BAYLISS–STARLING PRIZE LECTURE

New perspectives concerning feedback influences on cardiorespiratory control during rhythmic exercise and on exercise performance

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Abstract The cardioaccelerator and ventilatory responses to rhythmic exercise in the human are commonly viewed as being mediated predominantly via feedforward 'central command' mechanisms, with contributions from locomotor muscle afferents to the sympathetically mediated pressor response. We have assessed the relative contributions of three types of feedback afferents on the cardiorespiratory response to voluntary, rhythmic exercise by inhibiting their normal 'tonic' activity in healthy animals and humans and in chronic heart failure. Transient inhibition of the *carotid chemoreceptors* during moderate intensity exercise reduced muscle sympathetic nerve activity (MSNA) and increased limb vascular conductance and blood flow; and reducing the normal level of *respiratory muscle work* during heavier intensity exercise increased limb vascular conductance and blood flow. These cardiorespiratory effects were prevented via ganglionic blockade and were enhanced in chronic heart failure and in hypoxia. Blockade of μ opioid sensitive locomotor muscle afferents, with preservation of central motor output via intrathecal fentanyl: (a) reduced the mean arterial blood pressure (MAP), heart rate and ventilatory responses to all steady state exercise intensities; and (b) during sustained high intensity exercise, reduced O_2 transport, increased central motor output and end-exercise muscle fatigue and reduced endurance performance. We propose that these three afferent reflexes – probably acting in concert with feedforward central command – contribute significantly to preserving O_2 transport to locomotor and to respiratory muscles during exercise. Locomotor muscle afferents also appear to provide feedback concerning the metabolic state of the muscle to influence central motor output, thereby limiting peripheral fatigue development.

Jerome A. Dempsey was born and educated in Canada (Universities of Western Ontario and Alberta) and received his PhD at the University of Wisconsin – Madison. He has been on the faculty at UW Medical School since 1968, Director of the John Rankin Laboratory of Pulmonary Medicine since 1981 and is currently Professor Emeritus. His major interests have included the respiratory physiology, pathophysiology and cardiorespiratory interactions attending exercise, hypoxia, sleep, obesity, COPD and CHF, and the training of pre- and postdoctoral fellows and undergraduates.

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On behalf of the 68 pre- and postdoctoral fellows and many undergraduate alumni of the John Rankin Laboratory of Pulmonary Medicine, University of Wisconsin and the dedicated UW faculty who served our laboratory's research and educational missions I humbly accept this distinguished award, named after two of our profession's greatest scientists and innovators. I am especially grateful that several Rankin Laboratory alumni were in attendance at the London meeting for this 17th Bayliss–Starling lecture. Given the theme of our meeting it is relevant to recall Professor Starling's insights from his 1919 lecture delivered at the Royal Army Medical College into the neural mechanisms of blood flow regulation during exercise, beyond those which he had uncovered in the isolated heart (Starling, 1920): 'The extraordinary powers with which the heart muscle is endowed represent but the central fortress of the system and under normal conditions is protected from coming into play by the activation of the defending positions and outposts provided by the central nervous system and its servants.'

I am especially excited by the fact that two of the previous memorial lecturers were scientific heroes of mine and gentlemen with whom I had the opportunity to interact and to learn from. I refer specifically to Professor John Pappenheimer (1915–2007) (1982 lecturer) whose pioneering work on the physiological significance of the medullary chemoreceptors in the 1960s provided great impetus to our laboratory's research on cerebrospinal fluid pH regulation and chemoreceptor interactions. In addition, Ivan De Burgh Daly (1893–1974) (1966 lecturer) who together with his son Professor Michael De Burgh Daly (1922–2002) conducted two lifetimes of basic research into cardiorespiratory control, provided the basis for our attempts to apply these fundamental principles to problems of respiratory:cardiovascular interactions during exercise in healthy and diseased humans. Finally, I would especially like to recognize the personal influence provided by the late Brian Whipp (1937–2011). Brian and I were contemporaries who would meet almost annually at various meetings to share our findings on some aspect of respiratory control, often following a squash match. We were always highly critical of one another's theories and often agreed to disagree on many points, but I always learned something of significance from Brian's insights (and from his superior skills in squash) and am grateful for these wonderful memories.

Introduction

The primary controllers underlying cardiorespiratory responses to exercise have been the subject of fascination and debate among physiologists for over a century. The hyperpnoea accompanying rhythmic exercise represents the ultimate in homeostatic response owing to its *precision* in matching the rising CO_2 production and protecting alveolar and arterial gases, combined with its *efficiency* in minimizing the work and energy cost of respiratory muscles over the entire range of mild to maximum intensity exercise (Forster *et al.* 2012). The large selective increases in locomotor muscle blood flow during exercise require powerful local vasodilator mechanisms contained in active skeletal muscle vascular endothelium combined with simultaneous, sympathetically mediated vasoconstriction of vasculature within both active and inactive organs, so that blood pressure may be maintained along with high perfusion pressures (Andersen & Saltin, 1985; Rowell, 2004). Primary sources of increased sympathetic activity and inhibition of parasympathetic activity, as well as the drive to breathe, are known to include strong feedforward 'central command' influences that are activated along central neural pathways in parallel to motor command, as well as feedback from limb muscle afferents with a secondary modulating role for baroreceptors and chemoreceptors. The evidence for a strong contribution from central command mechanisms originating from several potential supra-pontine locations has been demonstrated convincingly in the decorticate cat model of 'fictive' locomotion (Eldridge *et al.* 1981; Waldrop & Iwamoto, 2006) and more recently in the human undergoing voluntary or 'imagined' exercise via the use of brain imaging techniques (Fink *et al.* 1995; Thornton *et al.* 2001) or the direct recording of local field potentials in a number of cortical nuclei (Green *et al.* 2007; Basnayake *et al.* 2012).

We now present evidence accumulating over the past two decades which implicates important feedback contributions to cardioventilatory control during exercise from three sources, namely the carotid chemoreceptors, the respiratory muscle metaboreceptors and the limb locomotor muscle group III–IV afferents. There is already sufficient evidence establishing that each of these reflexes, when stimulated *in isolation,* is capable of eliciting significant cardioventilatory responses akin to those present in voluntary whole body rhythmic exercise. However, the presence of redundant mechanisms and pathways available to protect the adequacy of gas transport during voluntary rhythmic exercise has confounded our ability to determine whether each of these sensory inputs is an obligatory cardiorespiratory controller. In our experiments we have addressed this question by using voluntary rhythmic exercise of a large muscle mass, which engages all potential feedforward and feedback mechanisms. On this background we superimposed a transient and/or steady state inhibition of the tonic afferent input from the specific receptor site(s) in question in order to determine the relative import of its contribution to cardiorespiratory control.

Carotid chemoreceptor contributions to sympathoexcitation/blood flow distribution

Research over the past decade has revealed a highly multifunctional, interactive and malleable carotid chemoreceptor. The carotid chemoreceptors are now known to respond sensitively to many circulating stimuli beyond oxygen, such as potassium, noradrenaline (norepinephrine), temperature, potassium, adenosine and glucose, and to play a major role in controlling sympathetic nerve activity as well as ventilation (Teppema & Dahan, 2010). They are also capable of upregulating their sensitivity in response to even relatively brief durations of arterial hypoxaemia (Nielsen *et al.* 1988; Wang *et al.* 2008) or reduced blood flow and sheer stress (Sun *et al.* 1999; Ding *et al.* 2011) and when stimulated, or inhibited, or denervated have also been shown to exert marked hyperadditive effects on medullary chemoreceptor CO_2 sensitivity (Dahan *et al.* 2007; Blain *et al.* 2010). A role for the carotid chemoreceptors in cardiorespiratory regulation during exercise has previously emphasized the ventilatory response. Most data to date, mainly from chemo-denervated preparations, suggest a potential role in mediating at least a portion of the hyperventilatory response to heavy exercise, i.e. when circulating metabolites have increased in arterial blood, although this contention remains controversial and confounded by the remodelling which occurs following denervation as well as the multiple potential systemic and central stimuli which undergo rapid changes under these conditions (Forster *et al.* 2012).

We have attempted to evaluate the carotid chemoreceptor's contribution to the increased sympathetic nerve activity and blood flow distribution accompanying exercise. First we examined these questions in the resting and exercising canine by transiently inhibiting the carotid sinus nerve activity using close intra-arterial infusions of hyperoxic saline or dopamine (Bisgard *et al.* 1979) with the catheter tip positioned at the bifurcation of the common carotid artery (Stickland *et al.* 2007). This transient inhibition of carotid sinus nerve activity caused immediate increases in limb vascular conductance and blood flow during mild intensity exercise in the healthy canine – but not at rest – and these effects were prevented by ganglionic blockade or carotid body denervation (see Fig. 1; Stickland *et al.* 2007).

In order to determine the relative contribution of the carotid chemoreceptors to the total sympathetic constraint we compared the maximum increase in limb vascular conductance with total systemic α receptor blockade *vs.* that achieved via carotid chemoreceptor inhibition alone (in the intact animal). As shown in Fig. 2, α blockade elicited a 50% increase in vascular conductance at rest and a 130% increase in conductance in mild intensity exercise, whereas carotid chemoreceptor inhibition by

itself accounted for none of the total sympathetic constraint at rest and about one-third of the total increase in vascular conductance during exercise. In the same dogs studied after several weeks of cardiac pacing at a high rate into heart failure, left ventricular ejection fraction fell from 55 to 18% and carotid chemosensitivity increased, as shown by a threefold increase in the ventilatory response to isocapnic hypoxia. The magnitude of the total sympathetic constraint in chronic heart failure (CHF) (*vs.* healthy) animals on limb vascular conductance increased more than fourfold at rest and during mild exercise. Under these conditions of enhanced chemoreceptor sensitivity in CHF, tonic activity from the chemoreceptors – even at rest – accounted for 20–25% of the enhanced sympathetic constraint of vascular conductance (Stickland *et al.* 2007).

A role for carotid chemoreceptor influences on sympathetic vasoconstriction in the healthy human was demonstrated by reductions in muscle sympathetic nerve
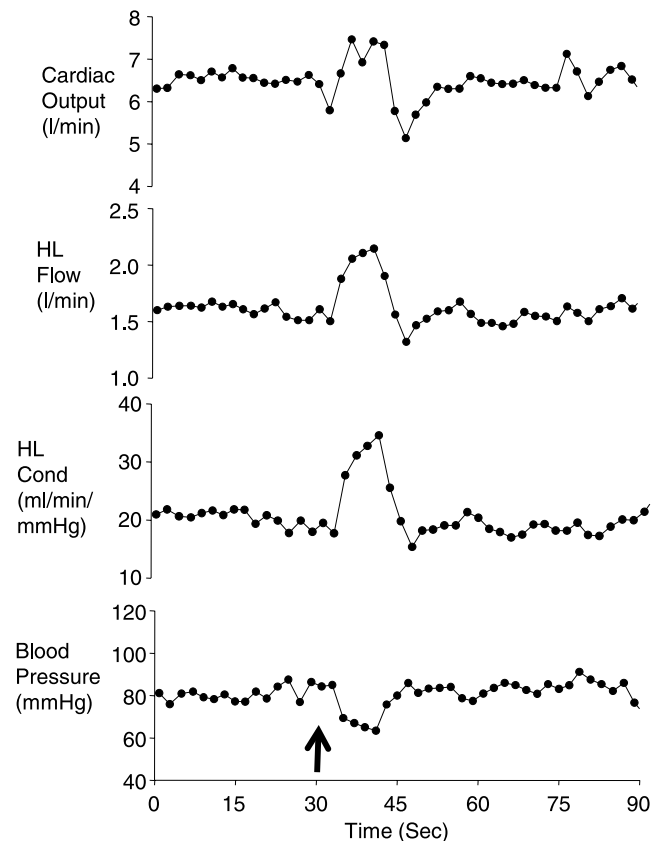
Figure 1. The effects of transient inhibition of carotid chemoreceptor tonic input on hind limb (HL) vascular conductance and blood flow during moderate intensity exercise in the healthy canine
The arrow indicates the time of the close carotid injection of 10 μg kg^{-1} dopamine. Carotid body denervation or α adrenergic receptor blockade with phentolamine prevented these cardiovascular effects of carotid body inhibition. Figure adapted from Stickland *et al.* (2007).
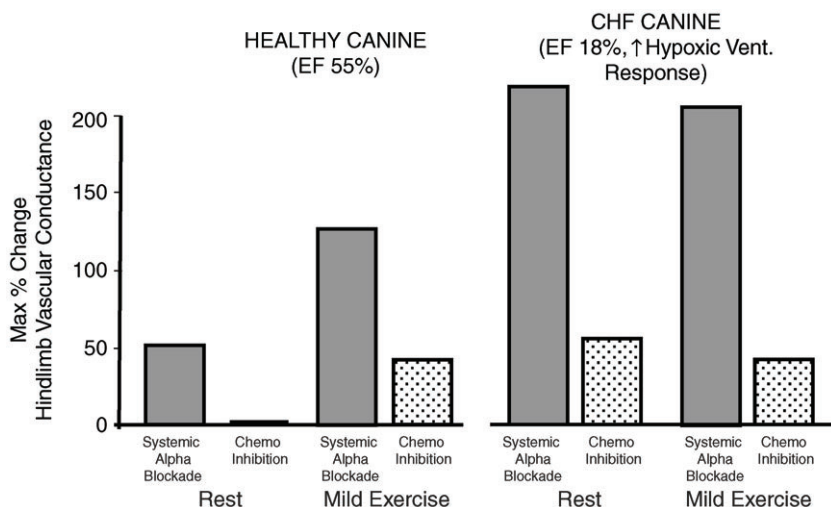
Figure 2. Effects of α blockade on hindlimb vascular conductance at rest and during mild intensity exercise
Note in the healthy canine, systemic α blockade increased limb vascular conductance by 50% at rest with no effect of chemoreceptor inhibition, *per se*, in the unblocked animal. During mild exercise the effect of α blockade on vascular conductance was increased 2.5-fold above that at rest and chemoreceptor inhibition contributed about one-third to the total increase in vascular conductance. The right-hand panel shows the effects of similar experiments in the same animals following 3 weeks of cardiac pacing to produce chronic heart failure (CHF). Note the left ventricular ejection fraction (EF) was reduced from 55% in health to 18% in the CHF animals and that the ventilatory response to isocapnic hypoxia was increased 3-fold in CHF *vs.* healthy animals. In the CHF animal the sympathetic blockade produced a 4-fold greater increase in limb vascular conductance both at rest and exercise and the influence of transient chemoreceptor inhibition was apparent even under resting conditions. Data obtained from Stickland *et al.* (2007).

activity (MSNA) elicited via transient inhaled hyperoxia during moderate intensity, rhythmic, arm exercise (Stickland *et al.* 2008; see Fig. 3). More recently a similar experiment in humans showed that transient chemoreceptor inhibition also increased limb vascular conductance and blood flow during mild intensity, single leg, rhythmic exercise (Stickland *et al.* 2011).

These findings provide evidence in support of a significant contribution of the tonic sensory input from the carotid chemoreceptors as one mechanism, among other feedback and feedforward mechanisms (see section below regarding respiratory and limb afferents), to provide a significant contribution to sympathetic vasoconstrictor outflow and blood flow distribution to working muscles across a wide range of exercise intensities. We do not yet understand the mechanisms underlying an apparent exercise-induced increase in carotid body sensitivity (Stickland *et al.* 2007), although a similar synergistic effect of mild intensity exercise on both ventilatory and MSNA responsiveness to acute hypoxia has been previously
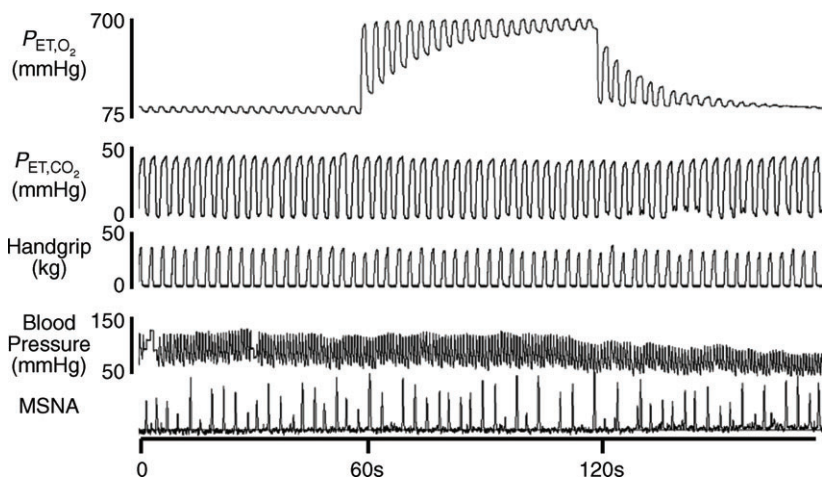
Figure 3. The effects in the healthy human of transient inhibition of the carotid chemoreceptor with 1.0 F_{IO_2} during rhythmic handgrip exercise (20 r.p.m. at 50% of maximum voluntary contraction)
Ventilation and P_{ET,CO_2} were voluntarily controlled via feedback by the subject during the transient hyperoxia intervention. Note ~15–20 s after P_{ET,O_2} rose above ~250 mmHg, MSNA frequency was markedly diminished and then returned to control levels as exercise was continued and normoxic conditions were restored. The reduction in MSNA with transient hyperoxia averaged 35% in 7 subjects. Data obtained from Stickland *et al.* (2008). Subsequently, transient hyperoxia was also shown to increase limb vascular conductance and blood flow during single leg kick exercise (Stickland *et al.* 2011).

reported (Weil *et al.* 1972; Seals *et al.* 1991). These data also demonstrate, using the CHF animal model, that carotid chemoreceptor hypersensitivity does occur under chronic conditions of reduced shear stress (Ding *et al.* 2011; Schultz, 2011) and this will elicit an enhanced contribution to sympathetically mediated vascular conductance and locomotor muscle blood flow both at rest and exercise.
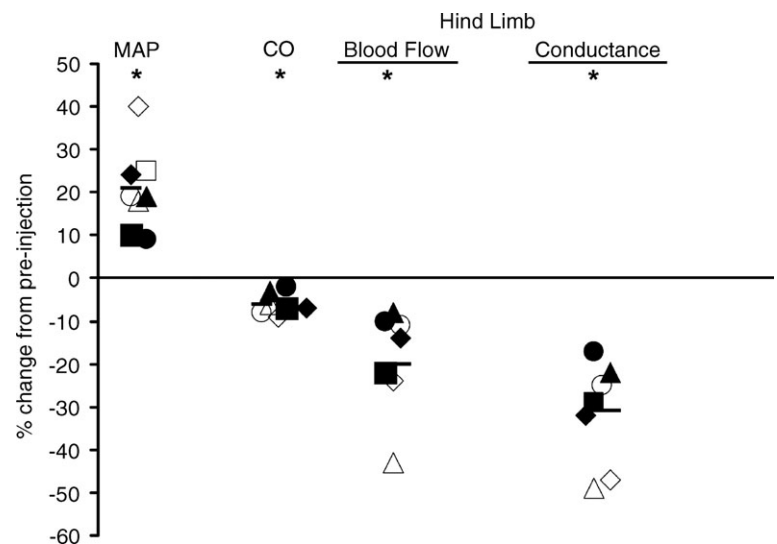
Respiratory muscle afferents

We now summarize evidence in support of the contributions of a respiratory muscle metaboreflex to blood flow distribution and exercise performance. First, we know that unmyelinated sensory fibres are abundant in the phrenic nerve (Duron, 1981) and unmyelinated group IV metaboreceptor afferents are activated when the diaphragm is fatigued in the anaesthetized animal (Jammes & Balzamo, 1992; Hill, 2000). Secondly, it has also been demonstrated that pharmacological or electrical stimulation of phrenic afferents in anaesthetized animals elicits vasoconstriction and reduces blood flow in selective vascular beds (Hussain *et al.* 1991). Thirdly, in the human at rest, fatiguing the diaphragm or expiratory muscles (via voluntary breathing efforts against resistance) will cause a time dependent increase in MSNA (St Croix *et al.* 2000; Derchak *et al.* 2002) and a reduction in vascular conductance and blood flow in the resting limb (Sheel *et al.* 2001, 2002). Furthermore, in the resting or mildly exercising canine, infusing lactic acid into the phrenic artery and diaphragm caused vasoconstriction and reduced blood flow in the contracting limb muscle and this vasoconstrictive effect was prevented via adrenergic blockade (Rodman *et al.* 2003; see Fig. 4).

Physiological and pathophysiological roles for the respiratory muscle metaboreflex. These types of data point strongly to the existence of a respiratory muscle metaboreflex capable of activating sympathetically mediated vasoconstriction in locomotor muscles, at least under conditions which elicit diaphragm fatigue and/or accumulation of anaerobic metabolites. However, to determine if there is a *physiological role* for this reflex during exercise it was important to reduce most of the respiratory muscle work *normally incurred* during exercise. When this was achieved with a unique servo-controlled mechanical ventilator while the subject exercised at >85% of the maximum rate of O_2 consumption ($\dot{V}_{O_2 max}$), we observed (a) that diaphragm fatigue was prevented (Babcock *et al.* 2002), and (b) that, relative to the control condition, vasodilatation and increased blood flow occurred in the exercising legs (despite a concomitant reduction in stroke volume and cardiac output) (Harms *et al.* 1997, 1998; see Fig. 5, top). It would follow that these apparent effects of respiratory muscle work on compromising limb blood flow and O_2 transport would also affect the rates at which locomotor muscle metabolites accumulated and limb muscle fatigue occurred during exercise (Barclay, 1986; Hogan *et al.* 1999). This possibility was assessed by measuring the force output of the quadriceps muscle (Q_{twitch}) in response to supermaximal magnetic stimulation of the femoral nerve before and immediately following cycling exercise (ΔQ_{twitch}). Indeed, unloading the inspiratory muscles: (a) prevented a significant amount of limb fatigue when comparisons of ΔQ_{twitch} were made between control and respiratory muscle unloaded conditions at identical heavy intensity work rates (>80% maximum (max) to exhaustion) for identical exercise durations (Romer *et al.* 2006; Amann *et al.* 2007), (b) increased exercise duration to exhaustion (by 14 ± 4%), and (c) significantly reduced perceived magnitude of 'limb discomfort' when exercise was carried out at a fixed, high intensity work load (Harms *et al.* 2000) (see Fig. 6).

Figure 4. Effects of acidification of the diaphragm via lactic acid injection into the phrenic artery during mild intensity exercise in the chronically instrumented canine ($n = 7$)
Note that the injection of lactic acid elicited an average increase in MAP of 20% and a decrease in limb vascular conductance of 30% and blood flow of 20%. These vasoconstrictor effects of diaphragm acidification were prevented via sympathetic blockade. Data obtained from Rodman *et al.* (2003). MAP, mean arterial blood pressure; CO, cardiac output.
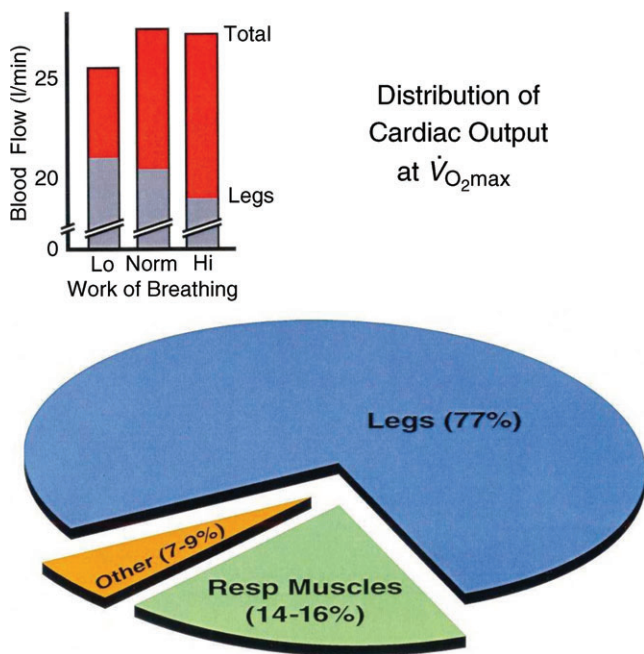
The relative importance of the respiratory muscle metaboreflex to blood flow distribution and limb fatigue during exercise was enhanced under conditions of increased ventilatory work and/or reduced blood flow – as occurs upon hypoxic exposure and/or in the presence of compromised cardiac output. For example, in health and during exercise in normoxia, unloading the respiratory muscles at work rates <75% of max had little effect on limb vascular resistance or limb fatigue or blood flow (Wetter *et al.* 1999; Amann *et al.* 2007). However, healthy subjects undergoing submaximal exercise in moderate hypoxia (arterial O_2 saturation,S_{aO_2} ∼80%) experienced substantial hyperventilation and unloading their inspiratory muscles alleviated the development of limb locomotor muscle fatigue by ∼30% and significantly prolonged exercise performance (Amann *et al.* 2007). So the increased work of breathing incurred by exercise in hypoxia contributed significantly to the limb fatigue and reduced exercise performance experienced in hypoxic environments.

Further, many CHF patients have an augmented, tachypnoeic ventilatory response to exercise combined with a limited availability of a reduced cardiac output

during exercise for respiratory and limb muscle perfusion. Accordingly, when the respiratory muscles in these patients were unloaded via mechanical ventilation during even very mild exercise intensities (<60% peak work rate), limb vascular conductance and blood flow were markedly increased (Miller *et al.* 2007; Olson *et al.* 2010; see note below). Similarly in chronic obstructive pulmonary disease (COPD) patients, exercise-induced quadriceps fatigue occurred when cycling exercise to exhaustion was carried out at only 50% of $\dot{V}_{O_2max}$, whereas no limb fatigue occurred under similar conditions in age matched controls. Unloading the inspiratory muscles in these COPD patients relieved a significant amount (but not all) of the limb fatigue induced during submaximal exercise (Amann *et al.* 2010*b*). These findings implicated a compromised aerobic capacity of limb locomotor muscles (Maltais *et al.* 2000) – in addition to reduced O_2 transport – as a significant contribution to exercise-induced limb fatigue in these highly sedentary COPD patients.

[In CHF patients, unloading the respiratory muscles via positive pressure mechanical ventilation – which reduces the left ventricular afterload – also *increased* stroke volume and cardiac output which accounted for a significant portion of the observed increase in limb blood flow, along with *increases* in limb vascular conductance (Miller *et al.* 2007; Olson *et al.* 2010). This increase in stroke volume contrasts sharply with the *reductions* in stroke volume experienced in healthy animals (Miller *et al.* 2007) and humans (Harms *et al.* 1997) when the negativity of inspiratory pleural pressure is reduced via positive pressure assist during exercise (see Fig. 5).]

Major questions remaining regarding the respiratory muscle metaboreflex. We propose that the evidence to date supports a significant role for respiratory muscle afferents in activating sympathetically mediated vasoconstriction and regulating blood flow distribution during sustained, high intensity exercise, with significant negative consequences for locomotor muscle fatigue and exercise performance. However, several major questions remain. For example, what mechanisms/conditions are required to trigger III–IV muscle afferents from inspiratory and expiratory muscles during exercise? (Also see section on limb afferents below.) Secondly, why, in the face of metaboreceptor induced increases in MSNA during exercise does the limb locomotor muscle vasculature constrict and the diaphragm vasculature dilate? Aacker and Laughlin showed in isolated vessels that noradrenaline-induced vasoconstriction was about twofold more sensitive in gastrocnemius muscle vasculature (in red *or* white muscle) than in the phrenic artery feed vessels (Aaker & Laughlin, 2002).

Thirdly, we know that altering the work of breathing during exercise will change blood flow to the exercising limb (see above) in the human but the presumption that

Figure 5. Average changes in cardiac output (total blood flow) and leg blood flow during maximum cycling exercise in fit athletes with superimposed increases and decreases in inspiratory muscle work
Limb vascular conductance and flow increased with reduced work of breathing and decreased with increased work of breathing. Stroke volume and cardiac output decreased with the reduced work of breathing and remained unchanged with increased work of breathing (also see bracketed note in the text on the quantification of respiratory muscle blood flow during exercise). Data adapted from Harms *et al.* (1997, 1998).

this also means increased blood flow to respiratory muscles has only been verified to date via microsphere data in the CHF rat model in whom reduced blood flow to the limb musculature during exercise occurred simultaneously with a greater blood flow to the diaphragm (Musch, 1993). The state of the art for measuring blood flow in respiratory muscles does not yet appear to be sufficiently sensitive or specific to test this hypothesis in exercising humans (Vogiatzis *et al.* 2008) and methods are not available for measuring diaphragmatic flow in humans. However, supramaximal phrenic nerve stimulation has been used in humans to show that the diaphragm fatigues during heavy intensity, sustained running exercise, but does not fatigue when comparable levels of respiratory muscle work are performed via voluntary hyperpnoea in the subject while *at rest*. We interpreted these contrasting effects of respiratory muscle work between rest and exercise to mean that locomotor muscles do indeed compete to a significant extent, for the available cardiac output during heavy intensity exercise (Babcock *et al.* 1995). This interpretation is supported by a similar experimental design which showed that the increase in intercostal muscle blood flow accompanying high levels of ventilation was substantially reduced during cycling *vs.* voluntary, isocapnic hyperpnoea at rest (Vogiatzis *et al.* 2009; see note below). Finally, in contrast to the sympathoexcitatory effects elicited by activating respiratory muscle afferents, the effects on the control of breathing are contradictory, showing either a stimulatory or inhibitory effect in anaesthetized animals (Road, 1990). When we acidified the diaphragm in the exercising dog we observed a small but consistent slowing of breathing frequency (Rodman *et al.* 2003).

[The quantification of respiratory muscle blood flow during exercise remains controversial. On the one hand, estimates of 'trunk and head' blood flow based on the difference between cardiac output and flow to the arms plus legs (measured with dye dilution via catheterization of the subclavian and femoral veins) suggest that the lumped structure of the head, neck, heart, abdomen viscera, kidney, respiratory muscles and gluteal muscles receives about 20% of the cardiac output (CO) and 15% of the $\dot{V}_{O_2}$ during maximum (max) upright cycling exercise (Calbet *et al.* 2007). On the other hand, higher estimates of respiratory muscle blood flow alone during max cycling exercise ranging from 8 to 10% in the untrained, to 14–16% in the well trained (at higher max work rates and minute ventilation, $\dot{V}_E$), were derived based on (a) the measured O_2 cost and increased cardiac output required while mimicking the work of breathing (WOB) achieved at max exercise (Anholm *et al.* 1987; Aaron *et al.* 1992*a,b*; Vogiatzis *et al.* 2008), (b) the reduction in CO obtained when the WOB was reduced via mechanical ventilation during max exercise (Harms *et al.* 1998; see Fig. 5, bottom pie chart), and (c) the total blood flow (measured directly via microsphere distribution) in all inspiratory and expiratory muscles during max treadmill running in

the pony (Manohar, 1986). One problem not yet addressed in any study is the identification of all muscles – in the chest wall, abdomen, upper back and shoulders – which are actually engaged (both dynamically and as fixators) in producing the hyperpnoea accompanying heavy intensity exercise.]

A logical extension of the evidence summarized above is to ask if specific respiratory muscle training will delay the rate of development of respiratory muscle fatigue during sustained exercise, and therefore also delay the activation of respiratory muscle metaboreflexes and blood flow redistribution during exercise? There is some limited evidence to date using adequately controlled studies to suggest that there might indeed be some small but significant effects of respiratory muscle training on reducing exercise-induced diaphragm fatigue (Verges *et al.* 2007), on alleviating calf muscle fatigue during plantar flexion exercise (McConnell & Lomax, 2006), on attenuating activation of the respiratory muscle reflex (Witt *et al.* 2007) and on exercise (time trial) performance (Romer *et al.* 2002). However, direct evidence for a benefit of respiratory muscle training on blood flow distribution during whole body high intensity exercise, i.e. when competition between locomotor and limb muscles for an increased share of cardiac output is at its peak, is not yet available.

Locomotor muscle afferents, exercise hyperpnoea, cardiovascular regulation and exercise performance

Cardioventilatory regulation. When studied in isolation, using muscle stimulation, i.e. without the central command mechanism of voluntary exercise, limb muscle group III–IV afferents clearly have the capability to increase respiratory motor output, ventilation, blood pressure and heart rate in anaesthetized animals or awake humans (Alam & Smirk, 1937, 1938; Asmussen *et al.* 1943; Coote *et al.* 1971; McCloskey & Mitchell, 1972; Tibes, 1977; Adams *et al.* 1984; Forster *et al.* 2012). Further, *in vitro* evidence in the isolated brain stem–spinal cord rodent preparation also supports an important role for muscle afferents in ventilatory control by demonstrating that lumbar locomotor networks can rhythmically entrain medullary respiratory neurons (Morin & Viala, 2002). However, over the past 100 plus years most supportive evidence in this regard is counteracted by negative findings which have questioned a clear, obligatory physiological role for these muscle afferents during voluntary, rhythmic exercise in humans. Note the following examples.

- There are an abundance of group III–IV afferent endings in limb muscle and they are activated by normal submaximal, rhythmic exercise (Pickar *et al.* 1994; Adreani *et al.* 1997) – but countering evidence claims muscle ischaemia (i.e. a mismatch between blood O_2
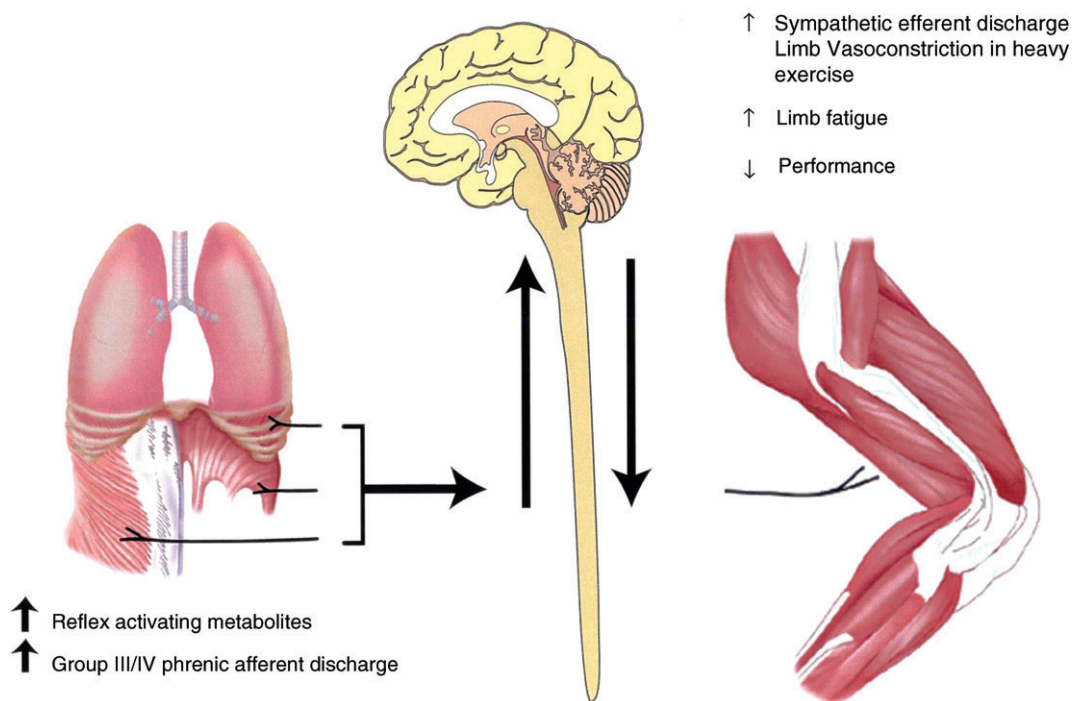
supply and demand), as opposed to a rhythmically contracting muscle with normally increased blood flow, is required to activate cardioventilatory responses via muscle afferents (Freund *et al.* 1979; Rowell, 2004). Furthermore, even imposed muscle ischaemia has been reported to elevate MAP – but to inhibit ventilation – during recovery from exercise (Dejours *et al.* 1957; Rowell *et al.* 1976; Eiken, 1987).

- Electrical stimulation of limb muscle contractions in intact humans elicits a cardioventilatory response comparable to that during physiological whole body voluntary exercise – at least over a modest range of increase in $\dot{V}_{O_2}$ above resting levels; however, spinal cord lesions do not always prevent these responses (Asmussen *et al.* 1943; Adams *et al.* 1984; Forster *et al.* 2012).

- Blocking opiate sensitive group III–IV afferents via intrathecal opiate agonists or epidural lidocaine (lignocaine) markedly reduced most of the cardio-ventilatory responses to induced static muscle contractions in anaesthetized cats (Hill & Kaufman, 1990; Meintjes *et al.* 1995) or limb vascular occlusion in exercising dogs (Pomeroy *et al.* 1986) or humans (Mitchell *et al.* 1989; Strange *et al.* 1993). However, afferent blockade with epidural lidocaine in the human

undergoing rhythmic (cycling) exercise has most often shown no effect and occasionally even an *increase* in ventilatory and/or heart rate responses, along with a small reduction in MAP (Hornbein *et al.* 1969; Fernandes *et al.* 1990; Strange *et al.* 1993; Kjaer *et al.* 1999; Smith *et al.* 2003; Amann *et al.* 2008).

So, the prevailing opinion was that central command, i.e. feedforward, plays the major, obligatory role in determining the ventilatory and cardioaccelerator responses to rhythmic exercise with limb afferents exerting some influence of significance on the sympathetically mediated increase in blood pressure (Kaufman, 2010). The negative findings in these last-mentioned epidural lidocaine experiments were especially damning to a 'physiological' or 'tonic' role for limb muscle afferents in voluntary, rhythmic exercise because they supposedly prevented the effect of any normally occurring, i.e. tonic, muscle afferent feedback. Apparently, the 'redundant' mechanisms remaining after blockade, i.e. primarily central command, were sufficiently dominant to completely overcome the loss of this feedback mechanism. However, an alternative explanation for these negative results is that epidural lidocaine also has a significant effect on efferent motor output (on the ventral horn of

Respiratory muscle metaboreflex

↑ Sympathetic efferent discharge Limb Vasoconstriction in heavy exercise

↑ Limb fatigue

↓ Performance

↑ Reflex activating metabolites

↑ Group III/IV phrenic afferent discharge

Figure 6

Schematic representation of the proposed respiratory muscle metaboreflex from the diaphragm and expiratory muscles activated by fatiguing contractions of these muscles and eliciting increased sympathetic discharge and limb vasoconstriction in heavy intensity exercise, with consequences to enhancing the rate of development of limb fatigue and reductions in exercise performance (see text). From Dempsey *et al.* (2006).

the spinal cord) in addition to its effects on sensory input (on the dorsal horn), thereby causing muscle weakness (Hornbein *et al.* 1969; Strange *et al.* 1993; Amann *et al.* 2008). In turn, as shown in experiments using partial muscle paralysis (Galbo *et al.* 1987), in order to achieve a given force output in the face of muscle weakness, more motor units must be recruited via augmented central command, thereby rendering this experimental approach inappropriate to determine the effects of afferent blockade, *per se*, on cardioventilatory responses to exercise.

Accordingly, to circumvent this problem we chose to employ intrathecal fentanyl, a μ opioid agonist, as a means of blocking opiate-sensitive limb muscle afferents, an approach which would also spare efferent motor output and muscle strength. Cephalad movement of fentanyl to the CNS is a critical potential confounder but we and others employed several tests to determine that the drug did not move beyond the cervical level following lumbar level injection (Amann *et al.* 2010*a*; Gagnon *et al.* 2012). As summarized in Fig. 7*A* and *B*, at all steady state exercise intensities during cycling, this partial blockade of limb muscle afferents caused significant reductions in breathing frequency and ventilation, heart rate and MAP. (These cardiorespiratory effects of afferent blockade were

originally revealed – serendipitously – as the byproduct of studies with the quite different aim of exploring the influence of muscle afferents on central motor output (Amann *et al.* 2008, 2009).) These afferents appear to mediate a highly significant portion of the normal cardio-ventilatory response as indicated by the observations in steady state exercise that (a) significant hypoventilation (+3 to 8 mmHg mean increases in end-tidal partial pressure of CO_2, P_{ET,CO_2}) persisted in the steady state throughout all exercise intensities despite (presumably) marked systemic and CNS acidosis (and even some hypoxaemia, i.e. minimum S_{aO_2} ~90% at peak work rate) which presented severe levels of central and peripheral chemoreceptor stimulation, and (b) sympathetic efferent outflow remained depressed throughout exercise, as evidenced by a relative hypotension and bradycardia, even in the face of substantial and persistent unloading of the baroreceptors. It also appeared as though the greatest relative effects of afferent blockade on cardioventilatory responses occurred during moderate intensity exercise, as the effect of blockade was diminished near maximal exercise intensities. This may reflect the addition of powerful overriding feedforward and (chemoreceptor) feedback stimuli, commensurate with the onset of heavy intensity, fatiguing exercise. Follow-up studies using
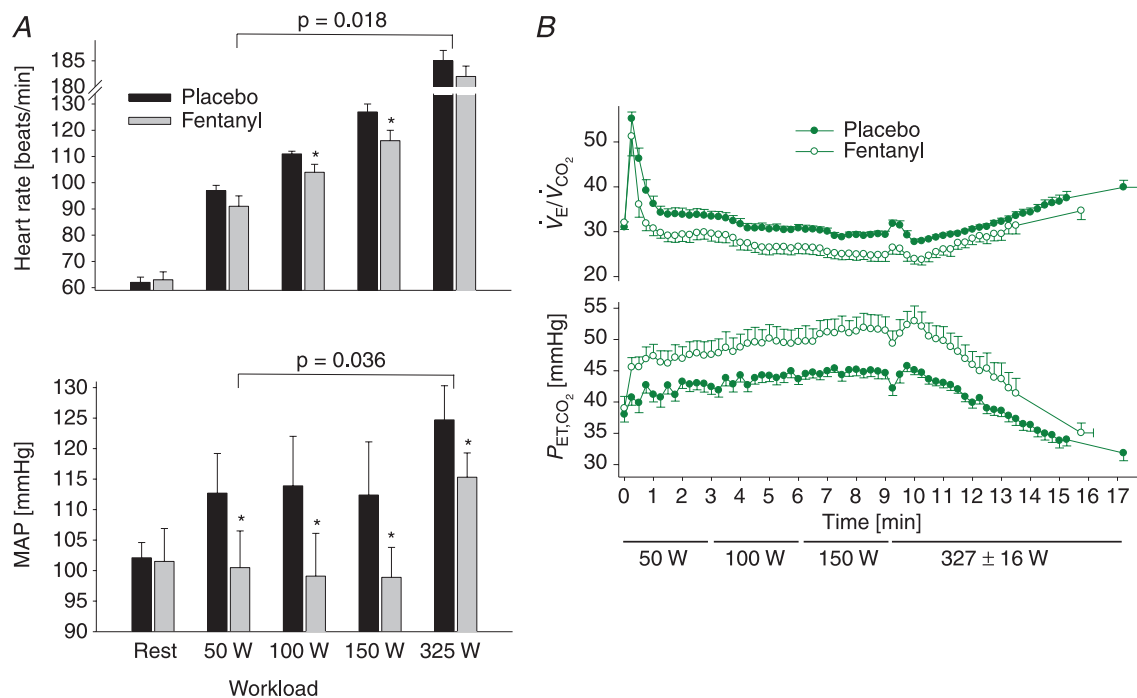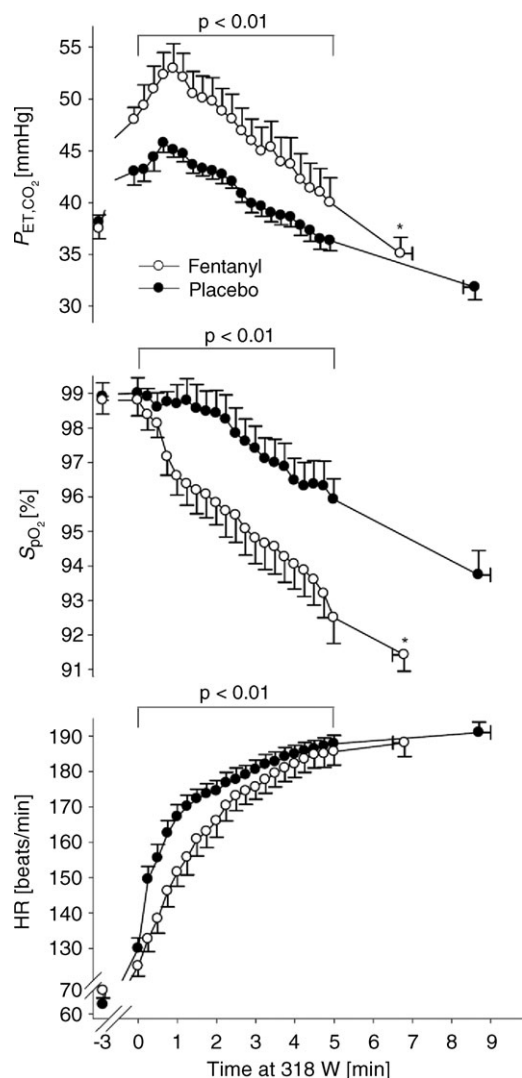

Figure 7
A, group mean effects of blockade of opiate sensitive limb muscle afferents on cardioacceleration and mean arterial blood pressure (MAP) at rest and during the steady state of voluntary rhythmic cycling exercise in healthy humans. Data obtained from Amann *et al*. (2010*a*). *B*, reduced steady state ventilation and CO_2 retention resulting from muscle afferent blockade via intrathecal fentanyl in healthy humans at mild to heavy steady state exercise intensities. Note the persistence of the hypoventilatory response, especially during moderate intensity exercise, despite the presence of increased chemoreceptor stimulation. From Amann *et al.* (2010*a*).

intrathecal fentanyl blockade during low intensity single leg kick exercise also showed significant reductions in cardiac output, and limb blood flow (Amann *et al.* 2011*a*). We emphasize that these observed cardioventilatory effects of blockade probably represent only a *partial* blockade of limb afferents, i.e. of only those pathways sensitive to μ opioid receptors (Hill & Kaufman, 1990).

This demonstration of significant effects of blockade of μ opioid-sensitive muscle afferents across varying intensities of steady-state rhythmic exercise (Fig. 8) confirms our previous findings during the highly variable work rates experienced during time-trial cycling exercise

Figure 8. Effects of afferent blockade from limb locomotor muscles during constant load heavy intensity exercise (80% max)
Note the increase in P_{ET,CO_2} secondary to a reduction in $\dot{V}_E/\dot{V}_{CO_2}$, the accompanying arterial O_2 desaturation and the reduced cardioacceleration accompanying afferent blockade during this heavy intensity exercise. Also note the 21% average reduction in exercise time to exhaustion. S_{pO_2}, pulse arterial blood O_2 saturation; HR, heart rate. Data from Amann *et al.* (2011*b*).

(Amann *et al.* 2009). Our findings are also consistent with the significant impact of cold blockade of group III–IV muscle afferents on the cardiovascular response to rhythmic electrical stimulation of limb muscles in the anaesthetized dog (Tibes, 1977) and with evidence in humans showing that muscle afferent blockade during rhythmic exercise significantly attenuated 'baroreceptor resetting' (Smith *et al.* 2003). However, these rather limited data to date need to be extended to larger numbers of subjects undergoing different intensities and durations of exercise. In such future assessments of the physiological role of muscle afferent feedback it will be important to recognize (a) the importance of the size of the exercising muscle mass utilized (Rowell & O'Leary, 1990), and (b) the potential importance of drug dosage on the effectiveness of afferent blockade, which remains untested and highly variable among the limited published reports to date (Amann *et al.* 2010*a*, 2011*a*, 2011*b*; Gagnon *et al.* 2012).

Interpreting the effects of afferent blockade. Fundamental questions remain regarding the interpretation of these findings of a suppressed cardioventilatory response to rhythmic exercise with blockade of opiate sensitive afferents and also of the strategies used to study the role of limb muscle afferents during physiological exercise. First, our finding that afferent blockade during rhythmic exercise attenuates the cardiorespiratory responses over a wide range of exercise intensities means that III–IV afferents and associated respiratory and/or sympathetic activities may be activated even under conditions where O_2 supply to locomotor muscles is clearly adequate relative to metabolic demand (Pickar *et al.* 1994; Adreani *et al.* 1997). Similarly, in human patients with myophosphorylase deficiency, increased MSNA accompanies isometric contractions even in the absence of muscle acidification (Vissing *et al.* 2001). It has also been documented that group III–IV afferents may be activated via accumulation of multiple muscle metabolites even at very moderate exercise intensities (Light *et al.* 2008) or via increased muscle blood flow and venous distention stimulating group III–IV fibres located in the blood vessel adventitia (Haouzi *et al.* 1999). The importance of venous distension was demonstrated during induced muscle contractions in anaesthetized animals by showing that impeding the circulation from the venous side alone stimulated ventilation, whereas impeding arterial inflow alone depressed ventilation (Haouzi *et al.* 2004). These contrasting effects question the physiological relevance of the common use of 'total' vascular occlusion techniques as a means of testing the effects of exercise-induced metaboreceptor stimulation (see above).

Secondly, our studies in humans do not distinguish between metabo- and mechanoreflex effects. While

discharge of type III mechanoreceptors clearly responds to mechanical distortion of their receptive fields (which usually occurs during the contraction phase of a step cycle), their sensitivity is also heightened by muscle metabolite accumulation (Hayes *et al.* 2006; Kaufman, 2012).

Thirdly, we question whether the cardiorespiratory effects of blocking spinal afferents represent primarily a direct effect on supraspinal input to the nucleus of the solitary tract and medullary cardiorespiratory neurons? . . .or are these predominantly interactive effects of muscle afferents on descending 'central command' at the level of the mid-brain or hypothalamus or even on descending output at the level of the spinal motor neurons (Garland & Kaufman, 1995; Gandevia, 2001). There is evidence, of both a neuroanatomical and functional nature, that significant interactions do indeed occur between muscle afferents and central command and that thin fibre muscle afferents to the medulla are 'gated' by descending inputs from central command. However, whether these inter- active effects are multiplicative or even hypoadditive (Waldrop *et al.* 1986; Degtyarenko & Kaufman, 2002; Plowey *et al.* 2002) has not yet been assessed under conditions of physiological exercise.

In summary, the accumulating evidence suggests that stimulation of cardioventilatory responses to rhythmic exercise via group III–IV afferents may be critically dependent on the presence of increased muscle blood flow, muscular contractions and/or descending influences from central command. Whether any of these mechanisms operates at a gain sufficient to account for a significant portion of the total (intact) cardiorespiratory response needs to be tested under physiological conditions. Given that these potential mechanisms all require an element of the 'exercising' state it may be misleading to use observations obtained at rest during the recovery phase following exercise – with or without vascular occlusion – to evaluate the relative contributions from muscle afferents to exercise-induced cardiorespiratory responses.

Limb afferents, peripheral/central 'fatigue' and exercise performance. Finally, we address the complex question of locomotor muscle afferent influences on endurance exercise performance. We based our approach to this question on the concept (Amann *et al.* 2010*a*, 2011*b*; Gandevia, 2001) (a) that the accumulation of muscle metabolites hastens the development of limb locomotor muscle fatigue during exercise (as determined by changes in supramaximal femoral nerve stimulation, ΔQ_{twitch} measured pre- *vs.* post-exercise), and (b) that these metabolite accumulations provide feedback via muscle afferents which engage sensory systems in the CNS and are responsible in part for the perceptual expression of 'muscle pressure and tiredness' leading to inhibition of

central motor command (Gandevia *et al.* 1996; Light *et al.* 2008; Taylor & Gandevia, 2008). That muscle afferents do indeed inhibit central motor output was clearly shown during a time trial cycling exercise in which experienced competitive cyclists in the presence of muscle afferent blockade voluntarily 'chose' much higher force outputs (*vs.* placebo) over the initial half of a time trial. This 'strategy', chosen in the absence of feedback, led to extreme limb fatigue developed over the second half of the trial as manifested via excessive quadriceps fatigue (i.e. increased ΔQ_{twitch}) at end-exercise (Amann *et al.* 2009).

We have now tested the effects of group III–IV muscle afferent blockade during constant load exercise to exhaustion at 85% $\dot{V}_{O_2max}$ and found that all cyclists reduced their performance time, an average of $21 \pm 4\%$ (Amann *et al.* 2011*b*). We believe this reduction in performance represents the net effect of two opposing influences of muscle afferents. On the one hand O_2 trans- port was reduced with blockade (*vs.* placebo) secondary to hypoventilation, arterial hypoxaemia and reduced heart rate and perfusion pressure (Amann *et al.* 2010*a*; see Fig. 8). On the other hand, central motor command was increased in the absence of afferent feedback, as indicated indirectly by the greater increase over time in quadriceps EMG during the constant load exercise test (see Fig. 9). This led to a 30% increase >placebo in limb fatigue (ΔQ_{twitch}) as measured at end-exercise even though the exercise duration was shorter in the blocked state *vs.* placebo. (The significance of this excessive fatigue at end-exercise is noted by the extreme difficulty subjects experienced with post-exercise ambulation.)

Our interpretation of this reduced performance plus the excessive muscle fatigue development at end-exercise with feedback blockade is as follows. First, the reduced O_2 transport induced by blockade (see Fig. 8) caused a sub- stantially faster rate of development of muscle fatigue at 80% of maximum exercise. Secondly, when an enhanced rate of peripheral fatigue development was previously produced experimentally in subjects with *intact feed- back* (by reducing the inspired O_2 fraction (F_{IO_2})) or causing pre-exercise specific limb fatigue; Amann *et al.* 2006) the exercise time was also shortened, but, unlike that in the subjects with limb afferent blockade, the end-exercise ΔQ_{twitch} was identical to that under control conditions. These correlative data point to the role of feed- back inhibition (in the intact subject) of central motor output in order to limit exercise duration, thereby pre- venting 'excessive' peripheral muscle fatigue. On the other hand, with afferent blockade and the absence of feedback inhibition from the limbs, the cyclist apparently 'pushes' himself to exercise for a longer duration, i.e. beyond the 'threshold' level of peripheral fatigue he usually achieves (when intact) at end-exercise.

This evidence supports the postulate that limb muscle afferents contribute to essential determinants of exercise

performance by (a) stimulating optimal cardioventilatory responses to preserve O_2 transport to working muscles, and (b) providing feedback of the metabolic status of the locomotor muscles to influence central motor command. We caution that we do not understand the mechanisms or even the specific sites of action in the higher CNS which underlie these afferent links between accumulation of muscle metabolites, afferent feedback and inhibition of central motor command (Gandevia, 2001). Nor do we intend to imply that a host of other influences – originating systemically or in the higher CNS – do not also provide important sources of limitation to exercise performance. Of course they do! (Amann & Secher, 2010; Marcora, 2010.)

Locomotor *vs*. respiratory muscle metaboreflexes. Finally, it is of interest to contrast the consequences of the respiratory *vs*. limb muscle afferents on O_2 transport and exercise performance. First, it appears as though they may have quite different thresholds of activation because activation of the respiratory muscle afferents and their cardiovascular sequelae (in health) appear to require sustained, heavy intensity, near-fatiguing contractions of the inspiratory and/or expiratory muscles whereas limb muscle afferents appear to be activated along with their cardiorespiratory sequelae during even moderate intensity rhythmic exercise. Furthermore, activating the respiratory muscle afferents precipitates sympathetic vasoconstriction and a selective effect which reduces blood flow and therefore O_2 transport to working limb locomotor muscles while local vasodilatation and increased blood flow (apparently) occurs in the respiratory musculature. So the fatiguing diaphragm presumably benefits from activation of its own metaboreflex but vasoconstricted limb muscles do not; the net effect is exacerbation of the rate of limb fatigue development leading to inhibition of central motor command, thereby reducing endurance exercise performance. On the other hand, in the healthy intact subject the cardiorespiratory consequences of activation of limb muscle afferents *promotes* increased systemic O_2 transport to meet metabolic requirements,

thereby delaying the development of limb fatigue and preserving exercise performance.

These positive effects of limb muscle afferents in health may not apply to chronic disease states such as CHF and COPD. These extremely sedentary patients have highly untrained, fatiguable locomotor muscles with reduced aerobic capacity (Maltais *et al.* 2000; Amann *et al.* 2010*b*). These conditions are likely to promote a highly sensitive muscle metaboreflex which in turn would contribute to abnormally high levels of tachypnoeic ventilation and ventilatory work as well as exacerbation of sympathetic vasoconstrictor activity during even low intensity exercise. Accordingly, Gagnon *et al.* (2012) have recently demonstrated that intrathecal fentanyl blockade in (non-hypoxaemic) COPD patients reduced their exercise-induced tachycardia and MAP, as well as their tachypnoea and dead space ventilation. As a result, hyperinflation and dyspnoea were reduced as were perceptions of limb fatigue in the presence of sensory blockade (*vs*. placebo) when comparisons were made at equal exercise time points. These effects of muscle afferent blockade in COPD patients produced substantial *improvements* in constant load exercise time to exhaustion and an increase in the magnitude of quadriceps fatigue at end-exercise.

Summary

Accumulating evidence in recent years reveals significant, complementary roles for three types of feedback mechanisms in controlling the cardiorespiratory responses to voluntary, rhythmic exercise in the intact human. We propose the following hypothesis.

- *Locomotor muscle afferents* provide an essential contribution to cardioacceleration, sympathetically mediated vasoconstriction and to hyperpnoea and arterial blood gas homeostasis over most exercise intensities.
- *Respiratory muscle metaboreflexes* activate sympathetically mediated, selective vasoconstriction of
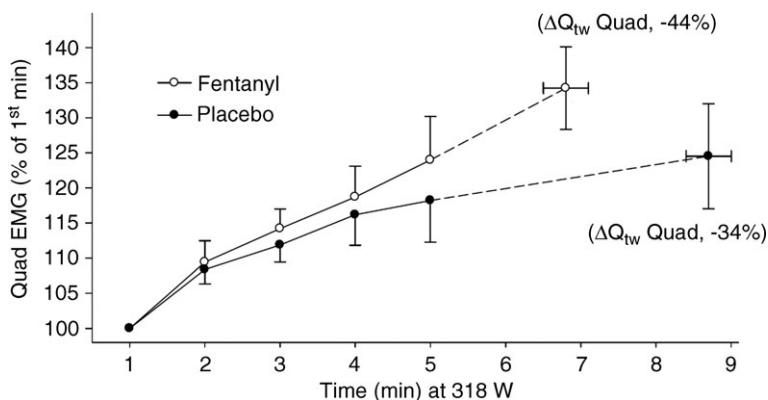
Figure 9. Effects of afferent blockade on an indirect estimate of 'central motor command' (quadriceps EMG) during prolonged heavy exercise to exhaustion
Note the increased EMG during muscle afferent blockade (*vs*. placebo) during the later stages of the exercise test. Also note the 25% greater limb fatigue (as measured by ΔQ_{twitch}) pre- to post-exercise, despite the shorter exercise time in the presence of locomotor muscle afferent blockade. Data from Amann *et al.* (2011*b*).

locomotor muscle vasculature, thereby contributing to blood flow redistribution between respiratory and locomotor muscles during heavy intensity exercise. The exact balance achieved in flow distribution between these two vascular beds (in healthy subjects) remains unknown.

- *Carotid chemoreceptors* contribute significantly to tonic levels of sympathetically mediated vasoconstriction and locomotor muscle blood flow during exercise of all intensities.

The exact stimuli responsible for activating limb muscle afferents or carotid chemoreceptors during mild to moderate intensities of rhythmic exercise remain unknown although there are some promising candidates. Further, the relative contribution of each of these reflex mechanisms to the total cardiorespiratory response and their interactive effects at the level of the central nervous system and/or spinal cord with ongoing central command mechanisms remains to be determined.

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