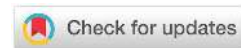


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Research Article

Validation of a wearable metabolic tracker (Breezing Pro™) for Resting Energy Expenditure (REE) measurement via Douglas bag method

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Abstract

Background and aims: Resting Energy Expenditure (REE) is one of the most important metabolic parameters since it accounts for 60%-70% of total energy expenditure (TEE) in a typical population and 80%-90% in a sedentary one. Accurate measurement of REE is essential for weight control, nutrition management, and disease treatment. Though metabolic carts, desktop metabolic analyzers, and portable metabolic trackers are available on the market to address certain needs of metabolic rate measurement, a stand-alone and truly wearable metabolic tracker that can provide comfortable and natural breathing experience (e.g. lacking of nose clips and mouthpieces) for nasal and pulmonary disease-restricted users is preferred. Here is featured a novel, wearable, stand-alone and fully integrated metabolic analyzer that overcomes the above-mentioned user experience barriers. The Breezing Pro™ determines REE based on the indirect calorimetry method. It measures both oxygen consumption (VO_2) and carbon dioxide production (VCO_2) rates using integrated colorimetric sensing technology. This work evaluates the accuracy of Breezing Pro™ device against the Douglas bag method, considered the gold standard of indirect calorimetry, and validates its functions as the world's first wearable and fully-integrated metabolic tracker, without the need for wearable accessories such as detector back packs, holders, and shoulder straps compared to other predecessors.

Methods: A total of 66 healthy subjects under resting conditions were simultaneously tested by Breezing Pro™ and the Douglas bag method. The exhalation rate (V_E), VO_2 , VCO_2 , and REE results from both methods were compared to evaluate the performance of the Breezing Pro™ device.

Results: The comparative correlation plots for V_E , VO_2 , VCO_2 , and REE indicate a strong correlation between the two methods for measuring the metabolic parameters previously described. R-squared correlation coefficients (R^2) between the data obtained were close to 0.9 for all parameters.

Conclusions: The accuracy of Breezing Pro™ for measuring V_E , VO_2 , VCO_2 , and REE has been validated using the gold standard Douglas Bag method. The results indicate that Breezing Pro™ is reliable for resting metabolic parameters measurements.

Introduction

Resting Energy Expenditure (REE), also called Resting Metabolic Rate (RMR), has been widely used for fitness,

nutrition and diseases study due to its high importance to body energy balance [1-6]. REE is the major component of the Total Energy Expenditure (TEE), determined by basal energy expenditure, diet induced thermogenesis and physical activity



[7]. REE is defined as the energy expenditure under resting conditions for the human body which allows the determination of the energy necessary for accurate nutrition assessment [8]. REE accounts for 60%–70% of Total Energy Expenditure (TEE) [1] and for 80%–90% in sedentary population [2,3], and it can be very different from person to person [7,9]. A recent study systematically compared calculated REE (using predictive equations) to measured REE (using an indirect calorimeter) and the difference shown to be as high as 900kcal/day [10]. Further, the study showed that overweight/obese populations had higher deviations from the predicted values in comparison with normal weight population which resulted from both the magnitude and the percentage of individuals with low measured REE values [8]. If an accurate REE is not known, a calorie intake and physical activity plan cannot be correctly made and can cause undesirable weight change. In fact, another study showed a weight gain of 40% of the participants whose caloric intake was determined with a predicative equation and demonstrated high adherence to caloric intake for ~50% of the study intervention days (6 months) [11]. Therefore, knowing REE value is important for effective weight control, nutrition management, and disease treatment. Additionally, a person's REE may vary over time due to different reasons [12–14].

Indirect calorimetry is a technique to determine energy expenditure by measuring the subject's oxygen consumption and carbon dioxide production rates and is a well-established approach for accurate assessment of REE [7]. A metabolic cart is the most widely used equipment for REE measurement, but it must be calibrated and manipulated by a professional. They are bulky equipment with specific maintenance and calibration protocols, including, but not limited to, flowmeter calibrations, replacement of internally embedded sensors and gas cylinder-based calibrations, culminating in a cumbersome and expensive procedure.

In order to facilitate a personalized REE measurement and provide a solution overcoming an unpleasant user experience, a truly integrated and comfortable metabolic tracker, with no external measuring units, has been developed. The truly wearable device, named Breezing Pro™ measures oxygen consumption (VO_2) and carbon dioxide production (VCO_2) rates in a duration of ten minutes via differential pressure-based flow sensor and integrated colorimetric chemical sensors, from which REE is determined using the well-known Weir equation [15].

In order to evaluate the accuracy and performance of this mobile indirect calorimeter, a comparative study was carried out using the Douglas bag method. A total of 66 both healthy subjects and subjects with pulmonary diseases, under resting conditions, were simultaneously tested with the Breezing Pro™ and the Douglas bag method. The $V_{E,T}$, VO_2 , VCO_2 , and REE results from both methods were compared to evaluate the performance of the Breezing Pro™ device. Statistical correlation analysis, such as linear regression, was used to establish a quantitative correlation between the data collected from the wearable indirect calorimeter and the Douglas bag method.

Materials and methods

Subjects

Two runs of studies were performed for the device validation. In the first run, 39 adults were tested, including 20 females and 19 males. 12 of the subjects were experiencing pulmonary diseases involving asthma or chronic obstructive pulmonary disease, among other related diagnoses. They were included with the aim of evaluating whether any discomfort resulting from the medical condition would impede the tests. In the second, 27 subjects were tested, comprising of 9 females and 18 males. Physical parameters of height, weight, ages and body mass indices (Body Mass Index, BMI, kg/m^2) for the patients are summarized in Tables 1,2, for the first run study and second run study, respectively.

All the subjects involved in the validation study participated voluntarily and signed informed consents.

The tests were carried out by Arizona State University (ASU) researchers at the Biodesign Institute or at a mobile setting located in a pulmonary associates' practice from December 2018 to May 2019. The study was approved by the Institutional Review Board of Arizona State University (IRB reference protocols # STUDY00006562).

The mobile indirect calorimeter Breezing Pro™ device

The wearable Breezing Pro™ device (Figure 1) is based on the same colorimetric sensing technology that portable Breezing® tracker device as was previously reported [16]. However, significant advantages improvements were introduced. The new Breezing Pro™ performs:

- A longer measurement (10 min) instead of 2 min, making the assessment more accurate.

Table 1: Summary of participating subjects (first run study).*

Gender	Subject #	Height (cm)	Weight (kg)	Age	BMI (Kg/m ²)
Women	20	163.2±6.7	68.7±17.8	31.9±14.1	25.6±5.3
		(152-174)	(44.5-105.3)	(26-74)	(18.2-34.9)
Men	19	174.0±9.7	82.7±24.7	45.7±20.5	27.2±6.9
		(155-198)	(48.8-120.7)	(22-92)	(15.1-41.8)
Total	39	168.3±9.8	75.6±22.6	43.4±17.6	26.4±6.2
		(152-198)	(44.5-120.7)	(22-74)	(15.1-41.8)

*Parameters including: mean ± standard deviation, and minimum and maximum values.

Table 2: Summary of participating subjects (second run study).*

Gender	Subject #	Height (cm)	Weight (kg)	Age	BMI (Kg/m ²)
Women	9	158.6±5.4	59.2±10.0	36.2±10.4	23.7±4.8
		(152-172)	(46.5-81.1)	(21-53)	(18.2-34.9)
Men	18	176.0±8.8	75.1±12.8	31.7±12.4	24.3±3.9
		(155-188)	(48.8-100.5)	(20-67)	(20.3-34.1)
Total	27	170.0±11.2	69.8±14.1	33.2±11.9	24.1±4.2
		(152-188)	(46.5-100.5)	(20-67)	(18.2-34.9)

*Parameters including: mean ± standard deviation and minimum and maximum values.



- A more comfortable measurement with a silicone mask instead of a nose clip and a mouthpiece; people that breath through their nose feel more comfortable with the new configuration. In fact, healthy and pulmonary disease patients could comfortably perform the test with positive feedback on the experience.
- A lower resistance to breathing, which is optimal for users with higher lung capacity.
- An app with cloud-based data management (see more detail below).

Similar to the previous Breezing® Tracker, the subjects inhale air from ambient and exhale into the device. The one-way valve systems (check-valve) on the mask separate the inhalation and exhalation processes. The data from the wearable device is also wirelessly transmitted to the customized app on the mobile device via Bluetooth. Headgear is provided to attach the indirect calorimeter device to the user making it a self-contained, easy to use, wearable metabolic analyzer.

The flow module of the Breezing Pro™ consists of a custom-made miniaturized Venturi tube and a differential pressure sensor. This design improves the accuracy of flow-rate measurement and minimizes backpressure to maintain a natural breathing condition for the subject.

The colorimetric sensor uses a one-time-use sensor chip for O₂ and CO₂ detection. Calibration factors for each individual sensor chip are encoded into a QR code, scanned by the app and transmitted to the Breezing Pro™ via Bluetooth.

The sensor cartridge is packed in sealed Mylar bags to prevent contact with moisture and ambient air thereby providing substantial shelf life. Immediately after opening a bag, the sensor cartridge is inserted into the Breezing Pro™ device for the REE measurement. Once the measurement is completed, the sensing probes on the sensor cartridge change color, after which the used sensor cartridge is discarded.

The mobile device application includes an account module, allowing a user to create, manage and edit user profiles for multiple patients, as well as data processing and management. The app also includes self-guided, pictorial user instructions enabling the user to perform the measurement with minimal training. The outputs from the measurement include exhalation volumes (V_E), REE, VO₂, VCO₂, respiratory quotient (RQ), breath frequency (BF), and tidal volume (TV).

Douglas bag for oxygen and carbon dioxide measure

The referenced Douglas bag method involved the measure of the O₂ and CO₂ concentrations in the subjects' breath. The O₂ and CO₂ concentrations were measured by a commercial electrochemical sensor (VTI Oxygen Analyzer, Vascular Technology, Nashua, NH 03062) and a commercial infrared sensor (Telaire 7001, GE, Goleta, CA) respectively, and both commercial sensor were modified with a Nafion drying tube and a pump (Parker) to bring the collected breath samples to the sensors.

The Breezing Pro™ was adapted in order to be connected in series with the Douglas bag. For this purpose, the front plastic grid in the front exterior portion of the device was removed, and the outlet of the flow channel was connected to a Douglas bag via a custom-made 3D printed adapter (Ultimaker). The 49 L bag was connected to the device during the second half portion of the 10-minute test period. Once the 10 min test was finished, the bag was disconnected from the Breezing Pro™ and a micro-pump was connected to deliver the sample to the O₂ and CO₂ sensors. After ~10 seconds of stabilization, the readings of the commercial O₂ and CO₂ sensors was recorded.

The metabolic parameters measured by Breezing Pro™ and Douglas bag method were analyzed, and the correlation was evaluated.

Douglas bag validation method for exhalation rate, V_E

In addition to validation for oxygen and carbon dioxide, the V_E parameter was also validated using a subset of the previously described subjects (N=13) and an independent set of tests. In this case, the V_E values were measured as the time to complete 49 L (maximum capacity of Douglas bag) exhaled breath at ambient conditions. Ambient conditions were measured and applied in the correction of breath volumes for reporting final V_E values under standard temperature, and pressure dry conditions (STPD). Data collected was analyzed and compared with V_E obtained from Breezing Pro™ device tests.

The calculations of VO₂ and VCO₂

The O₂ and CO₂ concentrations, together with V_E, were used to calculate the VO₂ and VCO₂ according to:

$$VO_2 = V_E \times (0.2093 - FO_{2,e}) \quad (1)$$

$$VCO_2 = V_E \times (FCO_{2,e} - FCO_{2,i}) \quad (2)$$

where 0.2093 is the fraction of inspired O₂, FO_{2,e} is the fraction of O₂ in the exhaled gas, FCO_{2,e} is the fraction of CO₂ in the exhaled gas; and FCO_{2,i} is the fraction of inspired CO₂ (0.0003-0.0004) respectively.

From VO₂ and VCO₂, REE was determined by the Weir equation [15],

$$REE \text{ (kcal/day)} = 1.44 \times [3.9 VO_2 + 1.1 \times VCO_2] \quad (3)$$

where REE represents the 24-hour energy expenditure under resting condition in kcal/day; the VO₂ and VCO₂ are in mL/min.

Resting Energy Expenditure Assessment Protocol

REE measurements were taken at resting state under specific conditions described below:

- No food or caffeine intake in the past 4 hours.
- No strenuous exercise performed for the past 12 hours.
- No moderate exercise performed 4 hours before the test.

All participants adhered to testing instructions and were rested for several minutes before the measurement. To assure resting conditions, heart rate was measured using a pulse oximeter.

Data and Statistical analysis

All data were reported as mean \pm SD, the parameters (V_E , VO_2 , VCO_2 and REE) compared by linear regression, and statistical analysis of the data performed using Excel. Furthermore, all the parameters collected were analyzed from paired t -tests to determine the statistical difference of the reading's two methods using Graphpad and Bland-Altman plots were also evaluated for all the tests.

Results

As seen from Figure 1, Breezing Pro™ offers a disposable mask attached to the metabolic analyzer (device body) and a headgear wrap to holds the entire self-contained wearable metabolic analyzer tight and stable to the user's head allowing breath collection.

Exhalation rate (V_E) validation

Before the clinical validation of VO_2 , VCO_2 and REE, the accuracy of the flow module of the Breezing Pro™ was evaluated to ensure the built-in flow calibration factors in the device remain accurate when measuring dynamic, real-time breath flow. As shown in Figure 2, the flow validation was studied for 13 subjects. The correlation slope obtained from the graph of Breezing Pro™ V_E vs Douglas bag method V_E was 1.015 and the R-squared correlation coefficients (R^2) was 0.8456 and a p value from the paired t -test was 0.4514. This result shows that the flow module of the Breezing Pro™ is accurate for real-time breath flow measurements.

VO_2 , VCO_2 and REE validation

Two different analyses were performed: First run study with 39 healthy subjects (Table 1); and a second run study with 27 subjects (Table 2).

First run study

The validation was conducted directly comparing the VO_2 and VCO_2 measures resulting from both oxygen and carbon dioxide concentrations measured by the Breezing Pro™ and Douglas bag methods.



Figure 1: Wearable Breezing Pro™ metabolic tracker.

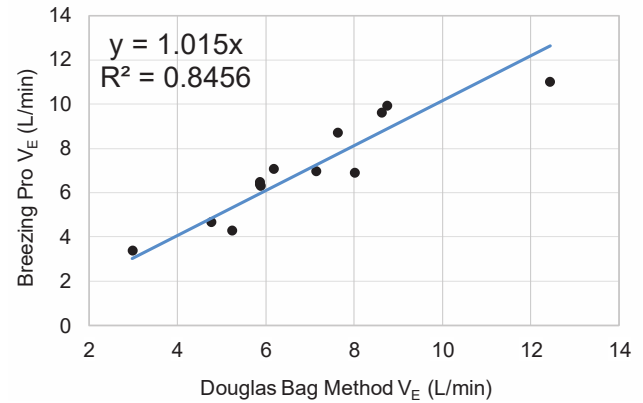


Figure 2: Flow independent validation (SPTD).

The first run study was performed on both healthy subjects and subjects with pulmonary diseases with the comparison for VO_2 , VCO_2 and REE measures shown in Figure 3. The sensor chips from the same production batch were used. These sensor chips share the same QR code and calibration factors. The graph represents the comparative correlations for both measurements carried out for a total of 44 tests (5 tests coming from multiple measurements of some of the 39 subjects). There is a strong correlation between the two methods, with correlation slopes close to 1.00 and R^2 to 0.9. See Table 3 for summarized parameters.

VO_2 measurements

Measured VO_2 were in the range of 174–382 mL/min. The linear fit comparison for the Breezing Pro™ oxygen consumption rates and the Douglas bag method values is shown in Figure 3A with values of 0.9779 and 0.8419 for the correlation slope and R^2 , respectively and a $p_{\text{paired } t\text{-test}}$ of 0.5764. The mean difference of -2.6 mL/min for VO_2 was calculated for both methods and indicates there is no significant difference. The individual VO_2 tests showed the difference is within \pm 26 mL/min.

VCO_2 measurements

In the case of measured VCO_2 , the range were between 141–380 mL/min. In Figure 3B. is shown the linear fit comparing the VCO_2 of the Breezing Pro™ and the Douglas bag method, with a slope of 1.0103, and a R^2 of 0.9107, and a $p_{\text{paired } t\text{-test}}$ of 0.3719. The mean difference of the measured VCO_2 between the two methods is +4 mL/min, indicating good agreement. The individual VCO_2 tests showed the difference is within \pm 19 mL/min.

REE measurements

The values for resting energy expenditure ranged from 1261 to 2846 kcal/day. In Figure 3C. is shown the linear fit comparing Breezing Pro™ REE values and the Douglas bag method with a slope of 0.9849 and R^2 of 0.874, and a $p_{\text{paired } t\text{-test}}$ of 0.7304. The mean difference of the measured REE between the two methods was -8.3 kcal/day, indicating no significant difference between them. The individual REE tests showed the difference is within \pm 165 kcal/day.

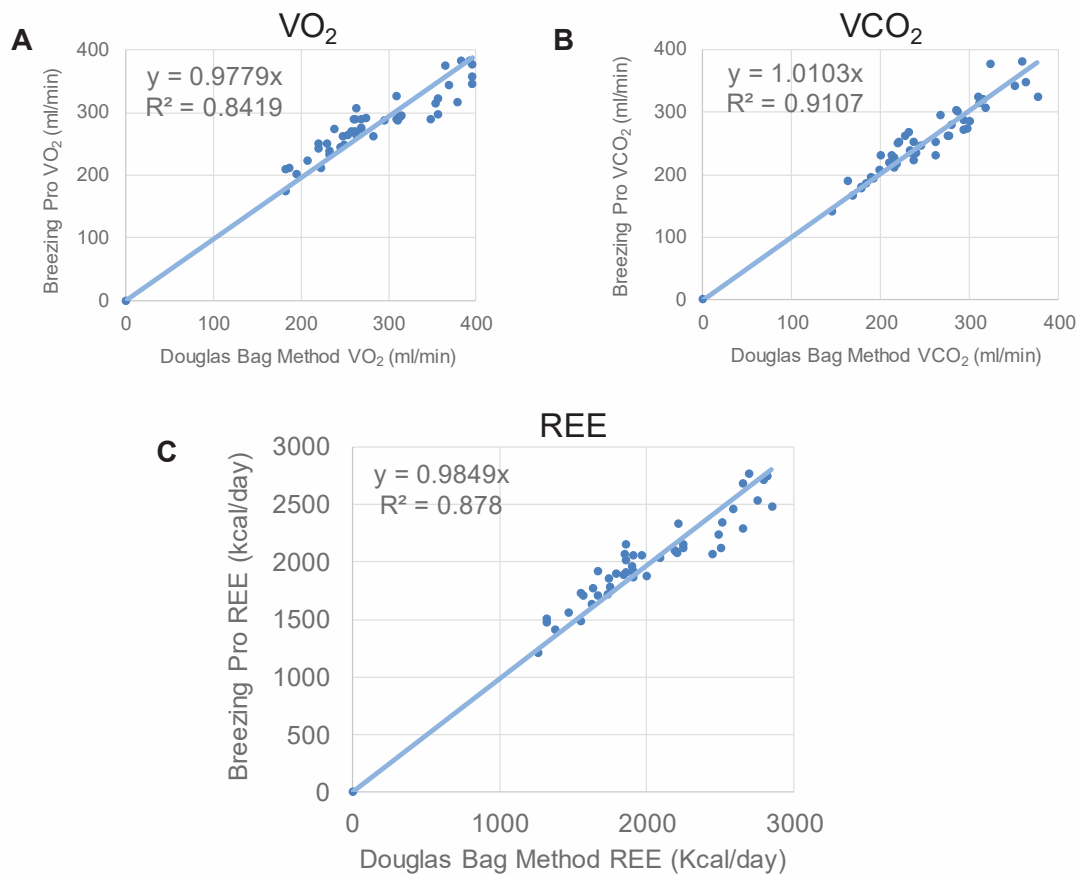


Figure 3: Validation Study: Breezing Pro™ vs. Douglas bag method (single sensor batch). N=39 subjects, 44 tests.

Second run study

The second study used four different batches of sensors and was performed in order to evidence the quality, reliability and repeatability of the data independently of the sensors batch. These sensor chips are from different production batches and have different QR codes and calibration factors. In this case, the study was performed on healthy subjects with the results shown in Figure 4. The graph represents the comparative correlations for both methods. As previously described, this study showed good correlation between them with a slope and R^2 close to 1.00 and 0.9, respectively. Parameter are summarized in Table 3.

VO₂ measurements

Measured VO₂ were in the range of 164–434 mL/min. The linear fit comparing the two methods had a slope of 1.0348 and a R^2 of 0.8989 (Figure 4A.) with a $p_{\text{paired } t\text{-test}}$ of 0.1017. The mean difference of the measured VO₂ between the Breezing Pro™ and Douglas bag method is -10 mL/min, indicating no significant difference. For each individual VO₂ test, the difference between two methods was within ± 31 mL/min.

VCO₂ measurements

The results for measured VCO₂ rates were in the range of 137–439 mL/min. Figure 4B. shows the linear fit comparing the two methods with a value of 0.9983 and 0.9252 for the slope and

Table 3: Summary and comparison between metabolic parameters determinate from Breezing Pro™ and Douglas Bag method.

	Single batch				Multiple batch			
	y	R ²	Mean Δ	SD	y	R ²	Mean Δ	SD
VO ₂	0.9779	0.8419	-2.6	±26	1.0348	0.8989	-10	±31
VCO ₂	1.0103	0.9107	4	±19	0.9983	0.9252	-0.2	±27
REE	0.9849	0.874	-8.3	±165	1.0282	0.9158	58	±205

- y = Correlation slope
 - R²= Squared correlation coefficient
 - Mean Δ±SD, mean difference of the measured between the two methods and standard deviation expressed for VO₂ or VCO₂ in mL/min, and kcal/day for REE.

R^2 , respectively and a $p_{\text{paired } t\text{-test}}$ of 0.9695. The mean difference of the measured VCO₂ between the Breezing Pro™ and Douglas bag method is -0.2 mL/min, indicating agreement between the two methods. For individual VCO₂ test, the difference between two methods is within ± 27 mL/min.

REE measurements

The values for measured REE ranged from 1143 to 3101 kcal/day. The linear fit comparing Breezing Pro™ REE values and the corresponding Douglas bag values had a slope of 1.0282 and an R^2 of 0.9158 (Figure 4C) and a $p_{\text{paired } t\text{-test}}$ of 0.1615. The mean difference of the measured REE between the Breezing Pro™ and Douglas bag method is 58 kcal/day, indicating no significant difference between the two methods.

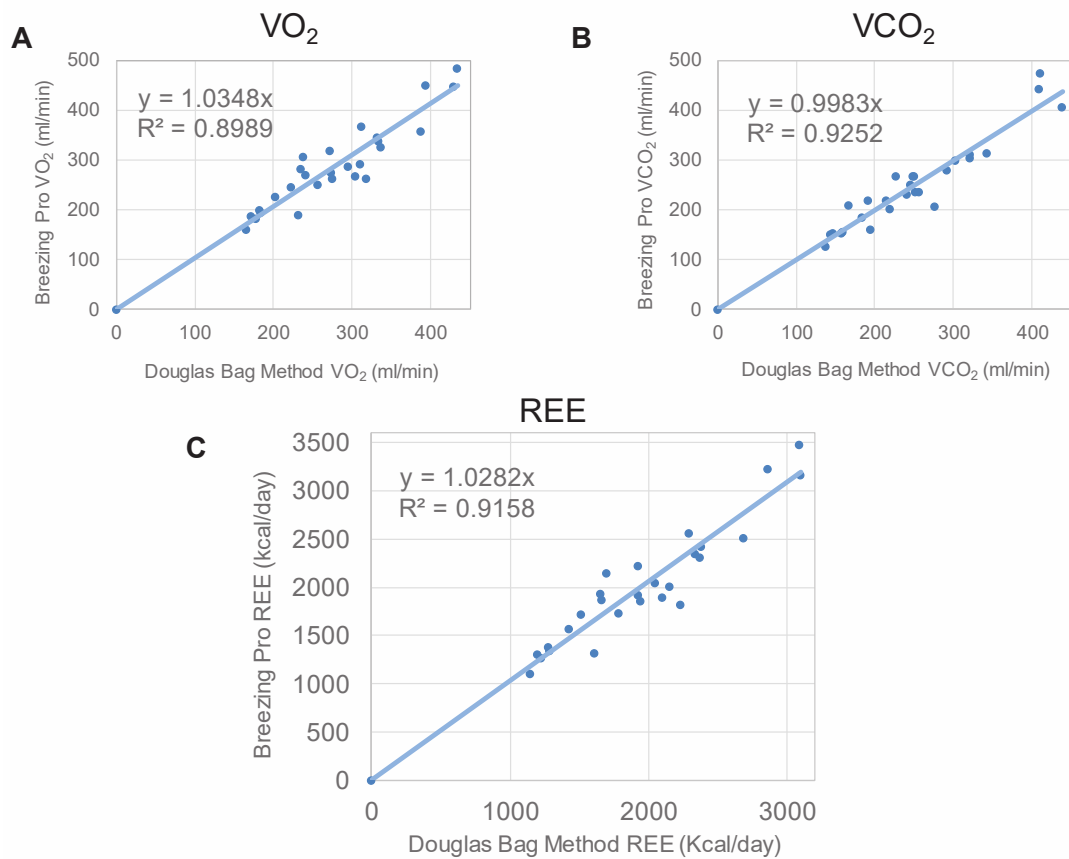


Figure 4: Validation Study: Breezing Pro™ vs. Douglas bag method (multiple sensor batch). N=27 subjects, 27 tests.

Bland-altman plots

As can be seen in Figure 5, the Bland-Altman plots were analyzed for diagnosis of VO_2 , VCO_2 and REE for all the tests including single and multiple batches. These graphs show agreement between Breezing Pro™ and the Douglas Bag Method, with relative errors within $\pm 15\%$ for the metabolic parameters for 95% of all the values.

In addition, over the range of 160-440 mL/min, the mean difference of VO_2 is 2.1 mL/min. For each individual test, the difference of the VO_2 between the two methods is within $\pm 9\%$. Measured VCO_2 presents a mean difference of 2.9 mL/min in the range of 140-440 mL/min, and each individual test gives a VCO_2 difference within $\pm 8\%$. Finally, the mean difference for REE is 16.1kcal/day in the range of 1100-3500 kcal/day. For individual REE test, the difference between two methods is within $\pm 8.3\%$. All these data were calculated over a total of 71 tests.

As for the entire 71 tests from 66 subjects, the overall means and standard deviations of REE are 2014 ± 466 Kcal/day for Breezing Pro™ and 1998 ± 493 Kcal/day for Douglas Bag Method. The difference of the mean REE between these two methods is only 16Kcal/day.

Pulmonary patients

A separated analysis was made over a subgroup of 12 subjects with pulmonary diseases. The aggravated cases which

means anormal breath frequency (BF), tidal volume (TV) and/or V_E were selected and are shown in Table 4. A normal human's BF, also called respiratory rate, for an adult at resting conditions is 12 to 20 breaths per minute, and is considered anormal when the value is under 12 or over 25 cycles per minute [17]. The reason of that anormal BF is due to various diseases, injuries, or use of narcotics or drug. On the other side, the normal range of TV for a resting and afebrile adult can be calculated as 7 to 9ml/kg. Also, normal V_E range are within 5 to 10L per min. Even considering these irregular values, the means and standard deviations of REE were 2248 ± 377 and 2291 ± 428 kcal/day for Breezing Pro™ and Douglas Bag, respectively. When comparing the two methods, the paired *t*-tests result was 0.7959 which means by conventional criteria, not to be

Table 4: Aggravated cases of pulmonary patients list.

Subject	BF ^a	TV ^b	TVn ^b	V_E^c	REE DB ^d	REE BP ^d	Diagnosis
1	16	726	520-670	11.6	2691	2769	CODP
2	10	498	677-871	4.7	1546	1488	Low oxygen saturation
3	10	693	737-948	7.4	1849	2067	smoker
4	10	1130	631-812	11.3	2816	2746	Cocci nodule
5	11	898	952-1224	9.4	2748	2534	Obstructive Sleep apnea

^a BF: Breath frequency expressed in breaths per min.

^b TV and TVn: expressed in ml.

^c V_E : units in L/min.

^d REE expressed in Kcal/day.

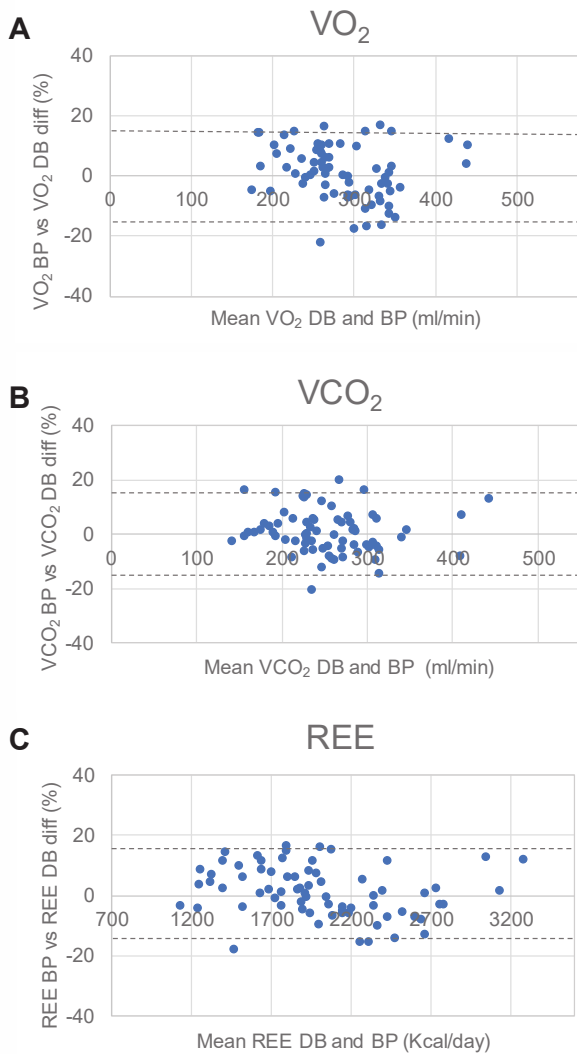


Figure 5: Bland-Altman plots corresponding to (A) VO_2 , (B) VCO_2 , and (C) REE for Breezing Pro™ (BP) and Douglas Bag (DB).

considered statistically significant.

Discussion

The wearable Breezing Pro™ metabolic tracker provides the user an easy way to conduct a breathing test where important parameters for weight management can be determined. The accuracy of those results is reflected in the measurements of VO_2 and VCO_2 and the validation using the gold standard method Douglas bag demonstrates the quality of the Breezing Pro™ measurements.

Breezing Pro™ metabolic tracker measures both the VO_2 and VCO_2 producing an actual RQ, instead of an assumed value of 0.85, to determine REE as do other metabolic devices on the market. This will improve the accuracy and help users to monitor their metabolism.

In addition, considering that typical practitioners use REE estimations, such as the Harris-Benedict equation, which potentially creates large errors [10,18], the Breezing Pro™ is

a more economical, yet accurate, alternative for personal and professional REE assessment.

According to previous studies, REE and energy expenditure levels can vary from person to person under different physical activity and diet conditions [19]. The improvements found in the Breezing Pro™ provides an accurate value for the final energy expenditure value for each individual study's participant in a user-friendly manner.

From the described results, it can be confirmed that V_E , VO_2 , VCO_2 and REE parameters measured by Breezing Pro™ were validated by Douglas bag method in a population of 66 subjects at resting conditions. All the comparative correlation plots for both studies indicated a strong correlation between the two methods, with correlation slopes close to 1.00 and R^2 to 0.9. In addition, paired t -tests performed results for both methods did not show any statistically significant difference ($p > 0.05$). In addition, data from Breezing Pro™ does not present difference when different batches of sensors are used.

As for a total number of 71 tests, the mean differences for all metabolic parameters (VO_2 , VCO_2 , and REE) have indicated that there is no noticeable difference between these two methods. The results from the validation test suggests the REE values measured by Breezing Pro™ are in perfect agreement with the values from the Douglas Bag Method.

Conclusion

This work demonstrates that a total of 66 subjects and 71 measurements were in excellent agreement with the gold standard method. The study validates the wearable Breezing Pro™ metabolic tracker as an accurate device for tracking metabolic parameters, which helps healthcare providers assess the metabolic health of their patients to develop personalized weight management programs with better clinical outcomes. In addition, since the Breezing Pro™ metabolic tracker is easy-to-use and calibration-free, it can eventually be used in home settings for personal fitness management. Finally, our systematic study has demonstrated that the Breezing Pro™ can be used for subjects with different personal profiles, such as different age, BMI, or health conditions.

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Authors' contributions

§ These authors contributed equally.

XX, SJM, DB, AQ, AG, and RR participated in the study design, data analysis, and manuscript preparation. SM, LB, and ML participated in the recruitment of subjects and collection of the data.

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