Chapter 2

Assessment of the Risk of Zinc Deficiency in Populations

2.1 Objectives of assessment

Assessing the nutritional status of a population is critical in developing nutrition intervention programs that enhance human health and well-being. The results of nutritional assessment efforts are necessary both to determine the presence and magnitude of particular nutrition problems and, when indicated by the results, to elicit public interest and garner resources for action. Assessment data are used to determine the level of risk of deficiency in the general population and thereby indicate the probable consequences for human health and productivity. Information derived from assessments is also used to identify specific segments of the population at elevated risk so that interventions may be targeted to those in greatest need, or to determine whether population-wide interventions are indicated. Assessments can also be used to monitor changes in nutritional conditions over time, thereby permitting decisions on the effectiveness of intervention programs and need for their continuation.

The following paragraphs review some general aspects of nutritional assessment. Available methods for the specific assessment of zinc status in individuals and populations are described in the subsequent sections.

Identifying high-risk groups

A major objective of nutritional assessment efforts is to identify high-risk groups who might be targeted by nutrition (and possibly other health) intervention programs. Targeting enables limited resources to be used most efficiently to reach those in need and to protect those who do not require the program from any possible adverse effects of the intervention. Risk groups can be defined in terms of almost any easily identifiable descriptor, which might include the following:
» Physiologic status, as defined by age group, phase of reproductive cycle (e.g., pregnant or lactating), or presence of illness (e.g., presence of persistent diarrhea, HIV infection);
» Political or geographic region of inhabitance (e.g., regions, districts, urban vs. rural, coastal vs. inland);
» Socioeconomic status (e.g., level of maternal education, income, employment, or access to health, water and sanitation services).

Information on the population's zinc status, or overall risk of zinc deficiency, should be disaggregated according to some of these possible risk factors, as appropriate, such that sub-populations at elevated risk may be identified. Physiologic factors associated with an increased risk of zinc deficiency are described in section 1.8 in chapter 1. As with most other nutrient deficiencies, the groups that appear to be most commonly affected by zinc deficiency are infants and young children, adolescents, women during pregnancy, and the elderly. Socioeconomic factors that may also be used to identify at-risk populations or sub-populations are described in section 2.5.

Applications for program evaluation

Nutritional assessments should be repeated periodically to determine changes in the population's status over time. Ideally, the same techniques that are used to examine the nutritional condition at baseline should be used consistently during follow up to facilitate interpretation of any differences that are encountered. Other indicators of environmental or economic conditions should be included in the assessment to determine whether changes in nutritional status likely occurred due to elimination of the underlying ecologic or socioeconomic causes of the problem or due to success of the program itself. Specific methods for the monitoring and evaluation of programs and possible indicators are provided in section 3.6 in chapter 3.

Importance of assessing both nutrition deficiency and excess

Nutritional abnormalities can be defined in terms of deficiency, excess, or imbalance of particular nutrients

or foods. Although nutrition interventions should be designed appropriately to provide the most favorable ratio of benefits to risks, even under the best of circumstances these programs may not be entirely free from possible adverse consequences. Thus, it is prudent to include an assessment of possible undesirable outcomes of these interventions as well as their positive impacts. In the case of zinc, excessive intake may result in abnormalities of copper, iron, and/or lipoprotein metabolism. It may not be feasible to include biochemical indices of copper status and lipoprotein profiles in large surveys. However, it is advisable to include these assessments in efficacy trials of zinc interventions programs to identify any possible adverse effects in the population of interest. Information on evaluating the risk of excessive zinc intake is given in section 2.6.

Individual versus population assessment

Nutritional assessment may focus on individuals or populations. A population is defined as any group of individuals who share a common trait, often nationality. Whereas assessment of individuals leads to case-specific treatment or counseling, assessment of populations is used to plan and evaluate population-based interventions. Thus, it is not critical for the population assessment techniques to provide certainty with regard to any particular individual's true status. This is an important distinction with regard to assessing zinc status, because while available techniques may misclassify some individuals, they may be appropriate for detecting populations at high risk of deficiency.

Assessment of zinc status in individuals will find application most often in clinical settings among those seeking medical attention for a health condition. In the context of lower-income countries, it is likely that diagnosis of isolated zinc deficiency will be rare, but rather will be found in association with a variety of health conditions for which primary treatment is being sought. For example, children presenting with severe malnutrition, diarrheal infections, or respiratory illnesses may be zinc depleted, in which case usual treatment strategies should ensure correction of the zinc deficiency state.

Population assessment is applied to a sample of the population of interest. This sample may be chosen in a number of different ways, but the sampling technique must select representative members of the whole population. Ideally, the assessment procedures used in population surveys should be simple, low-cost, and rapid, and any necessary equipment must be easily transportable. The primary goal of population-level assessment of zinc status is to characterize the degree of risk of deficiency in the population and the urgency with which the situation needs to be addressed, if at all.

Available methods for assessing risk of zinc deficiency in populations

As with other nutrients, a number of general techniques can be used to estimate the risk of zinc deficiency in individuals or in populations. These are categorized as the following:

1. The presence or prevalence of clinical outcomes of zinc deficiency (e.g., stunting, diarrhea), or other ecologic factors associated with risk of zinc deficiency or risk of inadequate zinc intakes;
2. Assessment of the adequacy of dietary zinc intakes in relation to theoretical requirements for absorbed zinc;
3. Biochemical measures of zinc concentration, activity of zinc-dependent enzymes, or other zinc-responsive biocomponents in biologic fluids or tissues, assessed in comparison to reference values or established cutoffs;
4. Measurement of functional responses following the intake of adequate supplemental zinc.

The selection of which risk indicators to use will depend on the specific objectives of the assessment and available resources. When applied, these indicators should be assessed in a representative sample of the target population. Ideally, several of these measures should be considered in combination, or at different stages of the assessment. The following sections provide details of the range of assessment methods currently available and their application and interpretation. The focus of these sections is the assessment of the risk of zinc deficiency in *populations*; applicability of these methods for assessments in individuals will be mentioned where appropriate. The assessment methods will be classified into two categories: (1) those that provide suggestive evidence of the risk of zinc deficiency based on existing data collected for other purposes (section 2.2); and (2) those that are applied specifically to estimate the risk of zinc deficiency in a population (section 2.3).

2.2 Suggestive evidence for the risk of zinc deficiency in populations

Certain health or ecologic conditions may be associated with, although not necessarily specific to, zinc deficiency. Nevertheless, these conditions may provide useful suggestive evidence that a population is at risk of zinc deficiency. For example, stunting (low height-for-age) among preschool children is a common clinical manifestation of zinc deficiency. Although other nutritional or environmental factors can also cause stunting, an elevated prevalence of this condition may be used as suggestive evidence of zinc deficiency in a population. Another kind of suggestive information is provided by national food balance sheets, which can

be used to assess the adequacy of zinc in national food supplies and to estimate the risk of inadequate zinc intake at the national level. Finally, the prevalence of iron-deficiency anemia is another type of suggestive information on the risk of zinc deficiency. Although iron deficiency does not cause zinc deficiency, both the distribution of iron and zinc in the food supply, and the dietary components that modify their absorption, are similar, suggesting a comparable risk for deficiency. Therefore rates of iron-deficiency anemia may be used as suggestive evidence of the risk of zinc deficiency.

A notable advantage of these proposed supporting data is that they may be compiled from existing sources of information and therefore be used as preliminary evidence for the likely presence of zinc deficiency in a given population. It must be recognized that these data are limited in that they cannot provide reliable estimates of the true proportion of the population at risk of zinc deficiency. In some cases, for example, the data may be available only at the national level or they may be representative of a selected sub-population group, such as children under five years of age. *Nonetheless, because these data are readily available for most countries, they can be used immediately by decision makers as a first step to assess the expected risk of zinc deficiency in the population and the degree of urgency with which to conduct more specific population assessments*. The rationale for use of these data and their application in assessing the risk of zinc deficiency in a population are described below.

2.2.1 Rates of stunting

It has been well established, both by studies in experimental animals and by human intervention trials, that zinc deficiency is growth-limiting. In a recent meta-analysis, the results of more than thirty community-based intervention trials completed in different parts of the world were examined to determine the overall magnitude of growth responses to zinc supplementation [1]. Notably, the responses to zinc supplementation were significantly greater in those studies that enrolled subjects with pre-existing nutritional stunting or underweight, defined respectively as height-for-age or weight-for-age Z-scores < –2 in relation to international reference data. By contrast, there were no significant effects of zinc supplementation in those studies that enrolled mostly children who were non-stunted and/or had adequate weight-for-age. These results indicate that children with low height-for-age or weight-for-age are likely to be zinc deficient, and they further suggest that the national prevalence of stunting or underweight among children under 5 years of age can be used as indirect indicators of a population's risk of zinc deficiency.

The aforementioned meta-analysis found no effect of zinc supplementation on weight-for-height indices, suggesting that zinc mostly affects linear growth. Thus, the rate of stunting, or low height-for-age, is probably the best anthropometric indicator of risk of zinc deficiency. The WHO considers national stunting rates of ≥ 20% to be a level of public health concern [2]. The same cutoff can be applied to indicate when there may be a substantial risk of zinc deficiency, in which case further assessment of zinc status should be considered. Data on the prevalence of stunting are collected routinely in many countries and are compiled in the WHO Global Data Base on Child Growth and Malnutrition [3]. Updated information is available on the Internet (http://www.who.int/nutgrowthdb/). Thus, countries can use this existing information to assess the likelihood that zinc deficiency is a local problem. The following map, reproduced from the WHO data (figure 2.1), indicates those countries where the prevalence of childhood stunting exceeds 20%, and specific data can be found in appendix 1.

2.2.2 Adequacy of zinc in the national food supply

Studies of dietary intake can be used to estimate the risk of inadequate zinc intake in a population. However, at present, information on zinc consumption has been obtained from representative samples of the national populations of very few lower-income countries. In lieu of available information on dietary zinc intake, a simple alternative method, based on the zinc content of the national food supply in relation to the population's theoretical zinc needs, can be applied to estimate the risk of inadequate zinc intake. The proposed method is described in this section.

Each year the Food and Agriculture Organization (FAO) of the United Nations publishes national food balance sheets (FBS), which currently provide data on the amounts of 95 food commodities available for human consumption in 176 countries. Despite the inherent weaknesses of this type of aggregated, national-level information, the FBS do provide reasonably accurate, frequently updated information that, with appropriate interpretation and considerable caution, can be used as an ecological indicator of the risk of inadequate zinc intake in the population. The general approach for estimating the amount of absorbable zinc in national food supplies and judging its adequacy has been described in detail elsewhere [4]. This earlier analysis has been updated for the present document to include more recent information from FBS for the period 1992–2000 and to account for the current IZiNCG revised estimates of the average physiologic requirements for absorbed zinc and the level of zinc absorption expected from different types of diets.

For these analyses, the amounts of each of the food commodities reported in the national FBS were multiplied by their zinc and phytate contents to determine the amounts of these food components that are avail-
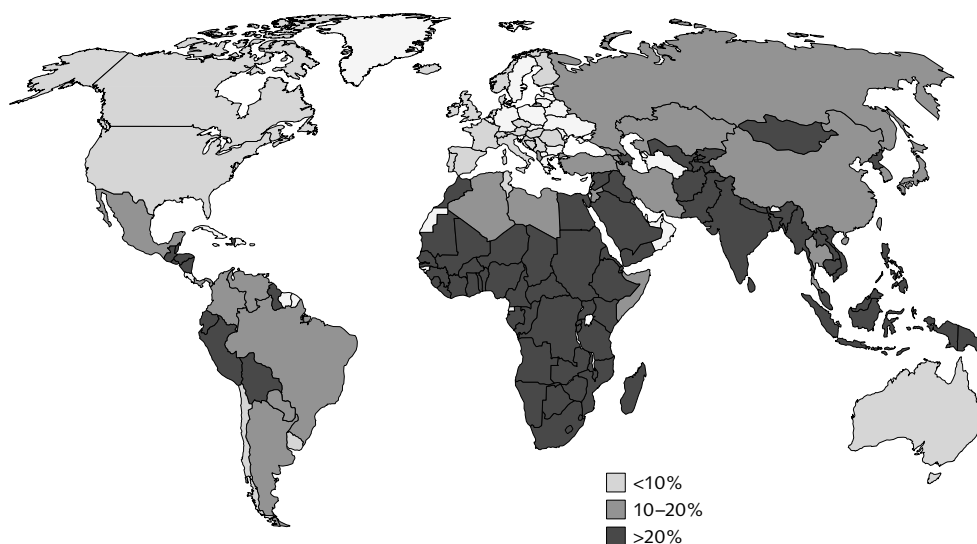
FIG. 2.1. Estimated prevalence of stunting (< –2 Z-score) among children under 5 years of age, by nation; adapted from the WHO Global Data Base on Child Growth and Malnutrition [3]

able for human consumption in each country. The amount of zinc that might be absorbed from these foods was then calculated, by using the amounts of zinc and phytate to estimate the country-specific mean fractional absorption of zinc, using the equation presented in section 1.6. The estimated mean daily per capita amount of absorbable zinc in the food supply was then compared with the mean physiologic requirement for absorbed zinc for the population (table 1.9), after weighting these theoretical requirements for the population's age and sex distribution. Finally, the proportion of the population at risk of inadequate zinc intake was estimated by calculating the percent of individuals whose intake of absorbable zinc is likely to provide less than their physiologic requirement, assuming the following: (1) that the mean intake of absorbable zinc in each country is the same as the mean daily per capita absorbable zinc content of the food supply; (2) that the habitual intake of absorbable zinc is normally distributed; and (3) that there is a 25% inter-individual coefficient of variation in intake [5]. This method is akin to the EAR cut-point method for estimating the adequacy of nutrient intakes in a population, as described in the recent FNB/IOM publication on dietary assessment [6]. The assumptions necessary to assure the validity of this method to estimate inadequate intakes in a population are described in further detail in section 2.3.1.

It is important to recognize that the accuracy of this approach is undermined to some extent by the lack of well-founded, quantitative information on the food processing techniques employed in different countries. For example, the extent of milling and fermentation of cereal staples markedly changes their zinc and phytate contents, and hence the estimates of the absorbable zinc

contents of these foods. Thus, several additional sets of assumptions had to be applied for the present analyses. In particular, with regard to wheat, the following were assumed: (1) that 90% of the wheat in the regions of North Africa and the eastern Mediterranean and South Asia is consumed as whole wheat and 10% is consumed as 75%-extraction white flour; (2) that 10% of the wheat in Latin America and the Caribbean and in sub-Saharan Africa is consumed as whole wheat and the rest is consumed as white flour; and (3) that in all other countries 1% of the wheat is consumed as whole wheat and the remainder as white flour. In all cases, it was assumed that whole-grain wheat is not fermented and 58.5% of the white flour is fermented with yeast, as is the practice in the United States [7]. In the case of maize, the following were assumed: (1) in Central America all available maize is processed into tortillas; (2) in West Africa all available maize is fermented; and (3) in all other countries maize is consumed as unfermented, unrefined maize. Finally, it was assumed that all rice is consumed as unfermented, milled, white rice and all other cereals are consumed as unfermented, whole-grain products. These assumptions are based on just a limited number of consultations with experts on national food supplies, so if more accurate information on food processing is available at the country level, the information presented herein should be revised accordingly. In a paper to be published separately, we examine in greater detail how modifying the assumptions regarding food processing techniques might affect the estimated percent of individuals at risk of inadequate zinc intake. Briefly, these estimates are affected to only a modest degree in all regions except the eastern Mediterranean, where the predicted percent of the population at risk of inadequate zinc

intake might range from 7% to 15%, and South Asia, where the predicted values might range from 24% to 34%, depending on the assumptions that are applied.

Table 2.1 displays regional data on the mean daily per capita availability of the following items in the food supply of 176 countries: total energy (kcal/day), percent of energy from animal-source foods, total zinc (mg/day), zinc density (mg/1000 kcal), percent of zinc from animal source foods, total phytate (mg/day), phytate:zinc molar ratio, estimated fractional absorption of zinc (percent of the mean daily per capita amount of available zinc), estimated absorbable zinc (i.e., the mean daily per capita total available zinc multiplied by the estimated fractional absorption of zinc, in mg/day), absorbable zinc as a percentage of the weighted daily mean population zinc requirement, and the estimated percent of the population at risk of inadequate zinc intake. The regions are ranked in descending order by the amount of absorbable zinc in the national food supplies.

The mean daily per capita amount of total zinc in the national food supply is about 15 mg/day in North Africa and the eastern Mediterranean, where nearly 50% of the food energy is derived from wheat (presumably, mostly whole-wheat) products, 11–12 mg/day in the countries of Europe, North America, China, and the western Pacific, and about 9–10 mg/day in the countries of Latin America and the Caribbean, South and Southeast Asia, and sub-Saharan Africa. The total zinc content of national food supplies is strongly associated with total energy content and percent of energy provided by animal source foods (data not shown). Notably, in the wealthier countries more than half the zinc is provided by animal source foods compared with just 15–25% in the poorer ones, leading to sizeable differences in the phytate:zinc molar ratios among regions and approximately 50% differences in the estimated amount of zinc that is likely to be absorbed from the available foods (table 2.1). Using the assumptions described above, it appears that fewer than 10% of the population of the Western Europe, North American, and North African/eastern Mediterranean regions is at risk of inadequate zinc intake compared with more than 25% of the population in Latin America and the Caribbean, South and Southeast Asia, and sub-Saharan Africa. Additional country-specific information on the absorbable zinc content of national food supplies is available in appendix 1.

Several concerns must be highlighted with regard to the interpretation of this information. First of all, the data base that was used to estimate the zinc and phytate contents of national food supplies contains information on a fairly limited number of foods, so in some cases information had to be imputed for the full range of commodities reported in the FBS. Secondly, national FBS provide information on food availability, not food consumption, so the actual within-country distribu-

tions of food intake, and hence zinc consumption, are uncertain. The assumption of 25% inter-individual variation in zinc intake that was applied is based on a single national nutrition survey conducted in the United Kingdom [8] and may vary across countries and age groups. Finally, the estimates of zinc absorption do not account for these inter-individual differences in intake, and they rely on additional assumptions regarding the types of food processing that are employed in different countries. For all these reasons, the estimates of the absolute percent of individuals at risk of inadequate zinc intake must be interpreted with a great deal of caution. Nevertheless, the rank order of risk level in different countries and regions should be reasonably reliable, so these results can be used to identify those settings where there is a greater likelihood of inadequate zinc intakes.

2.2.3 Rates of anemia

Although anemia may result from a variety of factors, about half of all cases are believed to be attributable to iron deficiency [9]. Iron and zinc have a similar distribution in the food supply, and some of the same food components similarly affect the absorption of both minerals. The richest sources of iron and zinc are meat and other animal flesh foods, and both are found in moderate concentrations in cereal grains, which have much lower proportions available for absorption. As the nutritional causes of iron deficiency and zinc deficiency are similar, high rates of iron-deficiency anemia may be used as suggestive evidence of the risk of zinc deficiency.

There are several data sets that demonstrate a positive correlation between anemia and indicators of the risk of zinc deficiency. For example, the prevalence of anemia is inversely correlated with the amount of absorbable zinc in national food supplies ($r = –0.47$, $p < 0.01$; Wuehler et al, unpublished data). In a study of New Zealand women ($n = 238$), serum zinc concentrations were modestly but significantly positively correlated with hemoglobin ($r = 0.182$; $p = 0.002$), and serum ferritin ($r = 0.10$; $p = 0.05$) among those not using oral contraceptive agents.* Significant correlations between serum zinc and hemoglobin ($r = 0.291$; $p < 0.05$) have also been noted in Italian adults [10]. In a study of pregnant Filipino women, hemoglobin and serum zinc were significantly correlated at 24 weeks ($r = 0.22$; $p < 0.001$), but not at 36 weeks of gestation [11]. Results from the recent National Nutrition Survey in Mexico [12] also demonstrated a significant correlation between hemoglobin and serum zinc concentration among women ($r = 0.221$; $p < 0.001$), and school-aged children ($r = 0.090$; $p < 0.05$), although not among preschool children ($r = –0.025$; $p > 0.05$). A lack of

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* RS Gibson, personal communication.

TABLE 2.1. Daily mean amounts of selected nutrients and food components in the national food supplies of 176 countries, by region (mean ± SD)[a,b] (See text for a detailed description of the analytic approach)

Variable	W. Europe	USA & Canada	E. Europe	N. Africa & E. Medit.	China, (+Hong Kong)	W. Pacific	Latin America & Carib.	South Asia	Southeast Asia	Sub-Saharan Africa	Total
Number of countries	20	2	27	17	1	12	35	6	10	46	176
Population (millions)	457	305	413	342	1,256	223	498	1,297	504	581	5875
Energy (kcals)	3,411 ± 129	3,602 ± 166	2,916 ± 277	2,796 ± 455	2,918	2,806 ± 267	2,781 ± 317	2,381 ± 106	2,626 ± 260	2,212 ± 411	2,755 ± 443
% of energy from animal sources	28.4 ± 8.0	27.9 ± 0.3	23.7 ± 4.4	9.9 ± 4.2	16.5	19.6 ± 7.4	18.2 ± 4.9	8.2 ± 3.5	8.4 ± 4.6	6.6 ± 5.5	14.9 ± 8.4
Zinc (mg)	12.4 ± 1.4	12.5 ± 0.5	10.6 ± 1.6	15.4 ± 2.2	12.4	11.3 ± 0.9	10.3 ± 2.0	10.8 ± 1.3	9.2 ± 0.9	9.4 ± 2.2	11.3 ± 2.1
Zinc per 1000 kcals (mg)	3.6 ± 0.4	3.5 ± 0.0	3.6 ± 0.3	5.5 ± 0.4	4.3	4.0 ± 0.3	3.7 ± 0.4	4.5 ± 0.4	3.5 ± 0.1	4.3 ± 0.8	4.1 ± 0.6
% of zinc from animal sources	56.2 ± 12.2	60.8 ± 1.6	49.6 ± 6.0	15.0 ± 6.8	37.2	39.4 ± 14.5	41.9 ± 11.5	10.9 ± 3.3	21.2 ± 11.2	15.1 ± 10.2	30.6 ± 18.4
Phytate (mg)	1,282 ± 271	1,338 ± 51	1,215 ± 292	3,877 ± 706	2,056	1,852 ± 482	2,030 ± 794	2,827 ± 265	2,231 ± 618	2,469 ± 668	2,221 ± 797
Phytate:zinc ratio	10.6 ± 3.4	10.6 ± 0.0	11.4 ± 2.5	24.9 ± 2.2	16.4	16.6 ± 5.2	19.8 ± 6.7	26.1 ± 1.3	23.8 ± 4.9	26.1 ± 4.0	19.8 ± 6.7
Fractional absorption, IZiNCG	0.24 ± 0.01	0.24 ± 0.00	0.26 ± 0.02	0.18 ± 0.01	0.22	0.23 ± 0.01	0.23 ± 0.03	0.21 ± 0.01	0.23 ± 0.02	0.22 ± 0.03	0.22 ± 0.02
Absorbable zinc, IZiNCG (mg)	3.0 ± 0.4	3.0 ± 0.1	2.7 ± 0.3	2.7 ± 0.2	2.7	2.6 ± 0.4	2.4 ± 0.4	2.2 ± 0.2	2.1 ± 0.1	2.1 ± 0.3	2.5 ± 0.4
% IZiNCG estimate of mean physiological requirement for absorbed zinc (adj. for phytate)	148 ± 14.6	149 ± 3.5	137 ± 13.3	151 ± 10.2	137	128 ± 18.2	126 ± 20.2	120 ± 10.5	113 ± 6.5	121 ± 15.2	131 ± 16.6
Estimated % of population at risk of inadequate zinc intake	10.9 ± 5.2	9.5 ± 1.3	16.2 ± 10.5	9.3 ± 3.6	14.1	22.1 ± 8.2	24.8 ± 12.0	26.7 ± 9.4	33.1 ± 5.9	28.2 ± 15.0	20.5 ± 11.4

a. Data on food availability obtained from FAO National Food Balance Sheets (http://apps.fao.org/page/collections?subset=nutrition). Demographic data obtained from United Nations' Women's Indicators and Statistics Database (Wistat CD-ROM version 3, 1994). China is considered as a separate region. China is considered as a separate region, hence no SD could be calculated.

b. SDs represent the dispersion of data for different countries within the same region. China is considered as a separate region, hence no SD could be calculated.

relationship between serum zinc and hemoglobin has been reported in some populations, such as pregnant Malawian women [13] and young Vietnamese children [14], and this may in part be attributed to the effect of confounding factors, such as the presence of concurrent infections, including malaria, on the biochemical indices. Thus, it must be recognized that the occurrence of anemia does not necessarily indicate the presence of zinc deficiency.

Anemia rates are often measured in large-scale population health surveys, so information is available for many countries, primarily for high-risk groups, such as women of childbearing age and young children. Where the prevalence of iron-deficiency anemia in any age group is considered to be high, further assessments of zinc deficiency are warranted. Current guidelines suggest that a prevalence of anemia > 40% is indicative of a severe public health problem requiring urgent corrective action [15] (http://www.who.int/nut/documents/ida_assessment_prevention_control.pdf). A global database of prevalence rates of anemia and iron deficiency is currently maintained by The Micronutrient Initiative (http://www.mn-net.org/idastat/). The United Nations Women's Indicators and Statistics Database (Wistat) provides data on anemia rates among pregnant and non-pregnant women in more than 200 countries and is available on compact disc (United Nations publications, New York/Geneva).

2.2.4 Composite index of the national risk of zinc deficiency, based on stunting rates and the adequacy of zinc in the national food supply

Two of the foregoing pieces of information that are suggestive of a population's risk of zinc deficiency—namely, the percent of preschool children who are stunted (low height-for-age) and the percent of individuals at risk of inadequate zinc intake (based on data obtained from national Food Balance Sheets)—use information that is already widely available and routinely published by the UN agencies. Therefore, these sources of information allow for immediate estimation of the risk of zinc deficiency in many countries. However, for the reasons stated above, neither of these pieces of information can provide a true estimate of the risk of zinc deficiency in a particular population. In an attempt to derive stronger inferences than might be possible from either one of these sources of information alone, the IZiNCG SC explored the possibility of combining both sets of information to construct a composite index of the national risk of zinc deficiency.

Preliminary analyses indicated that national-level data concerning the percent of preschool children who are stunted and the percent of individuals at risk of inadequate zinc intake are significantly correlated ($r = 0.60$, $p < 0.0001$), although there is considerable variability about the best-fit line (figure 2.2). Because

of this variability and the fact that these indicators only provide suggestive information on the risk of zinc deficiency, countries were then classified according to the combined set of information. As discussed previously (section 2.2.1), the WHO considers a rate of stunting ≥ 20% as indicative of a public health problem. With regard to the FBS information, a cutoff of 25% estimated prevalence of inadequate zinc intake was applied because of the multiple sources of uncertainty in using these data to estimate the risk of population zinc deficiency. Using the two sets of cutoffs, it is possible to identify a set of countries with a relatively high risk of zinc deficiency according to both indicators (figure 2.3). Likewise, the two sets of data can be combined to identify countries where the rate of stunting is less than 10% and the percent of individuals at risk of inadequate zinc intake is less than 15%, in which cases the risk of zinc deficiency is likely to be low. Finally, countries with intermediate rates of either stunting or prevalence of risk of inadequate intake can be considered to have an intermediate risk of zinc deficiency. According to this combined indicator, as shown in figure 2.3, selected countries in South and Southeast Asia, Southern Africa, Central America, and the Andean region appear to have the highest risk of zinc deficiency, and many other countries in these same regions are classified as having a moderate risk of zinc deficiency. Country-specific information on the available suggestive evidence of zinc deficiency and the combined indicator of risk is provided in appendix 1. Notably, approximately one third of the world's population live in countries identified as having a high risk of zinc deficiency and one half live in countries found to have a moderate risk of zinc deficiency.
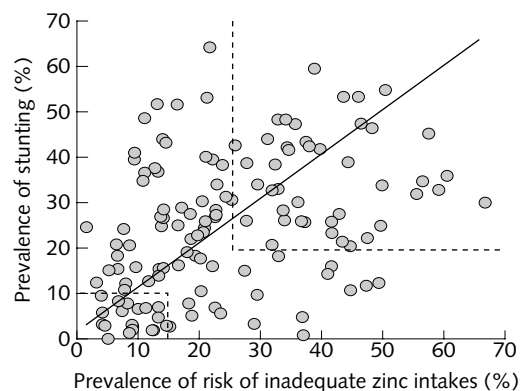

FIG. 2.2. Relationship between two sets of suggestive information concerning national risk of zinc deficiency: the prevalence of stunting (low height-for-age) in preschool children and the percentage of the population at risk of inadequate zinc intake (based on national food balance sheet data) ($r = 0.61$, $p < 0.001$).
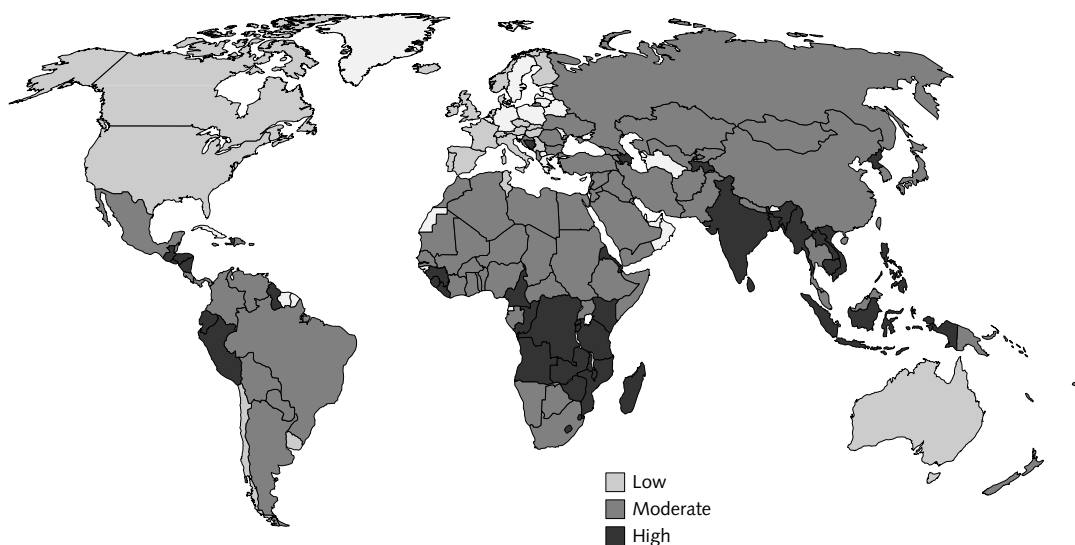
FIG 2.3. National risk of zinc deficiency based on combined information regarding the prevalence of childhood growth-stunting and the percent of individuals at risk of inadequate zinc intake

2.3 Methods to estimate the risk of zinc deficiency in populations

As indicated by the suggestive evidence described in section 2.2, zinc deficiency is expected to be widespread. However, to move forward with the development and evaluation of programs to improve population zinc status, it is necessary to derive more precise estimates of the magnitude of risk of zinc deficiency using more direct measures of a population's zinc status. Currently, national prevalence estimates of zinc deficiency based on direct measures are lacking for most countries. The methods for assessing the risk of population zinc deficiency that will be discussed in this section include assessment of dietary intakes of zinc, biochemical indicators of zinc status, and functional response to zinc supplementation. This section suggests methods for the direct measurement of zinc status, and possible approaches to the interpretation of results; application of standardized methods will assist in the comparison of information on indicators of zinc status of different populations.

2.3.1 Assessment of dietary zinc intakes

Because inadequate dietary intake of zinc is the most likely cause of zinc deficiency, dietary assessment is an important component in evaluating the risk of zinc deficiency. Information on the adequacy of dietary zinc intakes should be interpreted together with data derived from other assessment methods, such as biochemical assessment (section 2.3.2), to facilitate interpretation of the risk of zinc deficiency in the population. Standard dietary assessment methods can be applied to evaluate the adequacy of zinc intakes in populations, and to

support results of biochemical assessments. Further, information derived from dietary surveys is useful for determining the specific dietary causes of inadequate zinc intake and therefore to help identify appropriate food-based approaches to intervention.

It is unlikely that it will be feasible to develop large-scale population-based dietary surveys to assess intakes of a single nutrient in most countries. However, where national nutrition surveys are planned, assessment of zinc intakes should be included, particularly where suggestive evidence indicates an elevated risk of zinc deficiency in the population (section 2.2). Likewise, when such dietary surveys have already been completed, it should be possible to re-analyze the information on food intake to assess the adequacy of zinc intake. This section provides information that will help guide the choice of appropriate assessment methods and includes considerations for the design of dietary surveys and analysis of data where zinc is to be incorporated in the assessment.

Determining the objectives of the dietary survey

Before selecting specific dietary assessment methods and developing a survey design, the objectives of the dietary assessment study must be clearly defined. There are two possible approaches to collecting dietary survey data. The preferred approach is to estimate the prevalence of inadequate intakes based on the distribution of usual intakes in the population. In this case, the distribution of observed dietary intakes in a group must be corrected for intra-individual (day-to-day) variation in intake. The assessment must therefore cover at least two, preferably non-consecutive, days of dietary intakes for each individual, or for an appro-

priate sub-sample of individuals in the survey (i.e., at least 30–40 individuals; [6]). In the case where it is possible to estimate usual intakes for all individuals in the sample, the number of days of dietary data for each individual required to derive this estimate can be calculated using the information in box 2.1. A less desirable approach is to characterize mean intakes of a group, in which case dietary information for a one-day period is collected for each person in the survey sample.

This method is limited because only the mean intake can be estimated with certainty; the true distribution of usual intakes by the population is not known because variability due to intra-individual variation in intakes is not measured and therefore cannot be removed subsequently from the intake distribution. In both cases, each day of the week should be equally represented in the final sample.

The choice of survey approach will depend on the

BOX 2.1. Assessing the adequacy of individuals' intakes: an example

Source of data	n	Mean zinc intake (mg/day)	Intra-individual variance (%)	Inter-individual variance (%)	Variance ratio
Malawian women:					
2 × 24 hr recall	60	6.2	34	21	2.6
2 × 1-day weighed record	60	6.8	44	23	3.7
Ecuadorian women: 4 × 24 hr recalls	13	6.3	37	18	4.4
Ecuadorian men: 4 × 24 hr recalls	15	6.9	58	0	–

The ratio of the intra- to inter-subject variance, or variance ratio, can then be calculated. A ratio of 1.0 indicates that the intra-individual and inter-individual variances are equal, whereas a ratio > 1.0 indicates that the intra-individual variance is greater than the inter-individual variance [16]. In some cases, all or most of the intake variance is associated with intra-individual variation in intakes, despite the consumption of relatively monotonous diets.

After calculating the intra-individual variation using analysis of variance, the intra-individual coefficient of variation (CV_{intra}), can be determined as:

$$(\sqrt{\text{variance of intra-individual intake}} \div \text{mean intake}) \times 100\%$$

The CV_{intra} can then be used in the following equation to estimate the number of days required per subject to estimate an individual's zinc intake to within 20% of their true mean 95% of the time [17]:

$$n = (Z_\alpha CV_{intra}/D_0)^2$$

where: n = the number of days needed per subject
Z_α = the normal deviate for the percentage of times the measured value should be within a specified limit (i.e., 1.96 in the example below)
CV_{intra} = the intra-individual coefficient of variation
D_0 = the specified limit, as a percentage of long-term true intake (i.e., 20% in example given below modified from Willett [18]).

Example: To calculate the number of days needed to estimate a Malawian woman's zinc intake using 24 hr recalls to within 20% of the true mean, 95% of the time:

$$Z_\alpha = 1.96 \quad \text{and} \quad CV_{intra} = 34\%$$

then:

$$n = (1.96 \times 34\%/20\%)^2 = 11 \text{ days}$$

The mean dietary zinc intake data derived for the determined number of days can then be compared to the RDA appropriate for the sex, life stage, and usual diet type of the individual (table 1.10). Usual intakes well below the corresponding RDA indicate a risk that the individual's zinc requirements may not be met. Further assessment of clinical and biochemical status of the individual would be required to determine if zinc deficiency exists.

objectives of the survey, the expected application of the survey data, and the availability of resources. A survey designed to estimate usual intakes for individuals in the population will provide more accurate information on the distribution of usual dietary zinc intakes in the population and allow a greater capacity to do the following: (1) estimate the proportion of individuals in a population with inadequate intakes; (2) design specific, food-based zinc intervention programs (e.g., determining an adequate and safe level of zinc fortification for a specific food vehicle); and (3) evaluate the effectiveness of programs to improve the zinc status and health of the target group(s) in relation to changes in usual zinc intakes. In the case that usual intakes for each individual in the sample can be estimated from multiple days of dietary data, it will also be possible to assess correlations between zinc intakes and other indicators of zinc status. However, the survey design required to meet this objective will also require more resources (e.g., time, staffing, budget) and more intensive data analysis procedures.

Dietary surveys that only allow the mean population intake to be assessed may be carried out where resources for conducting surveys are limited. Mean population intake data can be used to identify foods that are the primary contributors of specific nutrients in the population and to identify possible food vehicles for use in fortification programs. However, such data cannot be used reliably to determine the prevalence of inadequate intakes in the population because data on the distribution of usual zinc intakes are not available. Instead, only a crude estimate of the risk of inadequate intakes in the population can be made. The choice of survey design will also have implications for data analysis, and this is discussed in further detail below.

Implementation of the dietary assessment protocol

There are four stages in the implementation of a dietary assessment protocol designed to evaluate the adequacy of zinc intakes in individuals or populations: (1) measurement of food intakes; (2) calculation of the nutrient and anti-nutrition contents of the foods eaten; (3) estimation of the proportion of dietary zinc available for absorption; and (4) comparison of the mean usual intake of absorbable zinc for the population, or the distribution of usual zinc intakes within the population, to appropriate requirement estimates to assess the adequacy of intakes.

Measurement of usual food intakes

Several quantitative methods for assessing usual dietary intakes of individuals exist: weighed food records, recalls, and semi-quantitative food frequency questionnaires [19, 20]. Of these, food records and recalls are designed to measure the quantity of each food consumed over a one-day period. By contrast, a food frequency questionnaire (FFQ) obtains retrospective information on the pattern of food consumption during a longer time period, and sometimes on the usual intakes of certain nutrients.

Weighed food records completed by trained research assistants in households have been used to collect reliable quantitative data on dietary intakes, including zinc, in lower-income countries [21]. Methods for collecting weighed food records are described in detail elsewhere [22]. Although this method is more time consuming and costly, has a higher respondent burden than other methods, and may increase the likelihood that respondents change their dietary intakes during the recording period, weighed food records are the most accurate method of determining actual intakes during the recording period.

Dietary recalls can be used for estimating zinc intakes among non-literate populations, provided that portion sizes of the staple can be recalled accurately [23, 24]. Proper training of field workers in recall interview techniques can minimize bias and non-response rates [25]. As well, several strategies can be used to reduce memory lapses and facilitate portion size estimates, including training respondents in the use of food picture charts, bowls, plates and utensils familiar to the locale, and samples of actual cooked or raw foods that are commonly consumed [24]. Recalls are suitable for areas where diets are not very diverse and are predominantly plant based, as is the case in many lower-income countries. Although some accuracy is compromised by their use, recalls are easier, faster, and less expensive than weighed food records and are less invasive, so that compliance is enhanced, and the tendency to alter food intake is reduced.

An interactive 24-hour recall method has been specially designed for measuring usual intakes of total and absorbable zinc in lower-income countries. The feasibility and the relative and concurrent validity of this method were tested in rural Malawi, in sub-Saharan Africa [13, 24]. Intakes of available zinc calculated from three interactive 24-hour recalls and indices of absorbable dietary zinc were significantly associated with hair zinc concentrations, confirming that this assessment method can provide valid estimates of the amount of zinc available for absorption at the individual level. Further validation of this method to estimate usual intakes of absorbable zinc by individuals in other populations would be useful. Details of this interactive 24-hour recall method for determining intakes and adequacy of absorbable and total zinc (and iron) are given in Gibson and Ferguson [26].

Semi-quantitative FFQs have not yet been validated for the estimation of usual zinc intakes by individuals. Unlike nutrients such as vitamin A and calcium, which are concentrated in a relatively small number of foods or specified food groups, zinc occurs in a wide range of plant-based food items as well as animal source foods

and therefore may be quantified less accurately using this method. Although FFQs may prove to be suitable for determining mean population intakes, more research is required on the validity of this technique for estimating usual intakes of absorbable zinc for individuals before it can be applied with confidence.

Calculating total zinc intakes and estimating dietary zinc absorption

Once the daily food intake has been measured, total zinc intakes can be calculated by multiplying the amount (g) of each food consumed by its zinc content (mg zinc/100 g). It is preferable to use local food composition data for calculating zinc intakes, when available, because the zinc content of locally grown plant based foods can vary according to soil conditions, agronomic practices, and local food processing and preparation techniques [27]. However, when local food composition tables are not available, data from regional or global tables can be used. Factors influencing the proportion of dietary zinc that is absorbed in the gut and the importance of considering these factors in assessing the adequacy of dietary zinc intakes are discussed in section 1.6 (chapter 1). Two different levels of zinc absorption were suggested to represent the estimated usual absorption of zinc based on diet type and the phytate: zinc molar ratio: (1) mixed diets or refined vegetarian diets were those with a phytate:zinc molar ratio ranging from 5 to 18, and having an estimated average zinc absorption of 27% (adult men), 35% (adult women), or 31% (children); (2) unrefined, cereal-based diets were those with a phytate:zinc molar ratio > 18, and having an estimated average zinc absorption of 19% (adult men), 26% (adult women), or 23% (children). As the EAR (and RDA) for zinc are dependent on the assumed level of zinc absorption, two different levels of EARs and RDAs are presented in section 1.6 for the two different levels of zinc absorption. It is thus useful to calculate total phytate intake, in addition to total zinc intake, from the dietary intake data, such that the phytate:zinc molar ratio can be calculated, as described in section 1.6. The diet type and phytate:zinc molar ratio can thus be used to select the most appropriate EAR for use in assessing the adequacy of zinc intakes by populations. Likewise, the appropriate RDA can be selected for assessing adequacy of an individual's intakes, as discussed in box 2.1.

At present, the amount of data available on the zinc content of foods is not as extensive as for some other nutrients, but is increasing. Also, very few local food composition tables contain values for the phytate content of local plant-based staple foods, which will make it difficult in many cases to quantify total dietary phytate intakes. Regional or national data centers of the International Network of Food Data Systems (INFOODS) may be contacted for information on the availability of data on the zinc and phytate content of local foods.

Data on the zinc content of foods are available from the US Department of Agriculture (USDA) food composition database, which may be downloaded from the Internet. Data on the phytate content of US foods are available on the University of Minnesota Nutrition Coordinating Center Nutrient Database; these data are updated regularly and the software system including the food composition database can be purchased from the Center. The phytate and zinc content of foods in several lower-income countries (Egypt, India, Indonesia, Kenya, Mexico, Senegal) derived from the International Minilist are available through the WorldFood Dietary Assessment System, 2.0. Although this database is not being updated, the dietary assessment software program can be downloaded free of charge from the INFOODS website (http://www.fao.org/infoods/software_worldfood_en.stm). Contact information for each of these resources is provided in appendix 2. Where adequate data on the phytate or zinc contents of foods are not available, it is preferable to determine these contents by direct analysis of locally acquired foods. When resources are not available to complete such laboratory analyses, the phytate content of foods may be extrapolated from the average phytate contents of common foods or food categories. A table of the phytate content of various foods is also provided in appendix 2.

The most commonly used method for analysis of zinc content of foods is flame atomic absorption spectrophotometry (AAS). Preparation of samples for analysis includes dry ashing to remove organic material followed by dilution with acid. Detailed methods can be found in Horwitz [28] and Aurand et al. [29]. Standard reference materials (SRM) suitable for food composition analysis are available from the National Institute of Standards and Technology (NIST; Gaithersburg, Maryland, USA) and Analytical Quality Control Services (Seibersdorf, Austria).

The adequacy of zinc intakes by individuals (as opposed to populations, or groups of individuals) can be estimated by comparing the usual intakes of the individual to the corresponding Recommended Dietary Allowance (RDA). The derivation of the RDAs for zinc is described in section 1.6, and the RDAs by sex and life-stage group, and by usual diet type, are summarized in table 1.10. To estimate the adequacy of zinc intakes by individuals it is first necessary to determine their usual intake of zinc, for which multiple days of dietary intake data are required.

To calculate the number of days required to assess usual zinc intakes of individuals, data on the intra-individual (within-subject) variation in zinc intakes are required. This may be derived from previously, or prospectively, collected data from a similar population group (i.e., similar age, gender, and socio-cultural group) for which more than one day of food intake data was collected for each individual [19]. To date,

very few estimates are available on the intra- and inter-individual variance for zinc intakes. Available data from lower-income countries are shown in box 2.1 (adapted from Gibson and Ferguson [30]).

The analysis of phytate content of foods on a wider scale is hindered by the present lack of a universally accepted laboratory method and certified reference material. Nonetheless, several methods exist and continue to be developed. The analytic method employed for phytic acid should preferably use high performance liquid chromatography (HPLC). This method is preferred because it can separately identify and quantify both the higher (hexa- and penta-inositol phosphates) and lower inositol phosphates [31]. Only the higher inositol phosphate forms (IP-6 and IP-5) compromise zinc absorption [32]. Use of the HPLC method is especially important for certain prepared foods that have undergone soaking, germination, and/or fermentation, because some enzymatic and non-enzymatic hydrolysis of hexa- and penta-inositol phosphates to lower inositol phosphates may occur [33].

Assessing the adequacy of zinc intakes

To evaluate the adequacy of dietary zinc in populations, intakes must be compared with an appropriate set of dietary reference values, taking into account the estimated percent absorption of dietary zinc. Although several different sets of recommended dietary intakes for zinc exist, comparison to a single set of recommendations is desirable to facilitate cross-comparison of dietary adequacy among populations. Currently available dietary recommendations for zinc intakes are described in section 1.6. The IZiNCG SC reviewed available information and presented a revised set of dietary recommendations for zinc (EARs, table 1.9); these recommendations take into account variation in intake requirements due to differences in estimated percent zinc absorption based on diet type, and are appropriate for international use. These recommendations can thus be used to assess the adequacy of dietary zinc intakes for different sex and life-stage groups. Information on the assessment of dietary zinc intakes for individuals and use of the RDA (table 1.10) is presented in box 2.1.

When the survey design allowed for the estimation of day-to-day (intra-individual) variation in intakes, the full probability approach may be used to estimate the risk of inadequate intakes in the population. It is beyond the scope of this document to describe the details of this method; for in-depth information on the theories and methods the reader is referred to the publications of the National Research Council [16] and the Food and Nutrition Board, Institute of Medicine [6]. To use this method, it is necessary that the distribution of the requirements is known and is symmetrical about the mean, and that the physiologic

requirements for the nutrient are independent of its intake. The coefficient of variation of zinc requirements has been estimated to be 12.5%, as described in section 1.6. In the case of zinc, independence of requirements and intakes can be assumed.*

The observed intake data must be adjusted to remove variability introduced by intra-individual variation. This can be done using specialized software programs (e.g., C-SIDE, Iowa State University, Department of Statistics and Statistical Laboratory, Ames, Iowa, USA), or other statistical software with appropriate programming; detailed information describing this statistical methodology can be found in the report of the National Research Council [16, 34, 35]. As distributions of dietary intake data are typically skewed, the data are mathematically transformed prior to the adjustment for intra-individual variation, and can be reverse transformed following adjustment. The corrected distribution of intakes is then compared to the distribution of the EAR, and the probability of individual intakes falling below the EAR is computed. When the overall probability of inadequate intake is ≥ 25%, it is considered that there is an elevated risk of zinc deficiency in the population.

As an alternative to the probability approach, a more simplified method to estimate the prevalence of inadequate intakes may be used, which is referred to as the EAR cut-point method. Theoretical aspects and application of this methodology have been described by Beaton [36] and the FNB/IOM [6]. Briefly, the requirements for use of this method are the same as those indicated for the probability approach, but include an additional requirement that the variability in intakes among individuals in a population is greater than the variability in requirements of individuals. The latter assumption is likely to be valid in most cases, as the CV of the distribution of population zinc intakes has been assumed to be 25% [5, 37] based on data from a survey in the United Kingdom [8], which greatly exceeds the assumed distribution of zinc requirements (i.e., 12.5%; section 1.6). Once a distribution for zinc

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* It is apparent from figure 1.4 that absorbed zinc intakes above the point where intake equals endogenous losses of zinc results in increased endogenous losses of zinc via the intestine, and therefore it is unlikely that increased intakes result from increased requirements. Although zinc requirements may become somewhat dependent on absorbed zinc intakes in the range of intakes just at or below the physiologic requirement for zinc as a result of homeostatic adaptations that reduce intestinal losses of endogenous zinc, the overall correlation between zinc intakes and zinc requirements across a range of dietary zinc intakes in a population is expected to be low (e.g., < 0.25–0.30), in which case any bias introduced by the dependency effect is likely to be minimal [6]. Nonetheless, it should be recognized that where a large proportion of individuals in a population have intakes near to the EAR, it is possible that either the probability approach or the EAR cut-point method will overestimate the proportion of individuals with inadequate intakes.

intakes with intra-individual variation removed is derived, as described above for the probability approach, the prevalence of inadequate intakes can simply be estimated by determining the proportion of individuals with intakes below the EAR. The accuracy of this method can approach that of the probability approach, particularly when the actual prevalence of the inadequate intakes is not very high or very low. Where ≥ 25% of individuals in the population have intakes less than the EAR, it may be considered that there is an elevated risk of zinc deficiency in the population. A hypothetical example of the estimation of the proportion of individuals with inadequate zinc intakes in a population of adult women is depicted in figure 2.4.

When usual zinc intakes of each individual in the sample have been determined, the mean intake data for each individual can be used and no further correction of the distribution is required. To estimate the prevalence of inadequate intakes, it is simply necessary to determine the proportion of individuals with usual intakes below the EAR.

For surveys that collected data for only a single day's intake by each individual, the true intra-individual variation and distribution of usual zinc intakes in that population are not known. In this case, it may be assumed that the CV of usual intakes by the population is equivalent to 25% [5, 37], as noted above. Assuming this distribution, the EAR cut-point method may be used to crudely estimate the proportion of individuals with inadequate intakes, as described above. The proportion of individuals in the population with intakes below the EAR can be determined using a cumulative distribution function, such as CDF.NORM in SPSS (SPSS, Inc., Chicago, IL, USA), where the SD of intakes is assumed to be 25% of the mean. However, caution must be used in the interpretation of these data because the true variability in usual population intakes is not known. The assumption of a 25% CV for population intakes would not be valid, for example, if the distribution of the observed (uncorrected) intakes had a CV of less than 25%. Where the true variability of usual intakes by a population greatly exceeds 25%, the prevalence of inadequate intakes will be underestimated.

Considerations for designing dietary assessments

A sample size estimate for a dietary survey can be made based on the anticipated prevalence of inadequate zinc intakes, and the desired precision of the estimate of inadequate intakes. The anticipated proportion of an individual's zinc intakes falling below the EAR may be derived from pre-existing dietary data, or from an indirect assessment of the adequacy of zinc in the food supply (section 2.2.2). The desired precision of the estimate will be represented by the width of the 95% confidence interval. In general, for a given confidence interval width, the required sample size will be higher where greater proportions of an individual's intakes are expected to fall below the requirement. However, where high proportions of inadequate intakes are anticipated (e.g., > 30%), wider confidence intervals may be acceptable, thus minimizing the required sample size. Sample sizes based on half-width 95% confidence intervals of 0.02–0.06, expressed as the proportion of an individual's intakes (i.e., the ability to determine the mean proportion within 0.02–0.06 on either side of the mean, with a 95% level of confidence), are given in table 2.2. In situations where it is intended to quantify the change in dietary zinc intakes from surveys repeated at intervals, narrower confidence intervals may be desirable.

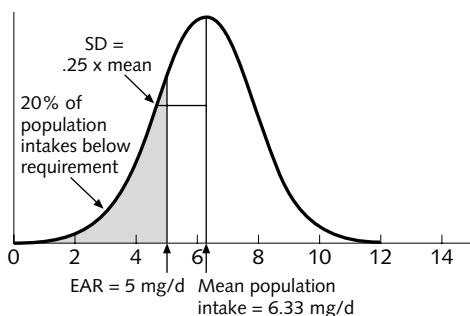Ultimately, the sample size for a large-scale dietary survey will often take into account the sample size

FIG. 2.4. Hypothetical, graphical representation of the estimation of the proportion of adult women with dietary zinc intakes below the estimated average requirement (EAR) for zinc from a typical mixed diet, assuming a mean intake of 6.3 mg/day and CV of the corrected distribution of usual intakes of 25%. Shaded area represents the percentage of the population (20%) with inadequate intakes.

TABLE 2.2. Estimated sample sizes for dietary surveys assessing usual zinc intakes of individuals within a population by anticipated proportion of individuals with inadequate intakes and width of 95% confidence interval

Estimated proportion[a]	Confidence interval (half-width)				
	± 0.02	± 0.03	± 0.04	± 0.05	± 0.06
0.05	457	203	115	73	51
0.10	865	385	217	139	97
0.15	1,225	545	307	196	137
0.20	1,537	683	385	246	171
0.25	1,801	801	451	289	201
0.30	2,017	897	505	323	225
0.40	2,305	1,025	577	369	257
0.50	2,401	1,068	601	385	267

a. Anticipated proportion of individuals with zinc intakes below the estimated average requirement (EAR)

needed to assess adequacy of dietary intakes for other nutrients, or other survey variables, and hence the final survey sample size may be dictated by those requirements. When designing population surveys, the sample size estimates should be applied to the different population strata, as appropriate. For example, samples may be selected to represent populations in different regions or districts or in urban versus rural areas. When collecting multiple days of intake data for individuals, non-adjacent days representative of the range of days to be studied should be selected to enhance statistical information. In studies of rural areas in lower-income countries, market days as well as weekend and weekdays should be proportionately included because the foods consumed can vary between market and non-market days [24].

Summary

The procedures for carrying out dietary surveys to estimate the adequacy of zinc intakes by populations are summarized in figure 2.5. The objective of the survey, and other factors such as the availability of resources for survey implementation will determine the survey design used. Weighed records or 24-hour recalls can be used to measure food intakes. Where the objective of the survey is only to estimate the adequacy of group mean zinc intakes, a single day of dietary intake data is collected. However, where the objective is to determine the proportion of individuals in the population with inadequate intakes of absorbable zinc, then at least two, non-consecutive days of dietary intake data are required for all individuals (or at least 30–40 individuals) in the survey sample. The usual diet type (e.g., mixed diet, refined vegetarian diet, or unrefined, cereal-based diet) and the phytate:zinc molar ratio of the diet, can be used to estimate an appropriate level of assumed zinc absorption, and thus to select the most appropriate set of EARs for determining the adequacy of zinc intakes by populations (section 1.6).

If the survey was designed to measure intra-individual variation in usual zinc intakes, the distribution of zinc intakes should be adjusted to remove this variation before estimating adequacy of zinc intakes. Using this corrected distribution, either the probability approach may be used to estimate the risk of individuals' intakes falling below the EAR, or the EAR cut-point method may be used to determine the proportion of individuals with intakes below the EAR. If the survey was not designed to estimate and correct for intra-individual variation in zinc intakes, the distribution of zinc intakes may be assumed to have a CV of 25%; the proportion of the population with intakes below the corresponding EAR can be crudely estimated using this assumption. Nonetheless, caution should be used in the interpretation of these data. For either study design, when ≥ 25% of individuals' intakes
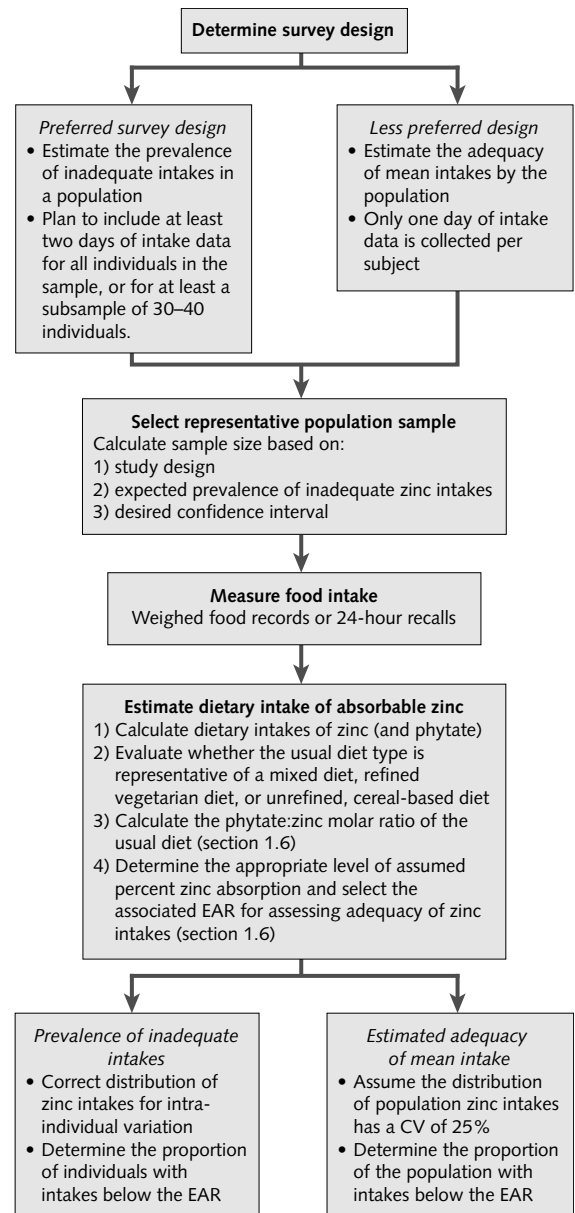

FIG. 2.5. Flowchart summarizing steps in assessing the dietary adequacy of zinc intakes in populations

fall below the EAR, the risk of zinc deficiency should be considered substantial. Nonetheless, to assist with the interpretation of the risk of zinc deficiency, it is recommended to combine estimates of adequacy of zinc intakes with data from biochemical assessments of zinc status (section 2.3.2).

2.3.2 Serum zinc concentration

Serum and plasma zinc concentrations are the most widely used biochemical markers of zinc status. Strictly

speaking, zinc concentrations measured in serum or plasma are not entirely comparable, largely as a consequence of the different collection and separation procedures used. Nevertheless, for the sake of simplicity, the following general discussions on circulating zinc concentrations will refer to serum zinc for both types of specimens. Although circulating zinc concentrations may have limitations in validity and reliability regarding the identification of mild to moderate zinc deficiency in individuals, several lines of evidence suggest that this index is useful in assessing zinc status at the population level [1, 38, 39]. In particular, the mean serum zinc concentration of groups of individuals responds as expected to dietary modifications and in association with functional outcomes following zinc supplementation. For example, in the United Kingdom National Diet and Nutrition Survey, there was a significant positive correlation between dietary zinc intakes, assessed by seven-day weighed food records, and fasting serum zinc concentration for girls aged 4 to 18 years, although not for boys [8]. In a study of New Zealand women not using oral contraceptives, there were significant negative correlations between serum zinc concentrations and intakes of dietary phytate and the phytate:zinc molar ratio [40]. An inverse correlation between serum zinc and dietary phytate: zinc molar ratios has also been reported in adolescent Canadian women consuming lacto-ovo vegetarian, semi-vegetarian, and omnivorous diets [41]. Further, in two experimentally controlled studies in which healthy adults were first fed omnivorous diets and then switched to vegetarian diets, measurable declines in serum zinc values on the vegetarian diets were observed [42, 43].

In an earlier meta-analysis of the effect of zinc supplementation on children's growth, which included studies of severely malnourished inpatients, the initial mean serum zinc concentration was inversely associated with the magnitude of the growth response to zinc supplementation [44]. However, this relationship was not statistically significant in an updated version of the meta-analysis that excluded severely malnourished children [1]. Low serum zinc concentration was found to be predictive of increased risk of diarrheal morbidity among Indian children [45]. In the aforementioned meta-analysis of the effect of zinc supplementation on children's growth, data on mean serum zinc concentration before and after the intervention were available for 15 studies [1]. In all but one study there was a positive response to zinc supplementation. The overall effect of zinc supplementation on serum zinc concentration was large (0.82 SD) and highly significant (p < 0.001). Thus, the population mean serum zinc concentration is a useful indicator of successful delivery and absorption of zinc supplements in children.

The following sections describe: (1) factors affecting the interpretation of serum zinc levels; (2) reference data for serum zinc concentration and the derivation of lower cutoffs for estimating the risk of zinc deficiency in populations; (3) technical considerations for the collection, preparation, storage, and analysis of serum samples for determination of zinc concentration; (4) quality control issues; and (5) considerations for survey design.

Factors affecting the interpretation of serum zinc concentration

Serum zinc concentrations fluctuate by as much as 20% during a 24-hour period [46], largely due to the effects of food ingestion. Following a meal, there is an immediate initial increase, after which the concentration declines progressively for the next 4 hours and then rises until food is eaten again. During an overnight fast, the concentration of serum zinc increases slightly, so the highest levels of the day are generally seen in the morning [47, 48]. However, diurnal variations in serum zinc concentration among fasted individuals have also been observed, whereby serum zinc decreased from morning to mid-afternoon and then began to rise again to morning levels [49].

Low serum zinc concentrations can occur in the presence of several conditions, representing a normal physiologic response and not necessarily indicative of low zinc status. Serum zinc concentrations are reduced during acute infections and inflammation, which is likely due to the redistribution of zinc from the plasma to the liver [50]; cytokines released during the acute phase response activate hepatic metallothionein synthesis [51], a metal-binding protein which appears to alter the hepatic uptake of zinc [52]. Elevated concentrations of C-reactive protein or other markers of the acute phase response can be used to indicate the presence of infection and should be considered in the interpretation of results. Stress and myocardial infarction also reduce serum zinc levels [53]. Because zinc is transported in plasma bound to albumin, diseases, such as cirrhosis and protein-energy malnutrition, that produce hypoalbuminemia result in lower serum zinc concentrations [54]. Hemodilution, as observed during pregnancy, oral contraceptive use, and other hormonal treatments, also results in a lower serum zinc concentration [55, 56]. On the other hand, conditions resulting in intrinsic or extrinsic hemolysis of blood cells can result in extremely high serum zinc levels because the concentration of intracellular zinc is considerably greater than in serum.

Reference data for serum zinc concentration and derivation of lower cut-offs

The largest population-based survey to include analysis of serum zinc concentrations in a presumably healthy,

non-malnourished, population was the United States National Health and Nutrition Examination Survey II (NHANES II: 1976–1980). NHANES II provides data for serum zinc concentrations in a representative sample of persons aged 3–74 years. Details of the NHANES II survey methodology, other data collected, and laboratory procedures have been previously reported [57, 58]. For both men and women, serum zinc values were lower in childhood, peaked during adolescence and young adulthood, and declined with age thereafter. From adolescence onwards, men had higher serum zinc values than did women, with the greatest differences occurring among adults aged 20–40 years. In the analysis of data originally reported by NHANES [58], serum zinc concentrations were described separately for three sets of samples collected: (1) in the morning from subjects in a fasted state ('AM Fasting'); (2) in the morning from subjects who were not asked to fast ('AM Other'); or (3) in the afternoon or evening ('PM'). As noted above, fasting state and diurnal variation are known to affect serum zinc concentrations. For each of the three sets of samples grouped by fasting state and time of day, the mean –2 SD was considered to represent the cut off level below which zinc deficiency is likely, and these values were reported as follows: AM Fasting, < 70 µg/dl (< 10.7 µmol/L); AM Other, < 65 µg/dl (< 9.9 µmol/L); and PM, 60 µg/dl (< 9.2 µmol/L). However, these proposed cutoffs did not take into account the known effects of age and sex on serum zinc concentrations, as reported in previous studies [59, 60]. Therefore, NHANES II data from a total of 14,770 individuals were reanalyzed for the present report to account for differences in reference values based on age and sex, as well as fasting status and time of day of sample collection. The results of this analysis are summarized here, and will be reported in detail elsewhere.

After eliminating data for 1307 subjects (13,463 remaining) for whom adequate information was not available, each of the four major variables (age, sex, time of day of blood sample collection [AM, PM, or Evening], and fasting status [≥ 8 hours fasted vs. fasting status unspecified or fasted < 8 hours]) were found to have significant main effects on serum zinc concentration (p < 0.0001; ANOVA). It is noteworthy that AM fasting samples were only collected from subjects 20 years and older. Data for an additional 1604 subjects were then excluded due to presence of conditions having a significant effect on serum zinc concentration, but where this effect may be independent of the subject's zinc status. These conditions were: low serum albumin (< 3.5 g/dl; p < 0.01); high white blood cell count (> 11.5 x 10^9/L; p < 0.001); currently pregnant (p < 0.0001) or lactating (females 14–42 yrs. only; p < 0.05); current use of oral contraceptives (females ≥ 13 years; p < 0.01), hormones (≥ 17 years; p < 0.01), or steroids (≥ 14 years; p = 0.059); and current diarrhea

(≤ 10 years; p < 0.05).

Data for the remaining 11,857 subjects were then used to develop smoothed curves for the 2.5th percentile of AM Fasting, AM Other, and PM collected samples for each sex. Age groups were formed in 5-year intervals, from 0–4 years up to 70–74 years, although data were only available for children 3–4 years of age in the first age group. These curves were then assessed for the need to establish different cutoff points based on sex, time of day of sampling and fasting status. Differences equivalent to 4.3 µg/dl serum zinc were considered to be meaningful; this allows for a margin of analytic error equivalent to a 5% coefficient of variation. Based on the latter criterion, separate reference curves for the 2.5th percentile were developed for each sex and, within each sex, for AM Fasting, AM Other, and PM (PM and Evening combined) sample collections (figure 2.6). Data for Evening samples were included with the PM samples as the differences between these curves in any age group were small. Within each of these curves, age
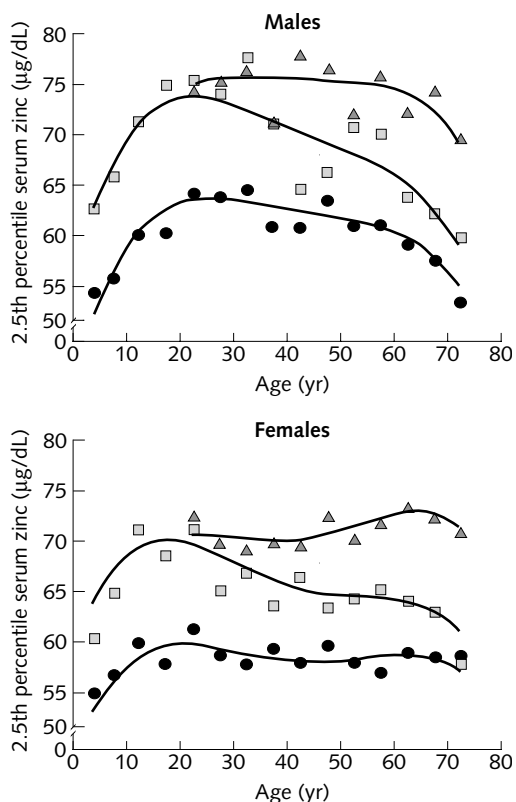

FIG. 2.6. 2.5th percentile of serum zinc concentration for males and females aged 3–74 years, by age and collection time/fasting status from NHANES II. Symbols represent the mid-point for five-year age intervals. Curves were fitted using a 4th order polynomial function for age in years. Data for each time of day and fasting status group are shown as: AM fasted ≥ 8 h (▲); AM non-fasted (□); PM/Evening non-fasted (●).

groupings with a difference of > 4.3 µg/dl were identified. The geometric means ± CV for these groups are given in table 2.3. The 2.5th percentile data for these groupings were then assessed for the need to establish different lower cutoffs by age groups. Appropriate suggested cutoffs for assessing serum zinc status are summarized in table 2.4.

The lower cutoffs for boys and girls aged 3–9 years were merged, as the differences between sexes were negligible. Although the 2.5th percentiles for serum zinc among males > 65 years are lower than for younger adult males, it would be prudent to apply the same cutoff established for males < 65 years, given the possibility that the decline in serum zinc concentration among males > 65 years is attributable to declining nutritional status [61].

Collection of morning fasting samples has previously been proposed as a standardized approach. However, it is recognized that in large population-based surveys this may be logistically very difficult and undesirable to implement, particularly where infants and young children are included. Further, there may be no apparent advantage to requesting fasting samples in a population survey in terms of reducing variability of serum zinc concentration due to meal effects, as the CVs for samples taken in presumably non-fasted samples are similar to those from fasting samples. Therefore, it is simply recommended that the time of blood collection and the fasting status (where fasting is considered to be > 8 hours since the last meal) of all subjects be recorded and serum zinc concentration for those individuals be
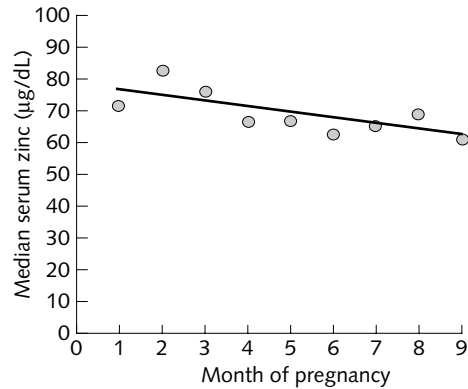

FIG. 2.7. Median serum zinc concentration by month of pregnancy derived from NHANES II (1976–1980)

TABLE 2.3. Geometric mean ± CV for serum zinc concentration by sex, time of collection/fasting status, and age

		Serum zinc concentration, µg/dl (µmol/L)[a] geometric mean ± CV		
Male	Age group (yr)	3–9	10–64	65+
	AM Fasting	na[b]	98 (15.0) ± 14%[c]	92 (14.1) ± 13%
	AM Other	85 (13.0) ± 14%	94 (14.4) ± 15%	82 (12.5) ± 15%
	PM	77 (11.8) ± 17%	82 (12.5) ± 15%	76 (11.6) ± 16%
Female	Age group (yr)	3–9	10–70+	
	AM Fasting	na[b]	90 (13.8) ± 13%[c]	
	AM Other	86 (13.2) ± 15%	86 (13.2) ± 14%	
	PM	75 (11.5) ± 15%	78 (11.9) ± 14%	

a. Conversion factor: µmol/L = µg/dl ÷ 6.54
b. na = not available
c. Based on data from subjects 20 years and older only

TABLE 2.4. Suggested lower cutoffs (2.5th percentile) for the assessment of serum zinc concentration in population studies, derived from NHANES II data

	Serum zinc concentration, µg/dl (µmol/L)[a]			
Age group	< 10 yr	≥ 10 yr		
		Females		
	Children	Non-pregnant	Pregnant[b]	Males
AM Fasting[c]	na[d]	70 (10.7)	1st trimester: 56 (8.6) 2nd/3rd trimester: 50 (7.6)	74 (11.3)
AM Other	65 (9.9)	66 (10.1)		70 (10.7)
PM	57 (8.7)	59 (9.0)		61 (9.3)

a. Conversion factor: µmol/L = µg/dl ÷ 6.54
b. Lower cutoffs given control for time of day/fasting status
c. Based on data from subjects 20 years and older only
d. na = not available

compared to the appropriate cutoff values, as given in table 2.4.

As noted above, serum zinc concentration is normally much lower in women during pregnancy. A regression line for the median serum zinc concentration by month of pregnancy from the NHANES II data indicates a distinct trend for declining serum zinc concentration throughout pregnancy (figure 2.7). However, the survey sample size for pregnant women is limited (n = 61) and, as a result, it is not possible to establish with reliability the 2.5th percentile from this distribution for each time of day/fasting group in each trimester of pregnancy. ANOVA of serum zinc indicated that the 2.5th percentile for the first trimester was 56 μg/dl. The 2.5th percentile for the second and third trimesters did not differ significantly from each other and the pooled value was 50 μg/dl.

It was not possible to derive a reliable estimate of the 2.5th percentile for lactating women due to the limited amount of data for this group (n = 23). Nonetheless, the mean serum zinc concentration of lactating women is not as low as during pregnancy. Until further reference data are available for this subgroup, it may be prudent to compare serum zinc concentrations to the lower cutoffs derived for non-pregnant women.

Given the relatively large number of women in the survey who were using oral contraceptive agents, 2.5th percentiles were also estimated for women up to 44 years of age in this sub-group. Women of childbearing age are a high-risk group for nutrient deficiencies and therefore are often over-sampled in surveys. Thus, there may be a large number of women included in surveys who are using oral contraceptives. The 2.5th percentiles for serum zinc for this group were 65, 61, 57, and 53 μg/dl for AM Fasting, AM Other, PM, and Evening samples, respectively. However, as the hormonal composition of the oral contraceptives used by women in NHANES II is not known, the applicability of the 2.5th percentile to users of different types of oral contraceptives is uncertain. Until further reference data on the effects of a variety of oral contraceptives on serum zinc concentration are available, these tentative lower cutoffs should be used with caution.

Unfortunately, the NHANES II survey did not provide reference data for children less than 3 years of age. Nevertheless, a few smaller studies have collected serum zinc data with the intent of establishing pediatric reference values for younger children [60, 62]. However, only the study of healthy Australian preschoolers by Karr and colleagues disaggregated the data for children less than 3 years of age. In this latter study, the 2.5th percentiles for serum zinc concentration reported were 59 and 52 μg/dl for children 9–23 months (n = 132) and 24–35 months (n = 109) of age, respectively, although the time of day of sampling, fasting state, or other possible confounders apparently were not considered for data collection or analysis. Nonetheless,

the 2.5th percentiles for the children 9–35 months of age were similar to those reported for children 3–5 years of age (52 μg/dl, n = 226) [62] and were intermediate to those found in the NHANES II survey for AM and PM collected samples among children (table 2.4). Therefore, until appropriate reference data are available for children less than 3 years of age, it appears reasonable to apply the same lower cutoffs presented for the 0–5 years age group, as derived from the NHANES II data.

In surveys that include an assessment of serum zinc concentration, information on possible confounding variables (i.e., current infection, pregnant or lactating, current use of oral contraceptives or other hormones or steroids) should be noted. It would be preferable to avoid sampling of subjects while they have current infections to minimize the confounding effects of infection. Data for women in their first or second/third trimester of pregnancy may be compared to the suggested cutoffs in table 2.4. Serum zinc data for lactating women and users of oral contraceptives, or other hormones/steroids should be interpreted with caution.

Technical considerations for collecting and analyzing serum zinc

The collection and preparation of biologic materials for zinc analysis should be performed in a controlled environment to ensure accurate assessment. Contamination of samples with adventitious sources of zinc can produce erroneously high results and several precautions should be taken to avoid this. Blood collection, separation, and preparation techniques can also affect zinc concentrations, and therefore standardized methods are also recommended in this section.

Contamination

Contamination of blood samples with adventitious sources of zinc will lead to false and inconsistent results during analysis of zinc concentration. Every surface with which blood comes into contact during collection, processing, and analysis, as well as dust or smoke in the air, are potential sources of contamination. Contaminant sources of zinc can also be introduced by the technician handling the blood, through sweat, fingernails or saliva (via sneezing or coughing), and transportation of dust particles. Specific measures to avoid contamination are described below.

Airborne particulate matter is a substantial source of zinc contamination. Therefore, sample preparation and analysis should be performed in an adequately controlled environment. The optimal laboratory environment for trace element analyses is a filtered air environment such as a laminar-flow class-100 clean room. However, less rigorous methods of providing a clean environment and minimizing environmental

sources of contamination have been found to be acceptable. Use of laminar flow boxes or hoods during sample processing is recommended, together with other practices for minimizing trace element contamination in laboratories, such as described by the National Bureau of Standards [63]. More recent information can be found in Iyengar [64].

All equipment used in blood collection, processing, and storage must be rendered trace element-free prior to use. Common sources of zinc contamination during blood sampling include improperly washed lab ware; rubber, which may be used for plunger tips in syringes or stoppers for blood collection tubes; lubricants used in blood collection tubes; anti-coagulants used for the separation of plasma; pipettes, pipette tips and storage containers; water, preservatives, and reagents [65–67]. Stainless steel needles are acceptable for zinc analyses. Siliconized needles, or polypropylene or Teflon catheters, are also acceptable options. Use of syringes with rubber tip plungers is not advised as the rubber is a source of contaminant zinc. For population-based surveys, trace element-free evacuated blood collection tubes that utilize siliconized, rather than rubber, stoppers are suggested. Polyethylene serum separators with polyethylene stoppers and olefin-oligomer have been recommended [64]. In general, disposable lab ware (e.g., storage containers, pipette tips) made of polyethylene or polypropylene is recommended. For the selection of appropriate equipment, manufacturers should be consulted as to which products are considered to be trace element-free.

Regardless of whether manufacturers specify equipment to be trace element-free, samples from each shipment should be pre-screened before use. A recommended screening procedure is to expose the equipment for 24 hours to solutions of standard reference materials for blood, serum, or plasma, with certified zinc content [64]. Analysis of these solutions for zinc should produce values within about 5% of the certified value to confirm that equipment is trace element-free. Disposable lab ware that contains detectable zinc, and all other lab ware used for the analyses, should be decontaminated; a suggested procedure is to immerse equipment for 24 hours in a chelating solution such as a 10–20% solution of ultrapure, concentrated hydrochloric or nitric acid, 1% disodium ethylene-diaminetetraacetate (EDTA), or Isoclean [68], followed by a thorough rinsing (3–4 times) with distilled, deionized water. Acid cleaning is preferred for gross contamination of glassware [69]. Suggested equipment and procedures for minimizing contamination are summarized in table 2.5.

Sample collection

Variation in serum zinc results may be caused by changes in intravascular pressure at the time of the blood draw, which varies with stress levels, position,

TABLE 2.5. Practices to eliminate adventitious zinc contamination in serum zinc analysis

> » Disposable polyethylene gloves, free of talc or other coatings, worn by those handling blood samples
> » Samples processed in laminar flow clean rooms, laminar flow hoods, or otherwise clean, dust and smoke-free laboratory
> » Stainless steel needles
> » Anti-coagulants (if separating plasma) that are pre-screened for zinc
> » Trace element-free polyethylene evacuated tubes, stoppers, and serum separators (should be pre-screened for zinc prior to use)
> » Pre-screened polyethylene processing and storage vials
> » All equipment (except pre-screened disposable equipment) decontaminated by washing procedures (soaked for 24 hours in ultrapure 10–20% HCl or HNO_3 solution and rinsed 3–4 times in distilled, deionized water)
> » All materials and equipment stored covered or sealed to avoid dust

and venous occlusion through use of a tourniquet. Intravascular pressure causes the outward movement of fluid into interstitial space, therefore increasing the concentration of serum proteins and zinc. To minimize this source of variation, it is recommended that the subject is generally free of stress, is in a seated position, and that the subject's arm is occluded with a tourniquet for a standardized length of time (i.e., about one minute). Typically blood is drawn from the antecubital vein. The subject's skin should first be cleaned, preferably with alcohol-soaked gauze pads, at the site of venipuncture to remove contaminant zinc from the skin surface.

Sample preparation: serum versus plasma

Either serum or plasma can be used for the analysis of circulating zinc concentrations. Notably, when blood samples were collected simultaneously from the same individuals and separated as either serum or plasma, the zinc concentrations were greater in serum than plasma [70, 71]. Differences between plasma and serum appear to be partly dependent on the time between collection and separation. Plasma is commonly separated shortly after collection, but for serum, adequate time is needed to allow samples to clot prior to separation. During this clotting time, zinc may be released from platelets. Both plasma and serum samples showed a linear increase of 6% in zinc concentration over the first 2 hours, after which only the plasma levels increased [70]. However, this increase in serum or plasma zinc concentration with time before separation is avoidable by storing the samples under refrigeration or on ice prior to separation [72].

Anti-coagulants, such as heparin or EDTA, required for the separation of plasma are potential sources of zinc contamination [73], and each batch must be tested

prior to use. Further, heparin has been shown to bind zinc selectively [74], and some anticoagulants (e.g., oxalate and EDTA) efficiently chelate metallic ions so that plasma values will be falsely low. Citrate can alter osmotic pressure and therefore causes changes in intracellular water content and changes in the apparent concentration of zinc in plasma following separation. To facilitate comparison among results from different studies or surveys, it is recommended that a single anticoagulant be chosen as the standard for use in plasma zinc analysis, and zinc-free heparin is suggested as the anticoagulant of choice.

The choice between using serum or plasma samples to measure zinc concentration in population surveys may ultimately be determined according to the preference of the analytic laboratory. If plasma is preferred, heparin is recommended as an anti-coagulant as noted above, but it should first be screened to ensure it does not contain contaminant levels of zinc. Some analysts prefer serum to plasma, as precipitates that form in plasma samples can be problematic due to clogging of the aspirator in atomic absorption spectrometry. Using serum also avoids the possibility of contamination by anti-coagulants, where this is a concern. A standardized clotting time of 30–40 minutes is recommended, with samples kept under refrigeration or on ice during this time. It is also noteworthy that serum samples were used to derive the reference data for circulating zinc concentration in NHANES II, as described above. Therefore, analysis of serum zinc in future surveys may be most appropriate for comparison to the available reference data.

During sample separation, collection tubes and centrifuge tubes should be closed with trace element-free stoppers, particularly during centrifugation where metal particles may be liberated. Centrifugation procedures should be adequate (e.g., 2000–3000 × g for 10–15 minutes) to remove all blood cells, as these contain higher concentrations of zinc and effectively serve as a source of contamination [64]. Samples that are obviously hemolyzed (i.e., red in color) should be discarded, as the zinc released from erythrocytes into the serum/plasma will produce falsely high results.

Sample storage

It is generally recommended that refrigeration (4° C) of plasma or serum samples is acceptable for short-term storage (i.e., 2–3 weeks) prior to analysis. For longer storage periods, samples should be kept frozen at −25° C or lower. In general, zinc will be stable in frozen samples for prolonged periods. However, long-term storage of samples before analysis can cause dehydration of the sample, especially if "frost-free" freezers are used. Dehydration increases the concentration of the analyte and the use of ice cubes in heat-sealed plastic bags along with the samples can

be used to prevent dehydration [75]. It is also advised to minimize the air space in sample tubes.

Analytic techniques

A number of different analytic methods can be employed for the measurement of zinc concentrations in serum samples. Flame atomic absorption spectrometry (FAAS) is most widely used. Others include graphite furnace atomic absorption spectrometry (GFAAS), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), Instrumental Neutron Activation Analysis (NAA), X-Ray Spectrometry (Proton-induced emission; PIXE), and Anodic Stripping Voltammetry (ASV). The suitability of different analytic methods for the analysis of serum zinc has been reviewed [5]; FAAS, ICP-AES, NAA, and PIXE have the capacity to produce high precision results (CV ~ 1%), whereas GFAAS has a lower precision (CV ~ 10%). FAAS is considered to be the most simple and practical technique that is suitable for use in lower-income country settings and capable of producing accurate results with proper staff training and application of quality control techniques. Ultimately, the choice of analytic technique will be dependent on the other potential uses for the equipment, taking into consideration the elemental analytes of interest, detection limits, and the sample matrix. It is beyond the scope of this document to review each of these techniques; more detailed information on these analytic methods and sample preparation procedures appropriate for each (e.g., sample dilution and/or digestion) can be found in Herber and Stoeppler [76] and Iyengar [64].

The majority of current FAAS methods are direct techniques in which the sample is diluted with deionized water [77], aqueous acid solution (e.g., 0.1 M HCl), organic alcohols (e.g., n-butanol or n-propanol), or with a signal enhancing mixture [64, 78, 79]. The main purpose of dilution is to reduce the solids content, and hence viscosity, of the plasma/serum sample to equal that of the standard solutions. Differences in viscosity affect the rate of aspiration of samples and hence will affect the FAAS readings. Reducing the solids content of the samples will also prevent blockage of the burner. Approaches that have been suggested to avoid analytic error due to differential viscosity between samples and standards are to use 5% aqueous glycerol solution as the solvent for the standards [80] or, preferably, to use 6% aqueous butanol or 10% aqueous propanol as the sample diluent in a 5-fold dilution [64, 78]. Dilutions of between 5- and 10-fold are suggested to minimize viscosity differences; as high as 20-fold have been suggested but they may cause added problems of weakened signals and decreased precision due to pipetting errors.

Quality control issues

In addition to applying adequate technical precautions, quality control procedures are required for accurate assessments of serum zinc concentration. The quality of results is highly dependent on the skill of the analyst and the laboratory practices employed to monitor accuracy and precision.

Two types of quality control procedures should be used. The first is a primary reference material, or a Standard Reference Material, with a certified, analyzed mean (± SD) zinc content. These standards should be used to validate the accuracy of analytic methodology and to assure the accuracy of results. Serum-based reference materials should be chosen (table 2.6) for consistency of the matrix. Accuracy can also be monitored through participation in external proficiency testing programs. Secondary reference materials, or carefully prepared 'in-house' or 'bench' controls such as a bulk sample of pooled serum should be used with every run to monitor precision. These can be prepared to cover the low, normal, and elevated levels in the normal range of serum zinc concentrations. This control measure is suitable for monitoring day-to-day variation, short-term "noise", and long-term "drift" or fluctuations of the instrument reading in the absence of a signal. Inherent in the use of quality control measures is the establishment of standards or tolerance limits for analyzed results. Precision is determined through the repeated analysis of reference materials, both within runs and between runs, and calculating the CV. A CV of 5% should be attainable for zinc using atomic absorption spectroscopy. Suggested quality assurance procedures and resources are summarized in table 2.6.

Due to the multiple methods available for sampling and analyzing serum zinc, it will be important to employ a standard method to improve the comparability of results within and among laboratories. Recommended standardized procedures for the collection, preparation, and analysis of serum zinc samples are summarized in table 2.7.

Considerations for survey design

Sample size determination

The sample size required to detect the prevalence of low serum zinc concentrations with a reasonable degree of precision is dependent to some extent on the anticipated prevalence of zinc deficiency. When the prevalence of zinc deficiency is anticipated to be low (< 5%), a narrow confidence interval is desirable to define prevalence more precisely. In this case, a large sample size is needed. Where a higher prevalence is expected, a wider confidence interval for the distribution of results is acceptable, and therefore a smaller sample size can be selected. Based on the distributions of serum zinc concentrations determined in the NHANES II survey, the confidence intervals and suggested survey sample sizes for expected low, moderate, and high prevalences of low serum zinc concentration are given in table 2.8. The range of sample size given for each anticipated level of prevalence reflects the range in percentage points of the confidence interval suggested.

TABLE 2.6. Quality assurance procedures and resources for the analysis of serum zinc concentration

» Certified standard reference material suitable for serum zinc analysis:
    Bovine serum, SRM 1598; National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA
    Animal blood, IAEA-A-13; International Atomic Energy Association (IAEA), Seibersdorf, Austria
» In-house or bench reference materials, such as pooled serum sample:
    Pooled sample should be analyzed against a certified standard reference material to establish its zinc content
    Samples should be prepared at the low, mid, and high range of normal serum zinc concentrations
    Pooled sample should be prepared in a large enough quantity to provide a single standard over a useful duration of time (e.g., two to three years for use in a monitoring program or on-going survey)
» Participation in an external proficiency testing program:
    College of American Pathologists Quality Assurance Service, New York, USA

TABLE 2.7. Summary of suggested procedures for serum zinc analysis

» Employ appropriate practices throughout the procedure to avoid adventitious trace element contamination (table 2.5).
Sample collection and preparation: » The subject should be seated » Clean subject's skin with alcohol at site of the antecubital vein » Restrict occlusion of subject's arm with tourniquet to about 1 minute » Draw blood using stainless steel needle, and collect into trace element-free evacuated blood collection tubes *without* anticoagulant for processing serum » Place blood sample in refrigerator or on ice and allow to clot 30–40 minutes » Centrifuge blood sample at 2000–3000 × g for 10–15 minutes » Discard any obviously hemolyzed samples » Store samples frozen unless they are to be analyzed immediately
Sample analysis: » Dilute sample 5- to 10-fold in solvents such as 6% aqueous butanol or 10% aqueous propanol » Read sample zinc concentration using Flame Atomic Absorption Spectrometry with appropriate standard dilutions, in-house quality controls, and standard reference materials (table 2.6)

TABLE 2.8. Suggested confidence intervals and estimated sample sizes for surveys of serum zinc concentration

Prevalence of low serum zinc concentration	Confidence interval (percentage points)	Sample size
Low (< 5%)	1–2	1,825–457
Moderate (5%–10%)	2–3	865–385
Moderately high (10%–20%)	3–4	683–307
High (> 20%)	4–5	505–289

Data analysis and interpretation

To determine the proportion of the population with low serum zinc status, it is first necessary to group data according to whether the blood drawn represented (1) morning fasting samples; (2) morning, non-fasting samples; or (3) afternoon samples. Based on these groupings, the result should be compared to the appropriate cutoff for age, sex, and physiologic status (table 2.4) and classified as either low or normal.

Information on expected prevalences of low serum zinc concentration in at-risk populations is somewhat limited. A recent National Nutrition Survey in Mexico indicated that 25% of preschool and school-aged children, and 30% of women had low serum zinc concentrations (defined as < 65 µg/dl in that survey) [12]. In rural areas, these prevalences reached 40% among children and 34% among women. Some community-based zinc supplementation trials from various populations have reported prevalences of low serum zinc. Examples include a study of 6–24 month old Vietnamese infants (n = 163) where the prevalence of low serum zinc (defined as < 70 µg/dl) was ~ 34% at baseline and dropped to < 10% after 3 months of supplementation with a concurrent increase in height-for-age Z-score among initially stunted children [14]. A group of Indian children 6–35 months of age (n = 609) had a prevalence of ~ 36% low plasma zinc (defined as < 60 µg/dl) concentration at baseline [80]. In the group receiving zinc supplements for four months, the prevalence was reduced to 11.6% with a concurrent reduction in the incidence of acute lower respiratory infection. A group of peri-urban 6- to 8-year-old Guatemalan school children (n = 155) had a prevalence of low plasma zinc concentration (defined as < 70 µg/dl) of 12.3% among boys and 1.5% among girls [81]. Following 3 months of supplementation, plasma zinc concentrations increased and the children demonstrated some body composition changes, without improvements in growth or other functional tests of zinc deficiency [82].

Although the above studies are not entirely comparable because of different sampling techniques and use of different cutoff points, they can be used to set tentative guidelines for taking action based on prevalences of low serum zinc concentrations. In populations with a prevalence of low serum zinc concentrations less than 10%, zinc deficiency would not be considered a public health concern warranting national level programs. A prevalence of between 10% and 20% for low serum zinc values warrants further assessment of results, as the slightly elevated prevalence suggests that some segments of the population may be at high risk of zinc deficiency. Programs targeted to high-risk groups may be necessary. Where the prevalence of low serum zinc values exceeds 20%, national level programs may be considered following further assessment to identify groups at elevated risk.

Prevalence rates should be examined according to possible risk factors to identify high-risk groups. Demographic variables to assess can include age groups, sex, location (e.g., region, district, urban/rural), as well as indicators of socioeconomic status (section 2.5). Appropriate statistical procedures, such as logistic regression or log-linear models, then may be used to identify associative risk factors for low zinc status for purposes of research or for development of more intensive, targeted public health programs.

Summary

Serum zinc concentration is the most widely used biochemical indicator of zinc status. Reference values and cutoffs suitable for most individuals have been suggested, taking into account age, sex, fasting status, and time of day of sample collection. Adequate information on all four of the latter variables is necessary so that results can be compared to the appropriate lower cutoff value. Several physiologic factors, such as low serum albumin, elevated white blood cell counts, pregnancy, lactation, and use of oral contraceptives or other hormones, can affect serum zinc levels and must be considered in the interpretation of results. Although these statistically derived lower cutoffs are suitable for estimating the risk of zinc deficiency in populations, further population-based studies are needed to validate the lower cutoffs against functional indices of zinc deficiency.

Either serum or plasma can be used to measure circulating zinc concentration. Proper measures to avoid contamination of samples with adventitious sources of zinc are essential to produce meaningful results. The use of serum samples avoids the need for anti-coagulants during blood collection, which can be a source of contamination. For separation of serum, a recommended, standardized procedure is to allow serum samples to clot, under refrigeration, for 30–40 minutes. Rigorous quality control measures are also required to produce accurate and precise results.

For population surveys, adequate sample sizes are needed to determine the prevalence of low serum zinc concentrations with a desired level of precision.

A prevalence of between 10% and 20% for low serum zinc suggests that some segments of the population may be at high risk of zinc deficiency. Where the prevalence of low serum zinc values exceeds 20%, national level programs may be considered. The assessment of serum zinc concentrations should be combined with other zinc status assessment methods, particularly with dietary zinc intake data, to strengthen the estimates of risk of zinc deficiency.

2.3.3 Hair zinc concentration

The zinc content of the hair shaft reflects the quantity of zinc that was available to the hair follicle during an earlier time interval, so hair zinc concentration has been proposed as a useful index of longer-term zinc status. During infancy and early childhood, hair zinc concentration declines from high neonatal values, reaching a minimum at about 2–3 years [83, 84]. These trends in hair zinc concentrations may arise from a gradual depletion of tissue zinc pools induced by rapid growth. During pre-pubertal years, hair zinc concentrations tend to increase from the lower levels of infancy and early childhood to reach a normal adult value of about 150 µg/g (2.3 µmol/g) [85]. Hair zinc concentrations differ markedly according to sex [86–88]. Boys have consistently lower hair zinc concentrations than girls of the same age, even when food consumption patterns and energy and nutrient intakes are comparable. Such sex differences in hair zinc concentrations may arise because of higher zinc requirements for boys or because of changes in growth hormone and testosterone concentrations [89]. Seasonal differences in hair zinc concentrations have also been described in a population in Canada [90]. Hair zinc values tend to be lower in the spring/summer. This may result from seasonal changes in linear growth velocity, such that rapid periods of growth lead to a gradual depletion of tissue zinc pools.

Significant relationships between hair zinc concentrations and functional outcomes of zinc status, such as impairments in linear and ponderal growth, taste acuity and appetite, as well as selected dietary zinc indices have frequently been observed [81, 84, 91–96]. The clinical signs of mild zinc deficiency have been associated with hair zinc values in infants and young children of less than 70 µg/g (1.07 µmol/g) in the spring or summer [84, 87] and less than 110 µg/g (1.68 µmol/g) in the winter [90]. Some studies have reported associations between hair zinc concentration and dietary zinc indices. For example, in adolescent Canadian women consuming omnivorous and vegetarian diets, high phytate intakes (r = –0.194; p = 0.03) and high phytate:zinc molar ratios (r = –0.215; p = 0.01) were negatively correlated with hair zinc concentration [97]. Similar negative correlations between hair zinc and phytate:zinc molar ratios

have been observed in Canadian [98] and Malawian preschool children [99]. Pregnant women in rural Malawi consuming diets with phytate:zinc molar ratios > 17 (the median) had lower hair zinc concentrations than those consuming diets with a phytate:zinc molar ratio < 17 [13].

The application of hair zinc concentrations as an indicator of zinc status has several advantages, and several limitations. Unlike serum zinc, concentrations of zinc in hair are more stable and not affected by diurnal variation, prolonged fasting, meal consumption, and acute infection. Collection of hair samples is less invasive than drawing blood, and may be more appropriate in some populations where collection of blood is not culturally acceptable, particularly from young children. Unlike blood, hair samples do not need to be processed in the field and refrigeration is not required.

The limitations of using hair zinc concentration as an index of zinc status include the limited availability of reference data, and some problems in the interpretation of results. The cutoffs currently used for infants and children may not apply to adolescents or adults. Controversy surrounds the interpretation of apparently normal or even high hair zinc concentrations in malnourished children with linear growth retardation. This has been attributed to a reduced rate of hair growth arising from a limited supply of zinc to the hair follicle [100–102]. An alternative explanation may be that the growth failure in these malnourished children was not induced by zinc deficiency *per se* but rather from environmental factors such as parasitic infections, morbidity, and/or deficiencies of other growth limiting nutrients [95]. As a result of the reduction in growth rate, requirements for zinc are also reduced, resulting in apparently normal or even high hair zinc concentrations. Therefore, although normal or high hair zinc concentrations do not necessarily indicate normal zinc status, low hair zinc concentrations would suggest that zinc status is not optimal.

Standardized procedures for collecting, washing, and analyzing hair samples are essential in all studies. Samples should be collected from close to the occipital portion of the scalp with stainless steel scissors, and only the proximal 1.0–1.5 cm of the hair strands retained for analysis. Care must be taken to remove all sources of adventitious contamination (e.g., nits and lice) prior to washing the hair samples according to a standard procedure. A non-ionic detergent (e.g., Actinox) with [102] or without acetone [103] can be used. Hair samples can be analyzed by instrumental neutron activation (INA) [83] or flame atomic absorption spectrophotometry [103]. Accuracy of the analytic methods can be assessed using a certified reference material for human hair (e.g., Community Bureau of Reference, Certified Reference Material #397; Institute for Reference Materials and Measurements, Retieseweg., B-2440 Geel, Belgium). Variations in

hair zinc concentrations due to age, sex, season, hair color, hair beauty treatments, growth rate, severity of malnutrition, and rate of hair growth have been described. The effects of these possible confounding factors must be considered in the interpretation of results.

Summary

Due to the lack of established cutoffs for most age groups and the uncertainties in interpreting results among malnourished children, the usefulness of hair zinc analysis in population zinc status assessment is presently limited. However, hair zinc concentrations may be useful as an alternative to serum zinc determinations when collection of blood samples from young children is not permitted. In this case, the proportion of young children with values less than 70 µg/g (1.07 µmol/g) can be used for samples collected in the spring/summer and the proportion less than 110 µg/g (1.68 µmol/g) can be used for those collected in the winter. A high proportion of infants or young children with hair zinc concentrations falling below these cutoffs may be used to support a diagnosis of zinc deficiency where it is suspected. Hair zinc concentrations may be useful for tracking trends in zinc status over time within a population. Further research would be useful to validate lower cutoffs for the assessment of zinc status in different age groups and seasons.

2.3.4 Other biochemical indicators of zinc status

Other biochemical indicators of zinc status described in this section are those that are still being explored and developed, and that merit further study of their usefulness as indicators of zinc status. These methods are presented for informational purposes only, and are not currently recommended for use in population assessments.

Enzymes and circulating proteins

Several zinc-dependent enzymes have been shown to be affected by zinc intake or zinc status in experimental animal models and human populations. These include plasma or serum alkaline phosphatase, 5'-nucleotidase, ribonuclease, lactic dehydrogenase, delta-aminolevulinic acid dehydrogenase and extracellular superoxide dismutase (EC-SOD). Results have been highly variable between studies and none of these has been shown consistently to reflect zinc status. Another common problem is specificity; several of these enzymes (e.g., alkaline phosphatase) are also affected by nutrients other than zinc. Metallothionein is a metal storage protein that is present in serum at a low concentration; the circulating concentration of metallothionein appears to correlate with zinc intake.

However, similar to several of the enzymes mentioned previously and to serum zinc, metaollothionein may be affected by other factors, such as infection and stress, although this has not been confirmed by direct studies. Because of these limitations and the relative difficulty of performing these assays outside the research laboratory, it is presently unlikely that they will be useful for assessment of zinc status at the population level.

Cells

Cells are more likely to reflect long-term zinc status than the rapidly turning over plasma pool. Various enzymes and binding proteins affected by zinc have been measured in cells, such as erythrocytes, leukocytes and platelets. Although results have been encouraging for some of these, they have received very little clinical use and there are no established reference values. Another limitation is the difficulty in separating the cells under field conditions and, for leukocytes and platelets, the large volume of blood required, which may lead to decreased participation rates and elimination of infants and children from studies.

Molecular techniques

Modern molecular techniques are being used increasingly to measure mRNA for proteins whose expression is regulated by metal ions, such as zinc. Usually, quantitative reverse-transcriptase polymerase chain reaction assays (RT-PCR) are used, but these require specialized equipment and procedures that are not yet widely available. Metallothionein mRNA in monocytes and erythrocytes has been shown to be affected by zinc intake [104, 105], but only a few studies have been performed and our knowledge regarding other factors, such as other metal ions, infection and stress, that may affect its expression is very limited.

Differential mRNA display and gene microarrays are likely to be helpful in identifying genes that are specifically altered by zinc status. However, it is not yet known whether this new technology will ultimately prove suitable for assessment of zinc status of individuals or populations.

Kinetic markers: pool sizes and turnover rates

Kinetic studies of zinc metabolism provide a powerful means of summarizing the integrated whole-body response to changes in zinc status. Kinetic parameters, estimated from a compartmental model, relate shifts in zinc absorption, excretion, cellular zinc fluxes, and the size of body pools with changes in zinc intake. Because the compartmental model integrates all of the adjustments in homeostasis, subtle changes in zinc status may be detected when the response of a single measurement is not evident. Several compartmental

models of zinc metabolism have been developed and used to study kinetic responses to severe zinc depletion in men [106, 107], moderate changes in zinc intakes [108], and zinc excess [109]. Parameters that seem to reflect changes in zinc status include the size of the exchangeable zinc pool(s), plasma fractional turnover rates, and total plasma zinc flux.

Exchangeable zinc pools (EZPs)

The zinc that is available for maintaining zinc-dependent functions is thought to be mobilized from small, rapidly exchanging zinc pools found primarily in the plasma and liver [108, 109]. The size of this pool can be estimated from the tracer-tracee disappearance curves using kinetic modeling software. The total exchangeable mass varies with the length of time over which the decay curves are measured. For example, if tracer disappearance is followed for 3 hours, the EZP mass is approximately 18 mg in healthy men; if the tracer disappearance is followed for 192 hours, or eight days, it is approximately 150 mg or about 10% of the whole-body zinc pool. A decline in one or more of the EZPs could be associated with a reduction in the zinc available for zinc-dependent functions, especially among rapidly turning over proteins [110]. If so, then EZP mass would provide a good indication of tissue zinc status.

EZP mass has been measured in individuals freely selecting their diets [108], in men fed zinc-depleted diets [106, 111], and in populations with chronically low zinc intakes. Among individuals freely selecting their zinc intake, EZP mass varied directly with dietary zinc, both in individuals [108] and populations [112]. Also, experimental acute, severe zinc depletion induced in adults by feeding a diet providing 0.23 mg zinc/day for five weeks [106] lowered total EZP by 36%. Plasma zinc concentrations declined 65% in that study suggesting that plasma zinc is more sensitive to severe zinc depletion than is EZP mass. When dietary zinc was reduced to a marginal level (4.6 mg/day) in a group of healthy men, EZP mass did not change [111]. Thus total EZP mass does not appear to be a good indicator of modest short-term changes in zinc intake. However, longer-term low intakes or acute zinc depletion causing a reduction in whole-body zinc content appears to cause a concomitant reduction in EZP.

EZP mass is correlated with fat-free mass in adults. This is expected since zinc is an integral part of the protein mass in lean tissue. Possibly, EZP would be more meaningful if it is expressed in terms of fat-free mass. Body size and body composition of individuals should be considered when the EZP values of individuals are compared.

Serum zinc turnover rates

The small amounts of zinc in the serum turn over rapidly in the body. To meet tissue needs, the rate of turnover is expected to increase when total body zinc is reduced, such as during zinc deficiency. Thus, serum zinc turnover rates may serve as an indicator of zinc status. With acute, severe zinc depletion in a group of healthy men, fractional plasma turnover increased from ~ 150 to ~ 200 times/day, but the total zinc flux declined from ~ 475 to ~ 230 mg due to a marked decline in plasma zinc concentrations [106]. This decline in the amount of zinc available to the tissues was associated with the onset of the clinical symptoms of zinc depletion in these men. One study reported that the fractional plasma zinc turnover was reduced in young women with low serum ferritin levels [113]. It was assumed that women with low iron status also had a low zinc status, and that this faster fractional zinc turnover reflected the poor zinc status. No other indicators of zinc status were reported, however. Fractional plasma zinc turnover rates were found to be 50% greater in zinc-deficient Egyptian subjects than in normal controls [114]. The use of fractional plasma turnover rates or total plasma zinc flux as a biomarker of zinc status needs further validation in humans.

In summary, kinetic measures of EZP and plasma zinc flux seem to reflect chronic zinc intakes of individuals and populations. These kinetic parameters may be useful in defining the status of individuals and populations that have marginal intakes leading to a loss of whole-body zinc. As these techniques are costly and intensive, however, they will not find use in population assessments. Rather, findings from small clinical-based studies using these techniques will contribute to our understanding of zinc requirements, zinc homeostasis, and the dietary and physiologic conditions that influence zinc status. Moreover, these techniques may ultimately prove useful to validate novel simpler techniques to assess zinc status as they become available.

2.4 Functional indicators: response to supplementation

Because of uncertainties in the interpretation of the foregoing techniques to assess zinc status, many recent studies have relied on the identification of a functional response to zinc supplementation as the basis for diagnosing preexisting zinc deficiency in the supplemented individuals (section 1.4). Use of a functional response to indicate deficiency requires randomly administering either zinc or placebo to members of the target population and comparing the responses in the two groups of subjects. Ideally, the subjects are selected to be a representative sample of a larger population, so inferences can be drawn for the population as a whole. Functional responses that have been used previously include physical growth, immune function and rates of specific infections, physical activity and performance on psychometric tests, and hormonal responses,

among others. The major disadvantage of using these functional responses to zinc supplementation as indicators of zinc deficiency is the long delay that is often required for the response to be detectable and the consequent high cost of this diagnostic technique. Appropriate efforts to ensure that the supplements are actually consumed contribute further to the cost of these assessments. Considerations for supplementation programs are covered in section 3.1.

2.5 Socioeconomic status indicators to identify high-risk groups

Social and economic factors are important underlying determinants of childhood morbidity, mortality and malnutrition, including micronutrient malnutrition. In the absence of clinical, biochemical, or dietary evidence of zinc deficiency, the general level of deprivation, as assessed through socioeconomic indicators, can be useful to inform on a population's potential vulnerability to zinc deficiency. These socioeconomic indicators can also be used to select priority areas or sub-populations for targeting interventions. Because of their unknown sensitivity and specificity, however, socioeconomic indicators should not be used for the monitoring and evaluation of interventions to address zinc or other nutrient deficiencies [115]. A few potentially useful socioeconomic indicators are suggested below.

Maternal education

Maternal education has been shown consistently to be critically important for child health, nutrition and survival [116, 117]. Although the precise mechanisms by which maternal education affects child outcomes are not fully understood, evidence from various countries indicates that childcare and feeding practices are key pathways [118–120]. Thus, lower maternal education is likely to lead to inadequate child feeding, hygiene and health-seeking behaviors, which in turn are likely to be associated with increased risk of zinc deficiency among children. Maternal education is also known to be highly correlated with socioeconomic status and household food security. Because maternal schooling is easier to measure and is less prone to recall bias than income or expenditure data, it may be a more useful overall proxy for poverty.

Indicators of maternal schooling could include the following:
» Rates of illiteracy among women;
» Mean number of years of schooling;
» Percentage of women having completed primary, secondary, or higher levels of schooling.

A cutoff point of 50% illiteracy among women 15–44 years of age has been suggested to define greater vulnerability to vitamin A deficiency, along with information on food availability and dietary patterns [115]. This cutoff point may also be useful to predict vulnerability to zinc deficiency, although it is important to recognize the arbitrary nature of this definition. In situations where the only information available is education of the head of household, it can also be used as an overall population level proxy for education because maternal education has been shown to correlate with the education level of the head of household.

Income

The association between poverty and child nutrition has long been recognized, and anthropometric indices of children under 5 years of age are often used as an indicator of socioeconomic development. Because poor populations often rely heavily on monotonous plant-based diets low in animal products and high in phytate content, poverty is bound to be associated with poor zinc status. Reliable income data are not widely available because they are difficult, time-consuming and expensive to collect. This is particularly true among populations relying on agriculture as their main means of subsistence, which constitutes a large proportion of the poor in lower-income countries. Similarly, collecting income data on individuals in urban populations who may have up to three different occupations, or who are self-employed or working in informal or even illegal activities, poses similar challenges in the assessment of income. Food expenditure data are often used as a proxy for income, but these data are also time-consuming and expensive to collect and are subject to recall bias. Economists have not yet agreed on a simple, reliable, proxy measure for income, but some commonly used alternatives are listed below:
» Number of household assets (e.g., furniture, television, electrical appliances, vehicles, etc.);
» Value of household assets (estimated value of assets possessed by the household);
» Type and quality of dwelling (e.g., whether dwelling is a room, apartment or house; whether the house is considered formal or informal; construction material of the walls, roof, floor; availability of water and sanitary services). (Because these variables are highly correlated, they are sometimes combined into an index using factor analysis or other data reduction approaches.);
» Availability of electricity (more useful at the community level, as the majority of households in a community are likely to have electricity if it is available);
» Access to land (for rural areas: land ownership, size of land, size of cultivated land).

Employment

In urban areas, where populations are highly dependent

on cash income, employment can be a useful indicator of standards of living. It may also be possible to rank different types of employment according to a salary scale. For example, unskilled laborers usually have lower wages than skilled laborers, and factory workers have lower salaries than office or bank employees. Thus, type of employment (sometimes referred to as 'functional groups') may be used as a crude indicator of socioeconomic status. Other potential indicators at the household level include the ratio of adults generating income per capita, and the gender of the head of household. Women-headed households are commonly found in urban areas and constitute a particularly vulnerable group because these women must assume complete responsibility for their households' livelihood and food security, and they often receive lower wages than do men for the same employment.

In rural areas, type of employment can be useful to predict income, but access to land is likely to be more important in agrarian societies. A potentially useful classification is subsistence versus cash-cropping agriculture to reflect differences in income. Similarly, the specific type of agricultural production that households are engaged in is relevant for the zinc supply and producers could usefully be classified into those who produce animal products (e.g., cattle-rearing, small animal husbandry, aquaculture) and those who do not.

Access to health, water, and sanitation services

Populations with poor access to health, water and sanitation are at increased risk of infectious diseases, which increases the risk of zinc deficiency. More remote and deprived populations often have poorer access to these services. Data on the percentage of the population with access to health, water and sanitation services are usually available at the national level and are often disaggregated at the provincial and district level and by urban and rural areas. The types of indicators typically available include the proportion of the population with access to the following:

» an adequate and safe water supply
» sanitary services
» health services

2.6 Indicators of the risk of excess zinc intake

The clinical symptoms of overt zinc toxicity and the biochemical changes that may represent subclinical zinc toxicity were described in detail in section 1.7 (zinc toxicity). Presently used indicators for excess zinc intakes include biochemical indicators of copper status, such as serum or plasma copper concentration and superoxide dismutase activity in erythrocytes [5, 37, 121]. Any statistically significant, negative change

in biochemical indicators of copper status has been interpreted to be indicative of zinc toxicity. However, the physiologic significance of small changes in these indicators has not been defined. Changes in copper status indices to identify a toxic effect of supplemental zinc should be evaluated with caution in populations where infections are common; supplemental zinc has well-documented effects on reducing the prevalence of certain infections (section 1.4), and a decline in copper status indicators may reflect the shift from elevated circulating copper concentrations that are normally observed during infections, back down to concentrations normally observed in a non-infectious state [122]. In such cases, it would be preferable to interpret changes in copper status in relation to the proportion of cases that fall outside the normal reference range or to restrict the analysis to uninfected individuals. Further research is required in this area.

Although supplemental zinc (50 mg zinc/day for 10 weeks) has been shown to decrease iron stores of healthy, adult subjects, as measured by serum ferritin [123], the use of iron status as a reliable and specific indicator of excess zinc intake may be problematic in many settings. This in large part would be due to the likely, frequent co-occurrence of zinc and iron deficiencies, and the subsequent suitability of including both minerals in the same supplement formulation, thus making it difficult to attribute changes in iron status to the intake of supplemental zinc. Nonetheless, whether zinc is given alone or together with iron, it is recommended that iron status be monitored to assure that no negative impact on iron status results.

Declines in the serum concentration of high density lipoproteins have also been observed to occur during zinc supplementation [124, 125] and this biochemical alteration may also reflect zinc toxicity. The mechanisms for, and possible pathological significance of these changes in lipoprotein metabolism are not well defined.

2.7 Summary

Several factors must be considered before deciding whether to initiate an intervention program to reduce the rate of zinc deficiency and what type of program(s) might be appropriate. The first, and most obvious, question is whether zinc deficiency does, in fact, occur in the population with a frequency or degree of severity that should be considered a public health problem. Unfortunately, because of the lack of easily interpretable indicators of zinc status, it is still difficult to classify countries definitively with regard to the prevalence of zinc deficiency. Nevertheless, as an interim measure, it is possible to assess the likelihood that zinc deficiency is an important public health problem by reviewing existing information on the

adequacy of zinc in the national food supply, the rate of stunting in preschool children, and other possibly contributing ecological factors. The suggested steps in conducting this assessment are summarized in the following paragraphs and the recommended responses to this information are shown in figure 2.8.

As described in section 2.2.4, when ≥ 25% of the population is at risk of inadequate zinc intake, based on the amount of absorbable zinc available in the national food supply, the population may be considered to have an elevated risk of zinc deficiency. Also, because childhood stunting seems to be zinc responsive, a prevalence of stunting ≥ 20% may indicate an increased likelihood of zinc deficiency. Thus, if a country has ≥ 25% of the population at risk of inadequate zinc intakes based on data from the national food supply or from direct assessments of dietary intakes, and ≥ 20% stunting among preschool children, the country should be considered at high risk of zinc deficiency (figures 2.2 and 2.3). By contrast, if the country has < 15% of the population at risk of inadequate zinc intakes, and < 10% prevalence of stunting, it is unlikely that zinc deficiency is a major public health problem. Those countries with either moderate rates of stunting (≥ 10%, < 20%) or moderately low prevalence of inadequate zinc intakes (≥ 15%, < 25%) are considered to have an intermediate level of risk of zinc deficiency. Further evidence to support determination of the level of risk in a population can be derived from indirect indicators of zinc status, such as rates of iron-deficiency anemia, or predominant diet types, as described in section 2.2.

The following initial assessments are recommended:

» If a country is decidedly at low risk of zinc deficiency, no further population-level intervention is likely to be necessary, although evaluation and treatment of selected high-risk individuals may still be indicated in clinical settings. Segments of the population who are at elevated risk for zinc deficiency may have to be identified using more detailed regional and local data on indicators of zinc status; isolated groups at elevated risk may be defined by their stage of lifecycle, place of inhabitance (e.g., state, district,

urban/rural), socioeconomic status, or other factors generally associated with disparity in nutritional status.

» When a country has an intermediate risk of zinc deficiency, further population assessment is appropriate. In this case, measurement of dietary zinc intake and/or serum zinc concentrations in a representative sample of the population is recommended. This will allow a more complete assessment of risk, and further information can be derived to identify segments of the population at highest risk and to guide the choice of appropriate intervention strategies.

» When a country has a high risk of zinc deficiency, either further assessment is indicated or planning for programmatic intervention may be initiated. If a decision is made to initiate an intervention program, further objective assessment is still desirable, so that baseline information will be available for future comparison.

A summary of possible indicators for assessing a population's risk of zinc deficiency and the suggested criteria for determining the magnitude of risk are presented in table 2.9. The range of possible indicators described relies on either suggestive information on the population's risk of zinc deficiency or on more objective measures of the population's zinc status. Using preexisting suggestive information on the national risk of zinc deficiency (namely, rates of childhood stunting and absorbable zinc contents of national food supplies) individual countries have been classified as having low, medium, or high risk of zinc deficiency, as shown in appendix 1.

There is an urgent need to develop better methods to assess the zinc status of individuals and populations and to evaluate the relationships of these biomarkers of zinc status to known functional consequences of zinc deficiency and excess. Pending the availability of such biomarkers, the risk of population zinc deficiency can be inferred from ecologic evidence, such as the absorbable zinc content of the food supply, rates of stunting, dietary zinc intake and possibly rates of anemia and other diseases. Research is needed to validate these proposed approaches to classify countries according to suggestive evidence of their risk of zinc deficiency against other markers of zinc status. With regard to dietary assessment, information is needed on the zinc and phytate contents of local foods; and simplified dietary methods, such as food frequency questionnaires or other techniques, should be developed and evaluated with regard to their ability to predict the risk of zinc deficiency. Although reference data are available for assessing the adequacy of serum zinc concentrations in relation to most age groups, sex, time of day and fasting status, additional information is still needed on appropriate cutoffs for children less than 3 years of age, elderly people, and pregnant and lactating women.
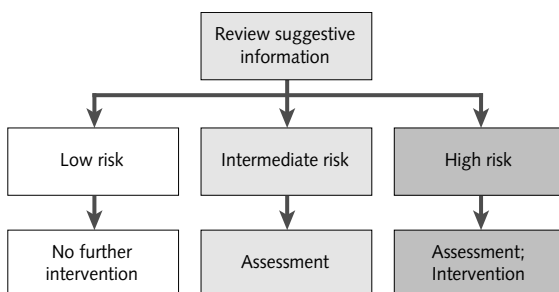

FIG. 2.8. Steps in assessment of population zinc status

TABLE 2.9. Summary of indicators for the assessment of zinc status in a population

Indicator category	Measurement variable	Variable unit criteria	Recommended cutoff to identify an elevated risk of zinc deficiency
Suggestive evidence (existing health/ecologic information)	Rates of stunting among children < 5 years	Length-for-age or height-for-age Z-score < –2	> 20%
	Absorbable zinc content in the food supply, based on national Food Balance Sheets	Estimated percent of population with access to absorbable zinc in food supply below the weighted mean physiologic requirement	> 25%
Dietary	Adequacy of population zinc intakes based on dietary surveys	Probability approach: Probability of zinc intakes below the EAR (table 1.9)	> 25% of population at risk of inadequate intakes
		EAR cut-point method: Proportion of individuals with intakes below the EAR (table 1.9)	> 25% of population with inadequate intakes
Biochemical	Plasma/serum zinc concentration	Prevalence of low concentrations compared to appropriate age, sex, fasting status, and time of day cut-off (table 2.4)	> 20% below cut-off
Response to zinc supplementation	Change in weight-for-age or height-for-age Z-score among a representative sample of children	Effect size compared to appropriate control group	> 0.2 SD units; p < .05

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