Free Radicals: The Pros and Cons of Antioxidants

Supplements and Tuning Up Metabolism[1–3]

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EXPANDED ABSTRACT

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Americans' intake of the 40 essential micronutrients (vitamins, minerals, and other biochemicals that humans require) is commonly thought to be adequate. Classic deficiency diseases, such as scurvy, beriberi, pernicious anemia, and rickets, are rare. The evidence suggests, however, that much chronic metabolic damage occurs at levels between the level that causes acute micronutrient deficiency disease and the recommended dietary allowances (RDAs).[5] In addition, the prevention of more subtle metabolic damage may not be addressed by current RDAs. When one input in the metabolic network is inadequate, repercussions are felt on a large number of systems and can lead to degenerative disease. This may, for example, result in an increase in DNA damage (and cancer), neuron decay (and cognitive dysfunction) or mitochondrial decay (and accelerated aging and degenerative diseases). The optimum amount of folic acid or zinc that truly is "required" is the amount that minimizes DNA damage and maximizes a healthy life span, which is higher than the amount needed to prevent acute deficiency disease. The requirements of the elderly for vitamins and metabolites are likely to differ from those of the young, but this issue has not been examined seriously. An optimal intake of micronutrients and metabolites also varies with genetic constitution. A tune-up of micronutrient metabolism should give a marked increase in health at little cost. It is a distortion of priorities for much of the world's population to have an inadequate intake of a vitamin or mineral, at great cost to health, when a year's supply of a daily multivitamin-mineral pill as insurance against deficiencies costs less than a few packs of cigarettes. The poor, in general, eat the worst diets and have the most to gain from multivitamin-mineral supplementation and improvement in diet.

Micronutrient malnutrition is related to energy intake

For most of human evolution, dietary energy shortage was likely to have limited population growth, and, because food was dilute and unprocessed, micronutrients may have been mostly adequate. In recent times, the introduction of the potato to Europe from South America in the late 1500s markedly increased the European population over the succeeding few centuries as the cultivation of potatoes spread and as cultivars were selected that thrived in each climate. "In 1845, close to 40% of the population of Ireland lived chiefly on potatoes. The emergence of the 'potato people' occurred against the background of the quadrupling of the population after 1700. . . . " (1). Rice clearly was the main factor enabling high population density in Asia. Now, carbohydrates and fats are remarkably inexpensive, and the United States has an obesity epidemic associated with micronutrient malnutrition (2).

Why micronutrients?

Although optimal nutrition is of benefit in many degenerative diseases and optimal nutrition clearly involves more than adequate micronutrients (e.g., fiber), there are important reasons for focusing on micronutrients and health, particularly DNA damage and mitochondrial damage (3). First, >20 y of effort to improve the American diet have not been notably successful with less-educated people, although this work must continue. A parallel approach that focuses on micronutrient malnutrition is overdue and might be more successful, because it should be easier to convince people to take a multivitamin-mineral pill as insurance against ill health than to change their diet appreciably. Second, a multivitamin-mineral pill is inexpensive, recognized as safe, and supplies the range of vitamins and minerals that a person requires, although not the essential (n-3) fatty acids. Inadequate intake of (n-3) fatty acids is

widespread (4). Fish-oil supplements with these essential fatty acids (EFAs) are available and inexpensive; evidence is mounting on their utility. Inadequate intake of both soluble and insoluble fiber is widespread (5). Fiber supplementation also is inexpensive.

Fortification of food is another useful approach, but it has been implemented very slowly, as with folate fortification. Moreover, fortification of food does not allow for differences between individuals. For example, menstruating women need more iron than do men or older women, who may be ingesting too much. This is why two types of vitamin pills are marketed: one with iron and one without. With more knowledge, it seems likely that a variety of multivitamin pills will be developed that reflect knowledge about different needs depending on age, sex, genetics, and the like (**Table 1**) (6).

DNA damage from vitamin and mineral deficiencies

DNA damage, which is a cause of cancer (although not the only one), is recognized as deleterious and can be assayed easily and relatively inexpensively in human white cells during intervention studies, in contrast to cancer. Our strategy in the laboratory is to use a variety of human cell lines in culture to cause growth limitation by deficiency of a particular micronutrient, then measure DNA damage by a variety of assays, followed by collaboration on human intervention studies. We also are developing improved assays for measuring DNA damage in humans. Deficiencies of vitamin B-12, folic acid, vitamin B-6, niacin, vitamin C, vitamin E, iron, or zinc appear to mimic radiation in damaging DNA by causing single- and double-strand breaks, oxidative lesions, or both. Half of the population may be deficient in at least one of these micronutrients (7). Micronutrient deficiency may explain in part why the quarter of the population that consumes the fewest fruits and vegetables (5 portions/d is advised) has about double the incidence rate for most types of cancer, compared with the quarter with the highest intake: 80% of American children and adolescents and 68% of adults do not consume 5 portions/d (7). A number of other degenerative diseases of aging also are associated with low fruit and vegetable intake.

Folate deficiency breaks chromosomes due to massive incor-

poration of uracil in human DNA (millions per cell) (8), with subsequent single-strand breaks in DNA formed during base-excision repair: 2 nearby single-strand breaks on opposite strands cause the chromosome to break. The level of folate at which high uracil and breaks are seen was present, before the recent flour supplementation, in 25% of the U.S. population and in close to one-half of the low-income urban minority population to poor diet. Deficiencies (<50% of RDA) of vitamins B-12 (10% of women; 5% of men) or B-6 (10% of U.S. population) also cause high levels of uracil incorporation in human DNA and chromosome breaks as indicated by our new evidence and as expected from mechanistic considerations (9,10). We currently are attempting to determine the level of these 3 vitamins that minimizes both nuclear and mitochondrial DNA damage in humans (8–10).

Folate, vitamin B-12, or vitamin B-6 deficiencies cause homocysteine accumulation, a risk factor for vascular diseases such as microangiopathy and macroangiopathy (11). Recent research points to an association of vascular dementia and Alzheimer's disease related to homocysteine (12). Multiple brain infarcts and vascular damage in the brain are caused by hypomethylation of DNA and proteins that expose neurons to greater damage and apoptosis (13,14). Many reports support the case that low intake of folate, vitamin B-12, and vitamin B-6, with accompanying homocysteine accumulation, contributes to Alzheimer's disease (15,16).

Iron deficiency (25% of women of menstruating age in the United States ingest <50% of RDA) causes oxidative damage to mitochondria and mitochondrial DNA in rats (17,18). Members of low-income groups tend to have the lowest levels and intake (19,20).

Iron deficiency causes decreased heme levels in the mitochondria, which results in dysfunctional mitochondria and neurodegeneration (21). Iron deficiency in the mitochondria appears at higher iron intakes than anemia (22). Many reports show that inadequate iron intake causes cognitive dysfunction in rats and humans (23) by altering metabolic processes such as mitochondrial electron transport and neurotransmitter synthesis and degradation. Iron deficiency causes neurological impairment in children (22), possibly through the effect on mitochondria (21). Comprehensive reviews compiled by Beard and Pollitt detail the effects of iron deficiency on psychomotor and cognitive development, low-birth-weight infants, and perinatal mortality (22,24–26). A large number of studies report beneficial effects of micronutrient supplementation on cognitive function in children (27). An analysis of data from the U.S. Women, Infants, and Children program, which promotes good nutrition in the low-income population, indicates that ~20% of the children in the program were anemic (Fernando Viteri, Department of Nutritional Sciences and Toxicology, University of California—Berkeley; personal communication).

Zinc deficiency (10% of the U.S. population ingests <50% of RDA) in human cells in culture causes oxidative DNA damage and inactivates Cu,Zn-superoxide dismutase, tumor suppressor protein p53 (a zinc protein), and oxidative DNA repair; these effects can multiply to cause severe genetic damage (28–30).

Zinc deficiency induces oxidative stress within the cell and damages DNA (3,21,28). Zinc deficiency in infants and children arrests cognitive development through altered attention level, motor development, and neuropsychological behavior (31). Many studies report that zinc repletion is beneficial in underdeveloped countries (32–37). Animal studies on rats and monkeys also show that zinc deficiency causes reduced cognitive function (38–40).

TABLE 1

Micronutrient deficiencies in U.S. individuals[1]

Nutrient	Population group	Current RDA	% Consuming < RDA	% Consuming <0.5 RDA
Mineral				
Iron	Women 20–30 y	18 mg	75	25
	Women 50+ y	8 mg	25	5
Zinc	Women 50+ y	8 mg	50	10
	Men 50+ y	11 mg	50	10
Vitamin				
Folate[2]	Women 20+ y	400 μg	75	50
	Men 20+ y	400 μg	75	25
B-6	Women 20+ y	1.5 mg	50	10
	Men 20+ y	1.7 mg	50	10
B-12	Women 20+ y	2.4 μg	25	10
	Men 20+ y	2.4 μg	10	5
C	Women 20+ y	75 mg	50	25
	Men 20+ y	90 mg	50	25

[1] Data adapted from Wakimoto & Block (6); dietary intakes include food fortification but not supplement use.
[2] Folate intake before U.S. fortification in 1998.

Common micronutrient deficiencies are likely to damage DNA by the same mechanism as radiation and many chemicals and appear to be much more important (7,30). Fenech (41) reviewed the need to set micronutrient requirements to minimize DNA damage. We compared radiation with folate deficiency to try to put risks in perspective (42). Low-income people are not served if huge resources are put into fighting minor hypothetical risks (30).

Delaying the mitochondrial decay of aging

Oxidative mitochondrial decay is a major contributor to aging (43–47). We are making progress in reversing some of this decay in old rats by feeding them the normal mitochondrial metabolites acetyl carnitine (ALC) and lipoic acid (LA) at high levels. The principle behind this effect appears to be that, with age, increased oxidative damage to protein causes a deformation of structure of key enzymes with a consequent lessening of affinity (K_m) or the enzyme substrate (48). The effect of age on the enzyme-binding affinity can be mimicked by reacting it with malondialdehyde (a lipid-peroxidation product that increases with age). Feeding the substrate ALC with LA, a mitochondrial antioxidant, restores the velocity of the reaction (K_m) for ALC transferase and mitochondrial function (48). In old rats (vs. young rats), mitochondrial membrane potential, cardiolipin level, respiratory control ratio, and cellular O_2 uptake are lower; oxidants or O_2, neuron RNA oxidation, and mutagenic aldehydes from lipid peroxidation are higher (45,48–55). Ambulatory activity and cognition decline with age (49,50). Feeding old rats ALC with LA for a few weeks restores mitochondrial function; lowers oxidants, neuron RNA oxidation, and mutagenic aldehydes; and increases ambulatory activity and cognition (as assayed with the Skinner box and Morris water maze) (48–50).

A recent meta-analysis of 21 double-blind clinical trials of acetyl carnitine in the treatment of mild cognitive impairment and mild Alzheimer's disease showed significant efficacy versus placebo (56). A meta-analysis of 4 clinical trails of lipoic acid for treatment of neuropathic deficits in diabetes showed significant efficacy versus placebo (57).

Common micronutrient deficiencies accelerate mitochondrial decay

Heme biosynthesis occurs predominantly in the mitochondria. Interfering with heme-a synthesis causes specific loss of complex IV with consequent release of oxidants (17,21). Iron deficiency (25% of menstruating women in the U.S. ingest <50% of RDA) also causes release of oxidants and mitochondrial decay (18), presumably through lack of heme (17). Biotin deficiency, which is quite common in the population (58,59), also causes defects in mitochondrial complex IV and oxidant leakage (Atamna, H. & Ames, B. N., unpublished data). There is evidence that deficiency of copper (58,60), a component of complex IV, or pantothenate (61) causes a decrease in complex IV. Zinc deficiency, which causes marked oxidative stress (29,30) is a component of δ-aminolevulinate dehydratase (62) and thus could lower heme-a and contribute to mitochondrial decay. These various deficiencies may lead to accelerated aging and neural decay (22).

Multivitamns in humans

Evidence is accumulating that a multivitamin-mineral supplement is good insurance and would markedly improve health (e.g., heart disease, cancer, immune function, and cataracts), particularly among low-income, young, elderly, or obese individuals (3,63–73). The caveat is, of course, that too much of many of the minerals (e.g., iron, zinc, copper, selenium, and some of the vitamins, such as vitamin A and β-carotene) is toxic, although taking a multivitamin-mineral supplement as insurance is not of concern. Mae West's dictum, "Too much of a good thing is wonderful," does not apply to micronutrients. Advice to take a multivitamin always should be coupled with advice to eat a good diet, as humans also need fiber, (n-3) fatty acids and other ingredients in a balanced diet (74).

Because it is so difficult to determine the exact levels of micronutrient intake in an individual's diet, the most direct approach to assaying nutrient effects would be to perform supplementation studies. Smaller clinical or metabolic studies of subpopulations of individuals with identified genetic alterations might be more valuable than large epidemiological studies that include several genotypes. Intervention studies that measure chromosome breaks or other damage in small numbers of people with a low intake of a micronutrient before, during, and after supplementation with the micronutrient might be the most successful research approach.

The K_m concept and metabolism

As many as one-third of all mutations in a gene increase the Michaelis constant K_m (i.e., decrease the binding affinity) of the corresponding enzyme for a coenzyme, and therefore decrease the rate of reaction. Thus, many of the carriers of 50 human genetic diseases that are caused by defective enzymes can be remedied or ameliorated by administering high doses of the B-vitamin component of the corresponding coenzyme, which increases levels of the coenzyme and at least partially restores enzymatic activity (75). Several single-nucleotide polymorphisms, in which the variant amino acid decreases coenzyme binding and thus enzymatic activity, may be remediable by increasing cellular concentrations of the cofactor through high-dose vitamin therapy. Examples include C 677 T/Ala222Val methylenetetrahydrofolate reductase (NADPH) and the cofactor FAD (in relation to cardiovascular disease, migraines, and rages), the C609T/Pro187Ser mutation in NAD(P):quinone oxidoreductase1 and FAD (in relation to cancer), the C131G/Ala44Gly mutation in glucose-6-phosphate-1-dehydrogenase and NADP (in relation to favism and hemolytic anemia), and the Glu487Lys mutation (present in one-half of Asians) in aldehyde dehydrogenase and NAD (in relation to alcohol intolerance, Alzheimer's disease, and cancer). The K_m concept may be relevant for mitochondrial aging as well as for human nutrition.

Public health

A metabolic tune-up is likely to have great health benefits, particularly for those with inadequate diets such as many low-income, young, elderly, or obese individuals, who need improvement the most, although this currently is not being addressed adequately by the medical community. The issues discussed here highlight the need to educate the public about the crucial importance of optimal nutrition and the potential health benefits of something as simple and affordable as a daily multivitamin-mineral supplement. Tuning up metabolism to maximize the human health span will require scientists, clinicians, and educators to abandon outdated paradigms of micronutrients merely preventing deficiency diseases and explore more meaningful ways to prevent chronic disease and achieve optimal health through optimal nutrition. It is becoming clear

that unbalanced diet will be the major contributor to ill health in the population, with smoking following close behind.

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