

## Historical Development of Nutrient and Calorimetry and Expired Gas Analysis Indirect Calorimetry

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Indirect calorimetry is the measurement of the amount of heat generated in an oxidation reaction by determining the intake or consumption of oxygen or by measuring the amount of carbon dioxide or nitrogen released and translating these quantities into a heat equivalent. In the last 20 years there has been significant development in both laboratory and computerized metabolic systems used in indirect calorimetry. In addition, there has been increased use of breath-by-breath EGAIC. Several researchers have suggested that breath-by-breath analysis, because of their practicality, could fulfill this need for a valid and reliable expired gas analysis indirect calorimetry instrument. It was hoped this investigation would determine the best validation for a precise measurement of breath-by-breath expired gas analysis indirect calorimetry. The problem with the available research is that few studies have examined the validity and reliability of all these different systems for breath-by-breath expired gas analysis indirect calorimetry. Therefore, there is a need to find out the most valid, reliable, and precise measurement of the breath-by-breath expired gas analysis indirect calorimetry.

**Key words** : Indirect calorimetry, heat equivalent, breath-by-breath, validity, reliability

### Introduction

When we exercise, our bodies use chemical energy derived from catabolism to cause muscle contraction. During this process we expend calories and generate mechanical power and work, as well as release heat.

Calorimetry is the science that quantifies the heat release from metabolism. There are two methods in calorimetry; direct calorimetry and indirect calorimetry. Direct calorimetry is the calorimetric method that directly measure heat dissipation from the body. Indirect calorimetry is the calorimetric method when heat dissipation is calculated from other measurements. Indirect calorimetry is divided into Closed-circuit indirect calorimetry that involves the recirculation of inhaled and exhaled air and Open-circuit indirect calorimetry that involves the inhalation of atmospheric air and measurement exhaled air [31].

The measurement of ventilation and expired gas analysis used as a method of Indirect calorimetry (expired gas analysis indirect calorimetry; EGAIC) is an old science, dating back to the pioneering work of Bischoff and Voit in 1860,

Rubner in the 1890's, and Atwater and Benedict in 1904 [1,18,30]. In 1842, the first law of bioenergetics helped scientists to quantify the total heat release from metabolism [30].

In 1860 Bischoff and Voit completed calculations on the caloric and respiratory gas exchange involved in the combustion of certain foods and pure nutrients [18]. The calorimeter used to combust food was called a bomb calorimeter. Researchers have found bomb calorimetry to be of value when studying the effects of diet, not only in laboratory animals, but also humans [30].

A German scientist named Rubner, established the clinical use of indirect calorimetry, and determined the caloric value of protein combustion in a bomb calorimeter, measured the energy release of dried urine and feces, and calculated the difference in energy release from the heat value of protein between bomb calorimetry and metabolism. Rubner's caloric equivalent values have been widely used in different types of carbohydrate, protein and fat molecules that are metabolized in the body [18]. Rubner's findings in 1904 were reproduced in human subjects by Atwater and Benedict using a more sophisticated closed-circuit respiration calorimeter [18,30].

The development of equipment and techniques allowing the measurement of oxygen consumption provided an in-

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direct means to quantify the metabolic intensity of steady-state exercise and to calculate changes in energy expenditure with changes in exercise intensity [30]. Expired gas analysis indirect calorimetry measures three variables; 1) ventilation ( $V_E$ ), 2) expired air  $O_2$  fraction ( $F_{E O_2}$ ), and 3) expired air  $CO_2$  fraction ( $F_{E CO_2}$ ). From these measurements, calculations are made for the rate of oxygen consumption ( $VO_2$ ) and carbon dioxide production ( $VCO_2$ ), and based on the data from bomb calorimetry and the correction to whole body metabolism provided by Rubner and Atwater, provides an indirect means to quantify biological energy expenditure expressed as Kcals [1,30].

Dr. Robert Robergs from the University of New Mexico developed software and hardware for breath-by-breath EGAIC, and was awarded a U.S. patent for this invention and preliminary validation (Mixing chamber and expired gas sampling for expired gas analysis indirect calorimetry, United States Patent 6,942,623). Such preliminary validation revealed numerous concerns about current validation procedures used in prior scientific investigations and of the validation of instruments and commercial systems used in EGAIC.

Several researchers have suggested that breath-by-breath analysis, because of their practicality, could fulfill this need for a valid and reliable expired gas analysis indirect calorimetry instrument. It was hoped this investigation would determine the best validation for a precise measurement of breath-by-breath expired gas analysis indirect calorimetry.

As such, this review will discuss the definition of indirect calorimetry, methods and instruments for indirect calorimetry, and validity and reliability of indirect calorimetry methods, respectively.

## Materials and Methods

### Definition of indirect calorimetry

The measurement of metabolism or metabolic rate has application in a varied number of fields including exercise physiology, physiology, biology, biochemistry, nutrition, fitness, cardiology, pulmonology, and physical therapy. The most common method of carrying out such determinations is by indirect calorimetry. Indirect calorimetry, the determination of airway carbon dioxide elimination ( $VCO_2$ ) and oxygen uptake ( $VO_2$ ), can be used to non-invasively detect non-steady state perturbations of gas kinetics and mirror tissue metabolism [11,32].

During at rest, indirect calorimetry determination on the effects of age, gender, body size, growth, disease, nutrition, and environment on metabolism are very useful. The resting metabolic rate per unit body mass is greater in males than in females, greater in children than in adults, greater in small individuals than in large individuals, and greater under extremes of heat and cold than under normal environment [6]. Also, the use of indirect calorimetry is becoming more widespread of clinical applications of the measurement of resting energy expenditure in normal individuals and for guiding daily nutrition support as a whole, in critical care medicine, including the assessment of the physical fitness of healthy and diseased individuals such as major trauma and sepsis, for the healthy or sick obese patient through the measurement of ventilation, and the fractions of oxygen and carbon dioxide in expired air [5,11,21,26,32].

These tests are usually conducted to (a) the maximal oxygen consumptions ( $VO_{2max}$ ), (b) an increase in the ventilator equivalent for oxygen (ventilation threshold, VT), (c) assessment of aerobic power, (d) the rate of change in  $VO_2$  for a given increase in exercise intensity ( $VO_2$  kinetics), (e) detection of cardiovascular and pulmonary diseases [2,7,20,13, 29,32].

Expired gas analysis indirect calorimetry is one of the most common tools in exercise physiology. It is used for various purposes including the assessment of aerobic power, determination of exercise intensity and the measurement of energy expenditure. All these systems need to calculate metabolic data are the fractional concentrations of oxygen ( $F_{E O_2}$ ) and carbon dioxide ( $F_{E CO_2}$ ) in expired air together with pulmonary ventilation (expired ( $V_E$ ) or inspired ( $V_I$ )). From these measurements, oxygen consumption ( $VO_2$ ), carbon dioxide production ( $VCO_2$ ), and the respiratory exchange ratio (RER) can be calculated. These calculations are based on the Haldane transformation, which is derived from the fact that nitrogen is physiologically inert.

$$VO_2 = \text{Inspired } O_2 - \text{Expired } O_2 = (V_I \times F_I O_2) - (V_E \times F_{E O_2})$$

$$VCO_2 = \text{Expired } CO_2 - \text{Inspired } O_2 = (V_E \times F_{E CO_2}) - (V_I \times F_I CO_2)$$

$F_I O_2$  is fixed, assuming a room air concentration of 20.95%.

$F_I CO_2$  is fixed, assuming a room air concentration of 0.03%.

While the science of EGAIC has remained largely unchanged for the last ten decades, the equipment and frequency of data collection and computations have changed enormously within the last 15 years. Today, computations of EGAIC are able to occur every breath, with breath-by-breath data collection and computation now the standard

in most commercial systems. Consequently, breath-by-breath EGAIC is now widely used in both professional practice and research in the clinical, basic and applied sciences.

#### Methods and equipments for indirect calorimetry

There are three methods of expired gas analysis indirect calorimetry (EGAIC); (1) manual Douglas bag, (2) fully automated breath-by-breath, and (3) semi-automated and fully automated mixing chamber. In the last 20 years there has been a significant development of both laboratory and computerized metabolic systems used in indirect calorimetry [8,19,29]. Methods were developed and validated based on automated repeated measurements of ventilation, typically from an electronically integrated dry gas flow meter, and expired gas fractions using electronic gas analyzers or a mass spectrometer [35].

The most basic of these techniques to collect and analyze expired gas is the Douglas bag (DB) method. While the DB method is considered to be the gold standard, it also has several disadvantages and its own limitations. For example, the time interval for Douglas bag requires careful analysis by researchers to reduce errors while measuring the content of the bag. In addition, there are considerable inconsistencies in using the Douglas bag assumptions that violate actual physiological function of the respiratory and collecting zones of the lung. Furthermore, the bags are made of PVC material, which is slightly permeable to the external air [3,7,8,12]. This mixing continues to the next expiration, as the first volume of air from the body is actually room air not alveolar air. There are currently no corrections for this mixing in any current method of indirect calorimetry. Some researchers have noted that it is very difficult to remove all the air from the Douglas Bag and air leaking out during the removal process [8]. According to some recent work of Bassett et al. [3], the time need to be flushed out the Douglas bag valve and tubing inflated oxygen and decreased carbon dioxide gas content of room air. This dead air space acts to decrease the overall sensitivity and accuracy of the system. Probably, the time needed to flush out the Douglas bag valve and tubing inflated oxygen and decreased carbon dioxide gas content of the sampled air.

Recently, increasing technology has seen the emergence of portable, lightweight and automated metabolic gas analysis systems; these systems are made breath-by-breath gas analysis of gas exchange practical and commonplace by using commercially available equipments. These systems allow

the continuous measurement of expired gas concentrations and ventilation right outside the mouth and then immediately display respiratory and metabolic data for each breath, and therefore markedly increase the efficiency of the gas analysis procedure. The use of these systems has allowed for very rapid gas analysis and ventilation measurement and is less time consuming than the DB technique [4,7,8,12,15,19,24,25,28,34]. However, various commercial these systems limitations to breath-by-breath systems have been advanced. Most commercial system limitations to breath-by-breath include some technical errors that can make these systems measurements inaccurate. Noguchi et al. [23] reported that the delay time between the on-line digital multiplication and integration of flow and fraction signals have sources of error such as the accuracy and reproducibility of flow and gas fraction measurements.

Recent gas analyzers are typically pressure and flow sensitive, therefore there must be near same flow resistance during calibration. Another concern is the failure of many computerized systems to correct for water vapor pressure ( $\text{PH}_2\text{O}$ ) in the expired air, as this pressure is different than in the calibration gas. Although mass- spectrometers can be altered to ignore the contribution of water vapor [10,15,19], most oxygen and carbon dioxide analyzers are sensitive to the presence of water vapor.

The reliability of breath-by-breath gas analysis systems will be influenced by the variability of each physiological measure. The total variability of a physiological measurement such as  $\text{VO}_2$  is the sum of the biological variability and the technical variability. The biological variability accounts for around 90% of the total variability, with only 10% less of the remaining variability coming from technical problems [19]. It is difficult to check the accuracy of a computerized system when subjects are at maximal aerobic power ( $\text{VO}_{2\text{max}}$ ) since the biological variability in  $\text{VO}_{2\text{max}}$  is about 5% [17].

Some commercial suppliers of EGAIC systems have promoted breath-by-breath EGAIC based on earlier systems that used a mixing chamber to mix expired air across a breath to derive a single representative value for each of  $\text{F}_{\text{E}}\text{O}_2$  and  $\text{F}_{\text{E}}\text{CO}_2$ . Most exercise laboratories are using automated systems to measure respiratory gas exchange with a mixing chamber which gives time averaged values for respiratory variables [3,12,19,27,34]. The mixing chamber also offers the advantage of presenting data in real time and is just as time-saving as the breath-by-breath analysis. The expired

gas from several breaths is mixed in a mixing chamber and a sample from this chamber gives an average expired gas concentration over those breaths. However, such systems have only been validated based on time averaged data using a criterion of the Douglas bag method [7,8,14]. Foss and Hallen [12] have assessed mixing chambers and have concluded that they should produce less error than the breath-by-breath analysis systems. The mixing bag may be constructed of any suitable material such as thin plastic that has sufficient compliance to expand with the pressure of exhalation. Furthermore, the Douglas bag method is not a suitable criterion method for breath-by-breath EGAIC. This problem has been recognized, and researchers have developed mechanical calibration systems from which numerous EGAIC validation studies have been completed [13,16]).

#### Validity and reliability of indirect calorimetry methods

There are a considerable number of automated gas analysis systems currently available either laboratory-based, semi-portable, or fully portable, yet relatively independent validity or reliability studies on these systems have not been reported to date. However, some groups of researchers have investigated the validity and reliability of various breath-by-breath analysis systems using a computerized metabolic system fitted with a mixing chamber [3,7-9,12,22,28], and a number of different approaches have been taken to assess breath-by-breath analysis function. Some studies have reported correlation coefficients between fast metabolic meas-

urement system (the Oxycon-Pro<sup>®</sup>) and Douglas bag method during low and high exercise intensities (Table 1).

Foss and Hallen [12] reported the  $\dot{V}O_2$  was 0.8% (0.03  $l \cdot min^{-1}$ ) lower with the Oxycon-Pro<sup>®</sup> than with the Douglas bag method with a coefficient of variation (CV) of 1.2% ( $p < 0.05$ ) and  $\dot{V}E$  was 1.8% lower with CV of 1.0% ( $p < 0.05$ ). Carter and Jeukendrup [7] used Oxycon-Pro<sup>®</sup> and Douglas bag method to assess the mean absolute values of  $\dot{V}O_2$ ,  $\dot{V}CO_2$  and RER. The results from testing 100 and 150 watts, showed very similar for the Oxycon-Pro<sup>®</sup> and Douglas bags. Therefore, the validity and reliability coefficients for the breath-by-breath analyses are high with validity coefficients as high as  $r = 0.994$  [8].

The high correlations between the values obtained from the Douglas bag method and the Oxycon-Pro<sup>®</sup> computerized metabolic system with mixing chamber for  $\dot{V}E$  ( $p < 0.001$ ,  $r^2 = 0.996$ ),  $\dot{V}O_2$  ( $p < 0.001$ ,  $r^2 = 0.957$ ) and  $\dot{V}CO_2$  ( $p < 0.001$ ,  $r^2 = 0.980$ ) [28].

Bassett et al. [3] used Truemax 2400 (ParvoMedics) and the Douglas bag method to assess the validity of inspiratory and expiratory methods of measuring gas exchange.  $F_{E}O_2$  was slightly lower (0.04%) with the computerized system, compared with the Douglas bag method ( $p < 0.01$ ).  $\dot{V}O_2$  was an average of 0.018  $l/min$  ( $p < 0.05$ ) higher for the inspired system compared with the Douglas bag.  $F_{E}CO_2$  was slightly lower (0.03%,  $p < 0.05$ ) for the expired system than the Douglas bag. Thus the Truemax 2400 system, using inspiratory or expiratory configurations, permitted extremely

Table 1. Validity and reliability of indirect calorimetry methods

Study	System	Outcome
Bassett et al. (2001)	Truemax 2400 (ParvoMedics)	Difference in $\dot{V}E$ , 0.018 $l/min$ higher $\dot{V}O_2$ with comp $F_{E}O_2$ lower (0.04%), $F_{E}CO_2$ lower (0.03%)
Carter & Jeukendrup (2002)	Oxycon-Pro <sup>®</sup>	$\dot{V}E$ : 6.6 - 7.4% (CV) $\dot{V}O_2$ : 4.7-6.5% (CV) $\dot{V}CO_2$ : 5.3-7.1% (CV)
Crouter, Antczak, Hudak, Della Valle, & Haas (2006)	TrueOne 2400 (ParvoMedics)	$\dot{V}E$ ( $r = 0.975$ , $p < 0.01$ ), $\dot{V}O_2$ ( $r = 0.994$ , $p < 0.01$ ) and $\dot{V}CO_2$ ( $r = 0.991$ , $p < 0.01$ )
Cullum, Welch, & Yates (1999)	Max-1 (Physio-dyne)	No significant differences in $\dot{V}O_2$ , $\dot{V}CO_2$ , $F_{E}O_2$ , and $F_{E}CO_2$
Foss & hallen (2005)	Oxycon-Pro <sup>®</sup>	Low CVs were found both $\dot{V}O_2$ (0.8%) and $\dot{V}E$ (1.8%)
Rietjens, Kuipers, Kester, & (2001)	Oxycon-Pro <sup>®</sup>	$\dot{V}E$ ( $r^2 = 0.996$ , $p < 0.001$ ), $\dot{V}O_2$ ( $r^2 = 0.957$ , $p < 0.001$ ) and $\dot{V}CO_2$ ( $r^2 = 0.980$ , $p < 0.001$ )
Storer, Bunnell, & Hand (1995)	Vmax (SensorMedics)	No significant difference Mean difference in $\dot{V}O_2$ (0.3%), $\dot{V}CO_2$ (1.8%), and $\dot{V}E$ (1.5%)
Yates & Cullum (2001)	Max-1 (Physio-dyne)	No significant difference 3.1% error at low flow rates and -6.1% error at high flows rates

precise measurements to be made in a less time-consuming manner than the Douglas bag technique. Similarly, Crouter et al. [8] used TrueOne 2400 metabolic cart against the Douglas bag method to assess the accuracy and reliability of the measurement of gas exchange. Reliability between days for  $V_E$  (CV 7.3 to 8.8%) was similar among devices.  $VO_2$  and  $VCO_2$  with the TrueOne 2400 (CV 4.7 to 5.7%) was more reliable compared to the Douglas bag (CV 5.3 to 6.0%). The TrueOne 2400 was not significantly different from the Douglas bag at rest or any work rate for  $V_E$ ,  $VO_2$ , or  $VCO_2$  ( $p \geq 0.05$ ). The reliability of the TrueOne 2400 is similar to other systems currently available, which have been shown to have good reliability [7,22]. The mean bias and 95% prediction intervals for the TrueOne 2400 in the current study are similar to those reported previously by Bassett et al. [3].

Two studies have reported the accuracy of measurement of gas exchange between Max-1 (Physio-dyne) and the criterion Douglas bag system. Cullum et al. [9] used Max-1 with the Douglas bag to assess the accuracy and reliability of measurement of gas exchange. Findings of this study indicate that there were no statistically significant differences between the systems either in  $VO_2$ ,  $VCO_2$ ,  $F_{E}O_2$ , or  $F_{E}CO_2$ . When averaged across the 4 workloads, the  $VO_2$  values from the Max-1 were 87 ml/min less than the Douglas bags (mean relative error of 3.3%,  $p=0.0528$ ).  $VO_2$  for the Max-1 demonstrated high repeatability, with an absolute error 64 ml/min (3.2%) which was slightly greater than the Douglas bag values 55 ml/min (2.5%). Yates and Cullum [36] also found that though there were no statistically significant differences between the Max-1 and the Douglas bag, although the automated system tended to produce  $VO_2$  values that, overall, underestimated the bag value by 2.9%. At low flow rates the error was around 3.1% and approximately -6.1% at high flows. Therefore, they concluded that the Max-1 was suitable system for measuring  $VO_2$ .

Simultaneous comparisons between the Vmax (SensorMedics) system and the Douglas bags with the mean differences in  $VO_2$ ,  $VCO_2$  and  $V_E$  were 0.3, 1.8 and 1.5%, respectively, with no statistically significant differences. They also concluded the Vmax was accurate over work rates ranging from 40 to 160 watts [33].

## Results

Indirect calorimetry methods have the potential to be used by exercise physiology, physiology, biochemistry, nu-

trition, cardiology for a number of different purposes. Breath-by-breath analysis systems use a computerized metabolic system fitted with a mixing chamber is well suited for metabolic measurements of  $VO_2$  and inspiratory or expiratory configuration.

Such preliminary validation revealed numerous concerns about current validation procedures used in prior scientific investigations and of the validation of instruments and commercial systems used in expired gas analysis indirect calorimetry. Consequently, there is a need to apply sound scientific principles to the re-investigation of validation procedures used in expired gas analysis indirect calorimetry, to develop appropriate methods of validation, and apply these validation techniques.

The problem with the available research is that few studies have examined the effects of validation for breath-by-breath expired gas analysis indirect calorimetry. Due to the difficulties in developing valid systems for breath-by-breath EGAIC, there is a need to improve valid and cost effective hardware and software options suited to breath-by-breath applications of EGAIC. Therefore, the need for the best validation for a precise measurement of the breath-by-breath expired gas analysis indirect calorimetry at this time.

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**초록 : 영양소와 열량측정법의 발달과정 및 간접 열량 측정법**윤병곤 · 김종원<sup>1</sup> · 김도연<sup>1\*</sup>(동의대학교 특수체육학과, <sup>1</sup>부산대학교 체육교육과)

간접 열량측정법은 호흡 시의 산소 소모량이나 이산화탄소나 질소의 배출량을 측정해 에너지 소모량을 측정하고, 산화작용의 열 방출량(칼로리소비)을 산출하여 측정한다. 지난 20년 내에 간접 열량측정법에서 사용된 laboratory와 computerized 시스템은 현저하게 발전되었으며, 특히 매 호흡의 변인의 산출을 가능하게 해주는 호흡당 시스템의 사용이 증가되었다. 많은 이전의 연구에서 호흡당 시스템은 간접 열량측정도구로 타당도와 신뢰도가 높아 실험에 적합하다고 평가 하였다. 본 연구는 간접 열량측정법의 가장 적합한 호흡당 시스템을 분석하고자 한다. 하지만 다른 모든 호흡당 간접열량측정계의 타당도와 신뢰도의 효과를 검증한 연구가 많지 않다는 문제점이 있다. 그러므로, 앞으로 연구에서는 호흡당 간접열량측정계의 가장 타당도와 신뢰도가 높은 적합한 측정도구가 필요하다고 사료된다.