Original Article

Micronutrients: Speculation on Inborn Errors, Nutrigenomics, Evolution, the Microbiome, and Nutritional Immunity

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Abstract

Many micronutrients or cofactors derived from micronutrients are highly reactive, hence their role in catalysis of reactions by enzymes. The concentration of cofactors has to be kept low to avoid unwanted reactions while allowing them to bind to the (apo)enzymes that need them. A new disorder causing B6-responsive epilepsy (proline synthetase cotranscribed bacterial homologue deficiency) is probably due to the absence of an important intracellular pyridoxal phosphate chaperone. The availability of some micronutrients varies by orders of magnitude in different geographical areas. Selenium is both essential and toxic, and during evolution, different populations have had to adapt to this differing availability. An "inborn error of metabolism (IEM)" in a low selenium area of China may be a selective advantage in a high selenium area and vice versa; the concept of nutrigenomics is an important one for micronutrients. The gut flora may make an important contribution to vitamin synthesis. This is difficult to study, but experiments can be undertaken with the nematode, *Caenorhabditis elegans* (with or without an IEM) and a single clone of *Escherichia coli* (with or without an IEM) as food and gut flora. This model shows that the gut microbiome can have profound influences on the folate cycle and associated vitamins. Our innate immune system makes use of the micronutrient requirements of pathogens and can deprive a pathogen of essential micronutrient(s) or expose it to toxic levels. It is not surprising, therefore, that some mutations affecting the way the host handles micronutrients can confer an advantage in resistance to infection and this may have acted as a selective advantage during evolution. This will be discussed by reference to the relationship of inborn errors to resistance to malaria. Conversely, other inborn errors of micronutrient metabolism are likely to make it more difficult for the host to use nutritional immunity to fight infection; this probably accounts for the list of infections that are more serious in patients with hereditary haemochromatosis.

Keywords

vitamins, trace elements, pyridoxal 5'-phosphate, selenium, malaria, hemochromatosis

Introduction

The inborn errors affecting micronutrient metabolism are a very important group as they can often be treated effectively, for example, by increasing the intake of the micronutrient or speeding its elimination. There is accumulating evidence that micronutrient availability has led to evolutionary adaptations making man better able to survive as he has moved to areas with a lower or higher micronutrient availability. Furthermore, natural selection has favored genetic variants that improve our ability to use micronutrients in the fight against infection, variants that either restrict the availability of a micronutrient to a pathogen or subject it to toxic levels—"nutritional immunity." When considering the availability of micronutrients, it is important to remember that the gut flora can synthesize vitamins and so a dietary deficiency may be compensated in the holobiome. This review will focus on inborn errors and polymorphisms affecting micronutrient metabolism in the holobiome starting with a recently described inborn error that illustrates the need to carefully regulate the concentration of a highly reactive micronutrient in our cells.

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The Reactivity of Pyridoxal Phosphate and Proline Synthetase Cotranscribed Bacterial Homologue Deficiency

Pyridoxal 5'-phosphate (PLP) is the active cofactor derived from all forms of vitamin B6 in the diet. It has a reactive aldehyde group, with the reactivity further enhanced by the phosphate group that prevents hydration of the aldehyde group (as occurs in pyridoxal) and by the aromatic ring that provides an electron sink. The aldehyde groups can react with amino and sulphydryl groups as well as the activated carbon atoms in Δ1-pyrroline-5-carboxylate (P5C) and Δ1-piperideine-6-carboxylate (P6C), compounds that accumulate in hyperprolinaemia type II and ALDH7A1 deficiency, respectively.[1-3] To prevent inappropriate reactions, it is estimated that the concentration of the free PLP is kept in the low micromolar range. Abundant proteins that can bind to PLP and keep the free PLP concentration low include albumin in plasma, hemoglobin in red cells, and glycogen phosphorylase in muscle. However, the cell must be able to deliver PLP to the apoenzymes that need it. We have found evidence to indicate that there is an intracellular protein that helps fulfill this role—proline synthetase cotranscribed bacterial homologue (PROSC) now renamed as pyridoxal phosphate binding protein.[4] Proline synthetase cotranscribed bacterial homologue was known to be a ubiquitously expressed protein that could bind PLP without any alteration in its quaternary structure and, while its structure was similar to that of alanine racemase, it had no apparent enzyme activity when tested with a range of amino acids. We had some discussions with Andrew Hanson as to whether it might prevent reaction of PLP with P6C and P5C. Then, at the 2012 SSIEM Meeting in Birmingham, Isa Holme and Niklas Darin told us of their studies on a family in which 2 siblings and a cousin had B6-responsive epilepsy and in whom mapping studies and exome analysis had shown a homozygous missense mutation in PROSC. We then sequenced PROSC in a cohort of 29 individuals with pyridoxine-responsive seizures of unknown cause and identified a further 4 patients with biallelic PROSC mutations.

The clinical features of the 7 patients thus identified[4] included features similar to those seen in other B6 disorders such as pyridoxamine phosphate oxidase (PNPO) deficiency—abnormal intrauterine movements (3/7), fetal distress (4/7), neonatal seizures (7/7) with burst suppression on electroencephalography (5/7), and a clear response to B6 (7/7). In addition, patients tended to have a birth head circumference below the 9th centile (4/7). Other features included systemic disease—lactic acidosis (4/7), anemia (2/7), and gastrointestinal dysfunction (3/7). Seizure control was somewhat better with PLP than pyridoxine (4/4), but most patients required an additional anticonvulsant for full seizure control (5/6). On follow-up, microcephaly was increasingly evident and most patients had a degree of developmental delay. Early computed tomography and magnetic resonance imaging (MRI) scans showed broad gyri and shallow sulci indicating an underdeveloped brain. Some patients had periventricular germinolytic cysts and others deep white matter edema and hemorrhages. Scans in childhood showed reduced myelination.

When we looked at the pretreatment cerebrospinal fluid (CSF) samples from the patients with PROSC mutations, we found low PLP levels and evidence of impaired activity of PLP-dependent enzymes such as aromatic amino acid decarboxylase (AADC)—elevated CSF concentrations of 3-orthomethyldopa, L-dopa, and/or 5-hydroxytryptophan—and urinary organic acid analysis showed raised vanillactate, also consistent with reduced AADC activity. These results confirmed the hypothesis that delivery of PLP to apo-AADC in the brain was impaired.

When PROSC-deficient fibroblasts were cultured in medium containing the usual amounts of fetal calf serum (a source of PLP) and supplementary pyridoxine, the cell content of PLP measured as nmol PLP per gram of cell protein was higher in the children with PROSC deficiency than in controls and a heterozygote. When the cell extract was fractionated into components with molecular weight below and above 3 kDa, the PLP content for PROSC-deficient patients was found to be increased in both fractions, suggesting that there was accumulation of PLP bound to both proteins and metabolites. This was compatible with the hypothesis that PROSC is an intracellular PLP binding protein preventing inappropriate reactions with both small and large molecules.

We then did some expression studies with Valerie de Crecy-Lagard, Laurence Prunetti, and Basma El Yacoubi from the University of Florida in Gainesville. They had shown that when *Escherichia coli* with a deletion of the PROSC homologue (ΔyggS) were cultured in a dish containing a pyridoxine-soaked disc, there was a ring of growth inhibition around the disc. Transfection with wild-type human *PROSC* abolished pyridoxine sensitivity. Human mutants affecting highly conserved regions of PROSC did not abolish *E coli* pyridoxine sensitivity. A missense mutant affecting an area not fully conserved across species did abolish *E coli* pyridoxine sensitivity. These studies provided further evidence that PROSC prevents toxic reactions of vitamin B6.

Plecko and colleagues have since shown that mutations in *PROSC* are also a cause of vitamin B6-dependent epilepsy in their cohort.[5] Onset of seizures in their patients was <10 days, and seizures were promptly controlled by pyridoxine in all patients, except one whose seizures had already been controlled by phenobarbitone. In contrast to our patients, all their patients were normocephalic with normal MRI scans and three out of four had normal IQ when assessed at 12.5, 15.5, and 30 years.

Geographical Variation in Micronutrient Availability

Deficiency of a micronutrient is a major world health problem. At least 2 billion people in the world are deficient in key vitamins and/or minerals, the commonest deficiencies being vitamin A, iodine, iron, and zinc.[6] In some areas of the world, there is also a risk of specific micronutrient toxicity. A good

example of this is selenium; availability of this micronutrient varies by orders of magnitude between different geographical areas. Even within China, there are areas where people are at risk of deficiency and areas where there is a greater at risk of toxicity. Deficiency can give rise to an impaired immune response, infertility, cognitive decline, increased mortality, and specific forms of heart disease (Keshan) and bone disease (Kashin-Beck). Excess selenium, on the other hand, can cause nausea and vomiting, nail discoloration, brittleness, and loss, hair loss, fatigue, irritability, and a foul garlic breath odor. As man migrated out of Africa 60 000 to 100 000 years ago, he encountered both high and low selenium environments.

White and coworkers looked at variants in selenium-related genes in individuals living in high- and low-selenium areas.[7] They found that there was a glutathione peroxidase allele with increased catalytic efficiency of selenium that was more common in individuals living in low-selenium areas than in individuals from higher selenium areas. They found many other variants in genes regulating selenium and in selenoproteins in out-of-Africa migrants compared to ancestral African genes. This leads us to the conclusion that what is an inborn error of metabolism (IEM) in one geographical area might be a selective advantage in another!

The Gut Flora as a Source of Micronutrients; The Microbiome

The gut flora may be very important in micronutrient metabolism; bacteria are able to synthesize many vitamins. Unfortunately, the interaction between the host and the gut flora is complicated and difficult to study. We were fortunate to be invited to participate in a series of experiments on an elegant model system devised by Filipe Gomes Cabreiro and colleagues working in another part of UCL.[8]

For the nematode *Caenorhabditis elegans*, bacteria constitute both a food and a source of a gut flora. It is relatively straightforward to study the effect of the interaction between a worm with or without a knockout of an enzyme involved in micronutrient metabolism and fed a single *E coli* strain with or without a knockout of an enzyme involved in micronutrient metabolism. We can't go into detail for all combinations tested but will give 1 example. The worms were treated with the anticancer drug, 5-fluorouracil (5FU), at varying concentrations and the 5FU toxicity was measured by the fraction of the nematode's eggs that hatched at that concentration of 5FU. The main way in which 5FU prevents cellular replication is by inhibition of thymidylate synthesis leading to a deficient conversion of deoxyuridine monophosphate to deoxythymidine monophosphate for DNA synthesis. Reduced folates stabilize the 5FdUMP-TS complex that formed and enhance 5FU toxicity. When the worm was fed bacteria with a defect in the de novo pathway for PLP synthesis, the *pdxJ* strain, the worm's PLP level was virtually undetectable, and the toxicity of 5FU was markedly reduced compared to that in worms fed wild-type bacteria. When the worm was fed supplementary pyridoxal, its PLP level could be restored and the normal high toxicity of 5FU was also restored. This confirms previous work that indicates that 5FU toxicity is closely linked to the activity of the folate cycle and hence to PLP levels. 5-Fluorouracil toxicity is reduced by PLP deficiency. The important point here is that bacterial micronutrient metabolism can have a profound effect on host metabolism, including the metabolism of xenobiotics.

Conversely, there is increasing evidence that host metabolism can affect the species of bacteria present in the mammalian gut and their metabolic pathways. Vitamin D, acting on the vitamin D receptor, affects the production of antimicrobial peptides by the mouse including the cathelicidin antimicrobial peptides, β-defensins, and the autophagy regulator ATG16L1.[9] When the vitamin D receptor is knocked out in the mouse, there are changes both in the species of bacteria found in the large bowel and in the expression of their genes. The pathways upregulated in the bacteria in the caecum of the mouse include those affecting other micronutrients including riboflavin, retinol, and others.[9]

Inborn Errors and Carrier States That May Increase Resistance to Malaria

We have mentioned the fact that inborn errors of micronutrient metabolism might increase or decrease an individual's ability to use nutritional immunity to overcome an infection. It is believed that the ability to survive infection with the malarial parasite has been an important selective advantage in evolution. We have listed a few common inborn errors (and carrier states) affecting micronutrient metabolism that may confer resistance to malaria. These include the following.

Genetic Variants Leading to Low Free Pyridoxal Phosphate in Erythrocytes

Americans of Afro-Caribbean origin have a high incidence of a polymorphism in the erythroid-specific promoter of pyridoxal kinase.[10] This leads to low activity of pyridoxal kinase and this is thought to confer resistance to malaria. Sickle hemoglobin binds PLP more avidly than normal β-hemoglobin.[11] This may reduce the availability of free PLP to the parasites in the red cells. Children with sickle cell trait have protection against malaria morbidity and mortality.[12] The R116Q variant in PNPO markedly reduces the synthesis of PLP from pyridoxine phosphate in red cells.[13] We have suggested that the relatively high frequency of this mutation may be explained by resistance to malaria.[14] In the Ferrara Province of Italy, where malaria was endemic in the 12th century, there is a high incidence of individuals with a low activity of both PNPO and glutathione reductase and this cannot be explained by dietary riboflavin intake: the postulated reason for the high incidence is that reduced enzyme activity confers resistance to malaria.[15]

A Disorder of the Folate Cycle

In the mouse, methylene tetrahydrofolate deficiency confers resistance to malaria[16] and of course there is a common allele

that reduces MTHFR activity in man, the C677T allele. Again resistance to malaria is suggested to be the selective advantage conferred by the C677T allele.

Evidence of Altered B6 and/or Folate Metabolism in Malaria

If we look at the metabolic profile of patients acutely infected with *Plasmodium falciparum*, one striking feature is hyperhomocysteinemia.[17] This suggests that the patient is trying to reduce the operation of the folate and remethylation cycles to impair 1-carbon metabolism in the parasite. This may indicate reduced availability of 1 of the 4 vitamins involved in the folate cycle.

Nutritional Immunity Affecting Other Micronutrients in Malaria

If we believe that the human host has improved the efficiency of its nutritional immunity during evolution where malaria acted as a selective pressure, we might expect evidence of upregulation of genes involved in micronutrient metabolism or transport during infection. Idaghdour and co-workers looked at whole-blood transcriptomes of *Plasmodium falciparum*-infected West African children.[18] Among the genes upregulated in the white cells of infected children were 2 genes affecting trace metal metabolism:

SLC39A8 is a transporter of manganese and zinc. It is known to be upregulated as a result of T-cell activation, especially at low zinc concentrations, and it is tempting to suggest that increased cellular uptake of zinc and/or manganese is playing a role in combating the infection with the parasite.

SCO1 is a copper chaperone, known to us because of its involvement in complex IV assembly. It is tempting to suggest that overexpression in response to *Plasmodium* infection exposes the parasite to either copper deficiency or copper toxicity.

Other Infections in Inborn Errors of Micronutrient Metabolism

We have tried to convince you that there is an important link between micronutrient metabolism and infection. If this is the case, we should see altered susceptibility to infection in our patients with inborn errors of micronutrient metabolism.

There is good evidence for this in hereditary hemochromatosis. Several papers have drawn attention to infections that appear to be more serious or more common in patients with hemochromatosis including those caused by *Listeria*, *Mucor*, and *Aspergillus* species.[19]

On the other hand, the Hfe knockout mouse is resistant to infection with the *Salmonella* that causes a typhoid fever-like illness in the mouse, *Salmonella typhimurium*. This has been attributed to impaired ability of the knockout mouse to restrict the parasite's access to intracellular iron.[20]

Conclusions

We hope we have convinced you that many micronutrients are reactive chemicals that the body handles with care, for example, through the use of binding proteins. Through evolution, we have learned to use micronutrients in the fight against infection. Ideally every individual (whether asymptomatic or with symptoms resulting from grossly deranged metabolism) should have a diet to suit their genome—"nutrigenomics." However, the dietary "prescription" needs to take account of geographical features; micronutrient availabilities differ in different parts of the world. The diet also needs to encourage the most beneficial gut flora. It may need to be modified to improve resistance to prevalent infections. Over long periods of time, evolution makes appropriate genomic adjustments to suit micronutrient availability and prevalent infections. An IEM can affect the individual's ability to use "nutritional immunity" to fight infection, for example, hereditary hemochromatosis confers resistance to some infections but susceptibility to others.

Authors' Note

All work done in our laboratory was performed with ethical approval. Specific approval details can be found in References 4, 8, 13 and 14.

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