NEW APPROACHES FOR CORRECTING ZINC DEFICIENCY WITHOUT ZINC FORTIFICATION

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Abstract

Purpose: Emphatically state that zinc deficiency is the most prevalent nutritional deficiency worldwide. Introduce the Phytate:Zinc Molar Ratio as a tool for predicting zinc deficiency. To introduce the consequences or degree of severity of zinc deficiency and the ease of assessing the deficiency by measuring zinc and phytate intakes, performing the appropriate calculations, and collecting quantifiable data. To provide evidence that the use of a naturally-occurring fungus will, when added to the diet, hydrolyze the phytate, rendering it harmless, and releasing the bound minerals, especially zinc for absorption into the body.

Design/methodology/approach: Obtain the Phytate:Zinc Molor Ratio of a variety of foods using High Performance Liquid Chromatography (HPLC) and Atomic Absorption Spectrophotometry (AASpec).

Findings: Any food with a Phytate:Zinc Molar Ratio greater than 10 contributes to zinc deficiency.

Originality/value: Introducing a new tool – the Phytate:Zinc Molar Ratio – for estimating and predicting zinc deficiency in animals and humans.

Key words: Zinc, Phytate, Phytase, Deficiency, Growth, Phytate:Zinc Molar Ratio, Nutrition

Paper type: Research paper

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INTRODUCTION

Phytate (IP6, inositol hexaphosphate) (Figure 1) constitutes approximately 90 per cent of the organically-bound phosphorus in plant seeds. Great improvements have been made in recent years in isolating and measuring the phytate content of various plant-based foods. Its structure and essentiality to provide phosphorus for all germinating seeds are clear, but its role in human metabolism is only now becoming fully understood. Phytate tightly binds zinc and other minerals, rendering it poorly available for absorption and reabsorption. Plants and certain animals such as ungulates (cows, etc.) produce and utilize the enzyme phytase, to hydrolyze phytate during normal metabolism, releasing the zinc for nourishment. However, humans and other monogastric animals are vulnerable to zinc-deficiency as a result of phytate binding, because monogastric animals do not possess the phytase enzyme (Oberleas and Harland, 2008). Humans, especially populations that are overly dependent upon high phytate plant-based diets, must somehow measure and compensate for the effect of phytate in their diet if they are to maintain proper zinc homeostasis. Both components, phytate and zinc, can be quantified in a diet, and a molar ratio can be calculated to estimate the zinc adequacy. In fact, the phytate:zinc molar relationship is the only one of all nutritional relationships which has been quantified by the derivation and introduction of a formula to calculate the physiological consequences of phytate (Figure 2). It has been determined that the critical ratio above which zinc deficiency is likely to occur is 10 (Lo *et al.*, 1981). In order to lower the phytate:zinc molar ratio, there are only two known methods. One is to increase the quantity of bioavailable zinc. This is accomplished either by fortification of foodstuffs or meals by the addition of a diversity of zinc salts (Oberleas and Harland, 2008), or the increased consumption of foods with a greater zinc content, ie: meat and other animal-based products. However, in many societies, neither of these options is feasible.

The only other way to lower the phytate:zinc molar ratio is to increase the bioavailability of zinc present in the existing diet. The authors submit that this is a vastly superior approach and can easily be accomplished by the introduction of the phytase enzyme into the 'normal' diet of a population, effectively hydrolyzing the phytate, rendering it less effective for complexing the zinc, and thus, making the zinc bioavailable.

BACKGROUND

Historically, zinc was considered to be required in such small amounts that zinc deficiency was considered improbable (Todd *et al.*, 1934). From the beginning of time, domesticated farm animals had access to soil, and 'normal' growth rates were experienced. The animals could stir the topsoil, obtaining adequate zinc and other minerals, and with the addition of meat, bone scraps and fishmeal, were well nourished. Farmers, thinking progressively, wanted to move their herds into barns where concrete slabs would be easier to clean. With the discovery, synthesis, and supplementation of vitamin B_{12} at this same time, soybean protein was projected to be utilized as the sole protein source. This combination of circumstances was quickly declared a disaster because the pigs (Oberleas *et al*,. 1962) developed a zinc-deficiency condition, parakeratosis (Figure 3). The first demonstration that phytate was involved in the onset of zinc deficiency was demonstrated by O'Dell and Savage (1960). It was later learned that only about 4 per cent of the phytate consumed in the diet of the animals had been hydrolyzed during passage through the gastrointestinal tract (Oberleas, 1964). The idea of adding the naturally-occurring phytase enzyme to the diets of monogastric animals was introduced to improve the utilization of phytate phosphorus. Successive and repeated animal studies found that the experimentation was so predictable that the mathematical phytate:zinc molar ratio based upon the amount of dietary phytate and the amount of dietary zinc could be used to predict zinc deficiency, and thus, predict the quantity of additional zinc needed to provide adequate zinc nourishment (Oberleas, 1975).

CHEMISTRY AND PHYSIOLOGY

Further studies in animals and humans revealed that 2 to 4 times as much zinc is secreted by the pancreas back into the duodenum as was consumed from dietary sources. This changed the focus from the bioavailability of ingested zinc, to zinc homeostasis, because the major effect of phytate is on the secreted pool of zinc rather than dietary absorption alone (Oberleas and Harland, 20ll). Zinc is the only essential divalent trace cation secreted via the pancreas (Montgomery, *et al.*, 1943), and is thus the divalent cation most affected by phytate (Oberleas and Chan, 1997). In animals, it has been conclusively demonstrated that zinc is intimately involved in cell division (Figure 4). Zinc serves as a co-factor in RNA polymerase and reverse transcriptase and in zinc-finger

proteins that are adducts to DNA which regulate the expression of DNA by serving as a receptor site for hormones and other exogenous factors. Thymidine kinase is the enzyme studied thus far that demonstrates the greatest sensitivity to zinc depletion, and is necessary for synthesis of DNA and thus cell division. The synthesis of zinc-finger proteins is the most sensitive metabolic zinc-dependent process in the body (Figure 5). Lactic dehydrogenase is another zinc-finger regulated enzyme that is of greater importance throughout adulthood (Chesters, 1992).

PUBLIC HEALTH CONSEQUENCES OF ZINC DEFICIENCY

In the early stages of zinc deficiency, there may be few easily observable symptoms that can be directly related to the deficiency, but early deficit of zinc can have serious long-term effects including, but not limited to stunted physical and mental growth, increased susceptibility to adventitious disease, decreased quality of life, and even decreased life expectancy.

At an early age, symptoms in infants include the heightened development of colds, influenza, pneumonia or other infectious diseases. Since phytate is a known contributor to zinc deficiency, and the earliest dry matter fed to most infants is prepared cereals high in phytate, increased susceptibility to these infections may begin within 2 weeks after the introduction of these cereals to a young infant. The mechanism of the effective physiology begins with atrophy of the thymus gland, the gland most sensitive to a zinc deficit. The thymus gland is the organ involved in the conversion of D lymphocytes to T lymphocytes (Miller *et al.*, 1968). The T lymphocytes scavenge the body for invasive bacteria or viruses, then initiate the production of antibodies to these invasive organisms to disrupt the continued invasion and consequences from the invasive organism. While this can occur at any age, it may be most devastating at a very young age. This effect has been corrected by Gerber Products Company, Fremont, MI, with the assistance of one of the authors and the application of the phytate:zinc molar ratio formula and the proper fortification of their line of infant cereals with zinc.

As the child ages, this zinc deficiency may be perpetuated by continually feeding unfortified cereals, peanut butter sandwiches and other plant-seed based products high in phytate following weaning beyond one year of age. The consequences are the same and the child will continue to be susceptible to secondary infections and a less than optimal growth rate.

Two enzymes that are early casualties to zinc depletion are thymidine kinase and lactic dehydrogenase. Neither of these enzymes contains zinc as an essential cofactor, but rather, are zinc-finger protein-regulated enzymes. Zinc-finger proteins were first described in 1983 (Hanas *et al.*, 1983). Zinc-finger proteins are peptides that contain clusters of amino acids (cysteine and/or histidine) in close proximity. These peptides complex zinc at critical locations associated with cysteine and/or histidine to form proteins with finger-like projections. Over 1500 zinc-finger proteins have been currently described with each having specific activities. These proteins attach themselves to DNA and alter the expression of a segment of that DNA. Segments of DNA that are thus altered may be specific for the production of a specific enzyme or hormone, and thus, if adequate zinc is lacking, that specific enzyme will not be synthesized. Thymidine kinase is essential for the phosphorylation of deoxythymidine for incorporation into DNA. Thus, inadequate zinc likewise decreases the synthesis of thymidine kinase enzyme and decreases the rate of DNA replication, consequently decreasing cell division and subsequent growth. Studies have demonstrated a correlation between high-phytate diets and reduced growth (Figure 6).

Lactic dehydrogenase is an enzyme that hydrolyzes lactic acid. Lactic acid accumulates in muscle tissue during strenuous activity and results in soreness and pain distress that may initially require several days for full recovery. With adequate lactic dehydrogenase activity, the muscle distress should be corrected overnight. Inadequate zinc negatively impacts lactic dehydrogenase activity, prolonging the suffering.

Osteoporosis, another malady that affects many people, mostly females in later life, is affected by zinc and copper deficits. Both of these minerals are bound by phytate in the diet.

There are at least four different collagenases in the body. All collagenases contain zinc as a cofactor. These enzymes synthesize collagen microfibrils. A mass of these microfibrils are soft and must be crosslinked to form a more rigid structure characteristic of mature collagen fibres. Cross-linking is accomplished by the enzyme lysyl oxidase, a copper-dependent enzyme. Cross-linking also occurs at lysine residues. Only new collagen fibres will accept osteocytes, a manganese dependent process. Calcium that is withdrawn to provide metabolic calcium is replaced only on new collagen. A zinc deficiency inhibits the healthy synthesis of collagen, contributing to the onset of osteoporosis.

There are more than 3000 different proteins that are dependent upon zinc. These proteins have a variety of activities with varied sensitivities. Among the more important are structural stability, enzymatic activity, and the creation of zinc-finger proteins (Andreini *et al.*, 2011).

AVAILABILITY OF PHYTASE

Phytase preparations are available today from the fungus *Aspergillus ficuum*, (NRRL), a genetically modified variety of *Aspergillus niger*, (NRRL 3135). *Aspergillus ficuum* produces the highest level of phytase activity among fungi and is currently the product of choice. It has an effective range of activity from pH 2.5 to pH 6, compatible with the physiology of the stomach and small intestine of most monogastric animals including man. Phytase activity is maintained at temperatures below 55 °C while temperatures above this are destructive.

In October 1993, Gist-Brocades of Delft, Netherlands, operating under the company name of ALKO Biotechnology Ltd., and recently acquired by DSM Nutritionals, genetically modified *Aspergillus niger* into *Aspergillus ficuum* and successfully submitted a GRAS (Generally Regarded As Safe) Affirmation Petition, establishing phytase as an acceptable human food ingredient. This firm has studied the enzyme for a number of years in cooperation with a development programme sponsored by the government of the Netherlands and has already achieved a commercial market for animal feed use. It is now sold in over 25 countries including the USA. BASF is another company marketing this feed additive under their brand name, NATUPHOS, a microbial phytase enzyme product suitable for monogastric animal feed (pigs, poultry and fish). Micronutrient powders, containing active phytases and consumed prior to a meal have also been shown to be effective in enhancing iron absorption in humans (Troesch *et al.*, 2009).

Negative consequences of exposure to phytase are thus far minimal. The same investigators above identified an occupational enzyme allergy in employees handling large quantities of the enzyme in a pre-mix factory producing animal food additives. After prolonged exposure to breathing airborne phytase, workers developed allergic sensitization to phytase, work-related asthma and other respiratory symptoms (Doeks *et al.*, 1999). Strict control measures at the workplace were instituted to keep airborne exposure low and to ensure that the risk of sensitization and work-related disease would be minimized.

With the phytate:zinc molar ratio available for evaluation of its efficacy and success, and the availability of phytase in a safe, human-consumable format, the opportunity is at hand to improve the health of millions of people worldwide by improving absorption of valuable trace minerals, saving billions of dollars in healthcare costs, all without incurring undesirable side effects (Pallauf and Rimbach, 1996).

CONCLUSION

Zinc deficiency is one of the world's most under-recognized public health concerns. Zinc is essential in more than 3000 metabolic functions involving structural stability and other protein activities (Andreini *et al.*, 2011). Zinc deficiency has been scientifically linked to reduced fertility, impaired fetal development, stunted physical and mental growth, and increased susceptibility to a host of diseases in both animals and humans. Zinc supplementation has been used for decades in animal studies and production, but with only limited success. A far more effective panacea is the introduction of the enzyme phytase into the diet to increase the bioavailability of dietary minerals, especially zinc.

Veterinarians and animal scientists associated with the University of Sydney in Australia are routinely feeding chickens and pigs phytase-based diets with satisfying consequences. The animals mature earlier, more consistently achieve full growth, and generally exhibit a lower incidence of disease. Collaborative studies are desperately needed to introduce these practices into the diets of humans with the goal of achieving similar results. *Aspergillus ficuum* has already been approved as GRAS by the United States Food and Drug Administration and can now be used for human phytase studies. Researchers should now band together to initiate experimental diets to gain efficacy.

Understandably, there are food manufacturers who are reluctant to experiment, knowing that this is a complex issue, both scientifically and politically, but zinc deficiency is universal in its impact upon the human species, and needs to be addressed. A severe zinc deficiency may produce severe consequences, but because a mild deficiency does not produce overt symptoms, comprehensive human studies have not been initiated. This is a world-wide problem which is not being adequately addressed and these studies are long overdue.

Nutritionists need to convey the ideas presented above not only to all of our colleagues in the health sciences as a scientific priority, but equally to all public policy-makers, especially those involved with populations dependent upon staple foods which are high in phytate. The use of phytase as an effective and readily available solution to zinc deficiency has been successfully demonstrated in animals for more than 20 years. It is time to give humans the same nutritional advantage. Reducing zinc deficiency would have enormous public health consequences and would reduce healthcare costs by billions of dollars world-wide while prolonging and improving the quality of human life.

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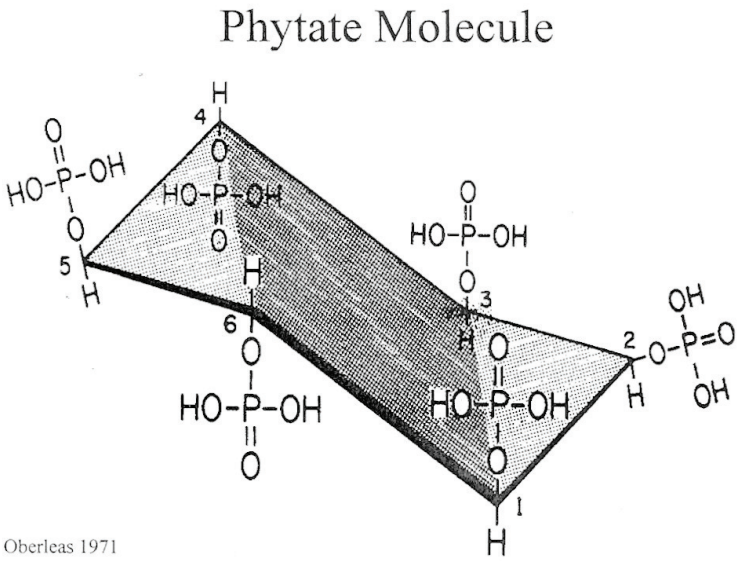

Figure 1. Phytate molecule. Oberleas, D. (1973).

Phytate:Zinc Molar Ratio

$$\frac{\dfrac{\text{g/kg phytate}}{660\ (\text{MW phytate})}}{\dfrac{\text{g/kg zinc}}{65.4\ (\text{Atomic weight zinc})}} = \text{Phytate:zinc molar ratio}$$

Oberleas 1975

Figure 2. Formula for calculation of phytate:zinc molar ratio. Oberleas, D. (1975).

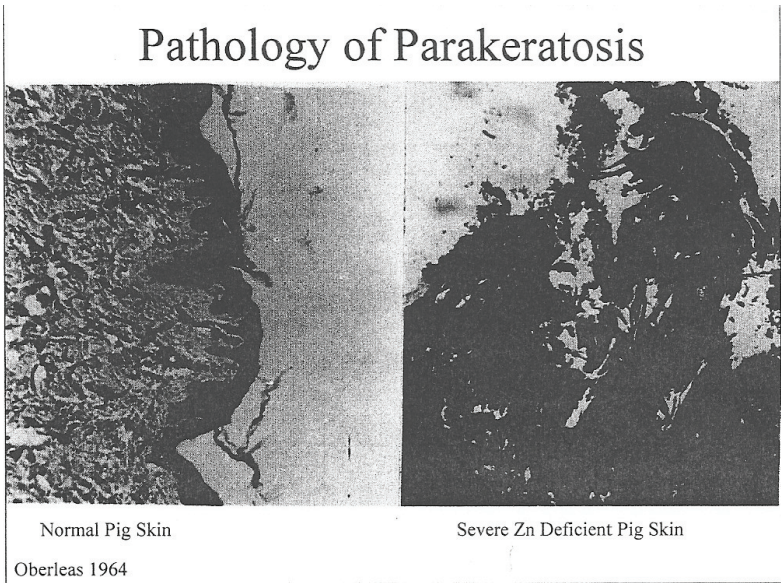

Figure 3. Normal and parakeratosis of pig skin. Kemkamp, H.C.H. (1953).

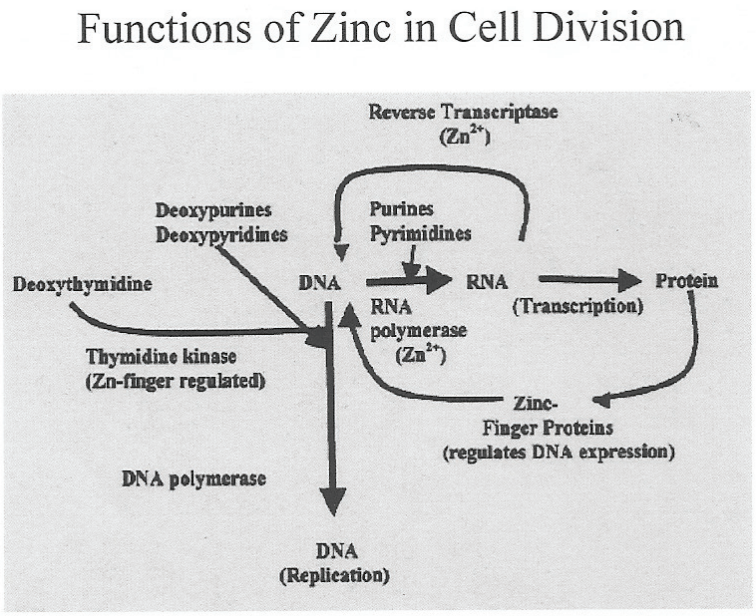

Figure 4. Schematic of relevant zinc required for cell division. Oberleas, D. & Harland, B.F. (2008).

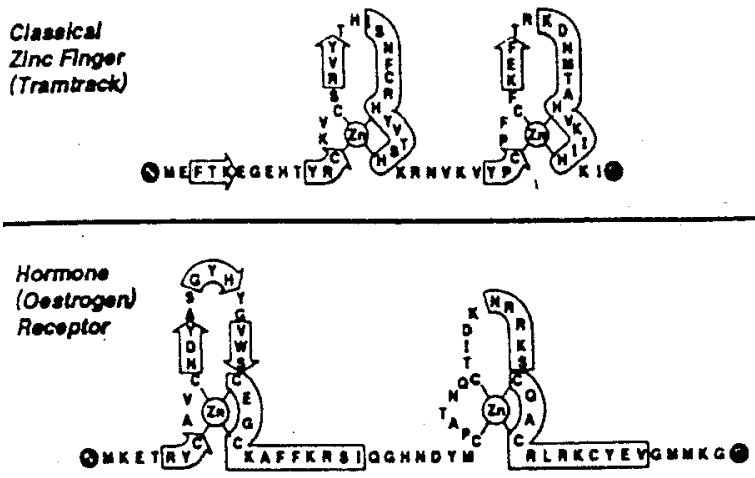

Figure 5. Zinc-Finger proteins. (Chesters, J.K. (1992).

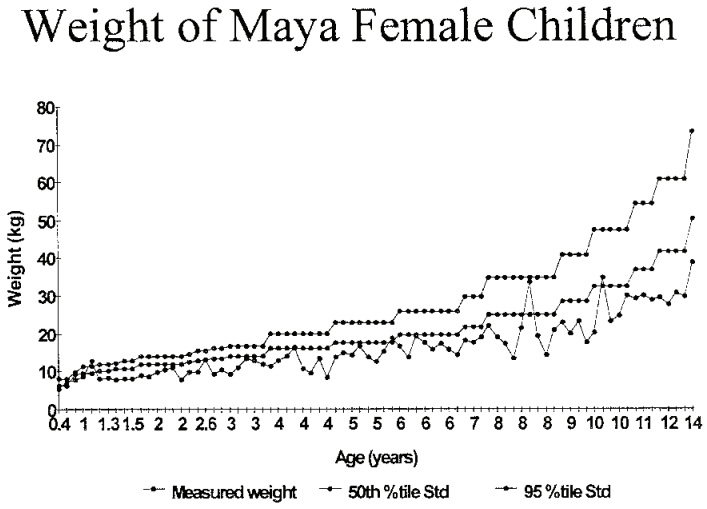

Figure 6. Weight of Maya female children. Oberleas, D. & Harland, B.F. (2008).

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