Nutritional Status Evaluation: Body Composition and Energy Balance

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Nutritional status is the anatomical, metabolic, and functional status of the body in relation to the availability of energy, nutrients, and other substances of nutritional interest; it strictly influences growth, body composition, health status, and the quality of life of the individual. In the elderly, body composition and skeletal muscle mass deficiency influence the risk of falls and the ability to lead an autonomous life. Moreover, diseases that modify the energy balance or nutrient intake can change body composition and nutritional status, and in so doing may worsen one's health status.

In obese people, a nutritional intervention will likely aim at reducing the percentage of body fat with maintenance of non-fat mass. In cases of *sarcopenic obesity*, understanding the body composition is of central importance as a lowering of the body's non-fat mass may mask increases in body fat (especially visceral fat) that are not brought into evidence with indicators such as body weight and body mass index, which may fall in the normal or overweight range. In such patients, the decrease in non-fat mass tends to affect the skeletal muscle and may result in functional deficits and a decrease in muscle strength. This condition is most commonly observed in the elderly or in patients with chronic inflammatory diseases such as chronic HIV infections. In these cases, an increased visceral fat may be associated with a lipoatrophy of the limbs [1, 2]. Recently, an abnormal distribution of adipose tissue has been also documented in anorexic patients after refeeding [3]. In all these cases, therapeutic intervention may be particularly complex and should aim at a modification of body composition through nutritional intervention, physical activity prescription, and also pharmacological therapy in selected cases.

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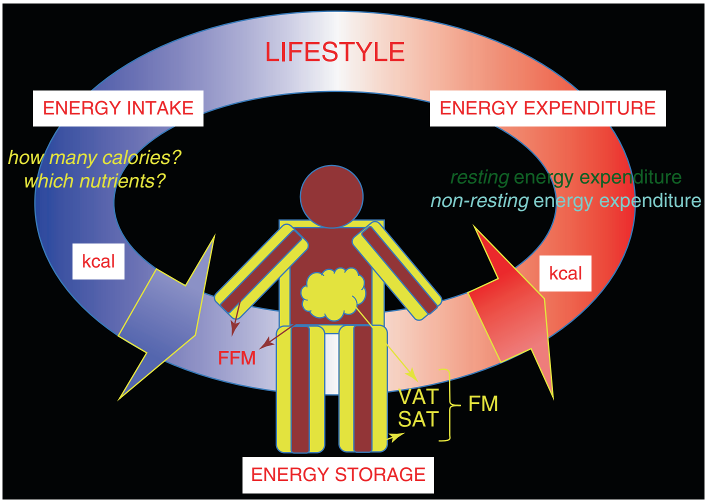

Fig. 16.1 Energy balance and body composition. *FFM* fat-free mass, *FM* fat mass, *VAT* visceral adipose tissue, *SAT* subcutaneous adipose tissue

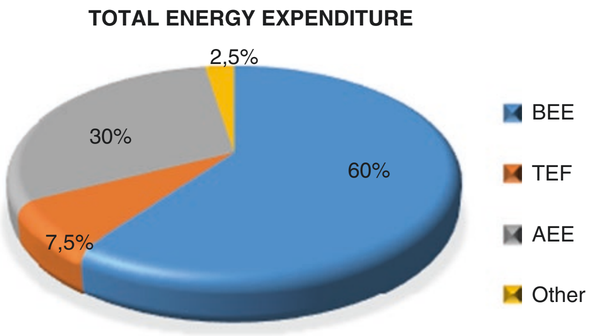

Fig. 16.2 TEE (total energy expenditure) in an average healthy sedentary man. *BEE* basal energy expenditure (or *BMR* basal metabolic rate), *TEF* thermic effect of food, *AEE* activity energy expenditure (PPA and NEAT)

Our nutritional status, in fact, is a manifestation of the effect of our lifestyle on our body. Our lifestyle, which includes eating habits, physical activity, and sleep, is in turn, strongly influenced by psychological, social, and economic factors. In particular, nutritional status is related to the quality and quantity of the components of our diet or *energy intake* (macronutrients, micronutrients, substances of nutritional interest) and to the quantity and quality of our *energy expenditure* (Fig. 16.1). If energy balance is positive, some of the excess can be stored as fat, whereas if energy balance is negative, not just the body's fat mass (FM) but also the body's fat-free mass (FFM) that forms visceral organs and skeletal muscle can be reduced. With time, the excess or deficit of energy can modify body composition and come to be seen in biochemical testing.

Total energy expenditure (TEE) is divided into *resting* and *non-resting* energy expenditure. Resting energy expenditure includes the *basal metabolic rate* (BMR), also termed basal energy expenditure (BEE), and the *thermic effect of food* (TEF) (Figs. 16.1 and 16.2). Non-resting energy expenditure, or *activity energy*

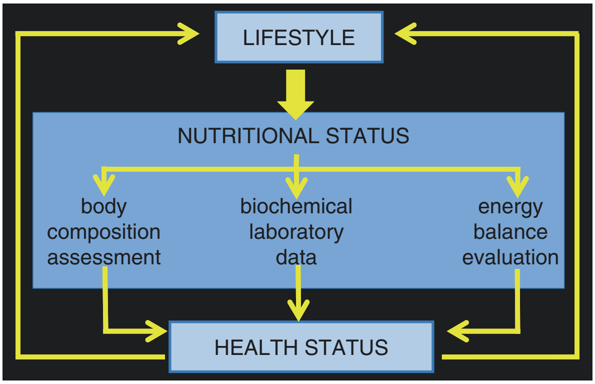

Fig. 16.3 Nutritional status, lifestyle, and health status relationship. *Body composition*, *energy balance,* and *biochemical laboratory data* represent the main biological variables through which it is possible to investigate nutritional status

expenditure (AEE), embraces *programmed physical activity* (PPA) and *non-exercise activity thermogenesis* or NEAT (i.e., the sum of *spontaneous physical activity* plus *occupational/leisure* activities) [4, 5]. BMR is the rate of energy expenditure by human body at rest while in the postabsorptive state and is a measure of energy spent for the ongoing function of the vital organs. As such, it is operative continuously and through temporal integration plays an important part in determining one's overall energy expenditure. Equally, the BMR determines the minimum energy requirement for maintaining ongoing vital organ function. It is therefore important to know one's BMR value before planning a diet therapy.

Since energy requirements depend on the absolute and relative size of the body components (mainly skeletal muscles, organs, and skin and to a much lesser extent adipose tissue), body composition has been recognized as a more suitable basis than body weight for estimating the basal metabolic rate.

Body composition, *energy balance,* and *biochemical laboratory data* represent the main biological variables through which it is possible to investigate the nutritional status (Fig. 16.3). In undernourished people, a nutritional status evaluation helps to quantify caloric and nutrient deficits that must be addressed in an appropriate diet therapy plan. The assessment of nutritional status similarly allows us to make the diagnosis and define the severity and clinical risks of associated overweight and obesity conditions, as well as to detect the existence of a reduced muscle mass (a condition named sarcopenia), investigate the causes of weight gain, prescribe the most appropriate treatment, and assess the results of a dietetic, medical, or surgical intervention over time. Nutritional status assessment in this context goes beyond appreciating the extent of energy imbalance to quantify the excess adipose tissue or fatness (*body fat percentage*) and to specify its regional distribution. The amount of excess white adipose tissue and its central or peripheral location (respectively, *visceral adipose tissue* or *subcutaneous adipose tissue*) are important issues related to cardio-metabolic risk in obese-overweight people. While generalized and abdominal obesities are both associated with greater risk of morbidity and mortality, abdominal obesity in particular has been associated with an increased risk of myocardial infarction, stroke, and premature death. Increased visceral adipose tissue is associated with a range of metabolic abnormalities related to the secretion of proinflammatory cytokines produced by adipose tissue. These adipokines, and in particular IL-6 and TNF, promote a chronic low-grade inflammatory state linked to the obesity-associated metabolic diseases [6]. These alterations cause decreased glucose tolerance, reduced insulin sensitivity, and adverse lipid profiles, which are risk factors for the development of metabolic syndrome, type 2 diabetes, cardiovascular diseases (CVD), stroke, and cancer.

The nutritional status should be always monitored in a patient with an increased cardio-metabolic risk, regardless of the body mass index (BMI) value that may be normal or increased. The same principle should also be applied in subjects with increased BMI although apparently healthy.

16.1 Body Composition

16.1.1 Body Composition Components: FFM and FM

FFM and FM are the two generic components used in models of body composition. FFM can be divided into a more metabolically active part, mainly formed by visceral organs, skeletal muscle, and skin and a part formed by the supporting tissues, bone, and body fluids whose metabolic demands are very low. Further stratification can be made as the visceral organs consume much more energy per unit of weight than skeletal muscle. The skeletal muscle component of FFM is considered to have a much lower resting metabolic rate than the visceral organs; nevertheless, it is more abundant and its mass can be modified by our lifestyle, mainly by physical activity. The FM is not metabolically inert but it certainly represents a minor component of the BMR. These considerations have led to the development of a number of models of body composition that differ in their complexity depending on how finely the subcomponents are divided and how they account for the influence of the varying proportions of the different components of FFM and/or tissue-specific metabolic rates. Validation and use of these models in the diagnosis and prognosis of obesity and its associated comorbidities require knowledge of the percentage of FM, the relative proportion of FFM and FM, and the body composition of specific segments of the body like the trunk and limbs. Various in vivo field and laboratory methodologies have been developed to estimate or measure the compartments that represent the different constituents of the human body [7, 8]. Both these aspects of nutritional status evaluation are discussed below.

16.1.2 Body Composition Models

The information obtained from complete dissections and analyses of the composition of human cadavers has led to the establishment of reference values; the development of the concept of the *Reference Man* [9] provides the bases for developing various models of human body composition.

In the *basic two-compartment model*, the body is divided into the *fat mass* that comprises body fat and the *fat-free mass*, including all the remaining tissues. Dividing the FFM into a *lean tissue mass* (LTM) and *bone mineral content* (BMC), in combination with the FM, yields a *three-compartment model* that is particularly interesting as it can be assessed by dual X-ray absorptiometry (DXA).

The elemental, the molecular, the cellular, the functional (or tissue model), and the whole-body models of body composition represent different *multi-compartment models* of progressively higher complexity. In the *molecular model*, the FFM is subdivided in its molecular constituents, water, proteins, minerals, and glycogen, while fat is the molecular component of the FM. The body cell mass in the *cellular model* is considered the metabolically active district responsible for energy consumption at rest, in the postabsorptive state, or BMR; the other components of the cellular model are extracellular fluids, extracellular solids, and fat.

The clinician is generally interested in measuring body composition in terms of FM and FFM as the bi-compartmental model of body composition is particularly well established for the diagnosis, treatment, and prognosis of diseases related to alterations of nutritional status.

16.1.3 Field Methods and Laboratory Measurements of FFM and FM

A variety of reference *laboratory methods* have entered use for body composition analysis, including densitometry, hydrometry, in vivo neutron activation analysis (IVNAA) [10], and DXA. Estimation equations have been compiled using values obtained by different *field methods* as anthropometry (weight, height, skinfold thickness, and circumference measurement) or conductance-based techniques like body impedance assessment (BIA); these estimate equations have been validated by comparison with data obtained by *gold standard* laboratory methods.

The different densities of the FM and FFM can be used to reexpress their mass fractions in the bi-compartmental model in terms of their relative volumes. The proportions of FM and FFM obtained from cadaver analyses have led to the creation of algorithms that indirectly estimates the percentage of body FM from body density (see *Siri equation* in Table 16.1). Body density in turn can be obtained by dividing body mass by body volume. While body mass is related to body weight, body volume cannot be so easily assessed. Water displacement is one possibility, but in practice, densitometry tends to rely on either underwater weighing or air-displacement plethysmography to evaluate body volume. *Underwater weighing* is a laboratory method that compares the weight of the subject in air to that obtained while they are entirely submerged in water. In *air-displacement plethysmography* on the other hand, the subject is placed in a closed air-filled chamber that is then subjected to a pressure change in order to calculate the volume of the body. Densitometry is probably the best laboratory approach to obtaining data for the two-component model for adults.

Table 16.1 Skinfold body density (BD) predictive equations

Skinfold equations		
Durnin and Womersley [11]	$n=481$ (209 M, 272 F), 16–72 years	*M*: $BD = 1.1765 - 0.0744 \times (\text{Log SKN})$
		SKN = biceps + triceps + subscapular + suprailiac
		F: $BD = 1.1567 - 0.0717 \times (\text{Log SKN})$
		SKN = biceps + triceps + subscapular + suprailiac
Jackson and Pollock 7 skinfolds [12, 13]	$n=308$ M, 18–61 years, $n=249$ F, 18–55 years	*M*: $BD = 1.112 - (0.00043499 \times SKN) + (0.00000055 \times \sqrt{SKN}) - (0.00028826 \times age)$
		SKN = chest + axilla + triceps + subscapular + abdominal + suprailiac + thigh
		F: $BD = 1.097 - (0.00046971 \times SKN) + (0.00000056 \times \sqrt{SKN}) - (0.00012828 \times age)$
		SKN = chest + axilla + triceps + subscapular + abdominal + suprailiac + thigh
Jackson and Pollock 3 skinfolds [12, 13]	$n=308$ M, 18–61 years, $n=249$ F, 18–55 years	*M*: $BD = 1.10938 - (0.0008267 \times SKN) + (0.0000016 \times \sqrt{SKN}) - (0.0002574 \times age)$
		SKN = chest + abdominal + thigh
		F: $BD = 1.0994921 - (0.0009929 \times SKN) + (0.0000023 \times \sqrt{SKN} - (0.0001392 \times age)$
		SKN = triceps + suprailiac + thigh
Forsyth and Sinning [14]	M, 19–22 years, male athletes	*M*: $BD = 1.103 - 0.00168 \times SKN^S - 0.00127 \times SKN^A$
		SKN^S = subscapular
		SKN^A = abdominal

SKN is the sum of the site-specific skinfold thickness measured in *mm*. The sites are specified for each equation. Age is expressed in years. BD represents the body density from which fat mass percentage (FM%) can be calculated applying the *Siri equation* [15]: $FM\% = (4.95)/BD - 4.50) \times 100$
M male, *F* female

Body density can be also estimated from the measurement of skinfold thickness with the use of population-specific equations (Table 16.1).

Dilution methods are based on the principle that the volume of a body compartment can be calculated as the ratio of a tracer dose dispensed orally or intravenously to its final concentration in that compartment after a certain period of time. Since on average in healthy adults the total body water (TBW) constitutes 73 % of the FFM, the latter can be easily calculated if TBW is measured. The measurement of TBW, or *hydrometry*, is a dilution method based on deuterium [16], a stable nonradioactive isotopic tracer of hydrogen. The value of FM can then be obtained by the difference of body mass and FFM. Similarly, extracellular water can be calculated with basic dilution techniques and a nonradioactive Br tracer administered orally.

Anthropometry-based methods are good field methods. Weight, BMI, skinfold thickness, and body circumference measurements are simple, quick, inexpensive, and portable techniques.

Body weight is more a dimension of body size than a measure of body composition. Nevertheless, weight as a function of height has an essential role in the evaluation of nutritional status in children and adults. Weight is the sum in kg of the FM and FFM. In the diagnosis of overweight and obesity, it is important to know the percentage of body FM rather than the absolute amount of fat in kg, and specialists encourage a definition of obesity based on percentage of body fat or fatness. Changes in body weight over time are more often due to changes in the amount of FM, as FFM is the most stable of the two components. Daily weight fluctuations are usually caused by variations in water content, such as in edema.

Body mass index or *BMI* is calculated by dividing weight (in kilograms) by height (in meters) squared. BMI is widely used as an index of the degree of obesity or undernutrition. It is better seen as a measure of fat content (FM in kg) than of fatness (% fat) however. BMI is a compositional index that has relevant clinical and prognostic value [17]. BMI values correlate with mortality rate [18] and have developed into an indicator of risk of diseases related to adiposity like premature death, cardiovascular diseases, high blood pressure, osteoarthritis, some cancers, and diabetes. It should be remembered however that although BMI has traditionally been used as a measure of body size and composition, abdominal fat mass can vary significantly within a narrow range of total body fat and BMI.

The most widely accepted classifications for overweight and obese people are those from the World Health Organization (WHO), based on BMI (Table 16.2).

Skinfold thickness and body circumferences. Skinfold thickness is a measure of subcutaneous adipose tissue. Skinfolds themselves may be compared with reference data or used in estimation equations to derive whole-body fatness (Table 16.1).

Skinfold measurements are taken at specific sites of the body. The subscapular, triceps, biceps, and suprailiac skinfold measurements are among the most commonly used sites. After pinching the skin and subcutaneous fat tissue away from the underlying muscle, the tester uses special skinfold calipers to measure the skinfold thickness in millimeters.

Skinfold thickness has the characteristics of a good field method. It is simple and quick, and the calipers are inexpensive and portable. Since estimation equations are

Table 16.2 Classification of overweight and obesity by BMI [19, 20]

Classification of overweight and obesity and associated disease risk			
Class	BMI	WC	Disease risk
Underweight	<18.5	M: <102 cm	–
		F: <88 cm	
		M: >102 cm	–
		F: >88 cm	
Normal[a]	18.5–24.9	M: <102 cm	–
		F: <88 cm	
		M: >102 cm	–
		F: >88 cm	
Overweight	25–29.9	M: <102 cm	Increased
		F: <88 cm	
		M: >102 cm	High
		F: >88 cm	
Obesity class I	30–34.9	M: <102 cm	High
		F: <88 cm	
		M: >102 cm	Very high
		F: >88 cm	
Obesity class II	35–39.9	M: <102 cm	Very high
		F: <88 cm	
		M: >102 cm	Very high
		F: >88 cm	
Obesity class III	>40	M: <102 cm	Extremely high
		F: <88 cm	
		M: >102 cm	Extremely high
		F: >88 cm	

The table shows the disease risk for type 2 diabetes, hypertension, and cardiovascular diseases associated with BMI and WC in Europid populations. Disease risk is relative to normal-weight and normal-waist circumference

In Caucasian population, a WC between 94.0 and 101.9 cm in men and 80.0–87.9 cm in women is associated with an *increased risk* of *metabolic complications* related to obesity; the risk is *substantially increased* if WC ≥ 102 cm in men and ≥ 80 cm in women or if WHR ≥ 0.90 in men and WHR ≥ 0.85 in women [19, 21]

[a]An increased *waist circumference* may denote an increased risk even in persons of *normal weight*

always specific to the population on which they have been validated (specificity concerns age, sex, ethnicity, technical origins), one must consider which of the available equations most closely fits the individual being measured. The seven- and three-skinfold equations of Jackson and Pollock [12, 13], the equations of Durnin and Womersley [11], and the equation of Forsyth and Sinning [14] are among the most used (Table 16.1).

Circumference measurement. Circumference measurement is another simple and useful anthropometric measure. The waist, hip, and mid-arm are the most frequent sites where circumferences are measured.

Waist circumference (*WC*) is measured at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest [19]. In other protocols, waist circumference has been measured at the top of the iliac crest [22], or at the level of the umbilicus or at the point of the minimal waist [23].

Hip circumference is taken around the widest portion of the buttocks.

Waist–hip ratio (*WHR*) is the waist circumference divided by the hip circumference.

Since waist circumference and waist–hip ratio reflect abdominal adiposity, high values for WC or WHR indicate abdominal fat accumulation [21]. They represent an additional measure of body fat distribution [24] and are a good predictor of cardio-metabolic risk [20] (Table 16.2). Unfortunately, universal cutoff points for WC, as for BMI, are not applicable as disease risk may differ between ethnic groups [19].

Mid-upper arm circumference (*MUAC*) is an index of nutritional status that, in conjunction with BMI, is particularly useful as a measure of chronic energy deficiency in children and hospital patients. It is highly correlated with BMI, but it does not need weight and height measuring apparatus. Used together with a triceps skinfold measurement, it is possible to obtain cross-sectional areas of adipose tissue and muscle at mid-upper arm level.

Body impedance assessment (*BIA*). The principle of body impedance assessment is that the resistance or impedance of the body to the flow of a weak alternating electric current (800 mA 50 kHz) is inversely proportional to the total body water (TBW). TBW composes 60 % of an adult body weight and about 73 % of the FFM of nonobese subjects [7, 25]. At low frequencies, current flows mainly through extracellular water, while at higher frequencies, it also penetrates cell membranes into intracellular water. Thus, *multifrequency instruments* can evaluate the extracellular and intracellular component of TBW.

The procedure of BIA involves the careful placement of the electrodes (signal and detecting electrodes) on the wrist and ankle. Simplified devices require merely that the subject stands barefooted on metal plates connected to the electrodes.

From appropriate well-validated estimation equations that include empirical impedance values and anthropometric variables such as height and weight, it is possible to calculate TBW and FFM, and hence FM. More involved BIA procedures give the possibility to estimate the composition of body segments, like upper and lower limbs.

BIA is a field body composition technique, it is quick and simple to use, and it has a good reproducibility. It is important to standardize the measurement conditions [26] and use appropriate well-validated estimation equations.

Dual X-ray absorptiometry (*DXA*). DXA is a method for *whole-body* and *regional body* composition analysis. DXA produces a bidimensional image of the entire human body. The principle behind DXA is in the differential degree of attenuation of X-ray beams of different energies by the soft tissues and bone. Subsequently, the procedure has been developed for non-osseous fat and lean tissue. This makes DXA well suited to describing the body in terms of *bone mineral content*, *lean tissue mass, and fat mass*, which can be used in the two-compartment model with the FFM divided into BMC and LTM. DXA technique may be used to describe the composition of the entire body or of its segments like the trunk and the limbs. Thus, DXA may be used to assess peripheral or central adipose tissue accumulation [3] or the amount of LTM in the limbs where it is mostly represented by skeletal muscle. An index obtained from the DXA has been used to diagnose sarcopenia [27].

DXA scan is relatively quick and is minimally invasive because the radiation doses are low.

The technique is quite precise, and it is increasingly used in medical practice to assess body composition even if debate continues about the status of DXA as a gold standard criterion for body composition [28].

While the DXA technique gives a bidimensional projection of body composition, whole-body techniques such as *computed tomography* (CT) and *magnetic resonance imaging* (MRI) can provide three-dimensional reconstruction. Both techniques can generate *cross-sectional volumes* and *images* of the body. CT and MRI identify FM and precisely describe its localization in terms of subcutaneous or visceral adipose tissue. CT exposes the subject to an X-ray dose greater than the DXA, and for this reason, it is more invasive and less frequently used for body composition studies than DXA. However, visceral fat distribution may be studied by a few scans at the lumbar level, limiting the dose of radiation to which the subject is exposed.

MRI does not require ionizing radiation and so is attractive for repeated measurements. It exploits the fact that elements such as hydrogen and carbon resonate in the presence of a magnetic field. After being excited by an oscillating magnetic field, the energy they dissipate is used to produce images that reflect the density of the source elements and properties of their physicochemical environment – for example, the presence of hydrogen in water or fat molecules. The disadvantages of MRI are the cost of the examination and the long scan time.

CT, MRI, and DXA are particularly helpful in regional measures of body composition allowing quantification of muscle mass and abdominal adipose tissue deposits [29–31]. Epicardial fat, a further risk factor for CVD, can be visualized by MRI and CT techniques [32].

16.2 Energy Balance

By energy balance, we mean the arithmetic difference between Ei (*energy intake*) and Eo (*energy output*).

$$\Delta E = \mathrm{Ei} - \mathrm{Eo}$$

In nutrition, energy is synonymous with food. Ei represents the algebraic sum of all the energy assimilated by a subject in 24 h, while Eo indicates the energy expended in the course of their physiological processes during his daily activities. We also have to consider ES (*energy storage*), which is the energy conserved by the organism during this process, especially when $\Delta E > 0$.

Under the first law of thermodynamics, energy cannot be created or destroyed, but it can be transformed from a form into another. This manifests in nutrition through biochemical processes as glycolysis, Krebs cycle, and mitochondrial respiratory chain. These reactions allow the transformation and the consequent availability of the energy conserved in food macronutrients. Each food item is composed of macromolecules that are metabolized by human cells in order to gain the energy necessary for cell metabolism and homeostasis.

In order to establish an accurate method to predict the single-person energy balance, it is fundamental to quantify the energy contained in different foods and their impact on human metabolism. Each food has a specific energy content, which is measured in *joules*. 1 J is the energy spent to move an object of a mass of 1 kg for 1 m. Although joule is the official unit of measure for energy in the SI (International System of Units), in nutrition, the kcal is the preferred unit of measure. *1 kcal*, approximately 4.2 kJ, is the energy needed to raise the temperature of 1 kg of water by 1 °C.

16.2.1 Energy Intake Evaluation

Accurate measurement of the daily Ei absorbed by an individual is essential to determining their energetic balance. There are two main approaches to this problem: retrospective methods and longitudinal ones.

Retrospective Methods

Retrospective methods consist principally of quantifying, in the most accurate manner, all the different foods eaten by patients in 24 h. *Food Frequency Questionnaires* (FFQs) are commonly used to absolve this task. Through a series of questions, FFQs gather an account of the typical daily diet of a patient. Modern FFQs may be integrated with pictures of different portions of various foods in order to increment the data accuracy.

A similar approach is the *24-h recall*, which consists of a series of questions aimed to obtain a reconstruction of the dietary intake of the day before the visit.

After data collection, the reported food intake has to be translated into a kcal intake in order to find the Ei. In this final step, the operator can take advantage of specific software packages that, together with accurate and validated food databases, give the total kcal content and the percentage content of the macronutrients and in some cases, even of the micronutrients of each food, to allow the cumulative quantities to be determined. It is fundamental for the reliability of the measurements made in these ways that validated databases be used (e.g., the INRAN food database in Italy), in which each food has been analyzed through standard chemical techniques. The retrospective methods are low cost and immediate, but do not lack disadvantages. The main problem is that the final data is only a reconstruction; the patient may have forgotten some information, could even voluntary fail to mention some food, and is unlikely to be accurate regarding portion size. For these and other reasons, the Ei obtained with retrospective methods is not very reliable. However, retrospective methods are useful for epidemiological studies.

Longitudinal Methods

Longitudinal methods give a more reliable value of an individual's Ei, so they are commonly used in clinics. A *food diary* is the most famous longitudinal method. This technique consists of making the individual write a diary in which he reports every meal with a detailed description of the quantity and the types of foods he consumes in a day. For more accurate data, the food diary should cover from 5 days to a whole week, including the weekend. In fact, it is not uncommon to find a radical diet modification during the weekend in some patients (especially in occidental culture). A food diary is also a good instrument to evaluate the psychological status associated with nutrition. In some diaries, the patient is asked to describe and report their emotional status during or after each meal. As in retrospective methods, the food data have to be transformed into micro and macronutrients and finally into kcals to find the Ei.

Although longitudinal techniques are more accurate than retrospective ones, they too have some disadvantages. First of all, patient compliance: by using a food diary, the physician or the nutritionist deeply enters into a person's life; this can be really problematic in patients affected by eating disorders with an important emotional component (e.g., anorexia, bulimia, binge eating syndrome). A food diary can be a very powerful technique for evaluating patient Ei, but it is important to recognize that a food diary's reliability is entirely based on patient compliance. A much-debated longitudinal technique is the *assisted* or *artificial nutrition*. It is the most invasive technique for Ei analysis. It is used for hospitalized patients as well as for the treatment of the severe form of anorexia. Subjects submitted to assisted nutrition are followed 24 h a day by high-qualified operators that control and report each food proposed to the patient. Considering that bromatological analysis of each food is performed a priori and each meal is accurately controlled, assisted nutrition is the most reliable technique to investigate Ei.

16.2.2 Energy Storage

An Ei major than Eo leads to an increment of ES. Overweight and obesity are strictly related to an altered mechanism of energy storage, which is mainly caused by an unhealthy and overabundant caloric intake, especially in the westernized world.

Extra energy is metabolized by human organism through biochemical processes and mainly transformed and stored into fat, glycogen, and proteins. While the energy conserved in glycogen and proteins has a physiological threshold, the quantity of exceeding energy that we can convert into fat is theoretically unlimited. This behavior seems to be a heritage from our past; throughout most of human evolution, the tendency to store calories as fat would likely have conferred an advantage by allowing energy storage during periods of abundance that enable survival during periods of prolonged caloric restriction, as well as providing greater energy stores to nourish the mother and fetus during and following pregnancy. According to

Lewis et al., the average US adult gains only 500–1,000 g of weight (approximately 2,000–2,500 kcal of stored energy) per year (more pronounced in older individuals, African Americans, Native Americans, and Hispanic Americans) despite ingestion of approximately 900,000–1,000,000 kcal/year [33]. Lewis' research points into focus an important data: energy storage is not only due to the simple difference between Ei and Eo, but this biochemical process is mastered and finely regulated by individual metabolic, neuroendocrine, and autonomic systems.

Rosenbaum and Leibel [34] investigated the mechanisms behind ES. In their study, they underline the importance of considering the bioenergetics physiologies and the hormonal impact on body weight. Several metabolic hormonal effects have been detected with leptin, involved in the hunger and satiety mechanism, playing a central role in energy storage. One must also consider the central roles of insulin, cortisol, thyroid hormones, and other important molecules implied in energy storage whose regulation is strictly correlated with the subject's nutritional behavior.

The many molecular mechanisms implicated in energy storage processes and their mutual interaction are one of the main causes of the potent *opposition* to the maintenance of reduced body weight. In addition to an increase of energy intake (increased appetite and less sense of satiety), this potent mechanism brings to a reduction of energy expenditure by an improvement of muscle efficiency especially at low levels of work, as during activities of daily life [4, 34].

16.2.3 Energy Expenditure Evaluation

Once we have investigated individual Ei, the estimation or even the measurement of the energy expenditure (Eo) is essential for the development of an efficient diet therapy.

Eo, also known as total energy expenditure (TEE), represents all the energy used by a single subject within 24 h, and it is measured in kcal/day.

In adult subjects, TEE can be represented by the following equation:

$$\text{TEE} = \text{BEE} + \text{TEF} + \text{AEE} + \text{other}$$

where BEE is the energy necessary for the conservation of vital functions (normally 60–70 % of TEE). Thermic effect of food (TEF) is the energy necessary for nutrient assimilation (5–10 % of TEE); this parameter becomes greater for a high protein intake. Finally, AEE represents the energy used during physical activity (20–35 % of TEE). AEE is comprehensive of PPA and NEAT [4]. PPA, or programmed physical activity, is the voluntary physical activity such as sport workouts, while NEAT embraces the energy expended for everything we do that is not sleeping, eating, or sports-like exercise. By the term *other* encompasses all remaining, minor metabolic components such as shiver reflex.

In children and adolescents, we also have to take into account the energy spent for physiological growth.

16.2.4 Estimate Equations

Since the start of the last century, several attempts at BMR prediction have been developed. In particular, predictive equations based on biological variables like weight, height, age, sex, or FFM have been assessed. Predictive equations are developed in healthy subjects by regression analysis of these body independent variables and BMR, the dependent variable measured by the *gold standard* method of direct calorimetry.

The most famous and oldest basal energy expenditure predictive equation was created by Harris and Benedict in 1919 [35] on the basis of weight, height, sex, and age. This equation was tested for the first time on 239 healthy individuals with different BMIs. The population was extended to 337 subjects in 1984, and after almost one century, this equation is still used (Table 16.3).

The Schofield equation with all its variants is another very popular and commonly used predictive equation [36]. The most common variants are the weight-based and the weight–height-based formulas. Its strength rises from the large number of samples used for the creation of the linear regression from which this equation was created: almost 11,000 healthy subjects from a variety of ethnic groups from all over the world. This equation can be considered reliable for normal-weight and overweight individuals [38] (Table 16.3).

Mifflin et al. [37] introduced FFM in their equation as an independent variable, but recent studies have demonstrated that the use of this parameter does not influence the reliability of the equation. Although this equation was not developed on a population as large as Schofield's (498 healthy patients vs. 11,000), the range of BMI used (from 17 to 42) makes it appropriate for obese subjects (Table 16.3).

There are many other predictive equations that essentially differ in the number of subjects used in validation, their ethnic group, age, and BMI (Table 16.3).

As calorimetry, the gold standard technique for measuring BMR, is not feasible in most dietetic settings, it is important to use the most accurate predictive equation to estimate BMR especially in overweight and obese persons.

The choice of the equation is fundamental; the subject analyzed using one of these formulas should have the same features as those used for developing the equation. For example, for obese and overweight German adults, there seem to be no accurate and reliable equations [38].

It is important to keep in mind that these formulas only give a predictive BMR value, which is often not reliable in pathophysiological conditions. In these cases, it is recommended to measure and not estimate the patients' BMR.

Predictive equations also do not take into account individual differences in physical activity level (PPA and NEAT); for example, according to Schofield equation, a professional athlete has the same BMR of a sedentary subject of the same weight and age. So, how can a nutritionist obtain a reliable TEE value from the estimated BMR in order to formulate an efficient diet therapy?

To overcome this gap, Food and Agriculture Organization (FAO) has produced a classification of *physical activity levels* (PALs) (Table 16.4) that, multiplied by BMR, allows to estimate TEE.

Table 16.3 BMR predictive equations

BMR predictive equations		
Harris and Benedict [35]	*n* = 239 (136 M, 103 F)	*M*: WT × 13.7516 + HTCM × 5.0033 − AGE × 6.755 + 66.473
		F: WT × 9.5634 + HTCM × 1.8496 – AGE × 4.6756 + 655.0955
Schofield weight and height [36]	*n* = 7,173, *n* = 4814 18 years, mean BMI of these 6 groups: 21–24; *n* = 3,388 Italians (47 %), *n* = 615 tropical residents, *n* = 322 Indian; 114 published studies, *n* = 7,173 subjects (11,000 values, includes group mean values); most European and North American subjects (Italian, closed-circuit calorimetry)	*M: Age 18–30 years*: 0.063 × WT − 0.042 × HTM + 2.953
		M: Age 30–60 years: 0.048 × WT − 0.011 × HTM + 3.67
		M: Age >60 years: 0.038 × WT + 4.068 × HTM − 3.491
		F: Age 18–30 years: 0.057 × WT + 1.148 × HTM + 0.411
		F: Age 30–60 years: 0.034 × WT + 0.006 × HTM + 3.53
		F: Age >60 years: 0.033 × WT + 1.917 × HTM + 0.074
Mifflin et al. [37]	*n* = 498 (251 M, 248 F), *n* = 264 normal weight (129 M, 135 F), *n* = 234 obese (122 M, 112 F), 19–78 years, BMI 17–42,	9.99 × WT + 6.25 × HTCM − 4.92 × AGE + 166 × SEX − 161
Mifflin et al. (FFM) [37]	*n* = 498 (251 M, 248 F), *n* = 264 normal weight (129 M, 135 F), *n* = 234 obese (122 M, 112 F), 19–78 years, BMI 17–42,	19.7 × FFM + 413

WT weight in kg, *HTCM* height in cm, *HTM* height in meters, *FFM* fat-free mass, *AGE* age in years; *SEX* (*M* = 1, *F* = 0), *BMR* basal metabolic rate, *M* male, *F* female

Table 16.4 Classification of lifestyles in relation to the intensity of habitual physical activity or PAL [39]

Category	PAL
Sedentary or light-activity lifestyle	1.40–1.69
Active or moderately active lifestyle	1.70–1.99
Vigorous or vigorously active lifestyle	2.00–2.40

A PAL of 2.40 or higher cannot be supported for a long period of time

$$\text{TEE} = \text{BMR} \times \text{PAL}$$

PALs are calculated on the basis of the energy expenditure of various activities, from sleeping (PAL = 1) to intense training (PAL equal to 15 or greater). In validation studies, indirect calorimetry was used as the *gold standard*, and the PAL of each activity was calculated in kcal/kg/h. In order to calculate the final daily PAL value (kcal/kg/day), it is necessary to sum the whole energy expenditure routine of each subject activity in 24 h [39]. PAL values that can be sustained for a long period of time by free-living adult populations range from about 1.40 to 2.40. According to the FAO, PAL values can be divided into three: sedentary, active, and vigorous. So, in the previous example, the final TEE difference between a sedentary subject and an athlete with the same age and weight becomes clear (sedentary TEE = BEE × 1.4 vs. athlete TEE = BEE × 2.4, 42 % TEE variation).

In conclusion, predictive equations corrected for physical activity levels may be suitable for estimating the healthy subject's TEE and for epidemiological studies, but not applicable to all patient categories.

16.2.5 Measurement Methods

Each of the components of TEE (BEE, TEF, AEE) is highly variable, and the total effect of these variances determines the variability in daily TEE among subjects. Measurements of TEE and their application must take into consideration the activity of the subject during the period of measurement in respect to routine daily life, and for this, free-living measurements may be preferred. From an alternative perspective, the measurement of energy expenditure can allow us to assess the relative thermic effects of various foods, nutrient compositions, pharmacological effects, and psychological components. Energy Expenditure can be measured using one of several approaches, including non-calorimetric techniques, direct calorimetry, and indirect calorimetry [40].

16.2.6 Non-calorimetric Approaches

As well as predictive equations, these methods measure or estimate TEE from variables related with energy expenditure. These techniques are often standardized against the calorimetric ones.

Doubly labeled water [41]: this method consists of monitoring carbon dioxide production and energy expenditure through the use of nonradioactive isotopes (D_2O^{18}).

This method is driven by the equilibrium between *body water*, O_2, and expired CO_2:

$$CO_2 + H_2O \leftrightarrow H_2CO_3$$

The body water is traced with isotope O^{18}, and over time, the concentration of marked O_2 in the organism will decrease while CO_2 is expired and body water is lost through urine and respiration.

Both O_2 and H_2 in body water are tagged with known amounts of tracers at the same time. The differences in the elimination rates of the O_2 and H_2 tracers are related to the elimination rate of CO_2. Double-labeled water is given to subjects orally after a sample of urine, saliva, or blood has been collected. A second sample is collected 7–21 days later, and tracer concentrations are determined through mass spectrometry. Body D_2 and O^{18} are measured over time and CO_2 and energy expenditure can be calculated with an error of 6–8 %. The percentage error decreases as the number of samples increases. This method is more indicated for epidemiological and research studies than ambulatory routine, mainly because of the high costs of reagents and the competence required for data analysis; however, it provides a good accuracy and it is applicable to free-living subjects.

Kinematic measurements. These methods are primarily used to estimate individual PPA and NEAT. Some techniques as *cine photography* are specific for confined spaces, while other ones, like *accelerometers* and *pedometers*, are useful for collecting data in *free-living subjects*. In particular, accelerometers detect body displacement electronically with varying degrees of sensitivity: uniaxial accelerometers in one axis and triaxial accelerometers in three axes. Kinematic techniques are generally not sufficient for accurately quantifying TEE, but an acceptable precision can be obtained through triaxial accelerometers [42]; in free-living subjects, data from these devices correlate well with the total daily energy expenditure, measured using doubly labeled water [43]. Triaxial accelerometer is a field method utilizable in ambulatory routine that is allowing us to gain important information about multiple components of a subject's lifestyle (e.g., information about sleep quality) in free-living conditions.

INDIRECT CALORIMETRY EQUATIONS

COMPLETE WEIR FORMULA

$BMR = [3.9 \times VO_2 + 1.1 \times VCO_2] \times 1.44 - 2.17 \times UN$

ABBREVIATED WEIR FORMULA

$BMR = [3.9 \times VO_2 + 1.1 \times VCO_2] \times 1.44$

LEGEND

VO_2 = oxygen consumption (mL/min)
VCO_2 = carbon dioxide production (mL/min)
UN = urinary nitrogen (g/d)
BMR = basal metabolic rate (kcal/d)

Fig. 16.4 Indirect calorimetry equations [45, 46]

16.2.7 Calorimetry Techniques

Calorimetry techniques are the most accurate methods that allow us to measure energy expenditure with a high rate of accuracy. Energy expenditure can be measured as the sum of work performed on the environment by the organism plus heat released during combustion of food. If the organism is at rest, energy expenditure equals the heat produced by oxidation of energy substrates in foods.

Direct Calorimetry

Direct calorimetry represents the *gold standard method* for energy expenditure measurement [44]. Direct calorimetry is based on the principle that all metabolic processes that occur in the body lead to the production of heat, a process known as thermogenesis.

This technique requires the use of a *metabolic chamber* that allows fine measurement of the heat lost from the human body. It is also suitable to evaluating the thermogenic variations induced by various foods and daily activities. The disadvantages are that the *metabolic chamber* is rather expensive and its use requires a full time technician.

Indirect Calorimetry

Indirect calorimetry is based on the principle that an organism produces energy by the oxidation of energy substrates in foods. The production of energy from these substrates takes place through known stoichiometric reactions in which oxygen is consumed and carbon dioxide produced. From the volume of oxygen consumed (VO_2), the volume of carbon dioxide (VCO_2) produced, and the excretion of urinary nitrogen, the *equation of Weir* [45, 46] (Fig. 16.4) gives the value of energy expenditure. The excretion of urinary nitrogen is often neglected in a simplified version of Weir equation with a minor error (Fig. 16.4). The method thus consists of collecting

Table 16.5 Respiratory quotient of various metabolic substrates

Metabolized substrate	Respiratory quotient (RQ)
Ethanol	0.67
Fat oxidation	0.71
Carbohydrate oxidation	1.0
Protein oxidation	0.82
Lipogenesis	1–1.2
After carbohydrate-based meal	1 ± 0.04
22 h after fatty-acid-based meal	0.71 ± 0.04
After mixed meal	0.86 ± 0.11

This table reports the RQ of various macronutrients. RQ is calculated through indirect calorimetry [46, 50]

expired air using either an airtight rigid structure or a portable flexible bag. There are various types of indirect calorimeters.

The Tissot gasometer [47] is a *rigid total collection system*. In this instrument, the subject has to expire through a mouthpiece and a nonreturn valve into a glass bell suspended over water. The test lasts for about 2 h, and the composition of the air in the bell is periodically analyzed.

Douglas bag [48] represents *a flexible collector system*, in which the patient expires through a mouthpiece into a polyvinyl chloride bag. Again, the volume of expired air is collected and analyzed (e.g., using a mass flow meter), and the concentration of oxygen and carbon dioxide are calculated. This technique is fast (20 min) and relatively cheap compared to other calorimetric methods. However, in order to obtain reliable results, it requires a good instrument maintenance and experienced operator.

Another group of calorimetric techniques is represented by *open-circuit indirect calorimeter systems*, which can be divided into two main groups [40]: *ventilated* open-circuit systems where the patient breaths into a container from which air is drawn and *expiratory collection systems* where a subject inspires from the atmosphere and expires via a nonreturn valve into a measurement unit. In the first case, the air can be collected through the use of mouthpiece, mask, transparent hood, or canopy. The expired air is drawn out through a pump, and the flow rate is accurately measured. The expired air is then mixed using a fan and/or mixing chamber, and a sample of the expired air is dried and analyzed for oxygen and/or carbon dioxide concentrations. This technique allows measurement in a brief lapse of time with a high accuracy rate.

An expiratory collection open-circuit system, on the contrary, has the advantage of being suitable for free-living analysis as many of these devices are portable and allow analysis to be performed over periods as long as 2 days.

Indirect calorimetric techniques can also provide another value: the *respiratory quotient* (RQ).

RQ [49] is calculated from the ratio:

$$RQ = VCO_{2\ \text{eliminated}} / VO_{2\ \text{consumed}}$$

This value is specific for each metabolized substrate, hence allowing the operator to know which is the nutrient mainly consumed by a subject (Table 16.5). Special care must be taken in interpreting the RQ value since there are many metabolic and non-metabolic causes that may result in alterations of the physiological range of RQ.

In order to accurately measure the basal metabolic rate, the subject should undergo 8 h of fasting, be awake, and be totally free of stressors. This last aspect is fundamental and takes on particular importance in anxious patients and children where the use of masks or canopies represents an important stressor input which could alter measurement reliability. To ensure patients are adequately relaxed, the experimental environment is therefore extremely important, and even steps such as the use of acoustic supports like classic music may be advisable. Moreover, the operator should also pay attention to the room thermal insulation since RQ is particularly sensible to temperature variations.

Indirect calorimetry represents a useful technique for the customization of a patient's energy requirements and nutrient supply when seeking to improve treatment outcomes in the clinical setting [51] especially in critically ill patients.

With advances in technology, indirect calorimetry has become easier to operate and less expensive, leading to a more widespread use of the instrument.

References

1. Cruz-Jentoft AJ et al (2010) Sarcopenia: European consensus on definition and diagnosis Report of the European Working Group on Sarcopenia in Older People. Age Ageing 39: 412–423
2. Scherzer R et al (2011) Decreased limb muscle and increased central adiposity are associated with 5-year all-cause mortality in HIV infection. AIDS Lond Engl 25:1405–1414
3. El Ghoch M et al (2014) Body composition, eating disorder psychopathology, and psychological distress in anorexia nervosa: a longitudinal study. Am J Clin Nutr 99:771–778
4. Levine JA (2004) Non-exercise activity thermogenesis (NEAT). Nutr Rev 62:S82–S97
5. Dulloo AG, Jacquet J, Montani J-P, Schutz Y (2012) Adaptive thermogenesis in human body weight regulation: more of a concept than a measurable entity? Obes Rev Off J Int Assoc Study Obes 13(Suppl 2):105–121
6. Ouchi N, Parker JL, Lugus JJ, Walsh K (2011) Adipokines in inflammation and metabolic disease. Nat Rev Immunol 11:85–97
7. Ellis KJ (2000) Human body composition: in vivo methods. Physiol Rev 80:649–680
8. Norgan N (2005) Laboratory and field measurements of body composition. Public Health Nutr 8:1108–1122
9. Snyder S et al (1975) Report of the Task Group on reference man: ICRP-23. Pergamon, Oxford
10. Mattsson S, Thomas BJ (2006) Development of methods for body composition studies. Phys Med Biol 51:R203–R228
11. Durnin JV, Womersley J (1974) Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 32:77–97
12. Jackson AS, Pollock ML (1978) Generalized equations for predicting body density of men. Br J Nutr 40:497–504
13. Jackson AS, Pollock ML, Ward A (1980) Generalized equations for predicting body density of women. Med Sci Sports Exerc 12:175–181

14. Forsyth HL, Sinning WE (1973) The anthropometric estimation of body density and lean body weight of male athletes. Med Sci Sports 5:174–180
15. Siri W (1956) The gross composition of the body. Adv Biol Med Phys 4:239–280.
16. Schoeller D (2005) Hydrometry. In: Heymsfield SB et al. (eds) Human body composition. Second ed. pp 35–50
17. Huxley R, Mendis S, Zheleznyakov E, Reddy S, Chan J (2010) Body mass index, waist circumference and waist:hip ratio as predictors of cardiovascular risk–a review of the literature. Eur J Clin Nutr 64:16–22
18. Allison DB, Zhu SK, Plankey M, Faith MS, Heo M (2002) Differential associations of body mass index and adiposity with all-cause mortality among men in the first and second National Health and Nutrition Examination Surveys (NHANES I and NHANES II) follow-up studies. Int J Obes Relat Metab Disord 26:410–416
19. WHO (2008) Waist circumference and waist–hip ratio: report of a WHO expert consultation. WHO, Geneva, pp 8–11
20. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary. Expert panel on the identification, evaluation, and treatment of overweight in adults (1998). Am J Clin Nutr 68:899–917
21. WHO (2000) Obesity: preventing and managing the global epidemic, vol 894, WHO technical report. WHO, Geneva
22. Westat Inc. (1998) Third National Health and Nutrition Examination Survey (NHANES III), 1988–94. ftp://ftp.cdc.gov/pub/health_statistics/NCHS/nhanes/nhanes3/2A/cff-acc.pdf
23. Ross R et al (2008) Does the relationship between waist circumference, morbidity and mortality depend on measurement protocol for waist circumference? Obes Rev Off J Int Assoc Study Obes 9:312–325
24. Björntorp P (1987) Fat cell distribution and metabolism. Ann N Y Acad Sci 499:66–72
25. Kotler DP et al (1999) Relative influences of sex, race, environment, and HIV infection on body composition in adults. Am J Clin Nutr 69:432–439
26. Heymsfield SB, Wang Z, Visser M, Gallagher D, Pierson RN Jr (1996) Techniques used in the measurement of body composition: an overview with emphasis on bioelectrical impedance analysis. Am J Clin Nutr 64:478S–484S
27. Baumgartner RN et al (1998) Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol 147:755–763
28. Barthe N, Braillon P, Ducassou D, Basse-Cathalinat B (1997) Comparison of two Hologic DXA systems (QDR 1000 and QDR 4500/A). Br J Radiol 70:728–739
29. Van der Kooy K, Seidell JC (1993) Techniques for the measurement of visceral fat: a practical guide. Int J Obes Relat Metab Disord 17:187–196
30. Mitsiopoulos N et al (1998) Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography. J Appl Physiol Bethesda Md 1985(85): 115–122
31. Fuller NJ et al (1999) Assessment of limb muscle and adipose tissue by dual-energy X-ray absorptiometry using magnetic resonance imaging for comparison. Int J Obes Relat Metab Disord 23:1295–1302
32. Marwan M, Achenbach S (2013) Quantification of epicardial fat by computed tomography: why, when and how? J Cardiovasc Comput Tomogr 7:3–10
33. Lewis CE et al (2000) Weight gain continues in the 1990s: 10-year trends in weight and overweight from the CARDIA study. Coronary artery risk development in young adults. Am J Epidemiol 151:1172–1181
34. Rosenbaum M, Leibel RL (2010) Adaptive thermogenesis in humans. Int J Obes 2005(34 Suppl 1):S47–S55
35. Harris J, Benedict F (1919) A biometric study of basal metabolism in man. Carnegie Institute of Washington, Washington, DC
36. Schofield WN (1985) Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr 39(Suppl 1):5–41

37. Mifflin MD et al (1990) A new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr 51:241–247
38. Weijs PJM (2008) Validity of predictive equations for resting energy expenditure in US and Dutch overweight and obese class I and II adults aged 18–65 y. Am J Clin Nutr 88:959–970
39. FAO (2001) Human energy requirements, Report of a joint FAO/WHO/UNU expert consultation. Food and nutrition technical report series. Rome, 17–24 Oct 2001
40. Levine JA (2005) Measurement of energy expenditure. Public Health Nutr 8:1123–1132
41. Black AE, Coward WA, Cole TJ, Prentice AM (1996) Human energy expenditure in affluent societies: an analysis of 574 doubly-labelled water measurements. Eur J Clin Nutr 50: 72–92
42. Bouten CV, Westerterp KR, Verduin M, Janssen JD (1994) Assessment of energy expenditure for physical activity using a triaxial accelerometer. Med Sci Sports Exerc 26:1516–1523
43. St-Onge M, Mignault D, Allison DB, Rabasa-Lhoret R (2007) Evaluation of a portable device to measure daily energy expenditure in free-living adults. Am J Clin Nutr 85:742–749
44. Webster JD, Welsh G, Pacy P, Garrow JS (1986) Description of a human direct calorimeter, with a note on the energy cost of clerical work. Br J Nutr 55:1–6
45. de Weir JBV (1949) New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 109:1–9
46. Matarese L, Fada R (1997) Indirect calorimetry: technical aspects. J Am Diet Assoc 97: S154–S160
47. Tissot J (1904) Nouvelle methode de mesure et d'inscription du debit et des movements respiratories de l'homme et des animaux. J Physiol Pathol Gen 6:688–700
48. De Groot G, Schreurs AW, van Ingen Schenau GJ (1983) A portable lightweight Douglas bag instrument for use during various types of exercise. Int J Sports Med 4:132–134
49. McClave SA et al (2003) Clinical use of the respiratory quotient obtained from indirect calorimetry. JPEN J Parenter Enteral Nutr 27:21–26
50. Compher C, Frankenfield D, Keim N, Roth-Yousey L (2006) Best practice methods to apply to measurement of resting metabolic rate in adults: a systematic review. J Am Diet Assoc 106:881–903
51. Haugen HA, Chan L-N, Li F (2007) Indirect calorimetry: a practical guide for clinicians. Nutr Clin Pract 22:377–388