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ORIGINAL ARTICLE

Biological variation of resting measures of ventilation and gas exchange in a large healthy cohort

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Abstract

Purpose Resting measures of ventilation and gas exchange are impacted by a variety of physiological stressors, such as those resulting from a research intervention or an extreme environment. However, the biological variation of these parameters, an important statistical consideration for identifying a meaningful physiological change, has not been quantified.

Methods We performed a retrospective analysis of 21 studies completed by the U.S. Army Research Institute of Environmental Medicine (USARIEM) from 1985 to present, totaling 411 healthy volunteers. First, we determined the intraindividual, interindividual, and analytic coefficients of variation (CV_I, CV_G, and CV_A, respectively) and subsequently the index of individuality and heterogeneity (II and IH, respectively). Second, when deemed appropriate via these outcomes, we defined the accompanying static and dynamic thresholds, beyond which a significant deviation from normal is indicated.

Results End-tidal partial pressure of oxygen ($P_{ET}O_2$) and the respiratory exchange ratio (RER) approached the II threshold required to be considered useful in the static assessment of physiological deviations from normal. $P_{ET}O_2$ and peripheral oxygen saturation (SpO_2) approached the IH threshold required to be considered useful in the dynamic assessment of physiological deviations from normal.

Conclusions This analysis identifies RER and $P_{ET}O_2$ as parameters that might be most useful when aiming to identify a meaningful ventilatory change following a research intervention or stressor. Alternatively, other parameters of ventilation and gas exchange, such as $P_{ET}CO_2$ and V_E, may be less useful for observing an anticipated physiological change.

Keywords Coefficient of variation · Reference change value · Decision level · Index of individuality · Index of heterogeneity

Abbreviations

AMS	Acute mountain sickness
BMI	Body mass index
BSA	Body surface area
CV_A	Analytic coefficient of variation
CV_G	Interindividual coefficient of variation
CV_I	Intraindividual coefficient of variation
DL	Decision level
HR	Heart rate
IH	Index of heterogeneity
II	Index of individuality
$P_{ET}CO_2$	End-tidal partial pressure of carbon dioxide
$P_{ET}O_2$	End-tidal partial pressure of oxygen
RCV	Reference change value
RER	Respiratory exchange ratio
SpO_2	Peripheral oxygen saturation
VCO_2	Carbon dioxide production
V_E	Minute ventilation
VO_2	Oxygen consumption

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Introduction

Understanding the biological variation of various physiological parameters is a crucial first step in determining which of those parameters might be useful in identifying a meaningful physiological alteration. For example, during a

research intervention or under an environmental stressor, the physiological response of interest can be best observed by focusing on those variables that are most promising from a statistical perspective. Biological variation analysis also provides specific information about typical between-person and within-person variation, which can inform between- and within-group a priori power analyses. Studies of biological variation are used routinely to understand normal biochemical ranges in clinical medicine (Fraser and Harris 1989). The application of biological variation has been used to better understand the most useful biomarkers of dehydration, sweating, breast cancer, and diabetes mellitus (Cheuvront et al. 2010; Kenefick et al. 2012; Lenters-Westra et al. 2014; Soletormos et al. 1993), as well as in sports training (Bagger et al. 2003; Koutedakis 1995; Nunes et al. 2010).

While predictive equations and clinical thresholds have been established and refined for measures of ventilation and gas exchange (Quanjer et al. 2012), a complete analysis of biological variation has only been investigated minimally within a broad and basic context (Consolazio et al. 1963). Nevertheless, measurement of ventilation and gas exchange is remarkably affordable, easy to perform, and relatively portable, making this testing an advantageous data source to investigate physiological changes in a variety of scenarios, particularly in research settings. With the addition of biological variation data, it would be possible to identify the specific ventilatory parameters that should be collected and analyzed whenever ventilation and gas exchange are of interest.

In healthy humans, ventilatory homeostasis is accomplished via detection of blood gases, neural integration, and autonomic control of respiratory rate and depth. However, ventilatory homeostasis is not achieved identically in all humans or identically in all circumstances; thus, there is a normal biological variation in ventilation and gas exchange among healthy adults. This biological variation is likely dependent on numerous factors including basal metabolic rate, hydration status, body temperature, acid–base balance, and emotional influences, as well as various interactions with the cardiovascular system (van den Aardweg and Karemaker 1991). External stressors can also yield changes in measures of ventilation and gas exchange. For example, previous research has demonstrated changes in minute ventilation (V_E) and $P_{ET}CO_2$ in healthy individuals who develop acute mountain sickness (AMS) during high-altitude exposure (Hackett et al. 1982; Moore et al. 1986). Further, the combination of $P_{ET}CO_2$, heart rate (HR), and oxygen consumption (VO_2) has been explored for the estimation of pulmonary vascular resistance during noninvasive exercise studies of heart failure patients (Taylor et al. 2013). Nevertheless, no study has systematically investigated the biological variation of these and other ventilation and gas exchange parameters, and thus the relative usefulness of various measures in a research setting is uncertain.

The present study aimed to determine the biological variation inherent to resting measures of ventilation and gas exchange, such that the statistical usefulness of these measures for identifying a meaningful physiological change following a stressor could be established. We hypothesized that analysis of biological variation would reveal at least one statistically useful candidate. This approach for establishing the usefulness of a given physiological parameter has been used successfully in closely related fields (Cheuvront et al. 2010; Slate and Turnbull 2000). These results will guide the use of ventilation and gas exchange parameters, providing insight into which measures, if any, have potential for identifying meaningful responses to various research interventions.

Methods

Retrospective data collection

This study was a retrospective analysis of data obtained from the U.S. Army Research Institute of Environmental Medicine (USARIEM) Mountain Medicine Database which includes studies starting in 1985. All studies included in this investigation were approved by the Institutional Review Board at USARIEM and volunteers provided their free and informed written and verbal voluntary consent before participating in the study. Investigators adhered to the policies for protection of human subjects as prescribed in DOD Instruction 3216.02 and the research was conducted in adherence with the provisions of 32 CFR Part 219.

Resting measures of ventilation and gas exchange

All studies included in the present analysis utilized a similar protocol for collecting and analyzing resting measures of ventilation and gas exchange (Vmax Encore 29 and similar, Vyaire Medical, Mettawa, IL, USA). This system utilizes a mass flow sensor alongside an electrochemical fuel cell for analysis of O_2 and a non-dispersed infrared thermopile analyzer for CO_2. The majority of measurements occurred in the morning following an overnight fast, and all data were collected in a thermoneutral, sea level environment (~ 20 °C, ~ 760 mmHg). The volunteer was seated in a comfortable position in a quiet space and instructed to relax with the mouthpiece in place for 10 or more min until at least 5 min of stable data was obtained. Outliers arising from coughing, swallowing, etc., were removed and the remaining data points averaged. In determining certain aspects of biological variation, analysis of ventilation and gas exchange data required that two or more measurements were collected in the same individual over 2 or more days.

Breath-by-breath ventilation and gas exchange variables collected included V_E, oxygen consumption (VO_2), carbon dioxide production (VCO_2), end-tidal partial pressure of oxygen ($P_{ET}O_2$), and $P_{ET}CO_2$. Additional variables were also calculated from the average values of these measures, including ventilatory efficiency for oxygen and carbon dioxide (V_E/VO_2 and V_E/VCO_2, respectively) as well as the respiratory exchange ratio (RER; VCO_2/VO_2). Furthermore, several variables were also normalized to body surface area (BSA) ($V_E$norm; V_E/BSA) or body mass (VO_2norm and VCO_2norm; VO_2/mass and VCO_2/mass). During the measurement of ventilation and gas exchange, SpO_2 and heart rate (HR) were also collected via a pulse oximeter (Nonin Medical Inc., Plymouth, MN, USA).

Biological variation

All analyses were performed in MATLAB R2017b (The Mathworks, Inc., Natick, MA, USA). Prior to performing any analysis, outliers within each resting measure of ventilation or gas exchange, defined as a value that was outside two standard deviations of the grand mean, were removed from the dataset. Then, the biological variation of each measure was investigated by taking into account the intraindividual (CV_I), inter-individual or group (CV_G), and analytic (CV_A) components of variation (Fraser and Harris 1989). First, the total variation within an individual ($CV_{T,within}$), which is a combination of CV_I and CV_A, was determined by analyzing all repeat trials available for each individual (in only those individuals who had at least two trials; $n=245$):

$$CV_{T,within} = \left(\frac{SD_{within}}{Mean_{within}}\right) \times 100. \quad (1)$$

The $CV_{T,within}$ for each volunteer was then averaged among all volunteers to determine the group CV_T:

$$CV_T = Mean(CV_{T,within}). \quad (2)$$

Next, CV_A, a single value for each ventilation or gas exchange measure, was assumed to account for half of the total intraindividual variation (CV_T) (Fraser 1983), such that CV_A, and by extension CV_I, could be calculated for each parameter:

$$CV_A = \frac{CV_T}{2}, \quad (3)$$

$$CV_I = \sqrt{CV_T^2 - CV_A^2}. \quad (4)$$

The total group variation among volunteers ($CV_{T,among}$), which is a combination of CV_G and CV_A, was then calculated for each measure (including all individuals, whether or not they had repeat trials; $n=411$):

$$CV_{T,among} = \left(\frac{SD_{among}}{Mean_{among}}\right) \times 100. \quad (5)$$

CV_G was then determined by accounting for the impact of CV_A:

$$CV_G = \sqrt{CV_{T,among}^2 - CV_A^2}. \quad (6)$$

Diagnostic value of ventilation and gas exchange measures

By assessing CV_A, CV_I, and CV_G in concert, the potential usefulness of a given ventilation or gas exchange measure in determining deviations from 'normal', both as a static value that can be compared to population-based reference intervals and as a dynamic change from baseline within an individual, can be investigated. First, the index of individuality (II), a measure of the parameter's ability to identify a static value as abnormal, can be calculated as follows (Harris 1974):

$$II = \frac{\sqrt{CV_A^2 + CV_I^2}}{CV_G}. \quad (7)$$

A low II (< 0.6) signifies high individuality, meaning that the measure is quite variable among subjects and thus less useful in identifying a deviation from population-based reference intervals within a given individual (i.e., abnormal). On the other hand, a high II (> 1.4) indicates a low individuality, such that the measure is relatively uniform among individuals and thus more powerful for identifying a deviation from normal within a given individual (Harris 1974).

The dynamic change from baseline (within an individual) that signifies a statistically significant difference, termed the reference change value (RCV), can also be calculated (Fraser et al. 1990):

$$RCV\,(\%) = \sqrt{2} \times 1.65 \times \sqrt{CV_A^2 + CV_I^2}, \quad (8)$$

$$RCV\,(\text{Units}) = \frac{RCV(\%) \times Mean_{among}}{100}. \quad (9)$$

In the RCV equation, 1.65 is the unidirectional z score for a one-sided test with a 95% probability level, where a significant finding suggests that the dynamic change in the resting measure of ventilation or gas exchange is abnormally increased or decreased (Fraser et al. 1990). However, the RCV is only valid if the CV_I is not heterogeneous within volunteers. Therefore, the index of heterogeneity (IH) is also calculated for each measure (Fraser and Harris 1989; Harris 1970):

$$IH = \frac{CV \text{ of } \sqrt{SD_A^2 + SD_I^2}}{\sqrt{\frac{2}{n-1}}}. \quad (10)$$

where SD_A and SD_I are the average SD of the repeat trials used to calculated CV_A and CV_I, respectively. However, the numerator of IH can be approximated to CV_T:

$$IH = \frac{CV_T}{\sqrt{\frac{2}{n-1}}}, \quad (11)$$

where n is the average number of repeat trials among volunteers, or 2.6 in the present study. In order for the RCV to be valid and thus useful in identifying a significant dynamic change in a ventilation or gas exchange measure, the IH must be below a certain threshold (less than 1.88 in the present study) (Fraser and Harris 1989; Harris 1970):

$$IH < \left(1 + 2 \times \frac{1}{\sqrt{2n}}\right). \quad (12)$$

Finally, a decision level, or threshold beyond which a static measure is considered abnormal (independent of a baseline value), can be determined. Importantly, whether the decision level is useful is dependent on II. Specifically, the parameter must demonstrate sufficiently low individuality and thus a sufficiently high II value, as discussed previously. For resting measures of ventilation and gas exchange that met or approached this II threshold criteria, the decision level (DL) for that parameter was calculated by adding the RCV to, or subtracting the RCV from, the grand mean, yielding a decision level for either an abnormal increase or decrease in the variable, respectively. For example, if an individual is hyperventilating, RER and $P_{ET}O_2$ would increase. However, the directionality of the change may differ based on the specific intervention or stressor (see "Direction of change in variable following a stressor"). Importantly, if a change in the variable is anticipated in both directions (or the directionality is unknown), a bidirectional z score of 1.96 must be used in Eq. 8 (instead of 1.65). Finally, a SD for the DL was also determined:

$$SD_{DL} = \sqrt{2} \times SD_I. \quad (13)$$

Results

Resting measures of ventilation and gas exchange

Twenty-one of thirty available studies fulfilled the inclusion criteria, resulting in 411 young healthy volunteers (123 females; mean ± SD; age: 24 ± 5 years, height: 174 ± 9 cm, weight: 74 ± 12 kg, BMI: 1.89 ± 0.19 kg m^{-2}, BSA: 24.5 ± 2.9 m^2). The grand mean and range of values among subjects for each resting measure of ventilation and gas exchange included in the present analysis are shown in Table 1; all volunteers were included in these averages, regardless of whether or not the individual had repeat trials (n = 411). Outliers, defined as a value outside two standard deviations of the grand mean, were removed. In general, all parameters were within the anticipated range for young healthy adults in the resting condition.

Biological variation

The three components of biological variation, including CV_A, CV_I, and CV_G, are shown in Table 2. For all components, a smaller value signifies that the parameter is less variable in that component. For each resting measure of ventilation and gas exchange, the CV_I is less than the CV_G, demonstrating that the variation within an individual is smaller than the variation among individuals—this is typical of most physiological parameters (Consolazio et al. 1963; Fraser 2001; Fraser and Harris 1989).

Diagnostic value of ventilation and gas exchange measures

By assessing the components of biological variation in concert, the individuality and heterogeneity of each parameter,

Table 1 Estimates for all potential resting measures of ventilation and gas exchange

	Measured (n = 411[a])	Range
V_E (l/min)	9.7 ± 1.8	5.5–14.2
V_E norm (l/min/m^2)	5.1 ± 0.9	3.1–7.3
VO_2 (ml/min)	286 ± 67	147–452
VCO_2 (ml/min)	242 ± 60	111–398
VO_2norm (ml/min/kg)	3.86 ± 0.73	2.25–5.78
VCO_2norm (ml/min/kg)	3.26 ± 0.70	1.78–5.05
$P_{ET}O_2$ (mmHg)	105.2 ± 4.1	95.3–115.9
$P_{ET}CO_2$ (mmHg)	38.7 ± 2.7	32.1–44.7
V_E/VO_2	33.6 ± 5.9	19.3–49.4
V_E/VCO_2	39.5 ± 6.6	23.2–57.7
RER	0.85 ± 0.07	0.68–1.03
HR (bpm)	65 ± 9	45–87
SpO_2 (%)	98 ± 1	96–100

V_E minute ventilation, $V_E norm$ minute ventilation normalized to body surface area, VO_2 oxygen consumption, VCO_2 carbon dioxide production, $VO_2 norm$ oxygen consumption normalized to body mass, $VCO_2 norm$ carbon dioxide production normalized to body mass, $P_{ET}O_2$ end-tidal partial pressure of oxygen, $P_{ET}CO_2$ end-tidal partial pressure of carbon dioxide, V_E/VO_2 ventilatory efficiency for oxygen (unitless), V_E/VCO_2 ventilatory efficiency for carbon dioxide (unitless), RER respiratory exchange ratio (VCO_2/VO_2, unitless), HR heart rate, SpO_2 peripheral blood oxygen saturation

Measured values are expressed as mean ± SD

[a]Prior to outlier removal, defined uniquely for each measure as ± 2 × SD, thus resulting in variable sample sizes among parameters

Table 2 Components of analytic variation (CV_A) and biological variation

Quantity	CV_A	CV_I ($n=245$[a])	CV_G ($n=411$[a])
V_E (l/min)	3.7	6.4	17.9
$V_E$norm (l/min/m^2)	3.8	6.6	16.5
VO_2 (ml/min)	3.4	5.8	23.2
VCO_2 (ml/min)	4.4	7.7	24.5
VO_2norm (ml/min/kg)	3.4	5.8	18.7
VCO_2norm (ml/min/kg)	4.3	7.5	20.9
$P_{ET}O_2$ (mmHg)	1.1	1.9	3.8
$P_{ET}CO_2$ (mmHg)	1.4	2.4	6.7
V_E/VO_2	3.4	5.8	17.2
V_E/VCO_2	2.7	4.8	16.6
RER	2.3	3.9	7.5
HR (bpm)	2.8	4.9	13.5
SpO_2 (%)	0.2	0.4	1.0

All values are percentages

CV_I intraindividual variation, *CV_G* interindividual variation, *V_E* minute ventilation, *$V_E$norm* minute ventilation normalized to body surface area, *VO_2* oxygen consumption, *VCO_2* carbon dioxide production, *VO_2norm* oxygen consumption normalized to body mass, *VCO_2norm* carbon dioxide production normalized to body mass, *$P_{ET}O_2$* end-tidal partial pressure of oxygen, *$P_{ET}CO_2$* end-tidal partial pressure of carbon dioxide, *V_E/VO_2* ventilatory efficiency for oxygen (unitless), *V_E/VCO_2* ventilatory efficiency for carbon dioxide (unitless), *RER* respiratory exchange ratio (VCO_2/VO_2, unitless), *HR* heart rate, *SpO_2* peripheral blood oxygen saturation

[a]Prior to outlier removal, defined uniquely for each measure as $\pm 2 \times$SD, thus resulting in variable sample sizes among parameters

and by extension the usefulness of each parameter in identifying both static and dynamic deviations from normal, was determined (Table 3). Two parameters met or approached an II above 0.6, including $P_{ET}O_2$ and RER. However, no parameter demonstrated low individuality (II > 1.40), or a strong ability to identify a static value as different from a population-based reference interval. However, for parameters that neared the threshold for II, the decision level for static assessment of a deviation from normal is included in Table 3. Similarly, two parameters met or approached an IH below 1.88, including $P_{ET}O_2$ and SpO_2. Therefore, these variables may be useful in identifying a deviation from baseline within an individual, i.e., the dynamic change.

Sex differences

To investigate potential sex differences, all analyses were repeated separately for males and females and compared to the present results. While there were some numerical differences, the variables identified as statistically promising with males and females combined remained so when males and females were studied separately. Therefore, the results for males and females have been combined in the present analysis.

Discussion

Key findings

Differential responses in resting measures of ventilation and gas exchange have previously been reported in individuals who are exposed to a variety of physiological stressors (Hackett et al. 1982; Moore et al. 1986; Taylor et al. 2013). This is the first study to determine the biological variation of these measures in a large cohort of young, healthy subjects, thus providing insight into which parameters demonstrate the most statistical usefulness for potential identification of a meaningful physiologic change. The major findings were as follows: (1) $P_{ET}O_2$ and RER met or approached the statistical threshold required to be considered useful in the static assessment of physiological changes; and (2) $P_{ET}O_2$ and SpO_2 met or approached the statistical threshold required to be considered useful in the dynamic assessment of physiological changes. These findings suggest that other ventilation and gas exchange parameters, such as V_E or $P_{ET}CO_2$, may be less sensitive to relatively small physiological perturbations given the substantial intraindividual variation in day-to-day values and the relatively large interindividual differences among healthy individuals. Nevertheless, the data presented here suggest novel potential for $P_{ET}O_2$ and RER, two parameters which are generally less utilized and should be investigated in future studies.

This is the first study to carefully and completely quantify the biological variation inherent to numerous resting measures of ventilation and gas exchange in young, healthy adults, thus providing insight as to whether a given parameter shows promise for detecting abnormal static and/or dynamic change values following a stressor. Similar quantification of biological variation and the accompanying diagnostic potential of a given parameter have been utilized in clinical practice, including hemoglobin A1c for the diagnosis of diabetes mellitus, blood markers used to monitor breast cancer, and dehydration assessment (Cheuvront et al. 2010; Fraser and Harris 1989; Lenters-Westra et al. 2014; Slate and Turnbull 2000; Soletormos et al. 1993), as well as in professional athletics (Lobigs et al. 2016) and sports training (Bagger et al. 2003; Koutedakis 1995; Nunes et al. 2010). The measurement of ventilation and gas exchange can be a semi-portable, affordable, and easy-to-perform method for use in various research capacities; thus, the present analysis aims to support future studies that hope to utilize these easily obtained parameters for understanding a physiological change.

Table 3 Indexes derived from analytic and biological variation data

Quantity	II	IH	RCV (%)	RCV (units)	Decision level (95%)	
					Increase expected (units)	Decrease expected (units)
V_E (l/min)	0.42	6.58	17.4	1.7		
$V_E$norm (l/min/m^2)	0.46	6.70	17.7	0.9		
VO_2 (ml/min)	0.29	5.97	15.8	45		
VCO_2 (ml/min)	0.36	7.84	20.7	50		
VO_2norm (ml/min/kg)	0.36	5.95	15.7	0.61		
VCO_2norm (ml/min/kg)	0.41	7.63	20.1	0.66		
$P_{ET}O_2$ (mmHg)	0.57[a]	1.91[b]	5.0	5.3[c]	110.5 ± 3.2[d]	99.9 ± 3.2[d]
$P_{ET}CO_2$ (mmHg)	0.42	2.48	6.5	2.5		
V_E/VO_2	0.39	5.96	15.7	5.3		
V_E/VCO_2	0.33	4.85	12.8	5.1		
RER	0.60[a]	3.98	10.5	0.09	0.94 ± 0.05[d]	0.76 ± 0.05[d]
HR (bpm)	0.42	5.02	13.2	9		
SpO_2 (%)	0.48	0.41[b]	1.1	1[c]		

II index of individuality, *IH* index of heterogeneity, *RCV* reference change value, V_E minute ventilation, $V_E norm$ minute ventilation normalized to body surface area, VO_2 oxygen consumption, VCO_2 carbon dioxide production, $VO_2 norm$ oxygen consumption normalized to body mass, $VCO_2 norm$ carbon dioxide production normalized to body mass, $P_{ET}O_2$ end-tidal partial pressure of oxygen, $P_{ET}CO_2$ end-tidal partial pressure of carbon dioxide, V_E/VO_2 ventilatory efficiency for oxygen (unitless), V_E/VCO_2 ventilatory efficiency for carbon dioxide (unitless), *RER* respiratory exchange ratio (VCO_2/VO_2, unitless), *HR* heart rate, SpO_2 peripheral blood oxygen saturation

[a]Met or approached an II between 0.6 and 1.4

[b]Met or approached an IH less than 1.88

[c]Potentially useful for dynamic assessment

[d]Potentially useful for static assessment

Our analysis of biological variation identified several potentially useful ventilation or gas exchange variables. RER was identified as being useful for the static assessment of a physiological change. Therefore, an RER threshold can be defined, above or below which (depending on the stressor) is considered 'abnormal' in the resting state. RER is the ratio of VCO_2 to VO_2 and is considered indicative of metabolic substrate utilization. Thus, it is perhaps not surprising that a variable that encompasses energy utilization could be helpful in identifying deviations from normal; nevertheless, RER is rarely utilized to diagnose or predict physiological changes. This potential of RER is a novel and interesting finding, and future studies should incorporate measures of RER to further explore its diagnostic and predictive potential.

$P_{ET}O_2$ was also identified as potentially useful in both the static and dynamic assessments of a physiological change. This finding was particularly surprising given that this variable's 'counterpart', $P_{ET}CO_2$, is generally utilized more extensively from a clinical perspective. Furthermore, it should be noted that $P_{ET}O_2$ was the only variable that demonstrated potential statistical usefulness for both absolute and change values, suggesting that both its set point as well as its dynamic response to a stressor may be indicative of a meaningful physiological perturbation. To our knowledge, $P_{ET}O_2$ is not recognized as a meaningful respiratory parameter, and therefore should also be investigated in future studies.

Direction of change in variable following a stressor

Defining a decision level, beyond which a static measurement can be considered 'abnormal', requires knowledge of whether the variable will increase or decrease in response to the physiological stressor of interest; thus, Table 3 provides a decision level for both scenarios. However, a research intervention or environmental stressor is likely expected to alter a given parameter in a specific direction. Therefore, it is important to keep in mind during analysis of biological variation that the RCV must be added or subtracted to the grand mean, depending on the direction of change, to best represent the dataset. If an increase or decrease in the value are both possible (or the directionality is unknown), a bidirectional z score of 1.96 (instead of 1.65 in Eq. 8) should be implemented in the calculation of the RCV, and the RCV must then be added to and subtracted from the grand mean to establish the 'normal' range.

The core measures of gas exchange included in this analysis were collected via a breath-by-breath system which utilizes the Auchincloss algorithm to estimate values of $\dot{V}O_2$ and $\dot{V}CO_2$ (Auchincloss et al. 1966). These estimations introduce an intrinsic error due to the inability to measure the alveolar volume (V_A) at the beginning of each breath (Cautero et al. 2003; di Prampero and Lafortuna 1989). While utilizing such software is standard practice in these systems, true breath-by-breath values are hard to measure precisely. Nevertheless, the present study of biological variation incorporated an analytical coefficient of variation (CV_A) term which can help account for the intrinsic error introduced by the system (Fraser and Harris 1989). More importantly, the reported value for each measured variable was calculated as an average over at least 5 min (at least ~ 50 breaths, but often many more). Additionally, RER, a crucial parameter given the present findings, was calculated directly from the 5-min average of $\dot{V}O_2$ and $\dot{V}CO_2$ (see "Resting measures of ventilation and gas exchange"), as opposed to breath-by-breath, such that we anticipate its value is very stable and can be considered steady state (Ferretti et al. 2017).

Limitations

This study included only young, healthy individuals, thus, it is possible that the resting ventilation and gas exchange measures reported presently, or the calculated coefficients of variation, may vary slightly in disease or with healthy aging. Nevertheless, a previous report regarding the methodology of biological variation calculations suggests that the CV_I remains valid even in chronic illness (Fraser and Harris 1989). Another limitation of the present investigation is the assumption that CV_A is half that of CV_I. Previous work has estimated the CV_A of RER as 2.9% (about 75% that of the CV_I reported presently), however, this previous report was also not able to directly calculate CV_A (Cooper et al. 2009). Finally, resting ventilation and gas exchange (particularly RER) can be greatly altered by factors such as time of day, stress, recent physical activity, and type as well as timing of prior food consumption. While the present study controlled for these variables, it may not always be possible to ensure these ideal conditions, particularly in extreme environmental settings.

Conclusions

In conclusion, $P_{ET}O_2$ and RER were identified as having potential usefulness in the static, and $P_{ET}O_2$ and SpO_2 in the dynamic assessment of deviations from normal. Therefore, future studies interested in ventilatory alterations should focus on the potential for $P_{ET}O_2$ and RER to identify a meaningful physiological change in response to an intervention or stressor. Alternatively, other measures such as V_E and $P_{ET}CO_2$ may not be ideal when assessing a relatively small physiological change, as these commonly cited parameters demonstrated substantial biological variation within and among individuals.

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Author contributions KEC, SNC, RMS, and RWK conceived and designed the research. KEC analyzed the data and wrote the manuscript. All authors read, edited, and approved the manuscript.

Compliance with ethical standards

Conflict of interest The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or reflecting the views of the Army or the Department of Defense. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations. No authors have any conflicts of interest to disclose. Approved for public release; distribution is unlimited.

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