

Critical Decisions

Continuing
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Dietitians (CPE)
and Respiratory
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Enhancing patient-specific care in the critically ill

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Indirect Calorimetry in the Ventilated Patient

Nutritional requirements are difficult to predict in mechanically ventilated patients, due to their disease processes, inflammatory response, and other variables. However, the development of proper nutrition support and its careful monitoring are vital to optimal patient outcomes. This monitoring requires the expertise of both respiratory therapy and nutritional support teams, and is best accomplished by using indirect calorimetry. Indirect calorimetry (IC) measures oxygen consumption and carbon dioxide production as a way of assessing energy expenditure and thus determining nutritional needs. The use of IC is preferred over estimations (predictive equations) whenever measuring patients is feasible. An understanding of the variables and potential confounders involved in IC is critical in assessing and interpreting its results. The three articles that follow examine IC from both respiratory therapy and nutrition support perspectives, offering the clinicians a comprehensive, interdisciplinary view of how the collective expertise of these groups and the ICU teams can use IC to the patient's greatest advantage. Practice scenarios and a discussion of various IC technologies are also included.

Technical Aspects of Indirect Calorimetry

By Richard D. Branson, MSc, RRT, FAARC, FCCM

Indirect calorimetry (IC) is the calculation of energy expenditure via measurement of oxygen consumption (VO_2) and carbon dioxide production (VCO_2). These determinations require precise measurements of inspired and expired gas concentrations and volume. Several technologies are available for these measurements. However, measurements themselves are not the "be all and end all" of indirect calorimetry. The respiratory therapist's (RT) background and expertise are essential to IC measurements because of his or her understanding of (1) how the measurements are derived, (2) what ICU interventions (e.g., mechanical ventilation, dialysis) can potentially confound the measurements, and (4) the effects of ventilator settings on IC. All this information is crucial for validating results, alerting the multidisciplinary ICU team to potential problems with measurement, helping nutritional support therapy best utilize the IC data, and detecting patient nutritional problems that can exacerbate respiratory-related problems.

Use and Interpretation of Indirect Calorimetry

By Jennifer A. Wooley, MS, RD, CNSD

Malnutrition is prevalent in critically ill patients. The metabolic stress of acute illness superimposed on malnutrition is associated with negative patient outcomes and increased healthcare costs. As part of a comprehensive nutrition assessment, determining energy expenditure by a registered dietitian provides the framework for creating and modifying nutritional support in critically ill patients. IC is the gold standard for determining energy expenditure. The measured REE is an objective, patient-specific caloric reference that serves as the most accurate method of determining energy expenditure. Protocols addressing IC methodology are necessary to ensure technical accuracy and clinically useful results. The measured REE should be the caloric target without the addition of stress or activity factors for nutrition support regimens in the ICU. Optimal nutrition intervention requires continuous evaluation of all pertinent clinical data and monitoring of each patient's response to metabolic stress and therapeutic nutrition interventions.

Ensuring Adequate Nutrition Support for the Ventilated Patient: An Interdisciplinary Approach

By Jorge Rodriguez, BSRC, RRT, and Sharla Tajchman, PharmD, BCPS, BCNSP

Nutrition Support Teams (NST) have a long-standing history of improving the quality of nutrition care in hospitalized patients requiring parenteral nutrition (PN). These multidisciplinary teams usually consist of some combination of a physician, nurse, dietician, and/or pharmacist, all specially trained in the area of nutrition support to manage the provision of specialized nutrition support within healthcare institutions. An important but often overlooked addition to any NST is a respiratory therapist (RT), who is trained to screen patients and perform indirect calorimetry. These individuals can provide significant insight into a patient's nutritional requirements via IC.

Technical Aspects of Indirect Calorimetry

By Richard D. Branson, MSc, RRT, FAARC, FCCM

Indirect calorimetry (IC) is the calculation of energy expenditure via measurement of oxygen consumption (VO_2) and carbon dioxide production (VCO_2). These determinations require precise measurements of inspired and expired gas concentrations and volume. Several technologies are available for these measurements. While measurements themselves are not the “be all and end all” of indirect calorimetry. The respiratory therapist’s (RT) background and expertise are essential to IC measurements because of his or her understanding of (1) how the measurements are derived, (2) what ICU interventions (e.g., mechanical ventilation, dialysis) can potentially confound the measurements, and (3) the effects of ventilator settings on IC. All this information is crucial for validating results, alerting the multidisciplinary ICU team to potential problems with measurement, helping nutritional support therapy best utilize the IC data, and detecting patient nutritional problems that can exacerbate respiratory-related problems.

How IC measurements are derived

The measurements of VO_2 and VCO_2 are converted to **energy expenditure** (Kcal/day) by application of the Weir equation:¹

$Energy\ expenditure\ (kcal) = [(VO_2\ L/min)(3.941) + (VCO_2\ L/min)(1.11)] 1,440\ min$
where VO_2 and VCO_2 are expressed in L/min and 1,440 is the number of minutes in a day.

A second important determination obtained from indirect calorimetry is the **respiratory quotient** (RQ), which is the ratio of the volume of CO_2 (VCO_2) given off by the body tissues to the volume of O_2 (VO_2) absorbed by them; this usually equals the corresponding volumes given off and taken up by the lungs. ($RQ = VCO_2 / VO_2$). In humans, RQ resides in a fairly narrow range (0.67 to 1.2)². Values outside this range suggest the presence of technical errors in measurement. The RQ can help determine net substrate utilization and can also serve as a quality control indicator. An $RQ \geq 1.0$ indicates lipogenesis; however, RQ alone cannot pinpoint which substrates are oxidized.

Determining energy expenditure and RQ helps

Conceptually, indirect calorimetry is simple: you measure the concentration and volume of gas inhaled and determine the difference from the concentration and volume exhaled (VO_2 and VCO_2).

in prescribing nutrition support for patients and modifying nutrition based on the results. This also provides the RT with valuable information about respiratory physiology. Thus, the two groups of professionals work hand in hand in interpreting and using the results. For example, malnutrition is common in patients with chronic lung disease, where excessive calories (particularly from glucose) may worsen hypercapnia. The measurement of oxygen consumption may also be helpful during weaning. In the ICU, indirect calorimetry is commonly accomplished with a stand alone-device (metabolic cart³) or newer ventilator technology that contains an integrated calorimeter (e.g., CareStation, GE Healthcare). Interpreting indirect calorimetry requires a thorough understanding of nutritional support, metabolic function, metabolic disorders, and respiratory physiology. Maximizing the utility of indirect calorimetry requires a team approach including a physician, nutritionist, and respiratory therapist.

Theory and Calculations

Conceptually, indirect calorimetry is simple: you measure the concentration and volume of gas inhaled and determine the difference from the concentration and volume exhaled (VO_2 and VCO_2). This seemingly straightforward concept is technically complex and fraught with potential errors. The required measurements are inspired (_I) and expired (_E) concentrations of oxygen (FIO_2 , FEO_2), carbon dioxide (F_{ICO_2} , F_{ECO_2}) and inspired and expired minute ventilation (V_I , V_E). In an ideal situation, VO_2 is calculated by the equation:

$$VO_2 = V_I(FIO_2) - V_E(F_{EO_2})$$

and VCO_2 by a similar equation:

$$VCO_2 = V_E(F_{ECO_2}) - V_I(F_{ICO_2})$$

However, because it is technically difficult to measure the small differences between V_I and V_E resulting from the respiratory exchange ratio (RER), V_I is commonly calculated using the Haldane transformation, which assumes that nitrogen (N_2), a relatively insoluble gas, is constant in both inspired and expired gases. Failure to account for the RER—and assuming that V_I equals V_E —can lead to clinically significant errors in VO_2 measurement.⁴

The denominator of the equation that accounts for RER is $1 - FIO_2$, so a measurement of $FIO_2 = 1.0$ is impossible ($1 - 1 = 0$). Because the denominator gets smaller as FIO_2 approaches 1.0, this magnifies small errors in gas concentration. Later sections of this article discuss the clinical ramifications of this in more detail.

Because CO_2 in inspired gases are negligible, the equation for VCO_2 can be simplified to:

$$VCO_2 = V_E(F_{ECO_2})$$

Technical Considerations of IC

Indirect calorimeters are generally easy to understand and operate. All calorimeters include gas analyzers (O_2 and CO_2) and a flow/volume measuring device. The analyzers must be capable of measuring minute changes in gas concentrations (0.001%) in room air and oxygen-enriched environments, as just described. The volume-measuring device must be capable of accurately measuring volumes across the clinically expected range. The difficult aspects of indirect calorimetry generally relate to the (1) patient interface, (2) presence of elevated inspired oxygen concentrations, and (3) sophistication of mechanical ventilators.

A host of variables must be taken into account to ensure accurate measurement: (1) the stability and absolute level of FIO_2 , (2) separation of inspired and expired gases, (3) complete collection of expired gases, and (4) handling of water vapor. These variables are summarized in Table 1 and are detailed in the following sections. In addition, the individual components of the system (e.g., gas analyzers and flow-measuring devices) must be calibrated and tested against known standards.

Effects of FIO_2

A number of authors^{8,9} describe in detail the untoward effects of elevated FIO_2 on VO_2 measurements. Error is introduced when FIO_2 approaches 1.0 and the denominator of the Haldane equation ($1 - FIO_2$) approaches zero. (Division by zero yields infinity.) Additionally, any error in measurement of gas concentrations is amplified as FIO_2 increases. A 1% error in FIO_2 measurement at an FIO_2 of 0.40 results in a 15% error in VO_2 measurement. At an FIO_2 of 0.80 or more, the same 1% error results in an error almost equal to the actual VO_2 value.⁴ At FIO_2 of 0.60–0.80, the error in measurement increases to 10–15%; VO_2 measurements at FIO_2 above 0.80 are highly suspect.

Several solutions to the problem of FIO_2 stability have been suggested. These include (1) accurate measuring of V_I with a volume-monitoring device at the airway, (2) improving O_2 sensor design, and (3) increasing the frequency with which samples are analyzed for gas concentrations.⁴⁻⁷

During IC measurements at elevated O_2 concentrations, the stability of the O_2 concentration is also critically important. Browning et al.¹⁰ demonstrated the deleterious effects that fluctuating FIO_2 has on metabolic measurements at elevated O_2 concentrations. Because many IC systems (i.e., metabolic carts) only measure FIO_2 periodically, FIO_2 changes between FIO_2 analysis and expired-gas collection will cause erroneous VO_2 measurements. Ventilators with IC capabilities built into them have the advantage of the ventilator flow/volume monitoring and gas analyzers being in phase. The continuous measurement of inspired and expired concentrations also helps limit the effects of fluctuations in delivered FIO_2 .

The greater the demand for flow (high minute volume, use of pressure control ventilation, and high PEEP), the greater the FIO_2 instability. The presence of patient ventilator dyssynchrony or the patient “fighting the ventilator” stresses the

Understanding ventilator operation and performance is the key to interfacing the IC and the ventilator.

ventilator’s flow delivery system and tends to increase FIO_2 variability. This underscores the importance of respiratory care practitioners in effectively using indirect calorimetry. Understanding ventilator operation and performance is the key to interfacing the IC and ventilator.

Placing a dry humidification chamber between the ventilator outlet and the humidifier has been shown to reduce FIO_2 fluctuation.¹¹⁻¹³ The dry chamber serves as an inspiratory mixing chamber, alleviating FIO_2 instability.

Effects of Anesthetic Gases

The presence of anesthetic gases invalidates open-circuit technique accuracy. Error is introduced into the Haldane equation when gases other than N_2 , O_2 , and CO_2 are present.^{14,15} Thus, IC should not be performed on post-operative patients until anesthetic agents are eliminated from their systems. Nitrous oxide diffuses into endotracheal tube cuffs during surgery but is usually gone after 3–4 hours.

Separation of Inspired and Expired Gas

Separation of inspired and expired gases is also crucial for measurement accuracy. This is particularly important in systems with any type of continuous flow. If continuous flow is allowed to enter the mixing chamber along with expired gases, then measured V_E will drastically increase; this will dilute gas concentrations. Generally speaking, if the bias flow for flow triggering exceeds 10 L/min, the measurement will be invalid. Non-rebreathing valves placed at the airway help solve this problem.^{16,17} Caution should be used to ensure that any valve system placed between the patient and ventilator does not adversely affect the work of breathing.¹⁸

Another complication of new ventilator technology is the so-called “active” exhalation valve. The active exhalation valve allows the patient to inhale or exhale during the ventilator breath. This increases patient comfort; however, exha-

lation during the inspiratory phase can confuse the calorimeter. The mixing of inspired and expired gases tends to result in falsely elevated V_E and VCO_2 , while causing RQ to fall—often to unphysiologic levels. When the calorimeter is built into the ventilator, it offers the RT a potential advantage of combining knowledge of ventilator operation and gas analysis.

The use of high levels of PEEP worsens FIO_2 stability, and it also may create unstable expiratory flow. In some instances, this results in a staggered or choppy expiratory flow. In these cases (usually PEEP > 12 cm H_2O), placement of a disposable PEEP valve in the tubing leading to the calorimeter can be helpful. The PEEP valve should be equivalent to set PEEP. Exercise caution if this is attempted, because the exhalation valve of many new ventilators is sensitive to pressure differentials across it; auto-triggering of the ventilator or ventilator malfunction could result. When indirect calorimetry is built into the ventilator, this problem is avoided.

System Leaks

During measurement, the ventilator circuit and tubing to the calorimeter must be leak-free. Any loss of gas to the environment or entrainment of room air results in erroneous data. All expired gases must also be collected. Patients with incompetent endotracheal tube cuffs, leaking chest tubes, or bronchopleural fistulas lose expired gases to the environment. Such leaks result in a decreased V_E and F_{ECO_2} and lead to underestimation of VO_2 , VCO_2 , and resting energy expenditure (REE).¹⁹ In this instance, the RQ may be correct; but as a rule, we are cautious in performing and interpreting indirect calorimetry in patients with leaks.

Sensors

Appropriate sensors that are capable of responding quickly to changes in gas concentrations but that also maintain reproducibility and stability are required. Paramagnetic oxygen sensors have a fast response time and are stable across a range of FIO_2 . CO_2 analyzers are generally infrared sensors with excellent stability and reliability. Volume-monitoring devices should be linear across a wide range of flows. Errors in measurement at either the low or high end of the volume range may make the device unsuitable for use in pediatrics and at high V_E .

Performing Energy Expenditure Measurements

What to Measure?

What is to be measured is resting energy expenditure (REE). As defined by Weissman et al.,²⁰ REE occurs when the patient is lying in

bed, awake, and aware of their surroundings. Of course, this cannot always be achieved with ICU patients. The objective is to make the measurement so that comparisons with future measurements made under the same conditions will be meaningful.

When to Measure?

Enteral or parenteral nutrition need not be stopped during the measurement, but it is important to prevent interaction between patient and caregivers or visitors during measurement. Weissman et al.²⁰ have shown significant alterations in VO_2 , VCO_2 , and REE during routine ICU procedures. Attempt to perform the measurement when the environment and attendants least disturb the patient. Timing is far more important than operator convenience; this underscores the importance of the respiratory therapist in making energy expenditure measurements.

Advanced ventilators containing an integrated IC module can generate real-time metabolic measurements, e.g., Engström Carestation, GE Healthcare. When an IC module is incorporated into a mechanical ventilator, the metabolic measurement may be easier to accomplish at the desired time, as there is no wait for the device. This type of ventilator also enables the therapist to view IC trends over several days, providing insight into the patient’s metabolic needs while nutrition support is provided and/or the patient’s medical condition changes. The disadvantage may be the cost of multiple devices integral to the ventilator.

Additional Considerations: physiological confounders

Blood filtration

Other factors can influence the accuracy of measuring energy expenditure. Avoid measurements during hemodialysis and peritoneal dialysis. In both situations, the filtration process removes CO_2 , resulting in inaccurate RQ and underestimation of total energy expenditure.²¹ Similarly, the use of continuous veno-venous hemofiltration (CVVH) in critically ill patients can obfuscate measurements because CO_2 is

Advanced ventilators containing an integrated IC module can generate real-time metabolic measurements.

removed through the filter. Patients typically receive CVVH 24 hours a day, making indirect calorimetry inaccurate and difficult to interpret. Because the body’s CO_2 stores are approximately 10x that of O_2 stores,²² CO_2 homeostasis may require several hours to be restored after dialysis ends. The use of bicarbonate in the dialysis fluid may reduce errors in indirect calorimetry, but this has not been studied systematically.

Hyperventilation

Hyperventilation can also alter measurements of VCO_2 , RQ, and REE.²³ The body stores approximately 16,000 mL of CO_2 ; of that, 13,000 mL is present in bone (relatively unavailable) versus what is stored elsewhere in the body. This reservoir of CO_2 can be tapped during hyperventilation. After a change in minute ventilation, up to 2 hours is required to establish a new equilibrium. Measurements of energy expenditure should be postponed until at least that long after changing the patient’s minute ventilation.²²⁻²⁴

How Long to Measure?

The short answer is “long enough.” More specifically, measure until the desired state is achieved. This is < 15 minutes in some patients and up to several hours in others. If the goal is to measure REE, then the measurement should continue until the resting state is maintained for 10 to 15 minutes. Only observation of the patient and subsequent comparison of the data

can reveal when REE is achieved.

In recent years, the idea of the “abbreviated” metabolic measurement has been advanced.²⁵⁻²⁷ Proponents have demonstrated that a steady-state period of 5 minutes is sufficient to predict 24-hour REE. This is in contrast to other authors who recommend from 30 minutes to 2 hours.²⁸⁻³⁰ Our own experience is that the stability of the patient and the presence or absence of all the confounding factors previously discussed dictate the time of measurement. The literature appears to support an abbreviated period of steady state lasting 5 minutes. Our own preference is to achieve a steady state of 5–10 minutes, during which the operator verifies the correct equipment setup and operation and observes the patient’s clinical condition. If you are measuring IC from a module built in to a patient’s ventilator, trending information over several days can help you verify the veracity of an “abbreviated” metabolic measurement.

How Often To Measure?

The frequency of measurement is also not completely clear. Weissman et al.³¹ found that most patients have a day-to-day REE variation of 5–15%. A smaller group of patients demonstrated REE variances as much as 50 to 60% from one day to the next. Weissman suggested measuring the more unstable patients frequently (2–3 times/wk) and the stable patients weekly. Unstable patients include those with spiking fevers, hemodynamic instability, and immediate postoperative patients. We preferentially rely on the nutritional support team’s expertise in identifying unstable patients and those not responding to current therapy as those who need more frequent measurements.

Infection control

Observe normal universal precautions during metabolic measurements. When using a stand-alone device, protect the system from the patient’s expired gas by using a filter at the inlet of the calorimeter.³² The filter should have a low resistance to gas flow and be changed frequently to prevent moisture buildup, which increases flow resistance. A system integral to the ventilator eliminates the concern of cross-contamination between patients.

Summary

Indirect calorimetry is a valuable tool to help assess energy expenditure in intensive care patients. A thorough understanding of the variables associated with IC measurements and what circumstances can confound results can help optimize patient care and minimize confusing or erroneous results.

Table 1. Impact of ventilator operation and ventilation issues on REE and RQ

	VO_2	VCO_2	REE	RQ
Leaks	Low	Low	Low	Unchanged
Unstable FIO_2	Low or high	Unchanged	Low or high	Low or high
$FIO_2 > 80\%$	Low or high	Unchanged	Low or high	Low or high
Mixing of inspired & expired gas (active exhalation valve)	Normal or high	Low	Normal or low	Low

Abbreviations used throughout this issue.

CVVH	Continuous veno-venous hemofiltration
FECO ₂	Fractional expired concentration of carbon dioxide
FEO ₂	Fractional expired concentration of oxygen
FICO ₂	Fractional inspired concentration of carbon dioxide
FIO ₂	Fractional inspired concentration of oxygen
N ₂	Nitrogen
NST	Nutrition support therapy
PEEP	Positive end-expiratory pressure
PN	Parenteral nutrition
POD	Post-operative day
REE	Resting energy expenditure
RER	Respiratory exchange ratio
RQ	Respiratory quotient
VCO ₂	Carbon dioxide production
V _E	Expired minute ventilation
V _I	Inspired minute ventilation
VO ₂	Oxygen consumption

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Use and Interpretation of Indirect Calorimetry

By Jennifer A. Wooley, MS, RD, CNSD

Malnutrition is prevalent in critically ill patients; it can be present prior to admission to the intensive care unit (ICU) or can develop over the course of the patient's critical illness.^{1,2} The role of nutrition support therapy (NST) in combating malnutrition in the hospitalized patient is well recognized. Nutritional status is vital to overall immune function and the ability to mount a stress response.³ The metabolic stress of acute illness superimposed on malnutrition is associated with negative patient outcomes and increased healthcare costs.^{4,5} Patients with a negative cumulative energy balance have a higher rate of ventilator dependence, remain in the ICU longer, and experience higher mortality rates than those with a positive energy balance.⁶⁻⁹

As part of a comprehensive nutrition assessment, determining energy expenditure by a registered dietitian provides the framework for creating and modifying NST in critically ill patients. There are two ways to calculate energy expenditure: by predictive equations (estimates) or measurement. The former is more convenient and is used more frequently—despite its well-documented limitations compared to measurement.¹⁰ Measurement of indirect calorimetry (IC) is the gold standard for determining energy expenditure in critically ill patients.¹

Estimation versus Measurement of Energy Expenditure

More than 200 predictive equations have been developed over the past 50–80 years. All of them fall short of including or accurately reflecting the multitude of variables present in patient populations, including body composition, nutrition status, life span, and ethnicity.¹¹ This underscores the problems inherent with such equations. Furthermore, no consensus exists on how clinicians select predictive equations, so results obtained from using them vary considerably from clinician to clinician.¹² Reportedly only 14% to 32% of patients receive nutrition regimens providing caloric delivery within 10% of their requirements when predictive equations are used.^{3,13}

IC is the most accurate method for determining energy expenditure,^{12,14-16} as it does not suffer from the limitations and endless variables that predictive equations do. IC-based nutrition regimens avoid negative consequences associated with under- and overfeeding. Underfeeding leads to deterioration of lean body mass, immunosuppression, poor wound healing and increased risk of nosocomial infection.¹⁷ Overfeeding leads to lipogenesis, hyperglycemia, and exacerbated respiratory failure by increasing oxygen (O₂) consumption and carbon dioxide (CO₂) production, which drives up minute ventilation.¹⁷

Despite its benefits, IC is underutilized in the clinical setting. This is partially due to its expense, poor insurance reimbursement for inpatients, and lack of clinical expertise necessary to conduct measurements, interpret results and successfully translate the information to patient care. IC is somewhat controversial even among nutrition support professionals, because no prospective randomized controlled trials have demonstrated that the use of IC directly improves patient outcomes.¹⁶ However, these obstacles can be overcome and the full benefits of IC can be realized by using a standardized approach to IC application and interpretation.

Basic principles of indirect calorimetry

Many authors have described the basic science of IC extensively.^{10,11,15,18-20} This article focuses on the practical application of IC in patient care. Indirect calorimetry calculates heat production through measurement of pulmonary gas exchange. Measurements include minute ventilation, temperature, and pressure because of their effect on gases. IC measures inspired and expired oxygen and carbon dioxide in order to calculate resting energy expenditure (REE) and respiratory quotient (RQ) using the abbreviated Weir equation,^{15,16,20} where:

$$\text{REE (Kcal/d)} = \left[(\text{VO}_2 \times 3.94) + (\text{VCO}_2 \times 1.11) \right] \times 1440 \text{ minutes/day}$$

$$\text{RQ} = \frac{\text{VCO}_2 \text{ (carbon dioxide production)}}{\text{VO}_2 \text{ (oxygen consumption)}}$$

Historically, a 24-hour urine collection to mea-

sure urinary nitrogen excretion was included in conducting IC measurement. This was an attempt to account for the contribution of protein substrates to energy expenditure. However, this is not necessary to ensure accuracy in measuring REE.^{10,11,18-21} Inaccurate UUN measurements have been shown to produce an error of only 1–2% in the measurement of true energy expenditure.^{19,21} Eliminating this cumbersome and often inaccurate step promotes both cost- and time-management efficiency related to IC.

Equipment options

When choosing equipment to perform IC, many options are available: (1) the traditional “metabolic cart,” (2) handheld devices, and (3) built-in metabolic modules that interface directly with a patient's mechanical ventilator to measure REE and RQ on a continuous basis. An interdisciplinary group including a physician, respiratory therapist, dietitian, nurse, and pharmacist should collectively make decisions regarding the selection of an indirect calorimeter. There are no hard and fast rules for IC equipment selection, but being able to calibrate and validate the device is essential.²²

Indications for indirect calorimetry

Indications for conducting IC include (1) the inability to estimate accurately caloric requirements (see Table 1), (2) an inadequate clinical response in the patient when using predictive equations, and (3) the presence of clinical signs suggesting either under- or overfeeding. Underfeeding impairs the regeneration of respiratory epithelium and is associated with muscular weakness.^{23,24} It may prolong ventilator dependence by failing to replete respiratory muscle strength and endurance.²³ In contrast, overfeeding exacerbates metabolic stress and also prolongs ventilator dependence by increasing the work of breathing.^{17,24,25-27}

Conducting indirect calorimetry measurements

IC is relatively easy to perform and is non-invasive. It can be conducted intermittently or continuously. “Snapshot” studies, more common than continuous monitoring, are done in 30 minutes or less and provide a mean REE and RQ that is extrapolated to a 24-hour day. Some technical factors can interfere with accurate measurements; for example, air leaks result in the loss of oxygen and carbon dioxide volumes, with an associated reduction in the measured REE.²¹

Interpreting information obtained from indirect calorimetry

Assessment of steady state

Two pieces of information are obtained from an IC study: measured REE and RQ. The first step in interpreting an IC study is to evaluate its validity.^{10,15,20,26} The respiratory therapist and the dietitian closely examine the methodology of IC measurement in assessing achievement of steady state.

The optimal duration of an IC measurement has not been well defined.²⁵⁻²⁷ Studies are performed until (1) a 5-minute “steady state” period is achieved or (2) a predetermined time (approximately 30 minutes) is assessed—if steady state is not achieved.^{16,31-35} Technically, steady state means that the patient’s ventilatory status, acid-base balance, and CO₂ production are stable with very little variation. Thus, steady state is a 5-minute period of “metabolic equilibrium,” where VO₂ and VCO₂ change by less than 10% or the coefficient of variation for the measured REE and RQ is less than 10%.^{10,11,33,36-38}

$$\text{Coefficient of Variation} = \frac{\text{Standard deviation}}{\text{Mean}}$$

If steady state is not achieved during the study, it may be helpful to compare predicted VO₂ and VCO₂ to measured levels in order to gain further insight into the trends observed in a patient. Clinical situations that can affect VO₂ and VCO₂ are outlined in Table 1. Normal adult VCO₂ = 2–3 mL/kg/min; normal adult VO₂ = 3–4 mL/kg/min.

Interpretation of, and adjustments to, measured REE

After an IC study has been assessed as being valid, the measured REE is used to design or alter a feeding regimen. If a patient’s REE is measured during fasting conditions, or if feedings are intermittent, an additional 5% may be added to the REE to account for the thermic effect of digestion.¹⁵ The majority of ICU patients are on continuous feedings; therefore, a thermogenesis factor is usually not necessary. Historically, the measured REE is multiplied by an activity factor and a stress factor to determine total energy expenditure (TEE).

The adjustment of measured REE to better estimate TEE has evolved significantly over the last 20 years. In a 2003 study, McClave and colleagues³⁹ found that adding stress and activity factors leads to overfeeding and should be abandoned. Adding 5–10% to measured REE as an “ICU activity factor” has been common practice for the typical ICU patient.^{28,40-46} However, this practice has been shown to reduce the accuracy by which the snapshot-measured REE correlates with the 24-hour TEE. In fact, a major advantage of IC is that it obviates the need

for use of additional factors. In the ICU, it is appropriate to feed at or below the measured REE without adding stress or activity factors as long as sufficient protein (1.5–2 gm/kg) is provided.^{39,47} To avoid the negative consequences of overfeeding, adding more than 130% of the measured REE is not recommended for ICU patients.^{10,28}

Interpretation of the RQ

Traditionally, RQ interpretation focuses on substrate utilization. Following this premise, an RQ < 0.85 suggests underfeeding. An RQ of 0.85–0.9 suggests mixed substrate utilization, indicating that the nutrition regimen is appropriate. An RQ > 1 suggests overfeeding and potential lipogenesis.²⁸

However, use caution when interpreting the RQ in this fashion. RQ does not always reflect substrate use. The RQ is subject to inter-patient variability, the patient’s stress response, underlying pulmonary disease, acid/base abnormalities and pharmacologic agents (e.g., propofol).⁴⁸ Because evidence has shown that the RQ is neither sensitive nor specific regarding substrate utilization, this author does not rely on it to determine the rate of caloric provision or nature of macronutrient composition.^{49,50} A more appropriate use for the RQ would be

Table 1. Factors affecting accuracy of caloric requirement estimates^{10,5,21,26}

- Multiple traumas
- Neurological trauma
- Burns
- Multi-system organ failure
- Sepsis
- Systemic inflammatory response syndrome
- Acute or chronic respiratory distress syndrome
- Use of paralytic agents or sedation
- Post-operative organ transplantation
- Large or multiple open wounds
- Malnutrition with altered body composition:
 - Underweight
 - Obesity
 - Limb amputation
 - Peripheral edema
 - Ascites

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to serve as a marker of test validity by gauging whether it is within normal human biological range of 0.67–1.3.^{15,20,22,47,50}

Designing the nutrition regimen

To design an appropriate feeding regimen, it is important to understand the evolution of a patient’s response to metabolic stress over time. During critical illness, there are three phases in the metabolic response to stress. The stress phase, also known as the ebb phase, typically lasts 12–24 hours. This phase is characterized by hemodynamic instability, hypometabolism, surging counterregulatory hormones, and insulin resistance.⁵¹⁻⁵³

The catabolic phase, or flow phase, generally lasts 7–10 days if uncomplicated, but it can last for weeks. Clinical manifestations of this phase include fever, hypercatabolism, gluconeogenesis, and increased oxygen demands. After the acute-phase response associated with the stress and catabolic phases resolves, the third phase, the anabolic phase, can last for months.⁵¹⁻⁵³

“Metabolic support” is the focus of nutrition intervention during the stress and catabolic phases. Patients are fed high-nitrogen feedings (1.5–2 gm/kg/day) at or below 100% of measured REE.^{2,10,39} The goal is to preserve lean body mass without the negative effects of overfeeding by matching nutrient delivery to expenditure. All caloric sources are included in the determination of caloric delivery; e.g., dextrose-containing medications and intravenous fluids, propofol, and dextrose in continuous renal replacement solutions.

As the patient moves into the anabolic phase, energy requirements tend to rise markedly. The emphasis of nutrition interventions shifts to “nutrition support,” with the goals being repletion and recovery. In this phase, patients are fed up to 130% of their measured REE with ongoing aggressive protein delivery (1.5–2 gm/kg/day).⁵³

Regardless of which phase a critically ill patient is in, the feeding regimen should meet the patient’s measured REE with total calories as opposed to non-protein calories using a protein/carbohydrate/fat ratio of approximately 15–20%, 50%, and 20–30% respectively.²

Follow-up measurements

The determination of energy expenditure, measured or predicted, is always a component of the nutrition care process for each ICU patient. It is beneficial if IC is used at least once; this gives the clinician an appreciation for the

degree of the patient's metabolic response to injury or illness.^{3,17} Follow-up measurements can be obtained in response to significant changes in a patient's clinical or metabolic status.^{10,15,28,54} However, consensus and guidelines in the literature are lacking for addressing a more systematic approach to the frequency of conducting IC.

Summary

Indirect calorimetry is the gold standard for determining energy expenditure in critically ill patients. The measured REE is an objective, patient-specific caloric reference that serves as the most accurate method of determining energy expenditure. Protocols addressing IC methodology are necessary to ensure technical accuracy and clinically useful results. The measured REE should be the caloric target without the addition of stress or activity factors for nutrition support regimens in the ICU. The RQ should be used primarily as an indicator of test validity. Optimal nutrition intervention requires continuous evaluation of all pertinent clinical data and careful monitoring of each patient's response to metabolic stress and therapeutic nutrition interventions.

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Ensuring Adequate Nutrition Support for the Ventilated Patient: An Interdisciplinary Approach

By Jorge Rodriguez, BSRC, RRT, and Sharla Tajchman, PharmD, BCPS, BCNSP

Mechanically ventilated patients have complex metabolic variations that make nutritional requirements difficult to predict. Evidence exists that provision of optimal nutrition support can have significant clinical impact in critically ill mechanically ventilated patients.¹⁻³ Thus, accurately determining a patient's energy requirements and administering the appropriate amount of nutrition support is a vital aspect of ICU care.

Metabolic Variations in Critically Ill Patients

Metabolic variations in mechanically ventilated patients can include increased energy expenditure and protein catabolism, leading to loss of lean body mass and muscle, the preferred endogenous energy source during periods of stress or critical illness. Adequate nutrition support is necessary to slow down or reverse muscle catabolism; however, determination of energy requirements in critically ill patients poses a difficult challenge.⁴ Controversy exists as to which predictive equations, if any, are appropriate for estimating nutritional needs in mechanically ventilated patients. Current consensus guidelines from A.S.P.E.N./SCCM for the provision of specialized nutrition support in adult critically ill patients recommend using indirect calorimetry (IC) where available to determine energy requirements in this patient population.⁵

Accurate determination of energy expenditure is essential to avoid feeding-associated adverse effects. Underfeeding may result in the development of malnutrition and its associated adverse outcomes such as delayed wound healing, increased infectious complications, longer duration of mechanical ventilation and ICU length of stay.^{3,6} Overfeeding the critically ill patient can lead to hyperglycemia, hepatic dysfunction, prolonged duration of mechanical ventilation, difficulty weaning, and fluid overload (including pulmonary edema and congestive heart failure).⁷

Sophisticated mechanical ventilators now have the ability to obtain real-time metabolic measurements by integrating a gas-analyzing module.

The Role of Nutrition Support Teams

Nutrition Support Teams (NST) have a long-standing history of improving the quality of nutrition care in hospitalized patients requiring parenteral nutrition (PN).⁸⁻⁹ These multidisciplinary teams usually consist of some combination of a physician, nurse, dietician, and/or pharmacist, all specially trained in the area of nutrition support to manage the provision of specialized nutrition support within healthcare institutions. An important but often overlooked addition to any NST is a respiratory therapist (RT), who is trained to screen patients and perform indirect calorimetry. These individuals can provide significant insight into a patient's nutritional requirements via IC.

The complex care of mechanically ventilated patients requires communication and collaboration among several multidisciplinary teams. The NST is a vital aspect of an ICU patient's care, and interaction with the ICU team begins with an initial consult to assess the patient for initiation of nutrition support. The team assesses the patient's nutritional status, weighs potential options for the provision of nutrition support, determines the most appropriate route of administration, and conveys their recommendations to the ICU team. The NST

is responsible for enteral feeding and PN initiation/management. Clinical studies show that the NST's regular follow-up and monitoring reduces the incidence of metabolic complications associated with specialized nutrition support (such as PN) and decreases the overall healthcare cost by deterring PN initiation when alternate nutrition routes are available.⁸⁻⁹

Indirect Calorimetry: Interpreting the Results

Specially trained RTs work very closely with the NST and ICU teams to evaluate all mechanically ventilated patients for metabolic measurement. Using an ICU-specific protocol, each mechanically ventilated patient is evaluated for IC eligibility. If no exclusion criteria are present, then the RT reviews the patient's medical history, ventilator settings, diagnostic imaging, hemodynamics, nutrition sources and any potential issues that may confound the IC results. The most accurate IC data are obtained during a steady-state time of respiratory and hemodynamic stability, so it is of the utmost importance that the patient remains in a calm and restful state during metabolic measurement. Do not perform IC studies during sedation holidays, periods of hemodynamic or respiratory instability, hemodialysis, during patient agitation, or shortly before or after painful procedures.¹⁰⁻¹¹

Sophisticated mechanical ventilators now have the ability to obtain real-time metabolic measurements by integrating a gas-analyzing module. Taking measurements of flow and gas concentrations proximal to the patient airway, these gas modules function as an extension of the ventilator. They are small, convenient, and have the ability to continuously measure metabolic data. In contrast, metabolic carts can be bulky and cumbersome. When needed for extended measurement times, the metabolic cart can be intrusive and hinder patient care.

Mechanical ventilators with integrated IC modules can display and store measured energy expenditure (MEE) and respiratory quotient (RQ) data, transfer the data to the patient's electronic medical record and allow the RT to assess metabolic measurements for validity. The RT obtains a 30- to 60-minute "snapshot" of metabolic measurement during hemodynamic and respiratory stability, while taking into consideration nutritional intake and disease processes, to report to the NST. Viewing IC trends over several days can provide insight into the patient's metabolic needs while nutrition support is provided and/or the patient's medical condition changes. IC measurements using 24 hours or more of data can be used to

evaluate MEE while accounting for the peaks and valleys of patient stress during ICU care.

Additionally, comprehension of the RQ and MEE relative to the patient’s demographics and acuity of illness are needed to validate the results. Nutritional intake must also be considered, as it takes 12–36 hours for the RQ to reflect changes in nutrition support. For example, a patient who has been mechanically ventilated for 3 days and has not received nutrition support will have an RQ near 0.70, due to utilization of fat stores in the absence of nutrition. After nutrition is initiated and advanced to goal, the RQ will move into the desired 0.85–0.95 range, showing a utilization of mixed fuel substrates.¹⁰⁻¹¹

After accurate IC measurements are obtained, the results, graphical representation, and any other pertinent information are conveyed to the NST for review and discussion. Communication between the RT and the NST is vital to validate IC results. The NST then uses the metabolic measurement as part of a complete nutrition assessment to formulate a nutrition regimen for the patient and make recommendations to the ICU team. The collective expertise of and exchange between the RT and the NST are crucial to the care of the mechanically ventilated patient.

Practice Scenarios: Performing indirect calorimetry

The following scenarios use these calculations to estimate ideal body weight (IBW) in kg:
 Males: $IBW = 50\text{ kg} + 2.3\text{ kg for each inch over 5 ft}$
 Females: $IBW = 45.5\text{ kg} + 2.3\text{ kg for each inch over 5 ft}$

ABW = actual body weight

Scenario 1:

GB, a 54-year old male (weight 77 kg; IBW 82 kg), was recently admitted to the medical ICU for acute respiratory failure that required emergent intubation and mechanical ventilation. A nasogastric tube was placed 24 hours after admission; and, per ICU protocol, IC was obtained on mechanical ventilation day 3. During the initial IC, the patient was noted to be hypertensive (160/89 mmHg), tachycardic (125 bpm), and tachypneic (35 bpm). The patient was within normal hemodynamic and respiratory parameters during subsequent metabolic measurements. Serial assessment of metabolic data is on Table 1.

Table 1. Scenario 1 Information

Mechanical Ventilation Day	MEE (KcalDay)	Respiratory Quotient (RQ)	Nutrition
3	2,355	0.85	Initiated
5	2,135	0.74	Not at goal
7	2,079	0.79	Goal rate
9	2,157	0.84	Goal rate

Table 2. Scenario 2 Information

Mechanical Ventilation Day	MEE (KcalDay)	Respiratory Quotient (RQ)	Nutrition
3 (30-minute steady state)	1,789	0.69	Initiated
6 (30-minute steady state)	1,636	0.70	Goal
6 (2-hour steady-state)	1,734	0.68	Goal

Question 1.1

What could cause the initial energy expenditure and RQ to be 2,355 kcal/day and 0.85, respectively?

1. The patient is not adequately sedated.
2. The patient may be in respiratory distress.
3. The patient is in pain.
4. All of the above

Consideration

Upon further investigation, the patient’s increased respiratory rate and minute ventilation were associated with a sedation holiday provided by the nurse. This information indicates hyperventilation due to patient agitation, movement, and lack of sedation, producing inaccurate data. Ideally, this scenario would more accurately render an RQ closer to 0.70, indicating utilization of fat stores and protein-muscle catabolism due to the lack of nutrition support since hospital admission. The RQ of 0.85 is actually the respiratory exchange ratio and does not reflect the patient’s metabolism. It is important to note that data obtained during steady-state conditions do not automatically ensure accuracy. The RT must use critical thinking and patient assessment skills to further validate any metabolic measurement.

Answer: 4

Hyperventilation due to agitation, pain, movement, lack of sedation, and respiratory distress will cause an increase in VCO_2 and VO_2 . This is the reason for the elevated MEE of 2,355 kcal/day and the measured RQ of 0.85, which would normally indicate mixed-fuel substrate utilization in an adequately fed patient.

Question 1.2

What can the RT do to help evaluate the measured EE of 2,355 kcal/day for accuracy?

1. Accept the results as accurate data because the patient had achieved steady-state conditions.
2. Obtain a longer metabolic measurement.
3. Reattempt IC after the patient is properly sedated and is exhibiting hemodynamic and respiratory stability.
4. Discuss results with NST to assess validity.

Consideration

Hyperventilation leads to increases in VCO_2 and VO_2 , which increases MEE and RQ. A longer IC measurement during hyperventilation does not ensure accurate data. Communication between the nurse and RT regarding patient sedation and comfort is crucial to achieve accurate IC results. After the patient is properly sedated and stable, another metabolic measurement can be evaluated. The RT is responsible for (1) obtaining the most accurate data possible while the patient is in the best state for metabolic measurement and (2) communicating this information to the NST.

Answer: 3

Using critical thinking and patient assessment skills, the RT should recognize that this patient is not in an ideal state for metabolic measurement. Properly sedating the patient and allowing hemodynamic and respiratory stabilization is necessary prior to another IC attempt. A longer IC measurement during this ideal time frame helps ensure accurate IC measurement.

Scenario 2

IM, a 70-year-old female (ABW 84.7 kg; IBW 50 kg), was admitted to the surgical ICU after abdominal surgery and was taken back to the OR 2 days postoperatively for repair of wound dehiscence. She was started on PN on post-operative day (POD) 3 for ileus. She remains intubated on pressure support ventilation with an FiO_2 of 0.3, PEEP 5 mm Hg and pressure support of 4 mm Hg. IC is performed on POD 3 and POD 6. The ICU attending physician questions why the RQ value continues to be low despite provision of goal nutrition support. On POD 6, trending of continuous metabolic data over a 2-hour period produces results similar to previous 30-minute measurements on post-op day 3. (See Table 2.)

Question 2.1

Based on the indirect calorimetry results, is IM getting adequate nutrition support?

1. No. Based on her MEE, the patient is not receiving enough calories from her PN.
2. No. Based on her RQ value, the patient is not receiving enough calories from her PN.
3. Unsure. A discussion with the NST is warranted to determine goal nutrition support.
4. Yes. The patient is overweight and does not need the calories determined by the MEE.

Considerations

During the 2-hour IC, the patient remained lethargic and slow to respond secondary to light sedation and analgesia. She was hemodynamically stable with a heart rate of 80–89 bpm and blood pressure of 135/87–143/89 mm Hg. Ventilator settings were minimal and minute ventilation (V_e) was 6.5–7.0 L/min with little variation. Average VO_2 and VCO_2 measurements were stable: 358 mL/kg/min and 244 mL/kg/min, respectively. The RQ was in physiologic range.

Answer: 3

The 2-hour IC measurement is very similar to the 30-minute measurement, showing little variation while the patient was in steady-state conditions. Due to the stable nature of the patient during the course of the study, there are no obvious reasons to suspect erroneous IC results. Discussion with the NST is warranted to validate the metabolic measurement. In IM's case, the NST is purposefully providing fewer dextrose calories and more protein calories due to her obesity. This strategy of providing hypocaloric/high protein nutrition support allows the patient to utilize her own adipose stores for fuel, minimizes adverse effects from infusing large amounts of dextrose, and ensures a positive nitrogen balance via provision of higher amounts of protein. The low RQ value is representative of lipolysis secondary to the nutrition support strategy.

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Mr. Rodriguez works in the University of Texas MD Anderson Cancer Center as a respiratory therapist. His past positions have included MD Anderson's emergency center and ICUs. Currently he is part of the Nutritional Support Team there. He also gives seminars on the use of integrated indirect calorimetry. At Clinical Nutrition Week 2011 in Vancouver, BC, he co-presented an abstract titled, "Current Nutrition Guidelines Underestimate Energy Requirements in Critically Ill Obese Cancer Patients," which garnered an abstract of distinction award and subsequently appeared in a recent issue of *Nutrition in Clinical Practice*.

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1. The accuracy of indirect calorimetry is unaffected by the inspired oxygen concentration.
 - A. True
 - B. False
2. Leaks in the system that prevent the collection of all the expired gas have what effect on REE and/or RQ
 - A. No effect on the REE and RQ
 - B. Overestimates the REE
 - C. Underestimates the REE
 - D. Overestimates the RQ
3. If indirect calorimetry is performed during renal dialysis, the measurement is invalidated by the loss of CO₂ in the dialysate.
 - A. True
 - B. False
4. The confounding factor related to the measurement of VO₂ at high FIO₂ is described by
 - A. The Haldane effect
 - B. The Bohr effect
 - C. The Henderson-Hasselback effect
 - D. Daltons' Law
5. What measurements are required for calculation of energy expenditure during open circuit indirect calorimetry?
 - A. VO₂ alone
 - B. VO₂ and VCO₂
 - C. VCO₂ alone
 - D. RQ and the respiratory exchange ratio
6. RQ is calculated using the formula VO₂/VCO₂.
 - A. True
 - B. False
7. Clinical scenarios in which IC is useful include:
 - A. Obese patients
 - B. Mechanically ventilated COPD patients
 - C. Septic patients
 - D. All of the above
8. IC should be conducted:
 - A. Once daily
 - B. Weekly
 - C. Whenever there is a significant change in the patient's clinical status
 - D. Monthly
9. Patient conditions that would benefit from permissive underfeeding include the following:
 - A. Refeeding syndrome
 - B. Sepsis
 - C. Class III obesity
 - D. All of the above
10. IC is inaccurate when patients require mechanical ventilation with:
 - A. > 60%FIO₂
 - B. 30% FIO₂
 - C. 40% FIO₂
 - D. 50 %FIO₂
11. The physiologic range for RQ is:
 - A. 0.67-1.3
 - B. 0.7-1.5
 - C. 0.85-1.9
 - D. 1.0-2.0



Answers

This program has been approved for 2.0 contact hours of continuing education (CRCE) by the American Association for Respiratory Care (AARC). AARC is accredited as an approver of continuing education in respiratory care.

This program has been approved by the commission on Dietetic Registration for 2.0 CPEs. To earn credit, do the following:

1. Read all the articles.
2. To earn 2.0 CRCEs or CPEs, you must achieve a score of 75% or more on the post-test. If you do not pass it the first time, you may re-take it once.
3. Complete the entire post-test and the participant evaluation.
4. The post-test may be taken online at www.saxetesting.com, or you may complete this page's test/participant evaluation and mail or fax it to the address listed on page 11.
5. If you use the paper version of this form, mark your answers clearly with an "X" in the box next to the correct answer. On the evaluation portion, clearly circle the most appropriate answer.
6. Answer forms must be postmarked by Dec. 31, 2012 (RTs); Nov. 30, 2013 (dietitians).
7. If you take this test online, you can print your certificate immediately upon successful completion. AARC members' results are automatically forward to the AARC for accreditation.
8. If you fax or mail this form, your results will be sent within 4–6 weeks after we receive them.

Participant's Evaluation (3 questions)
(Circle one answer for each question)

1. What is the highest degree you have earned? Diploma Associate Bachelor Masters Doctorate

Yes	Somewhat	Not at all
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2. Indicate to what degree the program met each of the following objectives:

Describe the principle of operation of open-circuit calorimeters for measuring energy expenditure.

Yes	Somewhat	Not at all
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List at least 4 common factors complicating the measurement of energy expenditure.

Yes	Somewhat	Not at all
------------	-----------------	-------------------

Provide solutions for each of those complicating factors.

Yes	Somewhat	Not at all
------------	-----------------	-------------------

Describe the interdisciplinary approach to interpreting IC results.

Yes	Somewhat	Not at all
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3. Please indicate your agreement with the following statement: "The content of this course was presented without bias of any product or drug."

Agree	Disagree
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1 <input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> C <input type="checkbox"/> D	7 <input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> C <input type="checkbox"/> D
2 <input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> C <input type="checkbox"/> D	8 <input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> C <input type="checkbox"/> D
3 <input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> C <input type="checkbox"/> D	9 <input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> C <input type="checkbox"/> D
4 <input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> C <input type="checkbox"/> D	10 <input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> C <input type="checkbox"/> D
5 <input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> C <input type="checkbox"/> D	11 <input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> C <input type="checkbox"/> D
6 <input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> C <input type="checkbox"/> D	12 <input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> C <input type="checkbox"/> D

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