

Chapter 8

Calcium Homeostasis

Introduction

Calcium has a number of critical roles in physiology. It is required for muscle contraction, as an enzyme co-factor, and as a second messenger. In order for these processes to function properly, extracellular calcium concentration must be maintained within a narrow range. Calcium is also a major component of bone; the bone can act as a reservoir to allow serum calcium levels to be maintained, but excessive demineralization of bone as a result can lead to severe problems in older individuals.

The symptoms of **hypocalcemia** depend on the severity of the deficit. In extreme cases, such as loss of parathyroid function, the initial result is hypersensitivity in the neuromuscular system. This degenerates into tremors and uncontrollable muscle contraction (hypocalcemic tetany), and convulsions. Death occurs as a result of contraction of the larynx and/or diaphragm preventing respiration. Milder hypocalcemia results in drying of the skin, dehydration (as a result of overstimulation of the intestine), and eventually decreased hair and fingernail growth, and bone abnormalities. The last is especially evident in children, along with defects in tooth formation. Prolonged hypocalcemia also results in mental function problems. Acute **hypercalcemia** (e.g. following an injection of calcium salts) can lead to cardiac arrest. Chronic hypercalcemia results in neurological and neuromuscular abnormalities, anorexia, vomiting, cardiac arrhythmia, renal failure, and, if untreated, death.

Maintaining serum calcium levels is a dynamic process, involving the interaction of a variety of different organs. The key theme of calcium related endocrinology is the maintenance of a constant calcium concentration in extracellular fluid. First, we will examine the process itself, and then turn to the methods used to control calcium levels.

Calcium Economy

The concentration of calcium in serum is fixed at 9.5 ± 1 mg/dL (~ 2.5 mM). About half of this is free in solution, with the remainder bound to serum albumin and other serum components, such as citrate. The total amount of calcium in the extracellular fluid is about 1 g.

A better figure for extracellular calcium concentration is 1.2 ± 0.1 mM; this value refers to the free (also called ionized) calcium concentration. As with other hormones, free calcium is the biologically active form. While free calcium is usually 50% of total calcium, allowing the total calcium measurement to be useful, altered serum composition (e.g., decreased albumin concentration during pregnancy) may cause an altered total, but not free, calcium level. Improved assay methods have resulted in increased usage of the more physiologically and medically relevant free calcium level.

Figure 1 shows the main compartments involved in calcium economy. The following discussion of calcium flux will use values based on a “standard 70 kg adult individual” maintaining homeostasis. The actual values for the flux will vary with the age and size of the individual.

The body is not capable of maintaining its supply of calcium without an external source. This source is the diet **(1)**. The recommended daily allowance of calcium is 1.0 to 1.2 grams. For the purposes of discussion, I will use 1.0 g/day. The route of the calcium into the body is absorption by the gut **(2)**. The gut is not very efficient, and only absorbs 30–40%, or 0.4 g. In addition, there are losses of calcium in the gut, as a result of processes such as cell sloughing and bile secretion **(3)**. These losses total about 0.2 g, leaving a net absorption of about 20% or 0.2 g, with the remaining total of 0.8 g lost in the feces **(4)**.

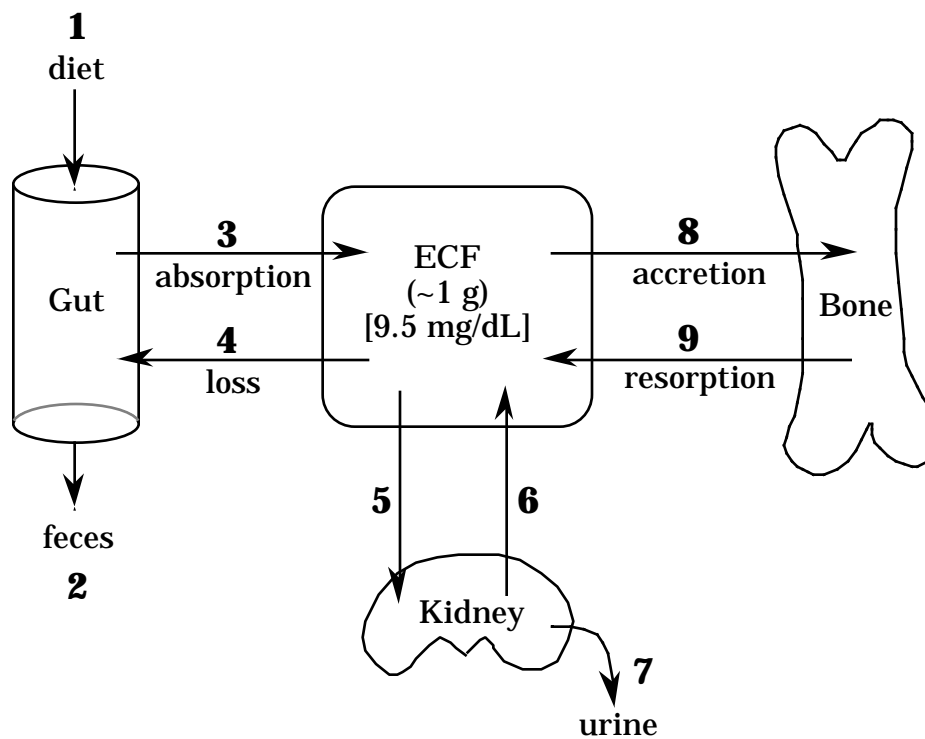


Figure 1. Calcium economy. This figure shows the various organs that contribute to maintaining extracellular calcium levels.

The calcium in the blood is filtered continuously through the kidneys, at a rate of between 6 and 10 grams/day **(5)**. The kidneys lose very little, reabsorbing about 97 to 98% of the total flow of calcium **(6)**. For the purposes of this example, and to maintain homeostasis, I will use a loss of 0.2 g/day in the urine **(7)** (1.0 g taken in, 0.8 g lost in feces, and 0.2 g lost in urine).

The bone is dynamic, and is continuously being remodeled. Most estimates indicate that about 0.5 g calcium is exchanged each day **(8,9)**. Note that this corresponds to about half of the total calcium in the extracellular fluid! The bone is the main

repository for calcium and contains about 1 kg of calcium. If the resorption of bone continued in the absence of accretion, all of the bone in the body would be gone in about 2000 days (~5.5 years). One of the most important functions of the calcium homeostatic machinery is the attempt to balance resorption and accretion of bone.

Calcium homeostasis is regulated by three hormones: parathyroid hormone (PTH), calcitonin and 1,25-dihydroxy-Vitamin D. PTH has three major effects: 1) PTH directly stimulates bone resorption; 2) PTH directly stimulates recovery of calcium in the kidney; and 3) PTH stimulates the production of 1,25-dihydroxy-Vitamin D from its precursors. Calcitonin has opposite effects to those of PTH in bone and kidney. Finally, 1,25-dihydroxy-Vitamin D stimulates calcium absorption in the gut, and has effects in bone and kidney as well.

The key to calcium homeostasis is the amount of calcium absorbed in the gut. There are always some losses in the feces, urine, and in sweat. The gut must compensate for these losses, because it is the only source of new material, and because renal compensation cannot meet a large challenge (the kidney is 97-98% efficient under normal conditions, leaving little room for improvement). The only other source of calcium to replace the losses due to excretion is bone. Bone resorption is the major protection against extracellular fluid calcium depletion; unfortunately, this can result in degradation of the bone, and eventually to a major health problem: osteoporosis (see Chapter 9).

Phosphorus Economy

The major mineral in bone is calcium phosphate. Calcium and phosphate must be deposited in bone and resorbed from bone together. Most phosphorus in the body is present as phosphate; as a result, phosphorus economy is closely related to that of calcium.

Absorption of phosphorus in the gut is quite efficient; about 80-90% of the phosphorus present in the diet is absorbed. The levels of phosphate in serum are controlled primarily by alterations in the rate of renal excretion of phosphate. In adults serum phosphorus concentration is about 3 to 4 mg/dL (the amount is stated as elemental phosphorus, although most is actually present as phosphate); it is higher in children (4-5 mg/dL). Most phosphate is free in solution, and acts as one of the major buffers that help maintain physiological pH. The levels of phosphate vary more than those of calcium due to lower degree of regulation.

Deficiency in phosphorus is uncommon. There are, however, several genetic disorders of phosphate metabolism that can result in altered calcium metabolism. Some examples of these disorders will be mentioned in Chapter 9.

Regulation of Parathyroid Hormone Release

PTH is an 84 amino acid peptide hormone, produced in the parathyroid gland as a pre-pro-peptide of 115 amino acids. The pro-peptide is processed to the mature form by proteases during its packaging into secretory vesicles.

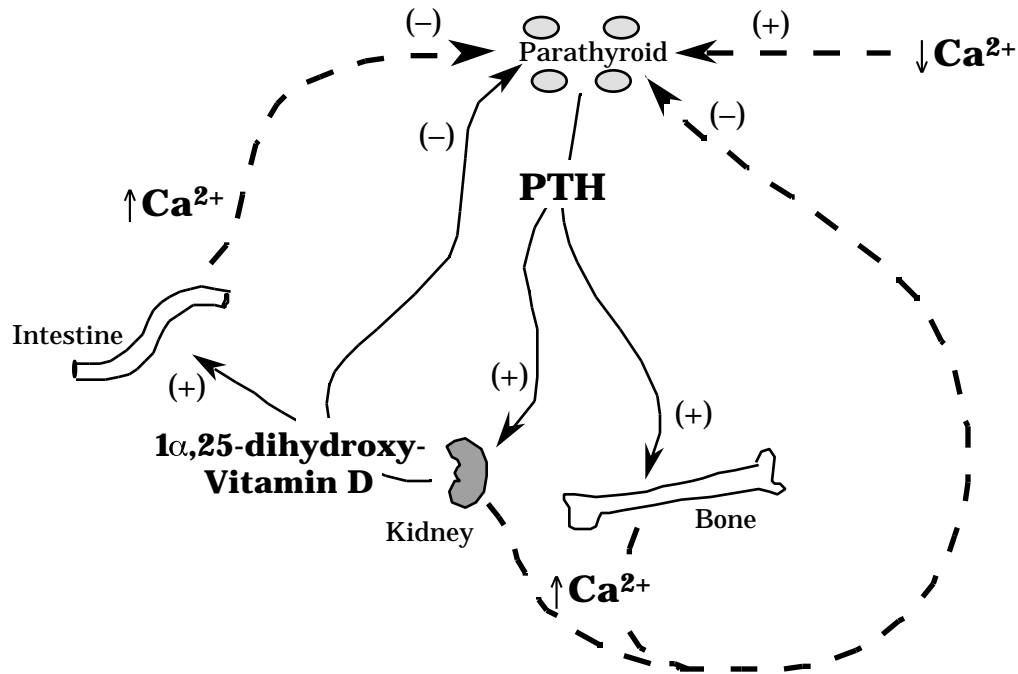


Figure 2. Control of PTH secretion.

Low serum calcium concentration results in rapidly increased PTH secretion (Figure 2); prolonged low serum calcium levels stimulate increased PTH synthesis, and eventually, parathyroid hyperplasia. In contrast, high serum calcium primarily increases PTH turnover in vesicles. As can be seen in Figure 3, PTH is constitutively released even at high serum calcium levels; however, a small decrease in calcium levels causes a rapid increase in PTH secretion, with a maximum reached when the serum levels reach 6–7 mg/dL. In addition to the effects of serum calcium, $1,25\text{-dihydroxy-Vitamin D}$ acts as a feedback inhibitor of PTH synthesis.

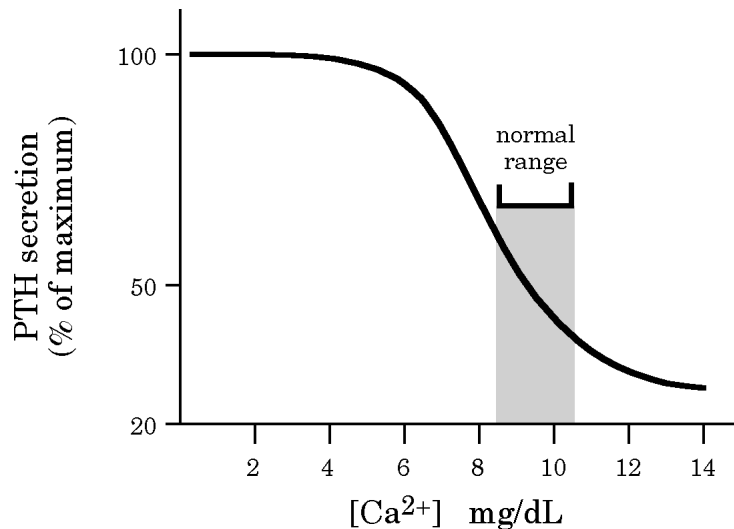


Figure 3. Variation in PTH release with calcium level. (Note that the y-axis begins above zero.)

The usual signal for release of secretory vesicle contents is an elevation in intracellular calcium, and this appears to be true for PTH as well. At first glance, this is somewhat paradoxical, because the signal for PTH release is a *decrease* in serum calcium. It works, because the normal cytoplasmic concentration of calcium is much lower than that in serum.

Recently, the parathyroid serum calcium sensor was cloned and found to be a G protein-linked receptor. On binding calcium, the calcium receptor stimulates phospholipase C via a G protein, resulting in a signal that causes a decrease in the rate of PTH secretion. Heterozygotes for inactive calcium receptor have familial hypocalciuric hypercalcemia, a disorder discovered in 1972 characterized by a higher than normal serum calcium, and lower than normal serum phosphate being "normal" for these individuals (the disorder is largely asymptomatic, and generally considered essentially benign). Homozygotes for the defective protein have neonatal severe hyperparathyroidism, which often requires parathyroidectomy. This implies that PTH secretion is actually constitutive, and under conditions of normal serum calcium must be suppressed to achieve a normal rate of secretion.

The calcium receptor is found in a number of tissues in addition to the parathyroid. In thyroid C cells, the calcium receptor is probably involved in regulation of calcitonin secretion. In kidney, the calcium receptor is thought to mediate the direct responses of the kidney to changes in serum calcium levels. The calcium receptor is also found in brain; its function in the CNS is currently under investigation. Mutations in the calcium receptor also affect calcium regulation in these tissues, in addition to their effects on PTH release.

Glucocorticoids stimulate PTH synthesis and release, although this may only be significant during prolonged stress or under conditions of pharmacological glucocorticoid administration. However, the effect of high levels of glucocorticoids on PTH release cannot be reversed by 1,25-dihydroxy-Vitamin D or by elevated serum calcium; this observation may explain, at least in part, the role of glucocorticoids in the etiology of osteoporosis.

PTH in Circulation

PTH has a short half-life of about 5 minutes. It is inactivated by proteolytic cleavage, particularly in the liver and kidney.

The calcium regulatory activity of PTH is confined to the first 34 amino acids. As a result of proteolysis of PTH in circulation, and of the fact that fragments of PTH are released by the parathyroid, PTH fragments are present in serum in much higher levels (up to 20-fold higher) than intact PTH. There appear to be separate receptors in bone and kidney for the C-terminal peptides, which are thought to have a separate, although probably related, activity.

PTH-related Peptide

Another peptide, functionally related to PTH, is called PTH-related peptide (PTHrP). PTHrP is transcribed from a gene on a different chromosome from that of PTH. There are actually three different forms of PTHrP, comprising 139, 141, or 173 amino acids (depending on differential mRNA splicing). The first 139 amino acids are identical among the three forms. The mature PTHrP protein product can bind to the PTH-receptor; amino acids 1-13 are 60% identical to PTH, and although

14-34 are somewhat less closely related to PTH, they still allow PTH receptor binding.

PTHrP is thought to act in an autocrine or paracrine fashion to locally regulate calcium levels. There is also some evidence that it is secreted by the parathyroid, possibly exclusively during fetal life. The fetus normally maintains serum calcium about 30% higher than adult levels; PTHrP appears to act to stimulate active transport of calcium across the placenta from the mother into the fetus via the placental calcium pump, and may be the primary circulating form of PTH during fetal life. In mice, homozygotes for the PTHrP knockout mutation are inviable, dying as a result of abnormal bone and cartilage development during embryogenesis. Although PTH and PTHrP are thought to bind to the same receptor and can substitute for each other in many functions, the effect of PTHrP on the placental calcium pump is specific for PTHrP; PTH has no effect on placental calcium transport. In addition, there is increasing evidence for regulatory roles for PTHrP in a variety of tissues during postnatal and adult life.

PTHrP is also secreted by a wide variety of tumors, resulting in the syndrome of **humoral hypercalcemia of malignancy** (see Chapter 9). The unregulated PTH-like activity of PTHrP causes severe problems as a result of bone degradation and elevated serum calcium levels, and acts as a marker for the presence of the tumor.

Recently, a new gene related to the PTH receptor was discovered. According to somewhat preliminary evidence, the new receptor responds exclusively to PTH. Thus, it is possible that response to PTH and PTHrP is tissue-dependent, depending on the relative levels of the different receptors.

Measuring PTH Levels

There are two major problems with measuring the amount of effective PTH: 1) the PTH-like response may be due to PTHrP, and 2) the many proteolytic products of PTH may interfere in the RIA for PTH. As a result, clinical laboratory reports of PTH levels must be viewed with caution.

PTH Actions

PTH is critically important for maintaining serum calcium levels: an individual lacking parathyroid function dies of hypocalcemic tetany in 48-72 hours unless treated.

Most actions of PTH are thought to be mediated by cAMP, although there is also evidence for PTH-stimulated phosphatidyl inositol hydrolysis in some tissues.

PTH increases the rate of turnover of bone and the rate of bone remodeling. This usually results in a net increase in serum calcium levels. However, the effect of PTH is *not* simply a breakdown of bone; remodeling and formation of bone also require PTH. Since receptors for PTH are present in the osteoblasts, not osteoclasts, PTH is

thought to act by inhibiting osteoblast differentiation and function, and by modulating cell-cell interactions which induce osteoclast differentiation and activity.

Two major cell types are involved in altering bone structure. **Osteoblasts** are responsible for bone synthesis and calcium deposition; **osteoclasts** are responsible for degradation of bone and calcium resorption.

The function of both osteoblasts and osteoclasts are under control of systemic hormones. In addition, the two cell types regulate both their own and their counterpart's activity by secreting a number of autocrine and paracrine factors.

Osteoblasts contain receptors for PTH and 1,25-dihydroxy-Vitamin D. The increased bone resorption associated with these hormones is thought to be due to release of a variety of stimulatory factors (such as IL-1, IL-6, TNF, and others) by the osteoblasts. Osteoclast precursors respond to these factors by differentiating into mature osteoclasts; mature osteoclasts respond by increasing their bone resorbing activity and delaying apoptosis.

Osteoclast activity is directly inhibited by calcitonin, and possibly by hypercalcemia. Osteoclast activity is also affected by a variety of inhibitory factors released by osteoblasts (e.g. TGF- β , IL-4, and nitric oxide). The estrogen and androgen inhibition of bone resorption is thought to be an indirect effect of action on the osteoblast; the sex steroids inhibit release of the stimulatory cytokines (especially IL-1, IL-6, and TNF), and induce release of osteoclast inhibitory factors. The inhibitory factors decrease differentiation of osteoclast precursors, and both decrease activity and induce apoptosis of mature osteoclasts.

Bone remodeling is a continuously occurring process, affecting 10-15% of the bone surface. As a result of mechanical stresses, hormonal signals, or other poorly characterized stimuli, osteoclast precursors migrate to the bone surface, fuse to become mature polynuclear osteoclasts, and begin enzymatically degrading the bone. This is followed by a replacement of the removed mineral and protein by osteoblasts. Remodeling is required for maintenance of bone structural strength; unfortunately, in adults, each remodeling cycle leaves slightly less bone than was originally present. The remodeling process is affected by a variety of different hormones. Chronically high levels of PTH, thyroid hormone, Vitamin D, and/or some other conditions result in increased bone resorption. Elevated levels of glucocorticoids decrease the efficiency of replacement of the bone. Estrogen deprivation in women increases the amount of bone that the osteoclasts remove, eventually to the point that the bone scaffold is perforated, and the osteoblasts are no longer able to repair the damage. Androgen deprivation in men decreases osteoblast activity. All of these hormonal conditions therefore increase the risk for the permanent weakening of the bone associated with osteoporosis.

The cytokines used for osteoblast/osteoclast communication also have (unrelated) effects in immune system cells (and in some other physiological processes). While there are exceptions (e.g., lymphocyte IL-6 release may result in damage to bone in rheumatoid arthritis), in general, these cytokines are released in small enough amounts that their effects are purely local.

In the kidney, PTH stimulates calcium reabsorption in the distal tubule, and stimulates phosphate excretion, by decreasing reabsorption. (Note that phosphate and calcium are coupled due to the release of both from degraded bone.) PTH also increases 1,25-dihydroxy-Vitamin D production by the kidney. PTH also increases calcium uptake in the gut, but these effects appear to be exclusively indirect, via increases in 1,25-dihydroxy-Vitamin D (see chapter 9).

Direct Regulatory Effects of Serum Calcium

Recent evidence suggests that the kidney, in addition to responding to PTH, directly senses serum calcium levels (probably due to the presence of the calcium receptor in parts of the kidney). Elevated serum calcium inhibits 1 α -hydroxylase activity. Elevated calcium levels also decrease both water and calcium reabsorption; the decreased water reabsorption is thought to allow increased urinary calcium excretion while reducing the risk of precipitation of calcium salts.

Elevated serum calcium appears to inhibit bone resorption. In part this is due to decreased PTH and increased calcitonin levels; however, some studies have shown a direct effect of serum calcium on osteoclasts.

Calcitonin

Calcitonin is a 32 amino acid peptide hormone synthesized and released by the parafollicular cells (C-cells) of the thyroid. Calcitonin is released in response to high serum calcium levels (and, for unknown reasons, in response to some gastrointestinal peptides). Like PTH, calcitonin has a short half-life of about 10 minutes.

The actions of calcitonin are opposite to those of PTH. In bone, calcitonin inhibits calcium resorption by inhibiting the function of mature osteoclasts and by inhibiting the differentiation of osteoclast precursor cells. In kidney, calcitonin inhibits the reabsorption of both calcium and phosphate.

Unlike PTH, however, calcitonin does not appear to have a strong physiological role. Thyroidectomized individuals require supplementation with thyroid hormone, but not calcitonin. C-cell tumors (thyroid medullary carcinomas) often secrete excess calcitonin, but these patients do not have problems with calcium balance as a result. The elevated calcitonin does act as a marker for these tumors, which may be its most useful clinical role.

Salmon calcitonin has a longer serum half-life than the human peptide, and has been used to treat Paget's disease and, with limited success, osteoporosis (see Chapter 9).

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