Vitamin K, an example of triage theory: is micronutrient inadequacy linked to diseases of aging?[1–3]

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ABSTRACT

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 nonhepatic 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. *Am J Clin Nutr* 2009;90:889–907.

INTRODUCTION

The triage theory (1, 2) posits that, when the availability of a micronutrient is inadequate, nature ensures that micronutrient-dependent functions required for short-term survival are protected at the expense of functions whose lack has only longer-term consequences, such as the diseases associated with aging. The triage theory is similar to the "disposable soma" theory of aging (3), which suggests that, as a result of natural selection, metabolic resources are preferentially allocated to functions necessary for reproductive survival at the expense of those required for survival beyond reproductive age. The triage theory is unique in that it proposes a mechanistic trigger for the reallocation of micronutrient-dependent metabolic resources and suggests that age-related disease is easily preventable by ensuring an adequate supply of micronutrients.

If the theory is correct, the implications for public health are enormous. Virtually every metabolic pathway includes one or more enzymes that require essential micronutrients for activity. Micronutrient intake below recommended concentrations, but not severe enough to cause overt clinical symptoms, is widespread not only in poor countries but also in the United States (especially in the poor, children, adolescents, the obese, and the elderly), in part because of the high consumption of calorie-rich, micronutrient-poor, unbalanced diets (1). Societal concern is low because no overt pathology has been associated with these levels of deficiency. But the triage theory suggests that insidious changes could be occurring, leading to an increased risk of diseases associated with aging.

A variety of both active and passive homeostatic mechanisms could be involved in triage. Homeostatic mechanisms to combat micronutrient deficiency include tissue redistribution in response to deficiency (4), normal micronutrient tissue distribution patterns that favor some tissues or cell types over others (5), activation of stress responses leading to protective metabolic changes in essential processes (6), up-regulation of transporters (7), and different cofactor binding constants for isoenzymes as observed for polymorphic variants of many enzymes that bind micronutrient cofactors with different affinities (8).

We have begun an analysis of available scientific evidence that is aimed at profiling the functional spectrum of individual micronutrients under different degrees of micronutrient adequacy

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to test the central predictions of the triage theory. Because some micronutrients are required for numerous functions (eg, zinc, iron, riboflavin, and niacin), our initial focus is on micronutrients required for relatively few functions (eg, biotin, folate, iodine, molybdenum, selenium, thiamin, vitamin B-12, vitamin C, and vitamin K). In this article, results are presented for vitamin K.

BACKGROUND

An analysis of vitamin K appears to be relatively straightforward compared with virtually all other micronutrients because it has a single known major function. Vitamin K in its reduced form is a cofactor for one enzyme, γ-glutamylcarboxylase, which is located in the endoplasmic reticulum of various tissues. The enzyme post-translationally γ-carboxylates certain glutamic acid residues in a number of vitamin K–dependent (VKD) proteins (9). γ-Carboxylation allows VKD proteins to bind calcium, is needed for proper folding of the Gla domain, and facilitates binding of Gla proteins to cell membranes (10, 11). It is required for the activity of coagulation and anticoagulation factors (12) and for osteocalcin binding to hydroxyapatite in bone (13) and is generally considered to be required for the function of growth arrest specific protein 6 (Gas6) and matrix Gla protein (Mgp) (14–16).

During γ-carboxylation, the reduced form of vitamin K is oxidized to the epoxide, which is then reduced by vitamin K epoxide reductase in a 2-step reaction to regenerate the active form (17), completing what is termed the *vitamin K cycle*. The common anticoagulant drug warfarin (Coumadin; Bristol-Myers Squibb Company, New York, NY) acts by inhibiting vitamin K epoxide reductase (9). For further general discussion of vitamin K and the vitamin K cycle, *see* several recent reviews (9, 18–22).

A potentially complicating issue for this analysis is that vitamin K is not a single entity but a family of structurally related molecules derived from different sources. Major molecular forms, their primary dietary sources, and their relative contributions to vitamin K activity are shown in **Table 1** (22–49). All molecules listed in Table 1 share the same methylated naphthoquinone nucleus (menadione) but have side chains of differing composition and length (eg, reference 22), which results in different potencies and absorption efficiencies (20).

Menadione, despite also being called vitamin K3, does not have vitamin K activity (30, 35) and has different chemical properties from the family of active vitamin Ks. Unlike phylloquinone and the menaquinones, menadione can undergo redox cycling leading to the production of reactive oxidants. It is used at high doses in cancer treatments to potentiate cell killing, usually in combination with radiation or chemotherapy (50–52).

As shown in Table 1, phylloquinone (vitamin K1) is considered to be the primary dietary source of vitamin K activity in humans (23–25, 36). It is the major focus both of the experimental literature and of this analysis.

MK-4 (also called menatetrenone) is of particular interest to this analysis because it is an endogenously produced form of vitamin K synthesized from vitamin K1 (22, 37, 40, 49, 53, 54) and may be more active than vitamin K1 in extrahepatic tissues (22, 42–44). For example, after intake by rats of a vitamin K1–enriched diet containing no MK-4, high concentrations relative to vitamin K1 are found in extrahepatic tissues (37, 55). MK-4 is also distinguished from vitamin K1 in that, in addition to its vitamin K activity, it has been shown at high doses to affect the expression of a number of genes (22). We discuss MK-4 in conjunction with its use at high doses (usually 45 mg/d) in treatment trials examining its effects on bone fragility and some cancers.

MK-7, which has substantial vitamin K activity (56, 57), is found in small quantities in liver mitochondria and in some other tissues (33, 58). Natto is a soybean product fermented with *Bacillus subtilis*, which is rich in MK-7 (59). MK-7 is a possibly important source of vitamin K in individuals in Asian cultures who regularly consume natto.

Thus, following expert opinion, we assume that vitamin K1 is the principal source of vitamin K activity in humans in the modern world, with the possible exception of individuals who regularly consume natto. During evolution, when mechanisms for dealing with micronutrient shortages were developed, vitamin K1

TABLE 1
Common vitamin K molecular forms, sources, and relative contributions to vitamin K activity[1]

Molecular form of vitamin K	Primary sources (reference number)	Relative dietary contribution to vitamin K activity (reference number)
Phylloquinone (vitamin K1)	Green leafy vegetables (23–25)	Major (24)
Dihydrophylloquinone	Partially hydrogenated oils (26) consumed in some processed foods (27)	Minor (28)
Long-chain menaquinones (eg, MK10-13)	Colon bacteria (eg, 22)	Minor (22, 29) [little vitamin K activity (30) and poorly absorbed (24)] Substantial amounts stored in liver mitochondria (31–34)
MK-4	Synthesized in animals from menadione, a vitamin K–inactive (30) animal feed additive (35), and consumed in some meats and cheeses (24, 36)	Minor as a direct dietary source of vitamin K unless diets are very unbalanced (36)
	Synthesized from vitamin K1 (22, 32, 37–41); menadione most likely an intermediate (49)	Probably major as a metabolic product of vitamin K1 in extra-hepatic tissues (22, 42–44)
MK-7	Colon and oral cavity bacteria (45, 46)	Minor [substantial vitamin K activity (30, 47), but small amounts in liver mitochondria and some extrahepatic tissues (33)].
	Natto (24, 35, 48)	Possibly major if large quantities of natto are regularly consumed (35)

[1] Menaquinones of other chain lengths are also found in the liver and in some other tissues in small amounts (eg, reference 29).

was most likely an even more dominant source of vitamin K activity, because leafy green vegetables were a major, although variable, component of the Paleolithic diet (60, 61).

METHODS

Standard search methods were used to retrieve published information, including PubMed, Google, and the ISI Web of Knowledge cited reference search tool (62). Together with published resources, a number of web-based data repositories were also used, particularly the Mouse Genomic Informatics (MGI) database (63), which was used to search for mouse knockout mutants; BRENDA: The Comprehensive Enzyme Information System (64) and Uniprot (65), 2 comprehensive protein information resources; SymAtlas (66), which contains tissue-specific gene expression information; and 2 resources of the National Library of Medicine (67): the Online Mendelian Inheritance in Man (OMIM) and Single Nucleotide Polymorphism (SNP) databases.

RESULTS

Known VKD-dependent proteins, tissue locations of γ-carboxylation, and functions are listed in the first 3 columns of **Table 2** (9, 12, 14–16, 18, 22, 42, 68–196). As shown, these VKD proteins form 3 general classes:

1) Four coagulation factors—prothrombin (FII), FX, FVII, and FIX—of which all but prothrombin are believed to be γ-carboxylated exclusively in the liver (9, 12, 22, 79). Limited extrahepatic expression of prothrombin mRNA has been reported in several tissues, including the brain (176, 177), kidney (178), and cornea (179). This expression appears to be in conjunction with regulatory functions of thrombin in addition to its role in the coagulation pathway (180).

2) Three anticoagulation regulatory proteins with some additional functions (proteins C, S, and Z) that are γ-carboxylated primarily in the liver but to some extent also in extrahepatic tissues (12, 18, 167, 168).

3) The remaining known VKD proteins—osteocalcin, Mgp, Gas6, the recently identified fasciclin I–like proteins periostin and transforming growth factor β–inducible protein (Tgfbi or βig-h3, keratoepithelin), and 4 proline-rich Gla transmembrane proteins (Prrg1-4)—which are not involved in coagulation and are γ-carboxylated exclusively or primarily in extrahepatic tissues (*see* references in Table 2).

Categorizing VKD proteins according to survivability of mouse knockouts

The degree of lethality of VKD protein mouse knockouts provides some indication of their relative necessity for survival. Mouse knockouts have been isolated for all known VKD proteins listed in Table 2, except protein S and Prrg1-4, and are listed in the fourth column of Table 2. As shown, knockout phenotypes for all 4 coagulation factors (FII, FX, FVII, and FIX) and for one of the anticoagulation factors (protein C) are either embryonic lethal or lethal soon after birth due to excessive bleeding (FII, FX, FVII, and FIX) or thrombosis (protein C), which suggests that these 5 proteins are critical for short-term survival. Not surprisingly, the γ-glutamylcarboxylase knockout is also lethal because of hemorrhaging (197). Knockout phenotypes for all other VKD proteins are nonlethal at least through weaning (protein Z, Mgp, osteocalcin, Gas6, Tgfbi, and periostin), which suggests that they are less critical for survival than the coagulation factors. Mgp is marginally included in the nonlethal category, because offspring survive weaning but usually die by 2 mo of age.

Thus, a major requirement of the triage theory—that the functional spectrum of individual micronutrients can roughly be categorized into functions required for survival in the short term (ie, survival for reproduction) and functions whose loss can be tolerated over the longer term—appears to be consistent with the above categorization based on the relative lethality of mouse knockout phenotypes. Not only do VKD-protein knockouts segregate into ''more lethal'' and ''less lethal categories'' but there is some logical consistency within these categories. That is, the most lethal knockouts are all coagulation or anticoagulation factors that are γ-carboxylated exclusively or primarily (protein C) in the liver. The nonlethal knockouts are all proteins not involved in coagulation that are exclusively or primarily γ-carboxylated in extrahepatic tissues, with the exception of one anticoagulation factor (protein Z), which is primarily γ-carboxylated in the liver.

Are VKD functions required for short-term survival more resistant to vitamin K scarcity than less critical functions?

The first major prediction of the triage theory is that micronutrient-dependent functions required for short-term survival will be more resistant to micronutrient inadequacy than less essential functions. Several experiments comparing the sensitivity of γ-carboxylation of the coagulation factor prothombin and the extrahepatic VKD protein osteocalcin to variations in vitamin K1 availability suggest that γ-carboxylation of the more essential VKD protein (prothrombin) is more resistant to vitamin K1 inadequacy than that of the less essential VKD protein (osteocalcin) (198–201). For example, over a study period of 84 d, vitamin K1 intake was first reduced and then progressively increased in a group of older women in a controlled dietary setting (199). Undercarboxylated prothrombin and undercarboxylated osteocalcin (uc-osteocalcin) were monitored, and although both increased during the depletion phase, uc-osteocalcin increased over 2 wk before there was a statistically significant increase in undercarboxylated prothrombin. Other experiments reinforce this result (198, 200, 201). For additional discussion of the osteocalcin experiments, *see* 2 reviews (22, 202). Studies directly comparing the sensitivity of the γ-carboxylation of other extrahepatic VKD proteins with that of prothrombin or other coagulation factors have not yet been published.

Although results of the experiments discussed above are suggestive, they are not definitive. Because prothrombin is primarily γ-carboxylated in the liver and osteocalcin in bone cells, it is not clear that forms circulating in the blood are the most direct measure of relative γ-carboxylation efficiencies under vitamin K deficiency. That blood concentrations of γ-carboxylation do not necessarily reflect tissue concentrations are shown by recent observations concerning Mgp, which is γ-carboxylated in chondrocytes (cartilage) and vascular smooth-muscle cells. Although undercarboxylated Mgp is accumulated in sclerotic lesions compared with healthy arteries, blood concentrations appear to be lower in patients with coronary or other diseases characterized by arterial calcification compared with healthy controls (14, 203–207).

TABLE 2
Vitamin K–dependent (VKD) proteins: tissue locations of γ-carboxylation, functions, and phenotypes of mouse knockout (KO) strains and known human mutants[1]

VKD protein	Tissue locations of γ-carboxylation	Functions	Mouse KO phenotypes	Human mutant phenotypes
Coagulation factors (general reviews: references 9, 12, 22, 79)				
Factor II (prothrombin)	Liver; limited EH (brain, kidney, cornea) (177–179)	Primarily coagulation factor; some regulatory functions (180).	Embryonic lethal: defects in yolk sac, internal bleeding, tissue necrosis; rarely, neonatal death (144, 154)	Complete deficiency lethal (98). Prothrombin thrombophilia: increased risk of VTE (74, 137).[2]
Factor X	Liver	Coagulation factor	Late embryonic or neonatal lethality—spontaneous bleeding (68, 86)	Absence of factor X lethal (147). Congenital factor X deficiency (rare)—abnormal bleeding (73, 112).
Factor VII	Liver	Coagulation factor	70% die within 24 h of birth from intraabdominal hemorrhaging (68, 140)	Abnormal bleeding: severe form is lethal (127)
Factor IX	Liver	Coagulation factor	Usually fatal; severe hemophilia (118, 120, 153)	Hemophilia B: rare X-linked hereditary disease. Gene deletion causes extreme disability by early adulthood (81, 129, 148).[3]
AC factors (general reviews: references 12 and 18)				
Protein C	Primarily liver (143) (15); some EH (eg, endothelial cells, keratinocytes)[4]	AC factor. Non-AC effects: antiinflammatory (90, 166, 167); autocrine growth factor (155); placental development (168, 169)	Neonatal death (consumptive coagulopathy) (110)	Homozygotes: severe neonatal purpura fulminans. Heterozygotes: increased risk of VTE (93, 113)
Protein S	Primarily liver (15, 101, 143); some EH (eg, osteoblasts (126), endothelial cells (91)	AC factor. Also ligand for TAM RTKs (72, 96, 100) and C4b-binding protein (183, 184)	No KO in MGI	Homozygotes: severe neonatal purpura fulminans (84, 125, 128). Heterozygotes: ≈10-fold increased risk of VTE (76, 93, 96), osteonecrosis (99, 136)
Protein Z	Primarily liver (143); some EH (eg, vascular) (149)	AC factor (114)[5]	No phenotypic abnormalities, but increased prothrombotic phenotype in factor V (Leiden) mice (158)	SNPs: normal AC activity (132)[6]
Other VKD proteins				
Mgp (42, 83, 138)	EH: primarily bone, cartilage, vascular tissue (14)[7]	Negative regulator of vascular calcification (42, 83, 138)[8]	Nonlethal to weaning. Death by 2 mo of age. Arterial and other calcification.[9]	Abnormal calcification (eg, Keutel syndrome, Singleton-Merten syndrome, SNPs)[10]

(*Continued*)

TABLE 2 (*Continued*)

VKD protein	Tissue locations of γ-carboxylation	Functions	Mouse KO phenotypes	Human mutant phenotypes
Osteocalcin (80, 102, 187)	EH: primarily osteoblasts (80, 185).[11]	ECM protein in bone (80, 135). Additional hormonal role for uc-osteocalcin in glucose homeotasis (92, 105, 119, 188).	Nonlethal. BMD loss, bone fragility after ovariectomy (78, 88); disturbed glucose homeostasis (119).[12]	SNPs: osteopenia (87), BMD in women (PM) (69, 82, 85, 107, 121, 139)[13]
Gas6 (15, 77, 100, 152)	EH (eg, smooth muscle, endothelial, NK cells) (15, 122, 134)	TAM RTK activating ligand[14]	Nonlethal. Apparently normal phenotype (70). Differential effects on progression of several experimentally induced pathologies (see text).	SNPs: association with stroke (189), acute coronary syndrome (190)
Tgfbi (βig-h3, keratoepithelin) (182)[15]	EH: widely expressed. Down-regulated in tumor cells (174, 182).	ECM protein. Promotes microtubule stability (173). Complex role in cancer.[16]	Nonlethal. Spontaneous cancer, chromosome abnormalities (174).[17]	Corneal dystrophy (175)
Periostin (165, 170, 172, 181)[18]	EH [eg, heart (171, 172), osteoblasts, tissues undergoing remodeling, tumor cells (170)]	ECM protein. Role in development (eg, bone, heart), wound healing. Complex role in cancer.[19]	Nonlethal. Developmental abnormalities [eg, latent heart valve disease (172, 186); poor recovery after induced myocardial infarction (162)].[20]	None specified in OMIM
Proline-rich Gla proteins 1–4	Variety of fetal and adult tissues [eg, spinal cord, thyroid (115, 116)]	Largely unknown[21]	No KO in MGI	No information in OMIM

[1] Several putative VKD proteins were not included in the table. Two fasciclin-like proteins in humans, stabilin-1 and stabilin-2, were recently shown to contain carboxylase recognition sites (CRS) but have not been further characterized (165). An additional Gla-rich protein (Grp) was recently isolated from sturgeon, with human homologs identified in an in silico analysis, but not further characterized (164). AC, anticoagulation; ECM, extracellular matrix; EH, extrahepatic; Gas6, growth arrest specific protein 6; MGI, Mouse Genomic Informatics; Mgp, matrix Gla protein; PM, postmenopausal; SNP, single nucleotide polymorphism; TAM RTKs, Tam, Axl, and Mer receptor tyrosine kinases; uc-osteocalcin, uncarboxylated osteocalcin; BMD, bone mineral density; VTE, venous thromoembolism; OMIM, Online Mendelian Inheritance in Man; Tgfbi, transforming growth factor β inducible protein. Information sources consisted of the published literature in MGI (63) and the OMIM (67) databases.

[2] Common allele resulting in elevated plasma concentrations of prothrombin.

[3] Classical hemophilia is hemophilia A, which is caused by factor VIII deficiency (133, 153). Factor VIII is not a VKD protein.

[4] Endothelial cells, keratinocytes, and some hematopoietic cells (166).

[5] Possibly other endothelial functions (149).

[6] Mixed results suggesting an inverse association between protein Z blood concentrations and ischemic stroke (149).

[7] Some synthesis also in heart, lung, and kidney (94).

[8] Mechanism may involve inhibition of bone morphogenetic protein (151, 157).

[9] Additional KO phenotypic characteristics include growth plate calcification resulting in small stature, osteopenia, and fractures (89, 123, 142).

[10] Keutel syndrome is a rare inherited disease resulting from mutations that inactivate Mgp. Clinical characteristics include abnormal arterial and cartilage calcification, peripheral pulmonary stenosis, and midfacial hypoplasia (108, 130, 131). Singleton-Merten syndrome clinical features include progressive aortic calcification, abnormal dentition, muscular weakness, and widened medullary cavities of bone; possible involvement of Mgp has been suggested (97, 150). Mgp polymorphic variants are associated with coronary artery calcification in healthy men (191), increased plaque calcification in myocardial infarction patients (104), tooth loss (106), kidney stones (95), pseudoxanthoma elaticum (103), and loss of BMD (146).

[11] Some expression in endothelial progenitor cells has recently been reported (185).

[12] Increased early rate of bone formation in KOs, but increased fragility compared with wild type after ovariectomy (78, 88). Disturbed glucose homeostasis includes decreased β cell proliferation, greater glucose intolerance, and insulin resistance (119).

[13] Evidence is mixed. Associations were not observed in premenopausal women (111, 141).

[14] Promotes innate immune response, cell survival, differentiation, required for natural killer cell maturation, and various brain functions (15, 100, 122).

[15] Tgfbi was recently discovered to contain CRS and γ-glutamic acid residues (165). The extent to which known functions of Tgfbi depend on γ-carboxylation is not yet known.

[16] ECM functions include cell adhesion and spreading. Although properties of Tgfbi appear to be tumor suppressive, in some cases, Tgfbi also appears to be recruited in some cases during tumor metastasis (192).

[17] Knockdown of Tgfbi results in mitotic spindle abnormalities (173) (*see* text).

[18] It has recently been discovered that periostin contains CRS and γ-glutamic acid residues (165). The extent to which known functions of periostin depend on γ-carboxylation is not yet known. An isoform of periostin has also been identified (193).

[19] Required for normal bone, heart, and dental ligament development; plays a role in remodeling processes after injury (161, 165, 171); and stimulates tumor progression (170) but appears to also have some tumor-suppressive properties (194–196).

[20] KO offspring are growth retarded with several additional developmental abnormalities, including periodontal disease (161, 163) and abnormal incisor eruption (160).

[21] One proline-rich Gla protein binds to YAP (117), a transcriptional coactivator identified as an oncogene (159).

What is the mechanism?

Because γ-carboxylation of the most essential VKD proteins (coagulation factors) is localized in the liver, and that of the less essential VKD proteins in the extrahepatic tissues, preferential tissue distribution of vitamin K1 to the liver is one mechanism that could facilitate better tolerance of coagulation factors to vitamin K1 scarcity than extrahepatic γ-carboxylation. Indeed, as is well documented, vitamin K1 preferentially accumulates in the liver of both humans (22, 83, 208) and rodents (55, 209). Orally administered vitamin K1 first dissolves in chylomicrons (lipoprotein particles), which are formed in the wall of the small intestine in response to dietary lipid intake, and on absorption rapidly enter the lymphatic circulation (210). Chylomicrons then undergo modification to become chylomicron remnants (CMRs) (211), which are smaller in size than chylomicrons, but retain dissolved lipid-soluble vitamins (212), such as vitamin K1. CMRs are primarily taken up by the liver (212–214), although some other tissues (215), including bone (216–219), have some capability of clearing CMRs. For example, mouse liver takes up ≈5 times more CMRs than bone (218). After vitamin K1 has been removed from CMRs in hepatic tissue, a portion can then reenter the systemic circulation in very-low-density lipoprotein particles secreted from the liver (22, 210, 220). Vitamin K1 in this form then reaches extrahepatic tissues (22, 212).

An additional mechanism by which γ-carboxylation of some VKD proteins could be favored over others could involve different binding affinities for the γ-glutamylcarboxylase. In fact, isolated peptides from the carboxylase recognition sites of various VKD proteins do bind with very different affinities to γ-glutamylcarboxylase (221). However, because of the modulatory effects of secondary binding sites, the actual differences in affinities of the intact VKD proteins for the γ-glutamylcarboxylase are not known, as recently discussed (9).

CONSEQUENCES FOR LONGTERM HEALTH OF DECREASED FUNCTIONALITY OF VKD PROTEINS

The second major prediction of the triage theory is that decreased functionality of VKD proteins not required for short-term survival can increase the risk of diseases associated with aging. In this section, 3 factors that modify VKD function are examined: genetic loss, dietary availability of vitamin K, and chronic anticoagulant therapy.

Genetic loss

Functional loss from genetic change is not expected to precisely mimic changes that might also be expected from vitamin K deficiency because mutations affect function throughout development and the life span. However, it is expected that functional loss resulting from mutations will to some extent prefigure categories of loss that might result from vitamin K deficiency. Below, functional changes resulting from genetic modification of VKD protein genes are briefly summarized, and interpretation of these changes from the perspective of the triage theory is discussed.

Mouse and human mutant phenotypes

These phenotypes are shown in the 4th and 5th columns of Table 2. Several points are evident from an examination of this table:

1) *Coagulation and anticoagulation factors II (prothrombin), X, VII, IX, protein C, and protein S.* The lethality of mouse knockouts is mirrored in human homozygous or deletion mutants that are also lethal or life threatening without treatment. Although a mouse knockout for protein S has not yet been isolated, it will probably prove to be lethal on the basis that homozygous human protein S mutants are characterized by severe neonatal purpura fulminans (125), which is similar to that observed in protein C homozygotes (109, 113). Less severe mutations that result in only partial loss of proteins C or S (93), or that result in overproduction of prothrombin (factor II) (74, 137), lead to increased risk of venous thrombosis, an age-associated condition. Less severe mutations in the other members of this class of VKD proteins result in lifelong, but not necessarily life-threatening, abnormal bleeding or thromboses.
2) *Mgp.* Keutel syndrome (108, 131) is the only known complete loss-of-function human mutation among VKD proteins with nonlethal knockouts. As in mice with Mgp knockouts, individuals with Keutel syndrome are born apparently normal but develop abnormal calcification early in life in multiple tissues, including arterial walls (130). Some evidence also suggests greater calcification in older healthy men (191) and myocardial infarction patients with Mgp polymorphisms (104), which is consistent with the known association of calcification with arterial disease (222).
3) *Osteocalcin.* As shown in Table 2, both mouse knockouts and human polymorphisms (the only human osteocalcin mutants so far identified) are characterized by increased bone fragility after estrogen loss. Note, however, the complexity of the mouse knockout phenotype compared with wild-type (ie, increased bone density before ovariectomy and greater bone fragility after ovariectomy) (88). To our knowledge, possible associations of osteocalcin polymorphisms with diabetes or other consequences of glucose dysregulation, as observed in mouse knockouts, have not yet been investigated in humans.
4) *Gas6.* As shown in Table 2, mouse knockouts appear to be normal but respond differently than wild type to several experimentally induced pathologic conditions. Some of these responses [eg, poor erythropoietic recovery from induced anemia (71), poor tissue repair after experimentally induced hepatitis (223), or greater loss of oligodendrocytes in a demyelination model (75)] suggest that wild-type Gas6 may protect against some disease processes. In contrast, other responses suggest the opposite [eg, decreased thrombus formation in a prothrombotic model (70, 145), less nephrotoxic nephritis in a disease model (156), and decreased inflammation in an induced atherosclerosis model (124)]. The multifaceted phenotype of Gas6 knockouts in these induced-disease models is not surprising given the wide range of Gas6-activated receptor tyrosine kinase (RTK) functions (Table 2).
5) *Protein Z.* There is limited information on protein Z, but the possible linkage of protein Z deficiency and its polymorphisms in humans to stroke and other arterial diseases (149) suggests that there could be a connection between protein Z deficiency and age-associated disease.
6) *Tgfbi.* The striking characteristics of Tgfbi knockouts are increased spontaneous cancer and mitotic abnormalities (174). In an important series of experiments, Ahmed et al (173) show that Tgfbi is required to maintain microtubule stability. Knockdown of Tgfbi causes mitotic spindle abnormalities and centrosome duplication (173). Genomic instability is associated with increased cancer risk (224) and also correlates with the risk of metastasis (174, 225). TGF-β, which induces Tgfbi, is known to suppress genomic instability (226) [inhibition of TGF-β increases centrosome aberration frequency,

tetraploidy, and aneuploidy (227)], but the mechanism is not known. Because expression of Tgfbi requires induction by TGF-β, it seems possible that Tgfbi maintenance of microtubule stability may be the mechanism by which TGF-β suppresses genomic instability. It is of interest that TGF-β null cells are also more sensitive to alkylating agents due to lack of expression of O^6-methylguanine DNA methyltransferase (228), suggesting that TGF-β induces the expression of this DNA repair enzyme. Although Tgfbi may act as a tumor suppressor, it also appears to promote extravasation (leakage of cancer cells into tissues from capillaries), an essential step in metastasis, at least in colon cancer (192). Hence, similar to TGF-β, which appears to transition from a tumor suppressor in the early stages of carcinogenesis to an oncogene in tumor progression (229–231), the role of Tgfbi in cancer may vary depending on the stage of carcinogenesis and tumor type. As shown in Table 2, the only known consequence in humans of mutations at the Tgfbi locus is corneal dystrophy. On the basis of the mouse knockout phenotype, however, it may be productive to examine polymorphism phenotypes and families with corneal dystrophy for evidence of genomic instability or increased cancer risk.

7) *Periostin*. The knockout phenotype suggests a role for periostin in cardiovascular health and recovery from myocardial infarction (162, 172). Periostin also appears to play a role in cancer progression. Periostin is, like Tgfbi, induced by TGF-β (172), and, like Tgfbi, periostin appears to have both tumor suppressive and oncogenic properties. For example, the rate of growth in periostin knockouts of tumors initiated by subcutaneous injection of several cancer cell lines was greater than in wild-type (194), which suggests tumor-suppressive properties (195, 196). On the other hand, periostin is well known to be up-regulated in a wide variety of metastatic tumors (232). Human periostin mutants have not yet been identified. However, it is of interest that it was recently reported (233) that periostin and Tgfbi are binding partners and that a Tgfbi mutation resulting in corneal dystrophy prevents their interaction. This raises the question of whether binding to periostin is required for Tgfbi effects on the mitotic spindle.

Triage theory perspective

The phenotypes discussed above raise several points that help to clarify the triage theory and bring it to bear in the context of disease progression. First, venous thrombosis is considered to be an age-related disease. Although the partial loss-of-function protein C and protein S mutants are linked to increased risk of this condition, the triage theory would predict that venous thrombosis would be unlikely to result from vitamin K deficiency because these 2 VKD proteins are required for short-term survival and are predominantly γ-carboxylated in the liver. Thus, the theory predicts that vitamin K deficiency would have to be severe enough to adversely affect coagulation function to interfere with their γ-carboxylation.

Second, in contrast, triage theory would predict that vitamin K deficiency that is not severe might be linked to bone fragility after menopause (osteocalcin), arterial calcification (Mgp), and genomic instability and cancer because these conditions are linked to genetic loss of VKD proteins not required for short-term survival. These proteins are γ-carboxylated exclusively in extrahepatic tissues and thus are likely to require higher intakes of vitamin K for maximum function compared with hepatic VKD proteins.

The complex phenotype of apparently normal Gas6 knockouts that is revealed in response to pathologic stress and the recruitment of Tgfbi and periostin in cancer progression provide a third example. These effects, within the context of ongoing pathology, are somewhat similar to "antagonistic pleiotropy," an evolutionary theory of aging suggested many years ago (234). Antagonistic pleiotropy suggests that some genes that have a positive function early in life may have a deleterious function late in life. In the Gas6 example, the health-promoting functions of Gas6, such as stabilizing platelet aggregates in hemostasis, can become disease promoting when recruited by an ongoing pathological process, such as thrombosis. Although the triage theory predicts that partial loss of Gas6 function due to vitamin K deficiency could lead to the *initiation* of age-associated disease in otherwise healthy individuals, it does not preclude the possibility that the partial loss of function could also lead to greater resistance to the *progression* of some diseases (*see* Discussion). Because Gas6 knockouts do not appear to have been followed into old age (*see* Table 2), it is not known whether they might have been more susceptible to the development of any age-associated diseases.

Dietary availability of vitamin K

As shown in **Table 3** (16, 22, 42, 52, 80, 102, 135, 204, 235–278), vitamin K deficiency has been linked to a variety of age-associated conditions, including loss of bone mineral density (BMD) or increased fracture risk (80, 102, 135, 235–252), arterial calcification or cardiovascular disease (16, 42, 253–262), cancer (22, 52, 263–268), insulin resistance (269, 270), osteoarthritis (80, 271), chronic kidney disease (42, 272–274) [frequently accompanied by vascular calcification (279)], and inflammation (22, 275). Bone-related conditions, arterial calcification or other cardiovascular conditions, and cancer have been most widely studied, and evidence linking them to vitamin K availability is briefly discussed below.

Bone-related conditions

For extensive discussion of this topic, *see* several reviews (80, 135, 235, 236, 280, 281). During pregnancy, a rare condition resulting in severe reduction of maternal vitamin K results in serious bone and cartilage abnormalities in the fetus (42, 237). In young girls, an association between low vitamin K1 status and several markers indicating increased bone turnover has also been reported (238). In adults, vitamin K deficiency has been linked to increased risk of bone-related conditions in many studies. Vitamin K1 intake (239–241), status (242, 243), or treatment (244); MK-4 treatment at pharmacologic doses (245); and MK-7-rich natto intake (246, 247) have all been linked positively to variables indicative of bone health (particularly decreased fracture risk, increased BMD, and bone mineral content).

Despite the considerable number of positive studies, most (80, 235, 236), although not all (135, 282), expert reviewers consider that a causal relation between vitamin K and bone health has not been shown, primarily because results of treatment trials have not been consistently reproducible. Several new treatment trials involving high doses of MK-4 (45 mg/d) (248–250) and vitamin K1 (500–1000 μg/d) (250–252) have appeared since those discussed by reviewers, and results of these trials are mixed as well. Thus, despite a large body of work linking vitamin K availability to bone health, a definitive causal relation has not been established in reproducible randomized controlled treatment trials.

TABLE 3
Age-related disease or conditions linked to dietary vitamin K inadequacy

	Representative references		
Age-associated condition	Studies	Reviews	Comments
Bone abnormalities (primarily increased fracture risk or loss of bone mineral density)	Positive: references 237–249 Negative: references 250–252	References 80, 102, 135, 235, 236	Specific endpoints (eg, bone mineral density) not consistently replicated Substantial design (eg, duration of treatment or follow-up) differences among studies
Arterial calcification or cardiovascular disease (atherosclerosis, other coronary heart disease)	Positive: references 255–260, 276 Negative: references 261, 262	References 16, 42, 253, 254	Most positive results are for menaquinone intake or vitamin K1 supplementation Confounding food components in vitamin K1–containing foods complicate interpretation of vitamin K1 intake study results
Insulin resistance	References 269, 270	Reference 269	Increase in insulin sensitivity in both a randomized vitamin K1 intervention trial and a cross-sectional, food-frequency questionnaire–based intake study
Osteoarthritis	References 271, 277	Reference 80	Low vitamin K1 status associated with osteoarthritis Results in a randomized intervention trial most evident in individuals deficient in vitamin K at baseline
Chronic kidney disease	Reference 272	References 42, 273, 274	Suboptimal vitamin K status and elevated undercarboxylated Mgp (204) in hemodialysis patients in one study; no association reported in another (278)
Inflammation	Reference 275	Reference 22	Vitamin K status inversely associated with several circulating markers of inflammation
Cancer	References 263–268	References 22, 52	Some case reports and several small pilot trials suggest that treatment with high doses of MK-4 may be therapeutic in patients with preexisting cancer. A prospective epidemiologic and nested case-control study of the same population presents marginally statistically significant results suggesting an inverse relation between menaquinone, but not vitamin K1 intake, and subsequent prostate cancer.

Although we do not disagree with this conclusion, note that there are substantial design (eg, duration of treatment, whether subjects were also treated with vitamin D and calcium, whether subjects had existing evidence of bone disease, outcome measures) and other protocol differences among trials that prevent precise comparisons. In addition, treatment trials usually extend over a relatively short time period (1–3 y) compared with dietary inadequacy, which may last for many years.

Arterial calcification and cardiovascular disease

Fewer studies have examined relations between vitamin K and cardiovascular health [*see* several recent reviews (16, 42, 254, 280)]. Cross-sectional (255, 256) and population follow-up (257, 258) studies from the Netherlands reported statistically significant inverse associations between total vitamin K intake (vitamin K1 + menaquinone) and aortic atherosclerosis (255) and between low menaquinone intake and coronary (256) and aortic (258) calcification and diagnosis of (257) or mortality from (258) coronary heart disease, and all-cause mortality (258).

We are aware of 2 vitamin K1 intervention trials that examined cardiovascular outcomes (259, 276). One trial (259) reported a positive effect of vitamin K1 supplementation (1000 μg/d) for 3 y in postmenopausal women on elasticity of the carotid artery, although significant changes were not detected in other indicators of arterial health, as pointed out by reviewers (254). A recent 3-y vitamin K1 (500 μg/d) supplementation study in older men and postmenopausal women did not measure carotid elasticity but did report less progression of coronary artery calcification that was statistically significant in subjects with $\geq$85% adherence to the supplement and in those with preexisting coronary artery calcification (276). It has proven more difficult to detect a relation between vitamin K1 and cardiovascular disease by using epidemiologic techniques for several reasons. Experts have pointed out that foods rich in vitamin K1 are also rich in other substances associated with heart health, so that it is difficult, if not impossible, to tease out any effect due specifically to vitamin K1 (254, 261, 262). In addition, the food-frequency questionnaires used in several studies from the Netherlands that indicated statistically significant inverse relations between menaquinone, but not vitamin K1, intake and calcification or coronary heart disease (256–258) only poorly reflected vitamin K1 intake. Furthermore, in these studies (256–258), vitamin K1 intakes were quite high, and the range of intakes was narrow. If an inverse relation was observed for menaquinone intake, one might expect that an inverse association would also be observed for vitamin K1 intake, provided that a well-validated food-frequency questionnaire was used and that the cohort examined had a wide range of vitamin K1 intake.

Important additional evidence linking vitamin K to cardiovascular health comes from 2 studies that observed a relation between vitamin K (vitamin K1 + vitamin K2) intake and serum concentrations of carboxylated osteocalcin with cardiovascular and bone health in the same individuals (255, 260). In the first case, both low vitamin K intake and high circulating uc-osteocalcin (possibly a surrogate indicating poor γ-carboxylation of Mgp) were associated with atherosclerosis (255). In the second case, women with both atherosclerosis and relatively high serum concentrations of uc-osteocalcin also had low BMD (260). The association of aortic calcification with indicators of poor bone health has been widely reported (eg, 283–286), and possible mechanisms have recently been reviewed (287). However, a recent large prospective cohort analysis did not find an association (288).

Cancer

Pharmacologic doses of MK-4 inhibit the growth of a number of cancer cell lines in vitro, and some in vivo evidence also suggests a therapeutic effect on cancer progression (22, 52). The likely mechanism is modulation of gene transcription, which may in part be mediated by the binding of MK-4 to the SXR/PXR receptor (the human and rodent, respectively, versions of receptors that bind steroids and a wide variety of molecules perceived by the organism as xenobiotics) (22, 289). It is not known whether these effects occur under normal physiologic conditions. Some case reports suggest that high-dose treatment with MK-4 in patients with different types of cancer may be therapeutic (52), and the possible protective effects of daily high-dose MK-4 treatment on the recurrence of hepatocellular carcinoma in successfully treated patients have been reported in several small trials (263–266). For additional discussion of some of these studies, *see* several reviews (22, 290, 291).

The only attempt of which we are aware to examine the possible relations between cancer incidence and vitamin K intake in normal physiologic ranges is a recent prospective epidemiologic analysis of a large cohort of >11,000 men (267). Marginally statistically significant evidence was provided that suggested an inverse relation between new cases of advanced prostate cancer diagnosed during a mean follow-up period of 8.6 y and dietary intake (determined by food-frequency questionnaire) of menaquinones but not of vitamin K1 (267). A subsequent nested case-control study using the same cohort (268) reported that higher ratios of uc-osteocalcin to total osteocalcin were also marginally associated with advanced or high-grade prostate cancer cases. This latter result was based on a single model-dependent *P* value that achieved only borderline significance and was most likely driven by outliers in the 4th quartile. Results of both of these studies are very weakly statistically significant and, in our opinion, are uncertain unless replicated in an independent cohort. As investigators discuss, interpretation is also complicated by the fact that MK-4 is endogenously synthesized from vitamin K1, and γ-carboxylation of uc-osteocalcin is well known to be sensitive to vitamin K1 intake (198–201).

Chronic anticoagulant therapy

Because warfarin blocks vitamin K epoxide reductase, it is expected that warfarin treatment will decrease γ-carboxylation of all VKD proteins, not just coagulation factors. Indeed, a broad spectrum of effects has been reported in humans and rodents, including effects of treatment on bone health, arterial calcification, and cancer, which are briefly described below.

Bone-related conditions

Use of warfarin during the early stages of pregnancy can lead to fetal warfarin syndrome, which is characterized by abnormal bone development and cartilage calcification (80, 253, 292), similar to the effects of severe maternal vitamin K deficiency on offspring discussed above (237). Ostopenia was reported in 52% of children and adolescents in long-term anticoagulant therapy (293). In adults, results of the significant number of studies examining possible linkages between warfarin-induced vitamin K deficiency and osteoporosis, loss of BMD, or increased fracture risk are mixed (80). However, as recently discussed in a thorough review of the field (135), association of anticoagulant use with poor bone health is most consistently reported in individuals in long-term therapy (eg, reference 294). Studies that failed to segregate subjects based on duration of therapy were generally negative [*see* a recent example (295)]. Adverse effects of chronic warfarin use in humans are reinforced by experiments in rats in which warfarin treatment of 4 mo decreased BMD and increased bone fragility after ovariectomy (296).

Arterial or aortic valve calcification

It is not clear whether the possibility of arterial calcification in fetal warfarin syndrome (discussed above) has been investigated. A rare adverse reaction to long-term warfarin therapy is tracheobronchial calcification (297), which may account for depressed arterial hemodynamics in patients at higher cardiovascular risk in long-term warfarin therapy (298). Several observational studies have also reported associations between aortic valve calcification and long-term anticoagulant therapy (205, 274, 299–301) in humans; warfarin treatment also induces arterial calcification in rats (43, 302). This effect may be exacerbated by the fact that warfarin also blocks the biosynthesis of MK-4 from vitamin K1 (43).

Cancer

As discussed above, there is a high rate of spontaneous cancer in mouse Tgfbi knockouts. Because warfarin treatment is expected to interfere with γ-carboxylation of Tgfbi, it is of interest to ask whether long-term warfarin treatment in humans increases cancer risk. Few studies have looked explicitly at this question because research on warfarin and cancer has been driven primarily by the fact that cancer triggers the coagulation cascade and is a major risk factor for venous thromboembolism (VTE) (303, 304), which can occur even before the clinical detection of cancer (305, 306). Cancer incidence after a VTE event has been tracked over the short term in many studies, and in fewer cases for periods longer than a year (305, 307–310). Results consistently suggest a much greater risk of occult cancers detected relatively soon after VTE, but a small increased risk persisting for $\geq$10 y (307, 310). Although very few studies have examined effects of warfarin use per se on cancer risk (311, 312), there is no indication to date that long-term warfarin use contributes to this residual risk, and in fact a recent report suggested that chronic warfarin treatment had a protective effect on urogenital cancer in men (313). Because of the complex relation between cancer and coagulation, it may be very difficult to design experiments to explicitly target any effects on cancer risk of anticoagulation therapy due to interference with γ-carboxylation of VKD proteins.

A body of evidence, although mixed, suggests that warfarin treatment of patients with metastatic cancer can increase survival, as recently reviewed (304, 311). Inhibitory effects of warfarin on γ-carboxylation of VKD proteins known to be recruited during metastasis—gas6, tgfbi, and periostin (discussed above)—should be included in discussions of possible mechanisms.

The fact that all 3 causes of loss (genetic, dietary intake, and anticoagulant therapy) are associated with increased bone fragility after estrogen loss (suggesting osteocalcin involvement) is compelling, despite poor reproducibility among treatment trials and other uncertainties discussed above. Similarly, linkage of all 3 types of loss to increased arterial calcification (suggesting Mgp involvement), which is known to be associated with cardiovascular disease (222), is also compelling, although fewer studies have been conducted. The variously health- or disease-promoting phenotypes of Gas6 knockouts when challenged by different induced pathologies raises the important point that loss of function may, in some circumstances, have health-promoting consequences. This point is reinforced by the recruitment of other VKD-dependent proteins, including coagulation factors, Tgfbi, and periostin in cancer progression. And finally, the propensity of Tgfbi mouse knockouts to develop spontaneous cancer suggests that further studies that examine possible linkages between vitamin K intake and cancer risk should be undertaken.

IS THERE A PUBLIC HEALTH PROBLEM?

In the United States, average intake of vitamin K1 is 70–80 μg/d (314), which is below the currently recommended Adequate Intake of 90–120 μg/d (25). Generally low intakes are also reported in Ireland (315, 316) and the United Kingdom (317–319), where the general guideline for vitamin K1 intake is $\approx$70 μg/d (1 $\mu g \cdot kg^{-1} \cdot d^{-1}$) (320).

Recommended intakes of vitamin K1 are based solely on amounts required to maintain coagulation function (25, 320). It is doubtful that average intakes, although below the Adequate Intake, hinder coagulation because the Adequate Intake includes a safety factor (25). However, concern has been expressed among some experts that current intake recommendations for vitamin K1 may not be high enough to ensure adequate function of VKD proteins not involved in coagulation (eg, references 253, 321, 322).

This concern is primarily based on the fact that, although prothrombin is essentially 100% γ-carboxylated under normal conditions (47), $\approx$10–40% of serum osteocalcin remains undercarboxylated (eg, references 251, 323); percentages are considerably higher in children (324). Interpretation of the significance of these results must take into account recent evidence that osteocalcin, in addition to its role as an extracellular matrix protein in bone, may, in an uncarboxylated form, also play a hormonal role in glucose homeostasis (92, 105, 119). Thus, the percentage of fully γ-carboxylated osteocalcin in blood that is required for optimal health cannot be assumed to be 100% and remains to be determined. Nevertheless, it is clear that γ-carboxylation of osteocalcin in blood is substantially more sensitive to vitamin K1 deficiency than prothrombin, as discussed above.

On the basis of triage theory, we would predict that γ-carboxylation of *all* extrahepatic VKD proteins (eg, Mgp, Gas6, Tgfbi, and periostin) is more sensitive to vitamin K1 deficiency than is prothrombin. Because, to the extent they have been studied, there are no known functions of the uncarboxylated forms of these proteins, concentrations of vitamin K1 intake required for their optimal γ-carboxylation may be a more helpful guideline for setting new intake recommendations than concentrations required for full γ-carboxylation of osteocalcin in blood. Clearly, more research is needed to quantify the percentage of γ-carboxylation required for optimal activity of these proteins and to better understand relations between circulating and tissue forms of proteins whose main activities are not in blood.

Some experts have suggested that revised recommendations should include consideration of MK-4 or MK-7 supplements as alternative sources of vitamin K in addition to vitamin K1 for optimal health (47). After dietary intake, both MK-4 and MK-7 are distributed more rapidly and more broadly to extrahepatic tissues than vitamin K1 and thus could circumvent the higher intake requirement of vitamin K1 due to its triage allocation to the liver. Observations supporting the use of these alternatives are briefly discussed below, along with some cautionary notes that suggest that safety studies should precede any change in official dietary intake recommendations.

MK-4 is more active than vitamin K1 in extrahepatic tissues (22, 42–44) and possibly has health-promoting effects (in addition to its role in γ-carboxylation) on the expression of certain genes (22, 325). In addition, some evidence (discussed above) suggests that normal dietary intakes of menaquinones (of which MK-4 is a major component) may be inversely related to the risk of cardiovascular disease (256–258). On the other hand, MK-4 is naturally synthesized from vitamin K1 in the body, and supplying additional MK-4 could alter this natural balance.

Of particular interest relevant to the possible use of MK-7 as an additional source of vitamin K are the decreased risks of fractures and bone loss among Japanese women consuming natto, which contains high concentrations of MK-7 (246, 247, 326) (*see* reference 47 for further discussion). Natto consumption is conceivably partially responsible for the dramatically lower prevalence of atherosclerosis (327–329) and bone fragility (330–332) in Japan compared with Western countries. A causal role for MK-7, however, is not definitive and interpretation of these studies should take into account the observation that, even in a region of Japan with relatively high natto consumption [Saitama Prefecture located in Kanto I region discussed by Yaegashi et al (326)], vitamin K1 is still the predominant dietary form of vitamin K (333). Although MK-7 has a longer half-life and accumulates to higher concentrations in serum than vitamin K1 (47), experts have also cautioned that intakes of MK-7 that are relatively low compared with vitamin K1 may interfere with anticoagulant therapy due to its potency (47).

A second area of possibly serious public health concern is functional vitamin K deficiency induced by long-term anticoagulant therapy with warfarin drugs, as previously discussed (22, 42, 135, 253). These drugs are among the most prescribed in the United States: >30 million prescriptions are dispensed each year (334). As discussed above, long-term warfarin use is linked to increased arterial calcification (299–301) and bone loss or fragility (80, 135). It seems desirable, as previously suggested

(42), to seriously explore the use of alternatives to warfarin that do not interfere with γ-carboxylation in extrahepatic tissues.

And finally, many individuals do not meet recommended intakes for more than one micronutrient. Suboptimal concentrations of other micronutrients could exacerbate effects of vitamin K deficiency. For example, both calcitriol, the active form of vitamin D, and vitamin A are known to modulate Mgp expression (42). Low vitamin D status, which is quite common (335), has been linked in several studies to vascular calcification, which could be due to effects on Mgp expression (336). Calcitriol also modulates expression of the osteocalcin gene (337).

DISCUSSION

In this article, vitamin K serves as an example to test the predictions of a new theory (the triage theory) that explains why modest micronutrient deficiencies may cause age-related diseases such as osteoporosis, cardiovascular disease, and cancer. The evidence presented here is consistent with a system that prioritizes the protection of VKD functions when vitamin K1 is scarce according to their essentiality for short-term survival at the expense of functions required to maintain long-term health. Our analysis highlights what appears to be the primary mechanism that accomplishes this prioritization: the separation of coagulation factors from less essential VKD proteins by localizing their γ-carboxylation in the liver, where ingested vitamin K1 is preferentially distributed.

An additional mechanism also suggests preferential protection of γ-carboxylation in the liver. As indicated above, long-chain menaquinones (and small amounts of shorter-chain menaquinones with vitamin K activity) are stored in mitochondria in the liver but not in the extrahepatic tissues. Although these menaquinones are not considered by most experts to contribute substantially to vitamin K activity, as discussed in the text, it is difficult to rule out some role for them, possibly as a back-up system when other sources of vitamin K are scarce, as originally suggested 16 y ago (338) and as recently reviewed (22).

Evidence supporting the 3 major predictions of the triage theory

The evidence discussed here is consistent with the major predictions of the triage theory, although there are some information gaps and uncertainties. Below, the strengths and weaknesses of the evidence relative to each of these predictions are briefly discussed.

Categories of essentiality for short-term survival according to mouse knockout lethality

To conduct an analysis from the triage theory perspective, the spectrum of functions of a particular micronutrient must be categorizable according to their degree of essentiality for short-term survival. Because information on mouse knockout phenotypes was available for almost all VKD proteins with known functions, we chose to use mouse knockout lethality as a categorizing guide. Although the use of mouse KO lethality to achieve this categorization appears reasonable, the method is not perfect. It cannot be assumed that essentiality in mice will always correlate with essentiality in humans. In this case, however, the similarity of mouse knockout phenotypes and human mutant phenotypes (Table 2) suggests that the method worked reasonably well. Furthermore, in a knockout mutant, essentiality for embryogenesis trumps later metabolic needs. Thus, knockouts of genes required for embryogenesis will be lethal, even if those genes are not required for short-term survival after development. This limitation does not appear to be a factor in the case of vitamin K because all lethal knockouts were coagulation or anticoagulation factors, which are known to be critical for hemostasis throughout life.

Comparative sensitivity of VKD protein γ-carboxylation to vitamin K1 availability

A key prediction of the triage theory is that VKD proteins required for short-term survival (coagulation factors) will be more resistant to loss of vitamin K1 than those that are less essential (Mgp, osteocalcin, Gas6, Tgfbi, and periostin). As discussed, γ-carboxylation of osteocalcin is more sensitive to decreased vitamin K1 availability than prothrombin, although these are the only pair of essential and nonessential VKD proteins for which results of studies comparing efficiencies of γ-carboxylation have been published. Experiments to compare the sensitivity of γ-carboxylation of the other extrahepatic VKD proteins to vitamin K1 availability relative to prothrombin or other coagulation factors are needed to further confirm this prediction.

Linkage of decreased functionality of VKD proteins to age-related conditions

The most important outcome of this part of the analysis is that genetic impairment in osteocalcin or Mgp in both mice and humans, limited vitamin K availability, and chronic warfarin therapy are all variously linked to the same set of age-associated conditions (bone deterioration and fragility late in life and arterial calcification or other cardiovascular conditions). Although both genetic impairment in mice and vitamin K1 deficiency are linked to glucose dysregulation or insulin resistance, to our knowledge, experiments have not been conducted to examine possible linkages of chronic warfarin therapy or human osteocalcin polymorphisms to this condition.

Tgfbi (βig-h3) is an extracellular matrix protein only recently recognized to be γ-carboxylated (165). The observations that its mouse knockout is characterized by spontaneous cancer (174) and its knockdown by mitotic spindle abnormalities and centrosome amplification (173) are potentially of great importance. As discussed above, few studies have been designed to specifically target possible linkages between vitamin K intake and cancer risk. Tgfbi mutations in humans are linked to corneal dystrophy (175), and examination of the many families identified with these mutations for possible evidence of mitotic abnormalities or increased cancer risk appears warranted.

Micronutrition in disease prevention compared with disease progression

The promotion of, or resistance to, the progression of different induced diseases in otherwise apparently normal Gas6 knockout mutants and the recruitment of Tgfbi and periostin, both of which have important health-promoting functions, to further the metastatic progression of cancer (192, 232) raises an important issue concerning the potentially contradictory roles of micronutrients in disease prevention compared with disease progression. These knockout phenotypes serve as reminders that "good" functions can be recruited by pathologic processes for "bad" ends (339). In addition to the examples cited in the text, some members of the TAM (Tam, Axl, Mer) subfamily of RTKs, of which Gas6 is the primary activating ligand, are up-regulated in cancer progression (122). In fact, there is ongoing research to develop chemotherapeutic strategies to inhibit these receptors (122). Modest vitamin K deficiency could offer a therapeutically effective nutritional alternative. The triage theory, supported by evidence discussed in this review, predicts that a relatively modest degree of vitamin K deficiency that is not severe enough to interfere with coagulation could be effective in retarding these recruitments because γ-carboxylation of these VKD proteins occurs extrahepatically. Possibly improved survival of cancer patients treated with warfarin is assumed to be due to prevention of cancer-induced VTE (311), but the possibility that inhibition of γ-carboxylation of extrahepatic VKD proteins recruited for cancer progression should not be discounted as a possible mechanism. A similar mechanism involving inhibition of gas6 function has been suggested to explain the protective effects of warfarin treatment against diabetic nephropathy in streptozotocin-induced diabetes in rodents (340) and in cyclophosphamide-induced diabetes in NOD (nonobese diabetic) mice (341).

In contrast, in cases where disease progression may be hindered by a functional Gas6 [demyelination (75) and recovery from chronic anemia (71), as discussed in the text], vitamin K supplementation could be beneficial. In the absence of ongoing disease, one would expect that modest vitamin K1 deficiency would adversely affect the broad spectrum of TAM RTK functions that are enabled by Gas6 binding, as well as the health-promoting functions of Tgfbi and periostin (*see* text and Table 2). Chronic warfarin therapy would also be expected to have an adverse effect on these extrahepatic functions and could have an even greater effect on Gas6 functions because it would be expected to impair γ-carboxylation of protein S (an alternate ligand for the TAM RTK receptors) as well as Gas6.

CONCLUSIONS

The functional spectrum of vitamin K viewed through the lens of the triage theory may provide a helpful way to think about the potential effects of vitamin K1 deficiency on age-associated disease. Vitamin K1 is an excellent example of a micronutrient for which the severe and immediate clinical consequences of deficiency (bleeding) have dominated its history. This is also the case for almost all other micronutrients—eg, vitamin C and scurvy, thiamine and beriberi, niacin and pellagra, or vitamin D and rickets. In recent years, more probing scientific investigation has begun to unearth subtle long-term health effects of modest deficiencies of many micronutrients, some of which we have previously discussed (eg, references 1, 342–346). The triage theory supplies a unifying framework explaining why a crop of diseases associated with aging is emerging for so many micronutrients. It is our hope that this analysis will stimulate further efforts to redefine micronutrient adequacy on the basis of long-term effects. Methods to determine optimal micronutrient intakes on the basis of long-term needs should allow recommended intakes to be set more accurately and with less reliance on uncertain safety factors. The result may be decreased intake recommendations for some micronutrients and increased recommendations for others. This greater certainty should stimulate more aggressive public health efforts to remedy deficiencies.

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