European Journal of Clinical Investigation (1995) **25**, 887–892

The reproducibility and comparability of tests of the peripheral chemoreflex: comparing the transient hypoxic ventilatory drive test and the single-breath carbon dioxide response test in healthy subjects

T. P. CHUA & A. J. S. COATS Department of Cardiac Medicine, Royal Brompton National Heart & Lung Institute, London, UK

Received 14 December 1994 and in revised form 26 June 1995; accepted 13 July 1995

Abstract. Both the transient hypoxic ventilatory drive test and the single-breath carbon dioxide (CO_2) response test have been used to assess peripheral chemoreflex sensitivity. We tested their comparability in 14 healthy adults (10 men, aged 31–73 years, mean 55·4 years). The within-subject reproducibility of both tests was also assessed ($n=7$ for each). The mean transient hypoxic ventilatory response was 0·287 ± 0·059 $l\,min^{-1}$ $(\%Sao_2)^{-1}$ (mean ± SEM, range 0·018–0·718) and single-breath CO_2 response was 0·276 ● 0·041 $l\,min^{-1}\,T^{-1}$ (range 0·081–0·501). Both tests were reproducible with a mean coefficient of variation of 20·1% and 17·7%, respectively. There was, however, no significant correlation between the results of the transient hypoxic and single-breath CO_2 tests when data were compared by linear regression analysis ($r=0·23$, $P=0·43$), suggesting that separate pathways of the peripheral chemoreflex existed for hypoxia and hypercapnia, respectively, and that these tests were specific for each. The authors conclude that these tests are reproducible but need to be used in combination for an adequate assessment of the peripheral chemoreflex.

Keywords. Peripheral chemoreflex, single-breath carbon dioxide, transient hypoxia, ventilatory responses.

Introduction

Peripheral chemoreceptor responsiveness is generally assessed by hypoxic testing, and three major techniques are available. These are the steady state [1], progressive [2,3] and transient hypoxic methods [4]. They all appear to correlate well with each other [5,6]. However, persistent hypoxia especially with the first two methods may result in the depression of ventilation owing to direct central effects. There are also the potential risks of hypoxic exposure with these tests. McClean *et al.* [7] reported the use of the single-breath carbon dioxide response test as an alternative method of assessing peripheral chemoreflex and highlighted the relative safety of the test. Abnormalities in regulation of ventilation may exist in certain lung and heart disorders [8–14], and the magnitude of respiratory drive may contribute to the degree of breathlessness. Because of the ease of performing the transient hypoxic and the single-breath carbon dioxide response tests, they are ideally suited as clinical tests of the peripheral chemoreflex. They may be useful in clinical practice to assess patients with chronic lung disease, asthma, heart diseases and unexplained dyspnoea. We are, however, unaware of any study in which the relation between the two tests is assessed. We therefore proceeded to examine the reproducibility of and the relationship between the tests.

Correspondence: A. J. S. Coats, Department of Cardiac Medicine, Royal Brompton National Heart & Lung Institute, Dovehouse Street, London SW3 6LY, UK.

Patients and methods

Fourteen healthy subjects, 10 men and four women, participated in this study. They were 31–73 years of age and were either staff members of the Royal Brompton National Heart & Lung Institute or friends of patients of the affiliated Royal Brompton National Heart & Lung Hospital. None had respiratory symptoms and all except two were non-smokers. All were told to avoid caffeinated products on the morning of the tests. Subject characteristics, including the results of spirometry tests, are summarized in Table 1.

All 14 subjects underwent both the transient hypoxic ventilatory drive test and the single-breath carbon dioxide response test on separate occasions. In addition, the transient hypoxic ventilatory drive test and the single-breath carbon dioxide response test were each repeated once within a month in seven subjects for the assessment of within-subject reproducibility. The study had been approved by the local ethics committee.

Table 1. Subject characteristics including results of spirometry tests

Number of subjects	Age (years)	Height (cm)	Weight (kg)	Body surface Area (m^2)	FEV_1 (% predicted)	FVC (% predicted)
14 (10 men)	55·4 ± 3·2 (31–73)	168·6 ± 2·3 (156–180)	71·8 ± 3·8 (45–93)	1.87 ± 0·05 (1·48–2·14)	104·4 ± 4·4 (82–140)	111·8 ± 4·7 (91–144)

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity. Body surface area is obtained from a standard height–weight–body surface area nomogram [15]. Values expressed as means ± SEM (range).

Transient hypoxic ventilatory drive test

The method of performing the transient hypoxic test was described by Edelman *et al.* [4]. This was performed while subjects were seated and after a period of quiet breathing. They were encouraged to relax in a quiet environment and were allowed to read. Each subject wore a noseclip and breathed through a pneumatic respiratory valve (Innovision, Odense, Denmark), which separated the expirate from the inspirate. The inspirate port was further connected to a T-valve placed behind the subject, and depending on the position of the T-valve, the subject breathed either room air or pure nitrogen from a 4-L reservoir bag. This bag was quietly refilled from a gas cylinder containing pure nitrogen with a valve mechanism (Intersurgical Complete Respiratory Systems, Wokingham, UK), which prevented overfilling and pressure from building up in the bag. Minute ventilation was measured breath by breath using a heated pneumotachograph by integrating the flow over one whole expiration and dividing by the duration of the breath. This was done on line. O_2 and CO_2 at the mouth were monitored continuously by mass spectrometry (Amis 2000, Innovision). The pneumotachometer and mass spectrometer were calibrated before each test. Arterial oxygen saturation was measured using a pulse oximeter (Model N-200E, Nellcor, Hayward, CA, USA) set at fast mode with a response time of 2–3 s and a lightweight ear-probe clipped gently on the subject's right ear lobe.

After the subject had breathed room air for several minutes, surreptitiously and without the subject's knowledge, the T-valve was turned during the expiratory phase of one breath so that pure nitrogen was inhaled for the next 2–8 breaths. This was repeated 10–15 times so as to provide a wide range of arterial oxygen saturations from 70% to 100%. End-tidal O_2 was monitored during the testing for safety reasons to prevent extreme hypoxia. Each transient was preceded by a period of air breathing, during which time oxygen saturation and end-tidal CO_2 were allowed to return to the subject's baseline. The average of the two largest consecutive breaths that gave the highest ventilation after the hypoxic stimulus was used to calculate maximal minute ventilation. A two-breath period was used for calculation of highest ventilation rather than a single breath to reduce data variability (unpublished observations and also noted by Edelman *et al.* [4]). This value was plotted against the lowest arterial oxygen desaturation reached for that period of nitrogen inhalation. The ventilatory response to transient hypoxia was expressed as the slope that related ventilation and arterial oxygen saturation, calculated by least-squares linear regression analysis, in terms of litres per minute per per cent O_2 saturation [$L\,min^{-1}$ $(\%Sao_2)^{-1}$].

Single-breath carbon dioxide response test

The method used was similar to that described by McClean *et al.* [7]. The apparatus including the T-valve was the same as for the transient hypoxic ventilatory drive test above. A smaller 2 L reservoir bag was used, and this was quietly refilled after each inhalation with a gas mixture containing 13% CO_2 in air instead of pure nitrogen. After a period of quiet breathing, the T-valve was again turned in a surreptitious manner during the expiratory phase of one breath such that the subject next inhaled a single breath of gas mixture high in CO_2 content. On average, 10 single breaths of CO_2 were administered at approximately 2 min intervals. As before, minute ventilation was measured breath by breath using a heated pneumotachograph and continuous monitoring of CO_2 was done at the mouth by mass spectrometry.

The mean of the minute ventilation of the preceding five breaths before the stimulus CO_2 breath was calculated and taken as the control ventilation [$\dot{V}$(C)]. The mean end-tidal fraction of CO_2 of these breaths was also calculated and taken as the control end-tidal fraction of CO_2 [F_{ETCO_2}(C)]. The response ventilation after the stimulus CO_2 breath [$\dot{V}$(S)] was calculated by averaging the two largest consecutive breaths but, unlike hypoxic testing, breaths during the first 20 s after the stimulus breath were excluded. This is because breaths beyond this time limit were assumed to be affected by changes in central chemoreceptor drive [7]. The end-tidal CO_2 concentration after the stimulus breath was considered the stimulus end-tidal fraction of CO_2 [F_{ETCO_2}(S)].

The single-breath CO_2 response was calculated using the parameters above as follows:

$$= \frac{\dot{V}(S) - \dot{V}(C)}{[F_{ETCO_2}(S) - F_{ETCO_2}(C)] \times (P_B - 47)}$$

Table 2. Results of transient hypoxic ventilatory drive and single-breath CO_2

Subject	Age (years)	Sex	Hypoxic ventilatory response [L min^{-1} (%Sao_2)$^{-1}$]	Single-breath CO_2 ventilatory response (L min^{-1} T^{-1})
1	50	F	0·182	0·390
2	64	F	0·082	0·110
3	64	M	0·615	0·268
4	56	M	0·183	0·092
5	58	F	0·186	0·081
6	31	M	0·236	0·495
7	69	M	0·222	0·446
8	42	M	0·299	0·182
9	64	M	0·265	0·232
10	41	M	0·168	0·501
11	45	M	0·018	0·270
12	58	M	0·718	0·309
13	61	F	0·157	0·083
14	73	M	0·687	0·406
Mean	55·4		0·287	0·276
SEM	3·2		0·059	0·041

where P_B is the atmospheric pressure in torr and 47 is the saturated water vapour pressure in torr. The mean of 10 responses was considered to be the subject's single-breath CO_2 response and expressed in litres per minute per torr (L min^{-1} T^{-1}).

Statistical analysis

Studies of reproducibility within subjects and the relation between the transient hypoxic and single-breath CO_2 tests were assessed by linear regression analysis. The coefficients of variation of the tests were also calculated. $P < 0·05$ was considered significant.

Results

The results of the transient hypoxic and single-breath CO_2 response tests are summarized in Table 2. The mean transient hypoxic ventilatory response was $0·287 \pm 0·059$ L min^{-1} (%Sao_2)$^{-1}$ and the mean single-breath CO_2 response $0·276 \pm 0·041$ L min^{-1} T^{-1}. The transient hypoxic responses correlated significantly with height ($r = 0·57$, $P = 0·03$) and body surface area ($r = 0·53$, $P = 0·049$), but not with weight ($r = 0·41$, $P = 0·14$). There was an association between single-breath CO_2 responses and height, although this did not achieve statistical significance ($r = 0·51$, $P = 0·06$). No correlation was found between single-breath CO_2 responses and weight ($r = 0·3$, $P = 0·75$) or body surface area ($r = 0·35$, $P = 0·21$). Neither transient hypoxic nor single-breath CO_2 responses correlated with age or spirometric measurements.

The reproducibility between two tests taken on different days is shown in Tables 3 and 4. The coefficient of variation calculated from the results of two transient hypoxic tests in seven subjects varied from 9·4% to 33% with a mean of 20·1% ±3·3%. Table 3 also shows the correlation coefficients obtained from the plots relating $\dot{V}_E$ and arterial oxygen desaturation, with a mean r of 0·75 for the first test and of 0·74 for the second. Ventilatory responses were linear in relation to arterial oxygen desaturation in all tests. Figure 1 shows a graphical representation of the reproducibility of the transient hypoxic test ($r = 0·96$, $P < 0·001$).

The mean coefficient of variation obtained from the two single-breath CO_2 tests in seven subjects was 17·7% ± 6·7% with a range of 3·9–56%. The high coefficient of variation of 56% seen in subject 2 may be the result of a low mean single-breath CO_2 response of this subject since the coefficient of variation is a measure of variability in relation to the magnitude of the mean. Figure 2 shows the results of the two single-breath CO_2 tests plotted graphically ($r = 0·84$, $P = 0·02$).

There was no significant correlation between the transient hypoxic and single-breath CO_2 responses, as shown in Fig. 3 ($r = 0·23$, $P = 0·43$).

Table 3. Reproducibility of transient hypoxic ventilatory drive test in seven subjects

Subject	Response 1 [L min^{-1} (%Sao_2)$^{-1}$]	r_1	Response 2 [L min^{-1} (%Sao_2)$^{-1}$]	r_2	Coefficient of variation (%)
4	0·183	0·93	0·138	0·50	19·8
6	0·237	0·58	0·172	0·79	22·5
7	0·222	0·74	0·148	0·66	28·3
8	0·299	0·79	0·481	0·77	33·0
10	0·168	0·66	0·195	0·61	10·5
12	0·718	0·82	0·820	0·91	9·4
14	0·687	0·89	0·894	0·84	18·5
Mean	0·359	0·77	0·407	0·73	20·1
SEM	0·090	0·05	0·125	0·05	3·3

r_1 and r_2 are the correlation coefficients, obtained by linear regression analysis from the plot relating $\dot{V}_E$ and arterial oxygen desaturation, during the first and second transient hypoxic ventilatory drive test, respectively.

Table 4. Reproducibility of single-breath CO_2 response test in seven subjects

Subject	Response 1 ($L\,min^{-1}\,T^{-1}$)	Response 2 ($L\,min^{-1}\,T^{-1}$)	Coefficient of variation (%)
1	0·390	0·369	3·9
2	0·110	0·255	56·2
6	0·495	0·412	12·9
7	0·446	0·410	5·9
8	0·182	0·233	17·4
9	0·232	0·271	11·0
10	0·501	0·635	16·7
Mean	0·337	0·369	17·7
SEM	0·060	0·052	6·7

Discussion

The results indicate that both tests are reproducible. We are not aware of any previous study of reproducibility of the transient hypoxic ventilatory drive test, although one study using the progressive hypoxic test based on the Rebuck & Campbell technique [3] showed a mean coefficient of variation of 23.1% (range 2·3–48·7%) [6]. This is similar to the mean coefficient of variation of 20.1% seen in our study. The mean coefficient of variation for the single-breath CO_2 response test was 17.7% in our study, and this is again compatible with the mean of 25% obtained by McClean *et al.* [7] with the single-breath CO_2 test.

We found that the transient hypoxic ventilatory responses correlated with height but not with age, compatible with the findings of Hirshman *et al.* [16] using the progressive hypoxic method. There was also significant correlation with body surface area. However, we were not able to demonstrate any correlation with weight, as they did, or with spirometric results. The reason for the lack of correlation with weight in our study is unclear. There appears to be no significant correlation between

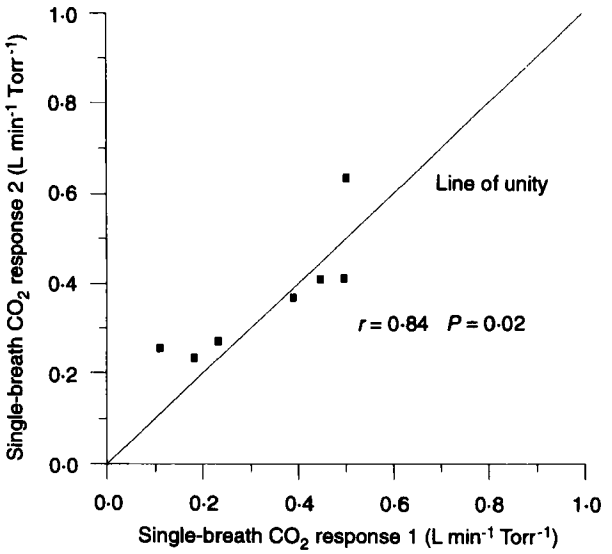

Figure 2. Reproducibility of the single-breath CO_2 response test.

single-breath CO_2 responses and age, height, weight, body surface area or spirometric results. The significance of this observation is not known but may point towards the divergence of the two responses.

We reasoned that if the transient hypoxic ventilatory drive and single-breath CO_2 response tests are assessments of the peripheral chemoreflex, it is likely that there is a significant correlation between the two tests. Our study, however, indicates that there is no correlation between the transient hypoxic and the single-breath CO_2 response tests. It has previously been shown that there is a positive correlation between progressive hypoxic response and CO_2 response by the *rebreathing technique* [17], but it must be borne in mind that the latter is a test of central chemoreceptors [18]. Furthermore, there appears to be no correlation between the single-breath CO_2

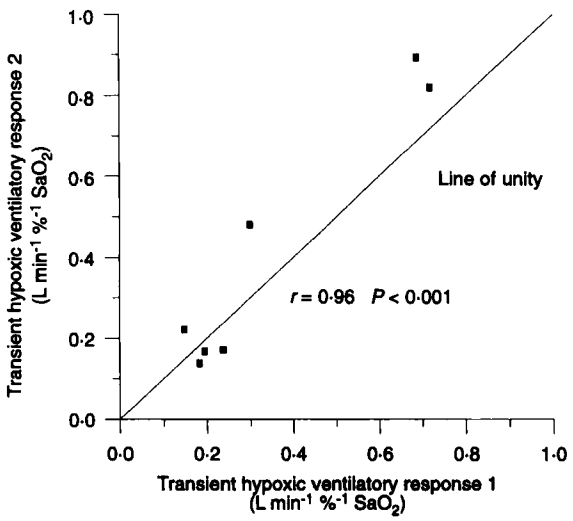

Figure 1. Reproducibility of the transient hypoxic ventilatory drive test.

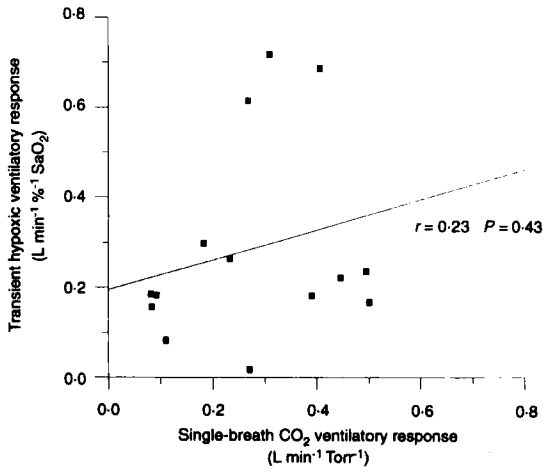

Figure 3. Scatter plot showing the absence of a significant correlation between the transient hypoxic and single-breath CO_2 ventilatory responses.

response and rebreathing CO_2 response [7]. It has also been demonstrated that nitrendipine, a dihydropyridine Ca^{2+}-channel blocker, inhibited the release of dopamine, a neurotransmitter in the carotid body chemoreceptors, by hypoxia but not by hypercapnia [19]. This suggests that, although hypoxic and peripheral hypercapnic responses are mediated by the carotid body, the pathways are partially separate, which may account for our observation. When P_{O_2} decreases, Ca^{2+} channels are activated with Ca^{2+} entry, causing the release of neurotransmitter. On the other hand, hypercapnia acts indirectly by altering intracellular pH [19,20]. As a result, there is active H^+ extrusion from the chemoreceptor cells in exchange for Na^+. The rise in the intracellular Na^+ concentration triggers the Na^+–Ca^{2+} exchanger so that Na^+ is in turn extruded in exchange for entry of Ca^{2+}, causing the release of neurotransmitter.

The other possibility which may explain the lack of correlation between the two tests lies in the methodology of the single-breath CO_2 test. The single breath of 13% CO_2 may not have provided an adequate stimulus for the complete transduction of a ventilatory response. CO_2 is thought to activate the carotid body chemoreceptors by its intracellular acidifying capacity, as discussed above. The peripheral chemoreceptor response to CO_2 is therefore to a large extent indirectly dependent on the effects of hypercapnia on intracellular pH. Thus, fundamental differences in the methodology of the two tests may also explain our observation. As we excluded the ventilatory responses for 20 s after the stimulus breath of CO_2, it is unlikely that the responses seen in our study were influenced by central chemoreceptors. In studies that involve inactivation of peripheral chemoreceptors, there may be lag of up to 60 s before a ventilatory response is seen after a hypercapnic stimulus [4,7,21]. In our study, typical responses to hypoxia and single-breath CO_2 were seen within 20 s after the stimulus.

In conclusion, we have found that both the transient hypoxic and the single-breath CO_2 response tests are reproducible. In view of the lack of correlation between the two tests, however, we suggest that both should be undertaken in the assessment of peripheral chemoreflex since the presence of a hypoxic response does not imply a similar peripheral hypercapnic response and vice versa. The single-breath CO_2 test on its own is not an adequate test of the peripheral chemoreflex and cannot be used to predict hypoxic chemosensitivity. These tests may be separately and independently abnormal in certain disease states such as chronic heart failure and cyanotic congenital heart disease.

Limitations of study

With transient hypoxic testing, both arterial oxygen desaturation and the resultant ventilatory response are fleeting in nature. The measurement of arterial oxygen desaturation and the use of a two-breath period to define maximal minute ventilation in such conditions, although necessary under the constraints of the method, may give rise to errors and hence potential study limitations. To minimize errors, we used a pulse oximeter with a fast response time and with documented accuracy of $< \pm 3\%$ compared with direct measurements of arterial blood samples [22,23]. Shaw *et al.* [6] have also previously shown that the transient hypoxic method, as used by us, can give as good an index of hypoxic chemosensitivity as the progressive method.

Some subjects felt an acid taste of the 13% CO_2, although none developed a cough. The acid taste may have affected ventilation and is a potential drawback of this method. However, the overall results of our single-breath CO_2 test compare favourably with previous data [7,24].

Acknowledgments

T.P.C. is a recipient of a British Heart Foundation Junior Research Fellowship. A.J.S.C. is the British Heart Foundation & Viscount Royston Senior Lecturer in Cardiomyopathy.

References

1 Cormack RS, Cunningham DJC, Gee JBL. The effect of carbon dioxide on the respiratory response to want of oxygen. Q J Exp Physiol 1957;42:323–34.
2 Weil JV, Byrne-Quinn E, Sodal IE *et al.* Hypoxic ventilatory drive in normal man. J Clin Invest 1970;49:1061–72.
3 Rebuck AS, Campbell EJM. A clinical method for assessing the ventilatory response to hypoxia. Am Rev Respir Dis 1974;109:345–50.
4 Edelman NH, Epstein PE, Lahiri S, Cherniack NS. Ventilatory responses to transient hypoxia and hypercapnia in man. Respir Physiol 1973;17:302–14.
5 Kronenberg R, Hamilton FN, Gabel R, Hickey R, Read DJC, Severinghaus J. Comparison of three methods for quantitating respiratory response to hypoxia in man. Respir Physiol 1972;16:109.
6 Shaw RA, Schonfeld SA, Whitcomb M. Progressive and transient hypoxic ventilatory drive tests in healthy subjects. Am Rev Respir Dis 1982;126:37–40.
7 McClean PA, Phillipson EA, Martinez D, Zamel N. Single breath of CO_2 as a clinical test of the peripheral chemoreflex. J Appl Physiol 1988;64:84–9.
8 Edelman NH, Lahiri S, Braudo L, Cherniack NS, Fishman AP. The blunted ventilatory response to hypoxia in cyanotic congenital heart disease. N Engl J Med 1970;282:405–11.
9 Zwillich CW, Sutton FD, Pierson DJ, Creagh EM, Weil JV. Decreased hypoxic ventilatory drive in the obesity-hypoventilation syndrome. Am J Med 1975;59:343–8.
10 Kronenberg RS, Drage CW, Stevenson JE. Acute respiratory failure and obesity with normal ventilatory response to carbon dioxide and absent hypoxic ventilatory drive. Am J Med 1977;62:772–6.
11 Woodcock AA, Gross ER, Gellert A, Shah S, Johnson M, Geddes DM. Effects of dihydrocodeine, alcohol and caffeine on breathlessness and exercise tolerance in patients with chronic obstructive lung disease and normal blood gases. N Engl J Med 1981;305:1611–6.
12 Rebuck AS, Slutsky AS. Control of breathing in diseases of the respiratory tract and lungs. In: Cherniack NS, Widdicombe JG, eds. Handbook of Physiology, Section 3: The Respiratory

System, Vol. 2, Control of Breathing. Bethesda, MD: American Physiological Society, 1986;771–91.
13 Hanly P, Zuberi N, Gray R. Pathogenesis of Cheyne–Stokes respiration in patients with congestive heart failure. Chest 1993;104:1079–84.
14 Kikuchi Y, Okabe S, Tamura G *et al.* Chemosensitivity and perception of dyspnea in patients with a history of near-fatal asthma. N Engl J Med 1994;330:1329–34.
15 Swanton RH. Cardiology, 3rd edn. Oxford: Blackwell Scientific Publications, 1994.
16 Hirshman CA, McCullough RE, Weil JV. Normal values for hypoxic and hypercapnic ventilatory drives in man. J Appl Physiol 1975;38:1095–8.
17 Rebuck AS, Kangalee M, Pengelly LD, Campbell EJM. Correlation of ventilatory responses to hypoxia and hypercapnia. J Appl Physiol 1973;35:173–7.
18 Dempsey JA. CO_2 response: stimulus definition and limitations. Chest 1976;70 (Suppl.):114–18.
19 Gonzalez C, Almaraz L, Obeso A, Rigual R. Carotid body chemoreceptors: from natural stimuli to sensory discharges. Physiol Rev 1994;74:829-98.
20 Donnelly, DF, Smith E, Dutton, RE. Carbon dioxide versus H ion as a chemoreceptor stimulus. Brain Res 1982;245: 136–8.
21 Gefland R, Lambertsen CJ. Dynamic respiratory response to abrupt change of inspired CO_2 at normal and high P_{O2}. J Appl Physiol 1973;35:903–13.
22 Hannhart B, Michalski H, Delorme N, Chapparo G, Polu J. Reliability of six pulse oximeters in chronic obstructive pulmonary disease. Chest 1991;99:842–6.
23 Barker SJ, Hyatt J, Shah NK, Kao J. The effect of sensor malpositioning on pulse oximeter accuracy during hypoxaemia. Anesthesiology 1993;79:248–54.
24 Sebert P, Barthelemy L, Mialon P. CO_2 chemoreflex drive of ventilation in man: effects of hyperoxia and sex differences. Respiration 1990;57:264–7.