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Concepts of indirect calorimetry on metabolic disorders: a narrative review

Conceitos da calorimetria indireta sobre distúrbios metabólicos: uma revisão narrativa

Rocio San Martin[1], Camila Fernanda Cunha Brandão[1,2], Marcia Varella Morandi Junqueira-Franco[1], Gizela Pedroso Junqueira[1], Fernando Bahdur Chueire[1], Joyce Cristina Santos de Oliveira[1], Selma Freire Carvalho da Cunha[1], Vivian Marques Miguel Suen[1], Julio Sergio Marchini[1]

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ABSTRACT: Introduction: Indirect calorimetry remains a gold standard in measuring resting energy expenditure in the clinical field. Through its measurements, it is possible to offers a patient's energy needs to maximize nutritional therapy benefits. However, the concepts and methodological basis of collected data can be difficult to be interpreted by users in clinical practice. Objective: To address the concepts of total daily energy expenditure and its components and present the methodological aspects of indirect calorimetry that can guide the clinical field. Method: Narrative bibliographic review using the electronic Pubmed (US National Library of Medicine), SCOPUS, and Scientific Electronic Library Online (SciELO) databases. The research was carried out in the period between 1905-2019, using the following identifiers in Health Sciences Descriptors: Basal Metabolism, Energy Metabolism and Indirect Calorimetry. We selected 55 researches published that presented contents related to the objectives of this study. Result: The total daily energy expenditure (TDEE) is comprised of three main components, such as physical activity (PA), thermic effect of food (TEF) and basal metabolic rate (BMR) and/or resting energy expenditure (REE). The REE is generally evaluated by indirect calorimetry, which also provides information on the respiratory coefficient (RQ) or oxidation of substrates. Its result varies depending on the existence of some metabolic disorders such as obesity or malnutrition. Therefore, the proper management of the methodological aspects of indirect calorimetry and its subsequent interpretation in metabolic disorders is essential to guarantee the results' quality. Conclusion: Energy expenditure concepts and the methodological basis of indirect calorimetry are relevant to providing individualized attention to patients with metabolic disorders. This review can be used as a practical guide, helping to understand the correct application of the indirect calorimetry technique in studies related to energy expenditure with an emphasis on metabolic disorders.

Keywords: Indirect calorimetry; Substrates oxidation; Energy expenditure; Respiratory quotient; Metabolic disorders.

RESUMO: Introdução: A calorimetria indireta continua sendo um padrão ouro na avaliação do gasto energético de repouso no campo clínico. Por meio de suas medições, é possível oferecer as necessidades energéticas de um paciente para maximizar os benefícios da terapia nutricional. No entanto, os conceitos e as bases metodológicas dos dados coletados podem ser dificultosos para serem interpretados pelos usuários na prática clínica. Objetivo: abordar os conceitos de gasto energético diário total e seus componentes, e, apresentar os aspectos metodológicos da calorimetria indireta que podem servir como guia no campo clínico. Método: Revisão bibliográfica narrativa, realizada pelas bases de dados eletrônicas Pubmed (US National Library of Medicine), SCOPUS e Scientific Electronic Library Online (SciELO). A pesquisa foi realizada no período entre 1905-2019, utilizando os seguintes identificadores em Descritores em Ciências da Saúde: Metabolismo Basal, Metabolismo Energético e Calorimetria Indireta. Foram selecionadas 55 pesquisas publicadas que apresentaram conteúdos relacionados aos objetivos deste estudo. Resultado: O gasto energético total diário (GETD) é composto por três componentes principais, tais como: atividade física (AF), efeito térmico dos alimentos (TEF) e taxa metabólica basal (TMB) e / ou gasto energético de repouso (GER). O GER é geralmente avaliado por calorimetria indireta, que também fornece informações sobre o coeficiente respiratório (CR) e oxidação de substratos, que pode variar de acordo com o metabolismo do paciente, como algum distúrbio metabólico, obesidade ou desnutrição. Portanto, o manejo adequado dos aspectos metodológicos da calorimetria indireta e sua posterior interpretação nos distúrbios metabólicos é fundamental para garantir a qualidade dos resultados. Conclusão: Os conceitos de gasto energético e as bases metodológicas da calorimetria indireta são relevantes para fornecer uma atenção individualizada aos pacientes com distúrbios metabólicos. As descrições desta revisão podem ser utilizadas como um guia prático, auxiliando a compreensão da aplicação correta da técnica de calorimetria indireta, em estudos relacionados ao gasto energético com ênfase nos distúrbios metabólicos.

Palavras-chave: Calorimetria indireta; Oxidação de substratos; Gasto energético; Quociente respiratório; Distúrbios metabólicos.

1. University of São Paulo, Ribeirão Preto Medical School, Division of Nutrology, Ribeirão Preto, SP, BR. ORCID: San Martin R - https://orcid.org/0000-0002-6288-904X; Brandao CFC - https://orcid.org/0000-0002-3330-8007; Junqueira-Franco MVM - https://orcid.org/0000-0002-7968-5799; Junqueira GP - https://orcid.org/0000-0003-4894-2550; Chueire FB - https://orcid.org/0000-0001-7663-0533; Oliveira JCS - https://orcid.org/0000-0003-1758-173X; Cunha SFC - https://orcid.org/0000-0002-4092-4502; Suen VMM - https://orcid.org/0000-0001-6165-5746; Marchini JS - https://orcid.org/0000-0001-9999-9149. E-mails**:** sansan.rocio@gmail.com, camilafbrandao@hotmail.com, mvmjf@hotmail.com, fbchueire@hotmail.com, joyce.coliveira@hotmail.com, sfreire@fmrp.usp.br, vmmsuen@fmrp.usp.br, jsmarchi@fmrp.usp.br.
2. State University of Minas Gerais, Divinopolis Division, Brazil.
Correspondence: Rocio San Martin. Avenida Bandeirantes, 3900 - Monte Alegre, Ribeirão Preto, São Paulo, BR. CEP: 14049-900.

INTRODUCTION

To accurately determine the energy expenditure of a patient is one of the nutritional assessment difficulties, especially in those with metabolic issues such as morbid obesity and malnutrition. Monitoring the physiological responses and their nutritional needs is a substantial clinical feature to avoid errors related to the delivery of nutritional needs, in particular with excess or shortage of calories. The evaluation of resting energy expenditure (REE) and the analysis of substrate oxidation depend on a complex methodology since correct assumptions must be made based on metabolic calculations. The gold standard for the resting energy expenditure measurements is indirect calorimetry (IC), which in the clinical practice, is the most accurate, reliable, and safe instrument[1]. The use of IC is already widespread, it is necessary to know its concepts, methodological basis and interpretation, in order to ensure the quality of the collected data. Therefore, it is essential to disclose all equations used in the principle of IC and interpretation of the results[2].

In this review, we introduce mainly the concepts of total daily energy expenditure (TDEE), and methodological aspects of the IC as a tool used to measure the resting energy expenditure, and the respiratory coefficients (RQ). This study's objective was to address a comprehensive overview of the concepts of energy expenditure and to present the methodological aspects of indirect calorimetry that might serve as guide in the clinical field.

MATERIAL AND METHODS

This is a narrative literature review, using the electronic databases Pubmed (US National Library of Medicine), SCOPUS, and Scientific Electronic Library Online (SciELO). The search for studies was carried out in the period between 1905-2019. Articles in Portuguese and English were selected using the following Health Sciences Descriptors, Basal Metabolism/physiology*, Energy Metabolism/physiology*, and Indirect Calorimetry. We used (*) to restrict searches to articles focused on the broadest MeSH term. This study's was to provide a general view of the concepts, methodological basis and interpretation of indirect calorimetry to facilitate understanding of the metabolic disorder's results in the clinical practice.

Among the search results, 52 articles that contributed to the objectives and relevance of the present study were selected. These articles were filtered by studies in humans and with focus on mathematical approaches. In this search, duplicate and studies that did not address the topic under study were excluded.

RESULTS

The search in the databases returned a total of 974 articles. Of these, 170 were duplicates, 120 were excluded after analysis of the title and abstract and then 630 articles were excluded after reading the full text. At the end of the exclusions, 52 articles were included in this study. This review narrates different topics as the concept of total daily energy expenditure and its components and how can be estimated. Then, we focused on the resting energy expenditure with emphasis in the indirect calorimetry principle and mathematical procedures. From the articles found it was possible to discuss the following main topics: total daily energy expenditure, total daily energy expenditure measurements, and resting energy expenditure measurements using indirect calorimetry.

TOTAL DAILY ENERGY EXPENDITURE

In all living organisms, there is an energetic cost of the physiological functions necessary to maintain the homeostasis. The total daily energy expenditure (TDEE) of an individual represents the energy that the body consumes. Three main components of energy balance determine TDEE: basal metabolic rate (BMR), physical activity (PA), and thermic effect of food (TEF). Other subcomponents may exist, such as the energy cost of the emotion, playing a slight role related to the energy balance[3].

First, the BMR is the minimum amount of energy that an organism requires to be alive. It constitutes 55% to 75% of the TDEE in the majority of sedentary adults; meanwhile, in physically active individuals, it is approximately 50%. It varies depending on body composition, fat mass (FM), and fat-free mass (FFM), as well as sex, age, and genetic factors[4]. It is essential to note differences among the terms BMR and REE, which in some publications have been considered as synonymous interchanged. However, BMR is measured post-absorptively (without food intake for at least 12 hours), in the supine position at complete rest under quiet ambient conditions, in a thermal-neutral temperature setting and darkened lightning in the morning after 8 hours of sleep and no exercise for the previous 24 h[3,5]. By contrast, REE can be performed at any time of the day, in the sitting or supine position, resting in advance at least 15 minutes. REE is almost 10% higher than BMR. The fast period for REE should be a minimum of 4 to 5 hours after a light food[6]. However, it depends on the energy load consumed; for example, if subjects are not able to fast as recommended, research suggests a small meal (<300 kcal) can be ingested, and REE can be measured 2 hours later[7].

Second, the PA can be categorized into exercise-related activity thermogenesis (EAT) and non-exercise activity thermogenesis (NEAT). Both vary widely within and between individuals. EAT refers to the level of physical activity, and it depends on if the person is sedentary or physically active[8]. NEAT corresponds to all the energy expended with occupation, leisure time activity, sitting,

standing, ambulation, playing the guitar, dancing, singing, and washing[3]. Some authors categorize NEAT into three main components: body posture, ambulation, and all other spontaneous movements, including small movements, especially of the hands and feet[9]. It has been estimated that PA ranges from approx. 15% in very sedentary individuals to up to 50% in highly active individuals[10]. EAT is believed to account for 15-30% of TDEE, [11,12] and NEAT is responsible for 6-10% of TDEE in individuals with a mainly sedentary lifestyle[3].

Finally, the TEF is related to food digestion, absorption, and storage and is a relatively stable component of total energy expenditure. The variance of TEF has been associated with nutrient composition and energy content of consumed foods[13]. The TEF represents nearly 10% of the TDEE.

How can the TDEE components be estimated?

There are different methodologies to assess the TDEE and each component (seen Table 1). To have a better understanding, we will separate them into:

- Total daily energy expenditure measurements;
- Physical activity measurements;
- Resting energy expenditure measurements.

Table 1: Summary of each component of the total daily energy expenditure

Components	Total daily energy expenditure (TDEE)	Basal metabolic rate (BMR)	Physical activity (PA)	Thermic effect of food (TEF)
Synonymous	Total Energy Expenditure (TEE) -Energy Expenditure (EE)	Resting Energy Expenditure (REE)	*Activity-related energy expenditure (AEE)*	Thermic effect of food (TEF); Diet-induced thermogenesis (DIT)
			Non-resting energy expenditure (NREE)	
Subcomponents		EE of the dream + EE of the maintenance of the wake	EE of voluntary exercise (EAT) + EE of spontaneous exercise (NEAT)	EE of food digestion + EE of food absorption + EE of food storage
Percentage of variation		55-75%	15- 30%	7 – 15%
Key determinants		body weight, height, fat-free mass, fat mass, gender, age, genetics, hormones/ Sympathetic Nervous System (SNS)	genetic, age, gender, environmental stimuli, intensity/duration of exercise	nutrients composition, age, obesity, insulin resistance, hormones/ SNS
Measurements methods	direct calorimetry, indirect calorimetry, doubly-labeled water, predictive equations	indirect calorimetry	accelerometry, heart rate monitors, pedometry, physical activity questionnaires, doubly-labeled water	indirect calorimetry

Legend: TEE: Total Energy Expenditure; EE: Energy Expenditure; REE: Resting Energy Expenditure; AEE: Activity-related Energy Expenditure; NREE: Non-Resting Energy Expenditure; TEF: Thermic Effect of Food; DIT: Diet-Induced Thermogenesis; EAT: Exercise-related Activity Thermogenesis; NEAT: Non-exercise Activity Thermogenesis; **SNS**: Sympathetic Nervous System.

TOTAL DAILY ENERGY EXPENDITURE MEASUREMENTS

The main approaches to measure the TDEE are direct calorimetry and non-calorimetric techniques, such as the doubly labeled water (DLW).

Direct Calorimetry

The direct calorimetry was one of the first procedures to measure TDEE based on the first law of thermodynamics. Which established that energy is not created, nor destroyed, but rather conserved and that the spent energy in the entire physiological process is dissipated as heat. Therefore, the TDEE can be measured directly by the heat production[14]. Direct calorimetry represents a technical challenge. Due requires measurement from all the heat transference, including radiation, convection, conduction, and evaporation. The major advantage is its easy use in free-living conditions, avoiding the problem of inhaling and exhaling the gas composition by the IC[15].

Non-calorimetric techniques: Doubly-Labeled-Water

The DLW technique approach has been widely recognized as a criterion for assessing TEE[16]. In the DLW technique, daily urine or saliva samples are collected over 7 to 14 days. After analyzed by isotopic mass spectrometry (IRMS) [17]. The stable isotopes, deuterium (^{2}H) and oxygen-18 (^{18}O) are administered orally via a drink of

water, and elimination of the isotopes from the body is tracked[18]. The difference between the elimination rates of ^{2}H and ^{18}O is equivalent to the rate of carbon dioxide production that can then be converted to average TDEE. One of the disadvantages of this technique, it requires the use of sophisticated laboratory-based equipment, and the cost of isotopes is higher, hindering the analysis of samples for large-scale studies. However, it is the only method to measure energy expenditure in any environment, especially concerning activity energy expenditure, where there is no interference with the behavior of the subjects[19].

Physical Activity Measurements

Research has demonstrated the benefits of PA and the negative consequences of sedentary behavior for physical and mental well-being[20]. Thus, PA has become increasingly prominent as an intervention tool[21]. Therefore, it is crucial to estimate this variable of the total energy expenditure. The main methods for measuring PA are summarized as follows:

Physical Activity Questionnaires

PA questionnaires are the most widely used approach. It can measure large numbers of participants at low cost, and they can be completed over a minimum of a 24-h period and up to 7-days[22]. However, it has some limitations to determine the intensity level of activity[23].

Heart Rate Monitoring

Heart rate (HR) monitoring is a physiological indicator of PA and energy expenditure, providing real-time data on the frequency, duration, and intensity of PA[24]. There is a significant linear correlation between HR and the rate of oxygen consumption (VO_2), which can then be extrapolated to predictions of energy expenditure or metabolic rate[25]. However, the slope of this heart rate VO_2 relationship varies across individuals partly due to age, sex, aerobic fitness, and movement efficiency. Thus, an individualized calibration procedure is required to use HR to predict energy expenditure. The major advantage of this method that it is very flexible in terms of duration of use is easy to administer and has an objective measurement[10].

Pedometers

Pedometers are a simple tool for measuring step counts that can be used to track daily PA, specifically walking. It is thought that the accumulation of 10 000 steps/day is an indicator of a healthy amount of PA[26]. Individuals who accumulate at least 10 000 steps/day are more likely to engage in at least 30 minutes of moderate-intensity PA than those who do not accumulate 10 000 steps/day[27]. The major advantages of pedometers are inexpensive, easy to use, and output data can be used to raise consciousness regarding the level of PA, including motivation to increase it, especially the NEAT[28].

Accelerometers

Recent advances in technology have allowed the development of accelerometers as one of the methods of PA energy and expenditure measurement. Accelerometers consist of the production of an electrical signal that is subsequently converted by processing units to produce an indication of movement and acceleration, which is defined as the rate of change in velocity over a given time. Therefore, the frequency, intensity, and duration of PA can be assessed as a function of body movement[29]. It has been reported that accelerometers are objective, practical, non-invasive, accurate, and reliable tools to quantify PA volume and intensity with minimal discomfort[22]. Accelerometers are more sophisticated and, therefore, superior to pedometers. A major advantage of the technique is the ability to quantify time spent in activities of different intensities. However, accelerometers have low sensitivity to sedentary activities and are unable to register static exercise[30].

RESTING ENERGY EXPENDITURE MEASUREMENTS

Predictive Equations

The REE is commonly measured with indirect calorimetry. However, REE predictive equations are commonly used as an alternative method. In the clinical practice, some of them are specific for certain population groups, including different weight status, height, age, sex, and body composition parameters or different ethnic groups[31,32]. However, some factors are affecting the REE that is not captured by predictive equations. In this regard, the REE is altered in patients with cardiometabolic diseases such as type 2 diabetes, hypertension, and sleep apnea[33]. For this reason, there are correction factors included in different predictive equations specific for patients with metabolic diseases[34].

Indirect Calorimetry Principle

The O_2 and CO_2 gas fractions are measured in the total expired gas volume by specific gas sensors. Transformed into values for VO_2 and VCO_2 in ml/min, and finally into values for REE in kcal (or kJ)/day; (1 kcal = 4.184 kJ). The Haldane transformation based on the relatively insoluble gas nitrogen (N_2) is constant in both inspired and expired gases. Assuming that only O_2 and CO_2 are exchanged in the lungs, and the rest of the respiratory gases (excluding water vapor) have the same volume[35]. Therefore, if there is no net nitrogen uptake, the inspired gas volume can be calculated, as seen in Table 2.

Table 2: Calculation of O_2 consumption (VO_2) and CO_2 production (VCO_2)

Inspired gases	Expired gases
$Fi_{O2} = Pi_{O2}/BP - 47$	$Fe_{O2} = Pe_{O2}/BP - 47$
$Fi_{CO2} = Pi_{O2}/BP - 47$	$Fe_{CO2} = Pe_{CO2}/BP - 47$
$Fi_{N2} = 1 - Fi_{O2} - Fi_{CO2}$	$Fe_{N2} = 1 - Fe_{O2} - Fe_{CO2}$
Conversion of Ve and Vi (ATPS) into Ve and Vi (BTPS):	
$Ve(ATPS) = Ve(BTPS) \times CF$	
$Vi(ATPS) = Ve(BTPS) \times Fe_{N2}/Fi_{N2}$ (Haldane transformation)	
Calculation of VO_2 and VCO_2 (liters/min):	
$V_{O2} = (Fi_{O2} \times Vi) - (Fe_{O2} \times Ve)$	
$V_{CO2} = (Fe_{CO2} \times Ve) - (Fi_{CO2} \times Vi)$	

FiO_2, FeO_2: fractional inspired and expired O_2 respectively (percentage; %). $FiCO_2$, $FeCO_2$: fractional inspired and expired CO_2, respectively (%). FiN_2, FeN_2: fractional inspired and expired nitrogen, respectively (%). PiO_2, PeO_2: partial pressures of inspired and expired O_2, respectively (millimeter of mercury, mmHg). $PiCO_2$, $PeCO_2$: partial pressures of inspired and expired CO_2, respectively (mmHg). Ve, Vi: inspired and expired gas volumes respectively (liters/min). BP: barometric pressure at sea level (760 mmHg). 47: partial pressure of water vapor at 37°*C* (mmHg). ATPS: ambient temperature and pressure saturated gas (degree Celsius (°C); mmHg). BTPS: body temperature and pressure saturated gas (°C; mmHg). CF: correction factor for 37°*C* (reduction of saturated gas volumes for those at body temperature: BTPS).
Adapted from da Rocha[36]

Calculation of substrate oxidation: stoichiometric equations

The IC method is based on the knowledge of the fixed ratio between the quantities of O_2 consumed and CO_2 produced. This ratio is called the respiratory quotient (RQ). Using a balanced chemical equation to calculate amounts of reactants and products is called stoichiometry. The RQ is calculated as the ratio of the volume of carbon dioxide (VCO_2) produced to the volume of oxygen (O_2) used: VCO_2/VO_2. The RQ, which typically ranges between 0.7 and 1.0 (Table 3), is an indicator of metabolic fuel or substrate used in tissues; it must be calculated under resting or steady-state exercise conditions. Carbohydrates (e.g., glucose) are oxidized through aerobic respiration using RQ, resulting in an equal ratio of CO_2 release and O_2 consumption. It implies that 100% of carbohydrates are consumed to produce ATP[37]. When fat (e.g., palmitic acid) is oxidized and measured using RQ, the outcome is reduced CO_2 production for every oxygen molecule consumed. When protein (e.g., albumin) is the respiratory substrates, it results in reduced CO_2 production for every oxygen molecule consumed[38] (Table 3).

Table 3: Respiratory quotients (RQ) for common food. A) Stoichiometry of glucose oxidation; B) palmitic acid and C) albumin

	Substrate	Equations	RQ
A	Carbohydrates (Glucose)	$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ + Energy	1.0
B	Lipid (Palmitic acid)	$C_{16}H_{32}O_2 + 23O_2 \rightarrow 16CO_2 + 16H_2O$ + Energy	0.7
C	Protein (Albumin)	$C_{72}H_{112}N_{18}O_{22}S + 77O_2 \rightarrow 63CO_2 + 38H_2O + S0_3 + 9CO(NH_2)_2$ + Energy	0.8

$C_6H_{12}O_6$: glucose; O_2: oxygen; CO_2: carbon dioxide; H_2O: water; $C_{16}H_{32}O_2$: palmitic acid; $C_{72}H_{112}N_{18}O_{22}S$: albumin; $S0_3$: Sulfur trioxide; $9CO(NH_2)_2$: urea

Lusk[39] and subsequently Weir[40] provided data and formulas that allow the development of a manual procedure by indirect calorimetry, without the need for special equipment and whose results are comparable to those observed by sophisticated methods. Table 4 shows the volumes of O_2 consumed, and CO_2 produced. As well as the amount of energy released in the form of heat (dG°), during combustion in a heat pump[40] of 1 mol of each of the three primary substrates (glucose, palmitate, and amino acids).

Writing the equations of the glucose, lipids, and protein oxidation reactions and expressed in grams (*g*) and liters (*l*), the reactions mentioned above (Table 3) would be as shown in (Table 5; equations 1,2,3). In turn, the amount of oxidized protein can be estimated from urinary nitrogen. Since nitrogen represents about 16% of the protein's weight. The 1 g of urinary nitrogen comes from 6.25 g of protein, and the oxidation equation can be rewritten as Protein = 6.25 × urinary nitrogen (N)[42] (Table 5;

equation 4). Solving equations 5 and 6, it can be calculated (equation 7 and 8). Therefore, knowing from the indirect calorimetry the consumption of O_2 (VO_2) and production of CO_2 (VCO_2), it is possible to estimate the amount of glucose and lipids oxidized by the organism. It should be noted that these calculations are based on the known stoichiometry of the oxidation of these substrates[41]. In the above equations, for example, the values resulting from glucose oxidation are used. The predominant form of oxidized carbohydrate is glycogen, and glycogen hydrolysis is considered to yield 1.11g of glucose per gram of glycogen. Therefore, the complete oxidation of glycogen would require 0.829 l O_2 (i.e., 0.746 × 1.11) and would produce 0.829 l of CO_2; thus, equation 7 would become in equation 9. Alternatively, the initial equation 7 can be used by multiplying its result by (1/11 = 0.9). The choice between glucose and glycogen as the predominant carbohydrate should be based on independent information on the physiological conditions of the study. For example, after one night, almost three-quarters of the plasma glucose turnover is derived from hepatic glycogenolysis, while this amount is substantially reduced in postprandial conditions[43].

Table 4: Energy balance for the main substrates

Oxidized substrate	dG°	Consumed O_2		Produced CO_2		RQ	ATP produced		ATP cost
1 mol	(kcal/mol)	(mol)	(L)	(mol)	(L)		(mol)	(kcal)	(Kcal/mol)
Glucose[a]	- 673	6	134	6	134	1.000	36	18.3	18.7
Palmitic acid	- 2.39	23	515	16	358	0.695	131	66.4	18.3
Aminoacids[b]	- 475	5.1	114	4.1	92	0.807	23	11.7	20.7

dG°: Gibbs free energy; negative reactions (-) exothermic reactions; RQ: respiratory quotient; L: liter; VO_2: volumetric oxygen consumption (L/min); VCO_2: volumetric carbon dioxide elimination (L/min). The coefficients used are those derived by Kleiber[41]. [a] Complete glucose oxidation yields 38 moles of ATP per mole, but 2 moles of ATP are used during glycolysis. [b] The complete oxidation of the amino acids yields 28.8 moles of ATP, but 5.8 moles are consumed in the process.

The determination of the use of substrates will be precise in metabolic stability in which it is possible to affirm that VCO_2 and VO_2 are the reflection of cellular oxidation and can be measured accurately. However, other metabolic processes require special considerations[44].

When the patient is under the process of lipogenesis, a respiratory ratio greater than 1 corresponds to a net synthesis of fat from carbohydrates[45]. The stoichiometry of glucose conversion in lipids, shows an endergonic reaction, with a 50% conversion that releases O_2 (Table 5; equation 10). Once this release of O_2 does not occur in vivo. It should be considered that the lipogenesis process occurs with glucose oxidation, which would result in the following stoichiometry (Table 5; equation 11). As can be seen, the RQ is, in this case, very high (RQ = 5.6). Therefore, the simultaneous occurrence of lipogenesis and oxidation of carbohydrates is reflected in respiratory ratios greater than 1. In these circumstances, equation 8 can be used to estimate the net lipid synthesis rate. In particular, a positive sign in the resolution of equation 8 indicates a net lipid synthesis, while a negative sign would indicate that lipid oxidation is superior to lipogenesis[46]. The most important is that in the case of considering the existence of a net lipogenesis with high carbohydrate contributions (e.g., in a situation of total parenteral nutrition), equation 7 would overestimate glucose oxidation in amounts equivalent to those used to synthesize fat[47]. Therefore, in these circumstances, the following equation is suggested for the calculation of glucose oxidation (equation 12). The preferential use of substrates in the process of gluconeogenesis and ketogenesis, characterized by having low respiratory ratios, can significantly affect the estimates made by the usual equations[48]. In the process of gluconeogenesis, although lactate, pyruvate, and glycerol are possible gluconeogenic substrates, they are not relevant in this context since their conversion to glucose does not imply gas exchange.

Alanine is the most abundant gluconeogenic precursor that can be effectively transformed into glucose in the liver. In a process in which CO_2 is produced without O_2 consumption and whose energy cost should presumably be covered from lipid oxidation. Approximately 0.1g of palmitate is oxidized for each gram of glucose, formed from alanine, to provide the energy cost of this endergonic reaction. Therefore, in those conditions where the gluconeogenic flow is significant, the usual equations for the calculation of the use of substrates will lead to a significant overestimation of glucose oxidation of equivalent magnitude to the novo synthesis of glucose from amino acids, at a very important overestimation of protein oxidation and an underestimation of about 10% in lipid oxidation[48].

In the ketogenesis, the production of ketone bodies is a metabolic process that requires oxygen. Therefore, if the ketone bodies are produced in excess with respect to their oxidation (as occurs in prolonged fasting or in diabetic ketoacidosis), gas exchange and the interpretation of the use of substrates can be affected. If the ketone bodies formed are retained or excreted in urine or through breathing, it is

possible to observe respiratory ratios below 0.7[48]. However, if the ketone bodies produced are subsequently derived towards their complete oxidation, the RQ of the total reaction will be identical to that of the complete oxidation of the precursor fatty acid. Gas exchange correction factors applied to the storage, excretion, and use of ketone bodies have been published[46].

However, in this situation of ketoacidosis, it is difficult to quantify the amount of ketone bodies formed in excess concerning their oxidation to try to correct the gas exchange[46] properly. Still, the measurements may also be altered by changes in the bicarbonate pool that occurred in order to compensate for metabolic acidosis. Once the oxidation rates of glucose, lipids, and carbohydrates have been calculated as described above, the EE can be calculated directly by taking into account the caloric equivalent of the three macronutrients (Table 5; equation 13), from Equations 4, 7 and 8 it follows equation 14. There are moderately different equations depending on the factors used by the different authors, but the most used has been the Weir equation[40]. In the literature, we can find the complete Weir formula (Table 5, equation 15), and the abbreviated Weir formula (Table 5, equation 16).

Table 5: Equations of macronutrients oxidation reactions and energy expenditure

Equations	Eq. No.
$1\,g\,glucose(G) + 0.746lO_2 \rightarrow 0.746lCO_2 + 0.6gH_2O$	(1)
$1\,g\,lipids(Lip) + 2.029lO_2 \rightarrow 1.430lCO_2 + 1.09gH_2O$	(2)
$1\,g\,protein(P) + 0.966lO_2 \rightarrow 0.782lCO_2 + 0.45gH_2O$	(3)
$1gN + 6.04lO_2 \rightarrow 4.89lCO_2 + 2.81gH_2O$	(4)
$VO_2 = 0.746G + 2.029Lip + 6.04N$	(5)
$VCO_2 = 0.746G + 1.430Lip + 4.89N$	(6)
$G = 4.55VCO_2 - 3.21VO_2 - 2.87N$	(7)
$Lip = 1.67(VO_2 - VCO_2) - 1.92N$	(8)
$Glycogen = 4.09VCO_2 - 2.88VO_2 - 2.59N$	(9)
$1g.G \rightarrow 0.52gLip + 0.31lO_2; dG° = +1.22\,kcal/g$	(10)
$1g.G + 0.045lO_2 \rightarrow 0.35gLip + 0.25lCO_2$	(11)
$G = 1.34(VCO_2 - 4.88N)$	(12)
$EE = 3.74G + 9.50Lip + 4.10P$	(13)
$EE = 3.91VO_2 + 1.10VCO_2 - 3.34N$	(14)
$REE = [3.941(VO_2) + 1.106(VCO_2)]x1440 - 2170UN$	(15)
$REE = [3.941(VO_2) + 1.106(VCO_2)]x1440$	(16)

g:grams; G:glucose; l:liter; Lip: lipids; P: protein; EE: energy expenditure; UN: urinary nitrogen; REE: resting energy expenditure (kcal/day); VO_2: volumetric oxygen consumption (L/min); VCO_2: volumetric carbon dioxide elimination (L/min).
*On average, the urea nitrogen excretion averages 0.075 mg/Kg/min of nitrogen, suggesting that the value of UN on equation 15, could be considered negligible[50].

Weir[40] used constants based on the works of Lusk[39] and other authors[49]. In the clinical practice, the use of the Weir abbreviated equation is valid, since the differences between the two are less than 1%-2%, for example, on average, the urea nitrogen excretion averages 0.075 mg/Kg/min of nitrogen, suggesting that the value of UN on equation 15, could be considered negligible[50]. The abbreviated equation of Weir does not include losses of urinary nitrogen. The primary determinant of the formula is VO_2 (a 5% error in its measurement will result in a 3.5% error in energy expenditure); a similar error in the measurement of VCO_2 only derives in a 1.1% error in the final determination. As already noted, a 100% error in the measurement of urea nitrogen will only represent a difference of 1% so that measurement can be neglected[40].

The estimated energy expenditure through IC is subject to the same assumptions and considerations as the calculation of substrate oxidation, even though its estimate

is much more robust. In fact, unlike what happens in the estimation of the use of substrates, the most important determinant of the estimation of the EE is the determination of VO_2. An error of 10% in the determination of VO_2, introduces a 7% error in the estimation of the EE, while an identical error of 10% in the determination of the VCO_2 only produces a 3% error in the estimation of the EE. In contrast, an error of up to 100% in the quantification of the urinary excretion of nitrogen, or even the non-determination of such measurement, will result in an error of only 2% in the estimated EE, which is equivalent to the error of the technique itself[51]. This is easily understood from the intuitive point of view since the EE is determined by the reactants and combustion products independent of the intermediate steps involved.

Special attention should be paid to the conditions of determination to avoid potential sources of error. For example, ambient humidity can alter gas concentrations and interfere with the analyzer response. Ensuring rest, normoventilation and tranquility of the individual is also indispensable and, in general, readings higher than 30 minutes are considered to yield greater precision than shorter readings[52]. This is due, in good part, to the fact that the body's CO_2 reserves are relatively large (unlike those of O_2), so that acute changes in its production can manifest itself with some delay in the expired CO_2 concentrations. Another critical point lies in the necessary initial calibration of the device by means of mixtures of known gases in order to correct any possible alteration in the sensitivity of the analyzers. The last consideration refers to the form of data expression. Since the main determinant of EE is body composition, this metabolic function should be normalized by metabolically active body mass in order to provide comparable data between individuals.

CONCLUSION

In conclusion, this review presents the different methods for analyzing and estimating energy expenditure, facilitating understanding in clinical practice. Indirect calorimetry provides reliable, non-invasive and accurate REE measurements, which is the largest component of TDEE. To estimate and correctly interpret the REE and QR data is very important to have in consideration the calculations of the he O_2 and CO_2 gas fractions. As well as the substrates, oxidation from carbohydrates, lipids and proteins to know if a patient is under some specific metabolic condition, as lipogenesis or lipolysis, depending in the QR value. Finally, this review can be used as a guide, helping to understand the indirect calorimetry technique in studies related to energy expenditure.

Summary of main points
• Total daily energy expenditure (TDEE) is composed of three main components, such as: physical activity (PA), thermal effect of food (TEF) and basal metabolic rate (BMR) and / or resting energy expenditure (REE).
• REE is generally assessed by indirect calorimetry, which also provides information on the respiratory coefficient (QR) and substrate oxidation
• Indirect calorimetry remains the gold standard in the evaluation of REE in the clinical field, to obtain caloric needs and define a precise goal for nutritional therapy.
• The REE may vary according to the patient's metabolism, such as some metabolic disorder, obesity or malnutrition.
• It is necessary to know the concepts of TDEE, its components, and the methodological aspects of indirect calorimetry to provide an adequate nutritional therapy in people with metabolic disorders.

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