**Human calorimetry: Energy expenditure and substrate utilization measurements using a respiratory chamber**

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**Abstract** Respiratory chambers are the current gold standard for assessing human energy expenditure and substrate utilization over a long period of time (several hours to several days), based on oxygen consumption, carbon dioxide production, and urinary nitrogen excretion. Analysis of human energy metabolism using a respiratory chamber provides information about the total energy expenditure (TEE), sleeping metabolic rate (SMR), resting metabolic rate, diet-induced thermogenesis (DIT), activity-induced thermogenesis (AIT), and substrate oxidation. In this review, we describe the theoretical underpinnings of the respiratory chamber, as well as the measurement reproducibility and applications as study endpoints for indirect calorimetry. In humans, the coefficients of variation in energy expenditure and substrate utilization were estimated by 24-h repeatability studies. Under the appropriate conditions, the coefficients of variation for TEE were 1% to 5%, SMR was around 1%, DIT was around 40%, AIT was around 10%, and substrate oxidation was around 5%. Factors that impact energy expenditure and substrate oxidation have been reported, and future weight changes can be predicted based on the 24-h respiratory quotient and substrate oxidation. As the 24-h energy expenditure and substrate oxidation are affected by the 24-h energy balance, it is important to consider the subject’s energy balance prior to and during calorimetry. Accurate measurements of energy and substrate balance (intake minus utilization) will contribute to a better understanding of the conditions that lead to changes in body weight. Properly obtaining measurements using a respiratory chamber requires a thorough understanding of the measurement principles and calculation methods, as well as an appropriate protocol.

**Keywords**: indirect calorimetry, coefficient of variance, energy balance, energy expenditure components, substrate oxidation

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**Introduction**

Interest in measuring energy metabolism has intensified worldwide in recent years due to the increase in obesity and diabetes research1. Elucidation of the pathogenesis of obesity and diabetes will require a detailed understanding of the role of energy metabolism2. The methods of measuring energy metabolism in humans include direct and indirect calorimetry. Indirect calorimetry has become mainstream, however, due to the relative convenience of the devices and the increase in the number of research facilities around the world. The use of Douglas bags, facemasks, ventilated hoods (canopies), and whole-body calorimeters for indirect measurements of respiratory gas exchange depends on the objective of the research. For short-term measurements of energy metabolism, Douglas bags, facemasks, and ventilated hoods are sufficient. For longer-term measurements, however, whole-body calorimetry is performed using a room-type metabolic chamber/respiratory chamber to measure energy metabolism by continuously monitoring oxygen (O2) and carbon dioxide (CO2) concentrations. Changes in the measured O2 and CO2 concentrations allow for a subject’s metabolism to be calculated as O2 consumption (VO2) and CO2 production (VCO2). VO2, VCO2, and urinary nitrogen excretion (N) allow for the calculation of energy expenditure as well as the simultaneous calculation of substrate utilization. The ability to measure energy expenditure and substrate utilization with a high degree of accuracy and continuously over a long period (~24 h) is the unique advantage of the respiratory chamber. The advantages and disadvantages of measurements using a metabolic chamber, described previously by Murgatroyd et al.3, are listed in Table 1. Although it has been nearly 20 years since the report

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by Murgatroyd et al. was published, the basic features remain essentially the same today. The objective of the present review is to describe the theory, methodology, and measurement variation of the respiratory chamber, and to discuss the current trends and future aims of research related to measurements using a respiratory chamber and their significance.

**Principles of measurements in a respiratory chamber**

Pettenkofer developed the first human respiratory chamber in the 19th century. Since then, significant progress has been made in terms of implementing more advanced gas analyzers, data acquisition systems, and computing to facilitate measurement and improve response time. The typical respiratory chamber is a small airtight room with a volume of 15,000 to 30,000 liters, and with strict control of the temperature, humidity, and air flow rate. The chamber contains facilities for living, such as a bed, desk, chair, TV, telephone, toilet, and washbasin (Fig. 1). Subjects are allowed to exercise using a treadmill, bicycle ergometer, or other equipment. Meals and beverages are passed to the subject through an air-locked pass box, and fecal and urine samples from the subject are passed out through another pass box. Subjects remain in the room from several hours to several days, and follow daily life activities similar to their free-living activities.

Respiratory chambers based on indirect calorimetry were recently developed to measure the energy expenditure and substrate oxidation in humans. Indirect calorimetry is a simple measurement method that quantitatively measures O₂ and CO₂ concentrations from a subject’s gas exchanges. Early respiratory chambers comprised a sealed system called a closed-circuit design, but the majority of modern devices employ an open-circuit design. The basic measurement concept of an open-circuit design respiratory chamber is the same as that of metabolic carts, such as facemask or hood systems. Outside air is continuously supplied to the respiratory chamber, mixed within the chamber so that it becomes uniform with the air breathed out by the subject, and drawn from the outlet. The flow rate, and O₂ and CO₂ concentrations at the inlet and outlet are continuously measured using a magnetic O₂ sensor, infrared CO₂ sensor, or mass spectrometer.

A detailed analysis of the gas (O₂ and CO₂) exchange in open-circuit indirect calorimetry was reported by Brown et al. in which they derived equations for calculating a subject’s VO₂ and VCO₂ in an open-circuit pull-type indirect calorimeter:

$$ R_G = F_0 f_{N_2} f_{O_2} / f_{N_2} + V_d / d(f_{O_2}) - f_{O_2} f_{N_2} V_d / d(f_{N_2}) $$
where $F$ is the flow rate in l/min, $f$ is fractional concentration, $R$ is rate of gas production in l/min, $t$ is time in min, $V$ is chamber volume in liters, $i$ is incoming, $o$ is outgoing, and $G$ is any gas (volume and flow rate were assumed to be corrected to standard temperature, pressure, dry [STPD]). Several groups have developed algorithms based on fundamental equations that yield an improved transient response and suppress measurement noise in the respiratory chamber. Various factors can introduce error into actual measurements, however, such as temperature, humidity, and air pressure. To eliminate these error factors, it is important to compare the results against standard values obtained with devices using a gas infusion test or alcohol combustion test.

### Calculation of energy expenditure and substrate utilization

The $\dot{V}O_2$, $\dot{V}CO_2$, and protein oxidation ($P$) estimated from $N$ of a subject ($P = 6.25*N$), while in the respiratory chamber, are used to calculate the subject’s energy expenditure and substrate utilization. Several formulas are available for determining a subject’s energy expenditure, but the most commonly used formula is that used by Weir et al.

Energy expenditure (kcal) = $3.941* \dot{V}O_2 (L) + 1.106* \dot{V}CO_2 (L) - 2.17*N (g)$

Protein correction is equal to a deduction of 1% when 12.3% of the total calories come from $P$ and, therefore, is estimated with the following formula:

Energy expenditure (kcal) = $3.9* \dot{V}O_2 (L) + 1.1* \dot{V}CO_2 (L)$

With the exception of special cases, $P$ is similar to protein intake, and thus this formula can be used in almost all cases. Another formula used to calculate energy expenditure is that developed by Brouwer et al.

The formula used to determine fat and carbohydrate (CHO) oxidation varies depending on the researcher; however, in general, the formulas are based on the respiratory quotient ($RQ = \dot{V}CO_2 / \dot{V}O_2$). The $RQ$ ranges from 0.7 to 1.0 in humans; CHO oxidation is greater as $RQ$ approaches 1.0, and fat oxidation is greater as $RQ$ approaches 0.7.

**Equation from Jequier**:  
CHO oxidation (g) = $4.113* \dot{V}CO_2 (L) - 2.907* \dot{V}O_2 (L) - 0.375*P (g)$  
Fat oxidation (g) = $1.689* \dot{V}O_2 (L) - 1.689* \dot{V}CO_2 (L) - 0.324*P (g)$

**Equation from Brouwer**:  
CHO oxidation (g) = $4.170* \dot{V}CO_2 (L) - 2.965* \dot{V}O_2 (L) - 0.390*P (g)$

Fat oxidation (g) = $1.718* \dot{V}O_2 (L) - 1.718* \dot{V}CO_2 (L) - 0.315*P (g)$

**Equation from Livesey & Elia**:
CHO oxidation (g) = $4.650* \dot{V}CO_2 (L) - 3.311* \dot{V}O_2 (L) - 3.518*N (g)$  
Fat oxidation (g) = $1.720* \dot{V}O_2 (L) - 1.720* \dot{V}CO_2 (L) - 1.776*N (g)$

**Equation from Ferrannini**:
CHO oxidation (g) = $4.55* \dot{V}CO_2 (L) - 3.21* \dot{V}O_2 (L) - 2.87*N (g)$  
Fat oxidation (g) = $1.67* \dot{V}O_2 (L) - 1.67* \dot{V}CO_2 (L) - 1.92*N (g)$

The difference between each formula is the difference between the $\dot{V}O_2$ and the $\dot{V}CO_2$ used for each nutrient in the tests. For example, the $RQ$ is the same for glucose and starch, but the $\dot{V}O_2$ and $\dot{V}CO_2$ differ. Samples containing fats and proteins also have different $\dot{V}O_2$ and $\dot{V}CO_2$ values. The equation for measuring substrate oxidation should be selected based on consideration of the dietary constituents. In addition, indirect calorimetry can be combined with other research methods. For example, tracer techniques can be used with a respiratory chamber to measure the oxidation turnover rate of various substrates.

### Assessment of physical activity in the respiratory chamber

Spontaneous physical activity of subjects in the respiratory chamber can be measured with a radar system based on the Doppler effect (Doppler radar) or microwave. The radar system records the amount of activity as the rate of activity per unit time over a certain interval rather than as a continuous record of activity intensity. Records of the amount of activity in the respiratory chamber are important for identifying abnormal activity in the chamber and for calculating the constituents of energy expenditure outlined below. In a number of recent cases, this radar system was replaced with, or run simultaneously with, an advanced accelerometer to measure the subject’s activity.

### Applications

**Total energy expenditure.** Total energy expenditure (TEE), also referred to as daily energy expenditure, is the simplest measurement value commonly applied in research using the respiratory chamber, and is considered one of the most appropriate endpoints for this measurement method. The reproducibility of TEE has been validated by repeated studies. The coefficient of variation (CV) obtained with repeated measurements from
an individual subject using a respiratory chamber over a 24-h period ranges from 1% to 5%. Spontaneous physical activity is limited in the respiratory chamber, which may explain the low CV of 24-h TEE²⁹. Although, the CV for spontaneous physical activity in the respiratory chamber is around 8% to 10%²¹,²⁷,³⁰, the CV for 24-h TEE is essentially the same after adjusting for day-to-day differences in spontaneous physical activity²⁸.

TEE measurement using a respiratory chamber is the most accurate method of measuring energy balance (energy intake minus energy expenditure) and is used to describe changes in body weight³¹,³². The features of the respiratory chamber are used to study the effects of controlling food quantity and quality, exercise, drugs or food ingredients, and environmental factors (temperature and humidity)³³. In addition to subjects suffering from obesity, identified by clear increases in body weight, TEE can also be measured to estimate changes in body weight in subjects with Alzheimer’s Disease³³, Huntington’s Disease³⁴, hyperthyroidism³⁵, diabetes³⁶, and a range of other diseases.

Components of total energy expenditure (resting metabolic rate, diet-induced thermogenesis, and activity-induced thermogenesis). Energy expenditure can be categorized into three main constituents based on information regarding the amount of activity simultaneously measured in the respiratory chamber: basal metabolic rate (BMR), diet-induced thermogenesis (DIT), and activity-induced thermogenesis (AIT)³⁷. A linear graph of energy expenditure, calculated over a 15- to 30-min time period, is plotted against the amount of activity measured using the radar system within the same time period, and the resting metabolic rate (RMR) is then obtained by extrapolating the interception point when the amount of activity is zero. AIT is calculated by subtracting the RMR from the TEE, while DIT is calculated by subtracting the BMR from the RMR (Fig. 2). BMR, the largest component of TEE comprising 60% to 70%, is the energy expenditure of an individual after a 12- to 14-h overnight fast during a period of physical rest in a thermoneutral environment, and reflects energy use for such basic functions as maintenance of the human body. Frequently, “resting energy expenditure” is measured in preference to BMR. Resting energy expenditure is quantitatively similar to the BMR, but is not subject to all the exacting requirements of BMR³⁸. BMR is usually measured using metabolic carts with hoods or masks, but there are reports that similar CV has been identified during BMR measurements using a respiratory chamber (CV = 5.0%)²⁴. Compared to TEE, however, BMR is less reproducible and has a significantly larger CV²⁹. The lower degree of reliability for BMR is thought to be related to the short time period of measurement³⁰.

DIT, a term commonly used to describe the thermic effect of food, is defined as the increase in energy expenditure after food ingestion, and is calculated using the measurement for resting energy expenditure after eating³⁹. DIT is measured using a respiratory chamber according to the method of Schutz et al.³⁷, as described above; the reproducibility of this method within individuals is 43% to 48%³¹,³⁰. The reproducibility is low compared to that of measurements obtained using a metabolic cart (6% to 30%)⁴¹,⁴², possibly related to methodologic issues. Measurements made using a respiratory chamber can be obtained over a long period of time and are based on a continuous stream of data. Therefore, this method is extremely valuable for obtaining DIT information, and improved DIT measurement methods were recently reported²²,⁴⁰.

AIT is the index of energy expenditure resulting from activity in a respiratory chamber. As the respiratory chamber has limited space, the AIT is lower compared to that during a normal non-restricted lifestyle; however, it is reported to correspond to activities conducted without any restrictions in day-to-day life⁴³. AIT can be categorized as exercise energy expenditure and non-exercise activity thermogenesis, which is derived from activities based on day-to-day life³⁹. Exercise energy expenditure involves measurements with many changes over a short period of time, so using a mask or Douglas bag is considered ideal; however, respiratory chambers are used to observe subsequent changes over a long period of time. Non-exercise activity thermogenesis is one of the constituents of energy expenditure that varies most between individual subjects and is considered a major factor for determining energy balance.

Sleeping Metabolic Rate. Measuring sleeping energy expenditure is one of the major features of a respiratory chamber. The sleeping energy expenditure of subjects can only be measured with a respiratory chamber under conditions that are similar to daily life without affect-
Sleeping energy expenditure decreases by around 10% compared to resting, likely due to the decrease in energy cost of arousal. Sleep among humans is generally categorized into rapid-eye-movement (REM) or non-rapid-eye-movement (NREM); and NREM sleep is categorized into stages 1 to 4. A constant relationship is observed between the categorized stages of sleep and energy expenditure. Studies using a facemask, ventilation hood, or small sleep-calorimeter have demonstrated lower energy expenditure during deep NREM sleep (stages 3 and 4) compared to that during REM or stages 1 and 2 energy expenditure during the time period when the amount of activity is the lowest, or as the morning energy expenditure when waking consciousness is at its lowest. Accordingly, the reproducibility of 24-h substrate oxidation in repeated measurements of an individual subject when dietary conditions are not controlled prior to measurements, is worse with a CV of 2.6% for RQ, 24.9% for fat oxidation, and 15.3% for CHO oxidation. It is important that subjects maintain their achieved energy balance when estimating substrate oxidation while in the respiratory chamber.

The most important information related to substrate oxidation in humans was reported by Zurlo et al., who stated that the 24-h RQ can be used to predict weight change. A 24-h RQ measurement, using a respiratory chamber in Pima Indians without diabetes, was positively correlated with body weight after 25 months; and subjects with low daily fat availability were predicted to have increased body weight in the future. Pannacciulli et al. also observed a correlation between 24-h RQ and CHO oxidation and subsequent amount of ingestion, and postulated this as the mechanism for increases in body weight.

A small positive energy balance (50 – 100 kcal) in free-living life can increase body weight over the long-term. The use of respiratory chambers to accurately measure energy balance can contribute to elucidating the conditions that lead to changes in body weight, including obesity. Measurements obtained using a respiratory chamber, however, are plagued by methodologic problems, excessive errors, and incorrect measurement results related to measurement protocols. To properly obtain measurements using a respiratory chamber, measurement principles and calculation methods must be thoroughly understood, and an appropriate protocol used. For over a century, energy expenditure measurements have been made using respiratory chambers, and the methods used continue to evolve. Proper utilization of these measurement devices and a better understanding of their disadvantages will facilitate new findings.

References
