

A simple breathing circuit to maintain isocapnia during measurements of the hypoxic ventilatory response

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Abstract

We report the development and testing of a simple breathing circuit that maintains isocapnia in human subjects during hypoxic hyperpnea. In addition, the circuit permits rapid switching between two gas mixtures with different partial pressures of oxygen. Eleven volunteers breathed repeated cycles of exposure to air (2 min of 21% O₂, balance N₂) and hypoxia (2 min of 8.3±0.1% O₂, balance N₂). Hypoxia induced significant increases in minute ventilation, breathing frequency and tidal volume ($P < 0.05$) that were consistent over repeated cycles of hypoxia ($P > 0.1$, one-way ANOVA). The system successfully maintained isocapnia in all subjects, with an average change in end-tidal CO₂ of only -0.2 mmHg during hyperventilation in hypoxia (range 0.4 to -0.8 mmHg). This system may be suitable for repeated tests of the hypoxic ventilatory response (HVR) and may prove useful for exploring intra- and inter-individual variability of HVR in humans.

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1. Introduction

The ventilatory response to hypoxia in humans is triphasic, with hypoxic exposure causing an initial rapid increase in both tidal volume (V_T, L) and breathing frequency (f_R, breaths min⁻¹)

above resting values (Weil and Zwillich, 1976; Easton et al., 1986). This initial acute response develops over a few seconds (Powell et al., 1998; Zhang and Robbins, 2000), and has been difficult to measure because of contamination of the data with those from later phases. During steady state hypoxic exposure lasting longer than 2–5 min, there is a second phase of decreased ventilation, termed hypoxic ventilatory decline (HVD, Easton et al., 1986; Powell et al., 1998). During this second phase, V_T returns towards resting values while f_R remains elevated for the entire hypoxic exposure

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(Easton et al., 1986). In experiments to measure the hypoxic ventilatory response (HVR), the imposed hypoxic challenge should be long enough for the full response to develop but short enough to preclude the development of HVD (Severinghaus, 1976; Khamnei and Robbins, 1990; Mou et al., 1995). In the third phase, called ventilatory acclimatisation to hypoxia (VAH), ventilation rises over orders of hours during hypoxia exposure (Powell et al., 1998).

Several different techniques have been used to measure HVR in humans. The earliest of these entailed exposing the subject to a gas mixture with a certain fraction of inspired O_2 and measuring expired ventilation (\dot{V}_E , $L \cdot \text{min}^{-1}$) after the subject's ventilatory parameters had reached steady state (Cormack et al., 1957; Lloyd et al., 1958). However, hypoxic exposures lasting more than 2 min induce varying degrees of HVD (Severinghaus, 1976; Cormack et al., 1957; Lloyd et al., 1958). Second, hypoxia may be induced by re-breathing of a fixed volume of air in a bag from which the CO_2 is partially removed to maintain the desired end-tidal CO_2 partial pressure (P_{ETCO_2} ; Rebuck and Campbell, 1974; Beall et al., 1997). This technique permits a single hypoxic exposure, but not the switching between hypoxic and normoxic mixtures that facilitate repeated measurements. Repeated measurements are important for assessing the considerable intra- and inter-individual variability in ventilatory parameters (Zhang and Robbins, 2000).

A third method, the end-tidal forcing system (Robbins et al., 1982; Howson et al., 1987), permits rapid changes of inhaled O_2 and CO_2 concentrations and yields valuable data on the time course of the HVR (Painter et al., 1993). Consequently, this system allows for repeated measurements of HVR within the same experiment without eliciting HVD, and also permits exposure of subjects to rapidly alternating cycles of hypoxia and normoxia for repeated measurements of HVR within the same experiment without eliciting HVD. However, it requires a chamber with a high gas turnover and complex computerised mixing equipment and is, therefore, costly and not portable.

Sommer et al. (1998) recently developed a technique that controls alveolar CO_2 concentration by providing a fixed flow of gas and a second flow of gas on demand. For the current study, we modified this circuit so that it permits rapid switching between inspired gases with different P_{O_2} 's over several cycles of alternating hypoxia and normoxia. Our main aim was to test the ability of the circuit to maintain isocapnia during changes between two gas mixtures of different O_2 concentrations (Fig. 1). Here, we report tests of this system's ability to keep subjects isocapnic when applying a square wave (SW) protocol similar to that described by Zhang and Robbins (2000), albeit without instantaneous attainment of hypoxia.

2. Methods

2.1. Subjects

Eleven healthy subjects (five male and six female) were asked to participate. Their mean body mass was 68.8 ± 11.0 kg (± 1 S.D.), mean height 173.6 ± 9.7 cm, and age 26.5 ± 5.6 years (Table 1). All experimental procedures were fully explained to them, verbally and in written form, before they signed a consent form. They 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. Isocapnia breathing circuit

The circuit described by Sommer et al. (1998) comprises a non-rebreathing valve, the inspiratory port of which is connected to two gas sources. One gas source (fresh gas, FG) is provided at a constant flow. A gas reservoir collects FG during exhalation and provides it for inhalation with the next breath. The second gas (termed reserve gas, RG) is provided via a demand regulator only if the ventilatory demands exceed the flow of FG,

where \dot{V}_A is the alveolar ventilation (L min^{-1}), or the ventilation that contributes to CO_2 exchange, the FGF is the FG flow (L min^{-1}), \dot{V}_E the expired ventilation during hyperpnea (L min^{-1}), and $P\bar{V}_{\text{CO}_2}$ and P_{RGCO_2} are the partial pressures of CO_2 of the mixed venous blood and the RG, respectively.

Eq. (1) has two theoretical shortcomings. First, the rationale for the use of $P\bar{V}_{\text{CO}_2}$ in the RG is not correct. To eliminate the effects of ventilation on arterial P_{CO_2} (P_{aCO_2}), the RG P_{CO_2} should be equal to that in the alveoli (P_{ACO_2}) or arterial blood. Indeed, Sommer et al. (1998) found that adjusting the CO_2 fraction of the RG (F_{RGCO_2}) to 5.5% (which corresponds approximately to a normal P_{ACO_2}) maintained isocapnic hypoxia better than F_{RGCO_2} of 6.5%, which corresponds more closely to $P\bar{V}_{\text{CO}_2}$. We found the same and, accordingly, have used $F_{\text{RGCO}_2} = 5.5\%$.

Second, Eq. (1) does not take into account anatomical dead space. Clearly, in the second term, the ventilation obtained from the RG will not be $(\dot{V}_E - \text{FGF})$ but $(\dot{V}_E - \dot{V}_{\text{Dan}} - \text{FGF})$, where \dot{V}_{Dan} is the minute ventilation of the anatomical dead space. A modification of the equation described by Sommer et al. (1998) taking these factors into account is:

$$\dot{V}_a = \text{FGF} + (\dot{V}_e - \dot{V}_{\text{aan}} - \text{FGF}) (P_{\text{aCO}_2} - P_{\text{rgCO}_2}) (P_{\text{aCO}_2})^{-1} \quad (2)$$

where the P_{ACO_2} is assumed to be equal to the P_{ETCO_2} .

To allow switching between normoxic (21% O_2 , balance N_2) and hypoxic (8% O_2 , balance N_2) gas mixtures while maintaining isocapnia, we added a duplicate circuit of FG and RG gas bottles containing hypoxic gas mixtures (Fig. 1). Consequently, we had two FG bottles containing different F_{O_2} 's (8.3 and 21%, balance N_2), and two RG bottles containing 5.5% CO_2 in addition to these two F_{O_2} 's (balance N_2). Our circuit worked as follows. The inspired gas was supplied from a 2 L reservoir filled continuously from a compressed gas cylinder containing normoxic (21% O_2 , balance N_2) or hypoxic ($8.3 \pm 0.1\%$ O_2 , balance N_2 , $n = 3$ bottles used) gas (Fig. 1). Flow into the bag was measured by a flow meter (Ohmeda, BOC

Healthcare, England) calibrated with air and with an 8% O_2 , 92% N_2 mixture using a water spirometer. Estimated flows were reproducible within 4%.

The flow of FG was set to equal each subject's \dot{V}_A (Eq. (1)), estimated as:

$$\dot{V}_A = \dot{V}_E 0.70 \quad (3)$$

where \dot{V}_E was measured during the initial 5 min period before the hypoxic–normoxic exposure, and 0.70 is the estimated fraction of \dot{V}_E contributing to gas exchange (Tortora and Grabowski, 1996). When \dot{V}_E was equal to or less than FGF, inspired gas consisted entirely of FG. When \dot{V}_E exceeded that supplied by the FG, the reservoir emptied and a low resistance demand valve (opening pressure -0.5 mbar; Oxidem 3000, Dräger Medizintechnik GmbH, Germany) opened to supply the RG with 5.5% CO_2 and the same F_{O_2} as the FG. This arrangement maintained the subject's P_{ETCO_2} at his or her normal resting values during both normoxia and hypoxia, despite the increases in ventilation produced by hypoxia.

2.3. Protocol

Experiments were conducted on each subject once, and female subjects were studied during the first 2 weeks of their menstrual cycles. Subjects were asked to refrain from drinking alcohol and caffeine-containing beverages from the day before the experiments. Before the study, each subject completed one or two preliminary experiments for familiarisation with the breathing circuit and the study protocol. The number of preliminary experiments was determined by each subject's comfort and ability to relax, indicated by stable and consistent resting values for \dot{V}_E and fR. On each study day, the HVR was measured using the SW protocol (Zhang and Robbins, 2000) modified as described below.

Before the start of the hypoxic–normoxic challenge, each subject was seated upright in front of the apparatus with his/her face level with the directional valve (see below and Fig. 1), and allowed to rest for 10–15 min. The subject was then fitted with an occlusive facemask (8930 Series, 47.2 ml dead space; Hans–Rudolph, Kan-

sas City, MO, USA) attached to a two-way directional valve (large two-way non-rebreathing valve, 2700 series; Hans–Rudolph). The seal of the mask was checked by asking the subject to occlude the mouthpiece with his or her hand and attempt to breathe. If breathing was possible, this meant that the facemask did not seal properly, and instead the subject breathed through a mouthpiece ($n = 3$) attached to the same directional valve with his/her nose occluded by a nose-clip. \dot{V}_E (L min⁻¹, BTPS), V_T (L, BTPS), and f_R (breaths min⁻¹) were measured by a metabolic system (MetaMaxTM, Cortex Biophysik GmbH, Leipzig, Germany) and average values recorded every 10 sec. There were no differences in ventilatory variables between subjects using the facemask and those using the mouthpiece ($P > 0.2$, two-tailed t -test). The metabolic system used a turbine volume transducer (Digital Triple-V, resolution 15 ml, accuracy 1.5%) to determine ventilatory rates, an infrared processor-controlled CO₂ analyser (accuracy 0.1%) and a zirconium processor-controlled O₂ analyser (accuracy 0.1%), all supplied by Cortex Biophysik GmbH. The P_{ETCO_2} (mmHg, BTPS) was sampled by a capnograph (MicrostreamTM, Microcap, Oridion Medical Ltd, Jerusalem, Israel) and average values were recorded every 5 sec.

For the first 5 min, the subject breathed air (21% O₂, balance N₂) supplied from a gas cylinder via a gas reservoir (Fig. 1). The subject's resting \dot{V}_E and P_{ETCO_2} were determined as averages during the last 2 min of this period. If these two variables were not stable during the final 2 min, the initial period was extended until stable values were attained.

Subjects were then exposed to 4 SWs of inspired O₂ that alternated every 120 sec between 8.3 (± 0.1) and 21% O₂. The period of each of the four waves was, therefore, 240 sec and longer than the 120 sec used by Zhang and Robbins (2000) because their dynamic end-tidal forcing system allows steady P_{ETO_2} values to develop within 5 sec (one to two breaths) of switching from normoxia to hypoxia. The P_{ETO_2} of subjects breathing through our circuit only stabilised 1 min after switching (Fig. 2). If inspired P_{O₂} is not dropped to extremely low values for one or two breaths at the

start of the hypoxic exposure, mixing of inhaled air with that in the dead space and the residual volume causes arterial P_{O₂} to lag behind inspired P_{O₂} (Anthonisen and Fleetham, 1987). Our subjects inhaled a gas mixture with a constant F_{IO_2} (8.3%; P_{O₂} = 60 mmHg) and therefore, required approximately 60 sec to reach steady P_{ETO_2} values, as did subjects in a recent study using a partial rebreathing circuit (Garcia et al., 2000). Thereafter, 50 sec at a steady, hypoxic P_{ETO_2} is long enough for full development of the acute HVR yet short enough to prevent a significant HVD (Mou et al., 1995; Zhang and Robbins, 2000).

Published studies using various protocols, some of which, like ours, do not instantaneously induce hypoxia, show that ventilatory decline begins 2–3 min after introduction of hypoxia (Severinghaus, 1976; Weil and Zwillich, 1976; Easton et al., 1986; Khamnei and Robbins, 1990; Bascom et al., 1992; Paterson et al., 1993; Powell et al., 1998; Garcia et al., 2000). Although Howard and Robbins (1994), Mou et al. (1995) recommend use of a 50 sec bout of hypoxia, the slower switching of our circuit meant that this would not have been long enough for our subjects to reach the required P_{AO_2} , and Fig. 2 in Howard and Robbins (1994) suggests that minute ventilation (\dot{V}_E) peaked 2 min after initial hypoxic exposure. We, therefore, chose a hypoxic interval of 120 sec to induce adequate hypoxia, causing significant desaturation of arterial blood (to a mean value of $82.5 \pm 5.5\%$) while reducing the risk of development of HVD. The total period incorporating one hypoxic and one normoxic interval was thus 240 sec. For each subject, we repeated this 240 sec hypoxia–normoxia cycle four times.

Subjects ' P_{ETCO_2} 's were maintained at normocapnic levels (± 1 mmHg). For each subject, normocapnia was determined during the final 2 min of the 5-min control period before the start of the alternating hypoxia–normoxia exposures.

2.4. Arterial O₂ saturation (SaO_2 , %)

SaO_2 was measured using a pulse oximeter (Nellcor N-395 Pulse Oximeter, Mallinkrodt, St Louis, MO, USA). Each subject was fitted with a forehead sensor (Nellcor RS10, Mallinkrodt) that

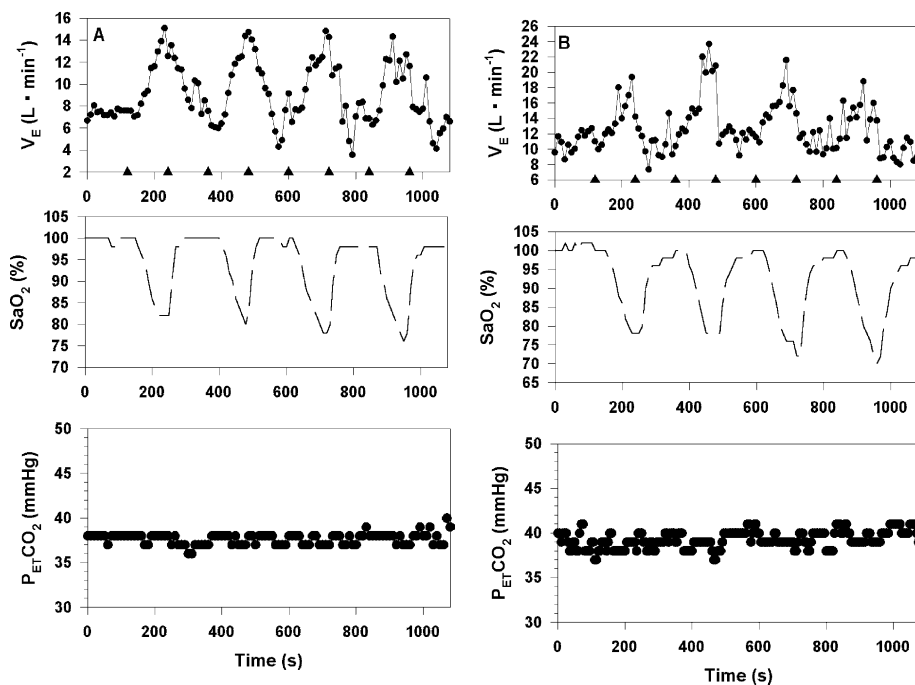


Fig. 2. Representative responses of minute ventilation (\dot{V}_E , L min^{-1} ; upper panel), arterial oxygen saturation (SaO₂, expressed as a percentage; middle panel), and end-tidal CO₂ (P_{ET}CO₂, mmHg; lower panel) to repeated 2 min bouts of exposure to air (21% O₂, balance N₂) and hypoxic gas (8.3% O₂, balance N₂) in a subject with a 'steady' response (A, subject ID 8, Table 1) and a subject with a variable response (B, subject ID 7, Table 1). Triangles on abscissa represent switch in gas mixture. \dot{V}_E and P_{ET}CO₂ are expressed in BTPS.

measured the SaO₂ (%), and heart rate; this provides reliable SaO₂ values both at rest and during exercise (Yamaya et al., 2002). In subjects undergoing 25 min of hypoxia, Garcia et al. (2000) compared SaO₂ measured in blood sampled from the radial artery with values obtained using a pulse oximeter, and found that the two methods yielded statistically indistinguishable values at SaO₂ levels 2–4% higher than the values in our study. In view of the invasive nature of the procedure, we therefore, chose not to sample arterial blood during our study. Analogue signals from the oximeter were sent to the metabolic system, where SaO₂ was recorded every 10 sec.

Other researchers (Clement and Robbins, 1993; Zhang and Robbins, 2000) commonly calculate SaO₂ from P_{ET}O₂ (Severinghaus, 1979), assuming that P_{ET}O₂ is equal to the PaO₂. This practice arises from concern that oximeters are not responsive enough to detect rapid changes in PaO₂. We

compared SaO₂ measured with our pulse oximeter with simultaneously measured P_{ET}O₂ and concluded that the values obtained from our oximeter accurately reflected P_{ET}O₂ (unpublished data).

2.5. Statistical analysis

All values are reported as mean \pm 1 standard deviation (S.D.), unless otherwise specified. Data from the start of the experiment up to the last 2 min of the initial control period were discarded. Resting values for each subject were calculated as means of each variable for the final 30 sec (60 sec for P_{ET}CO₂) of the initial control period, and for each of the hypoxia–normoxia cycles. Initially, means for each of the five normoxic and four hypoxic intervals for each subject were analysed separately.

Repeated measures ANOVA was used to determine if there were any differences in the mean

values of the respiratory variables with cycle number. Two-tailed Student's *t*-tests or, when appropriate, paired comparisons or Mann–Whitney were performed to determine differences in mean values between responses to the two gas mixtures (Zar, 1996). Statistical analysis was performed using the NCSS 2000 statistical package (NCSS statistical software, Kaysville, UT). Acceptance of significance was set to the $P < 0.05$ level, unless otherwise stated.

3. Results

In some subjects, \dot{V}_E , SaO_2 , and PET_{CO_2} were repeatable between each hypoxia–normoxia cycle (Fig. 2A), while others showed sudden changes in ventilatory patterns (Fig. 2B).

Over time, there were no significant differences in mean V_T ($P > 0.1$), f_R ($P > 0.1$), \dot{V}_E (Fig. 3, $P > 0.5$), SaO_2 ($P > 0.05$) or PET_{CO_2} ($P > 0.5$, repeated-measures ANOVA's done separately for normoxic and hypoxic intervals for all variables) between the different intervals of hypoxia or the different intervals of normoxia. Consequently, for each subject, the data for all the normoxic ($n = 5$) and hypoxic ($n = 4$) intervals were averaged and

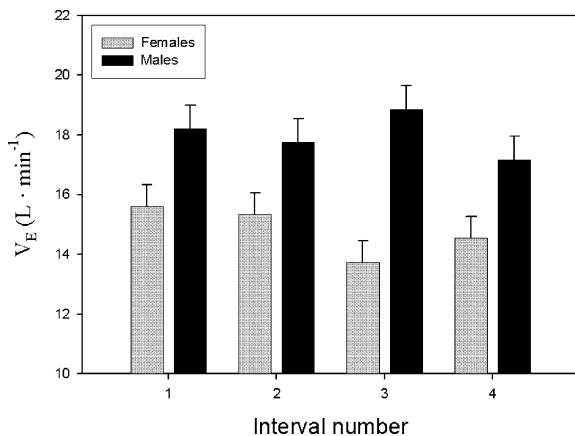


Fig. 3. Mean values for minute ventilation (\dot{V}_E , $\text{L} \cdot \text{min}^{-1}$) for male and female subjects for each of the four hypoxic intervals. There were no significant trends in relation to interval number ($P > 0.6$), but a significant between genders ($P < 0.05$, repeated measures ANOVA including gender as between factor). Vertical bars represent standard error of mean.

S.D. calculated for each subject (Table 2). Further comparisons between normoxia and hypoxia used a single mean value for each subject. Across all subjects, there were significant differences in V_T , f_R , \dot{V}_E , and SaO_2 between the normoxic and hypoxic intervals (Table 2). PET_{CO_2} within each subject did not differ significantly between normoxic ($n = 5$) and hypoxic ($n = 4$) intervals ($P > 0.1$, two-tailed *t*-test, Table 2).

The mean HVR ($\text{L} \cdot \text{min}^{-1} \%^{-1}$, $n = 4$) for each the four cycles was expressed as the change in \dot{V}_E ($\Delta \dot{V}_E$) divided by the corresponding change in SaO_2 (ΔSaO_2 , %, Table 3). The normoxic \dot{V}_E 's used for this calculation were the average values from the normoxic periods following a hypoxic exposure, i.e. the \dot{V}_E from the initial control period was not included. There were no systematic trends with repeated exposures to hypoxia and normoxia ($P > 0.3$, repeated measures ANOVA). The variability between subjects was assessed using the coefficient of variation (CV, Table 3) and this ranged between 60–76%.

3.1. Comparisons of PET_{CO_2} and \dot{V}_E during hyperventilation in normoxia, and hyperpnea in hypoxia

To compare changes in PET_{CO_2} with and without the addition of CO_2 , eight of our 11 subjects returned to repeat the test in 1 atm air without the addition of CO_2 (Table 4). We chose to do these additional experiments in normoxic air to avoid the discomfort of hyperventilation in combination with hypoxia. Subjects breathed normally for 5 min while we measured their resting \dot{V}_E and PET_{CO_2} . Each subject was then coached to raise voluntarily his or her \dot{V}_E to a level equivalent to that measured during his or her exposure to hypoxia, for 1 min. \dot{V}_E and PET_{CO_2} were measured and averaged during the last 30 sec of hyperventilation. This procedure was repeated three times, allowing enough time between each measurement for PET_{CO_2} to return to the subject's resting level. Averages of the three values of \dot{V}_E and PET_{CO_2} from each run were compared with corresponding values for each subject obtained during hypoxia.

PET_{CO_2} differed significantly before and after hyperventilation in normoxia ($P < 0.01$, paired

Table 2

Subject identification number (ID), and mean (± 1 S.D.) tidal volume (V_T), breathing frequency (f_R), expired minute ventilation (\dot{V}_E), during the last 30 sec during normoxia ($n = 5$) or hypoxia ($n = 4$), and mean end-tidal CO_2 partial pressure (P_{ETCO_2}) during the last 60 sec in normoxia ($n = 5$) or hypoxia ($n = 4$)

ID	Normoxia					Hypoxia				
	V_T (L)	f_R (breaths min^{-1})	\dot{V}_E (L min^{-1})	SaO_2 (%)	P_{ETCO_2} (mmHg)	V_T (L)	f_R (breaths min^{-1})	\dot{V}_E (L min^{-1})	SaO_2 (%)	P_{ETCO_2} (mmHg)
1	0.95 \pm 0.17	10.2 \pm 0.9	9.7 \pm 0.8	99.5 \pm 3.4	38.8 \pm 0.5	1.85 \pm 0.15	12.1 \pm 0.3	22.2 \pm 2.0	89.2 \pm 1.3	38.0 \pm 0.8
2	0.49 \pm 0.04	17.0 \pm 2.0	8.3 \pm 0.6	99.9 \pm 0.3	39.8 \pm 0.8	0.61 \pm 0.06	21.0 \pm 1.5	12.9 \pm 0.4	74.8 \pm 0.8	39.6 \pm 0.8
3	0.58 \pm 0.08	16.6 \pm 1.4	9.4 \pm 1.1	100.0 \pm 0.0	37.3 \pm 0.9	0.99 \pm 0.12	17.6 \pm 1.3	17.4 \pm 2.1	82.3 \pm 2.9	37.0 \pm 0.2
4	0.67 \pm 0.05	17.0 \pm 1.0	11.4 \pm 1.2	99.7 \pm 0.1	37.6 \pm 0.3	1.09 \pm 0.06	18.6 \pm 0.3	20.2 \pm 0.7	83.6 \pm 0.4	38.0 \pm 0.6
5	0.74 \pm 0.08	11.0 \pm 1.6	8.1 \pm 1.4	95.9 \pm 1.3	37.3 \pm 0.3	1.13 \pm 0.31	12.6 \pm 2.5	14.2 \pm 2.1	80.7 \pm 2.1	37.0 \pm 0.4
6	0.99 \pm 0.10	12.0 \pm 2.0	11.8 \pm 0.9	99.1 \pm 0.9	37.3 \pm 0.6	1.27 \pm 0.19	13.4 \pm 2.7	16.9 \pm 2.0	92.0 \pm 0.8	37.2 \pm 0.4
7	0.64 \pm 0.07	18.2 \pm 2.1	11.6 \pm 1.1	98.8 \pm 0.7	39.6 \pm 0.6	1.07 \pm 0.29	16.8 \pm 2.2	17.9 \pm 3.2	76.0 \pm 2.8	39.4 \pm 0.4
8	0.57 \pm 0.08	13.1 \pm 0.7	7.5 \pm 0.7	98.9 \pm 0.9	37.6 \pm 0.5	1.01 \pm 0.15	14.6 \pm 1.2	14.7 \pm 1.3	80.0 \pm 2.4	37.5 \pm 0.3
9	0.61 \pm 0.04	16.5 \pm 1.1	10.1 \pm 0.7	98.6 \pm 0.8	39.1 \pm 0.6	0.94 \pm 0.08	18.4 \pm 0.6	17.5 \pm 1.0	78.5 \pm 2.1	38.9 \pm 0.5
10	0.56 \pm 0.04	16.4 \pm 1.0	9.1 \pm 0.6	99.0 \pm 0.8	34.9 \pm 0.6	0.80 \pm 0.04	22.9 \pm 1.5	18.3 \pm 1.3	88.5 \pm 1.5	34.9 \pm 0.2
11	0.53 \pm 0.02	15.3 \pm 1.4	6.9 \pm 0.5	98.5 \pm 0.7	41.0 \pm 0.4	0.76 \pm 0.04	18.3 \pm 0.7	11.7 \pm 0.7	81.6 \pm 0.9	41.2 \pm 0.4
<i>P</i>						< 0.01	< 0.01	< 0.01	< 0.01	> 0.1
\bar{X}	0.67 \pm 0.17	14.8 \pm 2.8	9.4 \pm 1.7	98.9 \pm 1.1	38.2 \pm 1.7	1.05 \pm 0.32	16.9 \pm 3.5	16.7 \pm 3.1	82.5 \pm 5.5	38.1 \pm 1.7

\bar{X} , grand mean for all subjects. V_T , \dot{V}_E , and P_{ETCO_2} are given as BTPS. *P*-values represent paired two-tailed *t*-tests between the normoxia and hypoxia values.

Table 3

Mean HVR (L min^{-1} per percentage, ± 1 S.D.) for each of the four cycles of hypoxia (H1–H4) and normoxia (N1–N4) in all subjects, expressed as change in minute ventilation ($\Delta\dot{V}_E$, L min^{-1}) divided by change in arterial oxygen saturation (ΔSaO_2 , %)

Subject ID	HVR-response (L min^{-1} per percentage)				
	H1 vs. N1	H2 vs. N2	H3 vs. N3	H4 vs. N4	\bar{X}
1	-1.62	-1.36	-1.03	-1.01	-1.25 ± 0.29
2	-0.22	-0.22	-0.14	-0.17	-0.19 ± 0.04
3	-0.78	-0.39	-0.29	-0.45	-0.48 ± 0.21
4	-0.58	-0.57	-0.56	-0.48	-0.55 ± 0.04
5	-0.61	-0.50	-0.04	-0.52	-0.42 ± 0.26
6	-0.50	-0.48	-0.93	-0.79	-0.68 ± 0.22
7	-0.28	-0.46	-0.19	-0.20	-0.28 ± 0.12
8	-0.34	-0.49	-0.32	-0.27	-0.35 ± 0.09
9	-0.42	-0.28	-0.43	-0.32	-0.36 ± 0.07
10	-1.01	-1.00	-1.04	-1.02	-1.02 ± 0.02
11	-0.34	-0.36	-0.29	-0.34	-0.33 ± 0.03
CV	67	60	76	60	

\bar{X} , grand mean for all subjects; CV, coefficient of variation (S.D./mean).

two-tailed *t*-test) when the mean change was -7.6 ± 1.4 mmHg (range -6.1 to -10.3 mmHg), or $-20.4 \pm 4.3\%$ (Table 4). The mean decrease in PET_{CO_2} during hyperventilation in hypoxia with the addition of CO_2 was -0.2 ± 0.3 mmHg (range 0.4 to -0.8 mmHg, Table 2), or $-0.4 \pm 0.8\%$. The change in PET_{CO_2} of -20.4% (Table 4, $n = 8$) during hyperventilation in air was significantly different from the change of -0.4% during hyperpnea in hypoxia with the addition of CO_2 (Table 2, $n = 11$, Mann–Whitney, $P < 0.01$).

4. Discussion

The breathing circuit described here allowed us to adequately titrate the subjects' PET_{CO_2} s during rapid changes in inspired F_{O_2} . This circuit was modified from a simple design that maintains isocapnia during increases in ventilation (Sommer et al., 1998), by the addition of an extra gas reservoir that permitted us to switch quickly between normoxic and hypoxic gas mixtures. We were acutely aware of the need to avoid HVD

Table 4

Minute ventilation (\dot{V}_E , ± 1 S.D.) and end-tidal PCO_2 (PET_{CO_2}) at rest and during hyperventilation in air (without addition of CO_2 to maintain hypercapnia)

Subject ID	Rest		Hyperventilation	
	\dot{V}_E (L min^{-1})	PET_{CO_2} (mmHg)	\dot{V}_E (L min^{-1})	PET_{CO_2} (mmHg)
1	8.7 ± 1.1	36.4 ± 1.1	18.9 ± 0.7	28.6 ± 1.1
3	6.5 ± 1.8	36.6 ± 0.5	19.7 ± 3.5	29.7 ± 1.6
4	11.5 ± 0.8	38.9 ± 0.8	18.8 ± 1.2	31.8 ± 0.4
5	8.1 ± 1.8	37.7 ± 0.6	16.8 ± 0.8	27.4 ± 1.7
6	9.5 ± 1.6	34.8 ± 0.4	24.7 ± 1.5	25.6 ± 0.4
7	9.1 ± 0.8	39.4 ± 1.0	19.8 ± 3.0	33.1 ± 0.8
8	6.0 ± 0.5	37.5 ± 0.5	12.7 ± 1.0	31.4 ± 0.7
9	8.9 ± 0.6	39.5 ± 0.5	19.6 ± 1.1	32.0 ± 1.6
\bar{X}	8.5 ± 1.7	37.6 ± 1.6	18.9 ± 3.3	30.0 ± 2.6

Resting \dot{V}_E ($n = 13$) and PET_{CO_2} ($n = 25$) were averaged during the 2 min prior to the first hyperventilation; values of hyperventilating \dot{V}_E and PET_{CO_2} were averaged from three measurements during the last 30 sec. \bar{X} , grand mean for all subjects. All values given as BTPS.

during the slightly prolonged hypoxic intervals mandated by the lag in stabilisation of P_{ETCO_2} observed with our switching system.

Weiskopf and Gabel (1975), Weil and Zwillich (1976) and Easton et al. (1986) were among the first to report the development of HVD after more than 3 min of exposure to hypoxia. They reported an initial acute ventilatory increase during which both V_T and f_R rise significantly. Following this, V_T returns almost to the base line value while f_R remains elevated for the entire hypoxic exposure. The net effect is an initial rapid increase in \dot{V}_E followed after 2–5 min by a decline that nonetheless does not reach the resting value for the duration of the entire hypoxic exposure (Easton et al., 1986). Although our choice of hypoxic exposure falls within the period preceding development of HVD described by these authors, had our prolongation of the hypoxic exposure from 60 to 120 sec led to HVD we might have expected systematic changes in \dot{V}_E with progressive cycles of hypoxia, with V_T increasing less while f_R remained elevated. We saw no such changes, suggesting that the hypoxic exposure interval of 120 sec we used is short enough to preclude the development of HVD. Moreover, there were no systematic changes in the HVR itself with repeated exposures to hypoxia, further suggesting that our subjects' sensitivity to hypoxia was not altered. However, the slight non-significant decline ($P > 0.05$) in the Sa_{O_2} with repeated exposures to hypoxia and normoxia and the small sample size suggest that this protocol requires further study, and that repeated hypoxic exposures following the initial one be treated with caution.

The lack of systematic changes in respiratory variables with cycle number prompted us to pool the normoxic and hypoxic values (Table 2). These data demonstrate a significant increase in \dot{V}_E , V_T and f_R in hypoxia relative to normoxia. Over all subjects, there was a non-significant tendency towards a mean P_{ETCO_2} that was 0.2 mmHg higher in normoxia than in hypoxia, with the largest difference in any one subject being +0.8 mmHg. Ventilatory responses to decreased P_{CO_2} (Sahn et al., 1977; Ren and Robbins, 1999; Mahamed and Duffin, 2001) suggest that a decrease in alveolar P_{CO_2} of ~ 1 mmHg would elicit a change in \dot{V}_E of

approximately 3 L min^{-1} . Comparison of this value with the standard deviation around the increased \dot{V}_E resulting from hypoxia reported here ($0.4\text{--}3.2 \text{ L min}^{-1}$, Table 2) shows that the potential increase in \dot{V}_E due to stimulation of the CO_2 -sensitive central chemoreceptors is well within the confidence limits of our measurements. Moreover, separate analyses of the mean P_{ETCO_2} values during normoxia and hypoxia for each subject reveal that in no subject did P_{ETCO_2} differ in hypoxia versus normoxia ($P > 0.05$, two-tailed t -test).

Results from our second experiment on subjects coached to hyperventilate while breathing air show that P_{ETCO_2} changed substantially (~ 40 -fold) more during hyperventilation on air without the addition of supplementary CO_2 than during hypoxia when CO_2 was added via the circuit's demand valve (Fig. 1), a clear indication that the circuit satisfactorily compensated for changes in P_{ETCO_2} .

The non-significant decrease in P_{ETCO_2} of -0.2 mmHg in hypoxia relative to normoxia (Table 2) may be due to a slightly lower FR_{GCO_2} than in each subject's arterial blood, or to an FG flow slightly higher than resting \dot{V}_A . Both situations would lead to enhanced elimination of CO_2 from the blood into the lungs and thence the expired air. Maintenance of isocapnic eucapnia requires an FR_{GCO_2} that is not too low and an FG flow equal to \dot{V}_A . Moreover, the \dot{V}_E 's of several subjects declined to slightly less than their initial normoxic baseline values immediately after the switch from hypoxia and normoxia (Table 2; Fig. 2A). This result forced us to either increase the P_{ETCO_2} during the hypoxic interval or to maintain it at a level consistent with initial resting values. We chose to do the latter to maintain isocapnia.

The HVR values reported here are comparable to those obtained using the end-tidal forcing (Zhang and Robbins, 2000) and the rebreathing (Rebuck and Campbell, 1974; Beall et al., 1997) techniques. The CV between subjects was 70% (Table 3) and is consistent with data in the literature (Zhang and Robbins, 2000, 47%; Rebuck and Campbell, 1974, 72%). We conclude that the HVR can be measured using this circuit, but the inherent variation in HVR should be taken

into account and be discussed when this parameter is measured in future studies.

In summary, we have modified the breathing circuit described by Sommer et al. (1998) by adding an additional pair of FG and RG gas cylinders (Fig. 1). The original circuit described by Sommer et al. (1998) maintained isocapnia during increases in ventilation using a single gas mixture and employed one tank of FG and one of RG. Our modification facilitates changes in the inspired gas mixture that permit rapid switching between experimentally-induced normoxia and hypoxia in isocapnic human subjects. Our SW protocol comprising four cycles of normoxia and hypoxia each with a period of 240 sec appeared to avoid HVD, but the small sample size in this study and the trend towards a fall in SaO₂ with repeated cycles of hypoxia and normoxia suggest that further testing is justified. We conclude that this breathing circuit can be used for studies in which investigators wish to change the inspired gas mixture but maintain isocapnia, e.g. to measure the HVR. Investigators using such protocols may choose to restrict the number of hypoxic exposures to one period of 90–120 sec, and should be aware of the risk of development of HVD when choosing the time period of hypoxic exposure. Since it is simple, inexpensive to construct and maintain, and portable, the circuit is particularly well-suited for studies requiring large sample sizes or many repeated experiments, such as the assessments of variation in HVR which our data suggest are warranted, and field comparisons of HVR between human populations with differing degrees of altitude adaptation.

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