

The “triage theory”: micronutrient deficiencies cause insidious damage that accelerates age-associated chronic disease

The triage theory (Ames, BN (2006) PNAS 103-17589-94) posits that the spectrum of functions for a particular vitamin or mineral (V/M) are managed by the organism such that, when micronutrient availability is limited, functions required for short-term survival take precedence over functions whose loss can be better tolerated (e.g. by selection for micronutrient binding constants or targeted tissue distribution). Ames proposed that a consequence of this evolutionary adaptation is an increase in the risk of chronic diseases of aging when V/M availability is limited. That nature may have developed such a system is logically consistent with an important evolutionary theory that natural selection favors short-term survival for reproduction over long-term health [e.g, Kirkwood (2008) J Intern Med 2:117-27]. 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.

If the Triage theory is correct, the implications for public health are enormous. V/Ms are remarkably inexpensive. Intakes of a number of V/Ms are below levels considered adequate. These deficiencies are unusually widespread in poor countries, but also in the US population in all segments of society, especially the

Benefits of a Triage Analysis

1. Provides a rationale for why modest V/M deficiencies might increase risk of diseases of aging.
2. Offers a strategy for setting intake recommendations and for developing relevant biomarker assays.
3. Suggests where to look for mechanistic connections between MN deficiency and diseases.

poor, children, adolescents, the obese, and the elderly. In fact, it appears that only a small fraction of the population is adequate in all micronutrients. High consumption of calorie-rich, micronutrient-poor unbalanced diets exacerbates the problem. Yet, there is little societal concern because no overt pathologies have been associated with levels of deficiency that are not extreme. The triage theory predicts that more moderate levels of deficiency could be causing insidious changes that, over time, culminate in age-associated chronic disease.

A direct experimental test of the triage theory’s prediction of a causal relationship between long-term micronutrient deficiencies and chronic disease is virtually impossible to obtain in randomized controlled trials conducted over many years, as we have discussed (Ames et al (2007) Am J Clin Nutr 86:522-3). On the other hand, the central premise of the triage theory, that limited V/M availability favors functions required for short-term survival at the expense of those required for long-term health, can be tested.

VITAMIN K AND SELENIUM, TWO EXAMPLES OF TRIAGE THEORY

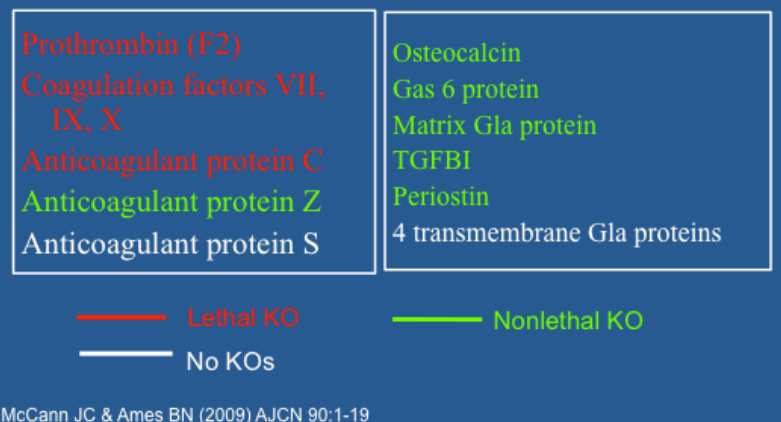
In these two studies, we applied a triage-based analysis to published evidence. The analyses were feasible because the number of proteins that require vitamin K or selenium are relatively few (14 for vitamin K and 24 for selenium), unlike V/Ms such as iron or zinc that are required for functions of hundreds of proteins. Brief summaries of these two studies are below.

A Triage-based Analysis – Four Key Questions

- 1. Can V/M dependent proteins be divided into more essential and less essential categories?**
 - Lethal and non-lethal mouse knock-out phenotypes were used to categorize proteins.
- 2. On V/M deficiency, are essential proteins protected at the expense of non-essential proteins?**
 - Human studies that, under different degrees of deficiency, measured functions of pairs of essential and nonessential proteins within the same tissues were analyzed.
- 3. Is modest V/M deficiency linked to diseases of aging?**
 - Prospective epidemiological studies examining associations between V/M intake and future disease risk were assessed.
- 4. Is there evidence these diseases are caused by dysfunction of non-essential proteins?**
 - Genes linked to human diseases and mechanistic studies were utilized.

Vitamin K [McCann & Ames (2009) Am J Clin Nutr 90:889-907]

Vitamin K is a cofactor for a single enzyme required for the γ -carboxylation of 14 different proteins, 10 with known functions. γ -carboxylation is required for these proteins to bind calcium, which is known to be required for protein function in almost all cases. Most of these 10 proteins are synthesized only in the liver and are required for coagulation. Only 3 of the 10 proteins are not involved in coagulation. In contrast to the coagulation factors, these proteins are γ -carboxylated in extra-hepatic tissues.



As shown in Figure 1, Mouse knockouts for almost all of the coagulation factors are lethal, suggesting these functions are required for short-term survival, in contrast to knockouts of the 3 extra-hepatic proteins, none of which is embryonic lethal.

Figure 1. The dichotomy between the embryonic lethality of coagulation factors, all predominantly γ -carboxylated in the liver, and the non-embryonic lethality of other vitK dependent proteins not involved in coagulation, all of which are predominantly γ -carboxylated in extra-hepatic tissues.

Higher dietary intakes of vitK1 are required for γ -carboxylation of the limited number of extra-hepatic proteins that have so far been examined than are required for γ -carboxylation of the hepatic proteins involved in coagulation.

As shown in Figure 2, dietary inadequacy of vitK1 is linked epidemiologically in some studies to several age-related chronic conditions (e.g., bone fractures, atherosclerosis, insulin resistance). And, phenotypes of the 3 non-embryonic lethal knockouts have characteristics of a similar set of chronic conditions (e.g., arterial calcification, skeletal abnormalities, glucose dysregulation).

Four Causes of Functional Deficiency of VKD-Proteins in Humans					
Non-lethal VKD-protein	Function	Mouse Knockout Phenotypes	Human Mutants	Anticoagulant Therapy	Modest VitK deficiency
Osteocalcin	Bone struct. Glucose homeostasis	Fragile bones Insulin resistance	BMD loss (SNPs)	Bone health (men/children/rats)	Bone health Insulin resistance
Matrix Gla Protein (Mgp)	Negative regulator of vascular calcification	Arterial calcification	Abnormal soft tissue calcification (Keutel Syndrome; SNPs)	Arterial calcification (humans/rats)	Arterial calcification

Figure 2. This figure summarizes results discussed in McCann & Ames (2009) Am.J Clin Nutr 90:889-907. The analysis combines mouse knock-out and human mutant phenotypes, effects of long-term warfarin (anticoagulant) therapy, and prospective epidemiological studies examining associations between vitK intake and disease incidence. The internal consistency of these results from diverse disciplines

The major dietary source of vitamin K (vitK1) is preferentially directed to the liver. Furthermore, it appears that vitK1 is mainly active in the liver and another form of vitamin K that can be synthesized from vitK1 is mainly active in the γ -carboxylation of extra-hepatic proteins.

Whether currently recommended intakes for vitK1 are sufficient for optimal carboxylation of the extra-hepatic proteins has been questioned (8). The average intakes of vitK1 in the United States and the United Kingdom are less even than currently recommended intakes (which are primarily based on levels required for adequate coagulation function). Thus, a large percent of the population may not be receiving sufficient vitK1 for optimal γ -carboxylation of proteins important for long-term bone and heart health.

Collectively, results form a coherent mechanistic picture consistent with triage theory. The localization of the γ -carboxylation of coagulation factors in the liver, and of other vitamin K dependent proteins to extra-hepatic tissues, sets up a dichotomy that takes advantage of the preferential distribution of vitK1 to the liver to preserve coagulation functions when vitamin K availability is scarce.

Selenium [McCann & Ames (2012) FASEB J 25:1793-1814]

In mammals, the great majority of dietary selenium (Se) is incorporated as selenocysteine during translation at the active site of 25 selenoproteins (SPs) in humans (24 in rodents). The substitution of Se for sulfur at the catalytic site has a major effect on enzyme potency (13) and can increase efficiency by ~100-fold (14, 15). SPs are required for life, as demonstrated by the lethality of mouse KOs unable to incorporate Se into SPs (16), and by severe impairment if SP synthesis is knocked out in specific tissues (e.g., refs. 17, 18). About a dozen SPs are well characterized, most of which are enzymes involved in redox regulatory pathways and maintenance of optimal redox status. This review focuses on 12 SPs with known mouse or human mutants with phenotypes that permit a classification of essential or nonessential.

Preferential protection against Se deficiency occurs between and within tissues. For example, Se is preferentially distributed to some tissues, such as the brain and reproductive tissues, at the expense of others.

A broad spectrum of both essential and nonessential SPs are present in these favored tissues and also in all other tissues, presenting a very different picture from vitamin K-dependent proteins, where all essential proteins are predominantly activated in the same tissue (liver).

Our triage analysis focused on within-tissue hierarchies of SP activities or concentrations measured *in vivo* in experiments where the sensitivities of essential and nonessential SPs to Se deficiency are directly compared. While these hierarchies have been widely discussed, this is the first systematic analysis.

Mouse knockout phenotypes were available for 13 SPs. Five (Gpx4, Txnrd1, Txnrd2, Dio3, and Sepp1) were classified as essential and 7 (Gpx1, Gpx 2, Gpx 3, Dio1, Dio2, Msrb1, and SelN) nonessential.

On modest Se deficiency, nonessential selenoprotein activities and concentrations are preferentially lost, with one exception (Dio1 in the thyroid, which we predict is conditionally essential). An example is in Figure 1.

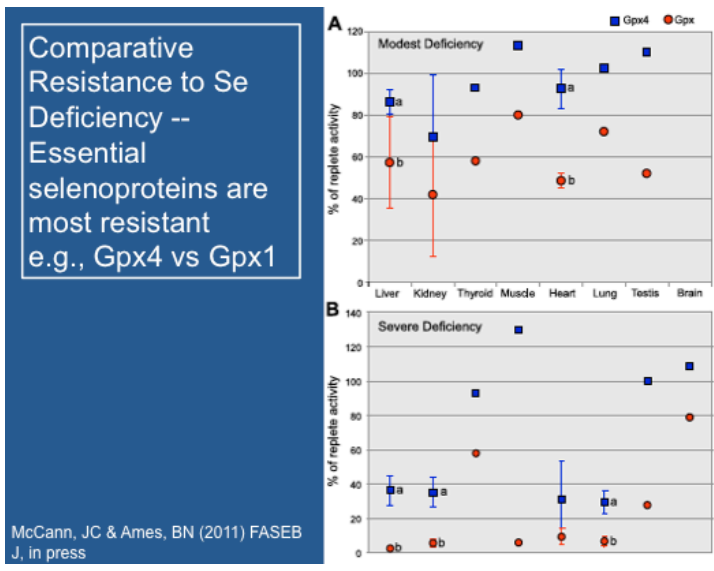


Figure 1. Sensitivity of Gpx4 and overall Gpx activities to modest and severe Se deficiency in multiple tissues. Essential SPs are represented by squares and nonessential SPs by circles: blue squares, Gpx4; red circles, overall Gpx activity (see text). When 1 experimental result was available in a given tissue, means \pm sd are plotted. Within a given tissue, means that do not share a common letter (*a*, *b*) are significantly different ($P < 0.05$). Points plotted without sds represent results of single experiments.

Mechanisms include the requirement of a special form of tRNA sensitive to Se deficiency for translation of nonessential selenoprotein mRNAs except Dio1, shown in Figure 2.

The same set of age-related diseases and conditions, including cancer, heart disease, and immune dysfunction, are prospectively associated with modest Se deficiency and also with genetic dysfunction of nonessential selenoproteins, suggesting that Se deficiency could be a causal factor, a possibility strengthened by mechanistic evidence.

Modest Se deficiency is common in many parts of the world; optimal intake could prevent future disease.

