Biomarkers of Trace Mineral Intake and Status[1]

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ABSTRACT The emerging public health importance of zinc and selenium and the continuing public health challenges of iron and iodine draw attention to the unmet need for improved biomarkers of trace element status. Currently available biomarkers of these four trace elements are critiqued including the outstanding lack of satisfactory biomarkers for the assessment of zinc intake and status. Other trace elements are reviewed briefly including copper, for which human dietary deficiencies and excesses have been documented, and chromium, which is of possible but unconfirmed public health significance. Evolving strategies of considerable potential include molecular techniques such as the measurement of metallothionein mRNA in lymphocytes as a biomarker of zinc status, an assay that can now be performed with a dried blood spot. The judicious application of tracer techniques also has a role in advancing the quality of zinc biomarkers. Also of special current interest is full definition of the potential of plasma-soluble transferrin receptor concentrations as the biomarker of choice for the detection of early functional iron deficiency. J. Nutr. 133: 948S–955S, 2003.

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There are currently nine trace minerals for which humans are considered to have a nutritional requirement: iron, zinc, copper, selenium, iodide, manganese, molybdenum, chromium and cobalt. Each of these elements contributes < 0.01% to the total body weight. Although it is recognized that a nutritional requirement for additional microminerals (e.g., boron) may be confirmed and that in one case (fluoride) it is partly a question of semantics, it is also true that the evidence for a nutritional requirement for some trace elements included in the "essential" list is still tenuous or limited. Cobalt, for example, is included only because an atom of this mineral is in the cyanocobalamin molecule; it will not be considered further in this paper. There is a wide range in apparent practical implications of these elements as nutrients in the likelihood and extent of clinical and/or public health importance of a nutrient deficiency or excess. There are also notable differences between the trace elements in the availability of biomarkers. Each of these factors influences the attention accorded to individual minerals.

Categories of biomarkers of mineral nutrient intake and status

Tissue concentrations. *Blood plasma or serum.* Despite the penchant for using plasma/serum samples in both clinical practice and epidemiologic research, of the trace minerals, only selenium assays in plasma/serum are a primary choice of biomarker (1,2). There are more sensitive biomarkers of iron status, and for other trace minerals, plasma/serum assays are of limited value. This may be attributable to homeostatic mechanisms that maintain plasma/serum levels when intake is marginal or inadequate (zinc), which results in lack of adequate sensitivity. Lack of specificity (copper, zinc and iron) may also compromise the value of plasma/serum levels as biomarkers. In other instances (manganese, molybdenum), the value of plasma/serum simply lacks adequate evaluation.

Cellular components of blood. Cellular blood components are used quite rarely and then primarily for research purposes. The combination of the demanding sample preparation and, in general, their limited and/or inadequately defined potential has restricted enthusiasm for these sample materials. Whole blood selenium is sensitive to intake of this micromineral and provides a longer-term biomarker (3), but plasma selenium is simpler to assay.

Erythrocyte-membrane zinc has been found to be sensitive to dietary zinc restriction in some (4) but not all (5) studies. This zinc is presumably part or whole of the smaller of two pools of erythrocyte zinc, which exchanges rapidly both in vitro and in vivo (6). Total leukocyte, lymphocyte (7) and especially neutrophil (8) zinc have all been reported to have value as biomarkers of zinc status. Although they have application in individual laboratories, these biomarkers lack adequate validation (5,9–11) and have not had widespread application. The potential for using

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monocytes to provide a molecular biomarker for zinc status is discussed (see *Zinc stores*).

Hair and others. For several of the trace minerals, hair analyses have provided a tantalizing potential biomarker. Inadequately substantiated commercial claims have unfortunately resulted in considerable disrepute for this sample material, which, although still requiring more extensive research, may provide certain useful insights into the long-term status of selected minerals (12). Group differences in hair zinc concentrations that are related to probable differences in intake of bioavailable zinc are a case in point (13,14).

Other tissue concentrations of minerals including urine are considered (see *Mineral homeostasis and metabolism*).

Mineral homeostasis and metabolism. To appreciate the potential value of excretion data and other parameters of mineral homeostasis as biomarkers of intake and status, it is helpful to review, at an organ or system level, the differences in mechanisms by which trace mineral homeostasis is maintained (**Table 1**). As an aside, the kidneys also have a primary role in the maintenance of major mineral homeostasis with the notable exception of calcium, for which the role of the kidneys is secondary to that of the intestine.

For those minerals for which the kidneys are not involved in maintaining homeostasis, measurement of urine excretion rates does not provide useful information on dietary intake. Nor do these measurements provide biomarkers of mineral status except possibly in certain pathological circumstances [for example, in hepatolenticular degeneration (Wilson's disease)] in which biliary excretion of copper is disrupted. Although important for investigating homeostasis in normal and abnormal circumstances, measurements of urine excretion rates do not provide acceptable biomarkers of intake or status for those minerals for which the kidneys have a relatively minor role in homeostasis. In contrast, urine excretion rates of trace minerals for which the kidneys have a prominent role in homeostasis such as iodide provide useful biomarkers of dietary intake of these minerals.

In those instances in which excretion of endogenous mineral is regulated by the gastrointestinal system including the liver and pancreas and for which the intestine is the major excretory route, measurements of endogenous mineral in feces theoretically provide a biomarker of dietary intake and status. The only mineral for which this has been the focus of significant research interest is zinc, and this is discussed further when this element is accorded individual attention (see *Zinc*).

Absorption of some minerals from the diet is also regulated to a greater or lesser extent, and fractional absorption varies inversely with the quantity of bioavailable mineral absorbed. As with intestinal excretion of endogenous mineral, these measurements require tracer techniques and metabolic collections including, to achieve maximal information, careful measurements of dietary intake.

Turnover rates [e.g., iodine (15)] and pool sizes [e.g., zinc (16)] measured with tracer techniques can also provide invaluable biomarkers of mineral status. Tracer techniques provide a powerful research tool but in general are too complex to be used as biomarkers for large populations under field conditions. They can, however, effectively complement large epidemiologic studies by providing more detailed information on a subset of the study population.

Understanding of homeostatic mechanisms at the subcellular and molecular levels is progressing rapidly and is starting to yield invaluable new biomarkers of mineral status and intake of bioavailable mineral. The most outstanding example is that of transferrin receptors, which can now be measured quite simply using a small plasma sample and which are strongly inversely correlated with the supply of iron that is reaching the cell membranes (17).

Body stores. Iron is the most notable example of a mineral that is stored by the body when intake of bioavailable iron is generous and released as required when intake is less adequate. Because of the potential of this mineral for oxidant damage, iron is sequestered tightly bound, primarily to ferritin in the liver. Circulating serum levels, though modest, have a strong positive correlation with tissue stores of ferritin and therefore provide a readily available biomarker (18). The body also appears to have the ability, though much more modest, to store zinc (16,19). (see *Zinc stores*).

Functional indices. Functional indices are of special putative practical value because they indicate when intake and status are sufficiently compromised to cause measurable disturbance of normal physiology and biochemistry. To be of value, these perturbations have to be more or less specific for deficiencies of a single micronutrient. Examples include a low hemoglobin, hematocrit and red cell morphology consistent with iron-deficiency anemia [though not alone totally reliable (20)]; low circulating concentrations of thyroid hormones that result from iodide deficiency (21) and low blood levels of selenium-dependent glutathione peroxidase (GPX3) in selenium deficiency (22).[4]

Response to increase in intake. For some minerals, biomarkers of intake and status have been and/or continue to be inadequate. In these circumstances, the detection or confirmation of a preexisting dietary deficiency and impaired nutritional status may depend on the outcome of carefully designed, randomized, controlled intervention studies with physiological quantities of the mineral under investigation. This approach is responsible for our recent appreciation of the global extent of zinc deficiency (23–25). A positive feature of this approach is that it links inadequate intakes with otherwise nonspecific disturbances of normal physiology and with otherwise nonspecific morbidity. Again, zinc provides an excellent example (26).

TABLE 1

Organs and systems that have a major physiological role in maintaining whole body trace mineral homeostasis

	Gastrointestinal tract		
Mineral	Absorption	Endogenous excretion	Kidneys
Iron	Single major site	—	—
Copper	Substantial	Major (liver)	—
Manganese	Major	Major (liver)	—
Zinc	Major	Major	Minor
Iodide	—	—	Major
Selenium	—	—	Major
Chromium	—	—	Major
Molybdenum	—	—	Major

Individual minerals

Iron. Reliable surrogate biomarkers of the total quantity of dietary iron are unavailable because of the wide variation in bioavailability and especially owing to the large difference in

[4] Abbreviations used: GPX3, glutathione peroxidase; MCV, mean corpuscular volume; PAM, peptidylglycine α-amidating monooxygenase; sTfR, serum transferrin receptor concentration.

absorption of heme iron versus inorganic iron. In contrast, a range of biomarkers is available that in combination allows for reliable assessment of iron status and, therefore, of the adequacy of iron intake, especially in noninfected, nonstressed individuals.

Biomarkers can distinguish three generally accepted levels of iron deficiency and are also useful in the evaluation of iron overload. The three levels of iron deficiency are depleted iron stores, early functional iron deficiency and iron-deficiency anemia.

Iron stores. Plasma/serum ferritin is the most sensitive indicator of iron stores. Iron that is not needed immediately is stored within cells as ferritin, and the relatively small quantities of ferritin in the plasma are proportional to the quantity stored intracellularly. In adults, each 1 μg ferritin/L plasma is proportional to ~8 mg of storage iron (18). Levels $\leq$12 μg ferritin/L plasma are indicative of the absence of iron stores. The normal distribution of serum ferritin is age and sex dependent. For example, each 1 μg ferritin/L plasma in young children is equivalent to ~14 mg of storage iron (18). The 3rd National Health and Nutrition Examination Survey provides detailed distribution data for normal subjects (27). For example, the 5th, 50th and 99th percentiles for men 19–30 y, women 19–30 y and children 1–3 y of age are, respectively, 36, 112 and 394; 7, 36 and 212 and 6, 23 and 116 μg ferritin/L plasma.

Whether high dietary iron levels can result in an abnormal elevation of ferritin levels is uncertain. Plasma ferritin within the normal range does, however, provide a useful biomarker of relatively high as well as inadequate iron intake. A possible association between relatively high plasma ferritin and cardiovascular disease in adult men was and continues to be the focus of extensive research (28–33). Associations were also reported between high serum ferritin and neoplasms, especially adenoma of the colon (34,35). Plasma ferritin is a key biomarker in screening for hereditary hemochromatosis: heterozygous individuals typically have a modest increase in plasma ferritin.

Ferritin is an acute phase protein, and plasma ferritin is increased in the presence of infections, inflammatory disorders and other disease states. Serum ferritin is also increased with ethanol consumption (36) and hyperglycemia (37) and is directly correlated with body mass index (27).

Plasma transferrin or total iron-binding capacity is elevated with storage-iron depletion before there is evidence of any effects of iron deficiency or erythropoiesis, but this is a less sensitive and reliable biomarker of iron stores than plasma ferritin. Transferrin is reduced during infection, inflammation and other stresses.

Early functional iron deficiency. Plasma-soluble serum transferrin receptor concentration (sTfR) increases in proportion to the extent of the functional iron deficit. It increases when the supply of iron to the bone marrow is marginal but not yet causing a measurable decline in hemoglobin. Although data are still quite limited, sTfR appears to be a sensitive and specific indicator of early iron deficiency (17,38). sTfR is produced by proteolytic cleavage of the extracellular domain of transferrin receptors on all cell surfaces and is released into the plasma in proportion to the number of transferrin receptors on cell surfaces, which is in turn proportional to the requirement for iron.

Serum transferrin saturation. Serum transferrin saturation is normally 30–35%. Levels $<$ 15% are indicative of an inadequate supply of iron to the bone marrow. In addition to inadequate stores, impaired release of iron stores due to chronic disease will depress transferrin saturation.

Erythrocyte protoporphyrin concentration. Erythrocyte protoporphyrin concentration (and the zinc protoporphyrin/heme ratio) are increased when there is an inadequate supply of iron to the developing red cell because of the synthesis and retention of more protoporphyrin than can be incorporated into hemoglobin. Any factors that reduce iron delivery or heme synthesis will elevate this ratio.

Iron deficiency anemia. *Hemoglobin and hematocrit.* Hemoglobin and hematocrit are extremely important because of the simplicity of measurements and especially because low values (anemia), if due to iron deficiency, are clearly indicative of a deficiency of this micronutrient that is of sufficient severity to cause impairment of normal physiology. These biomarkers are, however, neither specific for iron deficiency nor very sensitive for the detection of mild but functionally significant iron deficiency. In adults, levels $<$ 12–13 g hemoglobin/dL whole blood are indicative of anemia.

Red blood cell indices. Red blood cells in iron-deficiency anemia are microcytic with a low mean corpuscular volume (MCV) and low mean corpuscular hemoglobin. None of these changes are specific for iron deficiency. Iron deficiency is associated with increased variation in red cell width.

The precision of the laboratory diagnosis of iron-deficiency anemia can be improved by combining hemoglobin with other indices of iron status (39), specifically ferritin or MCV together with erythrocyte protoporphyrin and transferrin saturation. Two or more abnormal indices are indicative of iron deficiency. It is likely that these models will be superceded by sTfR assays.

Research priorities. For the diagnosis of iron deficiency, the most important immediate practical need is further evaluation of the promising potential of plasma sTfR assays.

Iodine. *Urine iodine.* Urine iodine is the standard method for assessing iodine status and intake worldwide (40). More than 90% of dietary iodine is excreted in the urine (41,42). Random urine samples have been found to be adequate for population screening, and there is little advantage in calculating urine iodine:creatinine ratios (43). Urine iodine excretion reflects intake within the past few days.

Thyroid function tests. The mean serum thyroid-stimulating hormone is increased in iodine deficiency, although absolute values may remain within the normal range (21). Serum thyroglobulin concentration correlates with urine iodine. Both of these assays can be undertaken with blood spot filter-paper technology. Thyroxine (T4) is also decreased during iodine deficiency, but considerable overlap with normal data limits its usefulness. A compensatory increase in triiodothronine (T3) may occur. Thyroid function tests provide useful functional biomarkers of longer-term iodine intake and iodide status. In individuals in noniodine-deficient populations, abnormal thyroid function tests are likely to be attributable to factors other than iodine deficiency.

Thyroid size. When assessed clinically or by ultrasonography, thyroid size provides a well-accepted biomarker of the early clinical response to iodine-deficient diets (40,44).

Iodine accumulation. Iodine accumulation and turnover provides a useful research tool. The fraction of a dose of radioactive iodine concentrated by the thyroid gland is inversely related to iodine status. Turnover studies based on the intravenous administration of ^{131}I have been used to calculate the average daily iodine requirement (15).

Zinc. Zinc is certainly not unique among the trace minerals for the paucity of adequate biomarkers. However, this has been especially frustrating in the case of this trace mineral because of the extent to which it has hindered studies of the epidemiology of zinc deficiency. As the global public health importance of zinc deficiency has attracted increasing attention during the past decade (23–26), so have the limitations of current biomarkers (45). Reasons for the lack of biomarkers include the

following: *1*) the small decrements in tissue zinc that are associated with zinc-deficient morbidity and impaired development (46). The speed with which growth is impaired after the introduction of a zinc-deficient diet in young animal models is significant in that it occurs before there is time for measurable decrease in tissue zinc concentrations (47); *2*) the effectiveness of homeostatic mechanisms in maintaining tissue, notably, from a biomarker perspective, circulating plasma/serum zinc concentrations; and *3*) the extraordinarily widespread dependence on zinc of so many aspects of biology including the involvement of this versatile metal in gene expression and a variety of aspects of cellular growth and replication. A corollary of this, as has been recognized for many years (48), is that the features of zinc deficiency (e.g., diminished growth velocity) including potential surrogate laboratory functional indices [e.g., impairment of multiple laboratory indices of immune status (49)], although of fundamental importance to human biology, are likely to be nonspecific.

Plasma zinc. Plasma zinc is currently the most widely used and accepted biomarker of zinc status despite poor sensitivity and imperfect specificity. Over a range of zinc intake and absorption values in adult women (including subjects with intake values $\leq$ 5 mg Zn/d), no correlation was observed between either intake or absorption and plasma zinc (50). This is in notable contrast to the positive correlation between dietary zinc (16), absorbed zinc (50,51) and estimates of the size of the combined relatively rapidly exchanging zinc pools. These observations suggest that homeostatic control of plasma zinc concentrations can occur while moderate changes are occurring in the zinc content of one or more of the pools of zinc that exchange rapidly with zinc in plasma. These may be in the form of a small readily exchangeable store that is quite possibly associated with metallothionein in hepatocytes and other organs. Other evidence for such control comes from studies of experimental human zinc deficiency (52,53). Mild growth-limiting zinc-deficiency states have been documented in young children with plasma zinc concentrations within the normal range, and increases in growth velocity with zinc supplementation have been observed without change in plasma zinc relative to placebo-treated controls (13). On the other hand, concentrations < 70 μg zinc/dL plasma have been found to be a useful predictor of growth response to zinc supplementation (23), and lower cutoffs have predicted beneficial effects of zinc supplements in the prevention and management of diarrhea (24). Plasma zinc is profoundly depressed in severe acute zinc-deficiency states, for example, in untreated acrodermatitis enteropathica (54).

Several studies have revealed no association between zinc intake and plasma zinc (55–58). On the other hand, some associations between intake of bioavailable zinc and plasma zinc have been observed. For example, levels vary inversely with phytate/zinc molar ratios in young Canadian women (59), and 20% of a group of young Canadian women had a plasma zinc level less than a lower cutoff of 10.07 μmol zinc/L plasma. The mean level of this group was significantly lower than that of a comparable group of young women in New Zealand who had a higher meat intake (60). Unusually high intakes that are achieved by use of zinc supplements can elevate plasma zinc concentrations above the defined normal range (61); therefore this marker may have utility for the detection of potentially toxic intakes.

The factor of greatest practical concern with respect to the specificity of plasma zinc as an index of zinc status is the decline that occurs as a recognized component of the acute phase response (62). Although it is impossible to exclude this effect as a cause of mild to moderate hypozincemia in the presence of infection or stress, this does not appear to negate the potential utility of this biomarker even when infection is present (63).

Attention to a number of items can reduce the "noise" factor (45). These include scrupulous care to avoid sample contamination, use of plasma rather than serum with prompt separation from red cells (64) and standardization of collection in relation to meals (65). Although no standard adjustment for albumin levels is possible on the basis of current data, low plasma zinc concentrations in the presence of hypoalbuminemia need to be interpreted with caution (66). Puzzling interlaboratory differences emphasize the need for diligent use of standard reference materials for what is a simple analysis by flame atomic absorption spectrophotometry.

Cellular components of blood. Zinc incorporated in carbonic anhydrase accounts for most of the erythrocyte zinc. This zinc has been generally regarded as not readily depleted, although low levels have been reported with some depletion studies (67) and further evaluation is required. Red cell membrane zinc, in contrast, may be sensitive to zinc depletion (4). The complexity of sample preparation is, however, likely to preclude widespread use of this biomarker; this is also true for monocyte, neutrophil or platelet zinc. Although the latter assays appear promising in the hands of some investigators (7,8), this is not a universal experience (10,45).

Hair zinc. Associations between low hair zinc levels and impaired growth velocity have been reported (68,69) including low hair zinc concentrations in young children who have had a growth response to zinc supplements (13,14). A relationship between dietary intake of bioavailable zinc and hair zinc is indicated by reports of low hair zinc in children whose habitual intake of phytate is high (70) or who eat little meat (71). Cutoff levels indicative of impaired zinc status or inadequate intake lack adequate definition.

Zinc excretion. Urine zinc excretion rates decline with severe dietary zinc restriction (72) and may reflect dietary zinc intake (or absorption) over a wide range of intake. Although further research is required, it appears unlikely that urine zinc will prove to be a sensitive index of zinc intake or status. Excretion of endogenous zinc in the feces is regulated in accord with changes in recent zinc absorption and zinc status. This regulation is important for the maintenance of zinc homeostasis (73). Measurement, however, requires tracer techniques and metabolic collections and is only applicable as a special research tool.

Zinc stores. Estimates of the quantity of zinc in relatively rapidly exchanging pools (i.e., those intermixing with zinc in plasma within 3 d) correlate positively with ingested (16) and especially absorbed (50,51,74) zinc. More extensive research is needed however to define the utility of this biomarker. This test requires intravenous administration of a stable isotope tracer and collection of small, approximately timed urine samples over several days.

Metallothionein monocyte messenger RNA has promising potential as a biomarker for zinc. Levels are markedly reduced with mild experimental dietary zinc restriction (75) and increase rapidly with zinc supplementation (76). Measurement is by an assay that employs reverse transcriptase–polymerase chain reaction and as is potentially very attractive for epidemiologic studies, can use a 50-μL dried blood spot on filter paper.

Activity of zinc-dependent enzymes. There have been expectations for many years that assays of the activity of selected zinc metalloenzymes would provide invaluable functional indices of zinc status and possible surrogate biomarkers of dietary zinc. Although some evidence for the utility of several enzyme assays has been reported, in no case have these received

adequate confirmation. Indeed, attempts to confirm their utility have often met with negative results. Among those that merit further attention are alkaline phosphatase (although this assay suffers from a lack of specificity); copper-zinc superoxide dismutase; and especially, lymphocyte 5′-nucleotidase (77,78), which again is not consistently depressed in experimental zinc deficiency. This enzyme is derived from the CD73 cell-surface markers of B and T cells.

Response to zinc supplementation. The most reliable and extensive data on the epidemiology of human zinc deficiency has been derived from studies of the effects of dietary zinc (as a single nutrient) supplementation under strictly randomized placebo-controlled conditions. For growth responses in particular, it is established that there is no pharmacologic effect of zinc, so that responses are indicative of an underlying zinc-deficiency state. Unfortunately recent studies have typically not included dietary data, which clearly precludes any utility as a biomarker of the adequacy of a specific level of dietary zinc associated with functional evidence of deficiency. Earlier studies of growth responses in North America did however include dietary data (13,14,79). Such studies are arduous; it is hoped that they can be increasingly replaced by more simple biomarkers in the future.

Research priorities. There is an compelling demand for improved zinc biomarkers. Priority research directions include *1*) molecular techniques that commence with thorough evaluation of the potential of metallothionein mRNA assays; and *2*) stable isotope tracer techniques, especially the measurement of exchangeable pool sizes.

Selenium. The quality of biomarkers of dietary intake of selenium and selenium status is quite favorable relative to that of most trace minerals. These biomarkers are in increasing demand principally because of the antioxidant role of this micromineral (80) and the strong epidemiological evidence of the links, which are primarily protective, between dietary intake and carcinogenesis (81). Identification of the major role of a geochemical deficiency of selenium in a large area of China in the etiology of Keshan disease (82) has also given impetus to research on selenium biomarkers.

Plasma selenium. In selenium-adequate conditions, ~8 μg selenium/dL plasma is accounted for by the physiological requirements of the selenoproteins GPX3 and selenoprotein P (1). Plasma selenium below these levels provides good indices of the severity of impaired selenium status and intake. At higher levels of intake, plasma selenium continues to increase, but the strength of the correlation with dietary intake depends on the chemical form of selenium in the diet (1). Typically this is principally selenomethionine, which is absorbed very efficiently but is not subject to homeostatic control (83). A high percentage of absorbed selenomethionine is incorporated into skeletal muscle, and the increase in muscle selenium with high intakes of selenomethionine may not be well reflected by plasma selenium.

Plasma selenium responds rapidly to selenium supplementation and is regarded as a biomarker of short-term selenium status (1), although in reasonably stable circumstances it also provides a biomarker of long-term intake. No acid digestion is required for assay by atomic absorption spectrophotometry with Zeeman background correction, and this procedure can be used for large-scale epidemiological surveys.

Whole blood, hair and nail selenium. Whole blood selenium is of potential value as a biomarker of relatively long-term selenium intake and status. Acid digestion is required before analysis and data are limited. Hair selenium has been used to assess selenium status in China (84), and nail (85) selenium assays have also been used to assess status. Several factors other than selenium nutritional status and intake can affect levels.

Plasma glutathione peroxidase. Plasma GPX3 activity provides a good functional index of dietary selenium intake and status when these are in the deficient range (22). Positive features include the provision of a functional marker for this micromineral, the availability of automated assay procedures and the lack of concern about sample contamination. A disadvantage is that values plateau when intake reaches and exceeds an optimal level. Other unfavorable aspects include the challenge of sample storage and interlaboratory variation in analytical data. Finally GPX3 activity can be affected by other nutrient deficiencies; hence low activity is not entirely specific for selenium deficiency (86).

Copper. *Plasma copper and ceruloplasmin.* Plasma copper and ceruloplasmin levels (measured either as the protein or as oxidase enzyme activity) are the most frequently used biomarkers of copper status. They are depressed in copper deficiency states. Levels plateau when copper intake reaches an adequate level, and these biomarkers do not reflect the magnitude of copper intake beyond this point.

Hepatic synthesis of ceruloplasmin depends on an adequate supply of copper. A high percentage of the copper circulating in plasma is bound to ceruloplasmin. Hence in turn the level of circulating copper is dependent on that of ceruloplasmin. Ceruloplasmin is an acute phase reactant and is elevated during infections, inflammatory processes and other stress circumstances. Notable among other factors that increase ceruloplasmin synthesis are estrogens, which result in an elevation of ceruloplasmin (and therefore of plasma copper) levels during pregnancy. In these circumstances, depression of these biomarkers by copper deficiency may not be apparent. Ceruloplasmin synthesis together with that of other hepatic proteins may be depressed by protein deficiency and result in low levels of circulating ceruloplasmin and copper that are not caused by copper deficiency. Levels of these biomarkers are also age dependent. Of special note are the low levels that are normal in the neonate and young infant and especially in the premature infant.

The value of these biomarkers is diminished by their lack of specificity, confounding factors (including physiological factors) that complicate the interpretation of data and by limited sensitivity. No reduction in levels of plasma copper or ceruloplasmin occurred in postmenopausal women that were fed a marginally low (0.57 mg copper/d) copper diet for >3 mo (87). In the same study, platelet copper and cytochrome *c* oxidase activity were reduced, which indicates that these other potentially more sensitive biomarkers merit research. A more severely copper-restricted experimental diet (0.38 mg copper/d) fed to healthy adult men for 6 wk did depress plasma copper and ceruloplasmin (88). Despite their limitations, these biomarkers are currently the most widely used biomarkers of copper status due to assay simplicity and long-term familiarity.

Copper enzyme activity. Research to identify better biomarkers of copper status has included some attention to the activities of several copper enzymes in addition to ceruloplasmin (89). Erythrocyte and extracellular superoxide dismutase activity is reduced with more severe copper restriction but confers no advantage over plasma copper because of lack of adequate sensitivity and specificity. Cytochrome *c* oxidase activity in platelets and leukocytes appears to offer a relatively sensitive biomarker (90), but these are not assays that are suitable for epidemiologic studies.

A simpler assay on a finger-stick plasma sample can be used to measure the activity of peptidylglycine α-amidating

monooxygenase (PAM) with and without the addition of copper in vitro. Studies in animal models and observations in humans suggest that the in vitro change in activity of PAM with the addition of copper has potential as a relatively sensitive biomarker (78,91).

Plasma diamine oxidase activity is also relatively sensitive to copper intake in that it increases with copper supplementation in normal subjects (88). Although other factors can affect activity, further evaluation is warranted.

Other functional indices and response to supplementation. The essentiality of copper for the maturation and signal-mediated activity of immune cells may provide potential for the development of novel biomarkers of copper status (89), although these are not likely to be readily applicable to epidemiologic studies. The response to copper supplementation in well-controlled studies may provide clues to inadequate copper intake in the absence of any detectable abnormalities of traditional biomarkers.

Research priorities. Further research is required to evaluate the potential of assays of activity of "new" copper enzymes, most notably PAM.

Other trace elements. *Manganese.* Plasma manganese concentrations reflect dietary manganese intake. Levels are decreased on a manganese-restricted diet (92) and have been found to be higher in women who consume 15 mg Mn/d compared with those of women who consume 1.7 mg Mn/d (93). There is no adequately confirmed evidence that manganese deficiency is either of clinical or public health concern. Excess manganese in the brain, which has been diagnosed especially in manganese miners, can cause severe extrapyramidal neurological and psychiatric disease. Manganese is excreted principally in the bile, and elevated plasma manganese concentrations have been documented in patients fed intravenously (including standard manganese additive) who have chronic obstructive liver disease (94).

Molybdenum. Only one case of apparent human molybdenum deficiency has been reported; this was in an individual with Crohn's disease who was on long-term intravenous nutrition (95). Biomarkers for molybdenum deficiency are decreased urinary levels of sulfate and uric acid with elevated sulfite, hypoxanthine and xanthine.

Chromium. Suggestive but poorly substantiated evidence that chromium deficiency may be widespread, especially in individuals with some degree of glucose intolerance, points to a need for reliable biomarkers of dietary chromium and chromium status. These are lacking. It has been concluded that plasma chromium is unlikely to offer a useful indicator in part because normal values are so near the limit of detection (96). There are conflicting data on the relationship of urine chromium excretion to dietary intake (97,98). On the one hand, excretion has been reported to be related to intake; on the other hand, a negative linear relationship has been observed between dietary chromium and the percentage excreted in urine, so that the absolute quantities excreted were identical at intakes of 10 and 40 μg chromium/d. Evidence for chromium deficiency is based on results of supplementation studies. Only one study in which controlled quantities of dietary chromium were given has been reported (99). The results of this study were complex, but they provided some evidence for deterioration in glucose tolerance with a severely chromium-restricted diet.

Research priorities. Because the lack of reliable biomarkers has had a major contributory role in the current uncertainties about the biological significance of chromium as an essential micromineral (and because of the putative importance of this biological role), this element is among those that merit priority research. Research needs include carefully designed intervention trials with small quantities of additional chromium and depletion trials coupled with careful measurements of dietary and urine chromium. Definitive research on biologically active forms of chromium is also required.

Conclusions. Deficiencies of iron, iodine, zinc and selenium are recognized public health problems either regionally or globally. Biomarkers of intake and/or status for iron, iodine and selenium have been adequate to support extensive epidemiologic research. Biomarkers for zinc are inadequate for this purpose, and recent progress in understanding the epidemiology of zinc deficiency has depended to a great extent on well-designed randomized trials of small dietary zinc supplements. Although probably of lesser public health importance, the identification of adequate biomarkers of copper status also merits attention. The need for a clearer definition of the essentiality of chromium is emphasized by the possible public health implications of chromium deficiency. Biomarkers of chromium intake and status would clearly facilitate this quest.

The emergence of new molecular techniques is especially promising for the development of a new generation of biomarkers. A notable example is provided by metallothionein mRNA analyses in lymphocytes as a biomarker of zinc status. Judicious application of zinc tracer (particularly stable isotope) techniques in random subgroups of populations also have potential value. Another outstanding example of a biomarker that requires further evaluation is the plasma concentration of soluble serum transferrin receptors. Despite the relative abundance of biomarkers of iron status, further evaluation of plasma sTfR promises to confirm the exceptional value of this assay in the detection of early functional iron deficiency.

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