INSTITUTE OF PHYSICS PUBLISHING PHYSIOLOGICAL MEASUREMENT

Physiol. Meas. **25** (2004) 1189–1197 PII: S0967-3334(04)77163-4

Measurement reliability of highly variable physiological responses to experimentally-manipulated gas fractions

John Terblanche[1], **Andreas Fahlman, Kathryn H Myburgh and Sue Jackson**

Department of Physiological Sciences, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa

E-mail: jst@sun.ac.za

Received 4 March 2004, accepted for publication 3 June 2004
Published 6 August 2004
Online at stacks.iop.org/PM/25/1189
doi:10.1088/0967-3334/25/5/009

Abstract

Ventilatory and cardiac responses to changing inhaled gas fractions are notoriously variable within individuals. Such variation can confound clinical diagnoses and hypotheses about human adaptation. In this study we use a cardiac (HHR) and a ventilatory (HVR) measure of physiological sensitivity to an experimentally manipulated oxygen concentration (8% O_2), to compare variation (a) within and between individuals, (b) within and between days and (c) within and between physiological parameters. To explore the sources of variation, we use the coefficient of variation (CV, %), repeatability (R, intra-class correlation coefficient, %) and repeated-measures analyses of variance. Both the HVR and the HHR are significantly repeatable (HVR: $R = 0.76$–0.92; HHR: $R = 0.35$–0.76) and equally variable within and between days. Its high R suggests that the HVR displays greater between-individual variation relative to within-individual variation than does the HHR. The HVR is thus a more reliable measure of physiological sensitivity to hypoxia than is the HHR. We suggest how these results may inform experimental design, and suggest how to avoid stochastic and experimental artefacts when investigating ventilatory and cardiac physiological responses to hypoxia.

Keywords: acute isocapnic hypoxic ventilatory response, isocapnic hypoxic heart rate response, repeatability, variability

[1] Present address: S.P.A.C.E. Research Group, Department of Zoology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa.

1. Introduction

Ventilatory and heart rate responses to hypoxic and hypercapnic conditions are known to vary within individuals (Sahn *et al* 1977, Zhang and Robbins 2000). In clinical circumstances under which it may be difficult for patients to return for re-testing, this makes estimation of such parameters difficult. The best way for investigators to measure parameters that exhibit high within-individual variability is repeated measurements, and repeated experiments exploring the relationship of within- to between-individual variability. Moreover, quantifying within-individual variability can provide valuable information regarding the precision of a measurement technique (Hopkins 2000). Some pertinent questions for the investigator of highly variable physiological parameters in the clinical or experimental setting are as follows:

(1) Is the within- and between-day variation in the parameter of interest similar? Should experiments be repeated on the same day, or single experiments repeated on different days?
(2) How many repeated experiments are necessary to obtain HVR and HHR values that are representative of patients' or subjects' physiological responses?
(3) Do all physiological responses display similar levels of variation within and between individuals?

We chose to investigate the variability in physiological responses to an altered gas composition for two reasons. First, from a clinical perspective, understanding physiological responses to experimentally-altered inhaled gas mixtures (e.g. hypoxia or hypercapnia) can provide insight into various pathological states, such as sleep apnoeas and other respiratory disorders (Neubauer 2001), and is applicable to anaesthesia and intensive care.

Second, variation in the acute hypoxic ventilatory (HVR) and heart rate responses (HHR) within and between days is poorly understood (Khoo 2001, but see Sahn *et al* 1977, Zhang and Robbins 2000). These two physiological measures are practical tools for demonstrating an individual's sensitivity to a reduction in the partial pressure of oxygen (Hochachka and Somero 2002).

The HVR is influenced by genetic factors (Weil 2003) as well as by a multitude of physiological factors (Powell *et al* 1998), and so it is not surprising that many studies have demonstrated that it varies considerably between individuals (e.g. Aitken *et al* 1986, García-Río *et al* 1998, Hirshman *et al* 1975, Kawakami *et al* 1981, Kronenberg *et al* 1972, Rebuck *et al* 1973, Sato *et al* 1996). HVR also varies within individuals when it is measured on different days (Sahn *et al* 1977, Zhang and Robbins 2000), because of changes in factors such as blood pH (Sahn *et al* 1977). We are aware of only one study partitioning within- and between-day variability in the HVR for the same subjects using the same testing protocol (Sahn *et al* 1977), showing that the former is greater than the latter. Some studies of the HVR use duplicate measurements on each subject (e.g., Beall *et al* 1997, Curran *et al* 1995, Kronenberg *et al* 1972, Zhuang *et al* 1993) that, although more representative than single measurements, do not permit variance partitioning within and between tests and individuals. Single measurements of the HVR for each individual (e.g., Hackett *et al* 1980) may not be representative of an individual's hypoxic sensitivity. Rigorous partitioning of sources of variation in this parameter, often used in comparative studies, will benefit future comparisons between studies and between populations.

Variation in physiological parameters may be quantified in different ways, some of which are more informative than others. The coefficient of variation (CV, the standard deviation divided by the mean) is commonly used as a measure of variation about the mean (Hopkins 2000), but may be biased by the relationship between the mean and the deviation around that

mean. A large mean usually has a large variation and vice versa (see Gaston and McArdle 1994 for further discussion), and this may be especially misleading in parameters which exhibit high between-individual variation, such as the HVR and HHR. To explore more thoroughly the variation in the HVR and HHR, we use the CV in conjunction with the repeatability (intra-class correlation coefficient, *R*) of these parameters. *R* informs us about variation between individuals relative to that within individuals, and is calculated as $MS_a/(MS_a + MS_w)$, where MS_a is the mean square among (between) individuals, and MS_w is that within individuals (Lessells and Boag 1987). We explore both within- and between-individual, and within- and between-day variation in these two physiological responses, to inform testing and experimental design for investigations of ventilatory and cardiovascular responses to acute hypoxia.

2. Methods

2.1. Subjects

Nine healthy subjects (five female and four male) participated voluntarily. All test procedures were fully explained to them, verbally and in written form, before they signed a consent form. Subjects understood that they were free to withdraw from the study at any time. Ethical approval for all procedures was granted by the Subcommittee C of the Research Committee of the University of Stellenbosch, which conforms to the internationally accepted ethical guidelines detailed in the Declaration of Helsinki.

2.2. Hypoxic ventilatory (HVR) and hypoxic heart rate (HHR) responses

To remove confounding effects of hypo-/hypercapnia, the isocapnic HVR and HHR were measured using the breathing circuit and square wave protocol described by Fahlman *et al* (2002). This circuit alternates hypoxic and normoxic inhaled gas mixtures while maintaining isocapnia. Each subject's end-tidal partial pressure of CO_2 ($P_{ET}CO_2$, at body temperature, pressure and saturation, BTPS) was maintained at his or her normocapnic level ($\pm$1 mmHg), determined as the average value during the initial baseline period for that subject (see below).

Each subject was seated upright in front of the breathing apparatus and allowed to rest for 10–15 min. The subject was then fitted with a face mask (Hans Rudolph Inc., Kansas City, MO, USA, 8930 Series) attached to a two-way directional valve (Large 2-way NRBV, 2700 series, Hans-Rudolph, Inc., Kansas City, MO, USA). The seal of the mask was ensured prior to each test. If the facemask did not seal properly, the subject breathed instead through a mouthpiece attached to the same directional valve with his or her nose occluded by a nose-clip. The total dead space of the circuit was measured to be $\sim$200 ml.

Expired minute volume ($\dot{V}_E$, l min^{-1} at standard temperature and pressure dry, STPD) was sampled by a metabolic system (MetaMax^{TM}, Cortex Biophysik GmbH, Leipzig, Germany) and average values recorded every 10 s and then converted to BTPS. $P_{ET}CO_2$ was sampled by a capnograph (Microstream^{TM}, Microcap, Oridion Medical Ltd, Jerusalem, Israel), and average values recorded every 5 s. The arterial oxygen saturation (SaO_2, %) was measured continuously and averaged every 10 s using a pulse oximeter (Nellcor N-395 Pulse Oximeter, Mallinkrodt, Inc., St Louis, MO, USA) with a forehead sensor (Nellcor RS10) as previously described (Fahlman *et al* 2002). Forehead sensors reduce inaccuracy in pulse oximetry associated with motion, and are a standard alternative to finger sensors on pulse oximeters used during exercise. Forehead sensor pulse oximetry has been shown to agree closely with blood gas analysis of simultaneously-collected arterial blood samples in exercising subjects exposed to the same level of hypoxia as those in our study (Yamaya *et al* 2002). In subjects undergoing 25 min of

hypoxia, Garcia *et al* (2000) found that SaO_2 values measured in blood sampled from the radial artery (and 2–4% higher than those in our study) were statistically indistinguishable from those obtained using a pulse oximeter. Direct sampling of arterial blood for measurement of SaO_2 is invasive, so we chose not to use this method. SaO_2 measured with our pulse oximeter was compared with simultaneously-measured $P_{ET}O_2$, and showed that the former values accurately reflected $P_{ET}O_2$ (J S Terblanche and A Fahlman, unpublished data).

For the first 5 min (the Pre period) of each test the subject breathed normoxic gas (21% O_2, balance N_2) and if the $\dot{V}_E$ and $P_{ET}CO_2$ were not stable during the final 2 min, this initial period was extended until stable values were attained. Only data from the last 2 min of the Pre period were used for analyses. Following the Pre period, subjects breathed hypoxic gas ($8.2 \pm 0.2\%$ O_2, balance N_2) for 120 s (H period). Because the switching between the two gas mixtures using this circuit is rapid (Fahlman *et al* 2002), this protocol was referred to as the square wave protocol and is similar in shape but not magnitude to that used by Zhang and Robbins (2000). The hypoxic period of 120 s is long enough for subjects' end-tidal PO_2 to reach steady state, hence for full development of the HVR (Fahlman *et al* 2002), yet short enough to prevent significant hypoxic ventilatory decline (HVD; Powell *et al* 1998, Zhang and Robbins 2000).

2.3. Protocol

Nine subjects (five females and four males) repeated three tests each separated by 60 min on a single day, and then repeated this complete series on three separate days (total $n = 81$ tests). Tidal volume responses to altered $PaCO_2$ show circadian sensitivity rhythms (Stephenson *et al* 2000), so we consistently tested each subject at the same time of the day, and randomly assigned subjects to testing either in the morning (07:00–09:00) or in the afternoon (12:00–14:00). Tests on different days were separated by at least 2 and at most 36 days. All female subjects were studied during the follicular phase of their menstrual cycles, because the menstrual cycle influences hypoxic sensitivity (Muza *et al* 2001). Ethanol and caffeine may influence hypoxic sensitivity (Sahn *et al* 1975, D'Urzo *et al* 1990), so subjects were asked to refrain from drinking alcohol and caffeine-containing beverages from the evening before the tests. Before the study, each subject completed one or two preliminary tests for familiarization with the breathing circuit and the study protocol. Before the first preliminary test, the subject's height (cm) and weight (kg) were measured. The number of preliminary tests for each subject was dictated by his or her comfort and ability to relax, evidenced by stable and consistent resting values for $\dot{V}_E$ and f_H (heart rate). During tests, which were conducted in a quiet room, subjects listened to music through headphones and the switching apparatus that controlled inhaled gas mixtures was concealed from them.

2.4. Data assessment and statistical analysis

All values are reported as means (± 1 standard deviation (SD)), unless otherwise specified. Data from the start of the test up to the last 2 min of the Pre period were discarded. The CV was reported as a percentage of the variable in question. The repeatability (R) was estimated as the intra-class correlation coefficient (Krebs 1999). The HVR is calculated as the change in ventilation from normoxia to hypoxia, expressed relative to a 1% change in oxygen saturation. The HHR is similarly the change in heart rate for a 1% change in oxygen saturation. The changes in $\dot{V}_E$ ($\Delta\dot{V}_E$) and f_H (Δf_H) in response to hypoxia are linearly related to the SaO_2 (Rebuck *et al* 1977, Easton *et al* 1986). Consequently, the HVR and HHR for each hypoxic interval were estimated as $\Delta\dot{V}_E \cdot \Delta SaO_2^{-1}$ (HVR, l min^{-1} %$^{-1}$, BTPS) and $\Delta f_H \cdot \Delta SaO_2^{-1}$

Table 1. Summary statistics for nine subjects each exposed to nine 2 min bouts of isocapnic hypoxia, as three tests on each of three separate days. Values for HVR (BTPS) and HHR are pooled means across all tests on all days. CV: coefficient of variation; *n*: number of subjects.

	Mean ± SD	CV (%)	*n*
Age (years)	25 ± 6	23	9
Mass (kg)	70.8 ± 11.5	16	9
Height (cm)	174.0 ± 10.0	6	9
HVR (L min^{-1} %$^{-1}$)	0.483 ± 0.316	65	9
HHR (beats min^{-1} %$^{-1}$)	0.97 ± 0.47	48	9

(HHR, beats min^{-1} %$^{-1}$), respectively, using the 30 s means for $\dot{V}_E$, f_H and SaO_2 from the normoxic (Pre) period.

2.5. Data analysis

The Kolmogorov–Smirnov test was used to detect departures of data from normality. *F*-tests were used to check for equal variances, and in the case of unequal variances, non-parametric tests (Mann–Whitney or Kruskal–Wallis) were used. We used repeated-measures ANOVAs to partition the variance in HVR and HHR. We used the NCSS 2000 statistical package (NCSS statistical software, Kaysville, Utah). Acceptance of significance was set to $P < 0.05$.

3. Results

3.1. Subjects

Summary statistics for subjects are presented in table 1. All subjects completed all three days of the study, and no subjects complained of any discomfort during breathing trials. Consistent with previous results obtained with this circuit (Fahlman *et al* 2002), each subject's $P_{ET}CO_2$ remained stable with minor fluctuations immediately after the switch between gas mixtures. During hypoxia, the breathing circuit maintained isocapnia to within 0.5 mmHg CO_2 of our subject's resting baseline (Pre) values. Indeed, ANOVA across all subjects showed no significant differences between $P_{ET}CO_2$ during the Pre interval and the H interval. There was no difference in either the f_H or $\dot{V}_E$ between tests performed on the same day or between days, suggesting that the period between tests was long enough to reverse any short-term acclimation effects during normoxia on these parameters (repeated-measures ANOVA, $P > 0.8$).

3.2. Variability of the HVR and HHR within and between days

Within subjects, the CV for HVR between all tests within each of three days ranged from 5 to 35% for all subjects but one, who showed a CV of > 80% for this parameter. There were no differences in the HHR or HVR with repeated tests on the same day or on different days (table 2).

Repeatabilities of both the HVR ($F_{8,72} = 44.77$, $P < 0.001$) and the HHR ($F_{8,72} = 10.14$, $P < 0.001$) were statistically significant. *R* did not differ within or between days for both the HVR and the HHR ($P > 0.4$, Kruskal–Wallis ANOVA; overlapping 95% confidence intervals), showing that variability was similar within and between days. Likewise, comparison of upper and lower 95% confidence limits showed that *R* of both the HVR and the HHR did not differ between male and female subjects, whether *R* was calculated within each subject for all three

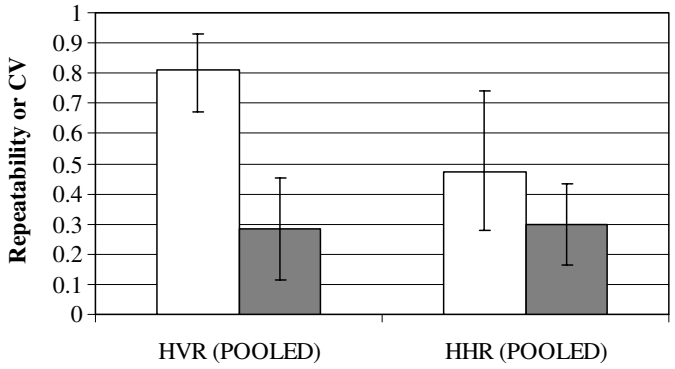

Figure 1. Repeatability of the HVR and HHR (R, open bars, error lines represent 95% CL) for all subjects ($n = 9$) for all tests and all days and CV for the same parameters (filled bars, ± 1 SD). R did not differ between HVR and HHR at the $P < 0.05$ level, because the 95% confidence limits overlap (Krebs 1999).

Table 2. Mean values for HVR and HHR (± 1 SD), showing similar variation in both parameters within and between days for all subjects. R (95% LCL − 95% UCL) did not differ within and between days, showing that variability at these two time scales is indistinguishable. We therefore pooled all tests across all days to test for gender effects (table 3).

Source of variation (days)	Response variable	Case			n
Within		Test 1	Test 2	Test 3	
	HVR	0.456 ± 0.278	0.482 ± 0.299	0.511 ± 0.373	27
	R of HVR	0.89 (0.72–0.97)	0.78 (0.49–0.94)	0.82 (0.57–0.95)	
	HHR	0.912 ± 0.536	1.016 ± 0.426	0.993 ± 0.451	27
	R of HHR	0.48 (0.08–0.83)	0.35 (0–0.76)	0.44 (0.04–0.81)	
Between		Day 1	Day 2	Day 3	
	HVR	0.442 ± 0.259	0.507 ± 0.339	0.501 ± 0.350	27
	R of HVR	0.76 (0.45–0.93)	0.92 (0.79–0.98)	0.90 (0.73–0.97)	
	HHR	0.912 ± 0.313	1.029 ± 0.621	0.980 ± 0.432	27
	R of HHR	0.48 (0.08–0.83)	0.76 (0.47–0.93)	0.65 (0.28–0.89)	

tests pooled for each day, or as nine independent tests per subject ($P > 0.05$). There was a trend for R of the HVR to exceed that of the HHR, with iterative calculations of confidence limits showing that although 70% confidence limits did not overlap, 80% confidence limits did (data not shown, 95% confidence limits given in figure 1). A trend with this significance level may warrant consideration in the design of experiments, which is the purpose of our comparison.

Lack of difference in variation within and between days led us to treat the nine separate values obtained for HVR and HHR for each subject across all tests on all days as repeated measures. HVR and HHR did not differ between genders and over time (repeated-measures ANOVA, $P > 0.15$ in all cases, table 3). The lack of significant interaction between test number and gender revealed that male and female subjects responded in the same way.

4. Discussion

Published studies suggest that inter-individual variation in the HVR is higher than intra-individual variation (Rebuck and Campbell 1974), and that within individuals, between-day

Table 3. HVR and HHR did not differ between genders and showed no trends across all tests over time: repeated-measures ANOVA (type I sums of squares). There was no significant interaction (∗) between time and gender. Unexplained variability is higher for the HHR than for the HVR.

	Effect	Gender	Error	Time (day and test pooled)	Time∗gender	Error
HVR	SS	1.402 64	4.349 99	0.097 36	0.095 95	0.942 80
	df	1	7	8	8	56
	MS	1.402 64	0.621 43	0.012 17	0.011 99	0.016 84
	F	2.257 13		0.722 85	0.712 38	
	P	0.176 700		0.670 566	0.679 500	
HHR	SS	0.266 33	9.065 71	0.457 04	0.724 93	7.136 88
	df	1	7	8	8	56
	MS	0.266 33	1.295 10	0.057 13	0.090 62	0.127 44
	F	0.205 65		0.448 27	0.711 03	
	P	0.663 924		0.886 533	0.680 651	

variation exceeds within-day (between-test) variation (Sahn *et al* 1977, Zhang and Robbins 2000). On different days, sleep deprivation can influence the HVR in a single individual (White *et al* 1983), and Zhang and Robbins (2000) suggested that the HVR varies physiologically over time. Our results revealed two main findings: (1) comparison of R values calculated within and between days showed that within- and between-day variability did not differ significantly for either the HVR or the HHR and (2) while the between-test variability (CV) of the HHR was similar to that of the HVR, the HHR was a less reliable indicator of hypoxic responsiveness, with a lower repeatability and therefore a higher within-individual variation relative to between-individual variation, by comparison with the HVR.

The fact that the HVR is significantly repeatable supports its usefulness in predicting the occurrence of chronic hypoxaemia in patients with, for instance, chronic obstructive pulmonary disease (Powell *et al* 1998). Hypoxic sensitivity is probably important in preventing hypoventilation and maintaining adequate arterial gas concentrations in acutely ill and/or sleep-deprived patients (White *et al* 1983), and its measurement is therefore of clinical importance.

Comparative evolutionary physiology requires an understanding of both within- and between-individual variability (Spicer and Gaston 1999). The advancement of hypotheses such as the origins of human adaptation to high altitudes (Beall *et al* 1997, Hochachka and Somero 2002; see also review by Powell 2003) requires rigorous assessment of the variability of a parameter or trait. Repeatability (R) is a measure of the proportion that between-individual variance contributes to the summed variance both between and within individuals (Falconer and MacKay 1996), and is usually expressed as a percentage. Traits with a high R are those most likely to respond to natural selection, and R may represent the upper limit to heritability within the trait of interest (Dohm 2002). The identification of such traits is useful to human biologists interested in adaptation to environmental conditions, such as high altitude. A statistically significant R, such as that obtained in both the HVR and HHR, suggests that these physiological responses are sufficiently repeatable for natural selection to operate upon them. The lower repeatability in the HHR, in conjunction with greater error mean squares in the repeated-measures analyses of variance, suggests that the measurement error in the HHR is greater than that of the HVR. The HHR in humans is probably of limited use as a measure of acclimation and adaptation to high altitude, and as a diagnostic indicator for pathological disorders. Naturally, the CV provided no such information regarding the nature of the variation within and between individuals in these parameters, but simply gives

an indication of the variance about the mean. Therefore, in future studies investigating the variation of physiological parameters, we strongly recommend the use of repeatability using the intra-class correlation coefficient approach.

5. Conclusions

For the testing of highly variable ventilatory and cardiac responses to experimentally-manipulated changes in inhaled gas fractions, testing protocols can select to use different days or different tests within the same day at the convenience of the researcher: single measurements should be avoided. We found that the cardiac responses to hypoxia are more subject to unexplained, residual error than are the ventilatory responses. We strongly recommend the use of repeated-measures when recording physiological variables with a standard deviation of greater than one-third of the mean, and further suggest that repeatability (R) be used to explore the nature of the variation in parameters of interest.

Acknowledgments

Alan Thomas, Nick Robinson, Shaun Thaysen and the staff of National Hyperbarics (Pty Ltd) helped with gas mixing. Peggy Schlie of Dräger (Medizintechnik GmbH, Germany) provided the Oxidem 3000 demand valve. Elrike Marais independently checked the repeatability calculations. We are grateful to all our subjects for their cooperation. Jaco Klok and various anonymous reviewers commented on an earlier version of this manuscript. This study was funded by the South African National Research Foundation (GUN 2047146), the Medical Research Council of South Africa and Stellenbosch University Research Sub-Committee B.

References

Aitken M L, Franklin J L, Pierson D J and Schoene R B 1986 Influence of body size and gender on control of ventilation *J. Appl. Physiol.* **60** 1894–9

Beall C M *et al* 1997 Ventilation and hypoxic ventilatory response of Tibetan and Aymara high altitude natives *Am. J. Phys. Anthropol.* **104** 427–47

Curran L S, Zhuang J, Droma T, Land L and Moore L G 1995 Hypoxic ventilatory responses in Tibetan residents of 4400 m compared with 3658 m *Respir. Physiol.* **100** 223–30

Dohm M R 2002 Repeatability estimates do not always set an upper limit to heritability *Funct. Ecol.* **16** 273–80

D'Urzo A D, Jhirad R, Jenne H, Avendano M A, Rubinstein I, D'Costa M, Goldstein R S and Rubenstein I 1990 Effect of caffeine on ventilatory responses to hypercapnia, hypoxia, and exercise in humans *J. Appl. Physiol.* **68** 322–8

Easton P A, Slykerman L J and Anthonisen N R 1986 Ventilatory response to sustained hypoxia in normal adults *J. Appl. Physiol.* **61** 906–11

Fahlman A, Fisher J A, Vesely A, Sasano H, Terblanche J, Jackson S and Myburgh K 2002 A simple breathing circuit to maintain isocapnia during measurements of the hypoxic ventilatory response *Respir. Physiol. Neurobiol.* **133** 259–70

Falconer D S and MacKay T F 1996 *Introduction to Quantitative Genetics* 4th edn (London: Longman Harlow)

Garcia N, Hopkins S R, Elliott A R, Aaron E A, Weinger M B and Powell F L 2000 Ventilatory response to 2 h sustained hypoxia in humans *Respir. Physiol.* **124** 11–22

García-Río F, Pino-García J M, Racionero M A, Terreros-Caro J G, Gómez-Mendieta M A, Prados C and Villasante C 1998 Long-term within-subject variability of inspiratory neural drive response to hypoxia *Chest* **114** 521–5

Gaston K J and McArdle B H 1994 The temporal variability of animal abundances: measures, methods and patterns *Phil. Trans. R. Soc.* B **345** 335–58

Hackett P H, Reeves J T, Reeves C D, Grover R F and Rennie D 1980 Control of breathing in Sherpas at low and high altitude *J. Appl. Physiol.* **49** 374–9

Hirshman C A, McCullough R E and Weil J V 1975 Normal values for hypoxic and hypercapnic ventilatory drives in man *J. Appl. Physiol.* **38** 1095–8

Hochachka P W and Somero G N 2002 *Biochemical Adaptation: Mechanism and Process in Physiological Evolution* (Oxford: Oxford University Press)

Hopkins W G 2000 Measures of reliability in sports medicine and science *Sports Med.* **30** 1–15

Kawakami Y, Yoshikawa T, Shida A and Asanuma Y 1981 Relationships between hypoxic and hypercapnic ventilatory responses in man *Jpn. J. Physiol.* **31** 357–68

Khoo M C K 2001 Respiratory instability and variability: why is respiration such a mess? *Frontiers in Modeling and Control of Breathing* ed C-S Poon and H Kazemi (New York: Kluwer/Plenum) pp 495–500

Krebs C J 1999 *Ecological Methodology* 2nd edn (Menlo Park: Benjamin–Cummings)

Kronenberg R, Hamilton F N, Gabel R, Hickey R, Read D J and Severinghaus J 1972 Comparison of three methods for quantitating respiratory response to hypoxia in man *Respir. Physiol.* **16** 109–125

Lessells C M and Boag P T 1987 Unrepeatable repeatabilities: a common mistake *Auk* **104** 116–21

Muza S R, Rock P B, Fulco C S, Zamudia S, Braun B, Cymerman A, Butterfield G E and Moore L G 2001 Women at altitude: ventilatory acclimatization at 4300 m *J. Appl. Physiol.* **91** 1791–9

Neubauer J A 2001 Physiological and pathophysiological responses to intermittent hypoxia *J. Appl. Physiol.* **90** 1593–9

Powell F L 2003 Functional genomics and the comparative physiology of hypoxia *Annu. Rev. Physiol.* **65** 203–30

Powell F L, Milsom W K and Mitchell G S 1998 Time domains of the hypoxic ventilatory response *Respir. Physiol.* **112** 123–34

Rebuck A S, Kangalee M, Pengelly L D and Campbell E J 1973 Correlation of ventilatory responses to hypoxia and hypercapnia *J. Appl. Physiol.* **35** 173–7

Rebuck A S, Slutsky A S and Mahutte C K 1977 A mathematical expression to describe the ventilatory response to hypoxia and hypercapnia *Respir. Physiol.* **31** 107–16

Sahn S A, Lakshminarayan S, Pierson D J and Weil J V 1975 Effect of ethanol on the ventilatory responses to oxygen and carbon dioxide in man *Clin. Sci. Mol. Med.* **49** 33–8

Sahn S A, Zwillich C W, Dick N, McCullough R E, Lakshminarayan S and Weil J V 1977 Variability of ventilatory responses to hypoxia and hypercapnia *J. Appl. Physiol.* **43** 1019–25

Sato F, Nishimura M, Igarashi T, Yamamoto M, Miyamoto K and Kawakami Y 1996 Effects of exercise and CO_2 inhalation on intersubject variability in ventilatory and heart rate responses to progressive hypoxia *Eur. Respir. J.* **9** 960–7

Spicer J I and Gaston K J 1999 *Physiological Diversity and its Ecological Implications* (Oxford: Blackwell)

Stephenson R, Mohan R M, Duffin J and Jarsky T M 2000 Circadian rhythms in the chemoreflex control of breathing *Am. J. Physiol.* **278** R282–6

Weil J V 2003 Variation in human ventilatory control—genetic influence of the hypoxic ventilatory response *Resp. Physiol. Neurobiol.* **135** 239–46

White D P, Douglas N J, Pickett C K, Zwillich C W and Weil J V 1983 Sleep deprivation and the control of ventilation *Am. Rev. Resp. Dis.* **128** 984–6

Yamaya Y, Bogaard H J, Wagner P D, Niizeki K and Hopkins S R 2002 Validity of pulse oximetry during maximal exercise in normoxia, hypoxia, and hyperoxia *J. Appl. Physiol.* **92** 162–8

Zhang S and Robbins P A 2000 Methodological and physiological variability within the ventilatory response to hypoxia in humans *J. Appl. Physiol.* **88** 1924–32

Zhuang J, Droma T, Sun S, Janes C, McCullough R E, McCullough R G, Cymerman A, Huang S Y, Reeves J T and Moore L G 1993 Hypoxic ventilatory responsiveness in Tibetan compared with Han residents of 3658 m *J. Appl. Physiol.* **74** 303–11