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# PHOSVITIN AND CALCIUM

THE RELATIONSHIP BETWEEN THE PRESENCE OF PHOSVITIN AND CALCIUM  
INTESTINAL ABSORPTION AND TRANSFORMATION INTO BONE MINERAL

ALEX HOLDCROFT, BSC., MM.  
ECOVATEC SOLUTIONS INC.  
31231 Wheel Ave., Abbotsford, B.C., CANADA, V2T6H1

## Executive Summary

**Ecovatec's revolutionary technology has unlocked the amazing potential of Phosvitin.** Ecovatec is the only known commercial producer of phosvitin in the world.

Phosvitin has been characterized by many scientific papers, and the literature consensus is that it has huge potential as a bio-active molecule, which are often used in nutraceutical, pharmaceutical, cosmetic, and food industries. Bioactivity is based on the ability of a compound to be "able to exert a biological effect at a physiological level, and it must be measurable and able to provide health benefits."<sup>1</sup>

Phosvitin can treat for **bone density or calcium deficiency** related health concerns as it can increase the **bioavailability of calcium** in the intestines. It can also increase the reaction rate of the formation of a bone mineral precursor that can be easily absorbed in the intestines to increase bone density and to create bone fillers and food supplements.

## Background

Phosvitin is the most highly phosphorylated protein found in nature and is derived from the egg yolks of vertebrates<sup>1</sup>. Proteins are made up of peptides, which are short chains of amino acids linked by peptide bonds. Bioactive peptides have shown the most promising potentials as therapeutic or health promoting agents<sup>2</sup>.

Serine is the major amino acid present in phosvitin (50%)<sup>1</sup>, and almost all the serine residues are phosphorylated. This phosphorylation gives phosvitin its extremely strong metal binding capacity and inhibits the bioavailability of metal ions<sup>1-4,7-9</sup>. When phosvitin is hydrolyzed into smaller peptides, it increases the bioavailability of calcium and iron as it inhibits the forming of insoluble calcium or iron phosphates.

Phosvitin is usually de-phosphorylated to open the tertiary protein structure before it is hydrolyzed to give it its nutraceutical applications as an antioxidant or pharmaceutical and food industry uses as a metal ion carrier or anti-inflammatory protein<sup>1-4,7-9</sup>. Phosvitin's phosphopeptides are also known to improve bone and dental health.<sup>1-3,6-8</sup>

*Samaraweers, et al* (2011)<sup>2</sup> and *Ren, et al* (2015)<sup>4</sup> both note that the potential use of phosvitin as a functional food ingredient is only hampered by lack of a scalable method of extraction. **Ecovatec Solutions Inc has eliminated this barrier.**

## Calcium's Functions in the Human Body

### Blood Calcium

Calcium ions freely circulate in the blood and participate in many important cellular functions. Calcium ions are involved in blood clotting factors, the function of nerves and muscles, and the tertiary structure of proteins.

### Conversion to Bone

When the concentration of calcium ions in the blood is high, parathyroid hormone (PTH) is released and starts a biological reaction which leads to the formation of bone mineral (increasing bone density). When calcium ion concentrations in the blood are low, the opposite occurs, and calcium ions are released from bone.

Calcium can be converted to hydroxyapatite (HAP) which is a precursor to the formation of bone mineral. This reaction can occur internally in the gut, or it can be created in the lab. This compound can then be ingested as a supplement or it can be used in its crystalline form as a filling for bones and teeth. It can also promote bone ingrowth in prosthetic implants. HAP is more easily absorbed by the gut than calcium and can be used instead of traditional calcium carbonate supplements.

### Absorption in the Intestines

Calcium is absorbed into the body with vitamin-D dependent calbindin proteins. When blood calcium levels are high, PTH inhibits the process of calcium absorption from the intestines, and the opposite occurs when levels are low. Only about 20% of the calcium ingested through diet and supplements is bioavailable to the body, the rest is excreted in the urine and feces.

### Calcium Disorders in Humans

Osteoporosis, osteomalacia, and rickets are bone disorders linked to calcium metabolism disorders. Calcium has also been suggested to have a role in preventing colorectal cancer, cardiovascular disease, high blood pressure, preeclampsia, and in promoting weight management<sup>5</sup>. Hypocalcemia and hypercalcemia are other serious blood calcium conditions. Too little calcium circulating in the blood is known as hypocalcemia and cause muscle spasms and paraesthesia.<sup>6</sup>

## Phosvitin in the Production of Bone Mineral<sup>7</sup>

When calcium (usually present as calcium phosphate) transforms into HAP, the reaction can be quite slow, often due to the lack of stabilizing molecules to bind to calcium in the intermediate reaction steps. *Zhang et al. (2017)*<sup>7</sup> found that the presence of phosvitin speeds this reaction by forming stable intermediate complexes with the calcium ions, providing nucleation sites for the crystal structure to form. This reaction can occur in the gut in the presence of ingested phosvitin, or it can occur in the lab to create HAP supplements or bone and teeth fillers.

### Research and Proof

*Zhang et al. (2017)*<sup>7</sup> measured the reaction time of calcium phase transformation to HAP by pH stat titration. They found that the control reaction took 3 hours, whereas it took 1 hour in the presence of native phosvitin, and over 6.5 hours in the presence of BSA (which retards the transformation). BSA is a protein commonly found in milk. Therefore, when calcium is ingested in milk, the conversion to HAP can be too slow for the body to absorb the HAP molecules. When phosvitin is ingested with calcium, the reaction to form HAP, the absorbable compound is 6.5x faster, allowing for greater calcium absorption. In a similar 2015 study, *Zhang et al.*<sup>8</sup> reported that the HAP formation time showed a 12x improvement when phosvitin was added compared to the control reaction.

The researchers also measured the reaction time with varying levels de-phosphorylation in phosvitin. De-phosphorylation refers to the removal of phosphate groups from the amino acid chains in phosvitin. In this case, this effect was noted to hydrolyze the protein as well. A positive correlation between de-phosphorylated phosvitin and increased reaction rate was observed.

## Phosvitin and the Solubility and Absorption of Calcium in the Gut<sup>9</sup>

Phosvitin can increase calcium absorption from the gut<sup>9</sup>. *Choi et al (2005)* researched the effectiveness of phosvitin peptides on enhancing the bioavailability of calcium and its accumulation in bones. They found that phosvitin's phosphoserine residues helped calcium remain soluble, which increases its bioavailability. This allows a greater portion of ingested calcium to be converted into bone mineral, increasing bone density. This may be because of the HAP compound formed in the gut and absorbed by the intestinal tissues.

### Research and Proof

*Choi et al (2005)*<sup>9</sup>, found that when infant rats were given a diet enriched with phosvitin for 4 weeks, they were shown to have higher calcium contents of their femurs and tibias. They measured statistically significant calcium absorption and accumulation in the bones of rats eating the phosvitin-enriched diet, compared to the control group. Interestingly, calcium absorption and accumulation were not correlated with the amount of phosvitin consumed, indicating that even a small amount of phosvitin in the diet can cause a difference in bone density.

*Ren et al (2015)*<sup>4</sup> discuss the properties of casein derived phosphopeptides (CPP), found in milk, and how they are known to play a key role in promoting calcium absorption and increase bone mass density and suppress bone mass resorption. Both the control and experimental groups in the 2005 Choi study were also supplied with 200mg of casein. The casein in the experimental diet was present in the ratio 160 mg casein: 1mg phosvitin. The experimental group showed 10-20% increases in the calcium present in the rat's leg bones. This indicates that a very small amount of phosvitin can make a large difference in calcium absorption. The properties that allow CPP to act to increase calcium absorption are its *15 phosphoserine residues, 3 of which are consecutive*.<sup>4</sup> Phosvitin phosphopeptides (PPP) have **123 phosphoserine residues, 113 of which are consecutive**.<sup>4</sup>

This study shows that with just a small ratio of phosvitin to available calcium, present in the food of these test animals, resulted in a significant increase in bone density versus a control group in just 4 weeks. With the recommended daily calcium intake for human adults being 1000-1200 mg, this equates to as little as 120mg of phosvitin daily to receive an equivalent ratio of phosvitin to calcium intake. If milk is being used as the primary source of calcium, then adding only 38mg of phosvitin per cup of milk provides this same ratio.

A 1995 study showed that patients who consumed HAP as a calcium supplement showed slower bone loss than those who consumed calcium carbonate<sup>10</sup> (the usual form found in supplements). Therefore, phosvitin can be used to increase the production speed of HAP, or it can be taken with calcium to significantly increase the HAP creation and absorption in the intestines.

## Preparation of Phosphopeptides Affects Solubility and Absorption of Calcium<sup>11</sup>

Zhong *et al.* (2016)<sup>11</sup> demonstrated a new way to prepare phosvitin phosphopeptides (PPP) using immobilized trypsin. An immobilized enzyme, such as trypsin, is an enzyme attached to an inert, insoluble material which will provide it increased resistance to changes in pH and temperature. It also allows the enzymes to be separated from the products at the end of the reaction and used again. This can potentially reduce the cost of preparing PPP, and therefore barriers to widescale use as a nutraceutical.

They found that immobilized trypsin has better stability and higher hydrolyzing activity on phosvitin. The resulting phosphopeptides were also demonstrated to be superior at calcium solubilizing than free trypsin.

These phosphopeptides were also tested *in vivo*. They found that the apparent absorption rate of calcium was much greater in rats fed immobilized trypsin phosphopeptides than free trypsin processed phosphopeptides, calcium chloride, and the control. The absorption rate increased from 30% to about 55-60% in about 1 week and was maintained over next 2 weeks. Blood serum calcium levels also increased by about 50-60% in the 3 weeks. This compares to the Choi *et al* study (2005) is discussed above where they used free trypsin and saw a marked increase in Ca solubility/absorption. This study shows that immobilized trypsin is even better for creating PPP.

They also showed that the bigger (1-3 kDa) phosphopeptides had a better binding ability than commercial casein. Very small fragments had very little binding ability. Therefore, both size of PPP and phosphorylation are critical to calcium binding.

### Practical Applications

These studies have real-world applications for individuals who are susceptible to bone loss, providing treatment options for osteoporosis or post-menopausal loss of bone density. It is recommended that adults consume 800-1200 mg of calcium daily, 20% of which is absorbed (about 200 mg). Supplements or nutritionally-enhanced beverages such as orange juice or milk could provide a much richer, bioavailable source of calcium if they also included de-phosphorylated phosvitin, as a greater percentage of the calcium consumed would be absorbed by the body.

### Sources

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