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# Variability of the ventilatory response to Duffin's modified hyperoxic and hypoxic rebreathing procedure in healthy awake humans

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ABSTRACT

We quantified the magnitude of within- and between-day, within-subject variability of the ventilatory response to Duffin's modified rebreathing procedure in 20 healthy humans. The $P_{ETCO_2}$ at which ventilation increased with progressive increases in $P_{ETCO_2}$ during rebreathing was identified as the ventilatory recruitment threshold ($VRT_{CO_2}$); the ventilatory response below and above the $VRT_{CO_2}$ was taken as an estimate of non-chemoreflex drives to breathe ($\dot{V}_{EB}$) and chemoreflex sensitivity ($\dot{V}_{ES}$), respectively. Within- and between-day intraclass correlation coefficients for each of these parameters were >0.60 (*range*: 0.62–0.93), indicating good-to-excellent test–retest reliability. Within- and between-day, within-subject coefficients of variation for hyperoxic and hypoxic $\dot{V}_{EB}$ (*range*: 24.6–30.7%) and $\dot{V}_{ES}$ (*range*: 18.5–32.7%) were relatively high but acceptable, while those for the $VRT_{CO_2}$ were very small (*range*: 3.0–3.8%). In conclusion, Duffin's modified rebreathing procedure, in both its hyperoxic and hypoxic form, is a highly reliable tool for measurement of chemoreflex and non-chemoreflex ventilatory control characteristics over short and long periods of time in healthy humans.



## 1. Introduction

Resting steady-state minute ventilation ($\dot{V}_E$) and arterial $P_{CO_2}$ ($P_{aCO_2}$) depend on central chemoreflex, peripheral chemoreflex and 'other' non-chemoreflex drives to breathe and their intersection with the metabolic hyperbola (Fig. 1) (Cunningham et al., 1986; Mahamed et al., 2001). Thus, any change in resting $\dot{V}_E$ and $P_{aCO_2}$ may be accounted for by a change in any one or combination of the following: metabolic rate ($\dot{V}_{CO_2}$); central and/or peripheral ventilatory chemoreflex sensitivity ($\dot{V}_{ES}$); central and/or peripheral chemoreflex ventilatory recruitment threshold for $CO_2$ ($VRT_{CO_2}$), secondary to alterations in arterial and central (or brain tissue) acid–base status; non-chemoreflex drives to breathe ($\dot{V}_{EB}$); and cerebral blood flow/cerebrovascular $CO_2$ reactivity (Duffin, 2005; Xie et al., 2006, 2008; Ainslie and Duffin, 2009) (Fig. 1).

Several techniques, including Read's (1967) original rebreathing procedure, the steady-state procedure either by end-tidal forcing (Robbins et al., 1982) or by prospective targeting (Slessarev et al., 2007) and the progressive isocapnic hypoxia procedure (Weil et al., 1970; Rebuck and Campbell, 1974), have been developed to estimate central and peripheral ventilatory chemoreflex sensitivity. However, only Duffin's modification of Read's rebreathing procedure (Casey et al., 1987; Duffin and McEvoy, 1988; Mohan and Duffin, 1997; Duffin et al., 2000), which includes 5-min of prior voluntary hyperventilation and maintenance of a constant (isoxic) hyperoxic or hypoxic end-tidal $P_{O_2}$ ($P_{ETO_2}$), permits measurement of (i) $\dot{V}_{EB}$ and (ii) both central and peripheral chemoreflex $\dot{V}_{ES}$ and $VRT_{CO_2}$. For simplicity and clarity, we propose the use of the term "*Duffin's modified rebreathing procedure*" to describe these tests.

The informed application of any experimental technique used to investigate a specific research question, including the interpretation of study results, requires a clear characterization of the variability associated with the physiological parameter(s) being measured over short (e.g., hours) and long (e.g., days, weeks, months) periods of time. Numerous published studies in awake humans have described the variability associated with measurement of central and peripheral chemoreflex sensitivity using either Read's rebreathing method (Read, 1967; Jennett, 1968; Strachova and Plum, 1973; Hirshman et al., 1975; Lederer et al., 1977; Sahn et al., 1977; Tobin et al., 1988; Berkenbosch et al., 1989; Beidlman et al., 1999; Spengler and Shea, 2001) or the steady-state procedure (Nishimura et al., 1991; Semple and McConnell, 1992) and the progressive isocapnic hypoxic procedure (Hirshman et al., 1975; Sahn et al., 1977; Nishimura et al., 1991; Garcia-Rio et al., 1998; Beidlman et al., 1999; Zhang and Robbins, 2000; Fahlman et al., 2002; Terblanche et al., 2004; Koehle et al., 2005), respectively.

However, only Mahamed and Duffin (2001) have reported on the variability of the ventilatory response to Duffin's modified

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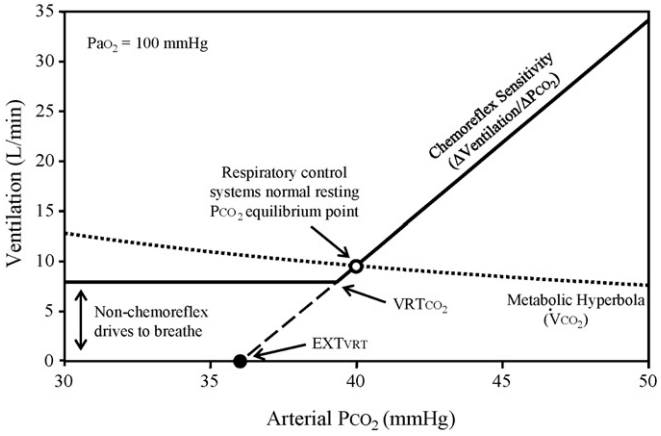


**Fig. 1.** Graphical representation of the determinants of the respiratory control systems *resting* $P_{CO_2}$ equilibrium point. Briefly, resting steady-state minute ventilation and arterial $P_{CO_2}$ depend on central and peripheral chemoreflex as well as '*other*' non-chemoreflex drives to breathe and their intersection with the metabolic hyperbola, which represents the relationship between ventilation and arterial $P_{CO_2}$ at a constant metabolic rate of carbon dioxide production ($\dot{V}_{CO_2}$). $P_{aO_2}$, partial pressure of oxygen in arterial blood; $VRT_{CO_2}$, ventilatory recruitment threshold for carbon dioxide; $EXT_{VRT}$, extrapolated ventilatory recruitment threshold for carbon dioxide (or parameter "*b*" in the Oxford nomenclature); $P_{CO_2}$, partial pressure of carbon dioxide; $\Delta$, change.

hyperoxic and hypoxic rebreathing tests measured once daily for 14 consecutive days in a small group of only 7 healthy volunteers. In that study, within-subject coefficients of variation (CV) over the 14 days for $\dot{V}_{EB}$ (86.6% and 83.3%) and $\dot{V}_{ES}$ (85.9% and 59.9%) were high, while those for the $VRT_{CO_2}$ were dramatically less (7.6% and 7.6%) for hyperoxic and hypoxic tests, respectively.

The purpose of the present study was to extend these observations by quantifying and comparing, for the first time, the magnitude of within- and between-day, within-subject variability of the ventilatory response to Duffin's modified hyperoxic and hypoxic rebreathing tests in 20 healthy, young, awake volunteers under strictly controlled experimental conditions.

## 2. Methods

### 2.1. Subjects

Twenty, healthy, young (20–30 years), regularly active, non-smoking, men ($n = 12$) and women ($n = 8$) with no history of cardiorespiratory disease completed this study. Female participants were nulliparous, eumennorheic (confirmed by serum progesterone ($[P_4]$) and 17β-estradiol ($[E_2]$) concentrations) and had not used oral contraceptives for ≥6 months prior to study participation. The study protocol and consent form were approved by the Queen's University and Affiliated Teaching Hospitals Health Sciences Human Research Ethics Board in accordance with the *Declaration of Helsinki*. All subjects provided written informed consent.

### 2.2. Experimental design and controls

This was a controlled, longitudinal study in which subjects visited the laboratory on 7 separate occasions (2 familiarization and 5 experimental visits) over a period of ~9 weeks (Fig. 2). During the first experimental visit (Day 0), subjects performed 4 hyperoxic and 4 hypoxic modified rebreathing tests (see below) in alternating order over a period of ~6 h. Subjects returned to the laboratory exactly 7, 14, 21 and 60 days thereafter to perform both a modified hyperoxic and hypoxic rebreathing test. Rebreathing trials were separated by ~30 min to avoid the development of respiratory muscle fatigue and to allow arterial and central acid–base status to return to normal resting levels.

Repeated tests on different days were conducted at the same time (±1 h) for each subject to minimize the possible influence of circadian rhythm on central and peripheral chemoreflex control characteristics (Spengler et al., 2000; Stephenson et al., 2000). Exposure to hypoxia, whether for (i) short or prolonged periods or (ii) repeated episodes produces alterations in the respiratory chemoreflexes, particularly the peripheral chemoreflex (Duffin and Mahamed, 2003; Duffin, 2007). On Day 0, therefore, we purposefully chose not to randomize the order of hyperoxic and hypoxic trials so as to minimize the potentially confounding effect that two, three or four consecutive hypoxic exposures may have had on the respiratory chemoreflexes and in this manner quantification of within- and between-subject variability of the ventilatory response to Duffin's modified rebreathing tests.

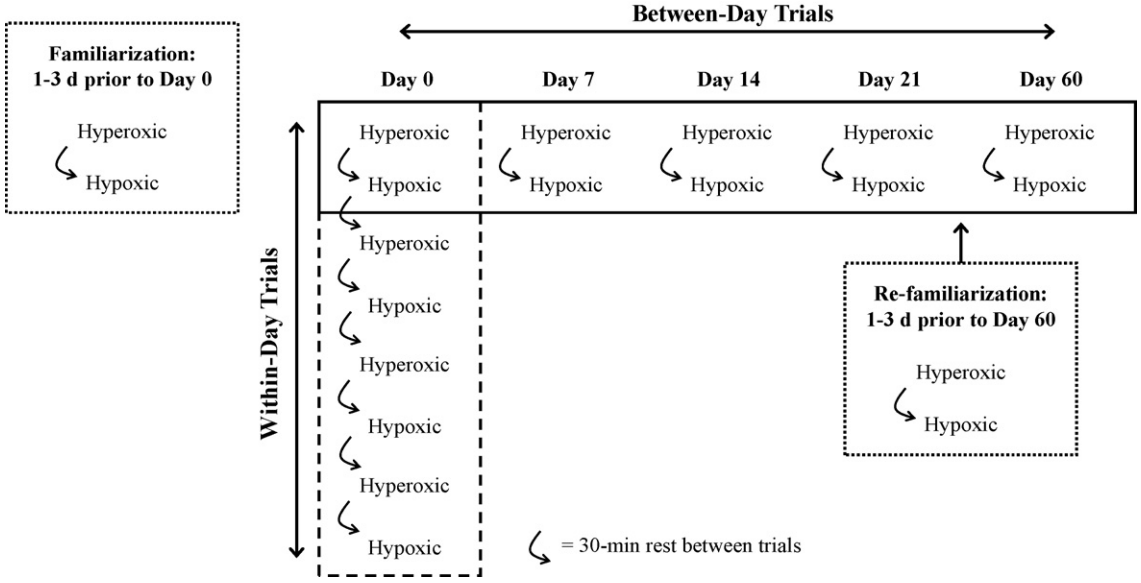


**Fig. 2.** Graphical representation of the experimental study design (refer to text for details).