COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

COT STATEMENT ON PHOSPHATE AND THE CALCIUM-PARATHYROID HORMONE AXIS

Introduction

1. Phosphorus was one of the nutrients considered in the recent report of the Expert Group on Vitamins and Minerals (EVM, 2003). The review related to phosphorus compounds, primarily phosphates, expressed as elemental phosphorus to allow comparison of exposures to different compounds. There were insufficient data to establish a Safe Upper Level but a guidance level of 250 mg/day supplemental phosphorus was established, in addition to the estimated maximum intake of phosphorus from food of 2110 mg/day. The guidance level took into account both the occurrence of osmotic diarrhoea in human volunteer studies and the possible adverse effects of phosphorus on the parathyroid hormone-calcium axis in subjects with hypovitaminosis D who would be vulnerable to the effects of hyperparathyroidism. The guidance level represents a supplemental intake which would not be expected to result in any adverse effects. The EVM noted that physiological changes in calcium and parathyroid hormone (PTH) levels have been associated with intakes of 1500 mg/day and above of supplemental phosphorus. An uncertainty factor of 3 was then applied to a NAOEL of 750 mg/day (Brixen *et al*., 1992) to allow for inter-individual variability, resulting in a guidance level of 250 mg/day phosphorus.

2. In the interests of maintaining choice and promoting consumer understanding, the Food Standards Agency is working with manufacturers to develop a labelling initiative, which would allow higher amounts of phosphorus (as phosphate) to be present in food supplements than those recommended by the EVM provided that appropriate advisory statements were also included.

3. The COT was asked to consider data on phosphate, PTH, calcium balance and bone health in detail, in order to allow the FSA to formulate the appropriate consumer advice. This review included new data which were not available to the EVM.

Background

4. Phosphorus is a non-metallic, group V element. It is most commonly found in its pentavalent form in combination with oxygen as phosphate (PO_4^{3-}).

5. Phosphorus is present in a wide variety of foods largely as phosphate. Particularly rich sources are red meats, dairy products, fish, poultry, bread and other cereal products. Food additives are also a significant source of phosphate (Calvo and Park, 1996). Phosphorus intakes in the UK in 2000 were estimated to be 1494 (mean) and 2381 (high level) mg/day in men and 1112 (mean) and 1763 (high level) mg/day in women (Henderson *et al*., 2003). These intakes had increased since the previous survey in 1986/6 (Gregory *et al*., 1990).

6. Food supplements provide doses of up to 1100 mg/day phosphorus (EVM, 2003), though 150 mg or less is more common (OTC, 2003). Tablet forms of supplement products may also contain calcium phosphates as fillers or diluents. It has been suggested that these could represent a dose of up to 250 mg phosphorus per day (personal communication, Proprietary Association of Great Britain). The phosphate may not be declared on the label as an active ingredient.

7. The solubility of the inorganic phosphate salts used in supplements is variable. Calcium phosphate is soluble at acid pH but much less soluble in neutral conditions (Carr and Shangraw, 1987), suggesting that it would be soluble in the stomach but less so in the small intestine. Salts such as sodium and potassium phosphate are much more soluble at neutral pH.

Phosphate: Absorption, distribution, metabolism and excretion

8. Phosphate is readily absorbed throughout the small intestine (Koo and Tsang, 1997). The majority of phosphate absorption occurs via passive diffusion but active transport, dependent on potassium and calcium ions, sodium-potassium ATPase and vitamin D, also occurs. Active transport of phosphate is regulated by PTH which promotes calcium absorption via increased synthesis of calcitriol. However, even at low levels of calcitriol, substantial phosphate absorption occurs, such that dietary phosphate content is the most important determinant of phosphate absorption. To some extent, the degree of phosphate absorption varies with need, being 90% in infants fed human milk with low phosphate levels and 60-70% in adults consuming a normal diet, but it is essentially linear over the normal physiological range (Lemann,1996).

9. Soluble phosphates from meat or milk are almost completely absorbed, whereas the phosphate present as phytate in certain vegetable fibres may not be (Koo and Tsang, 1997). Phosphate absorption is affected by calcium, with an insoluble calcium phosphate precipitate being formed in the intestine (Calvo and Heath, 1988; Calvo and Park, 1996). However it is likely that excess calcium affects phosphate absorption rather than phosphate affecting calcium absorption (IOM, 1996).

10. Phosphorous compounds are important constituents of body tissues, with over 85% being contained in the skeleton (Ilich and Kerstetter, 2000).

The quantities present in intracellular pools are small, but they are critical to many aspects of cell structure and function, being present in phospholipids, nucleic acids and the phosphoproteins required for mitochondrial function (Stoff, 1982). Phosphate is involved in the regulation of the intermediary metabolism of proteins, fats and particularly carbohydrates and may regulate a number of enzymatic reactions. Phosphate is also the source of the high energy bonds of ATP.

11. Phosphate is largely excreted in the urine. Plasma phosphate levels are maintained via phosphate reabsorption in the kidney by a sodium dependent active transporter. This is achieved both by a PTH dependent mechanism and by a second mechanism mediated by soluble factors or "phosphatonins". The latter pathway has not yet been fully characterised and the significance of it with respect to normal phosphate levels is unclear (Jan de Beur and Levine, 2002). There are few data on the effect of high phosphate levels on phosphatonins so the effect of dietary increase on this aspect of phosphate regulation is unclear.

Bone metabolism

12. Bone is an active tissue which undergoes a continual cycle of resorption and renewal via osteoclasts and osteoblasts respectively (COMA, 1998). When resorption exceeds renewal, loss of bone mass occurs eg osteoporosis. Calcium is extracted from bones by osteoclasts and laid down by osteoblasts in response to changes in serum calcium levels. This process is mediated by PTH and vitamin D.

Effects of high phosphate intakes on the calcium-PTH axis and markers of bone health

13. As noted previously, calcium and phosphate are thought to form an insoluble precipitate in the intestine reducing absorption. However, the data suggest that calcium is more likely to reduce phosphate absorption than vice versa, and that the reduction of calcium absorption by phosphate would be significant only where calcium levels were very low (IOM, 1997).

14. At high dietary intake, phosphate is also thought to react with calcium to form an insoluble complex in the plasma, thus reducing free (ionised) calcium levels. It has been argued that the resulting decrease in serum calcium causes an increase in PTH secretion and thus in the subsequent resorption in calcium from bone to maintain plasma calcium homeostasis. The increased PTH stimulates the synthesis of calcitriol (1,25-dihydroxy vitamin D) via the renal enzyme 1-alpha-hydroxylase, which then promotes calcium absorption from the gut. PTH secretion is then subject to feedback regulation by calcitriol and calcium. Phosphate may also have a direct effect on PTH at a post-transcriptional level, possibly by stabilising the PTH mRNA. The effects of high phosphate are difficult to distinguish from the effects of low calcium *per se* since this would also trigger increased levels of PTH.

15. Although increased PTH would be expected to increase bone resorption to release calcium, intermittent PTH treatment is used therapeutically to prevent osteoporotic bone loss and to stimulate bone formation. The mechanism for this paradoxical effect is unclear, but may involve the uncoupling of bone resorption and bone formation. PTH infusion has been shown to increase the number of collagen cross-links and it has been suggested that it may stimulate the activity and differentiation of osteoblast progenitors. Similarly, phosphate is used therapeutically to lower serum calcium and to "activate" bone remodelling; bone resorption is then stopped pharmacologically, so mineralisation without resorption occurs. The relationship between PTH and phosphate is complex and may be different in acute compared to chronic exposure.

16. It has however been argued that high phosphate levels may not have adverse effects on bone if calcium intakes are adequate, or until the Ca:P ratio becomes very low (IOM, 1996).

17. The data from human volunteer studies are conflicting. In acute human studies, single doses of 1-1.5 g phosphorus (as phosphates) result in changes in PTH, serum and urinary calcium and phosphate levels, but more specific markers of bone resorption such as deoxypyridilone and carboxy terminal telopeptide of type 1 collagen are generally unaffected. Markers indicating bone formation have been reported to decrease.

18. Repeat dose human studies, generally using phosphate supplements at doses of 1-3g/day or manipulating dietary phosphate content to a similar extent, have resulted in a variety of effects. These have included increased levels of PTH and related markers and decreased urinary calcium levels. However, specific bone resorption markers were generally unchanged; this may indicate that the studies concerned were not of a sufficient duration for adverse effects to be apparent (lasting generally 1 week to 1 month in contrast to the calcium resorption/deposition cycle in bone which takes 4-8 months) or that the changes were small changes reflecting calcium homeostasis. In 7 post-menopausal women taking 1g phosphorus/day as phosphate for up to 15 months, calcium balance was "improved" (Goldsmith *et al*., 1976). Similarly in a 4 month study by Heaney and Recker (1987) urinary calcium was decreased and there was no evidence of bone remodelling in 8 female volunteers given 1.1 g/day supplemental phosphorus. However neither of these studies looked at specific markers of bone formation or resorption.

19. It has also been noted that some human studies may not detect changes in PTH because of the sampling procedures used (frequently in the morning after an overnight fast) (Calvo and Park, 1996). PTH has a strong diurnal rhythm, peaking in the late afternoon and early evening. This rhythm can be altered by dietary manipulation.

Effects of high phosphorus intakes on bone health *in vivo*.

20. Epidemiological studies of subjects exposed to high dietary phosphate levels are conflicting. In some studies, high phosphate levels have been associated with increased fracture risk, while this is not apparent in other studies. Calcium intakes are low in the majority of the studies and it has been argued that the association of fracture risk may be with low calcium consumption or low calcium:phosphate ratio rather than high phosphorus *per se*.

21. Where studies have specifically investigated the effects of beverages containing the acidulant phosphoric acid on indicators of bone health such as bone mineral density or fracture risk, the results are also conflicting. Rather than phosphate having a direct effect on bone, it has been suggested that intake of such beverages is a surrogate for physical activity which increases beverage consumption for rehydration purposes (Wyshak *et al*., 1989), and increases risk of fracture due to enhanced activity (Petridou *et al*., 1997). Phosphate containing beverages may also displace milk in the diet, reducing calcium intake (McGartland *et al*., 2003). Other possibilities suggested have been that it is the caffeine, which increases calcium excretion (Heaney and Rafferty, 2001), rather than the phosphate content that may be having an effect on bone health (Garcia-Contreras *et al*., 2000).

21. In animal studies, increased phosphate or decreased Ca:P ratios have resulted in marked bone loss in a number of species; soft tissue calcification, particularly in the kidney, has also been observed. These studies have used doses of up to 3 g/day supplemental phosphate in dogs or up to 1.2% dietary phosphorus in mice. However, studies in primates have much less marked effects at comparable phosphate intakes and it has been argued that they may be a better model for humans than other laboratory species (Anderson *et al*., 1977). Minor osteoporotic changes have been observed microscopically in baboons given a diet with Ca:P ratios of 1:4 and 1:2.1, similar to that of the human diet (Pettifor *et al*., 1984). Where calcium concentrations have been increased, the effects of phosphate are partially offset and the bone loss induced by high phosphate and/or low calcium can be reversed by reducing phosphate and/or increasing calcium. It has been argued that the Ca: P ratio of animal diets is lower than that of human diets, making animals more susceptible to metastatic calcium and other adverse effects (IOM, 1996).

Discussion

22. The effects of high phosphate levels are difficult to distinguish from those of low calcium. However, the appropriate level of calcium in the diet is also unclear, with conflicting results obtained from both metabolic balance studies in human volunteers and epidemiological investigations (Kanis, 1994).

23. Many of the changes resulting from phosphate treatment appear to represent homeostatic mechanisms to maintain plasma calcium levels. However, as indicated in the animal studies, very high levels of phosphate or low Ca:P ratios can result in significant adverse effects on bone.

Conclusions

24. The Expert Group on Vitamins and Minerals was asked to advise on a level of phosphate supplementation that would not be expected to result in any type of adverse effect. Since the data were inadequate to establish a robust Safe Upper Level, a guidance level was established by applying appropriate uncertainty factors to the limited data available.

25. Numerous studies have shown that phosphate loads result in an increase in PTH levels and changes in plasma calcium levels. The EVM concluded that phosphorus could result in adverse effects on the parathyroid hormone-calcium axis and that subjects with hypovitaminosis D could be particularly vulnerable to hyperparathyroidism. The EVM noted that physiological changes in calcium and PTH levels were associated with intakes of 1500 mg/day and above supplemental phosphorus. An uncertainty factor of 3 was applied to a NAOEL of 750 mg/day to allow for inter-individual variability resulting in a guidance level of 250 mg/day supplemental phosphorus. In addition, this level would not be expected to result in adverse gastrointestinal effects.

26. We were asked to consider the relationship of phosphate intake and bone health in detail, and to advise on the levels of phosphate intake that might be associated with adverse effects on bone. Our consideration included new data on epidemiology and on the regulation of serum phosphate that were not available to the EVM . In the light of this *we conclude* that the elevated PTH levels associated with supplemental phosphorus intakes reflect a short term adjustment to maintain plasma calcium levels and do not necessarily represent an adverse effect of phosphate on bone health. The long term effects on bone health of elevated PTH resulting from high phosphate intakes are unknown.

27. Low calcium and vitamin D intakes are known to have adverse effects on bone health since calcium in bone is resorbed to maintain serum calcium levels. It is possible that high phosphate intakes may exacerbate such effects but this is uncertain.

28. Inorganic phosphate salts have different physiological properties. It is likely that salts such as di and tri calcium phosphates, which are less soluble and also provides a source of calcium, would be of less concern with regard to adverse effects on bone.

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