Defining the haemodynamic response to maximal exercise using novel beat-to-beat measurement methods

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Declaration

I, Adrian Elliott,

declare that this thesis is my own original work and that

no material contained in this thesis has been submitted

for any other academic award at this or any other university.

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DATE

Abstract

Strenuous exercise presents a significant challenge to the cardiovascular system, such that it is widely assumed that the heart largely governs short, high-intensity aerobic exercise performance. Despite considerable investigation of this topic, the haemodynamic responses to maximal exercise are still not well understood, mostly due to insufficient measurement methods unable to quantify the beat-to-beat response of the cardiovascular system during dynamic exercise. In this thesis, two novel approaches (bioreactance and pulse contour analysis calibrated by lithium dilution) for the continuous assessment of exercise haemodynamics in a healthy, trained population were evaluated. In study I, bioreactance was found to considerably underestimate cardiac output ($\dot{Q}$) in comparison with contemporaneous measurements with inert gas rebreathing. In studies II and III, we evaluated pulse contour analysis, calibrated by lithium indicator dilution. Our findings indicated that the timing of calibration was central to the accuracy of measurements made during exercise using this method, perhaps due to alterations in vascular compliance throughout exercise. In study IV, optimising the calibration of this method during exercise permitted the evaluation of the haemodynamic response to maximal and supramaximal (10% greater than maximal) exercise on a beat-to-beat basis, with the finding that cardiac power output, a measure of cardiac work, was higher during supramaximal exercise despite a similar $\dot{Q}$ and oxygen consumption ($\dot{V}O_2$) between the two workloads. This finding is important for the understanding of factors limiting exercise performance for it indicates that there is cardiac functional reserve at exhaustion during testing for VO_{2max}, thus indicating that the heart is unlikely to be responsible for the termination of exercise as it can be considered to be working submaximally.

CHAPTER 1: Review of the Literature

In the management of critically ill patients, haemodynamic monitoring provides a comprehensive overview of circulatory status that assists in diagnosis and subsequently, direction of appropriate therapy. Monitoring of cardiac output ($\dot{Q}$) and haemodynamic pressures are widely considered central to the management of surgical and intensive care patients, with the primary goal of maintaining adequate oxygen delivery (DO_2) to the tissues. However, recent advances relating to the benefits of haemodynamic monitoring support the demand for continuous monitoring rather than measurement by non-continuous methods. To enhance this approach, a number of haemodynamic monitoring devices, permitting the beat-to-beat measurement of $\dot{Q}$ and other haemodynamic variables, have been developed and assessed in the clinical setting (Alhashemi *et* al 2011).

In the exercise physiology laboratory, the measurement of cardiovascular function is also associated with the goal of assessing O_2 delivery to the exercising musculature. The measurement of $\dot{Q}$ is considered a key element in the study of exercise physiology (Warburton *et* al 1999a), where it provides valuable insight into the adaptations associated with physical training in athletes and clinical populations as well as examining the potential factors responsible for determining exercise performance. As in the critical care setting, continuous real-time monitoring of $\dot{Q}$ in the exercise setting is favourable to provide comprehensive assessment of the cardiovascular response to intense exercise. However, in healthy populations it is difficult to justify the use of invasive cardiac monitoring procedures (Warburton *et* al 1999b).

Despite a number of studies examining $\dot{Q}$ during exercise (Astrand *et* al 1964; Ekblom, & Hermansen 1968; Hermansen *et* al 1970; Higginbotham *et* al 1986; Stringer *et* al 1997; Mortensen *et* al 2005), there is little agreement on the preferred method for its measurement amongst the exercise physiology community. In addition, there is only limited data available on the accuracy and precision of available methods during high-intensity/maximal exercise, where the physiological demand placed on the heart and the vasculature is at its greatest.

Importantly, of those methods that are able to accurately reflect $\dot{Q}$ during exercise, few, if any, are capable of yielding real-time measurements in the moments preceding exhaustion. In the absence of accurate, beat-to-beat data during such exercise, the full picture of how the heart responds to fatiguing maximal exercise cannot be drawn. The consequence of these methodological limitations is that many of the key discussion points in exercise physiology, particularly relating to the physiological determinants of exercise performance, are compromised by the inability to accurately record cardiovascular function with adequate resolution during exercise to exhaustion.

PART I

Cardiovascular Responses to Exercise

Strenuous exercise presents a significant challenge to the human cardiovascular system, both in health and disease. Exercise performed for durations longer than 60 seconds, and thus considered primarily 'aerobic', is supported by energy created from the breakdown of glycogen and lipid stores for the regeneration of adenosine tri-phosphate (ATP), which is the source of energy for muscular contraction. Energy created via this pathway depends on the availability of O_2 within the mitochondria. From rest to exercise, the heart and vasculature must adjust rapidly to meet an elevated metabolic demand to ensure sufficient delivery of O_2 to the working muscles, without compromising the perfusion of other regions of the body, such as the brain and the myocardium itself. Additionally, the demand for sufficient O_2 delivery is coupled to the requirements to sustain arterial pressure, primarily by balancing the degree of vasodilatation by an increased $\dot{Q}$, and remove metabolic by-products, including carbon dioxide (CO_2).

Systemic O_2 Delivery

Both at rest and during exercise, systemic O_2 delivery is quantified by the product of blood flow from the left ventricle ($\dot{Q}$) and the content of O_2 within arterial blood (CaO_2). Oxygen delivery can be presented as: $DO_2 = \dot{Q} \times CaO_2$, where $\dot{Q}$ is the product of heart rate (HR) and the volume of blood ejected from the left ventricle per heartbeat (stroke volume, SV). The cardiovascular system responds to increasing exercise intensity by increasing systemic DO_2 in proportion to increased O_2 demand (Astrand *et* al 1964; Mortensen *et* al 2008), primarily by a rapid rise in $\dot{Q}$ so that, at maximal exercise, $\dot{Q}$ is approximately five times greater than that recorded at rest in healthy individuals (Astrand *et* al 1964; Ekblom, & Hermansen 1968; Hermansen *et* al 1970) with a similar magnitude increase for systemic DO_2 (Mortensen *et* al 2008) in the presence of only a minor elevation in CaO_2.

Cardiac Output

The first accurate measurements of $\dot{Q}$ at rest and during physical activity were made by Krogh & Lindhard (Krogh, & Lindhard 1912) using a nitrous oxide (N_2O) rebreathing method to calculate a resting $\dot{Q}$ of approximately 5 $L \bullet min^{-1}$ that rose to above 20 $L \bullet min^{-1}$ with heavy

exercise. In their seminal work investigating oxygen uptake ($\dot{V}O_2$) during running, Hill & Lupton (Hill, & Lupton 1923) postulated that a $\dot{V}O_2$ during exercise of ~4 L•min^{-1} would require a $\dot{Q}$ of ~30-40 L•min^{-1}. As early as 1955, evidence reviewed from studies recording $\dot{Q}$ during heavy exercise reported $\dot{Q}$ values commonly as high as 28 L•min^{-1} (Asmussen, & Nielsen 1955). Some of the highest $\dot{Q}$ values obtained in man were recorded by Ekblom & Hermansen (Ekblom, & Hermansen 1968) who obtained values well in excess of 30 L•min^{-1}, and as high as 42.3 L•min^{-1} during maximal treadmill exercise, albeit with $\dot{V}O_2$ values much higher than those observed by Hill & Lupton (Hill, & Lupton 1923). The $\dot{Q}$ response to heavy exercise was well characterised in a number of studies throughout the 1960's in both treadmill (Tabakin *et* al 1964; Hermansen *et* al 1970) and cycle exercise (Andrew *et* al 1966; Grimby *et* al 1966), with the common finding that $\dot{Q}$ increases to reach peak values of approximately 20-30 L•min^{-1} during exercise depending on the metabolic demand and aerobic capacity of the subject being studied. More recent studies have shown close agreement to the peak $\dot{Q}$ recorded in those earlier studies (Johnson *et* al 2000; Mortensen *et* al 2005; Calbet *et* al 2007; Jarvis *et* al 2007).

Heart Rate

Heart rate is controlled by both parasympathetic (PNS) and sympathetic nervous system (SNS) activity (Ekblom *et* al 1973), such that reciprocal changes in these two nervous system branches act to control output from the sinoatrial node, which regulates HR (Ekblom *et* al 1973). Increased parasympathetic activity decreases HR, whilst increases in sympathetic activity, raises HR. The increase in HR at the onset of exercise involves both parasympathetic withdrawal and elevated sympathetic activity, which most likely reflects activation of central command (O'Leary 1996), a feed-forward system based upon the notion that activation of the motor cortex results in parallel activation of pathways that descend into the area of the brainstem controlling PNS and SNS activity. In response to increasing work rate, HR rises further in an intensity-dependant manner, as a consequence of group III and IV afferent nerve feedback to the cardiovascular control centre in response to both mechanical (Coote *et* al 1971)

and metabolic stimulation (Fisher *et* al 2010) from the active muscles. Additionally, the arterial baroreflex, in response to blood pressure disturbances, is considered to further modulate the cardiovascular response to exercise (Raven *et* al 2006)

Stroke Volume

Stroke volume can be described as the volume difference between end-diastolic (EDV) and end-systolic (ESV) left ventricular volume. EDV is regulated primarily by ventricular size, LV filling pressure (preload) and ventricular compliance during diastole, whilst ESV is influenced by myocardial contractility and the ventricular pressure required for opening of the aortic valve (afterload). During progressive, upright exercise in healthy, untrained persons, SV increases with the onset of exercise until an exercise intensity of approximately 40-50% $\dot{V}O_{2max}$ before plateauing (Higginbotham *et* al 1986; Stringer *et* al 1997; Stringer *et* al 2005) or even declining (González-Alonso 2008) in the approach towards maximal exercise (Rowland 2009). As a consequence, the increase in $\dot{Q}$ with exercise intensity is driven primarily by rising heart rate. It is widely considered that the initial rise in SV with the onset of exercise reflects the actions of the skeletal muscle pump on venous return to increase EDV (Notarius, & Magder 1996) coupled to the sympathetically-driven increase in myocardial contractility (reduced ESV) (Poliner *et* al 1980). The subsequent levelling off of SV appears to be the consequence of a plateau in both EDV and ESV at moderate exercise intensities (Poliner *et* al 1980; Stöhr *et* al 2011).

Despite the evidence supporting a SV plateau during incremental exercise in healthy, untrained individuals, the hemodynamic responses observed in an endurance-trained cohort are less clear. Gledhill *et* al (Gledhill *et* al 1994) studied endurance-trained cyclists versus an untrained control group during incremental cycle exercise in which the SV of the trained participants increased in an intensity-dependant manner, peaking at approximately 180 mL at maximal exercise, whilst the SV of the untrained group remained stable at 120-125 mL throughout exercise. The authors also reported greater ventricular filling and emptying rates versus the untrained group throughout exercise to exhaustion in addition to an extended left ventricular

ejection time (LVET). Similarly, the SV response to incremental treadmill exercise in groups of trained versus untrained runners was examined (Zhou *et* al 2001), with the key finding that elite runners exhibit a linear increase in SV from rest to maximal exercise, whereas the SV of moderately trained and untrained runners, plateaus and decreases, respectively, from moderate to maximal exercise intensity. To examine the potential mechanisms underlying the enhanced SV response to increasing exercise intensity in trained individuals, Stickland *et* al (Stickland *et* al 2006) compared the SV and filling pressures of trained versus untrained males and found that left heart filling pressures were lower during exercise with similar or higher SV and EDV. These findings confirmed enhanced diastolic function and ventricular compliance in trained individuals, as reported elsewhere (Levine *et* al 1991). Echocardiographic evidence of enhanced diastolic function has been documented in trained athletes, including greater transmitral flow rates (early:late diastolic velocities (E:A) ratio) (Caso *et* al 2000; MacFarlane *et* al 1991; Palazzuoli *et* al 2002) and myocardial velocities (E_m:A_m ratio). However, transmitral flow velocities and myocardial velocities are largely influenced by heart rate and size, respectively (Rowland 2009). It is therefore uncertain whether these observations are the result of bradycardia / enhanced left ventricular size resulting from endurance training or whether they stem from actual diastolic adaptations in endurance-trained athletes.

Oxygen uptake

In describing the $\dot{V}O_2$ response to running, Hill & Lupton (Hill, & Lupton 1923) observed that 'the rate of oxygen intake.....increases as the speed increases, reaching a maximum' beyond which 'no further increase in oxygen intake can occur'. $\dot{V}O_2$ can be calculated from the Fick equation, which holds that $\dot{V}O_2$ is the product of $\dot{Q}$ and arterio-venous oxygen difference ((a-v)O_2diff). During exercise, $\dot{V}O_2$ increases rapidly at exercise onset following an exponential time course until $\dot{V}O_2$ matches the rate of O_2 demand. With increasing exercise intensity, $\dot{V}O_2$ continues to increase as a function of both increasing $\dot{Q}$ and widening of the (a-v)O_2diff (Stringer *et* al 1997; Sun *et* al 2000; Stringer *et* al 2005). Whilst (a-v)O_2diff appears to increase linearly with $\dot{V}O_2$ (Stringer *et* al 1997), the $\dot{Q}$-$\dot{V}O_2$ relationship differs between low-moderate

and high exercise, such that the slope of the relationship is greater at exercise intensities < 70% $\dot{V}O_{2max}$ and attenuated thereafter when assessed with both continuous (Stringer *et* al 1997; Stringer *et* al 2005; Mortensen *et* al 2005; Beck *et* al 2006) and discontinuous incremental exercise protocols (Astrand *et* al 1964; Trinity *et* al 2011). Previous studies have also shown in non-linearity in the $\dot{Q}$-$\dot{V}O_2$ relationship, where individuals with a negative curvature tend to have greater levels of aerobic fitness (Beck *et* al 2006).

In order to determine whether a true $\dot{V}O_{2max}$ can been attained during an incremental exercise test, several studies have measured $\dot{V}O_2$ during constant-load supramaximal exercise intensities, *i.e.* at an intensity above that achieved at $\dot{V}O_{2max}$. The consistent finding throughout these studies is that $\dot{V}O_2$ during a supramaximal bout does not differ to that measured at maximal exercise during a standard incremental test (Day *et* al 2003; Rossiter *et* al 2006; Hawkins *et* al 2007) and that this measure represents the true maximal $\dot{V}O_2$. A plateau in $\dot{Q}$ at or near maximal exercise intensity is possibly the explanation for the observed plateau that occurs in $\dot{V}O_2$ despite increasing work rate. However, the frequency at which participants are reported to exhibit a plateau in $\dot{V}O_2$ at the end of an incremental exercise test is variable with reports as low as 38% in elite athletes (Doherty *et* al 2003).

Skeletal Muscle Perfusion

This considerable hyperaemic response to exercise is likely dictated by a combination of neural, mechanical and/or metabolic factors (Joyner, & Wilkins 2007) that interact in a synergistic manner in an attempt to match muscle blood flow to metabolic demand whilst maintaining arterial pressure via the arterial baroreflex (Rowell 2004). The magnitude of the exercise hyperaemia appears most likely related to vasodilator substances released by muscle, rather than neural (i.e. sympathetic withdrawal, active vasodilation by sympathetic vasodilator fibres) or mechanical (muscle pump action) mechanisms [Joyner & Wilkins 2007). Two vasodilators that appear central to the hyperaemic response are nitric oxide and prostacyclin, both of which are stimulated by compounds released by contracting muscle (i.e. Adenosine, ATP) and by mechanically-induced signals, such as shear stress (Hellsten *et* al 2012).

However, despite over a century of investigations, the precise combination of factors explaining the 100-fold increase in muscle blood flow, are still elusive (Saltin *et* al 1998; Rowell 2004; Joyner, & Wilkins 2007; Hellsten *et* al 2012).

In the absence of clear causation, the study of regional and systemic circulatory capacities during maximal exercise remains of considerable interest to exercise physiologists examining the cardiovascular responses to maximal exercise. Early studies of skeletal muscle perfusion agreed that maximal $\dot{Q}$ was capable of a sufficient supply of blood to the active skeletal muscles whilst maintaining arterial pressure, even during maximal whole-body exercise (Mellander, & Johansson 1968). This view was based on studies reporting peak blood flow of 500-600 mL•kg muscle^{-1}•min^{-1} (Kjellmer 1964), which suggests that a $\dot{Q}$ of 25 L•min^{-1} could comfortably perfuse the 16-20 kg of active muscle mass in an average 175 cm tall male (Calbet, & Joyner 2010) during whole-body exercise, with a sufficient surplus of blood flow for vital tissues other than the active musculature. However, the values reported in these studies were based on xenon clearance or venous occlusion plethysmography, both of which tend to underestimate blood flow (Saltin 2007). More recently enhanced techniques have recorded blood flow of 2.5 L•kg muscle^{-1}•min^{-1} during leg extension exercise (Andersen, & Saltin 1985) and even higher (~4 L•kg muscle^{-1}•min^{-1}) in elite cyclists (Richardson *et* al 1995). Despite the lower hyperaemic response to arm exercise in the arm muscles (Volianitis *et* al 2003), one could postulate that whole-body exercise would demand a magnitude of perfusion that overwhelms the pumping capacity of the heart. A study of cross-country skiers during arm and leg exercise, both independently and in unison, provided the opportunity to study highly-trained humans reliant on both arm and leg exercise capacities (Calbet *et* al 2004). The findings of this study were that the combined maximal vascular conductance (VC) of the arms and legs, measured when each were working independently, exceeds the volume of maximal $\dot{Q}$ measured in the same participants during whole-body exercise. The conclusion drawn from these findings is that the vasodilatory capacities of the limb muscles during maximal whole-body exercise must be constrained to protect against a drop in mean arterial pressure (MAP), which remains stable throughout maximal exercise (Calbet *et* al 2004;

Mortensen *et* al 2008). It is therefore suggested that MAP is the primary regulated variable during maximal exercise (Secher, & Volianitis 2006).

Interaction of HR and SV in determining $\dot{Q}$ during exercise

The evidence presented in the sections above outline the typical response of HR, SV and $\dot{Q}$ to incremental exercise in healthy individuals. However, this approach to examining the cardiovascular responses to exercise presents a 'cardiocentric' viewpoint (Rowland 2005) that places the heart at the centre of determining circulatory flow in response to increasing metabolic demand with exercise. Several experimental models provide evidence that the heart plays a more passive role in the matching of $\dot{Q}$ to metabolic demand (Bada *et* al 2012).

When HR is increased in humans at rest and during exercise by atrial pacing, $\dot{Q}$ does not change due to a compensatory decline in SV (Bada *et* al 2012). Conversely, when the HR response to exercise is diminished under β-blockade, $\dot{Q}$ is maintained by an increase in SV (Wilmore *et* al 1985). These findings lend weight to the hypothesis that HR may not determine the $\dot{Q}$ response to exercise. Peripheral vasodilation in the active skeletal muscles via infusion of ATP increases $\dot{Q}$ to similar levels as that observed during one-legged knee extensor exercise, albeit with differing contributions of HR and SV to the $\dot{Q}$ response (González-Alonso *et* al 2008). During exercise, the elevated $\dot{Q}$ was driven primarily by HR whereas with graded arterial ATP infusion, greater increases in SV with only modest contribution from HR were responsible for the $\dot{Q}$ response. This same response is apparent when the heart is paced to increase HR (Bada *et* al 2012). The study of Gonzalez-Alonso *et* al (González-Alonso *et* al 2008) also examined the role of the muscle pump via passive exercise and thigh compression. Both interventions failed to account for the systemic peak hyperaemia during isolated leg exercise, suggesting that the contribution of the muscle pump to exercising hemodynamics is minimal compared to the effects of muscle vasodilation, as described elsewhere (Joyner, & Wilkins 2007). Nonetheless, the circulatory responses and limitations to exercise appear to reside in peripheral rather than central cardiac sites (Rowland 2005).

Blood Pressure

At the onset of exercise, a rapid increase in $\dot{Q}$ is paralleled by an increase in aortic and arterial blood pressure (Rowell 1993). The increase in systolic blood pressure (SBP) at the radial artery is enhanced due to pulse wave amplification compared to that recorded centrally (Rowell 1993; Sharman *et* al 2005). Regardless of measurement site, the magnitude increase in BP (< x2), even during maximal exercise, is comparably small considering the magnitude of $\dot{Q}$ increase (x5-6) commonly observed. This observation demonstrates that systemic vascular conductance (SVC) compensates for the rise in $\dot{Q}$ with exercise by minimising the elevation in BP per unit of flow. Indeed, the onset of exercise is accompanied by a large fall in peripheral vascular resistance, with a curvilinear decrease as work intensity increases (Otsuki *et* al 2006; Chantler *et* al 2011), that buffers the rise in MAP that would otherwise occur with large increases in blood flow. It is now widely accepted that arterial baroreflex control of BP remains active during exercise, in which it retains the ability to compensate for swings in arterial pressure during exercise by precise alterations in PNS and SNS activity, albeit at a higher operating range than under resting conditions (Raven *et* al 2006). During exercise, the arterial baroreflex appears to modulate arterial pressure primarily by adjustments to SVC rather than $\dot{Q}$ (Collins *et* al 2001; Ogoh *et* al 2003), thus lending support to the view that the circulatory responses to exercise are regulated primarily by peripheral sites. Although undoubtedly exerting mutual influence over one another, BP and blood flow cannot be regulated equally (Secher, & Volianitis 2006) during exercise. As outlined previously (Calbet *et* al 2007), if sufficient muscle mass (i.e. whole-body exercise) were to be recruited during maximal exercise, the large vasodilatory capacity of skeletal muscle (Andersen, & Saltin 1985) would result in a fall in BP due to insufficient $\dot{Q}$. To explain the active muscle vasodilation that occurs in the face of increasing SNS activity with increasing exercise intensity, the concept of 'functional sympatholysis' has been posited, which details a complete withdrawal of sympathetically-mediated vasoconstriction in contracting skeletal muscle during exercise (Remensnyder *et* al 1962). However, more recent evidence suggests that hypotension during high intensity/maximal exercise is avoided by way of tonic sympathetic vasoconstriction that

persists in contracting skeletal muscle (Tschakovsky *et* al 2002; Rowell 2004). Whilst some attenuation of blood flow would result from this protective mechanism, recent findings in animal preparations, suggests that functional sympatholysis may occur only in the smallest microvessels of skeletal muscle whilst tonic sympathetic control remains in the larger microvessels (VanTeeffelen, & Segal 2003). The implication for large muscle mass exercise in humans is that total flow to a contracting muscle would be constrained to protect against hypotension but the flow that is permitted would be directed to the most metabolically-stressed fibres (Calbet, & Joyner 2010).

Coronary Blood Flow

The haemodynamic adjustments with high-intensity exercise cause an approximately sixfold increase in $m\dot{V}O_2$, with approximately 60% of this due to an increase in HR and the remaining 40% split between increased myocardial contractility and left ventricular work (Duncker, & Bache 2008). The increase in $m\dot{V}O_2$ with exercise is met largely by a ~5-fold increase in coronary blood flow as coronary oxygen extraction is only able to increase modestly with exercise due to its high extraction fraction (70-80%) even under resting conditions (Duncker, & Bache 2008). Insight into the $m\dot{V}O_2$ demands of exercise can be determined from analysis of the rate-pressure product (RPP, HR x arterial pressure) or cardiac power output (CPO, $\dot{Q}$ x MAP x *k*, where *k* is a constant converting CPO to watts). Both measures correlate strongly with $m\dot{V}O_2$ (Kitamura *et* al 1972; Nelson *et* al 1974; Cooke *et* al 1998) and have been used extensively to quantify myocardial work during exercise. Despite the appearance of myocardial ischemia during exercise in patients with coronary heart disease, coronary blood flow appears adequate for the purposes of maximal exercise in healthy humans where exercise is terminated before the onset of any ischemia.

Maximal Oxygen Uptake ($\dot{V}O_{2max}$)

Maximal oxygen uptake ($\dot{V}O_{2max}$) is defined as the maximally attained rate at which O_2 can be taken up and utilised by the body during exercise (Bassett & Howley 2000). As a measure of the body's aerobic functioning (Day *et* al 2003), it remains the most widely used measure of an individual's integration of respiratory, cardiovascular and metabolic function. The highest $\dot{V}O_2$ attained during an incremental exercise test commonly forms part of the prognostic and diagnostic assessment of patients with cardiorespiratory disease (Arena, & Sietsema 2011), monitors systemic training adaptations (Burgomaster *et* al 2008) and is used to inform exercise prescription for training programs (da Cunha *et* al 2011). Despite its prominent role in cardiorespiratory assessment during exercise, there is still considerable debate regarding the factors limiting $\dot{V}O_{2max}$ in healthy humans (Saltin, & Calbet 2006; Spurway *et* al 2012).

The heart as the limiting factor for $\dot{V}O_{2max}$

Given the arrangement of the Fick equation, which states that $\dot{V}O_2$ is equal to the product of $\dot{Q}$ and (a-v)O_2diff, exercise physiologists have commonly assumed that the factors responsible for limiting the rise in $\dot{V}O_2$ during maximal exercise are primarily those affecting either, or both, blood flow and oxygen extraction (Mitchell *et* al 1958). The suggestion that systemic blood flow (*i.e.* $\dot{Q}$) limits $\dot{V}O_{2max}$ is largely derived from the strong linear relationship between maximal $\dot{Q}$ ($\dot{Q}_{max}$) and $\dot{V}O_{2max}$, where 5.9 (Astrand *et* al 1964) to 7.2 L of $\dot{Q}$ (Mitchell *et* al 1958) are required per litre of $\dot{V}O_{2max}$. Additionally, interventions that reduce $\dot{Q}_{max}$, including bed rest (Saltin *et* al 1968), β-blocker therapy (Pawelczyk *et* al 1992) and reduced blood volume (Krip *et* al 1997), are associated with lowering of $\dot{V}O_{2max}$. The apparent linkage between $\dot{V}O_{2max}$ and $\dot{Q}_{max}$ is strengthened by the observation that long-term aerobic training induced improvements in $\dot{V}O_{2max}$, are driven largely by a substantial improvements in $\dot{Q}_{max}$, with little adjustment in the (a-v)O_2diff, a finding confirmed by studies comparing trained persons versus their untrained counterparts (Ogawa *et* al 1992; Gledhill *et* al 1994; Stickland *et* al 2006). In a longitudinal endurance training study, Ekblom *et* al (Ekblom, & Hermansen

1968) reported significant improvements in $\dot{V}O_{2max}$ with a concomitant improvement in $\dot{Q}_{max}$ but not (a-v)O_2diff at maximal exercise.

Since maximal HR (HR_{max}) in athletes tends to be the same or lower (Rowell 1986) than that of untrained persons, it follows that any improvement in $\dot{Q}_{max}$ must arise from a similar magnitude increase in maximal SV (SV_{max}). Indeed, SV_{max} has been reported to be much higher in trained versus untrained humans (Ogawa *et* al 1992; Gledhill *et* al 1994; Zhou *et* al 2001; Stickland *et* al 2006) and following a period of endurance training (Ekblom, & Hermansen 1968). The enhanced SV_{max} in trained athletes has been shown to be largely due to enhanced diastolic function and compliance resulting in a large EDV (Levine *et* al 1991), with little or no change in ESV.

The concept underlying a circulatory limitation to $\dot{V}O_{2max}$ can be examined by assessing the cardiovascular responses to incremental and maximal exercise. As outlined in earlier sections, the $\dot{Q}$ and $\dot{V}O_2$ responses to submaximal incremental exercise have been well quantified and can generally be viewed as increasing linearly with increasing exercise intensity. However, above approximately 70% $\dot{V}O_{2max}$, the rate of $\dot{Q}$ increase slows (Stringer *et* al 2005; Trinity *et* al 2011) in proportion to the increase in $\dot{V}O_2$. At or near maximal exercise, $\dot{V}O_2$ often plateaus although the frequency of this phenomenon is debated (Doherty *et* al 2003). Nonetheless, the $\dot{V}O_{2max}$ achieved during an incremental test to exhaustion is commonly considered to represent the true $\dot{V}O_2$max (Levine 2008). Studies comparing the $\dot{V}O_2$ achieved during incremental exercise to exhaustion versus that measured during subsequent supramaximal exercise, have found no significant differences between the two values (Day *et* al 2003; Rossiter *et* al 2006; Foster *et* al 2007; Hawkins *et* al 2007; Brink-Elfegoun *et* al 2007b), indicating that $\dot{V}O_2$ is maximal prior to exhaustion during incremental exercise. The $\dot{Q}$ response to exercise approaching exhaustion has been shown to exhibit either a plateau (Mortensen *et* al 2005; Stringer *et* al 2005; Mortensen *et* al 2008) or continuous increase (Gledhill *et* al 1994; Ferguson *et* al 2001; Zhou *et* al 2001; Calbet *et* al 2007). Some of the variation between studies in $\dot{Q}$ responses to maximal exercise can be attributed to either participant conditioning

or measurement methods. However, measurement of $\dot{Q}$ during constant-load exercise to exhaustion reveals a decline in $\dot{Q}$ prior to exhaustion (Mortensen *et* al 2005; Mortensen *et* al 2008), coupled to a decrease in $\dot{V}O_2$ (Gonzalez-Alonso, & Calbet 2003; González-Alonso *et* al 2004). Taken together, these findings strengthen the concept of a circulatory limitation to $\dot{V}O_{2max}$. However, one must examine whether the apparent limitation in $\dot{Q}$, manifests as a reduction or plateau in locomotor blood flow during exercise to exhaustion.

Gonzalez-Alonso & Calbet (Gonzalez-Alonso, & Calbet 2003) looked to address whether locomotor blood flow limits $\dot{V}O_{2max}$ by examining participants during constant-load exercise eliciting $\dot{V}O_{2max}$ with and without heat stress. The findings of this study revealed a drop in $\dot{Q}$, MAP and leg blood flow (LBF) prior to exhaustion that was exacerbated by heat stress. Furthermore, as CaO_2 content remained stable throughout exercise, the drop in both systemic and locomotor blood flow reflected a reduced O_2 delivery to the working muscles that was the consequence of a falling SV. The decline in $\dot{Q}$ and LBF with normal core temperature paralleled a drop in both systemic $\dot{V}O_2$ and leg $\dot{V}O_2$, respectively. Similarly, Mortensen *et* al (Mortensen *et* al 2005) showed a decline in $\dot{Q}$ prior to exhaustion during constant-load maximal exercise, which was also attributed to a fall in SV. This study also incorporated an incremental cycle test to exhaustion and incremental one-legged knee extensor exercise. During the maximal cycle test, the rate of increase in LBF, $\dot{Q}$, O_2 delivery and consequently, leg $\dot{V}O_2$, was attenuated at higher exercise intensities, with plateaus reached at approximately 70-80% $\dot{V}O_{2max}$. In contrast, during incremental knee extensor exercise, LBF, O_2 delivery and leg $\dot{V}O_2$ increased linearly to exhaustion. The finding that the rate of increase in both systemic and locomotor blood flow is attenuated during large muscle mass exercise (cycling) compared to smaller muscle mass exercise (knee extensor exercise) agrees with studies published more recently (Mortensen *et* al 2008) and indicates that the circulatory system may be unable to sustain increasing blood flow as the intensity of exercise rises towards exhaustion. Interestingly, the later study by Mortensen *et* al (Mortensen *et* al 2008) also compared incremental cycle exercise to exhaustion with supramaximal cycling. A further key finding

was that $\dot{Q}$, LBF, systemic and locomotor blood flows were either the same or lower during supramaximal cycling, despite a greater workload. Likewise, studies comparing maximal and supramaximal (i.e. above the lowest power at which $\dot{V}O_{2max}$ is reached) whole-body exercise showed similar $\dot{V}O_2$ and $\dot{Q}$ between the two workloads (Brink-Elfegoun *et* al 2007b), which suggests that $\dot{Q}$ may also be maximal during exercise to $\dot{V}O_{2max}$ as no further increases are evident in workloads above this level.

The effects of a one-leg training model can also prove useful in determining whether LBF is constrained during two-legged/whole-body exercise (Saltin, & Calbet 2006). In a study by Saltin *et* al (Saltin *et* al 1976) one-leg endurance training increased the $\dot{V}O_{2peak}$ obtained during one-leg incremental exercise by 24%. Additionally, the untrained leg also improved by 6%. However, when incremental exercise was performed with both legs, $\dot{V}O_{2max}$ improved by only 11%. One would typically have expected two-legged $\dot{V}O_{2max}$ to improve by a magnitude close to the sum of the improvements in each leg individually. The observation that the improvement was less than expected suggests that insufficient perfusion during two-legged exercise may be the limiting factor for $\dot{V}O_{2max}$. To examine this further, Klausen *et* al (Klausen *et* al 1982) employed an 8-week one-leg training programme whilst measuring $\dot{V}O_2$, $\dot{Q}$ and LBF during one-leg and two-legged exercise following training. This study showed an improvement in one-leg and two-legged $\dot{V}O_{2max}$ by 19 and 11%, respectively. $\dot{Q}$ increased by 16 and 11%, respectively following training. However, leg blood flow increased by 22% following training in one-leg exercise but by only 1.4% during two-legged exercise. This data indicates that the leg blood flow during maximal two-legged exercise must be restricted by some mechanism, likely related to local vasoconstriction that operates to maintain MAP (Rowell 2004). The inability to sustain MAP with increasing locomotor blood flow suggests an insufficient circulation during maximal exercise.

The competition for available $\dot{Q}$ during exercise has been demonstrated experimentally in a number of studies aiming to 'challenge' the circulatory system under a range of conditions during exercise. Secher *et* al (Secher *et* al 1977) determined the circulatory effects of

superimposing arm exercise on leg exercise and vice versa. When arm and leg exercise was performed individually, $\dot{Q}$ was 14 L•min^{-1} and 21 L•min^{-1}, respectively. However, when arm exercise was superimposed onto leg exercise, $\dot{Q}$ rose only to 23 L•min^{-1}, whilst LBF declined from 12.4 L•min^{-1} to 10.5 L•min^{-1}. Despite an increase in leg (a-v)O_2diff from leg to combined exercise, leg $\dot{V}O_2$ also decreased slightly. This study indicates that LBF is constrained when the exercising muscle mass and metabolic demands are increased substantially. During this transition, MAP remained unchanged, indicating a potential neurogenic vasoconstrictor mechanism (Calbet, & Joyner 2010) in the exercising muscle that sacrifices blood flow to defend blood pressure. In a comprehensive study of blood flow to the legs and arms during cross-country skiing with highly-trained participants, Calbet *et* al (Calbet *et* al 2004) observed that arm blood flow during whole-body exercise was lower than that observed during maximal arm exercise alone. Moreover, the $\dot{Q}_{max}$ obtained in this study was lower than that expected on the basis of maximal limb blood flows observed during arm and leg exercise individually. The combined arm and leg maximal VC observed by Calbet *et* al exceed those obtained during whole-body maximal exercise, a constraint, which appears to defend a fall in MAP that would have occurred were the maximal VC of each limb reached during maximal whole-body exercise. Interestingly, the apparent competition for available $\dot{Q}$ during exercise does not seem to be limited to locomotor muscle groups. Increasing respiratory muscle work by an external obstruction has been found to reduce LBF during leg exercise (Harms *et* al 1997) but without any concomitant change in systemic $\dot{V}O_2$ or $\dot{Q}$ (Harms *et* al 1998), thus indicating a redistribution of $\dot{Q}$ during exercise when additional muscular work is applied.

During exercise involving a large muscle mass, where the potential hyperaemia is reported to exceed the pumping capacity of the heart (Andersen, & Saltin 1985; Calbet *et* al 2004; Calbet, & Joyner 2010), $\dot{Q}$ appears insufficient in its task of maintaining sufficient perfusion to the working muscles in addition to the core areas, including the myocardium and the central nervous system, without some constraint on the degree of exercise-induced vasodilation and distribution of blood flow. Previous studies have outlined a functional sympatholysis that

permits vasodilation in the active musculature in the face of increasing sympathetic activity with exercise (Calbet, & Joyner 2010). The evidence presented here that blood flow is restrained during large muscle mass exercise indicates a degree of sympathetic control that protects against hypotension during maximal exercise (Calbet *et* al 2004). However, the consequence of this mechanism is that exercise capacity and O_2 uptake are limited during high-intensity exercise, such that many notable authors have forwarded the view that, 'in healthy humans, $\dot{V}O_{2max}$ at sea level is limited by systemic oxygen delivery and especially by O_2 delivery to the locomotor muscles' (Saltin, & Calbet 2006).

O_2 transport pathways as the limiting factor for $\dot{V}O_{2max}$

In its most basic form, the Fick principle tends to support the view that $\dot{Q}$ limits $\dot{V}O_{2max}$, given that changes in $\dot{Q}_{max}$ account for most of the individual differences in $\dot{V}O_{2max}$ (Saltin, & Calbet 2006) and that the $\dot{V}O_2$ response to maximal exercise is proportional to alterations in O_2 delivery (Gonzalez-Alonso, & Calbet 2003). However, the pathway for O_2 from inspired air to skeletal muscle mitochondria represents an in-series transport system (Wagner 1996) where the O_2 flux at each step of the pathway must be the same, such that were one particular step to improve, so must all other steps for O_2 transport to improve. The capacity of mitochondrial respiration during exercise exceeds O_2 delivery (Boushel *et* al 2011). Therefore, any augmentation of O_2 transport should improve $\dot{V}O_{2max}$ (Wagner 2006). However, this viewpoint (Wagner 2006; Spurway *et* al 2012) argues that the heart is not the primary limiting factor, as forwarded elsewhere (Saltin, & Calbet 2006; Spurway *et* al 2012), but rather it is muscle O_2 conductance that dictates $\dot{V}O_{2max}$ (Wagner 1996; Wagner 2006). It is argued that raising $\dot{Q}$ alone reduces lung and muscle capillary transit time, resulting in a diffusion limitation that limits $\dot{V}O_2$. Any increase in $\dot{Q}$ must be accompanied by a similar improvement in muscle O_2 conductance for $\dot{V}O_{2max}$ to improve substantially. Despite some variation in the precise mechanisms, this viewpoint still supports the view that incremental exercise to $\dot{V}O_{2max}$ is limited by O_2 transport such that as exercise intensity rises, a healthy subject terminates

exercise at $\dot{V}O_{2max}$ because of severe functional alterations at the skeletal muscle, primarily due to a limitation in convective oxygen transport (Levine 2008).

The brain as the limiting factor for $\dot{V}O_{2max}$

An alternative view to those forwarded is one in which the brain plays a pivotal role in determining both $\dot{V}O_{2max}$ attained during incremental exercise testing and endurance exercise performance (Noakes 2000; Noakes, & St Clair Gibson 2004; Noakes *et* al 2005). This theory, termed the 'central governor model' (CGM) is built upon several 'creaking edifices' (Noakes 1997) in relation to both the testing for, and the factors limiting, $\dot{V}O_{2max}$.

The theory that cardiovascular limitations limit $\dot{V}O_{2max}$ proposes that exercise terminates due to the development of anaerobiosis in the active skeletal muscles that is the consequence of inadequate O_2 delivery (Bassett & Howley 1997; Bassett & Howley 2000). Subsequently, the measurement of a $\dot{V}O_2$ plateau during incremental exercise to exhaustion is considered a quantifiable parameter of the cardiorespiratory system's ability to maximally deliver O_2 that has achieved near-universal acceptance (Hawkins *et* al 2007). Despite this view, the plateau phenomenon is typically observed in less than 50% of incremental tests to exhaustion, and even less in highly trained endurance athletes (Doherty *et* al 2003), using either continuous or discontinuous protocols (Noakes, & St Clair Gibson 2004). Bassett & Howley (2000) contend that the evidence for a cardiovascular limitation to $\dot{V}O_{2max}$ does not rest entirely on the plateau in $\dot{V}O_2$ during maximal exercise. However, early work by Taylor *et* al (Taylor *et* al 1955) concludes that the existence of the plateau phenomenon proves that the cardiovascular system limits O_2 delivery and that skeletal muscle anaerobiosis limits maximal exercise performance. Indeed, many influential physiologists have used the existence of the plateau phenomenon to underpin the cardiovascular limitation theory (Mitchell *et* al 1958; Mitchell, & Blomqvist 1971; Wagner 1996). In challenging the classical theory, Noakes & St Clair Gibson (2004) argue that there is no logical reason to expect skeletal muscle anaerobiosis in the absence of a plateau. Furthermore, if the plateau phenomenon is the external marker of skeletal muscle anaerobiosis then it would be expected to occur in 100% of participants at exhaustion.

In a critical assessment of the classical cardiovascular limitation theory, Noakes & St Clair Gibson (Noakes, & St Clair Gibson 2004) provide several hallmark requirements for the theory to be accepted. Two of the most important with relevance to the testing for $\dot{V}O_{2max}$, are the requirements that $\dot{Q}$ must always be maximal at fatigue and that skeletal muscle anaerobiosis must develop at exhaustion during maximal exercise. A number of studies have shown a continuous, linear increase in $\dot{Q}$ up until exhaustion (Gledhill *et* al 1994; Krip *et* al 1997; Zhou *et* al 2001; Calbet *et* al 2007) without any evidence that $\dot{Q}$ has reached its maximum value. There appears to be minimal consensus on whether $\dot{Q}$ is maximal during exercise to exhaustion. In fact, the available evidence suggests that training adaptations enable endurance athletes to augment $\dot{Q}$ during maximal exercise (Gledhill *et* al 1994; Zhou *et* al 2001). Of particular interest to this discussion is the response of $\dot{Q}$ when O_2 availability is reduced both acutely and chronically, such as during conditions of hypoxia. Logically, one would predict that maximal $\dot{Q}$ should either remain the same as that measured during normoxia, or be even higher in an attempt to offset a reduced CaO_2. However, this may not be the case; Calbet *et* al (Calbet *et* al 2003) have previously shown that breathing 10.5% O_2 (equivalent to ~5300m altitude) caused a 17% reduction in peak $\dot{Q}$ compared to that achieved during normoxic maximal exercise. It can therefore be concluded that exercise in hypoxic conditions terminates at a submaximal $\dot{Q}$, a contradiction to the cardiovascular limitation theory (Noakes *et* al 2004). Furthermore, the maximal $\dot{Q}$ achieved under hypoxic conditions matches that measured at a similar absolute workload during normoxic exercise. Thus, there is little evidence from this data that the skeletal muscles, nor the heart, become hypoxic during exercise (Noakes, & St Clair Gibson 2004).

Whilst evidence from circulatory measurements during exercise provide conflicting evidence of the potential for skeletal muscle hypoxia/anaerobiosis during maximal exercise, studies examining sites of O_2 availability, including intracellular measurements of PO_2, have yet to provide evidence that skeletal muscle anaerobiosis develops during graded incremental exercise in humans. Richardson *et* al (Richardson *et* al 1998) examined lactate efflux and

intracellular muscle PO_2, determined by ^1H magnetic resonance spectroscopy (MRS), during single-leg extensor exercise to peak exercise, concluding that lactate efflux is unrelated to muscle cytoplasmic PO_2. The use of increasing lactate as an indicator of anaerobic metabolism during exercise is widely accepted, yet this data shows little evidence of skeletal muscle anaerobiosis in the presence of increasing lactate efflux. Likewise, Richardson *et* al (Richardson *et* al 2001) also concluded that from 50-60% of maximum work rate, intracellular PO_2, assessed by myoglobin desaturation, reaches a plateau that is then invariant with increasing work rate. Together, these findings conflict the traditional view that skeletal muscle anaerobiosis, due to inadequate O_2 delivery, limits exercise capacity. For this to be the limiting factor for exercise performance and $\dot{V}O_{2max}$, one should expect a progressive fall in intracellular oxygenation up until exhaustion during progressive incremental exercise. Perhaps one of the strongest arguments can be made for a central governor by examining the concept widely known as the 'lactate paradox' (West 1986) whereby blood lactate concentration is lower in acclimatised individuals exercising at altitude than that measured during exercise at sea-level. Why a marker of anaerobic metabolism should be lower in conditions of reduced O_2 availability is of great intrigue to those wishing to understand the processes leading of exercise-induced fatigue.

For fatigue during 'maximal' exercise to be the result of peripheral muscle fatigue resulting from the development of skeletal muscle anaerobiosis, to be true, Noakes & St Clair Gibson (2004) argue that there must be complete recruitment of all available motor units in the exercising limb. For if fatigue were to develop as the result of a metabolite-induced decline in contractile performance of individual muscle fibres, all other motor units must have already been recruited or otherwise contractile force would be sustained by the progressive recruitment of additional muscle fibres. These authors argue that, without maximal skeletal muscle recruitment, it remains possible that the proximate 'limitation' is the inability to recruit additional motor units (Noakes, & St Clair Gibson 2004). Indirectly supporting this interpretation is the well-documented disproportionate increase in surface EMG activity that occurs as an individual cross the anaerobic threshold during incremental exercise (Lucia *et* al

1999; Hug *et* al 2003). This observation shows that with the accumulation of fatiguing metabolites, EMG activity (*i.e.* skeletal muscle recruitment and activity) is increased, presumably to compensate for fatiguing motor units and thus maintaining exercise workload. Extending this knowledge to maximal exercise, it could reasonably be hypothesised that additional skeletal muscle recruitment should continue to occur with the progressive accumulation of fatigue that is predicted to occur at maximal exercise according to the cardiovascular limitation model.

During maximal exercise at altitude, fatigue occurs in the absence of complete motor unit recruitment (Kayser *et* al 1994) despite an accentuation of the O_2 delivery limitation by hypoxia. In fact, motor unit recruitment, inferred by integrated electromyographic (iEMG) activity, was lower at altitude that at sea level. Evidence from exercise trials in varying ambient temperatures (Tucker *et* al 2004), with changing arterial O_2 content (Amann *et* al 2006) and with pre-existing fatigue (Amann, & Calbet 2008) indicate that skeletal muscle recruitment is impaired during prolonged exercise, reaching <50% of that achieved during a maximal voluntary contraction. In the latter study, skeletal muscle activity (iEMG) correlated with CaO_2 such that activity was reduced during exercise in hypoxia compared with that with normoxia and hyperoxia. Likewise, during maximal exercise to exhaustion, exercise is terminated with submaximal skeletal muscle activity regardless of the duration of exercise required to reach exhaustion (Brink-Elfegoun *et* al 2007b). Together, these findings establish that fatigue in all forms of exercise occurs before the complete recruitment of skeletal muscle (Noakes 2012).

The critical analysis of the traditional view that exercise to exhaustion and $\dot{V}O_{2max}$ are limited by the cardiovascular system raises the question as to why exercise terminates without clear evidence of a maximal $\dot{Q}$ being attained and the active musculature being fully recruited? Data showing that cardiac function and skeletal muscle recruitment are both absolutely maximal and that homeostasis is lost at $\dot{V}O_{2max}$ would disprove the CGM (Noakes, & Marino 2009). Attempts to challenge the CGM have been made by groups comparing the circulatory (Brink-

Elfegoun *et* al 2007b) and neuromuscular (Brink-Elfegoun *et* al 2007a) responses to various exercise intensities eliciting $\dot{V}O_{2max}$. In their first study, Brink-Elfegoun *et* al (2007) showed that cardiac work, defined by calculation of the rate-pressure product, was higher during 'supramaximal' exercise (i.e. above that achieved during incremental exercise to $\dot{V}O_{2max}$) despite $\dot{V}O_2$ and $\dot{Q}$ remaining unchanged. Later, the same group showed that neuromuscular activity, defined using an EMG signal, also increased under similar circumstances without any concomitant change in $\dot{V}O_2$ (Brink-Elfegoun *et* al 2007a). Together, these findings suggest that at $\dot{V}O_{2max}$, cardiac work and neuromuscular activity increase with any further rise in work rate, despite the central governor model stating that muscle activation and, subsequently, cardiac work should be regulated at maximal exercise to avoid any significant loss of homeostasis. Noakes & Marino (2009) argue that the findings of Brink-Elfegoun *et* al (Brink-Elfegoun *et* al 2007a) are precisely those predicted by the central governor model; that $\dot{V}O_{2max}$ is achieved with a submaximal level of skeletal muscle recruitment and cardiac work, for in the trials performed at a lower intensity, yet still eliciting $\dot{V}O_{2max}$, there is evidence of reserve in both of these measurements. In support of this interpretation are the recent studies showing conventional methods for the assessment of $\dot{V}O_{2max}$ to give values *lower* than those achieved during modified testing protocols including a decremental exercise protocol (Beltrami *et* al 2012) and a self-paced protocol (Mauger 2013).

The CGM's function is not solely confined to regulation of exercise performance during the testing of $\dot{V}O_{2max}$ to exhaustion. Noakes (Noakes, & St Clair Gibson 2004; Noakes 2011; Noakes 2012) challenges the cardiovascular model to explain two common observations from human exercise performance: the pacing of exercise that appears the result of an anticipatory component and the increase in speed near the end of exercise, the so-called end-spurt. Neither of these performance characteristics can be explained by the cardiovascular model of exercise performance, which dictates that metabolites accumulating in the muscle are the causes of fatigue. If this were the case, Noakes (Noakes 2012) argues that exercise would commence at an unsustainable pace and then slow as these metabolites accumulate. Likewise, if exercise

performance were dictated as defined by the cardiovascular model, the 'end-spurt' would be a physiological impossibility, as metabolite-induced fatigue would prevent such an occurrence.

Briefly, the CGM proposes that the brain is central to the regulation of exercise, a view that is shared by neuroscientists examining fatigue who state that the mechanism(s) of muscle fatigue are 'not only because of peripheral changes at the level of the muscle but also because the central nervous system fails to drive the motoneurons adequately' (Gandevia 2001). Prior to exercise, the CGM proposes that feedforward control of central motor output determines the extent of skeletal muscle recruitment based on a number of factors including, but not limited to, knowledge of the exercise duration (Ansley *et* al 2004), prior experience (Mauger *et* al 2009), and the biological state of the athlete (Hettinga *et* al 2011). The commencement of exercise follows at an intensity that is deemed appropriate given the expected exercise duration. The chosen exercise intensity is always at a submaximal level of skeletal muscle recruitment (Amann *et* al 2006; Swart *et* al 2009). From herein, the exercise pace/intensity is continuously modified (Tucker *et* al 2006) by feedback from conscious sources including knowledge of the distance covered (Faulkner *et* al 2011) and the exercise endpoint (Swart *et* al 2009). In addition, subconscious feedback from a variety of sources contributes to the pacing of exercise, including feedback relating to arterial (Noakes & Marino 2007) and cerebral oxygenation (Rasmussen *et* al 2010), muscle glycogen stores (Lima-Silva *et* al 2010) and information relating to heat accumulation (Tucker *et* al 2006), amongst others. Based on these findings, the CGM dictates that human exercise performance is regulated to ensure that homeostasis is maintained and that no catastrophic biological failure can occur (Noakes, & St Clair Gibson 2004; Noakes 2012). In relation to testing for $\dot{V}O_{2max}$, skeletal muscle recruitment is regulated to avoid any such catastrophic failure, thus subsequently regulating the peak $\dot{Q}$ and $\dot{V}O_2$ that can be attained during incremental testing to exhaustion (Noakes 2008).

Summary

Almost a century following the initial evaluation of the cardiopulmonary responses to exercise, there is still little resolution regarding the factors governing short-term exercise performance.

The two primary models, the cardiovascular/anaerobic model and the Central Governor Model are still widely investigated by their respective supporters and opponents. This review of literature (part I) highlights the documented cardiovascular responses to exercise, as well as the opposing models on the limiting factor for $\dot{V}O_{2max}$ where either convective O_2 transport or the brain are the primary determinants of the maximal $\dot{V}O_2$ achieved during exercise to exhaustion.

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PART II

Methods of cardiac output assessment during exercise.

Introduction

The ability to measure cardiac output ($\dot{Q}$) during exercise offers important insight into the dynamics of blood flow and O_2 delivery. This has resulted in $\dot{Q}$ measurement being used to investigate topics ranging from limitations of maximal exercise capacity in athletes (Brink-Elfegoun *et* al 2007; Mortensen *et* al 2008) to the assessment of cardiac function in clinical patients with heart disease (Metra *et* al 1999; Bhella *et* al 2011; Jakovljevic *et* al 2010; Rosenblum *et* al 2010). However, measurement of $\dot{Q}$ during exercise is often difficult and complicated by inconsistent agreement between methods. Currently, there is no universally preferred technique for the measurement of $\dot{Q}$ during exercise although several invasive and non-invasive methods have been developed and used during submaximal and/or maximal exercise. The validity and reliability of many of these approaches have been evaluated elsewhere (Warburton *et* al 1999a; Warburton *et* al 1999b). However, in the last decade considerable advances have been made, with the development of new methods and the improvement of existing methods.

Evaluating methods of $\dot{Q}$ assessment

Gold standard for measuring $\dot{Q}$ during exercise

Ideally a measurement technique of $\dot{Q}$ during exercise would show good agreement with accepted methods, have a high resolution (i.e. track $\dot{Q}$ throughout exercise on a beat-by-beat basis) and be non-invasive. In addition, the measures would be precise and resistant to measurement error induced by exercise-related haemodynamic changes. Currently no such technique exists and the accuracy of methods in measuring $\dot{Q}$ is usually assessed against the 'gold standard' methods of direct Fick, dye-dilution and/or thermodilution (Warburton *et* al 1999a). Although these methods themselves are susceptible to measurement error during exercise (Warburton *et* al 1999a), a lack of alternative methods has ensured they continue to provide the standard against which all other methods are evaluated.

Validity & Reliability

Bias

Validity and reliability may be determined using bias and precision statistics (Bland & Altman 1986; Cecconi *et* al 2009). Validity, assessed by determining the bias between a method and a comparator, quantifies the agreement between an observed $\dot{Q}$ and the criterion method value. In this context, the 95% limits of agreement (± 2 Standard deviations (SD) of the mean bias) can be calculated to show the inter-subject variability in bias between two methods. Limits of agreement (LoA), expressed as a percentage of the mean $\dot{Q}$, within 30% are deemed acceptable (Critchley & Critchley 1999) representing low inter-subject variability. This review will adhere to the established manner of evaluating the validity of novel techniques in measuring $\dot{Q}$ during exercise; measurement error of new methods will be quantified by calculating the mean bias between the novel method and a standard method, expressed as a percentage of the standard value (see table 1).

Precision

Precision quantifies the random error of measurements and describes a method's ability to detect change in the variable it is measuring. Put simply, precision shows how close repeated measurements are to each other. Precision is calculated from the SD of repeated measurements (Hopkins 2000). Additionally, SD can be used to quantify the coefficient of variation (CV), where CV = SD/mean. This measurement serves to standardise the variability in repeated measures in relation to the mean $\dot{Q}$, which is particularly useful in exercise studies where the range of $\dot{Q}$ measured can be quite large compared to clinical studies. Defined as a percentage, precision can be presented as coefficient of error (CE) / 2, where CE = CV/$\sqrt{n}$ and n = number of replicates.

Least significant change

The least significant change (LSC) permits the judgment of whether an actual change in $\dot{Q}$ has occurred (95% confidence) or whether that change is the result of measurement variability. The LSC can be calculated using the following equation (Cecconi *et* al 2009):

LSC ($\dot{Q}$) = 1.96 CE x $\sqrt{2}$, or;

LSC ($\dot{Q}$) = 1.96 (CV/$\sqrt{n}$) x $\sqrt{2}$

Figure 1 depicts LSC determined by the CV of a method and the number of measurement replicates (Cecconi *et* al 2009). Exercise physiologists should carefully evaluate the precision of a method. For example, where a change in $\dot{Q}$ of ~10% is projected as the result of an exercise intervention, the CV of duplicated $\dot{Q}$ measurements should be ≤5% in order to be 95% confident that a recorded change of 10% is real or due to measurement artefact.

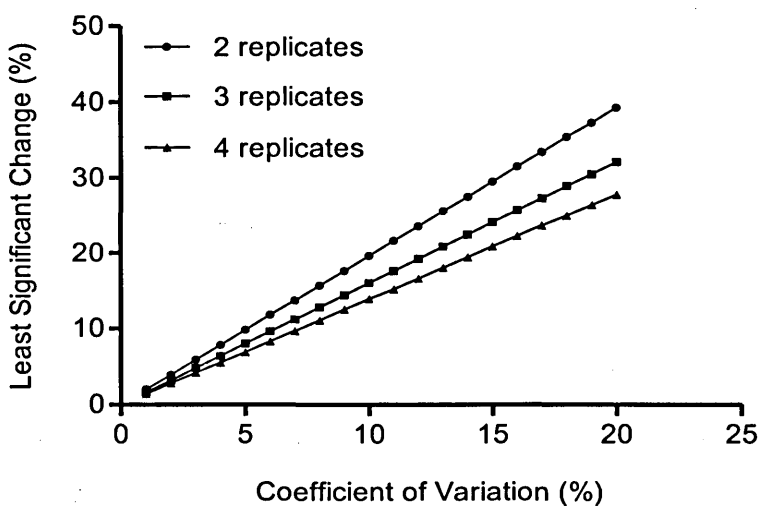

Figure 1. Relationship between the coefficient of variation (CV) of a method and the resulting least significant change (95% confidence) according to the number of replicates performed to determine CV.

While the method outlined above is statistically rigorous, employing 95% as a decision limit may be overly stringent in the exercise sciences, particularly in highly trained individuals

where physiological changes are typically small (Hopkins 2000). For example, a method with a CV of 5% (LSC = ± 10%, figure 1) indicates that for a measured increase of 10%, there is 97.5% probability that $\dot{Q}$ actually improved. Hopkins (2000) argues that half the 95% confidence intervals is a more realistic threshold for action such that, in the above example with a CV of 5%, a change of +7.8% would have 84% probability of an actual improvement. It is clear that the precision of a method is critical in drawing conclusions from changes in $\dot{Q}$.

Techniques for measuring $\dot{Q}$ during exercise

Direct Fick Measurement

The direct Fick method requires that measurement of O_2 concentration of both arterial (CaO_2) and mixed venous ($C\dot{V}O_2$) blood be obtained alongside measurement of $\dot{V}O_2$ during steady-state conditions. $\dot{Q}$ can subsequently be calculated according to the Fick equation, where:

$$\dot{Q} = \frac{VO_2}{CaO_2 - CvO_2}$$

In practice, measurement of both arterial and mixed-venous O_2 concentration requires invasive approaches. Arterial blood sampling requires direct cannulation of an artery whilst mixed-venous blood sampling demands pulmonary artery catheterisation (PAC), where a true measurement of mixed venous O_2 can be obtained without being susceptible to local variances in venous O_2 concentration.

The direct Fick method requires that measurements be obtained during a period of steady state (i.e. haemodynamic stability). Under such circumstances, direct Fick should provide exact measures of $\dot{Q}$. However, the measurement accuracy of the Fick method is largely influenced by the ability of the researcher and the participant to maintain a steady state (Warburton *et* al 1999a). Previous studies (Holmgren & Pernow 1960) have shown good reproducibility for this technique during supine mild exercise (CV = 5.2%). However, during vigorous to maximal exercise, where steady state conditions are seldom achieved, the accuracy and

reproducibility of the direct Fick method may be limited. Furthermore, the complex requirements for blood gas measurement and the inherent risk associated with PAC (Scheer *et* al 2002) render the direct Fick method largely unsuitable for the typical exercise physiology laboratory in the assessment of healthy participants. However, despite the apparent limitations, the direct Fick method remains the method of choice for a number of groups examining $\dot{Q}$ during both submaximal and maximal exercise (Calbet *et* al 2004; Mortensen *et* al 2005; Mortensen *et* al 2008).

Dye-Dilution Method

The dye-dilution method requires the intravenous bolus administration of a dye through a PAC or central venous line inserted proximal to the right atrium. Injection of the dye is followed by a rapid infusion of saline to ensure the full delivery of the dye into the venous circulation. The concentration of dye in arterial blood, once it has passed through the heart, is sampled through a densitometer for the calculation of a dye concentration-time curve. $\dot{Q}$ can subsequently be calculated by dividing the injected dye volume by the area under the concentration-time curve, such that a rapid rise and disappearance of the dye-dilution curve indicates a larger $\dot{Q}$.

The dye-dilution method is a relatively simpler approach to direct Fick measurement, primarily as direct cardiac catheterisation is not an absolute requirement. However, central venous cannulation remains an invasive approach for the determination of $\dot{Q}$ during exercise that possibly restricts its applicability for healthy participants. Nonetheless, measurement of $\dot{Q}$ with this approach has been widely employed to record $\dot{Q}$ and SV across a range of exercise intensity domains. Indeed, many of the early hallmark studies documenting the haemodynamic responses to submaximal and maximal exercise in humans incorporated this technique (Mitchell *et* al 1958; Chapman *et* al 1960; Astrand *et* al 1964; Tabakin *et* al 1964; Rowell *et* al 1966; Ekblom & Hermansen 1968; Hermansen *et* al 1970), whilst more recent studies continue to investigate the demands of exercise on the cardiovascular system using the dye-dilution method (Gonzalez-Alonso & Calbet 2003; Calbet *et* al 2007).

Thermodilution Method

The thermodilution method for the measurement of $\dot{Q}$ is based on the same principle as dye-dilution, except that instead of a dye injectate, a cold fluid is injected into the right atrium causing a decrease in blood temperature, which is measured by a thermistor placed in the pulmonary artery. Thermodilution is generally considered the clinical reference technique (Prabhu 2007) and offers several advantages over other invasive methods, including the potential for multiple consecutive measurements, as the marker is innocuous in the absence of any recirculation (Branthwaite & Bradley 1968; Warburton *et* al 1999a). Notable disadvantages include the potential loss of coolant through handling of the syringe, through the catheter and/or through the blood vessel wall (Mackenzie *et* al 1986).

Despite its common acceptance as a 'gold standard' measure in clinical practice, thermodilution has been reported to overestimate $\dot{Q}$ during rest (van Grondelle *et* al 1983) and light exercise (Espersen *et* al 1995) thus leaving a question mark over its place as an appropriate reference method. However, a more recent study (Jarvis *et* al 2007) showed thermodilution to agree closely with direct Fick at rest and during maximal exercise, but not submaximal exercise, where it tended to underestimate $\dot{Q}$. The authors concluded that, despite the potential disadvantages of thermodilution, the difference observed during submaximal exercise was more likely due to erroneous direct Fick measurements, particularly as CaO_2 measurements were determined by pulse oximetry rather than direct arterial measurements. At submaximal exercise levels, where the (a-v)O_2diff is relatively small, blood gas measurement error can be considerably detrimental to subsequent $\dot{Q}$ calculations (Jarvis *et* al 2007).

Gas Rebreathing Methods

Cardiac output measurement by foreign gas techniques is based on the principle that particular inert soluble gases enter the bloodstream from the lungs down the concentration gradient via rapid diffusion across the pulmonary blood-gas barrier. Diffusion of the gas is perfusion limited such that their rate of disappearance is directly proportional to pulmonary blood flow, which, in the absence of shunts, is equivalent to $\dot{Q}$. Foreign gas techniques can be broadly

described as either closed circuit (those requiring rebreathing) or an open circuit; traditionally, the most common gases used in these techniques are carbon dioxide (CO_2) and acetylene (C_2H_2).

Indirect Fick

Adolph Fick proposed that, using O_2, the principle of conservation of mass could be applied to the measurement of $\dot{Q}$, where the difference between the amount of O_2 in arterial blood and mixed venous blood, respectively, must be equal to the amount of oxygen being consumed. This approach is commonly known as the direct Fick method, where oxygen consumption is recorded by expired gas analysis and both arterial and mixed venous O_2 concentrations can be obtained by cannulation of a peripheral artery and the pulmonary artery respectively. Theoretically, by substituting O_2 with CO_2, and thus avoiding the potential complications of central catheterisation, $\dot{Q}$ can also be calculated according to the equation:

$$\dot{Q} = \frac{\dot{V}CO^2}{CvCO_2 - CaCO_2}$$

Sun *et al* (Sun *et* al 2000) compared estimates of $\dot{Q}$ using both O_2 (*i.e.* traditional direct Fick) and CO_2 as test gases. During incremental exercise in five participants, they found no error measurement between protocols supporting the principle of using CO_2 in a modified Fick equation. Significantly, by using CO_2 as the test gas, $\dot{Q}$ can be estimated non-invasively. Arterial CO_2 can be estimated by measuring end-tidal CO_2 in expired air, removing the necessity for arterial blood sampling. Mixed venous PCO_2 can be derived using either the equilibrium method (Collier 1956) or the exponential method (Defares 1958). Briefly, the equilibrium method begins with an initial high CO_2 in the rebreathing bag, which the subject rebreathes until a CO_2 equilibrium in the bag is reached. The equilibrium point is taken to represent mixed-venous PCO_2. The exponential method employs a low initial CO_2 concentration in the rebreathing bag that rises exponentially during rebreathing towards an asymptote that represents P_aCO_2. This approach is more practical during strenuous exercise, due to the reduced breathing discomfort that is associated with breathing a high concentration of CO_2, as per the equilibrium method. The exponential and equilibrium methods are two-step

methods; both require a measurement of steady-state gas exchange for the determination of VCO_2 before rebreathing commences. To overcome this Olszowka (Olszowka *et* al 2004) has developed a modified single-step method, based on an earlier single-step method (Farhi *et* al 1976), to minimise measurement duration and the reliance on CO_2 equilibration during rebreathing. Briefly, following expiration participants are switched to a CO_2-free gas containing 50% oxygen, 40% nitrogen and 10% argon for 15-25s rebreathing during exercise. Bag volume is determined so that end-tidal PCO_2 remains below that recorded during the last breath prior to rebreathing for 3-4 breaths during the manoeuvre. Using this method, $\dot{Q}$ is calculated according to the equation:

$$\dot{Q} = \left(\frac{\Delta \dot{V}CO^2}{CvCO_2 - CcCO_2}\right)(1/T)$$

where ΔVCO_2 is the amount of CO_2 added to the bag, $CcCO_2$ is the mean concentration of pulmonary end capillary CO_2 concentration during time T calculated using PCO_2 values recording during T. T is the time taken for PCO_2 to return to that recorded in the last breath prior to rebreathing.

The exponential CO_2 rebreathing method has shown good validity compared with dye dilution (Ferguson *et* al 1968), direct Fick (Reybrouck *et* al 1978) and thermodilution (Beekman *et* al 1984) during exercise (see (Warburton *et* al 1999a) for a complete review). The exponential method has demonstrated good reproducibility, particularly at high intensities, during incremental treadmill exercise in healthy participants (Cade *et* al 2004; Jakovljevic *et* al 2008). Jakovljevic *et* al (2008) did observe an overestimation of $\dot{Q}$ at rest, although agreement between the exponential method and an inert gas rebreathing method (see section 1.4) during exercise was good. Vanhees *et* al (Vanhees *et* al 2000) evaluated exponential and equilibrium methods of CO_2 rebreathing during exercise in 12 healthy males. Resting $\dot{Q}$ derived from the exponential method was significantly higher than the equilibrium method (supporting Jakovljevic's observations) and the equilibrium method showed greater reproducibility at rest. During exercise, reproducibility improved for both methods with increasing exercise intensity and both produced comparable values. Notable, however, was the greater frequency of

unpleasant side effects associated with the equilibrium method. More recently, Jarvis *et* al (Jarvis *et* al 2007) compared the single-step CO_2 rebreathing method to direct Fick, thermodilution and C_2H_2 rebreathing during submaximal and maximal exercise. They reported that single-step CO_2 rebreathing shows closer agreement with direct Fick and thermodilution than open-circuit C_2H_2 methods at both intensities.

Current evidence suggests that CO_2 rebreathing is a reproducible method for determining $\dot{Q}$ during exercise. Nonetheless, there are several limitations associated with these methods including the inability to record $\dot{Q}$ continuously and the potential effects of rebreathing manoeuvres on $\dot{Q}$ and subject discomfort during maximal exercise.

Acetylene (C_2H_2)

Closed-Circuit

Acetylene rebreathing methods have been reported to significantly underestimate $\dot{Q}$ during both submaximal and maximal exercise (Jarvis *et* al 2007). This is probably due to the reduced recirculation time of the rebreathing mixture, due to the increased blood flow. Previous reviews (Warburton *et* al 1999a; Warburton *et* al 1999b) have outlined the agreement between C_2H_2 rebreathing techniques with dye-dilution (8-12% mean bias) (Smyth *et* al 1984) and direct Fick (8% mean bias) (Liu *et* al 1997) during exercise. Whilst the closed circuit method has been widely used in exercise studies, open-circuit methods may offer several advantages; 1) increased subject comfort; 2) increased ease of measurement for both experimenter and subject; and 3) fewer perturbations in the partial pressure of arterial oxygen (PaO_2) and carbon dioxide ($PaCO_2$) content (Bell *et* al 2003).

Open-Circuit

Barker *et* al (Barker *et* al 1999) proposed a technique requiring participants to breathe from an open-circuit and measuring C_2H_2 uptake in a short-term, steady-state. In this non-rebreathing manoeuvre, participants breathe via a one-way valve from a gas containing 1% C_2H_2, 5% helium (He), 20.9% O_2, and 73% nitrogen (N_2). Concentrations of exhaled C_2H_2 and He are

recorded continuously for 20-25 breaths; along with minute ventilation (V_E) end-tidal (Alveolar, P_ACO_2) and mixed expired (P_ECO_2) PCO_2 from the breath-by-breath gas analysis, from which $\dot{Q}$ is subsequently calculated.

Barker's method compared favourably with direct Fick during exercise at 90% $\dot{V}O_{2max}$ in a small cohort of trained participants (Barker *et* al 1999). Furthermore, values obtained during exercise in 24 participants showed good construct validity and closely matched expected values based upon previously published data (Barker *et* al 1999). Subsequent studies have also demonstrated an acceptable test-retest reliability (CV < 4%) during intensive exercise (Dibski *et* al 2005).

Two alternative open-circuit methods were compared with direct Fick measurements of $\dot{Q}$ at-rest and during moderate through heavy exercise in six healthy participants (Johnson *et* al 2000). Both methods are advantageous in that they permit spontaneous breathing whilst the wash-in of C_2H_2 and He is conducted over eight breaths. The two methods differ in their algorithms for calculating $\dot{Q}$ (described elsewhere (Johnson *et* al 2000). Briefly, method one simplified the assumptions in predicting soluble gas uptake into the capillary during the breath cycle by including linear interpolation of end-expiratory gas measurement, to determine alveolar gas concentration during inspiration, and a basic assumption that the driving pressure for diffusion is constant during inspiration and expiration (Stout *et* al 1975; Gan *et* al 1993). Method two eliminated the need for assumptions based on the soluble gas uptake at the capillary during the breath cycle, primarily by using each data point acquired at 8 ms intervals to calculate alveolar gas uptake (Johnson *et* al 2000). Comparison of the two C_2H_2 methods and direct Fick were made at workloads ranging from 20 - 85% of peak power. Agreement between each respective C_2H_2 method and direct Fick at rest was good (% error = 1.8%). During moderate exercise, there were minimal differences between the C_2H_2 methods, although method one significantly underestimated $\dot{Q}$ at the highest exercise intensities. Close agreement was observed between method two and direct Fick throughout exercise (% bias = <3%). Both C_2H_2 methods showed good within-test and test-retest reproducibility throughout

exercise, comparing favourably to direct Fick. Further studies have supported the reliability of method two measurements during exercise in both young and old adults as well as confirming agreement with the closed circuit method (Bell *et* al 2003); this method has been widely used for the measurement of $\dot{Q}$ under a variety of resting (Charkoudian *et* al 2005) and exercising conditions (Beck *et* al 2006; Ridout *et* al 2010).

Novel Methods

Since the comprehensive Warburton reviews over a decade ago (Warburton *et* al 1999a; Warburton *et* al 1999b), several new approaches to inert gas rebreathing have been developed to obtain measures of $\dot{Q}$ during exercise. The earlier methods required a mass spectrometer, which is bulky, expensive and difficult to maintain. More recently, an infrared photoacoustic gas analyser (Innocor™, Innovision A/S, Denmark) has been developed, which measures the concentration of an inert tracer gas in the expired air. This method requires participants to rebreathe a gas mixture comprising 0.5% N_2O, 0.1% SF_6, 28% O_2 and the balance N_2, for approximately 30s at a volume equivalent to 30-40% of vital capacity. Breathing frequency is controlled at 20 breaths•min^{-1} during the rebreathing manoeuvre. Expired air is sampled continuously from the mouthpiece and $\dot{Q}$ is determined from the rate of N_2O disappearance (Becklake *et* al 1962).

Several studies have evaluated the Innocor™ inert gas rebreathing system (IGR) during exercise. In heart failure patients, IGR compares favourably with direct Fick and proved as reliable as thermodilution (Gabrielsen *et* al 2002). During incremental exercise in a similar cohort (up to ~ 11 L•min^{-1}), IGR showed close agreement with thermodilution and direct Fick, as well as high test-retest reliability (Agostoni *et* al 2005). In healthy participants, IGR showed good reliability and agreement with the exponential CO_2 rebreathing method (Jakovljevic *et* al 2008), thus supporting its use as a practical approach to non-invasive measurement of $\dot{Q}$ during exercise. Furthermore, IGR measurements of $\dot{Q}$ have been implemented in a range of exercise studies assessing healthy (Elliott *et* al 2010), cardiac

(Lang *et* al 2007; Jakovljevic *et* al 2011) and neurological (Jakovljevic *et* al 2012) populations.

Whilst IGR performs well against other methods, there are several disadvantages. Firstly, measurement of $\dot{Q}$ occurs over a 30s period, thus reducing the time resolution for $\dot{Q}$ monitoring. Secondly, the rebreathing manoeuvre occurs under tightly controlled conditions in which breathing rate is prescribed and tidal volume is regulated by the requirement to completely empty the rebreathing bag. These conditions may alter cardiac output or create a degree of breathing discomfort at maximal exercise intensities. Nonetheless, results obtained so far using this method are encouraging.

Doppler Echocardiography

Doppler echocardiography (DE) estimates SV from blood velocity and aortic cross-sectional area. This approach is non-invasive, less demanding on the subject and does not require cardiovascular steady-state (Rowland & Obert 2002). Briefly, the Doppler technique uses ultrasound waves emitted from a transducer at a fixed frequency, which are then reflected back at a different frequency; the relative change (or shift) in frequency is dependent on the blood flow velocity. Flow velocity can be calculated according to the following equation:

$$V = \frac{c(fr-ft)}{2ft(\cos\Phi)}$$

where V is velocity of blood, c is the speed of sound, f_r is the received frequency, f_t is the transmitted frequency, and Φ is the angle between the ultrasound beam and the direction of blood flow. The resulting Doppler signal is displayed as a plot of blood flow over time, with calculation of the resulting velocity-time integral (VTi) being used to determine SV according to the equation:

$$SV = VTi \times A$$

where A represents the cross sectional area of the aortic root, determined typically by echocardiography.

Previously, DE has been compared with several invasive comparators during exercise (Warburton *et* al 1999b) with mixed results regarding agreement during high intensity exercise. Despite these early findings, relatively few studies have since attempted to further determine the accuracy of DE to other accepted techniques for measurement of $\dot{Q}$ during exercise. Construct validity of DE has been determined by assessing the relationship between DE-derived $\dot{Q}$ and oxygen consumption ($\dot{V}O_2$) at different exercise intensities. Nottin *et* al (Nottin *et* al 2002) reported the slope of the $\dot{Q}$-$\dot{V}O_2$ equation (Table 2) to be similar (4.58) to that observed using direct Fick (4.6) (Johnson *et* al 2000) and dye dilution (4.82) (Smyth *et* al 1984). Furthermore, Vinet *et* al (Vinet *et* al 2001) reported values for the slope comparable to those obtained by open-circuit C_2H_2 methods (6.67 and 6.12, respectively) (Barker *et* al 1999; Dibski *et* al 2005) and CO_2 rebreathing (6.18) (Dibski *et* al 2005). Whilst this approach to assessing validity has been widely used, the range of values observed both within and between techniques limits its usefulness in determining accuracy.

The reliability of DE has been established in both children and adults during exercise. Rowland *et* al (Rowland *et* al 1998) showed good reliability in young adult males during maximal exercise (CV ~ 8%) for both $\dot{Q}$ and SV measurements. Nottin *et* al (Nottin *et* al 2000) found greater reproducibility for DE (CV = 5.2%) during maximal exercise in children, than for CO_2 rebreathing (CV = 11.7%]). Likewise, Vinet *et* al (Vinet *et* al 2001) concluded that DE is a reproducible method for determining $\dot{Q}$ and SV during exercise in both children and young adults. These consistent findings suggest that DE is a reliable method for assessing $\dot{Q}$.

It must be noted that traditional DE does not support continuous measurement of $\dot{Q}$ during exercise; however, recent technical developments suggest a continuous device may be feasible during exercise (Knobloch *et* al 2008; Knobloch *et* al 2009). More studies are required to determine the accuracy and reliability of this approach.

Whilst DE appears to be a reasonable method to employ for measuring $\dot{Q}$ during exercise, there are several potential sources of error and limitations that should be considered. Firstly,

transducer angulation, site of aortic cross-sectional measurement, changes in aortic diameter and non-uniformity of blood flow velocity are all possible causes of measurement error during exercise (Rowland & Obert 2002). Secondly, DE requires skilled operators for valid and reproducible measurements. Furthermore, obtaining measurements during exercise where there is body movement can be technically challenging.

Impedance Cardiography

Impedance cardiography (IC) is based on the assumption that thoracic aortic blood flow alters baseline thoracic impedance (Z_o), expressed as dZ/dt. IC operates by the emission of a low-voltage (2.5 - 4 mA), high frequency (70–100 kHz) alternating current through the thorax by two transmitting electrodes, subsequently recorded by two receiving electrodes. Changes in transthoracic electrical impedance are assumed to represent SV. SV can be calculated according to either the Kubicek (Kubicek *et* al 1970) or Sramek-Bernstein (Sramek *et* al 1983) formulas.

The Kubicek method makes five key assumptions; 1) the thorax acts as a cylinder; 2) the cylinder is homogenously perfused with blood of specific resistivity that varies with haematocrit; 3) the cylinder has steady-state mean base impedance; 4) the pulsatile variations in thoracic aortic blood flow cause pulsatile decreases in thoracic impedance; and 5) the ejection of blood assumes a square-wave pattern (Jensen *et* al 1995). SV is calculated as:

$$SV = p \bullet L^2/Z_o^2 \bullet (dZ/dt)_{max} \bullet LVET$$

where p is the resistivity of blood ($\Omega\bullet$cm), L is the distance between recording electrodes (cm), $(dZ/dt)_{max}$ is the maximum rate of change in the impedance signal during systole ($\Omega\bullet$cm), Z_o is baseline impedance (Ω) and LVET is left ventricular ejection time (sec).

The validity and reliability of IC using the Kubicek approach has been demonstrated in exercise studies using invasive comparators (Denniston *et* al 1976; TEO *et* al 1985). Although initial studies were encouraging, a number of concerns have been raised regarding the Kubicek

method for determining $\dot{Q}$, including the assumption that the thorax acts as a cylinder, difficulty determining p, and inconsistency in the measurement of L (Jensen *et* al 1995).

Sramek *et* al (Sramek *et* al 1983) advanced this approach by modelling the thorax as a truncated cone, such that SV can be calculated from:

$$SV = VEPT \bullet LVET \bullet \frac{\left(\frac{dZ}{dt}\right)max}{Z_0}$$

where VEPT (the volume of electrically participating tissue) = $(0.17 * \text{height})^3 / 4.3$ and LVET is left ventricular ejection time (sec).

Bernstein (Bernstein 1986) subsequently modified this formula to accommodate a correction factor (δ) for body habitus (i.e. ectomorph, mesomorph and endomorph) and body weight. Validation studies in healthy and clinical populations at rest using this approach were mixed (Jensen *et* al 1995). In patients with ischemic cardiomyopathies, Belardinelli *et* al (Belardinelli *et* al 1996) reported agreement between IC and both direct Fick and thermodilution during exercise. Similarly, Scherhag *et* al (Scherhag *et* al 2005) assessed IC versus thermodilution during exercise in patients with suspected coronary heart disease, showing a mean bias of 11.4 ± 29% during exercise, suggestive of an overestimation of $\dot{Q}$ measured by IC. However, a number of concerns have been raised regarding the Sramek-Bernstein formula, including the reliance on subject stature and mass for the calculation of L and potential gender differences regarding thorax size and body fat composition, which may explain the poor accuracy reports in many studies.

Physioflow™

More recently, a new IC device, the Physioflow™ (Manatec Biomedical, Macheren, France), has been developed. Alongside hardware improvements to boost signal-processing, modified algorithms eliminate the need for basal thoracic impedance (Z_O) or blood resistivity (p) measurements (Charloux *et* al 2000) as required in IC. In addition, exact electrode positioning is no longer crucial.

Several studies have examined Physioflow (IC_{pf}) validity during exercise. In forty respiratory patients, IC_{pf} closely correlated direct Fick measurements at rest and during low to moderate exercise ($r = 0.85$). During mild exercise, the mean difference in $\dot{Q}$ was 2.5% with reasonable limits of agreement of ±23.5%. During incremental exercise in healthy participants, Richard *et* al (Richard *et* al 2001) observed a correlation coefficient (r) of 0.94 for IC_{pf} versus direct Fick. The mean difference between the two techniques (IC_{pf} – Fick) was 2.8% (limits of agreement ± 24.2%). However, studies by Bougault *et* al (Bougault *et* al 2005) and Kemps *et* al (Kemps *et* al 2008) have shown systematic overestimation of $\dot{Q}$ in COPD (25-31% mean bias) and heart failure (HF) patients (42-53%) when compared to direct Fick during exercise. Both of the latter studies concluded that IC_{pf} is unsuitable for measuring $\dot{Q}$ during exercise in these patients. Whilst altered/oscillatory breathing experienced by both COPD and HF patients may influence $\dot{Q}$, perhaps a more likely candidate is overestimation of SV during the autocalibration procedure associated with this device (Kemps *et* al 2008). Evidence for the latter can be found in the data of Kemps *et* al (2008) who showed a relatively consistent bias throughout exercise, thus indicating that some factor prior to exercise led to the observed overestimation.

The reliability of IC_{pf} has also been evaluated during exercise in healthy adults and children. Richard *et* al (Richard *et* al 2001) assessed IC_{pf} reproducibility in repeated incremental exercise tests in adults. The mean difference between measurements was small (0.009 $L \bullet min^{-1}$), although the limits of agreement were wide (± 31%, approximately), which the authors attributed to the incremental test design where 1-minute steps were implemented. Welsman *et* al (Welsman *et* al 2005) compared maximal $\dot{Q}$ obtained from three tests to exhaustion in children aged 10-11 years. The CV was 9.3%, suggesting that IC_{pf} has acceptable reliability in this population.

Whilst IC is simple and non-invasive, the assumptions inherent in the algorithms of earlier devices, particularly with regards to modelling of the thoracic cavity, commonly results in observed inaccuracies with $\dot{Q}$ measurements obtained by this approach. Furthermore, to improve the trace quality of the impedance cardiograph, some authors have employed breath-

holds (Denniston *et* al 1976) or exercise pauses (TEO *et* al 1985) during measurement. These approaches should be of concern to the exercise physiologist since both procedures may markedly alter $\dot{Q}$ (Miles *et* al 1989). Despite some encouraging findings, the large overestimation of $\dot{Q}$ with IC_{pf} plus wide limits of agreement during exercise (Richard *et* al 2001) may preclude a wider acceptance of this method in exercise studies.

Bioreactance

Bioreactance offers an alternative to IC by analysing relative phase shifts due to changes in intrathoracic volume. This approach, relative to IC, yields an estimated 100-fold improvement in the signal to noise ratio (Squara *et* al 2007), whilst reducing measurement variability resulting from electrode placement, body size and electrical conductivity between the skin and electrodes (Keren *et* al 2007).

Briefly, this device (NiCOM, Cheetah Medical) consists of a high-frequency (75 kHz) sine wave generator and four dual-electrode stickers. Within each sticker, one electrode transmits a sine-wave, and the other receives the signal via a voltage input amplifier. Two paired stickers are applied to ventral and dorsal surfaces of the thorax and the signal from both sides is averaged to calculate $\dot{Q}$ (Keren *et* al 2007). In detecting the relative phase shift of the received signal ($d\Phi/dt_{max}$), SV is calculated from:

$$SV = C \bullet VET \bullet d\Phi/dt_{max}$$

where C is a constant of proportionality and VET is ventricular ejection time determined from the bioreactance signal and an integrated ECG signal.

In postoperative patients at rest, bioreactance values agree closely with those determined by thermodilution (Keren *et* al 2007; Squara *et* al 2007). Several studies have used bioreactance during exercise in heart failure patients (Myers *et* al 2007; Myers *et* al 2009; Maurer *et* al 2009). Despite reasonable accuracy at rest and during mild exercise, the evidence that at more strenuous exercise intensities, bioreactance appears to underestimate $\dot{Q}$, supports evidence from exercise studies in healthy individuals.

Elliott *et* al (Elliott *et* al 2010) compared bioreactance with IGR during incremental cycle exercise in healthy participants. Bioreactance systematically underestimated (19%) $\dot{Q}$ throughout exercise although this was attributed to the inability of the device to maintain a clear ECG signal during strenuous exercise. Jakovljevic *et* al (Jakovljevic *et* al 2012) compared bioreactance measures with those obtained by IC (Kubicek method) and predicted $\dot{Q}$ values using pulmonary gas exchange (Stringer *et* al 1997). These authors showed close agreement between methods during exercise up to heart rates of approximately 165bpm. At higher exercise intensities, IC values for $\dot{Q}$ were significantly lower than those observed for bioreactance and pulmonary gas exchange. In summary, bioreactance offers the opportunity to study real-time $\dot{Q}$ measurements during exercise non-invasively, yet the evidence published so far indicates potential loss of accuracy at higher exercise intensities.

Arterial Pressure Analysis

Modelflow

The Modelflow method (Wesseling *et* al 1993) calculates an aortic flow waveform from intra-arterial or finger pressure by simulating a non-linear three-element model of the aortic input impedance (Bogert & van Lieshout 2005), in which impedance to left ventricular outflow is determined by the aortic characteristic impedance (aZ_O) and arterial compliance (C) (Westerhof *et* al 1971). The third element, peripheral vascular resistance (PVR) is not considered a major determinant of systolic inflow (Wesseling *et* al 1993) and is calculated as the quotient of arterial pressure and $\dot{Q}$. aZ_O represents the aortic opposition to pulsatile flow from the contracting left ventricle, whilst C is defined as the change in volume (dV) per change in pressure (dP). Integration of the computed aortic flow waveform provides SV and subsequently $\dot{Q}$ when multiplied by HR. Continuous measurement of arterial pressure from the finger permits non-invasive, beat-to-beat measurements of $\dot{Q}$.

This model assumes that aortic cross-sectional area increases non-linearly with increasing pressure. The relationship between cross-sectional area and pressure is described by an algorithm in which maximal aortic diameter (A_{max}) during ejection is calculated from a

database, into which subject age and gender are input, from which the relationship is derived from data obtained from human aortas (Langewouters *et* al 1984). Variability of A_{max} from this estimation is considerable, which is reflected in the inaccuracy of absolute values with 'gold standard' comparators (Bogert & van Lieshout 2005). Modelflow values in both clinical and exercise populations are confined to tracking beat-to-beat changes in $\dot{Q}$, unless an appropriate reference technique can be employed for calibration (Jansen *et* al 2001), thus correcting for individual variations in A_{max}.

During incremental exercise to 60% peak power, Houtman *et* al (Houtman *et* al 1999) compared CO_2 rebreathing measurements with those obtained by Modelflow calibrated by CO_2 rebreathing. Modelflow and rebreathing measurements were only modestly correlated (r = 0.69), whilst the mean bias and wide limits of agreement (2.2 ± 7.6 L•min^{-1}) were considered too high for use during exercise. However, several limitations with this study deserve mention; firstly, measurements of $\dot{Q}$ by each method were not made simultaneously. Secondly, limitations with CO_2 as the reference method, including manoeuvre induced increases in $\dot{Q}$ and subject discomfort, may reduce the validity of these findings. During an incremental cycling test, Sugawara *et* al (Sugawara *et* al 2003) showed a mean bias ± limits of agreement (Modelflow-Doppler) of approximately 21 ± 39% (our calculation). Although Modelflow overestimates $\dot{Q}$ compared to DE, Sugawara *et* al noted that bias decreased with increasing exercise intensity (mean bias 25.1% to 16.2%). Furthermore, in a subset of participants the correlation coefficient of duplicate measurements was 0.9 with a mean difference of 0.5 L·min^{-1}, thus supporting the Modelflow as a reliable estimate of $\dot{Q}$. Tam *et* al (Tam *et* al 2004) compared Modelflow to open-circuit C_2H_2 measurement during mild exercise. Unsurprisingly, correlation with C_2H_2 uptake was improved (r = 0.93) when Modelflow was calibrated with the C_2H_2 method. Despite low bias (0.28 L•min^{-1}), the limits of agreement remained wide (±6.8 L•min^{-1}). The overestimation of $\dot{Q}$ may be partially explained by the difference between values obtained from the radial artery and finger pressure. Significantly higher $\dot{Q}$ was recorded at rest and during light exercise (Azabji Kenfack *et* al 2004) in finger pressure derived values,

which may account for the overestimation of $\dot{Q}$ reported previously (Houtman *et* al 1999; Sugawara *et* al 2003).

Although the Modelflow method presents a continuous, non-invasive method for measuring $\dot{Q}$, its application is confined to tracking changes throughout exercise. Where possible, radial artery pressure monitoring is favourable over finger pressure recording, although the invasiveness of arterial cannulation should be considered.

Arterial Pulse Contour Analysis (PCA)

The ejection of blood into the arterial conduit during systole causes fluctuations in arterial pressure around a mean value. The magnitude of this fluctuation is largely determined by the stroke volume. Despite the simple underlying principle, determining SV based on this concept is complicated by the non-linearity of arterial compliance across a range of pressures and the incidence of arterial pressure wave reflection. Due to the relationship between arterial pressure, stroke volume, arterial compliance and systemic vascular resistance, stroke volume can be calculated from an arterial pressure waveform if arterial compliance and systemic vascular resistance are known (Montenij *et* al 2011). If arterial compliance is assumed to be constant, $\dot{Q}$ can be determined from arterial pressure waveform analysis. Whilst the commercially available systems based on this technique differ in their pressure-volume conversion algorithms, these basic principles remain.

One such device that has been utilised during exercise is the PulseCO system (PulseCO™, Cambridge, UK), which calculates nominal SV from continuous measurement of intra-arterial pressure. Briefly, arterial pressure is corrected to a standardised volume waveform through the equation:

$$\Delta V / \Delta bp = \text{calibration} \times 250 \times e^{-k \cdot bp}$$

where V is volume, bp is blood pressure and k is the curve coefficient. The constant 250 represents the saturation value (mL), which is the maximal additional volume above that at atmospheric pressure that the aorta can fill. Autocorrelation of this standardised waveform

calculates the beat period plus a beat 'power factor', (root mean square), which is proportional to the nominal stroke volume (Rhodes & Sunderland 2005). Accuracy of this system is improved by the addition of an integrated calibration procedure (lithium indicator dilution), which corrects for arterial compliance and individual variation by altering the saturation volume.

Several alternative methods of determining $\dot{Q}$ by analysis of the arterial pressure waveform are available and used in clinical settings, including the thermodilution-calibrated PICCO™ system (Pulsion, Munich, Germany) and the uncalibrated FloTrac™ system (Edwards Lifesciences, Irvine, USA). Whilst both devices show agreement with 'gold standard' comparators (Montenij *et* al 2011), neither has been studied during exercise.

LiDCO™ System

The PulseCO™ system includes an incorporated lithium indicator dilution method for the conversion of nominal to absolute stroke volume. Briefly, lithium chloride is injected as a bolus, either centrally or peripherally, where it mixes with venous blood before transiting the heart, following which it is drawn through an ion-selective electrode attached to a peripheral artery (typically the radial artery). $\dot{Q}$ is calculated from the concentration-time curve, according to the equation:

$$\dot{Q} = (\text{LiCl dose (mM)} \times 60) / (\text{area under dilution curve (mM}\bullet\text{L}^{-1}\bullet\text{s}^{-1}) \times (1\text{-PCV}))$$

where PCV (packed cell volume) = haemoglobin ($g\bullet dl^{-1}$) / 33

The validity of the LiDCO system has been demonstrated widely in critically ill patients (Kurita *et* al 1997; Linton *et* al 2000; Cecconi *et* al 2010) and in liver transplant patients with $\dot{Q}$ > $8 L\bullet min^{-1}$ (Costa *et* al 2008). To date, only one study has examined this system during exercise. Kemps *et* al (Kemps *et* al 2008) showed close agreement with the direct Fick method during exercise in heart failure patients with a mean bias of 3% and limits of agreement of ±25% at peak exercise (mean $\dot{Q}$: 9.5 $L\bullet min^{-1}$). During exercise testing of trained cyclists, LiDCO calibration performed at rest appears to considerably overestimate $\dot{Q}$ during

subsequent exercise (Elliott *et* al 2012). Performing the calibration during exercise, preferably high intensity above the anaerobic threshold, improved the accuracy of this system during exercise.

The advantages of being able to record continuously are numerous although one should balance this with the requirement for arterial cannulation, which is often beyond the specialisation of an exercise laboratory.

Nexfin

The Nexfin CO-trek pulse contour method calculates beat-to-beat SV by dividing the area under the systolic portion of the arterial pressure curve by the aortic impedance (aZ_O), calculated similarly to the Modelflow method (Bogert *et* al 2010). Briefly, aZ_O is determined from a three-element Windkessel model, in which the non-linear effects of mean pressure and subject characteristics (i.e., age, height, weight and gender) are incorporated. The correction for subject characteristics is made from a database including finger and intra-arterial pressure together with thermodilution derived from participants undergoing surgery, head-up tilt and septic shock. Finger arterial pressure is recorded and transformed to reconstruct brachial arterial pressure waveforms that are subsequently used to determine beat-to-beat $\dot{Q}$.

Initial validation studies in cardiac patients show agreement with thermodilution for both intra-arterial and finger pressure-derived $\dot{Q}$, whilst the limits of agreement for both were within acceptable limits (Bogert *et* al 2010). To date, only one study has attempted to record exercising values using the Nexfin method (Bartels *et* al 2011); In 19 healthy participants, $\dot{Q}$ was determined by the Nexfin method and inert gas rebreathing during incremental exercise to a HR of 150 bpm. Both methods were highly correlated, with a mean bias of 3.6% (limits of agreement of ± 30.3%). In a subset of participants, incremental exercise was repeated until volitional exhaustion with Nexfin values compared to those obtained by the Stringer method (Stringer *et* al 1997) where a-vO^2 diff is estimated according to the exercise intensity (% $\dot{V}O_{2max}$). A mean bias (± LoA) of 4.7 ± 21.5% was observed indicating construct validity of this method. Further evaluation of this method is warranted against other accepted techniques.

Table 1. Summary of investigations (1999-2012) assessing the validity of Q measurement methods during exercise against 'gold standard' comparators.

Study	*n*	*Participants*	*Exercise Intensity*	*Comparator*	*r*	*Mean bias in Q (%)*[a]
C_2H_2 OpenCirc						
Barker *et* al (1999)	3	Well-trained	90% VO_{2max}	Fick	0.94	5.8% (1.3 L•min^{-1})
Johnson *et* al (2000)	6	Trained	20-85% VO_{2peak}	Fick	0.89	2.9% (0.5 L•min^{-1})[b, c]
C_2H_2 Rebreathe						
Jarvis *et* al (2007)	14	Active	Light Maximal	Fick	NA	18.8% (3.0 L•min^{-1})[c] 20.4% (4.9 L•min^{-1})[c]
Inert Gas Rebreathing						
Agostoni *et al* (2005)	20	CHF	Maximal	Fick	0.95	1.6% (0.2 L•min^{-1})[b]
CO_2 rebreathing (Single-Step)						
Jarvis *et* al (2007)	14	Active	Light Maximal	Fick	NA	8.1% (1.3 L•min^{-1}) 12.5% (3.0 L•min^{-1})
PhysioFlow™						
Charloux *et* al (2000)	32	COPD / SAS	Light	Fick	0.85	2.4% (0.26 L•min^{-1})
Bougalt *et* al (2005)	8	CHF	Incremental Intermittent	Fick	0.85 0.71	31% (3.2 L•min^{-1}) 25% (2.5 L•min^{-1})
Kemps *et* al (2008)	10	CHF	Light Moderate Peak	Fick	0.73[b]	53% (NA) 42% (NA) 47% (NA)
Impedance Cardiography						
Scherhag *et* al (2000)	20	Suspected CAD	Light	TD	0.93	11.4% (1.0 L•min^{-1})[b]
Pulse Contour Analysis (LiDCO™)						
Kemps *et* al (Kemps 2008)	10	CHF	Light Moderate Peak	Fick	0.90	-1 (NA) -1 (NA) 3 (NA)

[a] Calculated as [(method – comparator) / comparator * 100%], absolute difference in L•min^{-1} in parentheses
[b] averaged from all available exercise stages
[c] based on the best performing variation of the technique.
CAD = Coronary Artery Disease; C_2H_2 = Acetylene Method; CO_2 = Carbon dioxide; TD = Thermodilution; VO_2 = Oxygen Uptake; NA = Data not available; CHF = Chronic heart failure; COPD = Chronic obstructive pulmonary disease; SAS = Sleep Apnoea Syndrome

Table 2. Summary of investigations reporting the Q-$\dot{V}O_2$ relationship.

Study	Regression Equation	Subjects
Direct Fick		
Barker *et* al (1999)	$y = 4.71\ \dot{V}O_2 + 5.63$	Active
Johnson *et* al (2000)	$y = 4.6\ \dot{V}O_2 + 4.65$	Active & Untrained
C_2H_2 (OpenCirc)		
Barker *et* al (1999)	$y = 6.67\ \dot{V}O_2 + 2.38$	Trained
Bell *et* al (2003)	$y = 4.4\ \dot{V}O_2 + 7.8$	Active
Dibski *et* al (2005)	$y = 6.12\ \dot{V}O_2 + 3.0$	Active
CO_2 rebreathing		
Equilibrium Method		
Vanhees *et* al (2000)	$y = 5.52\ \dot{V}O_2 + 4.92$	Active
Dibski *et* al (2005)	$y = 6.18\ \dot{V}O_2 + 2.59$	Trained
Exponential Method		
Vanhees *et* al (2000)	$y = 5.03\ \dot{V}O_2 + 5.19$	Active
Inert Gas Rebreathing		
Elliott *et* al (2010)	$y = 4.43\ \dot{V}O_2 + 5.46$	Trained
Doppler Echocardiography		
Nottin *et* al (2002)	$y = 4.58\ \dot{V}O_2 + 6.24$	Active
PhysiolFlow™		
Charloux *et* al (2000)	$y = 7.17\ \dot{V}O_2 + 4.18$	Active
Welsman *et* al (2005)	$y = 6.32\ \dot{V}O_2 + 1.45$	Children 10-11 yrs
Bioreactance		
Elliott *et* al (2010)	$y = 3.6\ \dot{V}O_2 + 3.95$	Trained
Jakovljevic *et* al (2012)	$y = 4.56\ \dot{V}O_2 + 6.03$	Active

Summary

Despite their status as 'gold standard' measures of cardiac output, direct Fick and thermodilution are largely inappropriate for the measurement of $\dot{Q}$ during exercise, unless cardiac catheterisation is medically warranted. A number of minimally- or non-invasive measures have been developed to provide the exercise physiologist with haemodynamic data during exercise. Whilst many advances have been made, there is still considerable uncertainty regarding the acceptance of methods for the measurement of exercising $\dot{Q}$. Open circuit C_2H_2 methods perform reasonably well compared to 'gold standard' measures, although more data is required to support this view. Importantly, the precision of this method appears to be high during high-intensity exercise. Additionally, exercise physiologists should consider several important questions when investigating measurements obtained during exercise, including; a) Is the resolution of measurement sufficient to quantify the true cardiovascular adaptations to exercise; b) Is the technique precise enough to detect changes during exercise; and, c) Does the measurement procedure potentially influence hemodynamic variables during exercise?

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STUDIES

I. **Application and comparison of non-invasive methodologies for cardiac output assessment during exercise.**

Elliott, A., Hull, J.H., Nunan, D., Jakovljevic D., Brodie, D., and Ansley L. (2010). Application of bioreactance for cardiac output assessment during exercise in healthy individuals. *Eur J Appl Physiol*, **109**: 945-51.

II. **An evaluation of calibrated pulse contour analysis for cardiac output assessment during exercise in trained individuals.**

Unpublished findings.

III. **Optimising the measurement of cardiac output during exercise using lithium dilution and pulse contour analysis.**

Elliott, A., Skowno, J., Prabhu, M., and Ansley L. (2012). Measurement of cardiac output during exercise in healthy, trained humans using lithium dilution and pulse contour analysis. *Physiological Measurement*, **33,** p1691-1701

IV. **Evidence of cardiac functional reserve upon exhaustion during incremental exercise to determine $\dot{V}O_{2max}$.**

Elliott, A., Skowno, J., Prabhu, M., Noakes, T. D. and Ansley L. (2013). Evidence of cardiac functional reserve during incremental exercise to determine $\dot{V}O_{2max}$. *British Journal of Sports Medicine*, doi:10.1136/bjsports-2012-091752

STUDIES I, III, and IV CAN BE FOUND IN THEIR PUBLISHED FORM IN APPENDIX A.

CHAPTER 2:

STUDY I:

Application and comparison of non-invasive methodologies for cardiac output assessment during exercise.

Adapted from:

Elliott, A., Hull, J.H., Nunan, D., Jakovljevic D., Brodie, D., and Ansley L. (2010). Application of bioreactance for cardiac output assessment during exercise in healthy individuals. *Eur J Appl Physiol*, **109**: 945-51

Introduction

Measurement of cardiac output ($\dot{Q}$) during exercise provides a valuable insight into the cardiovascular response to physiological stress and is an important component in assessing the integrative cardiorespiratory response. A number of methods have been employed to evaluate $\dot{Q}$ during exercise (Warburton *et* al 1999a; Warburton *et* al 1999b) with varying degrees of accuracy. Invasive techniques, dependent upon vascular catheterisation and direct Fick calculation are generally regarded as the 'gold standard' (Warburton *et* al 1999a). However, the practical utility of these techniques is limited by the fact that they are expensive, require specialist expertise and are associated with inherent risks (e.g. vascular occlusion) (Scheer *et* al 2002).

A number of non-invasive techniques have therefore been evaluated in this setting and include methods employing gas rebreathing (Richard *et* al 2001), open-circuit acetylene breathing (Johnson *et* al 2000), the Modelflow method (Sugawara *et* al 2003) and bioimpedance cardiography (Richard *et* al 2001). The latter is based upon the principle that assessment of electrical conductance properties across the thorax can be used to calculate aortic blood flow. This technique is advantageous in the exercise setting given it is easy to apply, versatile and permits continuous, beat-to-beat monitoring of cardiac function (Warburton *et* al 1999b; Richard *et* al 2001). Bioimpedance $\dot{Q}$ has been validated with both dye-dilution (Denniston *et* al 1976) and direct Fick (Teo *et* al 1985) methodologies during exercise, although there have since been conflicting reports of accuracy (Warburton *et* al 1999b). Some of the discrepancy reported may arise from difficulty in obtaining signals sufficiently free of noise artefact and a need to precisely model thoracic dimensions and distance between electrode placements (Denniston *et* al 1976; Warburton *et* al 1999b). Furthermore, many studies have required recordings be obtained during a period of breath-holding or a pause in exercise to ensure accuracy (Teo *et* al 1985). Updated approaches to the use of bioimpedance (PhysioFlow™) have become available and shown reasonable agreement with direct Fick measurements during incremental exercise (Richard *et* al 2001). However, recent studies have shown poor validity of

this approach during exercise in both COPD (Bougault *et* al 2005) and chronic heart failure patients (Kemps *et* al 2008), which precludes its use for the routine assessment of absolute $\dot{Q}$ values during exercise.

A novel bioreactance based device (NICOM, Cheetah Medical, Delaware, USA), has become available (Keren *et* al 2007) offering the potential to address these deficiencies. In contrast to the *amplitude* based calculation of bioimpedance, bioreactance provides $\dot{Q}$ data from analysis of the *frequency* of relative phase shifts of electrical current injected across the thorax and as such is less subject to interference. It has been argued that this device yields a signal-to-noise ratio that is about 100-fold greater than bioimpedance (Squara 2008). Furthermore it is reported that signal magnitude is not affected by precision of electrode placement, body movement or respiratory excursion (Keren *et* al 2007b), all key points in the assessment of $\dot{Q}$ during dynamic exercise.

The bioreactance device has been found to have good precision in comparison with invasive methods at rest and in response to physiological challenge (e.g. fluid loading) (Keren *et* al 2007). In addition, Myers *et al* (Myers *et* al 2007) recently reported good construct validity of the bioreactance method during exercise as evidenced by a strong relationship between bioreactance determined $\dot{Q}$ ($\dot{Q}_{bio}$) and oxygen uptake ($\dot{V}O_2$) in patients referred with cardiac failure. In this setting the device was reported to be easy to use and provided consistent and reportedly valid results. To date, however the bioreactance device has not been evaluated during strenuous exercise at higher levels of $\dot{Q}$, in comparison with other accepted methodologies in healthy trained individuals.

The aim of this study was therefore to extend the previous application of this device by evaluating the practicality and utility of the bioreactance method for measuring cardiac output continuously during cycle ergometry across different exercise intensity domains in trained young individuals. In addition we assessed the level of agreement between the bioreactance and contemporaneous measures taken from an alternative non-invasive $\dot{Q}$ monitoring device;

based upon principles of inert gas rebreathing (Innocor, Innovision, Denmark) ($\dot{Q}_{rebreathe}$). This device has previously been validated against invasive techniques (Agostoni *et* al 2005) and been found to produce accurate, reproducible $\dot{Q}$ data during maximal cycle ergometry exercise (Jakovljevic *et* al 2008).

Methods

Participants

Fourteen healthy male recreationally trained cyclists (age 34 ± 8 years, body mass 80.5 ± 8.4 Kg, height 1.81 ± 0.4 m, $\dot{V}O_{2peak}$ 56.1 ± 5.7 ml•kg^{-1}•m^{-1}) were recruited to participate in the study. Participants provided written informed consent and the study was approved by the Kingston University Ethics Committee. All procedures were in accordance with the Declaration of Helsinki. Participants with a history of cardiac or respiratory disease were excluded.

Study Design

Participants were requested to attend the exercise physiology laboratory to perform an incremental maximal exercise test on an electromagnetically-braked cadence independent cycle ergometer (Velotron, Racermate Inc., USA). They abstained from exercise in the 24 hours preceding exercise and from caffeinated beverages, alcohol and tobacco on the day of testing. Following determination of height and body mass, participants were familiarised with the exercise equipment and protocol.

Exercise Protocol

After baseline resting measurement of $\dot{Q}$ (see *Cardiac Output Measurements*) participants commenced cycling at 150 watts (W) for five minutes followed by 200 W for a further five minutes. Power output was then incremented by 30 W every three minutes until volitional exhaustion (Figure 1). Active encouragement was given at all times throughout the test to encourage maximal volitional effort. The exercise test duration ranged from 16 to 28 minutes,

depending on the physical condition of the individual. Participants used their own cycling shoes and were permitted to self-select their cadence throughout the exercise protocol.

Throughout the exercise period breath-by-breath gas exchange data was collected continuously using an online system (Innocor, Innovision, Denmark) and averaged over 30 s epochs. Heart rate (HR) was recorded continuously using a telemetric monitor (Polar S610, Polar Elektro, Finland).

Cardiac Output Measurements

At rest, $\dot{Q}$ was determined as the mean of triplicate values obtained over a period of 5 mins rest whilst seated on the ergometer. During exercise, $\dot{Q}_{bio}$ was measured continuously (see below) with values recorded during the final minute of each exercise stage. Cardiac output measured by inert gas rebreathing ($\dot{Q}_{rebreathe}$) was measured at two timepoints; during the final minute of the 200 W stage and in the final exercise stage when participants indicated they were near to exhaustion but still remained able to comply with the rebreathing protocol (Figure 1). Cardiac index ($\dot{Q}$i) is reported as $\dot{Q}$ / body surface area.

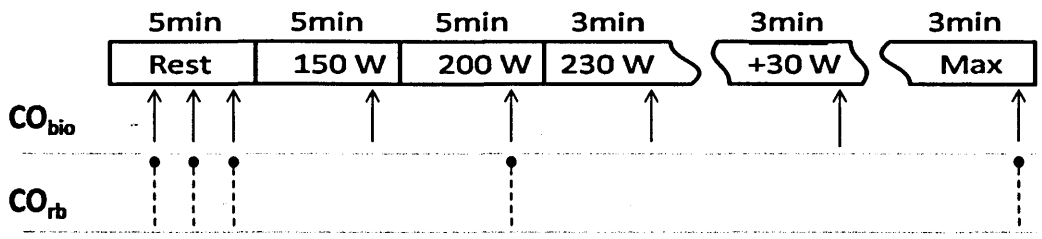

Figure 1. Timeline of study I protocol and measurements. Arrows indicate the measurement timepoints for each method, respectively.

Bioreactance cardiac output ($\dot{Q}_{bio}$)

The $\dot{Q}_{bio}$ (NICOM, Cheetah Medical, Delaware, USA) was determined as described elsewhere (Myers *et* al 2009). In brief, blood flow within the aorta was calculated based on an assessment

of the phase shifts in an alternating radiofrequency electrical current ($d\Phi/dt_{max}$) measured across the thorax. $\dot{Q}$ was subsequently determined from:

$$\dot{Q} = (C * VET * d\Phi/dt_{max}) * HR$$

where C is a constant of proportionality (based upon subject age, gender and body size) and VET is ventricular ejection time determined from the bioreactance and electrocardiograph signal.

Two surface dual-electrodes were applied over the trapezius muscle on either side of the upper torso and two on the lower posterior torso lateral to the margin of the latissimus dorsii musculature. Electrode connections were secured with medical tape and all connecting wires supported to ensure minimal movement artefact. The system was then auto-calibrated and an adequate electrocardiograph signal and bioreactance waveform was established prior to the acquisition of data. All $\dot{Q}_{bio}$ data was recorded continuously and averaged over 30s intervals to minimise beat-to-beat variability.

Inert Gas Rebreathing Cardiac Output ($\dot{Q}_{rebreathe}$)

The $\dot{Q}_{rebreathe}$ was determined using an inert gas rebreathing technique (Innocor, Innovision, Denmark). The system consists of a three-way respiratory valve with a mouthpiece and a rebreathing bag connected to a photoacoustic analyser. The device requires participants to inhale an inert gas mixture; consisting of 0.5% nitrous oxide (N_2O); 0.1% sulphur hexafluoride; (SF_6) and a 28% O_2 in nitrogen mixture in a closed system (i.e. with a nose clip *in situ*). In order to take a measurement, participants perform a specific rebreathing manoeuvre over a 30 second period during which participants are required to completely empty the rebreathing bag with each inspiration. The software then determined $\dot{Q}$ based upon the rate of decrease in the concentration of N_2O during the manoeuvre. The $\dot{Q}_{rebreathe}$ was taken as proportional to pulmonary blood flow with the assumption of an insignificant intra-pulmonary shunt.

Arterial-venous Oxygen Difference

The arterial-venous oxygen difference ((a-v)O_2diff) was calculated using a re-arrangement of the direct Fick equation:

$$(a\text{-}v)O_2diff(ml \bullet dl^{-1}) = 100 * (\dot{V}O_2\ (L \bullet min^{-1}) / \dot{Q}\ (L \bullet min^{-1}))$$

Data Analysis

Data are expressed as mean ± SD unless otherwise stated. Pearson's correlation coefficient was used to evaluate the relationship between $\dot{Q}$ and $\dot{V}O_2$ measures taken at contemporaneous timepoints. Linear regression analysis permitted the comparison of $\dot{Q}$-$\dot{V}O_2$ for each method against data obtained from previous studies with accepted methods. Differences between $\dot{Q}_{bio}$ and $\dot{Q}_{rebreathe}$ were compared using the method described by Bland and Altman (Bland & Altman 1986) with difference expressed as mean bias (upper and lower 95% limits of agreement) and a two-sided paired t-test to determine significance. Intra-class correlation coefficient (ICC) was also determined. All statistical analyses were performed on GraphPad (Prism v. 5, GraphPad Software Inc, USA) with a P value of < 0.05 considered significant.

Results

Rest

In all participants, high quality, stable bioreactance waves and electrocardiograph data were obtained with a high mean ICC, 0.94 ($P < 0.001$) for triplicate measures of $\dot{Q}_{bio}$ at rest. Similarly, all participants were able to adequately perform the rebreathing manoeuvre. The ICC of $\dot{Q}_{rebreathe}$ was lower at 0.61 ($P < 0.05$). Resting $\dot{Q}_{rebreathe}$ was greater than $\dot{Q}_{bio}$ ($P < 0.05$) with a mean bias of 1 $L \bullet min^{-1}$ (LoA 1.7 to -3.7 $L \bullet min^{-1}$) or -14.4% (25 to -54%).

Exercise

Due to inadequate rebreathing manoeuvre, we were unable to obtain a $\dot{Q}_{rebreathe}$ measurement in one participant (7%) at the 200 W stage, and in three participants (21%) at the maximal exercise stage. In contrast, the bioreactance device provided data continuously throughout exercise, with no evidence of electrode detachment. However, in nine participants (65%), the electrocardiograph trace was intermittently lost during exercise >160 bpm. Resulting in lower values for HR from the bioreactance device when compared with those obtained using a HR monitor.

Table 1. Cardiac output data at rest and during exercise.

Variable	Bioreactance	Inert Gas Rebreathing	Mean (SD) difference	*P*-value
At rest				
$\dot{Q}$ (L•min^{-1})	6.3 (1.2)	7.3 (1.3)	-1.0 (1.4)	0.019
$\dot{Q}$i (L•m^2•min^{-1})	3.1 (0.6)	3.6 (0.7)	-0.5 (0.7)	0.018
$\dot{V}O_2$ (L•min^{-1})	0.5 (0.1)	0.5 (0.1)	NA	NA
Calculated (a-v)O_2diff (ml•dl^{-1} O_2)	7.4 (2.0)	6.3 (1.0)	1.1 (1.5)	0.014
Estimated (a-v)O_2diff (ml•dl^{-1} O_2)	6.9		NA	NA
200 W data*				
$\dot{Q}$ (L•min^{-1})	16.2 (2.0)	20.4 (2.1)	-4.3 (2.9)	<0.001
$\dot{Q}$i (L•m^2•min^{-1})	8.0 (0.8)	10.1 (1.1)	-2.1 (1.5)	<0.001
ΔQ Rest – 200W (%)	156.5 (45.4)	188.3 (62.5)	-31.8 (78.3)	0.17
$\dot{V}O_2$ (L•min^{-1})	3.3 (0.4)	3.3 (0.4)	NA	NA
Calculated (a-v)O_2diff (ml•dl^{-1} O_2)	20.3 (3.2)	15.9 (1.8)	4.4 (3.0)	<0.001
Estimated (a-v)O_2diff (ml•dl^{-1} O_2)	13.8		NA	NA
Maximal exercise^				
$\dot{Q}$ (L•min^{-1})	19.3 (3.4)	23.3 (3.1)	-4.0 (3.5)	0.004
$\dot{Q}$i (L•m^2•min^{-1})	9.6 (1.5)	11.7 (1.1)	-2.1 (1.6)	0.001
ΔQ 200 W – MAX (%)	22.8 (15.8)	11.9 (13.7)	10.9 (14.5)	0.03
$\dot{V}O_2$ (L•min^{-1})	4.2 (0.5)	4.2 (0.5)	NA	NA
Calculated (a-v)O_2diff (ml•dl^{-1} O_2)	22.4 (3.9)	18.3 (1.6)	4.2 (3.9)	0.005
Estimated (a-v)O_2diff (ml•dl^{-1} O_2)	16.2		NA	NA

Data presented as mean (SD). Participants *n=13, ^n=11 for which contemporaneous data was available. Estimated (a-v)O_2diff determined from Stringer (Stringer *et* al 1997). **$\dot{Q}$;** cardiac output, **$\dot{Q}$i;** cardiac index, **$\dot{V}O_2$;** peak oxygen uptake, **(a-v)O_2diff;** arterial-venous oxygen difference.

Cardiac Output

Averaged exercise $\dot{Q}$ data are shown in Table 1. Individual $\dot{Q}$ and SV data are shown in figure 2. The $\dot{Q}$ data from the two devices were well correlated ($r = 0.93$; $P < 0.001$; Fig. 3), although the strength of this relationship was reduced when resting data was excluded ($r = 0.54$; $P < 0.01$). $\dot{Q}$ data obtained from both devices at rest, moderate and maximal exercise intensities were significantly different ($P < 0.05$, Table 1). When the data was presented as % change in Q from the previous stage, there was a significant difference ($P = 0.03$) between the two devices at maximal exercise only. Bland-Altman analysis (Fig. 4) revealed mean bias of -3.2 $L \bullet min^{-1}$ and limits of agreement from -9.0 to -2.6 $L \bullet min^{-1}$ (percentage bias = -20.3 ± 37.2%). For exercise comparisons alone, the mean bias was -4.3 $L \bullet min^{-1}$ and with limits of agreement from -10.1 to -1.52 $L \bullet min^{-1}$ (percentage bias = -21.5 ± 39.4%). $\dot{Q}$ data from both $\dot{Q}_{bio}$ and $\dot{Q}_{rebreathe}$ were well correlated with $\dot{V}O_2$ ($r = 0.84$ and 0.97, respectively; $P < 0.001$, Fig. 5), although the strength of this relationship was reduced ($r = 0.72$ and 0.75, respectively) when resting data was excluded. Analysis of the bioreactance measurement components revealed an increase in $d\Phi/dt_{max}$ from rest to maximal exercise (175.2 ± 42.5 to 457.9 ± 123.5 $ohm \bullet s^{-1}$), with a concomitant decrease in VET (276.6 ± 26.1 to 190 ± 17.4 ms).

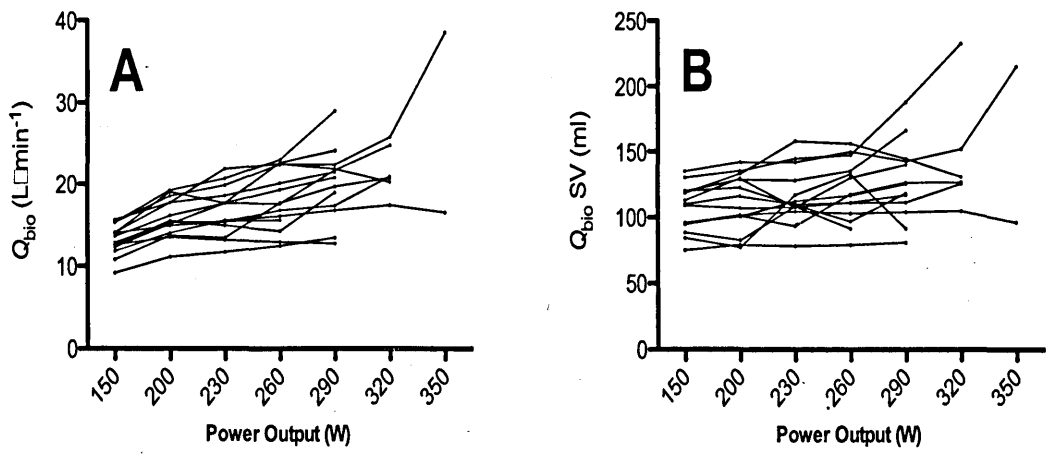

Figure 2. Individual cardiac output responses to incremental exercise measured by bioreactance ($\dot{Q}_{bio}$).

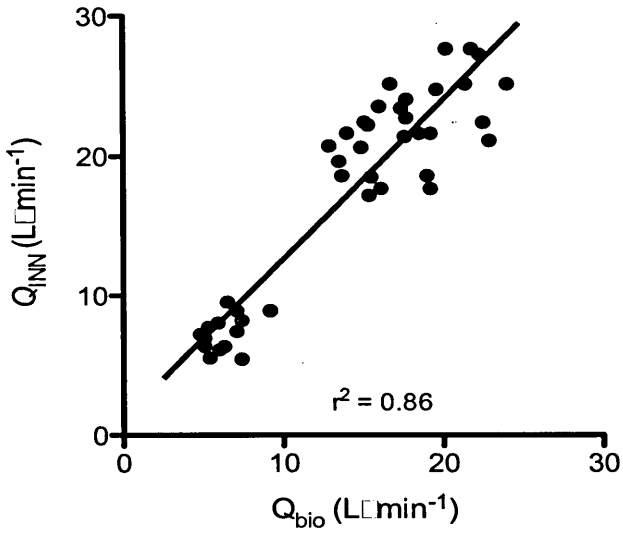

Figure 3. Scatterplot of $\dot{Q}_{bio}$ versus $\dot{Q}_{rebreathe}$ at rest and during exercise (n = 13)

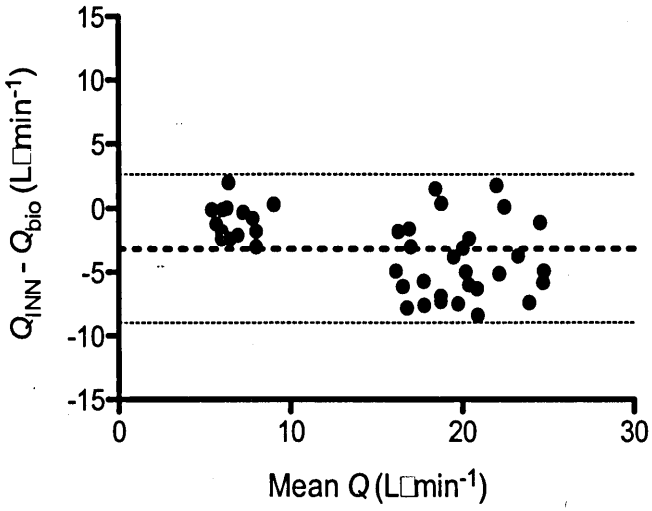

Figure 4. Bland-Altman analysis comparing difference between measurements of $\dot{Q}_{rebreathe}$ and $\dot{Q}_{bio}$ against the average of both measures (n = 13). Dark dashed line indicates mean bias. Light dashed lines represent 95% limits of agreement.

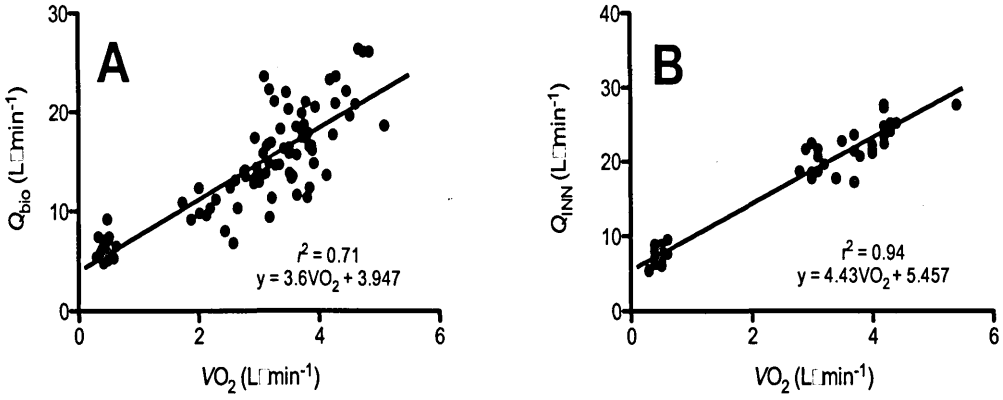

Figure 5. Relationship between cardiac output and oxygen uptake ($\dot{V}O_2$) for both $\dot{Q}_{bio}$ (**A**, n = 14) and $\dot{Q}_{rebreathe}$ (**B**, n = 13). Best fit regression lines are shown for both methods.

Discussion

The aim of this study was to evaluate the performance of a bioreactance device for the continuous measurement of $\dot{Q}$ at rest and during incremental cycle exercise in healthy trained volunteers. At rest, the device was simple to use, providing reliable, stable bioreactance and electrocardiograph waveform traces, whilst being comfortable for participants. We found a high intra-class correlation coefficient at rest. Furthermore, resting $\dot{Q}$ values using the bioreactance device were consistent with those reported elsewhere using invasive techniques in a healthy trained population (Stringer *et* al 1997; Sun *et* al 2000; Stickland *et* al 2006).

During incremental exercise, $\dot{Q}_{bio}$ paralleled increases in both power output whilst being closely tied to $\dot{V}O_2$. However, during exercise at higher intensities (HR > 160bpm), the quality of the electrocardiograph signal declined such that intermittent signal loss resulted in a discrepancy between the HR values obtained from the bioreactance device and contemporaneous measures from a separate heart rate monitor. This was despite meticulous preparation and fixation of the electrodes according to manufacturer's instructions and that described in previous reports using bioreactance during exercise (Myers *et* al 2007; Myers *et* al 2009). Additionally, we noted evidence of signal artefact with upper body movement during exercise, which was eliminated by the maintenance of a relatively stable upper body position.

Comparison of contemporaneous values of $\dot{Q}$ from both bioreactance and inert gas rebreathing revealed statistically significant differences. Furthermore, even when we determined the ability of each device to track changes in Q, we found a significant difference between the two devices at the highest exercise intensity, although not during submaximal exercise. Bland-Altman analysis (Figure 4) showed a mean difference of 3 $L \cdot min^{-1}$ (20.3%), with bioreactance providing consistently lower values than inert gas rebreathing. The relative magnitude of the difference between methods was similar at rest and during exercise. Previous studies have indicated the validity of the inert gas rebreathing approach to $\dot{Q}$ measurement during exercise

(Agostoni *et* al 2005; Jakovljevic *et* al 2008), thus indicating its role as a useful reference method.

In the absence of a 'gold standard' comparator, it is difficult to truly assess the validity of the bioreactance device for the measurement of true exercise $\dot{Q}$. In this context, construct validity can be determined by several approaches using the simultaneous measurement of $\dot{V}O_2$ (Rowland & Obert 2002). Calculation of the arterio-venous oxygen difference (a-v)O_2diff has been shown to be a useful surrogate indicator of the accuracy of a $\dot{Q}$ measurement method (Stringer *et* al 1997). Invasive studies have consistently demonstrated a progressive increase in oxygen extraction during incremental exercise proportional to $\dot{V}O_2$ (Stringer *et* al 1997; Mortensen *et* al 2005). This approach to determining construct validity was previously employed by Jakovljevic *et* al (Jakovljevic *et* al 2008) who found calculated (a-v)O_2diff at maximal exercise to be 16.4 ml•dl^{-1} O_2 using the inert gas rebreathing method. The (a-v)O_2diff calculated in this study with inert gas rebreathing was similar at peak exercise (18.3 ml•dl^{-1} O_2) to that reported previously using open-circuit acetylene uptake (Trinity *et* al 2011). Calculated (a-v)O_2diff obtained using $\dot{Q}_{bio}$ in this study was 22.4 ml•dl^{-1} O_2 at peak exercise, which in the presence of a normal haemoglobin (15 g•dl^{-1}) and arterial oxygen saturation (98%) would equate to a physiologically impossible systemic oxygen extraction of over 100% (Stringer *et* al 1997). Additionally, we observed the slope of the $\dot{Q}_{bio} - \dot{V}O_2$ relationship to be 3.6, which is lower than that observed from previous studies employing direct Fick (Johnson *et* al 2000), dye-dilution (Smyth *et* al 1984), and open-circuit acetylene breathing (Bell *et* al 2003) where the slope is typically observed to be >4.5. Overall, we would therefore expect $\dot{Q}$ values in this population to be larger than observed with bioreactance in this study and more in line with data from previous invasive studies indicating a peak $\dot{Q}$ of at least 27 L•min^{-1} for a similar peak $\dot{V}O_2$ (Teo *et* al 1985; Mortensen *et* al 2008).

The reason for this apparent underestimation of $\dot{Q}$ values during incremental cycle exercise is not immediately apparent, although studies recording $\dot{Q}$ during exercise with bioimpedance devices have reported evidence of underestimation of $\dot{Q}$ at higher exercise intensities (Richard

et al 2001). The bioreactance system determines $\dot{Q}$ as the product of HR, VET, $d\Phi/dt_{max}$ and a constant of proportionality. The manner in which these determinants interact to provide the final $\dot{Q}$ reading is commercially restricted, however it is worth noting that the device algorithm and invasive validation assessment has been reported in an older untrained population who were predominantly medically unwell at time of assessment (Keren *et* al 2007; Myers *et* al 2007; Squara *et* al 2007). This is the first time the device has been assessed in a healthy young population and our finding of systematic bias at rest and all exercise intensities suggests that alternative correction factors may be appropriate and improve measurement accuracy. The cardiovascular hemodynamic response to incremental exercise has been reported previously using invasive techniques (Stickland *et* al, 2006). Furthermore, similar studies utilising the bioreactance device (Myers *et* al, 2009) have only previously compared cardiac failure patients with 'normal' untrained participants reporting dyspnoea of non-cardiac origin and a limited cardiovascular and exercise reserve.

Additional reasons for the apparent underestimation of $\dot{Q}$ measurements include possible influence of interference / movement artefact, although in the present study we found adequate bioreactance trace quality during exercise, providing a stable upper body position was maintained. The discrepancy between bioreactance determined HR and the equivalent value from an alternative monitor at high intensity exercise may in at least part explain underestimation of maximal $\dot{Q}$ and is an important deficiency to be addressed.

Study Limitations

In assessing the efficacy of bioreactance for the measurement of $\dot{Q}$ throughout incremental exercise, we were unable to compare measurements obtained with an invasive 'gold standard' device, which would be desirable in drawing specific conclusions regarding the validity of this method. In assessing this approach, we chose to incorporate a relatively recent approach to the measurement of $\dot{Q}$ during exercise that has shown validity (Gabrielsen *et* al 2002; Agostoni *et* al 2005) and has been used widely during exercise (Jakovljevic *et* al 2008; Jakovljevic *et* al

2011; Jakovljevic *et* al 2012). This selection of study design was based on a lack of access to cardiac catheterisation facilities and an unfavourable risk-benefit ratio to the participants in this study. These limitations should not distract from the interpretation of these findings given that we were unable to determine construct and concurrent validity (Rowland & Obert 2002) by simultaneous measurement of pulmonary gas exchange and comparison with previously published studies using invasive techniques in a similar population. Indeed, the results presented here have permitted the evaluation of the performance of bioreactance during exercise without exposing participants to invasive testing.

Conclusion

The findings presented in this study suggest that whilst bioreactance offers a simple, versatile and cost-effective means of determining $\dot{Q}$ at rest and in clinical populations of limited exercise capacity (Agostoni *et* al 2005; Myers *et* al 2009), we found limited utility during strenuous exercise in this healthy trained population. The finding of a systematic underestimation of $\dot{Q}$ during exercise suggests further work is warranted before this approach can be recommended for the assessment of continuous hemodynamic measurements in healthy trained individuals during maximal exercise. Further studies should look to evaluate alternative methods for the recording of continuous hemodynamic parameters.

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CHAPTER 3:

Study II:

An evaluation of calibrated pulse contour analysis for cardiac output assessment during exercise in trained individuals.

Introduction

The measurement of $\dot{Q}$ is a key element in determining the cardiovascular response to exercise and to monitoring the changes in hemodynamic parameters associated with physical training, lifestyle or chronic disease (Warburton *et* al 1999). Furthermore, $\dot{Q}$ monitoring during exercise permits an evaluation of factors potentially limiting exercise performance and/or $\dot{V}O_{2max}$.

In the absence of invasive techniques incorporating cardiac catheterisation, $\dot{Q}$ is often difficult to measure accurately (Warburton *et* al 1999). In this context, direct Fick is often considered as the 'gold standard' method for determining $\dot{Q}$ during exercise. However, the direct Fick method is based on the assumption that a true cardiovascular steady-state is achieved during exercise, which is often not the case at near-maximal exercise intensities. Additionally, blood gases are often measured intermittently during exercise, precluding the recording of continuous measurements. However, this latter point has been somewhat addressed with the availability of continuous Fick measurements during exercise (Nakanishi *et* al 1993; Kemps *et* al 2008), although this approach has not been widely adopted or validated during strenuous exercise, where intermittent measurements are typically still employed (Brink-Elfegoun *et* al 2007; Mortensen *et* al 2008). Furthermore, there are a number of inherent risks associated with cardiac catheterisation, including ventricular arrhythmias and perforation on the pulmonary artery or right ventricle (Patel *et* al 1986) resulting in an unfavourable risk to benefit ratio for a healthy population where cardiac catheterisation is not clinically indicated.

An accurate, reliable, minimally-invasive method for determining $\dot{Q}$ continuously throughout exercise to exhaustion is desirable for exercise physiologists. Many studies investigating the cardiovascular determinants of O_2 delivery and exercise are limited by the lack of availability of accurate continuous measurements permitting the measurement of hemodynamic parameters in the periods immediately preceding and/or following a change in metabolic demand and/or exhaustion (Calbet *et* al 2007; Brink-Elfegoun *et* al 2007; Mortensen *et* al 2008). Despite technical advances in other measurements relating to exercise physiology, such as pulmonary

gas exchange and surface electromyography, all of which can be measured continuously during exercise, advances in $\dot{Q}$ measurement methods have not been made to the same degree.

A number of studies have attempted to evaluate non-invasive methods for measuring $\dot{Q}$ during exercise, including the Modelflow method (Sugawara *et* al 2003), impedance cardiography (Richard *et* al 2001), Doppler echocardiography (Rowland and Obert 2002), bioreactance (Elliott *et* al 2010) (study 1), acetylene uptake (Johnson *et* al 2000) and inert gas rebreathing methods (Jakovljevic *et* al 2008). Despite the multitude of methods available, there are few methods that have been extensively validated during strenuous exercise. Perhaps the most accepted non-invasive measure of $\dot{Q}$ is the acetylene method described in detail by Johnson *et* al (2000) and used in a number of studies during maximal exercise (Ridout *et* al 2010; Trinity *et* al 2012). However, this method only permits measurements at isolated timepoints, taking approximately 8s to perform.

An alternative measurement method is pulse contour analysis, which is based on the principle that SV can be continuously estimated by analysis of the arterial pressure waveform obtained from an arterial line (Alhashemi *et* al 2011). The LiDCO™ system uses a pulse pressure algorithm to track continuous changes in SV (PulseCO), which is calibrated using a lithium indicator dilution (LiDCO) technique (Linton *et* al 1993; Linton *et* al 1997). The pulse pressure algorithm is based on the assumption that the net power change in the arterial system during the heartbeat is the difference between the volume of blood entering the system (SV) and the volume of blood flowing out peripherally (Alhashemi *et* al 2011). This method assumes a linear relationship between netpower and netflow, once a correction is made for arterial compliance. To correct for individual variations, an incorporated lithium indicator dilution method converts nominal SV to absolute SV.

A number of studies in clinical populations have validated lithium dilution as a method for assessing $\dot{Q}$ in postoperative patients (Linton *et* al 1993; Linton *et* al 1997; Linton *et* al 2000; Garcia-Rodriguez *et* al 2002). Costa *et* al (2008) showed close agreement between thermodilution $\dot{Q}$ and both lithium dilution and calibrated PulseCO in patients with a high $\dot{Q}$

(~8 L•min^{-1}) following liver transplantation. To date, only one study has employed the LiDCO™ device during exercise. Kemps *et* al (2008) evaluated LiDCO™ measurements against those obtained with direct Fick during a symptom-limited maximal test in patients with chronic heart failure. Both at rest and during exercise, LiDCO showed good agreement with direct Fick values (bias 2% ± 28%) as well as closely tracking exercise-related within-patient changes of $\dot{Q}$ (Kemps *et* al 2008).

Given that the LiDCO system meets the desired criteria of also being continuous and accurate across a range of $\dot{Q}$, we therefore sought to assess its feasibility during exercise in healthy volunteers where $\dot{Q}$ easily surpasses the ~12 L•min^{-1} observed at peak exercise in previous studies using this system (Kemps *et* al 2008). Furthermore, we sought to determine whether lithium dilution calibration at rest differs from that obtained during exercise. To determine construct and concurrent validity, we compared $\dot{Q}$ values to contemporaneous measures of $\dot{V}O_2$, for comparison to previously published studies using invasive techniques.

Methods

Participants

Seven trained males (age 34.6 ± 7 years; body mass 82.9 ± 11 kg; $\dot{V}O_{2peak}$ 55.1 ± 5.2 ml•kg^{-1}•min^{-1}) were recruited to participate in the study. All participants were cyclists currently training for > 5 hours per week. Each subject provided written informed consent and the study was approved by the Kingston University Research Ethics Committee. Participants with a known history of cardiac or respiratory disease were excluded. Additionally, participants were prevented from volunteering if they were currently being prescribed muscle relaxants, had a lithium allergy, or were currently undergoing lithium therapy.

Study Protocols

Participants were asked to attend the laboratory on two occasions, 7-10 days apart, having abstained from caffeine, alcohol and strenuous exercise in the preceding 24 hours. During the initial visit, each subject completed an incremental exercise protocol (T1) on an electromagnetically braked cycle ergometer (Velotron, Racermate Inc.), set up to each participant's specifications, including alterations in saddle height, handlebar height and reach and crank length. This protocol consisted of three initial steady-state exercise stages, each lasting four minutes, at 100, 150 and 200 W, following which exercise intensity was increased at a rate of 0.5 W•s^{-1} in a ramp protocol, until volitional exhaustion. Participants were instructed to self-select their pedalling cadence and were encouraged to maintain this cadence throughout the duration of the incremental test. Verbal encouragement was provided throughout to ensure adequate motivation. Throughout the incremental exercise test, pulmonary gas exchange was recorded continuously (Jaeger Oxycon Pro, Hoecheberg, Germany) via a face mask fitted to the subject and averaged over 10-s epochs for the measurement of oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}E$) and respiratory exchange ratio (RER) calculated as $\dot{V}CO_2$ divided by $\dot{V}O_2$. $\dot{V}O_{2peak}$ was as the highest 10-s averaged value achieved at, or near, exhaustion.

During the second visit, participants performed an extended incremental protocol (T2) consisting of 10-min stages cycling at 40%, 60%, and 80% $\dot{V}O_{2peak}$ before completing a final 3-minute stage at 105% $\dot{V}O_{2peak}$ (MAX). Relative intensities were calculated as a percentage of the $\dot{V}O_{2peak}$ determined during T1. The workload during each exercise stage was determined by linear regression of the $\dot{V}O_2$-work rate relationship measured during the first three stages (100, 150, 200 W) of T1.

Cardiac output assessment

Throughout T2, continuous beat-to-beat $\dot{Q}$ was monitored by analysis of the radial artery pressure waveform (PulseCO™, LiDCO PLC, London, UK) coupled to a lithium indicator dilution calibration method incorporated into a single device (LiDCO™, LiDCO PLC, London, UK). Upon presenting to the laboratory for T2, a 21-gauge venous cannula was placed into a peripheral vein mid-way between the wrist and elbow of the right arm. A 20-gauge arterial cannula was then placed into the radial artery (Figure 1) of the left arm under local anaesthesia (2% Lidocaine) and connected to an ICU pressure monitor via a disposable pressure transducer (Philips M1567A, Philips, Germany), which was zeroed at ambient pressure at the level of the left ventricle. Once the pressure monitor is connected to the arterial line, it is slaved to the LiDCO monitor to obtain continuous pressure waveform data (Figure 2). Patency of the cannula was maintained by regular flushing of the arterial line with 10ml saline. The pulse contour analysis method subsequently calculates a nominal SV from a pressure-volume transform of the arterial pressure waveform. Nominal SV is converted to absolute SV using a lithium dilution method to calibrate the pulse waveform data.

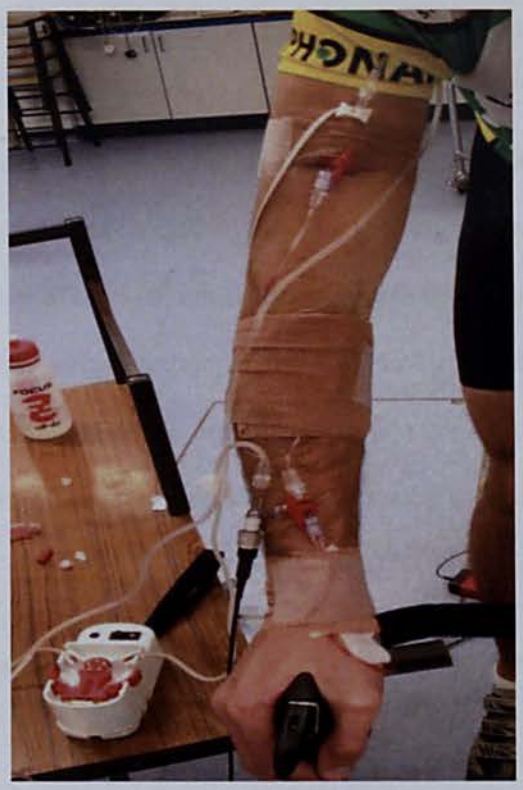

Figure 1. Experimental setup of the radial artery cannula connected to the lithium sensor and flow pump for the measurement of Q by lithium dilution.

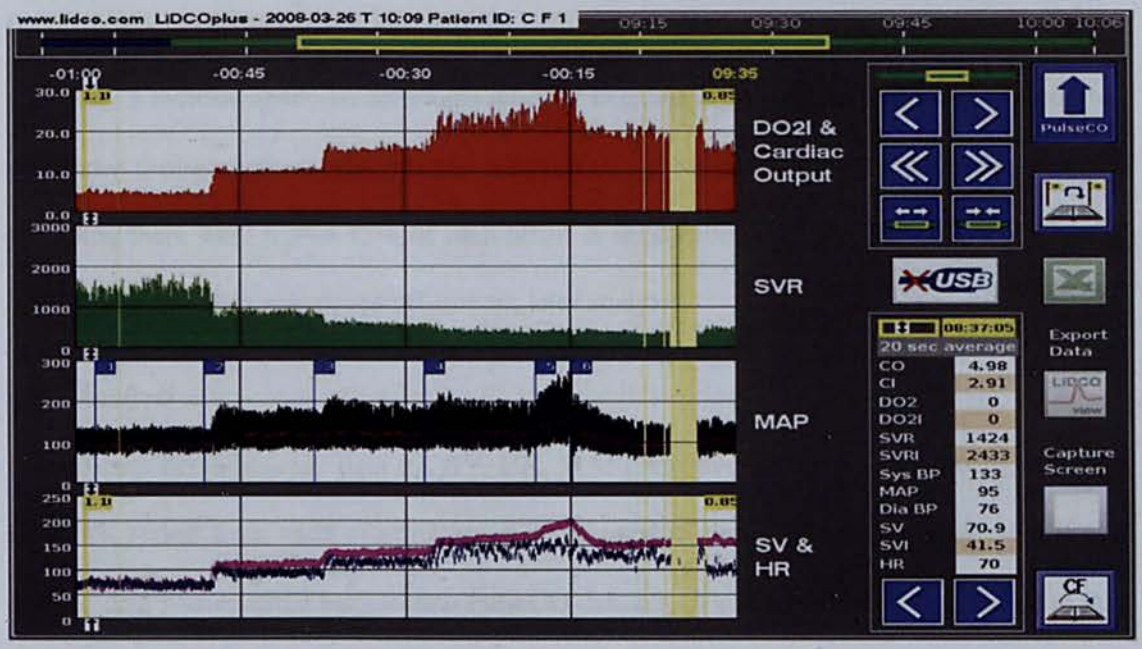

Figure 2. Typical profile of haemodynamic data obtained by the LiDCO™ device during an extended incremental exercise test.

Lithium dilution calibration

For the lithium dilution method, a bolus of lithium chloride (LiCl) was injected into the peripheral vein. To achieve this, the bolus of LiCl was parked into a 4ml extension tube attached to the venous cannula and flushed rapidly into the venous circulation with 20ml saline (NaCl). To ensure adequacy of the LiCl concentration-time curve, the bolus dose of LiCL was 3ml (0.45 mM). Following administration of the LiCl bolus, a lithium dilution curve was derived by drawing arterial blood past a lithium sensor at a constant flow rate using a follow pump. Cardiac output was calculated from the dilution curve according to the following equation:

$$\dot{Q}\ (L \bullet min^{-1}) = (LiCl\ dose\ (mM) \times 60) / (area\ under\ dilution\ curve\ (mM \bullet L^{-1} \bullet s^{-1}) \times (1\text{-}PCV))$$

Linton *et* al (1993)

where area is the integral of the primary indicator curve ($mM \bullet s^{-1}$) and PCV is packed cell volume, which is calculated as haemoglobin concentration ($g \bullet dl^{-1}$) divided by 34.

Upon establishment of a normal arterial pressure trace, baseline calibrations were performed in duplicate, 5 minutes apart. To determine whether exercise influenced the calibration factor, we performed further duplicate calibrations at 60% $\dot{V}O_{2peak}$. Successive calibrations were averaged providing there were within 15% of each other. A third calibration was performed if this was not the case. All data was stored off-line for later analysis.

Data Analysis

Beat-to-beat hemodynamic data ($\dot{Q}$, SV, HR, MAP) was averaged over 30s epochs, with the final 30s period from each exercise stage being used in the statistical analysis. All LiDCO derived data was reverted back to raw PulseCO data ($\dot{Q}_{raw}$) and subsequently compared to that obtained by multiplying $\dot{Q}_{raw}$ with the calibration factor obtained under resting conditions ($\dot{Q}_{restcal}$) and during exercise at 60% $\dot{V}O_{2peak}$ ($\dot{Q}_{excal}$). Linear regression was performed to assess the relationship between each $\dot{Q}$ method versus $\dot{V}O_2$. We also used $\dot{V}O_2$ data from each stage

to calculate the arterio-venous O_2 difference ((a-v)O_2diff) according to the Fick equation where:

$$(a\text{-}v)O_2diff = 100*(\dot{V}O_2\ (L\bullet min^{-1}) / \dot{Q}\ (L\bullet min^{-1}))$$

A Bland-Altman (Bland & Altman 1986) analysis was performed to assess the agreement between techniques by calculation of the mean bias and 95% limits of agreement. Differences between methods across exercise intensities were analysed using two-way ANOVA. Reliability of $\dot{Q}_{raw}$ and $\dot{Q}_{excal}$ was determined by a Bland-Altman comparison of duplicate T2 tests (T2(1) and T2(2)), completed 5-7 days part in a subset of three participants. A two-way ANOVA tested for significant differences between T2(1) and T2(2) across each exercise intensity. All data were analysed using GraphPad Prism (v5.0) and presented as mean ± SD unless otherwise indicated.

Results

We were able to collect $\dot{Q}_{raw}$ data continuously throughout exercise until exhaustion with only occasional dampening of the arterial pressure signal, which was rectified by saline flushing of the arterial line. Furthermore, we were able to obtain at least two adequate lithium dilution curves in all participants at rest and during exercise at 60% $\dot{V}O_{2peak}$.

Comparison between methods

Calibration factors ($\dot{Q}_{lithium} / \dot{Q}_{raw}$) obtained at rest and during exercise are shown in table 1. The mean calibration factor decreased by 25.7% from rest to exercise (1.67 ± 0.41 *versus* 1.24 ± 0.33, respectively). $\dot{Q}$ obtained with each calibration method is shown in Figure 1. $\dot{Q}$ recorded at rest was 5.1 ± 0.7, 8.6 ± 2.4, and 6.3 ± 1.7 L•min^{-1}, rising to 26.9 ± 2.5, 45.2 ± 13.6, and 32,7 ± 6.5 L•min^{-1} during maximal exercise for $\dot{Q}_{raw}$, $\dot{Q}_{restcal}$ and $\dot{Q}_{excal}$, respectively. Two-way ANOVA revealed a significant difference between calibration methods ($P < 0.01$) and an interactive effect that was significant (calibration method x exercise intensity; $P < 0.001$). Post-hoc testing revealed significant differences ($P < 0.05$) between $\dot{Q}_{raw}$ and $\dot{Q}_{restcal}$ at 60%,

80% and MAX. Significant differences were observed between $\dot{Q}_{excal}$ and $\dot{Q}_{restcal}$ at 80% and MAX ($P < 0.05$).

Table 1. Individual and mean calibration factors at rest and during exercise at 60% $\dot{V}O_{2peak}$. Calibration factors are shown as $\dot{Q}_{lithium} / \dot{Q}_{raw}$.

Subject	Resting Calibration Factor	Exercise Calibration Factor
1	1.71	1.77
2	2.20	1.20
3	1.46	1.02
4	2.11	0.96
5	1.36	1.38
6	1.80	1.53
7	1.05	0.85
Mean (SD)	**1.67 (0.41)**	**1.24 (0.33)**

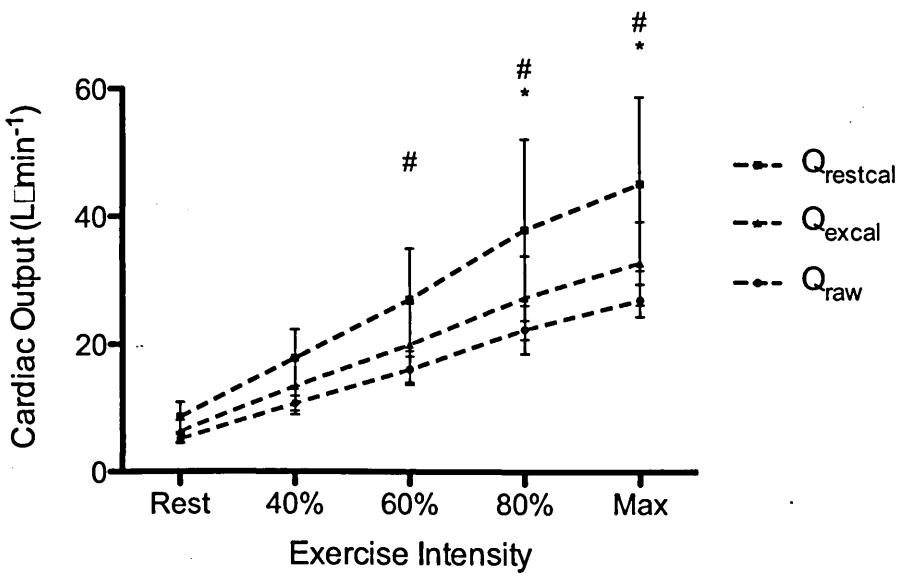

Figure 3. Cardiac output responses throughout T2 with each calibration method (n = 7). # indicates significant difference between $\dot{Q}_{raw}$ and $\dot{Q}_{restcal}$, * indicates significant difference between $\dot{Q}_{excal}$ and $\dot{Q}_{restcal}$.

Bland-Altman comparisons between each pair of methods are shown in Figure 2. The percentage bias between $\dot{Q}_{raw}$ and $\dot{Q}_{restcal}$ was 50.8% (-11.1 L•min^{-1}) with limits of agreement of ±85.6% (-29.7 to 7.6 L•min^{-1}). Between $\dot{Q}_{restcal}$ and $\dot{Q}_{excal}$, the mean bias was 31.1% (7.3 L•min^{-1}) with limits of agreement of ±82% (-12.0 to 26.7 L•min^{-1}). The mean bias between $\dot{Q}_{raw}$ and $\dot{Q}_{excal}$ was 20.5% (-3.7 L•min^{-1}) with limits of agreement of ± 60.9% (-14.8 to 7.3 L•min^{-1}).

Relationship with oxygen uptake

Figure 3 shows the least squares fit linear regression line between each calibration method and $\dot{V}O_2$. Pearson's correlation coefficients were r = 0.88, 0.84, and 0.61 for $\dot{Q}_{raw}$, $\dot{Q}_{restcal}$ and $\dot{Q}_{excal}$, respectively, against $\dot{V}O_2$. The linear regression equations for each $\dot{Q}$-$\dot{V}O_2$ relationship are shown in table 2, alongside those obtained in selected previous studies using alternative methods for determining $\dot{Q}$. The (a-v)O_2diff for each method during maximal exercise was 17.7 ± 1.7, 10.3 ± 2.4, and 14.1 ± 2.9 ml•dL^{-1} for $\dot{Q}_{raw}$, $\dot{Q}_{restcal}$ and $\dot{Q}_{excal}$, respectively.

Reliability

The test-retest reliability was determined in three participants for $\dot{Q}_{raw}$ and $\dot{Q}_{excal}$. The correlation between duplicate $\dot{Q}_{raw}$ and $\dot{Q}_{excal}$ measures was r = 0.99 and 0.98, respectively (Figure 5). There were no significant differences (P > 0.05) between T1 and T2 for either method. The mean bias between tests for $\dot{Q}_{raw}$ was 0.02 L•min^{-1} with 95% limits of agreement from -2.95 to 2.90 L•min^{-1}. For $\dot{Q}_{excal}$, the mean bias was 1.87 L•min^{-1} with limits of agreement from -6.40 to 2.66 L•min^{-1}.

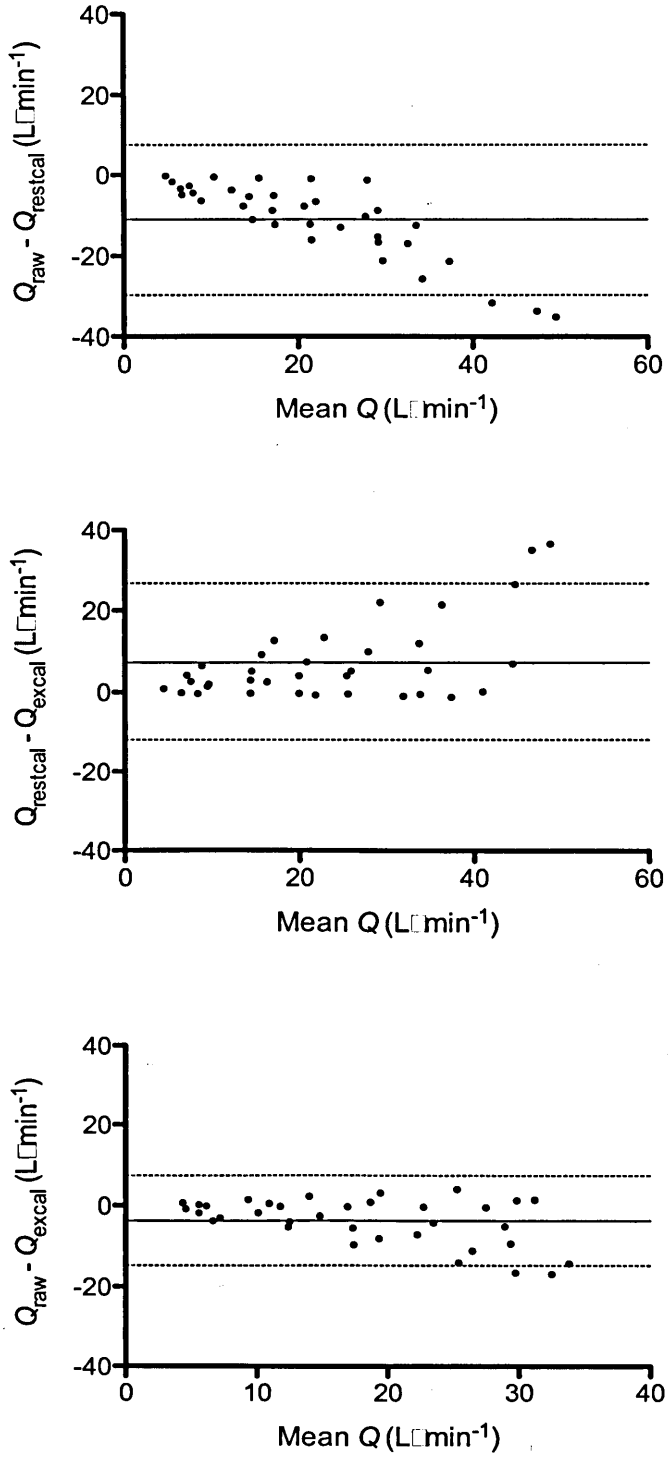

Figure 4. Bland-Altman plots comparing each pairing of $\dot{Q}_{raw}$, $\dot{Q}_{restcal}$ and $\dot{Q}_{excal}$ at rest and during exercise. Solid line represents mean bias with dashed lines showing the 95% limits of agreement.

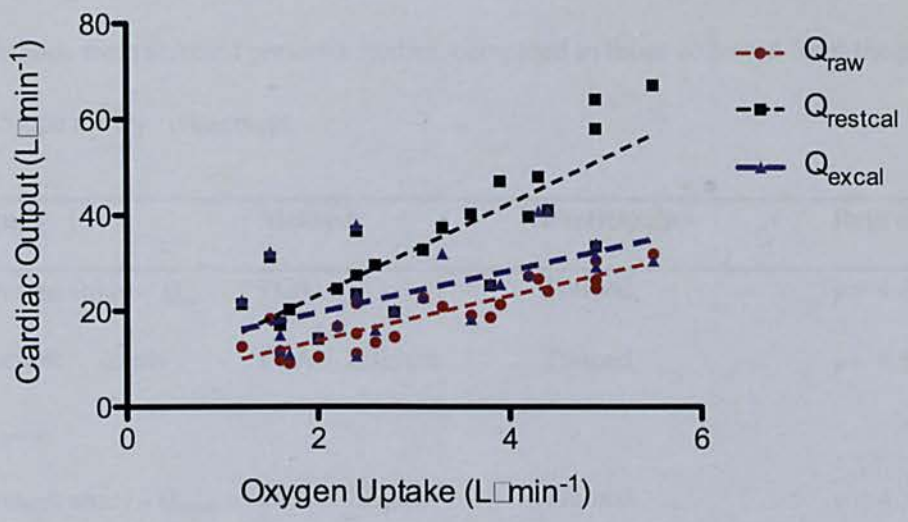

Figure 5. Scatterplot showing the least squares fit linear regression line for $\dot{Q}$-$\dot{V}O_2$ according to each calibration method (n = 6). Linear regression equations for each best-fit line are shown in table 2.

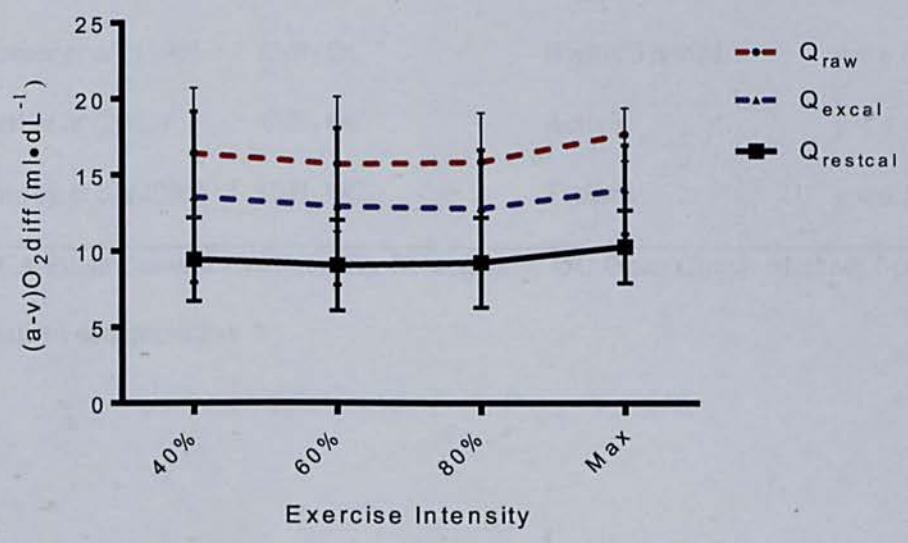

Figure 6. Plot showing the (a-v)O_2diff at each exercise intensity for each calibration method (n = 6)

Table 2. Regression equations quantifying the $\dot{Q}$-$\dot{V}O_2$ relationship with alternative accepted methods from selected previous studies, compared to those collected from the present study. $\dot{Q}$ = Slope x $\dot{V}O_2$ + y-intercept.

Study	Method	Participants	Regression Equation
Present study – $\dot{Q}_{raw}$	PCA	Trained	$y = 4.66\ \dot{V}O_2 + 4.54$
Present study – $\dot{Q}_{restcal}$	PCA + Lithium	Trained	$y = 9.57\ \dot{V}O_2 + 4.14$
Present study - $\dot{Q}_{excal}$	PCA + Lithium	Trained	$y = 4.39\ \dot{V}O_2 + 10.9$
Johnson *et al* (2000)	Fick	Active	$y = 4.60\ \dot{V}O_2 + 4.65$[a]
Astrand *et al* (1964)	Dye	Active	$y = 5.10\ \dot{V}O_2 + 4.15$[a]
Smyth *et al* (1984)	Dye	Active	$y = 4.82\ \dot{V}O_2 + 6.70$
Smyth *et al* (1984)	C_2H_2 Rb	Active	$y = 5.04\ \dot{V}O_2 + 4.67$
Gledhill *et al* (1994)	C_2H_2 Rb	Highly Trained	$y = 5.92\ \dot{V}O_2 + 7.18$
Barker *et al* (1999)	C_2H_2 OC	Highly Trained	$y = 6.67\ \dot{V}O_2 + 2.38$
Bell *et al* (2003)	C_2H_2 OC	Active	$y = 4.40\ \dot{V}O_2 + 7.80$
Dibsky *et al* (2005)	C_2H_2 OC	Trained	$y = 6.12\ \dot{V}O_2 + 3.00$

PCA Pulse Contour Analysis; **Rb** Rebreathing; **OC** Open Circuit Method; [a] calculated on the basis of data provided.

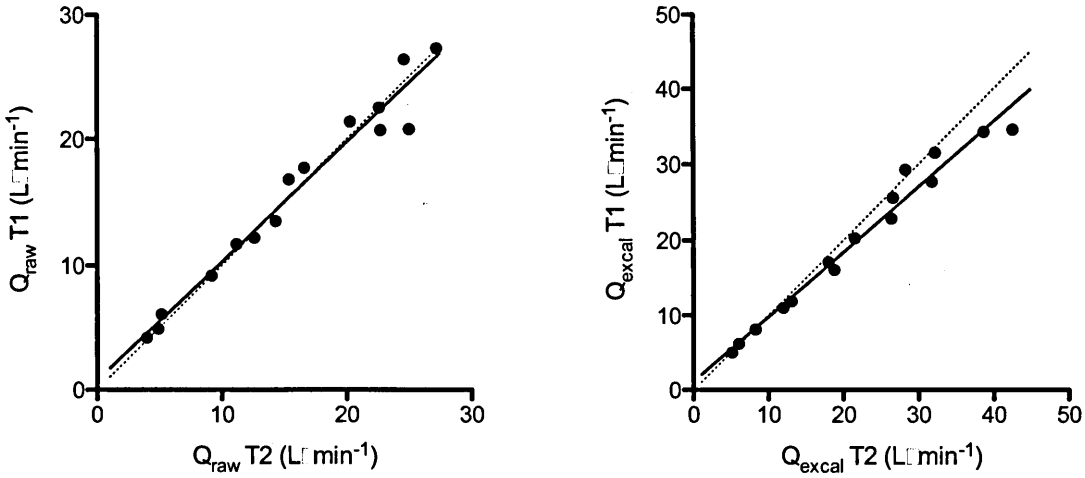

Figure 7. Scatterplot comparing cardiac output values from T1 and T2 (n = 3). Solid line represents the linear regression line. Dashed line represents the line of identity.

Discussion

The primary aims of this study were to evaluate the feasibility and practicality of using pulse contour analysis, calibrated with a lithium dilution method, for the measurement of $\dot{Q}$ during exercise in healthy, trained cyclists. In the absence of any previous studies employing this method during exercise in a healthy cohort, we also sought to determine the construct and concurrent validity of this method as well as assessing the reliability of measurements in a small sub-set of participants. Perhaps the most important finding of this study was that large differences exist in the calibration factors obtained at rest and during exercise. We observed an approximately 26% decrease in the mean calibration factor from rest to exercise, which was driven by a <15% decrease in 5 of 7 participants (71%), whilst 2 of 7 participants (29%) showed a <3% change. Consequently, significant differences were observed between $\dot{Q}_{\text{restcal}}$ and $\dot{Q}_{\text{excal}}$ at 80% $\dot{V}O_{2\text{peak}}$ and maximal exercise. Significant differences were also observed for $\dot{Q}_{\text{restcal}}$ versus $\dot{Q}_{\text{raw}}$, but not between $\dot{Q}_{\text{raw}}$ and $\dot{Q}_{\text{excal}}$.

Why the calibration factor should decrease with exercise is unclear, although examination of the algorithm incorporated into the LiDCO™ device for the purpose of $\dot{Q}_{\text{raw}}$ measurements provides some insight. This approach is based upon the assumption that a net power change in a heartbeat is the balance between the input of a mass of blood minus the blood lost to the periphery during the beat (Rhodes and Sunderland 2005). Underlying this is the assumption that following correction for arterial compliance, there is a linear relationship between net power and net flow. Thus, calibration by lithium dilution provides the opportunity to correct for individual differences in arterial compliance under resting conditions. As well as individual differences, our data suggests lithium dilution may also be used to correct for changes in arterial compliance that occur with exercise. Previous studies (Otsuki *et* al 2006; Otsuki *et* al 2008) have demonstrated the progressive decline in arterial compliance that occurs with increasing exercise intensity. Furthermore, Rhodes and Sunderland (2005) state that any potential drift or change in the calibration factor is limited to the extent that the arterial tree maximum volume (i.e. arterial compliance) can change over time. Thus, with any large change

in systemic haemodynamics (i.e. rest to exercise), a change in arterial compliance may necessitate a recalibration of the device.

In the absence of a 'gold standard' comparator, determining which calibration method provides the most accurate measurements of $\dot{Q}$ requires alternative approaches. We simultaneously measured $\dot{V}O_2$, which was used to determine construct validity of each method (Rowland and Obert, 2002). The linear regression of $\dot{Q}$ and $\dot{V}O_2$ (Figure 3, Table 1) for both $\dot{Q}_{raw}$ and $\dot{Q}_{excal}$ were characterised by a slope that was similar to that observed with both invasive (Smyth *et* al 1984; Johnson *et* al 2000) and non-invasive techniques (Smyth *et* al 1984; Bell *et* al 2003), although it should be noted that the strength of the correlation for $\dot{Q}_{excal}$ was lower than previously reported. The regression equation for $\dot{Q}_{restcal}$ yielded a slope in excess of 9, indicating a large overestimation of $\dot{Q}$ during exercise. Additionally, the (a-v)O_2diff was calculated with each $\dot{Q}$ method (Figure 4). $\dot{Q}_{raw}$ returned values at maximal exercise, which were in close agreement with those measured directly during maximal exercise using invasive methods (Stringer *et* al 1997; Mortensen *et* al 2008). Values obtained using $\dot{Q}_{excal}$ data showed slight overestimation of $\dot{Q}$, particularly at higher exercise intensities. Maximal (a-v)O_2diff using $\dot{Q}_{restcal}$ data provided further evidence of large overestimation by this method, where (a-v)O_2diff reached a mean peak of 10.3 ml•dL^{-1}, considerably lower than the 16-18.0 ml•dL^{-1} peak (a-v)O_2diff measured directly elsewhere (Gonzalez-Alonso and Calbet 2003; Stringer *et* al 1997; Mortensen *et* al 2008).

To date, only one study has evaluated the LiDCO™ system during exercise (Kemps *et* al 2008). Ten male chronic heart failure patients were assessed during symptom-limited incremental exercise. Whilst good agreement with direct Fick was observed in this study, there was some evidence of overestimation of $\dot{Q}$ values above 10 L•min^{-1}, which would equate approximately to the $\dot{Q}$ values we obtained from the 40% $\dot{V}O_{2peak}$ stage of exercise in T2 of the current study. These authors postulated that the apparent overestimation in the higher ranges was due to an overestimation of $\dot{Q}$ during the calibration procedure, which occurred at rest

only. This was primarily based on the observation that the relative change in $\dot{Q}$ in the higher ranges was similar for both LiDCO and direct Fick methods. It may be postulated from these findings that, were the subject group studied able to exercise to higher cardiac outputs, greater differences between methods might have been observed and that these differences are more attributable to greater changes in arterial compliance than observed in their current cohort.

Our findings of a good correlation and no significant differences between $\dot{Q}_{raw}$ and $\dot{Q}_{excal}$ between repeat exercise tests, supports the reliability of these methods. Although this analysis was performed in a small subset of three participants, it nonetheless represents the first evidence of the test-retest reliability of this device. We did, however, observe some degree of variability about the line of identity at higher exercise intensities, particularly with $\dot{Q}_{excal}$ data. Further data is required to establish the test-retest reliability of these methods.

Study Limitations

Several limitations with this study are worthy of mention; Firstly, we were unable to employ a 'gold standard' comparator to assess the true validity of calibrated pulse contour analysis. However, the benefits of applying invasive methods based on cardiac catheterisation to healthy participants are outweighed by the risks involved, including arrhythmias and perforation of the heart wall (Scheer *et* al 2002). Future studies should consider comparing the technologies included in this study to other accepted non-invasive methods such as acetylene uptake and/or Doppler echocardiography. Secondly, the experimental design did not include an assessment of whether the calibration factor changed with each increase in exercise intensity. We propose that further change in arterial compliance, as documented elsewhere (Otsuki *et* al 2006) may alter the calibration factor further, thus warranting further research into this aspect of device performance during exercise.

Conclusion

In conclusion, there are significant differences between the $\dot{Q}$ values obtained by each method, with $\dot{Q}_{restcal}$ yielding $\dot{Q}$ values substantially larger than those estimated according to previous

literature employing invasive techniques. We observed a decrease in the calibration factor from rest to exercise that limits the benefit or use of resting lithium dilution measurements for the calibration of pulse contour analysis during exercise. Further studies should seek to evaluate the behaviour of the calibration factor by lithium dilution, across a range of exercise intensities before this approach can be employed to assess systemic hemodynamic during stochastic exercise with varying workloads and cardiovascular demand. Nonetheless, this analysis offers an insightful first assessment of the LiDCO™ device during exercise, which suggests that lithium dilution calibrated pulse contour analysis, may provide reasonable estimates of $\dot{Q}$ during exercise providing that calibration is performed during exercise.

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CHAPTER 4:

Study III:

Optimising the measurement of cardiac output during exercise using lithium dilution and pulse contour analysis.

Adapted from:

Elliott, A., Skowno, J., Prabhu, M., and Ansley L. (2012). Measurement of cardiac output during exercise in healthy, trained humans using lithium dilution and pulse contour analysis. *Physiological Measurement*, **33**, p1691-1701

Introduction

The cardiac output ($\dot{Q}$) response to aerobic exercise yields valuable information regarding the patency of the cardiorespiratory system and its capacity to meet an increase in metabolic demand. Whilst the first accurate measures of $\dot{Q}$ during exercise were made almost 100 years ago, it was not until the landmark study of Astrand *et* al (Astrand *et* al 1964), using the dye-dilution technique, that $\dot{Q}$ was determined across a range of exercise intensities. It is commonly accepted that invasive methods requiring cardiac catheterisation, including direct Fick and/or thermodilution, represent the 'gold standard' methods to which all other techniques are compared (Warburton *et* al 1999a). However, the practicality of these techniques for measuring $\dot{Q}$ in healthy individuals is limited by the specialist expertise, facilities and equipment required and above all, the inherent risk associated with central or pulmonary artery catheterisation (Scheer *et* al 2002). Further concerns for the adoption of these methods is the lack of continuous measurement, whereby those methods typically measure systemic hemodynamic data at isolated time points, and the requirement for a cardiovascular steady state, which may limit their application to maximal exercise in individuals without compromised cardiovascular function.

Over the past 30 years, a range of techniques for the evaluation of $\dot{Q}$ have been evaluated during exercise that rely on either minimally or non-invasive approaches to measurement. These include open-circuit acetylene rebreathing (Johnson *et* al 2000), CO_2 rebreathing (Jakovljevic *et* al 2008); inert gas rebreathing (Elliott *et* al 2010), Doppler echocardiography (Christie *et* al 1987), impedance cardiography (Richard *et* al 2001), bioreactance (Elliott *et* al 2010) and the Modelflow method (Tam *et* al 2004). Whilst foreign gas rebreathing techniques are perhaps the most commonly employed non-invasive methods during exercise (Warburton *et* al 1999b), they are not without limitations including low time resolution, a commonly reported underestimation of $\dot{Q}$ during exercise (Jarvis *et* al 2007) and the possibility of ventilation mediated adjustments in $\dot{Q}$ (Stok *et* al 1999). Doppler echocardiography, which calculates stroke volume from aortic flow velocity and aortic outflow cross-sectional area

(Rowland & Obert 2002), has shown encouraging data but is methodologically limited during strenuous exercise as a Doppler probe needs to be held at a fixed angle to the aorta, a requirement that can be difficult to meet in the setting where upper body movements may be large. Continuous hemodynamic monitoring is possible with both the impedance cardiography and bioreactance methods during exercise. However, conflicting reports of its accuracy during exercise (Warburton *et* al 1999b; Elliott *et* al 2010) as well as wide limits of agreement when compared with direct Fick (Bougault *et* al 2005; Kemps *et* al 2008) raises questions over its validity during exercise.

A valid, minimally invasive method for the continuous measurement of $\dot{Q}$ and related systemic hemodynamic variables at rest and during exercise is particularly desirable for both clinicians and exercise physiologists alike. Techniques based upon arterial pressure analysis meet the requirements of being both minimally invasive and continuous measurements of $\dot{Q}$. The Modelflow method (Wesseling *et* al 1993), using either finger or intra-arterial pressure measurements has been assessed in a number of studies, in which agreement has been shown, conflictingly, as adequate (Sugawara *et* al 2003) or poor (Houtman *et* al 1999) with previously accepted methods as comparators. Whilst some attempt to modify this approach has been made (Bartels *et* al 2011), without a valid calibration technique, the systematic bias and wide limits of agreement preclude the use of this technique for recording absolute $\dot{Q}$ values during exercise.

An alternative method of recording $\dot{Q}$ based upon the analysis of the arterial pressure waveform, with lithium dilution as an incorporated calibration method (LiDCO™, Cambridge, UK), was assessed during exercise in study II. Previously, the only study to have used this device during exercise (Kemps *et* al 2008) was in chronic heart failure patients, where the LiDCO™ device showed close agreement with direct Fick measurements, albeit in a population with impaired left ventricular function resulting in peak $\dot{Q}$ values ~12 $L \bullet min^{-1}$. Accuracy and precision of this technique in the clinical setting has been widely reported (Kurita *et* al 1997; Linton *et* al 2000a; Cecconi *et* al 2010). In study II, we showed that this method appears to overestimate $\dot{Q}$ throughout exercise when the device is calibrated at rest.

Single point calibration during exercise provided a 'correction' for this error such that mean values obtained with exercise-calibrated pulse contour measurements showed reasonable construct and concurrent validity. Furthermore, we observed reasonable test-retest reliability in this method during exercise. Why the calibration factor should change during exercise is unclear, although we postulated that alterations in arterial compliance may play a role as calibration serves to correct for this element (Rhodes & Sunderland 2005). The only previous study of this device during exercise (Kemps *et* al 2008) incorporated only a single calibration under resting conditions prior to exercise. One might postulate that the trend towards overestimation of $\dot{Q}$ at the higher exercise intensities may be attributed to an alteration in the calibration factor with exercise-induced hemodynamic changes.

The aims of this study were threefold: first, to compare the values derived from PulseCO to those calibrated by lithium dilution; second, to confirm whether recalibration is warranted during exercise, and; thirdly, to determine the effect of increasing exercise intensity on the behaviour of the calibration factor. We hypothesised that the calibration factor would decrease with increasing exercise intensity.

Methods

Participants

Ten recreationally trained non-smoking male cyclists, whose characteristics are displayed in Table 1, volunteered to participate in the study. All participants were training for >3 hours per week at the time of the study. Exclusion criteria for participation included a history of cardiopulmonary disease, lithium allergy, and current lithium therapy. The protocol was explained in detail to the participants before they gave written informed consent. The study was approved by the local research ethics committees at Northumbria University and Kingston University. All procedures were performed in accordance with national (Hull *et* al 2008) and international (Declaration of Helsinki, 1964) guidelines.

Table 1. Characteristics of study participants (n = 10)

Subject Characteristic	Mean (SD)
Age (years)	39.7 (8.4)
Body Mass (Kg)	80.7 (10.2)
Height (cm)	179.2 (5.5)
Peak $\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	53.5 (5.8)

Study Design

Participants were asked to report to the laboratory after abstaining from heavy exercise, caffeine and alcohol in the 24 hours preceding testing. During their visit to the laboratory, participants performed two exercise trials, which were separated by one hour. Exercise trials were performed on an electromagnetically braked cycle ergometer (Velotron, Racermate Inc., USA) set up to each participant's specifications. Participants were allowed to self-select their cadence during each trial. The investigators provided verbal encouragement throughout each trial.

The visit to the laboratory comprised two exercise tests (Figure 1). The first trial (T1) consisted of an incremental protocol to exhaustion from which the power output at ventilatory threshold (VT) was determined as described previously (Beaver *et* al 1986). Briefly, following a 10-min period of seated rest on the ergometer, participants began cycling at 120 W for 6 min. Power output was subsequently increased by 30 W every 3 min until the participant reached volitional exhaustion. The workload during the second trial (T2) was derived from the VT identified in T1. During T2 participants completed four workload stages, each separated by a two-minute active recovery period, at 100 W. The initial three stages were all eight minutes in duration: stage one (Stage 1) was 120 W; stage two (Stage 2) was 80% of power at ventilatory threshold (<VT); and stage three (Stage 3) was the power at VT plus 30% of the difference between power at VT and peak power achieved during T1 (>VT). The final stage (Stage 4) was performed at a power output 10% above that the peak power achieved during T1 and continued until volitional fatigue. Pulse contour analysis of $\dot{Q}$ was calibrated at rest and at the

end of Stage 2 and Stage 3. The calibration factor derived at each calibration point was then applied to all preceding and succeeding values in order to assess the validity of single-point calibrations.

Throughout each trial pulmonary gas exchange was recorded continuously (Oxycon Pro, Jaeger) and stored off-line for later analysis. Participants wore a close-fitting oronasal facemask attached to a triple-V sensor (Oxycon, Jaeger) consisting of a gas analysis tube and flow turbine. The gas analyser was calibrated prior to each subject's visit using a gas with a known composition (16% O_2 : 4% CO_2). Calibration of the flow turbine was performed using a 3-L air syringe (Hans Rudolph) over a range of flow rates.

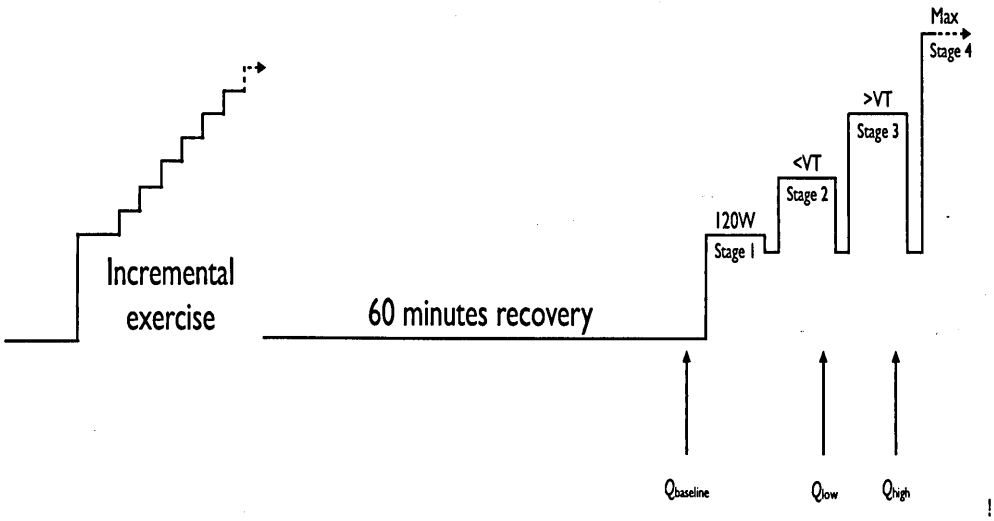

Figure 1. Protocol diagram depicting T1 and T2. Arrows indicate the timepoints for LiDCO calibrations

Cardiac output assessment

Cardiac output was monitored beat-by-beat throughout exercise by analysis of the arterial pressure waveform (PulseCO, Cambridge, UK) calibrated with lithium dilution (LiDCO, Cambridge, UK) at strategic timepoints. First, a 21-gauge venous cannula was placed into a peripheral vein mid-way between the wrist and elbow of the right arm. A 20-gauge arterial cannula was then placed into the radial artery of the left arm under local anaesthesia (2%

Lidocaine) and connected to an ICU pressure monitor (Hewlett Packard) using a disposable pressure transducer (Philips M1567A, Philips, Germany). The pressure monitor is slaved to the LiDCO™ monitor to obtain continuous pressure waveform data. The pulse-contour analysis method calculates a nominal stroke volume from a pressure-volume transform of the arterial pressure waveform. Nominal stroke volume is converted to absolute stroke volume using a lithium dilution method to calibrate the pulse waveform data.

Lithium dilution calibration

A bolus of lithium chloride (LiCl) was injected into the peripheral vein using either the technique recommended by the manufacturer (participants 1-5) or a modified technique (participants 6-10). The manufacturer guidelines recommended parking the bolus of lithium in a 4 mL extension tube and flushing the LiCl into the venous cannula with 20 mL saline. The bolus dose of lithium was increased from 2 mL (0.3 mM) to 3mL (0.45 mM) in some instances in order for the concentration-time calibration curve to reach the minimal threshold. This was usually necessary in larger participants (>80Kg) and when calibrations were performed at heavy workloads. In participants 1-5, we noted evidence of LiCl entry into the circulation when the lithium was parked in the tube prior to flushing. The modified technique eschewed parking the LiCl bolus in an extension tube and instead, the LiCl was injected from a separate 5ml syringe and flushed through by 20 mL saline. Using a saline and distilled-water model of bolus injection, we calculated the early entry of lithium into the circulation following the manufacturer's guidelines to be consistently around 9%. Therefore, we applied a correction factor to the lithium dilution-derived cardiac output determined in participants 1-5.

Following the administration of the LiCl bolus, a lithium dilution curve was derived by drawing arterial blood past a lithium sensor at a constant flow rate using a flow pump. The cardiac output was calculated from the dilution curve according to the following equation:

$$\dot{Q} = (\text{LiCl dose (mM)} \times 60) / (\text{area under dilution curve (mM}\bullet\text{L}^{-1}\bullet\text{s}^{-1}) \times (1\text{-PCV}))$$

(Linton *et* al 1993)

where PCV = haemoglobin ($g\bullet dl^{-1}$) / 33

Baseline calibrations were performed in duplicate prior to T2 (baseline). Additional calibrations were performed if the initial two calibrations did not agree to within 15%. During T2, duplicate calibrations were started at 5 minutes into each submaximal stage (<VT and >VT). During the maximal stage in T2 a single calibration was begun one minute into the stage.

The uncalibrated PulseCO values ($\dot{Q}_{raw}$) were determined by interpolating the mean averaged $\dot{Q}_{raw}$ values in the 10s preceding and succeeding the calibration period. This provided a representative value for $\dot{Q}_{raw}$ at the point of LiDCO determination. To assess the validity of a single calibration at each exercise intensity as well as multiple calibrations across exercise intensities, we compared the raw uncalibrated PulseCO data ($\dot{Q}_{raw}$), $\dot{Q}$ calibrated prior to exercise ($\dot{Q}_{baseline}$), during Stage 2 ($\dot{Q}_{low}$), during Stage 3 ($\dot{Q}_{high}$), and calibrations at each exercise intensity throughout T2 ($\dot{Q}_{exercise}$). Heart rate (HR) and arterial pressure was recorded directly by the LiDCO™ monitor from the arterial pressure trace.

Statistical Analysis

All data were analysed using GraphPad Prism (v5.0). Exercise data were taken from the T2 trial unless otherwise indicated. Linear regression analyses were performed for each $\dot{Q}$ method versus $\dot{V}O_2$. A Bland-Altman analysis was performed to assess the agreement between techniques. Differences between methods across exercise intensities were analysed using two-way ANOVA. Differences in $\dot{Q}$ values between exercise stages for each method were analysed using a repeated measures ANOVA with Bonferroni adjusted post-hoc analyses. All data are presented as mean ± standard deviation unless otherwise indicated.

Results

Successful calibrations were performed in duplicate at all resting and submaximal exercise stages. At rest, one subject required a third calibration due to lack of agreement between the first and second baseline calibrations. Because of aberrant shapes of the dilution curves ($n = 2$) and /or subject fatigue (n=1), 3 of 10 (30%) calibrations were unable to be performed at maximal exercise.

$\dot{Q}$ calculated using each method is presented in table 2. A two-way ANOVA revealed a significant difference ($P < 0.001$) between the calculation methods, with a significant interaction (Method x Intensity; $P < 0.0001$) effect. Post-hoc analyses revealed a significant difference ($P < 0.05$) between $\dot{Q}_{baseline}$ and all other calculation methods at each exercise stage, but not at rest. There were no significant differences observed between all the other methods ($P > 0.05$).

The mean bias between each method during exercise is outlined in table 3. The closest agreement with $\dot{Q}_{exercise}$ was observed for $\dot{Q}_{high}$ (4.8 ± 30.0%). The comparison of $\dot{Q}_{low}$ and $\dot{Q}_{high}$ was characterised by low bias and wide limits of agreement (1.0 ± 46.5%). The relative change in $\dot{Q}$ between exercise stages is shown in table 4 for $\dot{Q}_{raw}$ and $\dot{Q}_{exercise}$, respectively. A significant difference ($P<0.01$) was observed between methods for detecting the change in $\dot{Q}$ between rest and stage 2 and between stage 3 and stage 4, but not between stages 2 and 3.

The $\dot{Q}$ response to increasing exercise intensity differed between methods. $\dot{Q}_{raw}$ data showed a progressive significant increase (Figure 2, $P < 0.05$) at each successive exercise intensity. In contrast, $\dot{Q}_{baseline}$, $\dot{Q}_{low}$ and $\dot{Q}_{exercise}$ increased from rest but did not rise significantly between successive exercise intensities. $\dot{Q}_{high}$ showed no significant increase ($P < 0.05$) between Stage 3 and Stage 4. Each method was significantly correlated with $\dot{V}O_2$ during exercise (Figure 3), where the strongest correlation ($r = 0.82$; $P < 0.001$) was observed for $\dot{Q}_{high}$.

Table 2. Pulmonary gas exchange and systemic haemodynamic data at rest and each exercise stage. Haemodynamic data is corrected using the calibration factors obtained from baseline and at each submaximal exercise stage for comparison.

Variable	Calibration Factor	Rest	Stage 2	Stage 3	Stage 4
Power Output (W)	-	-	186 (24.8)	260 (31.5)	355 (38.4)
$\dot{V}O_2$ (L•min^{-1})	-	0.4 (0.09)	2.8 (0.4)	3.6 (0.5)	3.9 (0.5)
HR (beats•min^{-1})	-	84 (11)	151 (13)	174 (14)	181 (13)
$\dot{Q}_{raw}$ (L•min^{-1})	0.0	5.7 (1.3)	20.2 (3.1)#	24.1 (2.1)#	27.8 (3.3)#
$\dot{Q}_{baseline}$ (L•min^{-1})	1.66 (0.53)	9.3 (2.9)	33.2 (11.2)*#	40.0 (13.6)*	46.0 (15.1)*
$\dot{Q}_{low}$ (L•min^{-1})	1.08 (0.34)	6.2 (2.6)	21.6 (7.0)#	26.4 (9.4)	30.4 (11.0)
$\dot{Q}_{high}$ (L•min^{-1})	1.09 (0.24)	6.2 (1.5)	22.1 (5.4)#	26.5 (6.3)#	30.6 (7.6)
$\dot{Q}_{exercise}$ (L•min^{-1})	-	9.3 (3.0)	21.6 (7.0)#	26.5 (6.3)	27.7 (4.2)

Values are mean (SD); $\dot{V}O_2$, oxygen consumption; HR, heart rate. * Significant difference to other $\dot{Q}$ methods, # Significantly different from previous measurement

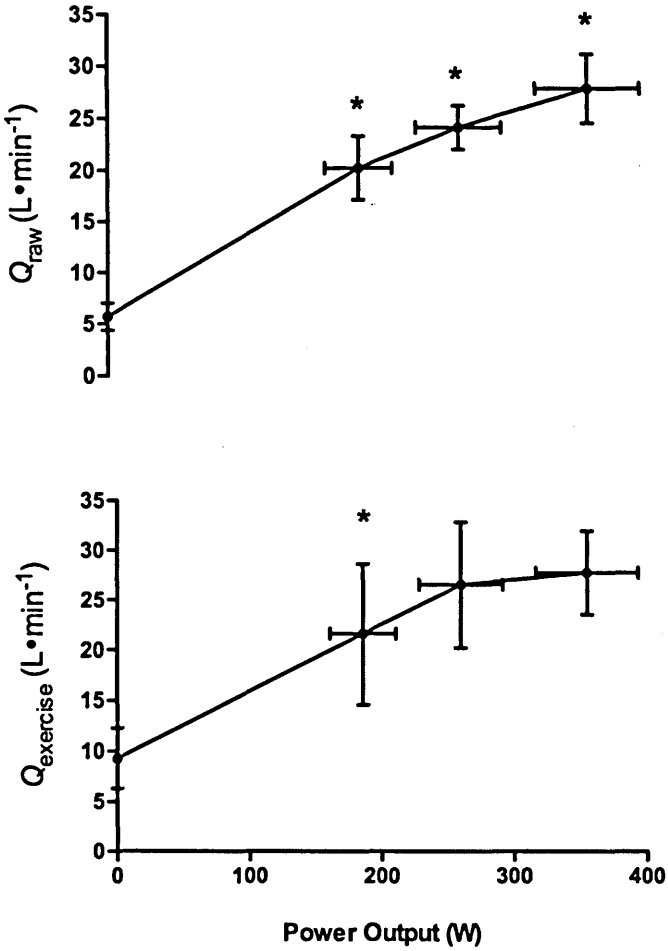

Figure 2. The mean cardiac output response of $\dot{Q}_{raw}$ and $\dot{Q}_{exercise}$ from rest to maximal exercise. * denotes significant difference compared to previous stage

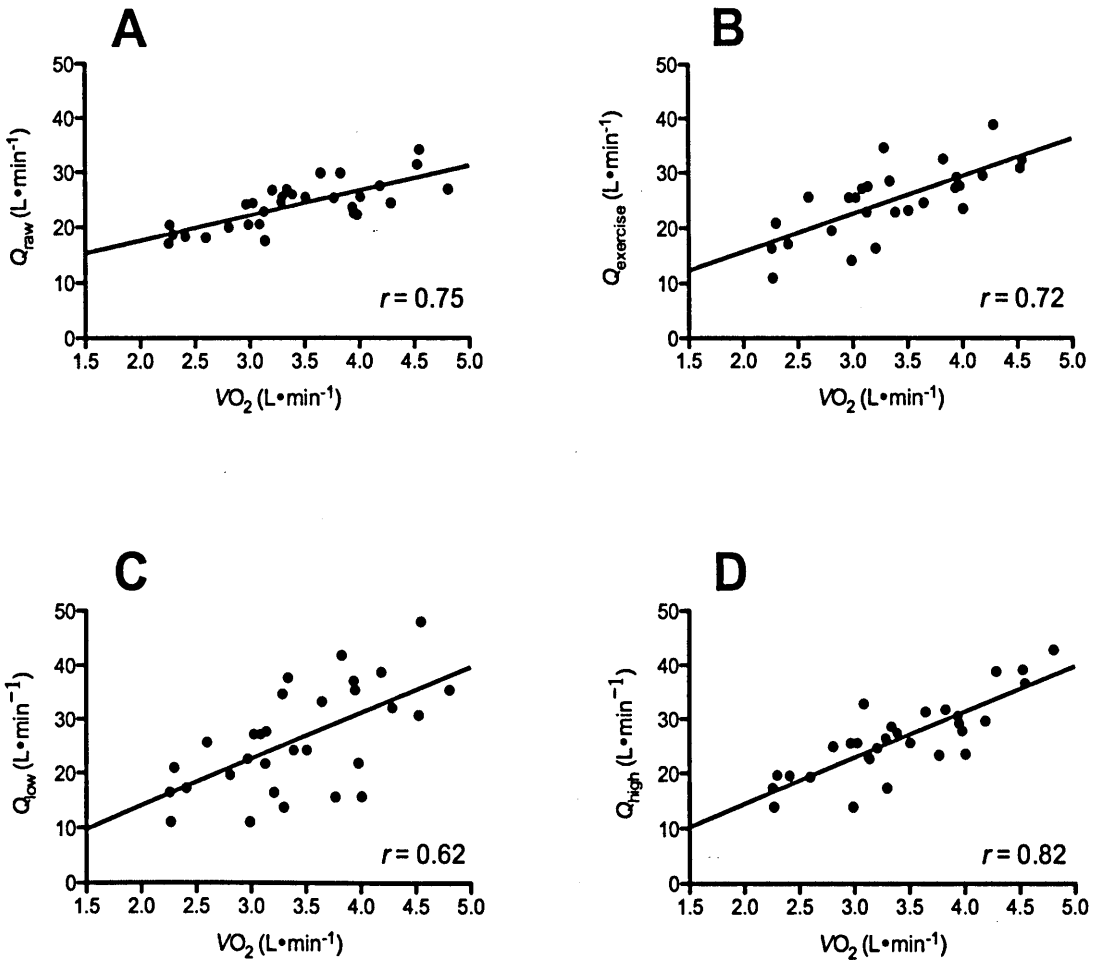

Figure 3. Correlation between $\dot{V}O_2$ and $\dot{Q}_{raw}$ (A), $\dot{Q}_{exercise}$ (B), $\dot{Q}_{low}$ (C) and $\dot{Q}_{high}$ (D) during exercise

Table 3. Mean bias (%) between $\dot{Q}$ calculation methods during exercise.

Method	$\dot{Q}_{raw}$	$\dot{Q}_{baseline}$	$\dot{Q}_{low}$	$\dot{Q}_{high}$	$\dot{Q}_{exercise}$
$\dot{Q}_{raw}$	-	49.3 (±77.6)	8.4 (±62.7)	9.4 (±42.5)	4.9 (±46.1)
$\dot{Q}_{restcal}$	49.3 (±77.6)	-	41.3 (±61.2)	40.3 (±51.5)	44.4 (±55.3)
$\dot{Q}_{low}$	8.4 (±62.7)	41.3 (±61.2)	-	1.0 (±46.5)	6.3 (±42.1)
$\dot{Q}_{high}$	9.4 (±42.5)	40.3 (±51.5)	1.0 (±46.5)	-	4.8 (±30.0)
$\dot{Q}_{exercise}$	4.9 (±46.1)	44.4 (±55.3)	6.3 (±42.1)	4.8 (±30.0)	-

All values are mean percentage bias (± LOA). LOA, Limits of agreement (1.96 x SD)

Table 4. Relative change (%) in Q between exercise stages for $\dot{Q}_{raw}$ and $\dot{Q}_{exercise}$

Method	Rest – Stage 2	Stage 2 – Stage 3	Stage 3 – Stage 4
$\dot{Q}_{raw}$	261.8 (52.5)	20.7 (16)	17.4 (12.7)
$\dot{Q}_{exercise}$	137 (53.3)*	26.8 (20.6)	2.1 (9.4)*

All values are mean (SD). * denotes significantly different from $\dot{Q}_{raw}$.

Discussion

The aim of this study was to evaluate the use of pulse contour analysis and lithium dilution for the determination of $\dot{Q}$ during upright cycle exercise in healthy, trained individuals. The comparison of $\dot{Q}$ derived at each exercise stage from uncalibrated pulse contour analysis ($\dot{Q}_{raw}$) and lithium dilution ($\dot{Q}_{exercise}$), respectively, showed low bias and wide limits of agreement. $\dot{Q}_{exercise}$ appeared to record values approximately 5% greater than those derived from $\dot{Q}_{raw}$ although the wide limits of agreement (> 40%) are outside acceptable clinical values (Critchley & Critchley 1999). In addition, we assessed whether the relative change in Q between exercise stages was similar for $\dot{Q}_{raw}$ and $\dot{Q}_{exercise}$. We found a significant difference between methods for the % change in Q from rest to exercise and between stage 3 and 4 but not between stages 2 and 3. Our findings also confirm those of study II, where calibration of $\dot{Q}_{raw}$ with a single lithium dilution determination under resting conditions ($\dot{Q}_{baseline}$), leads to large overestimation of $\dot{Q}$ recorded during subsequent exercise.

To investigate the apparent requirement of an exercise calibration, we sought to assess whether multiple calibration points at different intensity domains were necessary for stochastic exercise or if a single calibration point was sufficient. This is important for if recalibration is necessary for each shift in haemodynamic status, the practicality of this method becomes significantly impaired. In the only previous study of pulse contour analysis calibrated by lithium dilution during exercise (Kemps *et* al 2008), calibration was only performed at rest prior to exercise. Whilst these authors confirmed the validity of this approach versus the direct Fick method, one should note that the peak $\dot{Q}$ values recorded during exercise were ~12L•min^{-1}, considerably lower than that expected of a healthy individual during exercise (Stringer *et* al 1997). Furthermore, there was apparent evidence of an overestimation of $\dot{Q}$ from the LiDCO device at values in excess of 10L•min^{-1}. Perhaps the major finding of the study performed here is that calibrating at rest leads to a systematic overestimation of $\dot{Q}$ during exercise. Given that the peak $\dot{Q}$ values obtained in previous studies (Kemps *et* al 2008) were lower than that measured

at any exercise stage by participants in this study; it is unsurprising that this observation had no significant effect on the findings of Kemps *et* al (Kemps *et* al 2008). That resting calibration leads to the overestimation of $\dot{Q}$ during exercise, has significant implications for the application of this method during non-steady state exercise, where the exercise workload increases progressively. A major finding of this study is that a single-point calibration during exercise above the VT provides the closest agreement to multiple calibrations throughout an exercise bout ($\dot{Q}_{exercise}$). These data suggest that recalibration, whenever the intensity of exercise (and therefore haemodynamic stress) changes, may be unnecessary as long as a single-point calibration is performed during high-intensity exercise (i.e. above VT).

Interestingly, upon comparison of calibration factors we observe a decreasing calibration factor from rest to submaximal exercise. Furthermore, in participants where calibration was performed during maximal exercise, the calibration factor moved closer to 1.0, thus showing the calibrated $\dot{Q}$ becoming closer to the $\dot{Q}$ derived from raw PulseCO™ values (*i.e.* HR x nominal SV). Why this should occur is unclear although one explanation may be that it is an anomaly of the algorithm utilised by the system. Within the algorithm, the calibration factor serves to alter the assumed saturation volume, defined as the maximum additional volume (above that at atmospheric pressure) to which the aorta and arterial tree can fill (Rhodes & Sunderland 2005) thus correcting for differences in arterial compliance. A decrease in the calibration factor, therefore, suggests that the saturation volume during exercise may be approaching the 250 mL assumed in the PulseCO algorithm ($\dot{Q}_{raw}$). This may be a function of the decline in systemic arterial compliance that is observed with increasing exercise intensity (Otsuki *et* al 2006). Data from other studies have demonstrated the decrease in compliance (Otsuki *et* al 2008), which in this case would be corrected for by the reduced calibration factor. Furthermore, the PulseCO algorithm incorporates a curve coefficient that quantifies the compliance of the human aorta based upon published data from human cadaveric aortas (Rhodes & Sunderland 2005). Given the observation of increased aortic compliance in athletes

(Mohiaddin *et* al 1989) it is possible that structural adaptations resulting from chronic exercise training may alter this relationship.

Assessing the precision of our values in the absence of a gold standard comparator is difficult, although the use of such measurements as direct Fick during incremental exercise to exhaustion in healthy volunteers is invasive, technically difficult and impractical. Additionally, the lack of certainty regarding the validity of other non-invasive methods (Rowland & Obert 2002; Jarvis *et* al 2007; Kemps *et* al 2008) precludes the inclusion of an alternative comparator. The LiDCO™ system has been validated in a variety of clinical conditions (Linton *et* al 1997; Linton *et* al 2000b; Costa *et* al 2008; Mora *et* al 2011) and shows low bias and percentage error with $\dot{Q}$ obtained using a pulmonary artery catheter (PAC), when compared with other commercially available pulse contour analysis devices (Hadian *et* al 2010). The validity of lithium indicator dilution for the measurement of $\dot{Q}$ supports its place as the primary comparator (i.e. $\dot{Q}_{exercise}$) in this study. To date, no study has attempted to investigate the use of the LiDCO/PulseCO system during exercise in healthy individuals. Kemps *et* al (Kemps *et* al 2008) compared LiDCO values, calibrated at rest, with those from the continuous Fick method during incremental exercise in heart failure patients, although the $\dot{Q}$ values observed in this patient cohort were significantly lower (~50%) than those observed in our healthy, trained volunteers.

The use of minimally invasive, continuous $\dot{Q}$ measurements during exercise provides the unique opportunity to observe the cardiovascular responses to exercise in both healthy and diseased populations. Current 'gold standard' techniques are invasive and often unable to provide dynamic measurements throughout exercise, including up to the moment of volitional exhaustion where cardiac function may be maximal. The benefits of cardiopulmonary exercise testing, particularly in the clinical evaluation of heart and lung disease, are widely documented $\dot{Q}$ (Arena & Sietsema 2011). The continuous measurement of pulmonary gas exchange presents the valuable opportunity for using such data for both diagnostic and prognostic purposes. The addition of continuous $\dot{Q}$ would yield a valuable insight into the patterns of

oxygen delivery and cardiac function in both health and disease. Additionally, beat-to-beat measurements of $\dot{Q}$ during maximal exercise are highly desirable to the exercise physiologist wishing to examine how cardiovascular function may influence exercise performance. Previous studies have commonly relied on only one or two isolated measurements at maximal exercise (Stringer *et* al 2005; Mortensen *et* al 2005; Calbet *et* al 2007; Brink-Elfegoun *et* al 2007; Mortensen *et* al 2008), a limitation that may clearly restrict the understanding of exercising haemodynamics.

While our study is the first to document the use of pulse contour analysis with lithium dilution during exercise across a wide range of $\dot{Q}$, more investigation is required to assess the precision and accuracy of this technique. In particular, future studies should compare uncalibrated and calibrated $\dot{Q}$ values with those recorded using other commonly employed methods in this field, such as Doppler echocardiography and/or foreign gas rebreathing. In addition, an investigation into the accuracy of this technique in comparison with 'gold standard' techniques is desirable, although the practical restraints for such a study in healthy individuals are acknowledged. Whilst the LiDCO device is simpler and less invasive than other invasive methods, a prerequisite for measurement using this device remains the presence of a good quality radial artery pressure waveform. Additionally, the process of calibration using indicator dilution methods requires hemodynamic stability when the lithium dilution method is applied to correct the PulseCO data. To avoid any apparent mismatch, we interpolated the PulseCO data so that the LiDCO and PulseCO measures were exactly time aligned as the device default is to apply the calibration factor approximately 60 seconds following administration of the lithium chloride bolus.

In summary, pulse contour analysis provides a pragmatic approach to the measurement of $\dot{Q}$ during exercise providing that the system is calibrated by a lithium dilution method at least once during exercise. The findings of our study suggest that this should be performed at a high intensity above the ventilatory threshold. Further studies are warranted to assess the validity and reliability of this system during exercise in both healthy and clinical populations, although

these initial investigations in studies II and III warrant the inclusion of this method for the assessment of $\dot{Q}$ continuously during maximal exercise to exhaustion.

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CHAPTER 5:

Study IV:

Evidence of cardiac functional reserve upon exhaustion during incremental exercise to determine $\dot{V}O_{2max}$.

Adapted from:

Elliott, A., Skowno, J., Prabhu, M., Noakes, T. D. and Ansley L. (2013). Evidence of cardiac functional reserve during incremental exercise to determine $\dot{V}O_{2max}$. *British Journal of Sports Medicine*, doi:10.1136/bjsports-2012-091752.

Introduction

Maximal oxygen uptake ($\dot{V}O_{2max}$) is arguably the most researched parameter in exercise physiology. Its relationship with performance has been recognised since the pioneering work of Hill & Lupton (Hill & Lupton 1923) who concluded that oxygen uptake ($\dot{V}O_2$) reached a maximum level during peak physical work, since interpreted as a plateau in the $\dot{V}O_2$ response. Significant debate has surrounded the plateau phenomenon (Midgley *et* al 2007) although the concept of a truly maximal $\dot{V}O_2$, established by supramaximal testing to verify that obtained from incremental exercise, is generally accepted. (Rossiter *et* al 2006; Foster *et* al 2007; Hawkins *et* al 2007) However, there remains considerable debate regarding the factor(s) limiting $\dot{V}O_{2max}$. (Saltin & Calbet 2006; Wagner 2006; Noakes & Marino 2009; Gonzalez-Alonso & Mortensen 2009) Broadly, the most commonly suggested mechanisms are that either a circulatory (cardiac) limitation or a neural (central) regulation determines the $\dot{V}O_{2max}$ (Spurway *et* al 2012).

It is established that skeletal muscle perfusion capacity exceeds the pumping capacity of the heart. (Andersen & Saltin 1985; Calbet *et* al 2004) Secher *et* al (Secher *et* al 1977) showed a reduction in leg blood flow when arm exercise is superimposed on maximal two-leg exercise, supporting the theory that the capacity to supply O_2 during maximal exercise is limited thereby constraining oxidative metabolism and, consequently, exercise capacity. More recently a plateau in cardiac output ($\dot{Q}$) close to exhaustion during both incremental and constant load maximal exercise (Mortensen *et* al 2005; Mortensen *et* al 2008) has been demonstrated. This has been interpreted as further evidence that the circulation limits $\dot{V}O_{2max}$ as $\dot{Q}$ is no longer able to increase thus constraining $\dot{V}O_2$ as it reaches a plateau.

Opponents to the cardiac limitation theory propose that skeletal muscle recruitment is regulated through a central, neurally mediated mechanism during exhaustive exercise. (Noakes 1997; Noakes 2000; Noakes & St Clair Gibson 2004; Noakes & Marino 2009; Noakes 2012) Proponents of this theory argue that this ensures myocardial ischemia, or any other

catastrophic biological failure, is avoided by moderating the demand placed on the heart, thereby preventing the attainment of an absolute maximum. This theory is supported by the findings that, in trained athletes, $\dot{Q}$ continues to increase linearly up until exhaustion without a plateau. (Gledhill *et* al 1994; Zhou *et* al 2001; Calbet *et* al 2007)

Cardiac power output (CPO) is a measurement of cardiac function that incorporates both flow and pressure domains of the cardiovascular system and is measured as the product of $\dot{Q}$ and mean arterial pressure. (Cooke *et* al 1998) By measurement of CPO during maximal and supramaximal exercise, it becomes feasible to study the heart's ability to maintain circulation in the presence of increasing arterial pressure. During exercise at $\dot{V}O_{2max}$, the measurement of CPO allows the exercise physiologist to determine whether the work of the heart continues to increase at the exercise intensity resulting in exhaustion. Should an increase in CPO be observed during exercise above that achieved at $\dot{V}O_{2max}$, one might make one of two conclusions; that the heart is working submaximally during exercise at $\dot{V}O_{2max}$ (Noakes & Marino 2009) or, alternatively, that the circulation is absolutely maximal during exercise at $\dot{V}O_{2max}$ despite an increase in cardiac work. (Brink-Elfegoun *et* al 2007b)

To differentiate between the theories explaining the limitation to maximal exercise, Brink-Elfegoun *et* al (Brink-Elfegoun *et* al 2007b) designed an experiment in which two levels (100% and 110% $\dot{V}O_{2max}$) of whole-body exercise were performed. $\dot{V}O_2$ and $\dot{Q}$ were similar between workloads but blood pressure was significantly higher during exercise at 110% $\dot{V}O_2$max, resulting in increased cardiac work. The authors concluded that the greater cardiac *work* during supramaximal exercise indicates the absence of a central 'governor', for at such workloads, this neural governor would be regulating exercise to ensure there are no further increases in cardiac work and thus myocardial O_2 demand. This conclusion is challenged by Noakes & Marino (Noakes & Marino 2009) who argue that these findings show the heart to be working submaximally at $\dot{V}O_{2max}$ and that the higher work rate achieved during a supramaximal bout indicates a dissociation between $\dot{Q}$ and work rate, disproving the theory that $\dot{Q}$ regulates peak work rate and, consequently, $\dot{V}O_{2max}$ as is dictated by the cardiovascular limitation model.

In reframing the question, Noakes & Marino ask why exercise would terminate during the lower workload exercise, when there is clear evidence that the heart is working below its maximum potential.

Despite the extensive discussion on cardiac function and limitations during maximal exercise, CPO has not been measured continuously during maximal and supramaximal exercise, even in studies in which a plateau in $\dot{V}O_2$ (Rossiter *et* al 2006) and $\dot{Q}$ (Mortensen *et* al 2005; Mortensen *et* al 2008) have been observed. Continuous, beat-to-beat measurements afford the opportunity to examine peak $\dot{Q}$ and CPO, regardless of where it occurs during maximal exercise. Measuring these variables at isolated timepoints increases the chance that peak values are missed, thus leading to underestimation of both measurements. The aims of this study were therefore to evaluate the work of the heart during cycling exercise at maximal and supramaximal workloads. We hypothesized that the greater exercise workload would induce a greater circulatory and myocardial work demand, thus showing that the heart works submaximally at $\dot{V}O_{2max}$, confirming the finding of Brink-Elfegoun *et al* (Brink-Elfegoun *et* al 2007b)

Methods

Participants

Eight recreationally trained male cyclists, age 40.5 ± 9.2 years, body mass 80.5 ± 10.9 kg, height 178.8 ± 4.7 cm, $\dot{V}O_{2max}$ 53.7 ± 6.5 ml•kg^{-1}•min^{-1}, volunteered to participate in the study. All participants were training for >5 hours per week. Exclusion criteria for participation included a history of cardiopulmonary disease, lithium allergy, and current therapy with lithium or muscle relaxants. The protocol was explained to the participants before they gave written informed consent. The research ethics committees at Northumbria University and Kingston University approved the study. All procedures were performed in accordance with national (Hull *et* al 2008) and international (Declaration of Helsinki, 1964) guidelines.

Exercise Protocol

Participants reported to the laboratory on a single occasion after abstaining from caffeine, alcohol, and heavy exercise in the preceding 24 hours. Participants performed two exercise trials during their visit, each separated by one hour. Each trial, consisting of exercise conducted on an electromagnetically braked cycle ergometer (Velotron, Racermate Inc., USA), took place within an air-conditioned laboratory controlled to 22-23°C. The cycle ergometer was set up to each participant's specifications. Participants were allowed to self-select their cadence whilst remaining seated throughout each trial. The investigators provided consistent verbal encouragement throughout.

The first exercise trial (T1) consisted of an incremental protocol to exhaustion starting with 6 minutes at a power output of 120 W, increasing by 30 W every 3 min until the participant reached volitional exhaustion (MAX). Ventilatory threshold (VT) was determined according to the V-slope method of Beaver *et* al; (Beaver *et* al 1986) the workload for the second trial (T2) was derived from VT. During T2, participants each completed four exercise stages, each being separated by a two-minute recovery period at 100 W. The first three stages consisted of six minutes at 120 W followed by 8 minutes each at 80% of VT, and 30% of the difference

between power at VT and power at MAX. The final stage was performed at a power output 10% greater than that achieved during T1, until volitional fatigue (SUPRAMAX). The structure of T2 was designed so that the total exercise time was approximately similar to T1.

Measurements

Throughout each trial, pulmonary gas exchange was recorded continuously with an online gas analyser (Oxycon Pro, Jaeger, Germany). Participants wore a close fitting facemask connected to a tripe-V sensor (Jaeger, Germany) consisting of a flow turbine and gas sampling tube. Prior to each trial, the gas analyser was calibrated using gas of a known composition (16% O_2; 4% CO_2; balance Nitrogen). Volume calibration of the flow turbine was performed using a 3-L syringe over a range of flow rates.

$\dot{Q}$ was assessed continuously throughout each exercise by pulse contour analysis calibrated by an incorporated lithium dilution method (LiDCO, Cambridge, UK). The LiDCO™ device permits continuous hemodynamic recording by analysis of the radial artery pressure waveform (PulseCO™, Cambridge, UK), calibrated by lithium dilution (LiDCO™, Cambridge, UK). Lithium dilution shows good agreement with thermodilution, (Kurita *et* al 1997; Linton *et* al 1997) whilst continuous measurements have reported clinically acceptable accuracy and precision in critically ill patients. (Kurita *et* al 1997; Cecconi *et* al 2010; Linton *et* al 2000) During exercise, the LiDCO™ device compares favourably with direct Fick measurements during constant load and incremental exercise (Kemps *et* al 2008) and performs well during high-intensity exercise in trained cyclists. (Elliott *et* al, 2012)

Prior to T1, a 21-gauge cannula was placed into a peripheral vein mid-way between the wrist and elbow of the right arm. A 20-gauge arterial cannula was then placed into the radial artery of the left arm under local anaesthesia (2% Lidocaine) and connected to an ICU monitor (Hewlett Packard) via a disposable pressure transducer (Philips M1567A, Philips; Germany), zeroed to ambient pressure. The pressure monitor provides continuous arterial pressure waveform data to the LiDCO™ monitor. The LiDCO™ system calculates a nominal stroke

volume from a pressure-volume transformation of the arterial pressure waveform, which is then converted to absolute stroke volume using the incorporated lithium dilution method.

Lithium dilution calibration involved the administration of a lithium chloride bolus (0.3 – 0.45 mM) into the peripheral vein. The bolus was immediately followed by a 20 ml saline flush. A lithium dilution curve was subsequently derived by drawing arterial blood past a lithium sensor connected to the arterial cannula, at a constant flow rate using a flow pump. $\dot{Q}$ was calculated according to the following equation:

$$\dot{Q} = (\text{LiCl dose (mM)} \times 60) / (\text{area under dilution curve (mM}\bullet\text{L}^{-1}\bullet\text{s}^{-1}) \times (1\text{-PCV}))$$

(Linton *et* al 1993)

where PCV = haemoglobin ($g\bullet dl^{-1}$) / 33

Heart rate (HR) was calculated by the duration between subsequent pressure waveforms. Arterial pressure was recorded directly by the LiDCO™ monitor from the arterial pressure trace. LiDCO calibrations were performed during the SUPRAMAX workload of T2 and applied to all $\dot{Q}$ data. Calibration procedures began one minute into the SUPRAMAX stage. The pulse contour analysis data required for calibration with the lithium dilution method was obtained by interpolation of the mean values in the 10s preceding and succeeding the calibration period to ensure all data was time-matched. This calibration factor was applied retrospectively to all data obtained during the study.

Cardiac power output (CPO) and rate-pressure product (RPP) were calculated according to the following equations:

$$\text{CPO (W)} = \dot{Q} \bullet \text{MAP} \bullet k$$ (Cooke *et* al 1998)

where $k = 2.22 \times 10^{-3}$ and MAP = mean arterial pressure (mmHg).

$$\text{RPP (beats} \bullet \text{min}^{-1} \bullet \text{mmHg)} = \text{HR} \bullet \text{SBP}$$ (Nelson *et* al 1974)

where SBP = systolic blood pressure (mmHg)

MAP (mmHg) = DBP + 1/3 (SBP-DBP)

where DBP = diastolic blood pressure (mmHg)

Data Analysis

Hemodynamic and pulmonary gas exchange data were averaged over 10s epochs throughout both T1 and T2. Data for MAX and SUPRAMAX were taken at the time point of maximal $\dot{Q}$. Data analysis was performed with GraphPad Prism (Version 5). A paired *t*-test was performed to compare MAX and SUPRAMAX data. Statistical significance was determined at $P < 0.05$. All data are presented as means ± SD unless otherwise stated.

Results

The workloads for MAX and SUPRAMAX were 315 ± 39.3 W and 346.5 ± 43.2 W, respectively. Time to exhaustion was not significantly different between MAX and SUPRAMAX trials (176 ± 12.6 *versus* 170 ± 40.7 seconds, $P < 0.05$).

Table 1. Mean (SD) exercise data obtained from MAX and SUPRAMAX trials.

Variable	MAX ($n = 8$)	SUPRAMAX ($n = 8$)
Power Output (W)	315 (39.3)	346.5 (34.2)
$\dot{Q}$ (L•min^{-1})	28.7 (5.9)	29.4 (7.0)
$\dot{V}O_2$ (L•min^{-1})	4.26 (0.61)	4.26 (0.7)
SV (ml)	154.1 (30.9)	157.3 (34.4)
HR (bpm)	189 (10)	190 (13)
MAP (mmHg)	129 (11)	135 (12)*
SBP (mmHg)	222 (28)	224 (33)
DBP (mmHg)	78 (6)	81 (8)
CPO (W)	8.05 (1.9)	8.5 (2.1)
RPP (beats •min^{-1}•mmHg)	41, 625 (4347)	41, 456 (5489)

$\dot{Q}$ Cardiac Output; $\dot{V}O_2$ Oxygen Uptake; **SV** Stroke Volume; **HR** Heart Rate; **MAP** Mean Arterial Pressure; **SBP** Systolic Blood Pressure; **DBP Diastolic Blood Pressure; CPO** Cardiac Power Output; **RPP** Rate-Pressure Product; * indicates significantly different from MAX.

Peak exercise data are shown in table 1.There was no significant difference between MAX and SUPRAMAX (figure 1) for $\dot{Q}$ (28.7 *versus* 29.4 L•min^{-1}, 95% CI -2.6 to 1.4; $P = 0.48$), $\dot{V}O_2$ (4.26 *versus* 4.26 L•min^{-1}, 95% CI -0.18 to 0.17; $P = 0.96$), stroke volume (154.1 *versus* 157.3 ml, 95% CI -13.5 to 7.1; $P = 0.49$) or heart rate (188.9 *versus* 190.1 beats•min^{-1}, 95% CI-4.3 to 1.8; $P = 0.36$), respectively. MAP was significantly higher at peak exercise during the SUPRAMAX stage (129.1 *versus* 134.6 mmHg, 95% CI -10.1 to -0.9; $P = 0.03$), although both

systolic and diastolic blood pressure remained unchanged between workloads. Additionally, no significant differences were observed for $\dot{Q}$, SV, HR, MAP or $\dot{V}O_2$ between peak values and exhaustion during either exercise stage (Figure 2). Both $\dot{Q}$ ($\dot{Q}$/W; 91.6 ± 18.4 *versus* 85.3 ± 21.4 ml/W; 95% CI 1.24 to; 11.31 $P = 0.02$) and $\dot{V}O_2$ ($\dot{V}O_2$/W; 13.5 ± 1.1 *versus* 12.3 ± 1.5 ml/W; 95% CI 0.71 to 1.69; $P = 0.0007$) expressed per unit of power output, were significantly greater during the MAX stage.

The significantly greater MAP at the SUPRAMAX workload led to a tendency for a greater CPO (8.1 *versus* 8.5 W, 95% CI -0.92 to 0.02), although this did not reach statistical significance ($P = 0.06$). RPP was not significantly different between MAX and SUPRAMAX (41, 625 *versus* 41, 546 beats•min^{-1}•mmHg, 95% CI -3222 to 3378; $P = 0.96$).

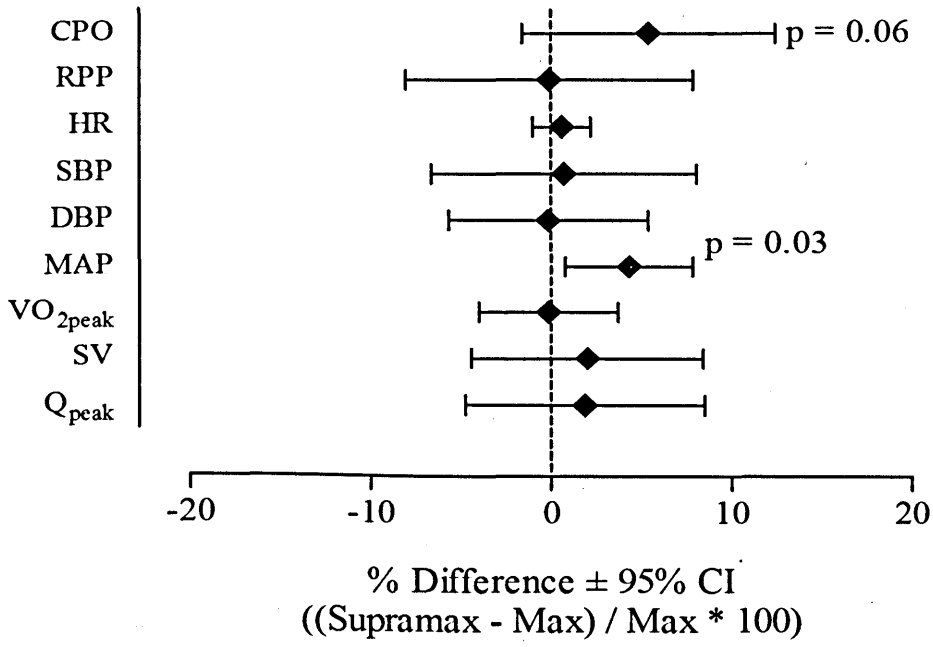

Figure 1. Forrest plot displaying mean % differences (± 95% CI) of measurements between SUPRAMAX and MAX exercise. All data are time aligned to measurement of peak cardiac output.

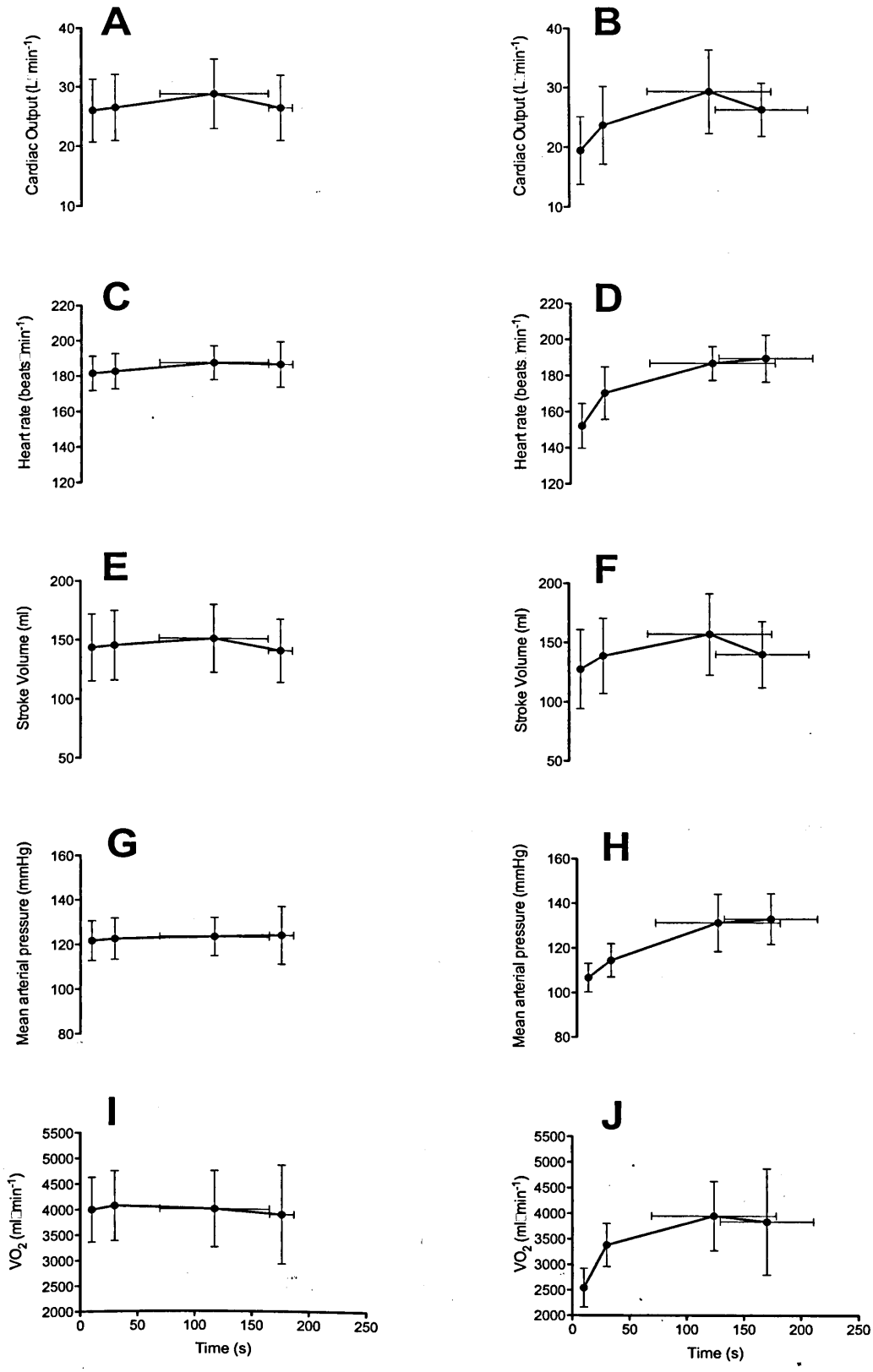

Figure 2. Systemic haemodynamics during MAX (A, C, E, G, I) and SUPRAMAX (B, D, F, H, J) exercise.

Discussion

The most significant finding from this study was that although $\dot{Q}$ was similar, the work performed by the heart, assessed by cardiac power output, was increased during supramaximal exercise compared with maximal exercise due to higher mean arterial pressure. Although this did not reach statistical significance, the 95% confidence intervals indicate a substantial increase that is likely to be of biological significance. This confirms previous findings (Brink-Elfegoun *et* al 2007b) during combined arm and leg exercise. These findings indicate an increase in the work performed by the heart during supramaximal exercise with a greater myocardial $\dot{V}O_2$. (Cooke *et* al 1998)

The observation that, at maximal exercise, further elevations in workload occur without any additional increase in $\dot{Q}$ and $\dot{V}O_2$ suggests that, in health, the attainment of $\dot{V}O_{2max}$ is accompanied by an attenuated increase in systemic blood flow, as frequently argued. (Mitchell *et* al 1958; Ekblom *et* al 1975; Gonzalez-Alonso & Calbet 2003; Calbet *et* al 2004; Saltin & Calbet 2006; Mortensen *et* al 2008) It is known that maximally vasodilated skeletal muscle in humans can accept a greater blood flow than the heart can supply (i.e. greater than $\dot{Q}_{max}$) as the peak hyperaemic response to single limb exercise indicates a vasodilatory capacity that outstrips the capacity of the heart when sufficient musculature is used. (Andersen & Saltin 1985; Secher *et* al 1977; Calbet *et* al 2004) Furthermore, a number of experimental manipulations of O_2 delivery have resulted in reduction of $\dot{V}O_{2max}$, (Ekblom *et* al 1975; Koskolou *et* al 1997; Calbet *et* al 2003) supporting the commonly accepted theory that O_2 delivery constrains $\dot{V}O_{2max}$ during exercise, a clear cause and effect according to those defending this model (Bassett & Howley 2000; Levine 2008; Ekblom 2009). However, the findings that the heart is able to increase its work output beyond that achieved during $\dot{V}O_{2max}$ testing, as previously shown, (Brink-Elfegoun *et* al 2007b) confirms that typical incremental exercise to $\dot{V}O_{2max}$, as observed during the MAX trial, terminates with some degree of cardiac functional reserve.

Our findings of a similar $\dot{V}O_{2max}$ between MAX and SUPRAMAX support the concept of a true 'testable' maximal $\dot{V}O_2$ observed by a number of groups comparing maximal and supramaximal exercise (Rossiter *et* al 2006; Hawkins *et* al 2007; Foster *et* al 2007) although alternative testing methods, such as a decremental test in which exercise intensity starts high and decreases (Beltrami *et* al 2012) or self paced incremental exercise (Mauger 2013), may yield higher $\dot{V}O_{2max}$ values. By the same principle, the absence of any increase in $\dot{Q}$ despite a greater workload during SUPRAMAX indicates that a maximal $\dot{Q}$ is achieved at the termination of incremental and constant-load exhaustive exercise, although the $\dot{Q}$ response to alternative testing methods producing higher $\dot{V}O_{2max}$, has not been investigated. The observation that $\dot{Q}$ fails to increase at or near maximal exercise has been demonstrated previously (Stringer *et* al 1997; Gonzalez-Alonso & Calbet 2003; González-Alonso *et* al 2004; Stringer *et* al 2005; Mortensen *et* al 2005; Brink-Elfegoun *et* al 2007b; Mortensen *et* al 2008) and is considered to be key evidence that $\dot{V}O_{2max}$ is regulated by the heart.

The theory of a central, neurally mediated limitation (Noakes 1997) predicts that the loss of homeostasis during exhaustive exercise is avoided by the actions of a centrally located 'governor' that limits skeletal muscle recruitment, thus reducing the likelihood of any homeostatic disturbances, including both metabolic and/or thermoregulatory regulation. (Noakes & St Clair Gibson 2004) Our findings that circulation appears maximal during exercise to exhaustion does not exclude the existence of a governor within the central nervous system (CNS) that anticipates significant homeostatic disturbance(s), therefore limiting any further increase in myocardial $\dot{V}O_2$ and potentially risking the onset of myocardial ischemia. The observation that the heart appears to be work submaximally during exercise eliciting $\dot{V}O_{2max}$ (Brink-Elfegoun *et* al 2007b) supports a central limitation theory by posing the question of why the heart does not work harder at $\dot{V}O_{2max}$ despite its apparent capacity to do so? (Noakes & Marino 2009) A system that is considered to be operating at its maximal capacity cannot be doing so if there is reserve in its function. One interpretation is that a

central regulator may limit myocardial O_2 demand by regulating additional skeletal muscle recruitment at the point of exhaustion during maximal exercise.

However, the greater workload during SUPRAMAX would require additional motor unit recruitment (Brink-Elfegoun *et* al 2007a; Levine 2008) that, in the view of the central governor theory, should be under regulation to constrain $\dot{Q}$. (Noakes & Marino 2009) It has been argued that it remains unclear how a central 'governor' would terminate exercise at $\dot{V}O_{2max}$ when there is evidence that greater skeletal muscle recruitment (Brink-Elfegoun *et* al 2007a) and workloads (Hawkins *et* al 2007) are achievable in the absence of significant homeostatic disturbances or myocardial ischemia. However, there are several points worth noting to address this question; Firstly, the central theory proposes that exercise terminates *before* there is maximal skeletal muscle activation, (Noakes & St Clair Gibson 2004) as shown elsewhere. (Brink-Elfegoun *et* al 2007a) Secondly, dissociation between $\dot{Q}$ and exercise power output appears during SUPRAMAX such that $\dot{Q}$/W is significantly lower than that measured during MAX, suggestive of an uncoupling between workload and cardiovascular function. This finding confirms that $\dot{Q}$ does not determine skeletal muscle work as is assumed by the cardiovascular limitation model. Finally, one should consider how supramaximal exercise as a separate effort, as conducted here and elsewhere (Rossiter *et* al 2006; Brink-Elfegoun *et* al 2007b; Hawkins *et* al 2007) influences this discussion. It is plausible that the degree of 'homeostatic disturbance' differs during a separate supramaximal bout. Indeed, Mortensen *et* al, (Mortensen *et* al 2008) with a similar experimental model, observed greater disturbances to blood pH, lactate, arterial O_2 saturation and body temperature during incremental exercise to $\dot{V}O_{2max}$ as compared with 'stand-alone' constant-load supramaximal exercise. One could postulate that supramaximal exercise performed separately provides no greater metabolic/homeostatic challenge than maximal exercise performed at the end of an incremental exercise protocol, with the consequence that constant-load supramaximal exercise requires little constraint from a central 'governor' in the initial stages as the perceived

physiological threat is lower than that seen during the maximal stage of an incremental protocol.

Whilst it is agreed that a maximal $\dot{V}O_2$ exists, this study and others (Beltrami *et* al 2012) suggest that cardiovascular function is not maximal during incremental exercise to exhaustion. Despite efforts to portray the $\dot{V}O_{2max}$ measured during incremental exercise as being limited by the circulation, (Levine 2008) the submaximal cardiac function observed during 'maximal' exercise in this study and others (Brink-Elfegoun *et* al 2007b) suggests that this form of exercise testing may not be a useful evaluation of exercise performance (Noakes 2011) or for the evaluation of maximal cardiac function in healthy humans as it is possibly more a function of the degree of work that the active skeletal muscle is able to perform under conditions of a precisely controlled central motor output. Instead, the measurement of $\dot{V}O_{2max}$ can perhaps be considered an indirect measure of the skeletal muscle recruitment that is permitted by the brain without significant risk of catastrophic biological failure. As such, the measurement of $\dot{V}O_{2max}$ may be primarily representative of the sensitivity of central motor command to afferent feedback, rather than a combination of both anticipatory (feed-forward) and feedback information.

Direct Fick and/or thermodilution are typically considered 'gold standard' methods for $\dot{Q}$ measurement during exercise. However, the technical difficulties and risk (Scheer *et* al 2002) associated with these methodologies renders them unsuitable for most exercise studies. Furthermore, previous studies employing these methods, and others, are typically only able to assess $\dot{Q}$ at one or two timepoints during maximal exercise, thus potentially recording submaximal values. Our method of assessment provides continuous measurements throughout exercise up until exhaustion (Elliott *et* al 2012), with only minimal risk and invasiveness. Therefore we were able to be absolutely sure that we obtained a maximal measure for $\dot{Q}$. Lithium dilution has proven accuracy in the clinical setting in critically ill patients, (Linton *et* al 1997; Hamilton *et* al 2002; Pittman *et* al 2005) patients with hyperdynamic circulation (Costa *et* al 2008) and exercising heart failure patients. (Kemps *et* al 2008) In this study, we

were able to successfully calibrate the device during the SUPRAMAX exercise stage, with this calibration factor being applied to all exercise data. Importantly, we were also able to obtain true peak $\dot{Q}$ and arterial pressure measurements, regardless of the timepoint at which they occurred. We believe this provides significant benefit when assessing the hemodynamic response to maximal exercise, which is typically of short duration.

Our study is not without limitations. Firstly, we chose to perform all testing in one session for practical reasons relating to the procedures associated with $\dot{Q}$ measurement. Likewise, we did not counterbalance the order of trials. This was to allow us to ensure that the SUPRAMAX exercise trial did not impact upon data obtained from a subsequent MAX trial. Both of these limitations mirror those relevant to the Brink-Elfegoun *et* al study (Brink-Elfegoun *et* al 2007b) therefore permitting comparisons between the two studies. Additionally, we chose upright cycling as our testing modality. Incremental cycle exercise is the common modality of maximal exercise testing for the determination of $\dot{V}O_{2max}$ and has been shown to elicit a plateau in $\dot{V}O_2$ suggestive of the true attainment of $\dot{V}O_{2max}$. Future studies should attempt to determine the cardiovascular responses to exercise in trials where recent evidence has shown the potential for increased $\dot{V}O_2$ during alternative protocols to those used in this study. (Beltrami *et* al 2012) We also acknowledge the limitations of $\dot{Q}$ measurement. We chose a method that permitted continuous measurements of $\dot{Q}$ throughout maximal exercise and therefore measurements in the period immediately preceding exhaustion. Pulse contour analysis with lithium dilution calibration has shown close agreement with gold standard methods during exercise. (Kemps *et* al 2008) Finally, this study's small sample size increased the chances of a type II error due to insufficient statistical power. Sample size was largely dictated by the requirement to perform relatively invasive procedures on healthy participants during strenuous exercise. Future studies attempting to address this research question should consider a statistical power analysis to determine the required sample size to detect the differences observed during this study and others.

In conclusion, this study shows that during two levels of maximal cycling exercise, differing by a workload of 10% and eliciting identical $\dot{V}O_{2max}$, cardiac work continues to increase despite $\dot{Q}$ remaining the same. These findings suggest that cycling exercise to $\dot{V}O_{2max}$ terminates with cardiac functional reserve, yet maximal $\dot{Q}$.

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CHAPTER 6: THESIS OVERVIEW

Purpose of the programme of study

The rationale underpinning this thesis was that despite over 50 years of investigation into the cardiovascular response to exercise, the limitations surrounding cardiac measurement techniques in providing high resolution, continuous, real-time, data of the cardiovascular variables during maximal exercise, has constrained our understanding of the factors that regulate exercise performance. Specifically, the absence of continuous recordings in the moments preceding fatigue has led to a reliance on isolated measurements during maximal exercise to describe the cardiac response to exhaustive exercise. The aim of this thesis was therefore to assess novel techniques of measuring $\dot{Q}$ during maximal and supramaximal exercise, namely: inert gas rebreathing; bioreactance; and pulse contour analysis (with the latter two providing continuous, beat-by-beat measurements) in order to identify a technique that may provide a comprehensive study of the haemodynamic response to maximal exercise to exhaustion. With a study population consisting of healthy, trained participants, the methods incorporated into this thesis focussed on minimally/non-invasive techniques appropriate to the exercise laboratory. In addition pulmonary gas exchange and other haemodynamic indices of cardiac work were measured throughout exercise, including the moments preceding exhaustion, in order to address the question as to whether $\dot{Q}$ and cardiac work are truly maximal during exercise to exhaustion, a central point of debate regarding the limiting factor(s) for $\dot{V}O_{2max}$.

Results

Comparison of Non-Invasive $\dot{Q}$ Measurements

In study I, successful measurements of $\dot{Q}$ at rest were obtained in all participants (n = 14) with both bioreactance and inert gas rebreathing methods. $\dot{Q}_{rebreathe}$ measurements were unsuccessful in one subject (7%) during submaximal exercise and in three participants (21%) during

maximal exercise. Bioreactance ($\dot{Q}_{bio}$) measurements were obtained throughout exercise in all participants, although a reliable ECG trace was lost intermittently during exercise with a HR >160 bpm. $\dot{Q}_{rebreathe}$ was significantly ($P<0.05$) greater than $\dot{Q}_{bio}$ at rest with a mean bias of 1 $L \cdot min^{-1}$ (95% Limits of Agreement -3.7 to 1.7 $L \bullet min^{-1}$), which translated to a percentage bias of -14.4% (LoA -54 to 25%). Similarly, $\dot{Q}_{rebreathe}$ remained significantly ($P<0.05$) greater than $\dot{Q}_{bio}$ during both submaximal (20.4 ± 2.1 *vs.* 16.2 ± 2.0 $L \cdot min^{-1}$) and maximal exercise (23.3 ± 3.1 vs. 19.3 ± 3.4 $L \cdot min^{-1}$). Throughout exercise, the mean bias was -4.26 $L \cdot min^{-1}$ (LoA -10.1 to -1.52 $L \cdot min^{-1}$), which relates to a percentage bias of -21.5%.

Construct validity of both measurement methods was assessed using the simultaneous measurement of $\dot{V}O_2$, which was used to rearrange the Fick equation to yield the (a-v)O_2diff, which is subsequently compared to that estimated using previous data. During submaximal exercise, the calculated (a-v)O_2diff was 20.3 ± 3.2 mlO_2/dl and 15.9 ± 1.8 mlO_2/dl for $\dot{Q}_{bio}$ and $\dot{Q}_{rebreathe}$, respectively, compared to 13.8 mlO_2/dl estimated by the Stringer *et* al (Stringer *et* al 1997) equation for a similar $\dot{V}O_2$. During maximal exercise, the calculated (a-v)O_2diff was 22.4 ± 3.9 $mlO_2 \bullet dL^{-1}$ and 18.3 ± 1.6 mlO_2/dl for $\dot{Q}_{bio}$ and $\dot{Q}_{rebreathe}$, respectively, compared to 16.2 mlO_2/dl estimated by the Stringer *et* al equation.

Evaluation of Pulse Contour Analysis and Lithium Dilution during Exercise

In study II, uncalibrated pulse contour analysis data ($\dot{Q}_{raw}$) was successfully collected throughout exercise with only occasional dampening of the arterial pressure signal, which was rectified by flushing the arterial cannula with saline. At rest and during submaximal exercise, at least two adequate lithium dilution curves were recorded in all participants ($n = 7$). Three $\dot{Q}$ datasets were recorded; $\dot{Q}_{raw}$, PCA calibrated at rest ($\dot{Q}_{restCal}$) and PCA calibrated during moderate intensity aerobic exercise ($\dot{Q}_{exCal}$). $\dot{Q}$ recorded at rest was 5.1 ± 0.7, 8.6 ± 2.4, and 6.3 ± 1.7 $L \cdot min^{-1}$, rising to 26.9 ± 2.5, 45.2 ± 13.6, and 32.7 ± 6.5 $L \cdot min^{-1}$ during maximal exercise for $\dot{Q}_{raw}$, $\dot{Q}_{restcal}$, and $\dot{Q}_{excal}$, respectively. There was a significant ($P<0.05$) difference between the three methods, with an interactive effect also observed for exercise intensity. $\dot{Q}_{restcal}$ was

significantly ($P<0.05$) higher than $\dot{Q}_{raw}$ at 60%, 80% and 105% $\dot{V}O_{2peak}$. $\dot{Q}_{restcal}$ was also significantly ($P<0.05$) higher than $\dot{Q}_{excal}$ at 80% and 105% $\dot{V}O_{2peak}$.

Bland-Altman analyses revealed a mean bias between $\dot{Q}_{raw}$ and $\dot{Q}_{restcal}$ of -11.1 L·min^{-1} ($\dot{Q}_{raw}$ - $\dot{Q}_{restcal}$) with limits of agreement of -29.7 to 7.6 L·min^{-1}. Presented as percentage bias, this equates to 50.8% ± 85.6%. Between $\dot{Q}_{restcal}$ and $\dot{Q}_{excal}$, the mean bias ($\dot{Q}_{restcal}$ - $\dot{Q}_{excal}$) was 7.3 L·min^{-1} with limits of agreement of -12.0 to 26.7 L·min^{-1} (% bias = 31.1% ± 82%). Between $\dot{Q}_{raw}$ and $\dot{Q}_{excal}$, the mean bias ($\dot{Q}_{raw}$ - $\dot{Q}_{exCal}$) was -3.7 L·min^{-1} with limits of agreement of -14.8 to 7.3 L·min^{-1} (% bias = 20.5% ± 60.9%). Test-retest reliability was determined in three participants for $\dot{Q}_{raw}$ and $\dot{Q}_{excal}$, with no significant ($P>0.05$) differences between repeat trials for either method. Duplicate $\dot{Q}_{raw}$ and $\dot{Q}_{excal}$ measures between trials were highly correlated (r = 0.99 and 0.98, respectively), with a mean bias between trials of 0.02 L·min^{-1} for $\dot{Q}_{raw}$ and 1.9 L·min^{-1} for $\dot{Q}_{excal}$.

Optimising Lithium Dilution & Pulse Contour Analysis Measurements

In study III, calibrations of $\dot{Q}_{raw}$ occurred across a range of exercise intensities using the lithium indicator dilution method. Successful calibrations were performed in all participants (n = 10) at rest and submaximal exercise. Because of aberrant lithium dilution curves (n = 2) and subject fatigue (n = 1), 3 of 10 (30%) calibrations were unable to be performed during supramaximal exercise.

Uncalibrated $\dot{Q}$ ($\dot{Q}_{raw}$) was measured continuously at rest and throughout exercise T1 and T2. Pulse contour calibrations were performed in T1 at rest (Cal_{rest}), during exercise below ventilatory threshold (<VT) (Cal_{low}), and during exercise above ventilatory threshold (>VT) (Cal_{high}). The calibrations at each if the timepoints were applied *post-hoc* to $\dot{Q}_{raw}$ to provide four complete data sets namely: $\dot{Q}_{restCal}$, $\dot{Q}_{low}$, $\dot{Q}_{high}$. A final data set ($\dot{Q}_{exercise}$) was derived from applying the corresponding calibration to the exercise epoch i.e. Cal_{rest} was applied to resting data, Cal_{low} was applied to all $\dot{Q}$ data obtained during exercise below the ventilatory threshold etcetera. $\dot{Q}_{restcal}$ was significantly ($P<0.05$) different to all other methods during exercise but not

at rest. There were no significant (P>0.05) differences between any other methods. Assuming $\dot{Q}_{exercise}$, which involves repeated calibrations at each workload, to be the most accurate method, the closest agreement was observed with $\dot{Q}_{high}$ (% bias ± LoA: 4.8 ± 30.0%). The comparison between $\dot{Q}_{low}$ and $\dot{Q}_{high}$ was characterised by low bias and wide limits of agreement (1.0 ± 46.5%).

Importantly, the $\dot{Q}$ response to incremental exercise was characterised differently according to the method employed. Data obtained using $\dot{Q}_{raw}$ showed a progressive significant (P<0.05) increase at each successive exercise intensity. In contrast, $\dot{Q}_{high}$ increased up until stage 3 (>VT) but did not increase further in the transition to SUPRAMAX. $\dot{Q}_{restcal}$, $\dot{Q}_{low}$ and $\dot{Q}_{exercise}$ increased in the transition from rest to exercise but showed no further significant increases thereafter.

Haemodynamic Responses to Exhaustive Maximal Exercise

In study IV, the power outputs for MAX (T1) and SUPRAMAX (T2) were 315 ± 39.3 W and 346.5 ± 43.2 W, respectively. Time to exhaustion was similar for both trials (176 ± 12.6s *versus* 170 ± 40.7s; P>0.05). Peak $\dot{V}O_2$ values measured during each trial were not significantly different (4.26 versus 4.26 L·min^{-1}; P>0.05).

There were no significant differences between MAX and SUPRAMAX for $\dot{Q}$ (28.7 *versus* 29.4 L•min^{-1}, 95% CI -2.6 to 1.4; P = 0.48), $\dot{V}O_2$ (4.26 *versus* 4.26 L•min^{-1}, 95% CI -0.18 to 0.17; P = 0.96), stroke volume (154.1 *versus* 157.3 ml, 95% CI -13.5 to 7.1; P = 0.49) or HR (188.9 *versus* 190.1 beats•min^{-1}, 95% CI-4.3 to 1.8; P = 0.36), respectively. MAP was significantly higher at peak exercise during the SUPRAMAX stage (129.1 *versus* 134.6 mmHg, 95% CI -10.1 to -0.9; P = 0.03), although both systolic and diastolic blood pressure remained unchanged between workloads. Additionally, no significant differences were observed for $\dot{Q}$, SV, HR, MAP or $\dot{V}O_2$ between peak values and exhaustion during either exercise stage. Both $\dot{Q}$ ($\dot{Q}$/W; 91.6 ± 18.4 *versus* 85.3 ± 21.4 mL/W; 95% CI 1.24 to; 11.31 P = 0.02) and $\dot{V}O_2$

($\dot{V}O_2$/W; 13.5 ± 1.1 *versus* 12.3 ± 1.5 ml/W; 95% CI 0.71 to 1.69; $P = 0.0007$) expressed per unit of power output, were significantly greater during the MAX stage.

The significantly greater MAP at the SUPRAMAX workload led to a tendency for a greater CPO (8.1 *versus* 8.5 W, 95% CI -0.92 to 0.02), although this did not reach statistical significance ($P = 0.06$). RPP was not significantly different between MAX and SUPRAMAX (41, 625 *versus* 41, 546 beats•min^{-1}•mmHg, 95% CI -3222 to 3378; $P = 0.96$).

Discussion

The series of experiments described within this thesis were conducted to evaluate potential approaches for the continuous beat-to-beat measurement of $\dot{Q}$ throughout maximal and supramaximal exercise to exhaustion. To address these aims we first evaluated three methods of $\dot{Q}$ assessment during exercise (pulse contour analysis calibrated with lithium dilution, bioreactance, and inert gas rebreathing), two of which provide continuous measurements. Based on these studies (I-III), $\dot{Q}$ was subsequently measured during two exercise protocols to exhaustion, using the preferred method, which gave the advantage of beat-to-beat measurements throughout intense exercise to exhaustion, and importantly, including the moments preceding fatigue. Additionally, we were able to continuously record arterial pressure and subsequently calculate cardiac work throughout two different workloads of maximal exercise. To our knowledge, there are no studies that have accurately quantified these measurements continuously throughout intense exercise and with this degree of resolution up until exhaustion.

The findings from study I, in which bioreactance ($\dot{Q}_{bio}$) and inert gas rebreathing ($\dot{Q}_{rebreathe}$) were compared for the measurement of $\dot{Q}$ during incremental exercise, showed a significant difference between the two methods. $\dot{Q}_{bio}$ consistently underestimated $\dot{Q}$ compared to $\dot{Q}_{rebreathe}$, during both rest and exercise. Previous studies during exercise in healthy volunteers have supported the accuracy of $\dot{Q}_{rebreathe}$. In the absence of a 'gold standard' comparator requiring highly invasive procedures, we determined construct validity by calculation of the (a-v)O_2diff from rearrangement of the Fick equation using simultaneously determined $\dot{V}O_2$ measurements (Rowland & Obert 2002). During peak exercise, the (a-v)O_2diff for $\dot{Q}_{rebreathe}$ reached 18.3 ml•dl^{-1} O_2, consistent with findings reported elsewhere in a similar cohort (Mortensen *et* al 2008). In contrast the (a-v)O_2diff difference from peak exercise for $\dot{Q}_{bio}$ reached 22.4 ml•dl^{-1} O_2, a physiological impossibility assuming normal arterial oxygenation and haemoglobin concentration. Additionally, the calculated slope of the $\dot{Q}$-$\dot{V}O_2$ relationship for $\dot{Q}_{bio}$ was lower

than values reported elsewhere with 'gold-standard' methods (Barker *et* al 1999; Johnson *et* al 2000). Together these results indicate that bioreactance leads to systematic underestimation of $\dot{Q}$ during exercise, which is exacerbated during high-intensity exercise by the intermittent loss of ECG signal that was also observed in this study.

Whilst the practicality of bioreactance for the determination of $\dot{Q}$ during exercise is attractive to the exercise physiologist, the lack of accuracy observed here indicates its unsuitability for tracking $\dot{Q}$ during maximal exercise. Whilst we were unable to specify the reason(s) for the apparent underestimation of $\dot{Q}$, one can speculate that validation of the device with predominantly cardiac populations (Keren *et* al 2007) indicates that the correction factors incorporated into the device algorithm may not be applicable for the healthy population. Furthermore, hardware issues relating to the ECG trace should be addressed to ensure an accurate HR measurement necessary for the calculation of $\dot{Q}$.

The development and evolution of methods that permit the monitoring of $\dot{Q}$ from analysis of the arterial pressure waveform, provides a potential approach to continuous $\dot{Q}$ measurement, providing that access to a peripheral artery is available, most commonly the radial artery. This approach is advantageous in being considerably less invasive than direct Fick or thermodilution, provides beat-to-beat monitoring, and allows the measurement of arterial pressure continuously to provide additional haemodynamic indices. In study II, we sought to evaluate the LiDCO™ system, which has the added benefit of an incorporated calibration technique using lithium indicator dilution. Despite comparing favourably with direct Fick during exercise in chronic heart failure patients (Kemps *et* al 2008), no studies have attempted to utilise this approach in healthy individuals at higher exercise intensities (*i.e.* $\dot{Q}$ >12 L·min^{-1}). In all participants, an adequate arterial pressure signal was obtained throughout exercise and lithium dilution curves were obtained at rest and during submaximal exercise, thus confirming the feasibility of obtaining $\dot{Q}$ measurements with this approach in a healthy population with exercising $\dot{Q}$ values above those previously reported (Kemps *et* al 2008).

A primary aim of this study was to evaluate whether the $\dot{Q}$ values obtained with this method are dependent on calibration and its timing. To achieve these aims, we compared raw pulse contour analysis values with those obtained when the raw data is calibrated at rest or during exercise. The degree to which calibration is influenced by haemodynamic changes with exercise is, to our knowledge, unexplored. Under resting conditions, $\dot{Q}$ was not statistically influenced by calibration or its timing (*i.e.* rest or exercise). However, following calibration at rest ($\dot{Q}_{restcal}$), $\dot{Q}$ values were ~30% higher throughout exercise than those obtained when the device was calibrated during submaximal exercise ($\dot{Q}_{excal}$). This indicates that the calibration factor used to 'correct' pulse contour analysis for differences in individual characteristics including arterial compliance, differed during exercise. This has important implications, as previous work with this technique did not recalibrate during exercise (Kemps *et* al 2008) despite data indicating that agreement with direct Fick tended to worsen at higher $\dot{Q}$ values. Our data indicates that the timing of calibration can statistically influence the values obtained during exercise, although it remains unclear whether there are any further changes within exercise. Rearrangement of the Fick equation using simultaneously determined $\dot{V}O_2$, plus analysis of the $\dot{Q}$-$\dot{V}O_2$ relationship, afforded the opportunity to evaluate construct validity of this method during exercise. These analyses confirmed a large overestimation of $\dot{Q}$ during exercise when the LiDCO™ device is calibrated under resting conditions where the $\dot{Q}$-$\dot{V}O_2$ slope (9.57) and the calculated (a-v)O_2diff (10.3 ml•dl^{-1} O_2) were considerably higher and lower, respectively, than data published using 'gold standard' methods (Johnson *et* al 2000; Stringer *et* al 2005).

To elucidate the reason for a change in the calibration factor (*i.e.* lithium indicator dilution $\dot{Q}$: $\dot{Q}_{raw}$) the device algorithm should be investigated. This algorithm makes the assumption that– following correction for arterial compliance, there is a linear relationship between net pressure change and net flow. The need to calibrate $\dot{Q}$ values obtained with pulse contour analysis is to provide the necessary correction for individual differences in systemic arterial compliance. Logically, an altered calibration factor is thus likely to depend, to a large extent, on the

decrease in arterial compliance that has been shown to occur with aerobic exercise (Otsuki *et* al 2006). Given that arterial compliance declines in a dose-dependent manner with increasing exercise intensity (Otsuki *et* al 2008), further study of the behaviour of device calibration at multiple exercise intensities was warranted prior to the device being employed for determination of $\dot{Q}$ during variable intensity exercise.

To optimise the practicality of the LiDCO™ device, we subsequently sought to determine whether multiple calibration points at different exercise intensity domains during stochastic exercise are required or whether a single calibration point during exercise was sufficient. No published studies have attempted to address this question, which is an essential one if lithium dilution/pulse contour analysis is to be considered for the assessment of $\dot{Q}$ during incremental exercise. Firstly, we were able to confirm the apparent overestimation of $\dot{Q}$ when pulse contour analysis is calibrated under resting conditions. To evaluate whether single calibration points during exercise are sufficient, we compared all datasets against the $\dot{Q}$ values obtained by calibrating repeatedly at each intensity of exercise ($\dot{Q}_{exercise}$). This data suggested that a single exercise calibration is sufficient providing it is performed at a level of work above the ventilatory threshold (Elliott *et* al 2012). In this domain, acceptable bias and limits of agreement (Critchley & Critchley 1999) characterised the comparison between single point calibration (>VT) and multiple calibrations. Furthermore, we also noted a trend for the calibration factor to decline with increasing exercise intensity, such that it approached 1.0 (*i.e.* closer to $\dot{Q}_{raw}$ values) in those participants in which a calibration was performed during maximal exercise. This trend further indicates the likely role of changing arterial compliance as the demand of exercise increases (Otsuki *et* al 2008; Chantler *et* al 2011). Importantly, for studies in which this method is used for the monitoring of $\dot{Q}$ throughout exercise, our data suggests that, where possible, a calibration at, or close to, the intensity at which exercise is to be performed, would be the optimal approach.

With a method of $\dot{Q}$ monitoring optimised for continuous measurements during high-intensity exercise it becomes possible to investigate the haemodynamic responses during exercise to

exhaustion, a topic still under considerable debate (Saltin & Calbet 2006; Ekblom 2009), and commonly represented by studies in which values obtained at isolated timepoints during maximal exercise (Gonzalez-Alonso & Calbet 2003; Mortensen *et* al 2005; Calbet *et* al 2007; Brink-Elfegoun *et* al 2007; Mortensen *et* al 2008) are used to infer the response to exercise across a period of non steady-state exercise with considerable cardiovascular demand.

Perhaps the most frequently posed question in exercise physiology is what limits intense endurance exercise performance and, in particular, the most commonly employed test to evaluate human exercise performance, the $\dot{V}O_{2max}$ test? Based on the model proposed by AV Hill in the 1920's (Hill & Lupton 1923), it is commonly stated that '$\dot{V}O_{2max}$ at sea-level is limited by systemic O_2 delivery ...' (Saltin & Calbet 2006, Page 75) and that '... the primary characteristic of elite endurance athlete ... is a large compliant heart with a compliant pericardium that can accommodate a lot of blood ... to take maximal advantage of the Starling mechanism to generate a large stroke volume' (Levine 2008, Page 31). Further supporting this view is the observation that in some studies (Mortensen *et* al 2005; Mortensen *et* al 2008), but not all (Gledhill *et* al 1994; Zhou *et* al 2001; Calbet *et* al 2007) $\dot{Q}$ appears to 'plateau' at, or close to, maximal exercise eliciting $\dot{V}O_{2max}$. A striking similarity between studies cited to support the cardiovascular limitation view is the measurement of $\dot{Q}$ during maximal exercise at isolated time points, often only once (Calbet *et* al 2007) or twice (Brink-Elfegoun *et* al 2007) without measurements in the moments preceding fatigue. Under such circumstances, it is entirely likely that an isolated measurement will miss the peak $\dot{Q}$ achieved during maximal exercise, thus leading to an underestimation of the $\dot{Q}$ response. The application of a method permitting continuous beat-to-beat measurements addresses any such limitations thus adding to the state of knowledge on this topic.

In study IV, our aim was to incorporate beat-to-beat measurements, optimised by the findings from studies 2 and 3, into the assessment of the haemodynamic response during exercise to exhaustion, including indices of myocardial work, which has yet to be documented beat-to-beat during strenuous exercise. In particular, we employed a protocol in which a typical

incremental test to exhaustion was employed, followed 60 mins later by a modified incremental protocol culminating in a bout of supramaximal exercise, similar to the verification protocols studied elsewhere (Day *et* al 2003; Rossiter *et* al 2006; Hawkins *et* al 2007).

The most significant finding from this study was that despite a similar $\dot{Q}$ between the two exercise bouts to exhaustion, the work of the heart, defined by cardiac power output, was greater during the supramaximal bout of exercise, primarily due to an elevated mean arterial pressure, which was significantly higher during supramaximal exercise. These findings agree with those published elsewhere during whole-body (arm + leg) exercise and confirm that the heart, despite a similar $\dot{Q}$ and $\dot{V}O_2$, is working harder with an increase m$\dot{V}O_2$ during supramaximal exercise. These findings are important for they challenge the commonly accepted view that an incremental test to exhaustion leads the heart to work maximally at $\dot{V}O_{2max}$, for this study and others (Brink-Elfegoun *et* al 2007) now show this not to be the case. Additionally, we are able to support the view that peak $\dot{V}O_2$ recorded during an incremental test to exhaustion, does represent a true attainable $\dot{V}O_{2max}$ in a healthy, suitably motivated individual, as supramaximal exercise failed to elicit a greater $\dot{V}O_2$, as also shown elsewhere (Day *et* al 2003; Rossiter *et* al 2006). The novelty of this study was that we were able to capture the true peak $\dot{Q}$ during each exercise bout. In light of these data it is perhaps unsurprising that we found $\dot{Q}$ to be similar in maximal and supramaximal intensity exercise. The obvious explanation for our findings is that $\dot{V}O_{2max}$ is therefore a function of the peak $\dot{Q}$ attainable during exercise, a conclusion supported by others (Saltin & Calbet 2006). The cardiovascular limitation model of $\dot{V}O_{2max}$ dictates that the attainment of maximal $\dot{Q}$, followed by skeletal muscle anaerobiosis and metabolite-induced fatigue limits exercise performance and $\dot{V}O_{2max}$. This model has been established by the observations that $\dot{Q}$ can appear to plateau at maximal exercise (Mortensen *et* al 2005), is insufficient to fully perfuse the active musculature during exercise (Andersen & Saltin 1985), and modifications in which proportionately influence $\dot{V}O_{2max}$ (Krip *et* al 1997; Saltin *et* al 1968). However, in study IV,

this view is challenged by the observation that exercise performed to $\dot{V}O_{2max}$ terminates with a degree of cardiac functional reserve as determined from CPO; in the MAX trial, exercise terminated with a CPO below that measured during supramaximal testing. For the cardiovascular limitation model to be accepted, one would expect that any available cardiac reserve would be fully employed in an attempt to augment $\dot{Q}$, which if unsuccessful, would lead to fatigue as postulated by this model. That this fails to occur suggests that some degree of cardiac function is with-held during incremental exercise to $\dot{V}O_{2max,}$ perhaps by some central, neutrally-mediated mechanism that acts to anticipate significant homeostatic disturbance (Noakes & St Clair Gibson 2004), which may potentially result in catastrophic biological failure, such as myocardial ischemia, which clearly does not occur in healthy individuals.

That a 'central governor' may be operational during incremental exercise to exhaustion leads to the question of how exercise at a greater workload is possible, such as during the supramaximal stage of study IV, if a central governor is deemed to be regulating the extent of skeletal muscle recruitment? In our study, and others (Mortensen *et* al 2008), the exercise protocol included an incremental exercise test lasting approximately 10-15 minutes, followed after 60 mins of rest by a supramaximal exercise bout to exhaustion. The data presented by Mortensen *et* al (Mortensen *et* al 2008) showed greater reductions in arterial O_2 saturation, blood pH coupled with higher lactate concentration following incremental exercise, thus indicating a more substantial metabolic disturbance during such exercise than during 'stand-alone' supramaximal exercise. Consequently, one can postulate that regulation by a central governor was not required until similar metabolic/homeostatic disturbances were reached, which appear not to occur early during supramaximal exercise. Furthermore, the very finding that $\dot{Q}$ does not change despite the achievement of a greater workload, demonstrates an uncoupling between skeletal muscle work and $\dot{Q}$, which in turn confirms that $\dot{Q}$ does not determine skeletal muscle activity.

Summary

The findings from the studies reported in this thesis shows that alternative approaches for the measurement of beat-to-beat $\dot{Q}$ are capable of recording the haemodynamic response during maximal exercise in healthy, trained volunteers and offer the opportunity to accurately track $\dot{Q}$ up until the moment of fatigue. Studies 1-3 offer the following conclusions:

- During incremental exercise to exhaustion, bioreactance appears to significantly underestimate $\dot{Q}$ in healthy, trained individuals, when compared to an inert gas rebreathing and values obtained from previous studies using accepted methods. Consequently, the accuracy of this approach was deemed insufficient for the beat-to-beat measurement of $\dot{Q}$ during strenuous exercise.
- Arterial pulse contour analysis, measured from the radial artery, provides nominal SV measurements throughout exercise to exhaustion with only minimal signal loss that is easily rectified by the flushing of saline through a cannula.
- Calibration of $\dot{Q}$ measurements derived from arterial pulse contour analysis using an incorporated lithium indicator dilution method, results in significantly overestimated $\dot{Q}$ values during exercise when the calibration procedure is performed at rest prior to exercise. We noted improved construct validity of $\dot{Q}$ when the calibration procedure is performed during submaximal exercise.
- A single calibration of pulse contour analysis with lithium dilution during exercise appears sufficient, providing that the calibration is performed above the ventilatory threshold, where possible. This approach gives acceptable bias and limits of agreement compared to performing multiple calibrations at each exercise intensity during incremental exercise testing. We further noted that the calibration factor used to correct the nominal SV obtained from pulse contour analysis (defined as lithium dilution $\dot{Q}$ divided by pulse contour analysis $\dot{Q}$) appears to decrease inversely with exercise intensity.

Using these findings, the haemodynamic response during exercise to exhaustion was characterised in study IV. The following conclusion was made regarding the cardiovascular responses to maximal exercise and their role in determining the limiting factors for exercise performance and $\dot{V}O_{2max}$.

- Incremental exercise to exhaustion terminates with cardiac functional reserve, as cardiac power output, measured beat-to-beat, was shown to be greater during supramaximal exercise. $\dot{Q}$ and $\dot{V}O_2$ were similar for each exercise protocol. This finding indicates that the heart is working submaximally during incremental exercise to exhaustion, a finding that refutes the commonly accepted view that the heart regulates exercise to $\dot{V}O_{2max}$

The series of studies presented here provides a detailed investigation into a method with the ability to quantify exercise haemodynamics on a beat-to-beat basis, without excessively invasive procedures lending inappropriate risk to the participant. Approaches such as these are essential for the full investigation of the cardiac response to maximal exercise and will undoubtedly shed further light on the factors that regulate short-term exercise performance, as we have done here by showing that cardiac power output is higher during supramaximal exercise, thus refuting the view that maximal exercise performance, and thus $\dot{V}O_{2max}$, is primarily governed by the heart.

Importantly, this series of studies opens up avenues for further investigation into a model in which the brain acts to centrally regulate exercise performance by defending homeostasis in the face of considerable cardiac and metabolic demands, such as during maximal exercise. That measurement of cardiac function with a similar time resolution to that of pulmonary gas exchange, surface electromyography, and arterial oxygenation, amongst others, is now possible will be of considerable benefit to those exploring this further. We also propose that this approach will permit the rigorous further investigation of the commonly held belief that the heart regulates exercise performance.

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