VALIDATION OF A PROPANE GAS CALIBRATION DEVICE FOR INDIRECT CALORIMETRIC SYSTEMS

by

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ABSTRACT

Indirect calorimetry (IC) estimates volumes of oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). From these volumes we calculate energy expenditure and respiratory exchange ratio which is used to estimate substrate utilization rates. The accuracy of IC systems is critical to detect small changes in metabolic data. Indirect calorimetry systems calibration is one way to ensure accurate data. The aim of this study was to validate a new calibration method using propane gas technique with three metabolic systems under environment with standardized conditions. A series of propane gas with different flow levels and ventilation rates were run on three different IC systems. The actual experimental $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated and compared to stoichiometry theoretical values. Results showed a linear relationship between gas volumes ($\dot{V}O_2$ and $\dot{V}CO_2$) and propane gas flows (99.6%, 99.2%, 94.8% for Sable, Moxus, and Jaeger systems respectively). In terms of system error, Jaeger system had significantly ($p < .001$) greater $\dot{V}O_2$ (M = -0.057, SE = .004), and $\dot{V}CO_2$ (M = -0.048, SE = .002) error compared to either the Sable ($\dot{V}O_2$, M = 0.044, SE = 0.004; $\dot{V}CO_2$, M = 0.024, SE = 0.002) or Moxus ($\dot{V}O2$, M = 0.046, SE = 0.004; $\dot{V}CO_2$, M = 0.025, SE = 0.002) systems. There were no significant differences between Sable or Moxus systems. In conclusion, propane gas technique is valid to calibrate Sable and Moxus systems but not Jaeger system.

Keywords: calibration, energy expenditure, Indirect calorimetry, propane gas.

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List of Abbreviations

BMR	Basal metabolic rate
BP	Barometric pressure
CO	Carbon monoxide
EE	Energy Expenditure
ET	Energy Transfer
FR	Flow Rate
IC	Indirect Calorimetry
MR	Metabolic Rate
N_2	Nitrogen
RER	Respiratory exchange ratio
TEF	Thermic effect of feeding
$\dot{V}_E$	Ventilation rate
$\dot{V}CO_2$	Rate of Carbon dioxide production
$\dot{V}O_2$	Rate of Oxygen Consumption
WVP	Water Vapor Pressure

Chapter I

Introduction

1.1 Background of study

The energy that human metabolism needs to maintain its vital function is obtained by the metabolism of diet macronutrients including carbohydrate, fat and protein) (Ferrannini, 1988). Because heat is transferred during metabolism, the metabolic rate (MR) is indicated by the rate of heat or energy transfer (ET) (Brooks, Fahey, & Baldwin, 2005). Accurate measurement of the rate of ET is necessary since it allows an understanding of the rate energy requirement for differing types of physical activity level in humans (da Rocha, Alves, & da Fonseca, 2006). The methodology of estimating the rate of energy transfer is called calorimetry in which a means of quantifying amount of heat released from the substrate level of phosphorylation, non-oxidative or aerobic oxidative metabolism food . By knowing the rate of heat transfer, basal metabolic rate (BMR) and the rate of energy expenditure (EE) can be determined (Brooks et al., 2005). Two main types of calorimetry are being used: direct calorimetry (DC) and indirect calorimetry (IC) (Lighton, 2008).

Direct calorimetry has an advantage of effectively measuring the amount of heat given off by body mass (Battley, 1995; Kaiyala & Ramsay, 2011). It involves direct determination of heat exchange between a living body and environment. An isolated, sealed and large chamber to allow some degree of activity, is the traditional means required to accomplish this assessment of the heat liberated from the human body. Although DC is considered as a gold standard method, it is not widely used due to its high cost and complexity (Pinheiro

Volp, Esteves de Oliveira, Duarte Moreira Alves, Esteves, & Bressan, 2011). An alternative to DC is the use of IC systems that estimate the rate of energy transfer via respirometry in a living organism. Basically, IC systems measure the rate of oxygen (O_2) uptake and rate of carbon dioxide (CO_2) production from expired air samples at rest or during activities (Leonard, 2012). Based on the concentration of these gases, IC systems provide information on the mix of metabolic fuels being oxidized and an estimation of the rate of EE via quantifying the rates of O_2 uptake ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) (Melanson et al., 2010). Furthermore, IC systems provide information about the metabolic response after the consumption of food by estimating thermic effect of food (TEF), the rate of energy expenditure after a 10-12 h fast for humans in a supine position (BMR), and during physical activity EE (Lam & Ravussin, 2017). Because IC system is relatively affordable and user-friendly, it has become the most common method used in research involving energy balance (Kaiyala & Ramsay, 2011) and greatly facilitated the measurement of the rate of EE in both laboratory and field settings (Leonard, 2012).

As noted earlier, because the body's rate of energy transfer from macronutrients is largely dependent on the breakdown of these energy substrates in the presence of a sufficient amount of O_2, the accurate determination of $\dot{V}O_2$ and $\dot{V}CO_2$ could provide an accurate way of estimating the rate of EE. Indeed, to ensure accurate IC systems, proper calibration techniques should be performed before testing subjects. The literature identified several methods to calibrate and to assess validity and reliability of IC systems such as the infusion of pure gaseous of CO_2 and nitrogen (N_2) (Schadewaldt, Nowotny, Straburger, Kotzka, & Roden, 2013), burning of alcohol (Cooper et al., 2009) or burning propane (Lighton, 2008). Propane gas is different than other reagents because it has good air-fuel mixing potential due to its low boiling temperature, and as a result produces less carbon monoxides and other volatile hydrocarbons (Lighton, 2008). Furthermore, burning propane gives a rate of O_2 consumption, a rate of CO_2 production and a RER that fall close to the range of rates and RERs that are evident for human; therefore, this method can be used to determine the accuracy, reliability, and validity of the IC systems (Lighton, 2008).

1.2 Purpose of study

Propane gas combustion can be a valuable calibrating methodology for metabolic carts . Therefore, this study was designed to assess the accuracy, validity, and reliability of three metabolic carts (Sable, Moxus, Jaeger) at different low metabolic rates through flows of propane gas.

1.3 Significance of study

Given the importance of determining the rate of EE and its impact on weight control interventions, testing the validity of calibration methods for metabolic cart is required to ensure the accuracy of the MR and, therefore, the calculation of caloric equivalents rates of substrate oxidation. To ensure an accurate nutritional intervention for weight control and sport nutrition, the IC systems should be valid and reliable. Improving calibration technique of metabolic carts will ensure better measurements of BMR and daily EE. This study is expected to provide objective measures to better evaluate the accuracy of metabolic carts that are used to assess patients' metabolic rates and for research purposes. Also, the results of the study may help guide future clinical research and practice towards better estimation of nutritional and physical activity recommendations for lifestyle changes and/or pharmacological interventions.

Chapter II

Review of Literature

2.1 Metabolism and heat production

The rate of heat production in a living organism depends in part on the biological oxidation of macronutrients from food such as carbohydrate, fat, and protein (Ferrannini, 1988). The basic unit of heat measurement is the calorie in which one calorie (1.0 calorie = 4.184 Joule) is defined as the amount of heat required to raise the temperature of 1.0 gram of water by 1°C (Brooks et al., 2005). In order to determine the rate of metabolism, the rate of heat production must be measured. This process is called calorimetry. Two main types of calorimeter are being used: the direct calorimetry (DC) and indirect calorimetry (IC) (Lighton, 2008).

Direct calorimetry can effectively estimates the amount of heat given off by the body mass (Battley, 1995; Kaiyala & Ramsay, 2011). It involves direct determination of heat exchange between a living body and its environment. By quantifying the change in temperature, measured usually from a water "jacket" in the calorimeter (chamber) wall, this corresponds to an increase or a decrease of the rate of heat transfer from a living organism (Simonson & DeFronzo, 1990). An isolated, sealed, and large enough chamber – to allow some degree of activity – is required to accomplish the measurement process (Pinheiro Volp et al., 2011). In the most common designs, the living body is surrounded by a metallic chamber that distributes the heat evenly and allows some air exchange with the environment (Lighton, 2008). It is acknowledged that DC is the most accurate method of quantifying ET because it relies on an easily measured temperature changes and it includes the heat transferred from all metabolic processes (Lighton, 2008). It is considered the gold standard because of its appropriate instrumentation and rigorous measurements (Kaiyala & Ramsay, 2011). However, DC it is not widely used because of its many disadvantages. First, DC requires the subject to be in a steady-state condition. Second, DC slowly responds to the changes in body temperature resulting from heat stored or lost from the body. Third, DC does not provide information about fuel oxidized (Simonson & DeFronzo, 1990), and finally, limits subject's mobility in addition to its complexity of operative procedures. (Pinheiro Volp et al., 2011).

For the reasons mentioned above, metabolic carts that employ IC have become the method of choice to estimate the rate of ET and substrate utilization in a living organism. Indirect calorimetry estimates the rate of O_2 uptake and CO_2 production in a sample of expired air using accurate gas analyzers and flow meters (Ferrannini, 1988). Because major part of body energy is generated by consuming O_2 (oxidative process), knowing the $\dot{V}O_2$ and $\dot{V}CO_2$ can yield a good estimate of rate of EE and the type of substrate that is catabolized (Simonson & DeFronzo, 1990). Thus, the methods of IC are more commonly used to quantify human rate of EE, particularly under field conditions (Leonard, 2012). Detailes about IC systems are listed in section number 2.3.

2.2 Historical development of calorimetry

Thinking about the relationship between life, air, and fire started a long time ago. It was known since prehistory that animal life depended on air, but what air was and how it supported life was unknown. The concept of quantifying the rate of heat transfer from a living organism was first proposed by the respiratory physiologist Antoine

Lavoisier and his wife, Marie-Anne-Pierrette Paulze, in the eighteenth-century. They invented the first direct calorimeter in which a living animal, the guinea pig, was kept inside a chamber which allowed the heat liberated from the animal's body to melt the icy water surrounding the chamber (Lighton, 2008). By measuring the amount of melted ice and knowing the amount of heat required to melt certain amount of ice (i.e. 80 kcal of heat is needed to melt one kilogram of ice), they were able to quantify the amount of heat released from that animal. Also, Lavoisier contributed to the understanding of the relationship between heat production and O_2. He was the first to recognize that a substance in the air consumed by living organisms was giving off heat. He named it oxygen (Brooks et al., 2005). Later in the nineteenth century, a new form of apparatus for measuring the respiratory gas exchanges of animals was invented by Haldane (1892). In this respirometer, the animal breathes O_2 from outside the device; therefore, it was called an open-circuit indirect calorimeter. In 1899, Atwater and Rosa developed an apparatus that determined the relationship between direct calorimetry and indirect calorimetry by measuring the rates of heat transfer, O_2 uptake and CO_2 production at the same time in resting individuals (Brooks et al., 2005). A few years later, Atwater and Benedict (1903) completed the work of Atwater and Rosa (1899) and demonstrated that direct and indirect calorimetry should yield identical rates of caloric expenditure based on the fact that large part of energy in the body is generated by the process of oxidation (Simonson & DeFronzo, 1990).

The next major advance in the field of metabolic measurement was the development of Douglas bag (DB) method, in 1911. In this method, the subject breathes through a mouthpiece connected with a two-way valve used to direct the expired air either into the atmosphere or into the bag for collection over an appropriate time interval (Douglas, 1911). After the gases collection period, the valve is turned to seal the bag and the expired air volume is measured using a spirometer. The measurement is conducted by analyzing a sample of the expired air using electronic gas analyzers to determine O_2 and CO_2 partial pressure at ATPS from which the rate of EE is calculated with an error of less than 3% (Cooper & Storer, 2001; Haugen, Chan, & Li, 2007; Levine, 2005). However, this error can substantially increase if the analysis of gases was inaccurate, if the bags were poorly maintained, and if the gases were collected by an untrained person (Levine, 2005). There are several advantages of DB method: (1) this method can accurately determine the respiratory gas exchanges at rest as well as during exercise, (2) the bags are portable and light in weight, (3) several sizes of the bags are available to suit the experiment duration, and finally (4) the apparatus can easily be used and fitted to the subject (Douglas, 1911). Although the DB method has been used to collect gases for many years, it has several limitations including it is time consuming due to the need of sampling and analysis of gases after collection. Also, with this method, rapid changes in ventilation ($\dot{V}_E$) cannot be detected because the device does not permit breath-by-breath data collection (Carter & Jeukendrup, 2002). However, for many respiratory physiology laboratories DB remains the gold standard for validating IC systems for accuracy in the determination of $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$ (Cooper & Storer, 2001).

While DB is considered the simplest method of gas collection, this method was replaced by more dynamic configuration metabolic carts that pass the expired air through connecting tubing into a mixing chamber and provide measurements using computer-controlled analog-to-digital signal processing (Cooper & Storer, 2001). Several metabolic carts are available, ranging from semi-automated mixing chamber systems to fully automated breath-by-breath measurement systems. In 1974, Wilmore and Costill described a semi-automated system in which the mixing chamber ensured thorough mixing of the alveolar air (exchange air) and dead space (air that does not take part in exchange process) for subsequent sampling. The expired air sample is pulled from the mixing chamber by a vacuum pump at a known flow rate into three small anesthesia bags. While manually controlled, one bag was filling mixed gas from the mixing chamber, the gas analyzers were sampling from the second bag and at the same time the third bag was evacuated (as cited in Cooper & Storer, 2001). However, the semi-automated systems average expired gas concentrations over a number of breaths and do not allow fast determination of $\dot{V}O_2$ and $\dot{V}CO_2$ especially during exercise conditions (Cooper & Storer, 2001). Therefore, these systems were replaced with fully automated breath-by-breath gas analysis systems.

In the 1970s, the first computer-based metabolic cart was presented for respiratory data collection and analysis. Beaver et al. (1973) described a computer-based system for analysis of breath-by-breath respiratory response to exercise and the graphical display of gas exchange variables (Beaver, Wasserman, & Whipp, 1973). Later in the same period, Pearce & Milhorn (1977) introduced a modified computer-based system for respiratory gas analysis similar to the Beaver et al (1973) system except that signals for respiratory variables are sent directly to a digital computer via an analog-to-digital converter for signal processing. In this system, respiratory frequency, minute ventilation, alveolar ventilation, dead space, O_2 and CO_2 partial pressures, and RER are measured and analysed on a breath-by-breath basis with mathematical equations implemented in the computer program. The data generated from this system were compared with other experimental data and found to be in a good agreement (Pearce & Milhorn Jr, 1977).

With the fast progression of technology, these metabolic carts became more sophisticated and accurate and allowed the continuous measurement of gas volumes and immediate display of this information on a computer screen (Carter & Jeukendrup, 2002). In these metabolic carts, gas volume measurement is accomplished by simultaneous sampling of the inspired and expired air at high frequency within a breath cycle. From the sampled gas concentrations, the $\dot{V}O_2$ and $\dot{V}CO_2$ are determined and the subsequent EE and substrate oxidation are calculated (Yoon, Kim, & Kim, 2010).

No doubt, the breath-by-breath system has allowed for a very rapid gas analysis and ventilation measurement with less time compared to the DB and semi-automated methods (Yoon et al., 2010). In addition, current automated metabolic carts include digital processing for data acquisition and a real-time display of physiological response variables. Furthermore, these systems are accurate and reliable, easy to use, portable, and affordable with product training and support from the manufacturer (Carter & Jeukendrup, 2002; Cooper & Storer, 2001). However, several limitations are associated with these systems including the problem of hyperventilation resulting from the breath of untrained subjects and the presence of water vapor pressure in the expired air; both are assumed to introduce errors to the calculations of gas volumes and EE. Also, some of the equipment used for gas collection such as mask and mouthpiece are impractical for use during measurements lasting for a long time (i.e. > 120 min) (Simonson & DeFronzo, 1990). These limitations are listed with more detail in the below section 2.3.

2.3 Indirect calorimetric systems

2.3.1 Theoretical background of indirect calorimetry

Indirect calorimetry (also called respiratory calorimetry) is a methodology to estimate the rate of heat transfer indirectly from respiratory gas exchanges. This method estimates rates of O_2 uptake and CO_2 production for resting individuals with RER between 0.7 and 1.0 (Ferrannini, 1988; Simonson & DeFronzo, 1990). For resting endotherms, the macronutrients of carbohydrate, fat, and protein are each metabolized in the presence of a sufficient amount of O_2 and releases energy or heat to maintain basic function of living organisms (Even, Mokhtarian, & Pele, 1994). In fact, macronutrients are formed of carbon-hydrogen bonds that undergo catabolic reactions through various metabolic pathways including the substrate level phosphrylation (without O_2) and aerobic (with the presence of O_2) . The aerobic pathway converges to the Krebs cycle (TCA) to produce ATP, CO_2, water (H_2O) and heat (Brooks et al., 2005). The oxidation of one mole of glucose requires 6 moles (134 L) of O_2, and releases 673 kcal resulting in a thermic effect of O_2 equal to 5.02 kcal•L^{-1}. Also, the oxidation of one mole of fat requires 23 moles (515 L) of O_2 to release 2398 kcal, however, depending on the type of fat oxidized, the longer fatty acids carbon chain, greater the energy transfer. Lastly, the oxidation of one mole of protein requires 5.1 moles (114 L) of O_2 to release 475 kcal. Overall, the thermic effect of O_2 is equal to 4.66 kcal•L^{-1} for fat (greater for longer fatty acids) and 4.17 kcal•L^{-1} for protein. Note that for the complete energy equivalent of protein, the amount of urea needs to be assessed for its energy content since it is not metabolized by the body (Ferrannini, 1988).

The ratio of $\dot{V}CO_2$ over $\dot{V}O_2$ ($\dot{V}CO_2$/ $\dot{V}O_2$) is called respiratory exchange ratio (RER). This ratio is important for respiratory calculations because its value represents the type of substrate being oxidized (Livesey & Elia, 1988). Typically, the RER value of 1 indicates a glucose oxidation, while the RER values of 0.81-0.88 and 0.7-0.74 indicate protein and fat oxidation respectively (Livesey & Elia, 1988). Because of these tight relationships between the rates of O_2 uptake, CO_2 production, and heat release, it becomes possible to make accurate and non-invasive measurements of energy production in a living organism simply by estimating its respiratory gas exchanges (Even et al., 1994). There are two main techniques used in indirect calorimetry: closed-circuit system and open-circuit system. These two techniques have some advantages as well as some limitations that may be important to understand before using indirect calorimetry.

2.3.2 Main types of Indirect calorimetry

2.3.2.1 Closed-circuit system

The closed-circuit system was the first technique used to measure respiratory gas exchanges and is used for calorimetric measurements in small animals (Simonson & DeFronzo, 1990). The process of gas measurement is accomplished by locating a living animal in a sealed, air-tight chamber in which the respiratory gas exchanges of the animal results in O_2 uptake and CO_2 and water production. Accordingly, the modified chamber pressure and air composition can be measured by different transducers depending on the complexity and the accuracy of the device (Even et al., 1994). To keep the animal alive for several hours or days, a controlled amount of O_2 is injected into the chamber and the CO_2 is scrubbed from the chamber. Next, $\dot{V}O_2$ uptake is determined by measuring the volumetric change from reservoir of O_2 over time (Branson & Johannigman, 2004).

The major advantages of this system are the high sensitivity because of its small size, the limited electronic requirements, and the low cost. These factors made a closed-circuit respiration system popular and is still widely used for small animals (Even et al., 1994). However, the main limitation of this system is the incomplete energy metabolism calculations because CO_2 is incompletely trapped by a substance (sodium hydroxide-NaOH) present in the chamber; therefore, RER cannot be measured and the computation of fuel oxidation cannot be performed as well (Even et al., 1994).

2.3.2.2 Open-circuit system

Open-circuit systems in use today can be classified as either pull system in which room air is pulled through the chamber at a constant flow rate, or push system in which room air is pushed through the respirometry chamber (Lighton, 2008). However, the literature has shown that most laboratories use a pull-type room calorimetry system (Melanson et al., 2010) for prolonged data collection and push-type system during short-duration exercise (20-min to 340-min).

In the open-circuit system, both ends of the respiratory system are open to the environment, and the room air is passed through the chamber at a constant flow-rate. In principle, open-circuit systems determine the rate of heat transfer from the flow rate of fresh air and respiratory gas concentrations at the chamber inlet and outlet. Classically, this system uses a fan or pump to pull fresh air into the chamber, pass the respiring animal, and out of the chamber through flow meter, therefore, creating a negative pressure inside the chamber to minimize escape of air through leaks (Even et al., 1994). At the chamber outlet, the air is collected, dried from the water vapor and directed to gas analyzers to determine the fractions of O_2 and CO_2. From the latter parameters, the $\dot{V}CO_2$, $\dot{V}O_2$, and RER are computed (Simonson & DeFronzo, 1990).

The open-circuit system can be applied in similar operation but different physical designs in which a whole body chamber houses the entire animal but restricts animal movement and are often very expensive; the alternative is using a ventilated hood that covers the subject's head or using a respiratory mask or a mouth piece (Storm, Hellwing, Nielsen, & Madsen, 2012). Open-circuit systems consist of a chamber collecting gases exhaled from the living organism, a ventilation supply of air, a gas sampling system, a water vapor pressure (desiccant dryer or water vapor pressure measuring device), barometric pressure and temperature control systems (Storm et al., 2012). However, some open-circuit systems include breath-by-breath metabolic carts with non-breathing valves which sample air from near the mouth. The interface between a metabolic cart and human or large animals (e.g. dogs, horses) collects the expiratory breath through canopy, mask, or mouthpiece (Simonson & DeFronzo, 1990).

The major advantage of the mask / mouthpiece over the canopy resides in its usefulness in recording metabolic rate during physical activity of all types and the freedom of movement it offers. Also, the short response time of airflow permits detection of changes in respiration and ventilation (Even et al., 1994). However, there are some limitations for using these interfaces. First, using mask / mouthpiece with untrained subjects can result in hyperventilation therefore affecting the fraction of gases and invalidating the subsequent calculations of rates of O_2 uptake, CO_2 production and EE. Nevertheless, the subject quickly acclimates to the interface to overcome such a problem. Second, using these types of interface is a little uncomfortable. Lastly, these interfaces are suited for short time measurements, but unreliable for studies collecting data over long periods (> 90 min) (Simonson & DeFronzo, 1990).

For long data collection periods, a plastic ventilated hood device can be implemented from which the expired air is pulled through the system using a pump. A flow-measuring device such as turbine, pressure transducer, or mass flow meter measures air volume, and standard temperature, dry and pressure (STDP) corrections are applied to $\dot{V}O_2$ and $\dot{V}CO_2$ rates (Simonson and DeFronzo, 1990). Lastly, a sample of the expired air is collected using a low-flow pump for the determination of fraction of gases. Typically, the canopy in the open-circuit system is ideal for measuring a subject's BMR and post mean thermic effect of feeding (Simonson & DeFronzo, 1990).

The open-circuit system has several advantages which include: convenient to use, accurate, and comfortable, and can be used for humans and animals with different sizes and metabolic rates or exercise intensities. In addition, open-circuit system is widely used for BMR measurements and for exercise testing because it allows a fast and real minute-to-minute analysis of respiratory gas exchanges (Even et al., 1994). However, the open-circuit system has many practical limitations. First, because of using very accurate gas analyzers, mass flow meters and electronic equipment, open-circuit systems are complex and expensive (Even et al., 1994). Second, FO_2 and FCO_2 and the subsequent calculations can be highly altered if 1-2 mmHg of water vapor was in the expired air (Simonson & DeFronzo, 1990). Therefore, water quantity must be measured in or removed from the air prior to further analysis. This can be accomplished by passing the expired air through a drying substance (e.g. calcium chloride, magnesium perchlorate) or by measuring the quantity of water in a volume of air to adjust PO_2 and PCO_2 (Simonson & DeFronzo, 1990). Third, usually, the air volume flowing into the hood is not equal to the volume flowing out (i.e., air volume flowing into plus exhaled air from the animal / human); therefore, a mass flow meter should be employed for accurate $\dot{V}O_2$ calculation (Even et al., 1994; Haugen et al., 2007; Simonson & DeFronzo, 1990).

2.3.3 Clinical benefits of indirect calorimetry

It is well established that EE is drastically altered and the metabolic responses are changed by the presence of illnesses and injuries. Therefore, it is quite important to accurately estimate the rate of EE to modify the total caloric needs in hospitalized patients (Haugen et al., 2007). The application of IC system provides reliable and useful measurements of EE that establish an accurate caloric intake required for the management of critically ill patients such as patients diagnosed with intestinal failure, metabolic syndrome, diabetes, hypo- and hyper-thyroidism, and cardiovascular disease (Carlsson & Burgerman, 1985; Ławiński et al., 2015). Indeed, information obtained from a metabolic cart is used for proper estimation of the nutritional needs that protect patients from under- or over-feeding and to optimize nutritional support (Norman, Pichard, Lochs, & Pirlich, 2008; Porter & Cohen, 1996; Samocha-Bonet et al., 2012).

2.3.4 Indirect calorimetry in research settings

Indirect calorimetry has been used in research for quite some time. In fact, it is considered a powerful research tool for studying metabolism with great emphasis on EE and substrate oxidation at rest and during exercise in both human and animals (Even et al., 1994; Ferrannini, 1988). Moreover, the IC system facilitates the generation of EE data specific to different medical conditions and diseases. This information assisted in refining clinical practice, modifying pharmacological options, and in changing clinical nutrition support (Haugen et al., 2007). In addition, metabolic carts are used as a gold standard method by comparing its results with some known caloric prediction equations such as Harris-Benedict and Mifflin-St Jeor equations (Haugen et al., 2007) and physical activity trackers (Alsubheen, George, Baker, Rohr, & Basset, 2016). Because IC system estimates the rate of EE and substrate oxidation, it can be a useful method in managing weight control. Scientists have shown that obese individuals are more likely to adhere to a diet programs that are based on a real metabolic measurements (Haugen et al., 2007). Furthermore, research has reported that early determination of energy metabolic rate can identify individuals who are prone to weight gain, and therefore, contribute to the control of the obesity epidemic. Valuable information about EE, BMR, TEF obtained with metabolic carts can efficiently help to predict obesity and design personal intervention programs of weight control (Lam & Ravussin, 2017). However, to ensure accurate research data and outcomes, IC systems must be assessed for its validity, reliability, and accuracy.

2.4 Validity, reliability and accuracy of indirect calorimetric system

As shown above, IC measurements are complex and sensitive to a wide range of errors during data collection. To obtain correct values of gas exchanges, first, the performance of gas analyzers, the flow-measuring device and the calibration gases all must be within appropriate range of accuracy. Second, the gas analyzers should assess the end-tidal air for O_2 and CO_2 partial pressures, and the inspired air must represent the true atmospheric O_2 concentration. Third, the temperature, barometric pressure, and humidity of the gases assessed by a metabolic cart must be monitored carefully. Lastly, the software for gas volume calculations must be accurate and correct (Nunn, Makita, & Royston, 1989).

It has been noticed that the new commercial metabolic carts have developed some error in all or part of the above mentioned aspects. Furthermore, it is not uncommon that different metabolic carts or even the same equipment provide different $\dot{V}O_2$ and $\dot{V}CO_2$ readings under the same conditions. Therefore, validation of their performance must be carefully examined using rapid and comprehensive methods that are easily undertaken by any user (Miodownik, Melendez, Carlon, & Burda, 1998; Nunn et al., 1989). Moreover, because errors gradually appear over time and could bias results, it is important to check IC system validity prior to each measurement and before and after the study period (Tøien, 2013).

The accuracy of IC gas analyzers typically refers to the closeness between the measured value and the “true” value. Since the “true” value cannot absolutely be determined, it is a common practice to use an accepted reference value (theoretical value) based on stoichiometry of a reference gas or gases. The reliability refers to reproducibility or repeatability of the readings of the gas analyzer under identical conditions. While the validity is the extent to which an instrument accurately measures the variable it claims to measure (Cooper & Storer, 2001).

The validation of a metabolic cart was originally performed by comparing its results with a DB method. However, this method had several limitations such as the time consuming and the need of highly qualified personnel to perform the validation process. Therefore, different techniques have been proposed to evaluate the accuracy, validity, and reliability of IC systems. The traditional technique for calibrating IC system requires to flow a known amount of calibration gas (CO_2, N_2) through O_2 and CO_2 analyzers at regular time intervals and then adjust analyzers control accordingly, however, this method can induce measurement error if the flow rate of gases lacks inaccurate. For instance, Schadewaldt et al. (2013) assessed the validity and reliability of a metabolic cart using a mass-flow meter regulator and infusion of pure gaseous of CO_2 and N_2. The authors reported that this method of calibration can be used to overcome a lack of accuracy problem in some metabolic carts that are based on flow-through respiratory measurements in canopy mode (Schadewaldt et al., 2013). However, to ensure accurate measurements, it is important to apply standard conditions when calibrating flow-measuring devices that also must be carefully maintained and recalibrated based on manufacturer's instructions (Fedak, Rome, & Seeherman, 1981; Lighton, 2008).

In fact, burning a certain amount of gas can simulate O_2 uptake and CO_2 and water produced by an animal or a human (Tøien, 2013). As a result, burning known amounts of pure alcohol (methanol, ethanol), butane or propane gas can provide a good alternative for the traditional flow of calibration gases. For example, Nunn et al. (1989) burned commercial butane in a closed-circuit IC system under the conditions of artificial ventilation and increased inspired O_2 concentration. At measured flow rates (4-15 $L \bullet min^{-1}$), they burned a certain amount of butane gas. Thus, 1 mol of butane is equivalent to 6.5 mol of O_2 and the respiratory exchange ratio (RER) is 0.61. They found that the combustion of butane provides a simple and very robust test of the IC system's performance. However, this method is not applicable for a mixture of gases containing nitrous oxide because its concentration decreases when burning butane, leading to wrong estimates of O_2 and CO_2 concentrations (Nunn et al., 1989). On the other hand, in the study of Marks et al. (1987), an open-circuit IC system was assessed during the combustion of a known mass of ethanol and methanol gases (alcohol burn) when they had a gas flow rate of 2-5 L•min^{-1} and measured the partial pressures of O_2, CO_2, and N_2. The authors found that the $\dot{V}O_2$ and $\dot{V}CO_2$ calculated from the known amount of alcohol combustion are within 5% from the true values. After conducting rigorous calculations, the authors concluded that the rate of combustion of a known mass of alcohol was a suitable laboratory method for validation of respiratory gas exchange measurements in the neonate (Marks et al., 1987).

In another study, Miodownik et al. (1998) described a new burning device with which a known amount of methanol was burned at flow rates of 0.2 to 4 L•min^{-1} and they calculated $\dot{V}O_2$, $\dot{V}CO_2$, and RER. In this methanol-burning lung model, the rate of methanol combustion was equal to the infusion rate of fuel over an extended range of O_2 concentrations. This method provides a continuously adjustable and quantitative volume of gases with RER of 0.667 and an error caused by CO and unburnt methanol of less than 0.005%. The authors concluded that this apparatus is simple to reproduce, accurate and valid to calibrate gas exchanges instrument. A major problem of this technique is the evaporation of alcohol that makes it difficult to simulate the low metabolic rates (Lighton, 2008; Miodownik et al., 1998).

Finally, burning propane gas was proposed by Lighton (2008) as a new method for validating IC systems. Propane gas differs from other reagents because it has good air-fuel mixing potential due to its low boiling temperature, and as a result produces less carbon monoxides and other volatile hydrocarbons (Lighton, 2008). Burning known amount of propane gas provides a check for both $\dot{V}O_2$ and $\dot{V}CO_2$; RER can be measured from the reaction as its value reaches 0.60. From these advantages, Melanson et al. (2010) reported that propane gas combustion is routinely used to calibrate IC systems in their laboratory (Melanson et al., 2010). Although propane gas appears to be the method implemented to calibrate metabolic carts, up to date, to our knowledge, there is no existing study assessing the validity, reliability, and accuracy of metabolic carts using propane gas technique.

2.6 Conclusion

Indirect calorimetry is the most commonly used method to estimates rate of EE in the clinical and research settings. By estimating respiratory gas exchanges, rates of O_2 uptake and CO_2 production, IC provides minute-by-minute EE data that makes it the most valuable tool to distinguish the various components of EE, that is, BMR, TEF, and physical activity EE. These measures also provide information on substrate oxidation rates by the body. Accurate measurement of the rate of EE is important for monitoring metabolic responses and nutrition status at rest and during exercise. The quality of recent commercial metabolic carts can permit highly valid and reliable measurements of $\dot{V}O_2$ and $\dot{V}CO_2$ to be made, yet extensive care must be taken in the maintenance and calibration of these IC systems to facilitate more accurate measurements. Different calibration techniques have been proposed to check the validity, reliability and accuracy of these systems such as passing known amounts of N_2 and CO_2 gases, burning alcohol (ethanol, methanol) or burning propane to simulate human/animal metabolism in which O_2 is consumed and CO_2 and water are produced. Although propane gas is used routinely to calibrate metabolic carts, to our knowledge, there is no existing study that examined the validity, reliability, and accuracy of IC systems using propane gas technique. Therefore, the aim of the present study was to assess the validity of a calibration procedure using propane gas combustion technique in three metabolic carts (Sable, Moxus, Jaeger).

Chapter III

Manuscript

3.1 Introduction

Of primary interest to use indirect calorimetry (IC) in human thermoregulation is the measurement of fractions of oxygen (FO_2) and carbon dioxide (FCO_2) for determination of metabolic rate (MR) and calculation of substrate partitioning of an individual in various environmental conditions – in resting state, during exercise, in cold exposure, or in hot environment (Power, 2012). To perform these measurements, chemical, electronic, and spectroscopic technologies have been developed. Indirect calorimetry systems integrate discrete electronic analyzers to record FO_2 and FCO_2 using computer-controlled analog-to-digital signal processing (Leonard, 2012). Several instrument configurations are available, ranging from very simple or semi-automated mixing chamber systems to highly sophisticated fully automated breath-by-breath measurement systems (Matarese, 1997). The expected accuracy and reliability of the instrument may differ from one manufacturer to another. Often, the user relies on the manufacturer's technical notes to calibrate their instrument. However, the calibrations must be performed in specific conditions for which the signal-noise ratio is optimized through proper calibration technique to detect small metabolic differences between the experimental and control conditions (Ferrannini, 1988; Lighton, 2008). Therefore, validating the results obtained from a flow-through respiratory (metabolic) system is a crucial operation.

The IC system is an important tool used in many areas of biological sciences, especially in human physiology and medical sciences (Lighton, 2008). Many techniques have been developed to determine the validity and reliability of IC systems but few were designed to generate accurate FO_2 and FCO_2 corresponding to human basal / resting metabolic rate. Techniques such as burning of methanol (Cooper et al., 2009), burning propane (Lighton, 2008) or diluting nitrogen (Fedak et al., 1981) are the most common techniques used by researchers. In fact, most of these techniques, implemented in the human physiology laboratory, rely on either oxygen uptake or respiratory exchange ratio (RER) values to validate IC systems. Lack of appropriate calibration and validation will lead to inaccurate volume of O_2 ($\dot{V}O_2$) and volume of CO_2 ($\dot{V}CO_2$) and, consequently, to errors in the calculation – from stoichiometry equations – of substrate partitioning and energy production (Ferrannini, 1988). One of the most important aspects of nutrition support and physical activity recommendations are based on the ability to determine and estimate, with high accuracy, human metabolic profile and rate of energy expenditure (EE) (da Rocha et al., 2006). For instance, a patient's nutritional status will be evaluated through changes in MR that reflect oxidation rates of carbohydrate, lipids, and proteins (Brooks et al., 2005). No doubt that the development of the IC system has greatly facilitated the measurement of MR, substrate partitioning and EE in both laboratory and field settings, and recent advances in gas exchange technologies have made assessment of MR promptly available (Haugen, Chan, & Li, 2007). However, accuracy and precision of the measurements under a variety of experimental and clinical conditions are important factors to consider when monitoring variations in energy production (Levine, Eberhardt, & Jensen, 1999). Therefore, it is important to enhance existing methodologies for calibrating IC systems and for determining O_2 and CO_2 detectors sensitivity. The aim of the present study was then to assess the validity and reliability of a new calibration method using propane gas technique in three metabolic carts (Sable, Moxus, and Jaeger).

3.2 Materials and methods

3.2.1 Equipment

Three IC systems that are currently available in our exercise physiology laboratory (Sable, Moxus and Jaeger) were assessed with an in-house built propane gas calibration device for their validity, reliability and accuracy. All IC systems were set as pull system flow measurement (negative pressure system). Prior to data collection, IC systems were calibrated with medically certified calibration gases (1% CO_2 and 100% N_2, for Sables system and 4% CO_2 and 16% O_2 for Moxus and Jaeger systems) and volumes were determined according to the manufacturer recommendations.

3.2.1.1 Technical characteristics of the indirect calorimetric systems

The Sable System (Sables Systems International, Las Vegas, NV, USA) is a modular IC consisting of a subsample pump (sub-sampler, SS4 – linearized mass flow meter ranging from 0-2000 ml•min^{-1}), a water vapor analyzer (RH-300 – resolution = 0.001% and full range = 0-100% RH non-condensing), a dual infrared carbon dioxide sensor [CA-10 Carbon Dioxide (accuracy = 1%; resolution = 0.00001%; full range = 0-10%; time response = 0.5 sec), a paramagnetic oxygen sensor [PA-10 Oxygen analyzers (accuracy = 0.1%; resolution = 0.0001%; full range = 0-100%; time response = 0.2 sec)] and an air mass flow generator and controller (FK-500 – accuracy = 0.05 L•min^{-1}; full range = 50-500 L•min^{-1}).

The automated gas analyzer, Moxus Modular Metabolic System (AEI technologies, IL, USA), consists of a turbine for determination of ventilation volume [VMM-400 (flow range = 0-800 L•min^{-1}; accuracy = ±1%)], a 4.2 L mixing chamber, a subsample pump (flow rate ranging from 10-500 mL•min^{-1}), a zirconia oxygen sensor [3A/I Oxygen analyzers (accuracy = ±0.01%; resolution = ±0.01%; full range = 0-100%; time response = 0.1 sec)] and a dual infrared carbon dioxide sensor [CD-3A Carbon Dioxide (accuracy = ±1%; resolution = 0.01%; full range = 0-15%; time response = 0.025 sec)]. The subsample air is dried using a nafion sample line embedded into a box containing desiccant.

The Jaeger system (Oxycon Pro, Hoechberg, Germany) is a quasi-modular IC consisting of a twin tube (Nafion sample line), a turbine volume transducer (flow range = 0-300 L•min^{-1}; accuracy = ±2%), a subsample pump (flow rate ranging from 200 to 220 ml•min^{-1}), a 6 L mixing chamber, a fuel-cell oxygen sensor (accuracy = 0.05%; resolution = 0.01%; full range = 0-25%; time response = 0.08 sec), a dual infrared carbon dioxide sensor (accuracy = 0.05%; resolution = 0.01%; full range = 0-15%; time response = 0.08 sec).

3.2.1.2 Propane gas calibration device

The in-house built propane gas calibration system consists of sequential connections. A tank of chemically pure (99%) propane gas (SPG-PROCHP6 – Air-Liquids Canada,) with a two-stage Western Medical gas regulator (model M1-940-PG, Westlake, Ohio) and its gas hose is connected to a one-way Matheson mass-flow transducer (model 8141) that is subsequently connected to a Matheson mass-flow controller, model 8240 (East Rutherford, NJ) and to a Bunsen burner – vertical metal tube of 60 mm high and 4 mm inside diameter (ID). The burner is located into a 2.4 L glass canopy that flows into a

0.4 L glass tubing. From there, the entire system is connected to IC by a 1.4-inch diameter hose (see Figure 1). The pressure in the gas-line flowing out of the cylinder is maintained at 10 psi. The regulator is fitted with a ¼ inch MNPT brass needle valve and a high pressure gas hose (4mm ID, 7mm outside diameter, OD) to prevent potential propane leak.

3.2.1.3 Mass Flow meters

Mass flow meters measure the molar mass of gas passing through them and the flow rates are expressed in STP-corrected volumes. Generally, all mass flow meters have an analog or digital output that can be recorded along with other data. This analog output varies from 0 to 5 V as the flow through the meter varies from 0% to 100% of full scale (Lighton, 2008). The advantages of the mass flow meter are direct gas mass flow measurements, high measuring accuracy, very low pressure drop, large span, no moving parts, no pressure and temperature compensation required, short response time, rugged construction, and easily sterilized (Lighton, 2008). The mass flow meter used in our study was the Matheson mass-flow controller, model 8240 (East Rutherford, NJ) which was validated using N_2 dilution technique as described by Fedak et al. (1981) and Lighton (2008). The data collection are detailed in the next section and its results are shown in the results section.

3.3 Data collection and reduction

3.3.1 Nitrogen dilution technique

Nitrogen dilution technique was implemented as described by Fedak et al. (1981) and Lighton (2008) using the in-house built propane gas device and the Sable system for validating purposes of the mass-flow meter. A series of N_2 gas flows (1000, 500,

250. 150 ml•min^{-1}) were randomly selected at two different ventilation rates (55 and 75 L•min^{-1}) and injected into the incurrent air connection of the Sable system. The actual experimental values of N_2 gas were compared to its theoretical value (Fedak et al., 1981; Lighton, 2008).

3.3.2 Propane gas data collection

A series of propane flow levels (200, 300, 400, 500, and 600 mL•min^{-1}) were selected and tested at two different ventilation rates (55 and 75 L•min^{-1}, for Sable and Moxus system; 20 and 40 L•min^{-1} for Jaeger system, according to manufacturer recommendation) via an air mass flow generator and controller (FK-500 – accuracy = 0.05 L•min^{-1}; full range = 50-500 L•min^{-1}). Three 30-min trials per flow level and per ventilation rate were randomly performed. All trials were conducted at one location and at the same time of the day. The fractions of oxygen (FO_2) and carbon dioxide (FCO_2), mass flow rate (FR), barometric pressure (BP), water vapor pressure (WVP), chamber temperature (T°C-Ch), and room temperature (T°C-Rm) were recorded through IC systems for the inspired air (F_i) baseline (15-min pre- and 15-min post-propane gas combustion) and the expired air (F_e) propane gas combustion.

3.3.3 Propane gas stoichiometry equations

Pure propane (C_3H_8) is an odourless, colorless and flammable gas. A complete combustion of one mole of 100% C_3H_8 produces three moles of CO_2 and 4 moles of H_2O for each five moles of O_2 consumed according to the stoichiometry reaction depicted in Equation 1.

$$C_3H_8 + 5O_2 \rightarrow 3CO_2 + 4H_2O \qquad \text{(Eq.1)}$$

Therefore, under standard pressure and temperature (STPD), 22.44 L of C_3H_8 would react with 112.2 L of O_2 for the reaction to be completed in order to produce 67.2 L of CO_2. At standard conditions, the molecular mass of 100% C_3H_8 is 44 g and one gram of propane would then require 2.55 L of O_2 (i.e. 112.2 L of O_2 to burn 44 g of propane) to produce 1.53 L CO_2, that is, 67.2L of CO_2 results from the burning of 44 g of propane. It can then be deduced that optimal C_3H_8 combustion results in RER equal to 0.600 (Lighton, 2008). For an accurate flow of gas, a mass flow meter was used in the present study, and the gas flow rate was calculated using the following formula:

Mass flow rate (g. min^{-1}) = volume flow rate x propane density

(propane density at 25^{o}C = 1.799 kg•m^{-3}, at 15^{o}C = 1.908 kg•m^{-3})

3.3.4 Flow-through respirometry equations

The fraction of gases (FO_2, and FCO_2,) and flow rate collected from the metabolic carts were first corrected for the effect of PH_2O, T°C (from the mixing chamber), and BP by computing the following equations (Lighton, 2008):

$$F'_iO_2 = FO_2 \times BP \div (BP - WVP) \qquad \text{(Eq.2)}$$

where $F'iO_2$, and FO_2 represent fraction of inspired air dry and moist oxygen, respectively.

$$F'_iCO_2 = FCO_2 \times BP \div (BP - WVP) \qquad \text{(Eq.3)}$$

where $F'iCO_2$, and FCO_2 represent fraction of inspired air dry and moist carbon dioxide, respectively.

$$FR' = FR \times (BP - WVP) \div BP \qquad \text{(Eq.4)}$$

where FR' and FR represents dry and moist air flow rate, respectively.

To correct for any drift in fraction of oxygen, the following equation was computed as:

$$((F_iO_{2i} + ((F_iO_{2f} - F_iO_{2i}) \times T_{ss} \div (T_f - T_i))) - F_eO_{2ss}) \times F\,R \qquad \text{(Eq.5)}$$

where F_iO_{2i} is the initial fractional amount of oxygen in the inspired air stream measured at equilibrium before each propane gas combustion (baseline pre-); F_iO_{2f} is the final fractional amount of oxygen in the inspired air stream measured at equilibrium after each propane gas combustion (baseline post-); T_{ss} is the time into each propane gas combustion at steady state; T_f is the time when final inspired oxygen fraction is measured; T_i is the time when initial inspired oxygen fraction is measured; F_eO_{2ss} is the mean fractional amount of oxygen in the expired air stream measured for each propane gas combustion at steady state for at least one minute at the end of each trial; FR is the mean gas flow rate in the canopy when steady state $F'O_2$ is measured.

The calculation of $\dot{V}O_2$ (the rate of O_2 uptake), $\dot{V}CO_2$ (rate of CO_2 production), and RER was performed using the following equations:

$$\dot{V}O_2 = FR_e \times (F'_eO_2 - 0.2094 \times F'_eCO_2) \div (1 - 0.2094) \qquad \text{(Eq.6)}$$

where FR_e is expired flow rate; F'_eO_2 stands for expired dry oxygen; and F'_eCO_2 for fraction of expired dry carbon dioxide. The constant (0.2094) stands for the fraction of O_2 in the room at sea level.

$$\dot{V}CO_2 = FR_e \times (F'_eCO_2 + F_iCO_2 \times F'_eO_2) \div (1 + F_iCO_2) \qquad \text{(Eq.7)}$$

where FR_e is expired flow rate; F'_eCO_2, fraction of expired dry carbon dioxide; F_iCO_2, fraction of inspired carbon dioxide; F'_eO_2, expired dry oxygen.

$$RER = \dot{V}CO_2 \div \dot{V}O_2 \qquad \text{(Eq.8)}$$

where RER is the quotient of $\dot{V}CO_2$ over $\dot{V}O_2$.

The metabolic data ($\dot{V}O_2$, $\dot{V}CO_2$, and RER) were truncated by 10 min (5 min at each end of expired air signal). Then, respirometry data were compared to the stoichiometry theoretical $\dot{V}O_2$ and $\dot{V}CO_2$ values, obtained through propane gas combustion under standard environment, to determine the validity, reliability and accuracy of the three different metabolic carts.

3.4 Statistical analysis

Statistical analyses were performed using SPSS, version 23 (SPSS Inc., Chicago, IL, USA). All values are reported as mean ± standard deviation, unless otherwise specified, and an alpha level (α) of 0.05 was used to indicate statistical significance. Tests for statistical assumptions (i.e., normality and homogeneity of variance) were performed, that is, the homogeneity of variance was tested using Levene's test and normality was tested using Kolmogorov-Smirnov test. First, descriptive statistics were conducted. Second, a series of one-way ANOVA was used to assess the effect of ventilation rates (55 $L{\bullet}min^{-1}$, 75 $L{\bullet}min^{-1}$ for Sable and Moxus , 20 $L{\bullet}min^{-1}$, 40 $L{\bullet}min^{-1}$, for Jaeger) on fraction of gases. Third, a linear regression analysis was performed to examine the linearity between the volumes of $\dot{V}O_2$ and $\dot{V}CO_2$, and propane flow levels for the three systems and to test the linearity of N_2 flow rates with $\dot{V}O_2$. Fourth, Bland-Altman plots followed by linear regressions were created to evaluate the mean difference (error) between systems outputs and the stoichiometry theoretical $\dot{V}O_2$, $\dot{V}CO_2$, RER values for all systems at all propane flows. Lastly, a series of two-factor [3 (IC) × 5 (flow rates)] ANOVAs were conducted to evaluate the effects of the three systems and the five propane flow levels for

$\dot{V}O_2$, $\dot{V}CO_2$, and RER error. A mixed models design was used with systems being a fixed effect and flow level a random effect. A corrected F-test was calculated (Neter, Kutner, Nachtsheim, & Wasserman, 1996) for the random factor as mixed models in SPSS incorrectly uses MS *from the interaction* as the error term (denominator). The correction is to use MS *error* as the denominator. SPSS correctly uses MS *error* in the F-test for the interaction, which is also considered random. SPSS correctly uses the MS *from the interaction* as the error term (denominator) for the fixed factor's F-test. Effect sizes were calculated for F-test: Omega-squared (Ω^2) was used for the fixed effect of system and rho (ρ) was used for the random effect of flow level as was the interaction effect. In case of significant interactions Tukey and Bonferroni post-hoc tests were applied.

3.5 Results

3.5.1 Exploratory and Descriptive Statistics

Descriptive statistics were performed on $\dot{V}O_2$, $\dot{V}CO_2$, and RER (see Table 1). Levene's test for testing homogeneity of variance ($\dot{V}O_2$, $\dot{V}CO_2$) for some propane flow levels were significant, therefore, the homogeneity of variance assumption of some of the data is violated. However, F-statistics is robust when there are not equal variances (Field, 2013). A test of normality (Kolmogorov-Smirnov) was performed within flow levels. The assumption of normality was not met for all data.

3.5.2 Regression analysis of N_2 flow rates with $\dot{V}O_2$ for the Sable system

Simple linear regression analyses were conducted to assess the linear relationship between N_2 flow rates and actual experimental $\dot{V}O_2$ values ($ml \bullet min^{-1}$) for the Sable system. Results showed a strong linear relationship between the actual experimental

$\dot{V}O_2$ values and the N_2 flow rates ($R_{adj}^2 = 0.996$; $\beta = 0.998$; 95% CI (0.174-0.183); $p < 0.001$).

3.5.3 Difference between ventilation rates among three systems

A series of 15 one-way ANOVAs [2 $\dot{V}E$ (55 L•min^{-1}, 75 L•min^{-1} for Sables and Moxus , 20 L•min^{-1}, 40 L•min^{-1}, for Jaeger) X 5 flow level (200, 300, 400, 500, 600 mL•min^{-1})] revealed no significant differences between the two $\dot{V}E$ rates at all propane flow levels for Sable, Moxus, and Jaeger systems, respectively. As $\dot{V}E$ rates were not significantly different from each other the data for $\dot{V}E$ rates were pooled (i.e., ventilation as a variable was ignored).

The average of the three experimental trials per propane flow level are shown in Table 1. The mean differences of the three systems were calculated by subtracting the actual experimental values from the stoichiometry theoretical values. The RER values were calculated by dividing $\dot{V}O_2$ / $\dot{V}CO_2$.

3.5.4 Linear relationship of $\dot{V}O_2$ and $\dot{V}CO_2$ by propane flow level per system

A linear regression analysis was conducted to assess the linearity between the $\dot{V}O_2$, $\dot{V}CO_2$ and the propane flow levels and to determine linear regression equations ($y = b_0 + b_x + \varepsilon$) for each system. Table 2 suggests that a linear relationship exists in the three systems. Statistically, R^2 represents the proportion of predicted data that is explained by the model around its mean. For the Sable system, 99.6 % of the variability in $\dot{V}O_2$ and $\dot{V}CO_2$ are explained by the propane flow levels with an error value of 0.4 %. The model of the Moxus system explains 99.2% of the variability in $\dot{V}O_2$ and 99.4% of the variability in $\dot{V}CO_2$ (the error values are 0.8% and 0.6% for the $\dot{V}O_2$ and $\dot{V}CO_2$, respectively). However, the Jaeger system had the worst linearity values as compared to other two systems (94.8%, error = 5.2, 94.2 %, error = 5.8 for $\dot{V}O_2$ and $\dot{V}CO_2$, respectively). The mean values of $\dot{V}O_2$ and $\dot{V}CO_2$ were lowest for the 200 ml•min^{-1} propane flow level and increased as propane flow level increased for the three systems (Figure 2).

3.5.5 Assessing error of $\dot{V}O_2$, $\dot{V}CO_2$, and RER for each system

To assess the relationship between the error in the system ($\dot{V}O_2$, $\dot{V}CO_2$, and RER) and the propane flow level, Bland-Altman plots were created and were followed by linear regression analysis for each system. As shown in Table 3, there was a weak, moderate, and strong linear relationship between the $\dot{V}O_2$, $\dot{V}CO_2$ errors and the propane flow level for the Sable, Moxus, and Jaeger systems, respectively. Also, $\dot{V}O_2$ and $\dot{V}CO_2$ errors of the Sable system had a non-significant *p*-value, which indicates that the Sable system had the lowest error in both volumes compared to the other two systems. However, although *p* values were significant for both Moxus and Jaeger systems, the $\dot{V}O_2$ and $\dot{V}CO_2$ errors of the Moxus system had a weaker relationship with propane flow level and therefore lower error compared to the Jaeger system. The Jaeger system had the highest error among the three systems.

A two-factor [3 (IC) × 5 (flow rates)] ANOVA was conducted to evaluate the effects of the three systems and the five propane flow levels for $\dot{V}O_2$, $\dot{V}CO_2$, and RER error. For $\dot{V}O_2$ error the results indicate a significant interaction between system and flow level ($F_{(8,75)}$ = 3.328, p = 0.0026). Approximately 26.6% (ρ = .266) of the variance in $\dot{V}O_2$ error is accounted for by the interaction factor. The results show a non-significant main effect of flow level on $\dot{V}O_2$ error ($F_{(4,75)}$ = 1.0714, p = 0.3767), and it accounts for only 0.2% (ρ =

.00286) of the variability in $\dot{V}O_2$ error. The main fixed effect of system was found to be significant ($F_{(2,8)} = 67.028$, $p < 0.001$). All else held constant, the fixed main effect of system accounts for approximately 83% ($\Omega^2 = .8293$) of the variability in $\dot{V}O_2$. The Jaeger system had significantly ($p < 0.001$) greater $\dot{V}O_2$ error ($M = -0.057$, $SE = .004$) compared to either the Sable ($M = 0.044$, $SE = 0.004$) or Moxus ($M = 0.046$, $SE = 0.004$) systems. There were no significant differences between Sable or Moxus systems (Figure 3).

The results for $\dot{V}CO_2$ indicate a significant interaction between systems and flow level ($F_{(8,75)} = 10.722$, $p < 0.001$). Approximately 54% ($\rho = .539$) of the variance in $\dot{V}CO_2$ is accounted for by the interaction factor. The results show a significant main effect of flow level on $\dot{V}CO_2$ ($F_{(4,75)} = 9.375$, $p < 0.001$) and it accounts for 14.8% ($\rho = .148$) of the variability in $\dot{V}CO_2$. The main fixed effect of system is also significant ($F_{(2,8)} = 34.966$, $p <$ 0.001). All else held constant, the fixed main effect of system accounts for approximately 89% ($\Omega^2 = .8947$) of the variability in $\dot{V}CO_2$. The Jaeger system had significantly ($p <$ 0.001) greater $\dot{V}CO_2$ error ($M = -0.048$, $SE = .002$) compared to either the Sable ($M =$ 0.024, $SE = 0.002$) or Moxus ($M = 0.025$, $SE = 0.002$) systems. There were no significant differences between Sable or Moxus systems (Figure 3).

The results for RER indicate a significant interaction between system and flow level ($F_{(8,75)}$ $= 3.332$, $p = 0.003$). Approximately 20% ($\rho = .2044$) of the variance in RER is accounted for by the interaction factor. The results show a significant main effect of flow level on RER ($F_{(4,75)} = 7.8125$, $p < 0.001$) and it accounts for 21.8% ($\rho = .2184$) of the variability in RER. The main fixed effect of system is also significant ($F_{(2,8)} = 4.993$, $p = 0.039$). All else held constant, the fixed main effect of system accounts for approximately 23.8% ($\Omega^2 =$ .2381) of the variability in RER. The Jaeger system had significantly ($p < 0.001$) greater

RER error (*M* = -0.028, *SE* = .003) compared to either the Sable (*M* = -.005, *SE* = 0.003) or Moxus (*M* = -.005, *SE* = 0.003) systems. There were no significant differences between Sable or Moxus systems (Figure 3).

3.6 Discussion

The propane gas technique described by Lighton (2008) is routinely used in physiology laboratories (Melanson et al., 2010; White et al., 1995), however, to our knowledge, there is no existing study assessing its effectiveness in calibrating metabolic carts. Therefore, the aim of the current study was to validate the propane gas as a technique to calibrate three metabolic carts: Sable, Moxus, and Jaeger. To validate this technique, we ran series of propane gas with different flow levels and ventilation rates (55 L•min^{-1}, 75 L•min^{-1} for Sables and Moxus , 20 L•min^{-1}, 40 L•min^{-1}, for Jaeger). The novelty of our experimental design resides in the use of a calibrated and validated propane gas mass flow meter (sections 3.3.1 and 3.6.1) which allows accurate monitoring of the flow of gaseous form of propane at any point of time. This differs from most of the proposed techniques that use the weighing method before and after burning gas. In addition, we flow propane gas with an accuracy of 10 ml•min^{-1} at a sampling rate of 1 Hz. Therefore, a variation of 10 ml•sec^{-1} can be documented and the quality of the IC systems output can be ascertained. The major outcome of the study revealed that flowing propane gas at the rate of a human basal metabolic rate shows differences in validation parameters between metabolic carts. Further, the propane system showed an agreement with the stoichiometric theoretical value (Table 2) suggesting that this agreement could be used as an indicator of the accuracy of O_2 and CO_2 analyzers in the Sable and Moxus systems.

3.6.1 Nitrogen dilution technique

Nitrogen dilution technique was used to calibrate the mass flow meter. The calibration technique corrects for possible errors caused by WVP (Fedak et al., 1981; Lighton, 2008). Our results revealed a strong linear relationship between the N_2 flow levels and $\dot{V}O_2$. Fedak et al (1981) pointed out that if O_2 analyzer has a linear output, then the drift caused by the WVP is negligible and thus, the mass flow meter is valid and provides accurate measurements.

3.6.2 Validity and accuracy of the indirect calorimetry systems

The accuracy of the IC system typically refers to the closeness between the measured value and the "true" value (stoichiometry theoretical value), while the validity is the extent to which an instruments accurately measures the variable it claims to measure (Cooper & Storer, 2001). After propane burning, regression analyses and ANOVA were performed on the calculated $\dot{V}O_2$, $\dot{V}CO_2$, and RER to determine the validity and the accuracy of the three metabolic carts. The combustion of propane gas at different flow levels and ventilation rates showed that the $\dot{V}O_2$ and $\dot{V}CO_2$ values were only influenced by the flow levels, therefore, the ventilation rates were pooled. As shown in Table 1, the percentage of difference between the experimental actual values and the theoretical values of the Sable and Moxus systems overestimated $\dot{V}O_2$ by 22.3% to 6.0%; 14.1% to 8.3%, and $\dot{V}CO_2$ by 22.7% to 5.7%; 14.9% to 8.1%, respectively. The lowest percentage of the differences was associated with higher flow levels indicating that both systems work more efficient at higher flow levels (i.e. work best at 600 ml•min^{-1}). Contrary, the Jaeger system underestimated $\dot{V}O_2$ by 14.4% to 13.0%, and $\dot{V}CO_2$ by 20.7% to 11.6%, and this percentage of difference was not influenced by the propane flow levels for both gas volumes. The propane gas technique showed a pronounced consistency with respect to RER values for both Sable and Moxus systems indicating that the oxidation of propane gas was not affected by the flow levels in contrast to the Jaeger system. Our study demonstrated that both Sable and Moxus systems responded more accurately to burning propane gas than Jaeger system.

The regression analysis of flow levels vs. actual experimental values revealed a strong linear relationship for the three systems; meaning that both $\dot{V}O_2$ and $\dot{V}CO_2$ increased with the increase of propane gas combustion (Table 2). However, when linear regression was performed on the $\dot{V}O_2$ and $\dot{V}CO_2$ errors (difference), Sable system provided the most accurate measurements evidenced by the least error values (i.e. R_{adj}^2 was the least with a non-significant *p* values (p= 0.37, p= 0.13) for $\dot{V}O_2$ and $\dot{V}CO_2$, respectively).

The two-way ANOVA revealed that both Sable and Moxus systems were similar in their response and had almost horizontal lines for $\dot{V}O_2$, $\dot{V}CO_2$, and RER (Figure 3) values indicating more accurate and valid measures compared to the Jaeger system. One reason for this difference in response could be the type of O_2 analyzer (sensor) used in the IC system. For instance, paramagnetic and zirconia oxygen analyzers are used in Sable and Moxus systems while fuel-cell oxygen analyzer is used in Jaeger system. Another reason for the difference in systems response might be the control of temperature change in O_2 analyzers. Technically, the paramagnetic oxygen analyzer has a temperature compensation circuitry that reduces the effect of temperature fluctuations, while a sudden change of temperature in the fuel-cell O_2 analyzer may create significant drift (Lighton, 2008).

3.6.3 Reliability of the indirect calorimetry systems

The reliability refers to reproducibility or repeatability of the readings of the gas analyzer under identical conditions (Cooper & Storer, 2001). The reliability of each IC system was determined by calculating the coefficient of variation (CV) of $\dot{V}O_2$ and $\dot{V}CO_2$. Our results showed that CV values of $\dot{V}O_2$ and $\dot{V}CO_2$ ranged from 1% to 5%, 1% to 4% for the Sable and Moxus systems, respectively, while the Jaeger system had the worst CV that ranged from 7% to 10% for both $\dot{V}O_2$ and $\dot{V}CO_2$. This indicates that the Sable and Moxus systems provided more reliable measures of $\dot{V}O_2$ and $\dot{V}CO_2$ compared to Jaeger system.

3.6.4 Propane gas technique compared to other techniques

Since no similar studies were conducted to validate propane gas for calibrating IC systems, no comparison can be performed. However, propane gas combustion technique is routinely performed in some human physiology laboratories to calibrate IC systems. For example, Melanson et al. (2010) conducted numerous propane gas combustion tests to calibrate Sable system in their laboratory. The authors reported that Sable system provided more than 98% of expected recovery of $\dot{V}O_2$ and $\dot{V}CO_2$ under two different temperature conditions (below 1°C and then above 1°C) (Melanson et al., 2010). However, the authors did not provide details about their propane combustion technique such as the amount of propane flowed pre- and post-burning using a mass flowmeter.

In a recent study, Rising et al. (2015) used propane gas to determine the accuracy of the Sable metabolic system. The Sable metabolic system was subjected to 10, one-hour propane (99.5% purity) combustion tests to simulate 24-hour metabolic measurements. The burn rate (0.15 g/min) was determined by obtaining the weight prior to and after completion of each combustion test using a calibrated analytical balance. After comparing the results of the actual combustion to the propane stoichiometry, Sable system was reported to underestimate $\dot{V}O_2$ while overestimating $\dot{V}CO_2$ (Rising, Whyte, Albu, & Pi-sunyer, 2015). These results are inconsistent with ours, in which $\dot{V}O_2$ and $\dot{V}CO_2$ were both overestimated. This discrepancy in the outcomes could be explained by the propane gas weighing technique, which is less accurate than using propane gas mass flow meter. Weighing gases before and after burning causes time lag between the two measurements (weighing) that limits the ability to document system linearity, to calculate sensor response time and; therefore, the sensitivity of the system cannot be determined.

Other commonly used techniques to calibrate IC systems include alcohol combustion and butane gas burning. These two methods are similar to the propane gas combustion in which human and animal metabolism can be simulated (i.e. O_2 is consumed and CO_2 and water are produced (Tøien, 2013)). For instance, Marks et al. (1987) calculated $\dot{V}O_2$ and $\dot{V}CO_2$ after burning known masses of ethanol and methanol gases (pure alcohol) in an open-circuit IC system. The authors reported that gas volumes differed by less than 5% from its true values, therefore, alcohol combustion is a valid technique for calibrating IC systems in neonate (Marks et al., 1987). Furthermore, a methanol-burning lung model was described by Miodownik et al. (1998) and showed that this technique is valid for IC calibration with an error of less than 0.005% caused by CO (carbon monoxide) gas production (Miodownik et al., 1998). Lastly, known amount of butane gas was burned in a closed-circuit IC system in which 1 mole of butane consumed 6.5 mole of O_2 and the RER was 0.61; the authors concluded that combustion of butane gas provides a simple and robust tests of the IC system performance (Nunn et al., 1989).

3.7 Methodological consideration

This present study had several limitations. Propane gas is odorless, therefore, it is difficult to detect gas leakage especially in closed area such as laboratory. This can result in an explosion hazard. However, ensuring a good maintenance of the propane apparatus and closing the cylinder tightly can reduce this hazard. In addition, with insufficient O_2 present, propane combustion produces CO_2, H_2O, in addition to CO gas which indicates incomplete combustion. However, it can be helpful to have a CO detector incorporated into the IC measuring systems, when calibrating with propane. Furthermore, mass-flow meter is affected by the thermal conductivity of the gas passing through them (Lighton, 2008); therefore, regular calibration is needed to ensure accurate gas flow rates. However, calibration can be performed using nitrogen dilution technique.

3.8 Conclusion

This study investigated the validity of a calibration method using propane gas combustion technique in three metabolic carts (Sable, Moxus, Jaeger). The accuracy of the IC systems output is important to ensure accurate determination of the rates of $\dot{V}O_2$ and $\dot{V}CO_2$ and EE for weight loss, sport nutrition and other research and clinical uses. We recommend using propane gas to calibrate indirect calorimetry systems. However, more work is needed to validate this technique and to perfect it. For instance, the propane gas must be used with other metabolic carts to assess different O_2 and CO_2 analyzers' accuracy.

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3.10 Declaration of interest

The authors report no declarations of interest.

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3.11 Figure captions

Figure 1::Schematic representation of the in-house built propane gas device utilized to assess the validity of three metabolic carts with the use of a validated and calibrated propane gas mass flow meter. Propane combustion tests were performed and the rates of oxygen uptake $\dot{V}O_2$ ($L \bullet min^{-1}$) and carbon dioxide production $\dot{V}CO_2$ ($L \bullet min^{-1}$) were compared to the corresponding stoichiometric theoretical rates.

Figure 2: Concurrent mean rates of oxygen uptake ($\dot{V}O_2$, $L \bullet min^{-1}$; top), carbon dioxide production ($\dot{V}CO_2$, $L \bullet min^{-1}$; middle), and respiratory exchange ratio (RER; bottom) measured during propane gas combustion tests with three metabolic carts: Sable system (dashed line), Moxus system (solid line), and Jaeger system (dotted line). Values are means ± SD.

Figure 3: Mean difference (delta) between the stoichiometric theoretical values and the actual experimental values of oxygen uptake ($\dot{V}O_2$, $L \bullet min^{-1-}$; top), carbon dioxide production ($\dot{V}CO_2$, $L \bullet min^{-1}$; middle) and respiratory exchange ratio (RER; bottom) measured during propane gas combustion tests with three indirect calorimetric systems: Sable system (dashed line), Moxus system (solid line), and Jaeger system (dotted line). Values are means ± SD.

Table 1: Descriptive statistics of the Sable, Moxus, and Jaeger metabolic carts including the SD, SE, CV of mean $\dot{V}O_2$ (ml•min^{-1}), $\dot{V}CO_2$ (ml•min^{-1}), and RER

System	PFL (ml•min^{-1})	$\dot{V}O_2$ (ml•min^{-1})						$\dot{V}CO_2$ (ml•min^{-1})						RER					
		MEV	SD	SE	CV	STV	MΔ (STV-MEV)	MEV	SD	SE	CV	STV	MΔ (STV-MEV)	MEV	SD	SE	CV	STV	MΔ (STV-MEV)
Sable	200	263	14.0	5.7	0.05	215	-48	158	7.9	3.2	0.05	129	-29	0.60	0.01	0.00	0.02	0.60	0.00
	300	363	6.9	2.8	0.02	323	-40	215	4.5	1.8	0.02	194	-21	0.59	0.01	0.00	0.02	0.60	0.01
	400	478	5.8	2.4	0.01	430	-48	284	5.0	2.0	0.02	258	-26	0.59	0.01	0.00	0.02	0.60	0.01
	500	585	12.6	5.1	0.02	538	-47	346	6.4	2.6	0.02	323	-23	0.59	0.01	0.00	0.02	0.60	0.01
	600	685	9.0	3.7	0.01	646	-39	409	4.9	2.0	0.01	387	-22	0.59	0.01	0.00	0.02	0.60	0.01
Moxus	200	245	5.8	2.4	0.02	215	-30	148	3.7	1.5	0.03	129	-19	0.60	0.02	0.01	0.03	0.60	0.00
	300	368	4.2	1.7	0.01	323	-45	217	3.8	1.6	0.02	194	-23	0.59	0.01	0.00	0.02	0.60	0.01
	400	486	14.6	6.0	0.03	430	-56	288	8.8	3.6	0.03	258	-30	0.59	0.01	0.01	0.02	0.60	0.01
	500	583	21.8	8.9	0.04	538	-45	346	11.5	4.7	0.03	323	-23	0.59	0.02	0.01	0.03	0.60	0.01
	600	700	12.6	5.1	0.02	646	-54	418	6.0	2.5	0.01	387	-31	0.59	0.01	0.00	0.02	0.60	0.00
Jaeger	200	187	19.4	8.0	0.1	215	28	114	8.2	3.3	0.07	129	15	0.61	0.05	0.02	0.08	0.60	-0.01
	300	281	26.5	10.8	0.1	323	43	163	11.9	4.9	0.07	194	31	0.58	0.03	0.01	0.05	0.60	0.02
	400	368	40.6	16.6	0.1	430	62	208	20.1	8.2	0.1	258	50	0.56	0.01	0.01	0.02	0.60	0.03
	500	474	31.9	13.0	0.07	538	64	262	17.7	7.2	0.07	323	61	0.55	0.01	0.00	0.02	0.60	0.04
	600	560	42.2	17.2	0.08	646	86	307	26.8	11.0	0.09	387	80	0.57	0.01	0.01	0.02	0.60	0.05

PFL: propane flow level, $\dot{V}O_2$: volume of oxygen, $\dot{V}CO_2$: volume of carbon dioxide, MEV: mean experimental values, SD: standard deviation, SE: standard error. CV: coefficient of variance, STV: stoichiometric theoretical values, MΔ: mean delta, RER: respiratory exchange ratio

Table 2: Linearity analysis of the Sable, Moxus, Jaeger metabolic carts including Beta, R^2, Confidence interval (CI), and p-value of $\dot{V}O_2$ (ml•min^{-1}) and $\dot{V}CO_2$ (ml•min^{-1}) errors

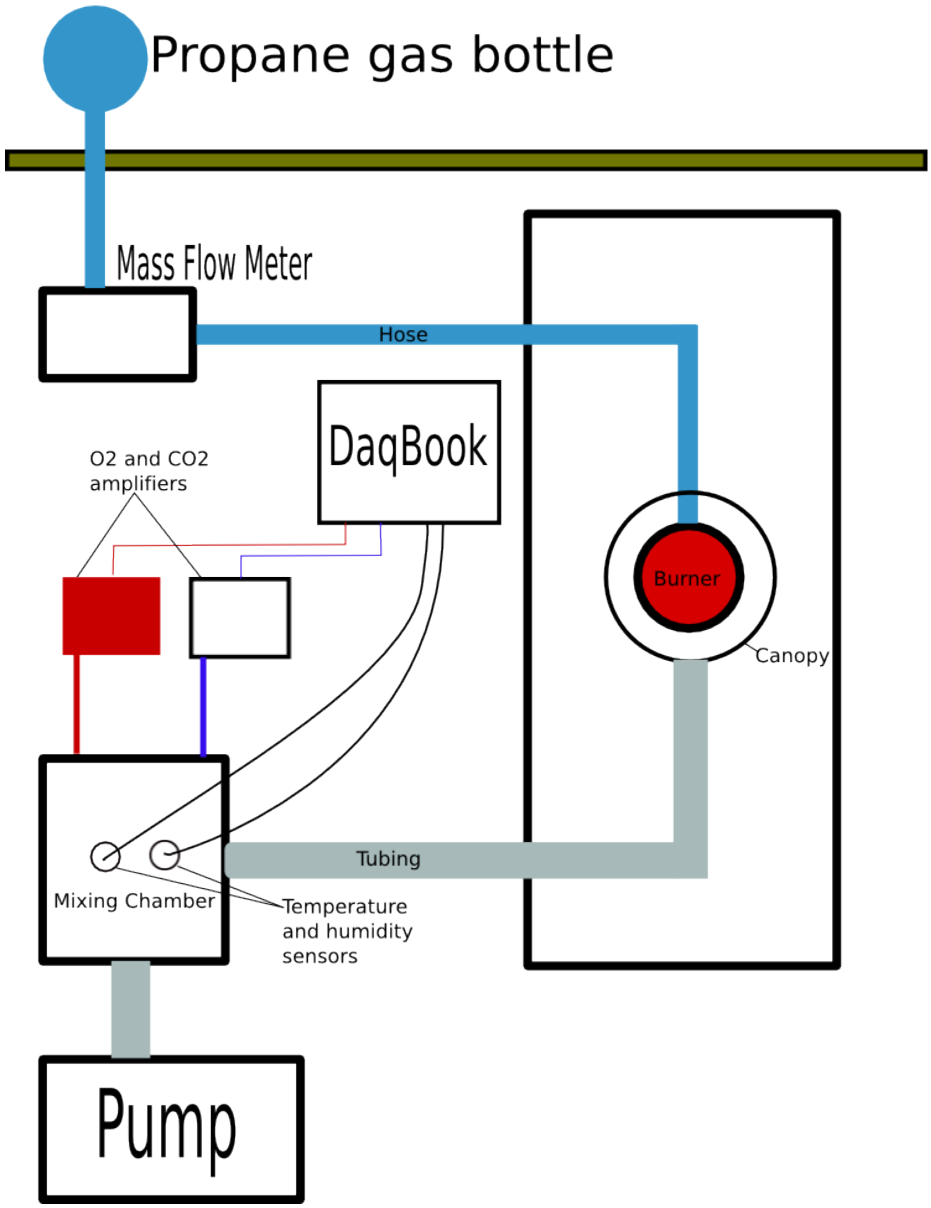
System	$\dot{V}O_2$ (ml•min^{-1}) Error				$\dot{V}CO_2$ (ml•min^{-1}) Error			
	β	R^2	95 % CI	*p*-value	β	R^2	95 % CI	*p*-value
Sable	0.998	0.996	(0.001, 0.001)	0.001	0.998	0.996	(0.001,0.001)	0.001
Moxus	0.996	0.992	(0.001, 0.001)	0.001	0.997	0.994	(0.001,0.001)	0.001
Jaeger	0.974	0.948	(0.001, 0.001)	0.001	0.944	0.942	(0.00, 0.001)	0.001

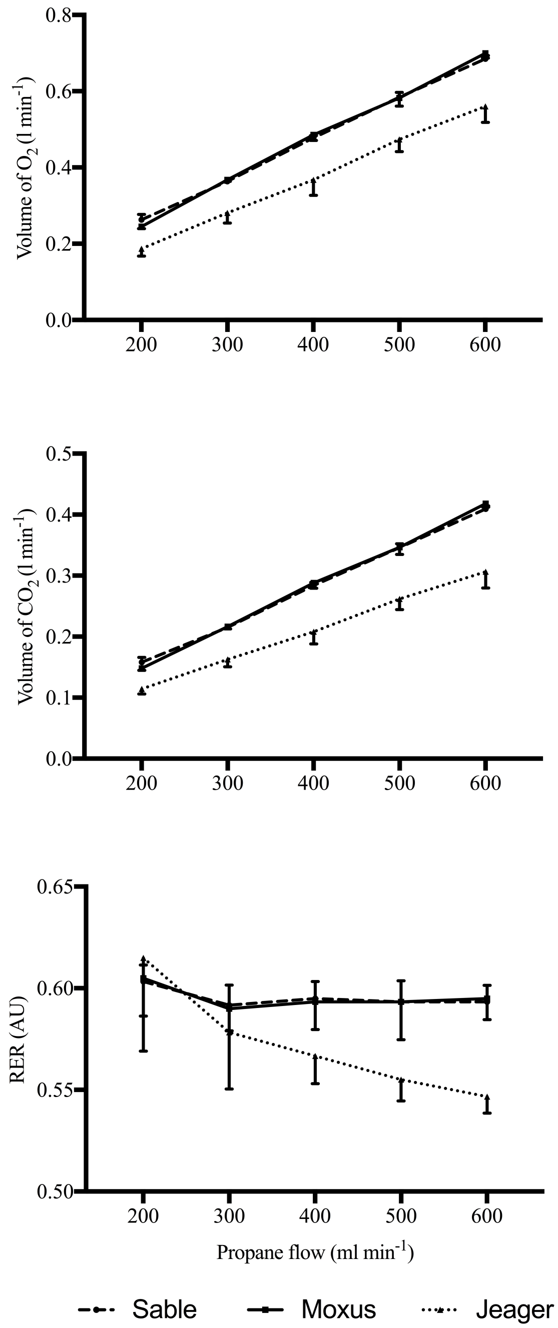
$\dot{V}O_2$: volume of oxygen, $\dot{V}CO_2$: volume of carbon dioxide

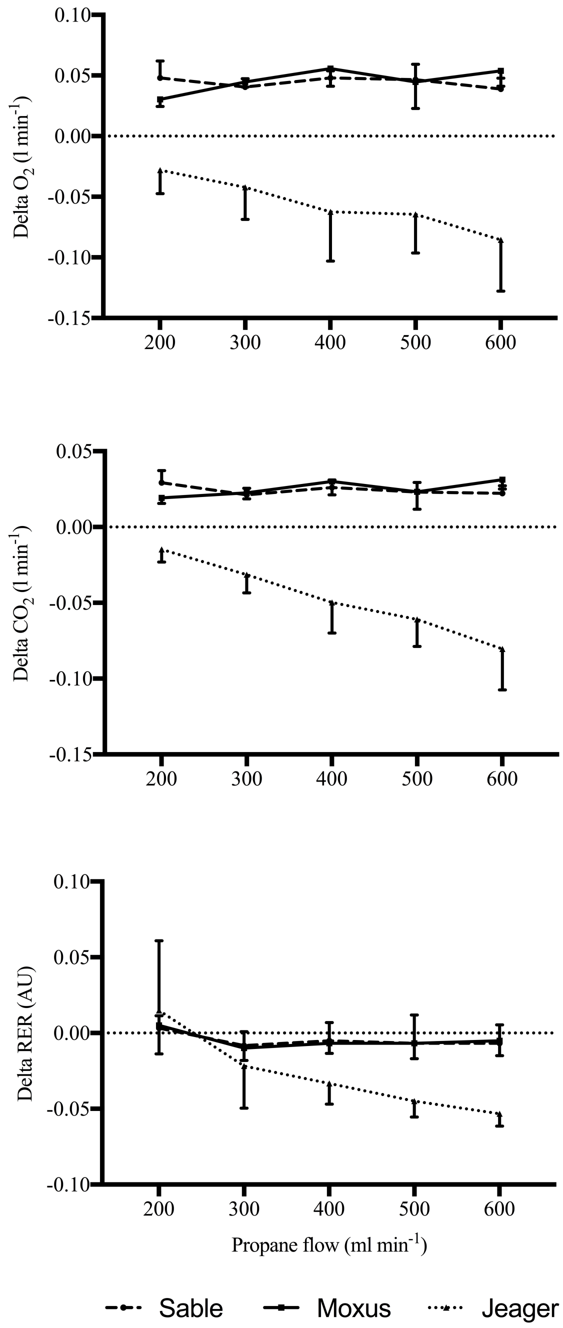
Table 3: Regression analysis of $\dot{V}O_2$, $\dot{V}CO_2$ and RER errors by propane flow levels which followed the Bland-Altman plots for each metabolic cart (Sable, Moxus, Jaeger)

System	$\dot{V}O_2$ (ml•min^{-1}) Error				$\dot{V}CO_2$ (ml•min^{-1}) Error				RER Error			
	β	SE	R_{adj}^2	t-value (*p*-value)	β	SE	R_{adj}^2	t-value (*p*-value)	β	SE	R_{adj}^2	t-value (*p*-value)
Sable	-0.168	0.000	-0.006	-0.903 (0.37)	-0.281	0.000	0.046	-1.552 (0.13)	-0.379	0.000	0.134	-3.848 (0.001)
Moxus	0.438	0.000	0.163	2.575 (0.02)	0.425	0.000	0.151	2.484 (0.02)	-0.162	0.000	-0.009	0.869 (0.4)
Jaeger	-0.536	0.000	0.262	-3.363 (0.002)	-0.807	0.000	0.639	-7.227 (0.001)	-0.679	0.000	0.441	-4.891 (0.001)

$\dot{V}O_2$: volume of oxygen, $\dot{V}CO_2$: volume of carbon dioxide, SE: standard error, RER: respiratory exchange ratio


Propane gas bottle
Mass Flow Meter
Hose
DaqBook
O2 and CO2
amplifiers
Burner
Canopy
Tubing
Mixing Chamber
Temperature
and humidity
sensors
Pump


0.8
0.6
0.4
0.2
0.0
Volume of O_2 (l min^{-1})
200
300
400
500
600
0.5
0.4
0.3
0.2
0.1
0.0
Volume of CO_2 (l min^{-1})
200
300
400
500
600
0.65
0.60
0.55
0.50
RER (AU)
200
300
400
500
600
Propane flow (ml min^{-1})
Sable
Moxus
Jeager


0.10
0.05
0.00
-0.05
-0.10
-0.15
Delta O2 (l min-1)
200
300
400
500
600
0.05
0.00
-0.05
-0.10
-0.15
Delta CO2 (l min-1)
200
300
400
500
600
0.10
0.05
0.00
-0.05
-0.10
Delta RER (AU)
200
300
400
500
600
Propane flow (ml min-1)
Sable
Moxus
Jeager

Chapter IV

Overall Summary of Study

Nutritional and physical activity recommendations are based on the ability to accurately determine and estimate MR and EE of humans (da Rocha et al., 2006). Information obtained from accurate EE values play a major role in human health and in the prevention of disease (Colberg et al., 2010). Different techniques have been established to measure EE such as DC and IC systems. Indirect calorimetry provides a measure of EE by estimating O_2 uptake and CO_2 production concentrations from the expired air samples (Leonard, 2012).

The validity, reliability and accuracy of these IC systems is based on the calibration methods (Lighton, 2008). Routinely, medical certified gases are used for calibrating IC systems, however, this method is expensive and the gases are delivered in very big cylinders. Therefore, other calibration techniques were developed for rapid, comprehensive and easy use methods such as alcohol combustion (Marks et al., 1987), burning butane (Nunn et al., 1989) or propane gas combustion (Lighton, 2008). Propane gas is routinely used in human physiology laboratories to calibrate IC systems (Melanson et al., 2010); however, to our knowledge, there is no existing study examine the validity of propane gas technique in assessing the validity, reliability and accuracy of IC systems. Propane gas is different than other gases because it has a good air-fuel mixing potential due to its low boiling temperature, and as a result produces less CO concentrations and other volatile hydrocarbons. Also, propane burning can simulate human metabolic rate because it consumes O_2 and produces CO_2 and water (Lighton, 2008). Therefore, this study was conducted to assess the validity of using propane gas technique in calibrating three metabolic carts: Sable, Moxus, and Jaeger.

A series of propane gas flow levels were randomly run through each IC system via an air mass flow generator and controller. The fractions of O_2 and CO_2 gases were collected and used to calculate $\dot{V}O_2$, $\dot{V}CO_2$, and RER in the Sable and Moxus systems while the volume of O_2 and CO_2 gases were obtained directly from the Jaeger system. Statistical analysis was performed on gas volumes and RER to assess the mean difference between the stoichiometry theoretical value and the actual experimental values. Also, linear regression was used to examine the relationship between propane flow levels and the actual experimental values, then between propane flow levels and the mean difference (error) values. Finally, a two-way ANOVA was performed to determine difference in response of the three systems.

The major outcome of the study revealed that flowing propane gas at the rate of a human basal metabolic rate shows differences between IC systems. Further, results revealed a remarkable agreement between propane gas technique and the stoichiometry theoretical values of the Sable and Moxus systems but not the Jaeger system which behaved differently. The error was the least in the Sable system, then the Moxus system, while the Jaeger system had the largest error. A lower error indicates accurate O_2 analyzers in the Sable and Moxus systems. Therefore, we recommend using propane gas technique to calibrate Sable and Moxus systems but not Jaeger system.

The present study provided evidence of the validity of using propane gas for assessing IC system validity, reliability and accuracy. Therefore, we highly recommend using propane gas technique because of its good air-fuel mixing potential due to a low boiling temperature, and as a result produces less CO. Also, propane gas is cheap and available in the market for easy use in contrast to the medical certified gases. The current study assessed three IC systems that are commonly used in most human physiology laboratories. Therefore, it provides a good reference for the procedures of propane gas calibration technique.

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