NUTRITIONAL ASSESSMENT: METHODS FOR SELECTED MICRONUTRIENTS AND CALCIUM

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Summary

Nutritional deficiency represents a continuum from the early to the late stages, which necessitates the use of different assessment methods involving subclinical and clinical indices. Selection of appropriate nutritional indices should be based upon the objectives and nature of certain factors such as population versus individual status, identification of areas/populations at risk, efficacy of intervention, tracking progress toward long-range goals, monitoring and evaluating the impact of programs, and so on. Indices used in nutritional assessment systems include: biological, ecological, and behavioral indices. Biological indices are derived from both static and functional laboratory tests and clinical measurements. Apparent clinical signs represent the last stage when the nutrient deficiency is severe. Clinical indices are useful for identifying the severity of the problem and measuring progress toward long-range goals to eradicate nutrient deficiencies. Static and functional laboratory tests are primarily used to detect subclinical nutrient deficiency states, and to confirm a clinical diagnosis. Static biochemical tests measure levels of the nutrients in biological specimens while functional biochemical tests determine the changes in the activities of enzymes dependent on a specific nutrient, or in the concentrations of specific blood components dependent on a given nutrient. Biochemical assessment provides useful information on the level of nutrients necessary to meet biological demands, bioavailability, and metabolism of nutrients, and on the impact of interventions.

Functional physiological tests assess the physiological performance of an individual in vivo such as immune competence, taste acuity, night blindness, muscle function, and work capacity. None of these functional physiological tests are specific and must be interpreted along with biochemical measurements. Behavioral indices, either qualitative or quantitative, are often included in nutrition surveillance systems used to monitor the impact of nutrition intervention programs and to track progress toward attainment of long-range goals. Ecological indicators include those related to socioeconomic status, dietary intakes, anthropometry, and illness/mortality. Because inadequate dietary intake precedes the other stages of nutritional deficiency, assessment of habitual food consumption pattern or nutrient intakes (qualitatively or quantitatively) is useful in identifying community at risk of inadequate intakes of nutrients and provides a basis for dietary intervention programs. The interpretation of nutritional assessment indices may be confounded by factors such as the impact of infection, concurrent nutrient deficiencies, seasonal variation, age, sex, disease states, and so on, in addition to sampling and measurement errors.

To evaluate nutritional assessment indices, the observed values can be compared with a reference distribution or reference limits or cutoff points. This article emphasizes biological indices (static and functional biochemical tests, physiological tests, clinical signs) to assess the status of the following nutrients: Vitamin B_2, folate, Vitamin B_{12}, Vitamin A, calcium, iron, iodine, zinc, and selenium. In a setting with limited resources, selection of inexpensive yet reliable indices can provide useful information on nutritional status. Clinical indices and simplified dietary assessment and field methods of functional, physiological, and behavioral indicators should first be considered. These indices should be accompanied by biochemical indices to identify the limiting nutrient.

Selection of both the appropriate design and indices, together with use of subsampling procedure, should be exercised, and use of local expertise and resources optimized.

1. Introduction

In the field of nutrition, a major challenge is how to identify individuals and/or populations who have nutritional problems. Appropriate nutritional assessment can provide the answer. Several texts and numerous journal articles have been written on the subject. This article does not attempt to duplicate such information nor cover assessment methods for every nutrient in detail. Instead, we highlight the following micronutrients which are of global importance: Vitamin B_2 (riboflavin), folate, Vitamin B_{12}, Vitamin A, calcium, and the trace elements iron, zinc, selenium, and iodine. The preferred methods of assessing the status of these nutrients at both the population and the individual level are described, commencing with the first stage in the development of a nutrient deficiency state: assessment of dietary intakes, followed by biochemical and functional indices and finally, clinical signs and symptoms. The application and interpretation of various indices used in research studies and/or public health programs are a major focus of this article. Finally, recommendations on how best to conduct nutritional assessment protocols in settings with both limited and sophisticated resources are indicated.

2. Stages in the Development of a Nutritional Deficiency

The development of a nutritional deficiency state represents a continuum from the early to late stages. Clinical assessment may readily detect the late or severe stages of nutritional deficiencies, for example, angular stomatitis for riboflavin deficiency, and eye lesions or xerophthalmia for Vitamin A deficiency. However, before such clinical signs become apparent, "subclinical" stages of deficiency develop as summarized below:

1. Nutritional deficiency is usually initiated by an inadequate dietary intake of one or more nutrients resulting from either a low content in indigenous food sources and/or the presence of exogenous factors that interfere with ingestion, absorption, and metabolism of the nutrient(s). This stage of nutritional deficiency usually can be identified by dietary assessment.
2. When inadequate intakes persist, the tissue stores become gradually depleted of that nutrient, resulting in low levels in certain body fluids and tissues, and/or in the activity of nutrient-dependent enzymes. Often, these changes can be detected by biochemical tests.
3. Following nutrient depletion of body fluids or tissues, functional changes occur. Functional tests provide a measure of the biological significance of a given nutrient because they assess the functional consequences of nutritional deficiency, for example, cognitive function for iron, taste acuity for zinc, and dark adaptation for Vitamin A.

Anthropometric indices such as weight for age, height for age, weight for height, and measurements such as mid-upper arm circumference and triceps skinfold, are especially useful in detecting a possible chronic imbalance of protein and energy, and can be used, in certain cases, to identify moderate and severe malnutrition (see *Nutritional Deficiency and Imbalances*). No single index of nutritional status provides a definitive diagnosis of all levels of nutritional deficiency. To assess the nutritional status of individuals or populations, a combination of dietary, biochemical, anthropometric, and clinical methods is considered the gold standard.

3. Choosing the Most Appropriate Nutritional Assessment Indices

Nutritional assessment systems can take one of three forms: surveys, surveillance, or screening. Each of these systems utilizes a variety of methods described in later sections. The methods, based on a series of dietary, laboratory, anthropometric, and clinical measurements, can be used either alone or more effectively in combination, depending on the available resources and the study objectives. Studies can be designed to meet objectives at both the population and individual levels. Possible objectives for population-based nutritional assessment systems include:

1. Determine the overall nutritional status of a population or sub-population.
2. Identify areas, populations, or subpopulations at high risk for chronic malnutrition.
3. Characterize the extent and nature of the malnutrition within the population or subpopulation.
4. Identify the causes of malnutrition within the population or subpopulation.
5. Design and target appropriate intervention programs to high-risk populations or subpopulations.
6. Monitor the progress of interventions programs.
7. Evaluate the efficacy and effectiveness of intervention programs.
8. Track progress toward attainment of long-range goals.

The first three of these objectives can be met by means of a *nutrition survey*, in which the nutritional status of a selected population or subpopulation is assessed cross-sectionally. Information from nutrition surveys can also be used to allocate resources to those population subgroups in need, and to formulate policies to improve the overall nutrition of the population. Nevertheless, such cross-sectional surveys are unlikely to provide information on the possible causes of malnutrition (i.e. objective 4), necessary for formulating and implementing nutrition intervention programs at the population or subpopulation level (objectives 5–7). Instead, *nutrition surveillance* must be carried out to accomplish these objectives. This involves monitoring the nutritional status of selected populations or specific at risk subpopulations over specified time periods.

Unlike nutrition surveys, nutrition surveillance data are collected, analyzed, and utilized over an extended period of time. Consequently, the data can be used to identify the possible causes of chronic and acute malnutrition, allowing appropriate nutrition intervention strategies to be developed, if required. Sometimes, only data for specific at-risk subpopulation groups, identified as at high risk in earlier nutrition surveys, are collected using this approach. Additional objectives that can be met from data collected via a nutrition surveillance system include the promotion of decisions by government concerning priorities and the disposal of resources, and the formulation of predictions on the basis of current trends. Nutritional surveillance is also used to assess the efficacy and effectiveness of nutrition intervention programs.

In some circumstances, the objective may be to identify only at-risk individuals who require intervention. This objective can be accomplished by using the third type of nutritional assessment system: *nutrition screening*. In this approach, an individual's measurements are compared with predetermined risks levels or cutoff points, with the aim of determining the proportion of individuals within the sample who are at risk for malnutrition. Screening programs are usually less comprehensive than surveys or surveillance studies.

Each of the nutritional assessment systems can also be used to assess nutritional status at the individual level. For example, screening can be initially carried out to identify frail elderly who require specialized nutritional management, followed by a more detailed and comprehensive baseline nutritional assessment to clarify and expand the nutritional diagnosis and establish the severity of the malnutrition. Next, a nutrition intervention regimen can be designed, based on the data collected in the initial survey. After implementing the intervention, the nutritional status of individuals can be monitored to establish their response to nutritional therapy. Depending on the study objectives and resources available, each of these nutritional assessment systems may utilize some or all the methods (i.e. dietary, laboratory, anthropometric, and clinical assessment) used for assessing nutritional status.

When a nutritional assessment system is to be implemented at the population level, it is important that sampling protocols are designed in consultation with a statistician. These should take into account the objectives of the study, and then be rigorously adhered to throughout the study to avoid systematic bias in the sample selection and to ensure that the sample is random and representative of the target population. Extrapolation of the results to the population at large will then be valid. Self-selection (i.e. survey participation by consent) produces unrepresentative samples and, sometimes, systematic bias. For example, only those with a higher level of education may volunteer to participate. In these circumstances, it is essential to identify the probable direction and magnitude of the bias arising from the sample design and nonresponse rate.

3.1. Nutritional Assessment Indices and Indicators

To facilitate interpretation of the data collected by nutritional assessment systems, the raw measurements are combined to form *indices* such as weight/(height)^2, equal to body mass index, and hematocrit/red-blood-cell-count, equal to the mean cell volume, or related to another measurement through the use of reference data such as weight for age, weight for height, hemoglobin concentration in relation to age, and so on. Frequently nutritional assessment indices are in turn converted to *indicators*, a term that relates to the use or application of nutritional assessment indices. Caution must be used when the term *nutritional indicator* is used, because many other conditioning factors, other than nutritional status, may impact on the variability of an indicator. For example, if the proportion of low birth weight infants is used as an indicator of maternal nutritional status, it is only a good nutritional indicator if a substantial proportion of its variability is indeed due to differences in maternal *nutritional* status per se, and not to other factors such as, for example, smoking during pregnancy. Other examples of nutritional indicators include breast milk Vitamin A concentration as an indicator of maternal Vitamin A status.

3.2. Criteria for Selecting Indices for use in Nutritional Assessment Systems

There are several important criteria that must be considered when choosing the most appropriate combination of indices to use in nutritional assessment systems. These criteria include:

1. Study objectives
2. Expected prevalence and severity of nutrient deficiency state
3. Technical feasibility
4. Acceptability
5. Cost
6. Performance:
    - validity; reliability; predictive value
    - sensitivity; specificity
7. Availability of reference data and/or cutoff points

Briefly, the choice of indices should take into account the study objectives, as discussed earlier, and the expected prevalence and severity of the nutrient deficiency states. The technical feasibility and acceptability of collecting and measuring the parameter of interest among the population or subpopulation group and its cost must also be considered.

For biological tissues and fluids, for example, rigorous precautions must be followed for sampling, transportation, preservation, and analysis, especially when samples are collected under field conditions for trace element analysis. Consideration should always be given to factors such as: ease of data/sample collection; sample storage and transport; transportability and ruggedness of field equipment; expertise available for collection and analysis; equipment available and its maintenance. The acceptability of work based on collecting blood samples, breast milk, hair, urine, and so on, will depend on the demographic and cultural context, and thus will vary among population groups and must be carefully considered by investigators. When the costs of conducting the measurements are taken into account, both capital and recurring costs should be included, as well as costs associated with training, personnel, and the maintenance of equipment.

The performance of the chosen index should be carefully considered in relation to the study objectives, and its validity, reliability, predictive value, and its sensitivity and specificity determined. Finally, to interpret the measurements, appropriate reference data and/or cutoff points are required. During nutrition interventions, it is also critical that the indices selected have the ability to respond over the range of improvement expected. The latter will depend on factors such as the type of intervention, severity of micronutrient malnutrition, and the age, sex, and physiological state of the target group. Ideally, in nutrition interventions, some of the indices should be measured at baseline and at selected points during the intervention so that the dose-response nature of the relationship can be characterized.

3.3. Classification of Indices

Indices used in nutritional assessment systems can be classified into: biological, ecological, and behavioral indices.

Biological indices are derived from both static and functional laboratory tests, and clinical measurements. The clinical measurements are most useful during the advanced stages of deficiency, when overt signs and symptoms of malnutrition exist. Because many of these clinical manifestations are nonspecific, they must frequently be interpreted in conjunction with biochemical or functional tests to confirm the clinical diagnosis. Examiner inconsistencies are often a source of error in clinical measurements, but can be minimized by training examiners and standardizing the criteria used to define the signs. In most cases, signs should be recorded as positive or negative and not in terms of grades of severity.

Static and functional laboratory tests are primarily used to detect subclinical nutrient deficiency states. Some static biochemical tests measure levels of the nutrient in biological fluids and tissues on the assumption that such tests reflect the total body nutrient content or the nutrient tissue store most sensitive to depletion. Other functional biochemical tests assess the consequences of the nutrient deficiency by measuring changes in the activities of a specific enzyme (e.g., alkaline phosphatase for zinc) or in the concentrations of specific blood components dependent on a given nutrient (e.g., hemoglobin for iron; retinol binding protein for Vitamin A). Functional physiological tests assess the physiological performance of an individual *in vivo* and may include tests of immune competence, taste acuity, night blindness (via vision restoration time for Vitamin A), muscle function, and work capacity. Growth and developmental responses such as lactation, sexual maturation, and cognition, also can be assessed. None of these functional physiological tests are specific and hence must be used in conjunction with biochemical tests to identify the nutrient deficiency involved.

Ecological indices can be subdivided into those related to socioeconomic status, dietary intakes, anthropometry, and illness/mortality. Socioeconomic status indices are derived from data collected by trained interviewers via questionnaires, observation, and sometimes, household interviews. Relevant variables include household composition, education/literacy, ethnicity, religion, income/employment and material resources, water supply and household sanitation, access to health and agricultural services, land ownership, and so on. Additional data can be collected on adequacy of food preparation equipment, extent of food reserves, cash-earning opportunities, and the percentage of household income spent on certain foods such as animal foods, fruits and vegetables.

Illness-related indices are often assessed by the recall of the number and duration of episodes of diarrhea, upper and lower respiratory infection, oral thrush, skin and mucous candidiasis, purulent conjunctivitis, fever, and so on over a pre-defined time period. More objective biological methods may be employed in which stool and urine specimens are examined for helminthic infection rates, and blood samples are collected for thick blood smears for malaria, total white cell count, or C-reactive protein. Inclusion of these indices is especially important, as infection may confound the interpretation of many of the indices used in nutritional assessment.

Changes in infant and toddler mortality rates may also be used as indicators. For example, in a recent meta-analysis, Vitamin A supplements were shown to result in a 23% reduction in mortality (95%, CI: 15% to 29%) for children up to 72 months of age. Indeed, reductions in mortality may be an earlier impact indicator than increased weight: when severely malnourished children are kept alive, their low weights have a marked effect on reducing the average weight gain. However, large sample sizes are required to assess changes in mortality rates, and the indicator is not as sensitive for children greater than 36 months of age.

Dietary indices, based on both quantitative and qualitative dietary assessment methods, are discussed later (see *Dietary Assessment*). They can be used to fulfill a variety of objectives, depending on the dietary assessment method used. These objectives may include an assessment at the individual or group level of the actual or usual intakes of nutrients, the use of specified foods, the pattern of food use, and the proportion of the population at risk to inadequate intakes of nutrients. The latter information is especially useful because it can be used to ascertain whether assessment using more invasive biological indices is warranted in a population or subpopulation. For some nutritional assessment protocols, dietary indices are used in conjunction with those designed to monitor knowledge, attitudes and practices, and reported food-related behaviors.

In intervention studies, dietary indicators can be used to measure exposure at selected intervals during the intervention, via monitoring changes in weaning food practices, food consumption patterns, and intakes of micronutrients and antinutrients.

Anthropometric indices assess growth and body composition and are used extensively in all nutritional assessment protocols, including nutrition intervention studies. The measurements can be performed relatively quickly, easily, and reliably using portable equipment, provided standardized methods and calibrated equipment are used. Details of anthropometric measurements are described in several international publications. Anthropometrists must be trained until acceptable levels of precision and accuracy are obtained, and their measurement procedures reviewed periodically. An accurate assessment of age, and for infants, information on birth weight, and if possible, birth length and gestational age, is also required for the interpretation of anthropometric measurements. In cases where age is unknown, construction of a locally relevant community-specific calendar may be helpful.

Growth indices based on length/height-for-age and weight-for-length are recommended by the World Health Organization (WHO) for evaluating the impact of nutrition interventions on children. In combination, these indices distinguish between stunting (low length/height-for-age) and wasting (low weight-for-length). Of these, length-for-age is more sensitive for measuring the impact of nutrition intervention strategies. In stunted populations with normal weight-for-length/height, nutrition interventions may have no impact on wasting indicators. Despite the WHO recommendations, weight expressed in relation to age is by far the most widely used anthropometric index in

Maternal and Child Health Clinics in less industrialized countries because of the difficulty of measuring length. Nevertheless, weight-for-age does not discriminate between wasting and stunting and underestimates the prevalence of malnutrition in stunted populations.

Body composition indices can also be derived from selected anthropometric measurements of skinfold thickness and mid-upper arm circumference. Mid-upper arm fat area and mid-upper-arm muscle area are derived from triceps skinfold and mid-upper arm circumference using standard equations, which provide indirect estimates of total body fat and lean body mass, respectively. Changes in arm fat area and arm muscle area can be used to monitor alterations in body fat and the protein reserves of the body.

Behavioral indices are often included in nutrition surveillance systems used to monitor the impact of nutrition intervention programs and to track progress toward attainment of long-range goals. They can be qualitative or quantitative. The former may assist in understanding how or why the activities of an intervention have or have not been successful and to identify those factors that have facilitated or impeded the behavior intention or behavior change process. They are based on ethnological and anthropological techniques involving in-depth interviews and focus groups, and observation through participation and systematic observation of practices.

Quantitative behavioral indicators can be used to monitor any changes in knowledge, attitudes, practices (KAP), and reported behaviors arising from exposure to and retention of nutrition communication and social marketing inputs that may be part of a nutrition intervention strategy: the underlying supposition being that such changes will lead to improved nutrient status. KAP surveys can also be used to ascertain why a program may not have had any impact. Such surveys are generally conducted at baseline and post-intervention, sometimes on a representative subsample of the target group. The instruments should be administered by a trained interviewer using a standardized format to minimize interviewer bias.

3.4. Impact of Confounders on Nutritional Assessment Indices

Possible confounders that must be considered when selecting and interpreting nutritional assessment indices can be classified into: biological; sampling; and measurement/analytical errors.

Biological confounders may include the impact of infection, concurrent nutrient deficiencies, seasonal variation, age, sex, ethnicity, physiological state, diurnal variation; presence of concomitant disease states; hereditary disorders, recent dietary intake, drugs, nutrient interactions, recent physical activity, hormonal status, and so on, on the performance of biological, ecological, and/or behavioral indices.

Sampling errors may include selection bias during collection procedures, particularly when nonprobability sampling is used. For example, ignoring persons who do not respond to the initial attempt to include them in the study (nonresponse bias), or sampling only persons attending an under-fives clinic and neglecting to include nonattendees. This is often critical in nutritional studies, because people who refuse to take part often have a nutritional status that differs widely from that of the responders. Collecting data only at one season of the year may introduce a seasonal bias, especially in developing countries, where nutritional status often varies markedly from the hungry to the harvest season. In clinical trials, historical events can often have a different impact on treatment group compared with the control group. Furthermore, a regression artifact may falsely suggest an improvement in the nutritional status of deficient persons without any intervention. Finally experimental mortality may occur in which dropouts have different characteristics than those who remain in the intervention.

Measurement and related errors include bias induced by systematic errors such as adventitious contamination—a serious problem in trace element analysis, dietary scales that always overestimate weight, skinfold calipers which systematically underestimate skinfolds, and a biochemical assay, for example, for Vitamin C, in foods which systematically underestimates the Vitamin C content because only the reduced form of Vitamin C (L-ascorbic acid) is measured. The accuracy and precision of the analytical method should always be carefully assessed. Interview and respondent bias may also reduce the accuracy of dietary assessment results. For example, a social desirability response bias may cause respondents to consistently underestimate alcohol consumption in a food frequency questionnaire.

Analytical sensitivity and analytical specificity also affect measurement errors. Recognition of the analytical sensitivity of a biochemical method is especially important when the nutrient of interest is present in low concentrations (e.g., ultratrace elements Cr, Mn, Ni). Routine procedures should be capable of analyzing the nutrient of interest at a level that is five times greater than the detection limit. Analytical specificity refers to the ability of an analytical method to measure only the substance of interest. Specific analytical methods do not generate false-positive results and have few or no interferences.

Frequently, the effects of these confounding factors can minimized or eliminated by

- standardizing the sampling and collection procedures for biological samples (e.g., blood, hair, urine)
- training the interviewers
- using calibrated and standardized equipment
- sometimes measuring the confounding factors concomitantly so that they can be taken into account in the statistical analyses

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Biographical Sketches

Emorn U. Wasantwisut is a graduate of Massachusetts Institute of Technology (US) from where she obtained her Ph.D. in Nutritional Biochemistry and Metabolism, following a Master's degree in Nutrition from Brigham Young University (US) and a Bachelor degree in Biochemistry from Chulalongkorn University (Thailand). She has been a member of the academic staff of the Institute of Nutrition, Mahidol University (INMU), Thailand, since 1985. During the year 1986–1987, she obtained her postdoctoral training at the Vitamin and Mineral Nutrition Laboratory, Beltsville Human Nutrition Research Center, US Department of Agriculture (Maryland, US). Her present position at INMU as Associate Professor and Deputy Director for Research and Academic Affairs allows her to gain management experiences on top of her research and teaching assignments. She teaches various courses for a Master's degree program of Applied Food and Nutrition for Development and Doctoral of Science program in Nutritional Sciences. Her research areas include nutritional assessment, bioavailability and metabolism of micronutrients (Vitamin A, zinc, iron), functional consequences of micronutrient deficiencies, micronutrient interaction, and food-based intervention in response to micronutrient malnutrition. She has published a number of technical papers in scientific journals, chapters in books, and proceedings. She has served as technical consultant to Thailand's Ministry of Public Health and various activities under international organizations such as WHO, UNICEF, IAEA, and US Agency for International Development. Her other current positions also include a Coordinator of Secretariat, Southeast Asia Nutrition Research-cum-Action Network as supported by WHO/SEARO and the National Coordinator for the IAEA Regional project on Measuring the Effectiveness of Multinutrient Supplementation.

Jorge L. Rosado obtained a Master's degree in Nutritional Biochemistry from the Massachusetts Institute of Technology (US) and a Ph.D. in Nutritional Sciences from the University of Connecticut (US) following a degree in Biochemical Engineering from the Monterrey Institute of Technology (Mexico). He has been involved in research activities with the National Institute of Nutrition in Mexico since 1983 where he holds a position as Senior Scientist. He has also been involved in teaching at the National University of Mexico over 10 years and is Professor at the University of Queretaro. His major areas of research includes the mechanism and physiological effects of nutrient absorption including dietary carbohydrates and minerals, the functional consequences of micronutrient deficiencies, and the development of formulated and fortified foods. His research has resulted in the publication of more than 60 scientific papers and several books and monographs. He has served as technical advisor for the development of several National Nutrition Programs in Mexico and other countries. He has also served as consultant for international companies in the area of food formulation and food fortification. He is on the editorial board of the *Journal of Nutrition*, *Nutrition Reviews*, and *Revista de Investigacion Clinica*. He has received national and international recognition including the National Prize in Food Research (1987), The Nestle Prize in Human Nutrition (1992), The Helen Keller Prize in Micronutrient Research (1995), The Alfonso Ribera Prize (1997), and the Nutrition Research Prize (1998).

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