jem
journal für ernährungsmedizin

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Journal für Ernährungsmedizin 2006; 8 (3), 18-28

Phytate – an undesirable constituent of plant-based foods?

Phytate is known for its negative effect on mineral uptake but on the other side new evidence shows that phytate reveals several positive effects on human health as well.

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ABSTRACT

Phytate (myo-inositol (1, 2, 3, 4, 5, 6) hexakisphosphate), a naturally compound formed during maturation of plant seeds and grains is a common constituent of plant-derived foods. The major concern about the presence of phytate in the diet is its negative effect on mineral uptake. Minerals of concern in this regard would include Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, Mn^{2+}, and Cu^{2+}. Especially zinc and iron deficiencies were reported as a consequence of high phytate intakes. In addition, a negative effect on the nutritional value of protein by dietary phytate is discussed. Consumption of phytate, however, seems not to have only negative aspects on human health. Dietary phytate was reported to prevent kidney stone formation, protect against diabetes mellitus, caries, atherosclerosis and coronary heart disease as well as against a variety of cancers. Furthermore, individual myo-inositol phosphate esters have been proposed to be metabolically active. D-myo-inositol (1, 2, 6) trisphosphate, for example, has been studied in respect to prevention of diabetes complications and treatment of chronic inflammations as well as cardiovascular diseases and due to its antiangiogenic and antitumour effects myo-inositol (1, 3, 4, 5, 6) pentakisphosphate was suggested as a promising compound for anticancer therapeutic strategies.

Keywords: **antinutrient, cancer, caries, coronary heart disease, diabetes mellitus, mineral availability, phytate, protein digestibility, renal lithiasis**

The proper chemical designation for phytic acid is myo-inositol (1, 2, 3, 4, 5, 6) hexakisphosphoric acid. Salts of phytic acid, designated as phytates, are found in plants, animals and soil. Phytate is ubiquitous among plant seeds and grains, comprising 0.5 to 5 percent (w/w) [1]. It is primarily present as a salt of the mono- and divalent cations K^+, Mg^{2+}, and Ca^{2+} and accumulates in the seeds during the ripening period. In dormant seeds phytate represents 60 to 90 percent of the total phosphate. Only a very small part of the myo-inositol phosphates exist as myo-inositol penta- and tetrakisphosphate of unknown isomeric state. Phytate is regarded as the primary storage form of both phosphate and inositol in plant seeds and grains [1]. In addition, phytate has been suggested to serve as a store of cations, of high energy phosphoryl groups, and, by chelating free iron, as a potent natural anti-oxidant [2, 3].

Because phytate is a naturally occurring compound formed during maturation of plant seeds and grains, it is a common constituent of plant-derived foods. Depending on the amount of plant-derived foods in the diet and the grade of food processing, the daily intake of phytate can be as high as 4500 mg [4]. In average, the daily intake of phytate was estimated to be 2000–2600 mg for vegetarian diets as well as diets of inhabitants of rural areas of developing countries and 150–1400 mg for mixed diets. Phytate behaves in a broad pH-region as a highly negatively charged ion and has therefore a tremendous affinity for food components with positive charge(s), such as minerals, trace elements and proteins [3, 5]. There is a large body of evidence that minerals are less available from foods of plant origin as compared to animal-based foods. Furthermore, phytate-phosphorus is less nutritionally available, since phytate is not hydrolysable quantitatively in the human gut [6]. Furthermore, it was demonstrated that phytate-protein interactions negatively affect protein digestibility in vitro and that the extent of this effect depends on the protein source [5], but a negative effect of phytate on the nutritional value of protein was not clearly confirmed in studies with simple-stomached animals [7]. Consumption of phytate, however, seems not to have only negative aspects on human health. Dietary phytate was reported to prevent kidney stone formation [8], protect against diabetes mellitus [9], caries [10], atherosclerosis and coronary heart disease [11] as well as against a variety of cancers [12].

Phytate as an antinutrient

The major concern about the presence of phytate in the diet is its negative effect on mineral uptake. Minerals of concern in this regard would include Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, Mn^{2+}, and Cu^{2+} [13, 14], but also a negative effect on the nutritional value of protein by dietary phytate is discussed [5].

▸ PHYTATE AND MINERAL INTERACTION

Phytate forms complexes with numerous divalent and trivalent metal cations. Stability and solubility of the metal cation-phytate complexes depends on the individual cation, the pH-value, the phytate:cation molar ratio, and the presence of other compounds in the solution [15]. Phytate has six reactive phosphate groups and meets the criterion of a chelating agent. In fact, a cation can bind to one or more phosphate group of a single phytate molecule or bridge two or more phytate molecules [3]. Most phytates tend to be more soluble at lower compared to higher pH-values [16]. Solubility of phytates increase at pH-values lower than 5.5–6.0 with Ca^{2+}, 7.2–8.0 with Mg^{2+} and 4.3–4.5 with Zn^{2+} as the counter ion. In contrast, ferric phytate is insoluble at pH values in the range of 1.0 to 3.5 at equimolar Fe^{3+}:phytate ratios and solubility increases above pH 4 [17]. Another important fact is the synergistic effect of secondary cations, among which Ca^{2+} has been most prominently mentioned [18]. Two cations may, when present simultaneously, act jointly to increase the quantity of phytate precipitation. For example, Ca^{2+} enhanced the incorporation or adsorption of Zn^{2+} into phytate by formation of a calcium-zinc phytate. The effect of Ca^{2+} on the amount of Zn^{2+} co-precipitating with phytate is dependent on the Zn^{2+}:phytate molar ratio. For high Zn^{2+}:phytate molar ratios, Ca^{2+} displaces Zn^{2+} from phytate binding sites and increases its solubility. The amount of free Zn^{2+} is directly proportional to the Ca^{2+}-concentration. For low Zn^{2+}:phytate molar ratios, Ca^{2+} potentiate the precipitation of Zn^{2+} as phytate. Thus, higher levels of Ca^{2+} result in a more extensive precipitation of the mixed phytates. Mg^{2+} also has been shown in vitro to potentiate the precipitation of Zn^{2+} in the presence of phytate, however, Mg^{2+} has been found to exert a less pronounced effect on Zn^{2+}-solubility than Ca^{2+} [18].

The knowledge about the interaction of partially phosphorylated myo-inositol phosphates with different cations is limited. Recent studies have shown that myo-inositol pentakis-, tetrakis- and trisphosphates have a lower capacity to bind cations at pH-values ranging from 5.0 to 7.0 [16, 19]. The capacity to bind cations was found to be a function of the number of phosphate groups on the myo-inositol ring. The cation-myo-inositol phosphate complexes are more soluble as the number of phosphate groups decreases. There is also some evidence for weaker complexes when phosphate groups are removed from phytate. In addition, the binding affinity of cations to myo-inositol phosphates has been shown to be affected by the distribution of the phosphate residues on the myo-inositol ring.

Effect of phytate on mineral availability

The formation of insoluble metal cation-phytate complexes at physiological pH-values is regarded as the major reason for a poor mineral availability, because these complexes are essentially non-absorbable from the gastrointestinal tract. Most studies have shown an inverse relationship between phytate content and mineral availability, although there are great differences in the behaviour of individual minerals. Zn^{2+} was reported to be the essential mineral most adversely affected by phytate [13, 14]. Zn^{2+}-deficiency in humans was first reported in 1963 in Egyptian boys whose diets consisted mainly of bread and beans [20]. These patients, who were characterised by dwarfism and hypogonadism, showed a response to dietary Zn^{2+}-supplementation. It became accepted that the presence of phytate in plant-based foods is an important factor in the reduction of Zn^{2+}-absorption. Phytate affects Zn^{2+}-absorption in a dose-dependent manner. There is, however, some lack of agreement among studies, particularly with respect to specific foods and their individual components. In addition, phytate was shown not only to depress the availability of dietary Zn^{2+}, but also to affect Zn^{2+}-homeostasis negatively [15]. A great deal of controversy exists regarding the effect of phytate on the availability of dietary iron [14, 21]. Much of this controversy may be due to the low absorption of iron in general, the presence of different iron-phytates with different solubility, and the existence of two types of food iron, heme and nonheme iron. Heme iron is better absorbed and its absorption is little affected by dietary factors; nonheme iron, however, is less easily absorbed, and its absorption is affected by other dietary factors. Since many human studies indicate that phytate has a very strong inhibitory effect on iron absorption, it is well accepted today, that phytate appears to be the major but not the only contributor to the reduction in iron availability in man [22]. Human studies also indicated that phytate inhibits Ca^{2+}-absorption, but the effect of phytate on Ca^{2+}-availability seems to be less pronounced compared to that on the availability of iron and particularly Zn^{2+} [14]. This may be due to the relatively high Ca^{2+}-content of plant-based foods, the capability of the bacterial flora in the colon to dephosphorylate phytate and the fact, that Ca^{2+} could be absorbed from the colon [23]. Relatively few studies have dealt with the effects of phytate on dietary Cu^{2+}, Mn^{2+} and Mg^{2+} utilisation. Phytate has been shown to decrease their bioavailability in in vivo studies, but it appears that the effect of phytate on Cu^{2+}, Mn^{2+} and Mg^{2+} availability is less marked than those for some other essential elements [13, 14].

The fact that phytate-phosphorus is poorly available to single-stomached living beings including man was already demonstrated [24, 25]. Phosphorus is absorbed as ortho-phosphate and therefore the utilisation of phytate-phosphorus by single-stomached living beings will largely depend on their capability to dephosphorylate phytate. It was already shown, that the human small intestine has only a very limited capability to hydrolyse phytate [26] due to the lack of endogenous phytate-degrading enzymes (phytases) and the limited microbial population in the upper part of the digestive tract.

Problems in identifying phytate as an inhibitor of mineral absorption

Reviewing the literature on the effect of dietary phytate on mineral availability revealed that the majority of the performed investigations resulted in a reduction in mineral ab-

sorption in the presence of phytate. Some studies, however, failed in detecting an effect of phytate on mineral availability and some studies showed even enhanced mineral uptake in the presence of phytate. The controversial results give an idea of the complexity of mineral absorption in the intestine. The differing types of experimental design may explain much of this controversy. In vitro studies can only incompletely simulate the physiological factors and physicochemical conditions affecting mineral availability and in vivo approaches also widely used in mineral availability studies are not easily comparable due to the existence of many factors that cannot be reproduced in the different experiments. In addition, part of the variability may arise from differences in the method of phytate analysis and experimental techniques to measure mineral bioavailability.

• Determination of phytate content

The method used for quantification of the phytate present in the experimental diets was shown to be one factor responsible for the variability of the results obtained in mineral availability studies. In the past, phytate was mainly quantified by addition of a controlled amount of Fe^{3+} to an acidic sample extract to precipitate the phytate [27]. Phytate is subsequently estimated either by determining the phosphate, inositol or iron content of the precipitate (direct method), or by measuring the excess iron in the supernatant (indirect method). These approaches are not specific for phytate due to the co-precipitation of partially phosphorylated myo-inositol phosphates [28] and should therefore be limited to the analysis of material which contains negligible amounts of phytate dephosphorylation products. If substantial amounts of partially phosphorylated myo-inositol phosphates are present such as in processed foods, the content of phytate will be overestimated by using phytate determination methods based on iron precipitation. More recently, high performance liquid chromatoraphy (HPLC) techniques have been introduced into phytate determination [28]. Among these ion-pair reverse-phase and anion-exchange chromatography are largely used today. These systems allow the simultaneous separation and quantification of myo-inositol tris- to hexakisphosphates (ion-pair reverse-phase chromatography) [29] or myo-inositol mono- to hexakisphosphates (anion-exchange chromatography) [30]. Furthermore, a number of isomer specific ion-exchange chromatography methods with gradient elution for the separation and quantification of myo-inositol phosphates in the picomolar range have been developed very recently [31–33].

During food processing and food digestion, phytate can be partially dephosphorylated to yield a large number of positional isomers of myo-inositol pentakis-, tetrakis-, tris-, bis-, and monophosphates. Therefore, processed foods and digesta may contain lower myo-inositol phosphates in substantial amounts. Solubility and stability of myo-inositol phosphate-mineral complexes, however, have been found to decrease as the number of phosphate residues on the myo-inositol ring decreases [16, 19]. Furthermore, removal of phosphate residues from phytate has been shown to result in a reduced impairment of intestinal uptake of essential dietary minerals [34–36]. In addition, phytate-bound phosphorus becomes available for utilisation after release from the myo-inositol ring. In isolated form only myo-inositol pentakisphosphate suppressed absorption of iron, Zn^{2+} and Ca^{2+} in humans, while myo-inositol tetrakis- and trisphosphates had no effect in the concentrations under investigation. In the presence of higher phosphorylated myo-inositol phosphates, however, myo-inositol tetrakis- and trisphosphates were shown to contribute to the negative effect of phytate on iron absorption [35]. Because a strong negative correlation was found between Zn^{2+}-absorption and the sum of myo-inositol tris- through hexakisphosphate from cereal and legume meals [37], such a contribution is probably also true for Zn^{2+}-absorption. Therefore, a reliable and accurate determination of the concentration of the individual myo-inositol phosphates in diets must be applied before any useful evaluation can be made for their physiological effects on mineral availability.

• Interaction of phytate in the gastrointestinal tract

Regarding mineral availability, solubility of phytate complexes is a critical and perhaps overriding issue. Complexes that are insoluble in the upper small intestine, where maximum mineral absorption normally occurs, are highly unlikely to provide absorbable essential elements. Thus, chemical interactions of phytate in the upper gastrointestinal tract are of particular concern. The form in which many minerals occur in foods is largely unknown, as is also the form in which they occur in the gut. Therefore, predicting the specific interactions of phytate in the gastrointestinal tract and the nutritional implications of these interactions is very difficult. As foods are ingested and the digesta travels through the gastrointestinal tract, phytate may continue to maintain associations developed during ripening or food processing. Because binding of phytate with minerals or proteins depends upon pH-value [3, 5], which changes from low pH in the stomach to about neutral in the upper small intestine, dietary phytate complexes may dissociate and phytate may form other chelates during its passage through the gastrointestinal tract.

• Total compositions of the diets

The total composition of the experimental diets greatly affects mineral availability. Mineral uptake depends on several dietary factors such as the total concentration of an individual mineral, mineral composition, phytate concentration as well as the concentration of food constituents which promote or inhibit mineral uptake. Phytate per se does not seem to have a direct adverse effect on mineral absorption, because the physiological concentration of a single mineral is generally not sufficient for formation of insoluble mineral-phytate complexes in the small intestine [38], but the Ca^{2+}-concentration in the diets is in general sufficiently high to completely precipitate phytate resulting in a co-precipitation of other minerals. Therefore, the Ca^{2+}-content of the diets is of vital importance for the result of studies on the

effect of phytate on mineral availability. Ca^{2+} clearly augments the adverse effects of phytate on mineral absorption, and several other dietary components show beneficial as well as adverse effects on mineral uptake [19]. The intake of meat and/or organic acids such as ascorbic acid, for example, effectively counteracted the inhibitory effect of phytate, whereas dietary fibre and polyphenols intensified it [39–41]. Both, phytate and fibre have a high potential binding capacity for minerals. Due to the fact, that both are generally present jointly in many foods, it is very difficult respectively impossible to quantify the contribution of either phytate or fibre to the demonstrated effect on mineral availability in studies with typical human diets.

Furthermore, the history of food processing was shown to affect the availability of phytate-phosphorus and phytate-associated cations. Depending on the manufacturing process reduction in phytate content of foods, destruction or alteration of food compounds promoting or depressing mineral uptake and inactivation of phytate-degrading enzymes present in the processed material may occur. Hydrolysis of phytate in the gastrointestinal tract of humans may be carried out by the action of phytate-degrading enzymes from three sources: dietary phytases, mucosal phytases of the small intestine and phytases from the bacterial flora in the colon. With the exception of Ca^{2+}, phytate degradation in the colon is not expected to affect mineral absorption significantly, because minerals are mainly absorbed in the upper small intestine. In addition only a very low phytate-degrading activity was demonstrated to occur in the human small intestine [26]. Thus, the human small intestine has only very limited ability to hydrolyse phytate. Dietary phytases, in contrast, are an important factor for phytate degradation during digestion. These enzymes have been shown to dephosphorylate dietary phytate in the human stomach [42]. Thus, inactivation of the endogenous dietary phytases during food processing results in only a limited degradation of phytate in the stomach accompanied by a lower availability of minerals and phytate-phosphorus. In addition, the level of dietary Ca^{2+} is of utmost importance for phytate dephosphorylation during digestion. A low Ca^{2+}-concentration favours phytate hydrolysis in the gut, whereas elevated dietary Ca^{2+}-levels decrease dietary phytate dephosphorylation [43].

The source of dietary phytate and minerals may also affect the results of mineral availability studies. Phytate present in plant-derived foods and phytate used to supplement experimental diets are not necessarily equal. In general sodium phytate is added to the experimental diets. Sodium phytate is capable of chelating the multivalent cations present in the experimental diets, whereas the phytate already present in the plant-based foods used to prepare the experimental diets already interacts with these cations. Therefore, increasing phytate content of the experimental diets by adding sodium phytate does not represent the situation of increasing phytate content by using diet components rich in phytate. Furthermore, the availability of minerals has mostly been examined after addition of ionic salts to the experimental diets. Results obtained under such conditions may not represent the level of absorption of minerals present in natural sources.

• Experimental species

The effect of phytate on mineral absorption seems also to be dependent on the experimental species used. Phytate degradation in the stomach and small intestine varies with the species. For example, rats have been suggested not to be a good model for assessing mineral absorption from phytate-containing foods due to the existence of rat intestinal phytase activity [44]. In addition, the age of the individuals is of importance, since it seems that phytate digestion decreases with age [19]. The ability of endogenous carriers in the intestinal mucosa to absorb essential minerals bound to phytate or other dietary substances as well as the mineral status and need of the individual also has to be considered. Adaptation to a high phytate intake is controversially discussed [16, 19, 45]. A long-term study did not show any human adaptation to a high phytate diet with respect to iron absorption, whereas the normal bone and teeth calcification throughout the world in several populations who depend almost exclusively on cereal diets suggests human adaptability towards high phytate consumption with respect to Ca^{2+}-absorption.

▸ PHYTATE-PROTEIN INTERACTION

Phytate interactions with proteins are pH-dependent [5]. At pH-values below the isoelectric point of the protein, the anionic phosphate groups of phytate bind strongly to the cationic groups of the protein to form insoluble complexes that dissolve only below pH 3.5. The α-NH_2 terminal group, the ε-NH_2 of lysine, the imidazole group of histidine and guanidyl group of arginine have been implicated as protein binding sites for phytate at low pH-values. These low pH protein-phytate complexes are disrupted by the competitive action of multivalent cations. Above the isoelectric point of the protein, both protein and phytate have a negative charge, but in the presence of multivalent cations, however, soluble protein-cation-phytate complexes occur. The major protein binding site for the ternary complex appears to be the non-protonated imidazole group of histidine, but the ionized carboxyl group of the protein are also suggested sites. These complexes may be disrupted by high ionic strength, high pH (>10), and high concentrations of the chelating agents.

Phytate and protein digestibility

Phytate is known to form complexes with proteins at both acidic and alkaline pH [5]. This interaction may effect changes in protein structure that can decrease enzymatic activity, protein solubility and proteolytic digestibility. However, the significance of protein-phytate complexes in nutrition is still under scrutiny. Strong evidence exists that phytate-protein interactions negatively affect protein digestibility in vitro and the extent of this effect depends on the protein source [5]. A negative effect of phytate on the nutritive value of protein, however, was not clearly confirmed in studies with simple-stomached animals [7]. While some have sug-

gested phytate does not affect protein digestibility, others have found an improvement in amino acid availability with decreasing levels of phytate. This difference may be at least partly due to the use of different protein sources. Of nutritional significance might be also the inhibition of digestive enzymes such as α-amylase [46, 47], lipase [48] or proteinases [49–51], such as pepsin, trypsin and chymotrypsin, by phytate as shown in in vitro studies. The inhibitory effect increases with the number of phosphate residues per myo-inositol molecule and the myo-inositol phosphate concentration. This inhibition may be due to the non-specific nature of phytate-protein interactions, the chelation of calcium ions which are essential for the activity of trypsin and α-amylase, or the interaction with the substrates of these enzymes. The inhibition of proteases may be partly responsible for the reduced protein digestibility. Phytate has also been considered to inhibit α-amylase in vivo as indicated by a negative relationship between phytate intake and blood glucose response [52]. Therefore, food rich in phytate has been considered to have great nutritional significance in the prevention and management of diabetes mellitus, one of the most common nutrition-dependent diseases in Western society.

Beneficial health effects of phytate

In the view of the above results, the evidence seems overwhelming that high intakes of phytate can have adverse effects on mineral uptake in humans. In the last years, however, some novel metabolic effects of phytate or some of its degradation products have been recognised. Dietary phytate was reported to prevent kidney stone formation [8], protect against diabetes mellitus [9], caries [10], atherosclerosis and coronary heart disease [11] as well as against a variety of cancers [12]. The levels of phytate and its dephosphorylation products in urine, plasma and other biological fluids are fluctuating with ingestion or deprivation of phytate in the human diet [53]. Therefore, the reduction in phytate intake in developed compared to developing countries might be one factor responsible for the increase in diseases typical for Western societies such as diabetes mellitus, renal lithiasis, cancer, atherosclerosis and coronary heart diseases. It was suggested that phytate exerts the beneficial effects in the gastrointestinal tract and other target tissues through its chelating ability, but additional mechanisms have also been discussed. Moreover, the potential beneficial effects of phytate in the prevention of severe poisoning should be considered. One to two percent calcium phytate in the diet has been found to protect against dietary Pb^{2+} in experimental animals and in human volunteers [54]. Furthermore, calcium phytate was capable of lowering blood Pb^{2+}-levels [55]. Thus, phytate seems to be a helpful means to counteract acute oral Pb^{2+}-toxicity. The effect of calcium phytate on acute Cd^{2+}-toxicity is still discussed controversially, but the majority of studies point to an improved Cd^{2+}-absorption in the presence of phytate [56, 57]. This may result in a Cd^{2+}-accumulation in liver and kidney.

PHYTATE AND DIABETES MELLITUS

Diabetes mellitus is one of the most common nutrition-dependent diseases in Western society. It may be caused by hyper-caloric diets with high percentage of quickly available carbohydrates. Foods that result in low blood glucose response have been shown to have great nutritional significance in the prevention and management of diabetes mellitus. In this regard phytate-rich foods are of interest, since a negative relationship between phytate intake and blood glucose response was reported [9, 52]. For example, phytate-enriched unleavened bread based on white flour reduced the in vitro starch digestibility besides flattening the glycemic response in five healthy volunteers in comparison with bread without phytate addition [52]. The in vitro reduction of starch digestion was positively correlated with the myo-inositol phosphate concentration and negatively with the number of phosphate groups on the myo-inositol ring. It has to be noted, that there are also studies which have not found an inhibition of α-amylase and starch digestion by phytate.

PHYTATE AND CORONARY HEART DISEASE

Heart disease is a leading cause of death in Western countries, yet it is low in Japan and developing countries. Elevated plasma cholesterol or more specifically, elevated LDL-cholesterol concentrations have been shown to be one of the risk factors. It has been proposed that dietary fibre or more specifically phytate, as a component of fibre, may influence the aetiology of heart disease [58]. Animal studies have demonstrated that dietary phytate supplementation resulted in significantly lowered serum cholesterol and triglyceride levels [11]. This effect was accompanied by decrease in serum zinc level and in zinc-copper ratio. Thus, the hypothesis was put forward that coronary heart disease is predominantly a disease of imbalance in regard to zinc and copper metabolism [59]. The hypothesis is also based on the production of hypercholesterolemia, which is a major factor in the aetiology of coronary heart disease, in rats fed a diet with a high ratio of zinc and copper [60]. It was thought that excess zinc in the diets resulted in decreased copper uptake from the small intestine, since both minerals compete for common mucosal carrier systems. As phytate preferentially binds zinc rather than copper [61], it was presumed that phytate exerts its effect probably by decreasing zinc without affecting copper absorption. It should be pointed out that the support for the preventive role of phytate in heart disease is based only on a few animal and in vitro studies. Results from human studies are still lacking.

PHYTATE AND RENAL LITHIASIS

The increase of renal stone incidence in northern Europe, North America, and Japan has been reported to be coincident with the industrial development of these countries, making dietary intake suspect. Epidemiological investigations found that there were substantial differences in renal stone incidences between white and black residents of South

Africa [62]. The major dietary difference is that, compared to the white population, blacks consumed large amounts of foods containing high levels of fibre and phytate. Furthermore, a high phytate diet has been used effectively to treat hypercalciuria and renal stone formation in humans [63]. In recent years, research on phytate as a potent inhibitor of renal stone formation has been intensified [8, 64, 65]. By comparing a group of active calcium oxalate stone formers with healthy people it was demonstrated that urinary phytate was significantly lower for stone formers [8]. Therefore, in vitro and in vivo experiments as well as clinical studies clearly demonstrate that phytate plays an important role in preventing the formation of calcium oxalate and calcium phosphate crystals, which function as nuclei for kidney stone development. Because excretion of low phytate amounts in the urine was shown to be an important risk factor in the development of renal calculi and urinary excretion of phytate decreased significantly after intake of a phytate-free diet [64], the importance of dietary phytate in maintaining adequate urinary levels to permit effective crystallization inhibition of calcium salts and consequently preventing renal stone development was demonstrated.

▸ PHYTATE AND CARIES

The higher incidence of caries in industrialised compared to developing countries was suggested to be nutrition-dependent. Phytate lowers the solubility of calcium, fluoride and phosphate, the major components of enamel [10]. Thus, teeth are more protected against the leading cause of caries, the attack of acids and bacteria. Furthermore, the very high affinity of phytate for hydroxyl apatite may prevent the formation of plaque and tartar.

▸ PHYTATE AND CANCER

The frequency of colonic cancer varies widely among human populations. It is a major cause of morbidity and mortality in Western society. The incidence of cancer, especially large intestinal cancer has been associated principally with dietary fat intake and is inversely related to the intake of dietary fibre. It was further suggested that the apparent relationship between fibre intake and rate of colonic cancer might arise from the fact that many fibre-rich foods contain large amounts of phytate and that this latter might be the critical protective element, since an inverse correlation between colon cancer and the intake of phytate-rich fibre foods, but not phytate-poor fibre foods has been shown [66]. A high phytate intake may also be an important factor in reducing the breast and prostate cancer mortality in man [12].

Both in vivo and in vitro experiments have shown striking anticancer effects of phytate. It was demonstrated that phytate is a broad-spectrum antineoplastic agent, affecting different cells and tissue systems [12]. Phytate inhibited the growth of human cell lines such as leukaemic haematopoietic K-562 cell line [67, 68], colon cancer HT-29 cell line [69], breast cancer cell lines [70], cervical cancer cell lines [71], prostate cancer cell lines [72–74], HepG2 haepatoma cell line [75], mesenchymal tumour cells [76], murine fibrosarcoma tumour cells [76], and rhabdomyosarcoma cells [77] in a dose- and time-dependent manner. However, cells from different origin have different sensitivity to phytate, suggesting that phytate may affect different cell types through different mechanisms of action. It was also demonstrated, that phytate has the portential to induce differentiation and maturation of malignant cells, which often results in reversion to the normal phenotype [68]. Phytate was further shown to increase differentiation of human colon carcinoma HT-29 cells [69, 78], prostate cancer cells [72], breast cancer cells [70], and rhabdomyosarcoma cells [77]. The effectiveness of phytate as a cancer preventive agent was also shown in colon cancer induced in rats and mice. Phytate was effective in a dose-dependent manner given either before or after carcinogen administration. The phytate-treated animals demonstrated a significantly lower tumour number and size. Studies using other experimental models showed that the antineoplastic properties of phytate were not restricted to the colon. Phytate significantly reduced experimental mammary carcinoma [79–83], skin papillomas [84], tumour size of metastatic fibrosarcoma and experimental lung metastases [76], growth of rhabdomyosarcoma cells [77], and regression of pre-existing liver cancers [75, 85]. In addition synergistic cancer inhibition by phytate when combined with inositol was demonstrated in several cancers in experimental animals [76, 81, 82, 86]. The in vivo experiments were performed either by adding phytate to the diet or by giving phytate via drinking water. Comparable of even stronger tumour inhibition was obtained with much lower concentrations of phytate when it was given in drinking water.

Mechanism of action

The mechanisms involved in the anticancer activity of phytate are not fully understood. It was suggested that phytate exerts the beneficial effects through its chelating ability, but additional mechanisms have also been discussed. Because several myo-inositol phosphates, including phytate, are present as intracellular molecules and because the second messenger D-myo-inositol (1,4,5) trisphosphate is bringing about a range of cellular functions including cell proliferation via mobilising intracellular Ca^{2+} [87], phytate was proposed to exert its anticancer effect by affecting cell signalling mechanisms in mammalian cells [68]. About 35 of the 63 possible myo-inositol phosphate isomers were identified in different types of cells [87]. Depending on cell type, that is different receptors, phosphatases, and kinases, myo-inositol phosphates were linked with different physiological effects, such as basic cell functions like secretion and contraction as well as functions like cell division, cell differentiation and cell death. Therefore, practically every myo-inositol phosphate isomer extracellularly present and may have a metabolic effect by activating receptors, by being metabolised by phosphatases and kinases or by acting as inhibitors of these intracellular proteins after being internalised by cells. An effect of extracellular phytate on the concentration of several in-

tracellular myo-inositol phosphate esters has already been demonstrated in human erythroleukemia cells [68]. Furthermore, it has been recently reported that highly negatively charged myo-inositol polyphosphates can cross the plasma membrane and be internalised by cells. Myo-inositol hexakisphosphate was shown to enter HeLa cells followed by an intracellular dephosphorylation to partially phosphorylated myo-inositol phosphates [71], whereas myo-inositol (1 ,3, 4, 5, 6) pentakisphosphate showed a quite slow turnover after internalisation by SKOV-3 cells [88]. It was suggested that the anticancer activity of phytate is actually due to its dephosphorylation to lower forms. Myo-inositol (1, 3, 4, 5, 6) pentakisphosphate inhibits specifically phosphatidylinositol 3-kinase, the enzyme catalysing the phosphorylation of inositol phospholipids at the D3 position to generate 3'-phosphorylated phosphoinositides [89], which act by recruiting specific signalling proteins to the plasma membrane [90]. Activation of phosphatidylinositol 3-kinase is a crucial step in some events leading to angiogenesis, the formation of a mature vasculature from a primitive vascular network [90]. Angiogenesis is involved in pathologies such as arteriosclerosis and tumour growth. The observed anticancer effects of phytate could be mediated through several other mechanisms. Besides affecting tumour cells, phytate can act on a host by restoring its immune system. Phytate augments natural killer cell activity in vitro and normalises the carcinogen-induced depression of natural killer cell activity in vivo [92]. The anti-oxidant role of phytate is known and widely accepted. The 1, 2, 3-trisphosphate grouping in phytate has a conformation that uniquely provides a specific interaction with iron to completely inhibit its capability to catalyse hydroxyl radical formation from the Fenton reaction [93]. Chelation of iron to the 1, 2, 3-trisphosphate grouping may also reduce the likelihood for iron-catalysed lipid peroxidation [94]. It is as yet uncertain whether physiological intakes of phytate can significantly improve the anti-oxidant status in man. The anticancer action of phytate may be further related to mineral binding ability or other positively charged compounds. By complexing Zn^{2+} and/or Mg^{2+}, phytate can affect activity of enzymes essential for DNA synthesis. Due to inhibition of starch digestion in the small intestine, undigested and unabsorbed starch will reach the colon where it may either contribute to faecal bulk and increase the dilution of potential carcinogens, or it may be fermented to short-chain fatty acids, which may subsequently decrease the colonic pH. The increased production of short-chain fatty acid, particularly butyrate, may play a protective role in colon carcinogenesis, because butyrate has been shown in several in vitro studies to slow down the growth rate of human colorectal cancer cell lines [95, 96]. Decreased pH has been suggested to be protective of colon carcinogenesis [97] by possibly causing alterations in the metabolic activity of colonic flora [98], altering bile acid metabolism [99] and inhibiting ammonia production and absorption [100].

Conclusion

The most severe effects attributable to phytate have occurred in populations with unrefined cereals and/or pulses as a major dietary component. Especially zinc and iron deficiencies were reported as a consequence of high phytate intakes [101, 102]. To reduce the risk for mineral deficiency in vulnerable groups such as child-bearing women, strictly vegetarians, inhabitants of developing countries, especially fast growing children, different strategies have been deve-

enzyme	*IP5-isomer*	*IP4-isomer*	*IP3-isomer*	*IP2-isomer*	*IP-isomer*	*references*
barley P1; P2, spelt D21, wheat PHY1; PHY2, rye, oat, rice, lupine L2	D-Ins(1, 2, 3, 5, 6)P^5	D-Ins(1, 2, 5, 6)P^4	D-Ins(1, 2, 6)P^3	D-Ins(1, 2)P^2	Ins(2)P	110–113
wheat F2	D-Ins(1, 2, 3, 5, 6)P^5	D-Ins(1, 2, 3, 6)P^4	Ins(1, 2, 3)P^3	D-Ins(1, 2)P^2	Ins(2)P	114
mung bean	D-Ins(1, 2, 3, 5, 6)P^5	D-Ins(1, 2, 3, 6)P^4	D-Ins(1, 2, 6)P^3/ Ins(1, 2, 3)P^3	D-Ins(2, 6)P^2/ D-Ins(1, 2)P^2	Ins(2)P	115
S. cerevisiae, Pseudomonas, lupine L11, lupine L12	D-Ins(1, 2, 4, 5, 6)P^5	D-Ins(1, 2, 5, 6)P^4	D-Ins(1, 2, 6)P^3	D-Ins(1, 2)P^2	Ins(2)P	113, 116, 117
E. coli	D-Ins(1, 2, 3, 4, 5)P^5	D-Ins(2, 3, 4, 5)P^4	Ins(2, 4, 5)P^3	Ins(2, 5)P^2	Ins(2)P	118
Paramecium	D-Ins(1, 2, 3, 4, 5)P^5	D-Ins(1, 2, 3, 4)P^4	Ins(1, 2, 3)P^3	D-Ins(2, 3)P^2		119
lily	D-Ins(1, 2, 3, 4, 6)P^5	D-Ins(1, 2, 3, 4)P^4/ D-Ins(1, 2, 3, 6)P^4	Ins(1, 2, 3)P^3			120
B. subtilis	D/L-Ins(1, 2, 3, 4, 5)P^5/ D/L-Ins(1, 2, 4, 5, 6)P^5	Ins(1, 2, 3, 5)P^4/ Ins(2, 4, 5, 6)P^4	Ins(1, 3, 5)P^3/ Ins(2, 4, 6)P^3			121
B. subtilis, B. amyloliquefaciens	D-Ins(1, 2, 4, 5, 6)P^5/ D/L-Ins(1, 2, 3, 4, 5)P^5	Ins(2, 4, 5, 6)P^4/ D-Ins(1, 2, 5, 6)P^4	Ins(2, 4, 6)P^3/ D-Ins(1, 2, 6)P^3			122
Pantoea agglomerans	D-Ins(1, 2, 4, 5, 6)P^5					123

Tab. 1: **Myo-inositol phosphate intermediates generated through enzymatic phytate degradation**

loped. The most widely recognised strategies for reducing micronutrient malnutrition are supplementation with pharmaceutical preparations, food fortification, dietary diversification and disease reduction [103]. For various reasons, none has been very successful. An alternative approach would be to increase the total level of micronutrients in the edible parts of stable crops while at the same time increasing the concentration of compounds which promote their uptake and/or decreasing the amount of compounds which inhibit their absorption either by plant breeding or by genetic engineering. Recently low phytate mutants in maize, barley, rice and soybeans were isolated [104] and their potential for improving the absorption of iron, Zn^{2+} and Ca^{2+} has been shown [105]. To improve rice as a source of iron, three proteins were expressed in the central endosperm of the rice seed: a Phaseolus phytoferritin, an endogenous cysteine-rich metallothionein-like protein, and a Aspergillus fumigatus phytase [106]. If properly targeted, overexpression of phytase during seed development can result in reduced phytate levels in the mature seed [107]. Enhanced levels of seed phytase may also contribute to an improvement in mineral absorption by reducing phytate levels in plant-based food during processing and digestion in the human stomach once a meal is consumed. In addition, phytate degradation during food processing could be optimised by adding exogenous phytases or by adjusting favourable conditions for the native plant or microbial phytases. Besides enzymatic degradation, non-enzymatic hydrolysis of phytate during food processing or physical separation of phytate-rich parts of the plant seed could result in reduced levels of phytate in the final foods. In general, the lower phytate levels must be paid for by a loss of valuable nutrients which are either removed together with the phytate-rich parts of the plant or destroyed by the strong acids or high temperatures needed for non-enzymatic phytate dephosphorylation. Enzymatic phytate degradation, however, occurs also under mild conditions and does not affect other food components. Up to now, phytases have been mainly, if not solely, used as an animal feed additive in diets largely for swine and poultry, and to some extent for fish. There is also a great potential for the use of phytases in processing and manufacturing of food for human consumption, but up to now, no phytase product for a relevant food application has found its way to the market.

Marked mineral deficiency syndromes attributed to phytate have not been identified in highly developed countries. Phytate intake does not necessarily result in mineral deficiency. The absorption of minerals depends on the total composition of the meal and in a balanced diet containing animal protein, a high phytate intake does not imply a risk of inadequate mineral supply. Therefore, the recommendation for increasing dietary fibre in Western diets would not be expected to have any adverse effect on mineral absorption. The higher phytate intake with whole-grain products will undoubtedly lead to a percentage decrease in mineral absorption, but the absolute amount of absorbed minerals may remain unchanged, because of the large amounts of minerals in these products. In addition, the impact of phytate on phosphorus availability can be considered of little consequence in man, since the phosphorus intakes are usually high, and phytate-phosphorus represents only a small portion of the total phosphorus in the diets. In addition, it has been suggested that dietary phytate may exhibit some beneficial health effects, such as prevention of kidney stone formation [8], protection against diabetes mellitus [9], caries [10], atherosclerosis and coronary heart disease [11] as well as against a variety of cancers [12]. Thus, it could be concluded that the beneficial health effects discussed in regard to phytate are more important for populations in highly developed countries than its antinutritive properties.

Since individual myo-inositol phosphate esters have been proposed to be metabolically active, a controlled dephosphorylation of phytate may result in individual food components maintaining human health and preventing chronic diseases. The number and distribution of the phosphate residues on the myo-inositol ring determines the metabolic effects triggered by the individual myo-inositol phosphate isomer. D-myo-inositol (1, 2, 6) trisphosphate, for example, has been studied in respect to prevention of diabetes complications and treatment of chronic inflammations as well as cardiovascular diseases [108, 109] and due to its antiangiogenic and antitumour effects myo-inositol (1, 3, 4, 5, 6) pentakisphosphate was suggested as a promising compound for anticancer therapeutic strategies [90]. So far enzymatic phytate dephosphorylation is the most promising approach to get access to an individual myo-inositol phosphate isomer. Different phytases may exhibit different phytate degradation pathways and therefore lead to the generation and accumulation of different myo-inositol phosphate intermediates. A summary of the so far established intermediates generated by the different phytases upon acting on phytate is given in table 1. If individual phytate degradation products are established to be metabolically active, phytases may find application in food processing to produce foods with improved nutritional value, health benefits and maintained sensory properties (functional foods). By adding phytase to the raw material, phytate will be degraded to metabolically active myo-inositol phosphates during food processing. To end up with foods with a reduced content of phytate and a regulated content and composition of partially phosphorylated myo-inositol phosphate esters with health benefits, phytate dephosphorylation during food processing has to be tightly controlled. An alternative could be to generate metabolically active myo-inositol phosphates as food supplements by using pure phytate as the source material.

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