High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme binding affinity (increased K_m): relevance to genetic disease and polymorphisms[1–3]

Bruce N Ames, Ilan Elson-Schwab, and Eli A Silver

ABSTRACT   As many as one-third of mutations in a gene result in the corresponding enzyme having an increased Michaelis constant, or K_m, (decreased binding affinity) for a coenzyme, resulting in a lower rate of reaction. 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 enzymatic activity. Several single-nucleotide polymorphisms, in which the variant amino acid reduces coenzyme binding and thus enzymatic activity, are likely to be remediable by raising cellular concentrations of the cofactor through high-dose vitamin therapy. Some examples include the alanine-to-valine substitution at codon 222 (Ala222→Val) [DNA: C-to-T substitution at nucleotide 677 (677C→T)] in methylenetetrahydrofolate reductase (NADPH) and the cofactor FAD (in relation to cardiovascular disease, migraines, and rages), the Pro187→Ser (DNA: 609C→T) mutation in NAD(P):quinone oxidoreductase 1 [NAD(P)H dehydrogenase (quinone)] and FAD (in relation to cancer), the Ala44→Gly (DNA: 131C→G) mutation in glucose-6-phosphate 1-dehydrogenase and NADP (in relation to favism and hemolytic anemia), and the Glu487→Lys mutation (present in one-half of Asians) in aldehyde dehydrogenase (NAD⁺) and NAD (in relation to alcohol intolerance, Alzheimer disease, and cancer).   *Am J Clin Nutr* 2002;75:616–58.

KEY WORDS   Genetic disease, therapeutic vitamin use, binding defect, favism, alcohol intolerance, autism, migraine headaches, single nucleotide polymorphisms, enzyme mutations, review

INTRODUCTION

High doses of vitamins are used to treat many inheritable human diseases. The molecular basis of disease arising from as many as one-third of the mutations in a gene is an increased Michaelis constant, or K_m, (decreased binding affinity) of an enzyme for the vitamin-derived coenzyme or substrate, which in turn lowers the rate of the reaction. The K_m is a measure of the binding affinity of an enzyme for its ligand (substrate or coenzyme) and is defined as the concentration of ligand required to fill one-half of the ligand binding sites. It is likely that therapeutic vitamin regimens increase intracellular ligand (cofactor) concentrations, thus activating a defective enzyme; this alleviates the primary defect and remediates the disease. We show in this review that ≈50 human genetic diseases involving defective enzymes can be remedied by high concentrations of the vitamin component of the coenzyme, and that this therapeutic technique can be applied in several other cases, including polymorphisms associated with disease risks, for which molecular evidence suggests that a mutation affects a coenzyme binding site.

The nutrients discussed in this review are pyridoxine (page 618); thiamine (page 625); riboflavin (page 627); niacin (page 632); biotin (page 637); cobalamin (page 638); folic acid (page 641); vitamin K (page 643); calciferol (page 645); tocopherol (page 645); tetrahydrobiopterin (page 646); *S*-adenosylmethionine (page 646); pantothenic acid (page 646); lipoic acid (page 647); carnitine (page 647); hormones, amino acids, and metals (page 648); and maxi B vitamins (page 649).

The proportion of mutations in a disease gene that is responsive to high concentrations of a vitamin or substrate may be one-third or greater (1–3). Determining the true percentage from the literature is difficult because exact response rates in patients are not always reported and much of the literature deals only with individual case reports. The true percentages depend on several factors, such as the nature of the enzyme, the degree of enzyme loss that results in a particular phenotype, how much a small conformational change disrupts the binding site of the particular enzyme, whether the binding site is a hot spot for mutations, and whether dietary administration of the biochemical raises its concentration in the cell. From what is known of enzyme structure, it seems plausible that, in addition to direct changes in the amino acids at the coenzyme binding site, some mutations affect the conformation of the protein, thus causing an indirect change in the binding site.

TABLE 1
Potentially vitamin-responsive polymorphisms[1]

Enzyme and EC no.	Cofactor	Nucleotide	Amino acid	Polymorphic frequency	Region where variant is found	Characterization
				%		
Methylenetetrahydrofolate reductase (NADPH) (1.5.1.20)	FAD	677C→T	Ala222→Val	TT = 10–20	Worldwide	Human enzyme shows decreased affinity for FAD
NAD(P):quinone oxidoreductase 1 (1.6.99.2)	FAD	609C→T	Pro187→Ser	TT = 4–20	—	FAD affinity is lowered
Short-chain acyl-CoA dehydrogenase (1.3.99.2)	FAD	625G→A	Gly209→Ser	$AA + AG$ = 35	Control population of an SCAD study	Mutation may affect FAD interaction
Aldehyde dehydrogenase (NAD⁺) (1.2.1.3)	NAD	—	Glu487→Lys	$KK + EK$ = 50	Asians worldwide	K_m (NAD) is increased 150-fold
Glucose-6-phosphate 1-dehydrogenase (1.1.1.49)	NADP	131C→G	Ala44→Gly	G = 11[2]	Rural south India	K_m (NADP) is increased 5-fold
Methionine synthase (2.1.1.13)	AdoCbl	2756A→G	Asp919→Gly	G = 15[2]	Control population of an MS study	Mutation is in the AdoCbl binding site
Folylpoly-γ-glutamate carboxypeptidase (3.4.19.9)	Folylpoly-γ-glutamates (dietary folates)	1561C→T	His475→Tyr	HY = 7.7 YY = 0.2	Control population of a dementia study	Enzyme activity is lowered 53%

[1] AdoCbl, adenosylcobalamin; E, glutamate; H, histidine; K, lysine; K_m, Michaelis constant; MS, methionine synthase; SCAD, short-chain acyl-CoA dehydrogenase; Y, tyrosine.
[2] Allelic frequencies.

An alternate form of a gene present in >1% of the population is called a polymorphism. Some polymorphisms that are associated with a phenotype have been shown to alter cofactor binding and affect a large percentage of the population (*see* **Table 1** for a list of the allelic frequencies of the polymorphisms discussed in this review). Our analysis of metabolic disease that affects cofactor binding, particularly as a result of polymorphic mutations, may present a novel rationale for high-dose vitamin therapy, perhaps hundreds of times the normal dietary reference intake (DRI) in some cases. This area should interest the entire health community because of the considerable percentage of the population affected by polymorphisms, many of which may have outlived their genetic usefulness. The setting of a DRI may become more complicated if a sizable percentage of the population in fact has a higher B-vitamin requirement because of a polymorphism. It seems likely that the examples listed in Table 1 will represent the beginning of a much longer list as genomics advances and awareness of remediable K_m mutants increases.

It also seems plausible that for each example of a genetic disease or polymorphism clearly involving derangement of metabolism, multiple forms of the disease exist that reflect slight increases in the enzyme K_m but that are not commonly thought of as genetic diseases. It seems likely that, as the generality of this phenomenon is appreciated, this approach will be found to be effective on a wider scale and with a larger variety of enzyme substrates and cofactors. The administration of high doses of vitamins may reverse, at least partially, many more genetic diseases than those described here [*see* Online Mendelian Inheritance in Man (OMIM) (4) for extensive, referenced reports on human genetic diseases]. To facilitate the collection and organization of similar data, we have created a forum on the Internet (www.KmMutants.org; 5) that will house dialogues on the data and ideas brought forth in this review; investigators in relevant disciplines are encouraged to correct or add to these discussions.

There are ≈40000 human genes. Of the 3870 enzymes catalogued in the ENZYME database (6), 860 (22%) use a cofactor. Any cofactor used by many enzymes is of particular interest, such as the 8 vitamin-derived coenzymes discussed in this review. Although high-dose vitamin remediation seems to be routinely tried for diseases involving enzymes dependent on pyridoxal-*P* (PLP) and thiamine pyrophosphate (TPP), some of the vitamins, such as riboflavin, pantothenate, folate, and niacin, deserve more attention. Thus, www.KmMutants.org is also intended for the input of physicians, who may examine the benefits of high-dose multivitamin treatment (*see* the section on maxi B vitamins) for mental or metabolic disorders of unknown cause or report side effects of vitamin treatment. Provided safe dosages are used (**Table 2**), there is potentially much benefit and possibly little harm in trying high-dose nutrient therapy because of the nominal cost, ease of application, and low level of risk. Most of the vitamins discussed here appear safe in relatively high doses because the body can discard excess.

Therapeutic remediation is contingent on increasing intracellular vitamin and cofactor concentrations; therefore, we present some data on plasma and tissue concentrations of coenzymes after the feeding of various amounts of vitamins (such information is sparse for some vitamins, especially at high doses). Data on plasma concentrations of amino acids after the administration of these metabolites would be desirable as well. Metals such as Mg^{2+}, Ca^{2+}, Zn^{2+}, Fe^{2+}, and K^+ are used by many enzymes, but the increases in concentration obtainable may be small because of toxicity limitations and physiologic regulation. For example, zinc is a cofactor for >300 proteins, but the human Zn^{2+} requirement is ≈10 mg/d and symptoms of toxicity can appear at 100 mg/d (10). Ascorbate concentrations are tightly regulated in young men and women and there is an upper limit on steady state plasma ascorbate concentrations of ≈80 μmol/L (11, 12). At the

TABLE 2
Dietary reference intakes (DRIs), tolerable upper intake levels (ULs), and mega-doses of nutrients discussed in this review

Nutrient	DRI[1]	UL[2]	Mega-dose[3]
Pyridoxine (vitamin B-6)	1.3 mg	100 mg	1000 mg
Thiamine (vitamin B-1)	1.1 mg	—	1000 mg
Riboflavin (vitamin B-2)	1.1 mg	—	400 mg
Niacin (vitamin B-3)	14 mg	35 mg	2000 mg
Biotin (vitamin B-7)	30 μg	—	100 000 μg
Cobalamin (vitamin B-12)	2.4 μg	—	1000 μg
Folic acid	400 μg	1000 μg	40 000 μg
Vitamin K	90 μg	—	45 000 μg
Calciferol (vitamin D)	5 μg	50 μg	5000 μg
Tocopherol (vitamin E)	15 mg	1000 mg	800 mg
Tetrahydrobiopterin	—	—	40 mg
S-Adenosylmethionine	—	—	800 mg
Pantothenic acid	5 mg	—	150 mg
Lipoic acid	—	—	300 mg
Carnitine	—	—	2000 mg
Thyroid hormone	—	—	1.75 mg
Serine	—	—	500 mg·kg^{-1}·d^{-1}
Glycine	—	—	200 mg·kg^{-1}·d^{-1}
Zinc	8 mg	40 mg	—
Potassium	2000 mg	—	—

[1] Daily values for female adults; values for males are similar. From references 7–9.

[2] The maximum daily nutrient intake that is likely to pose no risk of adverse effects. If no value is listed, either not enough data are available to establish a UL or no data are available.

[3] Upper doses used clinically as found in the literature; side effects were of less concern because of the disease severity but may accompany these mega-doses.

end of each vitamin section, we discuss the toxicity of the vitamin as well as data on raising tissue concentrations. Note that some reports discussed below did not ascertain the minimal necessary therapeutic level of treatment, but instead used high doses that were thought would produce the desired effect. This treatment strategy is obviously not feasible with all nutrients (because of possible toxicity). Additionally, it is likely that not all administered vitamin is absorbed at very high doses (*see* the discussion of tissue concentrations and toxicity in the section on riboflavin). In an ideal situation, the lowest adequate therapeutic dosage would be elucidated and used.

Nutritional interventions to improve health are likely to be a major benefit of the genomics era. Many coenzyme binding motifs have been characterized, and essential residues for binding have been elucidated. Structural data can be found at the beginning of many sections. It will soon be possible to identify the complete set of genes having cofactor binding sites and the polymorphisms that fall into these regions, with an end goal of using vitamins, and possibly amino acids, hormones, and minerals, to effect a metabolic "tune-up."

Support for some of the views discussed here can be found in the literature. It is clear that many individual researchers have recognized that high-dose vitamin treatment is effective in particular diseases because a mutation affects the affinity of an enzyme for its coenzyme. In particular, Linus Pauling (13) hypothesized in his review entitled *Orthomolecular Psychiatry* that much mental disease may be due to insufficient concentrations of particular biochemicals in the brain as the result of an inadequate intake of particular micronutrients and that some

brain dysfunction may be due to mutations that affect the K_m of enzymes: "The still greater disadvantage of low reaction rate for a mutated enzyme with K[m] only 0.01 could be overcome by a 200-fold increase in substrate concentration to [S] = 400. This mechanism of action of gene mutation is only one of several that lead to disadvantageous manifestations that could be overcome by an increase, perhaps a great increase, in the concentration of a vital substance in the body. These considerations obviously suggest a rationale for megavitamin therapy." More recently, high-dose pyridoxine therapy has been suggested as a treatment for improving dysphoric psychological states (eg, loneliness, anxiety, hostility, and depression) by stimulating the production of 2 pyridoxine-dependent neurotransmitters, serotonin and γ-aminobutyric acid (14).

Although he does not discuss binding defects, Roger Williams (15), another pioneer in the field of biochemical nutrition, also recognized that higher doses of vitamins may be necessary to accommodate for what he calls biochemical individuality: "Individuality in nutritional needs is the basis for the genetotrophic approach and for the belief that nutrition applied with due concern for individual genetic variations, which may be large, offers the solution to many baffling health problems. This certainly is close to the heart of applied biochemistry." [Human genetic variation appears greater than previously thought (16).] Williams' conclusions suggest that genetic and thus biochemical individuality necessitates much nutritional individuality. This is especially relevant in the dawning age of genomics, in which it will someday become routine to screen individuals for polymorphisms and thus treat persons more efficaciously by genotype, rather than just by phenotype.

It also appears that, during aging, oxidation deforms many proteins, thereby decreasing their affinity for their substrates or coenzymes (17). Mechanisms of protein deformation include direct protein oxidation, adduction of aldehydes from lipid peroxidation, and, in the case of membrane proteins, decreases in fluidity of oxidized membranes. This oxidative decay is particularly acute in mitochondria (18–20). Thus, feeding high amounts of several mitochondrial biochemicals may reverse some of the decay of aging (17, 21–26). Fourteen genetic diseases due to defective mitochondrial proteins are discussed in this review.

The impetus for this review arose while teaching an undergraduate laboratory course in which the students isolated bacterial mutants that grew on a complex medium but not on a minimal medium and characterized the defective gene and pathway. An appreciable percentage of mutant phenotypes could be explained by an increased K_m (decreased affinity) of an enzyme, which could then be remedied by higher concentrations of the coenzyme or substrate (27).

PYRIDOXINE (VITAMIN B-6)

The DRI for vitamin B-6 is 1.3 mg/d for adults (7). In the liver, pyridoxine and pyridoxal (an oxidized form of pyridoxine) are phosphorylated by pyridoxal kinase to form pyridoxine-*P* and PLP, the active cofactor form. Pyridoxine-*P* is oxidized to PLP by pyridoxine oxidase (7). PLP is utilized by 112 (3%) of the 3870 enzymes catalogued in the ENZYME database (6). The cofactor forms a covalent linkage (Schiff base) with a lysyl residue in the enzyme. This internal aldimine (enzyme-PLP) is converted to an external aldimine (substrate-PLP) when PLP is attacked by a substrate amino group. The PLP binding site has

been elucidated for some enzymes (28) and may be useful for probing genomic sequences for homology. The PLP-requiring enzymes discussed in this section are summarized in **Table 3**.

Ornithine aminotransferase: gyrate atrophy of the choroid and retina

Ornithine aminotransferase (OAT; ornithine–oxo-acid transaminase) is a PLP-dependent mitochondrial matrix protein that catalyzes the breakdown of ornithine to δ-pyrroline-5-carboxylic acid, which is then converted into proline. Defects in OAT lead to gyrate atrophy of the choroid and retina, an autosomal recessive disease that affects persons of all ages (*see* OMIM 258870). The disease is characterized by slowly progressive chorioretinal degeneration leading to blindness. Ornithine accumulates 10- to 15-fold when the enzyme is defective and appears to be responsible for much of the pathology of gyrate atrophy (29). In the pyridoxine-responsive forms of the disease, for which doses ranged from 10 to 750 mg/d, it appears that the defective enzyme has a K_m defect for PLP; ornithine accumulation is decreased when patients are given high doses of pyridoxine.

The OAT activity in fibroblast extracts of a pyridoxine-responsive patient with the alanine-to-valine substitution at codon 222 (Ala226→Val) increased from 9 to 44 nmol product·mg^{-1}·h^{-1} when the concentration of PLP in the assay was increased to 600 μmol/L. The K_m from the cell line of a second patient with the same Ala226→Val mutation was 122 μmol/L (control K_m: 6 μmol/L). Similar to cells from the second patient, Chinese hamster cells expressing an OAT complementary DNA (cDNA) producing the Ala226→Val protein also exhibited increased OAT activity with the addition of pyridoxine (30).

Vitamin B-6–responsive and -nonresponsive patients with gyrate atrophy were shown to have different point mutations resulting in single amino acid changes in the mature enzyme (31). After incubation with 40 μmol PLP/L, OAT activity increased substantially more in fibroblasts from carriers of the pyridoxine-responsive variant than in fibroblasts from control subjects and nonresponsive patients (32). These investigators concluded that "the greater increases in activity seen in pyridoxine-responsive cells when PLP was added to the assay suggest both that the holoenzyme content in these cells is decreased owing to low affinity and that PLP binding to the apoenzyme occurs at a higher concentration."

In another study, 3 patients responded to oral vitamin B-6 (600–750 mg/d) with a decrease in serum ornithine and a return to normal of reduced concentrations of serum lysine. Lower doses of vitamin B-6 (18–30 mg/d) appeared to work just as well as the high doses (33).

In another study of 9 patients with gyrate atrophy (34), 4 patients responded to pyridoxine, which lowered serum ornithine by ≥50% in 3 cases. The K_m (of OAT for PLP) was 23 μmol/L in the control subjects, 23 μmol/L in the pyridoxine-nonresponsive patients, and 168 μmol/L in the pyridoxine-responsive patients. This higher K_m for pyridoxine-responsive patients could be explained by mutations in the binding site that severely reduce coenzyme affinity, whereas nonresponsive patients may harbor more severe mutations that affect a different area of the enzyme.

In a study of Japanese patients in which 1 of 7 patients (all with different mutations) responded to vitamin B-6, the pyridoxine-responsive mutation was found to be Thr181→Met, but the affinity for PLP was not measured (35). In another Japanese study, one patient (of 3) responded to vitamin B-6 (300–600 mg/d) with a

60% reduction in serum ornithine concentrations. OAT activity in the fibroblasts from this patient increased up to 25% of normal levels in the presence of 2000 μmol PLP/L, although no significant improvement was observed in acuity or visual field. Thus, vitamin B-6 responsiveness may be due to a mutation in OAT that results in a high K_m for PLP (36, 37). A Glu318→Lys mutation of the *OAT* gene was found in 3 heterozygous patients and 1 homozygous patient, all of whom were vitamin B-6-responsive according to previous in vivo and in vitro studies. Dose-dependent effects of the Glu318→Lys allele were observed in the homozygotes and heterozygotes in *1*) OAT activity, *2*) increase of OAT activity in the presence of PLP, and *3*) apparent K_m for PLP with these values approximately doubled in the homozygous individual compared with the heterozygotes. Thus, the highest residual level of OAT activity and mildness of clinical disease correlated directly with the higher number of the mutant Glu318→Lys allele found in the homozygous patient (38).

Many case reports of gyrate atrophy exist; as of 1995, pyridoxine-responsiveness had been observed in 7 of the ≈150 total documented cases (7/150 = 5%) (39). Whether pyridoxine treatment was actually attempted in each of these cases is unclear. Thus the true response rate may be higher or lower than 5%.

Cystathionine β-synthase: homocystinuria

Cystathionine β-synthase (CBS) of the transsulfuration pathway catalyzes the PLP-dependent condensation of homocysteine and serine to form cystathionine. Individuals carrying a defective form of this enzyme (*see* OMIM 236200) accumulate homocysteine in the blood and urine and display a wide range of symptoms that appear to be due to homocysteine toxicity, including mental retardation, vascular and skeletal problems, and optic lens dislocation. Barber and Spaeth (40) were the first to report pyridoxine-responsiveness with a complete return to normal of the patient's methionine and homocysteine concentrations in plasma and urine. They speculated that "if the deficient enzymatic activity were due to decreased affinity of a defective apoenzyme for its cofactor, activity might be restored by increasing the intracellular concentration of pyridoxal phosphate" (40).

Kim and Rosenberg (41) showed that CBS activity was 5% of that of control subjects in pyridoxine-responsive homocystinuric patients, who had markedly elevated plasma and urinary concentrations of methionine and homocystine. The mutant synthases had a 20-fold lower affinity for PLP. A 2- to 3-fold increase in the K_m for homocysteine and serine was found in one vitamin B-6-responsive patient, although the K_m for PLP was not measured. The maximum reaction rate (*V*max) was also reduced. It was suggested that pharmacologic doses of pyridoxine led to increased cellular concentrations of PLP and increased enzymatic activity (41).

One group showed cell lines from pyridoxine-responsive patients to have higher K_m values for PLP (155, 145, 195, and 200 μmol/L) than control values (52, 52, and 85 μmol/L), whereas nonresponsive patients had the highest values (990 and 4000 μmol/L). It was noted that, in general, about one-half of CBS-deficient patients respond to pyridoxine with a lowering of homocysteine and serine concentrations to normal (42). A 21-y-old pyridoxine-responsive individual had a 3- to 4-fold elevated apparent K_m of CBS for PLP as measured in fibroblast extracts (43). An Ala114→Val substitution was present in this individual, which is only 5 residues away from the lysine residue, Lys119, that binds PLP. These investigators concluded that in vivo

TABLE 3
Enzymes that use a pyridoxal-*P* (PLP) cofactor[1]

Defective enzyme and EC no.	Localization	Reaction catalyzed	Disease or condition	OMIM no.	Inheritance
Ornithine aminotransferase (2.6.1.13)	Mitochondrial matrix protein	Ornithine + α-ketoglutarate → Δ-pyrroline-5-carboxylate + glutamate	Gyrate atrophy of choroid and retina, degrading sight to eventual blindness	258870	Autosomal recessive
Cystathionine β-synthase (4.2.1.22)	Cytoplasmic	Homocysteine + serine → cystathionine	Homocystinuria, optic lens dislocation, osteoporosis, skeletal abnormalities, and mental retardation	236200	Autosomal recessive
Erythroid specific δ-aminolevulinic acid synthase (2.3.1.37)	Mitochondrial	Glycine + succinyl-CoA → δ-aminolevulinic acid + CoA + CO_2	X-linked sideroblastic anemia	301300	X-linked recessive
Kynureninase (3.7.1.3)	Cytoplasmic	(Hydroxy-)kynurenine + H_2O → (hydroxy-) anthranilic acid + alanine	Mental retardation	236800	Autosomal recessive
Glutamic acid decarboxylase (4.1.1.15)	Cytoplasmic	Glutamic acid → GABA	Infantile seizures unresponsive to typical anticonvulsants	266100	Autosomal recessive
γ-Cystathionase (4.4.1.1)	Cytoplasmic	Cystathionine → cysteine + α-ketobutyrate	Mental retardation, convulsions, thrombocytopenia, nephrogenic diabetes insipidus, and diabetes mellitus	219500	Autosomal recessive
Alanine–glyoxylate aminotransferase (2.6.1.44)	Peroxisomal	Alanine + glyoxylate → pyruvate + glycine	Hyperoxaluria, kidney (calcium oxalate) deposits, and renal failure	259900	Autosomal recessive
Aromatic-L-amino-acid decarboxylase (4.1.1.28)	Cytoplasmic	L-DOPA → dopamine + CO_2; 5-hydroxytryptophan → serotonin + CO_2	Serotonin and dopamine deficiency, developmental delay, hypotonia, oculogyric crises, and irritability	107930	Autosomal recessive
Housekeeping δ-aminolevulinic acid synthase (2.3.1.37)	Mitochondrial	Glycine + succinyl-CoA → δ-aminolevulinic acid + CoA + CO_2	Sideroblastic anemia	125290	Autosomal recessive
β-Alanine–α-ketoglutarate transaminase (2.6.1.19)	—	β-Alanine → malonic semialdehyde	Cohen syndrome, hypotonia, midchildhood obesity, mental deficiency, and facial, oral, ocular, and limb anomaly	216550	Autosomal recessive
Enzyme involved with neurotransmitter metabolism (?)	—	—	Autism	209850	—

[1] DOPA, dihydroxyphenylalanine; GABA, γ-aminobutyric acid; OMIM, Online Mendelian Inheritance in Man (4).

responsiveness in individuals with some residual CBS activity is related both to the affinity of the mutant aposynthase for PLP and to the capacity of cells to accumulate PLP (43).

In one report (44), a G-to-A substitution at nucleotide 797 (797G→A; amino acid substitution: Arg266→Lys) was found in most pyridoxine-responsive patients. Seven of 12 patients were responsive to pyridoxine (40–900 mg/d), which greatly decreased total plasma homocysteine.

Pyridoxine (50–1000 mg/d) markedly reduced homocysteine excretion in a group of pyridoxine-responsive patients: patient 1, 867 to 10 μmol/d; patient 2, 1021 to 79 μmol/d; patient 3, 15 μmol/L (blood concentration) to undetectable concentrations; and patient 4, plasma amino acids reverted to normal (45). It appears that a missense mutation (Ile278→Thr) is common (41%) in pyridoxine-responsive patients and that patients who are responsive to pyridoxine usually have a milder clinical phenotype than do nonresponsive patients.

The idea that pyridoxine-responsive patients have an increased K_m was supported in a review of the mechanism of pyridoxine-responsive disorders (46). A review of the CBS deficiencies (2) found 629 patients in the literature: 231 (37%) were vitamin B-6–responsive, 231 (37%) were vitamin B-6–nonresponsive, 67 (11%) were intermediate in response, and 100 (16%) had not been classified. A decade later, the field was reviewed again and it was suggested that dosages of pyridoxine of 500 mg/d for 2 y appear to be safe, but that 1000 mg/d should not be exceeded (47).

A database of mutations in CBS (48) lists and maps >100 pathogenic mutations (including >70 missense mutations) that span all 7 exons of the CBS gene. Although the Schiff-base forming lysine has been assigned to nucleotide 119 in exon 3, it is difficult to say which domains are responsible for PLP binding. Serine should be tested clinically in addition to pyridoxine for treating patients, with the use of homocysteine concentrations as a measure of efficacy, because the K_m of CBS for serine was shown to be increased in several cases (41). Oral serine administration (500 mg·kg^{-1}·d^{-1}) raises serine concentrations in plasma and cerebrospinal fluid (49), although very high doses (1400 mg·kg^{-1}·d^{-1}) can result in adverse effects (50).

Vitamin B-6-therapy may be valuable in more than just severe homozygous CBS-deficient cases: heterozygous parents of CBS-deficient patients also have significantly increased homocysteine concentrations (51). Increased homocysteine is a risk factor for cardiovascular disease (52). Heterozygosity for CBS deficiency may be present in 1% or 2% of the population.

Erythroid specific δ-aminolevulinic acid synthase: X-linked sideroblastic anemia

Erythroid specific δ-aminolevulinic acid synthase (ALAS2; 5-aminolevulinate synthase), with its PLP cofactor, is located in the mitochondria of animal cells and catalyzes the condensation of glycine and succinyl-CoA to form δ-aminolevulinic acid, the first and rate-limiting step in the series of reactions that makes heme for incorporation into hemoglobin. Defects in ALAS2 are responsible for the most common inherited form of sideroblastic anemia, which is X-linked (see OMIM 301300). Because iron is transported to the mitochondria whether or not it is combined with heme, deficiencies in heme lead to iron deposits in erythroblast mitochondria and increased ringed sideroblasts in the marrow (53). About one-third of patients with sideroblastic anemia respond to pyridoxine (1), with doses ranging from 50 to 600 mg/d.

Three generations in a family originally described by Cooley in 1945 were found to have an ALAS2 gene with an A-to-C point mutation that results in an ALAS2 variant with reduced activation by PLP. The specific activity of the mutant enzyme was ≈26% of normal in the presence of 5 μmol PLP/L. The PLP cofactor activated or stabilized the purified mutant enzyme in vitro, consistent with the pyridoxine-responsive anemia in affected patients. It was hypothesized that the mutation alters the local secondary structure and possibly perturbs the overall conformation, thus decreasing stability, reducing the affinity for PLP, or both (54).

A point mutation (663G→A) in the ALAS2 gene of an 8-mo-old Japanese male led to pyridoxine-responsive sideroblastic anemia. The activity of the mutant enzyme (Arg204→Gln) expressed in vitro was 15% of that of the control; with the addition of PLP, the activity of the mutant enzyme increased to 35% (55).

Among 6 other cases (1 patient and 5 kindreds), 4 had an amino acid substitution at a PLP binding site of ALAS2 that reduced the affinity of ALAS2 for its coenzyme (1, 54, 56–58). One of the ALAS2 mutations, Gly291→Ser, reduced enzyme activity to 10% of normal; enzymatic activity was increased with the addition of PLP in vitro (57). In another mutation, Thr388→Ser, activity was decreased to ≈50% of wild-type but was raised by pyridoxine supplementation (1). A mutation found in a highly conserved region of exon 9, Ile471→Asn, was found in a 30-y-old Chinese man with the pyridoxine-responsive form of XLSA (56). Prokaryotic expression of the normal and mutant cDNAs showed that the mutant construct had lower enzymatic activity than did the normal enzyme and required higher concentrations of PLP to achieve maximal activation. The amino acid substitution occurred in the exon containing the putative PLP binding site, which may account for the reduced ability of the enzyme to catalyze the formation of δ-aminolevulinic acid. Another study showed that a large number of probands have mutations in exon 9, the exon containing the PLP binding site (59). Furuyama et al (60) reported an ALAS2 mutation that results in 12% of normal ALAS activity, which increases to 25% in the presence of PLP.

A novel missense mutation in the ALAS2 gene, 1754A→G, in a patient with 53% ALAS activity had 20% activity when expressed in bacteria but 32% in the presence of PLP. Although the mutation, which results in the substitution of glycine for serine, lies outside of exon 9, it is possible that it induces a conformational change that may alter PLP binding to the protein (61). Other mutations located outside exon 9 have also been reported to influence PLP binding (53).

Two cases that appeared late in life have also been analyzed (58). A 77-y-old man and an 81-y-old woman with initial diagnoses of refractory anemia with ringed sideroblasts (which is typically unresponsive to pyridoxine) were found to respond very well to pyridoxine (100 mg/d in the man and 600 mg/d in the woman); hemoglobin concentrations increased in both patients after treatment. The mutations Lys299→Gln and Ala172→Thr were found in the man and woman, respectively. The Ala172→Thr mutation resulted in decreased in vitro stability of bone marrow ALAS2 activity. ALAS2 from both patients showed marked thermolability. Addition of PLP in vitro stabilized the mutant enzymes, which is consistent with the observed in vivo response to pyridoxine. This late-onset form can be distinguished from refractory anemia and ringed sideroblasts by microcytosis, pyridoxine responsiveness, and ALAS2 mutations. These findings emphasize the need to consider all elderly patients with microcytic sideroblastic anemia as candidates for ALAS2 defects, especially if pyridoxine-responsiveness is demonstrated. These investigators concluded, "A decline in PLP

availability or metabolism may have precipitated the late onset of XLSA in these patients. An age-related decline in pyridoxine metabolism in combination with a reduced vitamin intake has been described in elderly populations" (58).

It appears that supplementation with glycine, as well as pyridoxine, may be beneficial in new patients and that supplementation with glycine may be beneficial in patients who do not respond to pyridoxine, because the K_m for glycine may be affected in some ALAS mutations. For example, the Gly142→Cys constructed mutant has a 4-fold increased K_m for glycine (62). If a patient had such a mutation, increased plasma glycine concentrations might increase ALAS activity. One report showed an increase in plasma and cerebrospinal fluid glycine after the administration of 200 mg·kg^{-1}·d^{-1} (49).

Kynureninase: xanthurenic aciduria and mental retardation

Kynureninase, a PLP-requiring enzyme involved in tryptophan degradation, catalyzes the conversion of kynurenine and 3-hydroxykynurenine to anthranilic acid and 3-hydroxyanthranilic acid, respectively (*see* OMIM 236800). Mutations in the kynureninase gene cause mental retardation in children and an excessive urinary output of 3-hydroxykynurenine and kynurenine (and their metabolites, xanthurenic and kynurenic acids). The condition was normalized in 2 children with pyridoxine doses of ≤30 mg/d. Significantly decreased kynureninase activity in a liver biopsy sample was markedly increased with the addition of PLP, suggesting that a mutation caused a modification in the binding site of the coenzyme (63). A follow-up study confirmed that the defective enzyme was a K_m mutant (64). (See the discussion of autism in this section.)

Glutamic acid decarboxylase: seizures in newborns and intelligence quotient deficits

Glutamic acid decarboxylase (GAD; glutamate decarboxylase), a PLP enzyme, converts glutamic acid, an excitatory amino acid, to γ-aminobutyric acid, the most important inhibitory neurotransmitter in the central nervous system (up to one-third of synapses in the brain use γ-aminobutyric acid as an inhibitory signal). Defects in GAD result in seizures in newborns (*see* OMIM 266100), but it is not clear whether the seizures are due to too little γ-aminobutyric acid or too much glutamic acid (65). Intravenous injection of 100–200 mg pyridoxine generally stops the seizures (66). One infant with pyridoxine-dependent seizures was shown to have decreased γ-aminobutyric acid production; the seizures stopped within 5 min of the administration of 100 mg pyridoxine. More than 50 cases of pyridoxine-dependent seizures have been reported since 1954 (67).

In one study of 28 infants with seizures, 3 infants had the pyridoxine-responsive phenotype (68). In a study of 120 infants with documented repeated and intractable seizures, only 2 infants responded to pyridoxine administration, suggesting that either only a small percentage of seizures are responsive or that there are many other causes of seizures that are not due to mutations in this vitamin B-6 enzyme (69). There appear to be other enzyme defects that can lower PLP and cause pyridoxine-dependent seizures: one patient had decreased γ-aminobutyric acid and increased glutamic acid in the brain, but no significant difference in GAD activity was found between the patient and control subjects, and PLP concentrations were markedly reduced (70).

In a cell line from an infant with pyridoxine-dependent seizures, GAD activity was increased when the enzyme was incubated with high PLP. The investigators speculated that the metabolic abnormality in this disorder may be a binding abnormality between GAD apoenzyme and PLP (71).

A 13-y-old child who died with seizures in progress had elevated glutamic acid and decreased γ-aminobutyric acid concentrations in the frontal and occipital cortices but not in the spinal cord; concentrations of all other amino acids, except for cystathionine, were normal. PLP was reduced in the frontal cortex, and GAD activity comparable to that of control subjects was detected when the PLP concentration was >50 μmol/L (70). Response rates from 2 reports give a cumulative pyridoxine-responsiveness of 3%, although seizures may be due to many different defective genes (68, 69).

Intelligence quotients are decreased in pyridoxine-dependent GAD patients, suggesting that the amount of pyridoxine administered should be adjusted to optimally retain intellectual capacity, and not just to stop seizures. A prospective open study found that an increased dose of pyridoxine was associated with an improvement in intelligence quotient. It was suggested that pyridoxine dependency has a wider range of clinical features than classic neonatal seizures and causes specific impairments of higher function, some of which may be reversible by vitamin B-6 therapy (72).

A treatment of asthma, theophylline, depresses PLP concentrations, and may cause seizures by decreasing γ-aminobutyric acid production. Pyridoxine treatment reduces theophylline-induced seizures in both mice and rabbits (73).

A linkage analysis study of 2 families argued that pyridoxine-dependent seizures are not due to a K_m mutation because the base pair substitutions found in the patients' enzymes were also found in control subjects, and different maternal alleles were passed on to 2 affected children in one family (74). However, some forms of pyridoxine-dependent seizures, which are likely a disease of multiple etiologies, are probably due to K_m defects affecting PLP binding by GAD (75).

γ-Cystathionase: cystathioninuria, mental retardation, and diabetes

After the formation of cystathionine by CBS, another PLP enzyme in the transsulfuration pathway, γ-cystathionase (cystathionine γ-lyase), converts cystathionine into cysteine and α-ketobutyrate, completing the transfer of sulfur from homocysteine to cysteine. Enzymatic defects (*see* OMIM 219500) result in cystathionine accumulation in the urine and tissues. The clinical features can include mental retardation, convulsions, thrombocytopenia, nephrogenic diabetes insipidus, and diabetes mellitus. High-dose pyridoxine therapy can markedly reduce concentrations of cystathionine in the urine and blood of deficient patients; it was suggested that vitamin B-6 responsiveness "can best be explained by a structural alteration of the apoenzyme, resulting in failure to combine normally with the coenzyme" (76). This binding theory is supported by others: "The B-6-responsive form results from the synthesis of an aberrant enzyme protein exhibiting altered interaction with the coenzyme, thereby resulting in an inherited increase in the requirement for vitamin B-6" (77). A high percentage of the cases can be ameliorated by supplementation with pyridoxine, which is associated with a reactivation of the defective enzyme and a major decrease in urinary cystathionine excretion; 33 of 37 cases (89%) were found to be pyridoxine-responsive (47). This high percentage is puzzling; one possible explanation is that more severe mutant genes cause

lethality and that most of the remaining genes code for a protein with a partial activity and increased K_m.

Alanine–glyoxylate aminotransferase: hyperoxaluria and renal failure

Alanine–glyoxylate aminotransferase is a liver-specific enzyme that uses a PLP cofactor to transfer the amino group from alanine to glyoxylate, forming serine and pyruvate. A primary hyperoxaluria (*see* OMIM 259900) caused by a functional deficiency of the peroxisomal alanine–glyoxylate aminotransferase results in an accumulation of glyoxylate that is converted to oxalate, resulting in renal deposits of calcium oxalate and renal failure. In one study, large doses of pyridoxine reduced urinary oxalate excretion in 2 of 3 patients with primary hyperoxaluria (78). Posttreatment oxalate concentrations were between pretreatment and control concentrations, and the effect of pyridoxine was maintained for 6 mo.

A review of hyperoxaluria indicates that pharmacologic doses of pyridoxine are of benefit and that a K_m mutant may be responsible. Pyridoxine treatment may overcome the effects of mutations in the gene encoding alanine–glyoxylate aminotransferase that might interfere with cofactor binding (79). It is suggested that as many as 30% of patients with type I primary hyperoxaluria respond to pyridoxine (80). A review of pyridoxine treatment, which discussed 2 recent reports including 18 patients, stated that 50% of patients are unresponsive to pyridoxine, whereas oxaluria is normalized in 20% of patients and somewhat reduced (but not to normal concentrations) in the remaining 30% (81).

Physicians may consider treating with alanine in addition to pyridoxine to determine the optimum cocktail for minimizing oxalate accumulation. We have not seen any reports in which plasma alanine concentrations were measured after the administration of high doses.

Aromatic-L-amino-acid decarboxylase: developmental delay

Aromatic-L-amino acid decarboxylase (AAD; *see* OMIM 107930) is a homodimeric PLP-containing enzyme synthesizing 2 important neurotransmitters: dopamine and serotonin (82). After the hydroxylation of tyrosine to form dihydroxyphenylalanine, catalyzed by tyrosine hydroxylase, AAD decarboxylates dihydroxyphenylalanine to form dopamine. Dopamine is sequentially broken down to dihydroxyphenylacetaldehyde by monoamine oxidase B [amine oxidase (flavin-containing)], to dihydroxyphenylacetic acid by aldehyde dehydrogenase, and finally to homovanillic acid by catechol *O*-methyltransferase. Tryptophan 5-monooxygenase produces 5-hydroxytryptophan, which is also decarboxylated by AAD to give rise to serotonin. Serotonin is broken down to 5-hydroxyindoleacetic acid. AAD deficiency is an autosomal recessive inborn metabolic disorder characterized by combined serotonin and dopamine deficiency.

The first reported cases of AAD deficiency were monozygotic twins with extreme hypotonia and oculogyric crises (83). AAD activity was severely reduced and concentrations of dihydroxyphenylalanine and 5-hydroxytryptophan were elevated in cerebrospinal fluid, plasma, and urine. Pyridoxine (100 mg/d) lowered dihydroxyphenylalanine concentrations in cerebrospinal fluid, but treatment with either bromocriptine or tranylcypromine was required for clinical improvement. Another AAD-deficient patient, with similar presentation, also had greatly reduced activity of AAD in plasma (84). Similar to the first reported cases, combined treatment with pyridoxine, bromocriptine, and tranylcypromine produced some clinical improvement. Several other cases of AAD deficiency have apparently benefited from high-dose pyridoxine treatment (85).

Housekeeping δ-aminolevulinic acid synthase: sideroblastic anemia

The mapping of a second δ-aminolevulinic acid synthase (5-aminolevulinate synthase) gene, *ALAS1*, to an autosome, chromosome 3, rules it out as the site of the primary defect in X-linked sideroblastic anemia. It was concluded that this gene is a housekeeping form of ALAS (*see* OMIM 125290) because it is expressed in all cell types including erythroid cells; thus, the gene is designated *ALAS1* to distinguish it from the red cell–specific form, *ALAS2* (86).

In a study of 20 patients with sideroblastic anemia, 3 patients showed low ALAS activity that was corrected by PLP in vitro, and 2 other patients were found to be responsive to pyridoxine (20 mg/d) (87). When 1 of these 2 patients was taken off pyridoxine, ALAS activity, as measured in bone marrow, fell markedly unless PLP was added in vitro. Additionally, the K_m of the enzyme for PLP was substantially greater (2.5 times) than that of a control sample. However, it is unclear whether these are ALAS1 or ALAS2 defects.

A 70-y-old who exhibited an attack of polymorphic, hypochronic anemia, with increased serum iron and numerous ringed sideroblasts in the bone marrow, was determined to have pyridoxine-responsive primary acquired sideroblastic anemia (88). Administration of pyridoxine (initially 200 mg/d, then 600 mg/d) caused a complete remission of all hematologic abnormalities. ALAS activity was increased to 50% of control with 600 mg pyridoxine/d. The activity could be further increased to 100% of control in vitro with 1000 μmol/L PLP. This defect could also be in ALAS1 or ALAS2.

β-Alanine α-ketoglutarate transaminase: Cohen syndrome

β-Alanine α-ketoglutarate transaminase (AKT; 4-aminobutyrate aminotransferase) is involved in the formation of malonic semialdehyde from β-alanine. Children with AKT deficiency have Cohen syndrome (*see* OMIM 216550), which involves hypotonia, midchildhood obesity, mental deficiency, and facial, oral, ocular, and limb anomalies. A case report of a girl with features of the syndrome reported a response to 100 mg pyridoxine/d for 1 mo, with a normalization of electroencephalogram and a subsiding of lethargy. The girl was hospitalized once when she missed a week of pyridoxine treatment, but reinstatement of the treatment resulted in more improvement. Cultured skin fibroblasts from the girl showed a toxic response to β-alanine with a 50% reduction in growth. The addition of 100 μmol pyridoxine/L to the cells abolished the toxic effects and increased AKT activity more than 2-fold (89).

Autism

Autism (a developmental disorder that involves impaired social interactions and deviant behavior) and its associated behaviors are thought to affect 5 in 10000 individuals [and as many as 1 in 300 in some US communities (90)]. Autism may be due to defects in a PLP-requiring enzyme or enzymes involved in the metabolism of serotonin and dopamine, although a genetic link to a vitamin B-6–requiring enzyme has not been established. The most replicated clinical sign of autism is an elevation of whole-blood serotonin (5-hydroxytryptamine), which is found in

>30% of patients (91). Increased concentrations of homovanillic acid, a breakdown product of dopamine, have also been found in several autistic patients. Pyridoxine therapy has been reported to be successful in autism, raising the possibility that a PLP-requiring enzyme might be defective in those patients responsive to vitamin B-6. (PLP is a coenzyme that forms a Schiff base with an amino group in its catalytic action, so that enzymes with PLP metabolize amino acids or other amines, such as dopamine and serotonin.) The only PLP-requiring enzyme directly involved with the synthesis or degradation of dopamine and serotonin is AAD (ie, dihydroxyphenylalanine decarboxylase). The finding in some vitamin B-6–responsive patients, namely elevated homovanillic acid that is at least partially reversible with pyridoxine therapy (92), does not suggest a defect in this enzyme. Additionally, cases of AAD deficiency have been reported in the literature (*see* the discussion of AAD above) and result only in a very severe inborn metabolic disorder involving deficient concentrations of dopamine and serotonin.

It remains to be seen whether other enzymes in the metabolic pathways of these neurotransmitters may be responsible for the various forms of autism that involve altered neurotransmitter metabolism. Autism is diagnosed by clinical, not biochemical, indexes. Thus, if different autistic patients harbor mutations in different metabolic enzymes, it may be possible to reverse the effects of autism by targeting a treatment to each individual patient. In addition to PLP, the coenzymes FAD, NAD, *S*-adenosylmethionine, tetrahydrobiopterin, and ascorbate are used by enzymes in serotonin and dopamine metabolism.

Because so little is known about the biochemical basis of this condition, it is difficult to associate a treatment response with a particular biochemical or physiologic pathway; however, there have been many reports of successful treatment of autism with pyridoxine. In a survey involving 4000 questionnaires completed by parents of autistic children, high-dose vitamin B-6 and magnesium treatment ($n = 318$) elicited the best response; for every parent reporting behavioral worsening with the treatment, 8.5 parents reported behavioral improvement. The next best results were with the acetylcholine precursor, deanol ($n = 121$); 1.8 parents reported a favorable response for every 1 patient who reported worsening (93).

Sixteen autistic patients previously shown to respond to vitamin B-6 treatment were reassessed and given vitamin B-6 or a placebo in a double-blind study (94). Behavior deteriorated significantly during B-6 withdrawal, and 11 of 15 children behaved better when given ≈300 mg vitamin B-6/d. The authors speculated that vitamin B-6 therapy may correct, or partially correct, a tryptophan-related metabolic error because of a marked increase in serotonin efflux from platelets of autistic children and because large doses of vitamin B-6 elevate serotonin concentrations (95).

A double-blind trial involving 60 autistic children found that vitamin B-6 (30 mg pyridoxine hydrochloride·kg^{-1}·d^{-1} up to 1 g/d) and magnesium (10–15 mg·kg^{-1}·d^{-1}) were more helpful than either supplement alone in ameliorating the various effects of autism. Patients receiving the combined treatment showed a significant ($P < 0.02$) decrease in homovanillic acid excretion (from 6.6 to 4.4 μmol/mmol creatine) and significant clinical improvement (96).

Tryptophan metabolism was studied in 19 children with various forms of psychosis including autism. Four children (including at least one who was autistic) who had abnormal tryptophan metabolite ratios were treated with 30 mg pyridoxine/d, whereupon biochemical features normalized (97). It was thought that these children had kynureninase defects because the kynureninase reaction required greater than normal amounts of PLP to proceed normally (*see* the discussion of kynureninase above).

More than a dozen other reports (with up to 190 participants) since 1965 and a review of controlled trials (98) have reported improvements in autistic patients with vitamin B-6 and often magnesium supplementation (99–102), although the conclusion that pyridoxine is an effective treatment of autism has been challenged: "interpretation of these positive findings needs to be tempered because of methodological shortcomings inherent in many of the studies" (92). A rebuttal (103) to this critique leaves the matter somewhat unresolved. Evidence supporting the hypothesis that defects in enzymes involved in neurotransmitter biosynthesis may be responsible for some forms of autism comes from a study showing that tetrahydrobiopterin, the cofactor for tyrosine and tryptophan hydroxylases, elicited behavioral improvements in 6 children with autism (104).

Tardive dyskinesia

The long-term use of neuroleptic drugs for the attenuation of psychotic disorders such as schizophrenia can lead to tardive dyskinesia, a neurologic movement disorder characterized by rapid, repetitive, uncontrolled movements. There may be >1 million cases of tardive dyskinesia in the United States today and there is some speculation that deranged metabolism of amino acid–derived neurotransmitters is responsible for the disease. The involvement of PLP in dopamine, serotonin, and γ-aminobutyric acid metabolism may be the reason for the first clinical applications of pyridoxine in the treatment of tardive dyskinesia; pyridoxine-responsiveness has been reported.

A double-blind, placebo-controlled crossover study found high doses of pyridoxine (≤400 mg/d) to be effective in reducing symptoms of tardive dyskinesia in patients with schizophrenia (105). Pyridoxine or placebo was added to the normal neuroleptic treatment of all 15 patients in the study for 4 wk at a time, split by a 1-wk washout period. Pyridoxine treatment invoked improvements in both the dyskinetic movement and Parkinsonian subscales with returns to baseline with removal from pyridoxine. An earlier pilot study by the same group showed significant clinical improvement in 4 of 5 tardive dyskinesia patients given 100 mg pyridoxine/d on top of their normal treatment (106). Three of the responders also showed significant improvement on the brief psychiatric rating scale.

The relation between tardive dyskinesia susceptibility and polymorphisms in dopamine and serotonin receptor genes has been a focus of exploration. Homozygosity for the Ser9→Gly polymorphism in the dopamine D3 receptor was higher in schizophrenics with tardive dyskinesia (22%) than without (4%), suggesting that the glycine allele may be a risk factor for developing tardive dyskinesia (107). A similar study supports the involvement of Ser9→Gly in tardive dyskinesia risk, although the presence of tardive dyskinesia was higher in heterozygotes than in either homozygous group (108). The Thr102→Cys polymorphism in the serotonin type 2A receptor gene has also been investigated, although contradictory results leave the matter unresolved as to which allele may be associated with schizophrenia, tardive dyskinesia, or both. A polymorphism might code for a receptor that has a decreased neurotransmitter binding and the capacity to be stimulated by a pyridoxine-induced increase of neurotransmitter level (95), but

before any such hypothesis is taken seriously more detailed biochemical evidence is necessary.

Tissue concentrations and toxicity

Pyridoxine's active role in ameliorating many cases of genetic disease involving enzymes that require a PLP cofactor is clear. Plasma PLP concentrations correlate well with tissue PLP concentrations in rats (109), and thus serve as a good indicator of vitamin B-6 status. There is a linear relation between vitamin B-6 intake and plasma concentrations of PLP (up to an intake of 3 mg/d in humans, which correlates with a plasma concentration of 60 nmol/L) (7). This proportional relation has been shown to hold even at 25 mg/d, resulting in a plasma PLP concentration of 200 nmol/L (110). A double-blind study investigating high-dose vitamin B-6 treatment of tardive dyskinesia showed that baseline plasma PLP (49 nmol/L) could be raised >14 times (690 nmol/L) safely with 400 mg/d pyridoxine (105). A rat study referenced in the DRI publication showed that extremely large doses are well absorbed (7).

The higher concentrations of PLP likely facilitate apoenzyme-coenzyme interaction, and hence higher enzymatic activity, although it should be noted that numerical discrepancies do exist in the literature. Normal serum PLP concentrations appear to be ≈60 nmol/L, whereas control K_m concentrations have been described in the μmol/L range for some enzymes (eg, OAT and CBS).

An upper limit exists as to pyridoxine administration. Although dosages in the hundreds of milligrams have been safely applied, reports exist of neurotoxic effects with very high vitamin B-6 usage. One review advises avoiding doses >1000 mg pyridoxine/d (47). The tolerable upper intake level (UL) of pyridoxine for normal use is 100 mg/d (7); however, the severity of some genetic diseases has reasonably prompted physicians to prescribe higher doses.

THIAMINE (VITAMIN B-1)

The DRIs for thiamine for men and women are 1.2 and 1.1 mg/d, respectively (7). Thiamine is phosphorylated to form TPP, the cofactor used by many enzymes. The crystal structure of at least one of these enzymes has been solved (111) and critical residues in the TPP binding site have been identified. The thiamine-dependent enzymes discussed in this section are summarized in **Table 4**.

Branched-chain α-ketoacid dehydrogenase: maple syrup urine disease (ketoacidosis, mental retardation, and ataxia)

The branched-chain α-ketoacid dehydrogenase (BCKAD) multienzyme mitochondrial complex is composed of 3 subunits: an E1 component (TPP-dependent decarboxylase) containing α and β subunits, an E2 component (lipoate-containing acyl-transferase), and an E3 component (FAD- and NAD-containing dihydrolipoyl dehydrogenase), the latter of which is also a component of pyruvate and α-ketoglutarate dehydrogenases. BCKAD is responsible for the oxidative decarboxylation of α-ketoacids of the 3 branched-chain amino acids valine, leucine, and isoleucine. Genetic defects in the complex cause maple syrup urine disease (*see* OMIM 248600), which involves ketoacidosis, mental retardation, ataxia, and sometimes blindness as a result of the accumulation of α-keto acids.

In 1985, thiamine responsiveness was reported in 12 patients who were fed thiamine in doses ranging from 10 to 1000 mg/d (112). The accumulation of ketoacids was shown to return to normal after thiamine feeding (113). In one thiamine-responsive maple syrup urine disease cell line (WG-34) the K_m for TPP (as measured via BCKAD decarboxylation activity) was found to be 16 times higher than normal (114). The sequence of the WG-34 mutant has been determined and unexpectedly, the dihydrolipoamide acyltransferase component of the complex was found to be altered. It is possible that the presence of a normal E2 is essential for the efficient binding of TPP to E1 (115). Other genes from thiamine-responsive patients have been sequenced and the E2 subunit was found to be altered in ≥2 other patients (116–118). It appears that mutations in E2 are responsible for the thiamine-responsive versions of maple syrup urine disease and it has been suggested that this E2 defect impairs the E1-E2 interaction where the TPP molecule must bind, thus increasing the cell's requirement for thiamine and TPP (116).

The crystal structure of the TPP binding portion of the BCKAD complex has been determined as well as the effects of various maple syrup urine disease mutations on the enzyme. One mutation (E1α N222S) increased the K_m for TPP in a nonresponsive patient. The other 3 mutations, which are described as affecting cofactor binding, all resulted in nonresponsive maple syrup urine disease. Another residue, E1β N126, which is altered in some patients with maple syrup urine disease, affects interface interaction in the complex and may be involved with subunit association and K⁺ binding (111). (*See* the discussion of potassium in the section on hormones, amino acids, and metals.) It remains to be seen whether some of the other mutations, specifically in the intermediate or thiamine-responsive patients, also result in an increased K_m for TPP.

In 9 thiamine-dependent cases, the decarboxylation activity (which is a measurement of overall BCKAD activity) ranged from 3% to 40% of normal (119). In one case, the mutant enzyme was shown to be heat labile and stabilized by increased TPP (120). No adequate reports of the percentage of cases that are remediable by thiamine are available. Although the enzyme complex also uses NAD, FAD, and dihydrolipoic acid as cofactors in addition to the Mg^{2+} salt of TPP, it appears that the therapeutic application of niacin, riboflavin, and lipoic acid has not been attempted. NAD, CoA, and Mg^{2+} were tried in cell culture but were ineffective (121).

In one case, supplementation with oral thiamine reversed the blindness that sometimes accompanies the disease (122). Another noteworthy thiamine-responsive case involved a compound heterozygote with a large deletion and a 1002G→A transition at an exon 8 splice site that resulted in exon skipping and the transcription of different length mRNAs (117). The mechanism for this thiamine response remains to be explained.

The data suggest that combination therapy with thiamine, lipoic acid, riboflavin, nicotinamide, and adequate potassium [for which the recommended dietary allowance intake is 2000 mg/d (9)] may be optimal for the initial treatment of patients with maple syrup urine disease. Potassium might be beneficial because K⁺ is required for the stabilization of E1 by TPP. The stabilizing effect of K⁺ on BCKAD was shown in the rat liver BCKAD enzyme (123) as well as in the human E1β protein (124). Both groups observed a dependence of enzyme activity on the concentration of potassium salts.

Pyruvate decarboxylase: Leigh disease (lactic acidosis, ataxia, and mental retardation)

Pyruvate decarboxylase is part of the pyruvate dehydrogenase multienzyme mitochondrial complex (PDHC) that uses TPP, lipoic

acid, CoA, FAD, and NADH coenzymes to catalyze the conversion of pyruvate to acetyl-CoA (*see* OMIM 312170). The gene encoding the E1α peptide of the E1 subunit (pyruvate decarboxylase), which binds TPP, is located on the X chromosome. Genetic defects in the complex can lead to lethal lactic acidosis, psychomotor retardation, central nervous system damage, ataxia, muscle fiber atrophy, and developmental delay (125).

X-linked genetic defects in PDHC cause pyruvate and lactate accumulation and encephalomyelopathy. Twenty-six patients responded to high intakes of thiamine, ranging from 20 to 3000 mg/d. In 2 sisters (126), lipoic acid (100 mg/d) plus thiamine (3000 mg/d) were found to give the best remediation. In several cases, the mutation was shown to increase the K_m of the E1 subunit for TPP and reduce the Vmax (127, 128). In several cases in which lactate was measured, thiamine lowered lactate concentrations significantly (127, 129, 130).

In a study of 13 thiamine-responsive PDHC-deficient patients, some had a decreased affinity of PDHC for TPP that was responsive to TPP, whereas the PDHC activity of others increased at high TPP concentrations with no statement about enzyme affinity (131). Another group of patients with lactic acidemia and muscle fiber atrophy had TPP-responsive PDHC enzymes (1.82 and 2.63 nmol·min^{-1}·mg protein^{-1} with 400 μmol/L TPP compared with 0.28 and 0.02 nmol·min^{-1}·mg protein^{-1}, respectively, with 0.1 μmol/L TPP) (132).

A female infant with West syndrome (a unique epileptic syndrome with frequently poor prognosis and spasms associated with elevated blood and cerebrospinal fluid lactate concentrations) had thiamine-responsive PDHC deficiency (133). Lactate concentrations were lowered and symptoms disappeared when the infant was administered dichloroacetate and high doses of thiamine (500 mg/d). The patient carried the mutation Gly89→Ser in exon 3 of her PDHC *E1α* gene, resulting in a decreased affinity for TPP. PDHC activity was activated in vitro with the addition of TPP. Gly89→Ser, along with 4 other mutations in thiamine-responsive PDHC-deficient patients (His44→Arg, Arg88→Ser, Arg263→Gly, and Val389→fs) are in regions outside the TPP binding site in exon 6 (133). However, these mutations may affect overall protein conformation and indirectly decrease cofactor binding affinity. Pyridoxine was shown to be effective in several cases of West syndrome (134).

Three point mutations in E1-deficient patients were recreated in vitro. One mutation, Met181→Val, exhibited a 250-fold increased K_m for TPP and, in addition to another studied mutation (Pro188→Leu) is involved with TPP binding. It was mentioned that an aspartate and an asparagine residue form H-bonds with and coordinate the divalent cation (Mg^{2+} or Ca^{2+}). This cation interacts with the oxygen groups of the pyrophosphate portion of TPP (135). Thus, encouraging adequate intake of magnesium and calcium in PDHC-deficient patients by physicians seems reasonable. The Met181→Val mutation also raised the K_m for pyruvate 3-fold (135). Physicians might consider treating PDHC-deficient patients with precursors to all of the cofactors used by the enzyme complex: thiamine, lipoic acid, pantothenate, riboflavin, and niacin.

Thiamine transporter, thiamine pyrophosphokinase, and α-ketoglutarate dehydrogenase: thiamine-responsive megaloblastic anemia

Thiamine-responsive megaloblastic anemia (*see* OMIM 249270) can be caused by defects in a putative thiamine transporter, thiamine pyrophosphokinase (TPK), and α-ketoglutarate dehydro-

genase [KGDH; oxoglutarate dehydrogenase (lipoamide)]. The putative thiamine transporter, encoded by *SLC19A2*, is homologous to reduced folate carrier proteins and may bring thiamine into cells. TPK is responsible for the phosphorylation of thiamine to TPP cofactor. KGDH is one of the thiamine-dependent dehydrogenases that binds TPP by an E1 carboxylase (*see* also the discussions of BCKAD and PDHC above).

Mutations in SLC19A2 (136, 137) and defects in TPK (138, 139) and KGDH (140, 141) have all been found in patients with thiamine-responsive megaloblastic anemia. Thiamine-responsive megaloblastic anemia, first described by Rogers et al (142) in 1969, is an autosomal recessive condition with an early onset and is characterized by the triad of megaloblastic anemia, diabetes mellitus, and sensorineural deafness.

Mutations in the gene for the putative thiamine transporter, SLC19A2 (*see* OMIM 603941), were found in all affected individuals in 6 families with thiamine-responsive megaloblastic anemia (136). Another study found similar results and supports the putative role of SLC19A2 in some forms of thiamine-responsive megaloblastic anemia (137). There is evidence that there is a low-affinity thiamine transporter and that this transporter is responsible for the clinical thiamine-responsiveness, partially correcting for the decreased intracellular thiamine concentrations that result from the defective high-affinity transporter, SLC19A2 (143). The low-affinity version may be the recently identified thiamine transporter SLC19A3 (144). Such a bypass would not involve overcoming a K_m defect. Both thiamine transport and TPK were thought to be the enzymes affected in a group of 7 patients with thiamine-responsive megaloblastic anemia (143).

TPK activity was reduced in a patient with thiamine-responsive megaloblastic anemia in whom 60 d of thiamine therapy (50 mg/d) normalized concentrations of free and phosphorylated thiamine (139). Thiamine responsiveness was found in 2 similar cases of thiamine-responsive megaloblastic anemia with deficient TPK activity (138). After thiamine ingestion (75 mg/d), which raised erythrocyte TPP concentrations 1.5- and 2-fold in the patients, hematologic findings returned to normal and insulin requirements decreased by 66%. (*See* OMIM 606370.)

Deficient KGDH activity (*see* OMIM 203740) in one patient with thiamine-responsive megaloblastic anemia was stimulated by TPP titration. Near normal activity was reached with 0.75 μmol TPP/L, whereas control subjects were not responsive (140). The KGDH activity in another patient was 2% of that of a control subject, and a defect in binding of TPP to the KGDH complex was suggested (141). Because the KGDH complex uses other coenzymes including lipoic acid, CoA, FAD, and NAD, patients may benefit initially from a high-dose mixture of thiamine, lipoic acid, pantothenate, riboflavin, and niacin, but controlled clinical investigations are needed to validate or reject this hypothesis.

Oxidation of α-ketoglutarate, pyruvate + malate, and malate + palmitate: lactic acidosis and cardiomyopathy

Cardiomyopathy and lactic acid accumulation in a neonate was remedied by feeding thiamine (50 mg/d), carnitine (2 g/d), and riboflavin (50 mg/d), which reversed the high blood lactate concentrations and other symptoms. The patient showed a deficiency in the oxidation of all substrates tested: pyruvate, α-ketoglutarate, and palmitate. After freezing and thawing and addition of essential cofactors (TPP, CoA-SH, NAD), the activities of the ketoacid dehydrogenases became normal. The apparent deficiency may have been caused by a primary deficiency in

one of the cofactors or by a defect at the level of thiamine. Although the precise metabolic defect was not assessed, it was concluded that the patient was responsive to thiamine (145). A similar case (146) was also reversed by thiamine (50 mg/d).

Tissue concentrations and toxicity

The effectiveness of thiamine administration in these diseases involving several mutant genes seems clear. It has been shown that a 10-mg dose of thiamine raised serum thiamine concentrations to 24 nmol/L; concentrations returned to baseline (17 nmol/L) 6 h later (147). With higher pharmacologic doses, namely, repetitive 250-mg amounts taken orally and 500 mg/d given intramuscularly, nearly 1 wk was required for steady state plasma concentrations to be reached (148). It seems apparent that thiamine administration raises both TPP and thiamine concentrations in serum, but we have not found documentation of this.

There is no defined UL for thiamine because of its relative safety. Adverse effects of thiamine have been documented, although they appear to be rare. For example, in a study of 989 patients, 100 mg thiamine hydrochloride/d given intravenously resulted in a burning effect at the injection site in 11 patients and pruritus in 1 (149).

RIBOFLAVIN (VITAMIN B-2)

The DRI for riboflavin is 1.3 mg/d for men and 1.1 mg/d for women (7). Riboflavin kinase synthesizes flavin mononucleotide (FMN) from ATP and riboflavin. Flavin adenine dinucleotide (FAD) is synthesized by the subsequent adenylation of FMN by FAD synthetase. A flavin-containing cofactor, FAD or FMN, is utilized by 151 (4%) of the 3870 enzymes catalogued in the ENZYME database (6). In addition to the identification of protein motifs (eg, Rossmann folds) involved with nonadenine binding of adenylate-containing cofactors (including FAD and NAD), a common adenine moiety binding motif was recently found in a large group of FAD binding proteins (150). The pyrophosphate moiety may be most important for FAD recognition because of a strongly conserved pyrophosphate binding motif found in FAD binding protein families (151). The following enzymes requiring flavin coenzymes are summarized in **Table 5**.

Methylenetetrahydrofolate reductase (NADPH): homocysteinemia, cardiovascular disease, migraine, neural tube defects, Down syndrome, diabetic nephropathy, congenital cardiac malformations, dementia, and male infertility

Human MTHFR (also discussed in the section on folic acid) uses both NADP and FAD cofactors to catalyze the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (*see* OMIM 236250). The latter is the predominant circulatory form of folate and the main carbon donor for the remethylation of homocysteine to methionine. The 677C→T (Ala222→Val) polymorphism (*TT* genotype occurring in 10–20% of the population) in the human *MTHFR* gene results in a thermolabile enzyme with reduced activity (152). Individuals with the polymorphism have a larger pool of 5,10-methylenetetrahydrofolate and are at a lower risk of developing chromosome breaks and cancer (153) and sperm dysfunction (154) when folic acid intake is marginal. The variant enzyme, however, results in a smaller pool of 5-methyltetrahydrofolate and an accumulation of homocysteine (155), which has been associated with an elevated risk of car-

diovascular disease (51, 52). A higher frequency of the 677C→T polymorphism has been associated with cardiovascular disease (156), neural tube defects (NTDs) (157), Down syndrome (158), migraine (159), diabetic nephropathy (160), congenital cardiac malformations (161), dementia (162), male infertility (163), and other conditions.

Although individuals with the polymorphism and many of the associated conditions have typically been treated with folic acid, the precursor to the MTHFR substrate, riboflavin, the FAD precursor vitamin, may prove to be of additional benefit because the primary defect in the 677C→T mutated enzyme is altered FAD binding. The human 677C→T mutation (164) and its *Escherichia coli* homologue (165) were found to alter the structure of MTHFR and lower the binding affinity for the FAD cofactor, while not affecting the K_m for folate. It was also found that the addition of FAD to crude extracts of human lymphocytes and of recombinant human MTHFR expressed in *E. coli* protects both wild-type and mutant enzymes, and that the protection is more dramatic with the mutant enzyme (165). The reduced ability to bind FAD was abolished under very high concentrations (μmol/L) of folate (normal serum range is nmol/L), and this mechanism was offered as the basis for the effectiveness of folic acid therapy in lowering homocysteine in those with the polymorphism. This suggests that feeding high doses of riboflavin to raise the concentrations of FAD might be of additional benefit to folic acid in lowering homocysteine concentrations in persons homozygous for the 677C→T mutation.

The maintenance of adequate riboflavin status is likely important for homocysteine and methylation metabolism, as suggested by the results of a rat study that showed MTHFR to be sensitive to both severe and moderate riboflavin deficiency (166). The 677C→T variant may be even more sensitive. Additionally, plasma riboflavin was found to be inversely related to plasma total homocysteine in a recent study in Norway of 423 healthy blood donors with adequate B-vitamin intake (167). Plasma total homocysteine, serum folate, serum cobalamin, serum creatinine, and *MTHFR* 677C→T genotype were measured, as well as both FMN and FAD. Riboflavin was found to be an independent determinant of total homocysteine status: total homocysteine was 1.4 μmol/L higher in the lowest than in the highest riboflavin quartile (*P* = 0.008). The riboflavin–total homocysteine relation was modified by genotype (*P* = 0.004) and was essentially confined to subjects with the 677C→T polymorphism in the *MTHFR* gene. Those with the *CC* genotype did not show the correlation, whereas those heterozygous and homozygous for this common polymorphism (in this study, 9% were *TT* and 43% were *CT*) did show a riboflavin determinacy of homocysteine status. It was suggested that subjects with the *T* allele may require higher concentrations of FAD for maximal catalytic activity. The altered interaction between FAD and MTHFR suggests that high-dose riboflavin treatment should be studied in *TT* individuals, even those who have normal vitamin intakes, who might benefit by lowered homocysteine concentrations in the blood via the stimulation of MTHFR and thus a lowered risk of cardiovascular and other diseases. Another study showed an inverse correlation between the intake of several B vitamins, including riboflavin, and plasma homocysteine in atherosclerotic patients and control subjects (168). *MTHFR* genotype was not considered in this study.

A positive correlation was found between the 677C→T polymorphism and migraine (159), which was shown to be responsive

TABLE 4
Enzymes that use a thiamine pyrophosphate (TPP) cofactor[1]

Defective enzyme and EC no.	Localization	Reaction catalyzed	Disease or condition	OMIM no.	Inheritance
Branched-chain α-ketoacid dehydrogenase (1.2.4.4)	Mitochondrial matrix	α-Keto acids → acyl-CoA + CO_2	Maple syrup urine disease (branched-chain ketoaciduria) and buildup of BCAAs (leucine, isoleucine, and valine) in blood and urine	248600	Autosomal recessive
Pyruvate decarboxylase (4.1.1.1)	Mitochondrial matrix	Pyruvate → acetyl-CoA + CO_2	Leigh disease and lactate and pyruvate buildup in serum	312170	X-linked
Thiamine transporter SLC19A2	Integral membrane protein	—	Megaloblastic anemia, diabetes mellitus, and sensorineural deafness	603941	Autosomal recessive
Thiamine pyrophosphokinase (2.7.6.2)	Cytoplasmic?	ATP + thiamine → AMP + TPP	Megaloblastic anemia, diabetes mellitus, and sensorineural deafness	606370	Autosomal recessive
α-Ketoglutarate dehydrogenase (1.2.4.2)	Mitochondrial matrix	α-Ketoglutarate + CoA + NAD → succinyl-CoA + CO_2 + NADH	Megaloblastic anemia, diabetes mellitus, and sensorineural deafness	203740	Autosomal recessive

[1] BCAA, branched-chain amino acid; OMIM, Online Medelian Inheritance in Man (4).

TABLE 5
Enzymes that use an FAD or FMN (riboflavin) cofactor[1]

Defective enzyme and EC no.	Localization	Reaction catalyzed	Disease or condition	OMIM no.	Inheritance
Methylenetetrahydrofolate reductase (NADPH) (1.5.1.20)	Cytoplasmic	5,10-Methylene-THF + NADPH → 5-methyl-THF + NADP	Homocysteinemia, cardiovascular disease, migraine, diabetic nephropathy, and congenital cardiac malformations	236250	Autosomal recessive
NAD(P);quinone oxidoreductase 1 (1.6.99.2)	Cytoplasmic	Reduction of quinones and quinonoid compounds to hydroquinones	Risk of leukemia and urothelial tumor	125860	Autosomal recessive
Protoporphyrinogen oxidase (1.3.3.4)	Bound to inner mitochondrial membrane	Protoporphyrinogen IX + O_2 → protoporphyrin IX + H_2O_2	Variegate porphyria and neuropsychiatric complications, including motor neuropathy	176200	Autosomal dominant
Electron-transferring-flavoprotein and electron-transferring-flavoprotein ubiquinone oxidoreductase (1.5.5.1)	Inner mitochondrial membrane (ETF-QO)	Electron flow: reduced acyl-CoA dehydrogenases → ETF → ETF-QO	Glutaric aciduria type II, myopathy, metabolic acidosis, and hypoglycemia	231680, 231675	Autosomal recessive
Peroxisomal glutaryl-CoA oxidase	Peroxisomal		Glutaric aciduria type III	231690	Autosomal recessive
Short-chain, medium-chain, and long-chain acyl-CoA dehydrogenases (1.3.99.2, 1.3.99.3, and 1.3.99.13)	Mitochondrial matrix	Acyl-CoA + ETF → 2,3-dehydroacyl-CoA + reduced ETF	Multiple acyl-CoA dehydrogenase deficiency, seizures, failure to thrive, metabolic acidosis, and neuromuscular disorders	201470, 201450, 201460	Autosomal recessive
Mitochondrial complex I (1.6.5.3)	Mitochondrial membrane	Electron transport enzyme	Complex I deficiency and myopathy	252010	Autosomal recessive
Complex I (mitochondrial transfer RNA Leu mutations)	Mitochondrial membrane	Electron transport enzyme	Complex I deficiency and MELAS	590050	Mitochondrial

[1] ETF, electron-transferring-flavoprotein dehydrogenase; ETF-QO, electron-transferring-flavoprotein ubiquinone oxidoreductase; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; OMIM, Online Mendelian Inheritance in Man (4); THF, tetrahydrofolate.

to high-dose riboflavin treatment (400 mg/d), with a significant reduction in attack frequency (169). Those homozygous for the *T* allele were at a considerably greater risk of migraine than were control subjects, suggesting that homocysteine, an excitatory amino acid, is a risk factor for migraine. Seventy-four patients with migraine were compared with 261 healthy control subjects. The frequency of the *TT* genotype among the control subjects (9.6%) was significantly lower than in the migraine patients with aura (9/22, or 40.9%; *P* < 0.0001). No significant difference was found between the control subjects and the patients with migraine without aura or with tension-type headaches (159). In a randomized controlled study of 55 patients, more patients taking 400 mg riboflavin/d improved by ≥50% than did those taking a placebo: 59% of the riboflavin group responded to treatment with fewer migraine days compared with 15% for the placebo group (*P* = 0.002), 56% of patients in the riboflavin group responded to treatment with a decrease in attack frequency compared with 19% for the placebo group (*P* = 0.01), and 41% of the riboflavin patients improved on the migraine index compared with 8% for the placebo group (*P* = 0.01) (169). The patients with aura made up 22% of the group but were not differentiated in the analysis. This confirmed a previous open study.

Riboflavin and β-blockers were similarly tested for the prevention of headaches (170). Intensity dependence of auditory evoked cortical potentials, a measurement of cortical information processing, was found to be decreased after treatment with β-blockers. Riboflavin had a clinical efficacy similar to that of β-blockers in the patients, but it did not change cortical information processing. Headache frequency decreased significantly in both patient groups (*P* < 0.05). Eight of 15 patients responded to riboflavin (meaning a reduction in attack frequency by >50%). It would be of interest to compare *MTHFR* genotype with riboflavin responsiveness in these migraine patients, although such a study has not been done (J Schoenen, personal communication, 2001).

NTDs, which are among the most common genetic malformations, have traditionally been prevented with periconceptional folic acid supplementation. It is estimated that the fraction of NTDs due to the *TT* genotype in Ireland is 11.4% (157). Homocysteinemia has been implicated in NTDs, and although some studies (171) found no correlation between 677C→T and NTDs, another study (157) of 271 NTD cases and 242 controls, found a higher prevalence of the *TT* genotype in the cases (18.8%) than in the controls (8.3%; *P* = 0.0005). These findings suggest that raising MTHFR activity through riboflavin administration may complement the action of periconceptional folic acid, especially because most women carrying affected embryos have plasma and red blood cell folate concentrations well above the clinically deficient range (172). Supporting evidence for this hypothesis may come from 2 Hungarian studies that showed a multivitamin (containing 800 μg folate) was more efficient than was folate alone (≈6 mg) in preventing the first occurrence of an NTD (173).

A positive relation between elevated homocysteine and cardiovascular disease risk has been clearly established. However, most of the >20 studies looking at the relation of the 677C→T polymorphism to cardiovascular disease risk failed to find a positive correlation. This failure has been attributed to a lack of statistical power to detect the added risk due to the mild homocysteine elevation associated with the *TT* genotype (174). A positive correlation of cardiovascular disease with the *TT* genotype was found in patients with end-stage renal disease (175),

familial hypercholesterolemia (176), and cardiovascular disease alone (156, 177, 178).

Two studies, involving 57 and 157 cases, respectively, showed the 677C→T mutation to be more prevalent among mothers of children with Down Syndrome than among control mothers (with odds ratios of 2.6 and 1.9, respectively) (158, 179). Trisomy 21 is due to maternal nondisjunction 93% of the time, and it is possible that the maternal polymorphism leads to altered folate metabolism, DNA hypomethylation, and abnormal chromosomal segregation. In fact, a positive correlation between the 677C→T genotype and DNA hypomethylation was been found in leukocytes of individuals with the polymorphism (180). DNA methylation was directly and significantly related to red blood cell folate concentrations in persons with the *TT* genotype, but not in those with wild-type MTHFR.

The 677C→T polymorphism and concomitant hyperhomocysteinemia are also associated with diabetic nephropathy in patients with serum folate concentrations <15.4 nmol/L (*P* = 0.02) (160). Congenital cardiac malformations may be connected with the 677C→T polymorphism as well: 26 pregnancies complicated by fetal cardiac defects had higher amniotic homocysteine concentrations and a higher incidence of the 677C→T polymorphism than found in 116 normal pregnancies (161). *TT* homozygosity is more common in infants with congenital heart disease than in control infants (181). Additionally, a higher incidence of *TT* was found in patients with dementia (25%) than in control subjects (12%) (162). When only study participants with hyperhomocysteinemia (concentrations ≥15 μmol/L) were considered, the percentage of *TT* in the dementia group rose to 43% (compared with 14% in the control group), suggesting that 677C→T and homocysteine are risk factors for dementia.

Other mutations in MTHFR also seem to affect FAD binding and may be remedied with high-dose riboflavin treatment. The *E. coli* homologue of the human mutation, Arg157→Gln, displays defective flavin binding (165). Two other mutations in MTHFR, 985C→T and 1015C→T, identified in 2 patients may also decrease the binding of the FAD cofactor (182). Rosenblatt and Erbe previously studied these 2 patients and found that reductase activity was much less stable at elevated temperatures in the absence of added FAD than with the addition of 72 μmol FAD/L (183). They concluded that, "There is a mutationally induced structural defect in the aporeductase as the basis for the observed alteration in thermostability, presumably reflecting reduced ability to bind the FAD cofactor" (183).

The *TT* genotype has been associated with increased homocysteine concentrations (especially in persons with low plasma folate) (184, 185). Clinical trials of the interventions of folate, vitamins B-6 and B-12, and high-dose riboflavin treatment would be of interest in patients with the 677C→T polymorphism or any of the accompanying conditions including migraine and diabetic nephropathy.

NAD(P):quinone oxidoreductase 1: urothelial tumor risk, leukemia risk, benzene-induced hematoxicity risk

NAD(P):quinone oxidoreductase 1 [NQO1; NAD(P)H dehydrogenase (quinone)] utilizes NAD and FAD cofactors to catalyze the 2-electron reduction of quinones and quinonoid compounds to hydroquinones (*see* OMIM 125860). Normal NQO1 activity is involved in both detoxification and chemoprotection as well as in the bioactivation of some compounds, including cytotoxic antitumor agents. A polymorphic mutation in NQO1, 609C→T

(*TT* frequency: 4–20%), which results in a Pro187→Ser amino acid substitution, has been associated with an increased risk of urothelial tumors, therapy-related acute myeloid leukemia, cutaneous basal cell carcinomas, pediatric leukemias, and the development of benzene-induced hematoxicity in exposed workers (186).

The data concerning an association between lung cancer risk and NQO1 genotype are contradictory. Some studies found the wild-type allele (C609) to be overrepresented in lung cancer cases relative to control subjects (187, 188), suggesting a chemoprotective role of the polymorphism (T609). In contrast, other studies found either the opposite to be true (189, 190) or that no correlation exists (191). The latter study was the largest of the NQO1 genotype and lung cancer risk studies to date, comprising 814 lung cancer patients and 1123 control subjects.

The polymorphism results in reduced amounts of the NQO1 protein, possibly as the result of to an accelerated degradation via the ubiquitin pathway. The mutant expressed in *E. coli* has between 2% and 4% of the activity of the wild-type enzyme (186). The cause of both of these observations is likely to be an aberrant binding of FAD by the mutant enzyme. The Pro187→Ser mutation disturbs the structure of the central parallel β-sheet (192), resulting in a reduction in binding affinity for the FAD cofactor (193). Others found that NQO1 activity can be measured only in the presence of increased concentrations of FAD, confirming that the impairment of activity in the Pro187→Ser enzyme is due to lowered FAD affinity (Ivonne Rietjens, unpublished observations, 2001).

These data suggest that individuals with the NQO1 polymorphism might benefit from high-dose riboflavin treatment by reductions in cancer risk. Further studies should be done to verify or reject this theory.

Protoporphyrinogen oxidase: variegate porphyria and motor neuropathy

Protoporphyrinogen oxidase, a mitochondrial flavoprotein, catalyzes the oxygen-dependent oxidation of protoporphyrinogen IX to protoporphyrin IX, the penultimate step in the heme biosynthetic pathway. Protoporphyrinogen oxidase deficiency results in variegate porphyria (*see* OMIM 176200), which involves various neuropsychiatric symptoms, including bulbar paralysis, quadriplegia, and motor neuropathy. Protoporphyrinogen oxidase shares significant homologies with several oxidases (eg, monoamine oxidases) that contain an FAD binding motif at the amino terminus (194). The Arg59→Trp mutation, one of fewer than a dozen mutations reported in the gene encoding protoporphyrinogen oxidase to date and common in South Africa [because of to a 17th century Dutch immigrant founder effect (195)], affects the FAD binding motif and is suspected to alter the FAD binding affinity of protoporphyrinogen oxidase. A similarity is postulated to X-linked sideroblastic anemia, which has been successfully treated with pyridoxine (*see* the discussion of ALAS2 in the section on pyridoxine). A similar approach with riboflavin supplementation may be useful in the treatment of persons with variegate porphyria whose mutations affect the FAD binding region of protoporphyrinogen oxidase (194).

Electron-transferring-flavoprotein and electron-transferring-flavoprotein ubiquinone oxidoreductase: glutaric aciduria type II and myopathy

Electron-transferring-flavoprotein [ETF; which contains an α (*see* OMIM 231680) and a β subunit (*see* OMIM 130410)] and electron-transferring-flavoprotein ubiquinone oxidoreductase (ETF-QO, *see* OMIM 231675) are 2 mitochondrial proteins that use FAD coenzymes. The enzymes mediate the transfer of electrons from mitochondrial flavoprotein dehydrogenases (*see* the discussion of short-chain, medium-chain, and long-chain acyl-CoA dehydrogenases below) to ubiquinone. Metabolic diseases characterized by defects in the mitochondrial oxidation of acyl-CoA esters involved in the metabolism of fatty acids and branched-chain amino acids are often ameliorated by feeding high riboflavin.

Glutaric aciduria II is characterized clinically by hypoglycemia, metabolic acidosis, myopathy, and stridor and biochemically by the accumulation of metabolites, such as glutaric acid. Electron transfer from 9 primary flavoprotein dehydrogenases to the main respiratory chain is impaired in this disease. In most cases, the disorder is due to a deficiency of either ETF or ETF-QO; treatment with oral riboflavin (100–300 mg/d) has been particularly effective in a few patients. It has been suggested that increased FAD concentrations might help some patients overcome a defect in coenzyme binding by ETF or ETF-QO (196).

The crystal structure of human ETF was solved to study 2 mutations seen in patients with glutaric aciduria II: αThr266→Met and αGly116→Arg, the former being the most common in ETF-deficient patients (197). The structure shows αThr266 to be within hydrogen-bonding distance of the N-5 of the FAD cofactor; the C-4 carbonyl oxygen of FAD resides in similar proximity to the amide nitrogen of αThr266 (198). The αThr266→Met mutant alters the flavin environment. Salazar et al (197) concluded, "The loss of the hydrogen bond at N(5) of the flavin and the altered flavin binding increase the thermodynamic stability of the flavin semiquinone by 10-fold relative to the semiquinone of wild-type ETF....However, kcat/K_m [a measure of catalytic activity] of ETF-QO in a coupled acyl-CoA:ubiquinone reductase assay with oxidized [αThr266→Met] ETF as substrate is reduced 33-fold."

A 29-y-old woman with aciduria who suffered from headaches, depression, and seizures responded markedly to riboflavin (100 mg/d) (199). Cultured fibroblasts collected before treatment showed residual oxidation of palmitate of between 66% and 52% of control fibroblasts. These investigators concluded, "The biochemical response to riboflavin we observed is consistent with the stabilization of a defective ETF or ETF-QO by increased levels of intramitochondrial FAD" (199). Five ETF-QO mutations identified in four patients with glutaric aciduria II were rare and resulted in a total lack of enzyme activity (200).

Cell lines from patients with glutaric aciduria II showed significantly lower mitochondrial oxidation of glutarate and ETF activity (201). The addition of FAD increased ETF activity from 4% to 21% of control in a cell line from one patient. The increase in ETF activity in this cell line may have resulted from FAD binding to an ETF apoenzyme with a lowered affinity for the cofactor, thus partially restoring enzymatic activity.

A child with brain damage induced by glutaric aciduria II responded to riboflavin therapy at the age of 4 y with consistent and rapid improvement (202). Several other cases of aciduria that responded to riboflavin have been reported in the literature, though the exact enzymatic defect is not always clear (203, 204).

Glutaric aciduria II was assessed through clinical follow-ups in 7 patients, 2 with the neonatal-onset form with congenital anomalies, 3 with the neonatal-onset form without congenital anomalies, and 2 with the late-onset form (205). The neonatal

form frequently results in rapid death. All 7 patients received a diet low in fat and protein in addition to oral riboflavin and carnitine. The results were promising for the late-onset disease, because the 2 children appeared to be growing normally and experiencing complications only with the consumption of food rich in fat or protein. One of the patients with the neonatal-onset form without congenital anomalies responded clinically and biochemically to intravenous carnitine (200–300 mg·kg^{-1}·d^{-1}) and oral riboflavin (100 mg·kg^{-1}·d^{-1}).

A 470T→G transversion was identified in the α subunit of a patient's ETF whose cultured cells showed ETFα deficiency (206). Reduced ETFβ was found in the cells despite its being synthesized at a normal rate. It was suggested that ETFα confers stability on ETFβ when they bond and that the instability of the mutant ETFα could be attributed to its inability to bind with ETFβ because of drastic conformational changes. In another study, a defect in ETFβ biosynthesis in a patient with glutaric aciduria II was revealed by pulse labeling techniques (207).

Peroxisomal glutaryl-CoA oxidase: glutaric aciduria type III

Investigation of cultured skin fibroblasts showed that the defect in a girl who responded to riboflavin was in peroxisomal glutaryl-CoA oxidase (*see* OMIM 231690) and not in ETF or ETF-QO, as in most patients with glutaric aciduria. Glutaric aciduria III in this patient was caused by a peroxisomal rather than by a mitochondrial dysfunction (208).

Short-chain, medium-chain, and long-chain acyl-CoA dehydrogenases: multiple acyl-CoA dehydrogenase deficiency, seizures, and neuromuscular disorders

Short-chain (SCAD; butyryl-CoA dehydrogenase), medium-chain (MCAD; acyl-CoA dehydrogenase), and long-chain acyl-CoA dehydrogenases (*see* OMIM 201470, 201450, and 201460) use FAD to catalyze the first steps in the β-oxidation of acyl-CoA substrates, which result in the transfer of electrons to ETF. Defects in the 3 acyl-CoA dehydrogenases, in addition to ETF and ETF-QO defects, have been implicated in multiple acyl-CoA dehydrogenase deficiency. All 5 are mitochondrial proteins. The resulting urinary accumulation of ethylmalonate, methylsuccinate, and butyrylglycine is associated with neuromuscular dysfunction and seizures.

Epileptic seizures were reported in a child carrying a Gly209→Ser mutant SCAD, resulting from the DNA mutation 625G→A (209). This SCAD polymorphism has been postulated to lower FAD affinity and occurs in ≈35% of individuals (*AA* = 4% of control population, *AG* = 31% of control population). The symptoms disappeared, with rapid and permanent improvement of the child's condition, with the administration of 25 mg riboflavin/kg, later lowered to 10 mg·kg^{-1}·d^{-1}. The authors speculated that the amino acid change affects the folding efficiency of the variant SCAD or influences the interaction of SCAD with its FAD cofactor.

In another case report, SCAD and MCAD activities were 35% of normal in a 12-y-old girl (210). Western blot analysis showed the absence of SCAD and decreased MCAD but normal amounts of ETF. Although unresponsive to carnitine, the patient showed a marked improvement with 100 mg riboflavin/d with a concomitant normalization of SCAD activity and a reappearance of SCAD protein in Western blots. MCAD activity and protein amounts remained low. It is possible that a mutation decreased the stability and that riboflavin increased enzyme stability. Although it is

also possible that the patient had an altered riboflavin metabolism resulting in lower mitochondrial FAD concentrations, the authors speculated that "the different effects of riboflavin deficiency on SCAD, MCAD, and possibly ETF and [ETF-QO] could be explained by the different affinities of FAD for the flavoprotein apoenzymes" (210). An FAD-related acyl-CoA dehydrogenation defect was also suspected in a patient with MCAD and SCAD deficiency who responded to 3 × 100 mg riboflavin/d (211).

An 11-mo-old boy with a mild variant of multiple acyl-CoA dehydrogenase deficiency (ethylmalonic-adipic aciduria) received 200 mg riboflavin/d, leading to dramatic clinical improvement with a restoration of normal respiration and an increase in muscular tone within 2 mo (212). Riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency was confirmed in cultured fibroblasts, which showed increased enzymatic activity in the presence of 1 g riboflavin/L (265 nmol/L).

Another case report described a male infant diagnosed with multiple acyl-CoA dehydrogenase deficiency who exhibited marked improvement through the administration of riboflavin (100 mg/d) and L-carnitine (1000 mg/d) (213). The child showed normal development (up to the age of 10 y) and was suspected of harboring an ETF-QO deficiency, although defects in SCAD, MCAD, and long-chain acyl-CoA dehydrogenase could also be responsible.

Mitochondrial complex I: myopathy

Defects in mitochondrial complex I [NADH dehydrogenase (ubiquinone)] cause myopathies (*see* OMIM 252010). Decreased affinity for the FMN cofactor may explain the cases of riboflavin responsiveness.

In 5 patients with a mitochondrial myopathy associated with a complex I deficiency, riboflavin (35–60 mg/d) was effective in 3 patients, with a normalization of enzymatic activity (214). In cultured fibroblasts from a patient with an Arg228→Gln mutation in the complex I subunit encoded by the nuclear *NDUFS2* gene, the addition of riboflavin was able to significantly increase ATP production (215). Because the *NDUFS2*-encoded protein does not contain an FMN binding site, it was suggested that the mutation either interfered with the interaction between a flavoprotein and FMN or that riboflavin has a general stabilizing effect on complex I. In a survey of 9 patients with complex I deficiency, the myopathy of 1 patient dramatically improved during treatment with riboflavin (9 mg/d) and L-carnitine (216). Complex I activity rose 17-fold to normal levels after 7 mo of therapy. Several other cases of riboflavin-responsive complex I myopathies have likewise been reported (217, 218).

Mitochondrial defects were analyzed in 3 patients from a large consanguineous family and in 1 unrelated patient who all had exercise intolerance since early childhood and complex I deficiencies (218). Supplementation with 100 mg riboflavin/d resolved many clinical complications. A biopsy taken in one of the patients after 2 y of riboflavin therapy showed an increase in complex I activity from 16% to 47% of control.

Mitochondrial transfer RNA leucine (UUR): complex I deficiency, MELAS syndrome, migraine, and myopathy

A 32-mo-old patient with a complex I deficiency, an associated myopathy, and a 3250T→C mutation in the gene for mitochondrial transfer RNA (tRNA) leucine (UUR) (*see* OMIM 590050; also discussed in the section on niacin) had a sustained clinical response to riboflavin (50 mg/d) (219). The authors refer to reports of riboflavin being effective in 11 patients with complex I

deficiency, although the underlying cause was not known in these cases. Another patient with a mutation (3243A→G) in the same mitochondrial gene for tRNA leucine (UUR), who initially presented with MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) syndrome at age 19, had previously responded to a mixture of riboflavin and nicotinamide, although the vitamins were not tested individually (220).

Thirteen electron transport chain proteins are coded for and synthesized in the mitochondria, of which 7 contribute to complex I. If the tRNA mutations incur a poor fidelity of leucine incorporation during mitochondrial translation, it is plausible that complex I would be more prone to defects than would other complexes. Another possible explanation for the specific complex I defect is that there is a critical leucine in a cofactor binding site of complex I that is incorporated into the complex I peptide with only moderate fidelity. FMN is an important cofactor for complex I, which could explain why riboflavin responsiveness has been reported in patients with these tRNA mutations.

Of the 3 mitochondrial encephalomyopathies [MELAS, myoclonus epilepsy associated with ragged-red fibers (MERRF), and chronic progressive external ophthalmoplegia (CPEO)] the mitochondrial mutation 3243A→G appears to be specific to MELAS patients; it was found in 26 of 31 MELAS patients and in 1 of 29 CPEO patients and was absent in 5 MERRF patients and 50 control subjects (221). Migraine can be a prominent feature in patients affected by mitochondriopathies such as MELAS syndrome and has been treated with high doses of riboflavin (169; *see* also the discussion of MTHFR above).

Methionine synthase reductase: homocystinuria and mental retardation

See the discussion in the section on cobalamin.

Dihydrolipoamide dehydrogenase: lactic acidosis

See the discussion in the section on lipoic acid.

Tissue concentrations and toxicity

No UL has been defined for riboflavin intake because there have been few reports of adverse effects with doses in the hundreds of milligrams (7). A migraine study mentioned above recorded 2 adverse effects of 400 mg riboflavin/d—diarrhea and polyuria—in 2 of 28 patients (169).

The rate of absorption of riboflavin is proportional to intake and increases when riboflavin is ingested along with other foods (7). In a small group of cirrhosis patients, a single 40-mg oral dose of riboflavin was shown to raise plasma riboflavin 13.7-fold and flavocoenzymes 1.4-fold over baseline (222). A randomized clinical trial determined that the maximum amount of riboflavin that can be absorbed from a single oral dose is 27 mg for adults (223). Thus, the percentage of high doses of vitamins that is actually taken up in plasma or cells depends strongly on mode and frequency of dosage delivery.

NIACIN (VITAMIN B-3)

The DRI for niacin is 16 mg niacin equivalents/d for men and 14 mg equivalents/d for women (7), where 1 niacin equivalent is 1 mg niacin obtained through the diet or the metabolism of tryptophan. The term *niacin* is often used synonymously with *nicotinic acid*. Nicotinamide, the amide form of nicotinic acid, is a building block for both nicotinamide adenine dinucleotide (NAD)

and nicotinamide adenine dinucleotide phosphate (NADP) (7). Rossmann folds are β strands connected by α helix crossover elements and compose nucleotide binding sites. They appear to be the most important structure for NAD(P) binding in at least one group of NAD(P)-dependent proteins. Common motifs include the GxxxGxG phosphate binding pattern of glycines (where x is any amino acid), a ribose binding aspartate, and a nicotinamide-ribose binding YxxxK motif. The adenine binding motif found in many FAD binding proteins was found in only a few NAD(P) binding proteins (150). The following NAD-dependent enzymes are summarized in **Table 6**.

Mitochondrial aldehyde dehydrogenase (NAD⁺): alcohol intolerance and flushed face in Asians, alcohol-induced vasospastic angina, Alzheimer disease, and oral, esophageal, and stomach cancer

Mitochondrial aldehyde dehydrogenase (NAD⁺) (ALDH2) catalyzes the NAD-dependent oxidation of acetaldehyde, which is formed by the oxidation of ethanol by alcohol dehydrogenase (*see* OMIM 100650). The single genetic factor most strongly correlated with reduced alcohol consumption and incidence of alcoholism is the naturally occurring variant of ALDH2, which contains a Glu487→Lys substitution. The Lys487 allele of *ALDH2* (also called *ALDH2*2*) is found in ≈50% of the Asian population and is associated with a phenotypic loss of ALDH2 activity in both heterozygotes and homozygotes. ALDH2-deficient individuals exhibit an adverse response to ethanol consumption, which is probably caused by elevated concentrations of blood acetaldehyde (224). The Glu487→Lys variant has been shown to exhibit a 150-fold increase in K_m for its NAD cofactor (225).

High blood acetaldehyde is potentially carcinogenic and neurotoxic, and the correlation between Glu487→Lys and several cancers has been well established: deficient ALDH2 activity due to the polymorphism has been associated with oral cancer, esophageal cancer, stomach cancer, Alzheimer disease, and alcohol-induced vasospastic angina.

ALDH2 and glutathione transferase M 1 polymorphisms were studied in 191 patients with oral cancer and in 121 control subjects without oral cancer who had a history of alcohol use (226). The incidences of inactive ALDH2 and glutathione transferase M 1 in the cancer group with an alcohol-drinking habit were 34.2% and 67.5%, higher than in the noncancer group with an alcohol-drinking habit (15.1% and 45.5%, respectively). Another study found the *ALDH2*2* allele to be overrepresented in Japanese alcoholics with esophageal, stomach, and oropharyngolaryngeal cancers (227), further illustrating the risk of developing cancer in individuals harboring the variant ALDH2 enzyme.

The polymorphism is also a risk factor for late-onset Alzheimer disease. The frequency of the *ALDH2*2* allele is 48.1% in late-onset Alzheimer disease patients (n = 447) compared with 37.4% in control subjects matched by sex, age, and region (P = 0.001) (228). The added risk was present in both men (P = 0.01) and women (P = 0.02). In addition, the *APOE*E4* allele of the apolipoprotein E gene was confirmed as an independent risk factor for late-onset Alzheimer disease (P = 0.002). The odds ratio for late-onset Alzheimer disease in carriers of the *ALDH2*2* allele was almost twice that in noncarriers. Among patients homozygous for the *APOE*E4* allele, the age at onset of late-onset Alzheimer disease was significantly lower in those with than in those without the *ALDH2*2* allele. In addition, dosage of the *ALDH2*2* allele significantly affected age at onset

TABLE 6
Enzymes that use an NAD or NADP (niacin) cofactor[1]

Defective enzyme and EC no.	Localization	Reaction catalyzed	Disease or condition	OMIM no.	Inheritance
Aldehyde dehydrogenase (NAD$^+$) (1.2.1.3)	Mitochondrial matrix	Aldehyde + NAD + H$_2$O $\rightarrow$ acid + NADH	Cancer risk from high blood acetaldehyde	100650	Autosomal dominant
Glucose-6-phosphate 1-dehydrogenase (1.1.1.49)	Cytoplasmic	Glucose-6-phosphate + NADP $\rightarrow$ 6-phosphogluconolactone + NADPH	Oxidation-induced hemolytic anemia, favism	305900	X-linked
Complex I (mitochondrial transfer RNA mutations) (1.6.5.3)	Mitochondrial membrane	Electron transport enzyme	Complex I deficiency, elevated blood lactate, and pyruvate	590050	Mitochondrial
Dihydropteridine reductase (1.6.99.7)	Cytoplasmic?	NAD(P)H + BH$_2$ $\rightarrow$ NAD(P) + BH$_4$	Phenylketonuria II, hyperphenylalaninemia, and cognitive dysfunction	261630	Autosomal recessive
Long-chain-3-hydroxyacyl-CoA dehydrogenase (1.1.1.211)	Mitochondrial matrix	3-Hydroxyacyl-CoA + NAD $\rightarrow$ 3-oxoacyl-CoA + NADH	β-Oxidation defect, hypoglycemia, cardiomyopathy, and sudden death	600890	Autosomal

[1] BH$_2$, dihydrobiopterin; BH$_4$, tetrahydrobiopterin; OMIM, Online Mendelian Inheritance in Man (4).

of patients homozygous for the *APOE*4* allele. It would be of interest to determine whether long-term niacin use could prevent or delay the onset of Alzheimer disease or relieve present Alzheimer disease in patients with the polymorphism.

Alcohol ingestion induced anginal attacks in 16 of 66 patients without the Glu487→Lys mutation, in 8 of 22 heterozygotes, and in 1 of 3 patients homozygous for the mutation (229). The intervals between alcohol ingestion and the onset of attacks were shorter in *ALDH2*2* homozygotes (0.17 h) and heterozygotes (1.5 ± 0.6 h) than *ALDH2*1* (ie, Glu487 allele) homozygotes (5.4 ± 0.6 h). The amount of ethanol that induced the attacks was significantly greater in control subjects (96.1 mL) than in *ALDH2*2* homozygotes (11 mL) and heterozygotes (42.5 mL). Although the frequency of anginal attacks induced by alcohol ingestion did not differ between the ALDH-deficient and normal homozygotes, anginal attacks were induced in the ALDH-deficient patients by a smaller amount of alcohol. Thus, niacin intake may help to ameliorate some of the deleterious effects of alcohol consumption such as anginal attacks.

With use of concentrations of the glycated hemoglobin as an assay, the Glu487→Lys polymorphism was also suggested to be a risk factor for hyperglycemia in persons with diabetes (230, 231). However, the acetaldehyde adduct of hemoglobin may cochromatograph with glycated hemoglobin and has not been ruled out as the cause of the findings. The amount of hemoglobin adducts formed is a function of the concentration and number of exposures to acetaldehyde (232).

A significant genetic difference was found in the *ALDH2* gene between a group of Japanese patients with alcoholic pancreatitis and control subjects (233). The frequency of the *ALDH2*1* allele was found to be 0.681 and that of the *ALDH2*2* allele to be 0.319 in the control subjects; these values were 0.935 and 0.065 in the patients, respectively. Most of the patients (27 of 31) were *ALDH2*1/2*1*, only 4 were *ALDH2*1/2*2*, and none of the patients were *ALDH2*2/2*2*. These results indicate that genetic polymorphism of the *ALDH2* gene decreases the risk of becoming an alcoholic and hence getting pancreatitis.

The Glu487→Lys polymorphism results in an ALDH2 enzyme with 8% enzymatic activity (234) and a 150-fold increased K_m for NAD (225). Administration of nicotinic acid (235) or nicotinamide (236), which have been shown to increase intracellular NAD concentrations, might reverse or prevent the carcinogenic and other effects of the polymorphism. A 6-y case-control study in Italy with >1000 participants found niacin intake to be inversely correlated with esophageal cancer risk (237). Similar investigations might be done on a population of Asian descent.

Glucose-6-phosphate 1-dehydrogenase: hemolytic anemia and favism

Glucose-6-phosphate 1-dehydrogenase (G6PD; *see* OMIM 305900) is an X-linked cytosolic enzyme that generates NADPH in the oxidative branch of the pentose phosphate pathway. Reduced NADPH is a key electron donor in reductive biosynthetic reactions and in the defense against oxidizing agents, possibly through the maintenance of reduced glutathione concentrations. Additionally, G6PD expression is enhanced by general oxidative stress (238).

G6PD is one of the most polymorphic enzymes and G6PD deficiency is the single most common metabolic disorder, with an estimated 400 million persons thought to be affected worldwide (239). This is because many defects in conserved regions of G6PD, which result in a reduced enzyme activity, confer an increased resistance to malarial infection. Although some forms of G6PD deficiency are asymptomatic, others result in neonatal jaundice, chronic nonspherocytic anemia, acute episodic hemolytic anemia (caused by oxidative stress such as ingestion of fava beans), drug-induced hemolysis, and hemolysis induced by infections. A mild phenotype, characterized by neonatal jaundice, favism, and hemolytic anemia, has become common in the world (240), arising in regions of past malaria risk such as Africa, India, the Mediterranean, and Southeast Asia (241).

G6PD mutations compromise the body's ability to protect against oxidative stress, as shown by the hemolytic response by many deficient individuals to fava beans, which are known to contain the oxidants vicine and divicine. Red blood cells in particular are susceptible to lysis mediated by oxidative stress because of their lack of mitochondria and subsequent reliance on G6PD and one other pentose phosphate pathway enzyme for the production of NADPH. Tobacco smoke is known to contain several oxidants (242) and may be a source of deleterious oxidative stress on persons around the world who are G6PD deficient. Because at least one common variant of G6PD has been shown to have a decreased binding affinity for NADP, it might follow that nicotinic acid or nicotinamide administration to raise intracellular NAD and NADP concentrations would strengthen the body's reductive capacity and reverse the deleterious effects of the deficiency. Although NAD concentrations have been shown to be increased by high concentrations of nicotinic acid (235) and nicotinamide (236), NADP concentrations do not appear to have been examined.

Defects in G6PD usually result in reduced enzymatic activity and a lower ratio of NADPH to NADP. Although some defects, including the Mediterranean polymorphism Ser188→Phe, do not affect cofactor binding (243), many G6PD defects, including at least one polymorphism, result in an increased K_m for NADP and directly alter the NADP binding site. That polymorphism (G6PD Orissa, or Ala44→Gly) was found in a rural region in southern India and resulted in an enzyme with ≈15% activity and a 5-fold increased K_m for NADP (59 compared with 12 μmol/L) (244). Of 677 males screened in the Orissa region, 81 (12%) were G6PD deficient. Twenty-eight of the 81 G6PD-deficient men were further characterized, and 25 of them (89%) had an Ala44→Gly substitution. This gives a polymorphic frequency of 11%.

A 514C→T missense mutation resulting in a Pro172→Ser amino acid substitution was found in a woman with chronic nonspherocytic hemolytic anemia (245). G6PD activity in this woman was 15% of normal in cultured skin fibroblasts, and the K_m for NADP was raised nearly 4-fold (51 compared with 14 μmol/L). When the mutant G6PD protein was expressed and purified from *E. coli*, activity was still decreased and K_m increased. Another (rare) mutation, G6PD Santiago de Cuba Gly447→Arg, results in an enzyme with increased K_m (43 compared with 3–5 μmol/L) (243).

Many other residues have been implicated as residing in the NADP binding site because mutants either had an increased K_m for NADP (G6PD Riverside Gly410→Cys) or could be (re)activated by high concentrations of NADP (G6PD Iowa Lys386→Glu), or both (G6PD Tomah Cys385→Arg, G6PD Beverly Hills Arg387→His, and G6PD Nashville Arg393→His) (246, 247). These results implicated residues 385–393 in the binding site. The structure of human G6PD, which was recently

solved, confirms that these residues are likely responsible for cofactor binding (248). In particular, mutations at 389, 393, 394, and 398 reside near the structural NADP molecule. It seems clear that mutations at other sites could also alter protein conformation so as to disrupt a cofactor binding site, which may explain their altered cofactor binding.

Searches in the BLAST database (249) suggested that amino acids 29–210 are likely responsible for an NADP binding or recognition site and that this region is well conserved over a diverse set of organisms (data not shown). Two well-conserved residues (Ser188 and Ala44) that are affected by polymorphisms—G6PD Mediterranean Ser188→Phe (one of the most common polymorphisms) and G6PD Orissa Ala44→Gly (which increases the K_m for NADPH 5-fold)—fall into this putative NADP binding site.

Mitochondrial transfer RNA leucine (UUR): MELAS syndrome

A tRNA mutation could result in a defective complex I via the incorporation of a critical leucine residue in complex I with low fidelity. Alternatively, because complex I depends on more mitochondrial-encoded components than any other complex, it is possible that the nonspecific low-fidelity of leucine incorporation would affect complex I more than the other complexes.

A MELAS patient with a mutation at nucleotide 3243 of the mitochondrial gene for tRNA leucine (UUR) (*see* OMIM 590050; also discussed in the section on riboflavin) was given nicotinamide therapy (1 g 4 times/d), which resulted in large reductions in blood lactate (40%) and pyruvate (50%) by day 3 of treatment (although the clinical effect of the treatment was temporary and the patient eventually died) (236). Blood NAD concentrations (a measure of intracellular erythrocyte concentration) were raised 24-fold by the sixth week of treatment, and it is speculated that the NAD increase with nicotinamide administration was probably universal because it occurred in a time- and dose-dependent manner in cultured fibroblasts from both the patient and the control subjects. The authors hypothesized that there was a complex I defect leading to an altered interaction between complex I and NADH that was responsible for the decreased complex I activity in the patient. The K_m of complex I for NADH in skeletal muscle from the patient was similar to that in the control subjects; thus, nicotinamide supplementation was effective in this condition because of an enhancement of complex I activity with normal K_m. In another case, a patient with the tRNA leucine (UUR) mutation at mitochondrial DNA base pair 3243 who initially presented with MELAS syndrome at the age of 19 y was clinically responsive to a mixture of riboflavin and nicotinamide, although the vitamins were not tested individually (220).

Dihydropteridine reductase: phenylketonuria II, hyperphenylalaninemia, and cognitive dysfunction

Dihydropteridine reductase (DHPR) utilizes the NAD-NADH redox system to catalyze the recycling of dihydrobiopterin to tetrahydrobiopterin. The tetrahydrobiopterin cofactor is used by the phenylalanine, tyrosine and tryptophan hydroxylases, which are necessary for dopamine and serotonin synthesis as well as nitric oxide synthase. Phenylalanine hydroxylase, when defective, causes classic phenylketonuria (type I).

The biochemical features of DHPR deficiency (*see* OMIM 261630), a disorder of biopterin metabolism resulting from defects in DHPR, involves hyperphenylalaninemia due to a block in the conversion of phenylalanine to tyrosine that is less severe than that of classic phenylketonuria. DHPR deficiency also involves deficient concentrations of various neurotransmitters in the central nervous system, causing severe neurologic symptoms. This is because of the reduced availability of tetrahydrobiopterin for tyrosine and tryptophan hydroxylases, as well as the competitive inhibition of these 2 hydroxylases by 7,8-dihydrobiopterin (N Blau, unpublished observations, 2001), which is the rearrangement product of normal dihydrobiopterin (6,7-dihydrobiopterin). Tetrahydrobiopterin deficiency may be an underappreciated cause of hyperphenylalaninemia and phenylketonuria; thus, it would be of interest to know how many children treated for phenylketonuria actually have the atypical form of the disease that is due to DHPR deficiency.

More than 20 mutations in DHPR have been assigned to each of the 7 exons, with polymorphisms assigned to exons 3 and 4 (250). Two mutations in exon 1 could affect the ability of DHPR to bind the NAD cofactor: the first, Leu14→Pro, results in a nonconservative substitution within the βαβ structure (Rossmann fold) required for NADH binding and is suggested to result in an unstable protein subject to rapid degradation. Another mutation, Gly17→Val, resides in the highly conserved motif involved in NADH binding (251). At least one of these mutations results in no detectable immunoprecipitation and a severe phenotype. It is plausible that niacin administration could raise NADH concentrations to stabilize these cofactor binding mutants or overcome K_m defects caused by similar mutations.

NADH directly increases the catalytic activity of DHPR in rat PC 12 cells (252), and it appears that activation of the defective enzyme via increased concentrations of NADH may serve as an additional benefit in humans. It is plausible that niacin-remediable defects in DHPR occur in humans and that they are the result of a decreased affinity for NADH.

Treatment of phenylalanine hydroxylase deficiency with tetrahydrobiopterin, which bypasses the DHPR reaction, has been successful (253), which affirms that proper tetrahydrobiopterin availability is essential for this pathway. However, tetrahydrobiopterin therapy is not useful in DHPR deficiency because DHPR is involved in the recycling, and not biosynthesis, of tetrahydrobiopterin. Thus, bypassing the DHPR reaction with tetrahydrobiopterin therapy would require equimolar amounts of phenylalanine. On the other hand, increasing intracellular NADH concentrations would hypothetically increase the tetrahydrobiopterin recycling activity of DHPR and this could alleviate the reduced availability of tetrahydrobiopterin.

In a study of 88 infants treated for phenylketonuria, between 48% and 80% of subjects had intakes of preformed niacin, but not a variety of other vitamins, below two-thirds of the 1968 recommended dietary allowance (254).

Long-chain-3-hydroxyacyl-CoA dehydrogenase α subunit: hypoglycemia, cardiomyopathy, and sudden death

The mitochondrial trifunctional protein, composed of 4 α and 4 β subunits, is responsible for the last 3 steps of the β-oxidation of long-chain fatty acids, which is the main source of energy in the heart. The α subunit contains the long-chain-3-hydroxyacyl-CoA dehydrogenase (LCHAD), which catalyzes the following NAD-dependent reaction: R-CHOH-CH$_2$-CO-S-CoA + NAD → R-CO-CH$_2$-CO-S-CoA + NADH + H$^+$.

Mitochondrial trifunctional protein deficiency resulting from impaired LCHAD activity (*see* OMIM 600890) is the second most common inborn error of fatty acid metabolism, second only

to MCAD deficiency (discussed in the section on riboflavin). Clinical complications include hypoglycemia, cardiomyopathy, and sudden death. In addition, severe maternal illness (eg, acute fatty liver) during pregnancy often accompanies mitochondrial trifunctional protein deficiency in the fetus (255).

The DNA mutation 1528G→C in the α subunit, resulting in an Glu510→Gln substitution (Glu474→Gln in some papers), was found to be directly responsible for the loss of LCHAD activity and subsequent illness, although it does not seem to affect overall conformation or subunit conformation because no difference in molecular weight was found between wild-type and mutant proteins. The allele frequency of G1528 was found to be 87% in 34 LCHAD-deficient patients, and there is speculation that the 1528G→C mutation affects the active site of the dehydrogenase domain of mitochondrial trifunctional protein (256).

We have assigned the Glu510→Gln mutation to the likely NAD binding domain (amino acids 358–542) by querying the Conserved Domain Database of the National Center for Biotechnology Information (257) with the LCHAD α subunit protein sequence. These results were verified by aligning the protein sequence of LCHAD α with that of the short-chain enzyme, SCHAD [the first 200 amino acids of which are responsible for NAD binding (258)], by using the Pairwise BLAST tool (249). Amino acids 27–314 of SCHAD lined up with amino acids 361–640 of LCHAD with 33% identity, 52% similarity, and 3% gaps. Residue Glu510 is conserved in the 2 enzymes. Although it appears that kinetic studies have not been performed on LCHAD, it would be of interest to study the NAD binding affinity of the mutant enzyme and to test the responsiveness of LCHAD-deficient patients to therapy with nicotinic acid or nicotinamide, pre- or postnatal.

Hyperlipidemia and heart disease

Patients with hyperlipidemia, an inheritable set of disorders involving altered lipid metabolism, were studied for response to colestipol, lovastatin, simvastatin, niacin, and placebo (259). (Statins are inhibitors of the cholesterol biosynthetic enzyme, 3-hydroxy-3-methylglutaryl CoA reductase.) Although niacin was not tested alone (but rather in conjunction with colestipol or simvastatin), it appears to have improved HDL, triacylglycerol, and cholesterol concentrations in patients.

Combined niacin and statin use has been recommended because of success in clinical trials for the reduction in cardiovascular events and improvement in progression or regression of coronary lesions (260). The niacin-statin treatment regimen appears to provide a unique combination of marked LDL-cholesterol reduction along with favorable changes in HDL cholesterol, lipoprotein(a), and triacylglycerol. A review of the use of niacin to prevent cardiovascular disease and related complications gives evidence of multiple trials of successful treatment with niacin, including the Coronary Drug Project, the largest of the trials studied, which concluded that niacin monotherapy leads to significant decreases in recurrent myocardial infarctions and cerebrovascular events (261).

The benefits of niacin treatment in the lowering of LDL cholesterol was tested in a randomized, controlled, double-blind study involving 201 men and women with elevated LDL-cholesterol values (in the 75th to 95th percentiles) (262). Four treatment groups (receiving daily niacin doses of 2000, 1500, 1250, and 1000 mg) were compared with placebo and diet-treated control groups. The groups given 2000 and 1500 mg had significant reductions in LDL cholesterol (26% and 19.3%, respectively),

total cholesterol (18.4% and 13.3%), and the ratio of total to HDL cholesterol (20.4% and.4%) when compared with the placebo and diet-treated control groups. Smaller improvements were seen in HDL-cholesterol and triacylglycerol concentrations. Blood chemistry monitoring indicated that a reduction in LDL-cholesterol concentration strongly correlated with an increase in baseline concentrations of some enzymes for niacin-treated subjects.

Schizophrenia

"It is supposed that the favorable therapeutic effects of nicotinamide, nicotinic acid and their active biological form—NAD—are realized due to the mechanisms of their functioning in the nervous system, for treating schizophrenia, epilepsy and other diseases of the nervous system" (263). Hoffer (264) and Pauling (265) review literature pertaining to the use of various forms of niacin to treat schizophrenia. They found several studies in which success was reported with niacin therapy. However, these conclusions have been criticized (266) for failure of the investigators to support their claims with evidence from double-blind and placebo-controlled studies, which are necessary to ascertain the efficacy of vitamin treatment of schizophrenia. *See* also the discussion of MTHFR in the section on folic acid.

Chronic fatigue syndrome

Eight of 26 patients (31%) with chronic fatigue syndrome responded favorably to NADH treatment as opposed to only 2 of 26 control subjects (8%) (267). Although NADH therapy appears to be helpful in some patients, the cause of this syndrome is unknown and clinical assessment is sometimes difficult.

Necrobiosis lipoidica

Nicotinamide treatment improved 8 of 13 patients with this granulomatous condition, which involves abnormalities in dermal collagen, vascular supply of the skin, and immunologic responses (268).

Dihydrolipoamide dehydrogenase: lactic acidosis

See the discussion in the section on lipoic acid.

Methionine synthase reductase: homocystinuria and mental retardation

See the discussion in the section on cobalamin.

Tissue concentrations and toxicity

The DRI manual enumerates various adverse effects of supplemental niacin use, but these effects are usually associated with doses of nicotinic acid of ≥1500 mg/d (7). It appears that nicotinamide produces fewer side effects than nicotinic acid. This difference could be due to study bias, however, if significantly fewer studies with nicotinamide have been performed.

Niacin administration raises NAD concentrations in rodents. In mice, the relation of niacin concentration in the diet to NAD in skin fits a logarithmic function, suggesting that NAD content approaches saturation at 0.5–1.0% niacin (g niacin/kg diet) supplementation (269). In rats, 2 wk of dietary nicotinic acid supplementation (500 and 1000 mg/kg diet) caused elevated concentrations of NAD in the blood, liver, heart, and kidney, whereas nicotinamide caused elevated concentrations only in the blood and liver, compared with controls fed a diet containing 30 mg nicotinic acid/kg. Both nicotinic acid and nicotinamide, at 1000 mg/kg diet, cause elevations in liver NAD, by 44% and 43%,

respectively (270). As mentioned above, nicotinamide has been shown to increase human intracellular NAD concentrations (236). Doses of nicotinic acid of 100 mg/d raise human lymphocyte NAD concentrations 5 times above baseline (235). At higher concentrations, passive diffusion predominates, with doses of 3–4 g niacin almost completely absorbed (271).

BIOTIN (VITAMIN B-7)

The DRI for biotin is 30 µg/d (7).

Holocarboxylase synthetase: multiple carboxylase deficiency, organic aciduria, seizures, and ataxia

Holocarboxylase synthetase (HCS), or biotin–[propionyl-CoA-carboxylase (ATP-hydrolysing)] ligase (**Table 7**), catalyzes *1*) the formation of biotinyl-AMP from biotin and ATP, and *2*) the transfer of biotin from biotinyl-AMP to enzymatically inactive apocarboxylases to form active holocarboxylases (272). Specifically, HCS catalyzes the biotinylation of the 4 biotin-dependent carboxylases found in humans: the mitochondrial propionyl-CoA carboxylase, pyruvate carboxylase, β-methylcrotonyl-CoA carboxylase, and the cytosolic acetyl-CoA carboxylase.

Multiple carboxylase deficiency due to HCS deficiency (*see* OMIM 253270) presents in children anywhere from the time of birth to 15 mo of age. Symptoms include organic aciduria, feeding difficulties, neurologic abnormalities (subependymal cysts, hypotonia, impaired consciousness, seizures, and ataxia), and cutaneous changes (rash and alopecia). Supplemental biotin (10 mg/d, compared with an DRI of 30 µg/d) can commonly provide sufficient substrate to increase HCS enzymatic function (when the K_m is increased and the Vmax is decreased) and thereby permit biotinylation of the 4 carboxylases (273).

Cowan et al (274) reported the first biotin-responsive patient, who displayed acidurias reflecting deficiencies in multiple carboxylase enzymes. Oral biotin treatment (10 mg/d) significantly decreased urine acid concentrations. The same group was the first to diagnose and treat an HCS deficiency case prenatally (275). An elevated K_m (>100 times) of HCS for biotin as well as a depressed Vmax were found in fibroblasts from the child.

Two other patients with HCS deficiency had K_m values 14 and 28 times greater than normal at 7.2 and 3.7 µmol/L (compared with the control value of 0.260 µmol/L) ($n = 5$). The Vmax of the enzyme from the patients was also significantly lower than that of the control subjects (276).

Analysis was performed on a mutant, Val550→Met, which resides in the putative biotin binding site (277). In fibroblasts transfected with the Val550→Met cDNA, the K_m for biotin (0.943 µmol/L) was larger than the value found for the wild-type cDNA (0.145 µmol/L). Additionally, the Vmax decreased to 10 pmol·min^{-1}·mg^{-1} in the mutant compared with a wild-type Vmax of 60 pmol·min^{-1}·mg^{-1}.

Of 6 different point mutations analyzed in the *HCS* gene, 2 are frequent among patients with multiple carboxylase deficiency: Val550→Met and Arg508→Trp (which appears to be spread worldwide across ethnic groups). Dupuis et al (278) reported that "four of the mutations cluster in the putative biotin-binding domain as deduced from the corresponding *E. coli* enzyme and consistent with an explanation for biotin-responsiveness based on altered affinity for biotin. The two others may define an additional domain involved in biotin-binding or biotin-mediated stabilization of the protein."

Dupuis et al (278) characterized the Arg508→Trp mutation, found in 4 (3 heterozygous, 1 homozygous) of 9 multiple carboxylase deficiency patients screened, as residing in the biotin binding site. "We anticipate that the four mutations in the biotin-binding region of HCS will account for the high K_m for biotin measured in patients with neonatal MCD. For example, two of the patient fibroblast lines we studied, JRi and MC, had a reported K_m of 0.346 and 0.048 µmol/L, respectively, compared with 0.015 µmol/L for the normal enzyme. JRi was found to have an [Arg508→Trp] mutation and MC was found to have a [Val550→Met] mutation (in each case, the second mutation has yet to be identified). While it is premature to conclude that these mutations are causative of the elevated K_m, their location in the biotin-binding region and the conservation of three of the four mutations among human, *Paracoccus denitrificans*, *E. coli, Bacillus subtilis*, *Salmonella typhimurium*, mouse and yeast biotin ligases is consistent with this notion" (278).

Of 7 HCS mutations analyzed, 2 (Gly581→Ser and delThr610) were found to reside in the putative biotin binding region of HCS and resulted in increased K_m values by 45-fold and 3-fold, respectively. The other 5 mutations were outside the biotin binding region. Administering biotin to Gly581→Ser mutant cells in culture increased propionyl-CoA carboxylase activity to control levels, whereas such treatment did not affect other mutant lines that had mutations outside the putative biotin binding domain (279).

By prenatal diagnosis, a 33-fold elevated K_m for biotin was found in a fetus (K_m patient, 0.221 µmol/L; control subject, 0.007 µmol/L). The mother was given 10 mg biotin/d and the newborn, who was clinically well, was maintained on biotin treatment after birth at 20 mg/d (280).

The K_m measured from amniocytes of a woman pregnant with another HCS-deficient child diagnosed prenatally was 12 times greater than control, and Vmax was 2% of control (281). Biotin responsiveness was shown in vitro, with the restoration of carboxylase activities to 51–58% of normal. The infant's K_m was increased as well to 0.060 µmol/L (control: 0.007 µmol/L). The mother was treated prenatally and the infant was clinically well at birth.

Five biotin-responsive patients with a defect in holocarboxylase synthesis were reported by Suormala et al (282). Enzyme activities and K_m were measured and clear evidence was presented that many HCS cases respond to biotin because of a K_m defect in the enzyme. In 3 patients, normalization of biochemical indexes required doses of 20–40 mg/d. The fourth patient required a dose of 100 mg biotin/d before her skin rash disappeared, but she remained mentally retarded and showed slightly elevated urinary organic acid excretion. The results in the 5 patients suggest a primary defect in HCS resulting from a decreased affinity for biotin, in one patient combined with a decreased Vmax.

A new polymorphism, 1121C→T, was identified in the mutational analysis of 7 patients with HCS deficiency (283). Note also that there are many known mutations in HCS that do not affect the biotin binding site; thus, an altered K_m would not explain those cases.

Tissue concentrations and toxicity

There seems to be good evidence that pharmacologic doses of biotin increase biotin concentrations in tissues and plasma (284). Normal plasma and whole-blood biotin concentrations are ≈2 nmol/L (277). The serum biotin concentration of one patient

taking 20 mg biotin/d was raised to 4.8 μmol/L, which was 4.5 times greater than the K_m for biotin of his HCS enzyme (285). More generally, 10 mg oral biotin daily is believed to produce concentrations of biotin in plasma of 1.3 μmol/L, well above the K_m for biotin of HCS of most patients with HCS deficiency (285). Lower dosages (≥600 μg/d) used in another study raised plasma biotin concentrations 7-fold (from 0.3 to 2.8 nmol/L), whereas concentrations of the "quantitatively most important biotin metabolite, bisnorbiotin," were raised 15.5 times in plasma post-supplementation (286).

No UL for biotin has been set. Toxicity has not been reported in patients receiving daily doses of ≤200 mg orally and ≤20 mg intravenously for the treatment of biotin-responsive inborn errors of metabolism and acquired biotin deficiency (7).

COBALAMIN (VITAMIN B-12)

The DRI for vitamin B-12 is 2.4 μg/d for adults (7). Cobalamin is the precursor to methylcobalamin and adenosylcobalamin, the bioactive cofactor forms of cobalamin. Cobalamin-dependent enzymes are listed in **Table 8**.

Methylmalonyl-CoA mutase: methylmalonic aciduria and cognitive dysfunction

Methylmalonyl-CoA mutase is a mitochondrial enzyme that requires adenosylcobalamin to catalyze the isomerization of methylmalonyl-CoA to succinyl-CoA. Deficiency of methylmalonyl-CoA mutase leads to methylmalonic aciduria (*see* OMIM 251000). Symptoms include multiple episodes of life-threatening organic acidosis and hyperammonemia associated with low-normal intelligence in the first years of life. Patients often respond to pharmacologic supplements of cobalamin (cyanocobalamin or hydroxycobalamin) leading to a reduction in methylmalonate accumulation. It is possible that from one-third to one-half of mutations in methylmalonyl-CoA mutase confer a reduced ability to bind cobalamin cofactor. A newborn screening program identified 17 children with methylmalonic aciduria, of whom 7 (41%) were cobalamin responsive (287).

Enzymes from fibroblasts of 4 remediable patients had elevated K_m values for adenosylcobalamin, suggesting a perturbation in cofactor binding (3). The nonremediable genetic defects constitute about two-thirds of those found and the remediable defects about one-third (3). The latter defective proteins retain some enzyme activity (2–75% of control) and have an increased K_m for adenosylcobalamin of ≈200–5000 times normal (288–290).

Kinetic analysis of one cobalamin-responsive patient showed abnormal binding of the coenzyme, adenosylcobalamin, for its methylmalonyl-CoA mutase apoenzyme, ie, a K_m of 38 mmol/L compared with the control K_m of 0.015 mmol/L. A decreased Vmax (14% of control) was found as well. It was concluded that the defect was "at the [adenosylcobalamin]-binding site since the K_m for the substrate, [methylmalonyl-CoA], is similar to controls whereas the K_m for [adenosylcobalamin] binding differs by 2,600-fold" (290).

In an examination of the fibroblasts from 2 other patients with a cobalamin-remediable phenotype, a decreased affinity of the mutant enzyme for adenosylcobalamin was found. The K_m values of the mutant enzymes for adenosylcobalamin were 280 and 17 mmol/L, compared with control K_m values of 0.06–0.07 μmol/L (an ≈2000-fold increase in K_m). The Vmax of both enzymes was decreased to 20% and 5% of control (288).

Two types of mutations have been described, those leading to no detectable activity (mut⁰), which are not corrected by excess cobalamin, and those exhibiting residual activity (mut⁻), which are corrected by excess cobalamin. X-ray structure analyses showed many of the latter mutations to be in the cobalamin binding site of the enzyme (291). This observation is consistent with a K_m mutation as an explanation for the cobalamin-dependent phenotype.

Altered enzymes without detectable residual mutase activity (<0.1%) were found in those patients not responsive to cobalamin (289). None of 7 mut⁰ mutant lines examined had any detectable mutase activity, even when assayed in 1 mmol adenosylcobalamin/L (a value >10000 times the control K_m for adenosylcobalamin). The 7 mutant lines with mut⁻ activity (≈0.5–50% of control) showed an increased activity in cell extracts with hydroxycobalamin supplementation. These latter altered enzymes had a 50- to 5000-fold elevated K_m for adenosylcobalamin and one mutase examined turned over at a rate 3–4 times higher than that of the control enzyme when the cells were grown in hydroxycobalamin-supplemented medium. Six, and possibly all 7, of these had an elevated K_m for adenosylcobalamin ranging from 2 to 290 μmol/L (control K_m: 0.04–0.08 μmol/L). The Vmax was also decreased: 7–725 pmol·min⁻¹·mg⁻¹ compared with control values of 1053–1827 pmol·min⁻¹·mg⁻¹. Five of 8 mutase heterozygotes examined expressed some mutase activity with reduced affinity for cofactor (289).

In another study of cell lines from patients with methylmalonic aciduria, 3 of 4 exhibited cobalamin-responsiveness. The Gly717→Val mutant enzyme was expressed in cell culture and was found to have a 1000-fold higher K_m for adenosylcobalamin and an increase in activity in response to high concentrations of cobalamin. Four novel mutations described are near the carboxyl end of the protein and are hypothesized to reside in the adenosylcobalamin binding site (292, 293).

Methionine synthase: homocystinuria and neurologic dysfunction

Methionine synthase (5-methyltetrahydrofolate–homocysteine *S*-methyltransferase) catalyzes the cobalamin-dependent methylation of homocysteine, using 5-methyltetrahydrofolate as the methyl donor (*see* OMIM 156570). Defects in methionine synthase result in hyperhomocysteinemia and are implicated as the lesion in the cblG complementation group of disorders in cobalamin metabolism (294).

The cblG group generally has reduced methionine synthase activity even under optimal conditions; thus, primary defects in the catalytic subunit of the enzyme may be responsible for this subgroup. The cblG group shows biochemical heterogeneity with respect to the binding of cellular cobalamin to methionine synthase. In extracts of cell lines from most patients, the methyltransferase binds ≈75% of cellular cobalamin, even though little of it is methylcobalamin. In a few lines, however, the methyltransferase is devoid of bound cobalamin of any form. This suggests the presence of mutations in the cobalamin binding domain of the methyltransferase, strengthening the possibility that the cblG group results from primary deficiencies in the methionine synthase apoenzyme (295). Hydroxycobalamin should be instituted (1 mg/d intramuscularly initially, then tapered to 1–3 mg/wk) as soon as the disorder is diagnosed (295).

Early treatment of methylcobalamin deficiency may prevent major neurologic complications of these diseases. One child who

TABLE 7
Enzymes that use a biotin cofactor[1]

Defective enzyme and EC no.	Localization	Reaction catalyzed	Disease or condition	OMIM no.	Inheritance
Holocarboxylase synthetase (6.3.4.10)	Cytoplasmic and mitochondrial	Apocarboxylases + biotin → holocarboxylases	Multiple carboxylase deficiency, metabolic acidosis, hypotonia, seizures, and lethargy (sometimes developmental delay or coma)	253270	Autosomal recessive

[1] OMIM, Online Mendelian Inheritance in Man (4).

TABLE 8
Enzymes that use an adenosylcobalamin or methylcobalamin cofactor[1]

Defective enzyme and EC no.	Localization	Reaction catalyzed	Disease or condition	OMIM no.	Inheritance
Methylmalonyl-CoA mutase (5.4.99.2)	Mitochondrial	Isomerization of methylmalonyl-CoA → succinyl-CoA	Methylmalonic acidemia, metabolic ketoacidosis, and cognitive dysfunction	251000	Autosomal recessive
Methionine synthase (2.1.1.13)	Cytoplasmic	Homocysteine + 5-methyl-THF → methionine + THF	Homocystinuria, failure to thrive, and neurologic complications	156570	Autosomal recessive
Methionine synthase reductase (2.1.1.135)	Cytoplasmic?	MS–cob(II)alamin + NADPH + SAM → MS-methylcob(I)alamin + S-adenosylhomocysteine + NADP	Homocystinuria and mental retardation	602568	Autosomal

[1] MS, methionine synthase; OMIM, Online Mendelian Inheritance in Man (4); SAM, S-adenosylmethionine; THF, tetrahydrofolate.

received hydroxycobalamin therapy before and after birth developed cognitively normally (296).

Of 2 patients described in another study, the first had greatly diminished steady state levels of methionine synthase mRNA (297). The biochemical data on the second patient's cell line (cblG WG1892) implicated mutations in the carboxyl-terminal *S*-adenosylmethionine binding domain and the intermediate cobalamin binding domain. Two mutations were detected in cblG WG1892: the conversion of a conserved proline (1173) to a leucine residue and a deletion of an isoleucine residue (881). The investigators concluded, "The crystal structure of the C-terminal domain of the *E. coli* methionine synthase predicts that the proline to leucine mutation could disrupt activation since it is embedded in a sequence that makes direct contacts with the bound *S*-adenosylmethionine. Deletion of isoleucine in the B12-binding domain would result in shortening of a β-sheet. Our data provide the first evidence for mutations in the methionine synthase gene being culpable for the cblG phenotype. In addition, they suggest directly that mutations in methionine synthase can lead to elevated homocysteine, implicated both in neural tube defects and in cardiovascular diseases" (297).

Two methionine synthase mutations that are candidates for causing the cblG disease are located in the vicinity of the cobalamin binding domain: one is the same deletion of isoleucine 881 mentioned above; the other is amino acid substitution His920→Asp. A polymorphism, Asp919→Gly (resulting from 2756A→G, mutant allele frequency 15%), was identified at an adjacent residue, and thus may also be near or in the cobalamin binding site (298). The polymorphism has been associated with lower plasma homocysteine concentrations (299, 300), which is puzzling and suggests that this polymorphism, which has been postulated to modify an amino acid on a helix involved with cofactor binding, is an activating mutation.

Of 2 unrelated boys with a cblG defect due to methionine synthase deficiency, one improved immediately on switching to hydroxycobalamin from cyanocobalamin (which caused respiratory depression and lethargy in the patient). It appears that treatment with intramuscular injections of hydroxycobalamin alleviates megaloblastic anemia and stabilizes neurologic deterioration in children with the cblG defect but may not completely correct hypotonia and developmental delay or improve the anorexia or poor weight gain associated with cblG disease (301).

Ample evidence seems to suggest that defects in methionine synthase can account for some cblG patients. Some mutations appear to affect cobalamin binding and thus serve as an explanation for the response to cobalamin in some patients. There is also evidence that the cblG complementation group is heterogeneous (302).

Methionine synthase reductase: homocystinuria and mental retardation

Methionine synthase reductase [MSR; (methionine synthase)–cobalamin methyltransferase (cob(II)alamin reducing)] is responsible for the reductive methylation and reactivation of methionine synthase with *S*-adenosylmethionine as a methyl donor (*see* OMIM 602568). MSR is a member of the ferredoxin-NADP reductase family of electron transferases, containing the FMN, FAD, and NADPH binding sites necessary to maintain methionine synthase in its functional state.

MSR deficiency is associated with the cblE complementation group of cobalamin deficiencies. Over time, the highly reactive cobalamin(I) cofactor of methionine synthase is oxidized to the inert cobalamin(II) form, rendering the enzyme inactive (294). Symptoms of MSR defects include microcephaly, psychomotor retardation, episodic reduced consciousness, megaloblastic anemia, increased plasma free homocysteine (>20 μmol/L), low plasma methionine (<10 μmol/L), and increased excretion of formiminoglutamate.

In one case report, a female patient with MSR deficiency was treated with several vitamins and cofactors and her clinical progress was followed for 17 y (303). With high-dose folic acid treatment, biochemical abnormalities such as formiminoglutamate excretion and homocystinuria nearly normalized, but clinical and hematologic abnormalities remained. When folate was replaced by methylcobalamin, alertness, motor function, speech, and electroencephalogram results improved and biochemical features were similar but mean corpuscular volume increased. The best control of symptoms was observed with a combination of folate and methylcobalamin. At the age of 17 y, the patient remained severely mentally retarded. In cultured fibroblasts, methionine synthesis was reduced to 0.03 nmol·mg^{-1}·16 h^{-1} compared with control values of 2.4–6.9 nmol·mg^{-1}·16 h^{-1}. Complementation studies indicated the cblE defect. These studies suggest a role for folate in addition to cobalamin in treatment (303).

The standard therapy consists of parenterally administered hydroxycobalamin (1–3 mg/wk) but not cyanocobalamin, and sometimes the additional administration of folic acid and betaine. The oldest known patient was first diagnosed with cblE disease at the age of 25 y (304). He had megaloblastic anemia at the age of 7 wk, which was treated with hydroxycobalamin (500 μg/d) and folic acid (5 mg/d) for 5 d, resulting in a prompt rise in hemoglobin concentrations even though megaloblastosis persisted. The therapy was continued with 1 mg cyanocobalamin intramuscularly every 8 wk and 5 mg folic acid /d orally. During the following months, he showed progressive neurologic deficits including developmental delay, pigmentary retinopathy, nystagmus, and seizures. Despite intensified therapy, the neurologic symptoms progressed. The therapy was changed to parenteral administration of hydroxycobalamin (1 mg twice a week), which, over a period of 5 mo, resulted in a normalization of methionine concentrations and a reduction in homocysteine concentrations but did not influence the neurologic symptoms.

Characterization of defects in cblE patients showed several mutations in the gene encoding MSR. Of the 11 mutations identified in one study, 3 were nonsense mutations (294). The remaining 8 mutations were found throughout the coding region and involved substitutions or in-frame disruptions of the coding sequence. Of 7 mutations not identified in the control population, 3 were located in the vicinity of the proposed FMN binding site and 2 more were found to be associated with the FAD and NADPH binding regions.

Cobalamin treatment seems to bypass the genetic defect because MSR does not use a cobalamin cofactor. We suggest that physicians consider the benefits of riboflavin, the precursor to FMN and FAD, and niacin, the precursor of NADP, in addition to cobalamin and folate when treating patients with cobalamin disorders.

Tissue concentrations and toxicity

There is no defined UL for cobalamin and cyanocobalamin (the form used in the United States and Canada). Therapy has resulted in few adverse effects with doses ≤5000 μg/d (7).

FOLIC ACID

The DRI for folic acid is 400 μg/d (7). The crystal structure of a folate binding enzyme (dihydrofolate reductase) complexed with folate has been solved to 2.3 Å and residues that directly interact with folate have been identified (305). The folate-dependent enzymes are summarized in **Table 9**.

Methylenetetrahydrofolate reductase (NADPH): homocysteinemia, schizophrenia, rages, depression, central nervous system dysfunction, and neural tube defects

MTHFR (also discussed in the section on riboflavin) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (*see* OMIM 236250). The latter is the predominant circulatory form of folate and the main carbon donor for the remethylation of homocysteine to methionine. Patients with severe MTHFR deficiency (0–20% residual activity) present in infancy or adolescence with developmental delay, motor and gait dysfunction, seizures, schizophrenic disturbances, and other neurologic abnormalities; they are also at risk of vascular complications. MTHFR mutations, including the 677C→T polymorphism, lead to elevated plasma homocysteine concentrations, a risk factor for vascular disease and possibly schizophrenia.

Recurrent episodes of folate-responsive schizophrenic-like behavior were documented in a mildly retarded adolescent girl with homocystinuria and homocysteinemia without hypermethioninemia who lacked the habitus associated with CBS deficiency (306). Enzymes involved in homocysteine-methionine metabolism were shown to be normal. A defect in the ability to reduce 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate was shown and MTHFR activity was 18% of control values. The girl was treated with oral folic acid and all her psychotic behavior ceased. Supplementation with folic acid (20 mg/d) for 14 d led to a decrease in homocysteine excretion and progressive improvement in intellectual function over the next 3 mo without further medication. The girl left the hospital without medication and was readmitted later, at which point folic acid and vitamin B-6 induced major improvements again. This is one of 4 patients described since 1975 (307).

Two children who were found to have homocystinuria after they were examined for rages and seizures were initially thought to have a biochemical defect in the conversion of homocysteine to methionine. They both responded favorably to low-dose folic acid (0.8–3 mg/d) with a decrease in urinary homocystine and other improvements, but the benefits only lasted several months, at which time homocystine concentrations increased and rages resumed. In one case, double the amount of folate and a low-protein diet caused improvement; in the other, betaine and a low-protein diet were effective. The authors suggested that the biochemical defect was a deficient MTHFR activity that is partially responsive to folate therapy (308).

The K_m of the *E. coli* mutant protein homologous to the human 677C→T variant was measured and found to not differ significantly from controls (165). FAD binding was affected—likely as a result of an increased K_m for the coenzyme—but the decreased affinity of MTHFR for FAD was abolished under conditions of high folate. It was thus hypothesized that folic acid therapy lowers homocysteine in *TT* individuals by increasing enzyme affinity for FAD. FAD binding is similarly impaired in the recombinant human enzyme, which was recently purified (164). The more severe cases discussed above may have responded to folate

through increases in enzyme affinity for FAD or by overcoming a decreased affinity for the folate substrate itself.

Another reason for lowering homocysteine concentrations by riboflavin, vitamin B-12, folate, and vitamin B-6 supplementation is the reduction of anger and hostility. Positive and significant associations were reported between hostility and homocysteine concentrations in both men and women and between anger and homocysteine concentrations in men (309).

The 677C→T polymorphism in *MTHFR* was examined in persons with schizophrenia, major depression, and bipolar disorder (310). The *TT* variant was found in 12% of 419 control subjects, 21% of 297 patients with schizophrenia ($P < 0.0006$; $P < 0.002$ after Bonferroni correction), 28% of 32 patients with major depression ($P < 0.06$; $P < 0.02$ after Bonferroni correction), and 13% of 40 patients with bipolar disorder (NS). The authors pointed out that the oxidation product of homocysteine, homocysteic acid, exerts potent excitatory effects (310). Joober et al (311) also found an overrepresentation of the *TT* variant in persons with schizophrenia who responded to neuroleptics compared with that in control subjects (311).

In another study, high homocysteine concentrations were found in 9 of 20 patients with schizophrenia (312). The thermolabile 677C→T polymorphism was screened for in a follow-up study of 11 patients with high homocysteine concentrations. Seven of the 11 patients, 6 males and 1 female, had the homozygous *TT* genotype. One male patient was heterozygous and all 3 normal homozygotes were females. In the patients who were homozygous for the polymorphism, homocysteine concentrations did not respond to vitamin B-12 but were normalized by folate supplementation. In the healthy homozygotes, however, homocysteine concentrations were reduced by vitamin B-12 alone. It was concluded that homozygosity for thermolabile MTHFR may be a risk factor for schizophrenia-like psychosis, and that this risk might be reduced by folate supplementation (313). In a small study (314), homocysteine was significantly higher in patients with schizophrenia who had low serum folate concentrations ($n = 6$) than in control subjects with low serum folate concentrations ($n = 8$).

Two reports did not find a link between schizophrenia, the 677C→T polymorphism, and hyperhomocysteinemia. In one report, no significant difference in the frequency of *TT* individuals was found between the 343 patients with schizophrenia and 258 control subjects studied. It was concluded that the 677C→T polymorphism is unlikely to have played a major role in the pathogenesis of schizophrenia or affective disorders in the sample population (315). Another group found no significant differences in plasma homocysteine concentrations between the 210 patients with schizophrenia and 218 control subjects studied. The distributions of the *T* allele and *TT* genotype frequencies were similar in both groups (40% and 15%). Thus, it was concluded that impaired homocysteine metabolism is unlikely to play a role in schizophrenia (316).

We suggest that clinical trials of B vitamin therapy (including folate and riboflavin) in relation to schizophrenia and rages are warranted on the basis of the association of higher homocysteine concentrations with anger (309) and schizophrenia (312) and the association of the *TT* genotype with schizophrenia (310). Homocysteine accumulation can be an indicator of a defective enzyme in the methylation pathway and treatment with vitamin precursors of substrates and cofactors in that pathway such as riboflavin, vitamin B-12, folate, and vitamin B-6 may be beneficial in managing rages and schizophrenia.

Folate is a well-established measure for preventing NTDs. Various studies have implicated the 677C→T mutation and high homocysteine in NTDs and others have shown a decrease in NTD prevalence in mothers who take folic acid perinatally (317). Although the etiology of NTDs is likely multifactorial and 677C→T alone is certainly not responsible for NTDs, a possible explanation for folate-responsiveness in individuals with the polymorphism could relate to the fact that incubation of the variant enzyme with high concentrations of folate abolishes the reduced FAD binding capacity of 677C→T MTHFR (165). It is unclear whether perinatal use of riboflavin, the precursor of FAD, would be of additional benefit for some mothers at risk of delivering a child with an NTD.

Methionine synthase: homocystinuria and neurologic dysfunction

One patient with 36% of normal residual methionine synthase activity improved significantly with folic acid treatment (318). (*See* OMIM 156570 and the section on cobalamin for further information on methionine synthase.)

Dihydrofolate reductase: megaloblastic anemia and neurologic symptoms

Dihydrofolate reductase uses NADPH to catalyze the successive reductions of folate to 7,8-dihydrofolate to 5,6,7,8-tetrahydrofolate (*see* OMIM 126060). In one patient with megaloblastic anemia and decreased enzymatic activity, oral folic acid (5 mg/d) resulted in a sustained 3-y remission (500 μg/d had no effect) (319). When folate therapy was discontinued, the patient relapsed.

Dihydrofolate reductase deficiency was reported in 3 children presenting with a megaloblastic anemia shortly after birth (320). A deoxyuridine suppression test was abnormal in 2 of the children and was only corrected with folinic acid, 5-formyltetrahydrofolate. A hematologic response was also evident after folinic acid therapy, although this therapy may bypass the defect.

Glutamate formiminotransferase: mental retardation

Glutamate formiminotransferase uses PLP to transfer the formimino group from formimino glutamate to tetrahydrofolate to form 5-formyltetrahydrofolate (folinic acid). A defect in this enzyme results in excretion of formimino glutamate (*see* OMIM 229100). Four of 5 original patients reported by Arakawa (321) had mental and physical retardation; enzymatic activity ranged from 14% to 54% of normal.

Five additional patients were reported with the deficiency; one was a 42-y-old woman whose elevated urinary concentrations of formiminoglutarate fell to normal with 30 mg folic acid/d and who improved with continued folate therapy (322). As measured by decreased excretion of formimino glutamate, 2 of 8 patients responded to treatment with high folic acid (323, 324).

Physicians might consider treatment with pyridoxine (cofactor precursor) in addition to folate (substrate precursor) in patients who present with a defect in glutamate formiminotransferase. Folinic acid therapy, which would bypass the metabolic defect, should be effective as well.

Folate membrane transport: dyserythropoiesis, central nervous system dysfunction, and megaloblastic anemia

Congenital malabsorption of folate (*see* OMIM 229050) results clinically in hypotonia, lethargy, seizures, megaloblastic ane-mia, mental retardation, and ataxia and biochemically in low folate concentrations in serum, red blood cells, and cere-brospinal fluid (320). A patient with hereditary dyserythropoiesis (without anemia) had reduced membrane transport of 5-methyltetrahydrofolate by red blood cells (325). Total uptake, uptake velocity, and maximal velocity of uptake were all significantly less than in control subjects. The patient's measured K_m was 0.27 μmol/L (Vmax 0.095 pmol·10^{-9} cells·min^{-1}) whereas that in control subjects was 0.50 μmol/L (Vmax 0.301 pmol·10^{-9} cells·min^{-1}). Although the Vmax was lower in the patient, the K_m did not appear to be the primary defect. However, the patient's daughter had an elevated K_m of 0.93 μmol/L, suggesting a reduced affinity of the transport system for 5-methyltetrahydrofolate.

Four cases of congenital malabsorption of folate with megaloblastic anemia, central nervous system abnormalities, and defective gastrointestinal absorption of folates responded at least partially to folic acid (40 mg oral) (322). A review of folate metabolic errors states that ≈12 cases of defective transport of folate across the intestine and the blood-brain barrier have been reported. High doses of oral folic acid (5–40 mg) or lower parenteral doses can reverse the hematologic abnormalities and digestive symptoms involved with the condition (320). A K_m explanation is not definitive because transport of folate through an alternative system at high concentrations has not been ruled out.

Seizures

Folinic acid has been used in treating early-onset intractable seizures (unresponsive to anticonvulsants and pyridoxine) and can elicit an immediate response (326).

Folylpoly-γ-glutamate carboxypeptidase: homocysteinemia

Folylpoly-γ-glutamate carboxypeptidase (γ-glutamyl hydrolase), an enzyme that is anchored to the intestinal brush border membrane, is responsible for cleaving terminal glutamate residues from folylpoly-γ-glutamates (*see* OMIM 600934), the predominant naturally occurring form of dietary folates. Inability to cleave glutamyl residues reduces the intestinal absorption of folates and decreases folate availability for the remethylation of homocysteine to methionine (*see* the discussion of methionine synthase in the section on cobalamin), resulting in hyperhomocysteinemia.

A C-to-T transition at base pair 1561 was found in 6 (8%) of 75 healthy individuals (327). The polymorphism causes a missense mutation, His475→Tyr that decreases protein activity by 53% as measured in transfected COS-7 cells. As expected, individuals with the polymorphism (heterozygotes) have lower serum folate and higher homocysteine (significant) concentrations and lower red blood cell folate concentrations (NS). Such individuals may benefit from folate supplementation through raised body folate concentrations and lowered homocysteine. This therapy would likely bypass the defect because supplements contain the monoglutamate form of folate and thus do not require the action of folylpoly-γ-glutamate carboxypeptidase. Nevertheless, it would be of interest to measure the kinetic properties of the enzyme as well as the ability of exogenous polyglutamate-folates to overcome the defect.

Tissue concentrations and toxicity

A UL for folate intake from supplements and fortified foods has been set at 1000 μg/d for adults and 300 μg/d for 2-y-olds

increasing to 800 μg/d for 16-y-olds (7), although higher amounts seem warranted in many cases. One patient mentioned above had serum folate concentrations of 185 mg/L (normal concentrations are ≈6 μg/L) and red blood cell folate concentrations of 591 mg/L (normal concentration: 160 μg/L) after 4 mo of supplementation with 12 mg folic acid/d (325). A recent report showed that 5 mg folate/d raises serum folate concentrations 6–7 times and red blood cell folate concentrations 2 times compared with placebo (328). Diets high in folate have also been shown to raise serum folate concentrations up to 85% (329). One study suggests that increasing plasma folates in individuals above the third quartile of folate intake is not feasible. In a double-blind, placebo-controlled study of 82 alcoholic subjects receiving 1 mg/d for 18.5 d, it was found that whole-blood folate in these individuals in the highest quartile of whole-blood folate (initially 3.73–7.70 nmol/g hemoglobin) could not be raised. However, subjects in the lowest 3 quartiles did show an increase with folate supplementation. The concentration in the lowest quartile (initially 0.71–2.06 nmol/g hemoglobin) was raised significantly by ≈0.8 nmol/g hemoglobin and the second lowest (initially 2.08–2.83 nmol/gm hemoglobin) by ≈0.75 nmol/g hemoglobin (330). It would be useful to test nonalcoholics as well (*see* the discussion of folate membrane transport above) and to measure tetrahydrofolate concentrations because alcohol interferes with folate absorption.

VITAMIN K

The adequate intake of vitamin K is 90 μg for women and 120 μg for men (8). The vitamin K–dependent proteins discussed below are summarized in **Table 10**.

γ-Glutamyl carboxylase: hemophilia

γ-Glutamyl carboxylase, with bound vitamin K in the presence of oxygen and carbon dioxide, converts glutamic acid residues to γ-carboxyglutamic acid residues on the amino-terminal regions of precursor forms of prothrombin (factor II) and factors VII, IX, and X (*see* OMIM 137167). These γ-carboxyglutamic acid residues are necessary for calcium-dependent phospholipid binding by the vitamin K–dependent clotting factors and are prerequisites for normal blood coagulation (331).

The binding sites for the γ-carboxylation recognition site containing propeptide and carboxylatable glutamate residues of a vitamin K–dependent substrate protein have been localized to the amino-terminal 250 residues of the enzyme. The carboxyl-terminal regions of the enzyme are important for conversion of vitamin K hydroquinone to vitamin K epoxide, a reaction that occurs concomitantly with carboxylation and is catalyzed by the vitamin K–dependent carboxylase. In addition, catalysis of vitamin K oxygenation by the enzyme is regulated by the availability of carboxylatable substrate (332).

The kinetic properties of a naturally occurring mutation in human γ-glutamyl carboxylase, Leu394→Arg, have been studied (333). The mutant has a 5-fold higher K_m (32.8 ± 5.4 μmol/L) for vitamin K hydroquinone, the reduced form of vitamin K, than does the control (7.0 ± 1.2 μmol/L). The coagulation activities of patients with combined deficiencies of vitamin K–dependent coagulation factors were partially corrected by the administration of vitamin K and vitamin K restored carboxylase activity from a low level to ≈33% of wild type (333). Thus, this seems to be a vitamin K–responsive K_m mutant.

An infant girl with abnormal bleeding and some skeletal abnormalities who showed deficiency of vitamin K–dependent clotting factors II (8% of normal), VII (22%), IX (28%), and X (15%) was put on treatment consisting of intramuscular injections of vitamin K and physiotherapy (331). After 7 d of treatment, the concentrations of factors II, VII, IX, and X rose to 60%, 65%, 112%, and 56% of controls, respectively. The patient was discharged with vitamin K injections (0.5 mg/kg body wt) every other day (later changed to 10 mg/d). Another patient with a Trp501→Ser mutation and deficiency of the same vitamin K–dependent factors was also responsive to vitamin K therapy (5 mg/d) and a binding defect was suspected: "the mutation may affect either the vitamin K-binding site or the propeptide-binding site" (334).

A patient congenitally deficient in factors II, VII, IX, and X was studied after a follow-up of 15 y (335). At birth, these factors, when determined by clotting assays, were undetectable. After therapy with vitamin K1, the clotting activity of these factors rose but never exceeded 18% of normal. Some molecules of the patient's prothrombin lacked the normal complement of γ-carboxyglutamic acid residues. It was suspected that this represents either a defective γ-carboxylation mechanism within the hepatocyte or faulty vitamin K transport; a K_m mutation in γ-glutamyl carboxylase affecting vitamin K binding would be a plausible explanation. Vitamin K therapy could be tried in cases of inborn errors of blood clotting.

Propeptide of factor IX: hemophilia

Blood clotting requires the posttranslational modification (γ-carboxylation) and proteolysis of several blood clotting factors. The γ-carboxylation reaction is performed by γ-glutamyl carboxylase in the presence of vitamin K, carbon dioxide, and oxygen and precedes cleavage. A proteolysis cascade is required for clotting factor activation, in which the cleavage of each factor activates a proteolytic activity in the subsequent protein so that the cascade can continue. The propeptide sequence of vitamin K–dependent proteins, such as factor IX propeptide, is a critical factor in the regulation of γ-carboxylation. One highly conserved residue of factor IX propeptide in particular, the alanine at position −10 (A-10), seems to influence the carboxylation reaction. A patient was investigated that had a 6346G→A transition in genomic DNA, resulting in a mutation, A-10T, in the factor IX propeptide (336). The mutation resulted in the carboxylating enzyme, γ-glutamyl carboxylase, having a 33-fold increased K_m for propeptide, as well as a less marked rise in K_m of the enzyme-propeptide complex for vitamin K. The K_m of the complex containing the wild-type propeptide was 4.2 μmol/L, whereas the K_m of the complex with the mutant A-10T propeptide for vitamin K was 9.3 μmol/L and the K_m of the complex with another mutant propeptide, A-10G, for vitamin K was 8.6 μmol/L. These authors concluded, "Thus, both enzyme-peptide complexes containing variant peptides have lower affinities for vitamin K than the complex containing the wild-type peptide, but the difference is not marked" (336). (*See* OMIM 306900.) It would be of interest to know whether patients would benefit from vitamin K therapy.

Tissue concentrations and toxicity

In vivo concentrations of vitamin K can be clinically manipulated (333). Plasma menaquinone-4 concentrations reached a

TABLE 9
Enzymes that use a folate cofactor or substrate[1]

Defective enzyme and EC no.	Localization	Reaction catalyzed	Disease or condition	OMIM no.	Inheritance
Methylenetetrahydrofolate reductase (NADPH) (1.5.1.20)	Cytoplasmic	5,10-Methylene-THF + NADPH → 5-methyl-THF + NADP	Homocystinuria, vascular complications, retardation, seizures, psychiatric problems, and neurologic abnormalities (including schizophrenia, rages, and depression)	236250	Autosomal recessive
Methionine synthase (2.1.1.13)	Cytoplasmic	Homocysteine + 5-methyl-THF → methionine + THF	Homocystinuria	156570	Autosomal recessive
Dihydrofolate reductase (1.5.1.3)	Cytoplasmic?	Dihydrofolate + NADPH → tetrahydrofolate + NADP	Megaloblastic anemia and neurologic abnormalities	126060	Autosomal recessive
Glutamate formiminotransferase (2.1.2.5)	Cytoplasmic	THF + N-formimino-glutamate → 5-formimino-THF + glutamate	Mental retardation	229100	Autosomal recessive
Folylpoly-γ-glutamate carboxypeptidase (3.4.17.21)	Extracellular and lysosomal	Cleaves terminal glutamate off folylpoly-γ-glutamates	Homocysteinemia	600934	—

[1] OMIM, Online Mendelian Inheritance in Man (4); THF, tetrahydrofolate.

TABLE 10
Enzymes that use a vitamin K cofactor[1]

Defective enzyme	Localization	Reaction catalyzed	Disease or condition	OMIM no.	Inheritance
γ-Glutamyl carboxylase	Integral membrane protein	Glutamic acid of propeptide → γ-carboxyglutamic acid	Hemophilia and decreased γ-carboxyglutamate and clotting factors	137167	Autosomal recessive
Propeptide of factor IX	Extracellular	Glutamic acid of propeptide → γ-carboxyglutamic acid	Hemophilia	306900	X-linked

[1] OMIM, Online Mendelian Inheritance in Man (4).

maximum (a 10-fold increase) at 3 h after administration of 4 mg menaquinone-4 to 6 infants aged 5 d (337).

CALCIFEROL (VITAMIN D)

The adequate intake of vitamin D (calciferol, or vitamin D_3) is 5 μg for middle-aged persons and 10 μg for older persons (338). Two successive hydroxylations of vitamin D in the liver and kidney produce the hormonally active form, calcitriol (1,25-dihydroxyvitamin D_3).

Vitamin D receptor: vitamin D–dependent rickets II

Calcitriol binds to the vitamin D receptor (*see* OMIM 601769), a nuclear transcription factor that regulates gene expression and is essential for the normal development of bone and the promotion of calcium transport across the small intestine. Defects in the vitamin D receptor lead to hypocalcemic vitamin D–dependent rickets, congenital total lipodystrophy, and persistent mullerian duct syndrome. The first known patient with a missense mutation in the vitamin D receptor hormone binding domain harbored a Arg271→Leu substitution and was not responsive to calcitriol (≤50 μg/d), most likely because of a 1000-fold decreased affinity of vitamin D receptor for calcitriol in vitro (339).

Other patients with missense mutations affecting calcitriol binding have, however, responded to high-dose vitamin D therapy (340–342). Sequence analysis of a hyporesponsive vitamin D receptor gene from one patient revealed a C-to-G transversion (His305→Gln) that caused an 8-fold decreased protein affinity for calcitriol (340). The patient was effectively treated with high doses (12.5 μg/d) of calcitriol. Two other point mutations, Ile314→Ser and Arg391→Cys, have been found that confer reduced calcitriol-dependent activation of vitamin D receptor (341). Vitamin D receptor with the Arg391→Cys mutation was partially activated by high concentrations of hormone in vitro, which was reflected in the only partial responsiveness of the patient to calcitriol. On the other hand, vitamin D receptor activity of the Ile314→Ser mutant was more easily rescued by calcitriol, and the patient with this mutation was almost completely cured by calcitriol therapy. A similar patient (aged 18 mo) with a decreased affinity of vitamin D receptor for calcitriol responded well to administered hormone (20 μg/d) with a resolution of serum calcium and phosphorous concentrations; marked clinical improvement, including the ability to stand and walk; and progressive healing of the rickets (342).

Three vitamin D receptor polymorphic alleles [polyA (long), *Bsm*I (*bb*), and *Taq*I (*TT*)] were found in higher frequencies in colon cancer patients than in control subjects and were thus associated with an increased rate of cancer (343). Such reports reinforce the need to decipher genotype-phenotype relations, with the ultimate goal of catering medical interventions to the needs of individuals. If increased concentrations of calcitriol could overcome a protein defect associated with cancer, vitamin D therapy could be important for reducing cancer risk associated with such defects in the vitamin D receptor.

In a study of the involvement of vitamin D deficiency (serum 25-hydroxyvitamin D_3 < 50 nmol/L) in prostate cancer development, young men (40–51 y old) with low serum vitamin D concentrations were at the greatest risk of developing prostate cancer (344). Cellular studies in prostate cancer cells suggested that vitamin D up-regulates androgen receptor expression, whereas androgens seem to up-regulate vitamin D receptor (344). If

defects in vitamin D receptor (or androgen receptor) also predispose individuals to prostate cancer, such an activation of receptors by vitamin D administration seems like a possible protective measure for preventing against the onset of cancer in individuals (especially young men) with vitamin D receptor defects.

By 1994, ≈50 cases of vitamin D receptor defects had been reported. Treatment is usually ≈20 μg/d of the bioactive form, calcitriol, or 5 mg/d of the dietary form, vitamin D_2, plus oral calcium and phosphate (345).

Calciferol 1α-hydroxylase: vitamin D–dependent rickets I

The mitochondrial cytochrome *P450c1α* gene codes for a calciferol 1α-hydroxylase (calcidiol 1-monoxygenase) that converts 25-hydroxyvitamin D_3 to the hormone calcitriol. A genetic deficiency in the renal proximal tubules causes a pseudo–vitamin D–deficiency called rickets (*see* OMIM 264700). The *P450c1α* gene was analyzed in 19 individuals from 17 families representing various ethnic groups (346). All patients had *P450c1α* mutations on both alleles. In the French Canadian population, among whom vitamin D–dependent rickets I is common, 9 of 10 alleles bore the haplotype 4-7-1 and carried the mutation 958ΔG. Patients are treated with a physiologic dose of calcitriol; thus, the hormone is replaced, but the remediation does not appear related to a change in K_m.

Morphea

Morphea is a skin-associated scleroderma, a disease of the connective tissue involving increased collagen synthesis and deposition in various organs and skin. A significant clinical improvement was observed in 3 patients with generalized morphea who were given 0.50–0.75 μg calcitriol/d (347). If the mechanism of action—possibly immunoregulatory or growth inhibitory—involved altered binding for the vitamin hormone, then it seems plausible that remediation of scleroderma or morphea with vitamin D results from raising the rate of some reaction in that pathway.

Tissue concentrations and toxicity

Data accumulated in the DRI manual suggest a direct relation between vitamin D intake and 25-hydroxyvitamin D concentrations. The UL of dietary vitamin D is 50 μg/d and adverse effects have been seen at concentrations ranging from 250 to 1250 μg/d (338).

TOCOPHEROL (VITAMIN E)

α-Tocopherol is the main form of the lipid-soluble vitamin E in animal tissues and plasma. The DRI for vitamin E is 15 mg/d (× 1.5 IU/mg = 22.5 IU/d) as α-tocopherol (348).

α-Tocopherol transfer protein: ataxia with isolated vitamin E deficiency

With structural similarity to other lipophilic vitamin binding proteins, α-tocopherol transfer protein (TTP), present in the liver and cerebellum, is responsible for the incorporation of α-tocopherol into lipoproteins and for the transport of α-tocopherol between membranes. An autosomal recessive disease characterized by ataxia with isolated vitamin E deficiency (AVED, *see* OMIM 277460) is caused by mutations in TTP. Patients with AVED retain normal intestinal absorption of vitamin E, but have defective incorporation of vitamin E into VLDLs by hepatic

cells. The disease involves abnormally low serum concentrations of α-tocopherol, absent tendon reflexes, cardiomyopathy, and intellectual decline. The prevention of neuronal damage associated with AVED is typically mediated by lifelong supplementation with high doses of vitamin E (800 mg/d), and other symptoms can be averted if therapy is started early enough.

In 3 individuals, vitamin E responsiveness was attributed to mutations in the gene encoding TTP (349). Two siblings were compound heterozygotes for a 421G→A transition (Glu141→Lys) and a 513-514 TT insertion. A third case from another family was homozygous for a 552G→A splice site mutation. Serum α-tocopherol concentrations dropped below normal with treatment withdrawal, confirming the impaired ability of AVED patients to conserve α-tocopherol in the body. Serum α-tocopherol concentrations were raised when treatment was reinstated.

The effectiveness of high-dose vitamin E treatment (800 mg/d) for 1 y was quantitatively measured in 24 AVED patients with use of the ataxia rating scale (350). All participants had the 744delA mutation in TTP, which is common to AVED patients in Tunisia. Mean scores on the ataxia rating scale decreased from 45 to 35 over the year, with statistically significant incremental drops at 3, 6, 9, and 12 mo. Serum vitamin E concentrations normalized as well.

The success of vitamin E may be attributed to either the nonenzymatic saturation of serum or the saturation of the hepatic TTP enzymes so as to accelerate vitamin E incorporation into VLDL. The latter scenario would likely entail the overcoming of an altered binding of TTP with vitamin E.

Tissue concentrations and toxicity

The UL of vitamin E on the basis of supplementation with α-tocopherol is 1000 mg/d (1500 IU) as a result of adverse effects including increased risk of hemorrhage (348). In 2 patients mentioned above, the reinstatement of α-tocopherol treatment showed a linear relation to serum α-tocopherol concentrations. The maximal dosage (40 mg·kg body wt^{-1}·d^{-1}) resulted in a plasma concentration >50 μmol/L (349).

TETRAHYDROBIOPTERIN

No DRI has been set for tetrahydrobiopterin.

Phenylalanine hydroxylase: phenylketonuria II (mild hyperphenylalaninemia)

Phenylalanine hydroxylase (PAH; phenylalanine 4-monoxygenase), the enzyme responsible for classic phenylketonuria (OMIM 261600), utilizes tetrahydrobiopterin to convert phenylalanine into tyrosine, a critical step in dopamine biosynthesis. Defects in PAH often lead to mental retardation as a result of the accumulation of phenylalanine and its neurotoxic metabolites.

Four patients with mild hyperphenylalaninemia (sometimes termed nonphenylketonuria hyperphenylalaninemia) responded to tetrahydrobiopterin therapy (5 or 10 mg/kg body wt) with a decrease in elevated serum phenylalanine concentrations (253). Because there were no abnormalities in urinary pteridine or DHPR activity, and mutations were detected in the *PAH* gene, it was presumed that these cases reflected a novel subtype of PAH deficiency responsive to cofactor supplementation. The authors suggested that the mutants "probably form mutant PAH with a high Michaelis-Menten constant K_m for tetrahydrobiopterin. It is likely that [tetrahydrobiopterin] supplementation increased the intracellular [tetrahydrobiopterin] concentration to restore

residual PAH activity and/or to stabilize the mutant PAH molecules" (253). The patients' protein defects were characterized in a screen of mutations typical of classic phenylketonuria. The patients, who were all compound heterozygotes, harbored mutations in PAH that are present in classic phenylketonuria. Other similar reports of successful tetrahydrobiopterin treatment support the view that tetrahydrobiopterin therapy is effective in some PAH-deficient patients because of K_m variants of the enzyme (351, 352). A 60-mg dose of tetrahydrobiopterin (20 mg/kg) was used to significantly decrease blood phenylalanine in one of the patients, who was continued on 45 mg/d (352).

In Portugal, the third most common mutation in the *PAH* gene (8.9% of mutant alleles), Val388→Met, has a reduced enzymatic activity and a 3.7-fold increased K_m for tetrahydrobiopterin (82 μmol/L) as compared with the wild type (22 μmol/L) (353). Although the mutation does not seem to reside in the putative pterin binding motif, it does affect cofactor binding and thus may prove to be another tetrahydrobiopterin-remediable form.

A recent review (354) evaluated the 3 reports of tetrahydrobiopterin-responsiveness to date (253, 352, 355) and stated that tetrahydrobiopterin therapy is effective in some PAH-deficient patients because the primary defects affect cofactor binding. In fact, several of the PAH mutations in tetrahydrobiopterin-responsive individuals have been assigned to regions of cofactor interaction (354). It remains to be seen, however, what percentage of hyperphenylalaninemias are due to tetrahydrobiopterin-responsive variants of PAH. We suggest that tetrahydrobiopterin therapy be considered with any case of phenylketonuria that might not be a classic case. The PAH Mutation Analysis Consortium Database contains information on the hundreds of mutations found in PAH (356).

***S*-ADENOSYLMETHIONINE**

No DRI has been set for *S*-adenosylmethionine.

Guanidinoacetate *N*-methyltransferase

S-Adenosylmethionine, a common methyl donor, is used by guanidinoacetate *N*-methyltransferase (*see* OMIM 601240), which catalyzes the last step in creatine synthesis, the methylation of guanidoacetate to creatine. Guanidinoacetate *N*-methyltransferase deficiency is a rare inborn error of metabolism that leads to creatine deficiency. Creatine administration, which bypasses the metabolic defect, alleviates symptoms of guanidinoacetate *N*-methyltransferase deficiency (357). Because the concentration of guanidoacetate, which has neurotoxic effects, remains high after creatine supplementation, *S*-adenosylmethionine administration could be of additional benefit. It would also be useful to elucidate the primary defect of the guanidinoacetate *N*-methyltransferase protein.

PANTOTHENIC ACID

The DRI for pantothenic acid, the nutritional precursor of coenzyme A, is 5 mg/d for adults (7).

3-Methylglutaconic aciduria and cardiomyopathy

3-Methylglutaconate is an intermediate in the catabolism of leucine, but whereas the primary metabolic defect in type I 3-methylglutaconic aciduria has been described (namely, methylglutaconyl-CoA hydratase deficiency), no specific

defect in the type II presentation has as yet been identified (*see* OMIM 302060).

In one case report, a young boy presented with dilated cardiomyopathy, growth failure, neutropenia, low serum cholesterol, and increased urinary excretion of 3-methylglutaconic and 3-methylglutaric acids (358). At a point when the patient was moribund, large doses of pantothenic acid, a precursor of coenzyme A, produced a dramatic and sustained improvement in myocardial function, growth, neutrophil cell count, hypocholesterolemia, and hyperuricemia, which suggested that a limitation in the availability of coenzyme A was the fundamental pathologic process in this condition. The dose of pantothenate was increased from 15 mg/d at the beginning to 3×50 mg/d. It is unclear why the patient responded to pantothenate, but it is possible that he had a defect in a CoA-metabolizing enzyme such as pantothenic acid kinase.

In a study of 25 mothers of children with birth defects (psychomotor retardation of unknown cause and microcephaly), 6 were found to excrete large amounts of 3-methylglutaconic acid (16 times that of control subjects) and 3-methylglutaric acid (6 times that of control subjects) (359). It is thus possible that feeding pantothenic acid to women with high concentrations of 3-methylglutaconic and 3-methylglutaric acid might lower the excretion rate of the 2 acids, normalize metabolism, and prevent future birth defects.

Pantothenate kinase: Hallervorden-Spatz syndrome and pantothenate kinase–associated neurodegeneration

Pantothenate kinase is a cytosolic enzyme responsible for the first step in the biosynthesis of CoA from pantothenic acid (vitamin B-5). Four genes encoding pantothenate kinase have been identified: *PANK1* (expressed in heart, liver, kidney), *PANK2* (ubiquitous), *PANK3* (predominantly liver), and *PANK4* (ubiquitous, predominantly muscle). Mutations in *PANK2*, which is the most abundantly expressed form in the brain, were recently implicated in pantothenate kinase–associated neurodegeneration (*see* OMIM 234200), an autosomal recessive neurodegenerative disorder characterized clinically by dystonia and often optic atrophy or pigmentary retinopathy and biochemically by iron deposits in the basal ganglia and globus pallidus (360). The mutations identified in *PANK2* fall into exons 1C, 2, 3, 4, 5, and 6. Missense mutations resulting in nonconservative amino acid changes were found in 32 of 38 classical pantothenate kinase–associated neurodegeneration cases. All 17 mutations found in atypical cases were missense mutations. It seems plausible that some of these mutations will lower pantothenate kinase activity by affecting the affinity of the enzyme for pantothenate substrate. Such cases may prove to be responsive to high-pantothenate therapy.

Tissue concentrations and toxicity

No UL for pantothenic acid has been established because there have been no reports of adverse affects (7).

LIPOIC ACID

No DRI has been set for lipoic acid.

Dihydrolipoamide dehydrogenase: lactic acidosis, cerebral cortical atrophy, and hearing loss

Deficiency of dihydrolipoamide dehydrogenase, the E3 component of all 3 mitochondrial α-ketodehydrogenase complexes (pyruvate, α-ketoglutarate, and branched-chain α-ketoacid), results in decreased activity of the dehydrogenases and lactic acidosis (OMIM 246900). Lipoic acid is covalently linked to a lysine residue in PDHC, KGDH, and BCKAD (*see* the section on thiamine).

An 8-mo-old boy with severe lactic acidosis was found to have dihydrolipoamide dehydrogenase deficiency (361). Dihydrolipoamide dehydrogenase activity in the patient's fibroblasts was reduced to 20% of the control and kinetic studies found an increased K_m for NAD and NADH (382 and 83 μmol/L, respectively) in the patient compared with control subjects (201 and 56 μmol/L, respectively). No significant differences in the K_m for lipoamide were found between control subjects and the patient, although this measurement was not definitive. Acidosis could not be relieved by thiamine, biotin, bicarbonate, protein restriction, or a ketogenic diet. Oral administration of lipoic acid (25–50 mg/kg) produced marked improvements in lactic and pyruvic acidemia, and the child continued to do well 2 y later, with clinical improvements.

Two other patients with Friedreich ataxia and dihydrolipoamide dehydrogenase deficiency had an ≈4-fold increased K_m for both lipoamide substrate and NAD cofactor (362). It is unclear how the patients were treated. Future patients with a high K_m for NAD may benefit from high-dose niacin treatment; physicians should consider riboflavin treatment as well because the lesion in at least one case of dihydrolipoamide dehydrogenase deficiency was a 3–base pair deletion in the FAD cofactor binding region of E3 (363).

In a case of lipoate-responsive pyruvate dehydrogenase deficiency, PDHC and E1 activities were severely depressed in the patient. Lactate homeostasis responded to pharmacologic supplements of lipoic acid, but the child died at the age of 20 mo (364).

CARNITINE

No DRI has been set for carnitine.

Carnitine *O*-acyltransferase: fatty acid toxicity

Carnitine *O*-acyltransferase is responsible for transporting fatty acids into the mitochondria. An infant girl with defective carnitine *O*-acyltransferase died at 31 h of age with profound macrovesicular fatty infiltration of liver, kidney, and muscle found on postmortem examination, suggestive of a defect in fatty acid β-oxidation (365). Carnitine was not fed because the defect was found only on autopsy; thus, it is unclear whether the enzyme had a K_m defect remediable by carnitine feeding, but it seems likely that other altered enzymes will. With age, carnitine *O*-acyltransferase activity decreases and the K_m of carnitine *O*-acyltransferase for both carnitine and CoA increase in rat brain. Additionally, carnitine supplementation restores activity (17).

Carnitine transporter

Primary systemic carnitine deficiency is due to a defect in the specific high-affinity carnitine transporter, which is expressed in most tissues and is responsible for bringing carnitine into the cytosol. This carnitine uptake defect, which is characterized by progressive infantile-onset carnitine-responsive cardiomyopathy, weakness, recurrent hypoglycemic hypoketotic encephalopathy, and failure to thrive, was identified in 2 unrelated patients (366). Each patient was a compound heterozygote with both alleles mutated by deletions and insertions and responded dramatically to high-dose

carnitine supplementation. A low-affinity, high-concentration, nonspecific-diffusion uptake of carnitine into the cells was suspected, which bypassed the specific carrier-mediated transporter. Although these particular carnitine-responsive mutations were not likely to have affected the K_m, other mutations could.

HORMONES, AMINO ACIDS, AND METALS

There is evidence that some diseases and conditions may be associated with an altered metabolism or altered binding of amino acids, metals, and hormones. The following examples are not necessarily conclusive K_m remediable defects, but rather evidence of the wide array of genetic profiles and conditions that may fit the nutrient-remediable mold.

Thyroid hormone: neurologic defects

Similar to the case with the vitamin D receptor (*see* the section on calciferol), mutations have been found in the thyroid hormone receptor β (*see* OMIM 190160) that affect the affinity for hormone ligand. Such mutations lead to increased circulating concentrations of thyroid hormone with normal or elevated concentrations of thyroid-stimulating hormone in serum and defects in growth and neurologic development. One child with a Pro453→Thr missense mutation had significantly reduced triiodothyronine binding affinity and was treated successfully with triiodothyroacetic acid (367). This therapy has been successful in ≥8 other patients (368) and a 1994 review found 26 mutations localized to the hormone binding domain of the thyroid hormone receptor β (369). The ability of in vitro synthesized mutant proteins to bind triiodothyronine was moderately or markedly reduced and the ability to activate or repress target gene expression was impaired. A missense mutation found in affected heterozygotes of one family caused a 12-fold decreased affinity of receptor for ligand (370). The evidence suggests that a K_m mutation in the thyroid hormone receptor β gene may be overcome by administration of triiodothyroacetic acid. The thyroid hormone receptor β system may serve as a model for receptor mutations for other hormones.

Alanine and isoleucine: Huntington disease

Alanine and isoleucine were found to be significantly lower in the plasma and cerebrospinal fluid of 16 Huntington disease patients than in that of 21 age-matched control subjects (371). It was hypothesized that defects in cellular uptake or metabolism of neutral amino acids could be a consistent feature of Huntington disease. This suggests treatment with alanine and isoleucine if the defect is in transport and pyridoxine if the defect is in transamination.

Serine and glycine: 3-phosphoglycerate dehydrogenase deficiency, seizures, and microcephaly

3-Phosphoglycerate dehydrogenase deficiency (OMIM 601815) is an inborn error of serine biosynthesis. Patients are affected with congenital microcephaly, psychomotor retardation, and intractable seizures. In 2 siblings studied, L-serine of ≤500 mg·kg^{-1}·d^{-1} was not sufficient for seizure control (49). Addition of 200 mg glycine·kg^{-1}·d^{-1} resulted in the complete disappearance of seizures, however, and electroencephalographic abnormalities gradually resolved after 6 mo. Biochemical abnormalities in this disorder are found in the fasted state and consist of low concentrations of the amino acids serine and glycine in plasma and cerebrospinal fluid (49). This appears to be a replacement therapy for a deficiency of serine biosynthesis; thus, a K_m defect in 3-phosphoglycerate dehydrogenase seems unlikely, but further mechanistic work needs to be done. Clinicians should use caution, however, because higher doses of serine (1400 mg·kg^{-1}·d^{-1}) have caused adverse effects (50).

Zinc: familial amyotrophic lateral sclerosis and Alzheimer disease

Mutations in Cu/Zn superoxide dismutase (*see* OMIM 147450) cause 25% of familial amyotrophic lateral sclerosis. Several of the Cu/Zn superoxide dismutase mutants involved with familial amyotrophic lateral sclerosis have been found to have a decreased affinity for zinc (372, 373), up to 30-fold in some mutants (374). The loss of zinc from wild-type superoxide dismutase approximately doubles the efficiency of the enzyme for catalyzing peroxynitrite-mediated tyrosine nitration, suggesting that this gained function by superoxide dismutase in amyotrophic lateral sclerosis may be an indirect consequence of zinc loss. Nitration of protein-bound tyrosines is a permanent modification that can adversely affect protein function. Thus, the toxicity of superoxide dismutase mutants associated with amyotrophic lateral sclerosis may be related to enhanced catalysis of protein nitration subsequent to zinc loss (374). In a recent study, both wild-type and amyotrophic lateral sclerosis mutant superoxide dismutase protected motor neurons when replete with both copper and zinc. When the same proteins were made zinc deficient, all initiated apoptosis in the neurons (375).

Lammich et al (376) report endogenous α-secretase activity was inhibited by a dominant negative form of a disintegrin and metalloprotease, namely ADAM 10 (*see* OMIM 602192), with a point mutation in the zinc binding site. ADAM 10 is associated closely with an α-secretase activity; the authors found it to cleave a site within the amyloid β peptide sequence. It appears that increasing ADAM 10 expression or activity could possibly promote proteolytic cleavage of amyloid precursor protein within the amyloid β sequence, thus preventing the protein from being converted into insoluble amyloid β, the proteinaceous component of amyloid plaques in brains of patients with Alzheimer disease. Because a mutation in the zinc binding site was found, it might be possible to stimulate the enzyme and rate of reaction via zinc administration.

Although zinc is a component of hundreds of important enzymes, the range between adequacy and toxicity is narrow (10). It is unclear whether zinc interventions will be successful.

Potassium

Inosine 5′-monophosphate dehydrogenase (OMIM 146690) catalyzes the oxidation of inosine 5′-monophosphate to xanthosine 5′-monophosphate with the concomitant reduction of NAD to NADH. *E. coli* inosine 5′-monophosphate dehydrogenase is activated by several cations including potassium. K$^+$ increases the rate constant for the pre–steady state burst of NADH production, possibly by increasing the affinity of NAD. Three mutant enzymes have been identified that increase the value of K_m for K$^+$: Asp13→Ala, Asp50→Ala, and Glu469→Ala. Both Asp13 and Glu469 appear to interact with the K$^+$ binding site identified in Chinese hamster inosine 5′-monophosphate dehydrogenase (377). (*See* also the discussion of BCKAD in the section on thiamine.)

MAXI B VITAMINS

Health food and drug stores sell a variety of high-dose B vitamin pills called B50, B100, and similar formulations. The time-release B100 pill contains 100 mg each of thiamine, riboflavin, niacin, pyridoxine, and pantothenic acid; 100 μg each of vitamin B-12 and biotin; and 400 μg folate. Except for folate and biotin, which are at or near the DRIs, these amounts approximate the high intakes discussed in this review. Until now, there has been little general support for high-dose B vitamin intake, so the presence of these pills on the market is puzzling. This review suggests that for some persons there might be a benefit from high-dose B vitamin treatment, although when there is, it would be desirable to find out which vitamin is responsible and to optimize the dose.

CONCLUSION

High-dose vitamin therapies have been efficacious in ameliorating ≈50 genetic diseases. The diseases are usually due to variant enzymes with decreased affinity (increased K_m) for vitamin-derived cofactors. Feeding high doses of the vitamin raises the tissue cofactor concentrations and thereby increases the activity of the defective enzyme. Several polymorphisms in which the variant amino acid is at a coenzyme binding site result in reduced enzymatic activity, which is likely to be remediable by raising cellular cofactor concentrations through the administration of high doses of vitamins. Remediation would be useful if the primary reason for the selection of the polymorphism is no longer important and the polymorphism has deleterious side effects.

The examples discussed here are likely to represent only a small fraction of the total number of defective enzymes that would be responsive to therapeutic vitamins. It seems likely that many additional enzymes requiring PLP, TPP, NAD(P), FAD, or other cofactors will be found to have genetic variants that affect cofactor binding. For example, the ENZYME database includes >100 entries each for FAD- and PLP-requiring enzymes (6), but we have found documentation of binding defects in <12 enzymes for either cofactor. Thus, both with polymorphisms and with the range of mutations causing severe to mild effects on phenotype, high vitamin administration is a potential remedy, and individuals with additional information pertaining to the topics discussed in this review are encouraged to contribute to the growing body of knowledge at www.KmMutants.org. With the advent of genomics and individual polymorphism assessment, it will become possible to customize vitamin therapies to suit the genotypic, and thus more specific, needs of individuals, instead of treating the phenotype. For now, for many of the conditions discussed, high-dose B vitamin pills or high doses of individual vitamins are available to physicians as reasonably safe and potentially helpful therapies; however, the possibility of some accompanying side effects should not be discounted. ⌘

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