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Clamping end-tidal carbon dioxide during graded exercise with control of inspired oxygen

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ABSTRACT

Exercise- and hypoxia-induced hyperventilation decreases the partial pressure of end-tidal carbon dioxide (PET_{CO2}), which in turn exerts many physiological effects. Several breathing circuits that control PET_{CO2} have been previously described, but their designs are not satisfactory for exercise studies where changes in inspired oxygen (F_IO_2) may be desired. This study is the first report of a breathing system that can maintain PET_{CO2} constant within a single session of graded submaximal exercise and graded hypoxia. Thirteen fit and healthy subjects completed two bouts of exercise consisting of three 3 min stages on a cycle ergometer with increasing exercise intensity in normoxia (Part A; 142 ± 14, 167 ± 14, 192 ± 14 W) or with decreasing F_IO_2 at a constant exercise intensity (Part B; 21, 18, and 14%). One bout was a control (CON) where PET_{CO2} was not manipulated, while during the other bout the investigator clamped PET_{CO2} within 2 mmHg (CO_{2Clamp}) using sequential gas delivery (SGD). During the final 30 s of each exercise stage during CO_{2Clamp}, PET_{CO2} was successfully maintained in Part A (43 ± 4, 44 ± 4, 44 ± 3 mmHg; P = 0.44) and Part B (45 ± 3, 46 ± 3, 45 ± 3 mmHg; P = 0.68) despite the increases in ventilation due to exercise. These findings demonstrate that this SGD circuit can be used to maintain isocapania in exercising humans during progressively increasing exercise intensities and changing F_IO_2.

1. Introduction

Hyperventilation decreases the partial pressure of end-tidal carbon dioxide (PET_{CO2}), a measure that is frequently used to indicate the partial pressure of arterial CO_2 (P_aCO_2). CO_2 is not only a powerful cerebral vasodilator, which can alter blood flow to the brain (Battisti-Charbonney et al., 2011) but also stimulates the peripheral and central chemoreceptors, altering cardiorespiratory parameters such as heart rate and ventilation (Kaufman and Forster 1996; Mateika and Duffin 1995). Therefore, the ability to clamp PET_{CO2} can be useful in various experimental exercise paradigms exploring neuromuscular, respiratory, and cardiovascular physiology. For example, it is well established that hypoxia impairs exercise performance. However, hypoxia induces a concomitant decrease in the partial pressure of arterial oxygen (P_aO_2) and P_aCO_2 and both of these variables can independently influence various physiological systems that respond and adapt to acute exercise. As the hypoxia-induced reductions in P_aCO_2 are frequently not controlled in exercise research involving hypoxia manipulations (Amann et al., 2006; Amann et al., 2007; Goodall et al., 2010; Goodall et al., 2012; Millet et al., 2012; Torres-Peralta et al., 2014), caution should be applied in attributing an impaired exercise capacity to P_aO_2.

Several non-rebreathing (Fan et al., 2013; Olin et al., 2012; Subudhi et al., 2011) and partial rebreathing (Banzett et al., 2000; Olin et al., 2011; Slessarev et al., 2007) systems have been developed to clamp PET_{CO2} (Table 1). However, most systems cannot accommodate the high rates of ventilation associated with exercise, or can only be used when the fraction of inspired oxygen (F_IO_2) remains constant. Our research requires a system capable of clamping PET_{CO2} during whole body exercise, while delivering fluctuating levels of F_IO_2 within a single exercise bout. In this report we describe and validate the hardware, software, and signal processing components of a semi-automated system that can "clamp" PET_{CO2} in spontaneously breathing humans, during voluntary whole body exercise, with continuous and progressive changes in the level of inspired hypoxia.

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Table 1
Comparison of various methods used to clamp PET_{CO2}.

Citation	Control system	Functional state	$\dot{V}_E$ ($L \bullet min^{-1}$) range covered in report	Inspired oxygen intervention while maintaining isocapnia
Farra et al., 2016	SGD	Exercise (Submaximal GXT)	Range: 51–148	Fixed normoxia (F_IO_2 = 21%)
		Exercise (Intensity fixed at ~AeT)	Range: 45–114	Progressive hypoxia Step decreases in F_IO_2 (21, 18, 14%) within a session
Fan et al., 2013	Supplemental CO_2	Exercise (15 km TT)	L: 22 ± 3 H: ~140 ± 20	Fixed normoxia (F_IO_2 = 21%) or fixed hypoxia (F_IO_2 = 10%) Each F_IO_2 tested during separate sessions
Olin et al., 2012	Supplemental CO_2	Exercise (Maximal GXT)	Range: 27–185	Fixed normoxia (F_IO_2 = 21%)
Subudhi et al., 2011	Supplemental CO_2	Exercise (Maximal GXT)	Rest: 51 ± 8 W_{max}: 160 ± 26	Fixed normoxia (P_b = 630 mmHg) or fixed hypoxia (P_b = 425 mmHg) Each P_b was tested during separate sessions
Eβfeld et al., 1990	DEF	Mild exercise (Intensity adjusted between 40 W and 100 W)	L: 26 ± 3 H: 60 ± 14	Fixed normoxia (PET_{O2} = 130 mmHg)
Olin et al., 2011	SGD	Exercise (Maximal GXT)	Pre-exercise: 26 ± 7 W_{max}: 192 ± 37	Fixed normoxia (F_IO_2 = 21%)
Rupp et al., 2015	Supplemental CO_2	Exercise (Isometric Knee Extension)	Not reported	Fixed hypoxia (F_IO_2 ~ 10%; adjusted to maintain S_PO_2 at 80%)
Slessarev et al., 2007	SGD	Rest	Range: ~20–40	Progressive hyperoxia Increasing PET_{O2} (100, 200, and 300 mmHg) within single session
Banzett et al., 2000	SGD	Rest	Range: 10–40	Fixed normoxia (F_IO_2 = 21%)
Koehle et al., 2009	ETF	Rest	L: ~11 ± 1 H: ~28 ± 8	Progressive hypoxia Step changes in PET_{O2} (~105, 50, ~105 mmHg) within a session
Tymko et al., 2015	DEF	Rest	L: 13 ± 1 H: 37 ± 4	Progressive hypoxia Step changes in PET_{O2} (~100, 47, ~100 mmHg) within a session

DEF, Dynamic end tidal forcing; ETF, End tidal forcing; SGD, Sequential gas delivery; GXT, Graded exercise test; AeT, Aerobic threshold; L, Low; H, High; W_{max}, Maximum work rate; TT, Time trial; F_IO_2, Fraction of inspired oxygen; P_b, Barometric pressure; PET_{O2}, Partial pressure of end tidal oxygen.
When the investigators reported individual data, $\dot{V}_E$ response is presented as the range from the lowest to the highest observed. Otherwise, the low and high $\dot{V}_E$ responses are presented as mean ± SD.

2. Methods

2.1. System design and function

Our isocapnic hypoxia system (IHS) is depicted in Fig. 1. It is a partial rebreathing circuit that utilizes the principles of sequential gas delivery (SGD) to clamp PET_{CO2} (Fisher et al., 2016). The IHS maintains isocapnia by means of a 3-way breathing manifold comprised of an inspiratory valve, an expiratory valve, and a crossover valve. The crossover valve is weighted, and when open, allows gas to cross from the expiratory to the inspiratory limb. The inspiratory and expiratory limbs of the 3-way breathing manifold are individually connected to their respective 11 L reservoirs, with a corrugated tube that has a length of 86 cm and an internal diameter of 3.5 cm (Part # 1011-34, Vacumed). The plastic bags that served as the reservoirs (Model # Ziplock® 6714070293, S. C. Johnson & Son, United States) had a length and width of 38 cm. The inspiratory reservoir is composed of a single compartment, while the expiratory reservoir is separated into three, with the two outer partitions being open to the environment. This configuration allows the expired gas within the centre compartment to remain uncontaminated with room air, while allowing excess gas to be expelled. The tubes were inserted into the reservoirs approximately two-thirds of the length of the bag. These unions were sealed to protect the contents of the bags from the external environment. The portion of the tube inside the inspiratory reservoir was cut along the corrugations to prevent the obstruction of a single aperture during heavy inspiration.

The system clamps PET_{CO2} by controlling alveolar ventilation ($\dot{V}_A$), independent of a subject's minute ventilation ($\dot{V}_E$), by adjusting the flow of fresh gas into the inspiratory reservoir. During expiration, medical grade gas is delivered to the inspiratory limb of the breathing manifold, and exhaled gas enters the expiratory reservoir. At the start of inspiration, gas is drawn solely from the inspiratory reservoir. During inspiration, once the inspiratory reservoir is empty and the flow of fresh gas is inadequate to support $\dot{V}_E$, the crossover valve opens and previously expired gas supplements fresh gas for inspiration. As previously expired gas has already equilibrated with the blood in the pulmonary capillaries, it provides no gradient for gas exchange (Slessarev et al., 2007; Sommer et al., 1998; Somogyi et al., 2005) and so impedes CO_2 elimination. By adjusting the flow of fresh gas into the inspiratory reservoir, the IHS controls the amount of rebreathing for a given $\dot{V}_E$ and its associated impediment to gas exchange.

The flow of fresh gas into the inspiratory reservoir, as well as its composition, were controlled by custom computer software (LabView, National Instruments) regulating two independent mass flow controllers (MFC) (Model # 32907-77, Cole Parmer), with each MFC adjusting the flow of one source gas (Tank 1–21% O_2, balance nitrogen [N_2]; Tank 2–5% O_2, balance N_2). The software was designed to maintain isocapnia in two modes. First, *Manual Control*, in which the gas flow rate and inspirate composition are both adjusted by the investigator. Second, *Semi-automatic Control*, in which the gas flow