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Review

Optimal micronutrients delay mitochondrial decay and age-associated diseases

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ABSTRACT

Three of our research efforts are reviewed, which suggest that optimizing metabolism will delay aging and the diseases of aging in humans. (1) Research on delay of the mitochondrial decay of aging by supplementing rats with lipoic acid and acetyl carnitine. (2) The triage theory, which posits that modest micronutrient deficiencies (common in much of the population) accelerate molecular aging, including mitochondrial decay, and supportive evidence, including an analysis in depth of vitamin K, that suggests the importance of achieving optimal micronutrient intake for longevity. (3) The finding that decreased enzyme binding constants (increased Km) for coenzymes (or substrates) can result from protein deformation and loss of function due to loss of membrane fluidity with age, or to polymorphisms or mutation. The loss of enzyme function can be ameliorated by high doses of a B vitamin, which raises coenzyme levels, and indicates the importance of understanding the effects of age, or polymorphisms, on micronutrient requirements.

1. Lipoic acid and acetyl carnitine supplements decrease the mitochondrial decay of aging

Mitochondrial decay appears to be a major contributor to aging and its associated degenerative diseases including cancer and neural decay (Shigenaga et al., 1994; Beckman and Ames, 1998). Mitochondria from old rats compared with those from young rats generate increased amounts of oxidant by-products (Hagen et al., 2002a), and have decreased membrane potential, respiratory control ratio, cellular oxygen consumption, and cardiolipin (a key lipid found in mitochondria). Oxidative damage to DNA, RNA, proteins, and mitochondrial membrane lipids contributes to this decay (Hagen et al., 2002a,b; Liu et al., 2002a,b; Ames et al., 2003) and leads to functional decline of mitochondria, cells, tissues, and eventually organs such as the brain, with an accompanying loss of cognition and ambulatory activity (Hagen et al., 2002a,b; Liu et al., 2002a,b; Ames et al., 2003).

Decreased capacity to produce ATP and increased oxidant production are two properties of aging mitochondria supported by multiple lines of direct and indirect observations. First, the analysis of gene expression profiles in mice showed significant age-associated declines in the mRNA levels of mitochondrially encoded subunits of complexes I, III, IV and V in old compared to young mice (Manczak et al., 2005). Second, in addition to reduced gene expression, the levels of 4-HNE and carbonylated-mitochondrial proteins increase in aging tissues (Suh et al., 2003; Navarro and Boveris, 2004). Lastly, the activities of NADH-cytochrome *c* reductase (complexes I–III) exhibit approximately 30% decline in the aging rat liver and the brain when compared to young (Paradies et al., 1994; Desai et al., 1996; Navarro and Boveris, 2004). Similar declines in complex IV activity were also reported (Suh et al., 2003; Navarro and Boveris, 2004).

While the gross age-related declines in respiratory complex activities have been reported extensively, much less attention has been given to the extent of age-related changes in their substrate binding affinities. One study (Feuers, 1998) examined the substrate binding affinities of complexes I, III and IV in mitochondria isolated from the gastrocnemius muscles of young and old mice. A kinetic analysis of complex III revealed a significant 29% age-associated increase in the Km (decreased binding) for ubiquinone-2 (Feuers, 1998). These findings are congruent with more recent work (Moghaddas et al., 2003) that reported a defect in the ubiquinone-binding site of cytochrome *b* in complex III in the interfibrillary mitochondria isolated from old rats (Moghaddas et al., 2003). The resulting defect in ubiquinone-binding affinity is likely to increase superoxide production at this site. The specific post-translational modification responsible for the Km increase is currently unknown. The affinity of complex IV for reduced cytochrome *c* also exhibits a similar age-related loss but again the exact mechanisms for the increased Km is incompletely understood (Feuers, 1998). A decreased electron flux due in part to the reduced affinity of complex IV for cytochrome *C* would result in an enhanced rate of oxidant production at complex III.

The importance of optimizing metabolic function to prevent mitochondrial decay is illustrated by feeding the mitochondrial metabolites acetyl carnitine (ALC) (Gadaleta et al., 1990, 1998;

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Hagen et al., 1998) and lipoic acid (LA) (Hagen et al., 1999) to old rats. Carnitine is used for transporting fatty acids into the mitochondria; the main short-chain acyl-carnitine is ALC (Gatti et al., 1998). In humans at rest, ALC accounts for roughly a quarter of total carnitine in plasma and muscle and liver tissues (Gatti et al., 1998). LA is a mitochondrial coenzyme and is preferentially reduced in the mitochondria to a potent antioxidant. LA is also an effective inducer of the transcription factor Nrf2 which in turn induces the glutathione synthesis enzymes (Smith et al., 2004; Suh et al., 2004a,b). All together, Nrf2 induces over 200 phase-2 antioxidant and thiol-protective enzymes (Kwak et al., 2003; Wakabayashi et al., 2004). ALC and LA, when added as a supplement can act, in some cases synergistically, to restore much of the lost mitochondrial function in old rats (Hagen et al., 2002a,b; Liu et al., 2002a,b).

ALC increases mitochondrial DNA transcription, translation, and the mitochondrial transcription factor A (TFAM) (Gadaleta et al., 1998; Pesce et al., 2004). Both LA and ALC are effective at inducing PGC1-α, which in turn induces mitochondrial biogenesis, in a human neuroblastoma model of Parkinson's disease; the combination showing strong synergy (Zhang et al., 2010). A synergy of ALC and LA was shown in inducing PGC1-α, Nuclear Respiratory Factors (NRF1, NRF2), and mitochondrial biogenesis in a murine adipocyte model of diabetes (Shen et al., 2008b). LA and ALC with biotin and niacin increased mitochondrial biogenesis in diabetic, Goto-Kakizaki rats (Shen et al., 2008a). Mitochondrial biogenesis in relation to diabetes, insulin resistance and nutrients such as ALC and LA has been reviewed (Liu et al., 2009).

Brain function declines with age and is associated with diminishing mitochondrial integrity. Feeding ALC and LA to rats improves cognition and other functions (Hagen et al., 2002a,b; Liu et al., 2002a,b; Ames et al., 2003). The activities/kinetics of the mitochondrial complexes were examined in the brains of young and old rats as well as in old rats fed ALC and LA (Long et al., 2009). The brain mitochondria of old rats, compared with young rats, had significantly decreased endogenous antioxidants and superoxide dismutase activity; more oxidative damage to lipids and proteins; and decreased activities of complexes I, IV and V. Complex I showed a decrease in binding affinity (increase in Km) for substrates ubiquinone and NAD and a decrease in Vmax with age. Feeding LA/ALC to old rats partially restored age-associated mitochondrial dysfunction (Vmax, but not Km) to the levels of the young rats, though clearly not by changing Km. These results indicate that oxidative mitochondrial decay plays an important role in brain aging and that a combination of nutrients targeting mitochondria, such as LA/ALC, could ameliorate mitochondrial decay, perhaps through preventing mitochondrial oxidative damage and enhancing mitochondrial biogenesis; neither ALC or LA is a substrate for complex I.

The neuronal mitochondrial ultrastructural changes of young and old rats supplemented with ALC and LA, were analyzed using electron microscopy techniques (Aliev et al., 2009). Mitochondria were examined in each micrograph, and each structure was scored according to the degree of injury. Controls displayed an age-associated significant decrease in the number of intact mitochondria as well as an increase in mitochondria with broken cristae in the hippocampus as demonstrated by electron microscopic observations. Neuronal mitochondrial damage was associated with damage in vessel wall cells, especially vascular endothelial cells. Dietary supplementation of young and aged animals increased the proliferation of intact mitochondria and reduced the density of mitochondria associated with vacuoles and lipofuscin. Feeding old rats ALC and LA significantly reduced the number of severely damaged mitochondria and increased the number of intact mitochondria in the hippocampus. These results suggest that feeding ALC with LA ameliorates age-associated mitochondrial ultrastructural decay and are consistent with previous studies showing improved brain function. In an apoE4 mouse model of Alzheimer's disease ALC and LA ameliorated brain mitochondrial decay and improved cognition (Shenk et al., 2009). Feeding the combination of ALC and LA also improves cognition in old beagle dogs (Milgram et al., 2007).

Hypertensive aged humans show a significant decrease in blood pressure when fed ALC and LA in a double blind clinical trial (McMackin et al., 2007), though mitochondria were not examined. LA has also been shown to protect retinal pigment epithelial cells from oxidation, a model for macular degeneration (Volobouева et al., 2005; Jia et al., 2007); part of the protective mechanism appears to be improving mitochondrial function.

One possible mechanism of mitochondrial decay is that with age, stiffer membranes due to lipid oxidation or increased oxidative damage to mitochondrial proteins causes structural deformation of key enzymes such as carnitine acyl transferase that lowers their affinity for the enzyme substrate (Liu et al., 2002b). Feeding old rats the substrate ALC with LA for a few weeks decreases oxidative damage, allowing the synthesis of new carnitine acyl transferase with normal binding affinity (Km) (Liu et al., 2002b). This partially restores mitochondrial function, decreases oxidants, neuronal RNA oxidation, and mutagenic aldehydes, and increases rat ambulatory activity and cognition (Hagen et al., 2002a,b; Liu et al., 2002a,b). ALC and LA are not usually thought of as micronutrients, as they can be made in the body, but they are illustrative of many normal metabolites that may be beneficial in the elderly.

Prolla and colleagues (Park et al., 2009) used DNA microarrays to identify panels of transcriptional markers of aging that are differentially expressed in young vs. old mice of multiple inbred strains. They then fed the mice various metabolites, mostly antioxidants, to see if they would oppose these transcriptional markers of aging. ALC was as effective as caloric restriction in the heart and LA was as effective in the cerebellum. These experiments suggest that ALC + LA is an effective caloric restriction mimetic and that tuning up metabolism may help in slowing down the aging process. The tissue specific effects of caloric restriction mimetic agents suggest that a combinatory approach may be needed.

2. Triage theory suggests a cause of much preventable aging-associated disease

The "triage theory" (Ames, 2006; Ames and McCann, 2009) provides a unifying rationale for a causal link between deficiency of a micronutrient (~40 essential minerals, vitamins, amino acids and fatty acids) and the many degenerative diseases accompanying aging such as cancer, immune dysfunction, cognitive decline, cardiovascular disease, and stroke. These diseases might be delayed by an inexpensive micronutrient intervention (Ames and McCann, 2009).

Triage theory (Ames, 2006; Ames and McCann, 2009) posits that when a micronutrient is inadequate, nature selects for a rebalancing of metabolism (e.g. by selection for micronutrient binding constants) that ensures survival of the organism at the expense of metabolism whose lack has only longer term consequences, which I propose include chronic diseases of aging. That nature may have developed such a system is logically consistent with the consensus that natural selection favors short-term survival for reproduction over long-term health (Kirkwood, 2008). During evolution micronutrient shortages were likely to be very common, e.g. the 15 essential minerals are not distributed evenly on the earth; dietary sources and availability also fluctuated markedly (Eaton and Konner, 1985).

The triage theory predicts that optimizing intake of the ~40 essential micronutrients will reduce the risk of chronic diseases associated with aging and increase lifespan (Ames,

2006). Micronutrients are remarkably inexpensive. Micronutrient intakes below recommended levels are unusually widespread in poor countries, but also in the US population in all segments of society, especially the poor, children, adolescents, the obese, and the elderly. High consumption of calorie-rich, micronutrient-poor unbalanced diets exacerbates the problem (Ames, 2006). For example, about two-thirds of the U.S. population have inadequate intakes of magnesium (Ames, 2006), almost all African-Americans are extremely low in vitamin D (McCann and Ames, 2008), and much of the population is low in a variety of other micronutrients, (e.g. omega-3 fatty acids, potassium, calcium, vitamin C, vitamin E, vitamin K) (Moshfegh et al., 2005; Ames, 2006; McCann and Ames, 2009). There is little societal concern because no overt pathologies have been associated with marginal to moderate levels of deficiency. The triage theory predicts that the pathology is insidious, but we believe that it is measurable. We hypothesize that two of the many insidious but measurable consequences of micronutrient triage are increased DNA damage (future cancer) and mitochondrial decay (future cognitive dysfunction and accelerated brain aging). Others age-related diseases, such as cardiovascular disease and immune dysfunction (Ames and McCann, 2009) are increased by micronutrient deficiencies and are discussed elsewhere (Ames and McCann, 2009; McCann and Ames, 2009).

2.1. Vitamin K as an example of the utility of triage theory (McCann and Ames, 2009)

The triage theory posits that some functions of micronutrients (the ~40 essential vitamins, minerals, fatty acids, and amino acids) are restricted during shortage and that functions required for short-term survival take precedence over those that are less essential. Insidious changes accumulate as a consequence of restriction, which increases the risk of diseases of aging. For 16 known vitamin K-dependent (VKD) proteins, we evaluated the relative lethality of 11 known mouse knockout mutants to categorize essentiality. Results indicate that 5 VKD proteins that are required for coagulation had critical functions (knockouts were embryonic lethal), whereas the knockouts of 5 less critical VKD proteins [osteocalcin, matrix Gla protein (Mgp), growth arrest specific protein 6, transforming growth factor β-inducible protein (Tgfbi or βig-h3), and periostin] survived at least through weaning. The VKD γ-carboxylation of the 5 essential VKD proteins in the liver and the 5 nonessential proteins in non-hepatic tissues sets up a dichotomy that takes advantage of the preferential distribution of dietary vitamin K1 to the liver to preserve coagulation function when vitamin K1 is limiting. Genetic loss of less critical VKD proteins, dietary vitamin K inadequacy, human polymorphisms or mutations, and vitamin K deficiency induced by chronic anticoagulant (warfarin/coumadin) therapy are all linked to age-associated conditions: bone fragility after estrogen loss (osteocalcin) and arterial calcification linked to cardiovascular disease (Mgp). There is increased spontaneous cancer in Tgfbi mouse knockouts, and knockdown of Tgfbi causes mitotic spindle abnormalities. A triage perspective reinforces recommendations of some experts that much of the population and warfarin/coumadin patients may not receive sufficient vitamin K for optimal function of VKD proteins that are important to maintain long-term health (McCann and Ames, 2009).

2.2. DNA damage and mitochondrial decay

We hypothesize that two of the many insidious but measurable consequences of moderate micronutrient inadequacy are increased DNA damage (future cancer) and mitochondrial decay (future cognitive dysfunction and accelerated brain aging) as aspects of a triage response. These consequences are known to increase with age. In addition, evidence from our own work and that of others, as briefly reviewed below, indicates that sensitive assays targeted at these endpoints have a high likelihood of detecting changes in individuals with moderate micronutrient deficiencies.

(a) *DNA damage.* Deficiency in each of the 7 micronutrients (iron, magnesium, zinc, and vitamins B6, C, folic acid, and biotin) that we have so far examined results in increased DNA damage in humans, primary human cells in culture, or in rodents (Ho and Ames, 2002; Walter et al., 2002; Courtemanche et al., 2004b; Ames, 2006; Atamna et al., 2007; Killilea and Ames, 2008). Folate deficiency in human cells in culture was accompanied by cell cycle arrest in the S-phase, apoptosis and high uracil incorporation into DNA (Courtemanche et al., 2004a; Mashiyama et al., 2008). Others have shown that DNA damage occurs in humans who are moderately deficient in Fe, Zn, folate and B12, or choline (Everson et al., 1988; Courtemanche et al., 2004b; Ames, 2006; Fenech, 2007); and in rodents or human cell cultures for mostly severe deficiencies in Se, Cu, Ca, niacin, choline, pantothenate, riboflavin (Ames, 2006). Many of these and other micronutrient deficiencies, when studied epidemiologically are associated with cancer (Liaw et al., 1997; Prasad et al., 1998; Courtemanche et al., 2004b; Jaiswal and Narayan, 2004; Abnet et al., 2005; Ames, 2006; Fong et al., 2006; Holick, 2006; Chavarro et al., 2007; Dai et al., 2007; Franklin and Costello, 2007; Grau et al., 2007; Ishihara et al., 2007). A number of human intervention studies with micronutrients report a decrease in DNA damage or cancer (Everson et al., 1988; Fenech et al., 1998; Goh et al., 2007; Ribeiro et al., 2007), though more studies are needed to reach a definitive conclusion. To the extent that the DNA damage is caused by oxidants released from mitochondria, mtDNA will be damaged before nuclear DNA and should be more easily detected.

(b) *Mitochondrial oxidant release.* A large literature provides evidence that mitochondrial decay occurs with age, and results in increased production of mutagenic oxidant by-products of electron transport (Ames, 2006; Lezza et al., 2008; Ames and McCann, 2009). This decay appears to be a major contributor to both aging and its associated degenerative diseases, such as brain dysfunction, e.g. complex I and Parkinson's disease; complex IV and Alzheimer's disease (Ames, 2006). In mice or human cells in culture, we found that deficiencies in Zn (Ho and Ames, 2002), Fe (Walter et al., 2002), biotin (Atamna et al., 2007), or vitamin B6 resulted in increased mitochondrial oxidative decay (Ames, 2006). In all 4 cases, the mechanism appears to involve inhibition of heme synthesis which prevents complex IV formation (Walter et al., 2002; Ames, 2006; Atamna et al., 2007).

2.3. Cellular aging

Growth under modest deficiency of each of the three micronutrients so far examined (Ames, 2006): vitamin B6, magnesium (Killilea and Ames, 2008), and biotin (Atamna et al., 2007), increases the rate of cellular senescence; folate (Courtemanche et al., 2004b) and vitamin B6 (Askree and Ames, in preparation) deficiencies result in slowing of the cell cycle; telomere shortening has been observed with Mg (Killilea and Ames, 2008), the only micronutrient so far examined.

2.4. Some micronutrient deficiencies impair heme synthesis, which can result in oxidative stress, mitochondrial decay, DNA damage, and cell senescence

Seven micronutrients (biotin, pantothenate, pyridoxine, riboflavin, copper, iron, and zinc) are required for heme synthesis in

mitochondria. A deficiency in those tested causes a deficit of heme and therefore of complex IV, of which heme-a is an essential component (Richert and Schulman, 1959; Atamna et al., 2001, 2007; Atamna, 2004; Ames, 2005; Ames et al., 2005). This mechanism is compatible with, but does not prove, a triage response. The normal complement of complex IV keeps oxidants to a minimum; deficits of complex IV result in oxidant leakage, DNA damage, accelerated mitochondrial decay, and cellular aging (Atamna, 2004; Ames et al., 2005; Atamna et al., 2007). Deficiencies of iron, zinc, and biotin are discussed below.

(a) *Iron.* Iron deficiency is the most common micronutrient deficiency in the world, and anemia is widespread in underdeveloped countries (World Health Organization, 2001). Iron intake in US menstruating women is low; ~16% are below the EAR, the standard measure of inadequacy (Moshfegh et al., 2005). Hispanic women and the obese are at greater risk of being iron deficient (Nead et al., 2004). In humans, iron deficiency anemia is associated with poor cognitive development in toddlers (Hurtado et al., 1999; Lozoff et al., 2000; Grantham-McGregor and Ani, 2001; Beard and Connor, 2003; McCann and Ames, 2007), suggesting that iron deficiency in humans during critical periods of development harms the developing brain (Grantham-McGregor and Ani, 2001; Atamna et al., 2002; Beard and Connor, 2003). Severe iron deficiency causes loss of mitochondrial complex IV in selected regions in the brain of neonatal rats (de Deungria et al., 2000) as well as other changes in function, morphology, and physiology of the brain (Youdim and Yehuda, 2000; Beard and Connor, 2003). Iron deficiency in rats damages mitochondria and causes oxidant release, oxidative DNA damage, and decreased mitochondrial efficiency (Walter et al., 2002).

Functional iron deficiency also is associated with diminished immune function, and neuromuscular abnormalities (Viteri and Gonzalez, 2002; Failla, 2003). The primary measure used to identify iron deficiency in most human populations is a reduction in hemoglobin concentration to the point of anemia (malaria, HIV, and other nutrient deficiencies may also lead to anemia). The effects of iron deficiency occur along a continuum (Beard and Connor, 2003; Dallman, 1986) and subclinical iron deficiency may have deleterious effects on heme biosynthesis. Iron deficiency without anemia can also occur in newborns exposed to intrauterine hypoxia, such as infants of preeclamptic or diabetic mothers (Chockalingam et al., 1987). In such cases, iron is prioritized to erythroid and hemoglobin synthesis, putting the nonerythroid tissues at risk of iron deficiency and hence heme deficiency (Guiang et al., 1997; Rao and Georgieff, 2002). Dietary iron deficiency in the absence of anemia decreases aerobic capacity and physical work performance, which are improved by iron supplementation (Brutsaert et al., 2003). Iron deficiency has not been adequately studied as a possible risk factor for cancer and the results are discordant (Hercberg et al., 2005). However, many studies are looking for a monotonic relationship and do not take into account that one might expect cancer at levels of iron that are *both too low and too high* (Walter et al., 2002), as in hereditary hemochromatosis, a known risk factor for cancer (Elmberg et al., 2003). Both iron deficiency and excess iron (excess iron may cause zinc or copper deficiency) in mice cause oxidant leakage from mitochondria, oxidative mtDNA damage, and mitochondrial dysfunction (Walter et al., 2002). Iron accumulates with age and causes mitochondrial damage and early senescence in human cells in culture (Killilea et al., 2003) and in rats (Seo et al., 2008). Excess iron in human cells causes mitochondrial dysfunction, which can be ameliorated by ALC and LA (Lal et al., 2008).

(b) *Zinc.* Zinc inadequacy is common in adults, ~12% of whom are below the EAR (Moshfegh et al., 2005). In human cells in culture, zinc deficiency, mostly severe, causes complex IV deficiency and the release of oxidants, resulting in significant oxidative damage to DNA (Oteiza et al., 2000; Ho and Ames, 2002; Ho et al., 2003). Zinc deficiency also causes chromosome breaks in rats (Bell et al., 1975) and is associated with cancer in both rodents and humans (Fong et al., 2005). As discussed above, these observations reinforce the need to determine what degree of deficiency in humans results in DNA damage. We think it is likely that the trigger for decreased heme synthesis is the inactivation of the second enzyme of the pathway, δ-aminolevulinate dehydratase, which contains 8 atoms of zinc (Atamna, 2004; Jaffe, 2004). Zinc deficiency in human cells also inactivates other zinc-containing proteins such as the tumor suppressor protein p53 and the DNA base excision repair enzyme, apyrimidinic/apurinic endonuclease, with a resulting synergistic effect on genetic damage (Ho and Ames, 2002; Ho et al., 2003).

(c) *Biotin.* Biotin deficiency is more common than previously thought; ~40% of pregnant women who do not take a multivitamin show metabolic signs of deficiency (Mock, 2005). Marginal biotin deficiency is teratogenic in mice (Mock, 2005). Biotin is a prosthetic group in 4 biotin-dependent carboxylases (3 of which are solely present in mitochondria) that replenish intermediates in the tricarboxylic acid cycle (Mock, 1996). Biotin deficiency decreases the activity of these enzymes, leading to a decrease of 2 heme precursors, mitochondrial succinyl-CoA, and glycine, thus resulting in heme deficiency (Atamna et al., 2007). Biotin deficiency in normal human lung fibroblasts in culture caused a 40–50% decrease in heme content, oxidant release, premature senescence, and DNA damage (Atamna et al., 2007). The relationship of these effects to human intake needs to be determined (Stratton et al., 2006).

3. Enzymes lose binding affinity (increased Km) for coenzymes and substrates with mutation or age: a strategy for remediation with high dose vitamins

A review (Ames et al., 2002) showed that about 50 human genetic diseases due to defective enzymes can be remedied or ameliorated by the administration of high doses of the vitamin component of the corresponding coenzyme, which at least partially restores enzyme activity. Up to a quarter of mutations in a gene result in the corresponding enzyme having a decreased binding constant (increased Km) for a coenzyme resulting in a lower rate of reaction. The review points out that many of the B vitamins, given at levels 10–100 times the RDA, can raise coenzyme activity levels by an order of magnitude or more. Several single-nucleotide polymorphisms (SNPs) in the literature with a deleterious phenotype under some conditions decrease binding constants and are thus also likely to be remediable by raising cellular concentrations of the cofactor by high dose vitamin therapy. This review raises the issue of whether some appreciable percentage of the population may require a higher level of a particular vitamin or substrate for optimum function.

A follow-up review (Ames et al., 2006), points out that it is common for proteins to become deformed with age, e.g. by membrane proteins, if membranes become stiffer by oxidation, particularly in mitochondria. This raises the question whether high dose B vitamins may be useful in the elderly. Deformation of an enzyme commonly decreases binding affinity (increased Km) for its coenzyme or substrate. Enzyme substrates and vitamin precursors of coenzymes can be elevated by feeding and may enhance the activity of a deformed enzyme. B vitamins, for

example, are quite non-toxic and associated coenzymes can be raised 10-fold or more with a high dose supplementation (Ames et al., 2002). This raises the question whether the RDA for B vitamins should be reexamined in the old, as the levels set are based on experiments in the young. It also raises the question whether many metabolites, as well as vitamins, might be fed to improve functioning of enzymes in the old. The remediation of deformed enzymes, whether due to mutation or aging, is a field that shows promise and may be an inexpensive way to improve health.

4. Conclusion

The work on acetyl carnitine and lipoic acid in rodents and dogs suggests that decay of mitochondria leading to dementia and a variety of other diseases of aging in humans is not inevitable, but may be delayed by various interventions to improve metabolism. Understanding the mechanisms will suggest still other interventions. For example, if lipoic acid is effective because it induces the ~200 enzymes in the phase-2 defense system against oxidants, as seems likely, then the whole area of optimizing our various inducible defense systems for longevity by hormetic mechanisms becomes attractive and we are at the start of the discovery of many interventions.

If the triage hypothesis proves to be correct, as the vitamin K analysis suggests, it will demonstrate the importance of avoiding micronutrient malnutrition for a long and healthy life and change how people think about both nutrition and health. Most of the world's population, including that of the US, is inadequate in one or more micronutrients according to current intake recommendations. Yet, because there is no overt pathology associated with these levels of deficiency, there has been little public concern. The triage hypothesis framework may facilitate the discovery of sensitive and specific biomarkers of micronutrient insufficiencies that can be used to optimize metabolism at a personal and populational level. Current recommendations do not take into account the insidious biochemical consequences of metabolic triage. We think that we can show that insidious damage is indeed occurring at modest levels of deficiency and that this damage will increase the risk of cancer, cardiovascular disease, cognitive dysfunction and the other diseases associated with aging.

The genomic variability between individuals is being explored at a rapid rate, but true understanding on how to intervene awaits bringing nutrition, particularly micronutrients into the picture. We believe that the analysis of binding constants is the beginning of a large field that will make it possible to overcome a large class of deleterious genetic changes by nutritional interventions.

More attention to balanced diets and optimizing micronutrients could have a major effect on delaying the degenerative diseases of aging. Though a cautionary note is that too much of some micronutrients, such as iron (see above) or selenium (Waters et al., 2005), as well as too little, can be harmful.

Various lines of evidence reviewed here suggest that healthier lives are to be gained by optimizing our metabolism. My vision is that this will be done in the future by individuals measuring their own numbers from a finger prick of blood, and tuning up their metabolism by adjusting diet or taking supplements. The beginning of an age of true preventive medicine.

Conflict of interest

Dr. Ames is one of the founders of Juvenon (www.juvenon.com), a company that has licensed the University of California patent on acetyl carnitine + lipoic acid for rejuvenating old mitochondria (Ames and T. Hagen, inventors), sells acetyl carnitine + lipoic acid supplements, and does clinical trials on them. Ames founder's stock was put in a non-profit foundation at the founding in 1999. He is director of Juvenon's Scientific Advisory Board, but reimbursement for that from Juvenon is given to the foundation.

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