
NUTRITION ADVISORY GROUP HANDBOOK



ASSESSMENT OF NUTRITIONAL STATUS OF CAPTIVE AND FREE-RANGING ANIMALS

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The essence of nutritional assessment is to determine the adequacy of the diet so that risk of disease might be limited and productivity and longevity might be enhanced. Knowledge of nutritional status, whether of an individual or of an animal population, is important for evaluation of captive management or quality of the wild habitat. This technical paper reviews some of the techniques for assessing nutritional status and the challenges those assessments present.

Methods of Nutritional Assessment

To be useful, the methods used for nutritional assessment must be accurate and reproducible, within sustainable cost and convenience limits, and should identify small but significant changes in nutritional status.⁴² Related factors, such as genetic differences, homeostatic regulation, diurnal variation, stress of capture, infectious disease, and others must be considered because they influence the specificity of measurements and, in some cases, render them useless.

Several methods have been used. These include: (1) determination of nutrient intakes and evaluation of dietary husbandry, (2) measurement of anthropometric features and assignment of body

condition scores, (3) measurement of body fat as an estimate of energy reserves, (4) biochemical analyses of body fluids and tissues, and (5) clinical evaluation and postmortem examination.³¹ These techniques have been used with varying degrees of success, but interpretation of the findings is greatly limited by uncertainty concerning their meaning and lack of adequate reference data.

Considerable effort has been directed toward assessment of the nutritional status of humans and domestic and laboratory animals, but early work with wild animals was limited primarily to free-ranging cervids.¹⁹ More recent studies also involve birds, fish, reptiles, and other mammals. The principal indices of nutritional status in free-ranging animals have been measures of body fat (or energy) stores, although a number of papers include analyses for other substances.² Several techniques used successfully with humans have not been applied to wild animals due to high cost, limited availability and portability of instruments, and logistic difficulties in moving wild animals into specialized laboratories.

Determination of Energy & Nutrient Intakes & Evaluation of Dietary Husbandry

A measure of food intake provides the baseline for estimates of energy and nutrients potentially available to the consumer. For captive animals, setting the baseline is relatively simple and requires measurement of food consumption and composition. For free-ranging animals, gathering this information is more difficult, and there are many variables to consider. Direct observations of feeding behavior,⁵⁶ or examination of crop contents, stomach contents, or scat samples can provide insight into food item selection but not necessarily into the actual diet consumed. Also, these methods are time-consuming, are potentially limited to a single or a few observations of the same individual, may not account for rare food consumption events, and, at best, provide a limited amount of quantitative information regarding the diet.^{6,33,39} Diets in the wild often are characterized with regard to the types of foods consumed, but data describing the amounts consumed and the nutrient composition of that food are infrequently collected. Additionally, even if quantitative energy and nutrient intakes are determined, they provide little information about the proportions of ingested energy and nutrients that are retained.

Some energy is lost during digestion as combustible gases, as heat, and as undigested organic matter. Significant proportions of ingested nutrients also are lost in the feces. Likewise, not all of the absorbed energy and nutrients are retained, and there are measurable losses in the urine. Apparent digestibility and metabolizability of dietary energy and nutrients can be determined, but the facilities and techniques required are complicated and expensive, and wild animals do not readily adapt to this research environment. As a consequence, it is frequently necessary to make assumptions about digestive and metabolic efficiency from studies of model animals with similar gastrointestinal anatomy and physiology and eating similar foods.

Evaluation of the dietary husbandry of captive animals includes not just available nutrient supply but also consideration of the physical form of the diet, its suitability, and where and how frequently it is provided relative to the normal foraging behavior of the species. Special attention is required to ensure that all members of mixed-species exhibits are properly nourished. Related issues, such as the method of feeding and psychological well-being, dietary form and oral health, and feeding sites and times that will minimize animal conflicts, parasitism, and transmission of infectious disease, deserve high priority.

Anthropometric Measurements & Body Condition Scoring

The nutritional status of an animal can influence the physical dimensions and gross composition of the body. Systematic and objective visual appraisal of an animal can provide insight into the nutritional condition of that animal and the quantity and quality of its food supply, especially when comparative differences are large.^{11,15,21,45} Scoring systems based on body shape and prominence of skeletal features have been developed for several species and have been found useful in judging the adequacy of energy supplies. However, considerable change in fat reserves can occur without altering the external appearance of an animal, and small changes may be difficult to detect.¹⁵

Measurements of body mass, height, length, and/or girth in relation to age, sex, and physiologic state also can provide information about nutritional condition. For captive animals that are readily handled, many of these measurements are easily collected. However, for less tractable captives and their free-ranging counterparts, these measurements require physical or chemical restraint. Although body masses of captive and free-ranging animals have been measured,^{15,34} such measures should be performed on several animals more than once to account for variations between individuals and in the mass of food consumed and excreta voided. Heart girths coupled with other measurements^{5,24,55} have been used with varying success to estimate body mass and nutritional status. Other measures include antler beam diameters in cervids,⁴¹ feather dimensions in birds (ptilochronology),¹⁷ and distances between concentric rings on fish scales or in otoliths.⁷

Measurement of Body Fat as an Estimate of Energy Reserves

Measurements of total body fat provide an estimate of body energy stores, and over time allow for identification of accretion or depletion in response to differing energy intakes. Direct measures of body fat, such as kidney fat index, marrow fat, or gizzard fat, are invasive and may preclude subsequent samples on the same individual. Nevertheless, such techniques can be useful for assessing the nutritional status of populations.^{35,53} There is, however, potential for such measures (and those that follow) to be confounded by physiologically normal seasonal changes in the efficiency of dietary energy use, such as short-day induced fattening in preparation for winter in temperate zone cervids. Thus, such data must be interpreted with caution.^{1,26} Measurement of fat cell diameter (from biopsies of fat tissue) assumes that increases in body fat stores influence adipose cell size.⁴⁴

Several indirect measures of body fat have been used.^{14,43} These include ultrasound devices generating high-frequency sound waves that pass through skin and subcutaneous adipose tissue and reflect back from the adipose tissue-muscle interface, thus measuring subcutaneous fat thickness. Other indirect measures used in wild animals involve measurements of bioelectrical impedance of the body to transmission of a weak electrical current, and estimation of the body water pool by deuterium dilution, the former positively related, the latter inversely related to body fat. Although requiring expensive equipment, an inverse relationship has been demonstrated between body fatness and total body electrical conductivity in an electromagnetic field. Computerized tomography (CT), producing collimated X-ray scans, can be used to estimate the volume of fat-free and fat tissue in the body. Other methods include dual-energy X-ray absorptiometry (DEXA) and magnetic resonance imaging (MRI). All these non-invasive techniques provide more insight into the energy status of an animal or group of animals than simple measures of body mass or dimension, yet do not identify specific nutrient deficiencies or excesses. Thus, for more complete characterization of nutritional status, these techniques must be coupled with others.

Biochemical Analyses of Body Fluids and Tissues

Measurements of the concentrations of a nutrient, its metabolites, or related biomarkers in blood, urine, or tissues are commonly used in nutritional assessment.⁴⁸ Ideally, the information derived will provide an estimate of the total body content of a nutrient or the size of the tissue store that is most sensitive to depletion. However, nearly all measures have their limitations. Certain methods provide valid estimates of nutrient status for selected nutrients, but a variety of factors influence the usefulness of others, confounding their interpretation. Some of these factors include effects of species, sex, age, geographical location, season, year, habitat, capture and/or handling methods, reproductive status, disease, and dietary concentrations of interacting nutrients.¹⁵ In addition, depending upon the analysis desired, samples need to be collected and handled according to specific protocols to avoid nutrient degradation, metabolic conversion, or migration between cellular and fluid compartments.

Nutrient levels in blood, urine, or tissues may be difficult to interpret when based on a single assay of a single nutrient. Increases or decreases in nutrient levels may be a consequence of various disorders which can be accurately delineated only after clinical examination or after assessment of the intakes of other nutrients that are interactive with the nutrient in question. For example, elevated liver iron concentrations have been observed under conditions of high bioavailable iron intake, copper deficiency, high ascorbic acid intake, high citric acid intake, or chronic infection.^{16,52,61}

Concentrations of nutrients in urine tend to reflect recent nutrient intakes, and for some nutrients, urinary excretion may decline with decreased intakes considerably before body stores are depleted. Urine volume, and thus the concentration of urine metabolites, can change with the environment, intakes of water, and the type and quantity of food consumed. Use of a common urinary metabolite, such as creatinine, as an internal standard assumes that daily urinary creatinine excretion is constant for a given individual, and is related to muscle mass. Presumably, expression of the concentration of a nutrient, metabolite, or related biomarker in urine as a ratio to the concentration of creatinine will correct for diurnal variations and fluctuations in urine volume.¹³ Unfortunately, repeated urine collections from individual humans have shown that the coefficient of variation of daily creatinine excretion may range from 1 to 36%.⁶⁰

While physiologic samples may be systematically collected and appropriately handled, not all analyses are valid. Some biochemical tests differ considerably in their reproducibility. Nutrient levels may vary from sample to sample and reflect recent rather than long-term intakes. Biological fluid levels and function tests may vary even among similar individuals, consuming similar diets, and suffering from equally apparent degrees of nutritional depletion, suggesting that certain measures are individually characteristic. Thus, it may be necessary to examine samples from several individuals in a wild ecosystem or in a captive management program to accurately assess the adequacy and safety of the nutrient supply for that species.

Two types of tests are generally employed when analyzing body fluids or tissues: (1) static measurements of nutrient concentrations in fluids and tissues, and (2) functional measurements of secondary or tertiary metabolites or enzyme activities that are influenced by nutrient supply. As an example, a static test may identify low current thiamin intakes by finding very low thiamin concentrations in urine, whereas a functional test may find low transketolase activity in red blood cells, reflecting significant tissue thiamin depletion.

Biochemical analyses are available for many nutrients and related biomarkers in the body, and some of these follow. This list is long, but not exhaustive. When deciding which to use for any nutrient, one should critically examine the method for validity, accuracy, precision, sensitivity, specificity, and error rate. For some situations, it may be wise to be selective and to use specific biochemical techniques for the nutrient that is most likely to be the problem. For others, a broad biochemical screening may be required to identify the principal problem. In addition, some assays are much more expensive than others. It also may be appropriate to save and store samples for future evaluation when newer, more technologically advanced techniques might be available. Usually a combination of biochemical and physical assessment methods will prove more useful than biochemical methods alone.

Protein

Protein deficiency produces a decrease in serum protein levels, especially serum albumin, but these decreases are not particularly sensitive indices, nor are they specific for protein deficiency. Also, serum protein concentrations may be maintained for a considerable time, despite an extended period of inadequate protein intake. Many methods have been used to assess protein status, including urinary creatinine excretion, urinary 3-methylhistidine excretion, and serum concentrations of total protein, albumin, transferrin, retinol-binding protein, thyroxine-binding pre-albumin, somatomedin-C, and others.^{16,31}

Total serum albumin and determinations of other serum proteins are standard clinical procedures. Serum albumin, with a half-life of over 14 days, is not sensitive to short-term changes in protein status. It also can be affected by altered metabolism, specific deficiencies of amino acids, pregnancy, lactation, changes in capillary permeability, stress, fluid loss, inflammation, and strenuous exercise.²⁸ Although total serum protein is easily measured, it, too, is rather insensitive to dietary changes. It appears to be significantly depleted only in cases where clinical signs of protein malnutrition are already apparent.¹⁶ There is little question that protein-depleted individuals have impaired immunocompetency, but other nutrient deficiencies also produce such a deficit.

Blood urea nitrogen (BUN) appears not to be influenced by capture-related stress or gender, and is commonly used to assess protein status of simple-stomached animals. However, in ruminants, BUN alone is not an adequate indicator of protein status since energy level in the diet affects nitrogen reutilization.²⁹ For this reason, techniques to assess both protein and energy status must be used.

Taurine

A few animals (including domestic cats and possibly other felids and some neonatal primates) have a dietary requirement for the amino acid, taurine. In other species, it appears to be synthesized in the body from cysteine. For animals requiring exogenous sources of taurine, serum taurine may be a valuable determinant of status. The method involves high-pressure liquid chromatography (HPLC) in a procedure that is specific for several amino acids, often using an amino acid analyzer. Thus, concentrations of several amino acids may be determined simultaneously with taurine. Analyses of liver tissue for the presence of enzymes involved in the conversion of cysteine to taurine (such as cysteine sulfinic acid decarboxylase) may characterize the animal's capability for taurine synthesis and, thus, the need for a dietary source.³¹

Cholesterol, triacylglycerol, and lipoproteins

Analyses of serum cholesterol, triacylglycerol, and lipoproteins are not particularly useful in assessing energy status, but concentrations of these substances are influenced by dietary energy source.^{8,18,31} Low-density lipoprotein (LDL) cholesterol concentration is a valuable biomarker for risk of cardiovascular disease in humans, and may be of comparable importance for other species. This measure is of interest when comparing nonhuman primate values to those for humans or when comparing values for captive vs. free-ranging animals and proposed links with diet. Furthermore, in studies of large human populations, serum LDL-cholesterol concentrations have been correlated with total cholesterol concentrations. Thus, epidemiologic studies tend to link elevated total serum cholesterol concentrations with cardiovascular disease. Saturated fatty acids in the diet of humans increase total serum cholesterol and LDL-cholesterol and have been assigned an atherogenic potential. However, the effect seems largely restricted to the saturated fatty acids lauric (12:0), myristic (14:0), and palmitic (16:0), and the *trans* monounsaturated acids. Stearic acid (18:0) and the *cis* monounsaturated oleic acid (18:1) appear not to raise serum total cholesterol or LDL-cholesterol concentrations. Dietary carbohydrate, like oleic acid, does not raise total cholesterol or LDL-cholesterol, but does promote an increase in triacylglycerol levels and a decline in high-density lipoprotein (HDL) cholesterol. Triacylglycerol levels, like total cholesterol levels, have been positively related to risk of cardiovascular disease. Since unsaturated fatty acids appear not to have an atherogenic effect, but high consumption promotes obesity, additional research is needed to define the appropriate proportion of unsaturated fatty acids and carbohydrate in the human diet. The relevance of these findings to wild animals remains to be established.

In some domestic animals, hyperlipidemia has been noted after consumption of a high-fat meal, so serum samples, if taken for the above measurements, should be obtained under standard conditions or after fasting. This also is true for canids and felids even though most healthy dogs and cats appear not to exhibit hyperlipidemia when fed high-fat diets.⁸ The ruminant, however, normally consumes a low-fat diet, does not empty the rumen, and has a prolonged gastrointestinal emptying time, so a true fasting sample cannot be obtained.

Calcium (Ca)

Serum Ca concentrations are homeostatically regulated and remain remarkably constant over a wide range of Ca intakes.^{4,16,31,46} Low levels occur only after prolonged Ca deprivation or as a consequence of other pathology. For example, hypocalcemia may be seen in hypoparathyroidism, hypomagnesemia, or acute pancreatitis. High levels (hypercalcemia) may be seen in vitamin D intoxication, hyperparathyroidism, hyperthyroidism, or as a consequence of extended immobilization. In the latter case, Ca from atrophying bone is released into body fluids. Serum, rather than plasma, should be used for Ca assays because many anticoagulants react with Ca and thus introduce a hypocalcemic artifact.

Slightly more than half of the Ca in human plasma is bound, mostly to albumin, with smaller amounts complexed with citrate, bicarbonate, and phosphate. Somewhat less than half of plasma Ca is ionized, and attempts have been made to use this fraction to define disturbances in Ca metabolism.¹⁵ It can be measured with ion-specific electrodes, but samples need to be collected and stored anaerobically, and it may be necessary to adjust pH to a constant value before measurement. Declines in ionized serum Ca concentration may be seen in hypoparathyroidism and in vitamin D-deficiency rickets before declines in total serum Ca become apparent. However, there are a number of factors that can confound ionized Ca measurements, including elevated serum concentrations of Mg or Na.

Other methods, such as photon absorptiometry, have been used to evaluate bone mass or bone mineral density, and have proven particularly useful in identifying osteoporosis in humans. Although osteoporosis is a multifactorial disease, increasing Ca intake is an integral feature of both prevention and treatment. Photon absorptiometry should also have potential for noninvasive characterization of Ca status in other species, since bone density is highly dependent upon long-term Ca supply.

Because the vitamin D supply is so closely linked to Ca absorption and metabolism, determination of vitamin D status will assist in delineating Ca problems more clearly.⁴⁶

Phosphorus (P)

Determination of the inorganic P concentration in plasma or serum is the most common means of evaluating P status.⁴ However, in the human body, inorganic P comprises only about 52% of the P in blood, and blood P comprises only about 1% of the total body supply. In addition to a simple dietary P deficiency, numerous other factors affect plasma inorganic P concentrations.⁹ These factors include excessive dietary Ca, which interferes with P absorption, and inadequate supplies of the vitamin D metabolite, 1,25[OH]₂D₃, that promotes P absorption in the intestine, promotes bone resorption and release of Ca and P into the plasma, and suppresses parathyroid hormone secretion, thus enhancing P reabsorption in the renal tubules. Faulty renal P conservation can also be a consequence of renal disease, acute respiratory and metabolic acidosis, and abnormal concentrations of parathyroid hormone, calcitonin, and certain steroid hormones.⁴⁶ Hypophosphatemia can be seen without cellular phosphate depletion following glucose or fructose administration in certain nonruminant animals or in response to heavy feeding after starvation. In these instances, there are major shifts of P from extracellular fluids into the cells. Hemolyzed serum or plasma should not be used for inorganic P determinations because red cells contain 17 times more P than plasma.

Magnesium (Mg)

Insufficient dietary Mg will eventually lead to low circulating Mg. While it is the fourth most common cation in the body, Mg in plasma represents less than 1% of total body Mg, whereas the skeleton may contain over 60%. Homeostatic regulation of Mg balance is largely dependent upon the amount ingested and upon intestinal and renal absorption and excretion. These processes may be influenced by secretions of certain endocrine organs, such as the parathyroids, and by dietary concentrations of Ca, P, K, nitrogen, and certain organic acids. Bone Mg is moderately labile in young animals but quite stable in adults. Total and ultrafilterable (ionized) Mg levels in plasma or serum have been used to assess Mg status. Total serum Mg, as determined by atomic absorption spectrophotometry, has been most commonly used. However, because protein-bound Mg concentrations are subject to variations associated with changes in serum albumin supply or acid-base conditions, it has been proposed that an assay of ionized Mg using an ion-selective electrode is a more relevant determinant of Mg deficiency.³ There are few reference data for this measure in domestic or wild animals. It should be noted that impaired bioavailability of Mg has resulted in Mg deficiency in domestic ruminants grazing lush, early-growth pastures.^{40,46} Serum rather than plasma should be analyzed because anticoagulants may be contaminated with Mg. Hemolysis also must be avoided because red cells contain three times more Mg than does plasma.

Urinary Mg excretion over 24 hr is considered a useful measure of Mg status in humans who are otherwise normal, although three consecutive 24-hr collections have been recommended to minimize diurnal and day-to-day variations.

Sodium (Na)

Plasma Na concentration is controlled by renal glomerular filtration and tubular reabsorption.^{9,20} Water balance is closely related to body balance of this electrolyte, and extracellular fluid volume and sodium concentration are closely linked. Extracellular fluids contain about $\frac{1}{2}$ / $\frac{2}{3}$ of body Na. Bone contains the majority of the remainder. Na is linked with Cl in regulating acid-base balance. Blood assessment of Na status is limited in usefulness because of precise homeostatic control. Thus, urinary excretion and creatinine clearance ratios have been used to provide a general gauge of daily intake or of a deficiency of Na and other electrolytes. Alterations in plasma volume, as determined by packed cell volume and total plasma protein concentration, are used to identify changes in fluid and electrolyte concentration but are nonspecific for assessing the status of individual circulating minerals.

Chloride (Cl)

The state of body hydration and changes in water balance, influence the circulating status of Cl.^{9,20} In the body, Cl is closely linked with Na balance and acid-base regulation. Thus, unless there is an electrolyte:water imbalance, Cl levels remain relatively constant. Circulating Cl levels are not useful determinants of Cl status. As noted for Na, urinary Cl excretion and creatinine:Cl clearance ratios have been used to provide a general gauge of daily intake or of a deficiency of Cl.

Potassium (K)

K is the most abundant cation in the body. It is primarily intracellular, with 98% of the body's K supply so located. However, small deviations in extracellular concentrations of K can have serious consequences, such as sudden death due to cardiac dysfunction. K metabolism is coupled with Na in its movement into and out of most cells, and there is a precise homeostatic balance of K between intracellular and extracellular fluid compartments. Serum K concentration is influenced by all the factors that influence fluid exchange in the body, including dietary electrolyte intake, excretion, renal function, and acid-base balance.⁹ Thus, measurements of circulating K concentration do not necessarily assess K status. It is noteworthy that the Irish prior to the Potato Famine of 1848 were sustained by a diet consisting almost entirely of potatoes. This diet provided about 20-40 g of K/day. In contrast, the single ingestion of 25 g of K by a person who has been consuming a normal diet providing 2-4 g of K/day is associated with a life-threatening toxicity. Adaptation to a high-K diet by the Irish was apparently a consequence of an enhanced renal capacity to excrete K, allowing them to tolerate potentially lethal intakes. One might conclude, therefore, that urinary K concentrations or creatinine:K ratios might be useful in assessing intakes in relation to need. Whether this conclusion is correct requires testing.³⁸

Iron (Fe)

Microcytic, hypochromic anemia is a common sign of iron deficiency. Identification and characterization of this anemia requires determination of blood hemoglobin concentration, hematocrit (% packed red blood cell volume), and red cell counts. Red cell indices, such as mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration, are used to characterize the anemia.¹⁶ However, declines in hemoglobin and hematocrit values and red cell counts are fairly insensitive measures, and altered concentrations may not be detected until advanced stages of iron deficiency. In addition, iron depletion is associated with an increase in plasma iron-binding capacity, due to increased circulating concentrations of transferrin (an iron carrier), and a decline in the percent of transferrin molecules that are associated with iron (% saturation).

It appears that plasma or serum ferritin (an iron storage compound) concentrations may most sensitively reflect body iron stores and can identify deficiency, excess, or normal status. Although serum ferritin has been measured in many species, including dogs, cows, pigs, rats, horses, and cats, the test remains species specific and difficult to adapt to other species because the assay is an antibody-driven reaction (radioimmunoassay and enzyme-linked immunosorbent assay [ELISA]).⁵² Another ELISA test with similar limitations but with considerable promise is designed to measure serum concentrations of transferrin receptors. Serum concentrations increase with advancing tissue Fe depletion and appear to be unaffected by infections and inflammation.⁴⁸

Protoporphyrin is a heme precursor that normally exists in red cells in low concentration. However, when iron stores are nearly depleted, protoporphyrin IX concentrations in developing red cells increase because iron supplies are inadequate to complete production of the heme in hemoglobin. As a consequence, erythrocyte protoporphyrin IX concentrations have been used to assess Fe status. Direct measurements of iron in liver biopsies also have been made.

Copper (Cu)

One of the signs of Cu deficiency is microcytic, hypochromic anemia, believed to be due to the need for ceruloplasmin (a Cu-containing protein) to oxidize ferrous Fe to ferric Fe prior to its mobilization from Fe storage sites and its transport via transferrin to hematopoietic centers for hemoglobin synthesis. Blood Cu concentrations have been used as a first approximation of Cu status, but Cu concentrations in plasma or serum differ among species and among individual members of a species. In addition, plasma Cu concentrations have been shown to vary with age, exercise stress, and health status. In human pregnancy, plasma Cu levels nearly double just before parturition. Ceruloplasmin concentrations in the serum of vertebrates represent 60-95% of total serum Cu and also have been used to assess Cu status. Although still a subject of debate, it appears that ceruloplasmin has a role as a transport protein, moving Cu from the liver to functional sites in extrahepatic organs. Whether serum Cu or ceruloplasmin concentrations are assayed to assess Cu status, conclusions should be confirmed by the response to judicious supplementation with Cu. Promising, but not proven, are measurements of the activity of a red blood cell Cu-dependent enzyme, superoxide dismutase. The validity of using hair Cu concentrations as an assessment method is questionable because of the large number of factors other than Cu that affect values.^{16,22}

Zinc (Zn)

There is no simple, reliable biochemical measure of Zn status. Plasma or serum Zn concentrations remain the principal diagnostic measures, but they are seriously impaired by their response to various stressors. In addition to an association with consumption of markedly Zn-deficient diets, low plasma or serum values are seen in a wide range of infectious diseases and other stressful conditions.^{10,16}

In rats, metallothionein concentrations in plasma appear to depend largely on metallothionein concentrations in the liver, and they decrease in Zn deficiency and increase with stress. Thus, a low plasma value for both Zn and metallothionein would indicate Zn deficiency, whereas a low plasma Zn value and a high plasma metallothionein value would be characteristic of stress. Zn-deficient plasma also has been shown to contain an inactive form of thymulin. This form can be reactivated *in vitro*, and the difference in thymulin activity between unmodified and Zn-supplemented plasma may have diagnostic potential.¹²

Plasma alkaline phosphatase activity declines in Zn deficiency and is easy to assay, but its activity is influenced by many other factors.

Zn is lost from the body mainly in the feces, although urinary Zn concentrations may decline markedly in Zn deficiency. However, the variability of urinary Zn levels and their sensitivity to contamination makes them of limited value. Concentrations of Zn in hair also have been examined, but the rate of hair growth, length of hair, and the distance between the point of excision and the hair follicle influences the Zn-intake period which should be compared with hair Zn concentration. Additionally,

hair colors differ in their normal Zn concentration, and all hair samples are subject to contamination from the environment.¹⁶

Tentative conclusions concerning Zn status should be confirmed by the response to judicious use of a Zn supplement.¹⁰

Manganese (Mn)

Mn is found in the environment in quantities similar to Fe and Cu, but its concentration in animal tissues and fluids is much smaller. As a consequence, contamination of samples is very likely. In addition, analytical techniques are required that are very sensitive and of limited availability. Neutron activation analysis is the most reliable and sensitive, although atomic absorption spectrometry using a graphite furnace rather than a flame has been used with some success. Mn is rather uniformly distributed in the soft tissues, with the liver having the highest concentration. The skeleton accumulates substantial amounts, but there appears to be no mechanism for Mn release. The most sensitive noninvasive methods for assessment of status include analysis of serum, urine, or lymphocyte Mn concentrations, and determination of serum Mn-dependent superoxide dismutase activity. Evaluations of Mn status are seldom performed because of the difficulties described above and the low requirement for available Mn. In many species, this requirement may be as low as 1-2 mg/kg of dietary dry matter, and in poultry about 10 mg/kg. The higher NRC poultry requirements of 20 mg/kg for mature birds and 60 mg/kg for young, rapidly growing chicks are based on the poor bioavailability of Mn in many natural dietary ingredients.³⁰

Cobalt (Co)

Co is an integral part of the vitamin B₁₂ molecule and is assigned a separate essential nutrient role only in ruminants, in which Co is incorporated into vitamin B₁₂ by ruminal microorganisms. Assessments of Co status in sheep have involved measurements of serum concentrations of Co, which decrease in Co deficiency, and of serum homocysteine and methylmalonic acid, which increase in Co deficiency because of the need for B₁₂ to further their metabolism. Assays for Co require atomic absorption spectrometry, using a graphite furnace, or neutron activation analysis.⁵⁴

Iodine (I)

Goiter is an enlargement of the thyroid gland from any cause and is not limited to I deficiency. Urinary I relative to urinary creatinine excretion is a potentially useful index of I intake and has been commonly used in studies of human populations. However, variations in daily creatinine excretion make this technique less suitable for evaluation of I status of an individual. Other biochemical determinants of I deficiency include plasma concentrations of thyroid-stimulating hormone (TSH), which increase, and of tetraiodothyronine (T₄), which decrease. Radioactive iodine uptake by the thyroid gland also has been measured, and is more rapid and more complete in the iodine-deficient subject.^{16,31}

Selenium (Se)

Assessment of Se status is complicated by the varying sensitivities of the criteria chosen, by factors other than Se that influence the Se requirement (such as vitamin E supplies), by the form of Se consumed, and by historical Se intakes that may support greater or lesser reserves at the time Se status is assessed.⁵⁸ It is very difficult to establish Se status of an individual with certainty unless prior dietary history is taken into account. Much of tissue Se is very labile, and following transfer from Se-adequate or seleniferous diets to those that are low in this element, Se losses from the body are rapid initially and then slower. The metabolism of dietary Se presented as selenomethionine is different than that of the inorganic salts, selenite or selenate, that are commonly used as dietary Se supplements in Se-deficient regions. The urine is a major pathway of Se excretion for selenomethionine but is less so for inorganic forms.

Se concentrations in plasma, serum, or whole blood of animals fed diets containing organic Se forms (such as selenomethionine in plants) tend to increase as Se intakes increase. Se concentrations in plasma or serum of animals fed diets containing selenite or selenate tend to increase as inorganic Se intakes increase from deficient to barely adequate levels. Small increases in inorganic Se intakes throughout the adequate range tend to result in a plateau or slow rise in plasma or serum Se levels. When inorganic Se intakes begin to considerably exceed need, plasma or serum levels once again rise rapidly. Whole blood and red blood cell Se concentrations tend to follow the pattern of plasma or serum in response to increasing intakes of inorganic Se, except there is a lag phase, presumably associated with the long half-life of erythrocytes and Se incorporation into new cells during erythropoiesis. Urinary Se excretion increases remarkably as a percentage of Se intake when dietary inorganic Se concentrations exceed need.

Measurements of the activity of Se-containing enzymes, such as glutathione peroxidase (GSHPx), in whole blood also have been used to assess Se status. Whole blood GSHPx activities are highly correlated with whole blood Se concentrations in animals of low Se status, but when dietary Se intakes are adequate to high, these correlations are poor.¹⁶ Measurements of Se in hair and toenails of humans have been made, but incidental exposure to Se from the soil or products used in medications or washes may invalidate the determinations.

Vitamin A and carotenoids

Plasma or serum vitamin A (primarily retinol) concentrations have long been used as criteria of vitamin A status. These measures have proven useful in human studies when plasma or serum retinol concentrations were very low (<10 µg/dl) or very high (> 100 µg/dl). Very low levels are indicative of depletion of vitamin A reserves, and very high levels are suggestive of vitamin A intakes exceeding need. In normally nourished humans, ~90% of the vitamin A reserve is found in the liver as retinyl esters, and the liver also appears to be a major site for vitamin A storage in many other species.^{16,37} When liver reserves of humans (expressed as retinol) are adequate but not excessive (≥ 20 to ≤ 520 µg/g), plasma vitamin A concentrations tend to be homeostatically controlled in each individual at a level that is largely independent of total body reserves. Thus, there may be no significant difference in liver vitamin A reserves between two individuals with plasma retinol concentrations of 25 µg/dl and 50 µg/dl, respectively. In controlled depletion studies with swine that were initially vitamin A replete, liver vitamin

A concentrations (determined by biopsy) reached very low levels before evidence of depletion was evident in declining serum vitamin A concentrations. Liver samples can be obtained at necropsy or by liver biopsy. The latter involves some risk, as with all surgical procedures.²⁷

Elevated levels of retinyl esters in relation to free retinol are seen in the serum of humans when vitamin A intakes exceed the storage capacity of the liver, and may indicate that the body is converting excess vitamin A to a less toxic form.^{16,31} Since transient increases in serum retinyl esters also occur after ingestion of a vitamin A-rich meal or a vitamin A supplement, fasting blood samples should be used for assays.³⁷ However, it should be noted that in many carnivores, retinyl esters normally appear in the plasma in concentrations equal to or greater than those of retinol.

Radioimmuno- or electrophoretic assays of retinol-binding protein (RBP) have revealed declining plasma concentrations of total RBP when vitamin A intakes are low, and also a declining proportion of RBP bound to retinol (*holo*-RBP) as compared to unbound RBP (*apo*-RBP) or to total RBP. The relative dose response in the proportion of retinol bound to plasma RBP, when a vitamin A load is administered, also has been used to estimate vitamin A reserves.³⁷

Clinical tests that have been used to assess vitamin A status include measures of visual dark adaptation, and impression cytology of buccal or conjunctival surfaces that detects early decreases in the goblet cells as a consequence of vitamin A deficiency.¹⁶

It should be noted that, in addition to responding to vitamin A status, plasma vitamin A levels may be lowered by protein/calorie malnutrition, infections, and some parasites, and may be increased by estrogenic contraceptives and by kidney disease. Additionally, humans and a number of other species, absorb, circulate, and store carotenoids.⁵¹ It has been demonstrated that about 50 of approximately 600 carotenoids exhibit provitamin A activity, and certain of these can be converted to vitamin A in some species. Thus, identification and determination of carotenoids in the serum of species that consume, circulate, and store carotenoids can serve as a secondary index of vitamin A status.³⁷

Vitamin D

Vitamin D synthesis in the skin (upon exposure to solar irradiation or to appropriate wavelengths of artificial UV light [see Fact Sheet 002]) has been observed in most species studied. Thus, vitamin D deficiency is unlikely in most animals, if exposed to adequate sunlight, even when dietary vitamin D concentrations are low. However, cats and dogs appear to have limited epidermal vitamin D synthesis in response to solar irradiation and depend largely on dietary supplies.²⁵ It is possible, but unproven, that other felids and canids may share this characteristic.

Elevated serum alkaline phosphatase levels may be indicative of vitamin D deficiency, but such elevations are seen also in deficiencies of calcium and phosphorus. If vitamin D deficiencies are sufficiently extreme, serum concentrations of phosphorus and calcium decline, although the precise homeostatic regulation of calcium metabolism limits the likelihood that measures of this element in serum will be diagnostically useful.¹⁶

Measurements of serum vitamin D or its metabolites have been used to assess vitamin D status, and when appropriately conducted, can distinguish between D₂ and D₃ forms.⁵⁹ Vitamin D₃ in human blood has a half-life of about 24 hr, and serum concentrations tend to reflect the most recent exposure to sunlight or the most recent oral intakes of vitamin D₃. Calcidiol (25-hydroxycholecalciferol or 25[OH]D₃) in human blood has a half-life of about 3 weeks, and serum values tend to reflect the sum of

vitamin D₃ intakes and vitamin D₃ photobiogenesis over several weeks or months. The half-life of calcitriol (1,25-dihydroxycholecalciferol or 1,25[OH]₂D₃) in human blood is 4-6 hr. Because 25[OH]D₃ is so efficiently converted to 1,25[OH]₂D₃, a vitamin D-deficient individual who ingests a very small amount of vitamin D₃ or who has a very brief exposure to sunlight immediately pretest may have low, normal, or high serum concentrations of 1,25[OH]₂D₃ with low or undetectable concentrations of 25[OH]D₃. Detailed studies of the half-lives of vitamins D₂ or D₃ and their metabolites have been conducted in few other species. Nevertheless, serum concentrations of 25[OH]D (individually or the total of D₂ and D₃ forms) are commonly considered most useful in assessing vitamin D status.⁴⁷

It should be noted that, in addition to dietary vitamin D intakes and solar exposure, circulating concentrations of vitamin D metabolites may be influenced by disorders of the intestine, liver, kidneys, and parathyroids, certain drugs, alcohol, age, sex, and species.

Vitamin E

Vitamin E is a generic term for a group of eight, naturally occurring compounds (α -, β -, γ -, and δ -tocopherol, and α -, β -, γ -, and δ -tocotrienol) exhibiting qualitatively the biological activity of α -tocopherol. Because α -tocopherol is the most abundant vitamin E compound in animal tissues and has the highest vitamin E activity, it is common to assay for this compound rather than to perform the more difficult separation and quantification of all eight compounds.³¹

Retention of α -tocopherol in the liver shows a logarithmic response and has been used in bioassays. Turnover rates are rapid, and large amounts are not retained for extended periods. Consequently, there is a relatively high correlation among plasma, dietary intakes, and liver levels of α -tocopherol. However, there are major differences among species in normal circulating α -tocopherol levels, and different animals of the same species tend to exhibit individually characteristic plasma α -tocopherol concentrations.⁵⁰ If these issues are not considered and a single plasma sample is taken from only one individual, a diagnosis of vitamin E deficiency should be confirmed by other means.

Since plasma tocopherol levels may be correlated with total plasma lipids in humans whose plasma lipid levels range from hypo- to hyperlipidemic, relating plasma α -tocopherol to plasma lipids removes some of the variation associated with differences in circulating lipid levels among these individuals or over time.¹⁶ However, this technique, or relating plasma α -tocopherol concentrations to circulating cholesterol concentrations (a more convenient assay), does not consistently improve assessments of vitamin E status in humans with normal plasma lipid levels or in most other species that have been studied.³²

Analyses of red blood cells offer no advantage over plasma,³¹ but assays of α -tocopherol concentrations in platelets show some promise.¹⁶ Analyses of liver biopsies¹⁶ and of adipose tissue biopsies⁴⁷ have been conducted to assess body stores, but the answers are different due to the much longer half-life of α -tocopherol in adipose tissue. A hydrogen peroxide erythrocyte hemolysis test has been used to test the fragility of red blood cells, but it requires rigorous standardization and is less precise than measurement of blood tocopherol levels. Specific diagnostic tests that measure α -tocopherol concentrations in cell components that are particularly susceptible to free-radical peroxidative damage, such as microsomal membranes, would be particularly valuable but technically are very difficult to conduct.

Vitamin K

The term vitamin K is a generic descriptor of 2-methyl-1,4-naphthoquinone and derivatives that have antihemorrhagic activity. The principal active compound found in green leaves is phytylmenaquinone (phylloquinone or vitamin K₁). A series of active compounds produced by microorganisms, including those in the gastrointestinal tract, are known as prenylmenaquinones. A major metabolic role for vitamin K is promotion of the carboxylation of glutamyl residues to form γ -carboxyglutamyl residues. Methods that have been used for vitamin K assessment and that require marked deficiencies for expression include measures of plasma concentrations of one of the vitamin K-dependent clotting factors, prothrombin (factor II), factor VII, factor IX, or factor X.³¹ Whole blood clotting times used in early work are now considered insensitive and inaccurate and are not recommended. Phylloquinone can be measured in plasma but concentrations tend to reflect recent intakes. Functional tests of vitamin K status showing promise include measures of plasma concentrations of under- γ -carboxylated prothrombin and under- γ -carboxylated osteocalcin. Plasma concentrations of these compounds increase in response to a mild deficiency of vitamin K, and urinary concentrations of γ -carboxyglutamic acid decline.⁵⁷

Vitamin C

Serum levels of vitamin C (ascorbic acid) in species with a dietary requirement vary substantially and depend to a considerable extent on intake immediately preceding the assay.¹⁶ As a consequence, fasting serum samples are considered essential to the determination of vitamin C status in humans, and are used primarily to identify individuals with a chronically low intake. Urinary vitamin C excretion also reflects recent intake, but urine concentrations decline progressively with increasing depletion until they become undetectable in humans with scurvy. A relatively low renal threshold for vitamin C limits serum identification of high intakes of vitamin C because the excess spills from the blood into the urine. Metaphosphoric acid or trichloroacetic acid is added to samples before analysis to precipitate proteins and stabilize the vitamin C.

Thiamin

A common test involves estimation of thiamin excretion in urine, using small fractional samples collected in the field rather than a 24-hr collection. Thiamin concentrations in urine do not reflect body stores but relate best to current intakes, particularly those that are high. Urinary excretion decreases with decreasing thiamin intake until a critically low level is reached, after which, further decreases in intake have little effect. Thiamin concentrations of fractional urine samples from the same individual may vary substantially. Twenty-four hour samples are less variable, but the variability of fractional samples can be reduced by relating thiamin values to urinary creatinine concentration. Nevertheless, this is a relatively insensitive test of thiamin status.

Whole blood thiamin also can be determined. However, whole blood contains only about 0.8% of total body thiamin. Thus, concentrations are very low. In comparison, serum concentrations are lower still. If whole blood is used, hematocrit also must be determined so that the contribution from

varying proportions of red blood cells may be considered. Like urine samples, blood levels reflect immediately preceding intakes and are insensitive measures of status.

A functional enzyme test is more reliable and preferable. Transketolase is catalyzed by thiamin pyrophosphate (TPP), is present in red blood cells, and increases in activity in response to additions of TPP.³¹ This is the method used most commonly to assess thiamin status because erythrocytes are among the first tissues to be affected by thiamin depletion.¹⁶ However, assay of thiamin phosphorylated esters in plasma may be an even more sensitive test since plasma levels of these compounds decline before any change in erythrocyte transketolase activity occurs.⁴⁷

Riboflavin

Analyses of blood, erythrocytes, or serum are of limited value. Blood and erythrocyte riboflavin concentrations are insensitive to small changes in riboflavin status, and serum riboflavin levels reflect recent intakes and are too variable to be useful. Urinary riboflavin levels have similar weaknesses, and excretion is decreased by physical exertion and sleep, whereas elevated environmental temperatures, negative nitrogen balance, and restricted physical activity increase excretion.

Erythrocyte glutathione reductase activity coefficients appear to be most useful and are determined by comparing activity of the enzyme with added flavin adenine dinucleotide (FAD) versus activity without added FAD. This enzyme-stimulated test measures conversion of oxidized glutathione to reduced glutathione as an indicator of FAD availability.⁴⁷ In a riboflavin-deficient subject, FAD supplies are limited and erythrocyte glutathione reductase activity is low compared to riboflavin-adequate subjects. Thus, added FAD produces a greater relative response in enzyme activity in erythrocytes of riboflavin-deficient subjects.^{16,31}

Pantothenic acid

Because of the widespread occurrence of pantothenic acid in foods, deficiencies in humans are rare. Thus, tests to assess the status of this vitamin have not been well developed. Pantothenic acid excretion in urine appears to be related to recent intake.⁴⁷ Both free and coenzyme A-bound pantothenic acid can be found in whole blood, and low total (free plus bound) pantothenic acid concentration has been suggested as an indicator of low dietary intake. The majority of pantothenic acid in the erythrocyte is bound in coenzyme A, whereas that in serum is in the free form.³¹

Niacin

Niacin is a term used for two compounds with comparable vitamin activity, nicotinic acid and nicotinamide. Because niacin may be derived endogenously from the amino acid tryptophan in many species, a deficiency of both niacin and tryptophan can complicate conclusions. The principal end products of niacin metabolism are N^γ-methylnicotinamide and N^γ-methyl 2-pyridone-5-carboxylamide

(2-pyridone). Estimation of N'-methylnicotinamide in the urine is technically simple as compared to analysis of 2-pyridone. As a consequence, assay of the former compound has been a traditional method of assessing status despite recognition that pregnancy increases urinary excretion of N'-methylnicotinamide and diabetes decreases it. With modern HPLC techniques, assays of 2-pyridone are more easily accomplished. This is important because excretion of 2-pyridone is more severely reduced in marginal niacin deficiency than is excretion of N'-methylnicotinamide. Recent surveys of niacin status in humans have used measurements of the urinary excretion of both metabolites (with and without nicotinamide loading), expressing the values as a ratio of 2-pyridone to N'-methylnicotinamide.^{16,31,46}

Niacin levels in plasma are low and related to dietary intake rather than to niacin status. However, 2-pyridone levels in plasma may decline as a consequence of depletion. Ratios of nicotinamide adenine dinucleotide to nicotinamide adenine dinucleotide phosphate concentrations in erythrocytes or whole blood show promise, but because the coenzymes are so labile, the analysis must be initiated within seconds after drawing the blood sample.

Vitamin B₆

Vitamin B₆ is a generic term referring to structurally related compounds that have the vitamin activity of pyridoxine. Compounds found in the body include pyridoxine, pyridoxal, pyridoxamine, and the coenzymes, pyridoxal-5'-phosphate and pyridoxamine-5'-phosphate. Recommendations for direct assessment of vitamin B₆ status in humans involves estimation of vitamin B₆ intakes, and determination of at least two biochemical indices, one being pyridoxal-5'-phosphate in plasma. Other potentially useful indices include plasma pyridoxal and urinary 4-pyridoxic acid. Dietary protein consumption also should be assessed, since increased protein intakes, like vitamin B₆ deficiency, lower plasma pyridoxal-5'-phosphate concentrations.

Indirect assessment of vitamin B₆ status involves metabolic pathways or enzyme activities requiring pyridoxal-5'-phosphate as a coenzyme. These include assays of tryptophan metabolites in urine after an oral tryptophan dose, assays of methionine metabolites in urine after an oral methionine dose, and assay of transaminase activity in erythrocytes before and after addition of pyridoxal-5'-phosphate.³¹

Biotin

Although declines in biotin concentrations in whole blood, plasma, or serum have been used as indicators of impaired biotin status in humans, recent evidence has revealed that these measures are neither early nor sensitive. Urinary concentrations of biotin and bisnorbiotin tend to decrease with depletion, but will increase and then decline rapidly in response to a pulse oral biotin dose. Urinary concentrations of 3-hydroxyisovaleric acid increase with depletion, presumably due to a decline in activity of the biotin-dependent enzyme methyl-crotonyl-CoA carboxylase, and this method of assessment shows promise.³⁶

A requirement for biotin for activity of propionyl-CoA carboxylase is believed responsible for the accumulation of odd-chain fatty acids in the liver and blood of biotin-deficient subjects. Apparently,

the accumulation of propionyl-CoA leads to incorporation of this 3-carbon moiety rather than 2-carbon acetyl-CoA during fatty acid elongation.

Biotin assays are performed microbiologically, by avidin-binding techniques, or by measurement of fluorescent biotin derivatives. Bound blood biotin must be liberated by papain digestion or hydrolysis before assay with certain microorganisms, and a number of interfering compounds must be eliminated in most assays.³¹ Interpretation of all biotin assays may be complicated by microbial synthesis of biotin in the gastrointestinal tract and its subsequent absorption.

Folacin (folic acid) and vitamin B₁₂

Folacin deficiency results in anemia and may mask vitamin B₁₂ deficiency. Megaloblastic anemia (abnormally large red blood cells) is an indication of a long-term deficiency of either vitamin. Serum folate levels tend to reflect current status but do decline with declining tissue stores. Hemolysis results in elevated serum folate concentrations because levels of folate in red blood cells are normally at least 10 times those in serum. Acute renal failure and acute liver damage also result in elevated serum folate.

Folacin deficiency increases excretion of formiminoglutamic acid (FIGLU) in urine, but this increase also may be seen in B₁₂ deficiency.^{16,31} Polymorphonuclear leukocyte lobe counts have been used to identify folacin and vitamin B₁₂ deficiencies in humans (leukocyte nuclei have an increased number of segments; normal is 3 to 4 in humans), although these changes also occur in other conditions.⁹ The nonlinear inverse relationship between plasma folate and plasma homocysteine concentrations has proven useful in assessing folacin status. Above a certain plasma folate concentration, plasma homocysteine concentrations do not change. Below this folate level, plasma homocysteine concentrations are elevated in proportion to the decline in plasma folate. It should be noted that plasma homocysteine levels also ultimately rise after an extended period of vitamin B₁₂ depletion.^{23,49}

To distinguish between folacin and vitamin B₁₂ deficiencies, vitamin B₁₂ status must be determined. Assays of serum vitamin B₁₂ concentration have been routinely used, a measure that appears to be associated with body stores, particularly those in the liver.¹⁶ There also is a test that measures the differential suppression of [³H]thymidine incorporation into DNA by added deoxyuridine in vitamin B₁₂ or folacin deficiencies. This is an *in vitro* test that uses either bone marrow cells or, more recently, lymphocytes.¹⁶ Loading tests also have been found valuable for experimental discrimination between the two deficiencies. Oral loading with valine results in a greater increase in the excretion of methylmalonic acid in the urine of vitamin B₁₂-deficient animals. Oral loading with histidine produces greater urinary excretion of formiminoglutamic acid in folacin-deficient animals.⁴⁷ Unfortunately, these tests also are affected by other conditions. Holotranscobalamin II is a vitamin B₁₂-transport protein that delivers the vitamin to DNA-synthesizing tissues. Serum concentrations of this protein decline much before those of vitamin B₁₂ or the appearance of deficiency signs. As a consequence, assay of holotranscobalamin II is proving to be a useful early indicator of negative vitamin B₁₂ balance.²³

Choline

Plasma choline concentrations reflect immediate choline intake.³¹ Little information is available on assessing status, but the need for dietary choline may be influenced by the intake of other compounds

that can supply labile methyl groups, such as methionine, or of accessory factors, such as folacin and vitamin B₁₂.

Clinical and Postmortem Examinations

By performing physical examinations, signs suggestive of nutritional deficiencies or toxicities may be detected. However, some nutritional diseases may be difficult to visualize, or signs may become apparent only after many months or years of nutritional insult.⁶ Signs of certain nutritional disorders may be evident only after death, and confirmation requires careful postmortem examination, including histologic evaluation of tissue changes. Postmortem blood, urine, and tissue samples are usually of less value for biochemical analyses due to changes that occur after death, such as nutrient shifts between cells and serum, hemolysis, persistent metabolic reactions, or degradation of sensitive compounds. However, the gross and microscopic appearance of body tissues can still offer insight into nutritional status. Classic disease states such as metabolic bone disease, scurvy, and steatitis may be observed upon visual appraisal of a live animal or evinced after the animal has died and an examination of internal tissues performed. The role of a postmortem examination is to establish a provisional cause of death, but it also can help identify subclinical nutritional disorders that were not detectable in a clinical setting but which may have contributed to the animal's demise. Unfortunately, by the time clinical signs appear or diagnoses are made at necropsy, it is too late for the subject in question. Nevertheless, such findings are useful in correcting dietary mismanagement that, if uncorrected, might harm others.

Summary

Knowledge of the nutritional status of captive and free-ranging individuals and of animal populations is crucial to making informed decisions regarding management of various species and their ecosystems. The interpretation and use of biochemically derived nutritional indices is seriously limited by the paucity of adequate reference data for the many wild species of concern. Selected biochemical measures are listed in the Appendix, but they should be applied with great caution. The integration of findings from multiple assessment techniques is likely to provide a more dependable profile of nutritional status and to contribute more significantly to sound management decisions.

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Appendix

This appendix contains a summary list of potential biochemical methods for nutritional assessment, but no test should be considered definitive by itself (see relevant text). When attempting to identify the role of inadequate or excessive dietary nutrient concentrations as primary agents of disease, there is no substitute for a combination of techniques. These should include clinical physical examinations (including appropriate anthropometric measurements), complete necropsies, relevant biochemical tests, and a careful evaluation of nutrient intake and dietary husbandry. If possible, a suspected nutrient deficiency should be confirmed by measuring the response to judicious supplementation with the nutrient in question.

Protein – total serum protein or serum albumin (not sensitive to short-term changes in protein status and affected by other factors); blood urea nitrogen [BUN] (used often, but misleading, particularly in ruminants, without consideration of energy intake and nitrogen recycling)

Taurine – serum or plasma taurine (relevant for animals with a dietary taurine requirement)

Cholesterol/triacylglycerols/lipoproteins – elevated serum concentrations of LDL-cholesterol, total cholesterol, and triacylglycerols associated with increased risk of cardiovascular disease in humans; concentrations of interest for species and diet comparisons

Calcium (Ca) – serum Ca (under strict homeostatic control; low only in extreme deficiency; affected by P and vitamin D status); ionized serum Ca but not specific for Ca deficiency; consider alternative techniques such as diet evaluation and photon absorptiometry of bone

Phosphorus (P) -- serum inorganic P (affected by Ca and vitamin D status)

Magnesium (Mg) – total serum Mg; serum ionized Mg (few reference data); 24-hr urinary excretion

Sodium (Na) – 24-hr urinary Na clearance or creatinine:Na clearance ratios; serum Na of limited use because under strict homeostatic control

Chloride (Cl) – 24-hr urinary Cl clearance or creatinine:Cl clearance ratios; serum Cl of limited use because under strict homeostatic control

Potassium (K) -- 24-hr urinary K clearance or creatinine:K clearance ratios may be useful but not well tested; serum K of limited use because under strict homeostatic control

Iron (Fe) – serum Fe; serum ferritin (species specific assay); serum total Fe-binding capacity; percent saturation of serum iron-binding capacity; erythrocyte protoporphyrin IX concentration; liver Fe by biopsy

Copper (Cu) – serum Cu (poorly related to Cu status); red blood cell Cu-dependent superoxide dismutase activity promising

Zinc (Zn) – serum Zn (well regulated; low in extreme Zn deficiency but also declines in response to infections and to stress); plasma Zn and metallothionein concentrations

Manganese (Mn) – serum, urine, or lymphocyte Mn (difficult assay); serum Mn-dependent superoxide dismutase activity

Cobalt (Co) – serum Co, homocysteine, and methylmalonic acid (relevant only to ruminants)

Iodine (I) – plasma TSH and T₄; urinary I:creatinine ratio; radioiodine uptake by the thyroid

Selenium (Se) – serum Se or whole blood Se (interpretation requires knowledge of species response to inorganic or organic Se forms); whole blood GSHPx

Vitamin A and carotenoids – serum retinol and retinyl esters; response of serum retinol-binding protein to retinol dose; serum carotenoids, if present and diet is devoid of preformed vitamin A; liver retinyl esters by biopsy

Vitamin D -- serum 25[OH]D; interpretation may be assisted by serum PTH, Ca, inorganic P

Vitamin E -- serum α -tocopherol; liver and fat α -tocopheryl esters by biopsy

Vitamin K – plasma prothrombin (II), clotting factors VII, IX, X; plasma under- γ -carboxylated osteocalcin; plasma under- γ -carboxylated prothrombin; urinary γ -carboxyglutamic acid

Vitamin C – urinary vitamin C repeated over an extended time

Thiamin -- urinary thiamin (insensitive); erythrocyte transketolase response to thiamin pyrophosphate addition

Riboflavin -- erythrocyte riboflavin; erythrocyte glutathione reductase response to flavin adenine dinucleotide addition

Pantothenic acid – erythrocyte coenzyme A

Niacin -- urinary metabolites after a nicotinamide loading test; plasma 2-pyridone

Vitamin B₆ (pyridoxine) – plasma pyridoxal phosphate; urinary metabolites after a tryptophan or methionine loading test; erythrocyte transaminase activity before and after pyridoxal phosphate addition

Biotin – plasma or urinary biotin; urinary 3-hydroxyisovaleric acid; odd-carbon fatty acids in blood

Folacin and vitamin B₁₂ – erythrocyte folate; serum folate and homocysteine; serum vitamin B₁₂; leukocyte lobe count but must distinguish between the two nutrients; serum holotranscobalamin II

Choline – no useful test