160] Zinc concentrations in seminal plasma are hundreds of times greater than that in blood plasma, which suggests a specific function for this trace element in spermatogenesis and stability of spermatozoa.[150] Zinc concentrations are

correlated positively with sperm cell density, and lower zinc concentrations are found in infertile men compared with fertile men.[161] Zinc deficiency leads to increased oxidative damage to testicular cell DNA (as measured by oxo^{8}dG) and increased protein carbonyl content.[162]

A considerable literature in experimental animals and humans suggests that zinc deficiency slows growth and development of the neonate. Severe deficiency in animals is teratogenic.[154] In a pair-matched, double-blind, study in Chile of preschool boys of low socioeconomic status, those supplemented with 10 mg zinc/day grew significantly more rapidly than the placebo group.[163] This is consistent with earlier reports in the United States and other countries on growth stimulation of poor children supplemented with zinc.[150]

Zinc deficiency leads to alterations in brain development and growth.[144] Zinc deficiency in pregnant rats, at a level that does not impair the pregnancy or the growth of the pups, impairs cognitive function in adult offspring.[150] Zinc deficiency in adult rats impairs hippocampal and behavioral functions.[150] Several studies on monkeys show that maternal zinc deficiency leads to learning and behavioral disabilities in offspring.[150] Six studies in humans suggest that zinc deficiency leads to cognitive defects.[150]

Several animal and human studies indicate that mild zinc deficiency impairs the immune system.[150,164] The incidence of respiratory infections in a group of institutionalized elderly was decreased by over twofold ($p = <0.01$) when they were given a supplement of zinc (20 mg) plus selenium (100 mg) in a double-blind placebo study; in other studies very high doses of zinc (100–150 mg/day) had an adverse effect on the immune system.[165]

NIACIN

In the United States, 2.3% of the population consumes less than half the RDA for niacin.[5] The main dietary sources of niacin include meat and beans. Tryptophan from protein can also provide niacin equivalents.[166] Fifteen percent of some populations have been reported to be severely deficient.[167] Niacin contributes to the repair of DNA breaks by maintaining nicotinamide adenine dinucleotide levels for the poly-ADP ribose protective response to DNA damage;[168–170] deficiency compromises repair of DNA nicks and breaks and thus is expected to act synergistically with folate and antioxidant deficiencies in causing DNA damage and cancer.[171]

[**NOTE ADDED IN PROOF:** Several recent references that were added (173–175) are relevant to folate/B_{12}/B_6 deficiency.]

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