Validation of a novel oxygen consumption measurement technique in neonates

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A. Specific Aims/Objectives

To compare oxygen consumption (VO_2), carbon dioxide production (VCO_2), resting energy expenditure (REE), and respiratory quotient (RQ) using a novel and responsive technique to measurements made using a gold standard technique (i.e. Douglas bag) in neonates.

B. Background and Significance

The importance of measuring resting energy expenditure

Resting energy expenditure (REE) is defined as the number of calories the body expends daily during resting conditions. Currently, there are two ways of estimating REE in patients: (1) equations that estimate REE based on patient characteristics such as age, weight, and gender, and (2) indirect calorimetry (IC) which uses measured VO_2 and VCO_2 to calculate patient-specific REE. There are several equations available to mathematically estimate REE, however, these equations are substantially less accurate than IC. Prior studies have demonstrated that true REE is both over- and underestimated when relying on such equations due to variation in patient conditions, activity levels, and temperature (Rousing et al., 2016). In fact, recent studies comparing estimated REE to measured REE using IC in the pediatric ICU have found that a significant number of patients were being overfed when comparing caloric intake to measured energy needs (Dokken et al., 2013; Mehta et al., 2010). This may have detrimental effects on patient outcomes, including fluid overload, carbon dioxide overproduction, and feeding intolerance. Therefore, REE estimates based on IC are considered the gold standard for the clinical assessment of energy expenditure (Mehta et al., 2014). It has also been demonstrated that adequate nutrition in critically ill children improves outcomes and reduces mortality (Mehta et al., 2012), making the ability to measure VO_2 and VCO_2 vitally important to improving outcomes in the neonatal and pediatric ICU.

Additional benefits of measured oxygen consumption

In addition to measuring the caloric needs of critically ill children, oxygen consumption can be used in the direct Fick principle together with arterial and mixed venous oxygen contents for calculation of hemodynamic indices including cardiac index, systemic and pulmonary vascular resistance. In these calculations, accuracy is vital as an error in VO_2 will translate directly into an equivalent percentage error in cardiac output and systemic vascular resistance. At Boston Children's Hospital, VO2 is routinely estimated using the LaFarge equation (LaFarge, 1970) for use in the Fick equation, a practice which has been shown in multiple studies to overestimate VO2 relative to measured values (Li, 2013), particularly in neonates and infants in whom the magnitude of the error was up to 75%. Thus, direct measurement of VO_2 rather than its estimation is necessary in children with congenital heart disease undergoing cardiac catheterization, particularly in infants and children <3 years of age – who comprise the majority of congenital heart patients.

Measured oxygen consumption can also be used to identify derangements in oxygen delivery in the postoperative setting, as oxygen consumption becomes supply dependent at a critical threshold (Shoemaker, 1991, Shoemaker, 1983, Shoemaker, 1988) at which point oxygen delivery is dangerously low. Identifying these changes in VO_2 can alert the clinician to the presence of poor tissue oxygenation (Mills, 2016). Further, a decrease in measured VO2 in excess of 30% from baseline during pressure supported ventilation has been shown to be predictive of extubation failure in the intensive

care setting (Mehta et al., 2014). In order to apply this finding to infants and neonates, the ability to quantify oxygen consumption in the setting of tachypnea and low tidal volume respiration is vital.

Methods used to quantify oxygen consumption

Currently, all patients undergoing mechanical ventilation for any reason are monitored using a capnograph, a device which uses an adapter fitted onto the end of the endotracheal tube for the continuous monitoring of end-tidal carbon dioxide ($EtCO_2$), and other basic spirometric measures (e.g. tidal volume, VCO_2, lung compliance). There are several different capnograph devices in current use within BCH. However, none of these devices measure VO_2, which is a critical endpoint for the reasons described above. Our group has investigated two modules manufactured by GE (Datex Ohmeda, GE Healthcare, E-CAIOVX module) to monitor VO_2 in neonates and infants in both benchtop and clinical settings, finding it to be inaccurate and not clinically informative in the setting of infants and young children (Smallwood, 2012, Smallwood, 2016, Mills, 2016)

In principle, VO_2 measurements for indirect calorimetry in ventilated patients can be done in different ways, including breath-by-breath methods, mixing chamber/mixed expired methods and Douglas gas collection methods. However, measurements of oxygen consumption in newborns by any of these approaches is challenging for several reasons.

When applying VO_2 measurements on a **breath-by-breath** basis, respiratory flow and oxygen concentration are measured proximally during inspiration and expiration. The principle is to integrate the product of flow and concentration and calculate the difference between inspired and expired volumes of oxygen. Ultra-fast gas analyzer response time and perfect time alignment between flow and gas concentration signals are required to obtain accurate values, . The flowmeter typically responds instantaneously, whilst the side stream gas analyzer responds with a requisite delay due to the transport of gas from the sampling site to the gas detector through the gas sample line. Even small errors in time alignment can cause significant errors in the calculated VO_2. For the flowmeter to detect both inspired and expired flows it needs to be placed between the endotracheal tube and wye piece. This presents a problem in patients with a small breathing volume such as newborns as it affects anatomic dead space, a critical parameter. Also, flowmeters do not cope well with humidified ventilation systems because water tends to affect the detection mechanism. This causes calibration to change when measurements are conducted for long durations. The breath-by-breath method may be useful for short term measurements in patients over ~3 years of age, where an added dead space is acceptable and breathing frequency is relatively low. For smaller infants, particularly those with rapid respiratory rates, the method is impractical because of added dead space, gas analyzer response time, critical time alignment between flow and gas signals, and possible condensation of water vapor in the flowmeter..

Alternatives to the breath-by-breath method have been proposed such as using a mass spectrometer system and avoiding the use of flowmeters by a mixing chamber for the expired gas (Davies and Denison, 1979). Oxygen concentrations in the inspired gas and gas at the outlet of the mixing chamber are measured by the mass spectrometer. Minute ventilation is calculated by adding a small but constant flow of an inert gas, e.g. argon, to the inlet of the mixing chamber and measuring its concentration downstream at the outlet. This technique is the basis for the AMIS 2000 medical mass spectrometer, the only device which has proven reliable in newborns in several studies (Shekerdemian et al., 1996; Li et al., 2000; Li et al., 2003; Li et al., 2004; Li et al., 2006; Li et al., 2007; Li et

al., 2008; Li, 2013). Unfortunately, mass spectrometers are physically cumbersome and complex devices that are neither easy to use nor scalable in an ICU environment.

Most studies using indirect calorimetry have used the Deltatrac II Metabolic Monitor (Datex-Ohmeda, Helsinki, Finland, and later Sensormedics, CA, USA) which has become acknowledged as a gold standard reference tool and criterion method among indirect calorimetry devices based on several validation studies and years of use in clinical and research settings (McLellan et al., 2002; Singer et al., 2006; Cooper et al., 2009; Graf et al., 2013; Sundström et al., 2013; Ashcraft and Frankenfield, 2015; Black et al., 2015; Graf et al., 2015; Sundström Rehal et al., 2016). The monitor has been evaluated in several in vitro studies (Takala et al., 1989; Makita et al., 1990; Phang et al., 1990; Weissman et al., 1990; Shortland et al., 1992; Weyland et al., 1994; Tissot et al., 1995; Wells and Fuller, 1998; Behrends et al., 2001; Joosten et al., 2000; Melendez et al., 2001), and even though it has been regarded by many as a gold standard, results have been of varying quality. Moreover, few studies have tested the device in infants or at metabolic rates corresponding to infants (Shortland et al., 1992; Weyland et al., 1994; Behrends et al., 2000; Joosten et al., 2000).

The Deltatrac II is connected to the exhaust port of the ventilator and consists of an infrared CO_2 analyzer, a differential paramagnetic O_2 analyzer, and a constant flow generator. All expiratory gas is led through a mixing chamber and then drawn through a fixed-flow generator that dilutes the expired gas with room air to a total constant flow rate of typically 40 L/min for adults down to 3 L/min for babies. VCO_2 is calculated by multiplying the constant flow by the fraction of CO_2 in the diluted expiratory gas mixture at the output of the fixed-flow generator. VO_2 is derived from the inspiratory O_2 fraction and the respiratory quotient (RQ), which is calculated prior to dilution using the Haldane transformation. The results are expressed as an average and are regarded as a moving average of the previous 3–5 minutes depending on the patient's minute ventilation.

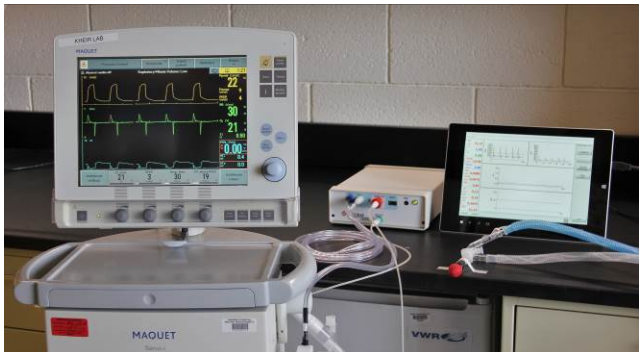
The production of both the AMIS2000 and Deltatrac have been discontinued, but several new devices have been introduced into the market that potentially could serve as a replacement. However, none of these commercially available devices are suitable for use in very small patients.

A novel technique for measuring oxygen consumption

Thia study represents a collaboration between the Boston Children's Hospital investigative team and InnoCC/Innovision in Denmark. Innovision is a company specialized in cardiopulmonary diagnostics based on advanced pulmonary gas exchange technologies. The company has been a leader in gold standard technologies for measurement of oxygen consumption, including the AMIS2000, the mass spectrometry based device described above. InnoCC is a wholly owned subsidiary to Innovision that has a license to exploit Innovision's technologies within the field of mechanically ventilated patients. The company has collaborated with the PI's Translational Research Laboratory at BCH for a couple of years. Peter Clemensen, who has a master of science degree in biomedical engineering, is employed by InnoCC/Innovision and has moved from Denmark to Boston to work as a visiting scientist at within the Translational Research Lab at BCH for the past year to advance the company's technologies for measuring oxygen consumption in neonates.

The current study aims to test a novel measurement apparatus for oxygen consumption (VO_2) measurements in newborns. While the equipment itself is novel, it connects to the ventilator circuitry in a noninvasive manner similar to that of FDA approved capnometry devices except that the sensor and gas sample line are **connected at the ventilator ports,** remote from the proximal Y-piece. This

important feature avoids the addition of dead space, and also the risks association with a weighted sensor attached to a small tracheal tube (risking displacement). The technique is based on sampling of inspired and mixed expired gas and measurement of inspiratory flow. Unlike other techniques, this technique should be suitable for use in mechanically ventilated pediatric patients where minute ventilation is low and respiratory rates are high.



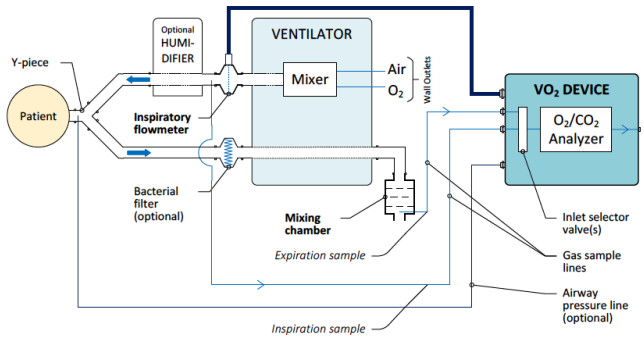


Figure 1. (Top) Image of the device, run using a standard tablet application, integrated with a standard Servo I ventilator. (Bottom) Schematic of the devices integration with Servo ventilation circuit, including a flowmeter, two gas sampling lines, and an optional airway pressure line.

The study device and a schematic of the Mixing Chamber setup are shown in **Figure 1**. The inspiratory **flowmeter** (differential pressure type pneumotach) is inserted at the inspiratory outlet of the ventilator prior to the humidifier so that it measures the flow of dry inspiratory gas. The flowmeter is connected to a differential pressure sensor in the VO_2 device using double-lumen rubber tubing. The VO_2 device also contains an **O_2 sensor** (laser diode sensor) with CO_2 module (NDIR infra-red sensor). Nafion gas sample tubes are connected between the de vice and the inspiratory limb for measurement of the inspired oxygen concentration and between the device and the exhaust port or mixing chamber outlet for measurement of mixed expired carbon dioxide and oxygen concentrations, respectively. A solenoid valve connected to both Nafion gas sample tubes inside the device is used to automatically alternate between inspiratory and expiratory gas measurements.

The design of the novel system is intended to overcome several small but compounding errors in oxygen measurements. First, ventilators are known to deliver fluctuating oxygen concentrations (F_IO_2) to different degrees (see **Figure 2**), which can have significant effects on metabolic measurements. By compensating measures of inspiratory flow of dry gas for FiO2-dependent changes in viscosity and flow-weighting F_IO_2 measurements (rather than simple time-weighted estimates, as other current instruments do), we believe it is possible to determine both inspired volume flow and average F_IO_2 accurately and thereby effectively make the novel system insensitive to fluctuations in F_IO_2 from the ventilator. Expired concentrations (F_EO_2 and F_ECO_2) are measured at the exhaust port of the ventilator in order to

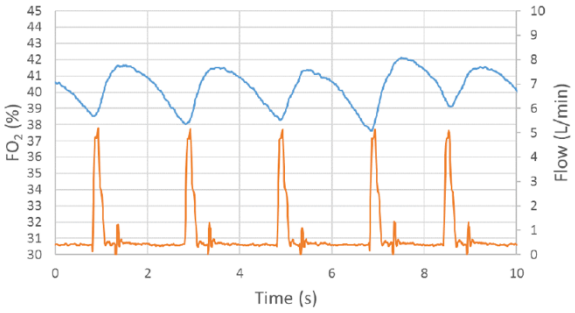


Figure 2. Measured fluctuations in FiO2 (blue line) during changes in measured inspiratory flow rate (orange line) using a standard Servo I ventilator and routine ventilatory flow rates. This finding renders the absolute precision of time alignment of flow and oxygen measurements mandatory.

obtain reliable average estimates. By combining inspired flow and F_IO_2 with time shifted F_EO_2 and F_ECO_2, it is possible to determine expired flow, VO_2, VCO_2, RQ and REE. The system also accounts for the presence bias flow (used to enable flow triggering of patient breaths in modern ventilators), and uses a lower sampling rate (60 mL/min) than any device on the market.

C. Preliminary Studies

As part of the preclinical validation of this device, we have recently completed both in vitro and in vivo interrogations of the device.

In vitro lung simulator experiments

To determine absolute measurement accuracy using our novel device, we created a lung simulator (**Figure 3**) consisting of artificial lungs in which VO_2 and VCO_2 are simulated with precision using a mass flow controller. Briefly, we created an in vitro system consisting of a silicone bellows reservoir including a minimum volume (i.e. FRC) and a tidal volume. To simulate metabolic gas exchange, a flow of a precision test gas with known fractional concentrations of CO_2 and O_2 (50% CO_2, balance O_2 with 0.03% absolute accuracy) is continuously injected at a constant rate (0-50 mL/min using a mass flow controller with an accuracy of ±0.2%). It should be noted that the lung simulator produces negative VO_2 (i.e. O_2 production). We designed this system to permit a standard ventilator to drive phasic respiration, to titrate F_IO_2, and to permit simultaneous humidication. This is more robust test of a respiratory module than we have previously described (Smallwood, 2016), which an active test lung and pistons to drive ventilation and a 21% CO_2/N_2 mixture for the infusion and displacement of oxygen.

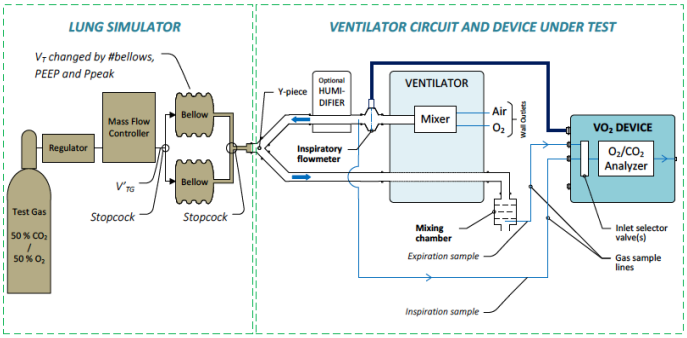


Figure 3. Lung simulator which permits the quantification of oxygen consumption and carbon dioxide production during phasic ventilation. Gold standard measures of each are provided by an inflow of precise volumes of oxygen and carbon dioxide production using a mass flow controller.

Using this setup, we have examined the performance of the novel device among a range of VO2, RR and FiO2 values (Table 1). Note that in all cases the error was <1 mL/min of VO2 (most were <0.5 mL/min), even when interrogated at RR as high as 80 breaths/minute, Tv as low as 10 mL, and FiO2 as high as 60%. These findings cover the clinically relevant range in infants and neonates, and provide significant optimism that this system will be able to accurately measure VO2. VCO2 is measured by

Ventilator settings			VO2		Error
FIO2	VT	RR	Set	Meas.	Abs.
%	mL	BPM	mL/min*	mL/min*	mL/min*
21	15	30	10	9.98	-0.02
21	10	30	5	4.90	-0.10
40	15	30	10	9.38	-0.62
40	10	30	5	4.72	-0.28
60	15	30	10	9.50	-0.50
60	10	30	5	5.10	0.10
40	10	20	5	4.62	-0.38
40	10	45	5	4.99	-0.01
40	10	60	5	4.73	-0.27
40	10	80	5	5.33	0.33

Table 1. Accuracy of novel device during lung simulator testing.

this system with even greater accuracy (since FiCO2 is always negligible), and we have elected not to show these data.

In vivo rat experiments

To simulate the performance of the device in extremely low birth weight infants, we compared measures of VO2 and VCO2 in Sprague Dawley (N=5, weight 662±61 g). Animals were intubated and the trachea sealed against the ETT using suture purse string. They were ventilated using a Servo I ventilator in SIMV pressure control with pressure support, PEEP of 5 cmH_2O, peak pressure 10 cmH_2O and mandatory rate of 30 bpm. F_IO_2 was maintained at 0.4. In each experiment, VO2 measurements were made under two conditions: 40% FiO2 provided by the ventilator as in clinical practice, and when provided using a certified gas mixture of 40% FiO2 with <±0.03% absolute accuracy. The latter condition was used to obviate the complicating factor of fluctuations in FiO2 as described above. In both conditions, device measurements were compared to those calculated using a Douglas bag method. Briefly, expired gases were collected within a 5 L non-diffusing gas collection bag connected to the exhaust port of the ventilator. The bag was flushed three times with expired gas and emptied. Then, measurements were performed with the device and gas collected simultaneously for comparison over ~6 minutes. The contents of the bag were subsequently analyzed, its volume measured with a calibrated syringe, and VO_2 and VCO_2 calculated using the Douglas technique. As shown in Table 2, differences between device estimates and reference measurements were below 0.4 mL/min in all cases, which would be clinically acceptable for even the smallest infants. During the completion of these experiments, we did not note any significant technical problems with the ventilator and its interaction with the ventilation of the animal.

Insp. source	**Mixing technique**		**Douglas bag**		**Difference**	
	VO2 mL/min	VCO2 mL/min	VO2 mL/min	VCO2 mL/min	VO2 mL/min	VCO2 mL/min
Avg.	7.84	6.46	8.17	6.24	-0.34	0.23
Vent.	8.00	6.71	8.35	6.51	-0.35	0.20
Tank	7.67	6.22	8.00	5.97	-0.33	0.25
N=5 (625-770 g)						

Table 2. Accuracy of novel device during in vivo testing.

Given these encouraging results, we would now like to test the novel device for accuracy and precision in newborn infants, comparing the results to those from the Douglas technique as described above.

D. Design and Methods

1. Study Design

This will be a single center, prospective, observational study comparing two methods of measuring VO_2, VCO_2 and REE in mechanically ventilated infants. We will choose patients who have the most significant clinical need and no current methods of measuring these endpoints, including preterm infants in the NICU and infants in the CICU. Briefly, consented patients will undergo measurements by two methods: (1) the novel method, in which inspired and expired oxygen will be measured using the

novel device, and VO_2, VCO_2, and REE outputted in real-time, and compared with (2) collected gas stored within gas-impermeable bags and analyzed post hoc for gas contents and volumes.

In this study, we have also elected to use a fixed FiO2 of 40% in order to permit us to, during a portion of the study, to utilize a certified gas mixture as a gas inlet for the ventilator, ensuring a precise input of FiO2. This step will remove an additional variable in our gold standard measurement (i.e. fluctuation of inspired gas fraction and the requisite perfect time alignment), improving our confidence in the reference standard even further.

(2) Study Population

Inclusion criteria.

1. Mechanically ventilated neonates and infants (<1 year of age).
2. Inpatients in the cardiac (8S) or neonatal (7N) intensive care unit.
3. Assent of patient's intensive care attending physician, including agreement to place patient on 40% oxygen for up to 60 minutes.
4. Written parental informed consent.

Exclusion criteria.

1. Hemodynamic or respiratory instability.
2. Patients on ECMO.
3. Clinically significant tracheo-esophageal fistula
4. Measured ETT leak >20% (i.e. difference between inspiratory and expiratory tidal volumes)

Recruitment Methods. The inpatient census of each ICU will be screened daily by a member of care study team. The attend physician of eligible patients will be approached to discuss study candidacy. The family members of appropriate patients (as per discussion with the ICU physician) will be approached for informed consent. We aim to recruit 30 patients.

(3) Description of Study Treatments or Exposures/Predictors

Essentially, this study will involve an ~1 hour, single point in time measurement of VO2, VCO2, REE and RQ using two methods to achieve a set FiO2 of 40%: standard FiO2 setting on the ventilator and a certified gas mixture. Responsive measurements will be compared with those from a Douglas bag. This is precisely the same experimental protocol we used for the rodent experiments above.

Following informed consent, completion of the study will be coordinated with members of the bedside nursing and respiratory care team. The device will be connected to the patient's ventilator circuit according to **Figure 4**, and will include the following.

(1) When the patient, bedside nurse and respiratory therapist are ready, the inspiratory limb will be temporarily disconnected for attachment of the pneumotach in-line. This takes 1-2 seconds and would be the same interruption in ventilation required to manually ventilate the patient, such that in most cases the patient will stay attached to the ventilator circuit. In our rodent experiments, we were able to complete this very efficiently with no detectable changes in patient condition.

(2) An inspiratory gas sampling connection will be placed into a luer locked side port in the inspiratory limb. This will draw a sample flow of 60 mL/min from the bias flow.

(3) Similarly, an expiratory gas sampling connection will be placed at the exhaust port of ventilator, distal to which a Douglas bag will be attached. In patients with a spontaneous respiratory rate <30, we will add an optional passive mixing chamber to ensure that the expired gas collected is completely mixed.

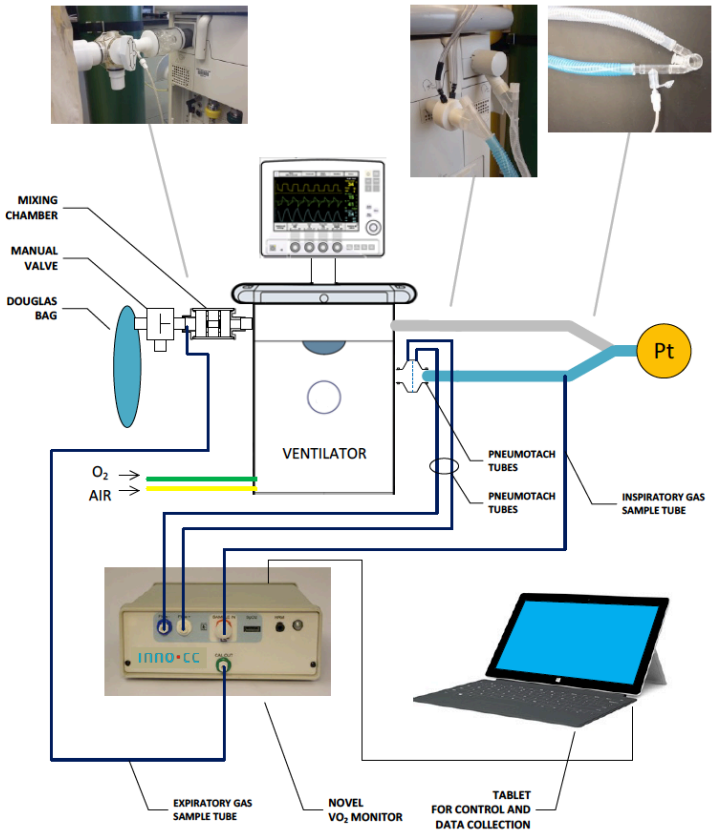


Figure 4. Schema of device attachments required as part of the study.

Following these connections, the patient will be allowed to stabilize on 40 % F_IO_2 administered in standard fashion using the ventilator for 10 minutes. During this time, we will make continuous and responsive measurements of VO2, VCO2, REE and RQ using the novel device. The same expired gases used create these measurements will be also be collected by the Douglas bag in timed fashion. As described above, the Douglas bag is a gas-impermeable, foil-lined, compliant bag routinely used for the collection of expired gas such as this. Following each timed collection, the bag will be disconnected, labeled, and stored for later analysis. Measurement will then be repeated twice in total.

Because of the fluctuations inherent in FiO2 measurements set by the ventilator (ref Figure 2 above), we will change the source of oxygen to a certified gas mixture (USP medical grade, oxygen concentration 40±0.03%) with the aid of the respiratory therapist. Measurements will then be completed twice using this inspired gas mixture, including the Douglas bag collections.

Following study end, all equipment will be carefully disconnected from the ventilator and removed from the bedside. Family and the care team notified of the end of study and of any adverse events. Data will be compared between replicates and the study team's best estimate (based on all available data) of VO2, VCO2, REE and RQ will be provided to the study team. A detailed timeline of these events is shown in Section 6 below.

(4) Definition of Primary and Secondary Outcomes/Endpoints

Primary endpoints

(i) VO_2, VCO_2, REE and RQ measured during steady state by novel device.
(ii) Same endpoints measured by Douglas technique.

Secondary endpoints

(iii) Estimated gestational age
(iv) Current body weight
(v) Tracheal tube size, presence of cuff, leak percentage (difference between inspired and expired tidal volumes)
(vi) Spirometry metrics
    a. Tidal volume
    b. Respiratory rate
    c. Baseline FiO2
    d. Minute ventilation
(vii) SBS score, total daily doses of opiates and benzodiazepines, current use of neuromuscular blocking agents
(viii) Temperature, heart rate, arterial blood pressure at study initiation and study termination
(ix) Adverse events
(x) Daily caloric intake on the day of study.

(5) Data Collection Methods, Assessments, Interventions and Schedule (what assessments performed, how often)

Outcome measurements
Measured VO_2, VCO_2, REE and RQ, as well as the endpoints listed above will be recorded at the bedside onto the data collection form.

Patient variables
Upon study enrollment, we will record the patient demographics, vital signs and treatments as described above.

Adverse events
The most important and significant potential adverse events will be related to the brief (i.e. 1-2 seconds) interruptions in the ventilator circuit. The risks will be lung derecruitment, a temporary decrease in oxyhemoglobin saturations, or potentially hemodynamic instability. We have mitigated this risk through our inclusion criteria, which will include patients who (in the opinion of the medical team) are able to tolerate an FiO2 of 0.4, which would exclude patients with severe lung disease. We will also exclude patients with minimal cardiopulmonary reserve in the opinion of the medical team.

Members of the study team will be present at the bedside for the duration of this study. We will monitor carefully for oxygen desaturation and for alterations in heart rhythm (e.g. bradycardia) or blood pressure from baseline.

Safety end points
Individual patient studies will be terminated for any of the following: (1) withdrawal of informed consent, (2) hypotension (defined as MABP<35 mmHg in term infants, and <30 mmHg in preterm infants), (3) oxygen desaturation (10% lower than baseline saturations at the beginning of the study). Any adverse events will be recorded on the data safety form and discussed with the data safety and monitoring board (DSMB) as per below.

Parameters for discontinuation of the research

We will meet with the DSMB following any adverse events (as above) and following the inclusion of the first 10 patients with our DSMB, which will include Ravi Thiagarajan, MD (Chief, Cardiovascular Intensive Care Unit) and James DiNardo, MD (Chief, Cardiac Anesthesia) to determine whether there is a need to modify the study protocol or to terminate the study early.

(6) Study Timeline

STEP	TIME (HH:MM)	TASK
1		Eligible patient screening (daily).
2		Approach treating team for assent.
3		Approach family for consent.
4		Arrange time with RN and RT.
5		Calibrate device and edit setup.
6	00:00	Attach device to inspiratory and expiratory limb of ventilator. Connect stopcock and collection bag to exhaust port.
7	00:02	Set F_IO_2 to 40%.
8	00:04	Flush Douglas bag 3 times and wait for steady-state.
9	00:10	Measure VO_2/VCO_2/REE using device and collect expired gas (Douglas method) for 10 minutes (TEST A1).
10	00:18	Disconnect Douglas bag and connect new bag.
11	00:18	Flush Douglas bag 3 times.
12	00:24	Measure VO_2/VCO_2/REE using device and collect expired gas (Douglas method) for 10 minutes (TEST A2).
13	00:32	Disconnect Douglas bag and connect new bag.
14	00:32	Change air inlet to certified 40% F_IO_2 tank.
15	00:33	Flush Douglas bag 3 times and wait for steady-state.
16	00:39	Measure VO_2/VCO_2/REE using device and collect expired gas (Douglas method) for 10 minutes (TEST B1).
17	00:47	Disconnect Douglas bag and connect new bag.
18	00:47	Flush Douglas bag 3 times.
19	00:55	Measure VO_2/VCO_2/REE using device and collect expired gas (Douglas method) for 10 minutes (TEST B2).
20	01:03	Change inlet to wall outlets. Set ventilator to pre-protocol F_IO_2 value.
21	01:04	Disconnect device. Discuss results with care team and adverse events with family.
22	01:05	End study.

E. Adverse Event Criteria and Reporting Procedures

We will meet with the DSMB following any adverse events (as above) and following the inclusion of the first 10 patients with our DSMB, which will include Ravi Thiagarajan, MD (Chief, Cardiovascular Intensive Care Unit) and James DiNardo, MD (Chief, Cardiac Anesthesia) to determine whether there is a need to modify the study protocol or to terminate the study early.

F. Data Management

Data management methods
Upon entry into the study, each patient will be assigned a unique patient identifier that is unique from their medical record number for the purpose of patient tracking. A spreadsheet which links each patient's UPI and BCH medical record number will be kept in a separate binder, locked in the PI's office. The UPI will be used to identify patients on all study-related materials and on the data collection forms.

Logistic considerations
At the time of study enrollment, baseline characteristics will be collected and recorded on a deidentified form. After informed consent is obtained by the family, the study device will be brought to the room by study personnel, and data recording will begin in real time and data will be collected and saved automatically by date and time. The data files will therefore not contain confidential information. Douglas bags will be labeled with date and time for later identification.

Using a study laptop, all requisite information will be entered into a Microsoft Excel spreadsheet. Data will be imported into the spreadsheet from data files saved automatically by the study device by a member of the research team, and annotated as necessary to clarify discussions with relevant parties. Data from Douglas bag analyses will be hand-entered or copied from raw data files by a member of the research team. These data will be double checked by a second member of the study team within 7 days.

G. Quality Control Method

Quality in the data collected by the oxygen consumption monitor will be ensured by following a calibration protocol prior to startup. Further, study personnel will be instructed to monitor for the degree of air leak from the respiratory system by looking at the difference between inspiratory and expiratory measured tidal volumes on the ventilator. If the air leak exceeds a value corresponding to the inspiratory gas sample flow, data will be excluded. VO_2 measurements are known to be unstable for several minutes following suctioning (Smallwood and Mehta, 2012), so we will exclude VO_2 data for 10 minutes following any ETT disconnect from analyses.

Quality in the transfer of data from the medical record to the spreadsheet will be ensured by an independent review of the primary data by a second member of the study team.

H. Data Analysis Plan

Each endpoint (i.e. VO2, VCO2, REE and RQ) will be compared between the gold standard (Douglas method using 40% FiO2) and the responsive device measurements will be compared using Bland Altman analysis. Bland-Altman plots will be used to graphically examine the magnitude of differences between the two methods in a pairwise fashion as a function of the measurement mean, and to see whether systematic variation exists. The plots will be examined to assess whether most measurements are within the calculated limits of agreement (± 2s, where s is the standard deviation of the observed Method 1 vs. Method 2 clinical measurement differences). For enhanced interpretability, the plots will also be constructed as function of the % difference between the two methods. For a quantitative assessment of agreement, the interclass correlation coefficient (ICC) will be estimated and its 95% confidence interval constructed. The ICC ranges from 0 to 1 and represents the proportion of the variation in the measurements that is due to differences between patients (as opposed to differences in measurement by the two methods being compared). If there is, as desired, little variation in measurement using the responsive device vs. Douglas measurement, then the ICC will be high. The anticipated ICC for this study is approximately 0.90. With a sample size of 30 patients, the width of the 95% confidence interval for the ICC will be 0.14 (that is, we can be 95% certain that the interval of width 0.14 contains the true ICC). If the ICC is larger than 0.90, then the width of the confidence interval for the planned sample size will be narrower.

I. Statistical Power and Sample Considerations

Based on the data achieved in rodents, the anticipated ICC for this study is approximately 0.90. With a sample size of 30 patients, the width of the 95% confidence interval for the ICC will be 0.14 (that is, we can be 95% certain that the interval of width 0.14 contains the true ICC). If the ICC is larger than 0.90, then the width of the confidence interval for the planned sample size will be narrower.

J. Risks and Discomforts

As described above, the most important and significant potential risks will be related to the brief (i.e. 1-2 seconds) interruptions in the ventilator circuit. These risks will be lung derecruitment, a temporary decrease in oxyhemoglobin saturations, or potentially hemodynamic instability. We have mitigated this risk through our inclusion criteria, which will include patients who (in the opinion of the medical team) are able to tolerate an FiO2 of 0.4, which would exclude patients with severe lung disease or a tenuous hemodynamic state. It is possible that the brief ventilator disconnect would cause the patient to cough or become more awake, though this should be well tolerated in study patients. We have minimized these risks by minimizing the number of variables (e.g. FiO2) we intend to study, and by selecting only hemodynamically stable mechanically ventilated patients. Members of the study team will be present at the bedside for the duration of this study and will monitor carefully for these risks and discomforts.

K. Potential benefits

Following termination of each patient's study, the study team will disclose the REE and RQ to the treating team based on the Douglas method. This information is not currently available by any means and may be helpful to titrate a patient's caloric intake to better meet their needs.

L. Study organization

The study team will be directed by John Kheir, MD who will oversee the screening and enrollment of patients, the performance of measurements, analysis of the data, and adjudication of any adverse events. Other members of the study team will be responsible for completion of these tasks under his direction.

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Paragraph for cover letter:

The study is a collaboration between Boston Children's Hospital and InnoCC/Innovision in Denmark. Innovision is a company specialized in cardiopulmonary diagnostics based on advanced pulmonary gas exchange technologies. The company has been a leader in gold standard technologies for measurement of oxygen consumption, including the AMIS2000, a mass spectrometry based device. InnoCC is a wholly owned subsidiary to Innovision that has a license to exploit Innovision's technologies within the field of mechanically ventilated patients. The company has collaborated with the Translational Research Laboratory at BCH for a couple of years. Peter Clemensen, who has a master of science degree in biomedical engineering, is employed by InnoCC/Innovision and has moved from Denmark to Boston to work as a visiting scientist at BCH for a year to advance the company's technologies for measuring oxygen consumption in neonates.