Introduction to Mineral Biochemistry

Minerals constitute one of two major classes of biologically critical micronutrients required for normal health and development of humans. The other class are the vitamins. Humans must consume both macronutrients (the major sources of calories: fats, carbohydrates, proteins) and micronutrients in order to maintain virtually all metabolic and developmental processes. There is a clear correlation between micronutrient deficit and the development of chronic metabolic disruption. This is quite clear in the Vitamins page which discusses numerous, potentially lethal, consequences of vitamin deficiency. Given the fact that many manufactured foods, consumed by most individuals in the developed world, are now supplemented with vitamins, deficiencies are less and less common. This is somewhat true for minerals these are not as rigorously supplemented in prepared foods to the extent of the vitamins. The functions of the minerals are numerous and either quite broad or highly specific. Minerals serve as ions required for nerve impulse transmission in the central and peripheral nervous systems. Minerals, as ions, serve as activators of complex biochemical reactions in most tissues with the role of calcium ions in the activation of cardiac and skeletal muscle activity being a prime example. Minerals also serve as required cofactors for many different types of enzymes involved in a vast array of critical biochemical reactions. The minerals considered as trace minerals function primarily as cofactors or regulators of enzyme function. The terminology of "trace" relates to the fact that these minerals are effective and necessary in only minute concentration.

Macro Minerals

Calcium: Ca^{2+}

Calcium ion (Ca^{2+}) is an extremely critical mineral required for a vast array of biochemical processes. Some of the most wide-spread functions for this ion are its requirements for neural signaling, cell proliferation, bone mineralization, cardiac function, muscle contraction, digestive system function, and secretory processes. In the context of Ca^{2+} in secretion, the ion is required for neurotransmitter release and hormone release from a number of different tissues. In addition, calcium is necessary for proper activity of a number of proteins involved in blood coagulation. Calcium concentrations in the blood are very tightly regulated within a narrow range. Within the blood over half of the Ca^{2+} is free while the rest is bound to albumin or complexed with other ions such as bicarbonate and phosphate. Calcium functions both intracellularly and extracellularly. As an intracellular ion, Ca^{2+} serves the role of a second messenger. The difference between the Ca^{2+} concentration outside the cell, within the interstitial fluids, is on the order of 12,000 times that of the free intracellular concentration. This difference creates an inwardly directed electrical gradient as well as allowing for dramatic influxes of the ion in response to a variety of cellular stimuli. Within the cell, most calcium is not free in the cytosol but is stored within the endoplasmic reticulum (ER) and other microsomal (membrane) compartments. This calcium is able to be rapidly

mobilized to the cytosol via the activation of ligand-gated ion channels. One of the most significant events resulting in intracellular calcium release is the plasma membrane receptor-mediated activation of phospholipase Cβ (PLCβ) in response to ligand (e.g. hormone) binding. Active PLCβ, in turn, hydrolyzes membrane phosphatidylinositol-4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). IP_3 binds to receptors in the ER, activating the inherent calcium channel of the receptor leading to the flooding of the cytosol with free calcium.

Humans express three distinct IP_3 receptors encoded by the ITPR1, ITPR2, and ITPR3 genes. The ITPR1 gene is located on chromosome 3p26.1 and is composed of 63 exons that generate three alternatively spliced mRNAs encoding three distinct isoforms of the receptor. ITPR1 isoform 1 is a 2710 amino acid protein, isoform 2 is a 2695 amino acid protein, and isoform 3 is a 2743 amino acid protein. The ITPR2 gene is located on chromosome 12p11.23 and is composed of 62 exons that encode a 2701 amino acid protein. The ITPR3 gene is located on chromosome 6p21.31 and is composed of 60 exons that encode a 2671 amino acid protein. Each of the IP_3 receptors possess a cytoplasmic N-terminal ligand-binding domain and is comprised of six membrane-spanning helices that forms the core of the ion pore. Once released, the free calcium interacts with a variety of proteins activating a series of biochemical reactions specific to the particular cell type and the signal initiating the calcium release.

Calcium exerts many of its biochemical effects by binding to Ca^{2+}-binding proteins, several of the most significant are outlined in the following Table. The vast majority of proteins, whose activities are controlled by Ca^{2+} binding, contain a structural motif referred to as the EF-hand. The EF-hand domain consists of two regions of α-helix linked by a short (usually 12 amino acids) loop region. These EF-hand proteins are found both intracellularly and extracellularly. The superfamily of human EF-hand domain containing proteins consists of 222 proteins with an additional subset of four actinin proteins included in the superfamily. The total number of proteins that bind calcium is beyond the scope of this discussion but several important examples of intracellular Ca^{2+}-binding proteins include the calmodulins, calcineurins, calbindins, and troponins, whereas important extracellular Ca^{2+}-binding proteins include the coagulation factors [II (prothrombin), VII, IX, X, protein C, protein S] and the cell-cell communication/adhesion proteins of the cadherin family.

Examples of Important Calcium-Binding and Regulated Proteins

Protein Name	Functions / Comments
Calbindins	refers to a family of Ca2+-binding proteins; original member identified in chickens as vitamin D-dependent calcium-binding protein and then called calbindin-D28K (encoded by the CALB1 gene); other members include calretinin (29kDa protein encoded by

	CALB2 gene) and calbindin-D9K (encoded by the S100G gene which is also referred to as CALB3); all members mediate Ca^{2+} transport across membranes; in humans the CALB1 encoded protein is involved in renal Ca^{2+} reabsorption; in humans the S100G (CALB3) encoded protein is required for mediating intestinal calcium absorption in response to hormonal action of calcitriol; CALB2 encodes a neural-specific Ca^{2+}-binding protein; S100G (CALB3) is a member of the S100 family of proteins of which there are 24 members each of which function in some capacity related to the regulation of proliferation, differentiation, apoptosis, Ca^{2+} homeostasis, energy metabolism, inflammation and migration/invasion
Calcineurins	these proteins are components of a Ca^{2+}-dependent serine/threonine phosphatase identified as protein phosphatase 3, PP3 (formerly PP2B); calcineurins consists of a catalytic subunit and a regulatory subunit, and a subunit of calmodulin; the catalytic subunit is encoded by one of three genes: PPP3CA (commonly called calcinuerin A, CALNA), PPP3BB (commonly called calcineurin B, CALNB), and PPP3CC (commonly called calcineurin); the regulatory subunit is encoded by one of two genes: PPP3R1 and PPP3R2; activity of the calcineurins also requires Zn^{2+} and Fe^{3+} binding to domains in the catalytic subunits; major cell types regulated by calcineurin activity are T cells, neural cells, and cardiac cells; within the brain the primary substrates for calcineurin activity are Ca^{2+} channels, the dephosphorylation of which leads to their inactivation, thereby modulating the release of various neurotransmitters; calcineurin is potently inhibited by the immunosuppressant drugs, cyclosporin A and FK506 (fujimycin)
Calmodulins	these proteins are regulatory subunits of numerous enzymes, particularly kinases; humans express three distinct calmodulin genes identified as CALM1, CALM2, and CALM3; the proteins possess four Ca^{2+}-binding sites; several kinase families are known to possess calmodulin subunits: glycogen synthase-glycogen phosphorylase kinase (PHK, composed of six subunits, the δ-subunit is calmodulin), myosin light-chain kinases (four isoforms: MYLK or MLCK in smooth muscle, MYLK2 in skeletal muscle, MYLK3 in cardiac muscle, and MYLK4), and the kinases termed Ca^{2+}/calmodulin (CaM)-dependent protein kinases (CaMK) which includes CaMKI, CaMKII, CaMKIII, and CaMKIV; CaMKIII is more commonly referred to as eEF-2 kinase (eEF-2K) involved in the regulation of protein synthesis; in addition to serving as calcium-sensing regulatory subunits of numerous kinases, calmodulins also regulate the activity of protein phosphatases (particularly PP3 as indicated above) and the nitric oxide synthases, NOS

Troponins	the troponins are actually heterotrimeric complexes of three distinct subunits: troponin C (TnC), troponin I (TnI), and troponin T (TnT); TnT and TnI exist in tissue specific isoforms with the cardiac muscle forms identified as cTnI and cTnT, whereas the skeletal muscle forms are skTnI and skTnT; TnC is the Ca2+-binding subunit whose role is to effect the Ca2+-dependent regulation of muscle contraction; TnI inhibits the ATPase activity of the actin-myosin complex of the thin filaments that control muscle fiber contraction; TnT binds tropomyosin, thereby regulating troponin complex interaction with thin filaments; measurement of plasma levels of cTnI is now considered the standard for determination of diseases/disorders related to cardiac function such as acute myocardial infarction (AMI)
PKC family	the protein kinase C (PKC) family of serine/threonine kinases is composed of several related enzymes (for a more detailed discussion go to the Signal Transduction page); PKC enzymes are divided into three subfamilies termed conventional (cPKC), novel (nPKC), and atypical (aPKC); it is only the conventional PKC subfamily of enzymes that is regulated by calcium ions

Chlorine

Chlorine (as chloride ion: Cl^-) is a major ion necessary for digestive processes as it is required for the formation of gastric acid (HCl) within the lumen of the stomach. The majority of the chloride ion in the body is found in the extracellular fluid compartment. Chloride ion represents approximately 3% of the total electrolyte composition of the human body. Chloride ion functions along with sodium ion (Na^+) and potassium ion (K^+) in the maintenance of electrolyte balance. Chloride ion is required for the function of several ligand-gated ion channels. Of particular importance is the role of Cl– in the function of the inhibitory neurotransmitter, GABA (γ-aminobutyric acid). The GABA-A receptor is a Cl– channel that, in response to GABA binding induces an inward flux of Cl– into the neuron.

Magnesium

Magnesium ion (Mg^{2+}) is an activator for more than 300 enzymes. All enzymes that utilize ATP as a substrate or as an allosteric regulator require Mg^{2+} ion for activity. Magnesium is a highly critical ion in the nucleus where it interacts with DNA, an interaction necessary for stabilization of DNA structure. With respect to the requirement for Mg^{2+} in ATP functions, essentially all of the ATP in the cell has Mg^{2+} bound to the phosphates. This Mg^{2+}:ATP complex allows ATP to more readily release the terminal phosphate (the γ-phosphate) when doing so to provide energy for cellular metabolism. Some of the nuclear enzymes that require Mg^{2+} for activity are DNA repair endonucleases (involved in nucleotide excision repair, NER and mismatch repair, MMR), topoisomerase II, and RNase H. Magnesium is also required for protein synthesis since it is necessary for the stabilization of the ribosomes. Magnesium is a required component of numerous signal transduction

pathways as a result of its role as a substrate (activator) of adenylate cyclase leading to the production of cAMP which in turn activates the serine/threonine kinase, PKA. Magnesium is also important in the processes of electrolyte transport across membranes which facilitates, among numerous metabolic processes, glucose uptake and metabolism, ATP production via mitochondrial oxidative phosphorylation, and the functioning of nerve transmission via stabilization of ATP in Na+/K+-ATPases. Another critical role for Mg2+ is in the formation of the mineral matrix of bone.

Phosphorous

Phosphorous is the most important systemic electrolyte acting as a significant buffer in the blood in the form of phosphate ion: PO_4^{3-} as well as the monobasic (HPO_4^{2-}) and dibasic ($H_2PO_4^-$) forms. In the context of biological systems, phosphate ion is commonly referred to as inorganic phosphate and written as P_i which is used to designate all phosphate ion forms. In addition to its role as a critical blood buffer, phosphate is required in the biosynthesis of cellular components, such as ATP, nucleic acids, phospholipids, and proteins, and is involved in many metabolic pathways, including energy transfer, protein activation, and carbon and amino acid metabolic processes. Phosphate is also required for bone mineralization, and is necessary for energy utilization. One of the most important metabolic reactions that requires Pi is the phosphorolytic cleavage of glucose from glycogen by the enzyme glycogen phosphorylase.

In order to carry out its functions in metabolic processes, serum and intracellular Pi levels are maintained within a narrow range via a complex interplay between intestinal absorption, bone storage, and intracellular exchange. Hormonal control of phosphate levels is exerted primarily via the actions of vitamin D and parathyroid hormone within the proximal tubules of the kidneys.

Potassium: K+

Potassium ion is a key circulating electrolyte as well as being involved in the regulation of ATP-dependent channels along with sodium ion. These channels are referred to as Na+/K+-ATPases and their primary function is in the regulation of electrochemical gradients between the inside of cells and the interstitial spaces particularly in the brain and the kidney tubule. Numerous other forms of potassium channels utilize this ion to regulate action potential propagation in the context of the transmission of nerve impulses in the brain and in the control of cardiac muscle and skeletal muscle activity. Potassium ions represent approximately 5% of the total electrolyte pool in the human body. The majority of potassium ion in the body is found intracellularly. The average intracellular potassium concentration in around 150mM, whereas the concentration of potassium in the blood is only around 3.5mM–5mM.

Sodium: Na^+

Sodium ion is a key circulating electrolyte and also functions in the regulation of Na+/K+-ATPases with potassium ion. Sodium ions represent approximately 2% of the total electrolyte composition in the human body. Along with chloride ion (Cl–) and potassium ion (K+), sodium ion is required for normal cellular

osmolarity, maintenance of normal water distribution and water balance in the body, and maintenance of normal acid-base balance. Sodium ions are also critical to the initiation of action potentials in the context of nerve transmission, cardiac muscle, and skeletal muslce activity. The majority of the total body sodium ion is found in the extracellular fluids. The intracellular Na+ concentration is around 10mM while the concentration in the blood is around 135mM–145mN. The functions of the Na^+/K^+-ATPases in the body are numerous with primary roles being in the processes of nerve transmission in the central and peripheral nervous systems, in the functioning of muscle cells, in particular cardiac muscle function, and in the regulation of fluid and ionic balance via the kidneys.

Sulfur

Sulfur has a primary function in amino acid metabolism (methionine and cysteine) but is also necessary for the modification of complex carbohydrates present in proteins (glycoproteins) and lipids (glycolipids), however, it should be noted that in this latter function the sulfur is donated from the amino acid methionine.

Trace Minerals

Copper

Copper is involved in the formation of red bloods cells, the synthesis of hemoglobin, and the formation of bone. Additional functions of copper are energy production, wound healing, taste sensation, skin and hair color. Copper is also involved in the proper processing of collagen and elastin via the action of the extracellular matrix-associated enzyme, lysyl oxidase. Thus, copper is critical to the proper production of connective tissue.

Important Copper-Dependent Enzymes

Enzyme Name	Gene	Functions
Ceruloplasmin	CP	major ferroxidase in the blood; each enzyme binds 6–7 Cu2+ (cupric) ions; plays a major role in ensuring no free iron in the circulation; oxidizes Fe2+ (ferrous) iron to Fe3+ (ferric) iron which can then be bound to transferrin, the major iron transporting protein in the blood; ceruloplasmin is often misrepresented as the major copper transporting protein of the blood due to the fact that up to 95% of copper in the blood is found in this enzyme, however, the major function of ceruloplasmin is as a ferroxidase not as a copper transporter; two CP isoforms generated via alternative mRNA splicing, one form is secreted the other is attached to the plasma membrane via a GPI linkage; secreted CP synthesized exclusively by the liver, the GPI-linked CP is expressed by numerous

		organs including the brain, liver, kidneys, and lungs; the GPI-linked CP is primarily responsible for iron efflux from tissues; aceruloplasminemia, due to defects in the CP gene, doesn't affect copper homeostasis but manifests with iron overload of a form referred to as hemosiderosis
Cytochrome c oxidase	13 genes	composed of 13 subunits that comprise the mitochondrial oxidative phosphorylation complex IV; mitochondrial genome harbors MT-CO1, MT-CO2, and MT-CO3 genes; nuclear genome harbors the other ten genes: COX4, COX5A, COX5B, COX6A, COX6B, COX6C, COX7A, COX7B, COX7C, COX8; functions to re-oxidized reduced cytochrome c while subsequently reducing molecular oxygen to water; the ferric (Fe^{3+}) iron in complex IV is the site of cyanide (CN^{-}) binding
Dopamine β-hydroxylase (dopamine β-monooxygenase)	DBH	involved in catecholamine synthesis, catalyzes hydroxylation of dopamine to norepinephrine; expression limited to adrenal medulla and post-ganglionic sympathetic neurons
Hephaestin	HEPH	functions as a ferroxidase (similar to ceruloplasmin); expression is limited to intestinal enterocytes; required for iron transport from intestinal enterocytes to the blood; dietary iron is transported from enterocytes to the blood via the action of ferroportin with simultaneous oxidation of Fe^{2+} (ferrous) iron to Fe^{3+} (ferric) iron by hephaestin; ensures the iron can be bound to transferrin for delivery to the tissues
Lysyl oxidase	LOX	catalyzes the oxidative deamination of the ε-amino group of lysine and hydroxylysine residues in collagens and lysine residues of elastin; results in cross-linking of protein forming fibrils
Methionine synthase (homocysteine methyltransferase)	MTR	official name is 5-methyltetrahydrofolate-homocysteine S-methyltransferase; catalyzes the conversion of homocysteine to methionine; is one of only two enzymes that require vitamin B12 (as methylcobalamin); as the name implies the enzyme also requires N5-methyl-THF for activity; defects in the MTR gene, or deficiency in either folate or B12 (or both), result in homocysteinemia/homocystinemia and macrocytic anemia
Cu-Zn Superoxide	SOD1	major cytoplasmic anti-oxidant enzyme; catalyzes

dismutase		conversion of superoxide free radicals to molecular oxygen (O2) and hydrogen peroxide (H2O2); the major mitochondrial superoxide dismutase (SOD2) is a manganese-dependent enzyme

Iodine

Iodine is required for the synthesis of the thyroid hormones and thus plays an important role in the regulation of energy metabolism via thyroid hormone functions.

Iron: Fe^{2+} and Fe^{3+}

Iron is the most abundant trace metal in the human body. Iron (as the ferrous ion, Fe2+) is a critical micronutrient with a major role in the transport of oxygen. Iron is the functional center of the heme moiety found in each of the protein subunit of hemoglobin. The function of Fe2+ is to coordinate the oxygen molecule into heme of hemoglobin so that it can be transported from the lungs to the tissues. Aside from its role in oxygen transport, iron is critical to the overall process of oxidative phosphorylation where it is also found in the heme of cytochromes and in the Fe-S (iron-sulfur) centers of the various complex of oxidative phosphorylation. Iron is the only metal in the human body that is toxic if allowed to remain free in the plasma or the fluid compartments of cells. The toxicity of free iron is related to its ability to rapidly generate the highly toxic hydroxyl free radical (HO·) via the Fenton reaction. For this reason there are extremely tight controls on overall iron homeostasis.

Several Iron-Dependent Enzymes

Enzyme Name	Gene Symbol	Functions / Comments
Aconitases	ACO1, ACO2	the protein encoded by ACO1 functions in the iron-mediated control of translation of the H-ferritin, L-ferritin, transferrin receptor, DMT1, ferroportin, ALAS2, and ACO2 mRNAs; the ACO2 encoded protein is involved in the TCA cycle
Alcohol dehydrogenases	7 different genes	belong to medium-chain dehydrogenase/reductase (MDR) superfamily; catalyze the oxidation of various alcohols to their corresponding aldehydes; important in the detoxification/metabolism of ethanol
Catalase	CAT	primary reaction is to detoxify the reactive oxygen species (ROS) hydrogen peroxide (H2O2) to water; can also oxidize certain alcohols to corresponding

		aldehydes
Cytochrome *c* reductase	multiple subunits including the Fe-S protein encoded by the UQCRFS1 gene	multisubunit component of oxidative phosphorylation Complex III; contains two cytochromes b (b-562 and b-566), cytochrome c1, and the Fe-S protein which is called the Rieske Fe-S protein after its discoverer J.S. Rieske; official name of this enzyme complex is ubiquinol-cytochrome c reductase
Lipoxygenases	ALOX5, ALOX12, ALOX15	all three lipoxygenases (5-LOX, 12-LOX, and 15-LOX) are involved in arachidonic acid oxidation during the synthesis of the leukotrienes and the lipoxins
Lysyl hydroxylases	PLOD1, PLOD2, PLOD3	official name for these enzymes is procollagen-lysine, 2-oxoglutarate 5-dioxygenase; PLOD1 is the major procollagen lysine hydroxylating enzyme; all 3 enzymes function as homodimers; PLOD2 and PLOD3 carry out hydroxylations in collagen-like proteins; mutations in PLOD1 are associated with Ehlers-Danlos syndrome (EDS) type VI, mutations in PLOD2 or PLOD3 are associated with EDS type VIB
NADH-ubiquinone reductase	multiple Fe-S subunit genes	multisubunit component of oxidative phosphorylation Complex I
Phenylalanine hydroxylase	PAH	catalyzes the conversion of phenylalanine to tyrosine; mutations in the PAH gene result in phenylketonuria, PKU
Prolyl 4-hydroxylase (two α, two β subunits)	three α subunit genes: P4HA1, P4HA2, P4HA3; β subunit gene: P4HB	catalyzes the formation of 4-hydroxyproline residues in procollagen
Ribonucleotide reductase	RRM1,	catalyzes the conversion

(contains 2 subunits)	RRM2	of ribonucleoside diphosphates to their corresponding deoxyribonucleotide diphosphates
Stearoyl-CoA desaturase	SCD	one of three fatty acid desaturases in humans; stearoyl-CoA desaturase is the rate-limiting enzyme catalyzing the synthesis of monounsaturated fatty acids (MUFAs), primarily oleate (18:1; a physiologically significant omega-9 fatty acid) and palmitoleate (16:1)
Succinate-ubiquinone reductase	multiple Fe-S subunit genes	multisubunit component of oxidative phosphorylation Complex II
Thyroid peroxidase	TPO	exclusively expressed in the thyroid gland; within the thyroid colloid TPO oxidizes I– to I+; reaction requires H2O2
Tryptophan hydroxylase	TPH2	initial enzyme in the conversion of tryptophan to the neurotransmitters, serotonin and melatonin
Tyrosine hydroxylase	TH	initial enzyme in the conversion of tyrosine to the catecholamines, dopamine, norepinephrine and epinephrine
Xanthine oxidase (derived from xanthine dehydrogenase)	XDH	also requires molybdenum for function; xanthine dehydrogenase can be converted to xanthine oxidase by reversible sulfhydryl oxidation or by irreversible proteolytic modification; catalyzes the conversion of hypoxanthine to xanthine and xanthine to uric acid in the catabolism and salvage of purine nucleotides

Manganese

Manganese is involved in reactions of protein and fat metabolism, promotes a healthy nervous system, and is necessary for digestive function, bone growth, and immune function. Maintenance of blood glucose levels is controlled in large part via the ability of the liver to produce glucose from precursor carbon atoms in the pathway of gluconeogenesis. Two of the enzymes of gluconeogenesis, pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK) require manganese for their activity. Within the liver, kidneys, and brain manganese is critical in the regulation of ammonium ion (NH_4^+) levels via its role activating glutamine synthetase. Within the liver, manganese plays an additional role in the regulation of NH_4^+ levels in the body via its activation of the urea cycle enzyme arginase. Manganese also serves as an important anti-oxidant mineral since it is

necessary for the proper function of mitochondrial superoxide dismutase (SOD2) which catalyzes the same reaction as that catalyzed by the cytosolic version, SOD1

Molybdenum

Molybdenum is primarily involved as a co-factor in oxidase enzymes such as xanthine dehydrogenase/oxidase necessary for purine nucleotide catabolism. Molybdenum is also a necessary cofactor in the detoxification reactions catalyzed by sulfite oxidase. Sulfite oxidase is the terminal enzyme in the pathways of the metabolism of sulfur-containing compounds such as the amino acid cysteine. The product of the sulfite oxidase reaction, sulfate, is then excreted.

Selenium

Selenium serves as a modifier of the activity of several enzymes through its incorporation into protein in the form of selenocysteine. Two critical re-dox enzyme families that require selenocysteine residues are the glutathione peroxidase and thioredoxin reductase families. Glutathione peroxidase is a critical enzyme involved in the protection of red blood cells from reactive oxygen species (ROS). This enzyme is a component of a re-dox system that also involves the enzyme glutathione reductase and NADPH as the terminal electron donor. This system is required for the continued reduction of oxidized glutathione (GSSG) and represents the single most significant system requiring continued glucose metabolism via the Pentose Phosphate Pathway in erythrocytes as the means for the production of the NADPH. Glutathione (GSH) becomes oxidized in the context of reducing various ROS and peroxides and to continue in this capacity the oxidized form needs to be continously reduced. Humans express eight different glutathione peroxidase genes identified as GPX1 through GPX8, with five of these enzymes (GPX1, GPX2, GPX3, GPX4, and GPX6) having been demonstrated to harbor selenocysteine residues. The enzyme encoded by the GPX1 gene (GPx1) is found in the cytosol of nearly all cell types in humans. GPx1 functions almost exclusively to reduce hydrogen peroxide (H_2O_2) to water. The protein encoded by the GPX3 gene, GPx3, is an extracellular enzyme found primarily in the plasma. The GPX4 encoded enzyme, GPx4, is localized to the intestines and is an extracellular enzyme as well. The GPX1 gene is located on chromsome 3p21.3 and is composed of 2 exons that generate two alternatively spliced mRNAs. The GPX1 coding region contains a polyalanine tract in the N-terminal region of the protein. There are several alleles of this gene that have five, six, or seven alanine repeats. The allele with five alanine repeats has been shown to be highly correlated to increased risk for development of breast cancer. The GPX2 gene is located on chromsome 14q24.1 and is composed of 4 exons. The GPX3 gene is located on chromsome 5q33.1 and is composed of 5 exons. The GPX4 gene is located on chromsome 19p13.3 and is composed of 8 exons. The GPX5 gene is located on chromsome 6p22.1 and is composed of 7 exons. The resultant GPX5 mRNA does not contain the canonical selenocysteine codon (UGA) and thus, the resulting protein does not contain a selenocysteine residue. Expression of the GPX5 gene is regulated by androgens and the gene is expressed exclusively in the epididymis in the male reproductive tract where the expressed protein, GPx5, is involved in

protecting spermatazoa membranes from the damaging effects of lipid peroxidation. The GPX6 gene is located on chromsome 6p22.1 and is composed of 5 exons. GPX6 expression is restricted to embryonic tissues and the adult olfactory system. The GPX7 gene is located on chromsome 1p32 and is composed of 3 exons. The GPX8 gene is located on chromsome 5q11.2 and is composed of 3 exons.

As the name of the enzyme implies, thioredoxin reductase is involved in the reduction of thioredoxin which itself is principally involved in the reduction of oxidized disulfide bonds in proteins. The reduction of these disulfide bonds results in oxidation of thioredoxin which then is reduced by thioredoxin reductase. The overall process, like the glutathione peroxidase system, requires NADPH as the terminal electron donor for the reduction process. A critically important reaction that is coupled to the thioredoxin system is the formation of deoxynucleotides. Humans contain three thioredoxin reductase genes that encode three distinct enzymes identified as TrxR1, TrxR2, and TrxR3. The TrxR1 enzyme is functional in the cytosol and is primarily involved in the maintenance of the ribonucleotide reductase system. The TrxR2 enzyme is functional in the mitochondria where it is principally involved in the detoxification of reactive oxygen species (ROS) produced in this organelle. TrxR3 is a testes-specific isoform of the enzyme. The TrxR1 enzyme is encoded by the TXNRD1 gene located on chromosome 12q23–q24.1 and is composed of 18 exons that generate several alternatively spliced mRNAs encoding five different isoforms of TrxR1. The TrxR2 enzyme is encoded by the TXNRD2 gene located on chromosome 22q11.21 and is composed of 19 exons that generate two alternatively spliced mRNAs resulting in two different isoforms of TrxR2. The TrxR3 enzyme is encoded by the TXNRD3 gene located on chromosome 3q21.3 and is composed of 16 exons that generate two alternatively spliced mRNAs resulting in two different isoforms of TrxR3.

The enzymes of the deiodinase family are also important selenocysteine-containing enzymes. Clinically relevant enzymes in this family are the thyroid deiodinases that are critical for the maturation and catabolism of the thyroid hormones. Humans express three different thyroid deiodinase genes identified as DIO1, DIO2, and DIO3. The enzyme encoded by the DIO1 gene, thryroxine deiodinase type I (also called iodothyronine deiodinase type I) is involved in the peripheral tissue conversion of thyroxine (T4) to bioactive form of thyroid hormone, tri-iodothyronine (T3). In addition to its role in the generation of T3, thyroxine deiodinase I is involved in the catabolism of thyroid hormones. The enzyme encoded by the DIO2 gene, iodothyronine deiodinase type II, is also involved in the conversion of T4 to T3 but does so within the thyroid gland itself. The activity of iodothyronine deiodinase II has been associated with the thyrotoxicosis of Graves disease. The enzyme encoded by the DIO3 gene is involved only in the inactivation (catabolism) of T3 and T4. Expression of the DIO3 gene is highest the female uterus during pregnancy and in fetal and neonatal tissue suggesting a role for this enzyme in the regulation of thyroid hormone levels and functions during early development. The DIO1 gene is located on chromosome 1p33–p32 and is composed of 4 exons that generate four alternatively spliced mRNAs. The DIO2

gene is located on chromosome 14q24.2–q24.3 and is composed of 6 exons that generate four alternatively spliced mRNAs. The DIO3 gene is located on chromosome 14q32 and is an intronless gene (is a single exon gene) that encodes a protein of 304 amino acids.

Selenium Toxicity

Given the significant role of selenium in the protection against the damaging effects of reactive oxygen and reactive nitrogen species, it might seem logical to consume large quantities of the metal as a protective prophylactic. However, this is definitely not a clinically sound approach. There is a very narrow clinically safe range for selenium intake, too little and there are serious clinical consequences, too much and some overlapping as well as a different set of serious clinical complications occur. Chronic selenium deficiency is associated with lethargy, dizziness, motor weakness and paresthesias, and an excess risk of amyotrophic lateral sclerosis. Selenium toxicity due to excess intake manifests most significantly with neurological impairment evidenced by ataxia, hypotonia, hyperreflexia, dyasthesia, and paralysis. Lethargy and dizziness are also common with selenium intoxication as for selenium deficiency. Additional CNS effects of selenium intoxication include localized or generalized tremors and convulsions. Many individuals suffering from selenium intoxication experience behavioral disturbances that can lead to suicidal ideation. The cardiovascular and respiratory systems are also impaired with selenium toxicity and can result in death due to respiratory failure and cardiac arrest. One characteristic feature associated with selenium intoxication is a garlic odor to the expired breath. This is similar to the consequences oif arsenic poisoning, therefore, in and of itself a garlic odor to the breath is not exclusively diagnostic for selenium intoxication.

Zinc: Zn^{2+}

After iron, zinc is the second most abundant trace metal in the human body. Zinc ion (Zn^{2+}) is found as a co-factor in over 300 different enzymes and thus is involved in a wide variety of biochemical processes. Zinc interacts with the hormone insulin to ensure proper function, thus, zinc participates in the regulation of blood glucose levels via insulin action. Zinc is necessary for the activity of a number of transcription factors such as those of the nuclear receptor (steroid and thyroid hormone receptor superfamily) family through its role in the formation of the structurally critical zinc finger domain that binds to DNA. Zinc also promotes wound healing, regulates immune function, serves as a co-factor for numerous antioxidant enzymes, and is necessary for protein synthesis and the processing of collagen.

Several Zinc-Dependent Enzymes

Enzyme Name	Gene Symbol	Functions
ALA dehydratase	ALAD	is the second enzyme in the pathway of heme

		biosynthesis, catalyzes the condensation of two molecules of δ-aminolevulinic acid (ALA) forming porphobilinogen
Alcohol dehydrogenases, ADH	7 different genes	belong to medium-chain dehydrogenase/reductase (MDR) superfamily; catalyze the oxidation of various alcohols to their corresponding aldehydes; important in the detoxification/metabolism of ethanol
Aldolases	ALDOA, ALDOB, ALDOC	catalyzes the hydrolysis of fructose-1,6-bisphosphatase (ALDOA) in the pathway of glycolysis; ALDOB is involved in the hepatic metabolism of fructose
Alkaline phosphatase, ALP	ALPL, ALPP, ALPI, ALPPL2	four different enzymes, three encoded by three different genes all clustered on chromosome 2, each of these three (ALPP, ALPI, ALPPL2) is tissue specific in expression, the non-tissue specific form of the enzyme is expressed from the ALPL gene on chromosome 1; each enzyme catalyzes the dephosphorylation of substrates in an alkaline environment; high amounts of ALP found in liver and bone; measurement for elevation in the blood is used in the overall diagnosis of liver or bone disease
Aspartate transcarbamoylase	CAD	a tri-functional enzyme that catalyzes the initial three rate-limiting reactions of pyrimidine nucleotide biosynthesis; the three activities are carbamoylphosphate synthetase 2, aspartate transcarbamoylase, and dihydroorotase
Carbonic anhydrases, CA	at least 12 different functional members	catalyze the formation of carbonic acid (H_2CO_3) from CO_2 and H_2O; see the Enzyme Kinetics page for more details
Histone deacetylases, HDACs	at least 18 different members of family	as the name implies, these enzymes remove acetyl groups from histones; the consequences of histone deacetylation are the silencing of transcription; the sirtuin (SIRT) proteins in humans also possess HDAC activity
Monoamine oxidases	MAOA, MAOB	catalyze the oxidation of monoamines; critical roles in the regulaiton of the catabolism of dopamine, serotonin, epinephrine, and norepinephrine; given these important functions MAO inhibitors (MAOIs) were used for several years as anti-depressants and anti-anxiety drugs; due to potential for excessive levels of

		epinephrine and norepinephrine MAOIs can cause hypertensive crisis
Phospholipase C (PLC)	13 enzymes in family	PLCβ and PLCγ most well characterized members of the family; each enzyme hydrolyzes membrane phospholipids, primarily polyphosphoinositols, at the bond where the phosphate is attached to the glycerol backbone
Pyridoxal kinase	PDXK	required for the formation of the cofactor form of vitamin B6: pyridoxal phosphate (PLP; also identified as pyridoxal-5-phosphate)
Pyruvate carboxylase	PC	first of two enzymes required for bypass 1 step of gluconeogenesis; catalyzes the formation of oxaloacetate from pyruvate and bicarbonate ion
Superoxide dismutase, Cu-Zn	SOD1	major cytoplasmic anti-oxidant enzyme; catalyzes conversion of superoxide free radicals to molecular oxygen (O2) or hydrogen peroxide (H2O2); the major mitochondrial superoxide dismutase (SOD2) is a manganese-dependent enzyme