Assessment of Mineral Status

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The scope of this review is restricted to the evaluation of the calcium, phosphorus, iron, and zinc status during pregnancy and lactation. It will cover laboratory indices of these minerals, excluding clinical signs and physical measurements.

CALCIUM STATUS

Calcium is the fifth most abundant element in the human body, with adults containing 1,000 to 1,200 g (1). More than 99% is found in the skeleton, associated with phosphorus, mostly in the form of poorly crystallized hydroxyapatite; Mg$^{2+}$, Na$^+$, CO$_3^-$, and citrate$^{3-}$ are also found in this crystalline phase. Bone is a dynamic tissue that undergoes constant turnover, and it is an essential component of Ca homeostasis. The Ca ion is involved in many other vital functions in the body, including blood coagulation, neuromuscular excitability, muscular contraction, cellular secretion processes, enzyme activation, and cell adhesion (2–5). The essential nature of many of these Ca functions, particularly those related to the nervous system, requires extremely tight control of Ca ion concentrations in extracellular fluids. An intricate mechanism controlling plasma Ca concentrations has evolved and includes the hormones parathyrin (parathyroid hormone), metabolites of vitamin D (mainly 1,25-dihydroxycholecalciferol), and calcitomin. They act independently or in concert to regulate intestinal uptake, renal reabsorption, and transfer of calcium out of and into the vast skeletal reservoir. Intracellular Ca concentration is also tightly controlled at a much lower concentration, around $10^{-7}$ to $10^{-8}$ mol/liter (5).

Calcium in blood is found almost exclusively in the plasma, in diffusible and nondiffusible forms. The latter is bound to protein, mostly albumin; the former, often called ultrafiltrable Ca, is mainly ionized Ca$^{2+}$, with some Ca complexed to bicarbonate, citrate, and phosphate. Typical values for Ca distribution in plasma are 43% to 46% protein bound; 45% to 47.5% ionic,
and 6.5% to 12% complexed (1,2). From the work of McLean and Hastings (6) it is widely accepted that ionized Ca$^{2+}$ is the physiologically active form which is hormonally controlled; some dissenting views exist, however, on the biological importance of complexed and protein-bound Ca (7,8). We first discuss total Ca and ionized Ca$^{2+}$ in blood as markers of Ca status before discussing the hormones involved in Ca homeostasis.

**Total Calcium in Plasma**

In humans, total Ca is found in concentrations of about 2.50 mmol/liter (10 mg/dl), with a normal range of 2.25 to 2.75 mmol/liter (9.0–11.0 mg/dl) (1). Normal ranges for plasma Ca depend on methods of measurement, and each laboratory should establish its own normal range. Levels of Ca are slightly higher in men than in women (9) and decrease with age (10). Posture (standing versus supine) (11) and stasis during sampling (12) have been shown to increase total Ca levels by 4.5% and 2.4%, respectively, with arm exercise increasing the stasis effect to 8.4%.

A large number of methods for plasma Ca determination have been published; this reflects the technical difficulties encountered. Earlier methods were based on the precipitation of Ca, for example as Ca oxalate, with subsequent titration of oxalate. Colorimetric methods were developed based on colored complexes between Ca and special dyes, such as O-cresol-phthalain complexone, calcein, and methylthymol blue. Magnesium interference is eliminated by the addition of γ-hydroxyquinoline. These methods have been widely adapted to automated analyzers. Commercial kits are also available for small batches of samples. Improved precision is achieved by replacing spectrophotometric or fluorometric detection of the Ca-dye complexes by titration with a chelating agent to remove Ca from the complex; ethylenediaminetetraacetic acid (EDTA) or ethyleneglycoltetraacetic acid (EGTA) are used as chelating agents. The method of choice for plasma Ca is atomic absorption spectrometry, which is accurate, precise, and rapid (14); it is not influenced by bilirubin, an important factor when working with neonate samples (15,16).

**Ionized Calcium in Plasma**

It is widely accepted that ionized Ca$^{2+}$ in plasma is the biologically active form of Ca involved in Ca homeostasis. The development of reliable commercial Ca-selective electrodes has made ionized Ca$^{2+}$ measurement a routine procedure in many laboratories. Technical problems, related to sampling and sample handling before and during analysis and to the electrodes themselves, make ionized Ca measurement more difficult than that of total Ca. Robertson and Marshall (5) have reviewed the measurement of ionized Ca
in body fluids, including blood, serum, and urine, and the interested reader is referred to them for more details.

Ionized Ca levels depend on fasting status (reviewed data conflict), on posture, in which these levels decrease in the supine position, on stasis, which is accompanied by an increase, as well as on acid-base status (hyperventilation and exercise). Data on difference in values for men and women are not consistent. Ionized Ca levels fall with age after 40 years; a slight rise has been observed after menopause. Ionized Ca levels fall slightly during pregnancy.

Published data on the normal range of ionized Ca, as reviewed by Robertson and Marshall (5), show very large variations, with mean values varying between 1.07 (0.93–1.20) mmol/liter and 1.26 (1.09–1.45) mmol/liter in serum and between 1.10 (1.02–1.18) mmol/liter and 1.16 (1.02–1.30) mmol/liter in blood. This points to the need for each laboratory to define its own normal range.

Over the years, many formulas have been devised to correct total Ca concentrations for variations of protein, albumin, and globulin concentrations and/or blood pH, and to calculate ionized Ca from total Ca [see, for example, White et al. (17)]. Many arguments have been presented for and against such corrections (5,17). Total Ca should probably be maintained as the primary index. Whenever abnormal Ca metabolism is suspected, however, direct measurement of ionized Ca is preferred; this includes, for example, disorders of acid-base balance, hemodialysis, renal failure, cirrhosis, myeloma, and endocrine disorders.

Measurement of dialyzable Ca has been compared to total Ca, albumin-adjusted Ca, and ionized Ca and has been proposed as an alternative to measurement of ionized Ca (18). This has been adapted to continuous-flow analyzers.

Calcium Homeostasis

Calcium homeostasis is essentially maintained by the hormones parathyrin (PTH), 1,25-dihydroxycholecalciferol (1,25-(OH)₂D₃), and calcitonin (CT) acting on Ca absorption through the intestinal wall, Ca reabsorption in the kidney, and Ca deposition and resorption in bones. The mechanisms of action of these hormones, their interactions, and their modulation by factors other than plasma ionized Ca are complex; only a very brief overview is presented here [for more details, the reader may refer to Bronner and Coburn (2)].

Parathyrin

Parathyrin is a polypeptide chain of 84 amino acid residues having a molecular weight of 9,500. It is synthesized in the parathyroid glands as prepro-
PTH, which is converted to pro-PTH and then to PTH within the glands by the successive removal of 31 and 6 amino acids at the NH$_2$ terminal. PTH is found in plasma as the intact hormone, with 84 amino acids and a short half-life, and as shorter peptides. The intact hormone is metabolized by the kidney and liver, with the subsequent appearance of shorter peptides: a carboxy terminal peptide, with a molecular weight of 7,000, a longer half-life, and without known biological activity; and amino terminal peptides with molecular weights of approximately 2,500, which have demonstrated biological activity in animal assays. The direct biological activity of these residues remains questionable in humans (19). Extracellular ionized Ca is the main regulator of PTH release, showing an inverse relationship. Extracellular Mg shows a similar regulating effect, albeit less efficient. Severe Mg deficiency, however, has been shown to inhibit PTH release and end-organ response to PTH.

**Biological effects of PTH on the kidney** are complex; the tubular reabsorption of Ca is increased, whereas phosphate reabsorption is decreased; urinary excretion of phosphate and cyclic adenosine monophosphate (cyclic AMP) are increased. The main action of PTH on bone is an increase in bone resorption, both by osteoblastic and osteocytic mechanisms, with concomitant release of Ca and P in the extracellular fluid. Finally, PTH acts indirectly on the intestinal absorption of Ca by increasing renal hydroxylation of 25-hydroxycholecalciferol (25-(OH)D$_3$) to 1,25-(OH)$_2$D$_3$.

Several *in vivo* and *in vitro* bioassays have been developed to quantitate PTH (2). Most of the recent work in humans has been done with radioimmunoassays (RIA). PTH metabolism and assay methods have also been reviewed (20). The presence in plasma, besides intact PTH, of PTH fragments with different half-lives and variable cross-reactivity has complicated the development and interpretation of RIA. So far, carboxy terminal and midmolecule assays have performed better in distinguishing normal from primary hyperparathyroid patients. Commercial kits are available, and the performance of some has been evaluated (21).

**Vitamin D**

Vitamin D occurs in two forms: ergocalciferol or vitamin D$_2$, of plant origin; and cholecalciferol, or vitamin D$_3$, produced in animals and humans by ultraviolet irradiation of 7-dehydrocholesterol in the skin. Both seem equally active in humans. Vitamin D is transported to the liver, where it undergoes the first hydroxylation at carbon 25. In terms of concentration, 25-(OH)D is the major circulating metabolite of vitamin D and the main parameter for the assessment of vitamin D status. In our latitudes, 85% to 90% of circulating 25-(OH)D is in the D$_3$ form. [For detailed information on vitamin D metabolism and activity, the reader is referred to Bronner and Coburn (2) and Fournier et al. (22).]
The metabolite 25-(OH)D$_3$ is transported to the kidney, where it is further hydroxylated to the hormone 1,25-(OH)$_2$D$_3$; although low in concentration, this is the most active metabolite of vitamin D. Other, less active metabolites, such as 24,25-(OH)$_2$D$_3$, are also produced whose metabolic functions are still unknown. With PTH, 1,25-(OH)$_2$D$_3$ is the main modulator of Ca and P metabolism; its production by the kidney is regulated primarily by PTH. Other activators (direct or indirect) include hypocalcemia, hypophosphatemia, calcitonin, prostaglandins, cyclic AMP, prolactin, growth hormone, insulin. Inhibitors include plasma Ca, vitamin D metabolites, particularly 1,25-(OH)$_2$D$_3$ itself, and thyroxin (22). The main biological action of 1,25-(OH)$_2$D$_3$ is exerted on the intestine, where it promotes the absorption of dietary Ca and P. It increases bone resorption and Ca and P release in the extracellular fluids. The direct action of 1,25-(OH)$_2$D$_3$ on bone mineralization, on the parathyroid glands, and on the kidneys is only partially understood.

If 25-(OH)D is the main parameter of vitamin D nutritional status, then 1,25-(OH)$_2$D$_3$ is the biologically active form, important for evaluating Ca and P metabolism. Circulating levels of total 25-(OH)D vary with the diet; 25-(OH)D$_3$, the major form of 25-(OH)D, depends on exposure to sunlight and varies with the seasons. Circulating 1,25-(OH)$_2$D is not influenced by diet, except in the case of severely deficient intake or overdose of vitamin D; a seasonal variation has been demonstrated for children (23).

Methodology for measurement of 25-(OH)D and 1,25-(OH)$_2$D has been reviewed (2,22). For 25-(OH)D high-performance liquid chromatography (HPLC) with ultraviolet detection is currently the method of choice (24). For 1,25-(OH)$_2$D, cytosol radioreceptor assays and radioimmunoassays are mostly used. Both methodologies require a sample purification step, because of cross-reactivity with the other vitamin D metabolites. Normal values for 25-(OH)D vary greatly depending on the level of food fortification with vitamin D and on the season; 1,25-(OH)$_2$D plasma levels do not show this variation. Interindividual (12) and interlaboratory variations, however, are considerable (25,26).

Vitamin D and its metabolites are transported in plasma by a common vitamin D-binding protein (DBP), which circulates mostly as apoprotein; its measurement is not necessary when evaluating vitamin D status. When alterations of plasma DBP levels may be expected, the measurement of 1,25-(OH)$_2$D should be accompanied with that of DBP; this is the case with liver cirrhosis and nephrotic syndrome and during pregnancy [see Fournier et al. (22), Chap. 4].

**Calcitonin**

Calcitonin is a peptide with 32 amino acid residues secreted by the parafollicular cells of the thyroid (2). The main modulator of calcitonin secretion
is plasma Ca; increased levels of Ca above a threshold of about 2.35 mmol/liter induce a proportional increase of calcitonin.

The primary action of calcitonin is to decrease plasma Ca and phosphate concentrations by inhibiting bone resorption and by increasing urinary excretion of Ca and P. These effects have been demonstrated in short-term studies. Over the long term, the physiological importance of calcitonin has not been clarified; thyroidectomized adults do not seem to develop skeletal abnormality.

Circulating calcitonin is measured by radioimmunoassay, preferably in plasma. The assay is particularly difficult, mainly due to the low concentration and the immunochemical heterogeneity of circulating calcitonin and to the variable susceptibility of the antisera to nonspecific interferences. These problems are described by Heath et al. (27,28). Immunoreactive calcitonin concentrations decline with age and are lower for women than for men. The main applications of the calcitonin assay are in research and in the diagnosis, treatment, and monitoring of medullary thyroid carcinoma.

Other Indices of Calcium Status

The measurement of urinary and fecal Ca may be included in the general evaluation of Ca status and homeostasis, the method of choice being atomic absorption. Under normal conditions, 70% to 90% of Ca is excreted in the feces and 10% to 30% in urine. Urinary Ca is increased in hyperparathyroidism, hyperthyroidism, acidemia, hypervitaminosis D, and idiopathic hypercalcemic renal tubular acidosis. Urinary Ca is decreased in hypoparathyroidism, vitamin D deficiency, hypothyroidism, and chronic nephritis. For evaluation of calciuria, dairy products should be withdrawn from the diet 2 to 3 days before the test and 24-hr urine should be collected during 3 days; daily urinary Ca excretion is usually below 250 mg in women and below 300 mg in men. Fecal Ca excretion may be increased in rickets and osteomalacia and in several malabsorption syndromes, such as sprue and celiac disease (22,29).

Plasma alkaline phosphatase of bone origin reflects osteoblastic activity; its activity is increased in rickets, osteomalacia, Paget’s disease, hyperparathyroidism, and bone metastases. Alkaline phosphatase may be determined with the method of Bessey, Lowry, and Brock, in which alkaline phosphatase hydrolyzes p-nitrophenyl phosphate to phosphoric acid and p-nitrophenol; the latter is quantified photometrically (22).

Urinary cyclic AMP (determined by radioimmunoassay) is used as an index of parathyroid function. Its usefulness in the diagnosis of hyperparathyroidism has been reviewed (30).

Urinary hydroxyproline excretion is a reflection of collagen turnover. The excretion is increased in hyperparathyroidism, hyperthyroidism, Paget’s dis-
ease, acromegaly, and bone metastases and is decreased during malnutrition. Dietary collagen intake must be controlled during this test (31); mean excretion has been reported to vary from 132 ± 12 to 354 ± 58 μmol/day, depending on dietary regimen in young adults (31).

Osteocalcin, or bone Gla protein, is a bone matrix protein with 49 amino acids that is found in small amounts in blood. Its value as a new index of bone metabolism is under investigation (32,33).

Abnormal Plasma Calcium

A detailed discussion of disorders of Ca metabolism is beyond the scope of this review. Some of the conditions affecting Ca levels have been reviewed elsewhere (22,29). Those related directly to nutritional causes are discussed elsewhere in this volume.

Hypocalcemia

The conditions and circumstances under which hypocalcemia (often associated with tetany) occurs include alkalemia (reflected in ionized Ca) due to vomiting, hyperventilation, or alkali intake; citrated blood transfusion; malabsorption (sprue, celiac disease, pancreatic and biliary insufficiency); lactose intolerance; rickets; osteomalacia; hypoparathyroidism (surgical, idiopathic, pseudohypoparathyroidism); chronic nephritis; anticonvulsions therapy, and severe protein malnutrition. Hypocalcemia is not uncommon in premature infants and in infants born to diabetic mothers.

Hypercalcemia

The conditions responsible include primary hyperparathyroidism, hypervitaminosis D, milk-alkali intoxication, multiple myeloma, some neoplastic diseases, immobilisation (during fast growth), and sarcoidosis.

Pregnancy and the Newborn

Pregnancy and lactation represent a major stress on Ca metabolism in women. The principal adaptive mechanism for preserving maternal homeostasis is a marked increase in Ca absorption. Calcium metabolism in pregnancy has been the object of several reviews (34–36) and is dealt with in detail by F. H. Glorieux, this volume. The influence of sex, race, age, season, and diet on vitamin D status (23) and on serum minerals, PTH, and calcitonin (37) has also been investigated in children from birth to 18 months of age.
PHOSPHORUS STATUS

Total body phosphorus (P) amounts to about 510 g in men and 400 g in women; about 85% is found in the skeleton and most of the remainder in soft tissues; plasma P is in equilibrium with bone and soft tissue P (38).

Plasma Phosphate

Total P in plasma is composed of lipid P, ester P, and inorganic phosphate (P_i), which is the fraction of interest to the clinical chemist: Its approximate distribution is 85% free orthophosphate (17% H_2PO_4^- and 68% HPO_4^{2-}), 5% complexed with Ca and Mg, and 10% bound to protein (38). Plasma P_i is in the range of 0.97 to 1.45 mmol/liter (3.0-4.5 mg/dl) in adults and 1.45 to 2.1 mmol/liter (4.5-6.5 mg/dl) in children [Latner (29), Chap. 6]; values expressed in milligrams per deciliter refer to the concentration of phosphorus, not to that of phosphate.

There is a general tendency toward a reciprocal relationship between Ca and P_i concentrations in plasma. Plasma P_i is not controlled by a homeostatic mechanism, as is Ca, and shows greater variation. The circadian rhythm of plasma P_i, with minimum values in the morning and maximum values in the evening, underlines the importance of blood-sampling time. Calcitropic hormones do influence plasma P_i, but their principal modulator is the Ca ion: 1,25-(OH)_2D increases intestinal P_i absorption, and PTH decreases renal tubular reabsorption of P_i. Renal excretion is the principal control mechanism of plasma P_i (38).

Inorganic phosphate is measured in plasma with a modification (13) of the Fiske and Subbarow (39) molybdenum blue procedure. Fasting samples should be taken in the morning; hemolysis should be strictly avoided, and plasma or serum should be separated rapidly from the red blood cells.

Plasma P_i tends to decrease during pregnancy; fetal levels are consistently higher than maternal levels: about 5.9 mg/dl in cord blood versus 4.4 mg/dl in maternal blood (36). The influence of sex, race, age, season, and diet on plasma P_i has also been investigated in children from birth to 18 months of age (37).

Plasma P_i is increased in conditions such as hypervitaminosis D, hypoparathyroidism, renal failure, and healing fractures. Hypophosphatemia is observed in rickets and osteomalacia, hyperparathyroidism, Fanconi syndrome, renal tubular acidosis, vitamin D-resistant rickets, celiac disease, sprue, and intravenous administration of glucose (29).

Urinary Phosphate

Urinary P is largely inorganic phosphate and is mostly a reflection of dietary intake (1). Marked circadian variation is observed, which parallels plasma
Pi. Urinary Pi is measured as for plasma Pi, with the molybdenum blue procedure.

Urinary Pi is increased in hyperparathyroidism, vitamin D-resistant rickets, Fanconi syndrome, and certain cases of heavy-metal poisoning. It is decreased in hypoparathyroidism and ordinary rickets.

**IRON STATUS**

Iron (Fe) deficiency is recognized as the most prevalent nutritional deficiency worldwide. The challenge of diagnosing mild cases of Fe deficiency in the community has led to the improvement of existing laboratory tests, particularly by automation, and to the application of additional tests, such as serum ferritin and erythrocyte protoporphyrin.

Excellent reviews of the problems of iron status and status assessment of infants, children, and women have been published (40–44), and a Nestlé Nutrition Workshop held in Manila in 1982 was devoted to iron nutrition in infancy and childhood (40). Dallman and Reeves [Stekel (40), pp. 11–44] have presented an extensive discussion of the laboratory diagnosis of Fe deficiency, which forms the basis of this section. The International Nutritional Anemia Consultative Group (INACG) has contributed excellent reports pertaining to Fe-deficiency anemia in infancy and childhood (ref. 42; French and Spanish translations in 1985), to Fe deficiency in women (ref. 43; French translation in 1983, Spanish in 1985), and to measurements of Fe status (44). For detailed aspects of these problems, the reader is referred to these publications, as well as to the work of Bothwell et al. (45) on iron metabolism in humans.

**Iron Metabolism**

Iron plays an important role in many metabolic functions of the body. As part of heme, Fe takes part in oxygen transport (hemoglobin) and in some tissue functions (myoglobin, cytochromes, catalase). As nonheme Fe, it is found in many enzymes (xanthine oxidase, phenylalanine hydroxylase). The human body contains small amounts of Fe, about 2.5 g in adult women, and 4 g in adult men. For an adult man of 75 kg, with a total Fe content of 3.8 g, about 2.3 g are found in red cells, about 1 g in storage complexes, about 0.5 g in tissues, and about 3 mg circulating in blood, bound to transferrin.

The economy of Fe is very tightly controlled; only 1 to 2 mg Fe is exchanged daily through dietary intake and excretion. An intricate system of recycling has been developed to spare Fe, which is constantly made available during the red blood cell (RBC) cycle (~120 days), to store it and to redistribute it where needed. Surplus Fe is stored in the body as ferritin and hemosiderin. Ferritin represents the soluble mobile fraction, and hemosi-
derin represents aggregated insoluble deposits. The proportions of the two forms of storage shifts toward hemosiderin when large concentrations of storage Fe are present. This stored Fe is readily available when needed for erythropoiesis or for key tissue functions. Ferritin is found in many body tissues and is mostly concentrated in liver, spleen, and bone marrow. Hemosiderin is found mostly in the reticuloendothelial system and in the hepatic parenchyma. For well-nourished adults, Fe stores amount to about 1,000 mg for men and about 300 mg for menstruating females.

Iron is transported in the plasma by a carrier protein, transferrin, which has two binding sites for Fe and is synthesized in the liver. Transferrin binds (ferric) Fe very strongly; its main Fe source is the reticuloendothelial system (destruction of senescent erythrocytes), and its main target is the erythropoietic apparatus.

When dietary intake and absorption of Fe do not cover the body’s requirements, deficiency develops. Three successive phases may be defined for convenience (45). The first phase, Fe depletion, describes the decrease of the body’s Fe stores without impairment of body functions. The second phase, Fe-deficient erythropoiesis, occurs when the Fe stores are depleted, and a reduced iron supply limits erythropoiesis, without overt anemia. The third phase, Fe-deficiency anemia, describes a more severe, prolonged Fe deficiency, with a marked reduction of blood hemoglobin levels.

Depletion of Iron Stores

When dietary intake does not meet body requirements over a certain length of time, depletion of stores develops to meet the demands of erythropoiesis. This is accompanied by a decreased concentration of circulating ferritin, with normal transferrin saturation (TS) and erythrocyte protoporphyrin (EP). When Fe stores are exhausted, TS saturation drops markedly; Fe supply to the bone marrow is limited, and Fe-deficient erythropoiesis develops.

Iron-Deficient Erythropoiesis

Serum ferritin concentration falls below 12 µg/liter (in uncomplicated deficiency), and circulating Fe levels are reduced. Serum Fe is decreased, and total iron-binding capacity (TIBC) is increased, resulting in a marked decline in TS. An increase in EP is observed (see later).

Iron-Deficiency Anemia

In the third, and final, stage of Fe deficiency, hemoglobin synthesis is depressed, generally accompanied by low serum ferritin, depressed TS, and
increased EP. In the early stages, RBCs are normocytic and normochromic; they are progressively replaced by microcytic and hypochromic RBCs.

This simple model is convenient for describing the development of Fe deficiency anemia and for identifying key indices. The analytical and biological variability of the Fe status indices, however, may not allow such a simple classification, since mild Fe-responsive anemia may well be present with normal ferritin or TS or EP (40,46).

Prevalence of Fe deficiency is highest in young children, adolescents, and pregnant women. The first screening tests to be applied are hemoglobin and/or hematocrit and mean corpuscular volume; confirmation tests will include ferritin, TS, and EP. A publication by the INACG describes in great detail the methodology of Fe status assessment (44). Each index is briefly reviewed next.

**Hemoglobin**

Hemoglobin (Hb) is the most frequently used screening test. It is not a very sensitive index for mild anemia, since Hb values for normal and deficient subjects overlap to a large extent. It is not very specific for Fe deficiency either, since anemia may be caused by other nutritional deficiencies (folate and B₁₂ deficiencies), by chronic infection, protein-energy malnutrition, and hemoglobinopathies. According to the method recommended by the International Committee for Standardization in Hematology, Hb is converted to cyanmethemoglobin and quantified spectrophotometrically (44).

For the interpretation of Hb, cutoff values have been recommended by WHO according to age, sex, and physiological state (47): 0.5 to 6 years, <11 g/dl; 6 to 14 years, <12 g/dl; adult men, <13 g/dl; adult women (nonpregnant), <12 g/dl; pregnant women, <11 g/dl. INACG recommends a slightly different age grouping for children and adolescents (44). In a clinical setting, it is recommended that developmental (percentile) curves be used, as reported by Dallman et al. (41). These cutoffs and percentile curves are all valid at sea level; Hb values should be corrected for altitude (~0.3 g Hb/dl increase for each percent decrease of arterial oxygen saturation) before applying the cutoffs (48).

**Hematocrit**

Hematocrit (Ht) is commonly measured with Hb, although both indices reflect similar information. On average, percent Ht is equivalent to Hb in grams per deciliter ÷ 2.9. It has been shown that they are not equivalent in detecting anemia in the same population (49). Hematocrit determination is simple, rapid, and requires little blood; it is performed by microcentrifugation or by calculation with an electronic counter. The INACG recommends the following cutoffs (sea level): 0.5 to 4 years, <32%; 5 to 10 years,
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<33%; 11 to 15 years, <35% for boys and <34% for girls; >15 years, <40% for men and <36% for women (44).

Mean corpuscular volume

When an electronic counter is available, mean corpuscular volume (MCV) should be included in the diagnosis. It may be useful to discriminate Fe-deficiency anemia from other anemias. Thalassemia minor and, sometimes, infectious and chronic diseases are also characterized by low MCV. The cutoff value for adults (both sexes) is 80 fl. Percentile curves for young children (0.5–15 years) have been published by Dallman et al. (41).

Transferrin saturation

In uncomplicated Fe deficiency, serum Fe is decreased and TIBC is increased; their ratio [transferrin saturation (TS)] maximizes the sensitivity. TS is more sensitive than Hb for detecting Fe deficiency. The analysis requires more blood, is time-consuming, and presents higher analytical variability; marked diurnal variation of serum Fe, with high values in the morning and low at night, decreases the precision. Specificity of TS is reduced by the low values found sometimes in inflammatory diseases. TIBC may sometimes discriminate Fe deficiency from inflammation, since TIBC is increased in the former condition (>400 µg/dl) and decreased in the latter. Cutoffs for TS, as recommended by the INACG, are 0.5 to 4 years, <0.12; 5 to 10 years, <0.14; 11 to 15 years, <0.16; >15 years, <0.15 (44).

Iron is measured in serum with or without deproteinization by colorimetric quantification after reaction with a chromogen, such as bathophenantroline or ferrozine (44). TIBC is measured by saturating transferrin with excess Fe, removing unbound Fe with magnesium carbonate, and measuring bound Fe as above (44). An alternative to TIBC is the measurement of transferrin (by immunological methods) with the subsequent calculation of Fe transport capacity, using an experimentally determined factor.

Erythrocyte protoporphyrin

Erythrocyte protoporphyrin (EP) is another useful index of Fe deficiency (50). It represents the final step in the synthesis of heme, just before inclusion of Fe. Deficient supply of Fe to bone marrow will produce an increase of EP. EP is more stable than TS toward changes in Fe status: TS may vary within hours, whereas EP response to Fe deprivation or treatment takes place over weeks. EP values are best expressed in terms of packed red cell volume or Hb concentration. Upper limits of normal, as recommended by the INACG (44), are for 0.5 to 4 years, 2.8 µg EP/g Hb or 80 µg EP/dl RBC; and for >4 years, 2.4 µg EP/g Hb or 70 µg EP/dl RBC. EP is not entirely specific for Fe deficiency since elevated or very high values may be observed.
in the presence of inflammation, lead intoxication, and, in the case of the rare genetic disorder, erythropoietic protoporphyria (45). Developmental changes during the first few months of life have been investigated (51,52).

Total EP is measured by acid extraction followed by fluorometric quantification (53). An alternative method uses a dedicated front-face hematofluorometer, which measures directly zinc protoporphyrin (µg/g Hb) in one drop of blood (54). Detailed descriptions and comparisons of these methods are presented elsewhere (44).

**Ferritin**

Serum ferritin is the most sensitive index of Fe status and is a good indicator of Fe stores: 1 µg/liter serum ferritin is equivalent to about 10 mg Fe stores (50). Serum ferritin levels lower than 10 to 12 µg/liter indicate total depletion of Fe stores. Serum ferritin may be increased, independent of Fe stores, in liver disease, inflammatory and infectious disease, and hemolytic and pernicious anemias (45).

Serum ferritin is measured by RIA, IRMA, or ELISA methods. In the past, normal ranges have varied greatly between commercial kits (55–57). The recent availability of an international standard of human liver ferritin should improve interlaboratory comparisons in the future. Serum ferritin levels during pregnancy and child development have been reviewed (42,43). Deficiency criteria recommended by the INACG are, for 0.5 to 15 years, < 10 µg/liter, and for > 15 years, < 12 µg/liter (44). When dealing with infants, it should be remembered that capillary samples give higher ferritin values than venous samples (58,59).

**Therapeutic trial**

Anemia due to Fe deficiency is the most common form of anemia encountered in clinical pediatrics. If subnormal hemoglobin values are observed, it may be more cost-effective to start a short, 1-month-long therapeutic trial with Fe, as suggested by Dallman and Reeves [Stekel (40)]. Iron supplementation may then be continued for 3 to 5 months if an Hb increase has been observed; otherwise, a more in-depth diagnosis should be performed (40).

The frequent association of anemia with inflammation and infection in the pediatric setting makes it worthwhile to supplement Hb determination with measurement of either erythrocyte sedimentation rate or one of the inflammatory proteins (acute-phase reactants) (60).

**Multiple biochemical criteria**

When working in the community, therapeutic trial with Fe is not practical. Use of Hb as the sole index of Fe status is inadequate, particularly in sit-
ATIONS OF LOW PREVALENCE OF Fe DEFICIENCY (44). INCLUSION OF TWO OR THREE ABNORMAL VALUES OF OTHER INDICATORS OF Fe STATUS (TS, EP, FERRITIN) PROVIDES A BETTER EVALUATION OF PREVALENCE (SEE REF. 44 FOR FURTHER DETAILS AND REFERENCES). ANOTHER APPROACH FOR EVALUATING THE Fe STATUS OF A POPULATION IS TO ESTIMATE THE DISTRIBUTION OF BODY Fe STORES BY USING COMBINATIONS OF BIOCHEMICAL INDICES (Hb, TS, EP, FERRITIN), AS SUGGESTED BY COOK AND FINCH (50) AND BY COOK AND CO- WORKERS (61). THIS MAKES THE DEFINITION OF PRECISE CUTOFFS LESS CRITICAL. THIS MODEL NEEDS FURTHER TESTING AMONG OTHER POPULATION GROUPS.

ZINC STATUS

Zinc (Zn) is found in all human tissues, and the total amount in the body has been estimated at 2 to 3 g (62). Zinc is associated with a large number of proteins and enzymes; many Zn-containing metalloenzymes have been identified.

Trace elements in the nutrition of children were the subject of a Nestlé Nutrition Workshop held in Munich in 1984. Many aspects of Zn nutrition during pregnancy, lactation, and perinatal development were discussed (63). Laboratory diagnosis was briefly reviewed by Prasad (63, pp. 20–21) and by Aggett for pregnancy and lactation (63, pp. 142–144).

Lack of specific and sensitive indices of Zn status has been a complicating factor in the evaluation of the extent of Zn deficiency in the general population and in groups at risk (children and pregnant women). Apart from plasma or serum Zn, many parameters have been proposed, including urinary Zn excretion, hair Zn, RBC Zn, leukocyte Zn, and the activity of some Zn-dependent enzymes (e.g., alkaline phosphatase in neutrophils and plasma, deoxythymidylate kinase in proliferating skin collagen) (63,65).

Serum or Plasma Zinc

Zinc circulates in plasma bound to albumin (60–70%) and α2-macroglobulin (30–40%); a small fraction is complexed with amino acids. Plasma Zn is the most often used index of Zn status; it may not, however, necessarily reflect tissue levels (64). A slowly decreasing level of plasma Zn has been observed in experimental Zn deficiency in humans (65). Changes are slow and small, however, and plasma Zn is not a reliable index of mild deficiency.

Plasma Zn may be affected by nonnutritional factors. In the presence of hypoalbuminemia, it is probably not a good measure of Zn status (66). Infection, inflammation, and acute stress (burns, myocardial infarction, surgery) depress plasma Zn (67). Plasma Zn also decreases during pregnancy (see later) and with age (68). Other conditions accompanied by low plasma Zn include acrodermatitis enteropathica, parenteral feeding without trace
element addition, chronic liver disease, Crohn's disease, and some types of cancer.

Fasting morning blood samples should be taken to minimize the influence of food intake and circadian rhythm. As for all other trace elements, great care must be taken to avoid contamination at all stages of sample handling. Hemolysis must be avoided, since the RBC Zn concentration is much higher than the plasma concentration. Either plasma or serum may be used for analysis (69). Zinc determination is performed by flame or flameless atomic absorption spectrometry (AAS) (70). Reported mean values in the literature vary between about 85 and 110 μg/dl; values below 70 μg/dl are considered low.

**Blood Cells**

Eighty percent of whole blood Zn is found in the RBCs, most of it associated with carbonic anhydrase. RBC Zn may be analyzed by flame AAS following nitric acid digestion. RBC Zn levels have been reported in the range 10 to 14 mg/liter RBC (71). In experimental Zn deficiency, RBC Zn is decreased (65); changes are slow to develop, due to the long life of RBC (120 days). RBC Zn is also reduced in protein energy malnutrition and sickle-cell disease; it has been reported to be normal in the presence of depressed plasma Zn in conditions such as diabetes, some liver diseases, and pulmonary tuberculosis (64). RBC Zn is therefore not a useful index of Zn status.

Leukocyte Zn has also been used to assess Zn status. In experimental Zn deficiency, leukocyte Zn is more depressed than RBC Zn (65). The use of leukocyte Zn as index of Zn status has been reviewed by Patrick and Dervish (72). Zn contents of the different cell fractions are quite different. Methods have been proposed to measure them; the more recent ones using cell separation on gradients of Percoll [Milne et al. (73)] and Ficoll-Hypaque [Purcell et al. (74)], followed by flame AAS, seem to achieve better separation of mononuclear leukocytes, polymorphonuclear leukocytes, and platelets. For healthy male and female adults, Milne et al. (73) report Zn contents of 0.48, 7.4, 5.1, and 1.13 ng/10⁶ cells in platelets, mononuclear and polymorphonuclear leukocytes, and RBCs, respectively (73). Interlaboratory values still differ greatly. Although promising, the value of leukocyte Zn as an index of Zn status still has to be demonstrated.

**Urinary Zinc**

Urinary Zn excretion has also been proposed as index of Zn status. As reviewed by Solomons (64), low urinary Zn excretion has been reported in experimental Zn deficiency, acrodermatitis enteropathica, and in Zn defi-
ciency in the Middle East. Hyperzincuria, however, may coexist with hypozincemia in cases of hepatic cirrhosis, acute viral hepatitis, sickle-cell disease, diabetes, postsurgical periods, and total parenteral nutrition. Moreover, during infection, intrahepatic sequestration of Zn may induce a decrease in Zn excretion. Urinary Zn excretion is therefore only of value in pure Zn deficiency.

**Hair Zinc**

The use of Zn content in hair as an index of Zn nutritional status presents the great advantage of easy, noninvasive sampling. Subject age, sex, hair growth, season, environmental Zn, and hair treatment *in vivo* are all confounding factors that may affect hair Zn concentration. Lack of clearly defined normal ranges seriously complicates interpretation of results. The limitation of trace element analyses of hair, particularly of Zn, has been thoroughly reviewed for the population at large (75,76) and for children (77). The conclusion is that much more work is needed in order to determine to what extent hair Zn may reflect Zn nutritional status.

**Other Tests**

Other approaches for evaluating Zn status include metabolic balance studies, measurement of the activity of Zn-dependent enzymes (e.g., alkaline phosphatase, thymidine kinase), as well as activity changes with Zn supplementation, and immune function (delayed hypersensitivity skin reaction, lymphocyte response to mitogens). These approaches are laborious. The reader may refer to Chandra (63) and Solomons (64) for further information. Zinc tolerance tests have been shown to be unreliable indicators of Zn status (78).

In conclusion, available laboratory methods are still of limited value in evaluating mild Zn deficiency. For children Zn supplementation trials with follow-up of linear growth and food intake remain the best approach, as described by Hambidge [Chandra (63), pp. 5–6].

**Pregnancy and Lactation**

Several reviews of Zn in human pregnancy have been published, and the reader may wish to refer to them for further information and original references (63,79–81).

Plasma Zn decreases during pregnancy, with late pregnancy values 10% to 50% lower than nonpregnant values, depending on the study (80). The decline occurs early in pregnancy and has been reported to extend over the
first two trimesters or over the entire pregnancy. Supplements of 15 to 25 mg Zn/day did not increase Zn levels in several studies (80); alkaline phosphatase did increase with supplementation in some studies (82). Zinc concentration in cord plasma has been reported to be 110% to 200% of maternal plasma concentration.

During prolonged lactation, plasma Zn of unsupplemented mothers has been shown to decrease, compared to that of mothers supplemented with 15 mg Zn daily (83).

Reduced leukocyte Zn concentration in infants with prolonged intrauterine growth retardation (IUGR) has been reported (80). Mothers of IUGR infants were found to have significantly lower polymorphonuclear and mononuclear leukocyte Zn levels at 24 to 48 hr postpartum (84). In the same study, smoking was associated with significantly lower polymorphonuclear leukocyte Zn levels.

REFERENCES


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