Optimum nutrition: thiamin, biotin and pantothenate

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The metabolism of glucose is deranged in thiamin deficiency, but once any deficiency has been corrected there is no further effect of increased thiamin intake on the ability to metabolize glucose through either pyruvate dehydrogenase (EC 1.2.4.1) and the citric acid cycle, or the pentose phosphate pathway, in which transketolase (EC 2.2.1.1) is the thiamin-dependent step. It has been suggested that the Wernicke-Korsakoff syndrome is associated with a genetic variant of transketolase which requires a higher than normal concentration of thiamin diphosphate for activity. This finding would suggest that there may be a group of the population who have a higher than average requirement for thiamin, but the evidence is not convincing. There are no estimates of biotin requirements, but either coenzyme saturation of erythrocyte pyruvate carboxylase, or the excretion of 3-hydroxy-isovalerate (perhaps after a test dose of leucine) could be used to assess requirements in depletion–repletion studies. Biotin deficiency leads to impaired glucose tolerance, but it is unlikely that glucose tolerance could be used to assess optimum biotin status, since other more common factors affect glucose tolerance to a greater extent. Plasma triacylglycerol and non-esterified fatty acids are moderately elevated in pantothenic acid deficiency. However, this is unlikely to be useful in assessing pantothenate status, since again, other more common factors affect plasma lipids. To date there are no biochemical indices of adequate pantothenate nutrition, and no estimates of requirements.

Thiamin status: Biotin status: Pantothenate status: Transketolase activation:
Erythrocyte pyruvate carboxylase: 3-Hydroxy-isovalerate excretion

In the absence of any clear definition of optimum nutrition, and given the impracticability of performing whole-life studies, we have to investigate the metabolic functions of the nutrients in order to determine which, if any, may provide markers of a level of intake that could be considered to be desirable or optimum, rather than being simply adequate to prevent deficiency. It is also relevant to consider whether inadequate intake of the nutrient in question may be sufficiently important to warrant investigation, or whether average intakes are so obviously greater than optimum levels of intake that further investigation is probably irrelevant.

Thiamin
Thiamin has two distinct functions. Thiamin diphosphate (TDP) is the coenzyme for pyruvate dehydrogenase (EC 1.2.4.1) and transketolase (EC 2.2.1.1) in carbohydrate metabolism, and ketoglutarate dehydrogenase (EC 1.2.4.2) in the citric acid cycle, and branched-chain keto-acid dehydrogenase (EC 1.2.4.4) in the metabolism of the branched-chain amino acids. Thiamin triphosphate (TTP) acts in nerves (and possibly also muscle) to activate a chloride ion channel.

Bettendorf et al. (1993b, 1994) showed that the formation of TTP in brain-membrane-vesicle preparations was correlated with chloride uptake. In neuroblastoma cells in culture the addition of TTP results in activation of the chloride ion channel, which is not reversed by washing out, suggesting that it acts by phosphorylation of the chloride-channel protein (Bettendorf et al. 1993a). Impaired formation of TTP may be a factor in the neurological signs of both beriberi and Wernicke-Korsakoff syndrome, although Thorner et al. (1980) reported that TTP was preserved in the brains of thiamin-deficient lambs, at the expense of TDP and free thiamin. There are no reports of TTP concentration in analyses of brains of patients with Wernicke-Korsakoff syndrome, presumably because of its instability post mortem, and at present there is no way of using TTP or membrane chloride permeability to assess thiamin nutritional status.

The role of TDP in pyruvate dehydrogenase was elucidated in the 1930s. Thiamin deficiency results in
impaired oxidation of pyruvate, and hence accumulation of lactate and pyruvate, especially after a glucose load and moderate exercise; for many years this process was the basis of assessed thiamin nutritional status (Horwitz & Kreisler, 1949). It is unlikely, however, that additional thiamin, above the amount required to prevent deficiency, will increase aerobic capacity and prevent the development of lactacidosis after vigorous exercise, or that lactic acidosis after exercise can be used as an index of optimum thiamin status. In maximum exertion the limiting factor is not the activity of pyruvate dehydrogenase, but the rate at which O₂ can be delivered to muscle. Furthermore, the activity of pyruvate dehydrogenase is regulated by phosphorylation and dephosphorylation in response to the intracellular NADH : NAD⁺, GABA after intracerebroventricular injection of [¹⁴C]glutamate. GABA undergoes decarboxylation directly or by way of glutamate. They showed that the GABA shunt (as assessed by the specific activity of succinate semialdehyde, and oxidation to succinate) was highly specific. This reduction in activity might be expected if there were reduced activity of this enzyme. Page et al. (1989) suggested that this lack of impairment was because the γ-aminobutyrate (GABA) shunt provides an alternative pathway. α-Ketoglutarate can undergo transamination to glutamate, followed by decarboxylation to GABA, which undergoes transamination to succinate semialdehyde, and oxidation to succinate. They demonstrated that in the brains of thiamin-deficient rats, there was significantly greater metabolic flux through the GABA shunt than through the specific activity of GABA after intracerebroventricular injection of [¹⁴C]glutamate (Krebs & Stryer, 1976). By contrast, Nixon et al. (1987) reported that the affinity of transketolase for TDP was approximately 10-fold lower in patients with the Wernicke-Korsakoff syndrome than in those from control subjects, suggesting that there may be a genetic polymorphism in transketolase that would result in a subgroup of the population having higher than average thiamin requirements. This finding is akin to the thiamin-responsive variant of maple-syrup urine disease, where the affinity of branched-chain keto-acid dehydrogenase is considerably lower than normal; in this case thiamin supplements of approximately 100 mg/d are required (Scrivert et al. 1971).

Support of the suggestion of a variant transketolase associated with the Wernicke-Korsakoff syndrome, Jeyasingham et al. (1987) reported that a significant number of elderly patients with acute dementia, as well as chronic alcoholics, showed not only a higher than normal transketolase activation coefficient (indicative of thiamin deficiency), but also showed a further increase in enzyme activity when erythrocyte lysates were incubated with 3 mmol thiamin, compared with the usual 0.3 mmol/l used for the transketolase activation test. By contrast, Nixon et al. (1984) reported no difference in the affinity of transketolase for TDP between patients with Wernicke-Korsakoff syndrome and controls. They demonstrated a variety of different patterns of electrophoretically-separable forms of transketolase in normal subjects; one pattern was found in thirty-nine of forty-two patients with the Wernicke-Korsakoff syndrome, but only eight of thirty-six control subjects. They demonstrated that multiple electrophoretically-separable forms of transketolase were not the result of variant alleles, tissue-specific isoenzymes or differential splicing of mRNA, and suggested that there were differences in either the assembly of the functional holoenzyme or post-synthetic modification...
of the protein. Support for this suggestion comes from the studies of Wang et al. (1997), who expressed the human transketolase gene in Escherichia coli, and noted that formation of the normal active enzyme required a cysteinsolic factor derived from human cells, and this factor was absent from, or inactive in, cells from a Wernicke-Korsakoff patient, which showed enhanced sensitivity to thiamin deficiency in culture.

Alzheimer’s disease

Perhaps partly because thiamin is effective in treating the Wernicke-Korsakoff syndrome, it has been used empirically in treatment of Alzheimer’s disease and other dementias. There is some evidence to suggest that thiamin deficiency may be a factor in Alzheimer’s disease. Mastrogacoma et al. (1993) reported low activity of α-ketoglutarate dehydrogenase in brains from patients with Alzheimer’s disease, and increased stimulation by TDP added in vitro, suggesting thiamin deficiency. Héroux et al. (1996) reported reduced activity of pyruvate and α-ketoglutarate dehydrogenases and transketolase, as well as thiamin mono- and diphosphatases, suggesting an impairment of thiamin metabolism. By contrast, Mastrogacoma et al. (1996) reported normal activities of thiamin-metabolizing enzymes, and normal concentrations of free thiamin and thiamin monophosphate in all three cortical regions in Alzheimer’s disease. They suggested that the 18–21% reduction in TDP was due to impaired phosphorylation as a result of impaired energy-yielding metabolism.

Mastrogacoma et al. (1995) noted a significant negative correlation between α-ketoglutarate dehydrogenase activity and the number of neurofibrillary tangles in the cortex. Calingasan et al. (1996) reported clusters of amyloid precursor-like protein in the brains of thiamin-deficient rats and mice, similar to those seen in the brains of patients with Alzheimer’s disease, but noted that there was no immunohistochemical evidence of amyloid precursor protein in the brains of patients with Wernicke-Korsakoff syndrome. There is little evidence that thiamin supplements have any beneficial effect in Alzheimer’s disease; a number of studies were summarized by Blass et al. (1992) who stated that ‘treatment with large doses of thiamin has not been beneficial, but the data are not wholly negative’. Nolan et al. (1991) reported no beneficial effect of 3 g thiamin/d over 12 months. In a preliminary report, Meadow et al. (1995) stated that there was a mild beneficial effect of 3.8 g thiamin/d, although there seems to have been no further report from this group. Mimori et al. (1996) reported a mild beneficial effect of 100 mg thiamin tetrahydrofurfuryl disulfide/d.

Biotin

Dietary deficiency of biotin is more or less unknown, apart from patients on long-term total parenteral nutrition, although deficiency can be induced by consuming relatively large amounts of uncooked egg-white, in which the protein avidin binds biotin tightly, preventing its absorption. Biotin depletion also occurs in epileptics treated with several of the commonly-used anticonvulsants (Krause et al. 1985). There are no estimates of biotin requirements, and no reference nutrient intake or recommended dietary allowance; the ‘safe and adequate range of intake’ is derived from observed average intakes, which are, obviously, more than adequate to prevent deficiency.

There is some evidence that inadequate biotin nutrition may be a factor in sudden infant death. Johnson et al. (1990) reported that the liver biotin content in children who had died from no known cause was approximately 75% of that in children who had died from a known cause. The fatty liver and kidney syndrome, which can cause sudden death of flocks of chicks, is associated with biotin deficiency (Bannister, 1976), and biotin deficiency can lead to skin and hoof lesions in pigs.

Biotin functions as the coenzyme for four carboxylases: pyruvate carboxylase (EC 6.4.1.1), acetyl-CoA carboxylase (EC 6.4.1.2), propionyl-CoA carboxylase (EC 6.4.1.3) and methylcrotonyl-CoA carboxylase (EC 6.4.1.4). Much of our knowledge of the metabolic disturbances that may be associated with biotin deficiency, and which might therefore provide sensitive markers of status, has come from studies of children with multiple carboxylase deficiency as a result of genetic diseases which lead to functional deficiency despite an adequate dietary intake. Holocarboxylase synthetase (EC 6.3.4.10) catalyses the covalent attachment of biotin to a lysyl residue in the carboxylase apo-enzymes; lack of this enzyme results in total absence of all four carboxylases, and is fatal in early life (Nyhan, 1987). When biotin-containing enzymes are catalysed, the biotin is released as biocytin (biotinyl ε-amino-lysine); this is normally hydrolysed by biotinidase (EC 3.5.1.12), and there is considerable conservation of the biotin released in this way. Children who lack biotinidase again suffer from multiple carboxylase deficiency, and can be treated with relatively large supplements of biotin, to replace that lost as biocytin rather than it being salvaged. However, they do suffer some long-term neurological damage (Hymes & Wolf, 1996).

In addition to its coenzyme role, biotin also has effects on gene expression; it reacts with, and binds covalently to, histones. Biotinidase seems to be important here, acting both as an intracellular biotin-binding protein and to catalyse the covalent attachment to histones (Hymes et al. 1995; Hymes & Wolf, 1996). There is some evidence that biotinylolation of histones is important in embryological development; biotin deficiency is strongly teratogenic in experimental animals (Watanabe & Endo, 1984; Watanabe et al. 1995).

Chauhan & Dakshinamurti (1991) have shown that biotin also acts, relatively specifically, to induce the synthesis of glucokinase in fasted rats. Glucokinase (EC 2.7.1.2) is an isoenzyme of hexokinase (EC 2.7.1.1) with a high Michaelis constant (Km) of 0.15 mmol/l; glucose in the liver, glucokinase is responsible for the increase in glucose uptake and metabolism in the fed state; hexokinase has a Km of 0.15 mmol/l, and is therefore saturated with glucose, and acting at a constant rate under all physiological conditions, whereas glucokinase, with a Km of 20 mmol/l, only has significant activity when the concentration of glucose in the portal blood rises after a meal (Stryer, 1995). Apart from the liver, the only other tissue in which glucokinase is known to be expressed is the β-islet cells of the pancreas. Frognal et al.
significantly less incorporation of $^{14}$C acetate into lipids, (1985) showed that in biotin-deficient chickens there was (Stryer, 1995). For gluconeogenesis or repletion of the citrate cycle CoA, which is an obligatory allosteric activator. It is only activity, since the enzyme is strictly regulated by acetyl-CoA, which there is 100% saturation of the enzyme, and hence an activation coefficient of 1.0. Pyruvate carboxylase is a key enzyme in gluconeogenesis, as well as being an important anaplerotic reaction for maintenance of an adequate supply of oxaloacetate for citrate cycle activity. Thus, impairment as a result of biotin deficiency might be expected to lead to fasting hypoglycaemia as a result of reduced gluconeogenesis, and ketosis as a result of lack of acetoacetate for citrate cycle activity. Fasting hypoglycaemia was proposed as the link between low biotin status and sudden infant death (Johnson et al. 1980), and impaired gluconeogenesis, which is corrected by biotin, is seen in chicks suffering from the fatty liver and kidney syndrome (Bannister, 1976). Impairment of pyruvate carboxylase activity leads to an accumulation of pyruvate, lactate and alanine (Hymen & Wold, 1996); however, this impairment cannot be exploited as a means of assessing optimum biotin status, since the same accumulation of pyruvate metabolites is seen in thiamin deficiency, as a result of impairment of pyruvate dehydrogenase. Furthermore, it is unlikely that intakes of biotin above those needed to prevent deficiency would increase pyruvate carboxylase activity, since the enzyme is strictly regulated by acetyl-CoA, which is an obligatory allosteric activator. It is only active when there is a need for synthesis of oxaloacetate for gluconeogenesis or repletion of the citrate cycle (Stryer, 1995).

Acetyl-CoA carboxylase is an essential enzyme for fatty acid synthesis and elongation, so impairment of its activity would be expected to affect acetate metabolism. Donaldson (1985) showed that in biotin-deficient chickens there was significantly less incorporation of $^{14}$C acetate into lipids, and a greater proportion of the dose was exhaled as $^{14}$CO$_2$. It is unlikely that this finding could be exploited as a test of optimum biotin nutritional status. Kopinski et al. (1989) showed that the impairment of fatty acid elongation in biotin-deficient pigs with natural desaturase activity led to an increase in the monenoic: saturated fatty acid value in liver lipids. While there may be similar effects on plasma lipid composition, this ratio is probably too susceptible to the effects of dietary fat intake to provide a useful index of biotin status.

Propanoyl-CoA arising from a number of sources, including the metabolism of isoleucine, methionine, the side-chain of cholesterol and (rare) dietary odd-chain fatty acids, is normally carboxylated by propanoyl-CoA carboxylase to yield methylmalonyl-CoA, which then undergoes isomerization to succinyl-CoA, a citrate cycle intermediate. Impairment of propanoyl-CoA carboxylase leads to urinary excretion of propionate and onward metabolites such as hydroxypropionate, propanoyl glycine and methyl citrate. Detection of these acids in urine may therefore provide an index of biotin deficiency; they are not normally detectable. Thus, it is possible that the ability to metabolize a test dose of $^{13}$C propionate may provide a sensitive index of status. Barschop et al. (1991) reported that control subjects exhaled between 46% and 70% of a test dose of $^{13}$C propionate as $^{13}$CO$_2$; however, apart from the endogenous sources of propionate, intestinal bacteria provide a considerable but variable amount, which would lead to unpredictable dilution of the isotope and confound interpretation of the results.

Propanoyl-CoA can also compete with acetyl-CoA for incorporation into fatty acids, leading to the formation of odd-chain fatty acids. Mock et al. (1988) reported an increased proportion of 15:0 and 17:0 fatty acids in plasma lipids of patients who were biotin deficient as a result of prolonged total parenteral nutrition, which was normalized by administration of biotin. Proud et al. (1990) showed that the proportion of all odd-chain fatty acids from 15:0 to 25:0 was increased in skin lipids from biotin-deficient rats. This finding suggests that measurement of odd-chain fatty acids in skin or serum lipids might be a useful index of optimum biotin status. However, dietary intake is probably more important than endogenous synthesis of odd-chain fatty acids. Watkins (1988) measured 17:0 levels in liver and heart triacylglycerol from biotin-deficient animals fed on four different dietary sources of fat: maize oil; hydrogenated soyabean oil; a mixture of triolein, tristearin and tripalmitin; spent restaurant grease. The differences due to dietary fat were considerably greater than those due to biotin deficiency.

Methylcrotonyl-CoA is an intermediate in the metabolism of leucine; it is normally carboxylated, leading eventually to the formation of acetyl-CoA and acetoacetate. Impairment of methylcrotonyl-CoA carboxylase activity leads to urinary excretion of methylcrotonate and two onward metabolites, methylcrotonyl glycine and 3-hydroxyisovalerate. The latter acid is an early marker of experimen- tal biotin deficiency in rats (Mock & Mock, 1992). 3-Hydroxyisovalerate is normally excreted in detectable amounts (some 25–42 µmol/24 h). In a human study in which avidin was fed in order to cause biotin depletion, Mock et al. (1997) showed that urinary 3-hydroxy-
isovalerate excretion increased 4-fold, while plasma biotin was still (just) within the reference range. Measurement of 3-hydroxy-isovalerate excretion, perhaps combined with a loading dose of its precursor leucine, may provide a sensitive test of optimum biotin nutritional status.

**Pantothenate**

As with biotin, pantothenate deficiency is more or less unknown. The so-called burning foot syndrome (nutritional melagia) in severely-malnourished prisoners of war in the Far East is often assumed to have been due to pantothenate deficiency, but for obvious reasons they were repleted with yeast extract as a source of all B-vitamins rather than being used for more precise experimental studies.

Early studies showed that there was loss of fur colour in black and brown rats fed on a pantothenate-deficient diet, and at one time it was known as the anti-grey hair factor. Despite the fact that there is no evidence that loss of hair colour with age in human subjects is related to pantothenate status, it is still added to shampoos. Matsumoto et al. (1994) reported that an early sign of pantothenate deficiency in rainbow trout (*Onchorhyncus mykiss*) is anorexia; neither lack of anorexia nor a voracious appetite could sensibly be used as an index of optimum nutrition.

Pantothenate is panto-yl β-alanine; the next higher homologue, pantoyl γ-aminobutyrate (pantoyl GABA, homopantothenate or hopanthenate), is used in Japan to enhance cognitive function, especially in Alzheimer’s disease. It acts via GABA receptors to increase acetylcholine release and cholinergic function in the cerebral cortex and hippocampus (Nakahara et al. 1985). A rare side effect is the development of hepatic encephalopathy (Noda et al. 1991), and the excretion of a variety of dicarboxylates which arise by way of CoA-independent ω- to ω-oxidation of fatty acids (Matsumoto et al. 1991). Both the encephalopathy and the dicarboxylic aciduria are reversed by pantothenate, suggesting that homopantothenate may cause pantothenate deficiency. If this suggestion is verified, dicarboxylic aciduria may provide a marker of pantothenate status. However, it is equally possible that the adverse effects are due to a toxic action of homopantothenate which is antagonized by pantothenate.

Pantothenate has two well-defined metabolic roles: in CoA in fatty acid oxidation acetate metabolism, cholesterol and steroid synthesis, and acetylation of drugs; as the prosthetic group of acyl-carrier protein in fatty acid synthesis and efficient conservation of phosphopantetheine (in the fasting state when there is a greater requirement for CoA). The availability of pantothenate is unlikely to affect the rate of incorporation into CoA, since the Km of the enzyme is 18 µmol/l, and even in severe experimental deficiency most tissues maintain a higher concentration. There is rapid turnover of the prosthetic group of acyl carrier protein (a half-life of the order of 6h) compared with a half-life of apo-acyl carrier protein (6-4d; Volpe & Vagelos, 1973). The phosphopantetheine released from acyl carrier protein is incorporated into CoA, which in turn activates apo-acyl carrier protein as required; presumably the enzymes responsible for this cycling of phosphopantetheine are under hormonal regulation.

Wittwer et al. (1990) showed that both plasma triacyl-glycerol and non-esterified fatty acids are moderately elevated in pantothenate-deficient rats; however, this finding is unlikely to provide the basis for a test of pantothenate status in human subjects.

**Summary**

Especially if there is a genetic polymorphism in transketolase that affects thiamin requirements, there is a need to re-examine current estimates of requirements; probably the only index of status that is available is the transketolase activation assay. While Thurnham (1981) questioned the physiological significance of marginal vitamin deficiency as determined by enzyme activation assays, transketolase activation is at least a marker that can be used to assess saturation of one physiological system. In the case of biotin, it is obviously desirable to have an estimate of requirements or desirable levels of intake; either saturation of erythrocyte pyruvate-CoA carboxylase with its coenzyme or, preferably, 3-hydroxy-isovalerate excretion (perhaps after a test dose of leucine) will provide an index of adequacy. There still seems to be no way of assessing pantothenate status.
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