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ANALYSIS INDIRECT CALORIMETRY

Doyeon Kim

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**VALIDATION OF IMPROVED HARDWARE AND
SOFTWARE FOR EXPIRED GAS ANALYSIS
INDIRECT CALORIMETRY**

BY

DO-YEON KIM

B.S., Physical Education, Kyungshung University, 2001
MS., Exercise and Sport Science, University of Utah, 2005

DISSERTATION

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Doctor of Philosophy
Physical Education, Sport and Exercise Science

The University of New Mexico
Albuquerque, New Mexico

August 2009

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ACKNOWLEDGMENTS

I would like to express my appreciation to those who helped me during the progress of my dissertation. I heartily acknowledge Dr. Robert Robergs, my advisor and committee chair, for continuing to encourage me through the last four years teaching and the long terms writing and rewriting this dissertation. I do thank you from my heart. Your guidance and professional style will remain with me during my life.

I do also would like to thank the members of my dissertation committee, Dr. Len Kravitz, Dr. Christine Mermier, and Dr. Carole Conn, for their invaluable guidance, advice, and support received from them which made this study more meaningful throughout this process.

To Dr. Len Kravitz, thank you for your interest, guidance and support. I will never forget your kind assistance and you have remained me as a kind professor.

To Dr. Christine Mermier, thank you for your guiding and assisting in my study. You will remain in my mind as a never-to-be forgotten teacher.

And I also thank Dr. Carole Conn, thank you for your advice and help in completing this dissertation.

Additionally, I would like to thank my family. To my sisters, Mi-hye and Na-yeong, and my brother-in-law, Jong-won, thank you for giving me immeasurable support and encouragement over the years. To my niece, Si-yeon, thank you for delighting me always. And to my uncle, aunt, and cousins, thank you for your encouraging me always.

Finally, to my parents, mom, Sool-hee Cha, and dad, Dr. Ki-bong Kim, thank you for your unwavering support and guidance that has made it possible for me to take the doctorate and to realize my dreams.

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ABSTRACT

The purpose of this investigation was to validate a new system of breath-by-breath expired gas analysis to both an artificial working model of lung ventilation and gas exchange as well as to the Douglas bag technique. In addition, comparisons will be made between expired fractions, ventilation, and computations of $\dot{V}O_2$, $\dot{V}CO_2$, and RER between the new system and a commercial mixing chamber system (ParvoMedics) for repeated measurements at rest, steady state and non-steady state cycle ergometry exercise. Post acquisition processing involved custom developed software (LabVIEW), where time to gas equilibration within the mixing bag was determined, as well as differences in equilibrated gas fractions. All testing procedures were repeated 5 times for parametric statistical analyses. Gas concentration (%) results for the compliant 2 L mixing bag was the only method to yield data not significantly different between alveolar and measured. Alveolar % oxygen was significantly lower than mixing bag, mixing chamber, and ParvoMedics. The most responsive method was the mixing bag, with significantly lower % gas data for oxygen for breaths 2 to 5 compared to the mixing chamber and ParvoMedics. The ParvoMedics and mixing bag yielded similar results after breath 6, but data were significantly higher than for alveolar air. The slope data for breaths 0 to breaths 2 was

significantly ($p < 0.05$) lower for the ParvoMedics system compared to the mixing bag and mixing chamber. The mean temporal distribution of 1 L ventilation maneuvers from the mixing bag turbine was 0.999 ± 0.142 L, with a range of 0.96 to 1.03 L. The mean ventilation (STPD) from the ParvoMedics (pneumotach) was significantly lower ($p = 0.0027$) than the mixing bag turbine. For V_E ($p = 0.097$), VO_2 ($p = 0.786$), and VCO_2 ($p = 0.178$) were not significantly different in the main effect for method and the Intensity x Method interaction (V_E : $p = 0.721$, VO_2 : $p = 0.059$, VCO_2 : $p = 0.406$). As expected, there was a significant difference for the intensity main effect ($p < 0.0001$). For F_{EO_2} ($p < 0.0001$) and F_{ECO_2} ($p < 0.0001$) there were significant findings for the main effects of intensity. However, the Intensity x Method interaction showed no significant differences in F_{EO_2} and F_{ECO_2} . RER was significantly different in the main effect for method ($p = 0.024$), intensity ($p = 0.0006$), and Intensity x Method interaction ($p = 0.005$). The expired oxygen and carbon dioxide had significant main effects and interactions ($p < 0.001$). All mean differences between alveolar and mouth end tidal gas % values across 6 breaths were significant ($p < 0.01$). The mean individual computed dead space volumes were 2.5 ± 0.13 L. The results suggested that the new 2 L mixing bag is capable of accurately reproducing specific gas fractions from reference calibration gas. The new 2 L mixing bag allowed expired air to wash out through the bag. This system, in combination with including anatomical dead space (ADS) as a factor in the determinations, gives more accurate measurements and calculations than a traditional mixing chamber. Additionally, the new mixing bag method has unique aspects that are advantageous to the operation and validity of the system. Although the new system is not used in commercial systems of expired gas analysis indirect calorimetry (EGAIC), this system provides enhanced accuracy and validity.

TABLE OF CONTENTS

Abstract.....	vi
List of Figures.....	x
List of Tables	xiii
CHAPTER I. Introduction.....	1
Purpose of the Study.....	6
Hypotheses	6
Scope of the Study.....	9
Limitations	10
Assumptions	10
Significance of the Study	10
Definition of Terms	12
Symbols and Abbreviations	14
CHAPTER II. Review of Related Literature.....	16
History of Calorimetry.....	16
Definition of Indirect Calorimetry.....	18
Methods and Equipments for Indirect Calorimetry.....	19
Validity and Reliability of Indirect Calorimetry Methods.....	24
CHAPTER III. Methods.....	28
Testing Procedures and Protocols, and Statistical Procedures.....	28
Hypothesis 1	29
Data Processing	33
Statistical Analyses	33
Hypothesis 2.....	34
Data Processing	37
Statistical Analyses.....	39

Hypothesis 3	40
Data Processing	43
Statistical Analyses	44
Hypothesis 4	44
Data Processing	46
Statistical Analyses	46
CHAPTER IV. Results	47
Hypothesis 1	47
Hypothesis 2	49
Hypothesis 3	57
Hypothesis 4	61
CHAPTER V. Discussion	64
Expired % gas values of O ₂ and CO ₂ for calibration gas	64
% O ₂ , CO ₂ , V _E , and slope in the expired air from the lung model	65
Averaged values for each of VO ₂ , VCO ₂ , and RER	68
V _E , F _E O ₂ , F _E CO ₂ , VO ₂ , VCO ₂ , and RER for the new system and ParvoMedics	68
% O ₂ and CO ₂ across 6 breaths of mimicked lung ventilation and gas exchange	70
Calculated data for the dead space of the modeled lung system	70
CHAPTER VI. Summary, Conclusions, and Recommendations.....	72
Summary	72
Conclusions	74
Recommendations.....	75
REFERENCES.....	76

List of Figures

Figure	
1. Photograph of the 2 L compliant mixing bag.....	29
2. Photograph of the equipment set-up for hypothesis 1 testing	30
3. Schematic of the equipment set-up for hypothesis 1 testing for other test conditions of this hypothesis	30
4. Photograph of the 25 L expired breathing bag used as a small Douglas bag	32
5. Photograph of the equipment set-up for testing the Douglas bag conditions of hypothesis 1.....	32
6. Schematic of the equipment set-up for hypothesis 1 testing for the 25 L expired breathing bag condition without the added low resistance tubing.....	33
7. Photograph of the equipment set-up for hypothesis 2 testing for the 2 L compliant mixing bag	34
8. Schematic of the equipment set-up for hypothesis 2 testing for the 2 L compliant mixing bag condition.....	35
9. Photograph of the equipment set-up for hypothesis 2 testing for the 5 L fixed volume mixing chamber and expired tubing.....	35
10. Schematic of the equipment set-up for hypothesis 2 testing for expired air flow to a traditional mixing chamber.....	36
11. Photograph of the equipment set-up for hypothesis 2 testing for the 25 L expired breathing bags	38
12. Schematic of the equipment set-up for 25 L expired breathing bag with 5 feet of low resistance tubing.....	38
13. Photograph of the custom developed system for EGAIC during the rest condition.	41

14. Photograph of the commercial (ParvoMedics) EGAIC system during the rest condition	41
15. Schematic of the custom developed system for EGAIC.....	42
16. Schematic of the commercial (ParvoMedics) EGAIC system	42
17. The subject exercising with analyses performed by the commercial (ParvoMedics) system for hypothesis 3 testing.....	43
18. Photograph of the adjusted lung model to suit profiling of changes in inspired and expired gas variables during inspiration and expiration	45
19. Schematic of the adjusted lung model to suit profiling of changes in inspired and expired gas variables during the expiration phase of data collection	45
20. The differences between % gas values for each of O ₂ and CO ₂ for calibration gas directed into three conditions of expired air collection	48
21. The changes over time (breath) and differences between methods for % oxygen from the mimicked lung model.....	51
22. The changes over time (breath) and differences between methods for % carbon dioxide from the mimicked lung model	51
23. The changes in initial slope for each of % oxygen and % carbon dioxide for breaths 0 to 2.....	53
24. The temporal distribution of 1 L ventilation maneuvers from the mixing bag turbine during testing of hypothesis 2	54
25. The changes over time (breath) for a VO ₂ , VCO ₂ and RER from the mimicked lung model	57
26. The mean ± SD data for ventilation (STPD) for the mixing bag and ParvoMedics methods.....	57
27. The mean ± SD data for ventilation (STPD), F _E O ₂ , and F _E CO ₂ for the	

New system and ParvoMedics methods	59
28. The mean \pm SD data for VO_2 , VCO_2 , and RER for the New system and ParvoMedics methods	60
29. The mean \pm SD data for % oxygen and % carbon dioxide across 6 breaths for the condition of mimicked lung ventilation and gas exchange.....	62
30. Calculated data for the dead space of the modeled lung system as built for hypothesis 4 testing	63

List of Tables

Table 1. Mean % gas values for each of O ₂ and CO ₂ for calibration gas	48
Table 2. Mean % oxygen from the mimicked lung model	50
Table 3. Mean % carbon dioxide from the mimicked lung model	50
Table 4. Mean slope data for breaths each of % oxygen and % carbon dioxide.....	52
Table 5. Mean changes over time (breath) for VO ₂	55
Table 6. Mean changes over time (breath) for VCO ₂	55
Table 7. Mean changes over time (breath) for RER.....	56
Table 8. Mean data for V _E , F _E O ₂ , F _E CO ₂ , VO ₂ , VCO ₂ , and RER for the new system.....	58
Table 9. Mean data for V _E , F _E O ₂ , F _E CO ₂ , VO ₂ , VCO ₂ , and RER for the ParvoMedics.....	58
Table 10. Mean % O ₂ and CO ₂ 6 breaths of mimicked lung ventilation and gas exchange.....	61

CHAPTER I

Introduction

Historical Development of Nutrition and Calorimetry

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

A calorimeter is a device used for calorimetry, the science of measuring the heat of chemical reactions or physical changes as well as heat capacity. Calorimetry has been around since the mid to late 1800s (Ainslie, Reilly, & Westerterp, 2003; Robergs & Keteyian, 2003). In 1842, the first law of bioenergetics helped scientists to quantify heat release from metabolic process (Robergs & Keteyian, 2003).

Bischoff and Voit in 1860 completed calculations on the caloric and respiratory gas exchange from the heat developed in burning the carbon and hydrogen elements of certain foods and pure nutrients (Lusk, 1909). This method is referred to as bomb calorimetry. Bomb calorimetry was an important advancement to understanding the energy value of foods. Researchers have found bomb calorimetry to be of value when studying the effects of diet, not only in laboratory animals, but also humans (Robergs & Keteyian, 2003).

While the direct measurement of heat production by the body can be accomplished, it is a procedure fraught with error, temporal insensitivity, and contamination by mechanical heat release during exercise that combine to make it an invalid option for the quantification of energy expenditure during exercise. Consequently, indirect methods of quantifying heat production and energy

expenditure have to be used for exercise applications.

Max Rubner in Germany, established the clinical use of indirect calorimetry, and determined the caloric value of protein combustion. He also measured the energy release of respiration, 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 determining the average fuel value of a mixed diet which were different types of protein, carbohydrate, and fat molecules that are metabolized in the body (Lusk, 1909). Rubner's findings in 1904 were reproduced in human subjects using a more sophisticated closed-circuit respiration calorimeter by Atwater and Benedict (Lusk, 1909; Robergs & Keteyian, 2003).

More recently, the development of more sophisticated equipment and alternative methods of both direct and indirect calorimetry were developed (Robergs & Keteyian, 2003). Direct calorimetry measures total heat loss from the body. This method is currently used to study basal metabolic rate (BMR) and daily energy expenditure, and to validate alternative indirect methods (indirect calorimetry) such as doubly labeled body water (Ainslie et al., 2003; Bisdee, James, & Shaw, 1989; Robergs & Keteyian, 2003).

For exercise applications, expired gas analysis indirect calorimetry is the standard method for providing highly accurate calculations of energy expenditure with high temporal resolution (for each breath) (Ainslie et al., 2003; Simonson & DeFronzo, 1990). The development of equipment and techniques have allowed breath-by-breath pulmonary gas exchange measurements, and direct field assessment of human performance during any kind of activity. Expired gas analysis indirect calorimetry measures three variables; 1) ventilation (V_E), 2) expired air O_2 fraction (F_{EO_2}), and 3) expired air CO_2 fraction (F_{ECO_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 (Ainslie et al., 2003; Robergs & Keteyian, 2003).

Expired gas Analysis Indirect Calorimetry

Expired gas analysis indirect calorimetry is the most common method used in exercise physiology labs for both quantifying metabolic rate and energy expenditure, and there are a number of commercial systems available to carry out the determination of the oxygen and carbon dioxide in exhaled air. All these systems need to calculate metabolic data are the fractional concentrations of oxygen (F_{EO_2}) and carbon dioxide (F_{ECO_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. The signals for O_2 , CO_2 and volume are aligned from which VO_2 and VCO_2 are calculated according to the Haldane transformation, where:

$$\text{VO}_2 = \text{Inspired O}_2 - \text{Expired O}_2 = (V_{\text{I}} \times F_{\text{IO}_2}) - (V_{\text{E}} \times F_{\text{EO}_2})$$

$$\text{VCO}_2 = \text{Expired CO}_2 - \text{Inspired O}_2 = (V_{\text{E}} \times F_{\text{ECO}_2}) - (V_{\text{I}} \times F_{\text{ICO}_2})$$

F_{IO_2} is fixed, assuming a room air concentration of 20.95%

F_{ICO_2} is fixed, assuming a room air concentration of 0.03%

$$\text{RER} = \text{VCO}_2 / \text{VO}_2$$

The three measured variables can then be used to assess metabolic rate and energy expenditure, and in so doing, also help in the detection of certain diseases such

as heart disease, lung disease, and peripheral vascular disease (Barnard & Sleight, 1995; Carter & Jeukendrup, 2002; Gore, Catcheside, French, Bennett, & Laforgia, 1997; Matarese, 1997; Robergs & Burnett, 2003; Rosenbaum, Kirby, & Breen, 2007).

While the science of expired gas analysis indirect calorimetry (EGAIC) has remained largely unchanged for the last 100 years, the equipment and frequency of data collection and computations have changed enormously (Crouter, Antczak, Hudak, Della Valle, & Haas, 2006; Macfarlane, 2001; Robergs & Burnett, 2003). 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 (Robergs & Burnett, 2003).

Computerized EGAIC systems have made gas exchange measurements easier and less time consuming, provide immediate display of data measurements and computations, and do this without compromising the accuracy based on validation to the Douglas bag method. In addition, as the technology becomes more sophisticated there is a movement towards using portable gas exchange systems for the purpose of obtaining real life or field-based measurements rather than laboratory measurements (Carter & Jeukendrup, 2002; Crouter et al., 2006; Foss & Hallen, 2005).

Despite all the electronic improvements to EGAIC, gas analyzers have several disadvantages, and their own sources of considerable errors can make breath-by-breath measurements inaccurate. The greatest error lies in the delay time between the expired gas and expiratory flow signals. The change in the gas concentration signal is delayed compared with the flow signal due to the time required to pump sampled gas to the electronic gas analyzers and the time involved in the operation of

the analyzers to measure gas fractions. Errors in the delay time between gas flow and gas analysis can cause errors in VO_2 measurements of up to a 30% at high breathing frequencies ($< 70 \text{ breaths}\cdot\text{min}^{-1}$) (Barnard & Sleigh, 1995; Beaver, Lamarra, & Wasserman, 1981; Gore, Clark, Shipp, Van Der Ploeg, & Withers, 2003; Hodges, Brodie, & Bromley, 2005; Proctor & Beck, 1996; Wagner, Horvath, Dahms, & Reed, 1973).

An alternative to breath-by-breath analysis is to use a computerized metabolic system with a mixing chamber (Bassett et al., 2001; Foss & Hallen; 2005; Macfarlane, 2001). Regardless of this, mixing chambers are now less common, and the issue of the delay time is not improved, but rather exacerbated to the added dead space tubing connecting expired air flow to the mixing chamber. Another concern is that with mixing chambers it is not possible to measure respiratory variables breath-by-breath. Secondly, systems with mixing chambers are more challenging to use due to the required maintenance of the mixing chamber and the connecting low resistance tubing. Thirdly, purchasing a system with mixing chamber typically adds a significant extra cost (Foss & Hallen; 2005). For example, Bassett et al. (2001) used a mixing chamber to validate a computerized metabolic system. They reported that the ParvoMedics method of measuring expired gas temperature resulted in V_E being overestimated by 2%. Because the gas cools as it moves away from the heated pneumotachometer, the mixing chamber temperature would have underestimated the actual gas temperature inside the pneumotachometer. However, errors of this magnitude would have only a minor effect on the calculation of oxygen consumption.

Dr. Robert Robergs from the University of New Mexico and the University of Western Sydney 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, September 13, 2005). 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. Consequently, there is a need to apply sound scientific principles to the re-investigation of validation procedures used in EGAIC, to develop appropriate methods of validation, and apply these validation techniques to this new invention.

Purpose of the Study

The purpose of this study was to validate a new system of breath-by-breath expired gas analysis to both an artificial working model of lung ventilation and gas exchange as well as to the Douglas bag technique. In addition, comparisons will be made between expired fractions, ventilation, and computations of $\dot{V}O_2$, $\dot{V}CO_2$ and RER between the new system and a commercial mixing chamber system (ParvoMedics, Salt Lake City, UT) for repeated measurements for each of rest and steady state cycle ergometry exercise.

Hypotheses

The following hypotheses were tested in this study.

Hypothesis 1: For controlled ventilation of a known gas mixture, V_E , F_{EO_2} and F_{ECO_2} will be identical to the recorded turbine ventilation and the constant gas fraction values from the calibration gas used to mimic alveolar air between each of the new compliant 2 L mixing bag system, and Douglas bag collections of expired air with and without 5 feet of additional low resistance tubing dead space.

Rationale: It is important to first show that the new 2 L mixing bag is capable

of accurately reproducing specific gas fractions from reference calibration gas. Similarly, it is important to document that the 2 L mixing bag is similar to the Douglas bag method under these experimental conditions.

While the small volume of dead space in the mouthpiece will slightly contaminate expired gas fractions, such error should be the same for the new 2 L mixing bag and a Douglas bag collection. Addition of 5 feet of expired tubing to the Douglas bag should not change this condition, as expired air will first be used to flush room air from the added tubing.

Hypothesis 2: For controlled ventilation and mimicked lung gas exchange, averaged values for each of V_E , $F_{E}O_2$, $F_{E}CO_2$, VO_2 , VCO_2 , and RER and the initial slope for the change in $F_{E}O_2$ and $F_{E}CO_2$, will differ between the new small mixing bag attached to the mouthpiece vs. a traditional mixing chamber connected to 5 feet of low resistance tubing, vs. the Douglas bag method, vs. a commercial automated system of indirect calorimetry (ParvoMedics).

Rationale: The new small mixing bag is attached to the mouthpiece and flow turbine. There is no added dead space volume involved in connecting a large and heavy fixed volume mixing chamber via low resistance tubing to the mouthpiece. In addition, the vent holes of the 2 L mixing bag allow expired air to flush through the bag. This enables the end tidal gas fractions to be better represented in the mixed gas signals from the 2 L mixing bag. Such characteristics will allow the new small mixing bag and the mouthpiece to be more accurate than a traditional mixing chamber.

Several studies have reported that there was no significant difference between the criterion vs. new systems in ventilation (Carter & Jeukendrup, 2002; Crouter et al., 2006; Cullum, Welch, & Yates, 1999; Engebretson, 1998; Meyer, Georg, Becker, & Kindermann, 2001; Rietjens, Kuipers, Kester, & Keizer, 2001; Storer, Bunnell, Hand,

& Grant, 1995; Yates & Cullum, 2001). In contrast, the study by Bassett et al. (2001) and Foss and Hallen (2005) demonstrated that ventilation from an automated system was lower compared to the Douglas bag method. However, the differences were so small as to be not physiologically significant.

Some studies have reported that there was no statistically significant difference between the systems either in $F_{E}O_2$, $F_{E}CO_2$, VO_2 , VCO_2 , or RER (Bassett et al., 2001; Carter & Jeukendrup; 2002; Cullum et al., 1999; Yates & Cullum, 2001). Other studies have found that there was no significant difference between the systems in VO_2 and VCO_2 (Crouter et al., 2006; Meyer et al., 2001; Pinnington, Wong, Tay, Green, & Dawson, 2001), but $F_{E}O_2$, $F_{E}CO_2$, and RER were significantly different from commercial systems and the Douglas bag method (Crouter et al., 2006; Engebretson, 1998; Foss & Hallen, 2005; Parr, Strath, Bassett, & Howley, 2001). In addition, some groups of researchers have investigated that there were significant differences between the systems in $F_{E}O_2$, $F_{E}CO_2$, VO_2 , VCO_2 , and RER (Hiilloskorpi, Manttari, Fogelholm, Pasanen, & Laukkanen, 1999; McLaughlin, King, Howley, Bassett, & Ainsworth, 2001; Pinnington et al., 2001).

Hypothesis 3: Use of the new system compared to a commercial system will yield the same values for V_E , $F_{E}O_2$, $F_{E}CO_2$, VO_2 , VCO_2 and RER at rest, during steady state exercise, and non-steady state exercise.

Rationale: This research project is unique in that it provides direct comparison of multiple methods of indirect calorimetry. In addition, the study challenges previously accepted assumptions about conducting zone gas mixing and computational accuracy in indirect calorimetry. As many of the hardware, software and adjustments to conducting zone mixing of the new system are not used in commercial systems of EGAIC, documentation of the validity of the new system yet

differences to commercial systems will be strong evidence of the invalidity of current commercial systems used for EGAIC.

Hypothesis 4: a) Mixed (integrated) and end tidal gas fractions for O₂ and CO₂ will be different compared to the calibration gas used to mimic alveolar air. b) The extent of mixing between alveolar (mimicked) and anatomical dead space air can be used to estimate the volume of the anatomical dead space.

Rationale: Sampling gas fractions from the lung model from an equivalent position of a subject's mouth will reveal the extent of alveolar air contamination by trapped air in the anatomical dead space. In addition, the volume of the anatomical dead space will be able to be determined from the extent of contamination of the calibration gas fractions by room air in the anatomical dead space. It is proposed that the main cause for differences between the method of EGAIC studied in this research concerns differences in the extent of anatomical dead space air contamination of alveolar air from the lung. This hypothesis will directly profile the contamination caused by this dead space air.

Scope of the Study

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. This study was designed to be the best validation for a precise measurement of a new system of breath-by-breath expired gas analysis indirect calorimetry.

A system for expired gas analysis indirect calorimetry including: (a) a new compliant mixing bag system, (b) a mouthpiece including a suitable one-way valve or a turbine that is connected to the new compliant mixing bag, and (c) improved software and hardware for improved expired gas sampling and subsequent

computations, will be validated to modeled lung function, the Douglas bag method, and a commonly used commercial automated system of EGAIC.

Limitations

This study was limited in the following ways:

1. Only one commercial indirect calorimetry system (ParvoMedics) was used in comparison with the new system.
2. There is no true gold standard system for validation comparison. It is for this reason that we devised a working model of lung function.
3. Only limited conditions of ventilation and criterion gas conditions will be used in the validation.

Assumptions

The following assumptions were made in this study:

1. The Haldane procedure uses a valid method for computing inspired ventilation from expired ventilation and gas fractions.
2. The accuracy in measuring non-physiological gas fraction conditions is the same as for true conditions.

Significance of the Study

In the last 20 years there has been a significant development of both laboratory and computerized metabolic systems used in indirect calorimetry. In addition, there has been increased use of breath-by-breath EGAIC (Crouter et al., 2006; Macfarlane, 2001; Robergs & Burnett, 2003). 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 a new system of breath-by-breath expired gas analysis indirect calorimetry.

Definition of Terms

The terms in this study have been operationally defined as follows:

Arterial-venous oxygen difference (a-vO₂ diff): The difference between the amount of oxygen returned in venous blood and the amount originally carried in arterial blood. Difference in oxygen content between arterial and venous blood.

Bomb Calorimeter: Instrument used to combust food and measure the VO₂, VCO₂, and heat release.

Breath-by-breath: The expression of a particular physiologic value averaged over one entire respiratory cycle.

Calorimeter: An instrument that measures heat release from the body.

Calorimetry: The measurement of body metabolism from heat release from the body.

Closed-Circuit Indirect Calorimetry: The calorimetric methods that involves the recirculation of inhaled and exhaled air, thus necessitating the removal of carbon dioxide and the replenishment of oxygen.

Direct Calorimetry: A calorimetric method that gauges the body's rate and quantity of energy production by direct measurement of the body's heat production.

Fick equation: The equation base on the Fick principle, where $VO_2 = Q \times a-vO_2\Delta$.

Haldane transformation: The use of equal inspired and expired nitrogen volumes to solve for either inspired or expired ventilatory volumes.

Indirect Calorimetry: A calorimetric method of estimating energy expenditure by measuring respiratory gases.

Maximal oxygen uptake (VO₂max): The maximal capacity for oxygen consumption by the body during maximal exertion. It is also known as aerobic power, maximal oxygen consumption, and cardiorespiratory endurance capacity.

Open-Circuit Indirect Calorimetry: The calorimetric methods that involve the

inhalation of atmospheric air and the sampling and measurement of exhaled air for respiratory gas analysis.

Respiratory exchange ratio (RER): The ratio of carbon dioxide production to oxygen consumption, as measured from expired gas analysis indirect calorimetry at the level of the lungs. V_{CO_2} / VO_2 measured from expired air for the lungs.

Respiratory quotient (RQ): The ratio of carbon dioxide production to oxygen consumption during metabolism. V_{CO_2} / VO_2 for the cell.

Respirometer: Instrument that quantifies the body's VO_2 and V_{CO_2} .

Tidal volume (TV): The amount of air inspired or expired during a normal breathing cycle. The volume of air ventilated into and out of the lungs with each breath.

Ventilation: The movement of air into or out of the lungs by bulk flow. (e.g., pulmonary or alveolar ventilation): external respiration.

Ventilation (Ventilatory) threshold (VT): The “breakpoint” at which pulmonary ventilation and carbon dioxide output begin to increase exponentially during an incremental exercise test.

Ventilatory equivalent for carbon dioxide (V_E / V_{CO_2}): The ratio of the volume of air ventilated (V_E) to the amount of carbon dioxide produced (V_{CO_2}).

Ventilatory equivalent for oxygen (V_E / VO_2): The ratio between the volume of air ventilated (V_E) and the amount of oxygen consumed (VO_2); indicates breathing economy.

Symbols and Abbreviations

Symbols and abbreviations used in this study are as follows:

ATPS: atmospheric temperature and pressure, saturated with water vapor

BMR: basal metabolic rate

BTPS: body temperature and pressure, saturated with water vapor

Δ : delta (cap): increment of change

Δ CO₂: CO₂ concentration in the expired air minus CO₂ concentration in the inspired air

Δ O₂: O₂ concentration in the inspired air minus O₂ concentration in the expired air

EE: energy expenditure

EGAIC: expired gas analysis indirect calorimetry

F_EO₂: fractional concentration of O₂ in expired gas

F_ECO₂: fractional concentration of CO₂ in expired gas

F_IO₂: fractional concentration of O₂ in inspired gas

F_ICO₂: fractional concentration of CO₂ in inspired gas

HR: heart rate

kPa: kilopascals

L: liter

L/min: liters per minute

mL/kg/min: milliliters per kilogram of body weight per minute

ml/min: milliliters per minute

min: minute

mL: milliliter

ms: milliseconds

PH₂O: water vapor pressure

Q (C.O.): cardiac output

RER: respiratory exchange ratio

RQ: respiratory quotient

s: second

STPD: standard temperature and pressure, dry air: 0°C, 760mmHg, dry

TV: tidal volume

VT: ventilation threshold

V_E : the volume of expired air per minute (expiratory volume)

$V_{E\max}$: maximal expiratory ventilation

V_E / V_{CO_2} : ventilatory equivalent for carbon dioxide

V_E / V_{O_2} : ventilatory equivalent for oxygen

V_I : the volume of inspired air per minute (inspiratory volume)

V_{O_2} : the volume of oxygen consumed per minute

V_{CO_2} : the volume of carbon dioxide produced per minute

$V_{O_2\max}$: maximal rate of oxygen consumption, maximal oxygen consumption

vs.: versus

W: watts

CHAPTER II

Review of Related Literature

This chapter contains the literature review and is organized into the following categories: (a) definition of indirect calorimetry, (b) methods and instruments for indirect calorimetry, (c) validity and reliability of indirect calorimetry methods.

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 (Robergs & Roberts, 1997).

The measurement of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) are the fundamental tools in the field of exercise physiology that are used to assess energy expenditure, aerobic capacity, exercise intensity and are also capable of detecting certain cardiorespiratory or ventilatory physiological abnormalities (Bassett et al., 2001; Carter & Jeukendrup, 2002; Hodges et al., 2005; Macfarlane, 2001).

History of Calorimetry

The development of automated metabolic gas analysis systems has facilitated the non-invasive determination of the ventilation threshold (VT) and cardiac output

(Q), respiratory gas exchange kinetics, and studies of outdoor activities. Although the fundamental principles behind the measurement of $\dot{V}O_2$ and $\dot{V}CO_2$ have not changed, the techniques used have, and some have almost turned full circle (Macfarlane, 2001).

Historically, gas exchange was measured by the Douglas bag method together with separate chemical analyses by early scientists (Bassett et al., 2001; Carter & Jeukendrup, 2002; Crouter et al., 2006; Foss & Hallen, 2005). However, the need for faster and more efficient techniques incited the development of semi-automated and fully-automated systems. In the 1960s, the measurement of inspired minute ventilation (V_I) became common and expired ventilation (V_E) values were calculated using the Haldane transformation of the Fick equation (Bassett et al., 2001). Wilmore and Costill (1974) described the semi-automated method, the measurement of the fractional concentrations of oxygen (F_{EO_2}) and carbon dioxide (F_{ECO_2}) in expired air was achieved by drawing representative gas samples from a mixing chamber into 2-liter latex bags for subsequent analysis. Today, most computerized metabolic systems have advanced to the point where systems normally use breath-by-breath analysis and measure the ventilation rate on the expired side (Bassett et al., 2001; Beaver et al., 1981; Carter & Jeukendrup, 2002; Crouter et al., 2006; Hodges et al., 2005; Foss & Hallen, 2005; Macfarlane, 2001; Proctor & Beck, 1996; Rietjens et al., 2001). In recent years, indirect calorimetry has largely become an automated systems procedure. It is important to establish the validity and reliability of all these different systems since this is not well known. Additional research is needed to better understand the correct methods of data processing for specific systems.

What is Indirect Calorimetry?

The amount of O₂ required to combust gram equivalents of carbohydrate, fat, and protein is extremely important. Glucose and fat metabolism depends on O₂ availability and produces CO₂ and water. The amount of O₂ and CO₂ exchanged in the lungs normally equals that used and released by body tissues. The method of estimating energy expenditure is called indirect calorimetry because heat production is not measured directly (Robergs & Keteyian, 2003; Wilmore & Costill, 2004). This method determines the metabolic rate and the net substrate utilization by humans based on the measurement of gas exchange such as whole body VO₂, VCO₂, and urinary nitrogen excretion (Brandi, Bertolini, & Calafa, 1997; Reid & Carlson, 1998). For individuals at rest, indirect calorimetry determination on the effects of body size, growth, disease, gender, drugs, nutrition, age, 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 the aged, greater in small individuals than in large ones, and greater under extremes of heat and cold than under normal conditions (Brooks, Fahey, White, & Baldwin, 2000).

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. This provides reliable, non-invasive and precise measurement of the body's metabolic activity through VO₂ and VCO₂ (da Rocha, Alves, & da Fonseca, 2006; Rosenbaum et al., 2007). Also, the use of indirect calorimetry has a wide range of clinical applications in critical care medicine, including the assessment of the physical fitness of healthy and diseased individuals and clinical nutrition support through the measurement of

ventilation, and the fractions of oxygen and carbon dioxide in expired air (Brandi et al., 1997; da Rocha et al., 2006; McClave & Snider, 1992; Reid & Carlson, 1998; Rosenbaum et al., 2007). These tests are usually conducted to (a) quantify the maximal rate of VO_2 ($\text{VO}_{2\text{max}}$), (b) indirectly assess the onset of exercise-induced acidosis (ventilation threshold, VT), (c) assessment of aerobic power, (d) determination of exercise intensity (VO_2 kinetics), (e) detection of cardiovascular and pulmonary diseases (Barnard & Sleigh, 1995; Carter & Jeukendrup, 2002; Matarese, 1997; Gore et al., 1997; Robergs & Burnett, 2003; Rosenbaum et al., 2007). However, during hard and prolonged exercise, indirect calorimetry may not provide a precise estimate of metabolic rate. However, determinations of O_2 consumption still provide important information about the cardioventilatory systems (Barnard & Sleigh, 1995; Brooks et al., 2000; Foss & Hallen, 2005; Gore et al., 1997; Noguchi, Ogushi, Yoshiya, Itakura, & Yamabayashi, 1982; Proctor & Beck, 1996).

Methods and Equipments for Indirect Calorimetry

There are three methods of expired gas analysis indirect calorimetry (EGAIC); (1) manual Douglas bag, (2) semi-automated and fully automated mixing chamber, and (3) fully automated breath-by-breath. Differences exist within and between methods for the equipment used to quantify ventilation, how values for expired fractions of oxygen and carbon dioxide are derived, and the frequency at which data is acquired and processed for computations for VO_2 , VCO_2 , and respiratory exchange ratio (RER).

Within the last 20 years, there has been a significant development of both laboratory and portable metabolic equipment used in indirect calorimetry has increased remarkably (Crouter et al., 2006; Macfarlane, 2001; Robergs & Burnett,

2003). Today, data are obtained, processed, and calculated within seconds, enabling the monitoring of changes during very small time intervals. Ventilation measurement is now performed by advanced electronics less than one-tenth the size of the original volume meters, and the response time of the electronic analyzers for O₂ and CO₂ is now as short as 100 ms. Since these improvements are combined with computer software and hardware advances that enable information to be processed at high rates, the automation of indirect calorimetry data collection is now a common feature of many advanced research and clinical exercise testing laboratories (Robergs & Burnett, 2003).

The most basic of these techniques to collect and analyze expired gas is the Douglas bag (DB) method, which has been in use for many years. Although this method is still considered to be the gold standard, it also has several disadvantages and its own limitations. For example, the time interval for Douglas bag expired air collection is much longer than now used in breath-by-breath applications of indirect calorimetry. 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 (Bassett et al., 2001; Carter & Jeukendrup, 2002; Crouter et al., 2006; Foss & Hallen, 2005). For example, after every expired breath, there is alveolar air within the conducting zone. Each subsequent inspiration then mixes room air with this dead space alveolar air. 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.

Over the past decade, increasing technological advances have resulted in the development of portable, lightweight and automated metabolic gas analysis systems; these systems are widespread internationally and most use breath-by-breath analysis. A breath-by-breath system measures airflow or volume continuously and simultaneously determines instantaneous expired O_2 and CO_2 concentration. They allow the measurement of expired gas concentrations and ventilation right outside the mouth and then immediately display respiratory and metabolic data for each breath. The use of these systems has allowed for very rapid gas analysis and ventilation measurement and is less time consuming than the DB technique (Beaver et al., 1981; Carter & Jeukendrup, 2002; Crouter et al., 2006; Foss & Hallen, 2005; Hodges et al., 2005; Macfarlane, 2001; Pinnington et al., 2001; Proctor & Beck, 1996; Rietjens et al., 2001; Wasserman, Hansen, Sue, Whipp, & Casaburi, 1994).

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 (P_{H_2O}) 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 (Davies, Hahn, Spiro, & Edwards, 1974; Hodges et al., 2005; Macfarlane, 2001), most oxygen and carbon dioxide analyzers are sensitive to the presence of water vapor. Additionally, failure to dry gas with desiccants will dilute the oxygen and carbon dioxide fractions and increase VO_2 (Withers & Gore, 2000). But even an additional 30% of water vapor will only lower 16.24% of a true gas fraction by $\sim 0.10\%$ with a resultant increase in 3% of VO_2 (Gore et al., 2003). Ignoring the effects of P_{H_2O} can lead to errors of up to 25% in the measurement of $F_{E}O_2$ (Beaver et al., 1981) and therefore have an important influence on the accurate calculation of VO_2 (Withers & Gore, 2000).

Noguchi et al. (1982) reported that 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. VO_2 and VCO_2 are obtained by multiplying flow and fraction signals during both expiration and inspiration. Consequently, compensation for barometric pressure, humidity, temperature, and gas composition of inspiratory gases should be performed to correct the respiratory volume for BTPS. Another problem is that breath-by-breath analysis of gas exchange is performed by measuring only expiratory flow and gas concentration without compensation for the effect of system dead space in most commercial systems (Beaver, Wasserman, & Whipp, 1973; Pearce, Milhorn, Holloman, & Reynolds, 1977). This dead space acts to decrease the overall sensitivity and accuracy of the system.

Myers, Walsh, Buchanan, and Froelicher (1989) have suggested that there is inherent error and causes of variability within the pulmonary gas exchange variables when performing gas exchange indirect calorimetry using the Medical Graphics Corporation 2001 system. The errors inherent in the measurements of V_E , F_{EO_2} and F_{ECO_2} can produce large errors when converting small time interval sampling to rates expressed relative to 1 min, and smoothing the record such as with the five point moving average (Beaver et al., 1981; Howley, Bassett, & Welch, 1995). Miscalculation of VO_2 can be caused by errors in the measurements of ventilation, fractions of oxygen and carbon dioxide, as well as ambient temperature and pressure. Of these possible errors, ventilation appears to be the most important (Mcfarlane, 2001).

The reliability of breath-by-breath gas analysis systems will be influenced by the variability of each physiological measure. The variability of a physiological

measurement such as VO_2 is the sum of the biological variability and the technical variability. Biological variability accounts for approximately 90% of the total variability, with only 10% or less of the remaining variability caused by technical problems (Mcfarlane, 2001). 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% (Katch, Sady, & Freedson, 1982). Armstrong and Costill (1985) have observed that the day-to-day variation of VO_2 and V_E measurements is 4.0% and 3.6% respectively, due to biological and technical error.

A number of breath-by-breath analysis systems use a computerized metabolic system fitted with a mixing chamber that mixes the dead space and alveolar gas to produce a gas that is representative of the mixed expired gas. Most exercise laboratories are using automated systems to measure respiratory gas exchange with a mixing chamber which gives time averaged values for respiratory variables (Bassett et al., 2001; Foss & Hallen; 2005; Macfarlane, 2001; Reybrouck, Deroost, & Hauwaert, 1992; Wasserman, Hansen, Sue, Whipp, & Casaburi, 1994). This is typically achieved by exhaling into a baffled chamber that mixes several breaths. An automated system using a mixing chamber can also be designed to record variables such as expired ventilation, mixed-expired CO_2 and O_2 , VO_2 and VCO_2 , heart rate (HR), and respiratory rate and to calculate variables periodically during exercise. Although these automated systems generally use a mixing chamber of fixed volume (~5 to 8 L), SensorMedics 2900 series, had the ability to change the volume according to the minute ventilation (Wasserman et al., 1994).

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. The gas concentrations together with ventilation measured in the gas flow give the respiratory data, reducing the difficulties associated with rapid analyzers and alignment of gas concentrations and ventilation. Thus, mixing chambers should be less error prone than the breath-by-breath analysis systems (Foss & Hallen; 2005).

Validity and Reliability of Indirect Calorimetry Methods

There are a considerable number of automated gas analysis systems currently available, 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 (Bassett et al., 2001; Carter & Jeukendrup, 2002; Crouter et al., 2006; Cullum et al., 1999; Foss & Hallen, 2005; Meyer et al., 2001; Rietjens et al., 2001; Storer et al., 1995; Yates & Cullum, 2001), 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 measurement system (the Oxycon-Pro[®]) and Douglas bag method during low and high exercise intensities.

Rietjens et al. (2001) reported high correlations between the values obtained from the Douglas bag method and the Oxycon-Pro[®] computerized metabolic system with mixing chamber for V_E ($r^2 = .996, p < .001$), VO_2 ($r^2 = .957, p < .001$) and VCO_2 ($r^2 = .980, p < .001$). Foss and Hallen (2005) also used 18 well-trained cyclists (21 ± 3 years) to check validity between the Oxycon-Pro[®] and the criterion Douglas bag method. The VO_2 was 0.8% ($0.03 \text{ L} \cdot \text{min}^{-1}$) lower with the Oxycon-Pro[®] than with the Douglas bag method with a coefficient of variation (CV) of 1.2% ($p < .05$). The lower

VO₂ was a result of a 1.8% lower V_E with CV of 1.0% ($p < .05$) and a 0.7% higher Delta O₂ (O₂ concentration in the inspired air minus O₂ concentration in the expired air) with CV of 0.8% ($p < .05$). Delta CO₂ (CO₂ concentration in the expired air minus CO₂ concentration in the inspired air) was 0.6% lower with the Oxycon-Pro[®] compared to the Douglas bag method with a CV of 1.4% ($p < .05$). Crouter et al. (2006) reported a correlation coefficient between the Douglas bag and TrueOne 2400 (ParvoMedics) computerized metabolic system with mixing chamber for V_E ($r = .975$, $p < .01$), VO₂ ($r = .994$, $p < .01$) and VCO₂ ($r = .991$, $p < .01$). In the Carter and Jeukendrup (2002) study, the mean absolute values of VO₂, VCO₂ and RER achieved from the 100W and 150W exercise testing were similar for the Oxycon-Pro[®] and Douglas bags. The Douglas bags and Oxycon-Pro[®] consistently produced small variations at both workloads, with a range of 1.3% to 6.5%. In summary, the validity and reliability coefficients for the breath-by-breath analyses are high with validity coefficients as high as $r = .994$ (Crouter et al., 2006).

Bassett et al. (2001) used Truemax 2400 (ParvoMedics) and the Douglas bag method to assess the validity of inspiratory and expiratory methods of measuring gas exchange. The results from testing 8 male participants (28 ± 6 years) at rest and up to 250 watts, showed extremely close agreement across all variables between both sides the inspired and expired systems compared with the Douglas bag method. F_EO₂ was slightly lower (0.04%) with the computerized system, compared with the Douglas bag method ($p < .01$). VO₂ was an average of 0.018 L/min ($p < .05$) higher for the inspired system compared with the Douglas bag. F_ECO₂ was slightly lower (0.03%, $p < .05$) for the expired system than the Douglas bag. The Truemax 2400 system, using inspiratory or expiratory configurations, permitted extremely precise measurements to be made in a less time-consuming manner than the Douglas bag method. Similarly,

Crouter et al. (2006) used TrueOne 2400 and Douglas bag to assess the accuracy and reliability of the measurement of gas exchange. The results from testing 10 healthy males (20 ± 1.7 years) at rest and during cycling at 50, 100, 150, 200, and 250 W. 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 ($p \geq .05$). The reliability of the TrueOne 2400 is similar to other systems currently available, which have been shown to have good reliability (Carter & Jeukendrup, 2002; Meyer et al., 2001). 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. (2001).

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. (1999) used Max-1 with the Douglas bag to assess the accuracy and reliability of measurement of gas exchange, using 19 males (18 to 47 years) over 4 workloads, from rest up to maximum exercise. Findings of this study indicate that there were no statistically significant differences between the systems either in VO_2 , VCO_2 , F_{EO_2} , or F_{ECO_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%, $p = .0528$). 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 (2001) 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 were done using 5 males (19 to 45 years), over 4 submaximal steady states work rates at each of 40, 80, 120, and 160 W. By work rates, 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 W (Storer et al., 1995).

In summary, indirect calorimetry methods have the potential to be used by exercise physiology, physiology, biochemistry, nutrition, 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 (Bassett et al., 2001; Carter & Jeukendrup, 2002; Crouter et al., 2006; Cullum et al., 1999; Foss & Hallen, 2005; Meyer et al., 2001; Rietjens et al., 2001; Storer et al., 1995).

CHAPTER III

Methods

We have devised a new method of breath-by-breath expired gas analysis indirect calorimetry. Currently, this new method, which involves new hardware and computer software, has not been validated to an artificial working model of lung ventilation and gas exchange, the Douglas bag technique, or any other commercial system. The methods followed in conducting this study are divided into the following sections: (a) testing procedures and protocols, (b) data processing, and (c) statistical analyses.

Testing Procedures and Protocols, and Statistical Procedures

We devised several approaches for validating the new system. Firstly, it must be recognized that no true gold standard exists in indirect calorimetry. While many investigators have used the Douglas bag method for this purpose, it differs in far too many ways from breath-by-breath sampling to be a suitable gold standard. As will also be explained, the Douglas bag method has broad assumptions regarding oversight of the potential air contamination to both inspired and expired gas fractions caused by the anatomical dead space or conducting zone of the lungs. Consequently, we have devised several approaches at validating components of the new system, as well the total system.

All data are presented as mean \pm standard deviation (SD) and all statistical analyses were completed using Statistica Software (StatSoft, Version 6.0, Tulsa, OK). All statistical tests used an alpha level set at $p < .05$. Statistical procedures were hypothesis specific, and are presented at the end of each hypothesis below.

1. Comparison between a 2 L Compliant Mixing Bag connected to the Expired-Side of the Mouthpiece vs. Douglas Bags.

Small volume (2 L ATPS) and semi-compliant mixing chambers were developed from plastic (0.05 mm) air breathing bags (VIASYS Healthcare, now Cardinal Health, Dublin, OH) by placement of two vent holes (4 mm radius) in each corner. The bags are manufactured with valve connectors that fit directly to indirect calorimetry T-valve assembly components (Hans Rudolph, Kansas City, MO) (Figure 1). A sample line was then fitted into the valve frame by drilling a hole and gluing a leur lock (female) fitting connected to an approximate 20 mm length of Tygon tubing sample line (ID=3/32 in, OD=5/32 in; Fisher Scientific Company, Pittsburgh, PA).



Figure 1. Photograph of the 2 L compliant mixing bag.

The 2 L mixing bag was connected to a T-valve apparatus, which in turn was connected (inspired side) to a 3-way valve (Figure 2 and 3). The three way valve was linearly connected to another T-valve apparatus at one end, and via 25 cm of tubing (ID=1 in, OD=1.25 in) to another 3-way valve connected to a 50 L Douglas bag. A gas sample line (ID=3/32 in, OD=5/32 in) connected the 2 L mixing bag to electronic CO₂ and O₂ gas analyzers connected in series to a gas flow pump (O₂=Model S-3A,

CO₂=CD-3A, pump=R1 flow controller, AEI Technologies, Pittsburgh, PA).

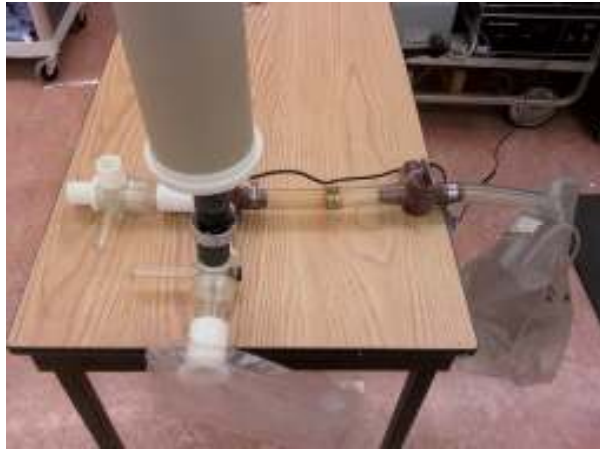


Figure 2. Photograph of the equipment set-up for hypothesis 1 testing.

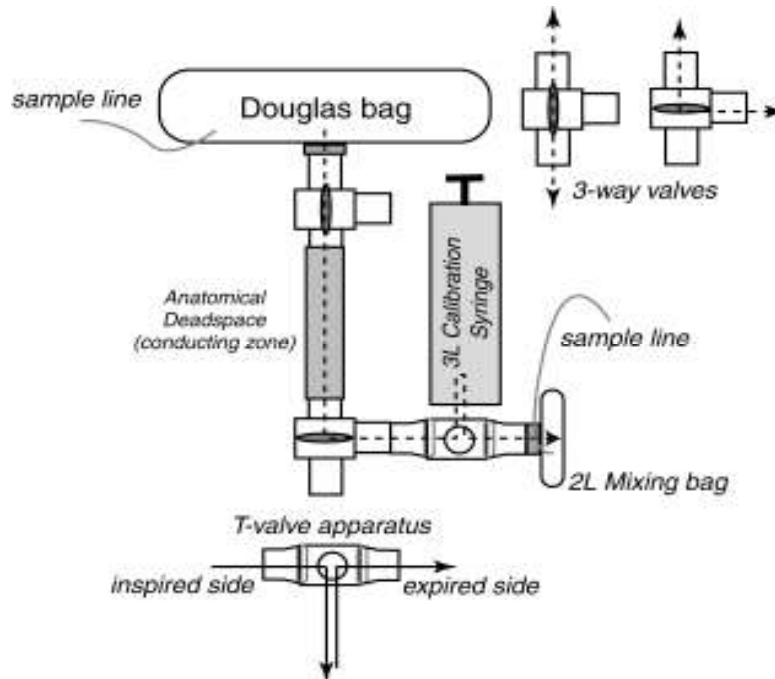


Figure 3. Schematic of the equipment set-up for hypothesis 1 testing. For other test conditions of this hypothesis, the 2 L mixing bag was replaced with a 25 L Douglas bag with and without 5 feet of added low resistance tubing.

Prior to data collection, the gas analyzers and flow turbine (bi-directional Universal Ventilation Meter, VacuMed, Ventura, CA) were calibrated. Gas analyzer calibration was performed using custom developed software (LabVIEW, National Instruments, Austin, TX) integrated to a computerized custom developed data acquisition system (National Instruments, Austin, TX). Calibration gases consisted of medical grade and certified calibration gas (5.1% CO₂, 15.11% O₂, balance N₂), room air (20.95% O₂, 0.03% CO₂, balance N₂), and 100% nitrogen (Argyle, Albuquerque, NM). Turbine calibration was performed using a 3 L calibration syringe (Hans Rudolph, Kansas City, MO).

Testing commenced by first filling the Douglas bag with known calibration gas (5.1% CO₂, 15.11% O₂, balance N₂, Argyle, Albuquerque, NM). The 2 L mixing bag and dead space tubing of the valve assembly was flushed with room air, and the gas sample line was connected to the 2 L mixing bag. A 3 L calibration syringe was connected to the T-valve assembly connected to the 2 L mixing bag. The collection software program was started, the flow pump was switched on, and both 3-way valves were opened for flow between the Douglas bag and mixing bag. Complete 3 L syringe maneuvers were performed at a rate of 10/min, equating to 30 L/min ATPS ventilation. Electronic (volts) signals for the analyzers and turbine were acquired continuously as 5 data point averages at 20 Hz and saved to a text file for latter processing. Testing continued until all calibration gas from the Douglas bag was emptied, which typically lasted approximately 2 min. This procedure was repeated 5 times for parametric statistical analyses.

Rather than using large volume commercial Douglas bags and risk incomplete air removal during volume measurement, we developed our own small volume bags using 25 L gas collection bags (VIASYS Healthcare, now Cardinal Health, Dublin, OH)

(Figures 4, 5, and 6). This gas sample bag was connected to a T-valve apparatus, which in turn was connected (expired side) to a 3-way valve, as previously described. The aforementioned procedures were repeated for the 25 L Douglas bag, with and without 5 feet of additional low resistance tubing. For these trials, Douglas bag collections occurred for 7 breaths, and involved the collection of 3 Douglas bag samples per trial.



Figure 4. Photograph of the 25 L expired breathing bag used as a small Douglas bag.



Figure 5. Photograph of the equipment set-up for testing the Douglas bag conditions of hypothesis 1.

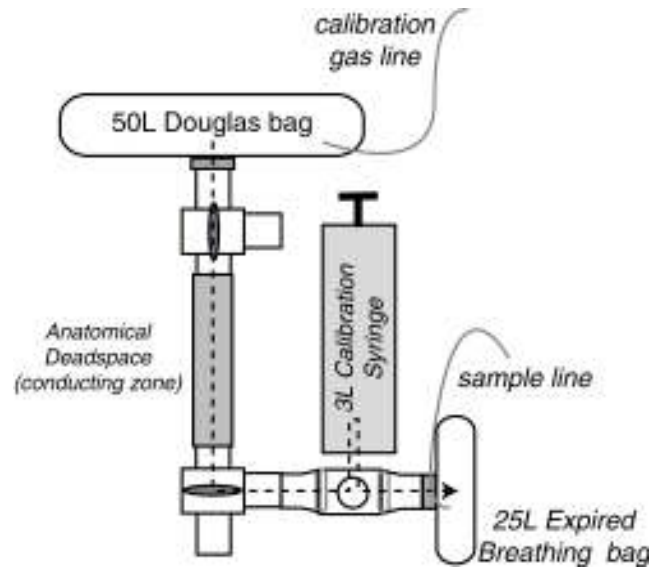


Figure 6. Schematic of the equipment set-up for hypothesis 1 testing for the 25 L expired breathing (Douglas) bag condition without the added low resistance tubing.

Data Processing

Post acquisition processing once again involved custom software in LabVIEW, where time to gas equilibration within the mixing bag was determined, as well as differences in equilibrated gas fractions between the mixing bag and Douglas bag.

Statistical Analyses

The expired gas fractions ($F_{E}O_2$ and $F_{E}CO_2$) aligned to the end of each expiration from start to equilibration were analyzed for differences across multiple ventilation maneuvers and to the calibration gas of the Douglas bag (with and without tubing) using 2-way repeated measures (method [3] and breath maneuvers [2]) ANOVA. Significant main or interaction effects were followed by simple effects contrasts and subsequent specific mean pair comparisons were performed by the Tukey's test.

2. Differences in Ventilation and Expired Gas Fractions between a Small Compliant Mixing Bag, a Traditional Large Fixed Volume Mixing Chamber, Douglas Bags, and a Commercial System of EGAIC during Conditions of Mimicked Ventilation and Lung Gas Exchange.

The 2 L mixing bag was connected to a T-valve apparatus, which in turn was connected (expired side) to a 3-way valve (Figures 7, 8, and 9). The three way valve was linearly connected to another T-valve apparatus at one end, and via 25 cm of tubing (ID=1 in, OD=1.25 in) to another 3-way valve connected to a 6 L mixing bag which mimicked lung alveolar air. A gas sample line (ID=3/32 in, OD=5/32 in) connected the 2 L mixing bag (expired side) to one set of O₂ and CO₂ analyzers and flow pump, and another sample line connected the 6 L lung model bag (mimicked alveolar gas fractions) to a second set of O₂ and CO₂ gas analyzers and flow pump (O₂=Model S-3A, CO₂=CD-3A, pump=R1 flow controller, AEI Technologies, Pittsburgh, PA). Another Tygon tubing sample line (ID=1/2 in, OD=3/4 in) was directly connected to a calibration gas tank (4.99% CO₂, 11.98% O₂, balance N₂), which in turn was connected to a 6 L mixing bag.



Figure 7. Photograph of the equipment set-up for hypothesis 2 testing for the 2 L compliant mixing bag.

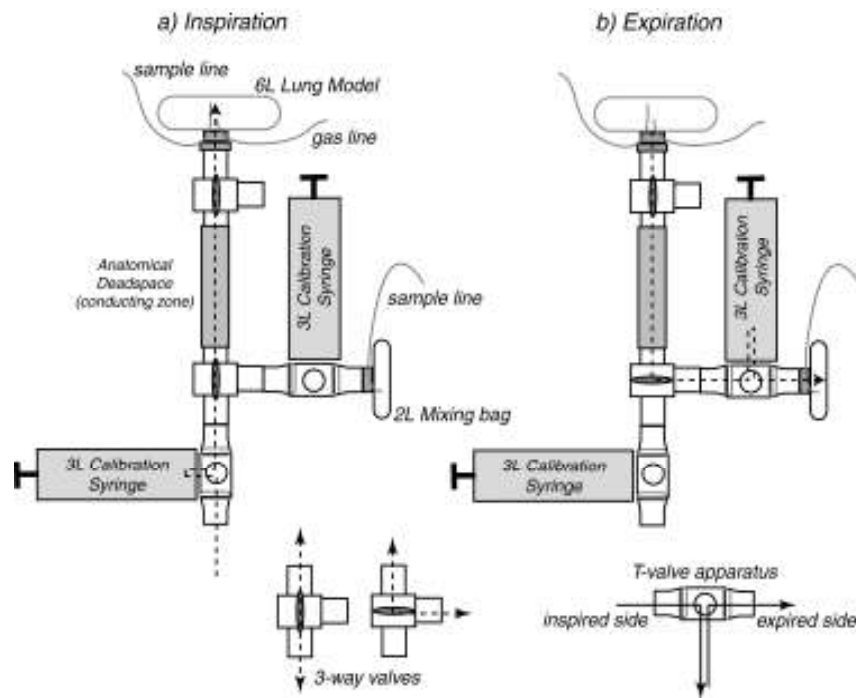


Figure 8. Schematic of the equipment set-up for hypothesis 2 testing for the 2 L compliant mixing bag condition.

Traditional large fixed volume mixing chamber: The equipment set-up was the same as described above, but instead of the 2 L mixing bag, a 5 L plastic traditional mixing chamber was connected to an approximate 5 feet of low resistance tubing to the T-valve apparatus (Figure 9 and 10).



Figure 9. Photograph of the equipment set-up for hypothesis 2 testing for the 5 L fixed volume mixing chamber and expired tubing.

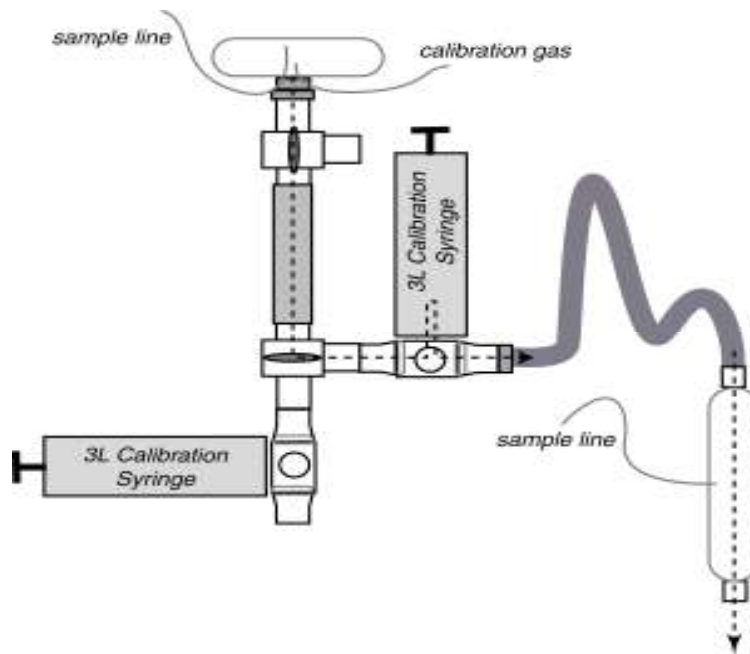


Figure 10. Schematic of the equipment set-up for hypothesis 2 testing for expired air flow to a traditional mixing chamber consisting of a 5 L cylindrical fixed volume mixing chamber connected to the T-valve via a 5 feet segment of low resistance tubing.

Testing commenced by first half filling the 6 L lung model bag with known calibration gas (4.99% CO₂, 11.98% O₂, balance N₂, Argyle, Albuquerque, NM). The 2 L mixing bag and dead space tubing of the valve assembly was flushed with room air, and the gas sample line was connected to the 2 L mixing bag. A 1 L calibration syringe was connected to an expired flow turbine and then to the T-valve assembly connected to the 2 L mixing bag, allowing air flow to mimic expiration from the 6 L lung model into the 2 L mixing bag. From the central three way valve, another T-valve apparatus was connected and another 1 L calibration syringe was connected to the T-valve assembly connected and an inspired air flow turbine.

To mimic pulmonary gas exchange and lung ventilation, calibration gas (4.99% CO₂, 11.98% O₂, balance N₂) was directly fed from the tank to the 6 L lung model at a pressure outflow of 10 kPa. The collection software program was started, the flow pumps were switched on, and the central 3-way valve was opened for flow

between the 6 L mixing bag and inspired turbine side. Complete 1 L syringe maneuvers were performed at a rate of 10/min, equating to 10 L/min ATPS ventilation. Between each successive maneuver the 3-way valve was adjusted to provide flow from the 6 L lung model to the expired side calibration syringe and 2 L mixing bag. The rate of these 1 L maneuvers was also 10/min. The 3-way valve was repeatedly adjusted between successive inspiration and expiration maneuvers. This was repeated for approximately 2 min, until well beyond equilibration of gas in the 2 L mixing bag and the 5 L fixed volume mixing chamber. Electronic (volts) signals for the analyzers and turbine were acquired continuously as 5 data point averages at 20 Hz and saved to a text file for latter processing. This procedure was completed 5 times for each mixing chamber, Douglas bags (Figures 11 and 12) and the commercial EGAIC system to suit parametric statistics.

Data Processing

Post acquisition processing once again involved custom software in LabVIEW, where time to gas equilibration within the mixing bag was determined, as well as differences in equilibrated gas fractions between the 6 L mixing bag (alveolar air), 2 L mixing bag, the 5 L fixed volume mixing chamber, Douglas bag collections, and averaged breath-by-breath data from a commercial system (ParvoMedics).



Figure 11. Photograph of the equipment set-up for hypothesis 2 testing for the 25 L expired breathing bags.

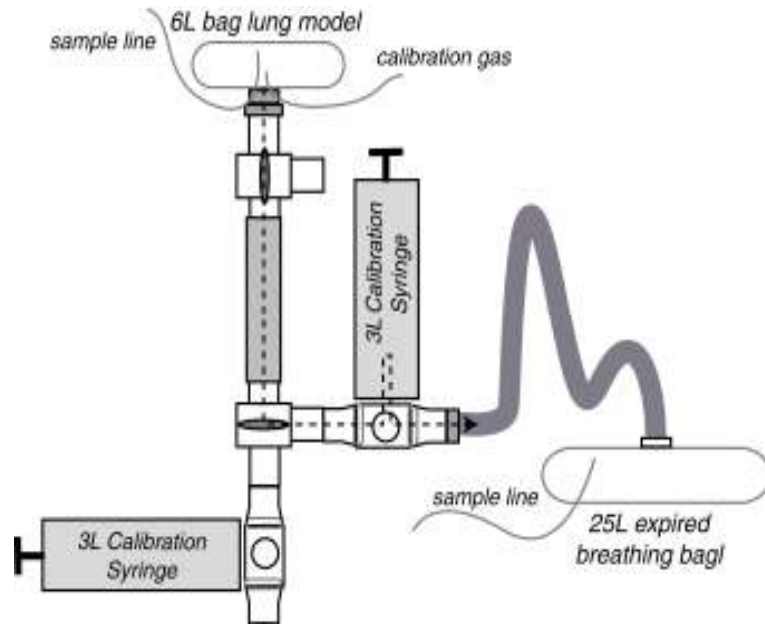


Figure 12. Schematic of the equipment set-up for 25 L expired breathing bag with 5 feet of low resistance tubing.

For the 2 L mixing bag, gas fraction signals were acquired by custom software (LabVIEW, National Instruments, Austin, TX) allowing the averaging of gas signals after equilibration for each of the first 11 breaths. The same software allowed detection of each tidal volume. For each breath, data values were entered into a results spreadsheet using Excel software (Microsoft, Seattle, WA). This procedure was

repeated for each of mixing bag, ParvoMedics, and 5 L mixing chamber, respectively. Gas fraction data was compared to mimicked alveolar gas fractions corresponding to each breath. As the ParvoMedics system assumed inspired gas fractions of 0.2094 and 0.03 for oxygen and carbon dioxide respectively, with no option for changing these on a breath-by-breath basis, these values were used in computations of $\dot{V}O_2$ and $\dot{V}CO_2$ for all methods. Water vapor pressure was assumed to be that of saturated air at room temperature, once again consistent with the ParvoMedics computation paradigm. Room temperature and atmospheric pressure were recorded for each trial condition and used to convert ATPS gas volumes to STPD.

As there were only 3 Douglas bag collections per condition and not 11, separate data processing had to occur for Douglas bag data. Gas fraction data from the Douglas bags were acquired from the data acquisition files using the aforementioned custom software and entered into the results spreadsheet file. For comparison to the other 3 methods, the Douglas bag data collections were compared to breaths 3, 7 and 11.

Statistical Analyses

The expired gas fractions (F_{EO_2} and F_{ECO_2}) aligned to the end of each expiration from start to equilibration were analyzed for differences across multiple ventilation maneuvers and between the 6 L lung mimicking bag and 2 L mixing bag using 2-way mixed design (repeated = maneuvers; between = gas bag) ANOVA. Significant main or interaction effects were followed by simple effects contrasts and subsequent specific mean pair comparisons were performed from selected contrast analyses due to violations of post-hoc analyses caused by the mixed design (between-within) ANOVA.

3. Comparison of the New System to a Commercial System for Rest, Steady State Exercise and Non-steady State Exercise.

The new system and commercial system (ParvoMedics, Salt Lake City, UT) were studied for 5 min each at rest, 100 watts, and 175 watts (Figures 13 to 17). During each condition, EGAIC was performed breath-by-breath with the new system with ventilation and gas fraction signals acquired as 5 data point averages at 20 Hz. Testing was conducted in the following sequence; rest, 100 watts, 175 watts. For the rest condition, testing was continuous between both systems, separated only by the calibration procedure for each system each trial. For the 100 watts condition, 5 min seated recovery separated each test session, and the sequence of system testing was alternated. For the 175 watts condition, 10 min of supine recovery separated each test session. For the exercise trials, the order of system testing alternated between each of the five repeated trials, starting with commercial followed by new, and always involving 100 watts followed by 175 watts. For example, the first series involved the commercial system at 100 watts, then 5 min of seated rest, followed by the new system at 100 watts, followed by 5 min of seated rest, followed by the commercial system at 175 watts, followed by 10 min of supine rest, followed by the new system at 175 watts, followed by 10 min of supine rest, followed by the new system at 100 watts, etc. This procedure was repeated 5 times for parametric statistical analyses.



Figure 13. Photograph of the custom developed system for EGAIC during the rest condition.



Figure 14. Photograph of the commercial (ParvoMedics) EGAIC system during the rest condition.

Calibration of the new system was performed as previously described. Calibration of the commercial system was fully automated and adhered to manufacturer guidelines based on (4.0% CO₂, 16.1% O₂, balance N₂) calibration gas and turbine calibration with a 3 L syringe.

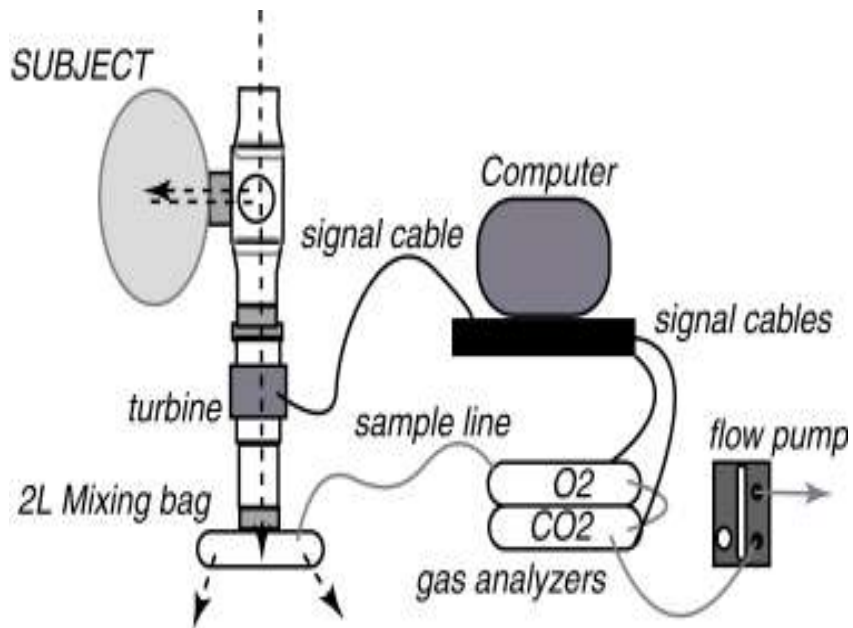


Figure 15. Schematic of the custom developed system for EGAIC.

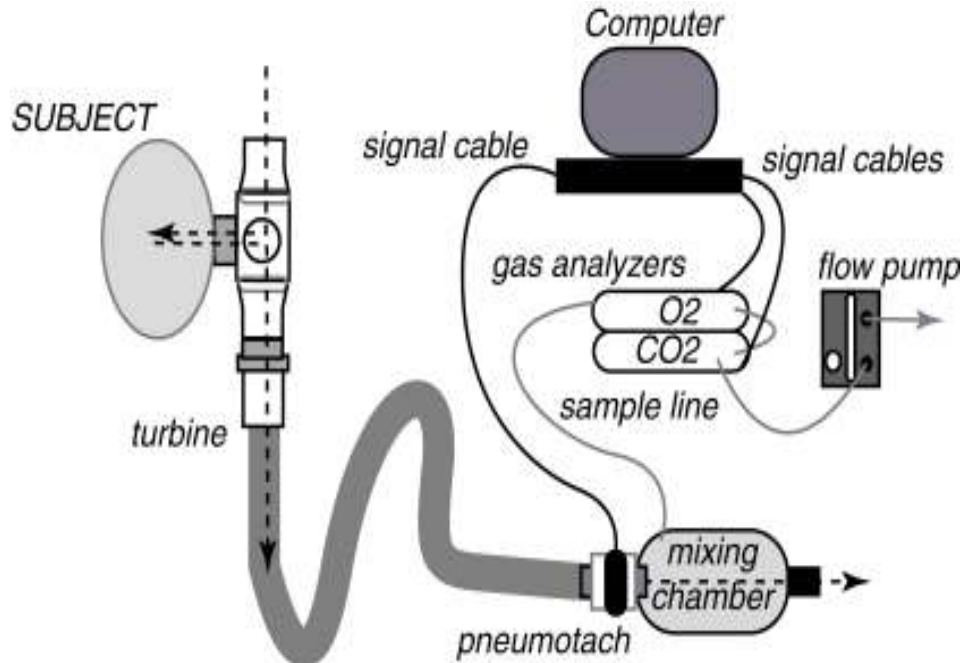


Figure 16. Schematic of the commercial (ParvoMedics) EGAIC system.



Figure 17. The subject exercising with analyses performed by the commercial (ParvoMedics) system for hypothesis 3 testing.

Data Processing

To determine EGAIC for the new system, the data were imported into a custom developed software program (LabVIEW, National Instruments, Austin, TX). The data were processed to acquire expired gas fractions across a 250 ms time interval immediately after the end of each end-exhalation. Tidal volume was obtained from the flow turbine and converted to ventilation rate after correction for breathing (expiration) frequency and gas condition conversion from ATPS to STPD. For both systems, post-acquisition EGAIC computation processing was performed using an 11 breathe running average to decrease breath-by-breath variability.

Steady state for each variable was quantified as the average of the last 2 min of data for each 5 min collection period. Variability for the two systems at steady state was quantified by the standard deviation of each measure of $F_{E}O_2$, $F_{E}CO_2$, V_E , VO_2 , VCO_2 and RER.

Statistical Analyses

Two-way mixed design ANOVA (Intensities (2 levels) vs Systems (2 levels)) was used to examine differences for each of V_E , $F_{E O_2}$, $F_{E CO_2}$, VO_2 , VCO_2 , and RER for steady state (rest, and 100 watts). For the 175 watts trial, the last 30 s average of data was used and analyzed using a paired t-test. For the 175 watts non-steady state condition, another analysis was also performed where time was used as a factor, and a two-way mixed design ANOVA was used to assess differences for Time (0, 0.5, 1, 1.5, 3 and 5 min) x System (2 levels).

4. Sampling Gas Fractions from the Lung Model from an Equivalent Position of a Subject's Mouth will reveal the Extent of Alveolar Air Contamination by trapped Air in the Anatomical Dead Space. In addition, the Volume of the Anatomical Dead Space will be able to be determined from Calibration Gas Fraction Contamination of the Anatomical Dead Space.

The model of the lung and conducting zone was modified by the placement of both inspired and expired turbines in series between the 6 L air breathing bag (lung model) and the 3-way valve (Figures 18 and 19). An expired breath gas sample line was connected to the inspired turbine, and another gas sample line was connected to the 6 L lung model. A gas flow line was connected to the 6 L lung model to provide a constant flow of calibration gas during data collection.

Prior to data collection, flow turbines and gas analyzers were calibrated as previously described. Immediately prior to data collection, approximately 2 L of calibration gas (4.99% CO_2 , 12.98% O_2 , balance N_2) was pumped into the 6 L lung model. The software program was started, the calibration gas flow was turned on, and the pumps for expired gas and alveolar sampling were switched on. Repeated 1 L

inspirations and expirations were performed as for hypothesis 2 until the 6 L lung model bag was filled. Continuous data collection occurred for ventilation, expired gas fractions and alveolar gas fractions, as for hypothesis 2. This procedure was repeated 5 times for parametric statistical analyses.



Figure 18. Photograph of the adjusted lung model to suit profiling of changes in inspired and expired gas variables during inspiration and expiration.

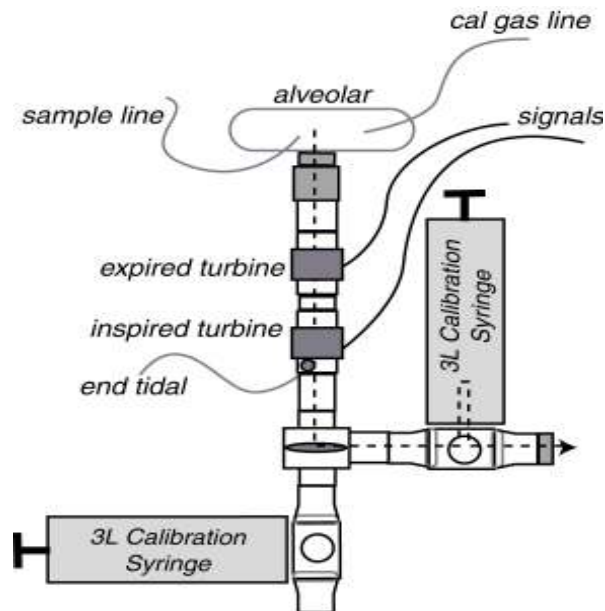


Figure 19. Schematic of the adjusted lung model to suit profiling of changes in inspired and expired gas variables during the expiration phase of data collection.

Data Processing

Data for each of the gas fractions from the end tidal and alveolar model sampling, and ventilation, were imported into a custom developed software program (LabVIEW, National Instruments, Austin, TX). The data were processed to acquire expired gas fractions during the last 50 ms of each expiration maneuver. Tidal volume was obtained from the flow turbine and converted to ventilation rate after correction for breathing (expiration) frequency and gas condition conversion from ATPS to STPD. Expired gas signal integration was not performed due to the high quality of the end-tidal signals decreasing the necessity for additional data processing, and the difficulty in accounting for the different delay factors in the integration of each of the oxygen and carbon dioxide signals.

Statistical Analyses

End tidal gas fractions were compared to reference gas fractions using a one-way ANOVA (end tidal vs. integrated signal vs. calibration gas fraction). Post hoc mean pair analyses were completed using the Tukey Test.

CHAPTER IV

Results

The results of this study are presented based on each of the four hypotheses.

Hypothesis 1: For controlled ventilation of a known gas mixture, V_E , $F_{E}O_2$ and $F_{E}CO_2$ will be identical to the recorded turbine ventilation and the constant gas fraction values from the calibration gas used to mimic alveolar air between each of the new compliant 2 L mixing bag system, and Douglas bag collections of expired air with and without 5 feet of additional low resistance tubing dead space.

All data for the comparison of turbine volumes vs. expected volumes vs. Douglas bag collections will be presented with Hypothesis II.

The summary of the mean values for the various measures of % gas values for each of oxygen and carbon dioxide for calibration gas is presented. A complete description of three conditions of expired air collection can be found in Table 1 and Figure 20.

Figure 20 presents % gas results for calibration air pumped into each method device using calibration syringes, compared to the calibration gas value for oxygen content. The compliant mixing bag was the only method to produce data not significantly different between measured and actual. Presumably, the room air contamination in the Douglas bag valve and tubing inflated oxygen gas content of the sampled air. Such contamination was exacerbated by the 5 feet of connection tubing.

Table 1. Mean % gas values for each of O₂ and CO₂ for calibration gas

Conditions	Oxygen		Carbon dioxide	
	Alveolar	Measured	Alveolar	Measured
Mixing Bag	14.96 ± 0.22	14.99 ± 0.13	5.27 ± 0.16	5.23 ± 0.09
Douglas Bag	14.97 ± 0.06	15.51 ± 0.12	5.36 ± 0.02	4.97 ± 0.24
DB + Tubing	15.16 ± 0.08	15.68 ± 0.27	5.12 ± 0.09	4.71 ± 0.18

Values are means ± SD. DB = Douglas Bag.

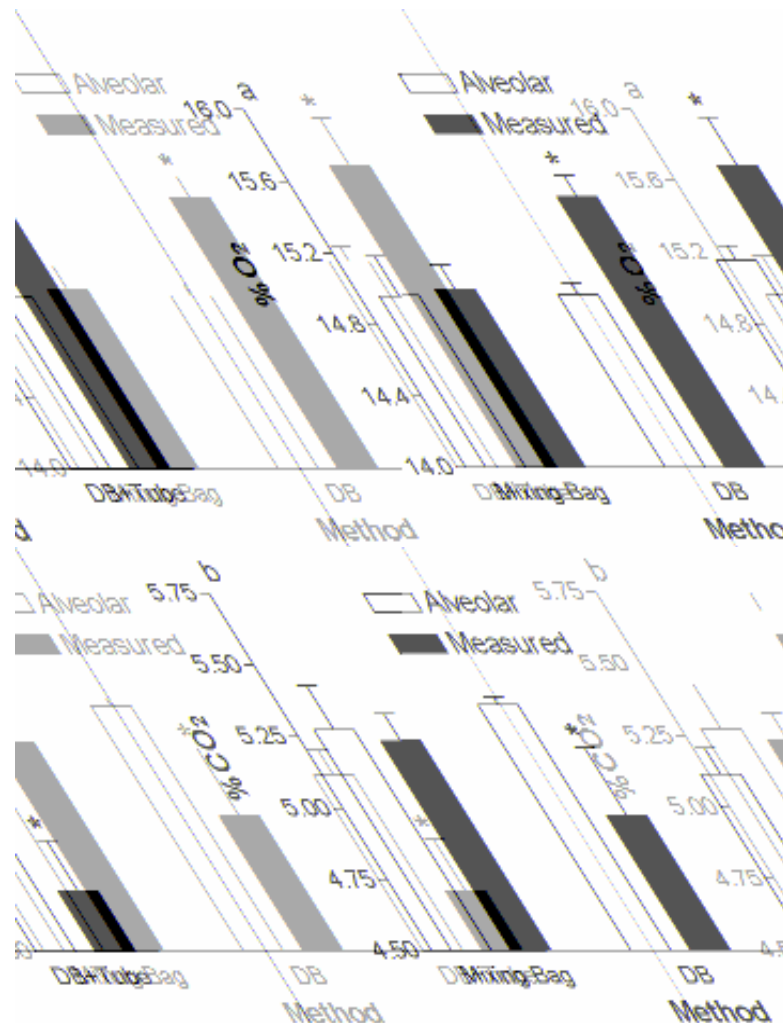


Figure 20. The differences between % gas values for each of a) oxygen and b) carbon dioxide for calibration gas directed into three methods of expired air collection (mixing bag, Douglas bag, Douglas bag + tubing) [* *p* < 0.05].

Hypothesis 2: For controlled ventilation and mimicked lung gas exchange, averaged values for each of V_E , $F_{E}O_2$, $F_{E}CO_2$, VO_2 , VCO_2 , and RER and the time to reach equilibration, will differ between the new small mixing bag attached to the mouthpiece vs. a traditional mixing chamber connected to 5 feet of low resistance tubing, vs. the Douglas bag method, vs. a commercial automated system of indirect calorimetry (ParvoMedics).

The descriptive characteristics of the mean % oxygen and % carbon dioxide from the mimicked lung model for the three methods of expired air collection (mixing bag, ParvoMedics, external mixing chamber) are presented in Tables 2 and 3.

Figure 21 and 22 present data for each of % oxygen and carbon dioxide gas in the expired air from the lung model, respectively. The change in gas composition of the alveolar air represents what the gas fraction data would be like if either of the methods has perfect precision on a breath-by-breath basis. Alveolar % oxygen gas was significantly lower than each method across all breaths. The most responsive method was the mixing bag, with significantly lower % gas data for oxygen for breaths 2 to 5 compared to the ParvoMedics and mixing chamber. The ParvoMedics and mixing bag yielded similar results after breath 6, but data were still significantly higher than for alveolar air.

Table 2. Mean % oxygen from the mimicked lung model

Breath	Mixing Bag	ParvoMedics	Chamber	Alveolar
1	18.20 ± 0.37	18.71 ± 0.04	20.56 ± 0.20	12.99 ± 0.62
2	17.72 ± 0.24	18.64 ± 0.04	19.94 ± 0.18	14.18 ± 0.42
3	17.41 ± 0.19	18.53 ± 0.05	19.37 ± 0.36	15.04 ± 0.32
4	17.32 ± 0.17	18.18 ± 0.15	18.96 ± 0.39	15.67 ± 0.27
5	17.35 ± 0.16	17.85 ± 0.23	18.73 ± 0.42	16.14 ± 0.22
6	17.46 ± 0.13	17.65 ± 0.25	18.57 ± 0.48	16.52 ± 0.18
7	17.58 ± 0.10	17.58 ± 0.25	18.49 ± 0.51	16.85 ± 0.15
8	17.72 ± 0.10	17.61 ± 0.23	18.45 ± 0.51	17.09 ± 0.13
9	17.85 ± 0.10	17.68 ± 0.20	18.47 ± 0.50	17.29 ± 0.10
10	17.98 ± 0.10	17.79 ± 0.17	18.51 ± 0.46	17.45 ± 0.12
11	18.10 ± 0.09	17.91 ± 0.14	18.51 ± 0.46	17.56 ± 0.11

Values are means ± *SD*

Table 3. Mean % carbon dioxide from the mimicked lung model

Breath	Mixing Bag	ParvoMedics	Chamber	Alveolar
1	1.55 ± 0.22	1.48 ± 0.02	0.29 ± 0.20	4.36 ± 0.40
2	1.71 ± 0.12	1.53 ± 0.02	0.63 ± 0.34	3.70 ± 0.27
3	1.96 ± 0.09	1.59 ± 0.02	0.99 ± 0.38	3.22 ± 0.20
4	2.01 ± 0.09	1.79 ± 0.09	1.18 ± 0.33	2.87 ± 0.16
5	1.98 ± 0.10	1.97 ± 0.12	1.30 ± 0.32	2.60 ± 0.13
6	1.93 ± 0.08	2.08 ± 0.14	1.38 ± 0.32	2.37 ± 0.11
7	1.86 ± 0.07	2.12 ± 0.13	1.42 ± 0.31	2.22 ± 0.08
8	1.79 ± 0.07	2.11 ± 0.12	1.43 ± 0.30	2.08 ± 0.08
9	1.72 ± 0.07	2.07 ± 0.11	1.42 ± 0.29	1.97 ± 0.07
10	1.64 ± 0.07	2.01 ± 0.09	1.40 ± 0.27	1.88 ± 0.07
11	1.58 ± 0.07	1.95 ± 0.08	1.40 ± 0.27	1.82 ± 0.09

Values are means ± *SD*

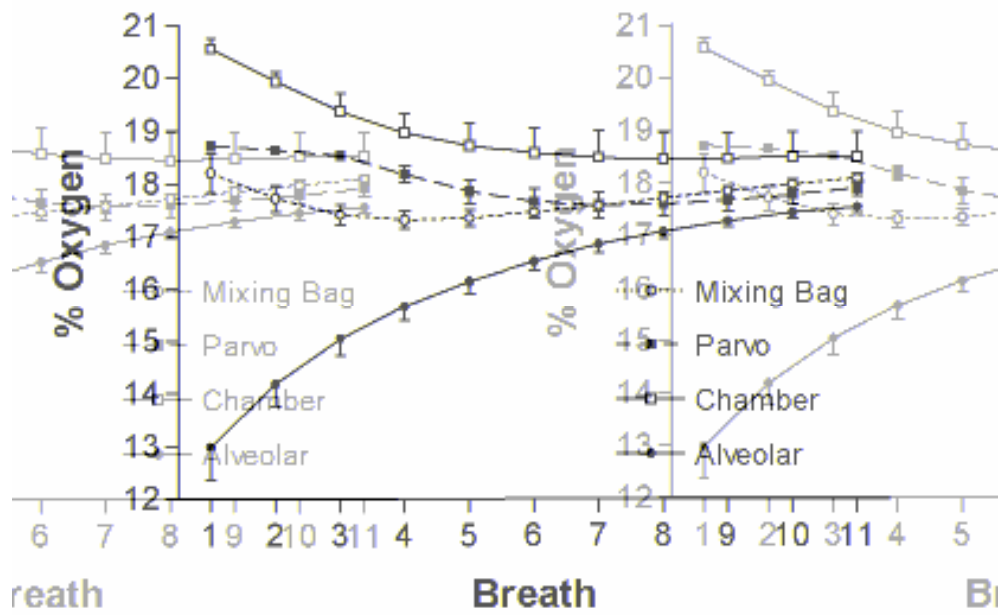


Figure 21. The changes over time (breath) and differences between methods for % oxygen from the mimicked lung model for the three methods of expired air collection (Mixing bag, ParvoMedics, External mixing chamber).

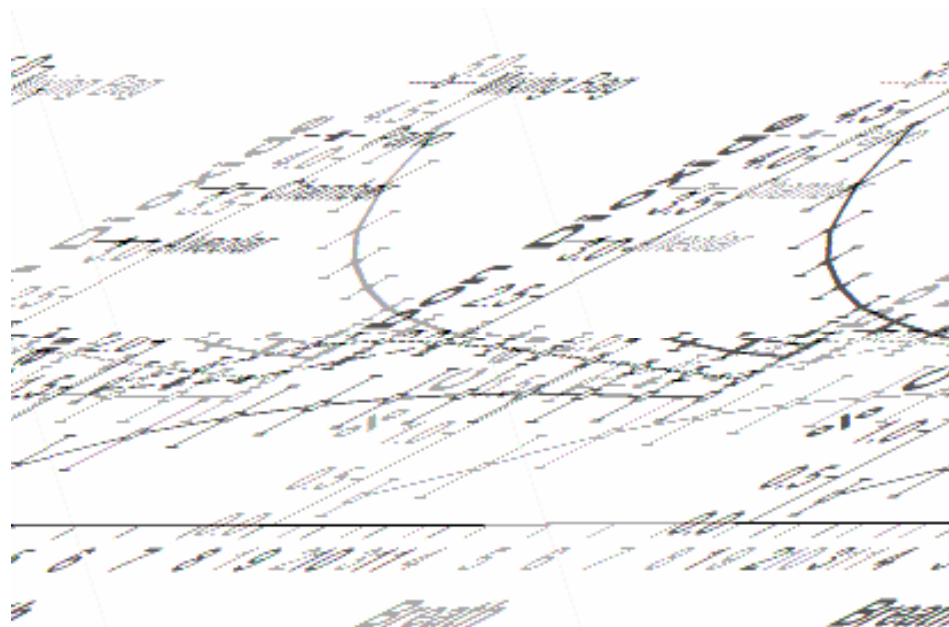


Figure 22. The changes over time (breath) and differences between methods for % carbon dioxide from the mimicked lung model for the three methods of expired air collection (Mixing bag, ParvoMedics, External mixing chamber).

The average changes in initial slope data for breaths each of % oxygen and % carbon dioxide are shown in Table 4.

Figure 23 presents the slope data for breaths 0 (start) to breath 2 for each of % oxygen and carbon in the sampled air. The data used are the first 3 data points for each method (other than alveolar) taken from the data sets used for Figures 21 and 22. The results clearly show a significantly lower response for the ParvoMedics system compared to the mixing bag and external mixing chamber for both oxygen and carbon dioxide. Combined with the data from Figures 21 and 22, there is evidence for inferior temporal sensitivity of the ParvoMedics system. Especially concerning is the increase in $F_{E}CO_2$ from breaths 3 to 7 in Figure 22. Such an exaggerated response does not occur for % oxygen (Figure 21) for this method, revealing that there might be a % carbon dioxide specific correction within the software for this system. This challenge cannot be verified from this study, as we did not directly sample expired air from the ParvoMedics mixing chamber.

Table 4. Mean slope data for breaths each of % oxygen and % carbon dioxide

Methods	Oxygen slopes	Carbon dioxide slopes
Mixing Bag	-0.40 ± 0.13	0.20 ± 0.09
ParvoMedics	0.07 ± 0.04	0.05 ± 0.02
Chamber	-0.44 ± 0.25	0.35 ± 0.15

Values are means \pm *SD*

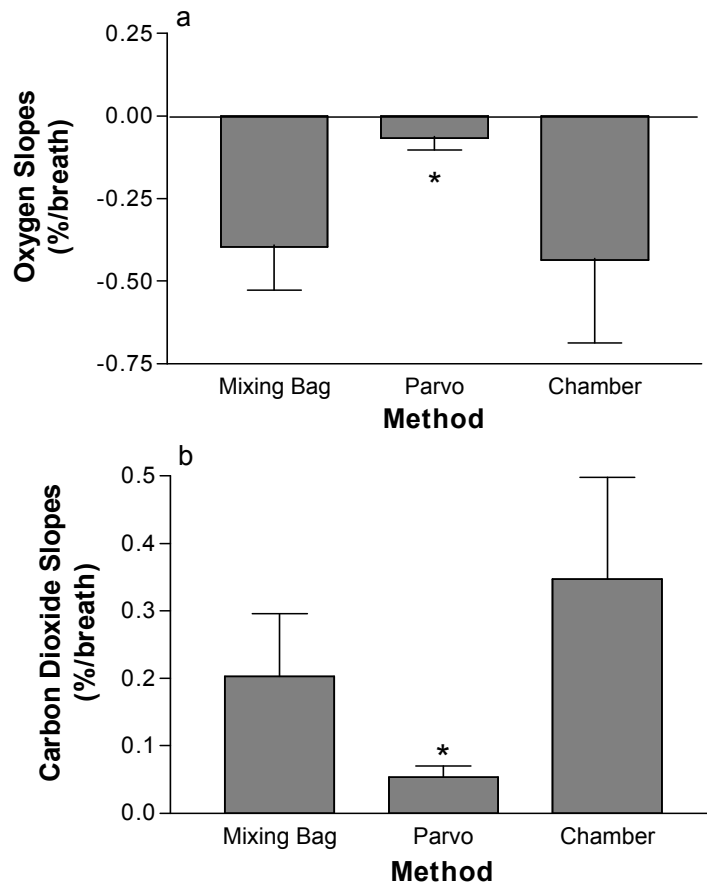


Figure 23. The changes in initial slope for each of a) % oxygen and b) % carbon dioxide for breaths 0 to 2 [* $p < 0.05$].

The results for tidal volume are presented in Figure 24 for the turbine used in the mixing bag trials. Rather than continue to analyze ventilation from small data sets, the ventilation results from the 1 L calibration syringe maneuvers of hypothesis 2 testing were combined resulting in 55 data points. Descriptive statistical analyses revealed a mean \pm SD of 0.999 ± 0.142 L, with a range of 0.96 to 1.03 L.

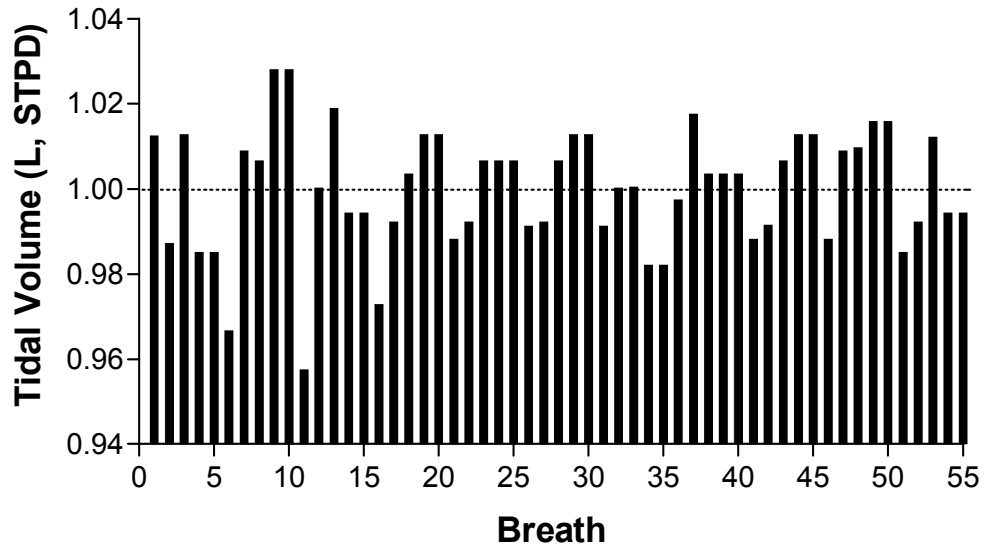


Figure 24. The temporal distribution of 1 L ventilation maneuvers from the mixing bag turbine during testing of hypothesis 2.

After each tidal volume was corrected to ventilation based on the 10 breath/min condition of controlled ventilation, data were computed for each of VO_2 , VCO_2 and RER. These results are presented in Tables 5, 6, 7 and Figure 25.

To assess the differences in ventilation between the mixing bag vs. ParvoMedics pneumotach, the 55 data points acquired for each method were compared using an unpaired t-test. Results are presented in Figure 26 and revealed a significantly lower ($p = 0.0027$) ventilation from the ParvoMedics compared to mixing bag turbine. Nevertheless, the mean difference between the methods was only 83 mL/min, which is physiologically insignificant at this ventilation rate, but would increase to 1.7 L/min at a ventilation rate of 140 L/min. Note the larger variability of ventilation from the ParvoMedics system (0.1 vs. 0.18 L/min).

Table 5. Mean changes over time (breath) for VO₂

Breath	Mixing Bag	ParvoMedics	Chamber
1	0.22 ± 0.03	0.19 ± 0.01	0.03 ± 0.02
2	0.27 ± 0.03	0.19 ± 0.01	0.08 ± 0.01
3	0.29 ± 0.02	0.19 ± 0.00	0.13 ± 0.03
4	0.30 ± 0.02	0.22 ± 0.01	0.16 ± 0.03
5	0.29 ± 0.01	0.25 ± 0.02	0.18 ± 0.03
6	0.29 ± 0.01	0.26 ± 0.02	0.20 ± 0.04
7	0.27 ± 0.01	0.27 ± 0.02	0.20 ± 0.04
8	0.27 ± 0.01	0.27 ± 0.02	0.20 ± 0.04
9	0.25 ± 0.01	0.26 ± 0.02	0.21 ± 0.04
10	0.24 ± 0.01	0.25 ± 0.02	0.20 ± 0.04
11	0.23 ± 0.01	0.24 ± 0.02	0.20 ± 0.04

Values are means ± *SD*

Table 6. Mean changes over time (breath) for VCO₂

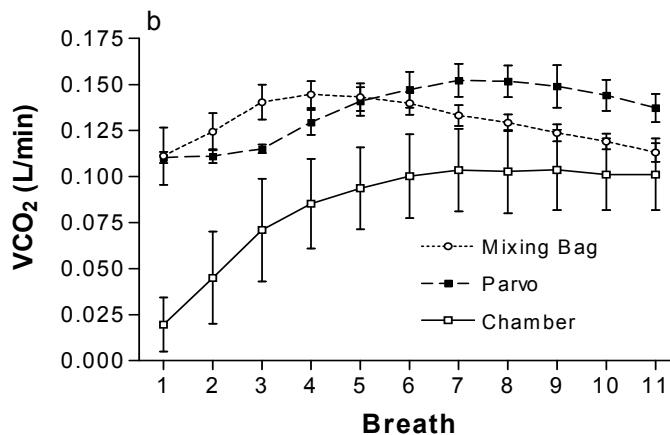
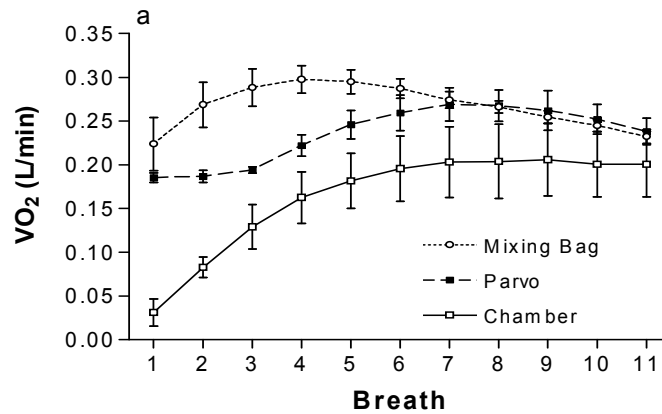
Breath	Mixing Bag	ParvoMedics	Chamber
1	0.11 ± 0.02	0.11 ± 0.00	0.02 ± 0.01
2	0.12 ± 0.01	0.11 ± 0.00	0.05 ± 0.03
3	0.14 ± 0.01	0.12 ± 0.00	0.07 ± 0.03
4	0.14 ± 0.01	0.13 ± 0.01	0.09 ± 0.02
5	0.14 ± 0.01	0.14 ± 0.01	0.09 ± 0.02
6	0.14 ± 0.01	0.15 ± 0.01	0.10 ± 0.02
7	0.13 ± 0.01	0.15 ± 0.01	0.10 ± 0.02
8	0.13 ± 0.00	0.15 ± 0.01	0.10 ± 0.02
9	0.12 ± 0.00	0.15 ± 0.01	0.10 ± 0.02
10	0.12 ± 0.00	0.14 ± 0.01	0.10 ± 0.02
11	0.11 ± 0.01	0.14 ± 0.01	0.10 ± 0.02

Values are means ± *SD*

Table 7. Mean changes over time (breath) for RER

Breath	Mixing Bag	ParvoMedics	Chamber
1	0.50 ± 0.03	0.60 ± 0.00	0.57 ± 0.08
2	0.47 ± 0.05	0.59 ± 0.00	0.53 ± 0.06
3	0.49 ± 0.00	0.59 ± 0.00	0.54 ± 0.09
4	0.49 ± 0.01	0.58 ± 0.00	0.52 ± 0.05
5	0.49 ± 0.01	0.57 ± 0.01	0.51 ± 0.03
6	0.49 ± 0.01	0.57 ± 0.01	0.51 ± 0.02
7	0.49 ± 0.01	0.57 ± 0.01	0.51 ± 0.01
8	0.49 ± 0.01	0.57 ± 0.01	0.50 ± 0.01
9	0.49 ± 0.01	0.57 ± 0.01	0.50 ± 0.01
10	0.49 ± 0.01	0.57 ± 0.01	0.50 ± 0.01
11	0.49 ± 0.01	0.58 ± 0.00	0.50 ± 0.01

Values are means ± *SD*



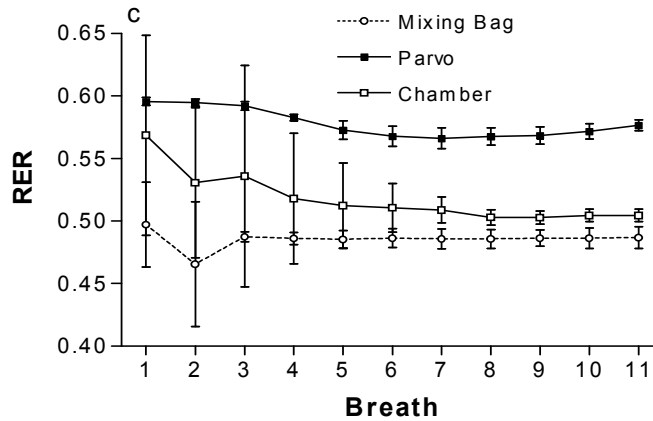


Figure 25. The changes over time (breath) for a) VO_2 , b) VCO_2 and c) RER from the mimicked lung model for the three methods of expired air collection (Mixing bag, ParvoMedics, External mixing chamber).



Figure 26. The mean \pm SD data for ventilation (STPD) for the mixing bag and ParvoMedics methods [$* p < 0.05$].

Hypothesis 3: Use of the new system compared to a commercial system will yield the same values for V_E , F_{EO_2} , F_{ECO_2} , VO_2 , VCO_2 and RER at rest, during steady state exercise, and non-steady state exercise.

The descriptive characteristics of the mean data for V_E , F_{EO_2} , F_{ECO_2} , VO_2 , VCO_2 , and RER for the new system and ParvoMedics are presented in Table 8 and 9.

Figure 27a, b, c presents the mean \pm SD data for the three measured variables of this hypothesis (a) V_E , b) F_{EO_2} , c) F_{ECO_2}). For V_E , there were no significant findings

for the main effect for method (new system vs. Parvomedics) ($p = 0.097$) and the Intensity x Method interaction ($p = 0.721$). As expected, there was a highly significant difference for the Intensity main effect ($p < 0.0001$). For $F_{E}O_2$, there were significant main effects for Intensity ($p < 0.0001$) and Method ($p = 0.0016$), and no significant findings for the Intensity x Method interaction ($p = 0.689$). $F_{E}O_2$ for the ParvoMedics was consistently and significantly higher than the new system method across all intensities. For $F_{E}CO_2$, there was a significant main effects for Intensity ($p < 0.0001$), but no significant findings for the main effect for Method ($p = 0.148$) or the Intensity x Method interaction ($p = 0.208$).

Table 8. Mean data for V_E , $F_{E}O_2$, $F_{E}CO_2$, VO_2 , VCO_2 , and RER for the new system

New system			
	Rest	100 W	175 W
V_E	5.51 ± 0.533	25.79 ± 0.86	51.31 ± 2.61
$F_{E}O_2$	0.17 ± 0.00	0.15 ± 0.00	0.16 ± 0.00
$F_{E}CO_2$	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
VO_2	0.24 ± 0.02	1.54 ± 0.03	2.39 ± 0.05
VCO_2	0.20 ± 0.03	1.35 ± 0.05	2.41 ± 0.07
RER	0.81 ± 0.06	0.88 ± 0.03	1.01 ± 0.02

Values are means \pm *SD*

Table 9. Mean data for V_E , $F_{E}O_2$, $F_{E}CO_2$, VO_2 , VCO_2 , and RER for the ParvoMedics

ParvoMedics			
	Rest	100 W	175 W
V_E	6.59 ± 0.90	27.22 ± 0.65	53.90 ± 2.54
$F_{E}O_2$	0.17 ± 0.00	0.16 ± 0.00	0.16 ± 0.00
$F_{E}CO_2$	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
VO_2	0.26 ± 0.05	1.54 ± 0.07	2.35 ± 0.05
VCO_2	0.25 ± 0.05	1.38 ± 0.04	2.43 ± 0.05
RER	0.94 ± 0.03	0.90 ± 0.07	1.03 ± 0.03

Values are means \pm *SD*

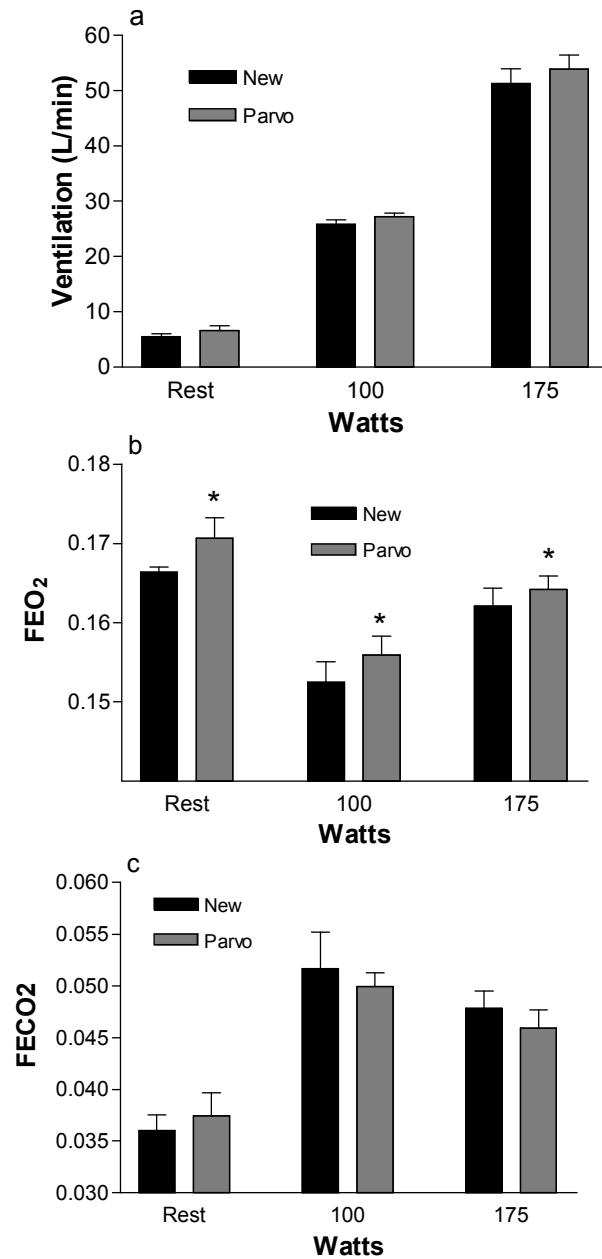


Figure 27. The mean \pm SD data for a) ventilation (STPD), b) $F_{E}O_2$, and c) $F_{E}CO_2$ for the New system and ParvoMedics methods [* $p < 0.05$].

Figure 28a, b, c presents the mean \pm SD data for the three calculated variables of this hypothesis (a) VO_2 , b) VCO_2 , and c) RER). For VO_2 , there were no significant findings for the main effect for method (New vs. ParvoMedics) ($p = 0.786$) and the Intensity x Method interaction ($p = 0.059$).

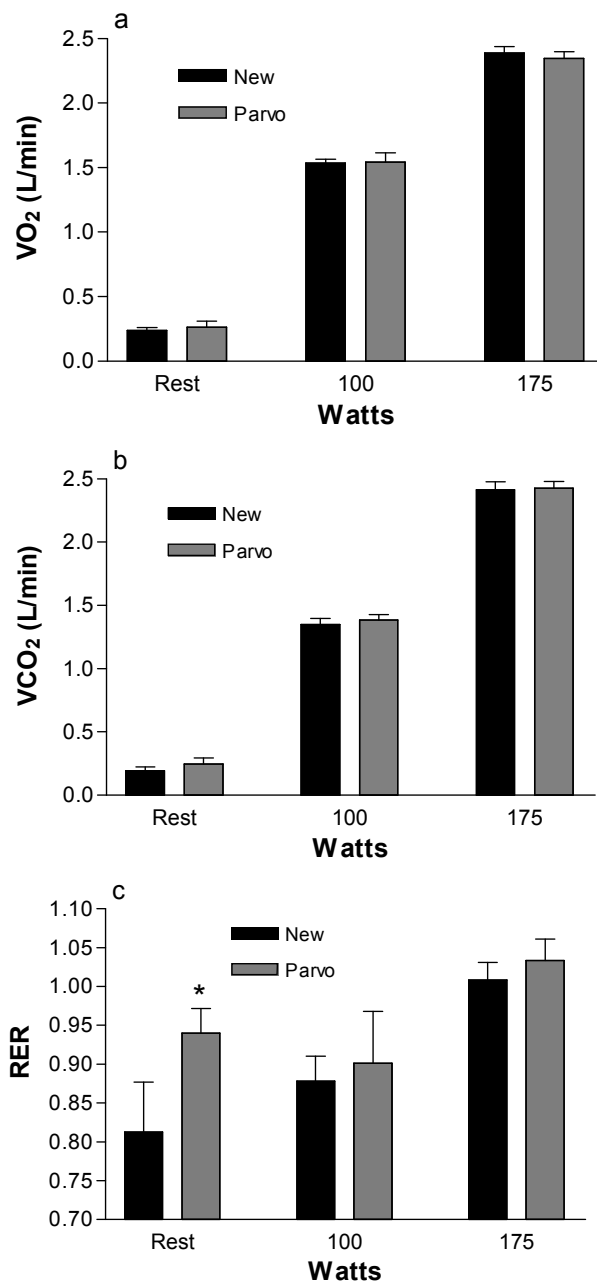


Figure 28. The mean \pm SD data for a) VO_2 , b) VCO_2 , and c) RER for the New system and ParvoMedics methods [$* p < 0.05$].

As expected, there was a highly significant difference for the Intensity main effect ($p < 0.0001$). For VCO_2 , there were no significant findings for the main effect for method (New vs. ParvoMedics) ($p = 0.178$) and the Intensity x Method interaction

($p = 0.406$). As expected, there was a highly significant difference for the Intensity main effect ($p < 0.0001$). For RER, there were significant findings for the main effect for Intensity ($p = 0.0006$), Method ($p = 0.024$), and a significant Intensity x Method interaction ($p = 0.005$). Post hoc analyses revealed the interaction was confined to the rest condition, where RER for the ParvoMedics was significantly higher than for the New system ($p = 0.001$).

Hypothesis 4: a) Mixed (integrated) and end tidal gas fractions for O₂ and CO₂ will be different compared to the calibration gas used to mimic alveolar air. b) The extent of mixing between alveolar (mimicked) and anatomical dead space air can be used to estimate the volume of the anatomical dead space.

Results of the two way mixed ANOVAs for each of expired oxygen and carbon dioxide were similar in that there were highly significant main effects and interactions ($p < 0.001$), where all mean differences between alveolar and mouth end tidal gas % values across 6 breaths were significant ($p < 0.01$). Mean \pm SD data for these data sets are presented in Table 10 and Figure 29a and b.

Table 10. Mean % O₂ and CO₂ 6 breaths of mimicked lung ventilation and gas exchange

Breath	Mouth	Alveolar
1	17.06 \pm 0.42	12.93 \pm 0.40
2	17.98 \pm 1.27	16.02 \pm 0.58
3	18.99 \pm 0.86	17.66 \pm 0.53
4	19.55 \pm 0.61	18.55 \pm 0.38
5	19.84 \pm 0.44	18.97 \pm 0.31
6	20.03 \pm 0.29	19.24 \pm 0.26

Values are means \pm SD

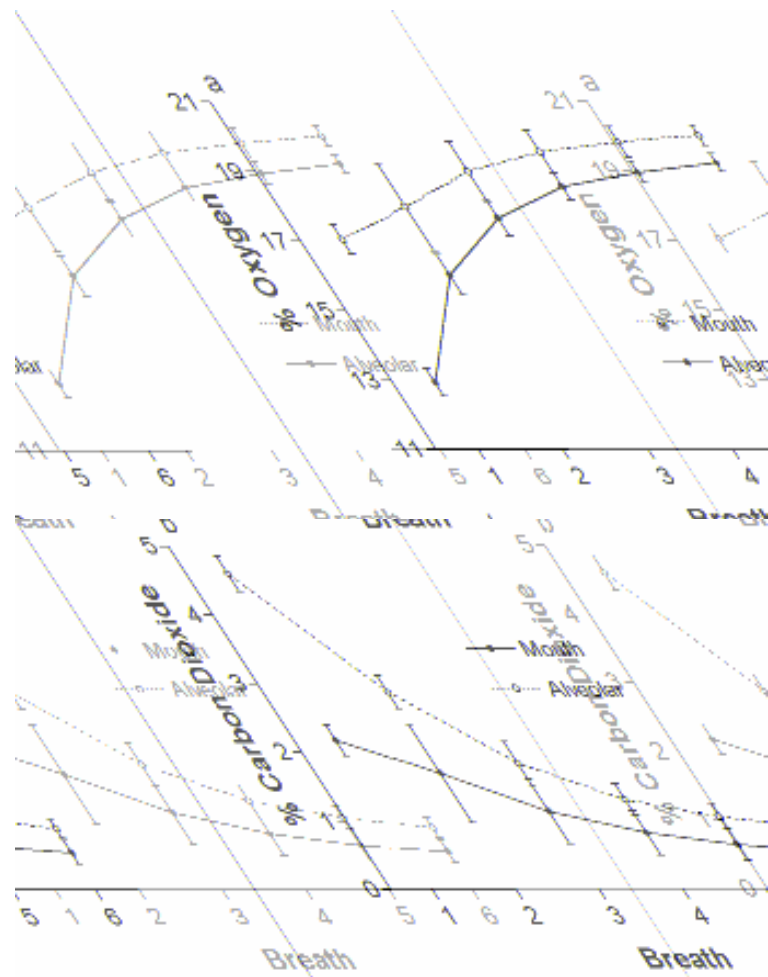


Figure 29. The mean \pm SD data for a) % Oxygen and b) % Carbon Dioxide across 6 breaths for the condition of mimicked lung ventilation and gas exchange. All mean comparisons between sampling site (alveolar vs. mouth) were significantly different.

The quality of the equilibrated “end tidal” data, and the continual change in alveolar gas conditions induced by the inflow of calibration gas prevented accurate integration of gas signals across each breath.

The combined data for ventilation air flow and changing gas % data for oxygen were used to compute the volume of the dead space within the system, which is a calculation that would reflect the anatomical dead space in an in-vivo system. The individual computed dead space volumes are presented in Figure 30. Data were very consistent, with mean \pm SD data being 2.5 ± 0.13 L.

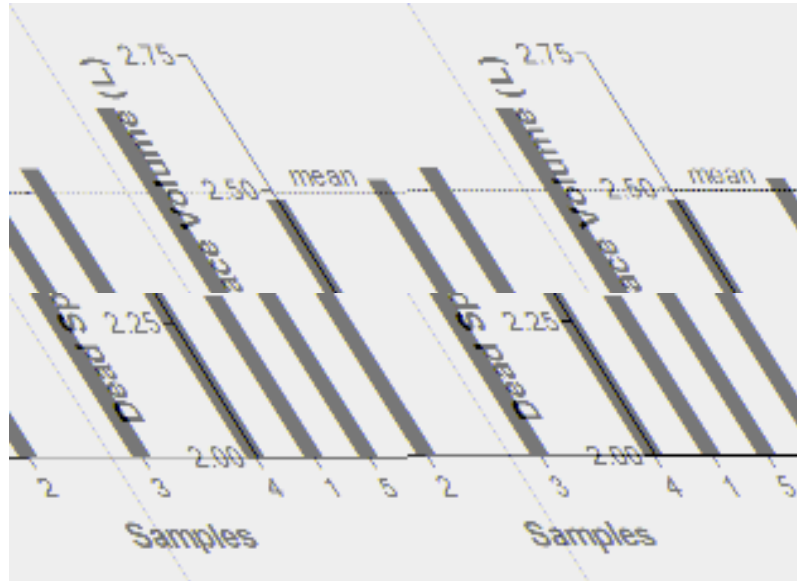


Figure 30. Calculated data for the dead space of the modeled lung system as built for hypothesis 4 testing.

CHAPTER V

Discussion

The discussion of the results is presented in the following sections: (a) Expired % gas values for O₂ and CO₂ for calibration gas, (b) % O₂, CO₂, V_E, and slope in the expired air from the lung model (c) averaged values for each of VO₂, VCO₂, and RER, (d) V_E, F_EO₂, F_ECO₂, VO₂, VCO₂, and RER for the new system and ParvoMedics, (e) % O₂ and CO₂ across 6 breaths of mimicked lung ventilation and gas exchange, (f) calculated data for the dead space of the modeled lung system.

Expired % gas values of O₂ and CO₂ for calibration gas

The primary purpose of this study was to compare turbine ventilation and the constant gas fraction values from the calibration gas used to mimic alveolar air between new compliant 2 L mixing bag and Douglas bag collections of expired air with and without 5 feet of low resistance tubing. The mean Douglas bag with tubing alveolar (15.16 ± 0.08) and measured (15.68 ± 0.27) % gas values of oxygen for calibration gas was significantly higher ($p < 0.05$) than the mean Douglas bag alveolar (14.97 ± 0.06), measured (15.51 ± 0.12), the mixing bag alveolar (14.96 ± 0.22), and measured (14.99 ± 0.13) method of expired air collection. However, the mean Douglas bag alveolar (5.36 ± 0.02) and measured (4.97 ± 0.24) % gas values of carbon dioxide for calibration gas was significantly higher ($p < 0.05$) than the mean Douglas bag with tubing alveolar (5.12 ± 0.09), measured (4.71 ± 0.18), mixing bag alveolar (5.27 ± 0.16), and measured (5.23 ± 0.09) method of expired air collection. The major finding in this study was that % gas fraction data from the compliant 2 L mixing bag was the only method to yield data not significantly different between

alveolar and measured. 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.

Other researchers have noted that it is very difficult to remove all air collected in the Douglas bag. According to some recent work of Bassett et al. (2001), the Douglas bag and expired collection tubing need to be flushed of room air. Specifically, Crouter et al. (2006) concluded that it is difficult to remove all the air from the Douglas bag and air leaking out during the removal process. This dead air space acts to decrease the overall sensitivity and accuracy of the system.

Foss and Hallen (2005) have descriptively 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. This would help to identify the compliant 2 L mixing bag was the only method to produce data not significantly different between alveolar and measured. This finding is important because it shows that the new 2 L mixing bag is capable of accurately reproducing specific gas fractions from reference calibration gas.

% O₂, CO₂, V_E, and slope in the expired air from the lung model

Many studies have examined the F_EO₂, F_ECO₂, and V_E between different expired gas analysis indirect calorimetry systems (Bassett et al., 2001; Carter & Jeukendrup: 2002; Crouter et al., 2006; Cullum et al., 1999; Foss & Hallen, 2005; Meyer et al., 2001; Pinnington et al., 2001; Yates & Cullum, 2001). In the present study, alveolar % oxygen was significantly lower than mixing bag, ParvoMedics, and mixing chamber method across all breaths. Previous studies have reported that there

was no statistically significant difference between the systems either in $F_{E}O_2$, $F_{E}CO_2$, and V_E (Bassett et al., 2001; Carter & Jeukendrup; 2002; Cullum et al., 1999; Engebretson, 1998; Rietjens et al., 2001; Yates & Cullum, 2001). However, some studies have shown that there were significant differences between the systems either in $F_{E}O_2$, $F_{E}CO_2$, and V_E (Hiilloskorpi et al., 1999; McLaughlin et al., 2001; Pinnington et al., 2001).

Interestingly, in this study, the most sensitive method was the mixing bag, with significantly lower % oxygen gas data for breaths 2 to 5 compared to the mixing chamber and ParvoMedics. The results of this investigation showed that the mixing bag and ParvoMedics provided similar data after breath 6, but results were still significantly higher than for actual air.

The results of this investigation revealed that significantly lower ($p = 0.0027$) ventilation (STPD) from the ParvoMedics (7.25 ± 0.18) compared to mixing bag turbine (7.34 ± 0.10 L/min). However, results indicated that the mean difference between the methods was only 83 mL/min. The results suggest that the ParvoMedics system may have meaningfully more variable ventilation compared to the mixing bag (0.1 vs. 0.18 L/min). Ventilation at rest was significantly lower for the ParvoMedics than the mixing bag, while ventilation was slightly higher for ParvoMedics compared to mixing bag during exercise. Presumably, the turbine used with the mixing bag is more sensitive at lower ventilation than the ParvoMedics pneumotach.

This finding regarding ventilation is in contrast to the findings of several researchers who demonstrated that there was no significant difference between the criterion vs. new systems in ventilation (Carter & Jeukendrup; 2002; Crouter et al., 2006; Cullum et al., 1999; Engebretson, 1998; Meyer et al., 2001; Rietjens et al., 2001; Storer et al., 1995; Yates & Cullum, 2001). In contrast, the study by Bassett et al.

(2001) and Foss and Hallen (2005) showed that ventilation from a computerized metabolic system was lower compared to the Douglas bag method. However, the differences were essentially physiologically insignificant at this ventilation rate.

The current investigation slope for each of % oxygen and % carbon dioxide for breaths 0 to 2 showed a significantly lower response for the ParvoMedics system compared to the mixing bag and mixing chamber. The second phase represented the continued increase in $F_{E}CO_2$ from breaths 3 to 7; however, we cannot explain this increase in $F_{E}CO_2$ and an increase in % oxygen for this method. The reason for this discrepancy is unclear, as we did not directly sample expired air from the ParvoMedics mixing chamber. However, such a discrepancy in gas responses may reveal a software adjustment embedded in the computations of the ParvoMedics.

This is a unique study as it is the first to measure differences in ventilation and expired gas fractions between a small compliant mixing bag, a traditional large fixed volume mixing chamber, Douglas bags, and the ParvoMedics during conditions of mimicked ventilation and lung gas exchange.

The new small mixing bag is directly connected to a mouthpiece and a suitable one-way valve such as flow turbine that is unique in providing expired moisture trapping capacity. The new 2 L mixing bag allow expired air to wash out through the bag. This system, in combination with including anatomical dead space (ADS) as a factor in the determinations, provides more accurate data than a traditional mixing chamber. This is the first study that shows averaged values for each of V_E , $F_{E}O_2$, $F_{E}CO_2$, VO_2 , VCO_2 , and RER and the initial slope for the change in $F_{E}O_2$, $F_{E}CO_2$ differ between a new small mixing bag attached to the mouthpiece vs. a traditional mixing chamber with 5 feet of tubing, vs. the Douglas bag method, vs. the ParvoMedics.

Averaged values for each of VO₂, VCO₂, and RER

This study showed the most responsive method was the mixing bag, with significantly higher VO₂ data for breaths 1 to 6 compared to the mixing chamber and ParvoMedics. The results of this investigation showed that the mixing bag and ParvoMedics yielded similar data after breath 6, but results were still significantly higher than for a mixing chamber.

The current study's data is in agreement with previous studies (Hiilloskorpi et al., 1999; McLaughlin et al., 2001; Pinnington et al., 2001) showing statistically significant differences between the systems in VO₂, VCO₂, and RER. However, some groups of researchers have investigated that there was no statistically significant difference between the systems either in VO₂, VCO₂, and RER (Bassett et al., 2001; Carter & Jeukendrup: 2002; Cullum et al., 1999).

V_E, F_EO₂, F_ECO₂, VO₂, VCO₂, and RER for the new system and ParvoMedics

An important procedure of this study was to compare the new system and a commercial system (ParvoMedics) values for V_E, F_EO₂, F_ECO₂, VO₂, VCO₂, and RER studied for 5 min each at rest, 100 watts, and 175 watts. There were various important findings of this study.

First, for V_E ($p = 0.097$), VO₂ ($p = 0.786$), and VCO₂ ($p = 0.178$) there was no significant difference in the main effect for method and the Intensity x Method interaction (V_E: $p = 0.721$, VO₂: $p = 0.059$, VCO₂: $p = 0.406$). These results showed that there was a highly significant difference for the Intensity main effect ($p < 0.0001$). This in accordance with Crouter et al. (2006), who showed that a commercial system (TrueOne 2400) was not significantly different from the Douglas bag at rest, 50, 100, 150, 200, and 250 W for V_E, VO₂, or VCO₂ ($p \geq 0.05$). The authors reported

that the reliability of the TrueOne 2400 have been shown to have good reliability. However, Storer et al. (1995) showed no difference in V_E , VO_2 , and VCO_2 . In this study, 5 males did 4 submaximal cycling steady states work rates at each of 40, 80, 120, and 160 W between the Vmax (SensorMedics) system and the Douglas bags.

The mean absolute values of V_E , VO_2 , and VCO_2 achieved from the 5 min each at rest, 100 watts, and 175 watts non-steady state exercise testing were similar for the new system and ParvoMedics. These results suggest that both the new system and ParvoMedics are valid systems for respiratory data for these three work rates. Thus, the findings from this study verify previous results that the new system is an accurate device for the measurement of V_E , VO_2 , and VCO_2 .

Secondly, for F_{EO_2} ($p < 0.0001$) and F_{ECO_2} ($p < 0.0001$) there were significant findings for the main effects for intensity. However, the Intensity x Method interaction was not significant for F_{EO_2} ($p = 0.689$) and F_{ECO_2} ($p = 0.208$) data as determined by two-way mixed design ANOVA analyses. The only discrepancy in the current study was consistently and significantly higher F_{EO_2} for the ParvoMedics compared to new system across all intensities. These findings also suggest that the new system tended to underestimate F_{EO_2} compared with ParvoMedics at rest and during 100 watts, and 175 watts cycling work rates.

Thirdly, for RER there were significant differences in the main effect for method ($p = 0.024$), Intensity ($p = 0.0006$), and Intensity x Method interaction ($p = 0.005$). This finding regarding RER is similar to the findings of Engebretson (1998) who demonstrated that there were significant differences for RER between a computerized breath-by-breath system and conventional bag collection system. In contrast, the study by Carter and Jeukendrup (2002) showed that RER from a commercial systems (Oxycon Pro and Oxycon Alpha) were similar compared to the

Douglas bag method. RER for the ParvoMedics was consistently and slightly higher than the new method across 100 W and 175 W, but significantly higher than the new method at rest. Consequently, there are serious concerns for the ParvoMedics systems to over estimate RER, and therefore reveal invalid data for computations of energy expenditure and macronutrient combustion.

% O₂ and CO₂ across 6 breaths of mimicked lung ventilation and gas exchange

We are the first to show that the model of the lung and conducting zone was modified by the placement of both inspired and expired turbines between the 6 L air breathing bag (lung model) and the 3-way valve (Figures 18 and 19). The major finding was a significant main effect and interactions ($p < 0.001$) for each of expired oxygen and carbon dioxide were similar. All mean differences between alveolar and mouth end tidal gas % values across 6 breaths were significant ($p < 0.01$). This study shows that the continual change in alveolar gas conditions caused by the inflow of calibration gas prevented accurate integration of each of the oxygen and carbon dioxide signals.

Calculated data for the dead space of the modeled lung system

Various commercial system limitations to expired gas analysis indirect calorimetry (EGAIC) have been advanced. Most commercial system limitations to EGAIC include factors dead air space including the typical size of the fixed volume mixing chamber, typically one to five liters. This dead air space performs to decrease the overall sensitivity and accuracy of the system.

In this study, data were very consistent, with mean \pm SD of 2.5 ± 0.13 L. The volume of ADS will be able to be determined from the extent of contamination of the

calibration gas fractions by room air in the ADS. The 6 L mixing bag system that was used to mimic lung function in this study functioned as a valid model of lung gas exchange and dead space anatomy. Future investigations are needed to study whether the extent of mixing between alveolar and anatomical dead space air can be used to estimate the volume of the ADS, and in turn correct for computations of expired gas analysis indirect calorimetry.

In conclusion, this is a unique study as it is the first to devise several approaches at validating components of the new system. This study shows that the mixing bag and the mouthpiece have unique features that are advantageous to the operation and validity of the system. Although the new system is not used in commercial systems of expired gas analysis indirect calorimetry (EGAIC), this system provides enhanced accuracy and validity.

CHAPTER VI

Summary, Conclusions, and Recommendations

Summary

The purpose of this study was to validate a new system of breath-by-breath expired gas analysis to both an artificial working model of lung ventilation and gas exchange to the Douglas bag technique. Additionally, comparisons were made between expired fractions, ventilation, and computations of $\dot{V}O_2$, $\dot{V}CO_2$ and RER between the new system and a commercial mixing chamber system (ParvoMedics) for repeated measurements for each of rest and steady state cycle ergometry exercise.

Prior to data collection, the gas analyzers and flow turbine were calibrated. Gas analyzer calibration was performed using custom developed software (LabVIEW) integrated to a computerized custom developed data acquisition system. Post acquisition processing involved in LabVIEW, where time to gas equilibration within the mixing bag was determined, as well as differences in equilibrated gas fractions. All testing procedures were repeated 5 times for parametric statistical analyses.

Percent gas results for the compliant 2 L mixing bag was the only method to yield data not significantly different between alveolar and measured. Alveolar % oxygen gas was significantly lower than mixing bag, ParvoMedics, and mixing chamber. The most responsive method was the mixing bag, with significantly lower % gas data for oxygen for breaths 2 to 5 compared to the ParvoMedics and mixing chamber. The ParvoMedics and mixing bag yielded similar results after breath 6, but data were significantly higher than for alveolar air. The slope data for breaths 0 to breaths 2 was significantly ($p < 0.05$) lower for the ParvoMedics system compared to

the mixing bag and mixing chamber.

The mean temporal distribution of 1 L ventilation maneuvers from the mixing bag turbine was 0.999 ± 0.142 L, with a range of 0.96 to 1.03 L. The mean ventilation (STPD) from the ParvoMedics was significantly lower ($p = 0.0027$) than the mixing bag turbine. V_E ($p = 0.097$), VO_2 ($p = 0.786$), and VCO_2 ($p = 0.178$) were not significantly different for the main effect for method and the Intensity x Method interaction (V_E : $p = 0.721$, VO_2 : $p = 0.059$, VCO_2 : $p = 0.406$). F_{EO_2} ($p < 0.0001$) and F_{ECO_2} ($p < 0.0001$) were significant for the main effects for intensity. However, the Intensity x Method interaction was not significant for F_{EO_2} and F_{ECO_2} . RER was not different in the main effect for method ($p = 0.024$), intensity ($p = 0.0006$), and the Intensity x Method interaction ($p = 0.005$). The expired oxygen and carbon dioxide were highly significant for main effects and interactions ($p < 0.001$). All mean differences between alveolar and mouth end tidal gas % values across 6 breaths were also significant ($p < 0.01$). The mean individual computed dead space volumes were 2.5 ± 0.13 L.

Therefore, the new 2 L mixing bag is capable of accurately reproducing specific gas fractions from reference calibration gas. The new 2 L mixing bag allows expired air to wash out through the bag. This system, even when the anatomical dead space (ADS) is not accounted for as a factor in the determinations, gives more accurate data than a traditional mixing chamber. The new mixing bag and the mouthpiece have unique aspects that are advantageous to the operation and validity of the system. Although the new system is not used in commercial systems of expired gas analysis indirect calorimetry (EGAIC), this system provides enhanced accuracy and validity.

Conclusions

Based on the analyses of the results, and within the limitations of the study, the following conclusions were drawn:

1. Percent gas results for the compliant 2 L mixing bag was the only method to yield data not significantly different between alveolar and measured.
2. Alveolar % oxygen gas was significantly lower than mixing bag, ParvoMedics, and mixing chamber. The most responsive method was the mixing bag, with significantly lower % gas data for oxygen for breaths 2 to 5 compared to the ParvoMedics and mixing chamber.
3. The slope data for breaths 0 to breaths 2 was a significantly lower response for the ParvoMedics system compared to the mixing bag and mixing chamber.
4. The mean temporal distribution of 1 L ventilation maneuvers from the mixing bag turbine was 0.999 ± 0.142 L, with a range of 0.96 to 1.03 L.
5. The mean ventilation (STPD) from the ParvoMedics was significantly lower than mixing bag turbine.
6. Computations for V_E , VO_2 , and VCO_2 were not significantly different between the ParvoMedics and the new 2 L mixing bag method for rest, 100 Watts and 175 Watts.
7. F_{EO_2} for the ParvoMedics was consistently and significantly higher than the new system across all intensities.
8. RER was higher at rest for the ParvoMedics, indicating that this method could be invalid for resting metabolic rate measurements without using ParvoMedics RMR software and hardware.

Recommendations

Future research should address the following points:

1. Further study involving several different commercial indirect calorimetry system comparisons with the new system, to increase the generalization of the findings.
2. Further study involving more subjects in several different age groups should be conducted during rest, steady state exercise, and non-steady state exercise between the new system and a commercial system, to increase the generalization of the findings.
3. Investigate whether limited conditions of ventilation and criterion gas conditions were used in the validation.
4. Further study involving the validation of new system measures assessed during different modes of exercise and under various environmental conditions.
5. Assessing differences between the methods during peak exercise intensities.

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