UNIT 7

Macrominerals

TABLE OF CONTENTS

I. OVERVIEW

The term macrominerals refers to those elements needed by the body in milligram quantities on a daily basis. The category includes sodium, potassium, chloride, calcium, phosphorus, and magnesium. While the body content of the first three is relatively small because of high turnover, the body content of calcium and phosphorus, by comparison, is relatively large. All of these serve as electrolytes and their critical use relates to this function. While they have a structural function as well, their roles as metabolic regulators is of primary importance. Each of these minerals will be discussed as they relate to each other.

II. SODIUM

Sodium is the major extracellular electrolyte. It has an atomic number of 23 and circulates as a fully dissociated ion due to its 1+ charge. It is thus fully water soluble. It has been estimated that the adult body contains 52 to 60 meq/kg (male) and 48 to 55 meq/kg (female). Thus, the average adult male would have about 83 to 97 g of sodium in his 70-kg body. Between 2/3 and 3/4 of this sodium is "fixed" in the mineral apatite of the bone. The remaining sodium comprises a pool which undergoes considerable flux as it participates in sodium-potassium exchange. The exchangeable sodium in the adult human body can be predicted using the following equation: Na_e (meq) = 163.2 (total body water) – 69 – Ke (meq). Ke is the exchangeable potassium which likewise can be predicted as follows: Ke (meq) = 150 (intracellular body $water_L$) + 4 (total body water – intracellular body water). Total body water can be determined (see *Advanced Nutrition: Macronutrients*, Unit 2) or estimated using one of several prediction equations. Sodium is primarily an extracellular ion and, as such, the serum will contain 136 to 145 meq/l. Normal sodium intake varies from less than 2 g to 10 g/day. Most of this sodium comes from table salt, NaCl. Foods rich in added salt are usually the snack foods such as potato chips, salted nuts, pretzels, and so forth. Processed foods have more added salt than nonprocessed foods because salt not only improves the taste of the finished product but also serves as a preservative. Luncheon meats, cheese spreads, pickles, relishes, catsup, canned and frozen vegetables, crackers, breads, and frozen desserts are but a few of the foods that contain more sodium as a processed food than in their raw or nonprocessed state. Every living thing contains sodium and, except for pure fats and carbohydrates, no major food source lacks this element. There is no RDA figure for sodium but there is an estimated minimum intake for healthy adults of 500 mg Na per day. These estimates presume that the individual has a moderately active life style and lives in a temperate environment. Heavy work in a hot, dry environment and a variety of medical conditions (see Table 1) will affect the need for sodium.

Table 1 Causes of Hypernatremia and Hyponatremia

Hypernatremia (serum Na > 150 meq/l)	Hyponatremia (serum Na < 135 meq/l)
Dehydration — excess sweating, deficient water intake	Cachexia (any wasting disease, i.e., cancer)
Excess solute loading	Anorexia nervosa
Diabetes insipidus (too little ADH)	Ulcerative colitis
Brain stem injury	Liver disease
	Congestive heart failure
	Ascites, edema
	Major trauma
	Severe infection
	Excess water intake
	Inappropriate ADH release
	Diarrhea
	Certain drugs (chlorothiazide, mercurial diuretics, etc.)
	Adrenalectomy

Table 2 Hormones Involved in the Regulation of Serum Sodium

Hormone	Function
Vasopressin (ADH)	Serves to stimulate the reabsorption of water by the glomerulus and renal convoluted tubule.
Atrial natriuretic hormone	Counteracts vasopressin and thus induces water loss, sodium loss, and potassium loss. It also decreases blood pressure and increases the glomerular filtration rate. Suppresses renin release and aldosterone release. Antagonizes angiotensin II and norepinephrine.
Renin	Catalyzes the conversion of inactive angiotensin I to active angiotensin II.
Angiotensin II	At low serum sodium levels it conserves sodium by stimulating its reabsorption. At high serum sodium levels it has the reverse effect. Stimulates vasoconstriction which increases blood pressure. Reduces water loss, decreases the glomerular filtration rate. Stimulates aldosterone release.
Aldosterone	Conserves sodium by increasing sodium resorption by the kidney.

While sodium intake can be highly variable, serum sodium is not. As described above, the normal range of serum sodium is quite small. Values in excess of 150 meq/l (hypernatremia) and below 135 meq/l (hyponatremia) are considered abnormal and are of clinical concern. There are several reasons why hypernatremia or hyponatremia can develop. These are summarized in Table 1. In normal individuals, the level of sodium in the serum is tightly controlled and this control is intertwined with the control of potassium concentration, chloride concentration, and water balance.

A. Regulation of Serum Sodium

The system which regulates sodium levels in the blood also is involved in the regulation of water balance, pH, and osmotic pressure. Both hormones and physical/chemical factors are involved. Table 2 lists the hormones and their roles in sodium balance as well as in water balance. The hormones listed are all involved indirectly or directly because of the need to regulate the osmotic pressure within and around the cells. Osmolality, that is, the concentration of solutes on each side of a semipermeable membrane, is maintained by the passage of water through that membrane. This osmolality is maintained at roughly 270 to 290 mosmol. The proteins, many of which can not pass through the plasma membrane, are part of this solute load. In the well-nourished, healthy individual, the intracellular and extracellular proteins are maintained at fairly constant levels. This leaves the small, fully ionized solutes (Na^+, K^+, Cl^-) as major determinants of the osmotic pressure of the system. Osmotic pressure is the physical force required to keep the osmolality on both sides of the membrane approximately equal. If the solute load on one side exceeds that of the other, water and the small solutes (Na^+, K^+, Cl^-) will pass through the membrane to equalize the concentration of solutes.

Since the number of particles determines osmotic pressure, substances which ionize affect osmotic pressure according to the degree of dissociation. Thus, fully dissociated NaCl produces the ions Na^+ and Cl^- in such a fashion that at 0.154 *M* there are 1.85 particles each of Na^+ and Cl^- which exert a pressure of 286 mosmol. There are several ways to measure osmolality in the laboratory using rather simple instruments called osmometers. For example, the osmolality of a solution can be determined by using the effect of a solute on the freezing point. The addition of 1 osmol to 1 l of water will depress the freezing point by 1.86°C.

Several of the hormones listed in Table 2 are released in response to changes in sodium concentration or to signals generated by osmoreceptors located in the anterolateral hypothalamus. The sodium ion, of all the circulating ions, is the most potent of the solutes activating the osmoreceptors, which in turn signal the release of hormones which regulate osmolality. Vasopressin (ADH) is one of these; aldosterone is another. When the osmoreceptors sense a change (increase) in the solute load, ADH release is stimulated. This results in an increase in renal water resorption and thus dilution of the solute load. Aldosterone release is stimulated by low serum sodium-high

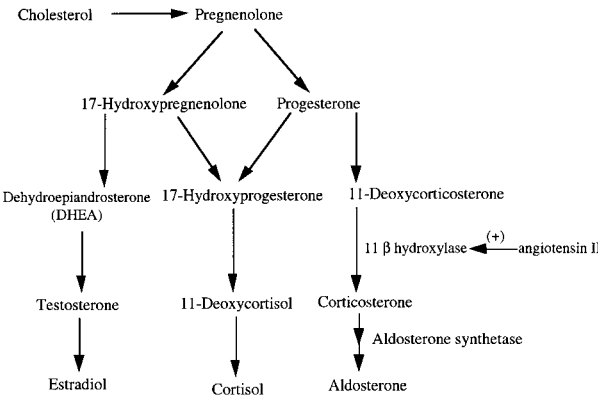

Figure 1 The steroid hormone biosynthetic pathway showing the synthesis of aldosterone.

potassium levels in the blood. Aldosterone, released by the adrenal cortex, functions in sodium conservation by increasing renal reabsorption of this ion. Of interest is the action of these ions at the level of the expression of the renin, angiotensin I, and aldosterone synthetase genes. At low sodium-high potassium levels, the transcription of these genes is stimulated. While more renin and angiotensin I synthesis has not been found with increasing mRNA, more aldosterone has been found. The reason for this difference in amounts of gene product has to do with the half-lives of these products. Renin and angiotensin I are made but are quickly degraded. Aldosterone does not disappear as readily. More messenger RNA is produced and more enzyme is synthesized.

The pathway for aldosterone synthesis (as part of the steroid hormone synthetic network) is shown in Figure 1. Aldosterone synthesis from corticosterone via the enzyme 11-β-hydroxylase (an enzyme that catalyzes 11-deoxycorticosterone conversion to corticosterone) is enhanced by angiotensin II. Angiotensin II release is stimulated by low sodium levels. Angiotensin II action requires the calcium ion and high potassium levels to facilitate calcium ion movement from outside to the inside of the cell. As aldosterone levels increase, the calcium ion facilitates its release by the cortex cell. Aldosterone then moves to the kidney where it stimulates electrolyte conservation. Figure 2 illustrates these hormone effects on electrolyte balance. Thus, the circle is completed. Sodium is regulated by several hormones and in turn regulates the synthesis of these hormones through effects on gene transcription. Sodium also stimulates the transcription of the genes for the cholesterol side chain lyase, a P450 enzyme, the gene for adrenodoxin and, in hypertensive animals, has been found to stimulate the transcription of the gene for endothelin 1. Endothelin 1 is an important vasoactive peptide that acts as a diuretic and as a natriuretic. It stimulates vasoconstriction and in genetic hypertension it seems to reduce sodium loss.

B. Function

Indirectly, the function of the sodium ion as a participant in the regulation of osmotic pressure has already been discussed as the regulation of serum sodium levels was presented. In addition to its function in this system, it also functions in nerve conduction, active transport both by the enterocyte and by other cell types, and plays a role in the formation of the mineral apatite of the bone (see Section VII.E.1). The common thread to its role in nerve conduction, active transport, and water balance is its function in the sodium-potassium ATPase. This enzyme, embedded in the plasma membrane of most cells, is perhaps the most thoroughly studied enzyme of the active transport systems. The cloning of the cDNAs encoding the subunits of this ATPase was achieved more than 10 years ago. The ATPase transmembrane protein was first isolated in 1957 and consists of two types of subunits: a 110-kDa nonglycosylated α-subunit that contains the enzyme's catalytic

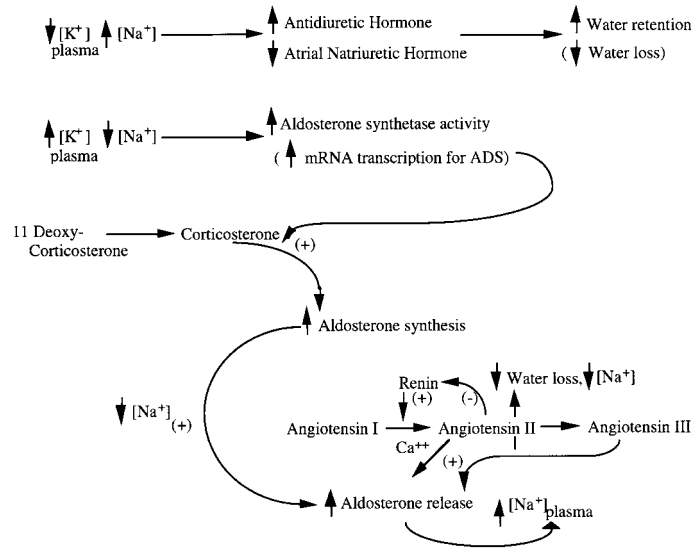

Figure 2 Schematic representation of how low-potassium high-sodium levels affect sodium conservation. Two systems are used. One involves the posterior pituitary which releases antidiuretic hormone (ADH). ADH stimulates water and sodium retention. The other system involves the adrenal cortex which is stimulated to release aldosterone, the mineralocorticoid responsible for Na^+ retention.

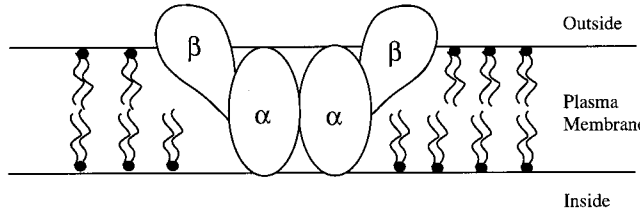

Figure 3 Structure of the Na^+K^+ ATPase.

activity and ion-binding site, and a 55-kDa glycoprotein β-subunit. The enzyme has two of each of these subunits. Figure 3 illustrates the structure of this enzyme. The glycoprotein probably plays a role in recognition of appropriate substrates, but this probable role is speculative. The ATPase is frequently called the Na^+K^+ pump because it pumps sodium out and, as potassium returns to the cell, there is a concomitant hydrolysis of ATP. The equation which describes this process is as follows:

$$3Na^+ \text{ (in)} + 2\ K^+ \text{ (out)} + ATP \leftrightarrow 3Na^+ \text{ (out)} + 2K^+ \text{ (in)} + ADP + Pi$$

The pump is an electrogenic system, illustrated in Figure 4, with the extrusion of three positively charged particles (the Na^+) in return for two negatively charged ones (the K^+). As an electrogenic system, the Na^+K^+ATPase generates an electrochemical potential gradient that is responsible for nerve action. Signal transmission along a nerve path occurs via a depolarization/repolarization scheme whereby potassium leaves the neuron and sodium enters (depolarization) and through ATPase activity the reverse occurs (repolarization). Much of the ATP that cells produce is used by this ATPase. In fact, in nerve cells up to 70% of their ATP production is consumed by the sodium pump as it functions in signal transmission.

Other cell types use the pump for other purposes. As mentioned, the active transport of needed nutrients into the enterocyte uses the pump. Muscle contraction/relaxation uses a Na^+K^+ ATPase

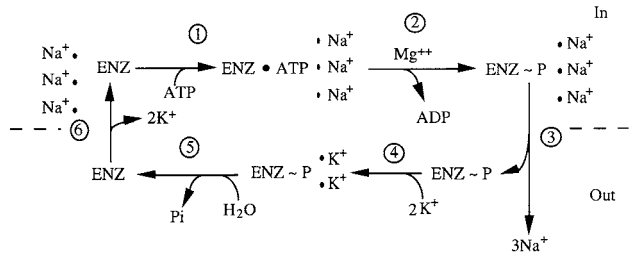

Figure 4 Mechanism of action of the Na^+K^+ ATPase. The ion exchange occurs via a series of reactions utilizing the α subunits of the enzyme which has an inward-facing high-affinity Na^+ binding site which reacts with ATP to form the activated ENZ~P only when Na^+ is bound to it. On the exterior aspect of the α subunit is a high-affinity K^+ binding site which will hydrolyze, releasing inorganic phosphate only when K^+ is bound to it. The obligatory sequence begins with the sodium ATP binding and ends with K^+ import into the cell. This sequence is numbered on the figure.

or pump. In the muscle there is an additional pump, the Na^+Ca^{2+} antiport system. This system pumps sodium out and allows calcium into the cell. Calcium triggers muscle contraction and so the two pump systems work together to regulate muscle action. When heart muscle degenerates, the clinician has several drugs that can be used to stimulate muscle action by inhibiting the Na^+K^+ ATPase. These drugs, called cardiac glycosides (digitalis, ouabain), inhibit the ATPase by binding to the α subunits (see Figure 2). This results in an increase in intracellular Na^+ which in turn stimulates the Na^+Ca^{2+} antiport system. The cell extrudes Na^+ and the resultant influx of Ca^{2+} triggers an increase in the force of the cardiac muscle contraction. The drug dose must be carefully monitored because too much Ca^{2+} influx could be lethal unless counteracted. The regulation of intracellular calcium levels together with its role in metabolic regulation is a topic of intense interest by nutritionists, biochemists, and physiologists. This topic is addressed below (see pages 164–166).

III. POTASSIUM

Potassium is the major intracellular electrolyte. It has an atomic weight of 39. The healthy young adult male has between 42 to 48 meq K^+ per kilogram of body weight or 2940 to 3360 meq in the 70-kg male. Persons with above average muscle mass, i.e., athletes, will have more body potassium than persons of average muscle mass. Virtually all the body potassium is exchangeable with the exception of small amounts that are irretrievably bound up in the bone mineral. Since potassium is primarily an intracellular ion, the number of cells in the body can be estimated using an infusion of the heavy isotope, K^{40}. The infused and exchangeable potassium equilibrates and by determining the dilution of the isotope one can determine cell number and mass while correcting for fat mass (see *Advanced Nutrition: Macronutrients*, Unit 2). The Na^+K^+ ATPase actively works to ensure that K^+ stays within the cell and that very little (3.5 to 5.0 meq/l) is present in extracellular fluid.

Just as all living things serve as sources of sodium, so too are they sources of potassium. Only highly refined food ingredients, i.e., pure sugars, fats, and oils, lack this essential nutrient. Especially good sources are orange juice, avocados, fish, and bananas. There is no RDA for potassium but an intake deemed safe and adequate is 2000 mg/day. Potassium passes freely from the gastrointestinal system into the enterocyte and thence into the body. Potassium is distributed in response to energy-dependent Na^+ redistribution. Almost all of the consumed potassium is excreted in the urine, with a very small amount found in the feces in healthy, normal adults. In persons experiencing diarrhea, however, the loss of potassium can be quite large and debilitating. If the diarrhea is of short duration (less than 12 hr) the body will compensate and the person will survive. However, should this condition persist, potassium supplementation will be needed. Such is the case with the disease,

Table 3 Causes of Hypokalemia and Hyperkalemia

Hypokalemia (Plasma Levels < 3.5 meq/l)	Hyperkalemia (Plasma Levels > 7 meq/l)
Vomiting in excess (loss of chloride)	Chronic renal failure
Diuretics that enhance K^+ loss	Addison's disease (no aldosterone)
Cushing's disease (excess steroids)	Major trauma, infection
Rehydration therapy without K^+	Metabolic acidosis
Chronic renal disease	
Metabolic alkalosis	
Diarrhea	

cholera, and for a number of the malabsorption syndromes. The plasma or serum level of potassium is not a reliable index of whole-body potassium status simply because potassium within the cell (not in the serum) is what is needed. Causes for concern with too little potassium (hypokalemia) or too much (hyperkalemia) have to do with muscle contractility. If hypokalemia persists the person could die of cardiac arrest. This occurs because too much K^+ has left the contractile unit and the heart muscle loses its ability to contract. Too much K^+ will have a similar effect; that is, the heart stops in diastole. Common causes of hypo- and hyperkalemia are listed in Table 3. The regulation of potassium balance follows that of sodium balance and its participation in the sodium potassium pump has already been discussed.

IV. CHLORIDE

Chloride (atomic weight 35.5) is the third leg upon which osmotic pressure and acid-base balance rests. Normal chloride levels in plasma are 100 to 106 meq/l and vary very little. The glomerular filtrate contains 108 meq/l and urine contains 138 meq/l. Sweat can contain as much as 40 meq/l but usually contains only trace amounts. The intracellular fluid contains very little Cl^- (~4 meq/l) whereas intestinal juice contains 69 to 127 meq/l. In instances of secretory diarrhea, the chloride content of the excrement can be as high as 45 meq/l. Most of the chloride in the intestinal tract does not appear in the feces of normal individuals. Rather, this ion recirculates as sodium and potassium are carried into the body. The main excretory pathway is urine. This ion is a member of the halogen family of elements that includes fluoride, iodide, and bromide in addition to chloride. Although very reactive, chloride is passively distributed throughout the body. As mentioned, it moves to replace anions lost to cells via other processes. It is the other half of table salt, NaCl, and as such is found in abundance in most foods. Dietary intake is in excess of that of sodium yet the usual plasma Na^+:Cl^- ratio is about 3:2. This imbalance is due to the passive nature of chloride transfer between water compartments and to the active system which serves to retain Na^+. As with Na^+ and K^+ there is no RDA, but an intake of 750 mg/day is deemed safe and adequate. Instances of below and above normal plasma levels of Cl^- are not diet related but are due to metabolic reasons usually related to Na^+ and K^+ homeostasis. Listed in Table 4 are reasons why hypochloremia and

Table 4 Causes of Hypochloremia and Hyperchloremia

Hypochloremia	Hyperchloremia
Increased extracellular water volume due to trauma and/or cachexia	Dehydration Brain stem injury Diabetes insipidus
Vomiting with large loss of gastric HCl	
Overuse of diuretics	Ureterointestinal anastomoses due to reabsorption of Cl^-
Overuse of adrenal steroids with retention of Na^+	
Chronic respiratory acidosis (high CO_2, low pH)	
Chronic renal disease, renal failure	

hyperchloremia develop. Note the similarities between these causes and those listed for sodium in Table 1 and those for potassium in Table 3.

A. Function

As an electronegative element, Cl^- is a good oxidizing agent. In typical reactions it is reduced to its electronegative form. One of its main functions is as an essential ingredient of the gastric acid, hydrochloric acid. Gastric juice contains 120 to 160 meq Cl^-/l. This is completely dissociated into a strong electron donor (H^+) and a strong electron acceptor (Cl^-).

HCl is produced by the parietal cells of the gastric mucosa. This mucosa, consisting of a variety of secreting cells, also release pepsinogen, which is activated by HCl and is the intrinsic factor needed for vitamin B_{12} absorption, and mucus. The mucus is a necessary protectant of the mucosa. Without it, HCl and the various proteases of the gastric secretions would digest the organ itself. The main function of HCl, aside from its role in the conversion of pepsin from pepsinogen, is as a bactericide. In the absence of HCl bacterial overgrowth occurs, with subsequent deleterious effects on gastrointestinal function.

The function of chloride, aside from its passive participation in electrolyte balance, has to do with hemoglobin and its function as a carrier for oxygen and carbon dioxide. As hemoglobin exchanges oxygen for carbon dioxide, the enzyme carbonic anhydrase catalyzes the formation of HCO_3^-. This carbonate ion diffuses out of the red cell in exchange for Cl^-. The Cl^- is bound more tightly to deoxyhemoglobin than to oxyhemoglobin. Hence the affinity of hemoglobin is directly proportional to the concentration of Cl^-. As mentioned, the carbonate ion, HCO_3^-, freely permeates the erythrocyte membrane so that once formed it equilibrates with the plasma. The need for charge neutrality on both sides of the red cell membrane requires that Cl^- flow into the erythrocyte to replace HCO_3^- as it leaves. This is called the chloride shift. Cations (Na^+, K^+) can not do this shifting, but Cl^- and HCO_3^- can. Because of the shift, the Cl^- ion in the venous blood erythrocyte is higher than in the arterial blood erythrocyte.

V. CALCIUM

A. Overview

Calcium is the fifth most abundant element in the body, exceeded only by carbon, hydrogen, oxygen, and nitrogen. It has an atomic weight of 40 and is the primary mineral in bones and teeth where it is present as hydroxyapatite [$3Ca_3(PO_4)_2 \cdot Ca(OH)_2$]. On a dry weight basis, bone contains about 150 mg calcium per gram. By comparison, soft tissue such as liver, muscle, or brain contains less than 35 μg calcium per gram. A normal 70-kg man will have about 22 g calcium per gram of fat-free tissue or a total of 1.54 kg. While the calcium in the teeth is seldom mobilized, that which is in the skeletal muscle is mobilized and replaced at a range of about 0.5 g/day. This daily turnover of calcium is essential to the maintenance of metabolic homeostasis because not only does calcium serve as a structural element, it also serves, in its ionized form, as an essential element in signal transduction. Of the total body calcium, 1% serves as an intracellular/intercellular messenger/regulator. Calcium mobilization and deposition change with age, diet, hormonal status, and physiological state. Bone calcium homeostasis is related to bone strength, and if mobilization exceeds deposition the bones will become porous (osteoporosis) and break easily.

B. Sources

The average daily calcium intake for adults in the U.S. ranges from 500 to 1200 mg. The range is quite broad because it depends on the percent of the diet that comes from dairy products. Milk,

Table 5 Food Sources of Calcium

Source	Calcium (mg/100 g)	Weight of Average Serving (g)
Skim milk	123	245
Whole milk	119	244
Ice cream	129	66
Yogurt	120	227
Oysters	45	84
Cheddar cheese	728	28
Spinach	135	90
Mustard greens	74	70
Broccoli	48	44
White bread	125	24
Whole wheat bread	72	25
Carrots	26	72
Potatoes	10	202
Winter squash	14	102
Egg	50	50
Hamburger	10	100
Hot dog	19	57

cheese, ice cream, yogurt, sour cream, buttermilk, and other fermented and nonfermented milk products can provide as much as 72% of the daily calcium intake. Nuts and whole grain products are also good sources of calcium while other foods are relatively poor sources of this mineral. Shown in Table 5 are a number of foods and their calcium content.

Some foods contain calcium-binding agents that reduce the availability of the calcium to the enterocyte. For example, some plant foods contain calcium in measurable quantities but these same foods also contain phytate, a six-carbon anomer of glucose having six phosphate groups. Phytate will bind calcium, reducing its availability for absorption. Phytate can be degraded by the enzyme phytase which, in turn, releases the bound calcium so that it is once again available for absorption. Unfortunately, this release occurs in the lower third of the intestine and in the large intestine, areas which are less active in terms of calcium absorption. Phytate binds other divalent ions (Zn, Fe, and Mg) as well, and has a similar effect on their absorption. In addition, oxalate and some tannins can have this effect.

1. Food Mixtures

Milk and milk products are excellent sources of calcium, as mentioned earlier. The reason why milk calcium absorption is so good is because of the type of carbohydrate (lactose) and protein found in these foods. Lactose favors calcium absorption. Casein, the main protein in milk, is a relatively small protein (23,000 Da) having numerous phosphorylated serine residues. These residues are negatively charged, thus enabling the protein to bind the calcium ions. Lactalbumin, another milk protein, is also a calcium-binding protein. In addition to calcium, it will also bind zinc. This is true for a number of proteins; those that bind one divalent ion will also bind other divalent ions. Hence, there could be a competition for binding that would affect the availability of the minerals involved. As the proteins are degraded by the digestive enzymes, these protein-bound minerals become available for uptake by the enterocyte. In mixtures of foods this availability can be enhanced or compromised, depending on the food mixture. As mentioned, cereal foods and green leafy vegetables contain oxalates or phytate that bind calcium. When these foods are mixed with dairy foods, one could anticipate a reduction in the availability of the calcium in the dairy food. In contrast, foods that are rich in vitamin C enhance calcium availability, probably due to the redox nature of ascorbic acid. This vitamin readily changes from an oxidized to a reduced form and assists not only calcium absorption but also assists in the absorption of iron.

Table 6 Food Components That Affect Calcium Absorption

Component	Effect
Alcohol	↓
Ascorbic acid	↓↑
Cellulose	↓
Fat[a]	↑↓
Fiber	↓
Lactose	↑
Medium-chain triglycerides	↑
Oxalates	↓
Pectin	↑↓
Phytate	↓
Protein[b]	↑↓
Sodium alginate	↓
Uronic acid	↓

[a] In cases of steatorrhea, calcium absorption is reduced.
[b] Certain proteins, e.g., those in milk, enhance calcium availability while others, e.g., those in plants, reduce it.

Foods and food mixtures that provide calcium and phosphorus together in a ratio of 2:1 to 1:2 optimize calcium absorption. Both minerals are actively transported and yet do not share a single transport mechanism (see below). When the food mixtures are unbalanced with respect to this ratio then calcium uptake will be impaired. Table 6 summarizes the influence of food components on calcium availability.

C. Bioavailability, Absorption

In contrast to the macronutrients, the vitamins, and the electrolytes sodium, potassium, and chloride, not all of the calcium that is consumed is absorbed. The fraction which is absorbed can vary depending on the food source, the mixture of foods consumed, and the physiological status of the individual. That which is actually involved in biological processes is the bioavailable fraction. The determination of calcium bioavailability and absorption efficiency is a complicated process that involves careful food analysis to determine calcium content: careful measurements of the food consumed followed by feces and urine analysis of calcium excretion, corrected for calcium recycling. While the amount of calcium in the urine reflects the calcium that is absorbed, it does not reveal the amount of calcium that has been recycled. Likewise, the calcium in the feces reflects not only the calcium that was not absorbed from the food but also the calcium that was secreted into the intestine and not reabsorbed. The use of the calcium balance technique also does not measure the amount of calcium deposited in the bones and teeth, nor does it provide an estimate of the amount of calcium that is mobilized from these depots. However, the balance technique can be combined with tracer techniques which provide reasonably good estimates of absorption and bioavailability.

Bioavailability estimates can be obtained using isotopes of calcium as tracers. The heavy isotope Ca (^{46}Ca, ^{48}Ca) or the radioisotope calcium (^{45}Ca, ^{47}Ca) can be incorporated into a food and its presence in blood, urine, and feces monitored over time (flux). Flux is a term used to provide a time dimension to the movement of calcium. It can refer to a loss or a gain in concentration in a given volume or mass. If a plant food, the plant can be grown in a calcium isotope-enriched growth medium and the edible portions provided to the subject or animal. If an animal food, the animal either consumes an isotopically labeled feed or is infused with a solution of labeled $CaCl_2$. Knowing the total food calcium and the percent that was isotopically labeled will then allow for calculations

of intestinal uptake, recycling, excretion, storage, and use. The double isotope technique involves the consumption of a ^{45}Ca-enriched food followed by an infusion of $^{47}CaCl$ in the vein. ^{47}Ca has a short half-life (~4.7 days) while ^{45}Ca has a much longer one (~163.5 days). The stable isotopes (^{48}Ca, ^{49}Ca) can be substituted for the radioisotopes. The infused ^{47}Ca will be diluted in the blood by the calcium mobilized from the depots as well as by the absorbed calcium which also dilutes the food ^{45}Ca. The fraction that is absorbed is calculated as follows:

$$J_{ms} = \frac{J_{max}\left[Ca_L^{2+}\right] + A\left[Ca_L^{2+}\right]}{K_T + \left[Ca_L^{2+}\right]} \quad (1)$$

where: J_{ms} = the amount of calcium that has entered the body from the lumen; it is equal to the appearance of ^{45}Ca (corrected for nonlabeled calcium) in the blood
J_{max} = maximum saturable flux
Ca_L^{2+} = lumen, $J_{max}/2$ is observed
A = diffusion constant

In practice, absorption = the fraction of oral ^{45}Ca in urine/fraction of ^{47}Ca in urine. The use of ^{47}Ca assumes that the infused calcium will behave as though it had been absorbed. In each instance the measurement is a single time point. Urine is collected for 24 hr after isotope administration. To obtain an estimate of recycling the investigator will collect blood, urine, and feces at intervals over a 2- to 4-day period, monitor the dilution of the consumed and infused labels, and using this information calculate rate constants for each calcium pool. Absorption can also be calculated from the feces using the loss of the consumed label to estimate the absorption. However, since there is considerable individual variation in gut passage time which can influence calcium absorption, feces must be collected for periods of up to 12 days. Given the shorter half-life of ^{47}Ca that is infused to provide the estimate of recycling, this method for determining apparent absorption is not as popular.

Combining the use of both radioactive and stable isotopes also has some problems. The stable isotope ^{48}Ca degrades to ^{49}Ca with the emission of a γ-ray. ^{49}Ca has a half-life of 8.8 min. ^{46}Ca converts to ^{47}Ca which has radioactivity. The use of heavy isotopes require the use of mass spectrometry to detect their presence while liquid scintillation or gamma counters are needed for detecting the radioisotopes. Small animals such as the rat, mouse, and chicken with their smaller body sizes, faster rates of growth, and faster metabolic rates, reduce the expense and increase the number of measurements as well as the validity of these measurements due to their genetic homogeneity.

1. Apparent Absorption

Apparent absorption is the fraction of the consumed calcium that disappears from the gastrointestinal tract. Thus, it is the net movement of calcium from the lumen into the animal. This movement can be tracked *in vitro* using an intestinal segment and a calcium isotope. Phenol red is used as a marker of absorbing cells. The apparent absorption can be calculated using the following equation:

$$\text{Apparent Absorption} = \frac{V\left[{}^{40}Ca_i - \left({}^{40}Ca_f\right)(PRR)\right]}{L \text{ or } W} \quad (2)$$

The subscripts i and f refer to initial and final concentrations of calcium in micromoles per milliliter. PRR is the ratio of phenol red in the initial and final samples. L is the length and W is the weight

Table 7 Apparent Absorption of Calcium *In Vitro* by Duodenal Loops from Normal Rats

Volume perfused	240 ml
Duration of perfusion	2 hr
Concentration of calcium	0.8 m*M*
Concentration of ^{45}Ca (specific activity, 2000 μCi/mg)	12 μCi/l
Length of intestine, cm	87
Weight of intestine, g	5.4
Mucosal dry weight, mg/cm	7.2
Net absorption, μmol/hr	
Per cm segment length	0.140
Per g dry weight mucosa	20.1
Lumen to plasma flux	
Per cm segment length	0.196
Per g dry weight mucosa	27.6

of the intestinal segment, and V is the volume perfused in milliliters. The lumen to plasma flux is unidirectional and can be calculated as follows:

$$\text{Lumen to Plasma Flux} = \frac{V\left[{}^{45}Ca_i - \left({}^{45}Ca_f\right)(PRR)\right]}{\left[\left(SA_i - SA_f\right)/2\right]W} \tag{3}$$

In this calculation SA refers to the specific activity of the calcium isotope and W is the dry weight of the intestinal segment. All the other definitions are the same as in Equation 2. These equations have been successfully used to calculate calcium absorption in normal rats, as shown in Table 7.

2. Physiological Status

Absorption efficiency is greater in the young, growing individual than in the mature adult, which in turn is greater than in the aged individual. As well, there are gender differences due in part to hormonal status. Postmenopausal females have a less efficient calcium absorption than premenopausal females. Men, both young and mature, have a greater calcium absorption efficiency than females of the same age. Testosterone has been shown to enhance calcium absorption. Calcium absorption is impaired in vitamin D-deficient individuals as well as in persons with insulin-dependent diabetes mellitus. Clearly the endocrine status of the individual has effects on calcium uptake and this, in turn, can influence calcium status in terms of bone structure and calcium content.

3. Mechanisms of Absorption

Only 20 to 50% of ingested calcium is absorbed yet this mineral is so important to metabolism that two mechanisms exist for its absorption. One of these is vitamin D dependent while the other is not. The latter is one that uses passive diffusion as the means for calcium entry into the enterocyte. However, once in the enterocyte the mechanism for its disposal is vitamin D dependent. The former is an energy-dependent active transport system and is illustrated in Figure 5. This is a saturable system which operates actively when calcium is in short supply in the diet. Thus, when the diet is rich in calcium this system is far less active than when a calcium-poor diet is consumed. Because both systems are operative, a wide range of apparent calcium absorption exists. In the active system, calcium diffuses across the brush border down its thermodynamic gradient into the cell as free unbound Ca^{2+}. This calcium is then bound by an intracellular protein called calbindin D_{9k} which serves to maintain the level of calcium in the cell at a low, nontoxic level. Calcium released from

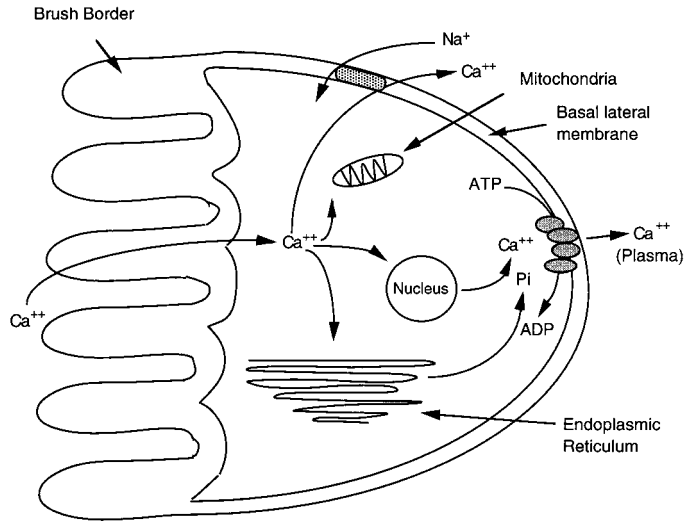

Figure 5 Calcium absorption by the enterocyte. Both the $Ca^{2+}Na^{+}$ exchange and the energy-dependent systems are shown.

calbindin enters and leaves the subcell compartments such as the mitochondria. This occurs in an orderly oscillatory manner. The calcium ion leaves the enterocyte either in exchange for sodium or is extruded from the cell by a calcium-activated ATPase. This ATPase has been found in both the brush border and at the basal-lateral membrane. As calcium is extruded, ATP is cleaved to ADP and Pi. Hence the energy dependence of this system. Because the sodium that is exchanged for calcium must be actively removed from the intracellular compartment by the $Na^{+}K^{+}$ ATPase, this process is also part of this energy-dependent system.

Absorption of calcium by the enterocyte is but one of the roles of vitamin D. Other roles include renal calcium conservation, intracellular calcium movement, bone calcium deposition, as well as bone calcium mobilization. Calcium homeostasis is thus one of the main functions of vitamin D. Vitamin D or 1,25-dihydroxycholecalciferol, has these effects on calcium homeostasis due to its effects on the synthesis of calcium-binding proteins (Table 8). In the intestine the protein of interest is calbindin D_{9k}. Vitamin D binds to its cognate receptor in the enterocyte and this steroid-receptor complex migrates to the nucleus where it binds to a specific DNA sequence which encodes the protein, calbindin D_{9k}. Studies of vitamin D-deficient animals have shown a rapid induction of calbindin mRNA transcription when the missing vitamin was provided. This was followed by a restoration of normal calcium absorption in these repleted animals. In addition to the rapid increase in transcription was an effect of the vitamin on calbindin mRNA half-life. There was an increase in this half-life which indicates a vitamin effect on mRNA stability. Thus, the vitamin both stimulates transcription and has a post-transcriptional effect on calbindin synthesis.

Vitamin D is not the only hormone involved in calbindin synthesis. Testosterone, growth hormone, progesterone, insulin-like growth factor 1, and estrogen augment, while glucocorticoids inhibit the synthesis of calbindin D_{9k}. Calbindin D_{28k}, another vitamin D-dependent calcium-binding protein in the enterocyte and renal cell, has its synthesis regulated by retinoic acid. In the brain this calcium-binding protein is not regulated by vitamin D. The multiplicity of hormonal controls of calbindin synthesis explains in part the gender and age differences in calcium absorption, and indeed help to elucidate the reasons why postmenopausal females are more at risk for calcium inadequacy than are young, growing males and females. Those hormones that have a positive effect on calbindin D synthesis are the same hormones that are missing (or in short supply) in the postmenopausal female. Should calbindin D_{9k} synthesis be inadequate, calcium absorption will decline. As well, calbindin D_{28k}, an important calcium-binding protein in the renal cell responsible

Table 8 Calcium Binding Proteins

Protein	Function
α-Lactalbumin	Carries calcium in milk
Casein	Carries calcium in milk
Calmodulin	Serves as major intracellular calcium receptor; activates cyclic nucleotidephosphodiesterase
Calbindin D_{9k} and D_{28k}	Facilitates intracellular Ca^{2+} translocation
Osteocalcin	Essential for calcium deposition in bone
$Ca^{2+}Mg^{2+}$ ATPase	Essential to movement of calcium across membranes
Prothrombin	Essential to blood clot formation
Calcitonin	Inhibits osteoclast-mediated bone resorption Regulates blood calcium levels by preventing hypercalcemia
Parathyroid hormone	Stimulates calcitonin synthesis, bone Ca resorption, renal Ca conservation
Albumin	Carries calcium in the blood
Globulin	Carries calcium in the blood
Osteopontin	Essential for calcium mobilization from bone
Troponin C	Muscle contraction
Alkaline phosphatase	Mineralization of bone
Sialoprotein	Embryonic bone growth
GLA-rich clotting proteins	Binds calcium in the coagulation cascade (see vitamin K)
Villin, gelsolin	Cytoskeleton stabilization

for renal calcium reabsorption, could also be synthesized at a reduced rate and thus calcium conservation is reduced. The two calbindins are subject to nearly the same hormonal influences. With decreased calcium absorption and conservation bone calcium mobilization increases, with the result of an age/gender-related loss in bone calcium, osteoporosis.

D. Calcium Transport, Blood Calcium Regulation

The concentration of calcium in the plasma is the result of three processes which are integrated so as to maintain a constancy of calcium in the circulation. Blood calcium is regulated at 100 mg/l (2.50 mmol/l) across the life span, although in late adulthood there may be a small (10%) decline due to an age related decline in the total calcium-binding capacity of the serum proteins. Most (80%) of the blood calcium bound to protein is carried by albumin; the remainder is bound to a variety of globulins. About 60% of blood calcium circulates as the free ion or as an ion complex. Calcium levels in the blood are regulated mainly by three hormones: active vitamin D (1,25-dihydroxycholecalciferol), calcitonin, and parathyroid hormone (PTH), a hormone released by the parathyroid glands. These glands are embedded in the thyroid gland and are stimulated by falling calcium levels to release PTH. PTH acts on the bone and the kidneys. In the kidneys PTH reduces calcium loss by stimulating its reabsorption. In the bone PTH stimulates calcium release. It also inhibits collagen synthesis by osteoblasts. Osteoblasts, through the synthesis of collagen, provide the organic matrix on which the minerals of the bone are deposited. Osteoclasts, in contrast, are those bone cells responsible for bone resorption. PTH stimulates osteoclast activity indirectly because PTH stimulates renal inorganic phosphate loss. Since phosphate is the counterion of Ca^{2+} in the bone, phosphate loss causes $Ca_5(PO_4)_3OH$ to leach out of the bone and thus raise serum calcium levels. Finally, PTH has one other effect. It stimulates the synthesis of 1,25-dihydroxy-cholecalciferol which in turn stimulates intestinal calcium uptake. This active form of vitamin D, in turn, enhances the PTH effect on bone calcium mobilization. Thus, PTH has a major role in blood calcium regulation and it has its effects at several different sites.

Counteracting PTH is calcitonin. Calcitonin, a single chain of 32 amino acids, is synthesized by C cells in the thyroid gland. The gene for its synthesis has been localized to chromosome 11 and the expression of this gene is tissue specific. Calcitonin inhibits osteoclast-mediated bone resorption. Calcitonin also inhibits the activation of vitamin D and renal calcium conservation.

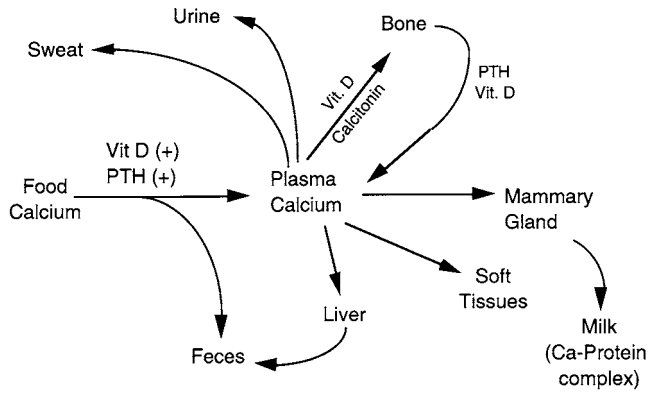

Figure 6 Pathways of calcium use.

Altogether, PTH, vitamin D, and calcitonin regulate blood calcium levels such that there is little variation in normal individuals. Should blood levels fall below the 2.2 to 2.5 mmol/l level, calcium tetany will occur. In newly lactating females this is called "milk fever." It can quickly be reversed with an infusion of a calcium lactate or gluconate. Milk fever develops because of a great demand for calcium by the mammary gland. When the demand exceeds the elasticity of the blood calcium homeostatic system, hypocalcemia results. As mentioned, this can be temporary and reversible with appropriate treatment. Failure to treat can be fatal.

Hypocalcemia due to underactive parathyroid glands or to chronic renal failure, vitamin D deficiency, or hypomagnesemia, is more difficult to manage because the underlying causes are more difficult. Hormone replacement, renal transplant, or correction of blood magnesium levels are the usual strategies followed. With respect to the low blood magnesium, this is one of the consequences of alcoholism. Excess ethanol intake can interfere with the intake and use of magnesium, which in turn results in a loss of responsiveness of osteoclasts to PTH. This interrupts or interferes with the homeostatic mechanisms needed to control blood calcium levels. The pathways of calcium use are illustrated in Figure 6.

E. Function

1. Bone Mineralization

About 99% of the total body calcium is found in the bones and teeth. This calcium is part of a mineral complex that is deposited on an organic matrix comprised primarily of type I collagen. This collagen has a unique amino acid composition consisting of large amounts of glycine, proline, and hydroxyproline. A single molecule of type I collagen has a molecular mass of ~285 kDa, a width of ~14 Å and a length of ~300 Å. There are at least 17 different polypeptides that comprise the collagen molecule. The polypeptides used vary throughout the body and each collagen uses at least three of them. Collagen is about 30 to 33% glycine with another 15 to 30% of the amino acid residues as proline and 3-, 4-, or 5-hydroxyproline. Collagen is a left-handed triple helix stabilized by hydrogen bonding. These bonds may involve bridging water molecules between the hydroxyprolines. The collagen fibrils are also held together by covalent cross linking. These cross links are between the side chains of lysine and histidine and the linkage is catalyzed by the copper-dependent enzyme, lysyl oxidase. Up to four side chains can be covalently bonded to each other. All in all, the collagen provides a network of fibers upon which the crystals of hydroxyapatite [$Ca_3(PO_4)_3OH$] are deposited. The hydroxyapatite is by no means pure calcium phosphate. Some ions (magnesium, iron, sodium, and chloride) are adsorbed onto the surface of the hydroxyapatite crystallites while other ions (strontium, fluoride, and carbonate) are incorporated into the mineral lattice. The presence

of these other ions affects the chemical and physical properties of the calcified tissue. Solubility, for example, is decreased when strontium and fluoride ions are present. Hardness is enhanced by the presence of fluoride.

Bone formation begins in the embryo and continues throughout life. The nature of the process and the cells involved change as the individual ages. Osteoprogenitor cells in early development synthesize the extracellular matrix described above and also regulate the flux of minerals into that matrix. As the calcified tissue begins to form, osteoclasts appear on the surface of this tissue, and osteoblasts connected to one another by long processes are totally surrounded by mineralizing matrix. Each type of mineralized tissue has some unique properties but all share several histologic features in the early mineralization process. Hunziker has described this process in the epiphysial growth plate during the ossification of the endochondral cartilage as it becomes bone.

Initial mineral deposition occurs at discrete sites on membrane-bound bodies (matrix vesicles) in the extracellular matrix. These initial deposits are diffuse and lack orientation. The mineral crystals proliferate and mineralization proceeds filling the longitudinal, but not the transverse, septa. Changes in the activities of enzymes which catalyze the hydrolysis of phosphate esters and those which catalyze certain proteolytic reactions follow or accompany this mineralization. These reactions are a prerequisite to vascular invasion. Following vascular invasion, lamellar bone is formed by osteoblasts directly on the surface of the preexisting mineralized cartilage. These osteoblasts secrete type I collagen with very little proteoglycan and few extracellular matrix vesicles. This process is repeated over and over until the bone has finished growing. At this point the growth plate closes and the mature length and shape of the bone is apparent. The bone, however, is not at all metabolically inert. It continues to lose and gain mineral matter; that is, it is continuously remodeled through the action of the osteoblasts which synthesize the collagen matrix, and the osteoclasts which are stimulated by PTH to reabsorb calcium (and other minerals) in times of need. While the number of osteoblasts declines with age, the number of osteoclasts increases, especially in postmenopausal females. This helps to explain some of the age-related loss in bone mineral that occurs in aging females. Osteoclasts act first during bone remodeling by producing cavities on either the cortical or cancellous (trabecullar) bone surfaces. When these cavities develop osteoblasts are recruited for bone remineralization, thereby filling (or refilling) the cavities. The bone matrix reforms and remineralizes as described above, resulting in new bone formation. The remodeling process is an ongoing one with rates of resorption equaling the rates of new bone formation as long as the hormones controlling each process are in balance and as long as the nutrients needed to support this ongoing process are provided. Figures 7 and 8 illustrate calcium turnover in bone and the influence of hormones on this process.

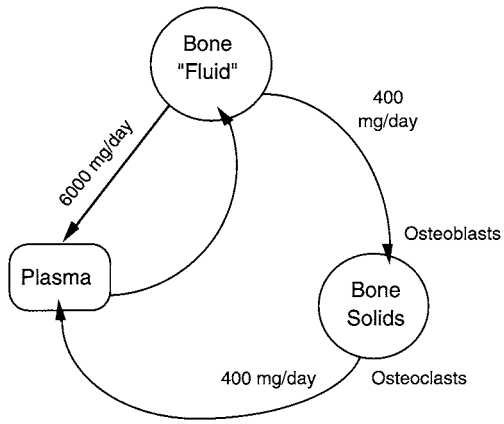

Figure 7 Calcium turnover in bone.

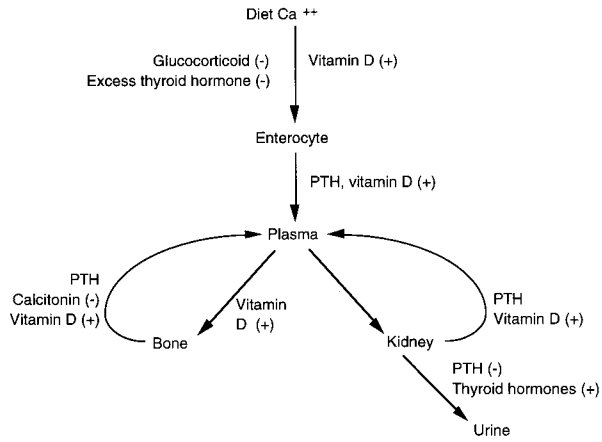

Figure 8 Hormones that influence bone remodeling.

Bone mass can remain constant for many decades. However, once the hormone balance changes this constancy changes. In females, bone mass declines by an estimated 1 to 2% per year after menopause. In senile men, bone mass loss also occurs. In fact, both senile males and females experience about a 1% loss per year. Not only is there a loss in bone mass but there is a loss in structural integrity. The bones lose their mineral apatite and become porous (osteoporosis) and as well lose the architecture upon which the mineral has rested. The very compact cortical portions of the bone disappear, leaving a fragile, largely trabecular bone. The result of these changes is a fragile skeletal system subject to nontrauma-related fracture.

While the importance of appropriate hormone balance (PTH, calcitonin, active vitamin D, and estrogen) can not be overemphasized, it should also be recognized that dietary calcium (as well as phosphorus and other nutrients) plays an important role in the maintenance of bone mass. Intakes at or exceeding 800 mg/day have been shown to counteract the age-related loss in bone mass.

2. Cell Signaling

Metabolic regulation and the integration of a variety of metabolic pathways and cell systems depend largely on the communications the cells, and the organelles within cells, have within and between each other. Signaling systems exist that orchestrate this communication. An integral part of these signaling systems is the calcium ion. Although less than 1% of the total body calcium store serves this function, its importance can not be overemphasized. The flux of calcium from one compartment to another plays a vital role in metabolic regulation. This flux is facilitated by an intracellular calcium-binding protein called calmodulin. Calmodulin contains four Ca^{2+}-binding sites with affinities in the micromolar range. It is ubiquitous in all eukaryotic cells and mediates many of calcium's effects. Among these are the activation of phosphodiesterase, a component of the cAMP second messenger system and the stimulation of renal Ca^{2+}, Mg^{2+} ATPase. Phosphodiesterase catalyzes the conversion of cAMP to 5′-AMP and requires calcium as a cofactor. Calcium is translocated from its storage site on the endoplasmic reticulum by calmodulin to the interior aspect of the plasma membrane, whereupon it is released to serve as a cofactor for phosphodiesterase. This is illustrated in Figure 9.

A similar mechanism exists for the action of calcium in another cell second messenger system, the phosphatidylinositol system (see Unit 5, Section III for a diagram of this system). In this instance, phospholipase C, a membrane-bound protein, is activated by the binding of an external compound, for example, a hormone, to its cognate receptor. Phospholipase C catalyzes the release of inositol-1,4,5-phosphate from phosphatidylinositol, one of the plasma membrane's phospholipids.

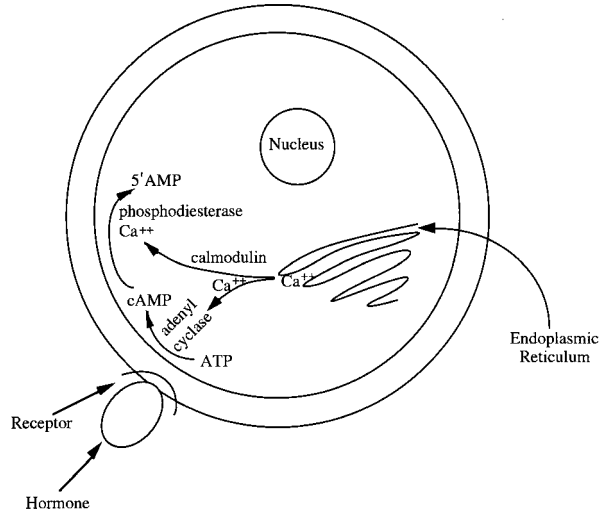

Figure 9 Calmodulin moves Ca^{2+} from the endoplasmic reticulum to serve as a cofactor in reactions catalyzed by phosphodiesterase and adenyl cyclase in the cyclic AMP second messenger system.

Diacylglycerol (DAG) and inositol-1,4,5-phosphate then migrate forward into the cytoplasm. DAG binds to protein kinase C and activates it with the help of the calcium ion. Inositol-1,4,5-phosphate, in the meantime, migrates to the endoplasmic reticulum, stimulating the release of more calcium which in turn further stimulates protein kinase C. Protein kinase C catalyzes the phosphorylation of a variety of proteins. Some of these are enzymes that must be phosphorylated to increase or decrease their metabolic activity whereas others are necessary for secretion processes, i.e., gastric acid release or hormone release, or for substrate uptake such as glucose transport, or for any of a number of energy-driven processes.

Calcium and the cAMP signaling system together with the phosphatidylinositol signaling system have been shown to explain the action of many hormones and cell regulators. Figure 10 illustrates these two systems. Angiotensin, cholecystokinin, acetylcholine, insulin-like growth factors, insulin, and glucagon are but a few hormones whose mode of action involve the calcium ion in one or the other of these signaling systems. There is considerable cross talk between these systems as a result of their mutual need for Ca^{2+}.

In addition to its role in these second messenger systems, Ca^{2+} flux between the cytoplasm and mitochondria regulates mitochondrial activity. Ca^{2+} uptake by mitochondria is energetically less demanding than its export. Uptake followed by active export is an oscillatory process. Ca^{2+} flows in until a 200 μM concentration is reached, whereupon it is actively pumped out using the energy of ATP. This active system costs about 63% of the ATP hydrolysis energy. There are three separate Ca^{2+} transport mechanisms at work (Figure 11). One is the calcium uniporter. The uniporter rapidly sequesters external Ca^{2+} into the mitochondrial matrix and in a similar fashion also sequesters Fe^{2+}, Sr^{2+}, Mn^{2+}, Ba^{2+}, and Pb^{2+}. (Mg^{2+} uptake is much slower than Ca^{2+} and probably is not mediated by this uniporter system.) The other two Ca^{2+} transport mechanisms facilitate Ca^{2+} efflux rather than influx. There is a sodium-independent and a sodium-dependent mechanism. The latter is the more powerful of the two for facilitating Ca^{2+} efflux. Its velocity is much higher than the sodium-independent mechanism. Altogether, these three mechanisms function to control Ca^{2+} flux and hence mitochondrial metabolism, particularly oxidative phosphorylation.

The mitochondrial Ca^{2+} cycle is designed to regulate intramitochondrial Ca^{2+} levels and to relay changes in cytosolic Ca^{2+} to the mitochondrial matrix. Surges in cytosolic Ca^{2+} via the cell signaling systems activate a variety of ATP-requiring reactions. In turn, as ATP is used, its metabolic end

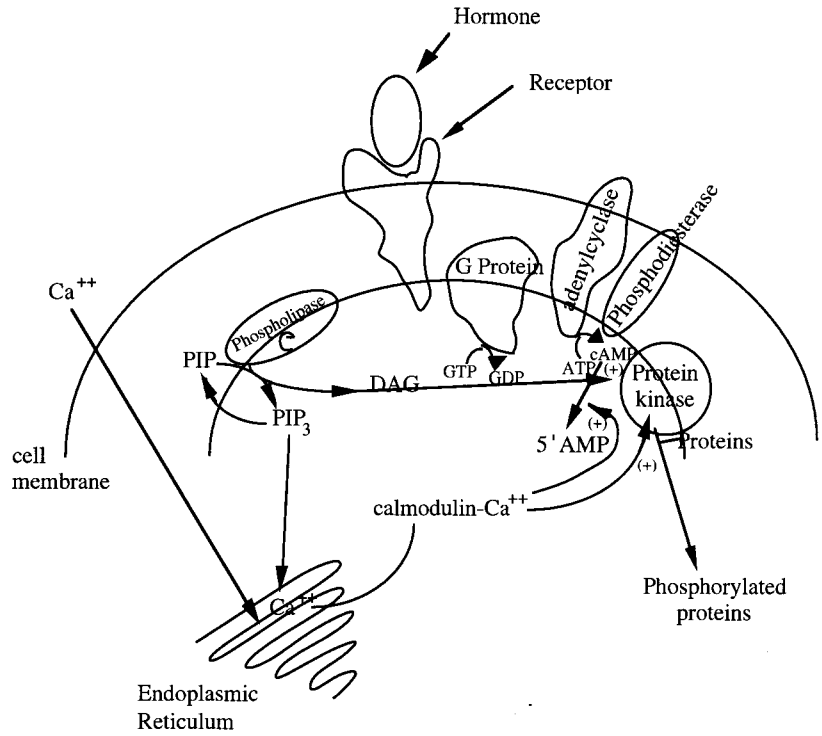

Figure 10 Integration of cAMP and PIP second messenger systems showing the role of Ca^{2+} in both.

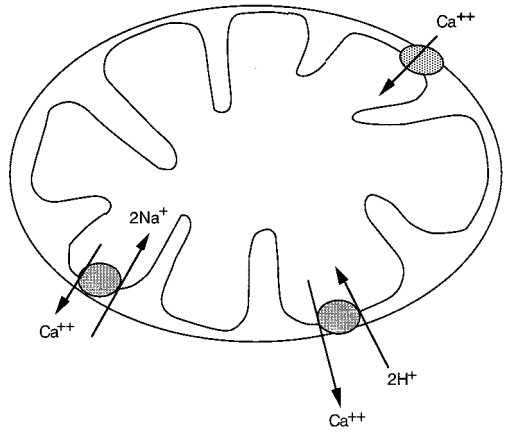

Figure 11 Ca^{2+} flux in the mitochondrial compartment.

product, ADP or AMP, is transported back into the mitochondria whereupon it is rephosphorylated to ATP and exported (along with the Ca^{2+}) to the cytosol. This multifaceted system provides flexibility and responsivity to changing cellular environments and has considerable control strength with respect to the balance of catabolic and anabolic metabolic pathways. In this instance, Ca^{2+} is more than a mere signal, it is a key element in metabolic control. Shown in Table 9 are some of the many reactions or reaction sequences activated by Ca^{2+}.

Table 9 Ca^{2+} as a Metabolic Regulator: Reactions or Reaction Sequences Stimulated by Ca^{2+}

Fatty acid oxidation
Amino acid transport into hepatocytes
Citric acid cycle (isocitrate dehydrogenase, α-ketoglutarate dehydrogenase)
Pyruvate dehydrogenase
ATP-Mg/Pi carrier (mitochondrial carrier)
Glucose-stimulated insulin release
Phosphodiesterase
Stimulation of olfactory neurons
Trypsinogen conversion to trypsin
Pancreatic α-amylase activation
Pancreatic phospholipase A_2
Hydrolysis of troponin in muscle to tropomyosin
Phospholipase C
Blood clotting (binding of calcium to GLA-rich proteins)

3. Calcium and Cell Death

Just as Ca^{2+} is important to the regulation of cell metabolism, it has another role: it can mediate the death of a cell or a group of cells. When the integrity of the cell's membrane is breached through injury, sepsis, chemical insults, or anoxia, the normal flux of Ca^{2+} from storage depots to sites of use and return is interrupted. Just as the extracellular Ca^{2+} is carefully regulated so as not to exceed that very narrow range of 2.2 to 2.5 mmol, so too is the intracellular Ca^{2+} concentration. As described above, Ca^{2+} flows into the mitochondria but is actively exported. If the injury to the cell interrupts this active, energy-driven export, the Ca^{2+} will continue to flow into the mitochondria and raise the ionic concentration of the mitochondrial matrix. This has negative effects on oxidative phosphorylation and the stage is set for a downward spiral towards mitochondrial dysfunction. Ca^{2+} influx into the cytosol as well as the nucleus also occurs because it is energetically downward under these conditions. When sufficient calcium accumulates, the cell dies. While the loss of one cell is not devastating, the loss of many cells can be and is. Of particular concern is the accumulation of Ca^{2+} by interconnected muscle such as the heart. Muscle contraction is a Ca^{2+}-mediated event. Ca^{2+} flows into the myocyte mitochondria and is pumped out using ATP. If Ca^{2+} accumulates in the myocyte and is not actively pumped out, the myocyte dies.

Counteracting this Ca^{2+} movement in cells under duress is a class of pharmaceutical agents called calcium blockers. These drugs postpone cell death by interfering with Ca^{2+} influx. Since their discovery, they have been very useful in the management of hypertension and heart disease, and have been found useful in several other settings. Calcium ionophores, agents that facilitate Ca^{2+} flux, have also been discovered. The one most widely used is A23187. Two molecules of this compound surround Ca^{2+} and because the agent is lipophilic it is able to passively cross the plasma membrane and deliver the Ca^{2+} to the cytosol.

Other functions of calcium, particularly those relating to clot formation and embryonic development, have been discussed in Unit 3, Sections I and IV. In these sections, the synthesis of specific proteins that bind calcium and that perform specific roles in the processes of interest are described.

4. Muscle Contraction

The muscle cell has a unique calcium storage site called the sarcoplasmic reticulum. This reticulum is similar to that found in other cell types but is highly specialized. It contains ribosomes and many of the same enzymes found in the endoplasmic reticulum of other cell types but, in addition, also contains large quantities of calcium-activated ATPase. Approximately 75% of the sarcoplasmic reticulum is ATPase. The ATPase also requires magnesium as its name indicates:

$Ca^{2+}Mg^{2+}$ATPase. It serves as a calcium pump using the energy released by ATP to drive the calcium ion from the cytosol to the sarcoplasmic reticulum. There the calcium resides until needed for muscle contraction. Upon receipt of a signal to contract, a Ca^{2+} channel in the sarcoplasmic reticulum is opened and Ca^{2+} flows into the cytoplasm of the muscle cell, whereupon it binds to troponin, a contractile protein of skeletal and cardiac muscle. This protein is a long, rod-like structure that extends over the length of the muscle fiber. When Ca^{2+} binds to it, it changes its shape, becoming shorter. The muscle cell contains two other filaments, actin and myosin, which interact when troponin shortens due to Ca^{2+} binding. When troponin is in the relaxed state these two filaments are too far apart to interact.

Muscles are signaled to contract by a wave of depolarization-repolarization flowing down the muscle fiber from its point of contact at the neuromuscular junction. During depolarization of skeletal muscle, extracellular Na^{+} flows into the cell and potentiates the Ca^{2+} release from the sarcoplasmic reticulum. In the heart muscle, with its slightly different muscle fiber organization, the signal for contraction is generated by the AV sinus node on the right side of the heart. This signal is regularly spaced and results in depolarization-repolarization just as happens in skeletal muscle. However, with depolarization Ca^{2+} flows into the cytosol from the extracellular fluid as well as from the sarcoplasmic reticulum. This has the result of increasing the strength of the contraction because more calcium is present. During repolarization Ca^{2+} is then pumped via the Ca^{2+} Mg^{2+} pump back into the sarcoplasmic reticulum. In heart muscle there are many more mitochondria providing ATP than are found in skeletal muscle, hence the need for Ca^{2+} by the heart muscle is greater than that of skeletal muscle.

In contrast to the heart and skeletal muscle, smooth muscle does not contract and relax strongly. These muscles can sustain contraction for a longer period of time and are far less dependent on calcium for contractile strength. Rather, the chief role of calcium in this muscle type is that of serving in the various hormone-mediated cell signaling systems.

F. Deficiency

Considering the vital role of vitamin D and other micronutrients in determining calcium status, it is truly difficult to produce a "pure" calcium-deficient state. Calcium deficiency does occur but it is usually due to other factors: lack of vitamin D activation, loss of estrogen production, adrenal dysfunction, parathyroid gland dysfunction, and so forth. If any of these conditions develop then signs of calcium deficiency do indeed occur. These signs include inadequate bone calcification and growth in children and weak porous bones in adults (osteoporosis). Rickets in children and osteomalacia in adults, characterized by malformed, poorly calcified bones, is more of a disease of inadequate vitamin intake than one of inadequate calcium intake. Should blood calcium levels fall acutely, calcium tetany will result, and unless calcium is provided quickly by the intravenous route, death will ensue.

G. Recommended Dietary Allowance

Because calcium absorption is dependent on so many different factors there has been vigorous discussion among experts as to what the recommended intake of calcium should be. In addition, there are a number of dietary factors (excess protein, calcium:phosphorus ratio, calcium:magnesium ratio, oxalates, and phytates) that increase calcium loss from the system and these are hard to quantitate such that an age-appropriate recommendation can be made. Several diseases stem from or affect calcium use and these must be considered. For example, hypertension has been linked to inadequate dairy calcium intake. Critical experiments that would irrefutably support such a linkage have yet to be conducted, nonetheless there are suggestions arising from population studies that those who consume calcium by way of dairy foods have less hypertension than those who avoid dairy foods. Follow-up studies in hypertensive rats have shown that increasing the dietary calcium reduces the hypertensive state. Calcium supplementation studies of pregnant women have shown

Table 10 Recommended Dietary Allowances for Calcium

Group		Intake (mg/day)
	Birth–6 months	400
	6–12 months	600
	1–3 years	800
	4–6 years	800
	7–10 years	800
Males	11–14	1200
	15–18	1200
	19–24	1200
	25–50	800
	51+	800
Females	11–14	1200
	15–18	1200
	19–24	1200
	25–50	800
	51+	800
Pregnancy	1200	
Lactation	1200	

a significant benefit with respect to a reduction in systolic and diastolic blood pressure and pre-eclampsia. Pregnancy-associated hypertension appears to be ameliorated by such supplementation.

As mentioned above, vitamin D and the estrogen status affect calcium use. Increasing the calcium intake can compensate for a reduction in absorption efficiency and thus the postmenopausal female could benefit. Note that older people have a larger RDA for calcium than young adults. Physical activity is another factor influencing calcium retention. Bedridden individuals tend to lose calcium, whereas the person who maintains a moderately active life style optimizes his/her calcium retention.

On the basis of the information currently available, the NIH Consensus Conference estimated the following optimal intakes for humans:

Birth–6 months, 400 mg/day
6–12 months, 600 mg/day
1–5 years, 800 mg/day
6–10 years, 800 to 1200 mg/day
Adolescents and young adults, 1200 to 1500 mg/day
Females age 25–50, 1000 mg/day
Postmenopausal females on hormone replacement therapy, 1000 mg/day
Postmenopausal females without hormone replacement therapy, 1500 mg/day
Males age 25–65, 1000 mg/day
After age 65, both males and females, 1500 mg/day.

These guidelines were based on calcium in the diet plus that provided by supplements. These recommendations differ from the 1989 RDAs in that for older people the intake recommendation is higher and postmenopausal women are divided into two groups: those having estrogen replacement and those without. Table 10 provides the RDAs of the Food and Nutrition Board, National Academy of Sciences.

VI. PHOSPHORUS

A. Overview, Recommended Dietary Allowance

Calcium and phosphorus are essential minerals that are usually considered together because the formation of bone and the uptake of calcium for this purpose is closely tied to an optimal

Table 11 Recommended Dietary Allowances for Phosphorus

Group		Intake (mg/day)
	Birth–6 months	300
	6–12 months	500
	1–3 years	800
	4–6 years	800
	7–10 years	800
Males	11–14	1200
	15–18	1200
	19–24	1200
	25–50	800
	51+	800
Females	11–14	1200
	15–18	1200
	19–24	1200
	25–50	800
	51+	800
	Pregnancy	1200
	Lactation	1200

calcium:phosphorus ratio of 1:2 to 2:1. However, phosphorus, like calcium, has other functions in addition to bone formation. Since bone formation was discussed in the preceding section concerning calcium, this section will present information about other aspects of the need for phosphorus.

Phosphorus is a member of Group V in the 4th period of the periodic table. It has an atomic number of 15 and an atomic weight of 31. There are no heavy isotopes but there are some useful radioisotopes. ^{32}P is the radioisotope most frequently used in biological systems. It has a short half-life (14.3 days).

Little free phosphorus is found in the living body. Most phosphorus is in the form of phosphate PO_4^{3-}. Phosphate is comprised of a central atom of phosphorus surrounded by four atoms of oxygen. At pH 7, hydrogen is joined to the phosphorus and oxygen to form HPO_4^{2-}. At low pH it is phosphoric acid, H_3PO_4. Other phosphates include H_3PO_4, $H_2PO_4^-$, and PO_4^{3-}. Phosphate in the free form is called inorganic phosphate with the abbreviation Pi. Phosphates are widely distributed in nature and, thus, a deficiency due to inadequate intake is highly unlikely. Soft drinks, processed foods, and foods of animal origin are excellent sources of phosphate. The usual intake of humans consuming a mixed diet is about 1 g/day for females and 1.5 g/day for males. Even though a deficiency is unlikely, a recommended dietary allowance very close to that of calcium is available, as shown in Table 11.

Symptoms of deficiency have been observed in premature infants fed a low-phosphorus milk. These symptoms include anorexia, muscle weakness, rickets, impaired growth, and bone pain. Adults consuming large amounts of aluminum oxide antacids and a low-phosphate diet can also develop some of these same symptoms. Rickets, of course, is only seen in the young. There is a genetic disease related to phosphate deficiency carried on the X chromosome that phenotypes as phosphate deficiency (X-linked hypophosphatemia). It is a dominant trait that is more severe in males than in females since males have only one X chromosome while females have two. The above symptoms can be ameliorated by phosphate supplements, and it is clear from biochemical studies using tissues from these patients that many of their symptoms are due to phosphate-related deficits in intermediary metabolism. The bone pain, poor skeletal growth, and mineralization are due to a lack of phosphate and hence hydroxyapatite for deposition in the bone matrix. Phosphates are readily absorbed with little loss except that which is tightly bound in indigestible portions of food. Phytic acid, a hexose containing six phosphate groups that is a component of some plant foods, can form insoluble salts with calcium, magnesium, and iron, rendering these minerals unavailable for absorption. However, there is a phytase lower in the intestinal tract that will

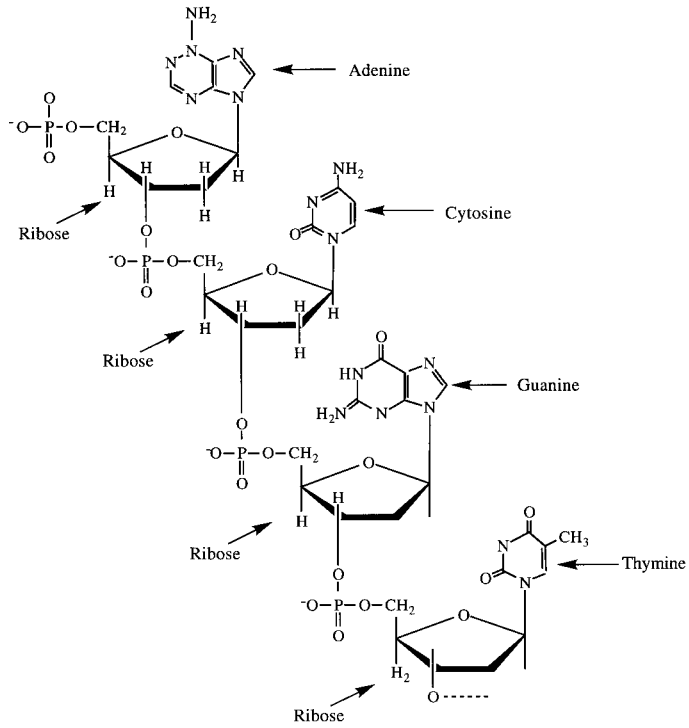

Figure 12 The bases which comprise the DNA polynucleotide chain are joined together by phosphodiester bonds using ribose as the common link between the bases.

dephosphorylate phytic acid, and when this happens these minerals are released. Usually, this happens too far away from their absorption sites to be of benefit.

Phosphorus as phosphate is readily absorbed via an active, saturable, sodium-dependent mechanism. Calcitriol (active vitamin D) facilitates the absorption as it facilitates calcium uptake. Of the total body pool of phosphorus (~750 g), about 600 g is found in the bones and teeth; 1 g is found in the extracellular compartment while the remainder (~150 g) is found within the cells. Excretion is via the urine although up to 25% of the food phosphorus can appear in the feces. Renal conservation of phosphorus is the main mechanism for phosphorus homeostasis. Vitamin D, glucocorticoids, and growth hormone enhance, while estrogen, thyroid hormones, parathyroid hormones, and elevated plasma Ca^{2+} levels inhibit renal phosphate conservation.

B. Function

A principal use for phosphorus is as the anion in hydroxyapatite used in bone mineralization. However, more important is the role of phosphate in intermediary metabolism. It is a crucial part of the genetic material DNA and RNA, of phospholipids, phosphoproteins, the adenine nucleotides (ATP, ADP, AMP), guanine nucleotides (GTP, GDP, GMP), and of the second messenger systems, and it plays a critical role in all anabolic and catabolic pathways. In the genetic material the purine and pyrimidine bases are linked together by deoxyribose or ribose and phosphate groups as shown in Figure 12.

In this structure the phosphate group serves as the links between the bases and because of the polarity of the phosphate group it serves to stabilize the structure. The DNA polymer is a very stable structure under nonenzymatic conditions. It owes its stability to the fact that the phosphate group can bind the bases yet still have sufficient polarity to retain a negative charge, thus repelling other negatively charged molecules such as peroxides. Of course, the phosphate group is not the

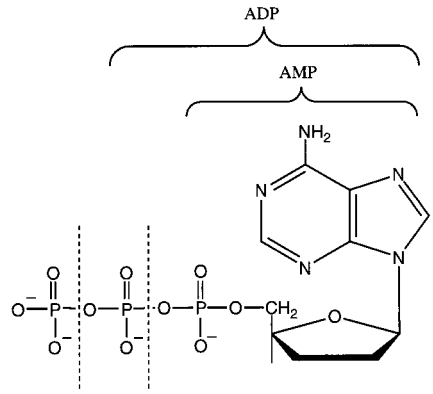

Figure 13 Structure of ATP. AMP has only one phosphate group; ADP has two.

only negatively charged group in the DNA molecule. Hydrogen bonding leaves some atoms vulnerable to attack but, overall, the phosphate group is the important group when nonenzymatic degradation is considered.

The ATP molecule (Figure 13) is another important use of phosphate. The bonds that hold the phosphate groups to the ribose and thence to the adenine are called high-energy bonds represented by the symbol ~. When broken, these bonds release about twice the energy of a normal bond. As such, ATP and its related high-energy compounds GTP and CTP are the keystones of energy transfer from one metabolite to another. A full discussion of ATP synthesis and degradation can be found in Unit 3 in the first volume of this text, *Advanced Nutrition: Macronutrients*.

The phosphorylates of intermediates in both catabolic and anabolic pathways are important uses of phosphate. Again, because of its unique structure it provides a necessary electronegative charge that creates a vulnerable position in the molecule to which it is attached. For example, glucose could not be metabolized to pyruvate unless it was phosphorylated. The glucokinase (or hexokinase) using the high-energy phosphate group from ATP phosphorylates glucose at carbon 6. In so doing the molecule becomes somewhat unstable such that in the next few steps it can be rearranged to form fructose-6-phosphate (a five-member ring).

This structure now has a vulnerable carbon at position 1 and the next step is to attach another phosphate group at this position. With two strong electronegative centers, one at each end of the molecule, this six-carbon structure can be split in half to form two phosphorylated three-carbon structures, and on it goes. The phosphate group is reused over and over and in each use it provides a means for subsequent reactions in metabolism, only to be discarded by the resultant metabolic product. Its highly charged structure thus is its reason for use and reuse.

Use and reuse also applies to the role of phosphate in the structure and function of the membrane phospholipids. Phospholipids have both a lipophilic and a lipophobic portion in their structure. The lipophilic portion (see Figure 14) is the fatty acid portion while the phosphatidylated compounds provide the lipophobic (or hydrophilic) portion. This structure is absolutely required for the functional attributes of the phospholipids that are part of the lipid-carrying blood proteins and that are

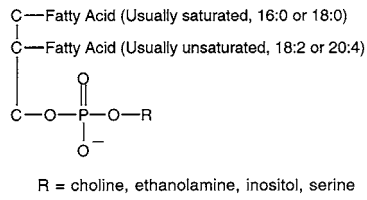

Figure 14 Structures of phospholipids.

important components of the membranes. The membranes (see *Advanced Nutrition: Macronutrients,* Unit 6) serve as geographical barriers around the cells and organelles. As such, some materials are refused entry into the cell or organelle while other materials are either embedded in these phospholipids and held there or are allowed to pass into or out of the cell or organelle. Lipid-soluble materials can diffuse through the lipophilic portion of the phospholipid while lipophobic materials are excluded unless carried through by one of the embedded proteins. The charge contributed by the phosphate group in the phospholipid is what holds the proteins in place and allows it to permit entry or exit of lipophobic metabolites and substrates. It should be noted, however, that highly charged metabolites such as the phosphorylated intermediates do not cross membranes. They are repelled by the phosphate group of the phospholipids.

From the above discussion of the function of phosphorus in living systems it is easy to understand why phosphorus is so widely distributed in the food supply. Every living thing must have phosphorus in its cells or it would not survive. It is as essential to life as oxygen, carbon, and nitrogen.

VII. MAGNESIUM

A. Overview

Magnesium is an alkaline earth metal with an atomic number of 12 and an atomic weight of 24. It is in Group II of the 3rd period of the periodic table. Magnesium has two naturally occurring isotopes, Mg^{25} and Mg^{26}, and seven radioisotopes. Mg^{28} is the most commonly used radioisotope with a half-life of 21 hr.

Magnesium is a very abundant divalent cation in living systems. As such its distribution in the food supply is broad. Both vegetables and meats are good sources of magnesium while milk and milk products are relatively poor sources of this mineral. Magnesium stabilizes mammalian membranes and in plants this mineral is ionically bound in the center of the chlorophyll molecule. In addition, magnesium is a cofactor in almost all phosphorylation reactions involving ATP. Because of the universality of its presence in the food supply, deficiency states are unlikely to develop in persons consuming a variety of foods.

B. Absorption, Metabolism, Excretion

Magnesium is absorbed by both passive diffusion and active transport. While there are two systems for Mg^{2+} uptake, neither is particularly efficient. Between 30 and 70% of that consumed in food is absorbed. When food is supplemented with Mg^{2+} the percent absorbed falls. Thus, a meal containing 40 mg in food will result in 28 mg actually entering the enterocyte and appearing in the blood, whereas in an enriched Mg^{2+}-containing meal (40 mg + 920 mg magnesium salt) only 11 to 14% will be absorbed, or 105 to 134 mg. The usual 300 mg Mg^{2+} intake has an apparent absorption of about 100 mg, a 33% efficiency.

Magnesium is recirculated via biliary secretion into the intestinal contents. The recirculated mineral can be reabsorbed and in times of need this reabsorption can be very efficient. If not reabsorbed this magnesium will be excreted via the feces. The usual excretory route for absorbed magnesium is via urine. In fact, the renal absorption mechanism is the main means for regulating magnesium status. About 100 mg of magnesium is lost via the urine per day by normal adults consuming about 300 mg/day in the food. In times of need, magnesium reabsorption by the renal tubules will occur and urinary magnesium levels will fall. In the deficient state the level will fall to zero as the deficiency proceeds.

After magnesium is absorbed, it circulates throughout the body; about 30 to 35% of the circulating magnesium is protein bound while the remaining circulates as magnesium salts (13% as citrate or

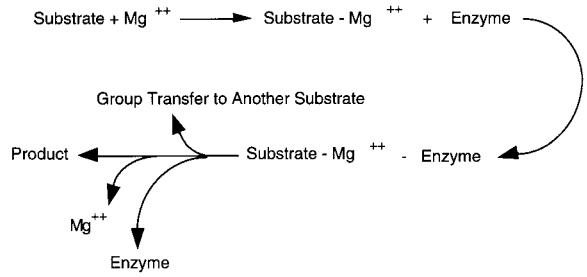

Figure 15 Use of Mg^{2+} as a cofactor in metabolic reactions.

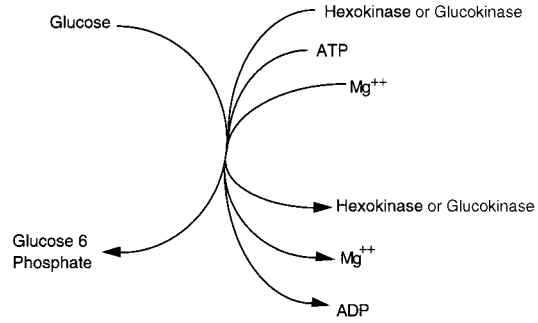

Figure 16 Role of Mg^{2+} as a cofactor in the phosphorylation of glucose.

phosphate complexes) or as free magnesium (~55%). Magnesium is principally an intracellular ion occupying a central role in intermediary metabolism, as described in the next section.

C. Function

More than 300 metabolic reactions require magnesium as a cofactor. The role of magnesium is one of forming a labile association with a substrate, allowing the enzyme to complex with it, and then once a product is formed, the product, the magnesium, and the enzyme separate for further use. This is illustrated in Figure 15.

Mg^{2+} reduces the high negative charge of the substrate (usually ATP) by chelate formation with two phosphate groups (the β and γ P). The adenine ring is not involved. Actually, the above reaction sequence occurs concurrent with the transfer of the liberated phosphate group to another substrate such as glucose. This coupled reaction sequence is facilitated by the enzymes hexokinase or glucokinase. Any kinase, however, will use this same reaction sequence, as will a number of other reactions involving cleavage of a high-energy bond and the use of that energy to activate via attachment of a reactive electronegative group to a formerly inactive metabolic substrate or intermediate. An example of this is shown in Figure 16 which illustrates the role of Mg^{2+} in the phosphorylation of glucose. Some coupled reactions use more than one Mg^{2+}. Enolase, for example, uses four Mg^{2+}. The Mg^{2+} ion binds to enolase and activates it by keeping it in its active conformation. Magnesium is probably binding to the sulfur group of sulfur-containing amino acids, thus favoring the reaction by polarizing the P-O bonds. Conformational change likely explains the Mg^{2+} in many of the enzymes in which it is a required cofactor for activation. In contrast, Mg^{2+} can be an inhibitor; it can bind with tyrosyl residues. If these residues are part of the active site of an enzyme then the enzyme will be less active. Actually, the binding of Mg^{2+} to the tyrosyl residue of albumin accounts for its transport in the blood. Much (32%) of the magnesium in the blood is carried by albumin.

Phospholipids form complexes with both Mg^{2+} and Ca^{2+}. These phospholipid complexes are integral parts of the various membranes in the cell (plasma membrane, endoplasmic reticulum, mitochondrial membrane, and nuclear membrane). The degree to which these minerals are held by the membrane depends on the type of phospholipid in that membrane. The phospholipids are not uniformly distributed. The plasma membrane, for example, has very little cardiolipin, while the mitochondrial membrane has very little phosphatidylserine (see Unit 6 in *Advanced Nutrition: Macronutrients*). Highly negative phospholipids will attract and bind more Ca^{2+} and Mg^{2+} than phospholipids having a lesser charge. In addition, Ca^{2+}, due to its stronger charge, will be more attracted than will Mg^{2+}. Nonetheless, the attraction of these minerals lends a stabilizing effect to an otherwise labile and mobile membrane constituent.

Bone accounts for 60 to 65% of the total magnesium in the body. It is deposited in the bone matrix along with calcium phosphate as part of the mineral apatite. Hormones which affect calcium deposition and mobilization also affect magnesium deposition and mobilization.

D. Deficiency

Deficiency states have been produced in animals under stringent magnesium exclusion conditions. As early as 1926 mice were shown to develop magnesium deficiency by use of a magnesium-deficient diet. Cattle, and later, rats, were also made deficient. The first clinical evidence in humans appeared in 1934 and subsequent studies have shown that persons experiencing prolonged malabsorption, renal dysfunction or failure, alcoholism, and a number of endocrine disorders, can become magnesium deficient. This deficiency is characterized by very low blood levels of magnesium and neuromuscular symptoms such as muscle spasms, twitching, muscle fasciculations, tremor, personality changes, anorexia, nausea, and vomiting. Acutely deficient individuals may have convulsions and lapse into coma. In almost every instance of clinically evident magnesium deficiency there has been a clinically important predisposing condition as cited above. Marginally deficient states have also been suggested in patients with normal serum or blood levels but depleted tissue levels of magnesium. In these patients the symptoms are varied and it may be difficult to assign magnesium deficiency as their cause unless tissue samples (muscle) are obtained and assayed. A positive magnesium balance of 1 meq/kg can be demonstrated in such individuals. Persons at risk are those with renal disease or malabsorption syndrome which, in turn, reduces magnesium conservation in the former and decreases magnesium absorption in the latter. Chronic ulcerative colitis and chronic granulomatous enteritis are the most common causes of the diarrhea of the malabsorption syndrome. Other causes are gluten-induced enteropathy, celiac disease, and unrecognized lactose intolerance. Frequently, magnesium deficiency is accompanied by hypocalcemia. This appears to be due to a failure of bone to exchange calcium for magnesium. The osteoclast receptor for PTH loses its responsiveness to this hormone with the resultant loss in active bone resorption. This means that there is a failure in the system used to maintain blood calcium levels and also that magnesium is a key feature in the PTH-calcium regulatory pathway. Once magnesium is restored, the sensitivity of the osteoclast receptor to PTH is reinstated and the serum calcium returns to normal levels.

Magnesium deficiency also has effects on the vitamin D-calcium relationship. Active 1,25-dihydroxycholecalciferol is not as active in promoting intestinal calcium uptake in the absence of magnesium. Even though neither ion is absorbed very efficiently, this efficiency is further reduced when one or the other is absent or in short supply. Both are poorly absorbed in vitamin D-deficient states because vitamin D mediates the uptake of both.

The use of mercurial or thiazide diuretics in the management of hypertension can result in excess magnesium loss. These diuretics interfere with renal magnesium conservation. They also enhance potassium loss as do some other drugs, e.g., furosemide, ethacrynic acid, goutamicin, cabenicillin, cisplatin, and amphotericin B. Many of these are cytotoxic drugs used in cancer

Table 12 Recommended Dietary Allowances for Magnesium

Age Group		Magnesium (mg/day)
	Birth–6 months	40
	7–12 months	60
	1–3 years	80
	4–6 years	120
	7–10 years	170
Males	11–14 years	270
	15–18 years	400
	19–24 years	350
	25–50 years	350
	51+ years	350
Females	11–14 years	280
	15–18 years	300
	19–24 years	280
	25–50 years	280
	51+ years	280
Pregnancy		320
Lactation	1st 6 months	355
	7–12 months	340

chemotherapy and, as such, are used over a short time span. While minerals are lost through the use of these drugs, they can be replaced from the body store or through supplementation. Many physicians using these therapies may elect to supplement the patient's diet after the chemotherapeutic regimen is complete, anticipating that the cancer cell also needs magnesium. Chemotherapy consists of using highly toxic materials, anticipating that the fast-growing cancer cell will be more likely to take up the drug and die than will the normal cell. Part of the drug action is to interfere with cell replication which, in turn, requires magnesium. If the cancer cell becomes magnesium deficient then the drug will be a more effective chemotoxic agent.

Magnesium deficiency in rats has been shown to result in elevated blood lipids as well as in proliferation of smooth muscle cells. These responses are key elements in the atherogenic process and it has been proposed that a relative magnesium deficiency in humans could pave the way for atherosclerosis or other degenerative diseases. However, other studies of alcoholism, renal disease, and various endocrinopathies have shown that these conditions must be in place prior to the development of magnesium deficiency. Thus, atherosclerosis could occur as a secondary complication of alcoholism or renal disease or diabetes, etc. and this secondary complication might develop as a result of an induced magnesium deficiency.

E. Recommended Dietary Allowance

As mentioned, magnesium is widely distributed in the food supply and thus a deficiency in an individual having access to a variety of foods is unlikely. Nonetheless, the Food and Nutrition Board of the National Academy of Sciences has recommended intakes based on age and gender of 40 to 400 mg/day, as detailed in Table 12. As mentioned in Section D, above, certain predisposing diseases may affect either magnesium absorption or reabsorption by the kidney. In these circumstances magnesium status may be negatively affected and an intake above that recommended as desirable may be needed. Assessment of that need, however, is imperative before an increased intake is undertaken. Excess intake can be toxic, with symptoms similar to those of uremia (nausea, vomiting, hypotension). Other responses to excess intake include changes in heart action (bradycardia, vasodilation) and CNS function.

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