The fallacy of the calcium-phosphorus product

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Scattered through the practice of medicine are dogmas with little or no scientific basis. One of these is the product of the serum calcium and phosphorus concentrations, the so-called calcium-phosphorus product or Ca × P. The assumption that ectopic calcification will occur when the product of the serum calcium and phosphorus concentrations exceeds a particular threshold has become standard practice in nephrology even though there is little scientific basis. Experimental support is lacking, the chemistry underlying the use of the product is oversimplified and the concept that ectopic calcification is simply the result of supersaturation is biologically flawed. The evidence that the Ca × P is an independent risk factor for mortality and morbidity is also questionable. Although ectopic calcification can occur in many sites, this review will focus on vascular calcification, as it is the most common site and the site most likely to affect patient outcomes.

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HISTORY OF THE CALCIUM-PHOSPHORUS PRODUCT

In 1917, Binger[1] showed that infusion of phosphate produced tetany in dogs, demonstrating for the first time the inverse relationship between circulating calcium and phosphate concentrations. The assumption that this was due to the precipitation of calcium phosphate was incorporated into early studies of bone formation. In the first mention of the calcium-phosphorus product (Ca × P), Howland and Kramer[2] showed that active rickets in children and rats was present when Ca × P was less than 35 mg^2/dl^2 and absent when the product was above 40 mg^2/dl^2. However, this product implied that bone resulted from a simple second order reaction between calcium and phosphate ions, which was not consistent with the stoichiometry of $Ca_3(PO_4)_2$ or of calcium and phosphate in bone. Clarification came from Shear and Kramer,[3] who showed that precipitation of calcium and phosphate from physiologic saline solutions was a second order reaction consistent with the formation of $CaHPO_4$ and governed by a simple solubility product of the concentrations of Ca^{2+} and HPO_4. Subsequent studies using addition of phosphate to human serum *in vitro* or intravenous infusion of phosphate in animals demonstrated a constant Ca × P equivalent to the solubility product for $CaHPO_4$,[4] and similar results were obtained in humans 30 years later.[5] Most recently, the concept that Ca × P might be a useful clinical parameter has been embraced by epidemiologists, who have shown correlations between Ca × P and outcomes in end-stage renal disease. However, it has never been demonstrated that exceeding the solubility of $CaHPO_4$ in plasma leads to ectopic calcification or that reducing the Ca × P alters outcomes in patients. Despite this, the Ca × P was incorporated into the Kidney Disease Outcome Quality Initiative (KDOQI) as an 'evidence-based' guideline.[6]

THE CHEMISTRY OF ECTOPIC CALCIFICATION

Chemical analyses of ectopic calcification in uremia has revealed at least three forms: magnesium whitlockite $(CaMg)_3(PO_4)_2$, carbonate-substituted hydroxyapatite $(CaMg)_{10}(PO_4CO_3)_6(OH)_2$, and amorphous (noncrystalline) calcium phosphate.[7–9] Apatite is the principal component of periarticular and vascular calcifications but whitlockite can occur in vessels under specific conditions.[9] The solution chemistry of hydroxyapatite and the calcium and phosphate that comprise it is complex and cannot be described by a simple Ca × P. It is often stated that the concentrations of Ca^{2+} and HPO_4^{2-} in normal human plasma exceed the

solubility product for hydroxyapatite and that normal plasma is supersaturated with Ca^{2+} and HPO_4^{2-}. Although the former is correct, the latter is certainly not. The explanation for this apparent paradox is that hydroxyapatite does not form directly from Ca^{2+} and HPO_4^{2-} in solution. Instead, solid $CaHPO_4$ is formed, followed by gradual, spontaneous hydrolysis to hydroxyapatite.[10] The solubility product (K_{sp}, based on activities not concentrations) for $CaHPO_4$ is 2.3×10^{-7} M^2 at 37°C and pH 7.4 in a physiologic salt solution[10] or in ultrafiltered serum,[11] which exceeds the product of the Ca^{2+} and HPO_4^{2-} activities in normal serum of approximately 1×10^{-7} M^2.[10,12] On the other hand, plasma Ca × P is much greater than that the K_{sp} of less than 10^{-50} M^2 for hydroxyapatite.[12] Thus, formation of hydroxyapatite from solutions of calcium and phosphate requires at least two chemical reactions: (1) formation of $CaHPO_4$, which requires supraphysiologic concentrations of the ions and is readily reversible under physiologic conditions and (2) conversion of $CaHPO_4$ to hydroxyapatite, which is essentially irreversible at physiologic pH and ion concentrations.[7,10] This explains why we do not turn into stone yet our bones do not spontaneously dissolve. Of note, only the first reaction is governed by Ca × P, and problems arise in establishing a specific Ca × P threshold (K_{sp}) *in vivo*.

The first problem is that total Ca or P in plasma may not accurately reflect Ca^{2+} or HPO_4^{2-}, the relevant ions. The 'P' is actually HPO_4^{2-} and $H_2PO_4^-$, and the proportions vary with pH, even in the physiologic range. At pH 7.4, approximately 80% of the phosphate is HPO_4^{2-}. In addition to ionized calcium, the 'Ca' also consists of complexed calcium (primarily to citrate and bicarbonate) and protein-bound calcium (primarily to albumin). As the concentrations of these compounds vary widely in our patients and their interaction with calcium depends on pH, which also varies, the correlation between total calcium and ionized calcium is poor at best. An additional complication is that physiologic salt solutions are not ideal solutions. This means that we must use activities and not concentrations in order to account for the colligative properties of the various ions. The solubility of calcium phosphate is governed by the product of the activities of Ca^{2+} and HPO_4^{2-}, not the concentrations. Activity coefficients (the proportion of the concentration that is 'active') vary widely, from 0.06 for PO_4^{3-} to 0.62 for $H_2PO_4^-$.[10] For Ca^{2+} and HPO_4^{2-}, the values are 0.36 and 0.23, respectively. Clearly, any attempt to correlate the product of total serum Ca and total serum phosphorus with a true solubility product is futile.

Even if we were able to determine the activities of Ca^{2+} and HPO_4^{2-} in plasma, what value for the product should we use? Assuming that 47% of total serum calcium is ionized[13] and that 81% of total phosphorus is HPO_4^{2-}, and assuming the aforementioned activity coefficients, the Ca × P in plasma would have to be above 90 mg^2/dl^2 to exceed the *in vitro* solubility of $CaHPO_4$.[10] However, an even higher product is required for spontaneous precipitation of $CaHPO_4$[10] because of the formation of colloidal $CaHPO_4$ that remains soluble.[4]

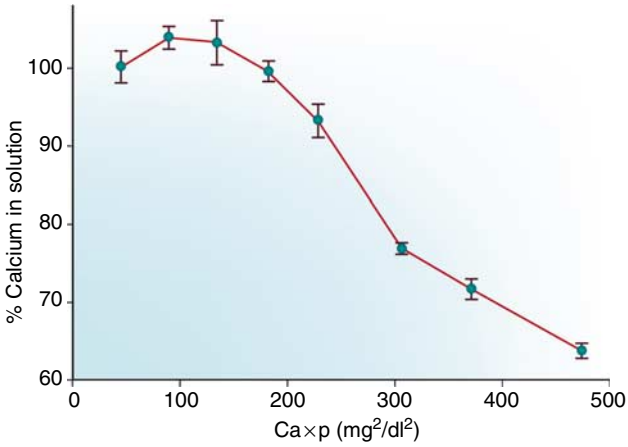

Figure 1 | Precipitation of calcium phosphate in human plasma. Plasma was anticoagulated with 15 U/ml heparin and incubated at 37°C in 5% CO_2. Tracer ^{45}Ca was added followed by sequential additions of $CaCl_2$ and buffered sodium phosphate from 1 M solutions. Samples were taken 15 min after each addition and centrifuged at 16 000 *g* for 3 min. Radioactivity in the supernatant was counted. Values are the means ± s.e. of triplicate determinations.

To determine this product, the author's plasma was incubated with increasing amounts of calcium and phosphate. As shown in Figure 1, precipitation did not occur until the Ca × P exceeded 200 mg^2/dl^2. Thus, a Ca × P at which $CaHPO_4$ precipitates in plasma is almost never achieved in our patients.

THE BIOLOGY OF ECTOPIC CALCIFICATION

So, what actually happens when the solubility of $CaHPO_4$ is exceeded *in vivo*? It was shown many years ago that the $CaHPO_4$ formed in supersaturated serum *in vitro* and plasma *in vivo* does not precipitate and remains in solution in a colloidal form,[4] probably bound to proteins, in particular fetuin.[14] The colloidal $CaHPO_4$ rapidly disappears from the plasma, even when large amounts are formed, because of rapid uptake by the reticuloendothelial system, specifically Kupfer cells and splenic phagocytes.[15,16] This process also occurs in interstitial fluid, with uptake of colloidal $CaHPO_4$ by regional lymph nodes, and would seemingly prevent precipitates of $CaHPO_4$ from forming in tissues. It is likely that this uptake represents endocytosis of fetuin–$CaHPO_4$ complexes.[17] The granules of $CaHPO_4$ in the reticulo-endothelial cells subsequently disappear, presumably due to dissolution. Whether this process can be overwhelmed and what happens when plasma is chronically supersaturated is not known. Presumably the same process occurs in humans but there are no data.

Oral or intravenous phosphate was a common treatment for hypercalcemia before the advent of other therapies and resulted in rapid decreases in circulating Ca concentrations. The fate of the $CaHPO_4$ that was presumably formed is unknown but the treatments were well tolerated. In the seminal study by Goldsmith and Ingbar,[18] autopsies were performed on seven of the 10 patients with severe

hypercalcemia who received phosphate. Although ectopic calcification was present in five patients, it was felt to be consistent with the magnitude and duration of the hypercalcemia and could not be ascribed to the phosphate treatment. Two patients who received repeated phosphate treatments (one of them intravenously) showed no ectopic calcification. Notably, in light of the recent focus on vascular calcification, arterial calcification was not found in any patient. One patient had calcification along a vein where extravasation of the phosphate solution had occurred. Recently, it has been reported that large doses of oral phosphate associated with bowel cleansing can result in nephrocalcinosis and renal failure.[19,20] However, this results from intratubular calcium phosphate deposition and appears to be unrelated to circulating levels of Ca or P.

Further evidence that the Ca × P has little to do with vascular calcification comes from conditions, such as diabetes and aging, in which vascular calcification occurs in the absence of hypercalcemia or hyperphosphatemia. The most dramatic example is infantile arterial calcification, which results from the lack of an enzyme that produces extracellular pyrophosphate, a potent inhibitor of hydroxyapatite formation.[21] These children develop severe vascular calcification in the absence of hypercalcemia or hyperphosphatemia and die by the age of 2 years. Absence of other proteins also results in extensive arterial calcification.[22,23] It is clear then that vascular calcification can occur at physiologic calcium and phosphate levels that are well below the precipitation point of $CaHPO_4$ and that we would all die of vascular calcification if it was not for processes normally in place to inhibit it. The fact that calcification can occur at such a low Ca × P is not surprising when one considers that bone is continually being formed under these conditions. This is accomplished by creating a microenvironment in which $CaHPO_4$ is less soluble and where pyrophosphate is removed by phosphatases.[24] In arteries, such a microenvironment may be created by elastin and glycoaminoglycans, which bind calcium and calcium salts.

Although hyperphosphatemia could clearly promote ectopic calcification, the tendency of vascular smooth muscle to calcify under normal conditions suggests that vascular calcification in renal failure has more to do with the lack of inhibitors than an elevated Ca × P. Consistent with this, circulating pyrophosphate levels are reduced in hemodialysis patients[25] and hydrolysis of pyrophosphate is increased in uremic vessels as a result of upregulation of alkaline phosphatase (P Garg, K Lomashvili, and WC O'Neill, *Journal of the American Society of Nephrology* 2005; **16**: 53, abstract). Whether other inhibitors are altered is unknown. Alternatively, others have proposed that osteogenic transformation of smooth muscle in uremia is responsible,[26,27] but a direct role in vascular calcification has yet to be demonstrated.

Does the Ca × P play any role in vascular calcification? This was examined in the author's laboratory using a model of medial calcification in cultured rat aorta. When aortas were cultured for up to 3 weeks in high concentrations of calcium and phosphate equivalent to a Ca × P in plasma well above 120 mg^2/dl^2, no calcification occurred,[28] confirming that an elevated Ca × P alone is not sufficient. This is because pyrophosphate, a potent inhibitor of hydroxyapatite formation, is produced by the vessels, and calcification occurs only when pyrophosphate is eliminated enzymatically. This calcification requires an elevation of both calcium and phosphate, but when the concentrations were varied to maintain a constant product,[29] calcification ranged from extensive to none (Figure 2). Surprisingly, calcification varied directly with the calcium concentration and inversely with phosphate concentration, suggesting that calcium may be the more important parameter. Although the model is far from perfect, this represents the most direct test to date of the role of Ca × P and it failed to correlate with vascular calcification.

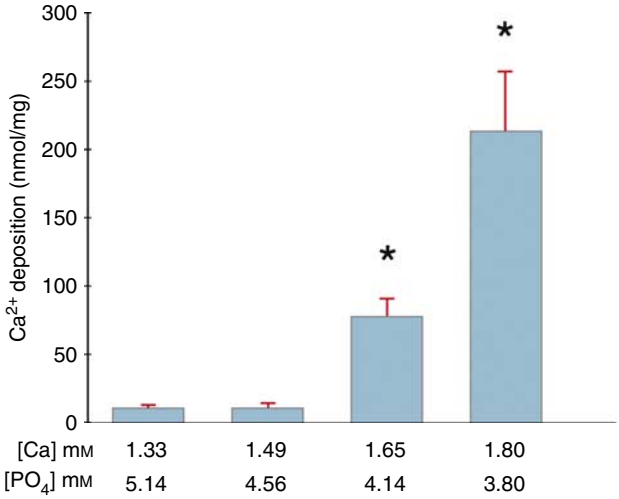

Figure 2 | Calcification of rat aortas cultured in serum-free medium with alkaline phosphatase at varying [Ca] and $[PO_4]$ with Ca × P kept constant at 6.84 $mmol^2/l^2$. Error bars are standard errors. *$P < 0.001$ versus 1.33 mM calcium and 5.14 mM phosphate. Adapted from Lomashvili *et al.*[29] with permission.

THE EPIDEMIOLOGY OF Ca × P

There are abundant epidemiologic data showing correlations between the Ca × P and cardiovascular outcomes or mortality. However, this does not prove causality and the medical literature is replete with negative intervention trials based on solid epidemiologic data. Although the KDOQI guideline that the Ca × P should be maintained below 55 mg^2/dl^2 is labeled as evidence-based, actually there are no data to support this. First, the studies are primarily cross-sectional and certainly do not indicate that keeping the product below 55 will improve outcomes. Second, the correlation between Ca × P and outcomes may have nothing to do with ectopic calcification. Lastly, it is questionable whether the Ca × P is an independent risk factor at all, as most of the variability in this parameter is accounted for by serum phosphorus, which is clearly associated with poor outcomes. In the large study cited in the KDOQI guidelines, hyperphosphatemia carried a slightly greater risk than elevated Ca × P.[30] This was also true

in an even larger follow-up study of 40 000 dialysis patients, despite the fact that serum Ca concentration was positively associated with death.[31]

It is also not clear that Ca × P is a risk factor for vascular calcification. In most cross-sectional analyses of patients with renal failure, vascular calcification has not correlated with Ca × P[32–37] and in almost all of these studies, there was no correlation with serum Ca or phosphorus levels as well. Univariate associations between Ca × P found in other studies either disappeared or became weaker in multivariate analyses.[38,39] In the largest cross-sectional study, both serum Ca and serum P concentrations were strongly associated with coronary calcification but Ca × P was not analyzed.[40] More importantly, longitudinal studies have not shown any correlation between progression of coronary calcification and Ca × P in patients with renal failure.[34,41–44] In a trial of sevelamer versus calcium-based phosphate binders, changes in calcification correlated with Ca × P only in the latter group.[45] The correlation was similar to that for Ca or P alone and was not apparent in multivariate analyses. The slower progression of calcification with sevelamer was not associated with a reduction in Ca × P.

SUMMARY

The Ca × P is a grossly oversimplified and scientifically flawed approach to the problem of ectopic calcification and there is no convincing evidence that it is a clinically useful parameter. Precipitation of $CaHPO_4$ does not occur in plasma until the Ca × P is at least three times the KDOQI threshold of 55 mg^2/dl^2, and there is no evidence that such precipitation is actually harmful. Like bone formation, ectopic calcification is a complex process that is governed as much or more by biology than by physical chemistry. Although hyperphosphatemia and calcium balance appear to contribute, vascular calcification is determined primarily by factors in the microenvironment of the vessel wall that both inhibit and promote it, not by spontaneous precipitation of calcium phosphate.

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