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An interactive 24-hour recall for assessing the adequacy of iron and zinc intakes in developing countries

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Preface

Purpose of the manual

Deficiencies of iron and zinc are a widespread public health concern. Dietary inadequacies of these two micronutrients are likely to occur in developing countries where staple diets are predominantly plant-based, and consumption of animal protein foods such as red meat, poultry, and fish is often small because of economic, cultural and religious constraints. As a result the amount of iron and zinc available for absorption from such diets is often low. This manual has been written as a tool for collecting, analyzing, and interpreting dietary data on intakes of iron and zinc. Such data are essential for identifying groups at high risk of dietary inadequacies of iron and zinc and for implementing intervention programs for combating deficiencies of these micronutrients and evaluating their effectiveness.

A simplified semi-quantitative dietary method has been developed by the International Vitamin A Consultative Group and by Helen Keller International to identify groups at risk for suboptimal intake and thus deficiency of vitamin A. However, this method is not appropriate for assessing intakes of iron and zinc and evaluating their adequacy in relation to nutrient reference levels. To accomplish these objectives, a quantitative dietary method must be used that has the ability to measure actual or usual intakes of iron and zinc at an individual or group level, and intakes of dietary modifiers known to influence the bioavailability of these micronutrients must also be measured. This manual contains practical guidelines and procedures for carrying out an interactive 24-hour recall method that has been especially modified to collect such information on rural populations in developing countries. Recall data collected for 1 day for each individual can be used for assessing or comparing average intakes of iron and zinc for one or more groups. Alternatively, recall data collected for 2 or more days on at least a sub-sample of individuals can be used to determine the proportion of the population at risk to inadequate intakes of iron and zinc, and to examine associations between dietary and other variables measured on the same individuals.

Users of the manual

The manual has been written as a tool for program planners, experienced health professionals, and nutritionists wishing to measure intakes of iron and zinc and evaluate their adequacy in developing countries. It may also be useful for training nutritionists and nutritional epidemiologists in developing countries. For some aspects of the manual, users may require the specialized assistance of persons with training in epidemiology or statistics. The manual contains step-by-step protocols for training health and nutrition field workers in how to design a dietary protocol and to collect valid data on iron and zinc intakes. Emphasis is given throughout the manual to the importance of collecting the correct type of dietary information to accomplish the purposes of the study. Depending on the dietary protocol adopted, the dietary intake data obtained could be used to design nutrition intervention programs targeted at the most vulnerable groups (e.g., pregnant women), estimate the prevalence of inadequate intakes for developing national food policies (e.g., fortification), examine relationships between dietary indexes for iron and zinc and health and disease outcomes, and identify certain food patterns associated with inadequate intakes of iron and zinc so that effective nutrition education and food-based intervention programs can be planned. The manual can also be used by experts from a range of disciplines such as rural extension, women's health, sociology, adult education, epidemiology, and agriculture, as well as nutrition and public health. Such an interdisciplinary team is essential for designing effective nutrition interventions.

Organization of the manual

The manual is divided into 11 chapters in the order required to plan, design, prepare and conduct the dietary survey, and then analyze and interpret the data. Each chapter lists the key objectives at the beginning and covers a distinct step in the dietary survey methodology. Within each chapter, there is a series of numbered task boxes highlighting the critical steps required to accomplish each task. Program planners and health and nutrition professionals will find it helpful to read the background information on each

task whereas health and nutrition field workers can simply follow the steps in the task boxes in sequential order. Cross referencing has been incorporated throughout the text to assist the reader and emphasize the relationships among chapters.

Chapter 1 describes the main features of iron and zinc deficiency and emphasizes the importance of defining the purpose of the survey because of its effect on the study design and sample size. Details of how the 24-hour recall method was modified and validated for use with rural populations in developing countries are given. Chapter 2 provides some basic guidelines to nonprobability and probability sampling strategies that can be used to select the participants, although the reader is advised to seek the advice of a person with training in sampling techniques before starting the survey. Chapter 3 outlines the methods for determining the sample size and number of recall days, depending on the study objectives, although readers may wish to consult with a statistician before finalizing the sample size. Chapter 4 provides a step-by-step guide on how to prepare for the survey using the interactive 24-hour recall method and includes details on fostering community participation, ethical considerations, and assembling the necessary equipment. Protocols for selecting and training the interviewers and pilot testing the recall method are also included. A detailed discussion of the four stages used to conduct the interactive 24-hour recall is given in Chapter 5. Because the quality of the food intake measurements collected by the interactive 24-hour recall depends on its validity and reproducibility, both of which vary with the population group under study, Chapter 6 describes how these attributes can be measured before carrying out the actual dietary survey. Again, it may be helpful to consult a statistician before carrying out studies designed to assess the validity and reproducibility of the interactive 24-hour recall on the study population.

After the interactive 24-hour recall is conducted, the next stage is to convert the intakes of foods to nutrients by using either a food composition table or a nutrient database stored on a computer. In many developing countries a local food composition table or nutrient database must first be compiled, as discussed in Chapter 7, before intakes of nutrients and antinutrients can be calculated (Chapter 8). Nutrient intakes calculated in this way represent the maximum amount available to the body. For iron and zinc, however, the amount actually absorbed and utilized by the body can be considerably lower than the determined values and depends on the bioavailability of these micronutrients. Chapter 9 describes how to estimate the bioavailability of iron and zinc from mathematical models—termed algorithms—based on knowledge of the major food sources of iron and zinc as well as those dietary modifiers known to influence their absorption. Finally, Chapter 10 presents ways to evaluate the adequacy of iron and zinc intakes by comparison with nutrient reference levels and Chapter 11 provides a brief guide to the selection of statistical techniques appropriate for the analysis of the nutrient intake data collected. The reader must consult other statistical texts for a more detailed discussion of these techniques.

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Chapter 1 | Introduction

What will you learn from this chapter?

- Why we need a dietary tool to assess risk of inadequate intakes of iron and zinc;
- How the interactive 24-hour recall was developed and validated; and
- How to design the dietary survey to suit the purposes of your study.

The United Nations has urged that priority be given to programs in developing countries for the prevention of micronutrient malnutrition—sometimes referred to as "hidden hunger". The micronutrients that have been emphasized are iodine, vitamin A, iron and, more recently, zinc (United Nations 1997, Hotz and Brown, 2004). Deficiencies of these micronutrients are estimated to affect the health, mental and physical function, and survival of more than two billion people worldwide. Women of childbearing age—especially those who are pregnant or lactating—and children are most at risk of micronutrient deficiency. Iron deficiency has the highest rate of prevalence among nutritional deficiencies.

Nutritional deficiencies of iron and zinc are often widespread in developing countries, where staple diets are frequently plant-based and consumption of expensive flesh foods (i.e., red meat, poultry, and fish) is low. Flesh foods are rich sources of readily available iron and zinc, whereas plant-based foods—notably unrefined cereals, legumes, and nuts—contain high levels of phytate and, at times, polyphenols. These components vary in the degree to which they inhibit iron and zinc absorption (WHO/FAO 2004); their effects are discussed in detail in Chapter 9 of this manual. Given such variability, even when intake of micronutrients appears adequate, the amount of iron and zinc absorbed from such diets is often low, an unfortunate consequence because even moderate deficiencies of these two micronutrients have far-reaching effects on maternal, infant, and child health. Such hidden hunger contributes to pregnancy complications, low birthweight, impaired immune competence and cognitive function, maternal and infant morbidity, and growth failure in infancy and childhood.

Table 1.1 summarizes some of the characteristic features of iron and zinc deficiency during infancy and childhood, and their impact on pregnancy outcome. These two deficiencies have similar adverse effects and other features in common.

TABLE 1.1

The Characteristic Features of Iron and Zinc Deficiency

Iron Deficiency	Zinc Deficiency
• Anemia	• –
• Anorexia	• Anorexia
• Increased susceptibility to infection	• Increased susceptibility to infection
• Impaired growth	• Impaired growth
• Impaired cognitive function	• Impaired cognitive function
• Defects in thermoregulation	• Poor taste acuity
• Increased risk of pregnancy complications	• Increased risk of pregnancy complications
• Increased risk of low birthweight	• Possibly increased risk of low birthweight

1.1 A New Tool for Assessing Dietary Intakes of Iron and Zinc

The interactive dietary recall method described in this manual has been developed to fill the need for a rapid, non-invasive dietary tool, one with a low respondent burden and the ability to quantify daily intakes of iron and zinc for rural populations in developing countries. This new method can assess actual or usual intakes for an individual or a group, and can measure dietary enhancers and inhibitors known to influence the amount of iron and zinc absorbed, so that both total and available intakes of iron and zinc can be determined. Consequently, prevalence

estimates for inadequate intakes of iron and zinc can be assessed and used to predict risk of iron and zinc deficiency within the study group without the need to collect biological samples, such as blood. Ideally, dietary assessment should be combined with other biochemical indicators of nutritional status, although collecting for monitoring of the latter is often culturally unacceptable to rural populations in developing countries.

This new dietary tool can also be used to develop dietary strategies to enhance both intakes and bioavailability of iron and zinc in plant-based diets. Such dietary strategies involve changes in food selection patterns and in traditional methods for preparing and processing indigenous foods. Although such dietary strategies require long-term commitment, they may be more sustainable, culturally acceptable, economically feasible, and equitable than supplementation and fortification. They can also be used to alleviate several micronutrient deficiencies simultaneously without any risk of antagonistic nutrient interactions.

The use of weighed food records completed by trained research assistants working in households has been the most common way to collect quantitative dietary intake data in developing countries. This method is, however, time consuming, expensive, requires a high respondent burden, and can be disruptive to rural communities. We have modified the 24-hour recall method to assess intakes of iron and zinc and certain dietary components known to influence their absorption in population groups of developing countries. The modifications involve providing some group training on portion size estimation before the actual recall; supplying picture charts on the day before the recall for use as a checklist on the day the food is actually consumed and for comparison with the recall to reduce memory lapses; and providing bowls and plates for use on the recall days to help respondents visualize the amount of food consumed. The method also calls for weighing the portion sizes of salted replicas of actual staple foods consumed by the respondents (Ferguson et al. 1995).

We chose to modify the 24-hour recall method because it is easier, faster, and less expensive to use than the weighed method, and it is less invasive; thus, compliance is enhanced. The 24-hour recall is especially suitable for areas where diets are not very diverse and are predominantly plant-based. Research suggests that when the recalls are carried out by interviewers trained to anticipate and recognize potential sources of distortion and bias, respondent and interviewer biases can be minimized. Efforts can also be made to minimize the non-response rate by training the interviewer to convey warmth, understanding, and trust.

Errors due to memory lapses can be reduced by using a standardized interview protocol which includes probing questions and visual aids, such as salted replicas of the actual foods consumed. By repeating the 24-hour recall on at least 2 days on all the subjects, or on a representative sample of at least 30 to 40 individuals per stratum, it is possible to obtain an estimate of the distribution of usual intakes of individuals; and hence, the proportion at risk of inadequate intakes. Relationships between dietary indexes and other variables of nutritional status measured on the same individuals can be examined, provided it is feasible to obtain valid estimates of usual intakes on an individual basis by collecting recalls on multiple days.

Unfortunately, it is not possible to use even more rapid and simplified semi-quantitative food frequency questionnaires of the type used to identify groups at risk of inadequate intakes of vitamin A; and thus at risk for vitamin A deficiency (IVACG 1989, Rosen et al. 1994). Methods of this type are only appropriate for nutrients such as vitamin A and other dietary components (e.g., dietary fiber) that are concentrated in a relatively small number of foods or specific food groups. They are not suitable for quantifying intakes of iron and zinc, because both these micronutrients occur in a wide range of food items. On the basis of our experience in Africa, rural women have difficulty with the concept of reporting habitual food intakes over a pre-defined time period and find it easier to respond to specific questions related to the previous day.

The feasibility and relative validity of this interactive 24-hour recall method was tested on 60 pregnant women in rural southern Malawi. Relative validity was assessed by comparing the intakes from two 24-hour recalls (i.e., the test method) with those assessed using two weighed records (i.e., the reference method)

conducted on the same 2 days (Ferguson et al. 1995). There was good agreement between the average intakes of both iron and zinc obtained by the two methods, confirming that the method could be used to determine average intakes of iron and zinc for this group of rural Malawian women. Comparable estimates for the proportion of women at risk of inadequate intakes of iron and zinc were also obtained using the two methods.

To assess the concurrent validity of the interactive 24-hour recall method, several dietary variables of iron and zinc were calculated from three interactive 24-hour recalls collected for 152 pregnant women living in rural southern Malawi. These variables were compared with selected biochemical indexes of iron and zinc measured on these same women. The results indicated significant associations between indexes of available dietary zinc (e.g., phytate-to-zinc molar ratios) and hair zinc concentrations (Gibson and Huddle 1998) as well as between intakes of available iron and of iron from animal protein with two biochemical iron indexes: serum iron and percentage transferrin saturation. From these results it was concluded that the interactive recall data provided valid estimates of the amounts of dietary iron and zinc available for absorption in this group of rural Malawian women.

BOX 1.1

Determining Mean Usual Intakes of Groups or of Individuals

For groups, do you wish to determine mean usual intakes of groups to:

- Compare the average intakes of iron and zinc of different groups within the country?
- Identify foods that are the primary contributors of iron and zinc in the diets of different groups within the country?
- Carry out epidemiological studies to compare relationships between intakes of foods and iron and/or zinc in different groups with certain outcomes of health and nutritional status or susceptibility to disease at national and international levels?
- Provide time trends in consumption of foods and iron and/or zinc intakes of different groups within the country?
- Evaluate the effect of nutrition intervention programs, such as food fortification and dietary modification, by assessing changes in mean intakes of iron and zinc in the target group before and after intervention?
- Evaluate the effect of nutrition intervention programs by comparing the mean intakes of iron and zinc of an intervention and control group?

OR

For individuals, do you wish to estimate usual intakes of individuals to:

- Assess the prevalence of inadequate intakes of iron and zinc within a population to identify those most at risk?
- Evaluate the effect of a nutrition intervention by showing decreases in the percentage of individuals with inadequate intakes?
- Identify food consumption patterns associated with adequate versus inadequate intakes of iron and zinc; and hence, provide a basis for designing appropriate food assistance and nutrition education programs?
- Develop guidelines for food fortification to ensure that those most at risk are targeted?
- Develop national food policies?
- Monitor the effect of food fortification and evaluate its effectiveness on nutritional and health status or disease incidence?
- Investigate intakes of iron and zinc of individuals in relation to nutritional and health status and disease?
- Assist with individual diet counseling?

After defining the purpose of your survey, go to Box 2.1 to select the sampling strategy.

1.2 Defining the Purpose of Assessing Intakes

Before any dietary tool is used to measure iron and zinc intakes, the purpose of the survey must be defined, because the purpose affects the study design and sample size. Dietary intake data are collected for many reasons (Gibson 2005), as shown in the two broad categories delineated in Box 1.1. When the purpose involves characterizing the average intakes of iron and zinc of a group, dietary data from each individual for 1 day will suffice. When the purpose involves characterizing the usual intakes of iron and zinc for individuals, the dietary data must be collected from each individual for more than 1 day. The number of days will depend on the purpose of the study, the nutrient of interest and its day-to-day variability in intake, and the setting. (More details are provided in Chapter 3. See, e.g., Box 3.8 and Box 3.9.)

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Chapter 2 | Selecting the Sampling Design

What will you learn from this chapter?

- How to choose the sampling design to define the participants;
- How to perform non-probability sampling;
- How to avoid sampling bias; and
- How to perform five probability sampling methods.

In most dietary work, information on a large population is required. However, for practical reasons, only a limited number of subjects can actually be studied. Consequently, representative subjects (i.e., the sample) must be chosen and investigated, and the results used to supply information about the whole population. The technique of selecting a sample representative of the entire population of interest and of a size adequate for achieving the primary study objectives may require the specialized assistance of a person with training in sampling techniques. Some basic guidelines are provided here. Securing statistical support is helpful and especially important when designing national dietary surveys.

A major factor influencing the choice of the sampling design is the availability of a *sampling frame* (see Appendix A for a glossary of the terms used here and throughout this manual). Additional factors are time, resources, and logistical constraints. The sampling frame is usually a comprehensive list of all the individuals in the population from which the sample is to be chosen. In some circumstances, however, the sampling frame may consist of a list of districts, villages, institutions, or households—termed "sampling units"—rather than individuals per se. When a sampling frame is available, probability methods can be used. In circumstances when a sampling frame is not available, non-probability sampling methods must be used (Box 2.1).

BOX 2.1

Choosing the Sampling Design to Select the Participants

1. When a sampling frame exists, use probability sampling methods.
2. In the absence of a sampling frame, use non-probability sampling methods.

2.1 Non-probability Sampling

Two non-probability sampling methods are convenience sampling and quota sampling (see Box 2.2). Many clinic-based studies use non-probability sampling methods. Such methods normally do not result in samples that are representative of the entire study population. Hence, they should not be used when the aim is to generalize results from a sample to the entire study population. Dietary data derived from a non-probability sample will almost certainly be biased in relation to those of the general population (Hulley and Cummings 1988).

BOX 2.2

Planning A Non-probability Sampling Scheme

Note: The approach will vary depending on the population's characteristics.

1. When the population to be sampled is relatively homogeneous, use a convenience sample. This involves taking individuals into the study who happen to be available at the time of data collection.

OR

2. When the population to be sampled can be divided into a number of different classes or categories (e.g., based on age groups, ownership of land, occupation, etc.), then quota sampling may be more appropriate. This involves including a certain number of individuals from each category in the final sample.

Non-probability sampling is generally less expensive and more practical than probability sampling. The occurrence and strength of various biases, discussed below, determines whether or not this type of sample simulates a true random sample of the population.

2.2 Sources of Bias in Sampling

Several possible sources of bias can occur when non-probability sampling is used (Varkevisser et al. 1993). They include the following:

- Ignoring people who do not respond to an initial approach to include them in the study—the non-response bias. For dietary studies, this may in fact be critical because people who refuse to take part in the recall interviews may well have characteristics and diets that differ widely from those of the respondents.
- Studying only volunteers, who are often unrepresentative of most of the population.
- Sampling only those persons attending a clinic, school, or health center, and neglecting to include non-attendees.
- Collecting data at only one time of the year, which may introduce a seasonal bias because certain food-stuffs are often consumed only at one time of the year. Therefore, when studying dietary intakes, data should be collected during all seasons rather than just at one time.
- Selecting subjects who are accessible by road introduces the "tarmac" bias. Areas accessible by road are likely to be systematically different from those that are more difficult to reach.

2.3 Probability Sampling

Probability sampling is the recommended method for obtaining a representative sample with minimum bias and can be used when a sampling frame either already exists or can be compiled (Lemeshow et al. 1990). Five probability sampling methods are listed in Box 2.3, and described in Boxes 2.4-2.8. These include: simple random sampling (Box 2.4); systematic random sampling (Box 2.5); stratified random sampling (Box 2.6); cluster random sampling (Box 2.7); and multistage sampling (Box 2.8). Each method involves random selection procedures to ensure that each sampling unit has an equal probability of being sampled (Varkevisser et al. 1993, World Vision Canada 1996).

BOX 2.3

Planning an Appropriate Scheme for Probability Sampling

- If you wish to select the sample at random from an accurate listing of every unit of the accessible population, then carry out simple random sampling (Box 2.4).
- If you wish to select your sample by a periodic process, then carry out systematic random sampling (Box 2.5).
- If you wish to ensure that special subgroups are adequately represented in the final sample, then carry out stratified random sampling (Box 2.6).
- If you wish to ensure that a random sample is obtained from a population in which households or villages are geographically close to one another but for which a list of individual units is not available, then carry out cluster random sampling (Box 2.7).
- If the population is large, diverse, and covers a large geographic area in which people, families, or units are difficult to locate, then carry out multi-stage sampling (Box 2.8).

BOX 2.4

Conducting Simple Random Sampling

1. Identify or compile a list of all the sampling units in the population under investigation from which you want to draw a sample (e.g., households with a pregnant woman).
2. Assign a number consecutively to each unit starting at one.
3. Select numbers by using a table of random numbers (see Appendix B), a computer program that generates random numbers, or the lottery method. For this last method, write all the assigned numbers on slips of paper and put them into a box. Shake the box vigorously, and then pick the required number of slips of paper out of the box and record their numbers. The units corresponding to these numbers will comprise the sample.
4. Continue drawing random numbers until the required sample size is obtained. Only use one method per random sample.

BOX 2.5

Stages in Systematic Random Sampling

1. Select sampling units (e.g., persons) at defined sampling intervals from the sampling frame. To calculate the sampling interval, divide the total population by the required sample size.
2. Randomly choose the number of the first sampling unit (e.g., first person) to be selected by numbering slips of paper between 1 and the calculated sampling interval (e.g., 10). Next, blindly pick out one slip of paper and record its number (e.g., 6). This number will be the random starting point. Alternatively, use the random number table in Appendix B to select the number of the first sampling unit to be selected.
3. Start with the random starting point number, and then select every sampling unit by adding the sampling interval cumulatively. For example; the second person will be #16, the third person #26, and the fourth person #36 from the sampling frame.
4. Continue with this process until the required sample size is obtained.

BOX 2.6

Conducting Stratified Random Sampling

1. Divide the sampling frame (list of potential sampling units) into a number of subgroups (strata), according to characteristics within your study population of interest, such as sex, race, age, and education level.
2. Draw a random sample from each of these strata using simple random sampling techniques (see above) until the required sample size is obtained.

OR

3. Carry out systematic sampling to obtain the required sample size from each stratum.

BOX 2.7

Conducting Cluster Random Sampling

1. Prepare a sampling frame of clusters. A cluster may consist of geographic units (e.g., districts, villages) or organization units (e.g., health centers) which are geographically close together. Care must be taken to ensure that the clusters are heterogeneous with respect to the variables of interest.
2. Select the required number of clusters to survey by using simple random sampling (Box 2.4) or systematic sampling (Box 2.5).
3. Prepare a listing of individual sampling units (e.g., households) only within those clusters (e.g., villages) that have been selected.
4. Determine the number of individual sampling units within each cluster to survey. All the individual units or only a random sample may be surveyed.
5. When only a sample of individual sampling units is to be surveyed, select the required number within each cluster using simple random sampling (Box 2.4).
6. Alternatively, select the required number within each cluster using the random walk method (Box 2.9).

BOX 2.8

Conducting Multistage Sampling

1. Define each of the stages in the sampling design, its associated sampling units (known as "clusters"), and the number of clusters to be surveyed at each stage. Seek clusters that are heterogeneous with respect to the variables of interest.
2. For the first stage, choose the required number of clusters by using systematic sampling.
3. Develop a sampling frame for the clusters chosen by preparing a list of individual sampling units.
4. For the second stage, randomly select the required number of individual sampling units within each cluster using a random number table.
5. Repeat step 4 for each stage in the multistage procedure.

Note that when stratified sampling is used (Box 2.6), the sample is not necessarily representative of the actual population. The imbalance can be corrected, however, when the results are generalized to the whole study population by *weighting*. Procedures for applying sampling weights to the final dietary survey results must be carried out in consultation with a statistician.

Alternatively, a sampling strategy known as "proportional stratification" can be used to adjust the sampling before choosing the sample in order to simplify the data analysis, provided information on the size and a listing of the sampling units are available. The procedure ensures that communities, such as those with larger populations, have a proportionately greater chance of being selected than do smaller communities. When the population to be sampled is very large, diverse, and widely dispersed, it is impractical and costly to sample from all of its elements. Instead, groups of study units—referred to as *clusters*—form the study units from which a certain number are randomly selected (Box 2.7).

Sometimes two or more stages are used to select the clusters, a process referred to as "multistage sampling" (Box 2.8) (Varkevisser et al. 1993, Sullivan et al. 1995). For each stage of sampling, a different sampling frame is used. Note that for both cluster and multistage sampling, a sampling frame of individual units is not required for the whole population. Instead, a sampling frame of clusters can be used from which a listing of individual units is only required for clusters that are selected at the final stage. Consequently, the sample is easier to select.

Multistage sampling is frequently used for national dietary surveys. It typically involves sampling at four stages: at the provincial or similar level (stage one); at the district level (stage two); at the level of communities in each selected district (stage three); and at the household level in each chosen community (stage four). A random sample must be drawn at each stage. Use the random walk method to select the required sample when a listing of the individual sampling units within each cluster is not available (Box 2.9). The goal of multistage sampling is to ensure that all sampling units within a population have an equal probability of being sampled.

BOX 2.9

Using the Random Walk Method for Sampling Individual Units

1. Use the random walk method to select the required sample size when a listing of the individual sampling units within each cluster is not available. This involves going to the center of each village and randomly selecting a direction to walk towards the outer part of the village. This can be done by spinning a bottle or pen on the ground, and walking in the direction that the bottle or pen points.
2. Count all of the households from the central area to the edge of the village.
3. Randomly select a number from 1 to the total number of households counted. The number selected will be the first household to visit.
4. The second household selected will be the one whose front door is closest to the first household.
5. The third household will be the closest front door of the next household (excluding any households already visited).
6. Carry on in this way until the required number of households has been surveyed.
7. Alternatively, select every third or fifth household until you have the required number of households.

The likelihood that the final sample drawn by cluster and multistage sampling is representative of the total study population depends on the size, heterogeneity, and number of clusters selected in the first stage; the larger the number of clusters, the greater the chance that the sample will be representative. The ultimate sample size is usually a compromise between what is desirable and what is feasible and affordable.

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Chapter 3 | Designing the Dietary Protocol to Meet the Study Objectives

What will you learn from this chapter?

- Why the design of your dietary protocol depends on the study objectives;
- What objectives can be met with dietary data that give mean intakes for groups or usual intakes for individuals;
- What assumptions must be made when preparing to estimate the sample size;
- How your objectives determine your sample size and number of recall days that need to be studied; and
- How to calculate your sample size and define the number of recall days required.

The overall protocol of the interactive 24-hour recall survey depends on the objectives of the study. Two categories of objectives can be defined. Category 1 objectives involve collecting the dietary information for each individual in the study group over a 1-day period to characterize mean intakes of a group (see Box 3.1). Category 2 objectives involve defining the usual intake distribution of a group (Box 3.2, Objective 2a), or determining the usual intakes of individuals (Box 3.2, Objective 2b). To meet Objective 2a, the dietary data must be collected on more than one day, from at least a subsample of individuals in the study. Such data can be used to characterize the proportion at risk for inadequate intakes. If multiple recalls are collected from each individual to provide an estimate of usual intakes for the individual (Box 3.2, Objective 2b), then relationships between dietary variables and other indexes of nutritional status measured on the same individuals can also be examined (Gibson 2005).

BOX 3.1

Determining the Mean Usual Intakes of Dietary Variables for a Group

Category 1 objectives may include the following:

Objective 1a: Describing the mean usual intake of one or more nutrients in a group of individuals with a certain precision or confidence interval (e.g., the mean intakes of iron and zinc for a group of teenage women).

Objective 1b: Demonstrating a significant difference within a specified probability in the mean usual intakes between two groups. The groups may, for example, be persons living in rural and urban areas.

Objective 1c: Demonstrating a significant change over time in the mean usual intakes of a group from paired measurements taken at baseline and again after a pre-selected time period, often after an intervention.

Objective 1d: Demonstrating a significant change over time in the mean usual intakes of unpaired measurements on a group, taken at baseline and again after a pre-selected time period, perhaps in relation to an intervention.

BOX 3.2

Determining Usual Intakes of Dietary Variables for Individuals

Category 2 objectives may include the following:

Objective 2a: Determining the usual intake distribution of a group to assess with a certain degree of precision the proportion of individuals within the group at risk of inadequate intakes. An extension of this objective might involve demonstrating a significant change in the proportion of individuals in a group at risk of inadequate intakes before and after an intervention.

Objective 2b: Relating the usual intakes of individuals to other indexes of nutritional status measured in the same people. An extension of this objective might involve a comparison among groups.

3.1 Defining the Study Objectives

The objectives selected determine the number of respondents required for the study as well as the number of days for which the respondent completes the 24-hour recall. These numbers in turn may be affected by the extent of variation in nutrient intakes both between subjects (i.e., between- or inter-subject variation), and within one subject over time (i.e., within- or intra-subject variation). Both between-subject and within-subject variation depend on the nutrient of interest. Generally, for nutrients found in high concentrations in a few foods (e.g., vitamins A and D, sodium, and cholesterol), between-subject and within-subject variation is high: between-subject and within-subject variation tends to be lower for iron and zinc because they are more widely distributed in foods that are eaten on most days of the year.

The size of the between-subject variation in nutrient intakes determines the precision with which the mean intake of a group can be characterized. For most nutrients, between-subject variation in nutrient intakes is usually smaller than within-subject variation. As a result, the mean intakes of a group can generally be assessed more precisely than individual usual intakes. Nevertheless, to minimize the effect of between-subject variation on the group mean intakes, the sample size should be as large as possible, and the sample should be representative of the study group. In addition, all days of the week should be equally represented in the final sample. To achieve Category 2 objectives, whereby the usual intake distribution of a group (Objective 2a), or the usual intake of individuals (Objective 2b) is required, then the size of the within-subject variation is important. This means that the dietary data must be collected on more than one day. Non-consecutive days are recommended for repeating the measurements of 1-day nutrient intakes to eliminate the error introduced by dietary intakes on adjacent days being correlated within the individual (IOM 2000). In practice, the number of repeat days used in the final study design depends on the respondent burden and on time, budget, and personnel constraints (Gibson 2005).

3.2 Preparing to Estimate the Sample Size

When the objectives of your study have been clearly defined, the next step is to calculate the number of respondents (i.e., sample size n) and define the appropriate 24-hour recall schedule (i.e., number of recall days n_d). Before this step can be accomplished, certain basic principles underlying the calculation of sample size must be understood and planned for in advance; these are outlined below.

For all non-descriptive studies in which statistical tests of significance will be applied to compare dietary variables among groups (i.e., Objectives 1b, 1c, and 1d; and 2a and 2b), specific hypotheses must be defined at the outset of the study. Formulation of an appropriate null hypothesis and a related one- or two-tailed alternative hypothesis helps the investigator to focus on the primary objectives of the dietary study.

The null hypothesis (H_0) states there is no difference between the dietary variables in the groups or no association between the variables under study. The proposition that there is a difference or association is called the alternative hypothesis (H_1). A one-tailed alternative hypothesis states the direction of the difference between the groups which it is desired to detect. As an example, it may state that the mean intakes of iron and zinc for the urban adults will be higher than those for the rural adults. Similarly, it may specify the direction of the association between the variables, for example; that the intakes of available iron and zinc will be positively associated with socioeconomic status.

A one-tailed alternative hypothesis requires a smaller sample than a two-tailed test, but can detect differences only in one direction and should be used with care. A two-tailed test can detect differences in either direction (Hulley and Cummings 1988). The prediction that urban adults will have different mean intakes of iron and zinc—either higher or lower—than rural adults is an example of a two-tailed hypothesis.

In some circumstances, the conclusions reached by the investigator on the basis of the study data may in fact be wrong, possibly because the differences or associations observed were due to defects in the study design arising from bias. It must be stressed that it may be

impossible to remove bias in nutritional data during subsequent statistical analysis if the bias is a result of defects in the study design or a differential error in the data collection.

Sometimes, the differences or associations observed in a study sample may arise by chance alone, and are not true in the population as a whole. This may occur due to type I or type II errors. A type I error (false positive) arises when an investigator rejects a null hypothesis (H_0) that is actually true; whereas a type II error (false negative) is committed if the investigator fails to reject a null hypothesis that is actually false in the population. The maximum chance of making type I and type II errors in any study can be defined in advance by setting α and β, as shown in Box 3.3.

The range for α is conventionally between 0.01 and 0.10, and for β between 0.05 and 0.20. When α is set at 0.05, the investigator has set 5 percent as the maximum chance of incorrectly rejecting the null hypothesis. If desired, α can be reduced still further to 0.01 or even 0.001. The value of β is also set by the investigator in advance. A low β is especially important to avoid a type II error. When β is set at 0.20, this indicates that the investigator is willing to accept a 20 percent chance of missing a difference or association, resulting in a power of 0.80 (1-β. A power of 0.80 implies there is an 80 percent chance of finding a difference or association of that size when it really exists (Table 3.1).

Finally, the investigator must select an effect size often without knowing the actual magnitude of the difference or association. In the absence of a pilot study or results for comparable groups, the minimum size of the difference between the mean usual nutrient intakes of the two groups or the smallest degree of association that the investigator is interested in detecting (if present) is generally chosen (Hulley and Cummings 1988). Tables are used to find the necessary sample sizes for a given power when the expected variability in the mean values is known (Lwanga and Lemeshow 1991).

TABLE 3.1

Power and Significance Levels in One- and Two-tailed Tests

Power Required (β)	**Percentage**		
	75%	u=0.67	
	80%	u=0.84	
	90%	u=1.28	
	95%	u=1.64	
Significance Level (α)		**One-tailed**	**Two-tailed**
	10%	v=1.28	V=1.64
	5%	v=1.64	V=1.96
	1%	v=2.33	V=2.58
	0.5%	v=2.58	V=2.81

BOX 3.3

Estimating the Effect Size and Non-response Rate and Setting other Critical Parameters

- Obtain estimates of the mean(s) usual intake and standard deviation(s) of the nutrient(s) of interest for a comparable group matched by age and gender. These data are often taken from pilot study results or other studies in the literature.
- In all cases, decide on a non-response insurance factor to be used when calculating the number of respondents to be interviewed. Increase the sample size by this factor. Unless the study is particularly invasive, an allowance for 10 percent non-responders should be sufficient.

In all studies which involve investigating differences, changes, associations, and the testing of some hypotheses:

- State the null hypothesis and either a one- or two-tailed alternative hypothesis.
- Set α and β. The probability of committing a type I error (rejecting the null hypothesis when it is actually true) is defined as α. Another widely used name for α is the level of significance; it is often set at 0.05. It gives a 95 percent assurance that you will not get a significant result when you should not (i.e., you will not reject the null hypothesis when it is true). If the alternative hypothesis is one-tailed, use a one-tailed α; otherwise, use a two-tailed α. The probability of making a type II error (failing to reject the null hypothesis when it is actually false) is defined as β, and is often set at 0.20, giving a power (i.e., 1-β) of 0.80.
- Select a reasonable effect size.

For Category 1 objectives, the effect size is the expected difference between the mean usual nutrient intakes of the two groups. For a longitudinal study, it is the expected change in the mean usual nutrient intakes with time. Estimate the standard deviations of the changes. Try to find data from pilot study results or the literature to estimate the likely effect size.

For Category 2a objectives, the effect size is the expected difference in the prevalence of inadequate intakes between two groups. For an intervention study, it is the expected change in the prevalence of inadequate intakes before and after an intervention.

For Category 2b objectives, the effect size is estimated as the absolute value of the smallest correlation coefficient (*r*) that you would like to be able to detect.

BOX 3.4

Objective 1a. Describing the Mean Usual Intake for a Group with a Specified Precision

1. Choose an appropriate probability sampling method (Section 2.3) to randomly select the participants for the dietary survey to ensure that the final sample is representative of the population group under study.
2. Determine the number of subjects (n) to be studied by using data for the most critical nutrient. The calculation should be repeated if several nutrients are of interest, and the worst case (the largest n) should be used if possible.

   For example, assume the expected mean iron intake obtained from the litereature is 10mg/day, with an anticipated standard deviation (s) of 3mg/day. Let us assume that we want to be 95 percent confident that the true mean lies between 9.2 and 10.8mg/day (i.e., the confidence interval has limits which are 0.8mg/day on either side of the mean). A 95 percent confidence interval is calculated approximately as the mean ± 2 × e, where e is the standard error of the mean – a measure of the precision of the estimated mean. Hence, we require 2 × e = 0.8, giving 0.8/2 = 0.4.

   We can use the formula: $n = \frac{s^2}{e^2}$ where s is the standard deviation (i.e., a measure of the between-subject variance) of the nutrient intake of interest, and e is the desired standard error.

   Hence, $n = \frac{(3)^2}{(0.4)^2} = 56.25$, and so 56 subjects are required.

   Alternatively, if we wanted to be 99 percent confident that the true mean lies between 9.2 and 10.8mg/day, then more subjects must be studied. A 99 percent confidence interval is calculated approximately as the mean + 3 × e, hence we require 3 × e = 0.8, giving 0.8/3 = 0.27.

   Hence, $n = \frac{(3)^2}{(0.27)^2} = 123$, and 123 subjects are required.
3. Increase the sample size to allow for non-response.
4. Randomly assign a day for the interactive recall for each person selected for the sample in such a way that all days of the week are equally represented in the final sample.

3.3 Defining the Sample Size to Meet the Study Objectives

Once the study objectives have been clearly stated and the assumptions and estimates outlined in Box 3.3 have been made, the next step is to calculate an optimum sample size for the number of interactive 24-hour recalls required to meet these objectives. To characterize the mean usual intake of a study group (i.e., Objective 1a), the sample size is calculated using the procedure outlined in Box 3.4; dietary variables of each respondent are measured on 1 day only, provided all the days of the week are equally represented in the final study design. For epidemiological studies comparative data are at times required, for example; to determine whether the mean usual nutrient intakes of two groups of individuals within a country are significantly different (Objective 1b). Again, in such cases, nutrient intakes can be measured on 1 day only for each person, provided all days of the week are equally represented in the final study design. The procedure for defining the sample size is detailed in Box 3.5. At the conclusion of the dietary survey, the appropriate statistical analysis must be undertaken to ascertain whether the differences observed are significant (see Chapter 11 for more discussion on this topic).

BOX 3.5

Objective 1b: Demonstrating a Significant Difference in the Mean Usual Intakes of Two Groups

1. Choose an appropriate probability sampling method (Section 2.3) to randomly select the participants from the two target populations for the dietary survey, ensuring that the final sample is representative of the groups under study.
2. Determine the number of subjects (n) to be studied in each group using data for the most critical nutrient. The calculation should be calculated separately for each nutrient of interest and the worst case (the largest n) used.

   Use the following formula: $n = \frac{(u + v)^2 \times (s_1^2 + s_1^2)}{(m_1 - m_2)^2}$

   where u=0.67, corresponds to a β for the test of 75%; v=1.96, corresponds to an α of 5%, two-tailed test (Table 3.1); s_1 = the standard deviation of the nutrient of interest in group A; s_2 = the standard deviation in group B; and m_1 and m_2 are the corresponding means. For example, if the anticipated mean iron intake for village A is estimated as 12mg/day with a standard deviation of 4.5mg/day, and the mean iron intake for village B is estimated as 9mg/day with a standard deviation of 4mg/day, then

   $n = \frac{(0.67 + 1.96)^2 \times ((4.5)^2 + (4)^2)}{(12-9)^2} = 27.9$. Hence, 28 subjects are needed.
3. Set the null hypothesis (H_0) to be that there is no difference in the intake of the nutrient of interest between the two groups. Set the alternative hypothesis (H_1) to be that the intake of the nutrient of interest is different in the two groups (two-tailed test).
4. Increase the sample sizes to allow for non-response.
5. Schedule days for the interactive recall for each person selected for the sample in such a way that all days of the week are equally represented in both groups. If information is also required on the proportion of the two populations at risk of inadequate intakes, each subject—or at least a subsample—will need to be surveyed at least twice, preferably on nonconsecutive days (Objective 2a). If this is the case, the calculation for the sample size will also differ from that shown here.

Another design is that of the cohort study, in which dietary data are collected for the same participants at baseline and after a pre-selected time interval, for example; to track impact of a nutrition intervention following its introduction. (A cohort study is a useful alternative to comparing two groups, one of which received an intervention and the other did not.)

In cohort studies, the difference in the dietary variables following the intervention (i.e., the numerical difference between the intake on completion of the intervention and the intake at baseline) is considered as the outcome variable (Box 3.6). Use of this method often—although not always—permits a smaller sample size because of the smaller variation in the outcome variable.

BOX 3.6

Objective 1c: Demonstrating a Significant Change in Mean Usual Intakes following an Intervention in a Cohort Study Using Paired Data

1. Choose an appropriate probability sampling method (Section 2.3) for random selection of participants from the target population, ensuring that the final sample is representative of the population group under study.
2. Determine the number of subjects (n) to be studied by using data for the most critical nutrient. The calculation should be repeated if several nutrients are of interest, and the worst case (the largest n) should be used if possible.

   Use the formula: $n = \frac{2 \times (u + v)^2 \times s^2}{E^2}$ where u = 0.67, corresponds to a β for the test of 75%;

   v = 1.645, corresponds to a significance level of 5% for a one-tailed hypothesis or v=1.96 for a two-tailed hypothesis (Table 3.1); E = the expected mean change in the intake of the nutrient of interest; and s = the standard deviation of the change in the nutrient intake of interest. For example, if the mean iron intake is projected to increase by 0.5 mg/day with a standard deviation of 1.5mg/day, then

   $n = \frac{2 \times (0.67 + 1.645)^2 \times (1.5)^2}{(0.5)^2} = 96.4$. Hence, 97 subjects are needed.
3. Set the null hypothesis (H_0) to be that there was no change in the mean intake of the nutrient of interest over the intervention period.
4. Set the alternative hypothesis (H_1) to be either the mean intake of the nutrient of interest over the intervention period increased (one-tailed test), or the mean intake of the nutrient of interest changed (two-tailed test).
5. Increase the sample size to allow for non-response and, more importantly, for mobility of the target population; and thereby address the loss of subjects from the target population during the course of the study.
6. Select the days for the interactive recall for each person in the sample so that all days of the week are equally represented in the final sample, both at baseline and at the conclusion of the study. If information is also required on the proportion of the target population at risk of inadequate intakes, at either baseline or on completion of the intervention, each subject—or at least a subsample—will need to complete at least two dietary recalls at each time point (Objective 2a), and a different sample size calculation used. Otherwise, only one dietary recall per subject is needed at baseline, and one at the conclusion of the study.

Alternatively, the target population can be randomly sampled at baseline and again after a pre-selected time interval (e.g., after the completion of an intervention), and the change in mean usual nutrient intakes can be tested statistically. In such cases, the number of subjects on the two occasions is calculated as shown in Box 3.7 and, unlike in Box 3.6, the subjects studied at the end are not the same as those studied at the start of the intervention. However, this approach is less effective for detecting dietary change over time than using paired measurements (Varkevisser et al. 1993).

BOX 3.7

Objective 1d: Demonstrating a Significant Change in Mean Usual Intakes following an Intervention Using Unpaired Data

1. Choose an appropriate probability sampling method (Section 2.3) to randomly select the participants from the target populations. Two samples are needed, one to be studied prior to the intervention, and one following the intervention. Both samples must be representative of the population under study.
2. Determine the number of subjects (n) to be studied on each occasion by using data for the most critical nutrient. The calculation should be repeated if several nutrients are of interest, and the worst case (the largest n) used.

   Use the formula: $n = \frac{(u + v)^2 \times (s_1^2 + s_2^2)}{(m_1 - m_2)^2}$ where u = 0.67, corresponds to a power for the test of 75%;

   v=1.645, corresponds to an α of 5%, one-tailed test; s_1 is the standard deviation of the nutrient of interest at the start of the intervention; s_2 is the standard deviation at the conclusion of the intervention, and m_1 and m_2 are the corresponding means. Normally, s_1 will be assumed to be the same as s_2. For example, if the mean iron intake is estimated as 12mg/day and estimated to increase to 13mg/day with a standard deviation of 4mg/day both before and after the intervention, then

   $n = \frac{0.67 + 1.645)^2 \times (4^2 + 4^2)}{(12 - 13)^2} = 171.50$. Hence, 172 subjects are needed.
3. Set the null hypothesis (H_0) to be that there was no change in the mean intake of the nutrient of interest over the intervention period.
4. Set the alternative hypothesis (H_1) to be either that the mean intake of the nutrient of interest over the intervention period increased (one-tailed test); or set it so that the mean intake of the nutrient of interest changed (two-tailed test). In this case, use v = 1.96.
5. Increase the sample size to allow for non-response. Loss of subjects during the intervention is not an issue provided care is taken in selecting the post-intervention sample to make sure that the subjects participated fully in the intervention, and are not new arrivals; and that those who dropped out of the intervention were not different from those that participated.
6. Schedule days for the interactive recall for each person selected for the samples in such a way that all days of the week are equally represented, both at baseline and at the conclusion of the study. If information is also required on the proportion of the target population at risk of inadequate intakes, at either baseline or on completion of the intervention, each subject (or at least a subsample) will need to be surveyed at least twice (Objective 2a), and a different sample size calculation used. Otherwise, only one dietary recall per subject is needed at baseline and one at the conclusion of the study.

BOX 3.8

Objective 2a: Assessing the Proportion of the Study Group at Risk of Inadequate Intakes

1. Choose an appropriate probability sampling method (Section 2.3) to randomly select the participants for the dietary survey to ensure that the final sample is representative of the population group under study.
2. Determine the number of subjects (n) to be studied by using prevalence data for the most critical nutrient. Repeat if several nutrients are of interest and use the worst case, largest n.
3. Use the following to calculate the number of subjects required, assuming an estimated prevalence and desired precision (half the width of the 95 percent confidence interval).

Estimated Prevalence	Desired Precision					
	±0.02	±0.03	±0.04	±0.05	±0.06	±0.10
0.05	456	203	114	73	51	18
0.10	864	384	216	138	96	35
0.20	1537	683	384	246	171	61
0.25	1801	800	450	288	200	72
0.30	2017	896	504	323	224	81
0.40	2305	1024	576	369	256	92
0.50	2401	1067	600	384	267	96

4. Define the number of replicate days (n_d). Normally, two non-consecutive days will suffice on at least a representative subsample of subjects per stratum. IOM (2000) suggests 30–40 subjects per stratum.
5. Because food intakes vary with the day of the week, schedule the days for the interactive recall for each respondent in such a way that all days of the week are equally represented in the final sample. Conduct the interview for each replicate day on a different day of the week from the first interview.
6. Note that this table should not be used when the expected prevalence is very small or very large.

Note: The values given in the table will not give accurate results for when the expected prevalence is very small or very large.

To achieve the Category 2a Objective, whereby the distribution of usual intakes of a group are characterized in order to estimate the proportion at risk of inadequate intakes of a nutrient, intakes must be measured on more than 1 day: results obtained from only a single day may over- or underestimate the proportion of the population at risk of inadequate intakes. At least 2 non-consecutive days of f ood intake are needed, preferably from each individual, or at least from a representative subsample of individuals in the study. The Institute of Medicine (IOM) (2000) suggests replicate food intake data from 30 to 40 individuals per stratum should be collected (Box 3.8). Note that it is more important to have a minimum number of replicate observations than a minimum proportion of replicate observations (IOM 2000).

The spacing between the replicate observations must also be considered. Short periods between the two observations may increase the risk of changes in reported intakes due to a "learning' effect, whereas a long period increases the risk of drop-outs. De Henauw et al (2002) recommend a period of at least one month and not more than six months between observations, although in developing countries where seasonal changes in food intake may be greater, it is preferable that replicates be obtained within one month. The replicates should be allocated randomly within each population sub-group, and evenly spread over all days of the week. Care must also be taken to ensure that the replicate observations are conducted on a different day of the week from the first. In this way, all days of the week will be equally represented for each stratum, and

any day-of-the week effects on food and nutrient intakes taken into account. If it is not feasible to include all days of the week, then at least some weekdays, weekend days, and market days should be selected.

You will need to collect 24-hour recalls for even more days on each person if you wish to calculate the usual nutrient intake of an individual as, for example; when relationships among a number of variables (e.g., dietary and biochemical) are to be examined by using correlation or regression analysis (Objective 2b) (Box 3.9) (Beaton et al. 1979; Basiotis et al. 1987). To calculate the number of days required per person to determine usual intakes of an individual that can be used with confidence in this way, an estimate of the within-subject variation for each nutrient of interest is required. This can be obtained from the literature, preferably from an earlier study on a comparable group or a pilot study. This estimate may be expressed as the variance, s^2_w; standard deviation, s_w; or as the coefficient of variation (CV_w) expressed as a percentage:

$CV_w = s_w$ / mean level of intake × 100%

The within-subject coefficient of variation (expressed as a percentage) can then be used as shown in Box 3.9 to determine the number of days required per subject to estimate an individual's nutrient intake to within the limit specified (as a percentage) of the long-term true usual intake for that season. If a pilot study is undertaken in which replicate 24-hour recalls are conducted, then the actual CV_w for each nutrient of interest can be calculated. In this way, the estimate of the number of days required to measure usual intake of each of the nutrients of interest in an individual can be defined with the desired degree of precision. However, because of the respondent burden and cost, often only a maximum of 4 days per person is usually feasible, regardless of the extent of the within-subject variation for the nutrient under study. In such cases, the precision of the estimate of the usual nutrient intake for that individual should be calculated based on the number of days actually measured for each individual (see Chapter 11).

BOX 3.9

Objective 2b: Examining Relationships between Dietary and other Variables Measured on the Same Individuals

1. Choose an appropriate probability sampling method (Section 2.3) to randomly select the participants for the dietary survey to ensure that the final sample is representative of the population group under study.
2. Determine the number of individuals (n) required. This will depend on the expected correlation coefficient and whether the design involves examining the difference between two correlations (e.g., urban and rural subjects). Consult a statistician for this task, and use data for the most critical nutrient.
3. Define the maximum desired number of replicate days (n_d). This requires an estimate of the within-subject variation for each nutrient of interest. This should be obtained from the literature, preferably from an earlier study on a comparable group or a pilot study. This estimate may be expressed as the within-subject coefficient of variation CV_w (as a percentage): $CV_w = s_w$ / mean level of intake × 100%. Use this estimate to determine the number of replicate days per subject to estimate an individual's nutrient intake to within 20 percent of their true mean intake 95 percent of the time: $n = (Z_\alpha CV_w / D_0)^2$ where n is the number of days needed per subject; Z_α is 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_w is the within subject coefficient of variation (as a percentage); and D_0 is the specified limit (as a percentage of long-term true usual intake, i.e., 20 percent in the example). For example, to calculate the number of days needed to estimate a Malawian women's zinc intake using 24-hour recalls to within 20 percent of their true mean, 95 percent of the time: $Z_\alpha = 1.96$ and $CV_w = 34\%$, then

   $n = (1.96 \times 34\% / 20\%)^2 = 11$ days
4. Increase the sample size (n) to allow for non-response and subject fatigue during the multi-day survey.
5. Schedule the days for the interactive recall for each individual in such a way that all days of the week are equally represented in the final sample. If less than seven days are studied per individual, carefully choose the days to proportionately represent the expected day-to-day variability in nutrient intakes that will occur over a typical week. For example; one weekend day, one market day, and one non-market day should be included. If possible, the days should be non-consecutive.

Note: The sample size calculation methods presented here, and use of the values presented in Table 3.1, are exact only when there is no uncertainty in the standard deviations S_1 and S_2 and the sample means can be considered to be normally distributed, even if the standard deviations (i.e., S_x) are different. This will not introduce much error if the sample sizes are large (e.g., >30). For smaller samples, an approach using *T*-distributions should be used. When in doubt, a statistician should be consulted.

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Chapter 4 | Preparing for the Interactive 24-hour Recall

What will you learn from this chapter?

- How to foster community participation;
- How to obtain ethical approval and informed consent;
- How to train the interviewers;
- How to standardize the interactive 24-hour recall procedures; and
- How to pilot test the interactive 24-hour recall.

When carrying out dietary studies in communities in developing countries, a participatory research process is recommended. Participatory projects focus on building relationships with the community and involving them in the study design and implementation. Such an approach enhances community participation and thus, the quality of the dietary intake data collected. During the collection and recording of the food intake data using the 24-hour recall procedure, errors may arise that affect the quality of the dietary data. These may include the following:

- Respondent biases may occur when subjects underreport food intakes; purposefully mis-report facts, such as income or age; or overreport the consumption of meat and refined cereals. In many developing countries, meat and refined cereals are perceived as reflecting a higher social status than, for example, vegetables and unrefined cereals. The use of alcohol and tobacco may also be underreported due to cultural or religious beliefs, among other reasons.
- Interviewer biases may occur if different interviewers vary in the degree to which they probe for information (e.g., if they intentionally omit questions) or record responses incorrectly. Such bias may also arise when subjects respond differently to personal attributes of an interviewer (e.g., whether male or female; from the same community, or some other factor).
- Respondent memory lapses may result in the unintentional omission of foods or beverages consumed outside the home, such as that which is consumed on the street, or when visiting neighbors and friends. Respondent memory lapses may also result in foods being unintentionally added during the recall.
- Incorrect estimation of portion sizes may occur when respondents fail to quantify accurately the amount of food consumed. Alternatively, an interviewer may assume an answer such as an average serving size.
- Use of nutritional supplements such as multivitamins and minerals may be omitted, causing significant errors in the calculated intakes, particularly of some micronutrients.
- Computation errors may arise when portion size estimates are converted from household measures of volume (eg., cups, teaspoons) into grams.

Other errors can be introduced when the food composition values are compiled and when nutrient intakes are calculated. These errors and how to minimize them are discussed in Chapters 7 and 8, respectively. Quality control procedures should be incorporated during each stage of the measurement and calculation process to minimize errors (Gibson 2005). This chapter describes some procedures to improve the accuracy of the portion size estimates (Section 4.3); ways to standardize the 24-hour recall interviews (Sections 4.4 and 4.5); and how to train the interviewers for the interactive recall (Sections 4.7).

4.1 Fostering Community Participation

Participatory projects must begin with the formation of an organizational structure for community participation and ownership at the national, regional, district, and community levels within the country, as described in Box 4.1. Only when nutrition and health personnel at all these levels are involved will a dietary intake survey have the support and recognition required for it to proceed in that setting (World Vision Canada 1996).

At the national level, senior nutritionists and health professionals from governmental agencies such as the ministries of health and agriculture, and community

services; and institutions such as universities and other in-country public and non-governmental organizations (e.g., UNICEF, or Save the Children Fund) should be consulted by the principal investigators to gain support, and prevent overlapping of projects and community research fatigue that may occur if too many groups work in the same area. Securing approval for the project at the community level is usually the responsibility of the survey coordinator, a person with previous experience in dietary surveys who is ideally known to, and has the cooperation of, appropriate government agencies within the country.

Liaising with the community must be maintained throughout the entire survey to secure its continued support for the survey. This approach helps ensure that the community is sensitized to the survey from the outset. Community members are given an opportunity to raise any questions or concerns, and they are made aware of the importance of the survey and kept informed of its progress. They also are informed about where the information collected is likely to be used. Importantly, after completion of the survey, the community should be thanked and presented with the results in a manner appropriate to all those involved.

BOX 4.1

Initiating Liaison Activities

- Identify senior nutrition and health personnel at the national level with whom you wish to collaborate, and send them an introductory letter explaining the justification, objectives, and methodologies to be used, together with details of the funding sources.
- Arrange to meet for more detailed discussions with those who express an interest and are willing to support the project.
- Identify nutrition and health personnel at the regional level.
- Arrange to meet with them for more detailed discussions.
- Several months before the project is scheduled to start, identify key district officials representing ministries such as health, agriculture, and community development and possibly any non-governmental agencies working in the district.
- Obtain support from authorities at national, regional, and district levels.
- Set up a consultative committee of district officials.
- Arrange regular meetings with the consultative committee to report on the project activities and to seek advice about how best to work with the communities.
- Arrange a meeting of the principal investigators and research coordinator with the district officers and community leaders. The latter may include village headmen, councilors, religious leaders, leaders of political parties, traditional birth attendants, teachers, etc.
- Inform the local community leaders (religious, political, and cultural) of the purpose of the dietary survey and its relevance to the community, and obtain their approval for the project.
- Arrange to meet with all the field staff already living and working in the communities in the area where the dietary survey will be undertaken to inform them about the study.
- Invite the field staff to the project planning meetings.
- Arrange to hold regular workshops with the district officers, their own field staff, and the community leaders to plan the activities in the communities associated with the dietary survey.

4.2 Incorporating Ethical Considerations

Four areas that must be addressed when considering the ethical aspects of a dietary study are outlined below. These are: i) ethical approval (Box 4.2); ii) informed consent (Box 4.3); iii) confidentiality (Box 4.4); and iv) feedback to the families and the community (Box 4.5), as well as to key people at district, regional, and national levels. A useful source of information about ethical considerations can be found in the guidelines of the Council for International Organizations of Medical Sciences (CIOMS 1991).

BOX 4.2

Obtaining Ethical Approval

- Identify the local institutional review boards for research involving humans and obtain application forms for requesting ethical approval when available.
- Complete the application form, which will probably require such information as an outline of the overall purpose of the project, the rationale for the project, the specific objectives, a brief description of the proposed methodology, and procedures for providing feedback to the participants.
- Enclose copies of the participants' information letter and consent forms. (These forms can be read and explained to the participants as part of the process of acquiring informed consent.)
- Obtain necessary signatures from the sponsoring institution.
- Submit the original proposal and required number of copies to the appropriate human ethics committee for approval.

Ethical approval from the appropriate human ethics committee of the country must be obtained by the principal investigator before work begins. Because it may take some time for approval to be granted, the proposal must be submitted early to avoid any delays. In countries that do not have a human ethics committee, approval must be sought from the advisory or technical committee of the appropriate ministry. To safeguard the confidentiality of information collected, certain rules must be followed. Informed consent is essential for all surveys: participants and principal caregivers must understand all the procedures so that they can give informed consent. Human ethics committees generally have examples of the information that must be included in the information and consent forms. Some way of providing feedback to the participants, families, and the community must be established and implemented. Finally, a report of the completed project must be submitted to the appropriate ministry or institution (World Vision Canada 1996).

BOX 4.3

Obtaining Informed Consent

- Compile an information letter. The letter must contain information given in a way that the participants will understand about the nature and purpose of the research, the procedure and how long it will take, and any risk or discomfort involved. It must also contain information on who can access personal information and under what conditions access will occur, and how the eventual disposal of the data will be handled. The name and contact details of the investigators involved, along with an explicit offer to answer questions or provide further information, should also be included.
- Give each participant a copy of the information letter and explain the contents of the letter.
- Compile a consent form. This must make it clear that a participant understands the nature of the proposal and has had all his/her questions satisfactorily answered, including that the participant: is aware of what will become of the data at the end of the study, is aware of risks, is aware that withdrawal from the project can occur at any time without penalty, is aware that the data may be published, and is aware that his/her anonymity will be preserved.
- Obtain verbal and signed informed consent from each participant.

BOX 4.4

Maintaining Confidentiality

- Identify respondents by code numbers.
- Do not permit any unauthorized person(s), including family members, to see the completed questionnaires or the 24-hour recall forms.
- Store all forms securely.
- Ensure that persons conducting the interviews do not discuss the respondents' completed recalls with anyone except the field supervisor.

BOX 4.5

Planning Feedback to the Participants, Families, and Community

- Decide when planning the survey how feedback will be provided to the participants, families, and community.
- For example, organize a workshop in the community to present some of the study findings along with some comments on what they could mean for the participants, community, etc.

BOX 4.6

Assembling and Calibrating a Set of Local Utensils, and Compiling Photographs of Portion Sizes

1. Assemble a set of local utensils, such as glasses, bowls, and spoons. Calibrate the glasses by, for example, filling water to 1 cup (227mL) by using a standard measuring cup or a graduated measuring cylinder and scratching the level onto the surface of the glass with a metal scriber.
   - Repeat and calibrate for 1½ cups (340mL) and 2 cups (454mL).
   - Calibrate a plastic bowl for 1 cup, 1½ cups, and 2 cups, repeating as above.
   - Calibrate a variety of different-size spoons by using standard measuring spoons: e.g., one heaped tablespoon (20mL), one level tablespoon (15mL), one heaped teaspoon (10mL), half tablespoon (7.5mL), and one level teaspoon (5mL). Thread the handles of the spoons through a metal ring to prevent loss.
2. Compile a set of graduated photographs that depict portion sizes of key foods commonly consumed. Consider the following factors in relation to the format of the photographs: size of the image, number and range of portion sizes depicted, and the interval between portion sizes. Consult guidelines in of Nelson and Haraldsdöttir (1998) as a resource. Calibrate the average portions sizes depicted for each food.

4.3 Assembling and Calibrating Equipment

One of the major sources of error in dietary methods based on recall is the estimation of the amount eaten. Graduated food models have been developed to assist in quantifying food portions. The use of such models may prevent a direct response, whereby respondents tend to specify that they have consumed the average portion size represented by a standard food model presented to them. The graduated food models may be a collection of papier-mâché, wooden, modeling clay or play dough, foam rubber, or hardboard shapes of various volumes or surface areas, often accompanied by thickness indicators made up of wooden or hardboard squares. Increasingly, a series of photographs that portray the range of portion sizes consumed by the subjects of the study have been used to quantify portion sizes. The photos are often bound together in a photographic atlas. Practical guidelines on how to develop a photographic atlas are given in Nelson and Haraldsdöttir (1998).

A simpler approach, advocated when the diversity of the diet is not large, is to use salted replicas of actual locally prepared or cooked foods, instead of graduated food models. Portion sizes of the salted replicas said to be consumed can then be weighed directly on dietary scales. Actual foods such as fruits (e.g., oranges or mangoes), and vegetables (e.g., sweet potatoes,

avocados, and tomatoes) can also be used instead of food models (Ferguson et al. 1995). Examples of ways to estimate portion sizes for different types of foods are given in Section 5.4.

A graduated plastic cylinder (500mL graduated in 5mL increments), a graduated plastic jug (1000mL graduated in 50mL increments), and a set of calibrated plastic beakers (1000, 500, 250, 50mL) are also useful for estimating portion sizes of liquids or flowing solids (e.g., rice). These measurement aids can also be used to measure the volume by water displacement of clay or play dough which has been molded into the shape and size of irregularly shaped food items (Section 5.4).

A selection of local utensils such as glasses, mugs, cups, bowls, plates, and spoons should be purchased for estimating the amount of foods or beverages actually consumed, although it is always preferable to ask the respondents to supply their own utensils for the recall interviews, where possible (Section 5.4). Before they are used, all of these local utensils must be calibrated by using a standard measuring cup and water or a graduated measuring cylinder, as described in Box 4.6 (see also Appendix C, "Measurement Abbreviations and Small Volume Measures"). Glasses can be used to describe the volume of beverages or to estimate the amount taken from a jug. Mugs and cups can be used to determine the volume of any liquid served—e.g., soup, tea or coffee—whereas bowls can be used to assess the volume of soups, desserts, porridges, relishes, canned fruit, stew, etc. Spoons are useful for estimating small amounts of many kinds of spreads such as butter, margarine, and jam, as well as sugar, salt, salad dressings, and cooking oil.

Picture charts depicting the foods most often eaten in the study area during the season of the survey are also used in the interactive 24-hour recall method as a check on the staple foods actually consumed, and for comparison with the recall to reduce memory lapses. These charts can be prepared from drawings or photographs; one section of an example picture chart is given in Figure 4.1.

FIGURE 4.1

Section of an Example Picture Chart Showing the General Layout

Food Type	√	Food Type	√	Food Type	√
Pumpkin 					
Cup of tea 					
Banana 					
Apple 					
Onion 					
Tomato 					
Turnip 					
Pear 					
Mushroom 					
Carrot 					

All the equipment required for the recall must be assembled, including a picture chart of local foods (Figure 4.1), dietary scales, recall forms, and samples of locally consumed foods as described in Box 4.7. Some of this equipment may be available locally but certain items (e.g., dietary scales, a graduated plastic measuring cylinder and jug, and/or a set of plastic beakers) may need to be purchased in advance. The use of dietary scales that permit adjustment for the tare weight of the plate or bowl as well as for previously weighed food is often preferred. (See Appendix E for a list of suppliers for the dietary scales listed in Box 4.7.)

BOX 4.7

Obtaining Equipment

- Commission a local artist to prepare a picture chart depicting the staple foods of the study area, or take photographs of the staple foods (See Figure 4.1).
- Purchase large self-seal plastic bags for storing picture charts flat.
- Purchase clipboards, pens, pencils, pencil sharpeners, and erasers.
- Purchase dietary scales (accurate to within 1g and preferably with a tare feature) that are portable and easy to read and use. Examples are Hanson digital kitchen scales (supplied by Arden Forest, Warwickshire, UK) and Soehnle electronic digital scales (supplied by CMS Weighing Equipment, London, UK). In addition, purchase calibration weights and spare batteries for scales.
- Prepare samples of prepared or cooked commonly consumed staple foods. Add salt to preserve the food so that it may last for several days. Store in plastic containers.
- Purchase examples of seasonal fruits and vegetables (e.g., mangoes, guavas, sweet potatoes, and avocados). Alternatively, prepare some graduated food models from foam rubber, papier-mâché, wood, or play dough.
- Purchase a ruler; a set of standard measuring cups and spoons; a plastic 500mL measuring cylinder (graduated in 5mL increments); a plastic 1000mL measuring jug (graduated in 50mL increments); and a set of five plastic beakers (1000, 500, 250, 100, and 50mL).
- Photocopy dietary recall forms and picture charts.

Finally, data on the gram-equivalent weights of the portion sizes of staple foods and mixed dishes commonly consumed in the study area—to be measured by using the graduated food models—must be compiled. Details on how to compile these data are given in Section 5.5.

4.4 Translating and Pretesting

One of the first jobs for the survey coordinator is to arrange for the translation of all the questionnaires (including the information and consent forms) and instructions for the interview procedure into the local language (Box 4.8). After translation, the materials must be pretested by the survey coordinator in an area near the study site, using respondents who are similar to those who will participate in the actual survey (Box 4.9). Some of the pretesting can be carried out on the field staff if they are comparable to the participants.

BOX 4.8

Translation of all Questionnaires and Interview Instructions

1. Arrange for one person to translate all questionnaires and the accompanying interviewing instructions into the local language.
2. Ask another translator to independently translate the questionnaires etc. back into the original language (e.g., English) without reference to the originals.
3. Compare the two versions and discuss any ambiguities.
4. If the second translation recreates the original version, the translation is taken to be accurate.

The objective of the pretest is to identify potential problems and to check that:

- the respondents recognize the staple foods in the picture charts;
- the respondents are willing and able to answer the questions in the way they are asked;
- no questions are especially difficult to answer;
- the questions address sensitive issues appropriately;
- the questions are well understood by the respondents;
- the respondents have the same understanding of the questions that the interviewers and researchers have;

- the questionnaire and recall form are designed with adequate space for responses;
- the interview will not interfere with the respondents' ability to perform their necessary daily tasks; and
- the interview does not take too long.

Note the initial interviews may take a long time, especially during the practice sessions. This should not be a cause for concern because the interviewers will get faster with practice.

BOX 4.9

Pretesting the Translated Questionnaire and Interview Instructions

1. Select a field site and 10 respondents who are similar to the study participants.
2. Administer the translated questionnaire to these 10 respondents.
3. Show the respondents a picture chart depicting the staple foods of the area and ask them to identify the pictures in the chart.
4. Explain to the respondents how to mark the chart to indicate the foods they ate on the previous day and the time of consumption.
5. Ask the respondents to practice marking the picture chart next to the foods they consumed the previous day.
6. Conduct an interactive 24-hour recall interview (see Chapter 5) on the respondents following the translated instructions for the interview protocol.
7. Amend the translated questionnaires, picture charts, and interview instructions, as necessary.
8. Prepare the revised materials for the training course.

4.5 Instructing the Field Supervisors

The field supervisors selected must have had some previous experience in well-conducted surveys and in interview training. They must also have good organizational skills, understand the importance of adhering to the survey instructions, and be capable of ensuring that the interviewers follow instructions correctly.

One of the first jobs for the field supervisors is to obtain copies of local maps of the area, where possible, from the census bureau or other governmental agency. These maps can then be used to identify the sampling units from which the participants for the 24-hour recall survey are chosen. Field supervisors must be trained by the survey coordinator on how to select the participants according to the chosen sampling design; details of non-probability and probability sampling schemes are discussed in Sections 2.1 and 2.3. Field supervisors also have several other tasks that should be performed daily, many of which involve monitoring the quality of the recall interviews by the interviewers; these are outlined in Box 4.10.

BOX 4.10

Instructing the Field Supervisors

Instruct the field supervisors to:

- Obtain copies of local maps to assist in identifying sampling units.
- Meet with the community leaders to plan the activities in the communities associated with the dietary survey.
- Supply the interviewers with equipment, materials, and questionnaires.
- Select the participants for the dietary survey by using the chosen sampling design.
- Assign the participants to the recall interviewers.
- Monitor about 5 percent of the interviews held by each of the interviewers to ensure consistency in interview methods with the participants.
- Review recalls as they are completed to ensure they are neat and legible, all the information is being properly collected and recorded, and no information is missing.
- Investigate any high levels of nonresponse for interviewers.
- Check that interviewers are not replacing participants who are difficult to contact with other people or fabricating data.
- Answer questions and resolve problems daily, and give feedback to the interviewing team on the progress of the dietary survey.
- Ensure that the community is satisfied with the survey procedures throughout the period when the recall interviewers are in their area.
- Keep the interviewing team on schedule.
- Collect all the 24-hour dietary recall forms and give them to the survey coordinator at headquarters.

4.6 Selecting the Recall Interviewers

Before hiring the recall team, a selection process must be undertaken to ensure that all the team members will be suitable. A background in nutrition is not essential for conducting 24-hour recalls, provided adequate training is given. The recall staff must be selected by the survey coordinator and the field supervisor who have had some previous experience in well-conducted surveys and in interview training.

All of the team members should be open, personable, mature, nonjudgmental, and sensitive to people, and be able to develop friendly social relationships with the communities under study (Box 4.11). They should also be able to live and function in a rural environment. Generally, female interviewers are preferred, because they tend to have the best knowledge of the local food preparation and processing methods. Respondents often have an expectation that it is more appropriate to speak to women about food.

Caution should be exercised in the selection of people who are in a position of leadership or authority in the community as they are sometimes perceived as threatening by respondents, and should therefore not be hired. An exception to this recommendation of avoiding leaders may, for example; be the selection of some community leaders who are respected by the community and who make excellent interviewers.

The number of interviewers required depends on the size of the study sample (n) and its geographic spread, the number of recalls required per respondent, the timeframe over which the dietary data are to be collected, a typical day for a man or woman in the survey community, and other logistical factors (e.g., availability and timing of transportation). Each recall interview takes about 30–40 minutes. When the time required to travel to the recall site is also included, a maximum of eight recalls per day can generally be completed by one interviewer, depending on the travel

time (World Vision Canada 1996). In countries where the women spend all day in the fields, e.g., in agricultural activities, interviewers may only have time to complete one or two interviews per day without being perceived as a nuisance by the community.

BOX 4.11

Selection of Interviewer Team Members

Select interviewer team members who have the following characteristics. They should:

- Be literate and numerate, preferably with at least a high school education;
- Be fluent in the local language;
- Have a thorough knowledge of the local region and its food culture;
- Preferably have some previous field experience;
- Be able to establish and maintain an easy rapport with strangers;
- Be able to empathize with the participants;
- Be mature and have the ability to handle difficult situations;
- Be able to live in a rural environment, if such is required; and
- Preferably be from the respondents' social stratum and/or religious caste.

4.7 Training the Interviewers

Adequate training for the interviewers is critical because the success of the dietary survey depends on the commitment and skill of the recall team. The interviewing techniques should always be consistent, both among the interviewers and over time. When several interviewers are employed for the same survey, ensure that each interviewer conducts only one recall per respondent to minimize any interviewer bias on the recalled intakes. This means that when 2 or more non-consecutive days of food intake are collected, the repeated 24-hour recall interview must be conducted by a different interviewer.

The training sessions for the interviewers should be participatory and include discussions, small group exercises, and role-playing. They should focus on developing both the interpersonal and technical skills of the interviewers. All the interviewers should be informed of their ethical responsibilities during the conduct of the survey (see Section 4.2). Safety and health issues should also be addressed, especially if the survey is being conducted in urban slums or in rural areas. Document handouts should be prepared for the recall team and, if possible, audiovisual aids (e.g., overhead projection) should also be used for the training. Videos or DVDs designed to emphasize correct and incorrect interviewing procedures can also be used.

A training course of *at least* 7 days is necessary to train the field staff to carry out the recall interviews. The full duration of training required to achieve proficiency will vary depending on the skill level of the trainees. An example of a training schedule is shown in Table 4.1, and also described below.

On Day 1, the purpose of the interactive 24-hour recall is explained as well as the details about the salaries and working arrangements (Box 4.12). On Day 2, details on how to conduct the 24-hour recall interviews and any associated questionnaires are discussed, with some practice interviews on recording and describing the foods and drinks consumed (pass 1 and pass 2) using hypothetical menus etc. (Box 4.13). Days 3 and 4 focus on: methods to estimate portion sizes; how to complete the 3 passes of the interview protocol correctly; and practicing a 24-h recall interview on a partner (Box 4.14). On Day 5, instructions are given on how to complete the recipe forms and how to handle difficult scenarios, and on Day 6 a field exercise is conducted (Box 4.15).

TABLE 4.1

Example of a Schedule of Activities for a 7-day Training Workshop on the 24-h Recall Method

Day 1	Morning	Introduction to study and 24-hour recall method
	Afternoon	Interviewing techniques
Day 2	Morning	Description of multiple-pass 24-hour recall with a focus on Pass 1 and Pass 2
	Afternoon	In-class activity: Practice generating a list of foods (Pass 1) and description of foods (Pass 2) with hypothetical menus.
	Homework	Interview a friend to generate a list of foods (Pass 1) and food descriptions (Pass 2) consumed in previous 24 hours.
Day 3	Morning	Discuss homework and role play. Show video designed to emphasize correct and incorrect interviewing procedures.
	Afternoon	Estimate portion sizes consumed (Pass 3) using: salted replicas, measuring cups and spoons, graduated photographs, calibrating home utensils, play dough, and water displacement to estimate volume. Practice measuring specific gravities and weighing with a dietary scale using a 'tare'.
Day 4	Morning	Practice completing the '4 Passes' of the recall interview using examples given in class. Record all the details on the recall form.
	Afternoon	Carry out a complete multiple-pass 24-hour recall interview on a partner in class, and record the data on the 24-hour recall form.
Day 5	Morning	Practice completing a recipe form. Generate additional recipes in class, and complete the details on the recipe forms.
	Afternoon	Discuss how to handle difficult scenarios.
Day 6	All day	Conduct a field exercise. Practice calibrating household utensils in the home. Check the recall forms and return them to the data-processing headquarters.
Day 7	Morning	Review any difficulties encountered in the field.
	Afternoon	Select the best interviewers. Give out training certificates.

During the field exercise, the field supervisor should evaluate the performance of each interviewer based on these 12 criteria: general manner of the interviewer, introduction by the interviewer, use by the interviewer of nondirected questioning, privacy of the interview (where possible), pacing, manner of questioning, objectivity, probing, use of models, documentation, memory aids, and review of the recall (Dennis et al. 2003).

On Day 7, any difficulties encountered in the field should be discussed (Box 4.15). Always try to train more interviewers than needed. At the end of the course, select the best interviewers, ensuring that you have some as replacements, and provide everyone (including those not selected) with a training certificate.

Note that the training sessions must be repeated at intervals during the dietary survey to minimize any inconsistencies in the methodology arising from fatigue of the recall interviewers. In addition, throughout the survey, some 24-hour recalls conducted by each interviewer should be randomly selected for tape recording. These audiotapes should be evaluated by the field supervisor, as noted earlier, and the interviewers should be provided with immediate feedback and retrained promptly, if necessary.

BOX 4.12

Implementing Interviewer Training: Day 1

1. Introduce all recall interviewers.
2. Provide an outline of the study objective and methods.
3. Motivate recall interviewers by explaining the importance of the data and describing how it will be used.
4. Provide instructions on how to ensure that each respondent is randomly selected.
5. Give details of the working hours, pay, survey schedule, transportation, and living arrangements.

BOX 4.13

Implementing Interviewer Training: Day 2

1. Discuss the interviewing technique and explain:
   - how to gain the confidence of the respondent with a warm greeting, a diplomatic and professional demeanor, appropriate dress, and proper identification; and by having all the equipment prepared in advance;
   - how to focus on and empathize with the respondent;
   - how to be a skilled listener as well as questioner;
   - how to convey interest and understanding by expressions, gestures, or brief comments; and
   - how to have a nonjudgmental attitude (to avoid showing reactions to any answers by gestures, words or expressions).
2. Explain and discuss each step in the recall procedure.
3. Carry out a demonstration recall interview, emphasizing the following technical skills of:
   - establishing a pattern of questioning,
   - stimulating memory by retracing the activities of the respondent on the preceding day,
   - fixing the time frame as the day immediately before the recall interview,
   - focusing on the detail required in terms of describing the food itself and exactly how much was eaten,
   - probing without bias by using standard prompts to provide more detail,
   - ensuring completeness but never cross-examining,
   - avoiding quick assumptions and conclusions, e.g., by using silence and waiting, and
   - avoiding providing information for the respondent.
4. Demonstrate the probes used to elicit detailed descriptions of food and beverage items.
5. Role play Pass 1 and Pass 2 of recall interviews, and arrange for trainees to interview each other.
6. Practice generating and recording the information collected from Pass 1 and Pass 2 on the interactive 24-hour recall forms.
7. Assign homework. Interview a friend to generate a list of foods (Pass 1) and their description (Pass 2) consumed in the previous 24 hours. Record information on the 24-hour recall form.

BOX 4.14

Implementing Interviewer Training: Days 3 and 4

1. Discuss the homework. Show the video designed to emphasize correct and incorrect interviewing procedures; practice interviewing techniques through role playing.
2. Demonstrate different methods for estimating portion sizes, including the use of salted food replicas, actual foods, modeling clay or play dough, graduated food models or photographs, and calibrated household utensils. See Sections 5.4 and 5.5 for more specific details.
3. Practice completing the '4 Passes' of the recall using the examples given in class and record the details on the recall form.
4. Carry out a multiple-pass 24-hour recall interview on a partner and record the data on the recall form.
5. Tape the practice recall interviews, if possible, and evaluate them using the following criteria: manner of the interviewer, introduction, use of nondirected questioning, pacing, manner of questioning, objectivity, probing, use of tools to estimate portion sizes, documentation, memory aids, and review of the recall.

BOX 4.15

Implementing Interviewer Training: Days 5, 6, and 7

1. Practice completing a recipe form for a mixed dish. Generate additional recipes in class, and complete details on the recipe forms.
2. Discuss examples of hypothetical problems that interviewers might encounter, such as:
   - a home that is very dirty and unsanitary, but the respondent insists on offering you some of the family meal,
   - too many neighbors or children wanting to see what is going on,
   - the respondent insists on asking the interviewer how her neighbor answered,
   - another family member responds or corrects the respondent,
   - the respondent gives socially desirable answers,
   - a mother giving a 24-hour recall on her child insists on asking whether what her child ate is satisfactory,
   - children needing more than minimal attention,
   - a father who does not want a 24-hour recall performed on his child because of his religious beliefs,
   - visitors arrive during the interview,
   - a mother has been abused by her husband during the night and is reluctant to carry out the 24-hour recall on the appointed day,
   - a mother is ill on the recording day,
   - the recording day falls on a festival and the respondent is inebriated,
   - unexpected news arrives during the recall,
   - respondent has difficulty adhering to the format of the recall interview, and the household is empty.
3. Organize practice recall interviews in the field. Each trainee should complete at least five practice interviews in the field. Observe all the interviewers' practice sessions and provide the interviewer with feedback, including on how they handled situations such as those listed in point 2 above. ►

BOX 4.15 (cont'd)

4. Practice how to use the dietary scales to estimate the quantities of the salted replicas or actual foods consumed.
5. Practice calibrating household utensils in the home.
6. Explain how to check the recall forms and return them to the data-processing headquarters.
7. Review any difficulties encountered in the field.
8. Select the best interviewers.

4.8 Pilot Testing the Interactive 24-hour Recall

The final stage in the training is carrying out a pilot study (Box 4.16) before starting the actual survey work. The pilot study should be organized by the survey coordinator and carried out by the field supervisor with the assistance of the recall interviewers. The purpose of the pilot study is to identify any further problems that may be encountered by the interviewers before commencing the survey, and to determine how they should be handled. The pilot study is also useful for identifying discrepancies in the way interviewers adhere to the interview protocol, and record or interpret information. To enhance standardization, each interviewer should have one 24-hour recall interview taped during the pilot study. These audiotapes should be evaluated by the field supervisor, following the criteria noted earlier, and the interviewers provided with immediate feedback, and retrained promptly, if necessary. If major problems arise in the pilot study, the trial run should be repeated before the start of the study itself.

BOX 4.16

Pilot Test the Interactive 24-hour Recall

1. Select an area and a group comparable to that of the actual study, and identify two volunteers per interviewer.
2. Assign an interviewer to each pair of consenting volunteers.
3. On the day before the intake is to be assessed (i.e., two days prior to the recall), visit the home of each volunteer to explain again the purpose of the 24-hour recall and to distribute the bowl, plate, and picture chart.
4. Explain again the use of the bowl, plate, and picture calendar to each volunteer.
5. Set up an appointment with each volunteer to visit their home on the next day.
6. Conduct a 24-hour recall on the next day on each volunteer, following the procedures outlined in Sections 5.1-5.4. Arrange for one 24-hour recall interview by each interviewer to be taped.
7. Check the recall forms to ensure that all the information required has been recorded correctly, including the portion size consumed and recipes for mixed dishes, if required. Check to ensure that the letters "O" and "D"; the numbers 1 and 7 (using the crossed form of the numeral seven, as noted here); and the capital letter "L" are clearly distinguished.
8. Check the recall forms to ensure that the writing is neat and all numbers and letters are legible.
9. Meet with the field supervisor to check that the taped interview protocol was satisfactory, that the information is being properly collected and recorded, and no information is missing.

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Chapter 5 | Conducting the Interactive 24-hour Recall

What will you learn from this chapter?

- How to prepare the respondents for the recall;
- How to conduct the interactive 24-hour recall;
- How to estimate the amount consumed;
- How to convert portion sizes to weight equivalents; and
- How to review the 24-hour recall data collected.

The success of the 24-hour recall depends on the subject's memory, how well the respondent estimates the portion sizes consumed, the respondent's degree of motivation, and the skill and persistence of the interviewer. Research indicates that a four-stage, multiple-pass interviewing technique yields the most accurate data. This method is described in this chapter. Some modifications were made to this protocol to adapt it for use among rural populations in low-income countries where there may be limited ability to read or write. Details of these modifications include: training the respondents in small groups on portion size estimation before the actual recall; supplying the respondents with picture charts on the day before the recall to use as a checklist on the day the food is actually consumed and to compare with the recall to reduce memory lapses; providing the respondents with bowls and plates for use on the recall days to help them visualize the amount of food consumed; and weighing portion sizes of salted replicas of actual foods said to be consumed by the subjects (Ferguson et al. 1995).

Recall interviews can be conducted on adults and children over 8 years of age. Children between 4 and 8 years should be interviewed along with their primary caretaker, usually the mother, to ensure that foods eaten away from home are reported. For this younger age group, questions should always be directed toward the child (Sobo et al. 2000). In some cases, it may be necessary to interview several people if children are at school or play in the homes of friends or relatives to ensure foods eaten away from home are also reported.

Very often the interviewing proceeds as a consensus recall, with family members helping the respondent to remember the types and amounts of foods and beverages consumed. This consensus approach has been shown to increase the accuracy of dietary recalls of children living in the United States (Eck et al. 1989). Although more time-consuming, it is generally preferable to conduct the interviews in the subjects' homes when the person is not too busy, because the familiar environment encourages participation, improves the recall of foods consumed, and facilitates the recording of brand names and calibration of local household utensils by the interviewer (See Sections 5.4 and 5.5).

To minimize any interviewer bias on the recalled intakes for repeated recalls (i.e., more than one recall per person), each interviewer should conduct only one recall per person, as noted in Section 4.7. To ensure consistency among the recall interviewers, the field supervisor must maintain a close liaison with them and with the community during the entire survey period. To achieve good relations with the recall staff and the community members, the field supervisor should set up a mechanism whereby responses to any of their day-to-day queries and practical problems can be dealt with quickly and on a regular basis.

5.1 Preparing the Respondents for the Recalls

Respondents are more likely to feel at ease if the interviewer observes local forms of greeting and personal address, and is dressed in a similar fashion to the respondents (Cameron and van Staveren 1988). The respondents, usually women, can be informed about the recall and prepared for the interview in small groups, preferably by requesting that they meet in the local health center, school, or church on the day before the first recording day. These information sessions should be conducted by the field supervisor. Studies have shown that training respondents in techniques of estimating portion sizes improves the accuracy of their estimates. Therefore, when preparing the respondents for the recall, some training of portion size estimation, as well as instructions on how to complete the picture charts, should be given. Details of the steps to be covered during these training sessions are given in Box 5.1. This specialized training should be held two days before the recall interview day.

BOX 5.1

Preparing the Respondents for the Recall

1. Introduce yourself to the respondent; explain the object of the training session.
2. Explain the purpose of the 24-hour recall to the respondent (e.g., you are interested in finding out about everything eaten and drunk from midnight of one day until midnight the following day).
3. Explain what the respondents will be asked to do (e.g., complete a picture chart, use a separate bowl and plate for eating their food, and take part in a recall interview).
4. Give the respondent a picture chart, self-seal plastic bag, pencil, bowl, and plate.
5. Explain the use of the picture charts to each respondent. First show them the charts, and then ask the respondent to identify each of the foods in the pictures to make sure they are identified correctly.
6. Explain to the respondents why you will ask them to mark on the chart each food (including all ingredients in mixed dishes) eaten the next day. Then ask the respondent to practice this.
7. Give the respondent a new picture chart to use on the next day. Then instruct each respondent to mark the chart with a tick or check each time the food is eaten on the next day.
8. Ask the respondents to use the separate bowl and plate for eating their food on the next day to help them visualize more easily what and how much of each food they ate on that day.
9. Ask the respondent to remember to bring the bowl and plate to the recall interview held on the day after the recording day.
10. Explain the importance of following a usual eating pattern on the intake recording day: this will decrease the likelihood of altering eating behavior on the recall day.
11. Explain how the amount of each food they have eaten will be estimated. Show the respondents the dietary scales, salted replicates of actual foods, and the graduated food models that will be used.
12. Ask some of the respondents to do a test case; e.g., ask them to take the amount of porridge they ate at their morning meal, place it on one of the plates, and watch it being weighed.

5.2 Recalling the Foods and Drinks Consumed

For the first pass of the recall interview, a list of all the foods and drinks (including drinking water) consumed during the preceding 24-hour period is obtained. The interviewer should start by reestablishing a rapport with the respondent and follow this with a brief introduction about the purpose of the study, during which the name and identification of the interviewer should be given to the respondent (Box 5.2).

Respondents should be reminded that questions will cover all the food and beverages, including snacks, consumed during the preceding day, with emphasis on the pattern of eating. Stress to respondents that all responses will be confidential, and emphasize the importance of providing the correct information.

Neutral questions should be used throughout the interview, such as "When did you get up in the morning?" and "Did you eat or drink anything then?" Avoid asking questions about specific meals (e.g., breakfast, lunch, or supper) or about snacks. Respondents should be given sufficient time to consider their responses and to clarify answers where necessary. During the interview, the interviewer should keep an open mind and avoid showing signs of surprise, approval, or disapproval of the respondent's eating pattern. The interview must always be conducted with an open and pleasant manner with the aim of being friendly, diplomatic, empathetic, and determined, as appropriate (Gibson 1993).

BOX 5.2

Recalling the Foods and Drinks Consumed

1. On the day of the 24-hour recall interview, start the interview with the following: "I would like you to tell me what you had to eat or drink after you woke up yesterday morning. Did you eat that food at home? What did you have next and at what time?"
2. Proceed through the day, repeating these questions as necessary, and record each food or drink (including drinking water) consumed in column 3 of the 24-hour recall form (Table 5.1). Remember to probe for any snacks and drinks consumed between meals. Follow the example given in Table 5.1 to ensure you are recording the information correctly.
3. When you reach the end of the day, check the respondent's responses against the picture chart (see Figure 4.1 for an example). If a food has been mentioned but it is not recorded on the chart, probe for information: Did the respondent forget to write it down? Was it eaten away from home? Was there a mistake made in recording it on the picture chart? If a food is on the chart but not mentioned, probe to see whether the food was forgotten in the interview.

5.3 Describing the Foods and Drinks Consumed

In the second pass of the recall interview, the interviewer should go over, in chronological order, each of the responses made by the respondent in stage 1, probing for more specific descriptions of all the foods and drinks consumed, including cooking methods and (where possible or relevant) brand names (Box 5.3). Examples of probes that can be used to obtain detailed descriptions of specified foods are given in Table 5.2. At this stage, the interviewer should also ask if the respondent has remembered any additional items that were consumed but which were forgotten in the first pass.

BOX 5.3

Describing the Foods and Drinks Consumed

1. For each food and drink item in the recall, record the time and place of eating in the appropriate columns on the form.
2. Use the appropriate probes listed in Table 5.2 to obtain further descriptive information.
3. Record a detailed description of each food and drink item on the form. When commercial products are reported, information from the product label should also be recorded on the form in the appropriate columns.
4. For homemade mixed dishes only, record on the recipe form (Table 5.4) and on the 24-hour recall form (i.e., Table 5.1), where possible, the following additional details:
   - name of mixed dish (local and general);
   - descriptive list of all ingredients in descending order of quantity;
   - amount of each raw ingredient (excluding water) (see Box 5.10);
   - method of preparation and cooking;
   - total amount of cooked dish (in grams or mLs); and
   - amount of the mixed dish consumed by the respondent in the same units (record under "amount eaten").
5. Record the name, ID number, age, and sex of the respondent on the form.

Note: Herbs and spices should also be included because they are often an important source of micronutrients.

TABLE 5.1

Form for Recording the Interactive 24-hour Recall, with a Sample Recall for a 4-year-old Female

Interviewer: *Doreen* Scale no.: *12* Interview date: *6th June 2005* Day food eaten: *Thursday*	Location: *Nembya* Subject ID: *00455* Subject name: *Sandikonda*	Sex: *F* Age: *48 months* Weight: *14kg*

Time	Place eaten	Food or drink	Description, and cooking method	Amount eaten	Weight equivalent (g)	Food Code
7:30	Home	Porridge	Prepared with mgayewa- unrefined maize flour	267mL	315	
		Salt	Not iodized	1/2tsp	4	
9:15	Home	Sweet potatoes	Boiled in skins and skins removed	350g	350	
11:20	Home	Ground-nuts	Raw	60g	60	
12:15	Home	Corn-on-cob	Boiled	5cm	100	
14:00	Home	Nsima	Prepared with mgayewa- unrefined maize flour	335g		
14:00	Home	Fish relish	Boiled (recipe completed)	37g		
			Ingredients: dry usipa			
			Salt, not iodized			
4:05	Home	Sugar cane	Raw	14cm	76	
6:20	Home	Nsima	Prepared with ufa - processed maize flour	305g	305	

Probe for alcohol: Yes ❑ No ☑	Probe for sickness: Yes ❑ No ☑ If yes, did sickness affect appetite? Yes ❑ No ❑ If yes, how? Increase ❑ Decrease ❑
Was food intake unusual? Yes ❑ No ☑ If yes, how was it unusual?	Probe for tablets: Yes ☑ No ❑ Iron ❑ Vitamins ☑ Other supplements ❑ Anti-malaria ❑
Was it a feast day? Yes ❑ No ☑ Was it a market day? Yes ☑ No ❑ Was it a fasting day? Yes ❑ No ☑	

TABLE 5.2

EXAMPLES OF PROBES TO OBTAIN DETAILED DESCRIPTIONS OF SPECIFIED FOODS

Food Type	Required Detailed Information
Meat	Kind of meat; description of cut, raw or cooked weight, method of cooking, lean or lean plus fat, bone in or not (waste factor)
Fish and seafood	Kind of fish or seafood; raw or cooked weight; method of cooking; amount of bones, skin, or shell (waste factor)
Poultry	Kind of poultry; parts or pieces eaten (e.g., breast, thigh), raw or cooked weight, method of cooking, white or dark meat, meat plus skin or meat only, bones (waste factor)
Fats	Kind of fat, brand name (if possible)
Milk products	Kind of dairy product, brand name (if commercial product), percentage fat (as butter fat or milk fat), liquid vs. powdered milk
Cheese	Kind of cheese (whole milk hard cheese, fresh cheese, Swiss, cream, etc.), percentage fat (if known), brand name (if commercial product)
Bread, rolls	Type of grain (rye, whole wheat, etc.), homemade or bought, size: standard or unusual, toasted or not, topping and condiments, brand name (if commercial product)
Baked goods	Type of product, whether iced or not, homemade or commercial, type of filling
Cereal, pasta, or rice	Type of grain, whole or refined, milled or polished (for rice), brand name, raw or cooked weight, enriched or not, cereal plus milk (if dry quantity unknown), method of cooking
Vegetables	Fresh, frozen, or canned; peeled or unpeeled; method of cooking; topping (butter, etc.)
Fruits	Fresh, stewed, frozen, or canned; peeled or unpeeled; type of liquid (heavy, light): sweetened or unsweetened; waste factor (e.g., peel, stone)
Beverages, soup	Fresh or frozen; canned or bottled; fruit juice: sweetened or unsweetened; added vitamins or minerals (e.g., vitamin C); coffee: brewed, instant, decaffeinated, regular; soups: homemade or canned, dilutant (milk or water), proportion of dilutant : concentrate (e.g., 1:1), recipe; brand name (if commercial product)
Street foods from vendors	Food (e.g., French fries and chips), brand name (if commercial product), condiments added, method of cooking, vendor's name/location
Mixed dishes	Product name, homemade or commercial, recipe ingredients, cooking method
Herbs, spices	Name; fresh or dried

5.4 Estimating Portion Sizes

The third pass of the 24-hour recall interview—estimating portion sizes—is the most challenging part of the recall interview but also one of the most critical for ensuring high-quality results. Some examples of ways to estimate portion sizes consumed are summarized in Box 5.4; methods for specific food items are listed in Table 5.3. Tools that can be used in estimating portion sizes include: salted replicas or actual foods;

- local household utensils (e.g., glasses, cups, bowls, and spoons) calibrated for use;
- modeling clay or play dough molded into the correct size and shape of the food;
- tape measure to estimate linear dimensions (length, width etc.);
- graduated food models made from play dough, foam rubber, papier maché, etc.; and
- graduated portion-size photographs of foods.

Monetary value of purchased or street foods can also help in estimating portion sizes.

Actual foods or salted replicas are preferred for estimating portion sizes because the amount eaten is easier for the respondent to visualize. To prepare the salted replicas, first arrange for women in the study area to cook a selection of commonly consumed dietary staples of the area using local ingredients and cooking methods. Then add a few grams of salt to each prepared food item and transfer the food to a plastic container for storage. The addition of salt preserves these cooked foods for periods ranging from several days to several weeks. Some staple foods (e.g., rice) are best cooked fresh every morning before starting the recalls. Use of salted replicas or actual foods is especially recommended for foods that are major sources of the nutrients of interest (i.e., iron and zinc). In some developing countries, dietary staples can provide up to 80 percent of the daily supply of energy and nutrients in the diet (except for vitamin A and calcium).

TABLE 5.3

Examples of Methods that Can Be Used to Estimate the Portion Sizes of Selected Food Types Consumed

Food Type	Ways to Estimate Portion Size
Staple; boiled flour or roots, rice	Weigh equivalent amount of actual food *OR* salted replica of cooked food *OR* record weight or volume of clay replica *OR* use household measures (i.e., scoop, medium portion)
Roots and tubers or fresh maize, boiled or roasted*	Weigh equivalent amount of actual food either from household's store or from various sized roots and tubers that you carry (preferred method) *OR* measure length and circumference with a tape measure *OR* record as small, medium, or large
Porridges and soups	Measure equivalent volume in subject's own cup or bowl and weigh *OR* use household measures (e.g., cup or bowl)
Stews	Weigh equivalent amount of actual food *OR* salted food replica *OR* use household measures (i.e., cup, scoop, or medium portion)
Purchased foods (e.g., cakes and biscuits)	Record monetary value *OR* use household measures (e.g., cup, bowl, or piece)
Bread	Weigh actual food from supply that you carry (preferred method) *OR* measure length and thickness with a tape measure *OR* record as thin, medium, or thick slice
Fruits*	Weigh equivalent amount of actual fruit from household's store or from various sized fruits that you carry (preferred method) *OR* measure length and circumference with a tape measure *OR* record as small, medium, or large
Ground nuts*	Weigh equivalent amount of actual nuts from household's store or from those that you carry (preferred method) *OR* record monetary value *OR* record volume by using household measures (e.g., cup or bowl)
Meat or fish	Measure volume of clay model of equivalent size and shape *OR* measure length, width, and thickness with a tape measure *OR* buy and weigh a piece of equivalent size and shape (e.g., a chicken drumstick)

* Note that weights may need to be adjusted for inedible amounts (e.g., cobs, peel).

BOX 5.4

Estimating the Size of the Individual Portions by Direct Weighing and Using Clay or Play Dough Models

Start at the beginning of the itemized list of food and drinks already recorded during stage 1 on the 24-hour recall form (Table 5.1).

1. Ask the respondent to first visualize the amount of the first food consumed.
2. Adjust the dietary scale to zero, and then place the respondent's empty plate or bowl on the scale and adjust the scale to zero again.
3. Ask the respondent to measure, with the utensil usually used, the amount eaten (preferably using an actual food or salted replica) onto the weighed plate or weighed bowl.
4. Get confirmation of the amount eaten by asking: Did you eat all of this? Remove any leftovers, if necessary, and then weigh and record the amount consumed in column 4 of the recall form.
5. Use pieces or whole items of local cakes, buns, bread, sweet potato, fruits, etc. purchased from local markets. Weigh an equivalent amount consumed and record the amount in column 4 of the recall form.
6. Use clay or play dough molded into the correct shape and size for assessing the volume of items such as meat, fish, cheese, pieces of fruit, pumpkin, cassava, roots and tubers. Determine the volume of the clay or play dough model by the water displacement method (described in Box 5.6), and enter the volume in column 5. Later you can convert the volume to a weight equivalent (see Box 5.6) and enter the weight in column 6 of the recall form.
7. Record the number of dietary scales used for the recall interview at the top of the form.

Note: Follow the instructions given in Section 5.5 to convert portion sizes into weight equivalents.

Local household utensils consisting of a set of glasses, cups, and bowls, calibrated as described in Box 4.6, can also be used for estimating portion sizes of items such as beverages, soups, thin porridges, breakfast cereals, and stews (Box 5.5). For spreads, sugar, salt, oil, relishes, etc., different-sized local calibrated spoons can be used. The full range of sizes of the appropriate utensil should be presented to the respondent, who should then be asked to select the size of the utensil that most closely resembles the size used or the portion size consumed. Alternatively, respondents can be requested to bring their own household utensils to the recall interview, which must then be calibrated by the interviewer to determine their volume using a measuring cylinder or measuring jug.

Modeling clay or play dough can also be used to estimate the portion size eaten of items such as pieces of meat, fish, cheese, vegetables (e.g., pumpkin), fruits, cassava and other roots and tubers (Box 5.4).

Tape measures (nonflexible fiberglass) are useful for recording linear dimensions, for example; the length and circumference of sugarcane, maize cobs, bananas, and potatoes.

Graduated food models for some fruits and vegetables may help describe the size of mounds of foods, such as potatoes or rice. These graduated food models can be made out of play dough, clay, papier mâché, foam rubber or even dried beans. The full range of sizes of the appropriate model should be shown to the respondent, and the corresponding model number recorded in Table 5.1 under the column "amount eaten."

Graduated photographs that depict the range of portion sizes commonly consumed by the subjects of the survey can also be used. Again, the full range of sizes should be shown to the respondent, and the corresponding model number recorded in Table 5.1 under "amount eaten."

Monetary value can be used to estimate the amount consumed of take-away foods, some commercial foods, and street foods (Box 5.5). For take-away and street foods, the name (local and general) and description of the food, price, and shop or vendor from which it was purchased (if known) should be noted, and the portion size consumed should be estimated. For commercial foods, the price, brand name, name of food, and weight or size on the label should be recorded together with the number of items consumed. Alternatively, the fraction of the whole food consumed should be recorded.

BOX 5.5

Estimating the Size of the Individual Portions Using Food Models, Household Utensils, Counts, and Other Methods

1. If an actual food or salted replica is not available, display an appropriate range of graduated food models and ask the respondent to show you which model most closely represents the portion size consumed. Record the model number used in column 5 of Table 5.1.
2. For soups, thin porridges, and beverages, record quantities as volumes, preferably using the respondent's own bowl or cup calibrated with a graduated measuring cylinder of water. Alternatively, use the graduated glasses, cups, or bowls—calibrated in milliliters or fluid ounces. Record the volume in column 4 of the recall form.
3. For jam, sugar, salt, oil, spreads (e.g., butter or margarine), sauces, pickles, salad dressings and oil use the respondent's own spoon and calibrate it with a graduated measuring cylinder of water. Alternatively, use a variety of different-sized local calibrated spoons. Record the volume in column 4 of the recall form.
4. Use counts for eggs and slices of bread (noting thickness) and record in column 4 of the recall form.
5. For commercial foods in individual portions, record the weight or size on the label and number of items eaten; for those in multiple portions (e.g., meat pie), record the fraction of the whole eaten in column 4 of the recall form.
6. For street foods, record the vendor's name (if known) and the monetary value in column 4 of the recall form.

When the amount of each food and beverage listed on the recall form has been estimated following the guidelines discussed above, the final step in the third pass is to record details of the recipes of homemade mixed dishes consumed by the respondent. The recipe details required are specified in Box 5.3 and must be recorded on a separate recipe form (Table 5.4). Note that the total amount of each mixed dish after cooking and the amount of each cooked mixed dish consumed by the respondent must also be recorded on the recipe form. An estimate of the amount of the total cooked mixed dish (in mL) can be obtained by requesting the householder to indicate the level of the dish after cooking on the side of the cooking pot. The latter is then filled up to the level indicated with rice or water, and the volume of the rice or water measured with a calibrated jug. The amount of the cooked mixed dish eaten by the respondent (in mL) can be estimated in the same way, so that a proportion of the recipe consumed can be determined. Alternatively, the weight of the total cooked mixed dish and the weight of the portion consumed by the respondent can be weighed. For example, if the weight or volume of water shown for the total recipe was 800g (mL), and its weight or volume shown for the portion consumed was 150g (mL), then the proportion of each recipe ingredient consumed by the respondent was 150/800 = 0.19.

During the data coding stage (Section 8.2), the weight equivalents corresponding to each food item (or ingredient of a mixed dish) consumed by the respondent and the associated food and photograph model numbers (when applicable) must be recorded in columns 6 and 7, respectively, of the recall form shown in Table 5.1. Details on how to compile these weight equivalents for the foods, beverages, and ingredients of mixed dishes consumed are given in the next section.

TABLE 5.4

Example of a Completed Recipe Form Used to Calculate Weight of Raw Ingredients Consumed in a Mixed Dish

Subject's Name *Tom Jones* **Subject ID** *054* **M / F** *M*

Interview Date: <**mm/dd/yy**> *06 /12 /07*

Day of the week food eaten: *Monday (market day)*

Name of Interviewer: *Florence* **Name of mixed dish:** *Pumpkin leaf relish*

Amount eaten by respondent (g or mL): *120g*

Wt empty pot: *500* **(g)** **Wt cooked mixed dish + pot:** *750* **(g)**

Wt mixed dish : 250 **(g)** ***Or*** **Volume of cooked mixed dish:** **(mL)**

Proportion of mixed dish consumed by respondent: = *120 / 250 = 0.48*

Ingredient	Description of ingredient and cooking method	Amount of raw ingredient in recipe	Weight of raw ingredient in recipe (g)	Weight of raw ingredient consumed	Weight of cooked ingredient in recipe (g)	Weight of cooked ingredient consumed
Pumpkin leaf	Whole with stalks: chopped, stewed	4 cups	130g	62.4		
Tomatoes	Whole, chopped with seeds: stewed	¼ tomato	32g	15.4		
Groundnuts	Skins removed, chopped, stewed	25 items	20g	9.6		

5.5 Converting Portion Sizes to Weight Equivalents

Several procedures can be used to convert the portion sizes of foods consumed, estimated by using the methods described in Section 5.4, into weight equivalents. This task is generally undertaken by the survey coordinator. Details on these methods include:

- direct weighing—recording the weight in grams of actual foods or salted replicas directly using dietary scales,
- volume equivalent—recording the volume of water that is equivalent to the volume of the food or beverage item consumed and then converting the volume to grams by multiplying volume (in mLs) by the specific gravity (density) for the food or beverage item consumed,
- household measures—recording the portion sizes of food or beverage in household measures and converting to weight equivalents,
- clay or play dough models—measuring and recording the volume of a clay or play dough model identical in size and shape to the food item consumed, and then converting the volume into weight equivalents of the actual food,
- linear dimensions—measuring the linear dimensions (length, width, and thickness) of a food item with a non-stretch tape measure and then converting into weight equivalents of the actual food, and
- monetary value—converting the monetary value of a purchased food item into weight equivalents.

Direct weighing of foods such as soup, stew, porridge, or beverages is the easiest way to determine the weight equivalents of portion sizes consumed (see Section 5.4). Respondents are asked to serve a portion size of the food or salted replica identical in size to the amount they consumed, preferably into their own dishes (e.g., plate, bowl, or cup) by using their own utensils. After questioning and removal of any leftovers, the final portions consumed are weighed by the interviewers (Box 5.4) and the amount eaten recorded (in grams) directly in column 5 of the 24-hour recall form (Table 5.1).

Dietary scales can also be used to convert food items such as fruits, roots, tubers, etc., which have been recorded in column 4 of the 24-hour recall form as small, medium, or large, into weight equivalents. In such cases, the weights of multiple samples of small, medium, and large sizes of each food item must be weighed on the dietary scale, and the weights recorded. Note that if the food item contains an inedible portion (e.g., banana skin, mango stone), it is important to weigh the edible portion only (e.g., banana and mango flesh only). For example, to determine the weight of a small banana you should purchase several small bananas, skin them, and then weigh each banana and calculate the average weight of the edible portion of one small banana.

Volume equivalent is useful when the volume instead of the weight of the actual food, drink, or salted replica can be recorded (in milliliters) e.g., for a beverage or thin porridge, by either measuring directly into a graduated measuring cylinder or a calibrated utensil (e.g., calibrated feeding cup) (See also Box 4.6). Alternatively, a volume of water equivalent to the volume of actual food, drink, or salted replica can be measured by using a measuring cylinder or calibrated utensil. This volume must then be converted into weight equivalents of the actual food or beverage consumed using the specific gravity. The latter can be determined by weighing known volumes of the food or beverage prepared using a local recipe and applying the equation: specific gravity (g/mL) = mass (g)/ volume (mL). The amount consumed (in grams) is then recorded in column 5 of the 24-hour recall form (i.e., the form in Table 5.1).

Household measures used to record portion sizes (e.g., of soups, stews, drinks, rice, and gari) can be converted into weight equivalents by weighing an equivalent amount of each food or beverage and recording the weight. Depending on the importance of the specific food item in the diet, between five and ten samples of each food or beverage item should be weighed to derive an average household measure weight-equivalent conversion factor for a specific food item. For example, if the amount of rice consumed was recorded in cups on the 24-hour recall form, between five and ten samples of one cup of cooked rice (prepared using local cooking methods) should be weighed several times to yield an average conversion factor for one cup of cooked rice.

Clay or play dough models in the shape and size of small pieces of meat, fish, certain fruits, and cereal or root-based staples (e.g., *nsima* or cassava in Malawi; *fufu* and *banku* in Ghana) consumed by the respondent can be used to estimate portion sizes. Two methods can then be used to assess the weight equivalents of the portion sizes of foods represented by the clay or play dough models. The simplest method is to weigh an equivalent amount of the food, similar in size and shape to that of the clay or play dough model, and record the weight in column 6 of Table 5.1 (Box 5.4). Repeat this procedure with several samples of the same food item so that an average weight equivalent for the clay or play dough model of a specific food item can be derived. The number of repeats required depends on the importance of the food item in the diet.

The other method is to estimate the volume of the clay or play dough model by the water-displacement method, using either a graduated measuring cylinder, a set of calibrated glass beakers, or household utensils. For small shapes that will fit into a measuring cylinder, fill the cylinder with a known volume of water that will just cover the modeled food item, place the clay or play dough shape in the measuring cylinder, and record the new water level. The difference (in milliliters) between the two levels is equivalent to the volume of the shape; the volume difference is converted to a weight equivalent (Box 5.6) and this amount is recorded in column 6 of the 24-hour recall form (Table 5.1).

BOX 5.6

Deriving the Weight Equivalent for Clay or Play Dough Models of Irregularly Shaped Food Items

Follow these steps to derive the weight of such food items as meat, pieces of cereal, root-based staples, or fruit, etc.

1. Weigh equivalent amount of the food similar in size and shape to the clay or play dough model and record its weight (in grams).
2. Repeat this procedure using five to eight samples of the same food item and record the weight (in grams) of each sample.
3. Calculate an average weight equivalent for the clay model of that specific food item.

OR

4. Estimate the volume of the clay or play dough model by the water-displacement method:
   - For small clay or play dough models that will fit into a measuring cylinder (e.g., pieces of stiff maize porridge), fill the measuring cylinder with a known volume of water that will just cover the modeled food item and record the volume.
   - Place the clay or play dough shape in the measuring cylinder and record the new water level.
   - Calculate the difference (in milliliters) between the two water levels in the measuring cylinder. This difference is equivalent to the volume of the shape of the modeled food item.
5. To convert the volume of the modeled food item to weight equivalents:
   - Use published specific gravity data, if available.
   - Compile specific gravity data for clay or play dough models of each food item by: (a) purchasing five to eight samples of different weights of each food item; (b) determining the volume of each by water displacement; and (c) calculating a specific gravity factor for that food item using the formula: specific gravity (g/mL) = weight (g) / volume (mLs).
   - Derive a conversion factor from weight equivalents given in food composition tables.

For larger shapes, the water displacement method can be carried out using calibrated plastic beakers. Select an appropriately sized beaker and partially fill the beaker with water to a level that will just cover the modeled food item. Record the level of the water in millimeters. Then place the modeled food item into the water and note the new level. Again, the difference between the two levels will equal the volume of the modeled food item (in mLs).

Next you must convert the volumes of the food items to weight equivalents. Several methods can be used to compile these conversion factors, including:

- Published specific gravity data can be used to convert volumes into grams using the following formula: weight (g) = volume (mL) x specific gravity (g/mL) (See Appendix D);
- Nutrient composition tables that provide the weight (in grams) of a standard 8-ounce measuring cup for specific foods (e.g., Pennington and Church, 1985) can be used to derive conversion factors (8 ounces = 250mL; the weight of one cup of the product divided by 250 will give the conversion factor to grams for 1mL of the product); and
- Purchased samples (approximately five to eight) of different weights of each food item of interest (e.g., chicken breast) can be used to determine the volume by water displacement; the specific gravity factor for converting volume to grams for each sample can be calculated, and an average value that can be used for that specific food item can also be calculated.

Linear dimensions of certain food items (e.g., sugarcane, boiled or roasted corn, and raw or cooked plantain) can be used to determine the amount consumed. For example, sugarcane can be estimated by asking respondents to show you the length of the sugarcane eaten. To compile weight equivalent conversion factors for the edible portion of different sizes of a food, the food must first be cut into pieces of various lengths. Next the dimensions of each piece must be measured and its weight recorded before eating. Each piece of food must then be eaten, all the leftovers (e.g., fibrous material, cobs, and peels) must be weighed, and the weights recorded. The edible portions will equal the first weight minus the second weight. From these data, a conversion factor for a linear dimension can be developed. Table 5.5 gives an example using sugarcane. A similar process can be used for boiled or roasted corn-on-the-cob, or for pieces of meat. Detailed examples of this procedure are given in Box 5.7.

TABLE 5.5

Example of Calculating the Weight Equivalent Conversion Factor from the Linear Dimensions of Sugarcane

Length (cm)	Weight (g)	Weight of Leftovers (g)	Weight of Edible Portion (g)
13	105	42	63
12.5	90	40	50
23	235	103	132
12.5	110	40	70
13	115	49	66
25	260	115	145
25	268	110	158
20	210	92	118
19	180	90	90
12	100	44	56
14	122	49	73
11	117	51	66
Total 200cm			Total 1087g
The conversion factor (density or specific gravity) for each cm of edible portion of sugarcane eaten = 1087g ÷ 200cm = 5.4g/cm.			

Note this example: A respondent who ate 77cm of sugarcane would have eaten the equivalent of 77cm x 5.4g/cm = 416g of sugarcane. Therefore, 416g should be entered into column 5 of the 24-hour recall form (Table 5.1).

BOX 5.7

Deriving the Weight Equivalent from the Linear Dimensions of Edible Portions of Suitable Foods

1. Cut food item (e.g., sugarcane, corn cob, and banana) into pieces of various lengths.
2. Measure the length (in centimeters) of each piece by using a fiber-glass tape measure, and record the length.
3. Weigh each piece with a dietary scale and record its weight (in grams).
4. Eat the edible portion of each piece and then weigh the leftovers.
5. Subtract the weight of the leftovers from the raw weight of each piece, which will give the weight of the edible portion of each piece.
6. Calculate a weight equivalent conversion factor based on length.

Monetary value of food (e.g., candies, biscuits, groundnuts, and meals), or beverage items purchased from vendors can be used to estimate the amount consumed; corresponding equivalent weights can then be compiled (Box 5.8). From various vendors in the local area purchase samples of the foods, meals, or prepackaged foods that represent a single monetary value or a range of monetary values. The number of samples of each item to be purchased depends on the variability in the portion sizes sold. Seasonal differences as well as differences among vendors might also need to be taken into account.

BOX 5.8

Deriving the Weight Equivalent for Purchased Food Items (e.g., rice and beans)

1. Select three vendors in your study area.
2. Purchase three 30-cent portions of rice and bean from each vendor.
3. Weigh the portions and record their weight (in grams).
4. Add up the total weight of the rice and beans purchased from all three vendors.
5. Divide the total weight of rice and beans by the number of portions represented. This average represents the average weight of a 30-cent portion of rice and beans.

For purchased meals, a conversion factor can be developed for certain street food vendors or an average conversion factor for all vendors. An example of the latter is given in Table 5.6.

TABLE 5.6

Example of Calculating the Gram Equivalent Conversion Factors from the Cost of Purchased Portions of Rice and Beans

Vendor	Cost (cents)	Weight (g)
Charity	30	160
Charity	30	150
Charity	30	165
Stella	30	100
Stella	30	150
Stella	30	120
Joyce	30	185
Joyce	30	185
Joyce	30	180
		Total 1395g
Average weight/per 30 cent portion = 1395g / 9 x 30 cent portions = 155g / 30 cent portion		

Note: If a respondent ate 30 cents worth of rice and beans and did not know the vendor, then you would estimate that the respondent ate 155g of rice and beans and record 155g in column 6 of the 24-hour recall form (Table 5.1). In addition, it is important to purchase the same number of portions from each vendor when using this calculation. If this is not done, then first calculate an average portion per cost for each vendor. Next, average the average portions per cost across all vendors.

Compiling weight equivalents of ingredients consumed from mixed dishes can be carried out in two ways, depending on whether the food composition table available provides data on the nutrient composition of raw or cooked foods. If nutrient values for raw foods only are available, then the amount of each raw ingredient in the recipe can be converted to a weight equivalent using the methods outlined above. These "raw" weights are then used to estimate the proportion of each raw ingredient in the mixed dish, as shown in Table 5.7.

TABLE 5.7

Example of a Calculation of the Average Weight Equivalent Conversion Factors for Individual Raw Ingredients for Pumpkin Leaf Relish *

	Cooked Weight (g)	Pumpkin Leaf (g)	Proportion	Raw Tomato (g)	Proportion	Groundnut (g)	Proportion
Charity	250	130	0.52	32	0.13	20	0.08
Charity	300	111	0.37	63	0.21	36	0.12
Charity	400	184	0.46	112	0.28	40	0.10
Patience	350	168	0.48	46	0.13	21	0.06
Patience	375	188	0.50	52	0.14	26	0.07
Patience	450	230	0.51	45	0.10	40	0.09
Lydia	300	114	0.38	81	0.27	27	0.09
Lydia	380	167	0.44	84	0.22	27	0.07
Lydia	290	125	0.43	38	0.13	23	0.08
Rose	310	127	0.41	25	0.08	28	0.09
Rose	430	241	0.56	82	0.19	22	0.05
Rose	410	217	0.53	41	0.10	29	0.07
Average gram equivalent conversion factor			0.47		0.17		0.08

*This approach can be used when an individual recipe is not collected for a participant.
Note: If a person was said to consume 120g of pumpkin leaf relish, then the corresponding gram weight equivalents for the three *raw* ingredients consumed would be: 120 × 0.47 = 56g of leaves; 120 × 0.16 = 19g of tomato; and 120 × 0.08 = 10g of groundnut flour. These are the weights that should be entered into column 6 of Table 5.1. If the number of recipes provided by each participant is not identical, then another method must be used to calculate an average recipe. First calculate an average recipe for each respondent, then average these average recipes across all respondents.

The proportions are then used to calculate the weight of each raw ingredient in the homemade mixed dish recipe consumed by the respondent (See Table 5.8 and Box 5.9). The raw weight of each ingredient should be recorded both on the recipe form (Table 5.4) and on the 24-hour recall form, as shown in Table 5.1. This procedure, does not; however, take into account any weight changes that may arise from alterations in the water and fat content of the mixed dish after cooking, nor does it make any adjustment for nutrient losses or gains during cooking. Hence, if nutrient values for cooked foods are available, the weight of each *cooked* ingredient consumed by the respondent should also be calculated (Box 5.10), and recorded on the 24-hour recall form (Table 5.1). In this way, weight changes and nutrient losses or gains during cooking are taken into account when the nutrient intakes are calculated. Data on yield factors during cooking based on food preparation and cooking methods are available in the U.S. (Merrill et al. 1966; Matthews and Garrison 1975), Thailand (Banjong et al. (2001), and in Australia and New Zealand (Food Standards Australia New Zealand 2004). Additional data are available from: www.foodstandards.gov.au/_srcfiles/revised_NIP_User_guidejuly02.pdf

BOX 5.9

Estimating the Weight Equivalents of Ingredients Consumed from Household Mixed Dishes for Nutrient Composition Data Expressed as Raw Foods

1. Ask the respondent to name all the raw ingredients (excluding water) in the homemade mixed dish, and list these ingredients in column 1 of the recipe form (Table 5.4) and in column 3 of the 24-hour recall form (Table 5.1).
2. Record a description of each raw ingredient and the method of preparation and cooking in column 2 of the recipe form and in column 4 of the 24-hour recall form (Table 5.1), using the detailed descriptions outlined in Table 5.2.
3. Record the amount of each raw ingredient (excluding water) in the recipe in column 3 of the recipe form (Table 5.4) using the methods outlined in Table 5.3.
4. Convert the *amount* of each raw ingredient in the recipe into weight equivalents, where necessary, using the methods outlined in Boxes 5.4 to 5.8, and enter this raw weight equivalent into column 4 of the recipe form.
5. Ask the cook to indicate the volume of the homemade mixed dish after cooking by indicating the level on the side of the cooking pot. Fill the empty cooking pot up to the level indicated with rice or water, and measure the volume of the rice or water with a calibrated jug. Record the volume of the cooked mixed dish in mL at the top of the recipe form. Alternatively, weigh the cooking pot with and without the cooked mixed dish to yield the total weight of the cooked mixed dish (in g). Enter the total weight of the cooked mixed dish at the top of the recipe form.
6. Calculate the *proportion* of the raw ingredients in the homemade mixed dish recipe by dividing the weight (g) or volume (mL) of the mixed dish consumed by the respondent by the total cooked weight (g) or volume (mL) of the mixed dish. Record this proportion at the top of the recipe form.
7. Calculate the weight equivalent of each raw ingredient consumed by the respondent by multiplying the proportion recorded at the top of the form by the weight of each raw ingredient in the recipe. Enter these weight equivalents in column 5 of the recipe form and in column 6 of the 24-hour recall form (Table 5.1). Applying the example given in Table 5.4, if a person was said to consume 120g pumpkin leaf relish, then the proportion of the mixed dish consumed by the respondent = 120/250 = 0.48. Hence, the corresponding weight equivalents for the three *raw* ingredients consumed would be: 0.48 × 130 = 62.4g of pumpkin leaves; 0.48 × 32 = 15.4g tomato; and 0.48 × 20 = 9.6g pounded groundnut flour. These are the weights that should be entered into column 5 of the recipe form.

BOX 5.10

Estimating the Weight Equivalents of Ingredients Consumed from Household Mixed Dishes for Nutrient Composition Data Expressed as Cooked Foods

Where possible, it is preferable to express the weight equivalents of ingredients consumed in mixed dishes as cooked foods in order to take into account any weight changes that may arise after cooking. This approach of using cooked food allows for any nutrient value changes (such as losses or gains) during cooking to be taken into account. The steps involved are listed below.

1. Apply a weight change factor to adjust the weight of the raw ingredients to cooked weights. A table of weight change factors is given in Table 5.9. For example, if the weight of raw carrots in mixed dish = 55g and weight change factor for boiled carrots = -7 percent, then the loss of weight of carrots after boiling is: 55g = 55 × 7/100 = 3.85g. Therefore, the cooked weight of the carrots is: 55g – 3.85g = 51.15g. (Note: If the carrots had gained 7 percent weight, then 3.85g would have been added to the raw weight value). Enter this cooked weight in column 6 of the recipe form. Repeat this calculation for each raw ingredient listed in step 1, applying weight change factors as appropriate.
2. Calculate the *proportion* of the cooked ingredients in the homemade mixed dish recipe by dividing the weight or volume of the mixed dish consumed by the respondent by the total cooked weight (g) or volume (mL) of the mixed dish. Record this proportion at the top of the recipe form.
3. Calculate the weight equivalent of each cooked ingredient *consumed* by the respondent by multiplying the proportion recorded at the top of the form by the weight of each cooked ingredient in the recipe. Enter these weight equivalents in column 7 of the recipe form and in column 6 of the 24-hour recall form (Table 5.1).

TABLE 5.8

An Example of the Use of Conversion Factors for Mixed Dishes to Calculate the Weight of the Raw Ingredients Consumed

Food and Amount Consumed (g)	Ingredients	Fraction of Raw Ingredient in Cooked Food	Grams of Raw Ingredient Consumed
Pumpkin leaf relish 45g	Leaves	0.47	21.2
	Tomato	0.17	7.2
	Groundnut flour	0.08	3.6
	Salt	0.02	0.9
Groundnut soup 176g	Groundnut paste	0.09	15.8
	Tomato	0.06	10.6
	Onion	0.01	17.6
	Chili	0.01	1.8
Pigeon pea relish 24g	Dry peas	0.30	7.2
	Tomato	0.14	3.4
	Onion	0.01	0.2
	Salt	0.02	0.5
Dry small fish 37g	Dry fish	0.24	8.9
	Tomato	0.31	11.5
	Onion	0.02	0.7
	Salt	0.03	1.1
Maize porridge 240g	Maize flour	0.17	40.8
	Groundnut flour	0.03	7.2
	Sugar	0.04	9.6
	Salt	0.02	4.8

Note: The grams of raw ingredient consumed are calculated from the product of the amount of the mixed dish consumed multiplied by the fraction of the raw ingredient in the cooked food. Thus, the calculation does not take into account any weight changes that may arise from alterations in the water and fat content of the mixed dish after cooking, nor any adjustments arising from nutrient losses or gains during cooking.

BOX 5.11

Constructing Generic Recipe Data for Local Mixed Dishes, and Estimating Weight Equivalents of Ingredients Consumed from the Generic Recipes

1. Compile a list from the 24-hour recall form of all the mixed dishes for which weight equivalent data are required.
2. Arrange for five to ten women in the study area to cook the required mixed dish three times each.
3. List the raw ingredients of each mixed dish and their method of preparation on columns 1 and 2 of the recipe form (Table 5.4).
4. As each mixed dish is prepared, weigh each raw ingredient and subtract the weight of any inedible part.
5. Record the weight of the edible part of each raw ingredient in column 4 of the recipe form.
6. Weigh the empty cooking container and lid.
7. Cook each mixed dish using local cooking methods.
8. Weigh each cooked mixed dish, including the container and lid.
9. Subtract the weight of the cooking container and lid from the total weight to obtain the net weight of the cooked food. Record on the top of the recipe form.
10. Measure the volume (in mL) of the cooked dish using a graduated measuring jug. Calculate specific gravity of the cooked mixed dish: Specific gravity (g/mL) = mass (g)/ volume (mL)
11. Divide the weight of each raw ingredient for the mixed dish by the total weight of the cooked food. This gives the weight equivalent factor.
12. Average the weight equivalent factors from data obtained from the multiple samples of each dish cooked by the five to ten women (Table 5.7).
13. For nutrient composition data expressed as raw foods: Use the average weight equivalent factor for each raw ingredient to calculate the weight of each raw ingredient consumed by the respondent, as shown in Table 5.8.
14. Enter the weight of each raw ingredient consumed by the respondent into column 7 of the recipe form. This is the weight that must be transferred to column 6 of Table 5.1.
15. For nutrient composition data expressed as cooked foods: Use the average weight equivalent factor for each cooked ingredient to calculate the weight of each cooked ingredient consumed by the respondent, as shown in Box 5.10.
16. Enter the weight of each cooked ingredient consumed by the respondent into column 7 of the recipe form. This is the weight that must be transferred to column 6 of Table 5.1.

TABLE 5.9

Examples of Weight Change Factors and their Use

Food Type	Weight Change Factors (as a percentage)
Boiled rice	189
Grains (other than rice) simmered, e.g., maize, porridge	-13
Other grains (boiled)	54
Carrots & similar root vegetables	-7
Leaf & stalk vegetables, boiled	-15
Peas & edible podded peas	-7
Tomato, boiled	-22
Pumpkin, baked	-15
Squash, zucchini, boiled	-16
Other vegetables, boiled	
Corn-on-cob	-1
Corn kernels	-4
Onions, boiled	-10
Vegetable mixture, boiled	-11
Mature legumes and pulses	
Beans, dried, boiled	149
Chick peas, dried, boiled	163
Split lentils, dried, boiled	227
Whole lentils, dried, boiled	142
Split peas	150

Example: If weight of raw carrots = 55g; weight change factor for boiled carrots = -7 percent;
Then loss of weight of carrots after boiling = 55 × 7/100 = 3.85g;
Cooked weight of carrots = 55g – 3.85g = 51.15g.

(Data from Food Standards Australia New Zealand, 2004) Additional data are available from: www.foodstandards.gov.au/_srcfiles/revised_NIP_User_guidejuly02.pdf

Note that when respondents are unable to provide any details for mixed dishes such as are listed in Box 5.3 under no.4, data for a theoretical average recipe for each mixed dish should be used. These average recipes can be created from recipes collected from other participants. Alternatively, a generic recipe can be obtained by arranging for women in the study area to cook the required mixed dish. Instructions on how to construct average recipes for mixed dishes in this way are outlined in Box 5.11. In some cases, it may be necessary to have separate recipes for the same dish if the dry matter content differs (e.g., thin or thick maize porridge).

5.6 Reviewing the Recall Interview Data

In the final stage of the interview (i.e., pass four), the interviewer reviews the recall to ensure that all the items have been recorded correctly (Box 5.12). Finding and correcting errors at the time of the recall interview, when both the interviewer and the respondent are focused on the previous day's food, will yield more accurate information than any reviewing that occurs after the interviewers have returned to the office.

BOX 5.12

Reviewing and Completing the 24-hour Recall Interview Data

1. Read the following statement to the respondent: "I will read back to you what I have recorded to make sure that I have not made any mistakes."
2. Then read back all foods recalled from the beginning of the day until the end of the day. Ask the respondent if what was read is correct.
3. Scan the recall to ensure that a full and accurate description of all the foods eaten has been recorded together with portion sizes under the "amount eaten" column in Table 5.1.
4. Scan the recall to ensure that the yield, ingredients, quantities, and cooking methods were recorded for the homemade mixed dishes or a note made about the need to construct an average recipe as outlined in Box 7.3.
5. Ask the respondent whether vitamin and mineral supplements were taken. Record the brand names (if known), a complete description of the supplements, and the quantity consumed in the 24-hour recall period.
6. If appropriate, ask in a nonjudgmental manner about any alcohol consumed by reading the following statement: 'Did you have any alcoholic drinks during the day?'
7. Inquire whether the respondent ate anything during the night.
8. Ask the respondent whether the day of the recall represented a usual day; if it did not, ask how the day differed from usual.
9. Express thanks for the respondent's time and cooperation.
10. Ask the field supervisor to check the records again for completeness, readability, and any missing information.
11. Make a list of the foods consumed for which composition data are not available. Collect samples of these foods at the end of the survey for chemical analysis. Details of the protocols for sampling, transport, and handling of these food items are outlined in Section 7.6.

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Chapter 6 | Assessing the Validity and Reproducibility of the Interactive 24-hour Recall

What will you learn from this chapter?

- How to assess the relative validity of the interactive 24-hour recall; and
- How to assess the reproducibility of the interactive 24-hour recall.

The quality of the food intake measurements collected by the interactive 24-hour recall depends on the validity and reproducibility of the measurements. Validity is affected by systematic measurement errors whereas reproducibility is associated with random errors. Both types of measurement error can be minimized by incorporating quality control procedures at each stage of the measurement process. Systematic errors are, however; much more difficult to control than random measurement errors. Subjects may systematically misreport certain foods during the 24-hour recall or may eat atypically during the dietary survey, even though every effort is made to discourage this. Such systematic errors may be associated with only some respondents (e.g., obese or elderly subjects), specific interviewers, or certain foods (e.g., alcohol). Systematic errors are especially critical because they can introduce a clinically important/significant bias into the results that cannot be removed by statistical analysis, unless a calibration study has also been completed. For more details of calibration studies, the reader is advised to consult Kaaks and Riboli (1997).

Table 6.1 summarizes the major sources of error that may occur in a 24-hour recall compared with estimated and weighed food records. Training of the interviewers for the 24-hour recall is critical to ensure that the respondents do not modify their eating patterns during the recall period. When conducting the interview, both interpersonal and technical skills are important: for example, the interview must always be conducted with an open and pleasant manner, with the aim of being friendly, diplomatic, empathetic, and determined, as appropriate. Leading questions, judgmental comments, and direct questions about specific meals (e.g., breakfast, lunch, supper) or about snacks should be avoided. Ways to stimulate the memory of the respondent and avoid biased probing are some of the technical skills that must be applied during the interview.

TABLE 6.1

Sources of Error in Quantitative Dietary Assessment Techniques

Sources of Error	24-hour Recall	Estimated Record	Weighed Record
Omitting foods	Likely	Possible	Possible
Adding foods	Likely	Unlikely	Unlikely
Estimation of food weights	Likely	Likely	Unlikely
Estimation of frequency	Unlikely	Unlikely	Unlikely
Day-to-day variation	Likely	Likely	Likely
Making changes to diet	Unlikely	Possible	Likely
Coding errors	Likely	Likely	Likely

This table was adapted from Staveren and Burema (1985).

The errors itemized in Table 6.1 have been minimized in the interactive 24-hour recall by including certain quality control procedures and modifications in the recall interview to make it more suitable for use with people who do not read or write. These include:

- Training the interviewers and the respondents before the recall;
- Providing respondents with bowls and plates for the recall days, and discouraging them from eating from a common pot to help them visualize the amount of food consumed;
- Supplying picture charts depicting commonly consumed local staple food items to use as a checklist on the day the food is actually consumed and for comparison with the recall on the following day to reduce memory lapses;
- Using a standardized multiple-pass interviewing technique and questionnaire which gives the respondent more opportunities to recall foods initially forgotten;
- Calibrating a set of local household utensils for recording volumes;

- Using standardized probing questions specific for each staple food consumed;
- Preparing salted replicas of actual cooked staple foods prepared by local cooking methods to estimate portion sizes;
- Using actual samples of commonly eaten foods to estimate portion sizes;
- Using calibrated dietary scales accurate to at least 1g to weigh the portions of prepared foods actually consumed; and
- Using a variety of graduated food models and photographs to estimate portion sizes.

The direction and extent of any random and systematic measurement errors will vary with the population group and the nutrients studied. Therefore, before commencing the actual dietary survey, it is always preferable to check the validity and reproducibility of the interactive recall on a random sample of subjects who are representative of the population under study. All too often, volunteers who participate in validation studies are self-selected; for this reason, they may have different dietary habits than the study population (Riboli et al. 1997).

Other characteristics of subjects that may influence the outcome of a relative validity study and hence should be taken into account in the design of validation studies include overweight, a history of dieting or restrained eating, depression, body image, social desirability, age, and sex (Gibson 2005). Several studies have shown that the response of women to dietary assessment differs from that of men (Johnson et al. 1994). In addition, and not surprisingly, memory and conceptualization skills affect the response of younger respondents (especially those less than 8 years of age) and older individuals (Nelson 1997). Socioeconomic status and ethnicity may also affect the outcome, possibly through a link with dietary diversity (Kristal et al. 1997). The health status of subjects is also important, especially in case-control studies. Here attempts should be made to validate the dietary methods with representatives of both the cases and the healthy controls.

Validation of the interactive 24-hour recall on a group of rural pregnant Malawian women is outlined in Ferguson et al. (1995), and is described briefly in Chapter 1. A more detailed description of how to validate the interactive 24-hour recall method for your own study group and check its reproducibility is given below.

6.1 Assessing Relative Validity

Validity of a dietary method is defined as the degree to which a method measures what it is intended to measure. When a dietary method is designed to characterize usual food intakes within a free-living community, absolute validity cannot be measured because the truth is never known with absolute certainty. Subjects may eat atypically during the dietary period, even though every effort is made to discourage this. Therefore, only relative validity can be measured. In this approach, the test method is evaluated against another reference method chosen for its accepted accuracy, precision, and ability to measure food intake over the same time frame as the test method. In addition, errors in the chosen reference method should be independent of those in the test method. For example, both methods should not rely on memory or use the same method for estimating portion size. Finally, it is essential to carry out the measurements by both the test and reference methods on the same subjects.

The first step in the validation study is to select the reference method. For the interactive 24-hour recall, a weighed food record is the method of choice because it does not rely on memory and uses a different and more accurate method (i.e., weighing) for estimating portion sizes. Next, the number of weighed food record days required must be defined. This will be defined by the number and schedule of the 24-hour recall days collected, which in turn depends on the study objective and the nutrients of interest (further details are provided in Section 3.1). Finally, the sequence of administration of the 24-hour recalls and the weighed food records must be considered.

Some investigators recommend that the test method should be administered prior to the reference method in a validation study so as to mimic the situation that will actually take place in the proposed study, and avoid the act of completing the reference method from drawing the respondent's attention to their diets. Others suggest that the study population should be randomized to complete the test method (i.e., 24-hour recalls) before or after the reference method (weighed food records). In practice, however, in populations in developing countries that do not read or write, weighed food records are generally completed in the households by trained research assistants. In such household settings, this way of collecting the records is more likely to reflect the response of the respondent to the 24-hour recall than if the respondents were completing the records themselves. Hence, in such circumstances, the weighed food records can be carried out on the same subjects, and the recall days can correspond to those for the weighed record.

Care must be taken to ensure that the person conducting the 24-hour recall interview is not the same person as the one who performed the weighed food records. To minimize the effect of systematic errors associated with specific recall interviewers, respondents should be assigned randomly to the interviewers when repeated 24-hour recalls are performed. In this way, respondents will not usually be questioned by the same interviewer on all occasions.

For the weighed record, the trained research assistants must arrive at the household early in the morning (preferably before sunrise) on the scheduled survey day, and remain in the household all day until the evening meal is finished. During this time, the research assistants are instructed to weigh and record all food and beverages consumed by the respondent both in the household and away from home. Details of the methods of food preparation and cooking, description of foods, and brand names (if known) should also be recorded in Table 6.2. Guidelines on the detailed information required to describe the food items consumed are given in Table 5.2. For mixed dishes, the weight of the portion consumed by the respondent should be recorded, along with the weights and descriptions of all the raw ingredients, including flavors and spices used in the recipe, as well as the final total weight of the cooked mixed dish. These details are best recorded on a recipe form (see Table 5.4), as noted in Box 6.1.

BOX 6.1

Recording Mixed Dishes on the Recipe Form

1. Record the interviewer's name, date, day of the week, and subject identification code at the top of the recipe form.
2. Record the name of the mixed dish at the top of the recipe form.
3. Record the weight of the mixed dish consumed by the subject at the top of the form. This weight should also be recorded on the corresponding weighed record form under "amount eaten".
4. Record weight of the empty cooking pot on the form.
5. Record the name and a complete description of each ingredient in the mixed dish on a separate line of the recipe form.
6. Record the weight of the *edible* portion (i.e., as eaten) of each raw ingredient. For example, for ingredients such as bananas, only the banana with the skin removed (i.e., the edible portion) is weighed.
7. Record the final weight of the cooked mixed dish in the cooking pot on the recipe form.

Details of how to conduct a weighed food record are given in four steps:

i) how to assemble the equipment and prepare to collect the weighed food records (Box 6.2);

ii) how to record the foods and drinks consumed (Box 6.3);

iii) how to weigh the amounts using the cumulative weight technique (Box 6.4); and

iv) how to describe the foods and drinks consumed in the food record (Box 6.5).

BOX 6.2

Assembling the Equipment, and Preparing to Collect the Weighed Food Records

1. Secure robust dietary scales, accurate to within ±1g and weighing up to 1.5kg, so that a plate or bowl can be used when weighing the food to be eaten. It is preferable to use scales with a tare feature which allows resetting to zero to adjust for the weight of the plate or bowl as well as previously weighed food. Examples are Hanson Digital Kitchen Scales (supplied by Arden Forest, Warwickshire, UK) and Soehnle electronic digital scales (supplied by CMS Weighing Equipment Ltd., London, UK) (See Appendix E for suppliers).
2. Obtain a second set of dietary scales, accurate to within ±5g and weighing up to 10kg, for weighing the total amount of the cooked food in the family pot.
3. Assemble spare batteries for scales, clipboards, pens, pencils, pencil sharpeners, and erasers.
4. Photocopy forms for weighed dietary records.
5. Decide on the number and schedule of 24-hour recall days (i.e., number of weekdays, market, and weekend days) required by the study objectives (see Section 3.3). This in turn defines the number and schedule for the weighed records.
6. Train the recall interviewers in the use of weighed food records, first in a classroom setting, and then under field conditions.
7. Plan for interviewers to visit the households of the respondents who have agreed to participate in the validation study and again review the purpose of the study with them.
8. Plan for interviewers to arrive at the household early in the morning before sunrise on the scheduled survey days and to remain in the household all day until the evening meal has finished.
9. Distribute a picture chart, bowl, and plate to each respondent and explain how to complete the picture charts on the food recall days and how to use a separate bowl and plate for eating food on the specified recall recording days.

BOX 6.3

Recording the Foods and Drinks Consumed

1. Record the interviewer's name, dietary scale number, date, day of the week, and subject identification code at the top of each page of the weighed food record form (Table 6.2).
2. Record the age (in years), body weight (in kilograms), and sex of the subject on the same form.
3. On each scheduled food record day, record throughout the day what time (column 1), and where (column 2) each food and drink (including snacks) is consumed. Begin each new day with a new page of the form; use more than one page per day, if needed.
4. Record at what time and where (e.g., in the fields or at neighbors) any snacks, meals, or beverages are consumed away from home.
5. Record recipe details of each mixed dish on a separate recipe form (Table 5.4) and on the weighed record form (Table 6.2). For example, a pumpkin leaf relish would be recorded as pumpkin leaves, tomato, and onion, with each item on a separate line on the recipe form. Likewise, a beef curry would be recorded as stewing beef, oil, onion, green chili, green cardamom pods, a piece of ginger root, dried red chili, curry paste, ground coriander, ground cumin, salt, beef stock, tomato, with each on a separate line. Further details are given in Box 6.1.
6. If vitamin or mineral supplements were used, list the amount taken each day, brand, and label information on the last line of the weighed record form. If possible, the details should be read directly from the bottle or package used by the subject. Alternatively, the respondent can include the label with the completed record.

BOX 6.4

Weighing the Amounts Using the Cumulative Weight Technique

1. To use the dietary scales:
   - adjust the scale to zero;
   - place the plate or cup on the scale and adjust the scale to zero;
   - place the food or beverage on the weighed plate or in the weighed cup;
   - read and record the weight of the food or beverage in grams in the "amount served" column;
   - adjust the scale to zero;
   - add the next item of food, and record its weight;
   - again adjust the scale to zero; and
   - repeat this taring, weighing, and recording procedure until all the items in the meal have been measured.
2. If all the food on the respondents plate has not been eaten or if there is any waste, such as bones, apple cores, skin from potatoes, bananas, oranges etc., adjust the scale to zero and then put another plate on the scale and record its weight.
3. Put one item of leftover food or waste on the plate and record the weight in the "amount left" column of Table 6.2.
4. Adjust the scale to zero and then add the next leftover and record the total weight.
5. Repeat #4 until all the leftovers and waste have been weighed and recorded.
6. Deduct the weight of the leftovers and the waste from the amount served and enter the amount eaten in Table 6.2.

BOX 6.5

Describing the Foods and Drinks Consumed in the Weighed Food Record

1. Record a complete description of all the foods and drinks as they are consumed in column 3 of the form (Table 6.2). Refer to Table 5.2 to help you with the details for specific foods. For all foods include the following details:
   - name (local and general, if known);
   - method of cooking;
   - state of food (e.g., raw, or cooked, peeled, or unpeeled, refined or not);
   - brand names where applicable;
   - all condiments, herbs, and spices (e.g., sauces, salt, and pepper); and
   - label information and the brand name, if available.
2. Record a description of each raw ingredient in the recipe for all homemade mixed dishes on the recipe form (Table 5.4) and on the weighed record form (Table 6.2). Consult Box 6.1 for further details.

A weighed food record form is presented in Table 6.2. Finally, on the day after each weighed food record day, an interactive 24-hour recall interview must be conducted (Box 6.6).

BOX 6.6

Conducting the Interactive 24-hour Recall on the Day After Each Scheduled Weighed Record Day

On the day after each weighed record day, conduct an interactive 24-hour recall interview with the respondent following the format described in Sections 5.1 to 5.5.

The number of subjects required for the validation study can be calculated from prior knowledge of the estimates of the mean intake ($\bar{x}$) and within-individual variation (s^2) for the nutrients of interest for the interactive 24-hour recall and the weighed dietary record (Willett 1998). The reader is advised to consult a statistician before carrying out this step. In many cases, these data may not be available and in the absence of such information, a reasonable size for a validation study is 100 subjects for each demographic group (Willett 1990). Care must be taken to ensure that the subgroup for the validation study is representative of the population in which the methods are to be used.

TABLE 6.2

Weighed Food Record Form

Interviewer: Scale no.: Interview date: Day food eaten:			Location: Subject ID: Subject name:		Sex: Age: Weight:	
Time	**Place**	**Food or drink**	**Description and cooking method**	**Amount served (g)**	**Amount left (g)**	**Amount eaten (g)**
Probe for alcohol: Yes ❑ No ❑			Probe for sickness Yes ❑ No ❑ If yes, did sickness affect appetite? If yes, how? Increase ❑ Decrease ❑			
Was food intake unusual? Yes ❑ No ❑ If yes, how was it unusual?			Probe for tablets Yes ❑ No ❑ Iron ❑ Vitamins ❑ Other supplements ❑ Anti-malaria ❑			
Was it a feast day? Yes ❑ No ❑ Was it a market day? Yes ❑ No ❑ Was it a fasting day? Yes ❑ No ❑			Name of supplement (Record this from the label, if available)			

6.2 Statistical Assessment of Validity

Depending on the objectives of the dietary study (Section 3.1), several different statistical procedures can be used to assess the relative validity of the interactive 24-hour recall and are summarized in Box 6.7. They can be classified as those that assess the extent of the agreement between the test and reference methods on a group basis (Category 1 objectives, as noted in Box 3.1), and those that assess the extent of the agreement between the two different methods at the individual level (Category 2 objectives, listed in Box 3.2). Additional details of computer packages of statistical programs that can be used for analyzing both validity and reproducibility (discussed in Section 6.4) are provided in Chapter 11.

BOX 6.7

Using Statistical Methods to Assess the Relative Validity

Category 1 objectives: Methods to assess the extent of agreement on a group basis include:

1. **Comparison of means:** use a *t*-test (using log-transformations where appropriate).
2. **Comparison of medians:** use a Wilcoxon's signed rank sum test.

Category 2 objectives: Many methods are available to assess the extent of agreement on an individual basis. It is advisable to use more than one statistical method wherever possible.

1. **Correlation analysis** measures the strength of the relationship. Use when dietary data are measured as continuous variables. Three measures of correlation can be used: Pearson, Spearman, and intraclass.
2. **Cross-classification** is to be used for data ranked into broad categories (e.g., into thirds). Calculate the percentage of respondents classified into the same/opposite category by the two dietary methods. Use Cohen's weighted kappa statistic (k_w) to avoid the inclusion of agreement that occurs by chance.
3. **Bland-Altman analysis** is used to plot the mean difference and standard deviation of the difference between the two methods for each nutrient. The test does not make any assumptions about whether the test or reference method is best.
4. **Analysis of surrogate categories** can be used when there is only a single day of intake. Assign a respondent to a category (e.g., third) based on his/her nutrient intake from the 24-hour recall. Then calculate mean intake of each third based on intakes from weighed record. Test whether mean intakes of the thirds by the two methods differ using the ANOVA and Tukey's test.

When the study objective is to measure the extent of agreement on a group basis only (see Box 3.1), first calculate the intakes for each individual and then calculate the group mean and standard deviations for the intakes derived from the test and reference methods. A *t*-test can then be used to test whether the two means are statistically different at some predetermined probability level (see Section 11.3), provided the data are normally distributed. If, however, the distribution of nutrient intakes is skewed, attempts should be made to normalize the data before testing the mean. Generally, energy intakes are normally distributed whereas intakes of iron and zinc, and particularly vitamin A, are often skewed.

If the intake data are not amenable to simple log (either $\log_{10}$ or $\log_e$) transformation, the median (50th percentile) and selected percentile points (e.g., 25th and 75th percentiles) should be used to quantify the average intakes and their variability. In such cases, the Wilcoxon's signed rank sum test for paired data (Section 11.3) can then be used to test the comparability of the medians, and hence, the relative validity of the test method (Gibson 2005).

If differences between the means or medians for the test and reference methods are significant for multiple nutrients, and if the differences point all in the same direction, bias in the test method may be indicated. Alternatively, the means or medians for the test and reference methods may be similar—not significantly different—even when the relative validity at the level of the individual (for example, as measured by correlation) is poor. Plots of the test versus reference results for each nutrient or food group of interest should always be drawn to highlight these relationships.

To assess the validity of the dietary intake data collected at the individual level, several statistical methods are available, and it is advisable to consult a statistician. Usually more than one statistical approach is applied. Methods include correlation coefficients, regression analysis, contingency tables (cross-classification), mean difference and standard deviation of the difference, and analysis of surrogate categories (Margetts and Thompson 1995). These methods are summarized in Box 6.7 and also discussed below.

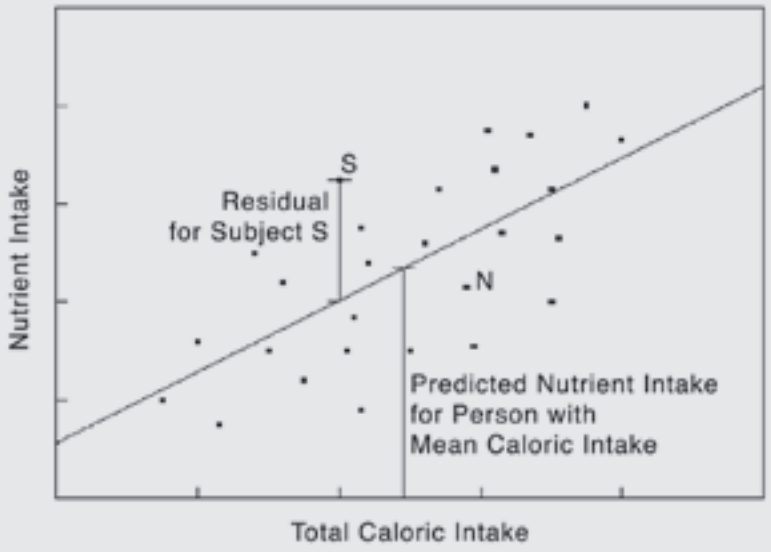
Correlation analysis is most commonly used to measure the strength of the relationship between intakes from the test and the reference dietary method at the individual level, provided intakes on multiple days have been collected. Usually Pearson correlation coefficients are calculated for normally distributed data; alternatively, transformations such as log (either $\log_{10}$ or $\log_e$) transformations can be performed to increase normality before the Pearson correlation coefficients are computed.

Several investigators have recommended energy-adjusting the nutrient intakes prior to correlation analysis for some validation studies. Such an approach may allow for the underreporting of intakes. Sometimes higher correlation coefficients result from applying an energy-adjustment. In its simplest form, the energy adjustment involves calculating the nutrient densities by dividing nutrient values for each subject by the energy content of the diet for that subject. These nutrient densities are then used instead of the original nutrient intake values. Data for both the test and reference methods may be transformed in this way before examining correlations.

An alternative and sometimes preferable procedure for energy-adjusting nutrient intakes is to use linear regression with total energy intake as the independent variable (x) and intake of the nutrient of interest as the dependent variable (y) (Willett 1998). In cases where the nutrient variables are skewed, they should be transformed to improve normality prior to their use in the regression. The energy-adjusted nutrient intake of each subject is determined by adding the residual—that is; the difference between the observed nutrient values for each subject and the values predicted from the regression equation—to the nutrient intake corresponding to mean energy intake of the study population (Figure 6.1). Data for both the test and reference methods may be recalculated in this way.

FIGURE 6.1

CALCULATION OF THE ENERGY-ADJUSTED INTAKE USING THE REGRESSION LINE WITH THE NUTRIENT OF INTEREST AS THE DEPENDENT VARIABLE.



Note: The residual for each subject is added to the predicted nutrient intake for a subject with an energy intake equal to the mean for the group. Subjects such as 'N' have negative residuals. See, e.g., Willett (1998).

Several limitations have been noted when using Pearson correlation coefficients as a measure of agreement in dietary validation studies. These limitations have been discussed in detail by Bland and Altman (1986). They include giving an overly optimistic or inflated measure of agreement between the test and reference method, and providing a measure of the strength of the relationship rather than a measure of the extent of the agreement. Further, the degree of correlation, and the calculated *r*, are affected by the characteristics of the study population. For example, when the between-subject variation in the measured nutrient intakes is large, then the correlation generated will be higher than that for a group with a more limited range of intakes, and thus give a lower between-subject variation. Such an effect may be apparent when comparing the strength of correlations between the test and reference method for males versus females. Because males tend to eat more than females, their nutrient intakes tend to have a wider range than females, resulting in an apparently higher correlation between intakes for the test and reference methods. However, the high correlation is spurious, and provides no indication as to whether the agreement between the test and reference method is better for the males or females.

When variables are not normally distributed, nonparametric correlation coefficients (e.g., the Spearman rank correlation) can be used, although the same limitations apply as those itemized for the Pearson correlation coefficients. Spearman rank correlation coefficients can also be used when the primary objective of the validation study is to investigate how well the test method ranks the subjects, rather than to assess the level of agreement between the test and reference methods.

The intraclass correlation (r_I) can also be used instead of the Pearson correlation coefficient because it is a better measure of association for interval measurements. The intraclass correlation takes into account the extent of the disagreement within pairs and the degree of correlation. Values for r_I are normally less than those for *r*; and values above 0.4 indicate agreement. Details are given in Nelson (1997).

In view of these limitations, it is not advisable to use only correlation analysis to assess the relative validity of a dietary assessment method. Other measures of agreement between the test and reference method must also be used. These may include contingency tables, mean and standard deviation of the difference, and analyses of surrogate categories.

Contingency tables (cross-classification) involve classifying subjects into broad categories, usually thirds (tertiles), fourths (quartiles), or fifths (quintiles) of intake by the test and reference method. The percentage of subjects correctly classified into the same category and grossly misclassified into the opposite category is calculated. This provides an indication of how well the 24-h recall method separates the subjects into classes of intake and thus provides an estimate of the relative validity of the test method.

Cross-classification does, however, have limitations. In particular, the percentage agreement will include agreement that occurs by chance. This limitation is best circumvented by using Cohen's weighted kappa statistic (k_w). However, the magnitude of k_w depends on the number of categories used and what weightings are applied, as well as the relative validity (Cohen 1968). Further, the values for k_w, like the correlation coefficient, also depend on the characteristics of the study population. Hence, the use of k_w is not recommended by all investigators.

Another approach, advocated by Bland and Altman (1986), uses the mean difference and standard deviation of the difference between the test and reference method for each nutrient, followed by the calculation of the 95 percent confidence limits (i.e., mean difference + 2 SDs) for the difference between the two methods. A judgment can then be made as to whether the agreement between the test and reference method is acceptable. This method does not make any assumptions about whether the test or reference method is better. However, when the dietary intakes of the test and reference methods do not correspond to the same day, then lack of agreement may be due to normal day-to-day variability in intakes. The respondent burden is often too high to collect data on a sufficient number of days to estimate an individual's "usual intake", which would minimize the bias.

Willett (1998) advocates the analysis of surrogate categories. This approach involves assigning individuals to a category (e.g., a quintile or quartile) according to the intake of a specific nutrient as estimated by the test method. Next, the mean intake in each quintile is calculated, using the nutrient intake for each subject as determined by the reference method. This gives an indication of the "true" or reference method nutrient intakes that are equivalent to the test method quintiles. One-way analysis of variance followed by Tukey's test can then be used to determine whether the mean intakes of the quintiles are statistically significantly different. If the test method is valid, the differences should be significantly different, and the means should change regularly from the top to the bottom category.

Because the analysis of surrogate categories involves calculating the mean intakes for a group—i.e., each quintile or quartile—it does not require multiple replicate days of intake per individual to represent the "truth". Even a single day of intake will provide unbiased estimates of the actual values for these categories. A discussion of the advantages and limitations of all these statistical approaches is given in Willett (1998).

6.3 Assessing Reproducibility

The interactive 24-hour recall method is considered reproducible (precise and reliable) if it gives very similar results when used repeatedly in the same situation. Reproducibility is a function of random measurement errors, uncertainty resulting from true variation in daily nutrient intakes, and variability introduced by a variety of other confounding factors (e.g., age and sex, season, chronic illness or dieting). Even if the random measurement errors and confounding factors are minimized, uncertainty in the estimation of usual nutrient intakes still remains. For example, although the dietary survey results from two separate occasions may disagree, the method may not have poor reproducibility: the food intakes may indeed have changed. Conversely, even if the dietary assessment method appears to have high reproducibility using a test-retest design, it does not necessarily produce the correct answers. Reproducibility may be high, even if some subjects consistently under- or overestimate the portion sizes consumed. Hence, a method may have good reproducibility but poor validity. In contrast, a method with good validity cannot have poor reproducibility (Nelson 1997).

Reproducibility is determined using a test-retest design in which the same dietary method is repeated on the same subjects after a pre-selected time interval (Box 6.8). The selection of the time interval depends on the time frame of the dietary method used. Care must be taken to avoid the second measurement being influenced by the earlier one through recollection of the first interview. The effects of season on changes in food habits over time must also be avoided. In low-income countries, the effects of season on food availability, and thus nutrient intakes, may be significant. An interval of about 2 weeks between the first and second set of recalls should address both of these problems.

BOX 6.8

Assessing the Reproducibility of the Interactive 24-hour Recall

1. Visit the households of the validation respondents, establish a rapport with them, and explain the purpose of the study.
2. On the day before the intake recording day, distribute the picture chart, bowl and plate to each respondent in his or her own home.
3. Instruct the respondents to use a separate bowl and plate for eating their food on the next day and show them how to complete the picture chart. Remind the respondent when the intake recording day starts and ends (i.e., from midnight tonight until midnight tomorrow).
4. Set up an appointment to visit the respondent in his or her home 2 days later for the recall interview.
5. On the day of the 24-hour recall interview, visit the household and conduct the 24-hour recall following the procedures outlined in Boxes 5.2 to 5.4.
6. At the end of the interview, check the recall for completeness and clarity (Box 5.5).
7. Set up another 24-hour recall appointment for another pre-selected day.
8. On the day before the intake recording day, distribute the picture chart, bowl, and plate to each respondent. Instruct the respondents on their use on the next day.
9. Two days later visit the household and conduct the 24-hour recall, again following the procedures outlined in Boxes 5.2 to 5.4.
10. At the end of the interview, check the recall for completeness and clarity (Box 5.5).
11. Repeat steps 7 to 10 until all the recalls are completed.
12. Exactly 2 weeks later, return to the same households and repeat steps 1 to 11, collecting the recalls on the same days of the week as those collected 2 weeks earlier.

In general, the reproducibility of a 24-hour recall method will depend on the population group under study, the nutrients of interest, the techniques used to measure the quantities of foods consumed, and the between-subject or within-subject variation.

6.4 Statistical Assessment of Reproducibility

The statistical assessment of reproducibility, like validity, can also be assessed on a group or individual basis. Again, *t*-tests, or the nonparametric Wilcoxon's signed rank test if the data are not distributed normally, are commonly used to assess agreement between nutrient intakes on a group basis. No significant difference between the means or the median intakes of the groups for the two sets of data (the first vs. second interviews) is taken to indicate agreement. However, the confounding effect of within-subject variation on usual nutrient intakes is not taken into account when a *t*-test or the Wilcoxon's signed rank test is used (see Chapter 11 for more discussion of this).

When within-subject variability is large relative to between-subject variation, the power of the *t*-test will be reduced. As a result, non-significant differences in group mean intakes may not necessarily indicate good reproducibility. Rather, they may instead indicate the confounding effect of large within-subject variation as judged by a large coefficient of variation (standard deviation divided by mean) (IOM 2000).

For testing individual agreement, the simplest method is to calculate the percentage of misclassification by comparing the number of pairs with exact agreement or agreement within a defined amount, as described in Section 6.2. This approach ignores the fact that a certain amount of agreement inevitably occurs by chance alone, but this limitation can be overcome by using Cohen's weighted kappa statistic (k_w), as noted in Section 6.2.

Another approach is to use either Pearson's or Spearman's correlation analysis, depending on the distribution of the data (as discussed in Section 6.2),

to assess agreement on an individual (within-pair) basis. Alternatively, intraclass correlation coefficients, which correct for the number of chance expected agreements, can also be calculated. High correlation coefficients relating nutrient intakes on the two separate occasions are taken as indicative of good overall agreement between the two sets of nutrient data.

Both parametric and nonparametric correlation coefficients quantify the extent of the linear trend relating the two sets of results, and not agreement. Additionally, sources of bias in one of the replicates may not be revealed by correlation analysis. For example, assume that results for the second replicate were exactly 10 percent higher than those obtained on the first occasion. Analysis will indicate perfect correlation (r=1.0) between the two replicates, but there is far from perfect agreement. A further limitation stressed by Altman et al. (1983), is that the correlation coefficients cannot be judged on a null hypothesis basis of no correlation; there is an *a priori* reason to believe that the methods are positively correlated (Gibson 2005). People tend to eat similar foods from day-to-day; hence, some agreement is to be expected.

None of the correlation procedures noted above takes into account the confounding effect of within-subject variation on usual nutrient intakes. Its effect, if it is large relative to between-subject variation, is to reduce the absolute value of the correlation coefficient relating pairs of individual nutrient intakes. Hence, the reproducibility of the method may be incorrectly interpreted. A method is available for correcting the calculated correlation coefficient, provided the sample size is at least 100. This method is discussed in Section 11.6.

Additional statistical tests that can be used to assess reproducibility include the mean and standard deviation of the difference and analysis of variance. Bland and Altman (1986) have recommended the use of the mean difference and standard deviation of the differences between the two replicates for comparing nutrient intakes at an individual level, as described in Section 6.2. This approach provides information immediately about the direction of bias. A plot of the individual differences against the mean level of intake can indicate if the bias is constant across all levels of intake. They also suggested calculating the 95 percent confidence limits for the difference between the two replicates, as noted in Section 6.2. A judgment can then be made as to whether the agreement reached between the two replicates is acceptable. Not all investigators, however, advocate the use of this approach.

The preferred statistical approach for estimating reproducibility of any dietary method involves analysis of variance (ANOVA). This procedure assesses the differences, if any, in the group mean intake of each nutrient between the replicates, and it can be used to identify and estimate between- and within-subject variability. The variance ratio (the ratio of within-subject to the between-subject variation) can then be calculated. Once estimates of the between- and within-subject variability have been calculated using ANOVA, they can be used to estimate either or both the standard error of the group mean intake $\sqrt{\frac{\sigma^2}{n} + \frac{\delta^2}{mn}}$, and the standard error of the individual's mean intake $\sqrt{\frac{\delta^2}{m}}$

where n is sample size, m is number of days measured, σ^2 is between-subject variance, and δ^2 = within-subject variance. It is advisable to seek assistance from a statistician when using analysis of variance in this way to estimate the with-subject and between-subject variation.

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Chapter 7 | Compiling a Local Food Composition Table

What will you learn from this chapter?

- How to compile the best nutrient estimates from existing food composition values;
- How to impute missing food composition values;
- How to calculate nutrient values for mixed dishes from recipes;
- How to check the quality of your food composition table; and
- How to select, sample, and prepare food samples for chemical analysis.

Food composition tables are used for converting food consumption data into energy and nutrient data. The food composition values ideally should represent the average composition of a particular foodstuff on a year-round nationwide basis. In practice, however, food composition values are often of variable quality and are derived from many different sources. Greenfield and Southgate (1992) present detailed guidelines on the production, management, and use of food composition data.

Food composition tables for evaluating the adequacy of iron and zinc intakes should contain iron and zinc values as well as values for dietary components known to influence the bioavailability of iron and zinc. Consequently, the tables should include values for the major known absorption enhancers—animal protein and ascorbic acid—and the major absorption inhibitors—calcium, phytic acid, dietary fiber (for energy and protein) and, if possible, polyphenols. Proximate nutrients such as total protein, fat, and carbohydrate as well as energy should also be included.

Readers of this manual working in developing countries are advised to consult an international dietary assessment system developed by the University of California at Berkeley now known as the WorldFood 2 Dietary Assessment System. (See Appendix E for supplier information). The dietary assessment system includes values for 53 nutrients and associated dietary components (including phytic acid and dietary fiber) for 1800 foods consumed in Egypt, Kenya, Mexico, Senegal, India, and Indonesia. It also includes a computer program that calculates energy and nutrient intakes (including total and available iron and zinc). (See Sections 9.1 and 9.2 for more detail.)

All the food composition values included have been carefully reviewed and represent the best estimates of the nutrient composition for each food item at the time the data were compiled; the sources are fully documented. The values were compiled from published food composition tables or imputed (i.e., derived from data for another form of the same food or for a similar food) when no suitable analytical data were available. There are no missing values. Food composition values for additional specific foods can be used to augment the WorldFood 2 Dietary Assessment System, if necessary.

Four methods can be used to augment existing food composition tables with values for local food items from a country or region. Options include:

- A best estimate can be compiled from other food composition tables or the published literature.
- Missing values can be estimated from data for similar foods (i.e., substitution of data).
- Nutrient values of mixed dishes can be calculated from recipes.
- Direct chemical analysis can be used for locally collected foods.

These methods are reported in detail in Murphy et al. (1991) and Rand et al. (1991), and are briefly summarized below.

7.1 Compiling the Best Food Composition Estimates

The food composition values presented in food composition tables are sometimes uneven in quality. The values for individual food items are drawn from a variety of sources ranging from the food industry, published and unpublished research, contract research, and government research laboratories. Consequently, care must be taken to ensure that the food composition values compiled for local food items represent the best estimates of the nutrient composition. Murphy et al. (1991) provide a detailed description of the development of a research food composition database for use in rural Kenya.

Reliable food composition tables contain a complete description of each food item, including its common name with local synonym and scientific taxonomic name, with variety listed too, when known. Information on the sampling and handling protocols, number of samples analyzed, and the methods and quality control procedures used for the nutrient analysis should also be included.

Discrepancies in the nutrient content for any given food will exist among food composition tables. Significant errors may be present as the result of:

- the use of inappropriate analytical methods for the analysis of the nutrient in the food;
- errors in the analytical procedure used in the analysis of the nutrient;
- the lack of standardized conversion factors for calculating energy and protein content of foods;
- inconsistencies in terminology used to express certain nutrients;
- incorrect description of individual food items; and
- inconsistencies resulting from genetic, environmental, food preparation, and processing factors.

To assess the reliability of food composition values in preexisting food composition tables, a rating system has been developed based on five quality criteria (Schubert et al. 1987). Where possible, when compiling the best estimates for local food items from preexisting food composition values, apply the same five quality criteria (Table 7.1) to generate a quality index (QI) for each nutrient value for a food item as outlined in Box 7.1. The quality criteria consist of the:

- number of samples on which values are based;
- the analytical method used;
- sample handling;
- sampling protocol; and
- analytical quality control.

Each quality criterion is further subdivided into four ratings ranging from 0 (unacceptable) to 3 (most desirable), as shown in Table 7.1.

TABLE 7.1

Details of Ratings for Quality Control Criteria Used to Assign a Quality Index (QI) to Each Value for a Specified Component and Food Item

Criteria	Rating = 3	Rating = 2	Rating = 1	Rating = 0
Number of samples	>10; standard deviation, standard error, or raw data	3–10	1–2; explicitly stated or not specified	–
Analytical method	Official or recommended method -full details; 95–105% recoveries; concentration of food component analyzed above detection limit of method	Some method details; incomplete validation studies for foods analyzed; 90–110% recoveries	Not official method and only partially described; 80–90% or > 110% recoveries	No documentation of method; no CRMs given; no validation
Sample handling	Complete documentation of procedures; analysis of edible portion only; validation of homogenization method; details of food preparation, storage, and moisture changes monitored	Pertinent procedures documented, including analysis of edible portion only; some details not reported	Limited description of procedures, including evidence of analysis of edible portion only	Totally inappropriate procedures or no documentation of criteria pertinent to food analyzed
Sampling protocol	Multiple geographical sampling with complete description; sample is representative of brands or varieties commonly consumed or commercially used	One or two geographic areas sampled; sample is representative	Sample representative of small percent of study population and/or origin not clear	Not described or sample not representative
Analytical quality control	Optimum accuracy and precision of method well documented	Documentation of accuracy and precision of method; acceptable accuracy and precision	Some description of mini-mally acceptable accuracy and precision of method	No documentation of accuracy and/or precision

Note that the Quality Index should be set at zero when three or more individual ratings are zero, or when the analytical method is rated as zero. Otherwise, the five ratings can be averaged for any food component in a specific food item. The final grand mean value equivalent to the best estimate for a food component for the food item of interest is represented by the average value derived from all the corresponding food composition data that scored a QI of 1.0 or greater (see Box 7.1).

BOX 7.1

Calculating the Best Estimate for Any Food Component in Any Food

1. Locate food composition data for the food items of interest, preferably from regions geographically and climatically comparable to your own.
2. Check to ensure that the names (i.e., common and scientific name, such as genus, species, variety) of the foods on which the data are based match as closely as possible the names of the food being considered.
3. Check the number of samples analyzed for the food item under consideration and whether the standard deviation, standard error, or raw data are given. Assign a rating based on the rating criteria given in Table 7.1.
4. Assess the adequacy of the analytical methodology used, and rate the results accordingly. See Section 7.7 for methods for trace element analysis.
5. Check whether sample handling is documented adequately and assign a rating accordingly.
6. Check whether details of the sampling protocol are included, such as details of geographical areas, brands, and varieties; assign a rating accordingly.
7. Assess whether analytical quality control procedures were adequate and rate accordingly. Refer to Sections 7.1 and 7.7 for additional information.
8. Assign a quality index (QI) for each set of ratings. QI is set at zero when more than three individual ratings are zero or when the analytical method is rated as zero; otherwise, average the five ratings.
9. Calculate the grand mean value for any given food component from all data with a QI of 1.0 or greater. Express in terms of per 100g edible portion of food.
10. The grand mean value represents the best estimate for any given food component for any given food.
11. Enter the best estimate item in your local food composition table together with the minimum and maximum concentration.
12. Record the number of acceptable studies used to determine the best estimate and the reference citations for each of the acceptable studies.

7.2 Missing Values in Food Composition Data Tables

Ideally, the food composition table will contain a value for each nutrient under study for every type of food (including types of preparation) eaten by any of the participants. Any missing values must be replaced with imputed nutrient values by using data for another form of the same food or a similar food. Zero values should be assigned when the nutrient is not present in any detectable amount in that food.

BOX 7.2

Imputing Missing Nutrient Values from Data for Similar Foods

Convert nutrient content from a dry weight to fresh weight basis:

1. Record the mean content for zinc in dried white beans (e.g., 3.67mg/100g).
2. Record the moisture content of dried beans (e.g., 8 percent).
3. Record the moisture content of fresh beans (e.g., 91 percent).
4. Calculate the zinc content of 100g fresh beans as follows:

$$\frac{\text{Zn content of dry food} \times (100 - \text{moisture content of fresh food})}{(100 - \text{moisture content of dry food})}$$

$$\frac{3.67 \times (100 - 91)}{(100 - 8)} = 0.36\text{mg}/100\text{g}$$

OR

Calculate the nutrient content of cooked food from data on raw food:

1. Record the mean content for zinc in dried white beans (e.g., 3.67mg/100g).
2. Record the cooked yield (as percentage) from dried white beans, using a yield of 240 percent for water retention taken from the *USDA Handbook No. 102* (Matthews and Garrison 1975) (i.e., 100g white beans yields 240g when cooked with water).
3. Record the average retention of zinc in the cooked beans (e.g., 90 percent, when taken from the USDA [2003a] or from a value from the country under study).
4. Calculate the zinc content of 100g cooked beans as follows:

$$\frac{3.67 \times 90\%}{240\%} = 1.38\text{mg}/100\text{g}$$

Adjust the nutrient content of a food for difference in moisture content:

1. Record the mean content for zinc in smoked haddock, steamed (e.g., 0.50mg/100g).
2. Record moisture content of smoked haddock, steamed from the same source (e.g., 71.5 percent).
3. Record moisture content of smoked haddock in *local* food composition table (e.g., 60.0 percent).
4. Adjust zinc content of smoked haddock to moisture value in *local* food composition table as follows:

$$\frac{\text{Zn content of smoked haddock} \times (100 - \text{moisture content of the } \textit{local} \text{ smoked haddock})}{(100 - \text{moisture content of haddock in the borrowed food composition table})}$$

$$\frac{0.50 \times (100 - 60.0)}{(100 - 71.5)} = 0.70\text{mg}/100\text{g}$$

Several procedures are available for imputing values (Murphy et al. 1991, Rand et al. 1991, Schakel et al. 1997). For example, data for cooked foods can be calculated from those for raw foods, and data for fresh weight can be derived from those for dry weight by using the procedures outlined in Box 7.2. For certain foods, the actual moisture content may be different from that shown in the food composition tables, a consequence of differences in local processing or storage conditions. In such circumstances, the nutrient values can be adjusted for the different moisture content. Moisture values are available in most food composition tables; an example of the calculations is shown in Box 7.2. For these calculations, information on refuse, yield, and nutrient retention is often required. Several sources of these data are available (Merrill et al. 1966, Paul and Southgate 1978, Matthews and Garrison 1975, Karmas and Harris 1987, Bergström 1994, Banjong et al. 2001, USDA 2003a). In the U.S. Department of Agriculture (USDA, 2003a) table, the retention of 25 vitamins, minerals, and alcohol during heating and food preparation is provided, along with factors for seven additional food components: folic acid, food folate, β-carotene, α-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin. This table can be accessed at the USDA website available at: www.nal.usda.gov/fnic/foodcomp/

All imputed food composition values should be documented in the augmented food composition table, and should include their source (with the specific journal citation) and the calculation procedure. A summary of the number of imputed values in relation to the total number of food values for the non-recipe foods should also be provided in the final food composition table.

7.3 Calculating Nutrient Values of Mixed Dishes

The most common method for calculating food composition values for mixed or multi-ingredient dishes is to calculate the nutrient content from recipe data, preferably by using nutrient values for the cooked individual ingredients, where appropriate (Box 7.3). The recipes selected should reflect food as prepared by the population or subgroup being studied and, if possible, should be collected directly from the respondents during the 24-hour recall interview and recorded on the special recipe form (Table 5.4), as discussed in Section 5.6.

When respondents are unable to provide the recipe details for mixed dishes, then data for an average recipe for each mixed dish consumed in the local study area should be used. In cases where average recipes for representative local mixed dishes are not available, arrangements should be made for five to ten women in the study area to cook each required recipe several times using locally available ingredients, as described in Section 5.7. Details on how to construct an average recipe for mixed dishes are given in Box 5.11. Torelm et al. (1996) describe the sources of variation in major nutrients and minerals that may occur when this approach is used.

Essential features of a recipe that must always be recorded on the recipe form (Table 5.4) are:

- a descriptive list of all ingredients, including flavors and spices;
- the method of preparation and cooking, including use of fats, oils, condiments etc.;
- the amount of each raw ingredient as edible portion;
- the final weight (or volume) of cooked food, if available; and
- the amount of the mixed dish consumed by the participant.

For the calculation of the nutrient values for the mixed dishes recorded on the recipe form, the amount of each raw ingredient must first be converted into weight equivalents using the methods outlined in Section 5.5. Next, the nutrient content of each raw ingredient must be calculated as either raw or cooked, depending on the nutrient composition data available, as described in Table 7.2. Once calculated, the nutrient content of each raw ingredient must then be adjusted by yield factors that account for both changes in its weight and any gains or losses in its nutrient content during cooking.

The yield and retention factors should be based on habitual cooking methods in the region. If local values are not available, however, then yield and nutrient retention factors from other sources must be used. Available sources include Merrill et al. (1966), Matthews and Garrison (1975), Paul and Southgate (1978),

Bergström (1994), Banjong et al. (2001), and USDA (2003a), as noted earlier. The final step involves converting the nutrient values for the total cooked recipe to values per 100g. An example of how to calculate the nutrient values of a mixed dish and the final weight of the cooked dish (if you do not have a measurement of this) is shown in Table 7.2. Computer programs are also available for these recipe calculations (Day 1980).

BOX 7.3

Calculating Missing Nutrient Values for Mixed Dishes from Recipes

1. Select or construct (see Box 5.11) an appropriate recipe.
2. Weigh each ingredient and subtract the weight of any inedible part to yield the weight of the edible portion.
3. If the nutrient data for *cooked* ingredients are available in the food composition table, calculate the cooked weights of the ingredients using local yield factors or those from Matthews and Garrison (1975) to adjust from raw to cooked weights, then compile nutrient content of the weight of each cooked ingredient. For more details, see Table 7.2 and Box 5.10, Step 1.

OR

4. a) If the nutrient data for cooked ingredients are not available, use the nutrient data for *uncooked* ingredients, and calculate the nutrient content of the weight of each uncooked ingredient. Then apply for each uncooked ingredient, local nutrient retention factors for nutrient losses or gains during preparation, cooking, or both, or those from Merrill et al. (1966), USDA (1984), or Bergström (1994). For more details, see Table 7.2.

   b) Calculate the total raw weight of the recipe before cooking by summing the weights of all edible portions of the raw ingredients (including water).

   c) Adjust the total raw recipe weight for changes in water and fat when the whole dish is cooked to obtain the final weight of the whole cooked dish. See Table 5.7 for examples of weight change factors after cooking.

5. Calculate the nutrient content of the total recipe by summing the nutrient values of the weight of each cooked ingredient.
6. Convert the nutrient values for the total cooked recipe to values per 100g. For more details, see Table 7.2.

From this discussion, it is apparent that the calculations of the nutrient content of cooked mixed dishes based on food composition values are estimates. The calculations normally rely on yield and nutrient retention factors taken from published tables, which are probably not a true reflection of the actual factors that vary according to the length and temperature of cooking, type of cooking equipment used, and surface area of food contact exposure, among other factors. (Rand et al. 1991). Where local foods are collected and analyzed directly for nutrient content, studies of nutrient retention for common cooking methods may also be conducted, as described in Section 7.7.

7.4 Checking the Quality of the Food Composition Table

After the local food composition table has been compiled, it must be checked to ensure its validity. Care must be taken to ensure that values correctly represent the levels of the food components in the foods, such as that foods and food components are carefully identified, origins of the data identified, and precision of the data is not misrepresented. Three levels of validity checks can be used to check the quality of the food composition table. These are:

- Level 1 - between or across nutrients, but within foods;
- Level 2 - between or across foods and within nutrients; and
- Level 3 - checks of the whole database.

An example of a check at Level 1 is to ensure that the sum of the carbohydrate components (starch, sugars, fiber) does not exceed total carbohydrates. Similar checks include calculating the energy content of each food from the protein, fat, and carbohydrate values and the Atwater conversion factors, and comparing these values with the recorded energy value; comparing the sum of fatty acids with the total fat; and comparing the sum of individual amino acids with total protein. Additional examples are shown in Box 7.5. Discrepancies between the values calculated from the algorithms and the recorded database values that fall outside acceptable ranges should be examined. Further details are provided by Buzzard et al. (1995).

Level 2 checks between or across foods can be used to verify that similar foods have comparable nutrient levels (e.g., calcium content of various milks). This task can be automated by setting edit limits which define the usual minimum and maximum nutrient values per 100g of food within a food group. Values that fall outside the limits are flagged and subsequently investigated for possible errors.

Level 3 checks of the whole database should be conducted. A diagnostic model developed by Hoover and Perloff (1984) can be used for this purpose, whereby the energy and nutrient content of a reference dietary record is calculated using the local food composition table and the results compared with those based on the USDA nutrient database (see Hoover and Perloff 1984).

It may also be useful to compare nutrient contents against those published in another reliable source. Where there is lack of concordance between values, verify that differences are real and not due to error.

TABLE 7.2

Example of Calculating the Nutrient Values of *Githeri* (bean and maize porridge) from Kenya

Recipe: Ingredients: maize, dry, raw, 2 Kimbo tins (1kg size); kidney beans, dry, raw, 1 Kimbo tin (1kg size); water 3 large Kimbo tins (2kg size) Note: Kimbo tins are used here as volume measures.

Combine ingredients and cook the mixture over a fire for 1 hour or longer.

In the following, it is assumed that the nutrient database includes values for the *raw* ingredients per 100g.

Step 1: Convert volume measures (Kimbo tins) to weight equivalents, and multiply the nutrient values (for raw ingredients per 100g) by the gram weight of the ingredient divided by 100. For example, for energy (kcal):
maize: (1402kJ/100g) × 1080g per tin × 2 tins = 32083kJ
beans: (1368kJ/100g) × 1000g per tin × 1 tin = 13682kJ

Step 2: For each food item, readjust the quantities of those nutrients that are lost during cooking using nutrient retention factors. As *githeri* is commonly cooked for one or more hours, there will be destruction of heat-labile vitamins (e.g., vitamin C and thiamin); some losses may exceed 50 percent. Add up the levels for each specified nutrient to obtain the totals for the recipe before cooking.

Step 3: Determine weight of recipe before cooking.
maize: 1080g per tin × 2 tins = 2160g
beans: 1000g per tin × 1 tin = 1000g
water: 2500g per tin × 3 tins = 7500g
total raw weight = 10,660g

Step 4: Determine weight of recipe after cooking (if you do not have a measurement for this) using yield factors. The average cooked weight of *githeri* is approximately 75 percent of its raw weight: 0.75 × 10,660g = 7995g cooked weight

Step 5: Determine the nutrient levels per 100g. Divide all the nutrient totals by the total cooked weight divided by 100 (i.e., 7995g/100) to give nutrients per 100g. For example, for energy: 45765kJ (from step 1)/(7995/100) = 572kJ/100g.

This table is based on Rand et al. 1991.

BOX 7.4

Checking the Quality of the Food Composition Table

Level 1

- Calculate the energy value of selected foods from the energy contributed from protein, carbohydrate, fat, and alcohol by using the generalized Atwater factors of 16.7kJ/g (4.0kcal/g) for protein, 16.7kJ/g (4.0kcal/g) for total carbohydrate, 37.7kJ/g (9.0kcal/g) for fat, and 29.3kJ (7.0kcal/g) for alcohol. Compare the value you calculate with the total energy value given in the food table.

Level 2

- Make a list of particular nutrients arranged by individual food type (e.g., various milks) and food groups. If any value deviates substantially (e.g., > 2 standard deviations) from other values in that food type or food group, ask a nutritionist to check whether the value is correct.

Level 3

- Check the entire nutrient database by calculating the nutrient content of a reference dietary record, available in Hoover and Perloff (1984). Compare your calculated results with those from a reliable nutrient database such as from the USDA. The USDA nutrient database is available on the following website: www.nal.usda.gov/fnic/foodcomp/ Verify that any of the differences noted come from true differences in the local food or new analysis and not from errors in compiling the food table.

7.5 Selecting Foods for Chemical Analysis

When nutrient composition values for a local staple food cannot be derived by the methods outlined in Sections 7.1 to 7.3, values must be obtained by direct chemical analysis. This approach is especially desirable for certain trace elements (for example; Se, I, Zn) because their content in plant-based staples is often dependent on local trace element levels in soil, agronomic practices (such as the amount and types of fertilizers used), methods of food preparation and processing, stage of maturity, and differences in species. Food analysis is, however, costly and time consuming, so a balance must be struck between making use of existing values (where possible) and carrying out chemical analysis.

A strategy has been proposed for selecting foods for food composition analysis. Priority should be given to analyzing foods that meet all four criteria outlined in Box 7.5.

BOX 7.5

Selecting Foods for Chemical Analysis

Choose foods for analysis, giving priority to those foods that:

- have inadequate or non-existent data for the food component of interest,
- have inadequate data on the concentration of the food component in foods as eaten,
- form a significant component of the diet of the study group, and
- contribute significantly to the intake of the dietary component in the local diet.

To assess whether the food contributes significantly to the overall intake of the nutrient under study, both the portion size and frequency of consumption of the particular food item by the population should be taken into account. To estimate the amount of each food consumed per day, the average daily frequency should be multiplied by the average portion size by the sex and age group of interest. This method takes into account food items that are consumed frequently but in small amounts. When differences in food processing, preparation, or cooking are not likely to affect the concentration of the food component of interest, food items prepared in different ways can be combined for analysis. Note that milling may markedly reduce both the phytate and mineral (including iron and zinc) content of cereal staples, whereas germination, fermentation, and soaking may all reduce the phytate content of cereals and legumes (see Section 7.7). Hence, it is essential to adjust the iron, zinc, and phytate values of any cereal or legume-based foods undergoing these food preparation and processing practices.

7.6 Food Sampling, Transport, and Handling Procedures

Once the foods have been selected for food composition analysis (see Box 7.5), a protocol must be set up to ensure that representative samples of each of the food items are collected. The sampling protocol will vary both with the food item and with the food component. Factors that must be considered when devising a sampling protocol specifically for trace element analysis include genetic variation, seasonality, region, the population consuming the food, and processing and preparation techniques. In addition, the preservation state of the food, its geographical source, ripening practices, the part of the plant or animal used, and fertilizer application should also be considered when devising a protocol for trace element analysis. The use of certain fertilizers may, for instance, increase the content of zinc, iodine and selenium (but not iron) of cereal grains grown on soils deficient in these trace elements. Norwegian investigators reported major differences when Finnish instead of Norwegian selenium food composition data were used to calculate daily selenium intakes (e.g., a discrepancy of 86 μg/d vs. 18 μg/d) (Ahola 1991).

Theoretically, a statistical formula should be used to estimate the number of primary food samples required to ensure the analyzed value is truly representative of the food item under consideration and has an acceptable level of precision. In practice, all the data required to perform these statistical calculations (i.e., mean, standard deviation, and level of acceptable error for the nutrient) are rarely available. Instead, at least 10 primary samples per food type are generally collected, each about 100 to 500g (Box 7.6). This number is thought to be large enough to reflect the variability in composition for most foods. In some cases where variation is thought to be small, 5 to 6 primary food samples may be collected.

BOX 7.6

Establishing a Protocol for Sampling and Preparing the Food Samples for Analyses

1. Purchase 10 primary samples of each food item (each weighing at least 450 grams, unless specified otherwise). Each food must be obtained at the same time of year as the survey and in the form in which it is generally purchased—either for preparation by householder, or ready to eat. For community studies, collect primary samples from local markets and vendors and from householders in the survey villages. For national surveys a more comprehensive sampling protocol must be devised that takes into account possible regional variations.
2. Prepare edible portions of each primary food sample by using traditional local preparation, processing, or cooking methods, as appropriate. This step is best done by local village women using local utensils.
3. When analysis is to be performed on a composite sample instead of on each primary food sample separately, homogenize edible portions of all primary samples of perishable food products (e.g., fruits, vegetables, tubers, and fish) together in a blender to form a composite sample. Blender should have a nonstick coating and be fitted with a blade with nonstick coating to avoid adventitious contamination. If necessary, a known volume of distilled, de-ionized water can be added to facilitate homogenization. However, if this step is done, care must be taken to record the volume of the water added, because it too must be taken into account when calculating the moisture content.
4. Withdraw an analytical sample (approximately 450g) of the blended composite sample for subsequent major, trace mineral, and phytate analysis. Freeze-dry this analytical sample and store in a trace element-free plastic bag or polyethylene bottle.
5. Grind edible portions of all primary samples of non-perishable products (e.g., cereals, legumes, nuts, and seeds) for trace element analysis in an agate ball mill (See Appendix E) to avoid adventitious trace element contamination.
6. When analysis is to be performed on a composite sample instead of on each primary food sample separately, combine ground primary samples to form a composite laboratory sample and place in the center of a large sheet of trace-element free polyethylene.
7. Roll the ground sample diagonally across the sheeting twice in all directions. Ensure that the pile of material is turned over rather than caused to slide.
8. Form the ground material into a circular pile and divide it into quarters. Save the two opposite quarters only.
9. Mix and quarter the two opposite quarters again as noted in steps 6–8. Repeat until a final analytical sample of approximately 450g remains.
10. Package final analytical sample of ground material in a sealed trace-element free polyethylene bag or container (See Appendix E).
11. Record the source and detailed description of each dried/freeze dried analytical sample, and store at between 4 to 8°C until analysis for trace minerals and other dietary components (e.g. phytic acid and dietary fiber).

To determine the nutrient composition of cooked foods, each primary food sample is prepared as eaten using local utensils and traditional preparation, processing, or cooking methods. These procedures are best done in the village by local women, and may begin with removal of inedible outer leaves, rind, seeds, pits or bones. Use of cast-iron rather than clay pots may increase the total iron content of the food. Hence, cast-iron pots should only be used if they are the actual local utensils of the study area to ensure that the chemical analysis is always performed on the food in the form in which it is usually prepared and consumed.

Although it is preferable for each of the 10 primary food samples per food type to be analyzed separately to provide information on variability within one food type, in practice; a single composite made up of the 10 primary samples can also be used for the analysis to reduce the analytical costs. In some cases the single

composite can be made up of specific proportions of different cultivars or brands to obtain a single generic value for the database. For certain large food items such as pumpkin, where the contents may vary markedly, the edible contents should be finely chopped and only a portion from each primary sample of pumpkin should be retained as the laboratory sample and prepared for analysis.

To obtain a composite sample for the laboratory, the edible portions of all the primary samples of each perishable item (e.g., fruits, vegetables, tubers, roots, and fish) must first be homogenized in a mechanical blender as noted in Box 7.6, and a representative analytical sample—known as the aliquot—should be preserved by freeze-drying for subsequent major, trace element, and phytate analysis. Care should be taken to record the original and freeze-dried weight of each analytical sample. Another portion of the analytical sample (representing approximately 2g dry material) should be dried at between 95 to 100°C in a vacuum oven to a constant weight to calculate the moisture content of the analytical sample. Alternatively, a portion of the analytical sample may be dried to constant weight in an ordinary oven at 135°C. Details on how to determine the moisture content are given in Box 7.7.

BOX 7.7

Determining the Moisture Content in Collected Food Samples

1. For perishable food products, withdraw a second portion of the blended homogenate (Step 3, Box 7.6) for moisture analysis.
2. Weigh two dry empty aluminum dishes and record their weight.
3. Place two portions of the blended homogenate (representing approximately 2g dried material) onto an aluminum sample dish and reweigh.
4. Calculate weight of sample in each dish.
5. Dry each sample at between 95 to 100°C under vacuum to a constant weight. Alternatively, dry in an ordinary oven at 135°C until constant weight.
6. Cool dried samples in a desiccator for at least 1 hour. Next, once they reach room temperature, reweigh and record the final weights.
7. Calculate the weights of each dried sample.
8. Calculate the moisture content of each sample using the following equation:

$$\frac{(\text{weight fresh sample} - \text{weight dried sample})}{\text{weight fresh sample}} \times 100\%$$

For each ground non-perishable food product, repeat steps 2 through 8 in duplicate.

For nonperishable food items such as cereal grains, legumes, nuts, seeds and so on, the edible portions of all the primary samples first are ground for trace element analysis in an agate ball mill to avoid adventitious trace element contamination (for suppliers see Appendix E). Next, the ground primary samples are combined to form a composite sample by placing the entire composite sample in the center of a large sheet of trace element-free polyethylene sheeting, and then rolling the sample diagonally across the sheeting twice in all directions. The direction of rolling is altered in such a way that the pile of ground material is turned over rather than caused to slide. After mixing the ground material sufficiently, the pile is made approximately circular and divided into quarters. Only the two opposite quarters are saved, and again mixed and

quartered, and the process repeated, yielding a final laboratory sample of approximately 450 grams (Box 7.6). A portion of the remainder of the sample is saved for a moisture determination (discussed above) and the rest is discarded.

A complete description of each laboratory sample must be recorded. Details must include common names with local synonyms and scientific taxonomic names, including variety when known. Information on the locality, time of year, season, and place of collection; condition of the food item; and state in which it was purchased (e.g., raw, prepared, or frozen) or the growing conditions and stage of growth should also be recorded. An example of a form that could be used is given in Table 7.3. The protocol that was used for the sample handling in the laboratory must also be well documented. Care must be taken to ensure that the food sample is handled appropriately both before and on arrival in the laboratory, and any adventitious sources of contamination (e.g., soil or dust) removed. To prevent changes due to excessive gain in moisture during transport, the primary samples should be packed in sealed plastic bottles or bags made from trace-element free polyethylene materials (for suppliers see Appendix E). More details on food sampling for nutrient analysis are given in Greenfield and Southgate (1992).

TABLE 7.3

Example of Form for the Collection and Handling of Food Samples for Chemical Analysis

Name of collector _____

Sample no. _____ Date _____

Town _____

Local name of food _____ English name _____

Scientific name _____

Description _____

(For cereals, note whether whole kernel, flour, meal, % extraction, etc. For edible plants, note with or without skin, stems, roots, seeds, rind, etc. For meat and fish, note cut and whether with or without bone, skin, or fat, etc.)

Total weight on collection _____ (g) Nature of: inedible _____ ; edible _____ portion

Weight of edible portion _____ (g) Weight of inedible portion _____ (g)

Condition when purchased. Exposed to sun Yes ❑ No ❑ Open market Yes ❑ No ❑

Harvested, how long days ❑ weeks ❑ years ❑ Length of storage days ❑ weeks ❑ years ❑

Growth conditions: Soil type _____

Fertilizer _____ Other _____

Frequency of use in the diet:

Every meal _____ Daily _____

Weekly _____ Monthly _____

Handling in the laboratory Date d/m/y __/__/__

Method of preparation as for consumption (type, time, temperature, particle size) _____

Weight before cooking (e.g., raw chicken) _____ (g)

Weight and type of ingredient added in cooking (e.g., fat used in roasting) _____

Weight and nature of edible portion of prepared food (e.g., flesh, skin) _____

Method of mixing and reduction (e.g., grinding, homogenizing in blender) _____

Details of preparation of composite sample (if relevant) _____

Types of analytical samples stored (e.g., freeze-dried, frozen) _____

Modified from Greenfield and Southgate. 1992.

7.7 Chemical Analyses of Food Samples

The analytical methods selected for food composition analysis must be generally accepted, reliable, and practical (Horwitz 2002). Consideration should always be given to using methods specified by the Association of Official Analytical Chemists (AOAC) International, such as those available at their website: www.aoac.org

Care must be taken to ensure that all the analytical samples are homogeneous and finely ground prior to analysis; freeze drying followed by crushing and grinding are commonly done. Alternatively, some fresh food samples that contain a large percentage of water can be homogenized in a blender, as noted in Box 7.6. Details of other homogenization methods are given in Lichon and James (1990). Care must be taken to avoid adventitious contamination during the preparation and analysis of laboratory samples for trace element analysis. Precautions include using an agate ball mill or agate pestle and mortar for grinding; a blender coated with nonstick trace-element free material and fitted with blades similarly coated; 18-mega-ohm deionized water; ultrapure reagents, acid-washed glassware, and trace-element-free polyethylene materials for sample preparation and analysis. Suppliers of trace element free polyethylene materials can be found in Appendix E.

The methods available for analyses of food components vary and have been classified as adequate, substantial, or conflicting according to their accuracy, precision, sensitivity, and cost-effectiveness. Methods available for the minerals calcium, magnesium, and phosphorus, and trace elements, such as copper and zinc, are classified as adequate; and those for iron and selenium as substantial, whereas those for iodine and manganese are categorized as conflicting because their accuracy is only considered fair, the analysis is slow, and the analytical cost is high.

Flame atomic absorption spectrophotometry (AAS) is the most widely used method for mineral and trace element analysis of food samples. Graphite furnace AAS is particularly suitable for ultratrace elements such as chromium and manganese. Several multielement methods for trace element analysis, including instrumental neutron activation analysis (INAA), X-ray fluorescence, and inductively coupled plasma spectroscopy (ICP) have been developed. For some (e.g., INAA and X-ray fluorescence), matrix effects are small or nonexistent, and treatment of the sample by ashing or digestion is usually unnecessary, reducing the risk of contamination. Efforts continue to increase both the precision and sensitivity of these multielement methods. For analyzing iron and zinc at the levels in foods, AAS is the recommended method. It is rapid, the least expensive, and easiest to run, and requires less specialized staff than do the multielement methods.

Before AAS can be carried out, the analytical sample must be ashed to remove the organic material; dry ashing techniques are preferred for trace mineral analysis. Details of the procedure are given in Box 7.8.

BOX 7.8

Ash Foods for Analysis

1. Grind each analytical sample to a fine homogenous powder using an acid-washed agate ball mill (See Appendix E), if necessary.
2. Include a sample of a suitable certified reference material of similar matrix to the sample and certified for the nutrient of interest, and a sample of a pooled food sample with each batch of analytical samples.
3. Carefully weigh, to the nearest milligram, duplicate portions of approximately 2 to 10g of ground analytical sample (amount depends on the trace element content of the sample) into weighed, acid-washed 100mL Pyrex beakers.
4. Place the beakers in the muffle furnace as close to the center as possible, and cover with an acid-washed Pyrex watch-glass. Slowly raise the temperature of the muffle furnace to prevent the samples igniting. Ash overnight at 450°C.
5. Remove the beakers from the muffle furnace and place in a desiccator for at least 1 hour to allow to cool. NB: the ash should be clean and white in appearance. If traces of carbon are still evident, cool the beaker and add a few drops of hyperpure nitric acid (See Appendix E) to the ash, and then return it to the muffle furnace.
6. Dissolve the ashed food samples in 5 to 10mL of 0.1M HCl, depending on the amount ashed (See Appendix E for HCl suppliers). Heat gently.
7. Quantitatively transfer the dissolved ash into a volumetric flask with double distilled de-ionized water.
8. Make up to volume with distilled de-ionized water.
9. Mix thoroughly.

Once the organic material is removed by ashing, the residue is dissolved in dilute acid in preparation for analysis by AAS or ICP. Details of these analytical methods can be found in Horwitz (2002) and are not included here. All the analytical methods must incorporate, where possible, an analytical quality assurance system using certified reference materials (CRMs) to control for accuracy. Alternatively, in-house quality control materials can be used, provided their trace element concentrations have been established previously using CRMs. Table 7.4 presents a list of CRMs suitable for food composition analysis together with the relevant suppliers.

To assess the adequacy of intakes of trace elements such as iron and zinc, food composition values for dietary modifiers known to influence their bioavailability must also be compiled. Of the important absorption inhibitors of iron and zinc, food composition values for polyphenols (for iron only), and phytic acid are often incomplete or vary widely among food composition tables for any given food, depending on the analytical method used. Selection of the most appropriate method for the analysis of phytic acid (inositol hexaphosphate) is critical. In the past, the method most frequently used was an anion-exchange column separation of phytate followed by acid hydrolysis and spectrophotometric determination of liberated inorganic phosphorus (Harland and Oberleas 1986). This method is not specific for inositol hexaphosphate but includes lower inositol phosphates as

TABLE 7.4

List of Certified Reference Materials Suitable for Food Composition Analysis

Certified Reference Materials 1549 Non-fat milk powder 1566a Oyster tissue 1567a Wheat flour 1568a Rice flour 1577b Bovine Liver Above certified for Ca, Cr, Cu, F, I, Fe, Mg, Mn, Ni, P, Se, V, and Zn	Available from: U.S. Dept of Commerce, Technology Administration, National Institute of Standards and Technology, Gaithersburg, MD, 20899-2322 USA Phone 301-975-6776 Fax: 301-948-3730 E-Mail: srminfo@nist.gov www.ts.nist.gov/srm/
Analytical Quality Control Samples V-8 Rye Flour (Ca, Cu, Fe, K, Mg, Mn, P, Rb, Zn A-11 Milk Powder (Ca, Cr, Cu, Fe, I, K, Mg, Mn, Na, P, Se, Zn) 350 - Tuna Fish (Cr, Cu, Fe, Mn, Ni, Se, Zn)	Available from: Analytical Quality Control Services, International Atomic Energy Authority, P.O. Box 100, A-1400, Vienna, Austria Tel: (+43)-1-2600-28226 Fax: (+43)-1-2600-28222 E-Mail: aqcs@iaea.org

well. This is a major disadvantage for germinated or fermented cereal or legume products. During germination or fermentation, enzymatic-induced phytate hydrolysis via endogenous or microbial phytase enzymes may occur. Only the higher inositol phosphates—hexa- and penta-inositol phosphates—inhibit zinc absorption (Lönnerdal et al. 1989), whereas hexa-, penta-, tetra-, and tri-inositol phosphates—inhibit iron absorption (Brune et al. 1992). Consequently, the preferred method for the analysis of inositol phosphates is that of high performance liquid chromatography (HPLC) (Lehrfeld 1989).

A variety of methods are also used to analyze dietary fiber, the selection depending on whether information on total dietary fiber or some of its individual components is required. Large intakes of dietary fiber, especially insoluble fiber, are known to increase fecal nitrogen excretion, resulting in a reduction in apparent protein digestibility of up to 10 percent. The WorldFood 2 Dietary Assessment System computes utilizable protein by adjusting intakes to account for both digestibility and amino acid score, using procedures outlined by the FAO, WHO and UNU in 1985. (FAO/WHO/UNU 1985) The relationships between the various forms and fractions of dietary fiber are summarized in Table 7.5.

The older methods for fiber analysis (e.g., classical Weende, neutral detergent fiber and acid detergent fiber methods) measure primarily the insoluble fibers (i.e., cellulose and lignin)—though not the water-soluble fibers (pectins, gums, and mucilages)—and some hemicelluloses. Alternative methods have been developed to measure total dietary fiber, including measurement of the soluble fraction. Total dietary fiber can be measured by the following methods: AOAC 985.29/AACC 32-05 or AOAC 991.43/AACC 32-07. These methods measure lignin, non-starch polysaccharides (NSP), and some resistant starch and inulin, and give similar, but not necessarily identical, results. Values for total dietary fiber in the USDA Nutrient Database for Standard Reference (USDA 2003b) and the food composition databases of continental Europe have been analyzed using the AOAC methods (Deharveng et al. 1999).

TABLE 7.5

Relationships among Different Dietary Fiber Fractions

<table>
<tr><td>Cellulose + Insoluble Noncellulosic Polysaccharides</td><td>Insoluble Fiber [a]</td><td rowspan="2">Englyst Fiber (nonstarch polysaccharides)</td><td rowspan="3">Southgate Fiber [b] (unavailable carbohydrate)</td></tr>
<tr><td>Soluble Noncellulosic Polysaccharides</td><td>Soluble Fiber</td></tr>
<tr><td colspan="3">Resistant Starch
Lignin</td></tr>
</table>

[a] Some methods of analysis also include lignin.
[b] Fiber determined by using Southgate's method (Southgate 1969) may differ from the sum of the fractions shown because it can include starch which is not necessarily the same as the resistant starch measured by the method of Englyst et al (Englyst et al. 1982).
This table was modified from the original one, as provided in Holland, Unwin and Buss in their third supplement to the 4th edition of McCance and Widdowson's *The Composition of Foods* in 1988. Reproduced with permission of the Royal Society of Chemistry on behalf of the Controller of Her Majesty's Stationery Office, United Kingdom.

Until recently, the United Kingdom had not used these AOAC methods for the analysis of dietary fiber. Instead, they used methods that measure NSP (Englyst et al. 1982; Englyst and Cummings, 1988). Non-starch polysaccharides (that is; Englyst fiber) are made up of insoluble and soluble forms of dietary fiber; resistant starches and lignin are excluded, as shown in Table 7.5. Hence, NSP values are generally lower than those based on the AOAC 985.29 or AOAC 991.43 methods. They are included in older editions of McCance and Widdowson's "*The Composition of Foods*" and in some of the published supplements (Holland et al. 1988).

In 1999, the United Kingdom Joint Safety and Standard Group recommended the adoption of AOAC 991.43 as the official U.K method for analyzing dietary fiber. Hence, since 1999, the method of analysis for dietary fiber used in the United Kingdom is consistent with that used in the United States and in continental Europe. Moreover, McCance and Widdowson's "*The Composition of Foods*'' 6th summary edition (Food Standards Agency 2002) contains dietary fiber values analyzed by AOAC 991.43. From this discussion, it is apparent that discrepancies in analytical methods used for dietary fiber values must be considered when

compiling food composition data to avoid any potential biases. Such inconsistencies must also be taken into account when comparing dietary fiber intakes among countries.

7.8 International Network of Food Data Systems

The International Network of Food Data Systems (INFOODS) was established by the United Nations University with the aim of creating an international standard for food analyses and the compilation of nutrient databases. The Food and Agriculture Organization (FAO) eventually joined UNU in its efforts in the promotion of INFOODS. The overall goal of INFOODS is to improve the quantity, quality, and availability of food composition data from all parts of the world, and to ensure that any country will be able to obtain adequate and reliable food composition data. INFOODS created a series of task forces to develop:

- standards and guidelines for collecting food composition data;
- standardized terminology and nomenclature so that food composition data can be understood and exchanged internationally;
- standards for data interchange;
- an international directory of existing databases; and
- a detailed description of the needs of database users.

INFOODS also created regional organizations, each of which maintains a regional database and works closely with others involved in compiling similar databases elsewhere in the world. Other activities of the regional organizations include the development of guidelines for food-sampling and analytical methods, preparation of reference materials for laboratory proficiency tests, and training support for use of appropriate software for the regional databases.

INFOODS operates three electronic information/communication activities via the Internet. These include the FOOD-COMP discussion list, the FOOD-TAG list, and an information server. Discussions on the FOOD-COMP list cover a wide range of topics, from sampling, sample preparation, and methodological details to naming conventions and data presentation formats and expressions. Participation involves communication via electronic mail. To join the list, send an email message to: infoods@infoods.unu.edu

Participation in the FOOD-TAG mailing list is by invitation or by special request to the INFOODS Secretariat. Participants on this list are responsible for assigning food code identifiers—known as "tagnames"—according to an established formula. The INFOODS resources can be accessed electronically at the Food and Agriculture Organization website at: www.fao.org/infoods/.

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Chapter 8 | Calculating Intakes of Nutrients and Antinutrients

What will you learn from this chapter?

- How to compile weight equivalents for raw ingredients of mixed dishes;
- How to code weight equivalents for raw ingredients of mixed dishes;
- How to select a suitable computerized nutrient analysis system for the 24-hour recall data;
- How to compile a coding manual and compute the nutrient intakes from the 24-hour recall data;
- How to calculate nutrient intakes manually using food composition tables; and
- How to prepare the data for statistical analysis.

Once the interactive 24-hour recall data has been collected and a suitable nutrient database or food composition table has been located or compiled, the next step is to calculate the intakes of nutrients and antinutrients. This step can be performed using a computer software package or by hand. Before this step is carried out, certain data quality control procedures must be undertaken to eliminate errors which may produce bias and thus affect the interpretation of the results. The errors may arise from incomplete recalls, foods or nutrient values that are missing from the nutrient database, or mistakes in coding food items. Major sources of coding errors include incorrect adjustment of portion sizes to weight equivalents, wrong or improbable weights of foods eaten, and insufficient information for coding ingredients of mixed dishes. Once the checking has been completed, duplicate copies of the cleaned dietary data should be made as back-ups, e.g., by copying onto a set of floppy diskettes, CD-ROMs, or other reliable media storage devices to guard against loss.

Procedures for calculating the weight of the raw or cooked ingredients of mixed dishes from recipes, coding the interactive 24-hour recalls, checking the coded data, and calculating the intakes of nutrient and antinutrients, are summarized below.

8.1 Compiling Weight Equivalents for Ingredients of Mixed Dishes from Recipes

The survey coordinator is responsible for supervising the calculation of the conversion factors for the weight equivalents from portion-size estimates (described in Section 5.5) as well as from ingredients for mixed dishes (Boxes 5.9 to 5.11). Recall interviewers are instructed to complete a recipe form (such as is provided in Table 5.4) for each mixed dish. The form provides a list of the names of the raw ingredients, detailed descriptions, and the amounts, together with the amount of the mixed dish consumed by the respondent. Once these details have been recorded, both the weight of the local mixed dish consumed by the respondent and its corresponding nutrient content can be calculated from either the raw or cooked ingredients by using the procedures in Table 7.2 and Box 7.3.

In some circumstances, the respondent cannot provide all the details required. In such cases, recipe data must be compiled and arrangements made for some women in the study area to cook the local mixed dish, as described in Box 5.11. Weight equivalents for the raw or cooked ingredients can be calculated from the recipes and used for converting the quantity of the local mixed dish specified as consumed in the 24-hour recall into the grams of individual raw or cooked ingredients in the portion size consumed by each respondent. Details of the procedure to be used when nutrient values are available for raw ingredients are provided in Box 5.9, with additional examples in Tables 5.7 and 5.8. When the nutrient database contains values for cooked foods, then adjustments must be made to the weight of each raw ingredient to take into account any alterations in weight after cooking, as described in Box 5.10.

8.2 Coding Weight Equivalents for Ingredients of Mixed Dishes

To reduce errors when coding ingredients for mixed dishes as weight equivalents, the survey coordinator is advised to supervise the construction of conversion-factor tables from the data compiled, as discussed in Section 8.1. Examples of how to tabulate these conversion factors and how to use them for calculating the grams of raw ingredients consumed for different portion sizes in various mixed dishes are given in Table 5.8,

where weighed recipe data have been collected and the amount of the cooked food consumed has been recorded. When the proportion of the total recipe consumed by the respondent has been recorded, then the weight (g) of each raw ingredient consumed by the respondent can be calculated by multiplying this proportion by the weight of each raw ingredient listed on the recipe form, as described in Box 5.9.

8.3 Compiling a Coding Manual and Computing Nutrient Intakes

Computer-stored nutrient databases are food composition tables transferred to, and maintained on, a computer. Such data banks can be revised and updated readily. They vary in size, comprehensiveness, how current they are, the units used to express portion size and nutrient content, and the source and reliability of the values for the food components listed. Food items in the nutrient data banks are usually identified by a numerical coding system that varies in complexity. Many commercially produced nutrient databases are available.

The nutrient database used in conjunction with a computer software package to calculate the nutrient intakes is usually referred to as a "nutrient analysis system". Before selecting a system, the reliability of the nutrient database, and the capabilities and validity of the computer program for calculating nutrient intakes, must be assessed. These features can be checked using the diagnostic tool developed by Hoover and Perloff (1984) and are listed in Box 8.1.

A nutrient analysis system—the WorldFood Dietary Assessment System 2.0 for use with a personal computer was developed at the University of California-Berkeley in 1997. Readers of this manual working in developing countries are advised to use this system, as described in Chapter 7. It is user-friendly and uses the International Mini-list nutrient database which contains food composition values for 1800 foods from six countries (Egypt, Kenya, Mexico, Senegal, India and Indonesia), and can also be modified to include food composition data for additional foods. Users specify the weight consumed (in grams), and the program provides data for 53 nutrients and antinutrients, including iron, zinc, dietary fiber, and phytate. The source of each food composition value is fully documented. The data are taken from published food composition tables, or imputed where necessary; there are no missing values. The program is designed to calculate intakes of available iron and zinc using the algorithms of Murphy et al. (1992) (see Sections 9.1 and 9.2). The WorldFood Dietary Assessment System 2.0 is available at the INFOODS website hosted by the Food and Agriculture Organization (FAO): www.fao.org/infoods/software_worldfood_en.stm. Details are also provided in Appendix E.

BOX 8.1

Selecting and Evaluating the Nutrient Analysis System

- Determine whether the nutrient analysis system and the accompanying database will run on your computer system. Do you have enough disk space and memory?
- Assess the adequacy of skills of those involved with the database development, management, and documentation.
- Check the source of the accompanying nutrient database.
- Investigate how missing and imputed nutrient data are documented.
- Check the completeness of the nutrient database, in terms of both its size and comprehensiveness in relation to the foods required.
- Determine the availability of values for nutrients and antinutrients for individual foods.
- Check that the software package provides the total nutrients per meal, per time interval (e.g., from 0700 to 1200 hr), and per day; average daily nutrient intake per day; average daily nutrient intakes per major food, food group, and food subgroup; average daily intake (in grams) of major foods, food groups, and food subgroups; average frequency of consumption of major foods, food groups, and food subgroups; and percent of energy from protein, fat, carbohydrate, and alcohol.
- Check that the computer program uses a number of significant digits sufficient to ensure that the weights of the ingredients and the amounts of the nutrients are appropriately reported.
- Insist on a demonstration or trial period for the software.

The first stage in using a nutrient analysis system involves coding the food intake data into a defined machine-readable form. Coding is not arbitrary, and will be defined either in the documentation that accompanies the program or in an appropriate food code handbook. To reduce gross errors in coding and ensure consistency among coders, formal coding procedures and rules must be developed by a nutritionist after examining the program documentation and traditional local food items consumed in the study area (Box 8.2) (Gibson 1993). It is essential that all coders make identical coding decisions throughout the data entry process. Care must be taken, for example to ensure that the correct codes are assigned to cereal flours with different extraction rates and to fortified versus unfortified foods.

Coding is best done by trained coders when the field work is still underway. It may involve assigning an identity code for each subject, numerical codes for the day and time of day, food identification codes, and codes for the amount of each edible portion of food consumed (Box 8.3). Sometimes the food identification codes are transferred to the 24-hour recall form by hand before they are entered into the computer.

BOX 8.2

Compiling a Coding Manual for the Dietary Data

1. Consult the coding manual which accompanies your nutrient analysis system. This normally includes an alphabetical list of foods described by generic and local names (including synonyms), and a numerical code list with food names and code numbers. Make copies of these lists for your own coding manual.
2. Add the local food names to the descriptive list of foods if local names differ from those stated in coding manual list.
3. Prepare a table to convert amounts of each food item measured using calibrated household utensils and graduated food models and photographs into weight equivalents (Section 5.5). Insert table in coding manual.
4. Prepare a list of foods missing from the coding manual, and a list of missing nutrients/antinutrients for specific foods. Insert list into coding manual.
5. Add missing food items, nutrients (e.g., zinc), and antinutrients (e.g., phytic acid) to the database using methods described in Sections 7.1 to 7.3, and Section 7.5. Ensure that the nutrient and antinutrient values added to the database are adjusted for any differences in moisture content. For each missing food and nutrient, document the source of the nutrient values in the coding manual.
6. Establish coding rules to follow when an incomplete description of a food is given. For example, when brand of cooking oil is not specified, always code for the type of oil most commonly consumed in the study area (e.g., palm oil). Alternatively, code for a weighted composite for oil based on the proportion of the population consuming each oil (e.g., 60 percent palm oil and 40 percent coconut oil).
7. Establish procedures in the coding manual for dealing with foods or beverages consumed but not listed in the food code handbook, and for which substitutions are available (e.g., use amaranth values for a local leaf).
8. Develop a form in the coding manual to record uncodable items (e.g., foods consumed which are not in the database).
9. Compile guidelines in the coding manual on correcting wrongly entered data.
10. List telephone numbers and e-mail addresses for contacting resource personnel (e.g., nutritionists and computing consultants).
11. Consult the lead nutritionist for the final decisions on the handling of all the uncodable items. Such decisions may include, for example; appropriate substitutions of one or more foods in which case an effort must be made to ensure that they resemble the item consumed as closely as possible. The same must be done for the amounts or proportions of ingredients for mixed dishes for which the actual amounts of raw ingredients are unknown. (See Section 8.1.) All of these final decisions should be stored in a cross-referenced file as they are made to facilitate standardization of future coding decisions.

BOX 8.3

Coding the Interactive 24-hour Recall Data

The precise details of the coding may vary according to the nutrient analysis system in use. An example of a typical procedure is given here.

1. Code for the participant identifier and the day.
2. Code for the time of day (if required) when the food items were consumed.
3. Code each food or beverage consumed, using its food identification code, if required, in the appropriate column of the 24-hour interactive recall form. When a food item cannot be coded according to any of the established procedures, document the item on the uncodable food form in the coding manual.
4. Code the actual amount of each edible portion of food consumed and enter this in the appropriate column of the 24-hour recall form. For the amount code, depending on the computer program, use either the actual amount consumed expressed in grams, or a decimal fraction of the amount given in the nutrient database for that particular food item.
5. Repeat steps 2 to 4 above for each food consumed until the complete dietary intake for that participant on that day is coded, then continue with the next day or start coding information for the next participant.
6. Uncodable food items should be handled by the supervising nutritionist who will be responsible for the decisions about the appropriate codes. Once assigned, the codes must be recorded in the coding manual so that the same code is always used for that specific uncodable food item.
7. When coding is complete, enter the coded information into the computer system. This may be done on-line or offline, depending on the software package used.
8. Check the input data for coding errors. Incorrect food codes can be rapidly identified if check digits are included in the food code. Check digits provide a simple method of improving the integrity of data by incorporating some redundancy into the encoding of the data; a program which is capable of dealing with check digits will be able to report when an impossible code has been entered.
9. Check the input data for weight errors. They can be more readily detected if the computer program flags those subjects whose daily intakes of energy and selected nutrients, foods, or portion sizes fall outside the 2-standard deviation limits for the data set; if such a situation occurs, checks should be made for weight errors in the coded data for the reported participants.
10. Carry out spot checks on subsamples of the stored data against the original data forms. If errors occur in more than 1 percent of the records, then all the data should be checked.
11. Select an appropriate software package to calculate the energy and nutrient intakes from the food intake data.

The food identification code is generally divided into several parts. The first part of the code may include four levels to describe the food group, major and minor food subgroups and, finally, the individual food. The code may also provide information on preserving or processing techniques, storage conditions, and so forth. There is no uniform coding system. Most national and regional databases use country-specific food classification systems based on national criteria. Hence, the food groups may be very specific. In low-income countries, the food groups used in the food code are often those used by the Food and Agriculture Organization in food balance sheets, and include cereal, oils and fats, milk and milk products, vegetables, fish and seafood, pulses and legumes, fruits, meat and offal, roots and tubers, sugar and honey, eggs, and other miscellaneous foods. Details of this FAO food balance sheet classification are available at: www.fao.org/es/ess/list.htm

Several other food classification systems are also used. Some of these group food items at the raw ingredient level; others describe foods mostly as consumed, whereas some describe foods both at the ingredient level and as consumed (e.g., Euro Food Groups (EFG) classification system) (Ireland et al. 2002). Table 8.1 provides details of the 33 food groups used by the EFG classification system.

TABLE 8.1

Example of the Euro Food Groups (EFG) Classification System

Euro Food Group	EFG Class
1	Bread and rolls
2	Breakfast cereals
3	Flour
4	Pasta
5	Bakery products
6	Rice and other cereal products
7	Sugar
8	Sugar products, excluding chocolate
9	Chocolate
10	Vegetable oils
11	Margarine and lipids of mixed origin
12	Butter and animal fats
13	Nuts
14	Pulses
15	Vegetables, excluding potatoes
16	Starchy roots or potatoes
17	Fruits
18	Fruit juices
19	Non-alcoholic beverages
20	Coffee, tea, cocoa powder
21	Beer
22	Wine
23	Other alcoholic beverages
24	Red meat and meat products
25	Poultry and poultry products
26	Offals
27	Fish and seafood
28	Milk
29	Cheese
30	Other milk products
31	Miscellaneous foods
32	Products for special nutritional use
33	

Adapted from Ireland et al. 2002.

When the WorldFood Dietary Assessment System 2.0 is used, food names rather than a numerical code are specified by the user.

Codes for the amount of each edible portion of food consumed will either be in terms of the actual amount consumed expressed in grams (which is the preferred system, and is used in the WorldFood Dietary Assessment System 2.0), or as a decimal fraction of the amount given in the nutrient database for that particular food item. For example, if the amount given for an avocado pear in the nutrient database is for one raw medium avocado pear without skin or stone but only one-half of a medium pear was consumed by a subject, then 0.5 would be the decimal fraction entered in column 3 of Table 8.1.

Useful references for data on average values for the weights of a variety of individual food items are given by Pennington and Church (1985) for the United States, and the Ministry of Agriculture, Fisheries and Food (1993) for the United Kingdom. The latter provides weights of specific discrete items (e.g. an apple, a hen's egg, etc.) as well as average portions of larger items (e.g., pasta or vegetables). Information on the specific gravities (g/mL) of commonly consumed dairy products and beverages that can be used to convert certain food items measured by volume into grams is also given. For example, to convert one dessert spoon of palm oil into grams, multiply the volume (i.e., 10ml) by the appropriate specific gravity (i.e. 0.890g/mL). This yields a weight of 8.9g for 10mL palm oil. Selected specific gravity data can be found in Appendix D.

The final stage in coding the food intake data is to check for any errors in the data set introduced during the measurement of the interactive 24-hour recalls or the coding and data-entry stages. Such errors may involve incorrect identification numbers assigned to participants; omissions of parts of meals, entire meals or days; mistakes in converting portion sizes to weight equivalents; incorrect food codes or weight of foods consumed; mistakes in calculating ingredients from a recipe; inconsistencies in coding for mixed dishes; and failure to follow the default coding rules. Some of the strategies for cross-checking may include incorporating checking digits in the food code so that incorrect food codes can be rapidly identified by the computer program.

Detection of weight errors for food items during the coding can be facilitated by including a routine in the computer program that flags subjects whose daily intakes of energy and selected nutrients, foods, or portion sizes fall outside the 2-standard deviation limits for the data set. Checks can then be made for weight errors in the coded data for these selected subjects. Duplicate coding of the recalls by independent coders is another strategy that is often used (Conway et al. 2005). Once the checking has been completed, the energy and nutrient intakes can be calculated with the chosen software, provided the food composition database is complete.

8.4 Manual Calculation of Nutrient Intakes

Manual calculation of nutrient intakes is carried out in two stages. In the first stage, the nutrient intake data are recorded separately for each respondent in Table 5.1, as described in Sections 5.2 to 5.5. Care must be taken to ensure that a complete description of all the ingredients of each mixed dish and the amounts eaten by the respondent are recorded both on the recipe form (Table 5.4) and in Table 5.1. Instructions for calculating the amount of each ingredient eaten are outlined in Section 5.4. These quantities should then be converted into weight equivalents, as outlined in Section 5.5 and entered on the recipe form (Table 5.4) and in column 6 of Table 5.1.

In the second stage, selected information from Table 5.1 must be transferred to Table 8.2 for each respondent for the manual calculation. The information transferred must include all the details entered in columns 3 and 4, and the weight equivalent (g) data in column 6. Details are also provided in Boxes 5.9 and 5.10. As noted in Section 8.3, investigators working in developing countries may wish to use the WorldFood Dietary Assessment System 2.0 because it contains a comprehensive set of food composition values, including data on the iron, zinc and phytate content of foods (per 100g). Care must be taken when using this system to ensure that the foods are labeled with names that are fully understood by users in the local region, and have descriptors sufficient for correctly matching the foods consumed in the interactive 24-hour recalls to those depicted in the food composition table.

In some older food composition tables, values are expressed in terms of the nutrient content of the edible portion of the foods per common household measures rather than per 100 grams. In such cases, the total sum of the edible portion of each food or beverage consumed during the 24-hour recall period must be converted into a decimal fraction of the amount given in the food composition table for that particular food item, and the results entered in column 3 of Table 8.2. When values are expressed per 100 grams, the total amount consumed in grams, entered in column 2, must also be converted into a fraction of 100 grams and entered into column 3. Next, the corresponding energy and nutrient values stated in the food composition table for each food type are multiplied by the fraction entered in column 3 before being entered into their respective columns in Table 8.2. The final step is to add up the amount of each nutrient and enter the sum in the ''total'' line. All hand-calculated nutrient intake data should be checked by another person before being finalized.

TABLE 8.2

Form for the Hand Calculation of Nutrient Intakes for Each Respondent

Day of the week														
Name of the Subject														
Interviewer						Location								
Interview date						Subject ID								
Day food eaten						Subject name								
						Age								
Description of food or beverage	**Amount in grams or household measures**	**Fraction**	**Energy KJ)**	**Protein (g)**	**Total fat (g)**	**Calcium (mg)**	**Iron (mg)**	**Zinc (mg)**	**Thiamin (mg)**	**Riboflavin (mg)**	**Niacin (NE)**	**Vitamin C (mg)**	**Phytate (mg)**	**Dietary Fiber (g)**
	Totals													
Probe for alcohol Yes ❑ No ❑						Probe for sickness Yes ❑ No ❑ If yes, did it affect appetite Yes ❑ No ❑ If yes, how? Increase ❑ Decrease ❑								
Was food intake unusual Yes ❑ No ❑ If yes, how was it unusual?						Probe for tablets Yes ❑ No ❑ Iron ❑ Malaria ❑ Vitamins ❑ Other supplements								
Increase ❑ Decrease ❑ Was it a feast day? Yes ❑ No ❑ Was it a market day? Yes ❑ No ❑														

Investigators may also wish to augment an existing food composition table, such as the WorldFood Dietary Assessment System 2.0, with nutrient and antinutrient values for selected local foods. In such cases, an existing up-to-date, country-specific food composition table containing a comprehensive list of foods and reliable food composition values should be used. Several food composition tables are available for Africa, Asia, and Latin America; these are listed in Appendix F. It is important to recognize the sources of error and discrepancies that may occur in these food composition tables (Section 7.1): their reliability and state of completeness vary, and many of the older tables will not contain zinc or phytate values. The reader is also advised to consult the *Journal of Food Composition and Analysis*, as this is an important source of reliable food composition values. Any missing values must be replaced with calculated or imputed numerical values by using the procedures outlined in Sections 7.2 and 7.3. Additional details about this process are also given in Murphy et al. (1991). To assess the reliability of any food composition tables used, users of this manual are advised to read the introductory text in the food composition tables that describes the sampling and methods used to derive the values.

BOX 8.4

Hand Calculating Intakes Using Food Composition Tables

1. Transfer the details of each food or beverage listed during the recall (including the ingredients of each mixed dish) from Table 5.1 (column 3) to column 1 of Table 8.2. Enter each food or beverage item on a new line.
2. Transfer the edible portion sizes of each food or beverage item consumed and recorded in column 5 of Table 5.1 as weight equivalents to column 2 of Table 8.2. Convert the weight equivalent in column 2 into a decimal fraction of the actual amount given in the food composition tables for that particular food item if the table values are expressed in household measures. For table values expressed per 100g (such as the WorldFood Dietary Assessment System 2.0), convert the total amount consumed in grams into a decimal fraction of 100g.
3. Record the appropriate decimal fraction in column 3 of Table 8.2.
4. Multiply the nutrient values for each food item in the food composition table by the corresponding decimal fraction in column 3, and enter these adjusted values in the appropriate nutrient columns in Table 8.2.
5. For mixed dishes for which nutrient values are available in the food composition table, record the name of the mixed dish in column 1, and the weight equivalent of the edible portion size consumed in grams in column 2 of Table 8.2. Repeat steps 3 to 5.

OR

6. For mixed dishes for which the nutrient values must be calculated from a recipe, record the name of the mixed dish in column 1, and the weight equivalent of the portion consumed by the respondent in grams in column 2. Then convert the total amount consumed in grams into a decimal fraction of 100g in column 3 of Table 8.2. Follow the calculation procedure in Box 7.3 to calculate the nutrient content of the mixed dish.
7. Repeat step 4, but use the calculated nutrient composition of the mixed dish.

OR

8. For mixed dishes in which the weight of each *raw* ingredient consumed has been recorded in grams (see Box 5.9), convert the raw weights into *cooked* weight equivalents using yield factors (Table 5.9), as shown in Box 5.10. Enter these cooked weight equivalents in column 2, and then convert them into a decimal fraction of 100g. Record the decimal fraction in column 3 of Table 8.2.
9. Repeat step 4 using the nutrient content of the *cooked* ingredients.
10. Repeat until all food items consumed by the respondent have been included.
11. After entering all the nutrient values for each food item and the mixed dishes listed, calculate the totals for energy and for each of the nutrients and antinutrients investigated. Record the totals in the last line of Table 8.2.
12. Repeat steps 1 through 5 and step 11 for each respondent.
13. Staple together any additional sheets and the recipe calculation sheets.

Readers may find the following sources (whose references are provided in full at the end of this chapter) helpful for augmenting an existing food composition table with selected iron, zinc, non-starch polysacccharide, dietary fiber, and phytate values (Sections 7.1 to 7.3):

- Abebe et al. (2007): information on zinc, iron, calcium, and phytate content for selected raw and prepared foods commonly consumed in southern Ethiopia.
- Brand Miller et al. (1993): data on one or more samples of 500 Australian bush foods with variable range of nutrients.
- Burlingame et al. (1994): iron, zinc, and non-starch polysaccharide values for New Zealand foods.
- Chan et al. (2007): iron, calcium, zinc, and phytate in cereals and legumes consumed in East Lombok, Indonesia.

- Dignan et al. (1994): iron, zinc, and non-starch polysaccharide values for Pacific Islands foods.
- Food Standards Agency (2002): iron, zinc, and non-starch polysaccharide values for the most commonly consumed foods in the United Kingdom.
- Holland et al. (1988): iron, zinc, non-starch polysaccharide, and selected phytic acid values for cereals and cereal products.
- Holland et al. (1992a): iron, zinc, non-starch polysaccharide, and selected phytic acid values for fruits and nuts.
- Holland et al. (1992b): iron, zinc, non-starch polysaccharide, and selected phytic acid values for vegetables, herbs and spices.
- Nutrition Coordinating Center, University of Minnesota (2007): database of foods and foodstuffs, and iron, zinc, non-starch polysaccharide, phytic and oxalic acid values.
- Siong et al. (1997): iron and selected zinc values for Malaysian foods.
- Umeta et al. (2005): zinc, iron, calcium, and selected phytic acid and tannin values for foods commonly consumed in Ethiopia.
- USDA (2003): iron, zinc, and dietary fiber values of foods in the United States.

The food composition table can also be supplemented with analyzed food composition values of local staple foods. The sampling and methods of preparation for analysis must follow the protocols outlined in Sections 7.5 to 7.7. As noted, INFOODS maintains an up-to-date computerized listing of food composition tables at: www.fao.org/infoods/

The directory is updated regularly and contains information on all the major national and international databases and food tables. INFOODS has also designed a system of standardized codes for nutritive and non-nutritive components of foods for international use that allows cross comparisons among studies (Klensin et al. 1989). Information about INFOODS may be obtained through the INFOODS Secretariat, Charles Street, PO Box 500, Boston, MA 02114-0500, USA, and via the Internet at www.fao.org/infoods/

8.5 Preparing the Data for Statistical Analysis

After calculating the energy and nutrient intakes, the next step is to examine descriptive statistics for each variable for all groups or selected groups of participants. List the five largest and five smallest values of every numeric variable or plot scatter diagrams or histograms. Outlying values are frequently very obvious on such diagrams. Such outliers may represent errors that have been previously overlooked in the data set. Values at the ends of the distribution should be examined carefully for plausibility. If implausibly low or high energy or nutrient intakes are detected, the recall information and calculations should be examined and corrected, as described in Section 8.3. For studies where dietary intake data have been collected on the same subjects on more than one occasion (as in Objective 1c), the internal consistency of the data can be checked by comparing the frequency distributions for the same variable collected on the two occasions (Hulley and Cummings 1988).

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Chapter 9 | Estimating Available Iron and Zinc Intakes

What will you learn from this chapter?

- Why we need to estimate intakes of available iron and zinc;
- How to use an algorithm to estimate intake of available iron; and
- How to use an algorithm to estimate intake of available zinc.

Nutrient intakes that have been calculated from food composition tables or determined by direct chemical analysis represent the maximum amount of the nutrient available to the body. For nutrients such as iron and zinc, the amount actually absorbed and used by the body is lower than the total intake and depends on the chemical form of the nutrient, nature of the food ingested, and composition of the diet. The current status of iron and zinc, and the physiological and health status of the individual are also known to affect the absorption and utilization of these two micronutrients (for example; if a woman is pregnant, this will have an impact).

The term "bioavailability" is defined as the proportion of the ingested nutrient that is absorbed and utilized through normal metabolic pathways (Hurrell 2002). It is influenced by both diet- and host-related factors. Many dietary components are known to modify the bioavailability of iron and zinc; some enhance absorption, and others inhibit absorption (Table 9.1). The chemical form of iron and zinc in foods also influences their bioavailability. Two forms of iron are present in foods: heme iron and non-heme iron. Heme iron is bound in a porphyrin ring, and is derived mainly from hemoglobin and myoglobin in meat, poultry, and fish. Non-heme iron is found primarily as iron salts in a wide variety of foods of both plant and animal origin, and as contaminant iron introduced by food processing and the soil. Each form of iron is absorbed by separate pathways, but once inside the mucosal cells of the small intestine, all iron enters a common pool. Heme iron is absorbed as the intact moiety, whereas non-heme iron is absorbed from the common pool within the gastrointestinal tract (WHO/FAO 2004).

Of the two forms of iron, heme iron is much more readily absorbed than non-heme iron. Absorption of heme iron depends on the iron status of the individual. Absorption of non-heme iron also depends on the individual's iron status but, in addition, is influenced by the iron content of the meal, and the simultaneous ingestion of the absorption modifiers of dietary iron listed in Table 9.1. Calcium is an exception because it inhibits the absorption of both heme and non-heme iron, although the precise mechanism by which it does this remains unclear.

TABLE 9.1

Dietary Modifiers Influencing Iron and Zinc Absorption

Non-heme Iron	Zinc
Enhancers	**Enhancers**
Meat, poultry, fish and other seafood	Meat, poultry, fish and other seafood, eggs, whey protein
Ascorbic acid	—
Other organic acids (citric, lactic, malic, tartaric)	Organic acids (citric, lactic, malic, tartaric)
Inhibitors	**Inhibitors**
Phytate	Phytate
Polyphenols (e.g., tannin)	—
Calcium	Calcium
Certain processed soy products	Certain processed soy products

The organic form of zinc tends to be more readily absorbed and less affected by dietary absorption modifiers than the inorganic form (Solomons et al. 1979). Of the absorption modifiers, both the type and amount of dietary protein influence the bioavailability of zinc. Increasing the amount of total protein enhances zinc absorption, and if the protein is from cellular animal sources, as shown in Table 9.1, the enhancing effect is even greater (Lönnerdal 2000). Organic acids produced during fermentation also have the potential to enhance zinc (and iron) absorption through the formation of soluble ligands in the gastrointestinal tract (Sandström 1997, Teucher et al. 2004). They may also complex some of the minerals bound to phytate molecules, rendering them more susceptible to hydrolysis via phytase

enzymes (see, for example, Maenz et al. 1999) while simultaneously generating a pH that optimizes the activity of intrinsic phytase from cereal or legume flours (Porres et al. 2001).

In contrast, phytic acid (*myo*-inositol hexaphosphate) as well as the salts—magnesium, calcium, or potassium—chelate metal irons, especially zinc (and iron and calcium) in the gastrointestinal tract, making them unavailable for absorption. Phytic acid also complexes endogenously secreted minerals such as zinc (and calcium), making them unavailable for reabsorption into the body (Sandström 1997). High amounts of calcium may exacerbate the inhibitory effect of phytate on zinc absorption by forming a calcium-zinc-phytate complex in the intestine that is even less soluble than phytate complexes formed by either ion alone.

Absorption of zinc, like iron, is also affected by the zinc content of the diet and the zinc status of the individual (Lönnerdal 2000). Direct measurements of the bioavailability of iron and zinc in the plant-based diets consumed in developing countries are limited, but some have been made by using radioactive and stable isotope techniques (FAO/WHO 1988, WHO 1996). As a result, bioavailability algorithms are frequently used to estimate the bioavailability of iron and zinc in whole diets. These are mathematical models that attempt to predict the bioavailability of iron and zinc by taking into account the form of the nutrient, presence of dietary enhancers and inhibitors, and the iron and zinc status of the individual. The algorithms then apply certain general principles to the complex dietary matrix of whole diets (Hunt 1996).

The accuracy of the bioavailability algorithms is limited by interactions known to occur between the enhancing and inhibiting factors in the whole diet. For example, when several absorption modifiers are contained in the same meal, their effects are probably not additive (Reddy et al. 2000). Furthermore, because most of the effects of the dietary modifiers on iron and zinc absorption have been calculated from the results of single test meals, their effects may be exaggerated compared to the extent of the enhancement or inhibition measured over several days (Cook et al. 1991). The magnitude of the effect of the absorption modifiers may also depend on the background dietary matrix (Hunt 1996). These findings emphasize that as new research findings emerge, the algorithms for iron and zinc will need to be revised on an ongoing basis. In general, for example, the effects of only some of the dietary modifiers listed in Table 9.1 have been taken into account in bioavailability algorithms for iron and zinc.

9.1 Algorithms for Estimating Intakes of Available Iron

Several algorithms are available for estimating intakes of available iron. The first algorithm was developed by Monsen et al. (1978), and later adapted by Murphy et al. (1992) to estimate iron bioavailability in diets from developing countries (see Box 9.1). In the algorithm of Murphy et al. (1992), heme iron absorption is assumed to be 25 percent, and to account for 40 percent of the iron in meat, poultry, and fish. The absorption of non-heme iron is assumed to be comparatively lower and to vary according to the amount of meat, poultry, and fish, ascorbic acid, and polyphenols consumed in each meal, as well as the level of iron stores of the individual. In Murphy's model, the predicted bioavailability of non-heme iron has been increased to account for the probable absence of iron stores (but no overt anemia) in children and women in developing countries.

BOX 9.1

Using Murphy's Model to Calculate Intakes of Available Iron (for Plant-based Diets)

Calculate available iron intakes from the data on mean daily iron intakes per individual using the algorithm below:

Available iron = (heme iron × heme iron availability factor) + (non-heme iron × non-heme iron availability factor × tea-coffee factor)

where heme iron is 40 percent of iron in meat, poultry, or fish; heme iron availability factor is 0.25; non-heme iron is all iron except heme iron; non-heme iron availability factor is 0.05* but depends on the average ascorbic acid and the meat, fish and poultry density of the diet (Table 9.2); and the tea-coffee factor ranges from 0.4 to 1.0, depending on the average number of cups of tea and coffee in the diet.

* Note that non-heme iron bioavailability is expressed as a percentage in Table 9.2. Hence, values in Table 9.2 must be divided by 100 before they are used in the model above.

To use this algorithm, quantitative data on the intake of heme iron, non-heme iron, and two enhancers—ascorbic acid; and protein from meat, fish, and poultry—are required. Ascorbic acid is the most potent enhancer of non-heme iron absorption, forming a soluble iron-ascorbate chelate in the acid milieu of the stomach which simultaneously prevents iron from forming a complex with phytate or tannin (Teucher et al. 2004). The enhancing effect of ascorbic acid is most apparent when consumption of muscle protein is low and in the presence of suboptimal iron status (Allen and Ahluwalia 1997). Although the mechanism whereby cellular animal protein enhances non-heme iron absorption is not clear, some 'meat factor' may be implicated, perhaps through the release of certain amino acids, such as oligo-ssccaharides, or possibly through cysteine-containing peptides during the digestion of cellular animal protein (Bjorn-Rasmussen and Hallberg 1979). Murphy et al. (1992) have applied cut-offs for the content of ascorbic acid and meat, fish, and poultry protein which are the same as those used by Monsen et al. (1978), except they are expressed per 4.18MJ (1000kcal), so that the same algorithm can be used for males and females across all age groups. The percentage levels for the estimated bioavailability of non-heme iron given for each classification (as shown in Table 9.2) approximate those of the typical meals of the low, medium, and high bioavailability categories of the algorithm developed by FAO and WHO (1988).

TABLE 9.2

Estimated Percent Bioavailability of Non-heme Iron for Iron-deficient, Non-anemic Persons with Differing Intakes of Meat, Fish, and Poultry (in g/4.18MJ) and Ascorbic Acid

	Meat, Fish, and Poultry Protein		
Ascorbic Acid (mg per 4.18MJ)	*< 9*	*9 to 27*	*> 27*
< 35	5	10	15
35 – 105	10	15	15
> 105	15	15	15

The algorithm of Murphy et al. (1992) can be corrected for the inhibitory effect of polyphenols from tea on non-heme iron absorption, as shown in Box 9.1. Polyphenols form insoluble iron-phenolic compounds; their inhibitory effect is independent of phytate and can be partly counteracted by the simultaneous consumption of ascorbic acid (WHO/FAO 2004). The correction factor applied depends on the average numbers of cups of tea or coffee per day. For tea, factors range from 1 if no tea is consumed to 0.40 for at least 600mL, and are based on the fact that a 200 to 250mL cup of normal-strength tea will reduce non-heme iron absorption at a meal by approximately 60 percent. Corresponding correction factors for coffee are in the range of 1 and 0.6. This algorithm can also be used to calculate the bioavailability of iron for each day if the food intake data by meal are not available. Estimates of available iron derived from day-based and meal-based results have proved very comparable (Murphy et al. 1996).

Nevertheless, the algorithm of Murphy et al. (1992) has several limitations. It assumes that 40 percent of the total iron in all muscle protein is heme iron, and that the bioavailability of heme iron is 25 percent. In fact, the actual heme content of flesh foods may range from 30 to 70 percent, and may differ—even in the same type of meat—depending on the raising and slaughtering practices in different countries. In addition, the algorithm does not take into account the effect of phytic acid and other substances, such as certain vegetable proteins (e.g., soybean protein), that are known to inhibit non-heme iron absorption (WHO/FAO 2004).

The computer program supplied with the WorldFood Dietary Assessment System 2.0 calculates intakes of available iron by using Murphy's model. Details of this program are provided in Appendix E.

Several alternative algorithms have been developed for calculating available iron, each of which takes into account differing numbers of dietary absorption modifiers. For example, Tseng et al. (1997) have refined Murphy's model so that non-heme iron absorption can be adjusted for the enhancing effects of meat, poultry, fish, and vitamin C, and the inhibitory effects of both tea and phytates in the diet. This algorithm has not had extensive use.

Phytate refers to phytic acid (*myo*-inositol hexaphosphate) as well as the salts: calcium, magnesium, or potassium phytate. It is the principal storage form of phosphorus in cereals, legumes and oil seeds. In the intestine, phytate acid forms insoluble chelates with iron (and zinc) that are unavailable for absorption, as noted earlier. Note that *myo*-inositol hexaphosphate can be hydrolyzed by certain food processing and preparation practices to lower myo-inositol phosphates (IP1 and IP2), which no longer form insoluble complexes with iron (Sandberg et al. 1999). Phytate begins to lose its inhibitory effect on iron absorption when phytate-to-iron molar ratios are less than 1.0:1.0, but it still inhibits iron absorption at ratios as low as 0.2:1.0 (Hurrell 2003). There is no reduction in the inhibitory effect of a high-phytate diet on iron absorption in long-term vegetarians, suggesting that no adaptation to such a diet occurs (Brune et al. 1989).

Reddy et al. (2000) have also developed an algorithm based on typical Western diets eaten by 86 subjects which takes into account the amount of animal tissue, phytic acid, and ascorbic acid. They reported that only 16.4 percent of the total variance in iron absorption (measured by extrinsic radio-iron labeling) was accounted for by these three dietary components, with the major portion being explained by the animal tissue and phytic acid contents of the meals. Non-heme iron, calcium, and polyphenols were not significant predictors of iron absorption. These results emphasize the relatively small influence of diet on the amount of iron absorbed compared to the more important, but often unknown, host-related factors.

Of all the algorithms available, the model of Hallberg and Hulthén (2000) is the most detailed, as it takes into account the effects of all the known enhancing and inhibiting factors on non-heme iron absorption as well as interactions between the different factors. Application of this more detailed model is, however, limited by the paucity of food composition data for the content of both phytate and iron-binding polyphenols in foods.

The Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO 1988) developed a semi-quantitative classification system for estimating iron bioavailability (summarized in Box 9.2) based on estimates of iron absorption from typical meals in Asia, India, Latin America and Western countries. The FAO/WHO system classifies these typical meals into three broad categories of iron bioavailability: 'low' (i.e., iron absorption of about 5 percent); intermediate (i.e., iron absorption of about 10 percent); and high (i.e., iron absorption of about 15 percent). The estimated iron absorption percentage will vary depending on the content of flesh versus plant-based foods (such as cereals, roots and/or tubers), together with the content of ascorbic acid-rich foods and tea or coffee intake. The FAO/WHO system does not, however, quantify these amounts.

BOX 9.2

Using the Food and Agriculture Organization/World Health Organization (FAO/WHO) Model to Estimate Iron Bioavailability

Use the data on major food sources of iron from the interactive 24-hour recalls to categorize the diets as having low, intermediate, or high bioavailability, as described below:

- **Low-bioavailability diet** (iron absorption of about 5 percent): a simple, monotonous diet containing cereals, roots, and/or tubers, and negligible quantities of meat, fish, or ascorbic acid-rich foods;
- **Intermediate-bioavailability diet** (iron absorption of about 10 percent): consists mainly of cereals, roots, and/or tubers, and minimal quantities of food of animal origin and ascorbic acid, both of which promote iron availability; and
- **High-bioavailability diet** (iron absorption of about 15 percent): a diversified diet containing generous quantities of meat, poultry, fish and/or foods containing high amounts of ascorbic acid.

The low-bioavailability diet specified by FAO and WHO (1988) contains a preponderance of foods that inhibit iron absorption (such as maize, beans, whole wheat flour, sorghum, etc.), and which are common in many parts of the developing world (for example; in the populations of Africa, Asia, and some areas of Latin America), particularly among lower socioeconomic groups. For these diets, the main meal has a high phytate content (that is, greater than 400mg), and contains less than 50g meat and less than 30g ascorbic acid. A low-bioavailability diet can be raised to an intermediate-bioavailability diet by increasing the intakes of absorption enhancers such as ascorbic acid and flesh foods at the main meal to intakes greater than 30mg and 50g, respectively.

The diets for most population groups in industrialized countries and in Latin American populations from socioeconomic groups of high status are within the high-bioavailability category. Such diets contain high proportions of meat (i.e., 100g per main meal) and vegetables containing ascorbic acid (i.e., more than 50mg ascorbic per main meal) and low proportions of phytates. Bioavailability may be reduced to the intermediate level if meals containing higher amounts of inhibitors of iron absorption, such as tea or coffee, are consumed regularly, as occurs, for example, in Costa Rica or Guatemala. Note that for some diets based almost exclusively on unrefined cereals, the bioavailability of iron may be as low as 1 to 2 percent, whereas for those with a high content of flesh foods it may reach as high as 20 to 25 percent.

The estimates of absorption given in Box 9.2 refer to non-anemic persons with normal iron transport (i.e., with normal hemoglobin concentrations) but no iron stores. When individuals have iron deficiency anemia (i.e., low hemoglobin concentration), absorption may be increased by 50 percent (i.e., increasing to percentages of 7.5, 15, and 22.5, respectively, in absorption for the low-, intermediate-, and high-bioavailability diets.) (FAO/WHO 1988).

In 2002, FAO and WHO revised their vitamin and mineral requirement estimates, but they still recommend the classification system shown in Box 9.2, and the use of two categories of bioavailability—5 percent and 10 percent—for diets in developing countries. For more Western food-type diets, FAO and WHO recommendations from both 2002 and 2004 propose two categories of bioavailability—12 percent and 15 percent—depending on the meat content of the diet.

Beard and colleagues (2007) have compared the performance of four of the quantitative algorithms discussed above (with the exception of Murphy's model) and two others (Du et al. 2000, Bhargava et al. 2001) in predicting an improvement in iron status of Filipino religious sisters over a period of 9 months. They concluded that all six algorithms underestimated iron absorption from these rice-based diets, when 250mg storage iron was assumed in these individuals. Overall median iron absorption was calculated to be 17.2 percent, based on change in serum ferritin compared to estimates predicted by the algorithms ranging from 2.6 to 7.3 percent. Clearly, as more comprehensive data become available on the absorption of iron from meals of differing compositions over a long period of time, new algorithms need to be developed to predict the bioavailability of iron from mixed diets, and the FAO/WHO classification system needs to be refined.

9.2 Algorithms for Estimating Intakes of Available Zinc

Three algorithms have been developed to estimate the bioavailability of zinc. The first is a semi-quantitative classification system developed by WHO (1996), and then adopted by both FAO and WHO in 2002 and in 2004. The system takes into account three dietary variables as important predictors of the bioavailability of zinc. These include one absorption enhancer, such as protein from meat, fish or poultry; and two absorption inhibitors; namely, high levels of calcium, particularly calcium salts, and the proportion of phytic acid to zinc in the whole diet. The calcium content of most plant-based diets is too low to have any detrimental effect. Exceptions are diets based on tortillas prepared with lime-soaked maize, diets of lacto-vegetarians, and possibly those diets of persons who chew betel nut with lime.

The FAO/WHO system of classifying diets into three broad categories of low-, moderate-, and high-zinc bioavailability is described in Box 9.3. (FAO/WHO 2002, WHO/FAO 2004)

BOX 9.3

Using the WHO Model to Estimate Zinc Bioavailability

Classify the diets based on the interactive 24-hour recalls into low-, moderate-, or high- bioavailability based on the following categories:

- **Low-bioavailability diet**—zinc absorption of about 15 percent;
- **Moderate-bioavailability diet**—zinc absorption of about 30 percent; and
- **High-bioavailability diet**—zinc absorption of about 50 percent.

Low-bioavailability diets are assumed to be associated with a zinc bioavailability of about 15 percent. They include:

- diets high in unrefined, unfermented and ungerminated cereal grain (e.g., flat breads and sorghum), especially when fortified with inorganic calcium salts and when intake of animal protein is negligible;
- diets in which the phytate-to-zinc molar ratio typically exceeds 15 (see Box 9.5) or 18 (see Table 9.3);
- diets in which high-phytate soy-protein products constitute the primary protein source;
- diets in which approximately 50 percent of the energy intake is accounted for either by one or a combination of the following high-phytate foods; namely, high-extraction-rate (≥90 percent) wheat, rice, maize grains and flours, oatmeal, and millet (chapatti flours and *tanok*); sorghum; cowpeas; pigeon peas; grams; kidney beans; blackeye beans; groundnut flours; and
- diets that have high intakes of inorganic calcium salts (more than 1g Ca^{++}/day) either as supplements or as adventitious contaminants (e.g., from calcareous geophagia), which potentiate the inhibitory effects of low-availability diets and low intakes of animal protein exacerbate these effects.

Moderate-bioavailability diets are mostly mixed diets with a zinc bioavailability of about 30 percent. They include:

- mixed diets containing animal or fish protein;
- lactoovovegetarian, ovovegetarian, or vegan diets not based primarily on unrefined cereal grains or high-extraction-rate flours;
- diets in which a phytate-to-zinc molar ratio of the total diet is within the range of 5 to 15 or not exceeding 10 if more than 50 percent of the energy intake is accounted for by unfermented, unrefined cereal grains and flours while the diet is fortified with inorganic calcium salts (more than 1g Ca^{++}/day); and
- modern diets in which the availability of zinc improves when the diet includes animal protein or other protein sources or milks.

High-bioavailability diets are mostly diets with an adequate protein content mainly from non-vegetable sources. They have a zinc bioavailability of about 50 percent, and include:

- refined diets low in cereal fiber, low in phytic acid content, and with a phytate-to-zinc (molar) ratio of less than 5; and
- adequate protein content mainly from non-vegetable sources such as meat and fish, and include semi-synthetic formula diets based on animal protein.

The second algorithm for available zinc is based on a modification of the WHO (1996) classification system, and was again developed by Murphy et al. (1992). It takes into account the content of animal protein and the content of the same two inhibitory factors—the proportion of phytic acid to zinc and calcium in the whole diet. For this algorithm, first the phytate-to-zinc molar ratio of the whole diet is calculated (Box 9.4). Phytic acid (myoinositol hexaphosphate) forms insoluble complexes with zinc (as well as non-heme iron) at the physiological pH conditions of the small intestine, making them unavailable for absorption. The inhibitory effect of phytate on zinc absorption follows a dose-dependent response, and the molar ratio of phyate-to-zinc in the diet (Box 9.4) can be used to estimate the proportion of absorbable zinc (Oberleas and Harland 1981).

BOX 9.4

Calculating the Phytate-to-Zinc Molar Ratio of the Diets

1. Calculate the total daily phytate intake (in milligrams).
2. Divide the total daily phytate by the molecular weight of phytate (660) to give the phytate intake in terms of millimoles.
3. Divide the total daily zinc intake in milligrams by the atomic weight of zinc (65.4) to give zinc intake in millimoles.
4. Divide the millimoles of phytate by the millimoles of zinc to find the phytate-to-zinc molar ratio.

Note: The atomic weight of iron = 55.85; the atomic weight of calcium = 40.08.

Appendix G gives a list of phytic acid values and phytate-to-zinc molar ratios of some plant-based foods and composite dishes consumed in Ghana and Malawi. Additional phytate values are available in Harland and Oberleas (1987). Only the hexa- and penta-phosphate esters of inositol significantly inhibit the bioavailability of zinc (Lönnerdal et al. 1989); they may also complex endogenously secreted zinc (Sandström 1997, Manary et al. 2000), making it unavailable for reabsorption into the body. Additional discussion of the analytical methods for zinc and phytate analysis can be found in Section 7.7.

Next, a zinc availability factor is assigned to the diet, depending on the phytate-to-zinc molar ratio, as shown in Box 9.5. Finally, where appropriate, the zinc availability factors are adjusted based on the animal protein and calcium consumed during the meal. If the animal protein content exceeds 16g / 1000kcal (4.82MJ), absorption is increased by 5 percentage points, whereas if the calcium content in the meal is between 500 and 750mg, then 5 percentage points are subtracted. The calcium content of most diets in developing countries is too low to have an effect on zinc bioavailability, so calcium is not generally included in the calculation of available zinc, as noted in Box 9.5. Even in Latin American countries where calcium intakes are often above 1g / day, phytic acid intakes are so high that any further reduction in zinc absorption is unlikely. Note there is some inconsistency in the range of phytate-to-zinc molar ratios and their associated zinc availability factors shown in Box 9.5 and those specified in the description of the WHO model.

BOX 9.5

Using Murphy's Model to Calculate Intakes of Available Zinc

Calculate intakes of available zinc from data on mean daily zinc intakes per individual using the algorithm below:

Available zinc (mg/day) = total zinc (mg/day) × zinc availability factor

where zinc availability factor is set at 0.10 if phytate-to-zinc ratio is greater than 30; 0.15 for ratios between 15 and 30; and 0.30 for ratios less than 15.

Note that calcium is not included in this model because the calcium content of most plant-based diets is too low to have an effect on zinc bioavailability.

The International Zinc Nutrition Consultative Group (IZiNCG) has developed a third algorithm for calculating available zinc based on measurements of zinc absorption in adults using only total diet studies. Studies using semi-purified diets or exogenous sources of zinc in the form of zinc salts were excluded (Hotz and Brown 2004). A logit regression model was used to describe the relationship between four dietary factors (zinc, phytate, protein, and calcium) and the percentage of the zinc intake absorbed. However, in the final model, only zinc and the phytate-to-zinc molar ratio were shown to be significant predictors of the percentage of zinc absorption in adults ($r^2 = 0.4$, $p < 0.001$). Neither calcium nor protein added significant predictive power. As a result, IZiNCG suggests that the phytate-to-zinc molar ratio should be used to define diet types with respect to zinc absorption. The group has divided diets into two categories based on their phytate-to-zinc molar ratios from which they derived estimates of fractional zinc absorption for adults using their prediction equation. These estimates are provided in Table 9.3. Note that the applicability of this model to children, pregnant or lactating women, or the elderly is unknown.

TABLE 9.3

International Zinc Nutrition Consultative Group (IZiNCG) Estimates of Dietary Zinc Absorption

Diet Types	Mixed or Refined Vegetarian	Unrefined, Cereal-based Diets
Phytate-to-zinc molar ratio	4–18	> 18
Zinc absorption: Adult males	26 percent	18 percent
Zinc absorption: Adult females	34 percent	25 percent

This table was modified from Hotz and Brown (2004).

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Chapter 10 | Evaluating Nutrient Intakes of Groups

What will you learn from this chapter?

- How nutrient reference levels are derived;
- How to use the WHO/FAO and IZiNCG requirements for iron and zinc;
- How to adjust the distribution of observed intakes to usual intakes;
- How to use the full probability approach to assess the prevalence of inadequate iron intakes in a group; and
- How to use the estimated average requirement (EAR) cut-point method to assess the prevalence of inadequate zinc intakes in a group.
- How to calculate the distribution of usual intakes from the distribution of observed intakes

The final stage in the dietary assessment protocol is to evaluate the nutrient intakes of the population in relation to nutrient reference levels. This is usually done by comparing the calculated nutrient intakes of the study group with tables of nutrient reference levels. Several tables of nutrient reference levels are available. When country-specific tables of nutrient reference levels are not available for a developing country, the WHO/FAO (2004) and the IZiNCG (Hotz and Brown 2004) requirements should be used.

Values for the nutrient reference levels are derived from measurements or calculations of nutrient requirements based on a number of individuals of the same age and sex. For certain life-stage groups (e.g., the elderly), the requirements are often extrapolated from measurements made on young adults. These measurements generate a distribution of requirements among individuals of similar age and sex. The requirements are generally considered to follow a normal distribution except for iron requirements in menstruating women, which are positively skewed because some women have high menstrual losses. For the normal distributions, the mean of the distribution represents the estimated average requirement for that particular group of individuals, and the standard deviation is a measure of the variability in the requirement. Hence, WHO/FAO (2004) defines the EAR as 'the average daily nutrient level that meets the needs of 50 percent of the "healthy" individuals in a particular age and gender group'. The EAR for iron for menstruating women is generally set at the fiftieth percentile because the iron requirements are skewed due to the high menstrual iron losses of some women.

Strenuous efforts are now made to establish the EAR for each nutrient because it is the basis for the multiple nutrient-based reference levels now in use in several countries. However, there is still no agreement by expert groups on the criteria used to define the requirements for many nutrients, including iron and zinc. Indeed, a range of criteria—selected on the basis of a careful review of the literature—are often used and the strengths and weaknesses of each of the sources of data considered. In some cases, the criterion for a specific nutrient may vary for different life-stage groups (e.g., adolescents or the elderly). The reliability of the data used to select the criterion (or criteria) to define the requirements varies with the nutrient.

For nutrients such as iron and zinc, both the nature of the diet ingested (Sections 9. 1 and 9.2) and certain host-related factors can affect their absorption and/or utilization (WHO/FAO 2004). In such cases, an adjustment must be made to the physiological requirement to yield a dietary requirement estimate. The dietary requirement is defined as "the requirement of the nutrient as ingested in a specified type of dietary pattern and under specified conditions of the host". Because both the diet- and host-related factors vary markedly across countries, it follows that the dietary requirement may differ among countries and expert groups, even if there is approximate agreement on the physiological requirements. Several expert groups, including WHO/FAO (2004) and IZiNCG (Hotz and Brown 2004), have established EARs for iron and zinc for different types of diets, such as unrefined cereal-based diets, refined vegetarian diets, or omnivorous diets.

Once the EAR and associated standard deviation for a nutrient for a group of healthy individuals in a specific life-stage and sex group has been set, then most expert groups define a nutrient reference level at two standard deviations above the EAR. For many nutrients, however, the standard deviation is unknown. In such cases, the standard deviation is calculated from the EAR and an assumed coefficient of variation (CV). For many nutrients a CV in the range of 10 to 12.5 percent of the mean requirement is often assumed, although this is not always the case.

The terminology used to define the reference level set at two standard deviations above the EAR varies among countries. The United Kingdom has adopted reference nutrient intake (RNI) (COMA 1991); and the United States and Canada use recommended dietary allowance (RDA) (IOM 2000a); whereas WHO and FAO (2004) prefer the term recommended nutrient intake (RNI). By definition, at this nutrient reference level, habitual intakes will cover the daily nutrient requirements of almost all (97.5 percent) of the healthy individuals in a specific life-stage and sex group. Hence, the nutrient reference level at two standard deviations above the EAR should *not* be used to evaluate the nutrient intakes of population groups. Use of this reference nutrient intake as a cutoff to calculate the proportion of individuals in the group with inadequate intakes will result in a serious overestimate of the proportion at risk (Murphy and Poos 2002). Instead, it should only be used for planning diets for individuals. When usual intake of a nutrient for an individual is at this level, risk of inadequacy to the individual is very low (2 to 3 percent) (Barr et al. 2002).

For some nutrients with important functions in humans, there are insufficient scientific data to establish an EAR. In such cases, the nutrient reference levels are often based on observed intakes for the nutrient, and these levels are judged to be adequate for the specific life-stage group but not so large as to cause undesirable effects. This is an approach often adopted for infants under 6 months old, when the nutrient reference levels are usually based on the varying content of the nutrient in breast milk and the average amount of breast milk consumed. Again, the terminology used for the reference levels set in this way varies.

The WHO/FAO approach (2004) has adopted the terminology "Acceptable Intake" for nutrients (e.g., vitamin E) for which data were considered insufficient to set an EAR, whereas the U.S. Food and Nutrition Board uses the term "Adequate Intake" (IOM 2000a). The United Kingdom (COMA 1991) has adopted the term Safe Intake (SI) for seven nutrients defined in this way.

Increasingly, many expert groups are defining tolerable upper-intake levels to help people avoid harm from ingesting too much of a nutrient. Exposure from food and fortified food products and sometimes water; supplements; and medications are considered, where relevant. These upper levels are based on risk-assessment methodologies similar to those used in toxicological studies (IOM 1998).

Upper Tolerable Nutrient Intake Levels are defined by WHO/FAO (2004) as "the maximum intake from food, water, and supplements that is unlikely to pose risk of adverse health effects from excess in almost all (97.5 percent) apparently healthy individuals in an age and sex-specific population group". In addition, they have defined an Upper Tolerable Nutrient Intake Level for some micronutrients, including zinc (but not iron). The U.S. Food and Nutrition Board has set Tolerable Upper Intake Levels (ULs) for 19 nutrients including iron and zinc, whereas the U.K. Expert Group on Vitamins and Minerals (EVM) has set safe upper levels for only nine micronutrients, but they do include iron and zinc.

Readers who are working in developing countries and who wish to evaluate energy intakes are advised to consult the FAO/WHO and United Nations University (UNU) system of classification (FAO/WHO/UNU 2004). In the 2004 U.N. report, the energy requirements for children and adolescents are based on total energy expenditure (TEE) measured with doubly labeled water and energy needs for growth. For adults, energy requirements are calculated from factorial estimates of habitual TEE that combine the time allocated to habitual activities and the energy cost of those activities. To account for differences in body size and composition, the energy costs of activities were expressed as multiples of basal metabolic rate, or physical activity ratios (PARs). Details of these calculations are available in the report by FAO and WHO together with UNU. (FAO/WHO/UNU 2004)

To evaluate protein intakes in developing countries, an earlier FAO/WHO/UNU publication, *Energy and Protein Requirements*, may be used (FAO/WHO/UNU 1985). Protein requirements for infants have also been compiled by Dewey et al. (1996) for the International Dietary Energy Consultative Group. Readers may wish to consult this alternative source for estimates of protein requirements for infants.

The methods used to evaluate nutrient intakes in a population group only provide an estimate of the prevalence of inadequate intakes. None of the methods actually identifies specific individuals in the population who have a nutrient deficiency. This can only be done if biochemical and clinical assessments are also carried out with the dietary investigation. Such uncertainty arises because the actual nutrient requirement of an individual is not known. Further, the nutrient-intake data recorded only approximate the individual's usual nutrient intake, because of normal day-to-day variation in the diet combined with measurement errors (see Chapter 6). The reliability of the risk estimate obtained depends on the method used for the evaluation of nutrient inadequacies. In the past, nutrient-based reference levels have not always been used correctly. Two new methods for evaluating the prevalence of inadequate nutrient intakes in population groups are described in Sections 10.3 and 10.4.

10.1 FAO/WHO Requirements for Iron and Zinc

The FAO and WHO have revised their nutrient requirements and these are available in two publications: *Human Vitamin and Mineral Requirements* (FAO/WHO 2002); and *Vitamin and Mineral Requirements in Human Nutrition* (WHO/FAO 2004). In both publications, the nutrient reference levels for iron and zinc are based on estimates that meet the normative requirements and are adapted from earlier reports (FAO/WHO 1988, WHO 1996). The normative requirement provides not only for the prevention of functional impairment but also for the maintenance of tissue stores (in the case of iron), or reserve capacity (in the case of zinc); thereby safeguarding against any future increase in requirements or shortfall in intakes. In the nutrient reference levels documented in the WHO/FAO (2004) report, estimated average requirements are only provided for a few micronutrients. WHO has calculated EARs based on the FAO/WHO (2002) Recommended Nutrient Intakes. These calculated values are available in Allen et al. (2006) and should be used to evaluate the prevalence of inadequate intakes of population groups (see Section 10.5).

As discussed earlier, the estimated bioavailability of iron and zinc from the habitual local diets must be taken into account when selecting the appropriate EAR for comparison with the usual intake data derived from the interactive 24-hour recalls. The dietary components known to affect the bioavailability of iron and zinc in local diets and considered in the FAO/WHO classification systems are discussed in detail in Sections 9.1 and 9.2. The amount of iron and zinc absorbed also depends on the iron and zinc status of the individual and certain host-related factors; these are discussed in detail in Gibson (2007).

Table 10.1 presents the normative calculated estimated average requirements for iron for certain life-stage groups adjusted for three levels of dietary iron bioavailability: 5, 10, and 15 percent. The WHO/FAO approach (2004) recommends using iron bioavailability figures of 5 and 10 percent for diets in developing countries. No figures are given for the EAR for dietary iron for pregnant women in this table, because iron balance during pregnancy is dependent on both the composition of the habitual diet and the amount of storage iron. Note also that no data are given for the EAR for iron for children aged 1 to 3 years; children aged 4 to 8 years; menstruating adolescents (aged 14 to 18 years); or menstruating women. The iron requirements for these life-stage groups are not normally distributed, mainly because of the skewed distribution of their iron losses, most notably menstrual losses (Allen et al. 2006).

TABLE 10.1

Calculated Average Requirements (EARs) for Iron (mg) for Selected Life-stage Groups and from Diets Differing in Iron Bioavailability

(based on FAO/WHO (2002) Recommended Nutrient Intakes)

Age (y)	Sex	15 Percent Bioavailability	10 Percent Bioavailability	5 Percent Bioavailability
1–3	M & F	See Table 10.4		
4–8	M & F	See Table 10.4		
14–18 menstruating	F	See Table 10.4		
19–50	M	7.2	10.8	21.6
19–50 menstruating	F	See Table 10.4		
Pregnant, second trimester	F	>40.0	>40.0	>40.0
Lactating, 0-3 months	W	7.8	11.7	23.4

Adapted from Allen et al. 2006.

The RNI values for dietary iron are not presented in Table 10.1, because they should not be used for evaluating intakes of population groups (Barr et al. 2002). Nevertheless, WHO/FAO's system (2004) does provide data on the RNIs for iron to meet the normative storage requirements from diets with four levels of iron bioavailability (5, 10, 12, and 15 percent). These are the nutrient reference levels to use for evaluating the iron intakes of individuals.

Table 10.2 presents the EARs for zinc according to whether the bioavailability of zinc in the habitual diet is assumed to be high (i.e., 50 percent), moderate (i.e., 30 percent), or low (i.e., 15 percent). The WHO/FAO report (2004) presents the EARs for zinc in terms of µg per kg body weight, but they have also been calculated from the FAO/WHO (2002) RNIs, and are presented in Allen et al. (2006). Details of the criteria used to classify habitual diets as high, moderate, or low for zinc bioavailability are given in Section 9.2.

The RNIs for zinc are also given for three levels of zinc bioavailability for specific life-stage groups; these too are available in the WHO/FAO report (2004). For these derivations, a coefficient of variation for the dietary zinc requirements of 25 percent was assumed. As a result, the RNIs for zinc are set at 150 percent of the EAR and should only be used for evaluating the zinc intakes of individuals, as noted earlier for iron (Barr et al. 2002).

TABLE 10.2

Calculated Average Requirements (EARs) for Zinc (mg) for Selected Life-stage Groups and from Diets Differing in Zinc Bioavailability

(based on FAO/WHO (2002) Recommended Nutrient Intakes)

Age (y)	Sex	High Bioavailability	Moderate Bioavailability	Low Bioavailability
1–3	M & F	2.0	3.4	6.9
4–6	M & F	2.4	4.0	8.0
19–50	F	2.5	4.1	8.2
19–50	M	3.5	5.8	11.7
Pregnant women Second trimester	F	3.5	5.8	11.7
Lactating women 0-3 months	F	4.8	7.9	15.8

Adapted from Allen et al. 2006.

10.2 IZiNCG Requirements for Zinc

The International Zinc Nutrition Consultative Group (IZiNCG) has also compiled Estimated Average Requirements for zinc (Hotz and Brown 2004). Again, the EAR for zinc is derived by dividing the physiological zinc requirement by the estimated absorption of zinc. IZiNCG adopted the factorial method to estimate the average physiological requirement for zinc for most age and physiological groups, as used earlier by WHO (1996) and the IOM (2002). The estimates for zinc absorption were also calculated using a similar conceptual approach to that used by both WHO/FAO/IAEA (International Atomic Energy Agency) Expert Consultations (WHO 1996; FAO/WHO 2002) and the U.S. Institute of Medicine (IOM 2001). Unlike WHO (1996), IZiNCG only included total diet studies of zinc absorption and not single-meal studies. Data from 15 studies were included in the final analyses: 11 were based on mixed diets; three on refined vegetarian diets; and one on an unrefined cereal-based diet. These diets had phytate-to-zinc molar ratios ranging from 4 to greater than 18.

Table 10.3 presents the estimated average requirements for zinc for all age, sex, and life-stage groups. The EARs are presented for both mixed/refined vegetarian diets and for unrefined, cereal-based diets, as reported by IZiNCG (Hotz and Brown 2004). Recommended dietary allowances (RDAs) for zinc for each sex and life-stage group were also compiled by IZiNCG. These were calculated based on the assumption that the coefficient of variation or CV of the distribution of requirements for zinc was 12.5 percent and not the 25 percent assumed by FAO/WHO (2002). Hence, the IZiNCG RDAs for zinc are equivalent to 125 percent of the corresponding EAR level. Again, the RDAs should only be used to evaluate the zinc intakes of individuals; hence, they are not presented here.

TABLE 10.3

Revised Estimated Average Requirement (EAR) for Zinc by Life-stage and Diet type

(as suggested by IZiNCG)

Age	Sex	Reference Body Weight (kg)	Revisions Suggested by IZiNCG for EAR for Zinc (mg/d)	
			Mixed or Refined Vegetarian Diets	Unrefined, Cereal-based Diets
6-11 mo	M & F	9	3	4
1–3 y	M & F	12	2	2
4–8 y	M & F	21	3	4
9–13 y	M & F	38	5	7
14–18 y	M	64	8	11
14–18 y	F	56	7	9
Pregnancy	F	-	9	12
Lactation	F	-	8	9
≥ 19 y	M	65	10	15
≥ 19 y	F	55	6	7
Pregnancy	F	-	8	10
Lactation	F	-	7	8

Adapted from Hotz and Brown (2004).

10.3 Incorrect Approaches for Evaluating Nutrient Intakes of Groups

In the past, some of the approaches used to evaluate nutrient intakes of groups have been incorrect. For example, the mean or median nutrient intake of the group has been compared (as a percentage) with the RNI (or equivalent). When the mean or median nutrient intake was ≥ RNI (or equivalent), the nutrient intake of the group was considered adequate. This approach should not be used, however, because it does not take into account the distribution of usual intakes, and the inferences made are misleading, as shown in Box 10.1. Even when the mean nutrient intake of a group equals the RNI (as shown in the example for vitamin B-6 intakes for women aged 51 to 70 years), the prevalence of inadequate intakes is in fact greater than 25 percent when the distribution of usual intakes is compared with the EAR. This discrepancy arises because of the wide variation in usual nutrient intakes (Otten et al. 2006). Indeed, to ensure a low prevalence of intakes below the EAR, the mean intake of the group should exceed the RNI, often by a considerable amount.

BOX 10.1

Inappropriate Use of the RNI to Assess Group Mean Intakes

1. The data below represent distribution of vitamin B-6 intakes for women aged 51-70 years adjusted for intra-subject variability from US National Health and Nutrition Examination Survey III 1988 to 1994 (NHANES III). The US EAR for vitamin B-6 is 1.3mg/d and the RDA is 1.5mg/d for women of this age group.
2. Selected percentiles of vitamin B-6 intake, women 51-70 y, NHANES III:

Percentile	5th	10th	15th	25th	50th	75th	85th	90th	95th
Vit. B-6 intake (mg/d)	0.92	1.02	1.11	1.24	1.51	1.90	2.13	2.31	2.65

3. When the median intake (1.15mg/d) for the group is compared with the RDA of 1.5mg/d, it may seem that inadequate intake of vitamin B-6 is not a problem.
4. However, when the distribution of usual intakes is compared with the EAR cutpoint, the EAR value (1.3mg/d) falls between the 25th percentile and the 50th percentile of usual intakes. Hence, more than 25 percent of usual intakes are below the EAR cutpoint.
5. The result is that the prevalence of inadequacy within the groups is greater than 25 percent (but less than 50 percent).

Adapted from Otten et al. (2006)

In another method, the RNI has been used as a cutoff value and the percentage of individuals with intakes below that value has been calculated. This method assumes that the RNI describes the requirement of all individuals of the same sex and age in the population. Yet by definition the RNI is set at an intake level that exceeds the requirements of 97.5 percent of the population. Hence, use of the RNI as a cutoff will result in a gross overestimate of the actual prevalence of inadequate intakes (Murphy and Poos, 2002). Instead, the correct methods for evaluating the prevalence of inadequacy in a group are the full probability approach (especially suitable for evaluating the iron intakes of children and menstruating women) and the EAR cutpoint method, further described below.

10.4 Full Probability Approach for Evaluating Iron Intakes

The full probability approach is a statistical method that was first described by Beaton (1972). It involves determining the probability of inadequacy of the usual nutrient intake level for each individual in the group, and then averaging these individual probabilities across the group to estimate the prevalence of inadequate intakes for the group. To use this method, information on both the distribution of usual nutrient intakes and the distribution of requirements in the group is needed. Because there is no information about the actual requirements of each individual, this procedure does not identify with certainty which individuals are "at risk''. Thus, it cannot be used to screen individuals at risk of nutrient inadequacy.

The full probability approach must be used to estimate the prevalence of inadequate intakes of iron for the following population groups: children aged 1 to 3 years; children aged 4 to 8 years; menstruating adolescent young women aged 14 to 18 years; and menstruating adult women, because the iron requirements of all these groups are not symmetrical about the EAR (IOM 2001). To adopt the probability approach for evaluating iron intakes, the following information is required:

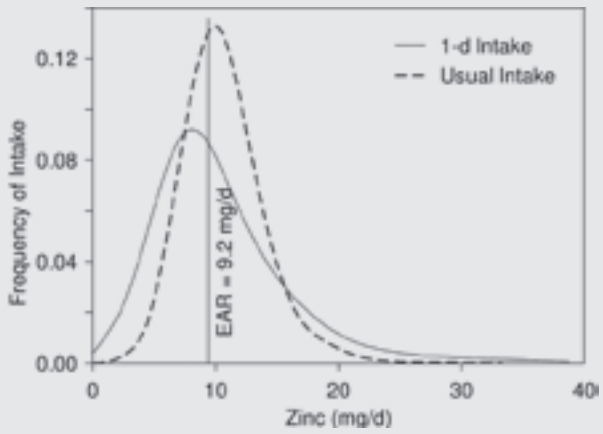
- The EAR for each nutrient data for the particular sex and life-stage group of individuals.
- The distribution of requirements for each nutrient among similar individuals: for most nutrients, this is not precisely known. In the absence of such information, the distribution of requirements for most nutrients is assumed to be symmetrical (not necessarily normal) with a coefficient of variation of 10 percent about the EAR. A notable exception is the iron requirement distributions for children and menstruating women, which are positively skewed.
- Reliable data on the distribution of usual intakes of the study group.
- Knowledge of the expected correlation between intakes and requirements among individuals: For all nutrients (except energy), this is assumed to be very low.

Reliable information on the distribution of usual intakes of the nutrient in the group can be obtained by adjusting the distribution of observed intakes statistically in an attempt to partially remove the effects of day-to-day variability in intakes within individuals (i.e., within-subject variation). The reader is advised to seek the advice of a statistician before adopting this approach. The adjustment process provides estimates of the distribution of usual nutrient intakes for the group, and can be performed using the method outlined by the U.S. National Research Council (NRC) (NRC 1986), or a more refined NRC approach developed by Nusser et al. (1996) which uses the software programs SAS and PRC IML. The program C-SIDE (Center for Survey Statistics and Methodology, Iowa State University) may also be used, and can be downloaded at: www.iastate.edu/

Figure 10.1 compares the adjusted distributions of usual zinc intakes with the observed zinc intakes for New Zealand adult females aged 19 to 50 years (Gibson et al. 2003). The intakes were obtained from single 24-hour recalls from each woman, and adjusted with replicate intake data from a subsample of these women using the refined NRC method. Note that the adjustment process yields a distribution with reduced variability that preserves the shape of the original distribution. Further, in this example, the proportion of women with adjusted intakes below the assumed EAR for zinc is markedly reduced, emphasizing that without the adjustment, the estimate obtained for the prevalence of inadequate zinc intakes would have been incorrect. Note that any bias arising from under- or overreporting of food intakes is not removed by this adjustment process.

FIGURE 10.1

Unadjusted and Adjusted Zinc Intakes



Adapted from Gibson et al. (2003).

In theory, intakes from a number of days are required. Fortunately, it is not necessary to collect multiple days' intakes for the entire study group but only for a representative subset. The U.S. Food and Nutrition Board (IOM 2000a) suggests that the representative subset should consist of at least 30 to 40 individuals per stratum, and that the repeated 24-hour recalls should be independent and made on nonconsecutive days. If the data can be collected only on consecutive days, then three 24-hour recalls should be collected. It is more important to have a minimum number of repeated observations in the subsample than a minimum proportion of repeated observations.

To carry out the statistical adjustment, the computer programs calculate the within- subject and between-subject variation for any given nutrient. Within-subject variation represents the day-to-day variation in the nutrient intakes within the same person, plus all sources of random measurement error that may occur.

The confounding effects of any measurement errors can be reduced by including appropriate quality control procedures during the collection of the food intake data, as described in Sections 4.3 to 4.6. Between-variation is a measure of the differences among individuals in intakes of a nutrient. If between-subject variation is large relative to within-subject variation, then individuals can be readily distinguished.

The ratio of within- to between-subject variation is known as the variance ratio. A variance ratio of 1.0 indicates that the within-subject and between-subject variances are equal, whereas a ratio of greater than 1.0 indicates that the between-subject variation is greater than the within-subject variation. Generally speaking, within-subject variation is larger than between-subject variation, especially in homogeneous populations. Variance ratios depend critically on factors such as age, season, gender, and sociocultural group as well as on the dietary methodology used, sample size, and number of measurement days over which the food intake of each participant was measured (Gibson 2005).

The calculations for the full probability approach can be performed by using a computer program. Details are given in Appendix H. Alternatively, for iron, a spreadsheet can be used for these calculations in conjunction with the data presented in Table 10.4, provided the distribution of observed intakes of iron has been adjusted statistically to yield information on the distribution of usual iron intakes. Table 10.4 provides data for the probability of inadequate intakes of iron (mg/d) for the population groups with iron requirements known to be asymmetrical about the EAR (IOM 2001). Different ranges of usual iron intakes are presented at three levels of bioavailability: low (5 percent), intermediate (10 percent), and high (15 percent). An example of how to use this table to estimate the prevalence of inadequate intakes of iron for menstruating women consuming a diet with 5 percent average bioavailability of iron is given in Table 10.5 and Box 10.2.

TABLE 10.4

Probability of Inadequate Iron Intakes for Different Age Groups at Different Ranges of Usual Intake (mg/d)

p for inadequacy[a]	Usual Intake of Children 1-3 y			Usual Intake of Children 4-8 y			Usual Intake of Females 14-18 y			Usual Intake of Menstruating Women		
	Bioavailability			Bioavailability			Bioavailability			Bioavailability		
	5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%
1.0	<3.6	<1.8	<1.3	<4.8	<2.4	<1.6	<16.2	<8.1	<5.4	<15.0	<7.5	<5.0
0.96	3.6-4.5	1.8-2.3	1.3-1.5	4.8-5.9	2.4-3.0	1.6-2.0	16.2-17.7	8.1-8.8	5.4-5.9	15.0-16.7	7.5-8.4	5.0-5.6
0.93	4.5-5.5	2.3-2.8	1.5-1.8	5.9-7.4	3.0-3.7	2.0-2.4	17.7-19.6	8.8-9.8	5.9-6.5	16.7-18.7	8.4-9.4	5.6-6.2
0.85	5.5-7.1	2.8-3.6	1.8-2.4	7.4-9.5	3.7-4.8	2.4-3.2	19.7-22.1	9.8-11.1	6.5-7.4	18.7-21.4	9.4-10.7	6.2-7.1
0.75	7.1-8.3	3.6-4.2	2.4-2.8	9.5-11.3	4.8-5.7	3.2-3.8	22.1-24.1	11.1-12.0	7.4-8.0	21.4-23.6	10.7-11.8	7.1-7.9
0.65	8.3-9.6	4.2-4.8	2.8-3.2	11.3-13.0	5.7-6.5	3.8-4.3	24.1-26.0	12.0-13.0	8.0-8.7	23.6-25.7	11.8-12.9	7.9-8.6
0.55	9.6-10.8	4.8-5.4	3.2-3.6	13.0-14.8	6.5-7.4	4.3-4.9	26.0-27.8	13.0-13.9	8.7-9.3	25.7-27.8	12.9-13.9	8.6-9.3
0.45	10.8-12.2	5.4-6.1	3.6-4.1	14.8-16.7	7.4-8.4	4.9-5.6	27.8-29.7	13.9-14.8	9.3-9.9	27.8-30.2	13.9-15.1	9.3-10.1
0.35	12.2-13.8	6.1-6.9	4.1-4.6	16.7-19.0	8.4-9.5	5.6-6.3	29.7-32.1	14.8-16.1	9.9-10.7	30.2-33.2	15.1-16.6	10.1-11.1
0.25	13.8-15.8	6.9-7.9	4.6-5.3	19.0-21.9	9.5-11.0	6.3-7.3	32.1-35.2	16.1-17.6	10.7-11.7	33.2-37.3	16.6-18.7	11.1-12.4
0.15	15.8-18.9	7.9-9.5	5.3-6.3	21.9-26.3	11.0-13.2	7.3-8.8	35.2-40.4	17.6-20.2	11.7-13.5	37.3-45.0	18.7-22.5	12.4-15.0
0.08	18.9-21.8	9.5-10.9	6.3-7.3	26.3-30.4	13.2-15.2	8.8-5.1	40.4-45.9	20.2-23.0	13.5-15.3	45.0-53.5	22.5-26.7	15.0-17.8
0.04	21.8-24.5	10.9-12.3	7.3-8.2	30.4-34.3	15.2-17.2	5.1-5.7	45.9-51.8	23.0-25.9	15.3-17.3	53.5-63.0	26.7-31.5	17.8-21.0
0	>24.5	>12.3	>8.2	>34.3	>17.2	>5.7	>51.8	>25.9	>17.3	>63.0	>31.5	>21.0

[a] Indicates that the probability that the requirement for iron is greater than the usual intake. For the purpose of assessing populations, a probability of 1 has been assigned to usual intakes that are below the 2.5th percentile of requirements, and a probability of 0 has been assigned to usual intakes that fall above the 97.5th percentile of requirements. Usual intakes should be adjusted for within-subject variation, as described in Section 10.4. Modified from Allen et al. (2006).

TABLE 10.5

Example of Calculations to Estimate the Prevalence of Inadequate Iron Intakes for Menstruating Women Consuming a Diet with 5 Percent Average Iron Bioavailability

Probability of Inadequacy[a]	Range of Intake with the Probability of Inadequacy (mg/d)	Percent of Menstruating Women with Intake in this Range (column total = 100%)	Prevalence of Inadequacy (=probability of inadequacy × % with intake in the range)
1.0	<15.0	2%	2%
0.96	15.0-16.7	10%	9.6%
0.93	16.7-18.7	10%	9.3%
0.85	18.7-21.4	10%	8.5%
0.75	21.4-23.6	15%	11.3%
0.65	23.6-25.7	20%	13%
0.55	25.7-27.8	10%	5.5%
0.45	27.8-30.2	8%	3.6%
0.35	30.2-33.2	5%	1.8%
0.25	33.2-37.3	5%	1.3%
0.15	37.3-45.0	3%	0.5%
0.08	45.0-53.5	2%	0.2%
0.04	53.5-63.0	0%	0%
0.00	>63.0	0%	0%
Total probability of inadequate intakes for menstruating women:			66.6%

[a] Indicates probability that iron requirement is greater than the usual intake. For the purpose of assessing populations, a probability of 1 has been assigned to usual intakes that are below the 2.5th percentile of requirements, and a probability of 0 has been assigned to usual intakes that fall above the 97.5th percentile of requirements. Usual intakes should be adjusted for within-subject variation, as described in Section 10.4. Adapted from Allen et al. (2006).

To perform the calculation, the first step is to determine the number of individuals within the group with usual intakes of iron in each of the 14 classes of a specified range of intake, as defined in Table 10.5, column 2. After converting this number to a percentage of the whole group (column 3), the percentage is then multiplied by the appropriate probability for each of the 14 classes (column 1) to give the prevalence of individuals (as a percentage of the total population from each class) who were likely to have iron intakes below their own requirements (column 4). The numerical probabilities in column 1 are derived from the area beneath the "normal" curve between the stated standard deviation limits. The sum of the percentages (column 4) gives the total percentage of individuals in the population who are at risk of an inadequate intake of iron. This sum represents the total probability of inadequate intakes of iron for the menstruating women in the group.

In the example given in Table 10.5, the percentage of women in the group with usual iron intakes within classes 1 to 14, respectively, are: 2%, 10%, 10%, 10%, 15%, 20%, 10%, 8%, 5%, 5%, 3%, 2%, 0%, and 0%. When multiplied by the appropriate probabilities for each class (Table 10.5, column 1), the percentage of the total population of individuals from each class likely to have iron intakes below their own requirements becomes 2%, 9.6%, 9.3%, 8.5%, 11.3%, 13%, 5.5%, 3.6%, 1.8%, 1.3%, 0.5%, 0.2%, 0%, and 0%, as shown. The sum of these percentages equals 66.6 percent, representing the prevalence of inadequate intakes for iron. Thus, 66.6 percent of this population of menstruating women are predicted to have intakes of iron below their own requirements (Box 10.2). This approach does not identify the specific women with inadequate intakes, as noted earlier.

BOX 10.2

Estimating the Prevalence of Inadequate Iron Intakes for Menstruating Women Using the Full Probability Approach

1. Correct the distribution of observed iron intakes for within-subject variation by using the program PC-SIDE to yield usual iron intakes.
2. Classify the individual usual intakes of iron within each class and enter this percentage for each class into column 2.
3. Calculate the percentage of individuals with usual intakes of iron within each class, and enter this percentage for each class into column 2.
4. Multiply the percentage for each class by the appropriate probability for each class (Table 10.5, column 1) to give the prevalence of inadequate intakes of iron (as a percentage) in each of the 14 classes. Enter this percentage for each class into column 4.
5. Add up the percentages across classes in column 4.
6. This is the percentage of the group who are predicted to have intakes for iron below their own requirements, and represents a probability estimate for the population as a whole, as long as the group is a representative sample of that population.

The full probability approach should not be used for energy. Energy intake is highly correlated with requirements among non-obese individuals (even after age, sex, and weight adjustments have been made), but data on the extent of this correlation is insufficient at the present time.

10.5 EAR Cutpoint Method

A short-cut to the probability approach has been developed by Beaton (1994) for assessing the prevalence of inadequate intakes in a group. This simpler version, known as the EAR "cutpoint method," does not require information on the exact requirement distribution. The EAR cutpoint method can be used provided the following conditions are met:

- Intakes and requirements of the nutrient are independent; thus, no correlation exists between usual intakes and requirements. (Such is assumed to be true for most nutrients, but is not true for energy.)
- The distribution of intakes in the population group must be more variable than the distribution of requirements (which is generally the case among groups of free-living individuals).
- The distribution of requirements in the group is symmetrical about the EAR (but this is not the case for the iron requirements of menstruating women and children aged 1 to 8 years).

BOX 10.3

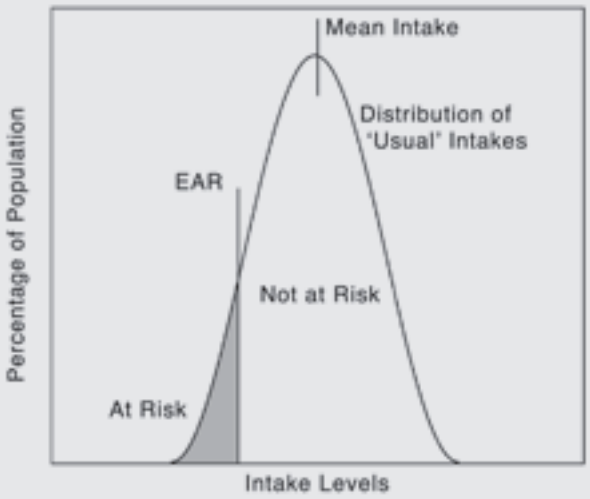
Estimating the Prevalence of Inadequate Intakes by Using the EAR Cutpoint Method

1. Select the appropriate EAR for age, sex, physiological state, and diet type from the table of reference nutrient intakes for your country, or the appropriate WHO/FAO or IZiNCG tables.
2. Correct the distribution of observed nutrient intakes for within-subject variation by using the program PC-SIDE.
3. Count the number of individuals in the group with usual intakes of the nutrient below the chosen EAR.
4. Calculate the percentage of individuals in the group who have intakes for any given nutrient that fall below the EAR value.

In this approach too, data on the usual nutrient intakes are required, and these are best obtained using the statistical adjustment described in Section 10.2. In this simplified version of the probability approach, the prevalence of inadequate intakes within the group is simply estimated by counting the number of individuals in the group with usual intakes below the EAR, instead of estimating the risk of inadequate intake levels of each individual separately. This prevalence is represented by the shaded area to the left of the EAR under the curve showing the distribution of usual intakes, as shown in Figure 10.2.

FIGURE 10.2

The EAR Cutpoint Method for Estimating the Proportion of Individuals with Intakes Below the EAR



Adapted from Gibson (2005).

The EAR cutpoint method can be used to assess the prevalence of inadequate intakes of zinc for the life-stage groups depicted in Table 10.2 and Table 10.3, but not for iron for children of certain ages and menstruating women, as noted above. IZiNCG suggests that when at least 25 percent of individuals in the population group have zinc intakes less than the EAR, there is an elevated risk of zinc deficiency in the population (Hotz and Brown 2004). The larger area to the right of the EAR represents the majority with usual intakes above the EAR. Further theoretical justification for the EAR cutpoint method can be found in Carriquiry (1999) and Otten et al. (2006).

This EAR cutpoint method is especially useful when the actual prevalence of inadequate intakes in the groups is close to 50 percent. As the true prevalence approaches zero or 100 percent, the performance of this method declines, even if the conditions listed above are met. Some examples of simulations used to assess the performance of the EAR cutpoint method in different situations are provided by the U.S. Institute of Medicine (IOM 2000a).

At present, the absence of reliable estimates of the EARs for all nutrients limits the applicability of the full probability approach and the EAR cutpoint method to estimating the prevalence of inadequacy for every nutrient. WHO only provides EARs for certain population groups (Allen et al. 2006). Table 10.6 summarizes the nutrients for which an EAR, RDA (or equivalent), Adequate Intake, and UL have been defined by WHO (Allen et al. 2006) and the U.S. Food and Nutrition Board (IOM, 2000b, 2000c, 2001). In the United Kingdom, EARs have been documented for iron, calcium, zinc, vitamin C, vitamin B12, folate, riboflavin, and vitamin A (COMA 1991).

10.6 Using 77 Percent of the RNI as a Cutoff Value

In some tables of nutrient reference levels, estimated average requirements (i.e., EAR) for nutrients are not specified, as noted earlier. In such cases, approximations for the estimated average requirements can be calculated, provided the RNIs or equivalent for each nutrient approximate the mean requirement estimate plus two standard deviations, with a specified coefficient of variation (NRC 1986). The proportion of the population with usual intakes below the derived EAR is then calculated, as described in Section 10.3.

BOX 10.4

Evaluating Mean Nutrient Intakes of Individuals as 77 Percent of the RNIs

1. Select the appropriate reference nutrient intake value for age, sex, physiological state, and diet type from the table of dietary reference values for your country, or use the WHO/FAO (2004) tables.
2. For each individual, calculate observed nutrient intakes from the interactive 24-hour recalls.
3. Correct the distribution of observed nutrient intakes for within-subject variation by using the program PC-SIDE.
4. Count the number of individuals in the group with usual intakes of each nutrient[a] that fall below 77 percent of the chosen RNI.
5. Calculate the percentage of individuals in the group who have usual intakes for each nutrient that fall below 77 percent of the chosen RNI.

[a] *Note:* This approach cannot be used for iron intakes of menstruating women or children aged 1 to 8 years.

Use of 77 percent of the RNI as a cutoff value assumes a coefficient of variation (CV) for the nutrient of 15 percent about the EAR. Such an assumption will yield a conservative estimate of nutrient inadequacy compared to that based on a CV for the nutrient of 10 percent about the EAR. Use of the latter CV corresponds to a cutoff of approximately 83 percent of the RDA instead of 77 percent; hence, it will yield a large percentage of the group likely to have inadequate intakes. This procedure cannot be used for calculating the EARs for iron, because the iron requirement distribution for certain population groups is positively skewed, as noted earlier.

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Chapter 11 | Statistical Analysis of Dietary Data

What will you learn from this chapter?

- How to design your data analyses to answer your research questions;
- How to use the confidence interval to assess how well the group mean intakes estimate the population mean;
- When to apply the unpaired and paired *t*-test and chi-square test; and
- Whether to use the chi-square test or correlation to examine relationships between variables.

The final stage in the dietary assessment protocol is to carry out the statistical analysis of the dietary survey data. A wide variety of statistical analysis methods can be used, depending on the objectives of the study. Two categories of objectives were defined in Section 3.1. Category 1 objectives involve collecting information on dietary indexes on a group basis. The information can be based on a single day's food intake from each individual in the study group, from which mean intakes for the group are calculated. For category 2 objectives, the distribution of usual intakes of dietary variables within a study group must be defined. This involves the collection of the dietary data on more than 1 day from at least a subsample of individuals within each stratum. Such data can then be used to determine the proportion at risk for inadequate intakes (i.e., Objective 2a). Alternatively, multiple recalls can be collected on each individual to assess relationships between dietary variables and other indexes of nutritional status measured on the same individuals (i.e., Objective 2b).

Before carrying out the statistical analyses, it is helpful to select and become familiar with the statistical procedures to be used. Two large packages of statistical programs for use with computers, the Statistical Analyses System (SAS) and the Statistical Program for the Social Sciences (SPSS), are available and can be used by most computer systems. A third statistical program–Stata Statistical Software (StataCorp 2005)–is especially useful for large surveys involving complex designs. Comprehensive manuals and on-line documentation for these three programs detail the mechanics of the statistical techniques. In addition, many books describe the packages, some of which provide background statistical theory, such as the book *Discovering Statistics Using SPSS* by Field (2005). A word-processing, database, and statistics program for public health on IBM-compatible microcomputers entitled "EpiInfo 2004, Version 6" has been produced by the U.S. Centers for Disease Control and Prevention (CDC) in collaboration with the Global Programme on AIDS at WHO. Both the manual and the program are in the public domain and may be freely copied, translated, and distributed. EpiInfo 2004 is available at: www.cdc.gov/epiinfo.

11.1 Assessing the Distribution of the Dietary Data

Many statistical tests make assumptions about the distribution of the dietary data. The best approach to starting the data analysis is to use descriptive statistics to examine the distribution of each dietary variable, one at a time. Descriptive statistics include measures of the central tendency of a variable, such as the mean, median, and mode; measures of dispersion, such as range, percentiles, and standard deviation; and descriptors of the shape of the distribution (e.g, skewness). A normal distribution is a bell-shaped curve with most of the values clustered near the mean, and a few values out near the tails. Data that are normally distributed are symmetrical around the mean, and the mean and median are numerically identical. When the distribution is skewed, the mean and median are markedly different. Data that are markedly skewed may need to be transformed before applying standard statistical procedures. The most frequently used transformation is to take $\log_{10}$ or $\log_e$; this is used where the skew is positive–i.e., the bulk of the values are at the lower end of the distribution with a "tail" of high values. Several statistical tests can be used to test for "normality". Examples include the Kolmorogov-Smirnoff test and the Cox test, details of which can be found in any standard statistical text. However, these tests tend to be oversensitive, and the distribution is usually examined visually for any obvious skewness. If the distributions cannot be normalized using a mathematical transformation, then non-parametric statistical procedures will need to be used.

11.2 Analyses Involving the Mean Intake of a Group

Dietary data collected to determine the mean intake of a group of people (i.e., Category 1 Objectives) can be based on a single recall day from each individual. Care must be taken, however, to ensure that all days of the week are equally represented in the final sample. Such data should not be used to determine the intake of each individual, but can be used for a variety of other objectives (Box 3.1). If the dietary data are found to be approximately normally distributed, then the mean intake for that variable is the best estimate of the central tendency and the standard deviation should be used to express the variability in the data (i.e., how much the measurements of the 1-day intakes differ, on average, from the mean in the population). For normally distributed data, about two-thirds of the values fall within one standard deviation, and about 95 percent of the measurements fall within two standard deviations of the mean.

In most cases, the dietary study is undertaken on a sample drawn from the population, rather than the whole population. Nevertheless, information on the dietary variables for the whole population is often required, and this is inferred from the data obtained from the sample. There is always uncertainty in the extrapolation of results from the sample to the whole population, and this uncertainty is expressed by calculating the confidence interval (CI), a measure of the precision of the sample estimates. The confidence interval is defined as the interval or range of values that most likely encompasses the true population value. The lower and upper limits of this interval are referred to as "confidence limits". The confidence interval is calculated from the sample mean and the standard error (SE). The standard error gives an estimate of the degree to which the sample mean varies from the true population mean. To calculate the standard error for the mean intake of a nutrient ($\bar{\chi}$), divide the standard deviation (s), by the square root of the sample size (n) (i.e., $SE = s/\sqrt{n}$). The lower and upper limits of the 95 percent confidence interval are then calculated as the sample mean ± 1.96 times the standard error.

That is, the lower $CI_{95} = \bar{\chi} - \frac{1.96s}{\sqrt{n}}$

and the upper $CI_{95} = \bar{\chi} + \frac{1.96s}{\sqrt{n}}$

(For a 90 percent confidence interval, replace 1.96 by 1.67.)

The larger the sample size, the smaller will be the standard error and the narrower the confidence interval and, as a result, the sample mean will be a better estimate of the population mean.

When the nutrient intake data are not normally distributed, as is the case for some micronutrients, it is better to present the median intake as a measure of the central tendency instead of the mean, because the median is not influenced by extreme values. The 25th and 75th percentiles should be used to express the variability in the data. Alternatively, the geometric mean and the 95 percent confidence interval can be presented if the distribution has been normalized with a log transformation.

11.3 Differences between Mean Intakes of Two or More Groups

In many dietary studies, the objective is often to determine differences between mean intakes of two population subgroups (e.g., urban and rural) studied at one point in time (i.e., Objective 1b) , and to assess whether the observed differences represent real differences and are not the result of chance. In cases where each set of observations is sampled from a population subgroup with a normal distribution, and provided the variances of the two population subgroups are the same, Student-Newman-Keuls ('Student's') two sample *t*-test can be used (see Box 11.1). To assess whether the variances are homogenous, an *F*-test can be used. Further information on the Student-Newman-Keuls two sample *t*-test can be found in any standard statistical text (see e.g., Altman 1991).

Alternatively, to compare mean intakes among two or more population subgroups, analysis of variance (ANOVA) can be used. The assumptions of this method are the same as those for the two sample *t*-test that each population subgroup is normally distributed, and that their variances are equal. To determine whether the means of all the subgroups are equal, the *F*-test can be used. The null hypothesis is rejected

when the *F*-ratio is greater than a specified critical value, and the means of each population subgroup are not equal. A further analysis is then required to find out which means are significantly different. Several *posteriori* tests can be used for multiple comparisons between means, including Duncan's multiple range test, the Scheffé test, the Student-Newman-Keuls test, and the method of least significant differences. These tests are available in most computer programs that include ANOVA (Beaglehole et al. 1993).

When the dietary variables are not normally distributed, attempts should first be made to normalize the data before testing the means. If the intake data are not amenable to simple log (either $\log_{10}$ or $\log_e$) transformation, then the median (50th percentile) should be used to express the central tendency of the two groups, and selected percentiles (e.g., 25th and 75th percentiles) to describe the variability. The nonparametric Wilcoxon's matched pairs signed-rank test or Mann-Whitney *U*-test should then be used to test for a significant difference between the two groups. For more than two groups, the Kruskal-Wallis one-way analysis of variance is the statistical method of choice, followed by the Mann-Whitney-*U posteriori* test to identify which means are significantly different. For details of these tests, consult a standard statistical text.

BOX 11.1

Choosing a Significance Test when Determining Differences between Groups

Sampling Method	Unpaired Observations	Paired Observations
Categorical nominal data Expected frequency >/= 5	Chi-square test	Sign test
Expected frequency <5	Fisher's exact test	McNemar's chi-square test
Numerical data Two groups (normally distributed data)	Student's two sample *t*-test	Paired *t*-test
Two groups (non-normally distributed data)	Wilcoxon two-sample or Mann-Whitney-*U* test	Wilcoxon signed rank test
More than two groups (normally distributed data)	Analysis of variance	-
More than two groups (non-normally distributed data)	Kruskal-Wallis	-

Different statistical procedures are used to demonstrate a significant change in the mean nutrient intakes over time when the same subjects have been studied at baseline and during follow-up (Objective 1c). In this case, the most commonly used statistical test for examining paired observations of normally distributed, numerical data, is the paired *t*-test (Box 11.1). This test should also be used when two different groups of subjects have been individually matched; for example, in a matched pair case-control study. In the event that the nutrient intakes for the paired data are not normally distributed, then the nonparametric Wilcoxon's matched pairs signed-rank test can be used (Box 11.1).

The confounding effect of within-subject variation on usual nutrient intakes is not taken into account when any of the statistical tests discussed above are used. When the within-subject variation is large relative to the between-subject variation, the power of all these statistical tests will be reduced. As a result, the lack of any significant differences in group mean intakes may be due to the confounding effect of large within-subject variation. This will be apparent in a large coefficient of variation (NRC 1986).

When assessing changes in the mean intakes of two groups measured at baseline and again after an intervention (Objective 1c), use of analysis of covariance (ANCOVA) is often used. Details are given in Vickers and Altman (2006). ANCOVA is unaffected by differences between the two groups at baseline, and has greater statistical power to detect a treatment effect than other statistical methods.

For studies in which the mean intakes at baseline and post-intervention are not measured on the same subjects at baseline and post-intervention (i.e., Objective 1d), analysis of variance (ANOVA) procedures are usually used.

11.4 Analyses of Proportion at Risk of Inadequate Intakes

As noted in Section 3.3, dietary data collected for two non-consecutive days on at least a representative subsample within each stratum of the respondents can be used to derive the distribution of usual intakes of foods and/or nutrients in the study group. The subsample in each stratum should consist of 30 to 40 individuals.

Note that it is more important to have a minimum number of repeated 24-hour recalls in the subsample than a minimum proportion of repeated 24-hour recalls (IOM 2000). The distribution of observed intakes can then be adjusted to represent those of usual intakes by removing the variability introduced by day-to-day variation in intakes within an individual (i.e., to remove the within-subject variation), as described in Section 10.5.

The adjustment process provides estimates of the usual nutrient intakes for each specified age- and sex-specific subgroup, as noted in Section 10.4, which can then be used to provide a more valid estimate of the proportion of the study group at risk of inadequate intakes (Objective 2a) by using either the full probability approach (Box 10.2) or the Estimated Average Requirement cutpoint method (Box 10.3).

With such data, the investigator may then wish to examine whether risk of inadequate intakes is associated with certain other variables (e.g., age, sex, household composition, education, socioeconomic status, or geographic area). This can be done by constructing simple cross-tabulation tables for determining the pattern of the association between the selected variables. For example, the number at risk of inadequate intakes of iron in children could be cross-tabulated with the following dichotomized variables: age (younger or older than 3 years), sex, household composition (at least four persons or fewer than four persons), geographic area (urban or rural), years of education of the primary caregiver (0 to 3 years, or 4 or more years), and so forth. Care must be taken when constructing the cross-tabulation tables to ensure that the categories of variables for the tables have no overlaps and no gaps, divisions in the variables have meaning in the context of the population, and that cell-sizes are expected to be approximately equal. Under these circumstances, the column and row counts correspond to the frequency counts for each variable, and the grand total in the table corresponds to the number of subjects in the sample.

The next step is to select an appropriate test for examining the magnitude of any association between the two variables in the 2 x 2 table. If the sample size exceeds 40, then the chi-square (χ^2) test can be used and the chi-square value for the 2 x 2 table calculated (Box 11.1). This test is based on measuring the difference between the observed frequencies in each of the cells and the expected frequencies if the null hypothesis (i.e., the hypothesis of no difference) were true. It involves three steps: calculating the chi-square value; using a chi-square table; and interpreting the result. Details of each of these steps can be found in any standard statistical text.

Note that the chi-square test, unlike the *t*-test, can also be used to compare more than two groups, such as the 2 x 3 (seen in Table 11.1), 3 x 3, and even larger tables. However, it should not be used when the expected frequency in any cell is less than five. In such cases, Fisher's exact test should be used (Box 11.1).

TABLE 11.1

An Example of the Use of a Contingency Table to Assess the Difference in the Risk of Inadequate Intakes of Iron in Subjects Living in an Urban vs. a Rural Setting

Risk of Inadequate Intake of Dietary Iron	Number of Urban Subjects	Number of Rural Subjects	Total Number of Subjects
Low risk of inadequate intake of iron	58 (35.8%)	35 (21.6%)	93 (57.4%)
Moderate risk of inadequate intakes of iron	11 (6.8%)	25 (15.4%)	36 (22.2%)
High risk of inadequate intakes of iron	10 (6.2%)	23 (14.2%)	33 (20.4%)
Total	79 (48.8%)	83 (51.2%)	162 (100.0%)

Table 11.1 shows the number of urban and rural subjects in each of three risk categories for inadequate intakes of iron, together with the percentages (%) of the total number of subjects in each category.

The calculated chi-square statistic for the data in Table 11.1 is 16.17, with 2 degrees of freedom. The probability of this distribution of risk of inadequate intakes of iron arising by chance is 0.00031. The rural group appears to be significantly more at risk to low intakes of iron than is the urban group.

In case-control and cohort studies, the relative risk (RR) and/or the odds ratio (OR) is often used to quantify the strength of an association between a risk factor

and the presence or absence of the condition or disease, although other explanations such as chance, bias, and confounding must also be considered. The RR is defined as the ratio of the risk of occurrence of a condition or disease (e.g., anemia) among individuals exposed to the risk factor (e.g., high risk of inadequate intake of available iron) to the risk of occurrence of a condition (e.g., anemia) among those unexposed (e.g., low risk of inadequate intakes of available iron). The odds ratio (OR) can be used to estimate the relative risk when the prevalence of the disease is low. It measures the odds of having the risk factor (e.g., inadequate intake of available iron) if the condition or disease (i.e., anemia) is present divided by the odds of having the risk factor if the condition or disease is not present.

Both the RR and the OR can take values between 0 and infinity. A value > 1 indicates that the risk or odds of the disease are greater when exposed to the risk factor (positive association); a value of 0 indicates no association, and a value < 1 indicates reduced risk or odds of the disease with exposure to the risk factor (negative association). Numerical differences in the RR and OR may occur but the values are always in the same direction. The 95th confidence interval for OR can also be calculated. Details of how to obtain estimates of relative risk and/or OR for unpaired and for paired observations can be found in Altman (1991).

11.5 Analyses of Interrelationships between Dietary and Other Variables

Associations among dietary variables and between dietary variables and biochemical, anthropometric measures on the same individuals can also be examined (i.e., Objective 2b), provided reliable estimates of the usual food and nutrient intakes of individuals have been obtained. This can only be achieved by obtaining multiple 24-hour recalls on each individual. The number of recall days required depends on the nutrient of interest, and the within-subject variation in nutrient intakes (Box 3.9). In practice, however; because of the respondent burden and cost, generally only a maximum of 4 days per individual is feasible, regardless of the extent of the within-subject variation for the nutrient under study, as noted in Section 3.3.

Dietary variables used in this way might include the intakes of selected foods, food groups, nutrients or antinutrients expressed per day, per kilogram body weight, or per megajoule of energy. Examples of additional variables that may be associated with variation in dietary intake include sex; age; geographical area; household composition; socioeconomic status; education; and anthropometric, biochemical, and physiological functional or clinical indexes.

If the two numerical variables of interest are both measured on a continuous scale (e.g., for each individual, mean daily intake of iron in milligrams per day and hemoglobin concentration in grams per liter), a useful first step in examining the association between the variables is to generate a scatter plot to show graphically the relationship of the two variables. Such a procedure is available in most computer statistics packages. The scatter plot can then be inspected visually to assess whether the relationship between the two variables is approximately linear. Caution must be used, however, because a relationship may appear linear where there is no relationship at all between the two variables but the population consists of two groups, one of which has a higher mean value on the two variables. If a linear relationship appears likely, then the magnitude of the linear association can be estimated using Pearson's correlation coefficient (r). This indicates the strength of the linear association between two continuous variables that are normally distributed, and ranges between –1.0 and +1.0. Negative values indicate that as one variable increases, the other decreases. The closer the absolute value of r is to 1.0, the stronger the association; the closer to 0, the weaker the association. Note for data that are not normally distributed, Spearman's nonparametric rank correlation coefficient can be used. The statistical test for the significance of the correlation coefficient involves testing whether the absolute value of the correlation coefficient is significantly greater than 0.

These correlation procedures do not take into account the confounding effect of within-subject variation on usual nutrient intakes (see Section 10.4). This effect, if it is large relative to between-subject variation, reduces the estimated value of the correlation coefficient

relating individual nutrient intakes to other variables measured on the same persons. The procedure for correcting the correlation coefficients is discussed in Section 11.6.

Alternatively, a classificatory approach is sometimes used to examine associations between dietary intakes and risk of chronic disease. This approach is often used when only a limited number of repeated 24-hour recalls (e.g., two) have been collected on each subject. In this approach, respondents are classified into categories, usually thirds (tertiles), fourths (quartiles), or fifths (quintiles), based on their intake of specific foods or nutrients. Relative risks can then be computed, for each of the four lower quintiles, to cite one example, by treating the uppermost quintile of intake as the reference quintile.

11.6 Correcting the Effects of Within-subject Measurement Errors

Random within-subject measurement error may decrease correlation and regression coefficients towards zero and bias relative risk estimates towards one such that important associations between diet and disease may be obscured. This phenomenon is referred to as "attenuation bias".

Correlation at the individual level between dietary data and other indexes of nutritional status such as anthropometric, biochemical, or clinical indexes are lowered by within-subject variation. The theoretical reduction in the absolute value of the correlation coefficient can be calculated from the ratio of within-subject to between-subject variation (i.e., the variance ratio) and the number of replicate observations (Table 11.2) provided the sample size is large (preferably above 100). For example, if the observed variance ratio is 2.0, as determined from three separate 24-hour recalls, the correlation coefficient *r* between the estimated intake and some biochemical parameter is 77 percent of the true correlation. This figure represents the theoretical attenuation factor from Table 11.2. Hence, the calculated correlation coefficient can be corrected by dividing by 0.77 before testing the significance of the *r* value (Box 11.2). With small sample sizes (i.e., fewer than 100), however, this correction is not advised, because the sampling error associated with the correlation coefficient may be too large (IOM 2000).

Attenuation may also reduce the significance of regression. Attenuation factors corresponding to different ratios of within- to between-subject variance

TABLE 11.2

Attenuation Factors for Simple Correlation Coefficients as Determined by the Number of Replicate Observations per Individual and the Variance Ratio

(the ratio of the within-subject to between-subject variances)

Variance Ratio	Number of Replicates per Individual					
	1	3	5	7	10	14
0.0	1.00	1.00	1.00	1.00	1.00	1.00
0.5	0.82	0.93	0.95	0.97	0.98	0.98
1.0	0.71	0.87	0.91	0.94	0.95	0.97
1.5	0.63	0.82	0.88	0.91	0.93	0.95
2.0	0.58	0.77	0.85	0.88	0.91	0.94
2.5	0.53	0.74	0.82	0.86	0.89	0.92
3.0	0.50	0.71	0.79	0.84	0.88	0.91
3.5	0.47	0.68	0.77	0.82	0.86	0.89
4.0	0.45	0.65	0.75	0.80	0.85	0.88
4.5	0.43	0.63	0.73	0.78	0.83	0.87
5.0	0.41	0.61	0.71	0.76	0.82	0.86

Adapted from a more complete data table provided by Anderson (1986).

and different numbers of measurements are also available for simple linear regression. As an example, if the variance ratio equals 2.0 and three measurements of dietary intake (e.g., three 24-hour recalls) are used, the regression coefficient of a biological variable on the estimated value of the dietary factor is 60 percent of the true coefficient. Such a correction must be made with caution, as noted for the correlation coefficients. The complete data tables for the attenuation factors for simple correlation and linear regression coefficients are available in Anderson's publication (1986).

BOX 11.2

Correcting the Correlation Coefficient by Using the Attenuation Factors

- Calculate the variance ratio (for the nutrient of interest) by using analysis of variance (NRC 1986).
- Determine the attenuation factor from Table 11.2.
- Divide the calculated correlation coefficient by the attenuation factor.
- Test the significance of the corrected *r* value using tables of significance for Pearson's correlation coefficient.

Several methods are also available to correct attenuated relative risk estimates for both continuous and categorical variables; however, these methods are beyond the scope of this manual. The reader is advised to consult Willett (1998) for further details about these techniques. The presence of other factors may also influence the relationship between two variables. In some cases, associations may be spurious due to chance (random error) and/or bias (systematic error). Alternatively, associations may be real but do not represent a cause-effect relationship. Instead, it is possible that the association may be either an effect-cause relationship, or an effect-effect relationship. An effect-cause relationship occurs when the outcome actually caused the predictor, rather than *vice versa*, and is often a problem in cross-sectional and case-control studies. An effect-effect relationship occurs when there is a confounding variable that is associated with the predictor variable and a cause of the outcome variable (Hulley and Cummings, 1988). The reader is advised to consult a standard epidemiology text for further details. Several strategies can be used to cope with confounding variables, depending on the design of the study. Details are given in Hulley and Cummings (1988). In cases where associations among multiple variables are to be examined, multivariate analysis techniques are necessary. For multivariate analyses, statistical correction of the attenuation arising from within-subject variation in nutrient intakes is not feasible. Application of such multivariate techniques requires the assistance of an experienced statistician. Increasingly, complex statistical models are being developed to overcome sources of measurement error in dietary intake data (Kipnis et al. 2002). Indeed, the impact of measurement error in dietary assessment on the design, analysis, and interpretation of nutritional assessment data is a topic of intensive research.

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Appendix A: Glossary of Terms

Adequate intake (AI): is used when an estimated average requirement (EAR) cannot be set because of the absence of definitive data. Adequate intakes are based on an approximation of the average nutrient intakes by a population group or subgroup that appears to be healthy. The AI is used as an intake goal for an individual.

Accuracy reflects the extent to which the measurement is close to the true value.

Alpha (α) is the probability of committing a type I error (i.e., an association due to random error).

Alternative hypothesis (H_1) is a statement of what the value of the parameter is in the population if the null hypothesis is not correct.

Analysis of variance refers to a statistical analysis that compares the averages of a continuous variable across more than two subgroups (e.g., dietary iron intakes by low, medium, and high socio-economic status).

Association refers to the relationship between two or more variables.

Beta (β) is the probability of making a type II error.

Bias is a condition resulting from defects in a study design that cause a result to depart from the true values in a consistent direction.

Bioavailability is defined as the proportion of a nutrient in food that is absorbed and utilized for normal metabolic and physiological functions or storage.

Bioavailability algorithm is a mathematical model that attempts to predict bioavailability by taking into account the form of the nutrient, presence of dietary modifiers, and nutrient status of the individual, where applicable.

Certified reference materials (CRMs) are used to test the accuracy of an analytical method by comparing analyzed values with the certified values supplied by the manufacturer.

Cluster sampling can be defined as any sampling plan that uses a frame consisting of clusters of enumeration units. Unlike strata, clusters should be as heterogeneous as possible.

Coding is a method used to convert the data gathered during the dietary survey into symbols appropriate for data analysis.

Coefficient of variation (as %) expresses the standard deviation as a percentage of the mean value. It is used to compare the precision of several variables.

Cohort study involves making repeated observations of the same individuals over time.

Community participation refers to a process whereby the community is involved in at least some stages in planning and carrying out the dietary survey. The participation may involve local advisory committees, volunteers or paid peer counselors, or volunteer or paid survey interviewers.

Concurrent validity of a dietary method is determined by comparing dietary intake results with those obtained from external variables such as biochemical indexes that are sensitive to dietary intake. In this way, the biochemical indexes play a role in calibrating and improving the dietary assessment tool.

Confidence interval is defined as the interval or range of values that most likely encompasses the true population value.

Confounding variable refers to a variable that is associated with the problem and with a possible cause of the problem. Such a variable may either strengthen or weaken the apparent relationship between the problem and possible cause.

Correlation is a statistic used for studying the strength of an association between two variables.

Cross-sectional dietary surveys involve studying the dietary intakes of individuals within a population at the same time.

Cutoff points are based on the relationships between the nutritional assessment indexes and functional impairment, clinical signs of deficiency, or both. They are used to establish the prevalence of malnutrition within a population or to identify and classify malnourished individuals.

Dependent variables describe or measure the problem under study.

Dietary modifiers are components in the diet that enhance or inhibit the absorption of a nutrient.

Effect size refers to the size of the difference sought.

Estimated average requirement (EAR) is the daily intake estimated to meet the requirements, as defined by a specified function or biochemical measurement of 50% of the individuals in a particular life-stage and sex.

Flat slope syndrome occurs in 24-hour recalls from the tendency for low food intakes to be overestimated and high food intakes to be underestimated.

Frequency distribution refers to the results grouped according to the frequency in each category.

Graduated food models assist in quantifying portions of foods consumed in recall dietary methods. They consist of a collection of papier-mâché, plastic, wooden, or hardboard shapes of various volumes or surface areas together with a series of thickness indicators that are used to assess the overall size and thickness of foods.

Hypothesis is a prediction of a relationship between one or more factors and the problem under study that can be tested.

Imputed food composition values are derived from data for another form of the same food or for a similar food. Examples include calculating data for cooked foods from raw foods or calculating foods on a fresh weight basis from their dry weight.

Independent variables describe or measure factors that are assumed to cause or at least to influence the problem.

Internal consistency is the concordance between two variables that measure the same general characteristic.

Interindividual variation describes the extent to which a particular parameter varies between individuals within a sample population.

Interviewer bias occurs if different interviewers probe for information to varying degrees, intentionally omit certain questions, or recall responses incorrectly.

Intraindividual variation describes the extent to which a particular parameter varies within one individual in a sample population.

Mean value ($\bar{\chi}$) is the average value for a particular variable.

Median value is that value of the variable, in an ordered list of values, that has an equal number of items on either side of it (i.e., the middle value).

Mode value refers to the result that occurs most often.

Multistage sampling involves sampling in two or more stages.

Non-probability sampling is based on a sampling plan that does not rely on formal random techniques to identify the units to be selected. Examples include convenience sampling and quota sampling.

Normal (or Guassian) distribution refers to a continuous symmetrical frequency distribution with a shape determined by its mean and standard deviation.

Normative requirement estimate represents the amount needed to prevent clinically detectable signs of functional impairment.

Null hypothesis (H_0) is a statement concerning the value of the population parameter. For instance, the statement might be that there is no difference between the dietary variables in the groups or no association between the variables under study.

Observer bias is a consistent distortion, conscious or unconscious, in the perception or reporting of the measurements by the observer.

Odds ratio measures the odds of having the risk factor if the condition or disease is present divided by the odds of having the risk factor if the condition or disease is not present.

Power of the sample refers to the probability of detecting a specified difference.

Power of a test refers to the probability of correctly rejecting the null hypothesis when it is false, commonly denoted by $1 - \beta$.

Precision refers to the degree to which a variable has nearly the same value when measured repeatedly.

Prevalence is a measure of the number of persons with inadequate intakes of a nutrient or with malnutrition or disease at a given time. Numerically, the prevalence is the proportion of individuals who have inadequate intakes, or who are really malnourished, or infected with the disease in question, divided by the sample population.

Probability approach is a statistical method for accurately establishing the proportion of people in a population who have nutrient intakes below their own individual requirements.

Probability sampling relies on formal random techniques to ensure that every element in the population has a known probability of being included in the sample.

Random measurement errors may occur when the same examiner repeats the measurements (within- or intra-examiner error) or when several different examiners repeat the same measurement (between- or inter-examiner error). Such errors reduce the precision of a measurement by increasing the variability about the mean. They can be minimized by incorporating standardized measurement techniques and using trained personnel.

Range is the difference between the highest and lowest data values in a population or sample.

Recommended dietary allowance (RDA) refers to the dietary intake level that is sufficient to meet the daily nutrient requirements of almost all (97 to 98 percent) of the individuals in a specific life-stage and sex. If the variation in requirement is well defined, it is set at two standard deviations above the EAR. The recommended dietary allowance is an appropriate goal for daily nutrient intakes of individuals.

Reference nutrient intake (RNI) (UK) or Recommended nutrient intake (WHO) refers to the dietary intake level that is sufficient to meet the daily nutrient requirements of almost all (97 to 98 percent) of the individuals in a specific life-stage and sex. If the variation in requirement is well defined, it is set at two standard deviations above the EAR. The reference nutrient intake is an appropriate goal for daily nutrient intakes of individuals. The term RNI is used interchangeably with RDA as used by the Food and Nutrition Board of the United States National Academy of Sciences.

Relative risk is defined as the risk of getting a condition or disease in the group with the risk factor divided by the risk of getting the condition or disease in the group without the risk factor.

Relative validity involves evaluating the test dietary method against another reference dietary method. The reference dietary method must be as accurate and precise as the test method and must be designed to measure similar parameters over the same time frame as the test method. For example, the relative validity of a single interactive 24-hour recall should not be assessed by comparison with a dietary history or a 7-day weighed record, but with a 1-day weighed dietary record.

Reproducibility refers to the degree to which repeated measurements of the same variable give the same value.

Respondent is any person who answers a set of questions in an interview or questionnaire. The responses by the individual are considered to pertain to that person alone and not to a wider population whom the person professes to represent.

Sampling frame consists of a list from which the sample can be selected. It may consist of a list of individuals in the population, households, districts, villages, schools etc.

Sensitivity is the ability of an index to identify and classify persons who are genuinely malnourished. An indicator with 100 percent sensitivity correctly identifies all those individuals who are genuinely malnourished: no malnourished persons are classified as well (i.e., there are no false negatives).

Simple random sampling is a sampling procedure in which every study unit has the same chance of being selected and every sample of the same size has the same chance of being chosen.

Specificity is the ability of an index to identify and classify persons who are genuinely well nourished. An indicator with 100 percent specificity correctly identifies all those individuals who are genuinely well nourished: no well-nourished persons are classified as ill (i.e., there are no false positives).

Social desirability response bias refers to the tendency to overreport the consumption of certain "good" foods and to under-report the consumption of "bad" items.

Standard error is a measure of the degree to which the sample mean varies from the population means. To calculate the standard error for the mean intake of a nutrient ($\bar{\chi}$), divide the standard deviation (s) by the square root of the sample size ($\sqrt{n}$).

Stratified random sampling is the process of breaking down the population into mutually exclusive and exhaustive strata, selecting a random sample from each stratum, and finally combining these into a single sample to estimate the population parameters.

Stratum is a subpopulation of the original population. The strata are formed on the basis of some known characteristic about the population (e.g., pregnancy), which is believed to be related to the variable of interest (e.g., biochemical iron status).

Subject bias is a consistent distortion of the measurement by the study subject.

Systematic measurement errors may be present in any measurement process. Such errors reduce the accuracy by introducing a bias that alters the mean or median value. Such errors have no effect on the variance and hence do not alter the precision of the measurement.

Test-retest consistency refers to the concordance among repeated measurements on a sample of subjects.

Two-tailed test refers to a statistical significance test in which deviations from the null hypothesis in either direction are considered. Use of a two-tailed test implies that the investigator was willing to consider deviations in either direction before data were collected.

Type I (or alpha [α]) error occurs whenever a null hypothesis which is true is incorrectly rejected. The probability of making a type I error may be controlled by the investigators and is denoted by α.

Type II (or beta [β]) error occurs when a null hypothesis was in fact false and should have been rejected. The probability of making a type II error is denoted by β.

Validity describes the degree to which any measurement or index measures what it is supposed to measure.

Variable is a characteristic of a person, object, or phenomenon that can take on different values.

Variables: background include such things as age, sex, education level, socioeconomic status, marital status, and religion. They are often related to a number of independent variables so that they influence the problem indirectly.

Variables: categorical are phenomena that are not suitable for quantification but instead are expressed as categories. For example, the variable sex has two values, male and female, that are distinct categories.

Variables: categorical nominal are phenomena that can be categorized but not scaled as there is no ranking order in the categories. Examples include sex or main food crops (such as maize, millet, rice), among other categories.

Variables: categorical ordinal are phenomena that can be categorized, and the categories can then be ranked in increasing or decreasing order. Examples include high income, middle income, and low income.

Variables: confounding distort an assumed relationship between two other variables. For example, the relationship between diet and nutritional status is influenced by the presence of infection.

Variables: continuous have quantified intervals on an infinite number of values (e.g., body weight).

Variables: dependent (or outcome variable) describe or measure the problem under study.

Variables: independent describe or measure factors that are assumed to cause or at least to influence the problem under study.

Variables: numerical are those that are expressed in numbers.

Variance ratio is a measure of the ratio of intra- to intersubject variation in the true usual intake of a nutrient.

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Appendix B: Random Number Table

Instructions for Using the Random Number Table

1. Decide how large a number you need. Is it a one-, two-, or three-digit number? For example if your sampling frame consists of 10 units, you must choose from the numbers 1 to 10 (inclusive) and you must draw a two-digit number to ensure that 10 has an equal chance of being chosen. Two-digit numbers will also be required for a sampling frame from 0 to 99 units; three digits are needed for a sampling frame of 0 to 999 units; four digits for sampling frame from 0 to 9999 units.
2. Decide beforehand whether you are going to read the page of numbers from left to right, right to left, or up the page, or down the page.
3. Without looking at the table and using a pencil, pen, stick—or even your finger—pick a single-digit number within the body of the table.
4. Let us assume that in this way you randomly decided to start at column 3, row 20, and that you decided to read the table from left to right. If you need a single-digit number, that number is 8; if you need a two-digit number, that number is 89, if you need a three-digit number, that number is 893; etc.
5. If the randomly selected number is too large, for example, you may need two-digit numbers from 0 to 50. Discard the first digit pair (89) and read the next pair of digits (31), again reading from left to right. Continue in this way until you have sufficient digit pairs for your purposes.
6. Proceed similarly if selecting three- and four-digit numbers, starting at the left margin of the next lower line of numbers after completing the first row.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	3	4	4	7	4	4	8	5	2	2	9	9	3	5	8	8	7	4	7	3
2	0	1	5	7	4	4	7	2	5	6	1	0	6	8	5	0	2	2	8	4
3	0	0	0	7	5	7	2	9	8	1	3	7	3	6	7	7	8	6	9	0
4	0	4	1	4	6	7	2	8	5	2	4	5	1	0	9	3	7	4	1	3
5	8	9	0	8	5	2	0	9	7	3	8	4	7	0	3	7	3	7	2	0
6	5	7	6	1	9	6	4	5	5	3	0	7	6	0	4	5	8	7	7	6
7	2	4	5	5	5	7	6	1	4	2	4	8	0	2	0	4	9	2	7	6
8	8	5	7	9	0	5	4	9	6	3	6	2	5	8	1	8	7	2	3	7
9	3	0	7	2	2	3	1	1	6	9	0	8	2	3	1	8	1	0	9	4
10	5	4	8	5	5	0	2	8	6	6	9	8	6	3	9	9	7	9	7	0
11	0	9	3	6	2	7	2	7	1	2	7	3	0	9	8	9	6	0	9	6
12	9	5	6	1	3	4	3	2	9	5	7	5	2	5	0	9	5	2	6	7
13	8	1	7	7	6	4	1	7	3	3	8	9	4	3	0	3	6	2	3	8
14	2	3	2	1	9	4	9	2	7	2	1	5	4	3	3	0	9	2	6	4
15	4	2	0	5	2	2	9	4	9	1	2	4	3	1	9	2	4	2	7	7
16	6	8	0	9	7	6	6	0	8	4	3	7	4	3	8	3	9	3	9	3
17	8	1	5	5	1	4	5	7	0	9	8	0	8	5	1	9	4	6	5	3
18	8	0	4	5	7	1	6	5	0	5	0	4	3	6	4	3	9	6	5	8
19	6	4	6	5	4	5	1	4	4	6	4	6	4	3	8	0	4	8	9	6
20	7	9	8	9	3	1	1	3	3	8	9	5	8	9	0	8	8	2	5	2
21	5	4	5	8	5	9	9	8	5	1	7	0	3	0	9	6	5	7	1	5
22	7	6	2	6	5	4	0	3	6	3	2	0	2	6	9	7	8	8	8	3
23	8	1	5	5	2	7	0	5	0	0	8	8	8	1	2	6	6	7	3	1
24	5	8	8	9	6	1	2	3	0	2	3	7	7	1	4	0	7	7	4	8
25	0	4	3	8	1	1	8	8	8	2	8	5	3	6	0	5	9	8	8	4
26	2	2	7	0	3	3	0	8	8	3	5	9	8	6	0	6	3	0	4	6
27	2	9	6	5	5	0	8	6	3	5	0	6	9	1	1	6	9	0	0	5
28	8	7	2	1	4	6	8	0	2	1	7	9	0	1	6	3	7	4	6	1
29	0	0	2	3	9	9	6	5	7	5	9	8	8	1	9	8	4	1	8	4
30	0	2	0	9	2	5	0	9	0	3	6	8	8	4	8	6	6	8	2	0
31	1	9	2	8	4	5	4	2	4	1	9	2	2	6	3	7	7	0	8	1
32	8	6	0	9	9	5	9	7	6	3	3	0	6	7	3	6	2	0	8	2
33	8	0	9	0	4	7	6	9	3	7	5	7	1	4	3	0	5	1	6	7
34	3	8	9	8	1	6	5	9	5	2	6	2	6	0	0	3	4	5	9	8
35	5	2	7	3	8	1	4	3	0	2	7	7	9	0	3	2	4	8	0	8
36	5	0	1	9	2	7	0	1	7	1	9	2	8	6	2	9	4	1	2	7
37	6	1	8	7	4	6	0	1	3	7	9	4	4	6	4	1	8	9	3	9
38	7	9	5	7	7	9	7	5	4	4	8	6	6	9	9	4	6	4	2	9
39	6	0	7	3	5	9	2	6	3	6	4	6	5	3	5	2	1	8	8	4
40	7	4	5	5	2	9	1	6	1	6	6	2	8	6	0	5	5	5	9	8

Appendix C: | Measurement Abbreviations and Small Volume Measures

cm	centimeter	mmol	millimol (10^{-3} mol)
fl oz	fluid ounces	mg	milligram (10^{-3}g)
g	gram	ng	nanogram (10^{-9}g)
g	gravitational constant	nm	nanometer (10^{-9}m)
in	inch	pg	picogram (10^{-12}g)
kcal	kilocalorie	T	tablespoon
L	liter	t	teaspoon
lb	pound	µg	microgram (10^{-6}g)
MJ	megajoule (10^{6} joules)	µL	microliter ((10^{-6}g)
mL	milliliter (10^{-3}L)	µmol	micromole (10^{-6} mol)
mm	millimeter (10^{-3}m)	w/v	weight for volume

1t	= ⅓T	= ⅙fl oz	= 4.9mL
3t	= 1T	= ½fl oz	= 14.8mL
2T	= ⅛ cup	= 1fl oz	= 29.6mL
4T	= ¼ cup	= 2fl oz	= 59.1mL
5 ⅓T	= ⅓ cup	= 2 ⅔fl oz	= 78.9mL
8T	= ½ cup	= 4fl oz	= 118.3mL
10 ⅔T	= ⅔ cup	= 5 ⅓fl oz	= 157.7mL
12T	= ¾ cup	= 6fl oz	= 177.4mL
14T	= ⅞ cup	= 7fl oz	= 207.0mL
16T	= 1 cup	= 8fl oz	= 236.6mL
1mL	= 0.034fl oz	= 1cc	= 0.001 liter
1 liter	= 34fl oz	= 1000mL	

Note: The U.S. and Canadian standard measuring spoons used above are slightly smaller in capacity than the equivalent United Kingdom standard measuring spoons.

Appendix D: Specific Gravity Data for Drinks and Other Liquids

Milk Products and Eggs	
Skimmed milk	1.036
Semiskimmed milk	1.034
Whole milk	1.031
Condensed milk (sweetened)	1.160
Evaporated milk (unsweetened)	1.066
Single cream	1.000
Whipping cream	0.990
Double cream	0.990
Yogurts	1.080*
Ice cream	0.550*
Eggs	1.020
Fats and Oils	
Palm oil	0.890
Other vegetable oils	0.918*
Selected Beverages	
Baby fruit juice	1.040
Baby fruit juice concentrate	1.320
Carbonated Drinks	
Barley crush	1.070
Cola	1.040
Fruit juice drink	1.040
Lemonade	1.020
Fruit Juice Drinks (ready to drink)	
Apple flavor	1.040
Citrus	1.040
Mixed fruit	1.030
Black currant	1.050
Mixed fruit with blackcurrant	1.040
Fruit Drink Concentrate	
Any fruit, not blackcurrant	1.100
Barley water	1.100
Mixed fruit	1.115
Black currant	1.280
Milk Drinks	
Chocolate skimmed milk drink	1.050
Other skimmed milk drinks	1.040
Mars milk	1.070
Milk shake	1.060
Yogurt drink with fruit	1.060

*variable; mean value quoted

References

Ministry of Agriculture, Fisheries and Food. 1993. *Food portion sizes.* 2nd ed. Her Majesty's Stationery Office, London.

Appendix E: Suppliers

Disclaimer: *This information is provided for the information of readers; it should not be taken as an endorsement by the authors or publisher of listed products, manufacturers, or suppliers. No significance should be attached to the absence from this list of any product, manufacturer, or supplier.*

Agate ball mill: for grinding dried food samples for trace element analysis

Brinkman Model MM,
Brinkman Instruments Division, Sybron, Canada Ltd.
6670 Campobello Rd.,
Mississauga, Ontario L5N 2L8, Canada
Phone: 1-(800)-263-8715 or 1-905-826-5525; Fax: 1-905-826-5424

Brinkman Instruments, Inc.,
One Cantiague Rd., P.O. Box 1019,
Westbury, New York, 11590-0207, USA
Phone: 1-(800)-645-3050 or 1-516-334-7500; Fax: 1-516-334-7506
E-mail: info@brinkmann.com

Certified reference materials: for food composition trace element analysis

Analytical Quality Control Services,
International Atomic Energy Authority,
P.O. Box 100, A-1400, Vienna, Austria
Phone: 43 2254-72251-226; Fax: 43 2254-73951-222
E-mail: AQCS@IAEA.org

Association of Official Analytical Chemists, Method validation programs
AOAC INTERNATIONAL
481 North Frederick Avenue, Suite 500,
Gaithersburg, Maryland 20877, USA
E-mail: webmaster@aoac.org

European Commission – Joint Research Centre,
Institute for Reference Materials and Measurements,
Reference Material Unit,
Retieseweg 111, B-2440 Geel, Belgium
Phone: 32 14 571 705; Fax: 32 14 590 406
E-mail: jrc-irmm-rm-sales@cec.eu.int

Laboratory of the Government Chemist (LGC),
Queens Rd, Teddington,
Middlesex, TW11 OLY, United Kingdom
Phone: 44 (0)20 8943 7000; Fax: 44 (0)20 8943 2767
E-mail: info@lgc.co.uk

National Institute of Standards and Technology (NIST),
Standard Reference Materials Program,
100 Bureau Drive, Stop 2322
Bldg. 202, Room 204,
Gaithersburg, Maryland 20899-2322, USA
Phone: 1-301-975-6776; Fax: 1-301-948-3730
E-mail: srminfo@nist.gov
www.ts.nist.gov/srm/

Dietary scales:

Hanson digital kitchen scales
Hanson (UK) Ltd
2 The Waterhouse, Waterhouse Street
Hemel Hempstead, Herts, HP1 1ES, United Kingdom
Phone: 44 (0)1442 270444
E-mail: Sales@hansonuk.com

Soehnle electronic digital scales
CMS Weighing Equipment Ltd.,
18 Camden High St., London, NW1, United Kingdom
Phone: 44-020-738-37030

Trace-element free polyethylene vials and containers etc.

Sarstedt, Inc, P.O. Box 468,
Newton, North Carolina 28658-0468, USA
Phone 1-704-465-4000; Fax: 1-704-465-4003
Sarstedt Canada
5655 Bois-Franc, St. Laurent, Quebec H4S 1B2, Canada
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Computer programs:

SIDE is run under SAS using PROC IML or a PC version C-SIDE which runs on a UNIX operating system. The program adjusts statistically the distribution of observed intakes to represent those of usual intakes. Copies of PC-SIDE can be downloaded from www.iastate.edu/

Epi Info, version 6. This is a word-processing, database, and statistics program for public health which runs on IBM-compatible microcomputers. It was produced by Dean et al. (1994) from the U.S. Centers for Disease Control and Prevention (CDC) in collaboration with the Global Programme on AIDS at WHO. Both the manual and the program are in the public domain and may be freely copied, translated, and distributed. Epi Info, version 6 is available from: www.cdc.gov/epiinfo/

WorldFood Dietary Assessment System 2.0. This is a nutrient analysis system used to calculate intakes of 53 nutrients. For use with an IBM compatible personal computer, it is user-friendly and uses the International Mini-list nutrient database which contains food composition values for 1800 foods from six countries (Egypt, Kenya, Mexico, Senegal, India and Indonesia), and can also be modified to include food composition data for additional foods. Users specify food names rather than a numerical code, together with the weight consumed (in grams). The source of each food composition value is fully documented. The data are taken from published food composition tables or, where necessary, imputed. There are no missing values. The program is designed to calculate intakes of total and available iron and zinc as well as phytate using the algorithms of Murphy et al. (1992) (See Sections 9.1 and 9.2). The nutrient database and an associated nutrient analysis computer program–the WorldFood Dietary Assessment System–are now in the public domain and can be downloaded over the Internet from INFOODS at: www.fao.org/infoods/

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Appendix G:
Zinc, Phytic Acid, and Phytate–to-Zinc ([phytate]/[Zn]) Molar Ratios of Some Foods and Composite Dishes from Ghana and Malawi

Food, and Scientific Name or Recipe	Zn†	Phy†	Phy/Zn	%H_2O
Cereals				
Maize flour, 95% extraction (*Zea mays* L.)	2.2	792	36	10
Maize flour, 65% extraction	0.9	211	23	10
Maize bran	3.7	1089	29	10
Maize dough	1.4	n.a	n.a	50
Sorghum flour (*Sorghum bicolor* L.) Moench)	1.4	446	32	10
Rice (*Oryza sativa*)	1.6	n.a	n.a	10
Legumes				
Ground nuts, boiled (*Arachis hypogaea* L.)	1.4	505	35	49
Ground nuts, flour	2.8	1297	45	8
Pigeon peas, fresh (*Cajanus cajan* L.) Millsp.)	0.9	255	27	63
Pigeon peas, dry	2.2	727	33	8
Kidney beans, fresh (*Phaseolus vulgaris* L.)	1.5	557	36	52
Cowpeas, boiled (*Vigna unguiculata* L. Walp.)	1.0	349	37	68
Lima beans, fresh (*Phaseolus lunatus* L.)	1.5	238	16	66
Bengal beans, fresh (*Stizolobium aterrimum* Piper & Tracey)	1.0	166	17	68
Vegetables (boiled)				
Pumpkin leaf (*Cucurbita maxima* Duch. ex Lam.)	0.7	34	5	89
Chinese cabbage (*Brassica chinensis* L.)	0.7	5	1	94
Okra leaf (*Hibiscus esculentus* (L.))	1.8	97	5	79
Okra (*Hibiscus esculentus* (L.))	0.5	13	3	91
Cassava leaf (*Manihot esculenta* Crantz)	1.2	42	3	78
Cocoyam leaves (*Xanthosoma* sp. Schott.)	0.6	19	3	88
Amaranth leaves (*Amaranth* sp. L.)	0.3	n.a	n.a	93
Roots and Plantain (boiled)				
Sweet potato (*Ipomoea batatas* L.)	0.2	10	5	70
Yam (*Dioscorea* sp. L.)	0.3	50	13	68
Cocoyam (*Xanthosoma* sp.)	0.5	37	7	60
Cassava (*Manihot* sp.)	0.3	54	18	65
Cassava dough, fermented	0.4	48	12	51
Gari: dry fermented cassava, not boiled	0.7	51	4	12
Plantain, ripe (*Musa paradisiaca* L.)	0.2	0	0	73
Plantain, unripe (*Musa paradisiaca* L.)	0.2	1	1	65
Water yam (*Dioscorea alata* L.)	0.2	26	16	72

Food, and Scientific Name or Recipe	Zn†	Phy†	Phy/Zn	%H_2O
Fruits				
Avocado pear (*Persea americana* Mill.)	0.3	11	3	78
Banana (*Musa paradisiaca* L.)	0.2	22	9	72
Mango, raw (*Mangifera indica* L.)	0.1	25	23	82
Composite Dishes - home-prepared snacks				
Chitumbawa (water, maize flour and pounded bananas formed into a round cake and fried in oil)	1.2	504	42	30
African bread (water, maize flour and bananas formed into a cake, in banana leaves and boiled)	0.3	102	37	70
African cake (mixture of water, maize flour and sugar baked in tin can)	1.2	297	26	45
Composite Dishes - staples				
Hausa porridge (thin porridge of corn flour)	0.1	25	25	94
Porridge of corn grits	0.1	23	23	88
Banku (boiled corn dough and cassava dough)	0.7	107	16	73
Ga kenkey (corn dough made into dumplings and boiled in banana leaves)	0.8	172	19	71
Fanti kenkey (corn dough made into dumplings and boiled in plantain leaves)	0.7	118	21	72
Fufu (pounded boiled cassava and plantain)	0.4	96	24	69
Composite Dishes - purchased meals				
Rice and stew (rice and SI‡)	0.6	118	21	68
Rice and beans (rice, cowpeas and SI)	0.5	107	18	70
Gari and beans (gari, cowpeas and SI)	0.9	178	22	59
Composite Dishes - soups				
Palmnut soup (water, palmnut cream and SI)	0.4	n.a	n.a	86
Groundnut soup (water, groundnut paste and SI)	0.8	81	10	88
Composite Dishes - stews				
Okra (okra and SI)	0.4	38	9	90
Bean (cowpeas and SI)	0.7	n.a	n.a	72

† = mg/100g wet weight. n.a = not analyzed. ‡ SI = standard ingredients: tomato, red peppers, salt, onion, fish; palm oil in stews, rice and beans, and gari and beans. Phy/Zn = [phytate]/[Zn] molar ratios. Phytate was analyzed by the standard AOAC method. Data provided is from Gibson (1994), Zinc in developing countries. *Nut Res Rev* 7: 151–173.

Appendix H:
Computer Program for Calculating the Probability of Inadequacy

The probability of inadequacy for an individual depends on placing the individual's observed intake within a distribution of requirements which is assumed to be normal. The area under the normal distribution, to the right of (i.e., above) the observed intake is then calculated. This can be done by first calculating the z-score of the observed intake:

$$z = \frac{\text{Observed intake} - \text{Mean requirement}}{\text{Standard deviation of requirement}}$$

Statistical tables of the standard normal distribution can then be consulted to determine the area to the right of z. This represents the probability that the intake is inadequate for the randomly selected person.

An alternative approach is to calculate the probability of inadequacy using a statistical function within an appropriate computer program. The function PROBNORM in SAS is suitable. The relevant equation is:

$$\text{Probability of inadequacy} = 1 - \text{PROBNORM}\left(\frac{\text{Observed intake} - \text{Mean requirement}}{\text{Standard deviation of requirement}}\right)$$

A third approach, if no PROBNORM or analogous function is available, is to calculate the individual probability of inadequacy using a small separate computer program. The segment of code for such a program written in BASIC would appear as follows:

```
1510    Z=(A(X) - NR)/(SD)
1515    IF Z<0 THEN Z=ABS(Z): VZ=1
1520    IF Z>10 THEN R=0: GOTO 1545
1525    D1=.0498673470: D2=.0211410061: D3=.0032776263:
        D4=.0000380036: D5=.0000488906: D6=.0000053830
1530    G=1+(D1*Z)+(D2*Z^2)+(D3*Z^3)+
                        (D4*Z^4)+(D5*Z^5)+(D6*Z^6)
1535    R=1/(2*G^16)
1540    R=INT(R*1000+0.5)/1000
1545    IF VZ <>0 then R=1-R:VZ=0
1550    R(X)=R: R=0
```

In this code A(X) is the intake for nutrient X; NR is the average requirement for nutrient X; and R and R(X) represent the calculated probability that the intake of nutrient X is inadequate for that individual.

Having calculated, using one of these three approaches, the probabilities that the intake of nutrient X is inadequate for all the individuals, the mean probability of inadequacy for the sample is calculated. This is the predicted prevalence of inadequate intakes for the sampled population (NRC 1986).

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