Part 5

Nutritional assessment

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29 Food analysis, food composition tables, and databases

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Food composition tables or databases are designed to describe the composition of the foods in the country of origin. They contain data on foods eaten on a regular basis by the population and generally include some less widely consumed foods that are unique to the culture or eaten on special occasions. The values for nutrient and nonnutrient constituents are based on chemical analyses, sometimes performed by the compiler of the tables (or databases) or in an associated laboratory. Some food composition values may be 'borrowed' from a major overseas food table, or represent estimated averages from reports in the literature. Alternatively, they may be imputed from analytical values existing for a similar food or derived from the ingredients of a mixed food. The origins of the nutrient composition values should be specified, although in practice this is not always done. The UK food composition tables (*McCance and Widdowson's: The composition of foods*) and the US data (*USDA Handbook, No. 8*) are widely used reference sources. The US data are also available as a nutrient database at the USDA internet site, which is updated regularly.

When compiling food composition data, there are two important considerations. First, food items must be relevant; sampling of an individual food should be representative of the types commonly consumed by the population on a year-round, nationwide basis and should be pertinent to the current food supply. Second, the food composition data must be of high quality; analyses of the foods should be conducted in a rigorous, scientific environment so that values are precise and accurate. Well-established food composition tables have evolved over many years and often combine old and new analytical methods from a variety of different sources. Clear and detailed documentation of sampling and analytical procedures at all stages is as critical as the choice of the analytical procedure itself, so that compilers of tables, faced with the challenge of inevitable changes in the food supply, can continue to evaluate the relevance of the item and quality of the data.

29.1 Sampling

How does one sample foods that are truly representative of a particular food item? Does the analyst just go out to the corner shop nearest the laboratory and buy some food or try to include the varieties of that food across the nation? Foods are ultimately based on parts of plants or animals that vary naturally according to many factors. For example, varieties of sweet potato differ widely in β-carotene content according to whether the flesh is orange, yellow, or white in colour. Seasonal variation can markedly influence water and vitamin content, and fruits and vegetables tend to increase their concentration of sugars as they ripen, a process that is highly temperature dependent. Fat depots in animal foods are also extremely variable according to degree of exercise, type of feed, and age of the animal. Guidelines for sampling protocols that take these variations into account are detailed by Greenfield and Southgate (2003). In general, the greater the natural variation in a particular food the larger the number of samples required. National food production figures may also indicate the types of foods most widely consumed and therefore most representative of the population.

When the food arrives in the laboratory for analysis, it must first be unambiguously identified with both scientific and local names. Full descriptions are required for the part of the animal or plant used, and its stage of maturity, size, shape, and form (Table 29.1). Any cooking or processing methods used in preparing the item must be documented and the edible portion must be carefully separated from inedible refuse. Analyses may then proceed in one of two directions. Individual samples of the same food from different locations can be analysed separately in order to provide information on the variation between samples as well as their average nutrient content. This approach, however, may be a luxury that many laboratories cannot afford. Alternatively, a composite sample can be prepared by pooling several individual subsamples of a food from many locations to give a single sample for analyses. Often, a weighting scheme is used to ensure that those varieties and/or locations where the food item is consumed more frequently are proportionately represented in the final composite. Whether derived from an individual or composite sample, the edible portion must be homogenized or ground thoroughly to ensure that the aliquot taken for analysis is representative of the original sample. For trace minerals, it is also important that the sample is not exposed to adventitious sources of contamination during the collection, homogenization, sample preparation, and analytical stages. Similarly, care must be taken that vitamins and other susceptible organic components are not degraded by air or light, for example.

29.2 Analysis

The ultimate aim of food tables or databases is to provide nutrient information on food components that are of nutritional importance to the health of the population, over their lifetime and in different disease states (Table 29.2). Some of the more common methods used to analyse food components are described below. It is important to document the accuracy and precision of all analytical methods and to use reference materials of a similar matrix to the food sample and certified for the nutrient of interest. Reference materials can be obtained from the US National Institute for Standards and Technology, Washington, DC, and the International Atomic Energy Agency, Vienna.

29.2.1 Moisture

Moisture (water) is the first component to be analysed in a food, and it is probably the single most important piece of food composition data.

Table 29.1 INFOODS guidelines for describing foods

Name and identification	Name of food in national language of the country
	Name in local language or dialect
	Nearest equivalent name in English, French, or Spanish
	Country or area in which sample of food was obtained
	Food group and code in database used in the country
	Food group and code for food in regional nutrient database
	Codex Alimentarius or INFOODS food indexing group
Description	Food source (common and scientific name)
	Variety, breed, strain
	Part of plant or animal
	Manufacturer's name and address
	Other ingredients (including additives)
	Food processing and/or preparation
	Preservation method
	Degree of cooking
	Agricultural production conditions
	Maturity or ripeness
	Storage conditions
	Grade
	Container and food contact surface
	Physical state, shape, or form
	Colour

Source: Truswell, A.S., Bateson, D.J., Madafiglio, K.C., Pennington, J.A.T., Rand, W.M., and Klensin, J.C. (1991) INFOODS guidelines for describing foods: A systematic approach to describing foods to facilitate international exchange of food composition data. *J Food Comp Anal*, **4**, 18–38.

Underestimation of water content will lead to an overestimation of other components that are subsequently determined in the dried food. Water can be gained or lost during the cooking process, with changes in the apparent content of an array of nutrients, as well as energy. Water content is an important preliminary consideration when comparing the nutrient content of similar items in tables from different countries, as may happen if a specific food is not available in the local database. Furthermore, a high moisture content, typical of many fresh fruits and vegetables, indicates a low-energy value, while the reverse is true for items of low-moisture content.

The moisture content in foods is determined by simply evaporating off the water and calculating the difference in weight at the beginning and end. Different methods are used. Water may be driven off in an oven at temperatures around its boiling point, provided the sample itself does not decompose or oxidize or contain other volatiles that would

Table 29.2 Food components of nutritional importance

Basic components[a]
Moisture
Energy
Protein, fat, and carbohydrate
Up to 13 vitamins, and 10 or more minerals or trace elements
More detailed profiles[b]
Fatty acid profile (up to 37 fatty acids)
Amino acid profile (around 18 amino acids)
Carbohydrate components (sugars, starches)
Dietary fibre and components (soluble, insoluble)
Alcohol
Optional components[c]
Cholesterol
Vitamin A-inactive carotenoids (lycopene, lutein, zeaxanthin, and others)
Organic acids (malic, citric, lactic, formic, oxalic, and salicylic acids)
Biologically active components (e.g. flavonoids and isoflavonoids)
Glycaemic index

[a]Essential for growth and maintenance of body tissues and always listed in food tables.

[b]Useful for research into diet and disease risk and usually included in well-established tables.

[c]Not essential nutrients but may influence nutritional status indirectly by exerting physiological effects. Not routinely listed in food tables but may be cited in appendix sections.

contribute to the weight loss. Alternatively, evaporation can be achieved at lower temperatures by vacuum drying under reduced pressure, or by freeze-drying. In practice, a wide range of methods varying in temperature, time interval, and sample preparation have evolved to optimize this apparently simple process.

29.2.2 Protein and amino acids

Protein in foods is determined indirectly by measuring the content of amino-nitrogen, an essential constituent of the amino acid units that combine to form proteins. For the amino-nitrogen assay, the food sample is digested in hot concentrated sulphuric acid to convert the nitrogen into ammonium ion, which is then quantified by either distillation and titration in the classic *Kjeldahl method* or by *spectrophotometric analysis*. The protein content in the original food is then estimated by multiplying the total nitrogen value by specific conversion factors for different foods, as shown in Table 29.3 (based on their amino acid composition). Alternatively, the general conversion factor of 6.25 is used because nitrogen is assumed to represent about 16% of the protein content.

From a nutritional viewpoint, the importance of a particular dietary protein lies in its ability to sustain growth or replenish tissues, functions that depend more on the quality of the protein (or pattern of content of indispensable amino acids) than the total amount (see Chapter 5). Gelatin, for example, is a food that comprises over 80% protein, one of the highest values listed in food composition tables, yet as the sole protein source it cannot sustain life no matter how much is eaten because it is deficient in the indispensable amino acid tryptophan. *Amino acids* are measured by hydrolysing the protein with strong acid or alkali to break down the peptide bonds, followed by separation and measurement of the free amino acids using ion-exchange chromatography. Food tables may list the amino acid composition of major foods in addition to the total amount of protein, either in the main tables or in an appendix. The profile of indispensable amino acids can then be compared with that of a reference protein, such as hen's egg (a protein known to be utilized very efficiently) for adults, or human milk for babies, to develop a measure of the protein quality, referred to as the amino acid score or chemical score. Table 29.4 shows the indispensable amino acids in two common food proteins compared with those in hen's egg.

Table 29.3 Sample calculations of protein content from nitrogen

Item (100 g)	Total nitrogen (g)		Conversion factor[a]		Protein (g)
Rump steak	3.02	×	6.25	=	18.9
White rice	1.23	×	5.95	=	7.3

[a]Conversion factors for converting nitrogen in foods to protein: milk and milk products, 6.38; eggs, meat, fish, 6.25; rice, 5.95; barley, oats, rye, whole wheat, 5.83; soybeans, 5.71; peanuts, brazil nuts, 5.46; almonds, 5.18.

Table 29.4 Content of indispensable amino acids in food proteins (mg amino acid per g of protein)

	Hen's egg	Cheese	Wheat flour
Isoleucine	54	67	42
Leucine	86	98	71
Lysine	70	74	20[a]
Methionine + cysteine	57	32	31
Phenylalanine + tyrosine	93	102	79
Threonine	47	37	28
Tryptophan	17	14	11
Valine	66	72	42

The nutritive value of a dietary protein can be estimated by comparing its pattern of indispensable amino acids with those in a reference protein such as hen's egg. The amino acid score is the content of the most limiting (inadequate) amino acid, as a percentage of the reference value for this amino acid.

[a]For wheat flour, the most limiting amino acid is lysine and the amino acid score is 20/70 or 28%. In real life, however, wheat flour (as bread) is frequently eaten with another protein (e.g. cheese) that has even more lysine than egg, so the amino acid score of whole meals is usually much higher than for individual foods. The limiting amino acid in an equal mixture of wheat and cheese protein is methionine + cysteine and the amino acid score is 55%.

29.2.3 Fat

A characteristic property of fats is their solubility in organic solvents such as *n*-hexane, petroleum ether, or chloroform. Estimation of fat in foods involves extraction with one of these solvents followed by evaporation of the solvent and weighing of the final fat residue.

The accuracy of this estimation depends on the type of fat as well as the mix of other components in the food. The traditional *Soxhlet method* tended to underestimate fat that was bound to other food components such as protein. To overcome this problem, the sample is now predigested in concentrated acid or alcoholic ammonia to release the bound portion before extraction. The fat residue may be further analysed in various ways. Thin-layer chromatography can separate the fat into lipid classes, including triglycerides, phospholipids, and sterols. If lipids are hydrolysed to liberate their fatty acids, these can be separated by gas–liquid chromatography. Fatty acid profiles of major foods are provided in several comprehensive food tables such as the *USDA Handbook, No. 8* and the New Zealand Food Composition Tables. Total saturated fatty acids (including branched-chain acids), monounsaturated (*cis* and *trans* together) and polyunsaturated fatty acids are given in a supplement to the UK's *McCance and Widdowson's: The composition of foods*.

The presentation of the fat content of foods differs among food tables. In addition to providing the total fat content, compilers may sum the individual fatty acids into groups: polyunsaturated,

monounsaturated, and saturated, and list each class separately, or they may present these as a ratio of polyunsaturated and monounsaturated fatty acids to that of saturated fatty acids, termed the PMS ratio. Alternatively, they may list each fatty acid according to its chain length and degree of saturation. The PMS ratio provides a crude estimate of overall atherogenic risk; the higher the proportion of unsaturates, the more favourable the ratio. For research purposes, however, the content of individual fatty acids is more useful because each may exert independent effects. (Chapters 4 and 21 discuss further the role of individual fatty acids in health and disease.)

29.2.4 Carbohydrates

Food composition tables vary widely in the methods used to measure carbohydrate. One approach is to estimate 'by difference', which defines carbohydrate as the difference between 100 and the sum of the weight percentages for protein, fat, water, and ash. However, inaccuracies arise using this approach because of the summation of the errors in estimating these four constituents. In addition, such values are of limited use because they do not distinguish between different carbohydrates, especially those that are available to the body (i.e. digested, absorbed, and utilized) and those that are unavailable. More accurate methods quantify the available and unavailable carbohydrate by direct measurements.

Available carbohydrate includes sugars (monosaccharides and disaccharides) and starches and dextrins (which are polysaccharides). Individual sugars can be extracted with aqueous alcohol and measured by high-performance liquid chromatography or using specific enzymatic colorimetric tests. Starches and dextrins, which are glucose polymers, are measured in the same way after an initial hydrolysis step to liberate free glucose. Food tables that report carbohydrate by direct analysis may list the monosaccharide equivalents because this is the form in which the carbohydrate is estimated. To obtain the actual values for disaccharides and starch (polysaccharide) in the food, these values should be divided by 1.05 and 1.10, respectively. A published database is also available for the glycaemic index (GI), which ranks carbohydrate-containing foods according to the degree of rise in blood glucose immediately after the food is consumed, compared with that of a standard food—either glucose or white bread (Atkinson *et al*., 2008). The GI data are derived from studies in which human subjects are fed a range of carbohydrate-containing foods and, therefore, provide a qualitative biological rather than quantitative chemical measure of carbohydrate intake.

Unavailable carbohydrate or dietary fibre is the mixture of plant components that are resistant to digestive enzymes in the human small bowel. With advances in food technology, the term has been expanded to include chemically synthesized, and also extracted fibre components; these components can be added for functional purposes to nonplant foods, such as dairy products, but are not usually listed as a separate nutrient in food composition databases. The chemical diversity of fibre makes it a very challenging and elusive component to analyse in the laboratory. Accordingly, a number of methods have evolved. All begin with the defatted, dried food sample but each method measures a different chemical fraction. The *Englyst method* is the most sensitive and perhaps the most useful from a nutritional perspective because it can distinguish between soluble and insoluble fibres, both of which have physiologically distinct effects in the body in relation to chronic diseases such as diabetes, heart disease, and cancer. In the Englyst method, starch is initially removed by digestion with strong amylases and then the constituent sugars of dietary fibre are measured directly after acid hydrolysis to produce the free sugars. This yields estimates of both soluble (pectin, gums, mucilages, and hemicelluloses) and insoluble (cellulose and other hemicelluloses) fibre components, collectively called nonstarch polysaccharides (NSPs). Lignin escapes detection because it is not a carbohydrate but a polymeric phenolic compound. The older *Southgate method* gives a higher value for dietary fibre content as it measures lignin, as well as NSPs.

Other methods are less precise because they measure fibre 'by difference' but they involve less

analytical work and so are more economical. In the widely used procedure of the *Association of Official Analytical Chemists* (AOAC), developed by Prosky *et al.* (1984), starch is first removed by enzymatic hydrolysis and the undigested residue is weighed, analysed for nitrogen, and then ashed. Protein and ash contents are then subtracted from the residue weight. *Van Soest's neutral detergent fibre* method measures only the insoluble cellulose and lignin, and not the soluble fibre; hence, this method underestimates total dietary fibre. The *crude fibre* method is the least accurate, involving rigorous treatment with boiling acid and alkali, which removes much of the dietary fibre itself.

A further complication in the analysis of dietary fibre is the recognition that a variable but small proportion of the dietary starch found in beans, wholegrains, potatoes (especially if eaten cold), or unripe bananas is not completely digested and is unavailable to the body for absorption. This starch, termed 'resistant starch', escapes digestion because it is physically inaccessible to the enzymes and, instead, it is probably fermented in the colon, thus behaving like soluble dietary fibre. Current values for dietary fibre in food tables do not include separate values for resistant starch, although the AOAC and Southgate methods include some of the resistant starch in the fibre value.

Different analytical methods can result in several-fold variations in the estimate of fibre for the same item, as shown in Table 29.5. Most food tables, however, are internally consistent in their choice of analytical method(s) and these should be stated clearly in the introductory section or in the main tables alongside the nutrient values. Special care should be taken when comparing carbohydrate values from different food composition tables. For reasons outlined above, values from those tables in which total carbohydrate is analysed by difference (i.e. including dietary fibre) are not compatible with others in which carbohydrate constituents are analysed directly.

29.2.5 Energy

The total energy of a food is measured by *bomb calorimetry* in which a sample of the food is burned with oxygen in a sealed chamber until completely oxidized. The heat released corresponds to the chemical or gross energy of the food. Food energy is reported in kilojoules (kJ) or kilocalories (kcal), where 1 kJ = 0.24 kcal or 1 kcal = 4.187 kJ. When a food is eaten, however, the energy-yielding components—protein, fat, carbohydrate, and (where present) alcohol—are oxidized by enzymatic processes within the body to provide energy, but not with 100% efficiency. Some energy is lost into the faeces as not all food components are fully absorbed from the digestive tract. Further energy is lost into urine since dietary protein, unlike carbohydrate and fat, is not completely oxidized by the body and its excretory product, urea, still retains some of the chemical energy from the original protein. The eminent American physiologist Atwater measured the energy losses into faeces and urine by a series of meticulous experiments in humans fed a mixture of foods. In his experiments, 92% of protein, 95% of fat, and 97% of carbohydrate were absorbed by the body, but for every gram of protein ingested, about one-quarter of its gross energy was lost into the urine.

Atwater's experiments, conducted at the turn of the nineteenth century, represent landmark studies from which the energy content of foods in today's food composition tables are derived. Atwater developed a system of four conversion factors, which represent the energy available in: (1) protein (17 kJ/g, 4 kcal/g); (2) fat (37 kJ/g, 9 kcal/g); (3) carbohydrate (16 kJ/g, 4 kcal/g); and (4) alcohol (29 kJ/g, 7 kcal/g), taking into account the estimated energy losses into faeces and urine. The value is slightly higher for starch (due to lower hydration) than that given for all carbohydrates, and slightly lower for sugars. These factors provide values for food energy as it is utilized by the body (i.e. metabolizable energy). The energy content of each food is calculated by first multiplying the weight of each component by its Atwater conversion factor, and second, summing the energy from each component to give a grand total for the food, as shown in Table 29.6. Note that the total weight of each nutrient is obtained by direct analysis of the food, as described previously (see also Chapter 5).

Table 29.5 Dietary fibre content of dried red kidney beans estimated by five different methods

Method	Fibre content (g/100 g)	Fibre components
Englyst[a]	15.7	Soluble + insoluble fibre (not lignin or resistant starch)
Southgate[a]	23.4	Soluble + insoluble fibre, lignin
AOAC (Prosky and Asp)[b]	21.5	Soluble + insoluble fibre, lignin
Neutral detergent fibre (Van Soest)[c]	10.4	Insoluble fibre only
Crude fibre[c]	6.2	Part of the insoluble fibre

Red kidney beans contain both soluble and insoluble dietary fibre. AOAC, Southgate, and Englyst methods measure total dietary fibre, but Englyst fibre is lower because it does not measure lignin or resistant starch. Neutral detergent fibre is lower because it does not measure soluble fibre. AOAC, Association of Official Analytical Chemists.

Sources: [a]Food Standards Agency (2002) *McCance and Widdowson's: The composition of foods*, 6th summary edition. Cambridge, Royal Society of Chemistry; [b]Food Standards Australia New Zealand (2007). *NUTTAB 2006 Australian food composition tables*. Canberra: Food Standards Australia New Zealand; [c]USDA (1986) *Composition of foods: Legumes and legume products*. Handbook No. 8–16. Washington, DC, US Department of Agriculture.

The biggest variable is the carbohydrate value and whether this includes fibre, because Atwater factors are applied to the total carbohydrate content irrespective of whether this is analysed directly or by difference. In practice, the value for energy is somewhere between the two extremes of 16 kcal/g for available carbohydrate and 0 kJ/g for unavailable carbohydrate. This is because a proportion of dietary fibre is fermented in the colon to short-chain fatty acids, which can be absorbed from the large bowel and oxidized for energy. Livesey (1991) has estimated that about half the dietary fibre can

Table 29.6 Calculation of metabolizable energy content in raw soy bean using Atwater conversion factors

Component	Weight (g/100 g)		Atwater factor (kJ (kcal)/g)		Energy content (kJ (kcal)/100 g)
Water	8	–	–	=	–
Protein	31	×	17 (4)	=	527 (124)
Fat	20	×	37 (9)	=	740 (180)
Available carbohydrate	7	×	16 (4)	=	112 (28)
Dietary fibre	20	–	Not *usually* included	=	–

The energy content of individual components is then summed to give a grand total of 1379 kJ/100 g or 332 kcal/100 g.

be utilized by the body in this way and proposed an average energy conversion factor of 8 kJ/g (2 kcal/g) for unavailable carbohydrates, about half the value for available carbohydrate. Estimates of the energy from fibre are included in some more recent databases, such as the Australian Food and Nutrient Database (Food Standards Australia New Zealand, 2008).

The energy conversion factors used today vary somewhat from Atwater's original factors. The German, British, Australian, and New Zealand food composition tables, for example, apply the same four factors to all foods, whereas the US and East Asian tables use a range of slightly differing conversion factors, rather lower for components in plant foods than in animal foods, reflecting Atwater's initial observation that the energy from plants was less available. The conversion factors selected to calculate energy should be specified in the introduction or appendix section of all food composition tables and the reader is referred to these for more in-depth information.

29.2.6 Inorganic nutrients and vitamins

The range of inorganic nutrients in foods including calcium, iron, magnesium, zinc, copper, manganese, potassium, and sodium can be determined by *flame atomic absorption spectrophotometry* (AAS), a method whereby a solution of the ashed or acid-digested food sample is sprayed into the flame of an atomic absorption spectrophotometer and quantified by the degree of absorption at a specified wavelength. For selenium, direct AAS with a Zeeman background correction is required. Graphite-furnace AAS is used for analysing the ultratrace elements, such as chromium, nickel, and manganese. Alternatively, all the minerals can be measured in the one sample using inductively coupled plasma spectrophotometry or X-ray fluorescence. Minerals are a very stable component of foods and these procedures can be highly accurate provided any interfering substances such as plant pigments or organic constituents have first been removed. This is achieved by reducing the food sample to a dry ash by thorough heating in a muffle furnace or by breaking down and oxidizing the organic components by wet ashing with boiling concentrated acids. For trace element analysis, precautions must be used to avoid adventitious contamination by the use of ultrapure acids, acid-washed glassware, plastic materials for sample preparation and analysis, and high-grade deionized water.

In contrast to the minerals, many of the vitamins in foods are not very stable. Riboflavin and vitamin A are sensitive to light; thiamin, folate, and vitamin C are sensitive to heat; and vitamin E to oxidation. Vitamins are either analysed by the traditional but more time-consuming microbiological methods or by newer, faster chemical techniques. *Microbiological assays* are conducted with a culture of organisms that have a specific growth requirement for the particular vitamin. The assumption is made, however, that the microorganism reacts in the same way as the human organism. Such methods are available for a wide range of B vitamins including thiamin, niacin, riboflavin, vitamins B_6 and B_{12}, folate, biotin, and pantothenic acid and have the advantage of estimating the total biological potential of the vitamin. Alternative chemical methods, such as *high-performance liquid chromatography* (HPLC), can be used for most of the vitamins. They require an initial extraction step to remove other components in the food, but are useful for separating and quantifying different chemical forms of the vitamin. It should be borne in mind that many of the existing values for vitamins, as well as minerals, in food tables were obtained with older, less specific colorimetric methods that may now be obsolete.

Values in food composition tables represent the total content of each mineral or vitamin in the food and do not address the complex problem of bioavailability, defined as the proportion of a nutrient that is actually absorbed from the food and utilized. When a vitamin exists in two or more forms that are utilized differently in the body, some food composition tables tabulate each form separately. Vitamin A, for example, has two major components obtained

from quite different food sources: preformed vitamin A (retinol), which is found in many animal products, and the provitamin A carotenoids derived from plants. The compiler may further attempt to calculate the overall potency of the vitamin in the body by summing the different forms, taking into account their relative biological activities. Some of the assumptions and calculations made in food tables regarding the different forms of niacin, folate, and vitamins A, C, D, and E are shown in Table 29.7.

29.2.7 Nonnutrient biologically active constituents

In addition to the nutrient components, such as protein, fat, carbohydrate, vitamins, and minerals, plant foods in particular contain an abundant array of chemical constituents or 'phytochemicals'. Examples of nutritional importance include the flavonoids, isoflavonoids, and nonvitamin A carotenoids. Although

Table 29.7 Presentation of different vitamin forms in food composition tables

Vitamin	Main forms	Unit of total vitamin activity
Niacin[a]	1. Preformed in foods (nicotinic acid + nicotamide) 2. Derived from tryptophan	Niacin equivalents (NE)
Folate[b]	1. Food folates 2. Folic acid enrichment	Dietary folate equivalents (DFE)
Vitamin A[c]	1. Retinol 2. Provitamin A carotenoids	Retinol equivalents (RE) or Retinol activity equivalents (RAE)
Vitamin C[d]	1. Ascorbic acid 2. Dehydroascorbic acid	Vitamin C
Vitamin D[e]	1. Vitamin D (cholecalciferol) 2. 25-Hydroxyvitamin D	Total vitamin D activity
Vitamin E[f]	1. Tocopherols (α, β, γ, δ) 2. Tocotrienols (α, β, γ, δ)	α-Tocopherol equivalents

[a]Because approximately 1% of protein is tryptophan and 1/60th tryptophan is converted to niacin in the body: NE (mg) = preformed niacin (mg) + dietary protein (g) × 0.16.

[b]Bioavailability of folate from food is about 50% from foods, 85% from fortified foods or as a supplement (consumed with food), or 100% as a supplement (on an empty stomach) (Institute of Medicine, 2006): 1 μg DFE = 1 μg food folate = 0.6 μg folic acid (taken with meals) = 0.5 μg folic acid (on empty stomach).

[c]Vitamin A can be expressed as RE, where β-carotene has one-sixth the activity of retinol and other carotenoids have one-twelfth the activity of retinol. To acknowledge lower reported availability from vegetable sources, some databases (e.g. Agricultural Research Service, 2010) now express vitamin A as RAE, where β-carotene has only one-twelfth the activity of retinol (Institute of Medicine, 2006): RAE(μg) = retinol (μg) + β-carotene (μg)/12 + (α-carotene + β-cryptoxanthin (μg)/24.

[d]Both forms have equal activity and are summed to give total vitamin C.

[e]Vitamin D in food is measured as natural cholecalciferol (or ergocalciferol) and 25-hydroxyvitamin D (an active circulating form in animals, and hence meats), which has about five times the activity of cholecalciferol (Food Standards Agency, 2002): total vitamin D activity = sum of cholecalciferol + 5 × 25-hydroxycholecalciferol (in meats).

[f]α-Tocopherol is the most abundant form, with over twice the activity of other tocopherols and tocotrienols. Activities of individual vitamin forms are cited in Food Standards Agency (2002).

not classed as essential nutrients in terms of preventing a specific nutritional deficiency, these constituents are biologically active in the body and are thought to contribute to optimal health and longevity (see Chapter 16).

Phytochemicals occur in relatively small mg or µg quantities per 100 g food. While precise HPLC assays are available for quantification, it is often difficult to estimate levels in foods accurately. For example, the concentration of isoflavonoids in soybeans, a rich natural source, shows up to six-fold variation. This reflects genetic differences and also the fact that isoflavonoids, unlike more stable structural components, such as proteins, are part of the plant's natural response to stress. Insect infestations or climatic considerations, including low temperature and high soil moisture, can trigger dramatic increases in isoflavonoid content. This natural variability means that the isoflavonoid content in the same variety of soybean or soy product available in local retail outlets could vary several-fold between different batches. Despite certain limitations, the identification and quantification of biologically active substances in plants is an area of intense current research. Databases for isoflavonoids and a range of other special-interest constituents have been collated by the Nutrient Data Laboratory at the US Department of Agriculture, and are available at the USDA internet site http://www.ars.usda.gov/nutrientdata

29.3 Compilation of food composition data

Compilation of food composition data either in the form of tables or computerized databases is a very large task. It requires painstaking inspection of a wide range of sources that use a variety of sampling and analytical procedures. Data analysed outside the compiler's laboratory must frequently be traced back to its source and any items without clear documentation discarded because there is no way to evaluate their quality. Values from different sources must be compared and statistical calculations made to provide a meaningful average for the nutrient content of a food. As food patterns within a population are constantly changing and evolving, data must also be scrutinized to determine its relevance in the current food supply.

Not only should data be accurate and relevant, but also the format must be clear so that the user may easily understand the data. Food items are listed alphabetically and usually grouped according to food groups with similar nutritional properties (e.g. vegetables, fruits, grains, meats, and dairy products) or by product use (e.g. snacks, desserts, and breakfast cereals). In cases where foods are collected or prepared with inedible matter, the percentage edible portion, sometimes expressed indirectly as percentage refuse, is also given. However, irrespective of the proportion of edible matter or the accustomed serving size, nutrient values for items are always presented in terms of 100 g edible portions. Consequently, this does not include the core or stone in fruit, or the bones in meat and chicken, but it does include optional materials such as certain vegetable skins and trimmable meat fat, unless specified otherwise. Most food tables cite both scientific and local names for each item, and some specify the number of food items analysed and whether a single or composite sample was used for analysis. Rarely do tables include the natural variation around the mean value, but rather provide a single mean representative value. The German (Souci *et al.*, 2008) and the US (Agricultural Research Service, 2009) tables are exceptions, citing the range (i.e. highest and lowest values known) or the standard error of the mean, respectively, in addition to the mean value.

Ideally, food composition tables or databases should include analyses for all food components of nutritional relevance to the potential user, whether this be a dietitian/nutritionist prescribing advice to a client, a research worker investigating certain nutrients in relation to disease risk, or a food manufacturer seeking accurate nutrient information on their products for the purposes of marketing and

food legislation. In practice, however, inclusion is determined more by the analytical resources and public health priorities of the country concerned. A wealth of analytical and descriptive information on food habits and customs already exists within different cultures. Yet, many of these data are not widely accessible outside the country, often because local names are idiosyncratic or culture-specific, making it difficult to identify the food. In this regard, the INFOODS guidelines (Table 29.1) were established to ensure that foods are named and described in a standardized manner with a view to facilitating interchange of food composition data at the international level.

Further Reading

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Useful Websites



To see topical and scientifically robust updates on nutrition associated with this textbook and web links to many of the journals in the Further Reading sections, please see the dedicated Online Resource Centre at **http://www.oxfordtextbooks.co.uk/orc/mann4e/**

US Department of Agriculture, Nutrient Data Laboratory home page: http://www.ars.usda.gov/ba/bhnrc/ndl

Souci-Fachmann-Kraut online database: http://www.sfk-online.net/cgi-bin/sfkstart.mysql?language=english



30 Dietary assessment

Jim Mann and Sheila Bingham*

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Dietary assessment is one of the specialized interests of nutritionists, used in surveillance of populations, nutritional epidemiology, clinical assessment, and experimental research. There are two basic approaches to estimating food intake: one principally concerned with determining the intake of populations, the other with assessing the diets of individuals. These approaches are not mutually exclusive, since methods for assessing dietary intake of individuals may be used for estimating intake of populations as happens in most national nutrition surveys which generally involve individual 24-hour recalls. This chapter describes the various methods used in the different contexts in which knowledge of food and nutrient intakes are required.

Methods used for assessing dietary intake in carefully controlled experimental studies will differ appreciably from those required in large, epidemiological studies. The chapter also considers the reliability of the various methods, taking into account the conflict between the need for accuracy to establish exactly where an individual lies within the overall distribution of foods and nutrients and the logistics of doing so when very large populations required for epidemiological studies are investigated.

30.1 Population estimates

Population estimates are needed principally for surveillance, for example, to assess intakes of a particular food or nutrient in relation to reference nutrient intakes (RNIs) and to determine changes over time. Such information would also be used during emergencies when food is in short supply, to make recommendations concerning usual diet, and in considering the case for fortification of foods on a national basis. Population estimates of dietary intake have also been compared with disease rates in different countries or populations or within the same country over time to identify clues as to possible nutritional causes of the disease. Population estimates may be derived from food balance sheets, household food surveys, national nutrition surveys, and, potentially, supermarket records.

Food balance sheets are based on national statistics of food produced, imported, and exported with factors for wastage included. The Food and Agricultural Organization (FAO) of the United Nations (http://faostat.fao.org/) collates these and they have been used extensively for monitoring changes in consumption and for population comparisons, linking, for example, population

*deceased

BOX 30.1 Food consumption at the national level

Food consumption at the national level is also called food moving into consumption, food disappearance data, and apparent food consumption. The food supply is calculated from estimates of domestic food production plus imports minus exports. Potential food diverted for farm animal feed, nonfood industrial use, and wastage at wholesale level are subtracted. The total is divided by the estimated population each year. These statistics are useful for monitoring changes in consumption of commodities and comparing countries' food habits. FAO food balance sheets are based on these statistics and available each year from some 175 countries. For many countries, these are the only regular measures of food intakes. However, these are macro figures. The calorie and nutrient numbers are around 25% (or more) above what individuals actually eat and drink, because there is wastage of food in homes and catering establishments, and food is fed to tourists and pets. They give no idea of distribution of food resources among regions, socioeconomic groups, or within the family.

estimates of fat and cardiovascular disease rates and population estimates of meat and fat and bowel and breast cancer rates (Box 30.1).

Household surveys are records kept by the householder of all food available to the family over 1 week, and the total food entering the household is divided by the number of people living there. This approach has been used in the UK in the National Food Survey, which has been running continuously for over 50 years as a combined food consumption and expenditure survey and which in recent years has taken into account food eaten away from home (http://www.defra.gov.uk/statistics/foodfarm/food/familyfood/nationalfoodsurvey/). From this, regional comparisons and secular trends in consumption are available, for example the trend towards a lower saturated fat composition of the diet in the UK.

When using data derived from food balance sheets or household surveys, it is not possible to compare the intake of food or nutrients in different age and sex groups with differential trends in disease incidence or risk factors, nor for individual data to be assessed. This is because the findings relating to, for example, children, the elderly, or males and females cannot be separated out from the overall population average data. Potentially, computerized supermarket records of sales or loyalty cards could be used to obtain regional information on nutritional consumption (Robertson *et al.*, 2004).

Typically the 24-hour recall (see Section 30.2 and Box 30.2) is used in *national nutrition surveys*. Although the information is derived from individuals, the aim is to describe the nutritional intake of the population. This requires the study of large numbers of people who are representative of the

BOX 30.2 National nutrition surveys

National nutrition surveys estimate food intake of individuals and may involve clinical examination and blood tests to further assess nutritional status. Although based on the intakes of individuals, such surveys provide the average and range intakes of foods and nutrients of different age and sex subgroups relating to the population from which the individuals are drawn. Examples are NHANES in the USA, National Diet and Nutrition Surveys in the UK, National Nutrition Survey in Australia (1995), and the National Nutrition Survey in New Zealand (2009). They have all been somewhat different. They identify nutrients for which intakes are lower than recommended on the one hand and frequency of overweight/obesity on the other. They also gather information about food usage as a basis for formulating and evaluating health policy and regulatory needs. The food intake method used has to be a 24-hour recall or food record because of the need to know the exact types of food people are eating.

population of interest in order to be reasonably confident that the survey yields an unbiased estimate of the usual mean intake of nutrients of interest. National nutrition surveys often involve a repeat 24-hour recall on a subsample of participants.

30.2 Individual methods

Several methods are available for measuring the dietary intake of individuals. They generally consist either of the collation of observations from a number of separate days' investigations, as in records, checklists, and 24-hour recalls, or attempts to obtain average intake by asking about the usual frequency of food consumption, as in the diet history and food frequency questionnaires (FFQs). In all methods of dietary assessment, some estimate of the quantity of food consumed is required, and for the determination of nutrient or other food component intake, either an appropriate description that can be matched with an entry in the food tables or an aliquot for chemical analysis (Box 30.3). Each of the methods is described briefly below and further details regarding equipment, protocols, uses, and limitations are available in the references in Further reading. Detailed examples of methods used in particular studies are shown on the web sites given.

30.2.1 Food records

Food records involve subjects being taught to describe and either weigh or estimate the amount of food immediately before eating and to record leftovers. Records are generally completed by the participant on sheets or booklets, which, in the case of estimated records, may include photographs to facilitate estimation of portion size. Cups, spoons, rulers, and scales may be provided to aid accurate description. Verbal records, with descriptions of amounts recorded on tape cassettes, have also been used, as have records incorporating bar codes from purchased foods. As this method is a record kept at the time of eating and does not involve participants attempting to remember if or how often a food has been eaten, it is generally regarded as providing the most reliable information regarding the dietary intake of individuals, provided sufficient days' observations are collected on each individual

BOX 30.3 Estimating individual food intake

Basically, four types of method are used to estimate individual food intakes:

- **Food diary or record** 'Please write down (and describe) everything you eat and drink (and estimate the amount) for the next 3 (4 or 7) days.' Amounts are usually recorded in household measures, but for more accuracy subjects can be provided with quick-reading scales to weigh food before it goes on the plate (and any leftovers).
- **24-hour recall** 'Tell me everything you had to eat and drink in the last 24 hours.' This is less subject to wishful thinking about what the subject feels they ought to have eaten. The weakness is that yesterday may have been an unusual day; 24-hour recalls can, however, be repeated.
- **Food frequency questionnaire** 'Do you eat meat/fish/bread/milk, etc. on average more than once a day, two or three times a week, once a week, once a month, etc.?' (usually filling in 100 to 150 lines on a questionnaire form) (Fig. 30.2).
- **Dietary history** 'What do you eat on a typical day and how does your food intake vary?' This requires a skilled and patient interviewer. Food models, cups, plates, and spoons are used to estimate portion sizes.

(see Section 30.4.2). In the past, it has been used for the purpose of validating other methods of dietary assessment, but this approach is now recognized to underestimate the extent of measurement error (see Section 30.4.6). Respondent burden is higher than with other methods, but the approach has been used in multicentre cross-sectional comparisons of representative population samples in which instructions to participants have been standardized among different centres (e.g. the Key's study of coronary heart disease (see Chapter 21) and other epidemiological settings such as the very large prospective studies of diet and health, for example a study of 25 000 people in the European Prospective Investigation of Cancer in Norfolk (EPIC Norfolk; see http://www.srl.cam.ac.uk/epic/nutmethod/). Fig. 30.1 shows an example from this website. Weighed records have also been used in surveillance procedures; for example, records from representative population samples have been routinely obtained by the UK Government National Diet and Nutrition Survey (http://www.food.gov.uk/science).

30.2.2 Twenty-four-hour recalls

This method is also a report of daily habits, but interviewed or written information about the previous day's intake, the 24-hour recall, is obtained. The participant has to remember the actual foods consumed and give information on portion weights from memory. Some information may be forgotten and descriptions of portion size are more difficult to supply, though the interviewers will often use food models or photographs as memory aids and to assist in quantifying portion size. Although the 24-hour recall may consist of a very simple written list completed by the participant, most 24-hour recalls have several stages or multiple passes, in which data are checked and verified by a skilled interviewer, and each recall may take about 40 minutes. The respondent burden for a single 24-hour recall is less than for several days of food records and the method is typically used for determining average usual intakes of a large population or group, for example in national nutrition surveys. For details of methods used in the USA NHANES survey, see http://www.cdc.gov/nchs/nhanes.htm. National nutrition surveys are typically based on a single 24-hour recall, sometimes with a repeat recall being undertaken on a subsample. However, when attempting to assess the diet of individuals using this method, multiple 24-hour recalls may be needed, with a subsequent increase in respondent burden, depending on the level of precision required and nutrient to be studied (see Section 30.4).

30.2.3 Food frequency questionnaires

FFQs are designed to assess long-term habits, over months or years, and may either comprise a relatively small list of foods that are the major sources of a limited group of nutrients of interest, or a longer list if a full dietary assessment is required. Participants usually complete the FFQ themselves, generally after receiving the FFQ in the post with detailed instructions regarding completion of the questionnaire. The length of the list of foods generally does not exceed 150 items. Various methods to assess portion sizes may be used, for example fitting average portion weights derived from other data to the respondents' chosen food and frequency selections. To assess the frequency of food consumption, accompanying the food list is a multiple response grid in which respondents attempt to estimate how often selected foods are eaten. Up to ten categories ranging from never or once a month or less, to six times per day is a usual format. Fig. 30.2 shows an example of a FFQ taken from EPIC Norfolk. Because responses are standardized, FFQs can be analysed in comparatively short periods of time so that large numbers of individuals can be investigated relatively inexpensively. The FFQ has been widely used in large epidemiological cohort studies to classify participants according to quantiles of intake of nutrients and disease incidence in each quantile examined, to identify food patterns associated with excessive or inadequate

DATE 2 3 1 0 1 9 9 3 DAY OF WEEK SATURDAY

BEFORE BREAKFAST

Food/drink	Description and preparation	Amount
Orange Squash	Robinsons whole orange—sweetened	1 Glass

BREAKFAST

Food/Drink	Description and Preparation	Amount
Beef Patty with onion	Homebaked cold Salt added	3a
Tea	Typhoo	1 Cup
Milk	S/Skimmed	1 Dessertspoon
Sugar	White	$1\frac{1}{2}$ Teaspoon

MID MORNING – between breakfast time and lunch time

Food/Drink	Description and Preparation	Amount
Coffee	Maxwell House, Instant $\frac{1}{2}$ Water/$\frac{1}{2}$ S/Skimmed milk	1 Mug
Sugar	White	$1\frac{1}{2}$ Teaspoons
Cake	Homemade Date Cake	16a

LUNCH

Food/drink	Description and preparation	Amount
Gammon Steak	Microwaved	6oz
Chips	Deep Fried in Oil (Crisp & Dry)	7a
Peas	Birds Eye (Frozen)	12a
Bread	Local Bakery White unsliced	$\frac{1}{2}$ Slice $\frac{1}{2}$ Thick
Apple Pie	Homemade	3B
Sugar	White—sprinkled on	1 Teaspoon
Custard	Birds—made with S/Skimmed milk	Small Fruit Dish

TEA – between lunch time and the evening meal

Food/Drink	Description and Preparation	Amount
Tea	Typhoo—tea bag	1 Mug
Milk	S/Skimmed	1 Dessertspoon
Sugar	White	$1\frac{1}{2}$ Teaspoons
Biscuit	Chocolate Digestive Fox's	1

Fig. 30.1 Example of a food diary.

Source: Reproduced with permission from EPIC Norfolk (http://www.srl.cam.ac.uk/).

PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
DRINKS	Never or less than once/month	1–3 per month	Once a week	2–4 per week	5–6 per week	Once a day	2–3 per day	4–5 per day	6+ per day
Tea (cup)								✓	
Coffee, instant or ground (cup)						✓			
Coffee, decaffeinated (cup)	✓								
Coffee whitener, eg. Coffee-mate (teaspoon)	✓								
Cocoa, hot chocolate (cup)						✓			
Horlicks, Ovaltine (cup)	✓								
Wine (glass)	✓								
Beer, lager or cider (half pint)	✓								
Port, sherry, vermouth, liqueurs (glass)	✓								
Spirits, eg. gin, brandy, whisky, vodka (single)	✓								
Low-calorie or diet fizzy soft drinks (glass)	✓								
Fizzy soft drinks, eg. Coca cola, lemonade (glass)						✓			
Pure fruit juice (100%) e.g. orange, apple juice (glass)	✓								
Fruit squash or cordial (glass)							✓		
FRUIT (1 fruit or medium serving) **For very seasonal fruits such as strawberries, please estimate your average use when the fruit is in season**									
Apples				✓					
Pears				✓					
Oranges, satsumas, mandarins		✓							
Grapefruit	✓								
Bananas			✓						
Grapes			✓						
Melon	✓								
Peaches, plums, apricots				✓					
Strawberries, raspberries, kiwi fruit						✓			
Tinned fruit		✓							
Dried fruit, eg. raisins, prunes	✓								
	Never or less than once/month	1–3 per month	Once a week	2–4 per week	5–6 per week	Once a day	2–3 per day	4–5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

Fig. 30.2 Example of a food frequency questionnaire. This is one of several different pages that have to be filled in.

Source: Reproduced with permission from EPIC Norfolk (http://www.srl.cam.ac.uk/).

intakes of nutrients, and to obtain descriptive information on usual intakes of foods. Although their use is increasing, some doubt has been expressed regarding the ability of FFQs to detect associations between diet and disease using this method (Kristal *et al.*, 2005).

30.2.4 Diet histories

The diet history is usually conducted by trained interviewers who record a 24-hour recall followed by more detailed information on usual foods consumed, portion sizes, recipes, and frequency of food consumption over the recent past. This method is less commonly used in epidemiological research due to the necessity for face-to-face interviews of up to 90 minutes, and consequent costs, but it is the most frequently used method for the assessment of diet by dieticians in the clinical context (see Gibson, 2005).

30.2.5 Checklists

The checklist is a record, to be completed for 7 days or more. However, the checklist method is a printed list of representative foods in which participants are asked to check off at the end of each day which foods they have eaten. This means that participants do not have to estimate how often the food is eaten, thus avoiding problems in the estimation of the frequency of food consumption that occur in the FFQs. Like the FFQs however, the foods can be precoded for rapid data entry and computerized linkage to food tables. In one published version, the checklist took the form of a booklet, which comprised one page of instructions, one of an example, and seven pages (one for each day over 1 week) of the checklist. When selecting foods, participants were asked to count half for a small portion and two for a large portion. A space was left to record foods not present on the printed list, but otherwise the list was precoded for nutrient analysis. The list of 160 foods was that used for a FFQ and, where possible, 'units' (slices, cups, etc.) were specified (Bingham *et al.*, 1994). This method has been further developed more recently (Lillegaard and Andersen, 2005).

30.2.6 Duplicate diets

If there is inadequate food table information, 'precise weighing' of duplicate diets may be necessary, for example if food composition tables with values for cooked foods are not available, in carefully controlled experiments when precise knowledge of nutrients and energy intake is required or if exposure to phytochemicals and contaminants are being investigated. Raw ingredients, the cooked food, meal, or snack, plus the individual portions are generally prepared in duplicate, one for consumption, the other for weighing and chemical analysis if required. Amounts not consumed are taken into account. This method is very labour-intensive compared with the records outlined above and it is usual for skilled field-workers to carry out this survey, rather than the subjects themselves (Bingham, 1987).

30.2.7 Retrospective assessment

Methods that are designed to assess recent past diet (the 24-hour recall, FFQs, and diet history) can in theory also be used to assess distant past diet, for example in case–control investigations where dietary habits before the onset of symptoms (and possible change in diet) are required. However, there is evidence to suggest that individuals cannot remember past diet and instead report present diet (Friedenreich *et al.*, 1992). This may introduce bias into case–control studies if dietary habits have changed as a result of the symptoms of the disease in question. For this reason, more weight is placed on results of prospective studies than retrospective case–control studies in nutritional epidemiology.

30.3 Calculation of nutrient intake

Once the primary data concerning foods consumed are obtained, the information is converted to nutrient intake using tables of food composition. In the past, this was generally done manually, perhaps with the assistance of a calculator, and the information obtained was generally restricted to a narrow

range of nutrients, for example energy and macronutrient consumption. Computerized databases of food composition revolutionized the amount of information that could be obtained, but in some data entry systems the matching of the description of the food consumed to the correct computer code has to be done manually, which leads to errors. In present-day surveys, necessitated partly by the growth in the variety of foods consumed, this procedure is now usually entirely computerized, usually by the investigators themselves. Programs are more expensive to run and develop for record or 24-hour recall methods than for FFQs, since at least 150 000 different food items are available in Westernized food supplies, all of which require estimation of portion size and individual computer coding (see http://www.srl.cam.ac.uk/epic/nutmethod/). Furthermore, most investigators will incorporate some means of calculating nutrient intake from individual recipes used in home cooking, since these can have a marked effect on some nutrients, for example on specific fatty acids. The checklist and FFQ methods require much simpler methods and considerably less coding time by the investigator, although much information on actual foods consumed is lost.

30.4 Measurement error in dietary assessments

Methods of measuring diet are associated with both random and systematic error. Both types of error can arise in the assessment of portion size, daily variation, frequency of food consumption, and failure to report usual diet, either because of changes in habits while taking part in an investigation or misreporting of food choice or amount. Error may also result from the use of food tables.

30.4.1 Assessment of portion size

Information about the weight of food consumed may be obtained either by asking subjects to weigh out individual items of food onto the plate as it is being served (weighed records), or requiring that portions of food be described in terms of household measures, volume models, photographs, average portions, units, or pack sizes (estimated records, diet histories, and 24-hour recalls). Errors are reduced when weights are obtained, but participants need to be given a set of scales accurate to 1–2 g with a capacity of 2 kg. Digital scales are now usually used and have replaced spring balances used in older surveys. Participants need instruction on the use of the scales and on the detail of information required, including description of recipes used (see Further reading). Estimated records are much easier for participants to complete, but conversion of descriptions of food into weights requires considerable investment by the investigator and may necessitate the determination of density of separate foods, as well as a detailed database of weights of foods equivalent to the photographs, models, package sizes, and household measures used. On balance, there appears to be little or no systematic bias in group averages of nutrients obtained by records with estimates of food, compared with group averages obtained by weighed dietary records. Nevertheless, despite the absence of overall bias in a population, the estimation of portion size rather than direct weighing is associated with imprecision at the individual level. In general, this is in the order of 50% (coefficient of variation) for foods, but less, about 20%, for nutrients, probably due to cancellation of error from the use of food tables. Models and photographs may incur less error in the estimation of portion weights, at least when compared with estimations from household measures and dimensions.

30.4.2 Daily variation

Individuals do not consume the same food from day to day and substantial error is introduced when diet is assessed from a single day's dietary investigation

in records or 24-hour recalls. Thus, daily variation is one of the main factors in reducing precision of individual estimates in either of these methods of assessing diet. The variability from day to day is closely related to the nutrient under study. The early descriptions of record techniques specified that subjects should be observed for 7 days, and this practice has been followed for over 60 years. Nevertheless, when only the average intake of a group of individuals is required for cross-sectional studies, it is difficult to justify gathering this amount of data, even for the more variable nutrients such as cholesterol or polyunsaturated fatty acids. A 1-day record or 24-hour recall collected from a large number of subjects may suffice for the assessment of group means, although it is generally more useful to undertake the assessment on at least 2 days in order to be able to estimate the within-subject component error.

Seven days is generally accepted as the minimum length of time required to gain precision in observations on each individual, although shorter periods of time with correction for the within-subject component error are under investigation. The actual number of records required to classify individuals in any specific population according to quantiles of nutrient intake will depend on the ratio of the average within-person daily variation and the between-person variation. Thus, whereas a 7-day record is probably sufficient to classify into thirds of the distribution for energy and energy-yielding nutrients, longer periods are necessary for items such as some vitamins and minerals and cholesterol.

30.4.3 Frequency of food consumption

Overestimation, compared with records of food consumption, particularly of vegetables but also of energy and energy-yielding nutrients, is a usual finding with FFQs. The cause of this is uncertain, but may result from the use of lists. Restriction of the choice of food into a comparatively short list of around 150 foods or fewer, means that error associated with estimation of amounts of single items is more likely to be biased than when the full variety of foods is analysed, as occurs, for example, in a 24-hour recall or record of food consumption. Participants using the FFQ may also have difficulty in choosing the correct category of how often food is consumed, so that overestimation occurs of the number of times foods are eaten over a defined period of time. FFQs routinely overestimate intake of fruits and vegetables.

30.4.4 Under-reporting

The term 'under-reporting' particularly applies to methods that attempt to assess total energy intake. This problem has been demonstrated by comparing the group average intake of energy from diet assessment methods with group energy expenditure estimated from body weight, or, more accurately, the doubly labelled water technique (see Section 30.5). Under-reporting has been documented with all methods of dietary assessment, including 24-hour recalls, weighed records, diet histories, and FFQs designed to assess total diet. Overweight individuals in particular are likely to under-report the amount they eat. Table 30.1 is an example showing that some but not all nutrients and foods are under-reported: protein, sugars, and fat, and foods such as cakes and sweets, tend to be under-reported but nutrients such as carotene, nonstarch polysaccharides and vitamin C, and vegetables are not.

The problem of under-reporting is particularly difficult when mean intakes are to be compared with reference nutrient intakes for surveillance and clinical work, or amounts of nutrients eaten by a different population or group (such as obese compared with lean individuals). Cut-offs based on estimated energy expenditure calculated from body weight have been devised, but they are imprecise when used in the absence of information on energy expenditure. Ideally, all dietary studies should include independent measures of validity (see Section 31.6 and Black, 2000).

30.4.5 Energy adjustment

Energy adjustment can be carried out by a variety of methods, including expressing results for

Table 30.1 Intakes of energy and macronutrients expressed as reported and after energy adjustment in individuals who under-reported dietary intake and those who did not, as judged by the urine to dietary nitrogen ratio

Nutrient	Valid records $n = 126$ (mean ± SE)	Under-reporters $n = 33$ (mean ± SE)	*P*-value
Reported intakes			
MJ	8.14 ± 0.04	6.65 ± 0.23	<0.001
Protein (g)	71 ± 0.2	60 ± 1.7	<0.001
Fat (g)	80 ± 0.3	62 ± 2.5	<0.001
Carbohydrate (g)	231 ± 4.3	191 ± 8.2	<0.001
Energy adjusted intakes			
Protein (g)	69 ± 0.8	67 ± 1.4	>0.05
Fat (g)	77 ± 0.9	75 ± 0.9	>0.05
Carbohydrate (g)	223 ± 2.7	225 ± 4.0	>0.05

nutrients as a percentage of the total energy, or using regression techniques (Willett, 1997). One reason for attempting to correct for energy intake in dietary assessments is to reduce extraneous variation from the general correlation of nutrients with total energy intake, brought about by differences in body size and hence (in sedentary populations) energy expenditure. In addition, the correlation between results from one method and another is sometimes improved by energy adjustment. Furthermore, although there are significant differences in absolute macronutrient intake between individuals who give valid records and those who do not, these differences are substantially reduced after energy adjustment, although the overall mean within a population is not altered. Table 30.1 shows reported and energy-adjusted intakes of fat, carbohydrate, and protein in a group of women. Differences between under-reporters and those who gave valid records were no longer significant after energy adjustment.

The effect of energy adjustment depends on the correlation between the nutrient concerned and energy intake, and also on the correlation between the errors of measurement for these two quantities. The latter is heavily dependent on the dietary method used. Hence, the relation between nutrient intakes derived from FFQs and weighed records can be much improved by energy adjustment, but to a lesser extent between nutrient intakes derived from weighed records and 24-hour recalls. Energy adjustment is inappropriate (and without effect) if there are zero correlations between energy intake and the nutrient concerned, for example in the case of some vitamins. More details of techniques for energy adjustment are given in Willett (1997).

30.4.6 Effects of measurement error

Measurement error is a serious problem in dietary assessment. The effect of measurement error may be to introduce bias, so that, for example, group mean intakes may be over- or underestimated when population intakes are investigated for comparison against recommended levels (see Section 30.4.4). In epidemiological research, individuals may be misclassified in the distribution of nutritional intakes so that a null or attenuated relationship may

be obtained and the true effect between diet and disease missed. For example, in a prospective study relating diet to breast cancer risk, diet was assessed using both a FFQ and a detailed 7-day diary of food and drink in 13 070 women in 1993–97. By 2002, there were 168 incident breast cancer cases for analysis. When their baseline dietary intake was compared with matched controls (four for each of the breast cancer cases), the hazard ratio for breast cancer for each quintile increase of energy-adjusted fat was strongly associated with saturated fat intake measured using the food diary (1.219 (95% CI 1.061–1.401), $P = 0.005$). However, with saturated fat measured using the FFQ, the comparable ratio was 1.100 (0.941–1.285, $P = 0.229$) (Bingham *et al.*, 2003).

Different methods of dietary assessment have different types of error structure, so that the magnitude of the error varies according to the method and may not always be predictable in different populations. In large prospective epidemiological studies, it is now common practice to correct for measurement error in the assessment of relative risk by regression calibration when the correction factors are derived by comparison of the method in use, such as a FFQ, with a 'reference' method, such as a record. This 'relative validation' relies on the assumptions that errors in the reference instrument are not correlated with both 'true' intake and errors in the method in use. However, errors associated with the method under investigation may be correlated with those of the reference method, so that correction for regression dilution is substantially underestimated (Day *et al.*, 2001; Schatzkin *et al.*, 2003). For example, an individual who under-reports using one dietary assessment method such as a food record will also do so with another, such as a FFQ. The validity of dietary assessment methods for determining intake of 15 priority micronutrients and n-3 fatty acids has recently been reviewed by the EURopean micronutrient RECommendations Aligned Network of Excellence (EURRECA) (Willett, 2009). The conclusion of this substantial report generally supports the view that in epidemiological studies, FFQs provide reasonable estimates of intake when their preference is compared with a presumed superior method or biomarker. Most correlations were regarded as being in the acceptable to good range ($r = 0.30$–0.70). Whether such correlations are acceptable and whether it is appropriate to compare one dietary intake method with another will continue to be debated. It is generally accepted that wherever possible and appropriate, biomarkers rather than 'relative validation studies' should be used to validate methods for assessing dietary intake.

30.5 Biomarkers

True estimates of food consumption can only be obtained by actually observing the activities of participants, or by developing some other independent way of assessing food intake. This has become possible with the advent of biological markers in biological specimens, such as blood, urine, or hair, that reflect intake sufficiently closely to act as objective indices of true intake (Bingham, 2002).

30.5.1 Types of biomarkers

There are four main classes of biomarkers used for assessment of the accuracy of dietary methods (Bingham, 2002). The most important are *recovery biomarkers*. These biomarkers have been tested under controlled conditions, usually in a metabolic suite, and have been shown to reflect an individual's intake of energy or of a particular nutrient with a high degree of accuracy. Thus, they provide a true gold standard against which another method of dietary assessment may be compared. Few such biomarkers of dietary intake have been identified; they include doubly labelled water as a measure of energy intake, used for example in the OPEN study (Schatzkin *et al.*, 2003) and markers of potassium and nitrogen in 24-hour urine collections (Bingham *et al.*, 1995; Bingham, 2002). A recent new category has been the *predictive biomarker* of 24-hour

urinary sucrose and fructose, which is closely correlated with intake of sugars, despite the very small fraction of intake that is present in urine collections (Tasevska *et al.*, 2005).

Several *concentration biomarkers* (including serum levels of some vitamins such as vitamin C and carotenoids) are available to compare with estimates of dietary intake. Concentration biomarkers cannot be related directly to absolute levels of intake but concentrations do correlate with intakes of corresponding foods or nutrients, although correlation coefficients are much lower (usually equal to less than 0.6) than that expected for recovery biomarkers. Results from a dietary intake method that agreed most closely with these biomarkers would be expected to yield more reliable estimates of intake than one that did not. Finally, *replacement biomarkers* may be used if databases of food composition for certain items are not available or considered to be inaccurate, for example iodine, aflatoxins, and phyto-oestrogens. As it is not usually possible to measure added salt or salt used in cooking, and as a consequence salt intake cannot be measured by dietary methods, 24-hour urine sodium is also an example of a replacement biomarker.

30.5.2 Validation of dietary methods with biomarkers

Biomarkers have been used to validate methods used for dietary assessments in both large-scale epidemiological studies designed to establish the nutritional aetiology of non-communicable diseases and in national nutrition surveys intended to evaluate population nutrient intakes. For example, in the UK National Diet and Nutrition Survey, pilot studies were carried out before the main survey in order to compare intakes of energy from the dietary assessment method and energy expenditure from doubly labelled water (see http://www.ons.gov.uk/ons/search/index.html?pageSize=50&newquery=enery+expenditure+and+doubly+labelled+water). In the USA, doubly labelled water has been used to assess 24-hour recalls and FFQ methods used in epidemiological studies (Schatzkin *et al.*, 2003). To assess the validity of several different methods of dietary assessment in UK cohorts of the EPIC study, 160 women were asked to complete 16 days of weighed-food records over 1 year, as four repeated 4-day records. The volunteers were also asked to provide eight 24-hour urine collections, as four repeated 2-day collections, and completeness of the urine collections was assessed using a specially developed marker. Correlations were greater between the biomarker 24-hour urine nitrogen and estimates of nitrogen intake from records ($r = 0.7$) than from estimates of intake from other methods, including FFQs ($r = 0.4$). A similar pattern was evident with the urinary potassium biomarker (Bingham *et al.*, 1995). This study showed that the 7-day food diary was associated with less measurement error and, consequently, estimates of diet from the 7-day food diary as well as a FFQ were obtained in the full cohort of 25 000 people in EPIC Norfolk (Black, 2000; Bingham *et al.*, 2001).

30.5.3 Calibration

Another way to reduce measurement error is to increase the heterogeneity of the population and pool results from different populations with diverse dietary practices so that it becomes easier to correctly classify intake of individuals in the distribution of food and nutrient intake. This was the approach adopted in the largest epidemiological study of diet, cancer, genetic factors, and health in the European Prospective Investigation of Cancer (EPIC). However, as diet was measured by country-specific questionnaires designed to capture local dietary habits and to provide high compliance, it was also necessary to calibrate results. A second dietary measurement was taken from an 8% random sample (36 000 individuals) of the cohort using a computerized, 24-hour diet recall method (EPIC SOFT) in order to calibrate the questionnaires. A total of 1103 volunteers of both genders from 12 centres also provided complete 24-hour urines for biomarker analysis, and the high, sex-partial Spearman correlation of 0.72 between mean urinary and dietary nitrogen suggests that confidence can be placed in the validity

of the calibration method used. When dietary intake was related to disease risk, calibration of the main method against the more detailed method strengthened associations considerably. Examples of the effect of calibration include the reduced risk of colorectal cancer associated with dietary fibre consumption and increased risk associated with red and processed meat consumption (Bingham and Riboli, 2004; Norat *et al.*, 2005).

Conclusion

Dietary assessment methods have enabled nutritional surveillance, facilitated clinical management of patients, and demonstrated powerful and sometimes causal associations between diet and disease which have led to nutrition recommendations with important public health implications. However, the limitations of the methods have not always been appreciated.

Many lessons about the ability to obtain accurate data have been learnt from incorporating appropriate statistical, biomarker, and epidemiological techniques into nutritional assessment. Use of biomarkers has shown that substantial attenuation of diet effects and loss of statistical power can occur in epidemiological studies in homogeneous populations when relatively inaccurate methods of dietary assessment are used. There has even been a suggestion that FFQs should be abandoned in large epidemiological studies (Kristal *et al.*, 2005). Attenuation and loss of power, together with genetic variation in response, could account for the inability of existing studies to show causal links between diet and chronic disease. Caution in the interpretation of null results from the epidemiological literature should be exercised. Failure to do so may account for some of the potentially misleading interpretation of the findings from cohort studies suggesting that saturated fatty acids may not be strongly related to coronary heart disease, as was previously believed to be the case. (Siri-Tarino *et al.*, 2010). This may also be the case in other types of research and in clinical studies. Some of this measurement error can be overcome by studying populations whose dietary habits are more heterogeneous than single populations, but biomarker studies suggest that improved, more detailed, and novel methods of dietary assessment will be necessary if further causal associations between diet and disease are to be established with any degree of certainty in future investigations.

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Useful Websites



To see topical and scientifically robust updates on nutrition associated with this textbook and web links to many of the journals in the Further Reading sections, please see the dedicated Online Resource Centre at **http://www.oxfordtextbooks.co.uk/orc/mann4e/**

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http://faostat.fao.org/
http://www.srl.cam.ac.uk/epic/nutmethod/
http://www.defra.gov.uk/statistics/foodfarm/food/familyfood/nationalfoodsurvey/



31 Assessment of nutritional status and biomarkers

Stewart Truswell

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31.1 Nutritional status versus dietary intake

Dietary intake estimation, described in the preceding chapter, cannot always prove that an individual or community is well nourished or poorly nourished—or overnourished. This has to be confirmed or established by one or more methods of examination.

Food intake can be distorted by intrusion of investigators; intake over a day or a few days may not represent intake over time. It is difficult to relate mixed dishes to lines in the food tables. Nutrients in food tables are only averages, often from another country and an earlier time. Not all nutrients are in the food tables. Nutrient reference intakes (recommended dietary intakes) may not be enough for everyone, especially if they have an illness. Then is an intake below the recommended dietary intake serious or covered by safety factors? Health professionals cannot rely on the history of quantity and type of food when assessing a patient's state of nourishment. You cannot diagnose obesity, overnutrition, from someone's dietary history, and undernourished people may not be able to tell you what they have and have not eaten for a variety of reasons.

Food intake measurement—really estimation—is ultimately *subjective*. It depends on the memory, cooperation, and honesty of individuals. Assessment of nutritional status is, by contrast, ultimately *objective*. A person's weight, height, and chemical concentration in blood or urine is measured by an outside observer and if a second and third observer repeats the measurement, they should obtain about the same result.

BOX 31.1 Nutritional status

Nutritional status is a multidimensional concept, a jargon term used by nutrition professionals. It ultimately means whether a person has functioning in their body enough, but not too much of all the nutrients, from calories down to the micronutrients. Nutritional status can be obvious, with wasting or obesity or only discovered with high technology chemical methods on samples of body fluids or a biopsy. Different aspects of nutritional status are the focus for hospital dietitians and clinicians, for public health workers and epidemiologists, and for nutrition researchers.

31.2 Uses of nutritional assessment

31.2.1 Evolution of assessment methods: looking for malnutrition

The scientific methods of assessing nutritional status were put together after World War II when there was widespread malnutrition across Europe. They were used to detect people who were poorly nourished. Nutrition surveys were done in communities considered at risk by nutrition specialists from Britain and North America.

In the 1950s, these methods were applied in the rest of the world, especially in less-developed countries (where kwashiorkor (Chapter 19) was rediscovered in 1952). The US Interdepartmental Committee for Nutrition in National Defense carried out surveys of military personnel and civilians between 1956 and 1967 in 26 countries that had alliances with the USA, each as a separate operation with its own report. The standardized methods for the nutritional surveys (national food supply, sampling, clinical examination, biochemical studies, and dietary data) were published in a manual in 1963. This enabled investigators to compare results between communities, to plan applied nutrition programmes, and to advise on national food and nutrition policy.

In the same year, the World Health Organization (WHO) Expert Committee on Medical Assessment of Nutritional Status commissioned Derek Jelliffe to prepare a standard guide for nutrition surveys everywhere in the world. He consulted 25 top nutrition experts in various countries and wrote a WHO monograph, *The assessment of the nutritional status of the community (with special reference to field surveys in developing regions of the world)* in 1966. This classic of nutrition literature is the foundation of examining people systematically to see whether they are malnourished.

Since the 1960s, the biggest change is that most of the 50 clinical signs of malnutrition in Jelliffe's book are little used today. They are rare in industrial countries, require experienced medical personnel to diagnose, and many have other causes as well as malnutrition. Pallor (anaemia), oedema, sore lips, inflamed tongue, and enlarged liver can all be due to poor nutrition, but other causes are more common in most countries. A small number of clinical signs are important in nutrition work but it depends where you are. Hair changes of kwashiorkor in a toddler suggest protein deficiency in deprived parts of Africa but not in the developed world. Bitôt's spots or the skin changes of pellagra are reliable signs of vitamin A deficiency or niacin deficiency, respectively, in places where these deficiencies are known to occur and when observed by an experienced clinician. Thyroid enlargement in teenagers (likely to be endemic goitre from iodine deficiency) and mottled teeth (likely to indicate mild excess of fluoride in early life) are reliable signs of nutritional status and so are some of the signs of rickets (enlarged radial epiphysis, beading of costochondral junction).

The two main types of methods used today for nutritional assessment are anthropometry, measuring weights and heights and other body measurements, and biochemical tests, usually on blood, sometimes on urine. These are described in detail in Sections 31.3 and 31.6. Professional staff have to be trained and paid to do accurate anthropometry and each particular biochemical measurement has a cost, so nutritional assessments can only be done where there is sufficient funding.

31.2.2 Parenteral and enteral nutrition (see Chapter 42)

Modern formulae for total parenteral nutrition, including balanced amino acids and safe intravenous lipid preparation, have been available and approved since 1977. Special nutrition support teams have been set up in major hospitals and there are international societies for the nutritional speciality of enteral and parenteral nutrition (ASPEN in America, ESPEN in Europe). For assessing and monitoring hospital patients' nutritional status, clinical teams use selections from the general

methods for nutritional assessment. Rapid biochemical tests are available for monitoring, but critically ill patients lying in bed with lines and tubes attached cannot be weighed so other anthropometric measurements have to be used. Chapter 42 discusses nutritional support for the hospitalized patient in more detail.

31.2.3 Overnutrition

With the increase of overweight and obesity in the last decade, there has naturally been a focus on reliable and accessible indicators of the amount and effects of a person's excess accumulated calories. The two established simple anthropometric measures are: (1) body mass index (BMI), where:

$$\text{BMI} = \text{weight}/\text{height}^2 \text{ (see Section 31.3.3)}$$

where weight is in kg and height is in metres; and (2) waist circumference (either alone or expressed against hip circumference). Reference numbers have been derived for different ages and nations for BMI and for men and women (for the waist measurement). Along with these physical measurements, biochemical tests can indicate if overweight is accompanied by metabolic abnormalities. The most usual are plasma low-density and high-density lipoprotein cholesterol, fasting triglycerides, and fasting or postprandial glucose.

31.2.4 Biomarkers to check, support, or replace some food intake estimates

Some biochemical tests are increasingly being used for this purpose in human nutrition experiments and epidemiological studies. Not many are suitable, but four good examples are:

- for checking protein intake (and hence roughly energy intake)—24-hour urinary nitrogen;
- to support change of type of fat—plasma fatty acid pattern, especially 18:2 ω-6;
- more reliable than estimating salt intake—24-hour urinary sodium;
- the only way to gauge iodine intake (because it varies greatly among foods)—urinary iodine.

31.3 Anthropometric assessment

The basic anthropometric measurements are simple, straightforward, inexpensive, and safe and anyone can do them. For research purposes, weight and height are measured more precisely and for clinical work some other measurements are made.

31.3.1 Body mass (body weight)

In affluent communities, most people know their body weight and many weigh themselves regularly on an electronic bathroom scale. These are not as accurate as health professionals require in a clinic or consulting room. The best weighing machines are beam balances with non-detachable weights (Fig. 31.1), but these are bulky and difficult to move. They should stand on a level, hard surface and be checked with a known weight regularly. People should be weighed to the nearest 0.1 kg, wearing minimal clothing. If changes in body weight are being followed, the measurement should if possible be made at the same time of day because meals, drink, a full bladder, and bowel action can all affect the reading. When people in a steady state are weighed repeatedly, the day-to-day fluctuation can be ±1.0 kg.

Nutritionists are very interested in the measurement of body weight and its interpretation, whether someone is overweight or underweight, and whether their weight is increasing or going down. They want as well to estimate what components

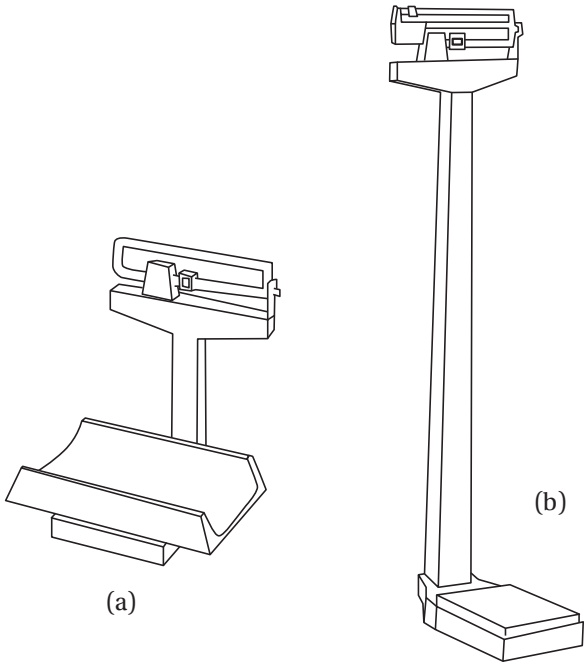


Fig. 31.1 Measurement of weight. (a) Paediatric scale for infants, and (b) beam balance for a child or adult.

Source: Gibson, R.S. (2005) *Principles of nutritional assessment*, 2nd edition. Oxford, Oxford University Press.

inside the body make up the weight and the change in weight.

Fig. 31.2 shows the different compartments in an average, healthy weight adult. About 20% of this average person is fat and the rest is fat-free mass. Women have more fat and less muscle than men. Of the fat-free mass, part is muscle (about 40% of total body weight), part is bones, and the rest is all the other organs, in descending order of weight, skin, blood, gut, liver, brain, lungs, heart, and so on. Chemically, the largest component is water, then there are about equal amounts of protein and fat (each around 20%) and the rest is bone mineral. In adults, weight gain is nearly all fat; weight loss from negative energy balance is of fat, but some fat-free mass, i.e. protein is lost as well.

When total body mass has been measured, we need to judge whether it is in the healthy range or whether he/she is too thin or too fat. First, an adjustment has to be made for size. A lean giant must weigh more than a dwarf. Weight has to be considered in terms of height (or stature). Different indices have been proposed and tested. Of all the possibilities, weight/height^2 has been found the most generally useful (Cole, 1991) as a direct measure of over- or underweight. It only requires two measurements, weight and height, and a simple calculation. This BMI is expressed in kg (for weight) and metres (for height), i.e. in SI units. Quite different numbers would be obtained if height were in centimetres. The Belgian mathematician Quetelet first recommended this index.

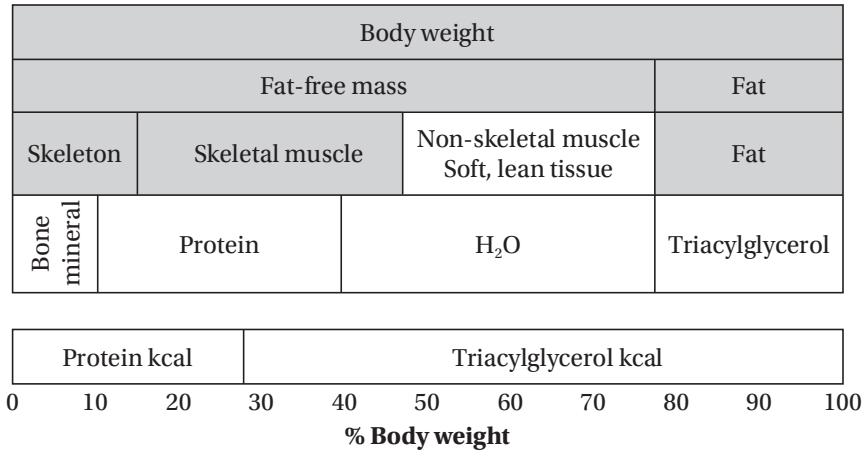


Fig. 31.2 Compartments of the body. Relationship between anthropometry (shaded), body composition, and energy reserves.

Source: Heymsfield, S.B. (1984) Anthropometric assessment of adult protein-energy malnutrition. In: Wright, R.A., Heymsfield, S.B., and McManus, C.B. (eds) *Nutritional assessment*. Boston, Blackwell Scientific.

31.3.2 Measurement of height (stature)

Height is more difficult to measure than weight. Consequently, people's heights are seldom measured and often not accurately remembered. For adults and children, a level floor and straight wall are needed (Fig. 31.3). The subject has to stand straight with buttocks, shoulders, and back of the head touching the wall, with heels flat and together, shoulders relaxed, and arms hanging down. The head should be erect and look straight forward, the lower border of the orbit in line with the external auditory meatus (the Frankfurt plane). The headpiece (a metal bar or wooden block) is lowered gently, pressing down the hair. Ideally, this headpiece should be on a sliding scale but if a loose object is used, such as a firm book, this must be kept horizontal. In practice, two people can better manage an accurate reading. For research work, a specially designed stadiometer can be obtained. There is a circadian variation in height. People are taller in the morning by 1–2 cm. Then, during the day, the intervertebral discs get somewhat compressed.

For very young children that cannot yet stand properly, their length is best measured lying supine on a specially designed measuring board. Two examiners are needed to position the infant correctly and comfortably.

For adults who are deformed (e.g. with scoliosis) or bedridden, estimates of what their height would be if they could stand straight can be made from *knee height* or *arm span* (right fingertips outstretched to tips of left fingers) or *demispan* (from sternal notch to webspace between middle and ring fingers). These measurements are used in geriatrics. Equations to give estimated height differ for gender and ethnic group.

31.3.3 Interpretation of body mass index

WHO and government health departments of the major countries have all adopted BMI as the

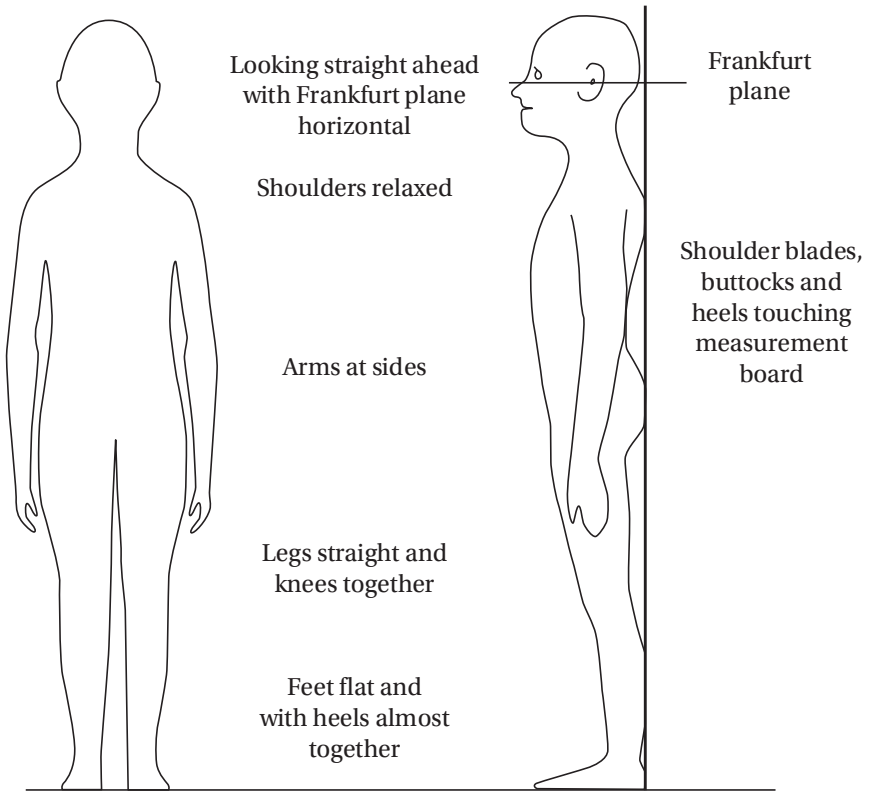


Fig. 31.3 Positioning of person for height measurement.

Source: Gibson, R.S. (2005) *Principles of nutritional assessment*. Oxford, Oxford University Press.

standard way of diagnosing overweight and obesity. With the same cut-offs for men and women, this is much simpler than the earlier tables of desirable weights for height, with different numbers for frame size and for gender. For those who are put off by, say,

$$65\ (\text{kg}) \div (1.73 \times 1.73\ (\text{metres})) \rightarrow 21.67\ \text{kg/m}^2$$

graphs like Fig. 31.4 are available.

Increased BMI indicates increased adiposity, but the correlation is not, of course, 100%. People with broad frame and weight lifters (with big muscles) can have a high BMI for their present body fat. In older people (see Chapter 35), muscle bulk declines and percentage of body fat increases. As BMI increases above 25 kg/m^2, mortality increases gently at first and then (above BMI 30 kg/m^2) more steeply (Fig. 31.5).

WHO classifies adults according to BMI:

- Underweight = <18.50 kg/m^2
- Normal range = 18.5–24.99 kg/m^2
- Overweight = 25.00–29.99 kg/m^2

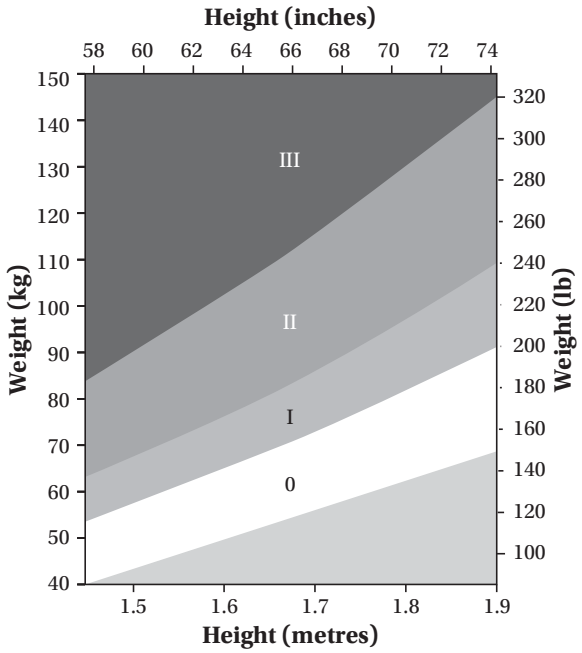


Fig. 31.4 Graph for reading BMI from weight (in kg or lb) and height (in metres or inches). Obesity grades I, II, and III have a BMI (kg/m^2) of 25–29.9, 30–40, and over 40, respectively.

Source: Garrow, J.S. (1981) *Treat obesity seriously*. Edinburgh, Churchill Livingstone.

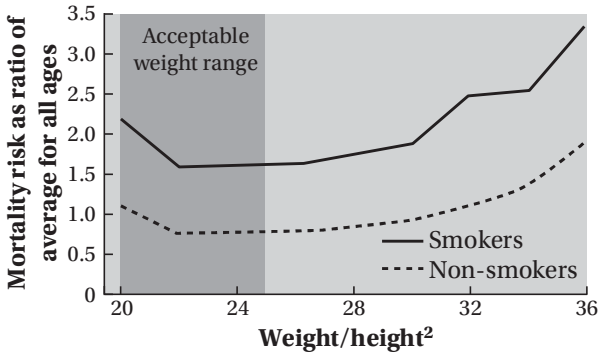


Fig. 31.5 Variations in mortality by body mass index (BMI) among 750 000 US men and women.

Source: Lew, E.A. and Garfinkel, L. (1979) Variations in mortality by weight among 750,000 men and women. *J Chron Dis*, **32**, 563–7.

- Obese = >30.00 kg/m^2
- Severely obese = >35.00 kg/m^2
- Very severely obese = >40.00 kg/m^2.

The value of these BMI levels probably has to be adjusted for ethnic groups because of differences in body composition and proportions. At a given BMI, South Asians and Indonesians have relatively more body fat, while Polynesians tend to have more muscle.

Assessing adult undernutrition, below a BMI of 18.5 kg/m^2, Ferro Luzzi *et al.* (1992) suggest that severity of chronic energy deficiency is moderate down to 17.0 kg/m^2, severe down to 16.0 kg/m^2, and very severe below this.

In children, the BMI that corresponds to start of overweight in adults (i.e. 25 kg/m^2) is lower. From surveys in different countries, Cole *et al.* (2000) estimate it should be 17.3 kg/m^2 at age 5, 19.9 kg/m^2 at age 10, and 23.6 kg/m^2 at age 15.

31.3.4 Waist circumference

The metabolic complications of overweight and obesity (see Chapter 17) have been found to be more likely if some of the adipose tissue is specially concentrated inside the abdomen (in the omentum, mesentery, etc.). This abdominal or visceral obesity can be estimated with a simple tape measure around the waist. Waist circumference correlates quite

closely with BMI. Some authorities prefer measurement of the waist/hip ratio to allow for people with a heavy frame, but it is more complicated to do and does not seem to have better predictive value.

Table 31.1 gives the WHO recommendations for Caucasians. The identification of risk using waist circumference is population-specific and depends on the levels of obesity and other risk factors for cardiovascular diseases and diabetes in the ethnic group.

31.3.5 Measuring children's growth

The primary index of growth, used universally, is *weight for age*. At any particular age, the infant's or the child's weight (unclothed) is compared against a reference, a sort of standard to see if its weight is at, below, or above the average. If it is far off the average, percentile lines on the reference graph will show how it compares with the reference population.

The most used weight-for-age set for older children is probably the US Centers for Disease Control and Prevention growth reference. Graphs for boys and girls aged 2–20 years are reproduced in Chapter 34.

For boys and girls from birth to 5 years there is a new WHO child growth standard (WHO, 2006). Infants are weighed more frequently, so a broader horizontal scale is needed; infants' stature is measured lying down and length is 1.0–2.0 cm longer than height. The reference subjects were healthy and included all ethnic groups, excluding those who had very low birth weights.

Table 31.1 WHO recommendations for Caucasians

Risk of metabolic complications	Waist circumference (cm)	
	Men	Women
Increased	≥94	≥80
Severely increased	≥102	≥88

In Britain, the current weight-for-age reference (Freeman *et al.*, 1995) is based on measurements of 25 000 white children between 1978 and 1990. These references replace earlier standards because more infants are now breast-fed and children have fewer infections and grow taller.

In weight-for-age graphs, the average is the 50th percentile, i.e. median of the reference sample. An individual's difference up or down from this median can be read firstly from the *percentile lines*. A second way used for distance from the median is the *standard deviation* or *Z-score* above or below the median. One SD below the median is a Z score minus one (–1). A third indicator used for undernutrition is not percentile of the reference children but *percentage of the median*, i.e. of the 50th percentile, the international standard. Below 80% of the median, a child is 'underweight'; this weight is near the 3rd percentile line and near a Z-score of –2. A child under 60% of the median is seriously underweight and has marasmus.

In developing countries, a simplified version of the weight-for-age graph; a 'Road to Health' card, can be kept by the mother for her child and brought back to the clinic on each visit (see Chapter 19).

There is little difference in weight for age of modern children of the privileged class between different countries and ethnic groups. The reference data can thus be used internationally. If a child is somewhat heavy for age (say 80th percentile) or light (say 20th percentile), this does not mean overnutrition or undernutrition. The child may be larger (taller) or smaller (shorter) than average. The weight has to be judged against the height (or length).

Length-for-age (for infants) and *stature-for-age* references will show if a child's longitudinal growth is taller or average, or shorter than the reference population. In developing countries, *stunting* is an important measure of poor nutrition and/or other adverse environment. It is usually defined as 2 standard deviations below the international median reference height for age, i.e. a Z-score of –2.

Excessive thinness or *wasting* is recognized anthropometrically from *weight-for-height* of 2 standard

deviations below the median for age, i.e. a Z-score of –2.

Prevalence of wasting, stunting, and underweight (as defined by WHO) in preschool children in different countries are given in Table 31.2. This shows the value of simple anthropometry in monitoring the nutrition situation in the world's young children.

In adolescence, the growth references have to be used cautiously because there is a growth spurt around the time of puberty followed by slowing of growth, and some girls mature early and some late, with about 5 years between their peaks (see Fig. 34.1). There is the same sort of range in peak height velocity in boys.

Table 31.2 Wasting, stunting and underweight in 0–5-year-old children, 2000–2006 (%)

	Wasting	Stunting	Underweight
Afghanistan	7	54	39
Bangladesh	13	43	48
Brazil	2	11	6
China	–	11	7
Cuba	2	5	4
Ethiopia	11	47	38
Guatemala	2	49	23
India	20	48	43
Kenya	6	30	20
South Africa	3	25	12
USA	0	1	2

Wasting, <2 SD below international reference median (WHO/NCHS) weight for height.

Stunting, <2 SD below reference median height-for-age.

Underweight, <2 SD below reference median weight-for-age.

Source: UNICEF (2008) *The state of the world's children*. New York, United Nations Children's Fund.

31.4 Estimating body composition: simple methods

The methods available that anyone can do measure body fat or muscle at one or more sites and from these total body fat and/or muscle can be estimated approximately. The following methods are used by clinical nutritionists.

31.4.1 Skinfold thickness

With special precision callipers (Fig. 31.6), a pinch of subcutaneous fat is gently taken up and the width measured. Caught between the jaws of the callipers is a double layer of fat and skin. This fold is measured in mm. Skinfolds could be measured at many sites, but the best established are:

- *triceps skinfold*: over the triceps muscle midway down the back of the upper arm;
- *biceps skinfold*: measured as a vertical fold midway down the front of the upper arm, over the biceps muscle;

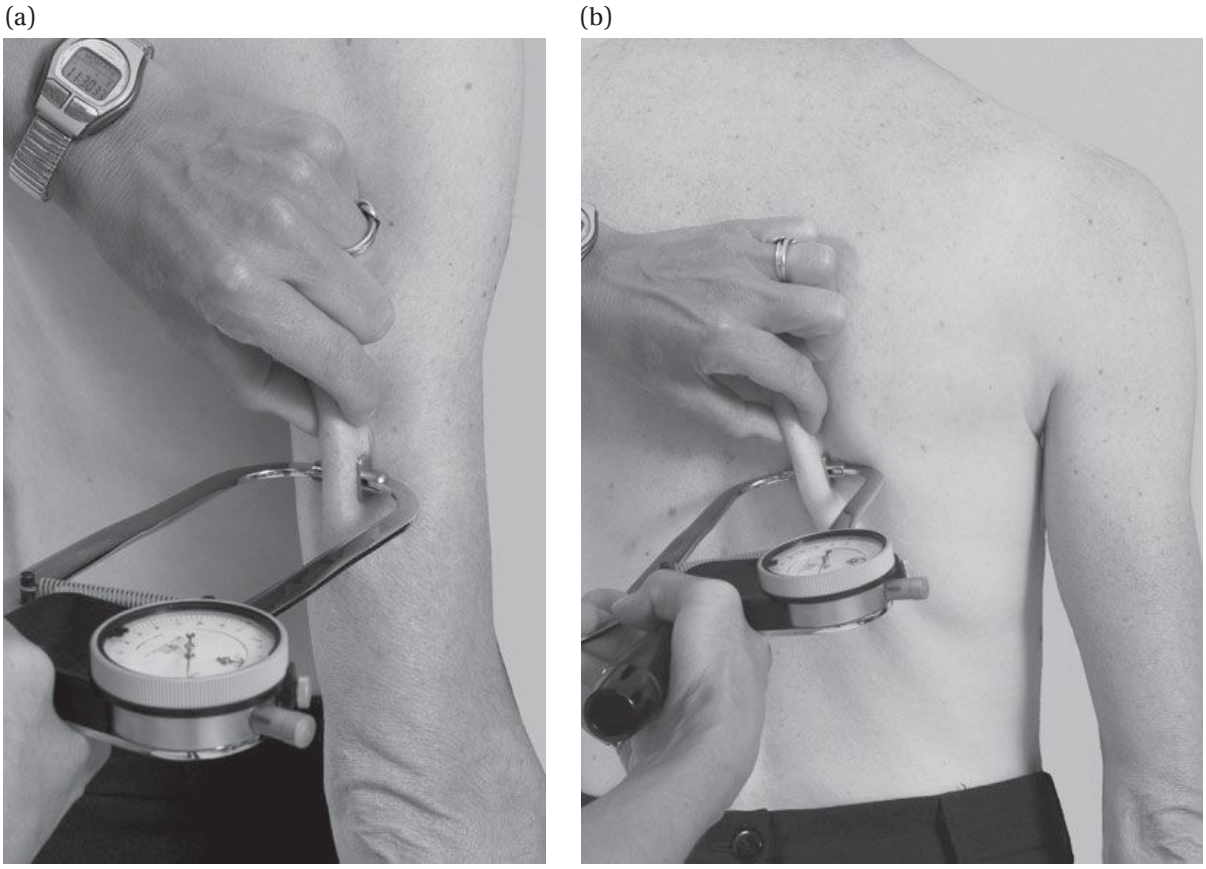


Fig. 31.6 Measuring skinfold thickness—triceps (left) and subscapular (right) sites with Holtain callipers.
Source: Truswell, A.S. (2003) *ABC of nutrition*. London, BMJ Books.

- *subscapular skinfold*: a vertical fold taken just below and lateral to the inferior angle of the scapular, with shoulder and arm relaxed;
- *suprailiac skinfold*: in the mid-axillary line immediately above the iliac crest, grasped obliquely.

The skinfold is first picked up between finger and thumb, clean away from the underlying muscle before closing the callipers on the fold.

Reference tables are published for triceps and subscapular skinfolds (see Gibson, 2005).

The above four skinfold sites are most commonly used partly because they are easily accessible and reference tables are available, partly because they were chosen by Durnin and Womensley (1974), who measured total body fat from body density by underwater weighing in 480 white men and women ranging in age and fatness. They then derived different equations to predict total body fat from the sum of four skinfolds in men and women at different ages. These were all arm or trunk skinfolds but the British Olympic Association recommends that addition of the anterior thigh skinfold improves the estimate of percentage body fat. In very fat people, skinfolds at some trunk sites are not practicable as it is impossible to take up and measure a fold or the fold is too large for the callipers.

31.4.2 Limb circumferences

By simply measuring the circumference of an arm or leg and skinfolds at the same level, it is possible to calculate the circumference or the cross-sectional area of the limb's muscle. An assumption for the area of bone inside the muscle section improves the muscle area for estimating total muscle in the body.

Mid-upper arm is the usual site. The other two sites are mid-thigh and mid-calf. The arm is not always accessible. Heymsfield and Baumgartner (2006) found that the sum of limb muscle area for arm, thigh, and calf predicted quite well the skeletal muscle measured by whole-body magnetic resonance imaging:

$$\text{Limb muscle circumference} = C - \pi \times SF$$

$$\text{Limb muscle area} = \frac{[C - \pi \times SF]^2}{4\pi}$$

where C is a limb circumference (upper arm, thigh, or calf) and SF is the skinfold taken at the same level.

The uncorrected mid-upper arm circumference reflects muscle and fat in the limb and is used for screening preschool children in the field where no weighing scales are available. A tape is used, marked at 12.5 and 13.5 cm. From 12 to 60 months of age, the arm circumference of healthy children, boys and girls, stays the same. A circumference over 13.5 cm is normal, between 12.5 and 13.5 cm suggests mild undernutrition, and under 12.5 cm indicates definite malnutrition.

31.5 High-technology methods for body composition

Total body water by isotope dilution This method uses a tracer dose of water, labelled with deuterium ^{2}H, tritium ^{3}H, or ^{18}O. After time for equilibration, the concentration of the label is measured in serum or urine.

Bone mineral density by DEXA Dual energy X-ray absorptiometry (DEXA) scanning is usually of the lumbar spine and hip with X-rays at two energies. There is relatively more attenuation of the lower voltage rays by more dense tissue (i.e. bone mineral). Results are related to average bone mineral density for age. All major hospitals have this equipment. DEXA can also be used to quantify fat versus other tissue in regions of the body.

Underwater weighing using Archimedes principle The subject is weighed in air (i.e. in the usual way) and again when completely submerged in water in a large tank. Body volume is given by the apparent loss of body weight in water (i.e. the difference between weight in air and in water), which corresponds to the displaced water. Weight (in air)/volume gives body density. Knowing that the density of fat is 0.90, the percentage body fat can be calculated. Adjustments have to be made for residual air in the lungs.

Bioelectrical impedance Relatively inexpensive equipment is used to measure the impedance of an electrical current (typically 800 microamps) passed between an electrode on the right foot and another on the right wrist, where there is a voltage sensor. The fat-free mass is a good conductor of electricity, while fat is not. The voltage drop between foot and wrist is greater in subjects with more fat. A computer calculates fat-free mass and fat when data on weight, height, age, gender, and level of physical activity are entered. A number of factors, including hydration state, meals, and length of limbs affect the reliability of the results.

Total body potassium, by counting ^{40}K This depends on the fact that 0.012% of potassium everywhere (and in our bodies) is the γ-emitting isotope ^{40}K. The amount of this can be counted in a whole-body counter from which background radiation has been screened with thick steel or lead. Potassium occurs in the body almost exclusively inside the cells so that from total body potassium the body's cell mass can be calculated.

Total body nitrogen by in vitro neutron activation analysis This is an ingenious method for determining total body nitrogen and hence total protein in the body ($N \times 6.25$). The patient is

'bombarded' while lying on a special table with a low neutron flux from a neutron source (such as californium-252). This converts a proportion of the nitrogen, ^{14}N, to a very short-lived state of ^{15}N, which emits a γ-ray at 10.83 MeV. This is counted with a gamma counter as the neutron source is moved over the subject's body on a motorized bed. The method is very expensive; only a few institutions can use it, and it gives a significant radiation dose.

Ultrasound This is harmless, not expensive, and has some uses, e.g. in quantifying the size of the thyroid gland.

Magnetic resonance imaging and computed tomography These have also been used for some research studies on body composition. Both use very expensive, bulky equipment.

31.6 Biochemical methods

A person may be ill from a qualitatively inadequate diet and yet their body measurements can be within normal limits. The right biochemical test would show the deficiency. Anthropometry mostly reflects undernutrition or overnutrition, too little or too much food energy. Biochemical tests are needed to demonstrate micronutrient status. On the other hand, there is no biochemical test on a body fluid that gives a measure of carbohydrate or total fat intake. Biochemical methods are an essential part of nutritional assessment. There are many more biochemical tests than anthropometric measurements. Each test costs money, for collecting the blood or urine, for the equipment, chemicals, and the skilled laboratory worker's time, and for reporting and interpreting the test. So tests have to be selected, based on the situation and the subject, so that they are likely to yield useful results.

31.6.1 Different situations for laboratory tests

Biochemical methods for nutritional assessment are used for several different purposes:

- to recognize acute malnutrition for which the clinical signs are non-specific, e.g. potassium deficiency;
- to confirm the clinical diagnosis of a deficiency disease, e.g. xerophthalmia, scurvy, beri-beri, rickets, Wernicke's encephalopathy, kwashiorkor;
- for monitoring nutritional management in intensive care, with parenteral nutrition and/or tube feeding;
- in haematological diagnosis, e.g. iron, folate, and vitamin B_{12} estimations;
- in community nutrition surveys, to detect subclinical micronutrient deficiency, e.g. iodine deficiency, iron deficiency;
- for checking validity of food intake measurement: 24-hour urinary nitrogen indicates protein intake in people with stable dietary pattern; carotenoids reflect fruit and vegetable intake;
- for reliability and convenience: biomarkers, for some nutrients, are more reliable and convenient than food intake estimations (e.g. 24-hour urinary sodium is better than trying to work out dietary salt);
- to demonstrate objectively the response to a nutrition education programme, e.g. reduction of plasma cholesterol or of urinary sodium;
- for biochemical confirmation of alcoholism;
- to diagnose nutritional supplement overdosing (e.g. with vitamin A, pyridoxine).

31.6.2 Stages of nutrient deficiency

When absorbed intake of a nutrient is less than the requirement, i.e. less than losses from metabolism

and excretion, the depletion goes through four stages:

1 reduced excretion of the nutrient, e.g. reduced urinary excretion, but body pool maintained;
2 body pool smaller but no disturbance of function;
3 biochemical signs of impaired function: reduced activity of an enzyme or cell depletion;
4 morphological changes and clinical signs of deficiency disease.

Obviously, many more people are found at stage 1 than are found with obvious classic deficiency disease. In other words, biochemical tests usually show *subclinical* nutritional deficiency; the subject may or may not be ill and an illness may or may not be due to the nutritional depletion.

31.6.3 Which biochemical tests

The principal biochemical tests for nutritional status are shown in Table 31.3. Most clinical biochemical laboratories are set up for only some of the methods in the table as a routine, but others could be set up in special circumstances, or alternatively a laboratory specializing in nutrition research could be asked. It is usually easy to find methods reflecting recent intake. Good methods for nutritional dysfunction or tissue depletion are not available for all nutrients. To estimate stores of a nutrient, the only method generally available is serum ferritin for iron stores.

31.6.4 Some problems with biochemical tests

Instability of the nutrient in vitro

Vitamin C is the outstanding example. Plasma should be acidified with metaphosphoric or trichloracetic acid and analysed the same day or kept for a few days at –80°C.

Chemical specificity of the method

Older methods were often not specific for the nutrient, e.g. DCIP or DNPH methods for vitamin C.

Other influences

Non-nutritional influences can significantly raise or lower plasma concentrations of most, if not all, nutrients. Plasma albumin goes down (without protein deficiency) with inflammatory disease or trauma. The liver switches to synthesis of 'acute-phase' plasma proteins and albumin may move to the extravascular space.

The relation between intake and plasma concentration

This shows important differences among nutrients (Fig. 31.7). Plasma selenium increases in the expected linear fashion but serum calcium is homeostatically maintained the same over the range of usual calcium intakes. Plasma retinol also stays the same over most vitamin A intakes but it does go down at very low intakes, so this test is useful in low-income communities. Vitamin C concentration plateaus at intakes of about 150 mg/day (see Chapter 14), so it is no higher in people who take megadoses than in those who eat a moderate amount of fruit.

Blood and urine tests cannot show calcium status

Calcium is not in Table 31.3. To estimate body calcium requires measurements of bone mineral.

Biochemical tests for zinc are also unreliable. Serum zinc is affected by age, gender, acute inflammation, time of day, fasting status, oral contraceptives, storage, haemolysis, zinc contamination of collecting tube, or anticoagulant. It therefore needs careful technique and interpretation.

31.6.5 Methods used

A previous edition of this book contained a table that gave the names of the methods then likely to be used for the different nutrients. However, such methods are constantly evolving, and the three main questions the nutritionist can ask the laboratory are: Is this specific for the nutrient? Is it a standard method? Is the laboratory experienced with using it?

Urine tests

These should if possible be made on 24-hour urine collections. This may be inconvenient

Table 31.3 Biochemical methods for diagnosing nutritional deficiencies

Nutrient	Indicating reduced intake	Indicating impaired function (IF) or cell depletion (CD)	Supplementary method
Protein	Urinary nitrogen	Plasma albumin (IF)	Fasting plasma amino acid pattern
Vitamin A	Plasma β-carotene	Plasma retinol	Relative dose–response
Thiamin	Urinary thiamin	Red cell transketolase and TTP effect (IF)	
Riboflavin	Urinary riboflavin	Red cell glutathione reductase and FAD effect (IF)	
Niacin	Urinary *N*′-methyl nicotinamide or 2-pyridone, or both	Red cell NAD/NADP ratio	Fasting plasma tryptophan
Vitamin B_6	Urinary 4-pyridoxic acid	Plasma pyridoxal 5′-phosphate	Urinary xanthurenic acid after tryptophan load
Folate	Plasma folate	Red cell folate (CD)	
Vitamin B_{12}	Plasma holotranscobalamin II	Plasma vitamin B_{12} Plasma methylmalonate	Schilling test
Vitamin C	Plasma ascorbate	Leukocyte ascorbate (CD)	Urinary ascorbate
Vitamin D	Plasma 25-hydroxy-vitamin D	Raised plasma alkaline phosphatase (bone isoenzyme) (IF)	Plasma 1,25-dihydroxy-vitamin D
Vitamin E	Ratio of plasma tocopherol to cholesterol + triglyceride	Red cell haemolysis with H_2O_2 *in vitro* (IF)	
Vitamin K	Plasma phylloquinone	Plasma prothrombin (IF)	Plasma des-γ-carboxy-prothrombin
Sodium	Urinary sodium	Plasma sodium	
Potassium	Urinary potassium	Plasma potassium	Total body potassium by counting ^{40}K
Iron	Plasma iron and transferrin	Plasma ferritin (CD)	Free erythrocyte protoporphyrin
Magnesium	Plasma magnesium	Red cell magnesium (CD)	
Iodine	Urinary (stable) iodine	Plasma thryroxine (IF)	Plasma TSH
Zinc	Plasma zinc	Red cell zinc	
Selenium	Plasma selenium	Red cell glutathione peroxidase	Toenail selenium
Fluoride	Urinary fluoride	Plasma ionic fluoride	(Bone fluoride)

^{40}K, natural radioactive potassium; FAD, flavin adenine dinucleotide; NAD, nicotinamide adenine dinucleotide; NADP, NAD phosphate; TPP, thiamin pyrophosphate; TSH, thyroid-stimulating hormone.

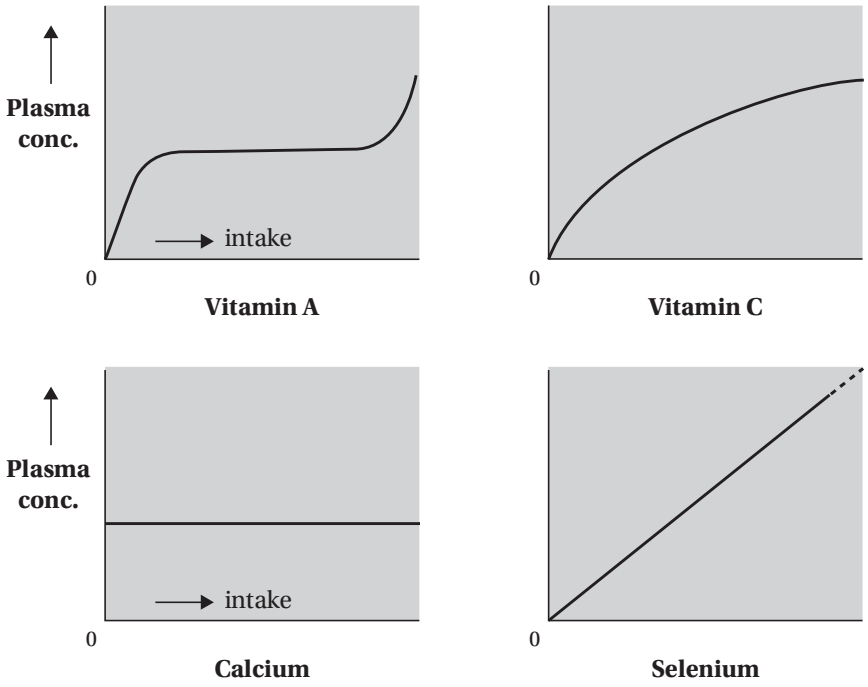


Fig. 31.7 Difference in relation of serum concentrations to intake of four nutrients: vitamin A, vitamin C, calcium, and selenium.

for the subjects; they have to carry a 2-litre bottle with them during the day. As second best, an early morning sample can be collected at home and the nutrient concentration related to the creatinine concentration, because creatinine excretion (in health) is assumed to be more or less constant from day to day. However, within subjects the coefficient of variation of 24-hour creatinine is 10% or more and it is larger between subjects because it depends on muscle mass and is increased after eating meat.

Hair is unreliable Researchers have sent hair samples from the same subjects for trace element analysis to several commercial laboratories and have received widely differing results. It is difficult to remove the many environmental contaminants that adhere to hair and the distribution of inorganics may not be uniform. Hair analysis may have some value in forensic work or detecting toxic exposure to heavy metals, but for nutritional work urine analysis is more reliable.

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