Effects of Salbutamol on Potassium and Exercise Performance

By

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ABSTRACT

Salbutamol is a β_2 adrenergic agonist widely used for the treatment of asthma and known to reduce plasma potassium concentration [K^+]. The International Olympic Committee had been concerned that salbutamol may give an inequitable advantage to athletes. However, little work has investigated whether salbutamol has beneficial effects on plasma [K^+], K^+ dynamics during exercise and how these potential changes may impact on exercise performance. This study investigated the effects of salbutamol on K^+ regulation at rest, during and following submaximal and high intensity continuous exercise, as well as during and following repeat sprint exercise. The impact on performance during repeat sprint exercise was also explored.

Seven healthy, recreationally-active men (age 23.29 ± 5.96 yr, height 178.03 ± 9.06 cm, body mass 72.23 ± 8.47 kg, mean ±SD) participated in the study which utilised a double-blind, randomised crossover design, with two trials separated by two weeks. Participants received either 1000 µg of salbutamol or a placebo via inhalation, 30 min prior to performing continuous cycle ergometer exercise comprising 5 min at 40% $\dot{V}O_{2peak}$, 5 min at 60% $\dot{V}O_{2peak}$ and then 90 seconds at 130% $\dot{V}O_{2peak}$. Then, after 20 min of recovery, participants performed repeat sprint exercise comprising three sets of five x 4 s sprints on a cycle ergometer, followed by a final 30 min of recovery. Radial arterial (a) and antecubital venous (v) blood samples were drawn throughout rest, during exercise and recovery and analysed for plasma [K^+], as well as other electrolytes and fluid shifts.

Salbutamol lowered arterial plasma [K^+] ($[K^+]_a$) during the initial rest period by ~0.35 mM at 20 min ($P < 0.001$) with no change in the placebo trial. Plasma $[K^+]_a$ increased

during continuous exercise, reaching ~6 mM during exercise at 130% $\dot{V}O_{2peak}$ ($P < 0.05$, compared to resting baseline); at 19 min of recovery plasma $[K^+]_a$ had returned to baseline values, with no significant differences between salbutamol and placebo trails. During repeat sprint, exercise plasma $[K^+]_a$ increased above baseline to ~5 mM during each set and decreased to below baseline during the 4.5 min of recovery after each set; plasma $[K^+]_a$ was lower than baseline with salbutamol after sets 1 and 2 ($P < 0.05$). Plasma $[K^+]_a$ returned to baseline within 1 min after repeat sprint exercise during both salbutamol and placebo trials. Plasma $[K^+]_a$ then decreased to below baseline at each of 2, 5, 10 and 30 min of recovery following salbutamol ($P < 0.05$) but did not differ from baseline after placebo.

Due to small differences in the baseline plasma $[K^+]_a$ between salbutamol and placebo trials, the change in plasma $[K^+]_a$ from baseline ($\Delta[K^+]_a$) was calculated for each of the salbutamol and placebo trials. During the initial rest period, $\Delta[K^+]_a$ was more negative at 20 min after salbutamol compared to placebo (-0.35 ± 0.32 vs -0.03 ± 0.06 mM, respectively, $P < 0.05$). The $\Delta[K^+]_a$ following salbutamol was smaller (less positive) during continuous exercise at 40% $\dot{V}O_{2peak}$ and at 130 $\dot{V}O_{2peak}$ compared to placebo (0.38 ± 0.17 vs 0.71 ± 0.39 mM, and 1.77 ± 0.77 vs 2.24 ± 0. mM, respectively, $P < 0.05$). The $\Delta[K^+]_a$ did not differ between salbutamol and placebo trials during repeat sprint exercise. The $\Delta[K^+]_a$ declined to below baseline at 2, 5, 10 and 30 min recovery following salbutamol ($P < 0.05$), whereas no difference was found with placebo. No differences in $\Delta[K^+]_a$ were found between salbutamol and placebo during repeat sprint exercise or during 30 min of recovery. The $\Delta[K^+]_a$ was also corrected for decline in plasma volume ($\Delta[K^+]_{a,corr}$). This analysis revealed $\Delta[K^+]_{a,corr}$ was more negative following salbutamol compared to placebo at 10, 20 and 30 min of rest, during

continuous exercise at 40% $\dot{V}O_{2peak}$ and at 60% $\dot{V}O_{2peak}$, as well as at 20 and 30 min of recovery ($P < 0.01$).

In contrast to $[K^+]_{a,}$ no differences in $[K^+]_v$ were found between salbutamol compared with placebo, during rest, continuous exercise, repeat sprint exercise or subsequent recovery. Despite these changes, salbutamol did not improve exercise performance during repeat sprint exercise, with no differences in work output found between salbutamol and placebo trials.

In conclusion, inhaled salbutamol decreased arterial plasma $[K^+]$ at rest, during continuous exercise and during recovery post-exercise, with these effects most evident after correcting for differences in baseline values and for fluid shifts. However, earlier lowering of $[K^+]_a$ had no effect on performance during repeat sprint exercise. The result from this study suggested that inhaled salbutamol maintain to be permitted in sport competition.

STUDENT DECLARATION

"I, ***Muath Altarawneh,*** declare that the Master by research thesis entitled, ***Effects of Salbutamol on Potassium and exercise performance*** is no more than 60.000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work".

Signature *Muath* Date 15/8/2013

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Lastly but most importantly, I dedicate this thesis to my dear deceased father, whom I deeply miss.

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LIST OF ABBREVIATIONS

K^+	Potassium ion
$[K^+]$	Potassium concentration
$[K^+]_a$	Arterial potassium concentration
$[K^+]_v$	Venous potassium concentration
$\Delta[K^+]$	change in potassium concentration
$\Delta[K^+]_{corr}$	change in potassium concentration corrected for fluid shifts
Na^+	Sodium ion
Lac^-	Lactate
$\dot{V}O_{2peak}$	peak oxygen consumption
Na^+, K^+-ATPase	Sodium-potassium Adenosine Triphosphatase
µg	Microgram
mM	$mmol.l^{-1}$

CHAPTER 1. INTRODUCTION

Potassium (K^+) is the most profuse cation in the body (Rastergar and Soleimani, 2001) and is essential for normal cellular function, including in the brain, nerves, kidney and skeletal muscle (Giebisch, 1998). The total body K^+ content in human adults ranges from 2,000 to in excess of 4,000 mmol, depending on body mass, with around 2% of total K^+ located in the extracellular space and about 98% intracellularly (Sejersted and Sjogaard, 2000; Giebisch, 1998). Skeletal muscles have the largest K^+ store in the body and thus play a key role in K^+ regulation (Sejersted and Sjogaard, 2000; Giebisch, 1998).

In response to muscular contraction, K^+ shifts from the intracellular to the extracellular space in contracting skeletal muscle and then diffuses into capillaries (Nielsen et al., 2004). Circulating potassium is extracted by resting muscle, thereby minimising the increase in plasma K^+ concentration ($[K^+]$) during exercise (Sejersted and Sjogaard, 2000). The plasma $[K^+]$ during exercise is related to the balance between the rate of release from and uptake of K^+ into skeletal muscle and other tissues (Lo et al., 2004; Clausen, 2003). Accumulation of potassium in muscle interstitium during exercise is argued to contribute substantially to muscle fatigue (Nielsen et al., 2004; Bangsbo et al., 1996). Hence, regulation of the intracellular and extracellular $[K^+]$ in muscle during exercise is essential for maintenance of muscle function and minimisation of fatigue (McKenna et al., 1996). In skeletal muscle, the Na^+, K^+- pump plays a key role in K^+ regulation and thus also cellular excitability and muscle function. Numerous hormones can modulate plasma $[K^+]$ via stimulation of the Na^+, K^+- pump (Juel et al., 2000b; Clausen, 2003) and therefore also have the potential to affect muscle function and

exercise performance. Specifically, β_2 adrenergic agonists can lower plasma [K^+] (Bia et al., 1986; Clausen, 2008a).

Salbutamol, a β_2 adrenergic agonist, is a common drug used to treat for symptoms of asthma attacks (Sears and Lötvall, 2005), due to its ability to stimulate the Na^+, K^+ -pump (Murdoch et al., 1991) and thus to relax smooth muscle and open airways (Shore and Drazen, 2003). The International Olympic Committee had been concerned that salbutamol may give an inequitable advantage to athletes. According to the World Anti-Doping Agency (WADA) Prohibited List 2011, salbutamol is allowed to be used as a legal treatment, if administered by inhalation and at a dose of not more than 1000 ng/mL and not exceeding 1600 μg over a 24 h period. Some studies have reported an improvement in exercise performance after salbutamol (Le Panse et al., 2007, Collomp et al., 2000b), whilst others have not (Goulbault et.al 2001, Arlettaz et al., 2009). However, little work has investigated whether salbutamol has beneficial effects on plasma [K^+], K^+ dynamics during exercise and how these potential changes may impact on exercise performance. Therefore this thesis investigates the effects of salbutamol via inhalation as most commonly used drugs, on arterial and venous [K^+] during continuous incremental exercise and repeated-sprint exercise, as well as on performance during repeated-sprint exercise.

CHAPTER 2. LITERATURE REVIEW

This literature review focuses on K^+ regulation during exercise, including the role of the Na^+, K^+-pump and examines the role of β_2 adrenergic agonists on plasma [K^+] regulation and also on exercise performance.

2.1 Regulation of K^+ in skeletal muscle: Na^+, K^+-ATPase

2.1.1 Overview of Na^+, K^+-ATPase

A key protein involved in cellular and systemic K^+ regulation is the sodium-potassium adenosine triphosphatase (Na^+, K^+-ATPase; sodium pump; Na^+, K^+-pump. EC 3.6.1.37). In vivo activity of the Na^+, K^+-pump is critical in controlling K^+ balance. Furthermore the Na^+, K^+-pump is a key target for salbutamol. Hence the structure and function of Na^+, K^+-pump is reviewed briefly here. The Na^+, K^+-pump is a ubiquitous membrane-associated protein complex that is expressed in most eukaryotic cells (Vague et al., 2004). The Na^+, K^+-pump was first discovered in 1957 by Jens Skou (Skou, 1957). The Na^+, K^+-pump maintains transcellular gradients for Na^+ and K^+, which play an important role in maintaining membrane potential of the cell (Jorgensen et al., 2003) and thus are essential to cellular membrane excitability as well as Ca^{++} and H^+ movements maintenance of osmotic balance, cell volume (Lingrel, 1992) and regulation of intracellular pH (Geering, 1990). The Na^+, K^+-pump is found in most tissues and especially including the brain, skeletal muscle and cardiac muscle (Mobasheri et al., 2000); in skeletal muscle, the Na^+, K^+-pumps are thought to be primarily localized in the sarcolemma and the T- tubules (Clausen, 2003).

2.1.2 Function of the Na^+, K^+-ATPase

The major function of the Na^+, K^+-pump is producing and maintaining steep transmembrane Na^+ and K^+ concentration gradients; it stimulates cellular uptake of K^+ and extrusion of Na^+ via hydrolyzing ATP generated from cellular glycolysis (Mobasheri et al., 2000). The Na^+, K^+-pump exchanges three Na^+ ions for two K^+ ions across the plasma membrane during each cycle of ATP hydrolysis (Floyd et al., 2010, Morth et al., 2007, Kaplan, 2005). The exchange of three Na^+ ions for two K^+ ions is electrogenic and thus Na^+, K^+-pump activity contributes to membrane potential hyperpolarization which is essential for muscle excitability (Clausen, 1996).

During muscle contraction, each action potential comprises an influx of Na^+ into muscle cells and efflux of K^+ from the cells, allowing immediate activation of muscle contraction. Repeated action potentials may therefore result in a large increase in extracellular [K^+] (Figure 2.1) (Clausen, 2010). The intracellular K^+ concentration ([K^+]) is maintained at around 120 – 140 mM by active K^+ pumping from the extracellular environment where the [K^+] concentration is around 5 mM. Through extrusion of Na^+, the Na^+, K^+-pump constrains the rise in intracellular [Na^+] that would otherwise occur with repeated action potentials (contraction). Thus the Na^+, K^+-pump maintains a low intracellular [Na^+]-to-[K^+] ratio in face of an inward concentration gradient for Na^+ and an outward gradient for K^+ and fluxes due to each action potential (Mobasheri et al., 2000).

2.1.3 Structure of the Na^+, K^+-ATPase

The Na^+, K^+-pump comprises a subgroup of oligomeric enzymes comprising α and β subunits (Kaplan, 2002, Scheiner Bobis, 2002) both of which are required for Na^+, K^+-pump function. A third γ subunit has also been identified in some tissues and in skeletal

muscle is identified as phospholemman (Figure 2.2) (Scheiner Bobis, 2002, McDonough et al., 2002, Morth et al., 2007, Floyd et al., 2010).

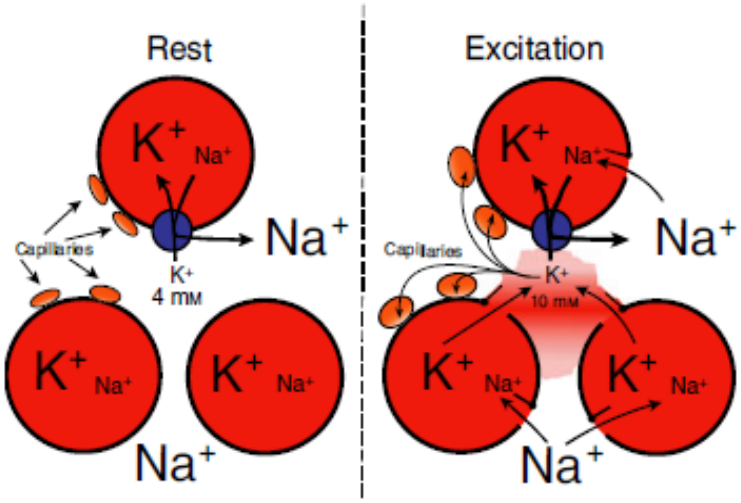

Figure 2.1 Na^+,K^+ exchange in resting and working muscle. Excitation is triggered by a rapid influx of Na^+ via the Na^+ channels, immediately followed by an almost equivalent efflux of K^+ via K^+ channels. This leads to an increase in extracellular K^+ depolarization of the muscle cells, loss of excitability and muscle fatigue. K^+ is cleared by diffusion into the surrounding interstitial water space and into the capillaries, which concomitantly undergo a dilatation, further favouring K^+ clearance. K^+ is also cleared by reaccumulation into the muscle cells via the Na^+, K^+ pumps. From (Clausen, 2010).

The α subunit spans the membrane 10 times forming trans-membrane domains (M1 to M10); both N- and C-termini are localized on the cytosolic side (Scheiner Bobis, 2002). The α subunit is composed of about 1000 amino acids (Kaplan, 2002) and exhibits a molecular mass of 110 to 113 kDa (Mobasheri et al., 2000, Kaplan, 2002, Floyd et al., 2010) which depends on the presence of different isoforms, referred to as α_1, α_2, α_3, or

α_4 (Scheiner Bobis, 2002). The α subunit is responsible for catalytic processes of the enzyme, and also for hydrolysis of ATP, and binding and transport of Na^+ and K^+ ions across the membrane.

The β subunit spans the membrane once and the N-terminus is localized on the intracellular side of the membrane (Scheiner Bobis, 2002), it is composed of about 370 amino acids (Kaplan, 2002). The β subunit is glycosylated and exhibits a molecular mass of about 40 to 60 kDa (Mobasheri et al., 2000, Kaplan, 2002, Scheiner Bobis, 2002, Floyd et al., 2010), which depends on the presence of different isoforms β_1, β_2 or β_3 (Scheiner Bobis, 2002). This subunit has regulatory effects on Na^+, K^+- pump activity and is necessary for functional maturation and is responsible for transporting the α subunit to the plasma membrane. Each of the Na^+, K^+- pump α_1, α_2, α_3, β_1, β_2, and β_3 isozymes are found in skeletal muscle, depending on fibre type and species.

The γ subunit has a relative mass of 7-11 kDa (Scheiner Bobis, 2002) and with 30 amino acids (Morth et al., 2007), and crosses the membrane only once (Mobasheri et al., 2000). The γ subunit also exerts a regulatory effect on Na^+, K^+- pump activity.

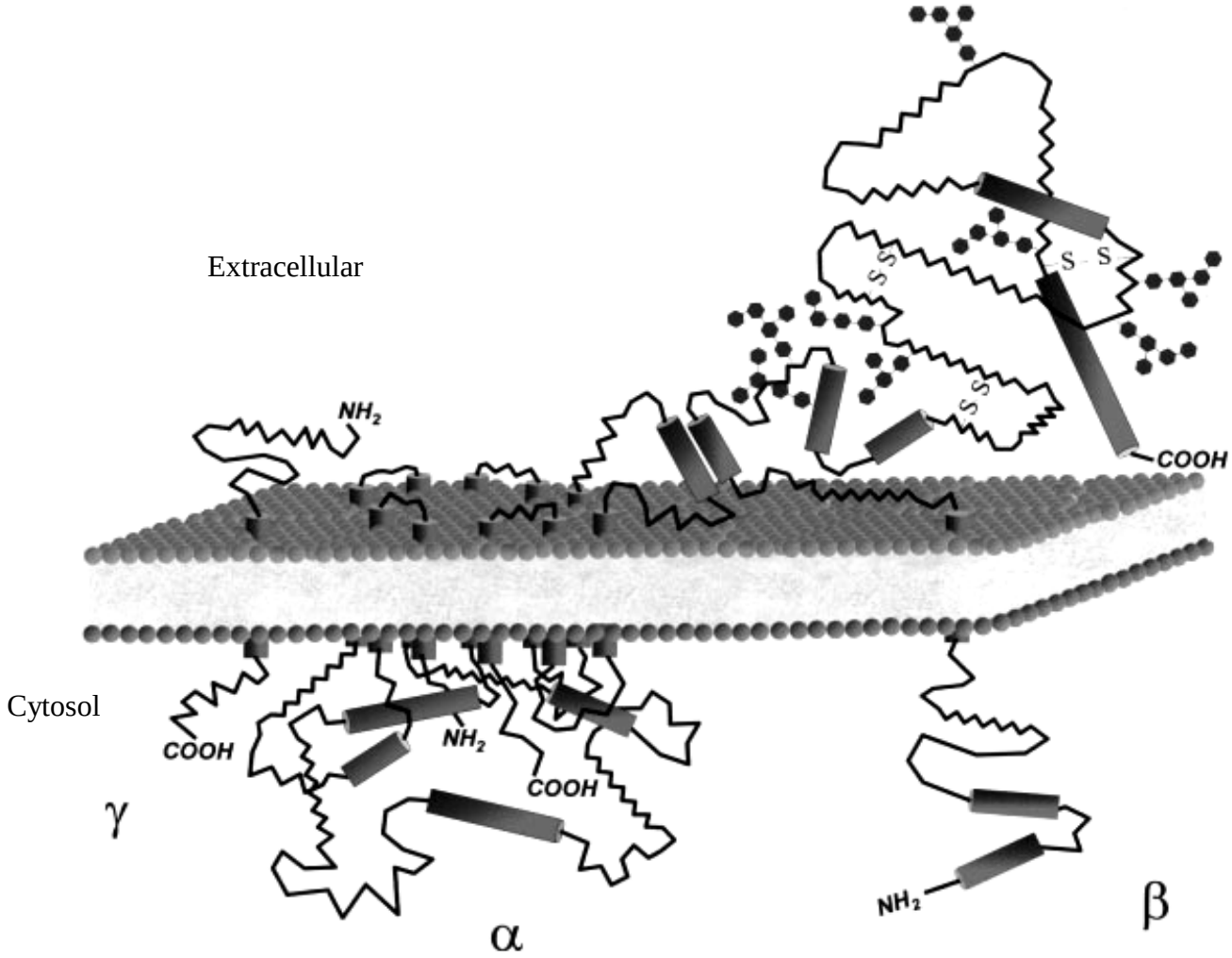

Figure 2.2 Putative three-dimensional model of the topological structure of the Na^+, K^+-ATPase. This model of the Na^+, K^+-ATPase depicts the catalytic α subunit spanning the plasma membrane 10 times and places both the NH_2 and COOH termini in the cytosol. The β subunit spans the plasma membrane once and its NH_2 terminus is in the cytosol. The β subunit contains 3 disulfide bridges (S-S) and may have three to eight potential sites for *N*-linked glycosylation (depending on the specific isoform). Areas with predicted α-helices and β-pleated sheets are shown as cylinders and zig-zags respectively. The γ subunit also spans the plasma membrane once and its COOH terminus is in the cytosol. From (Mobasheri et al., 2000).

2.1.4 Effects of exercise on the Na^+, K^+-pump

Muscle contraction causes an increase in the activity of Na^+, K^+-pump (Tsakiridis et al., 1996, Nielsen and Harrison, 1998; Clausen, 2008b). This may be in part due to an increased abundance of Na^+, K^+-pumps in the muscle plasma membrane. In rats the abundance of the Na^+, K^+ -pump α_2 subunit and of cavelolin-3 in the muscle plasma membrane increased by 36% and 19%, respectively during treadmill running (Kristensen et al., 2008). During 1 h low-intensity treadmill running in rats, the abundance of each of the α_1, α_2, β_1, and β_2 subunits abundance were increased by 19 – 32% in membranes from oxidative muscle fibers, whilst the α_1, α_2, and β_2 abundrce rose by 13 – 25 % in membranes from glycolytic muscle fibers (Juel et al., 2001). The skeletal muscle plasma membrane abundance of the catalytic α_1 and α_2 subunits of the Na^+, K^+ -pump in both red-type I/IIa and white-type IIa/IIb muscle increased after 1 h of treadmill running in rats (Tsakiridis et al., 1996).

Several studies have investigated exercise-induced changes in Na^+–K^+-ATPase subunit isoform protein abundance, using Western blot analyses and in Na^+, K^+-ATPase content using muscle [^{3}H] ouabain binding site analyses. In humans, during 16 hours of heavy intermittent cycling exercise, the muscle Na^+, K^+-pump α_2 abundance increased by 26% and α_3 by 29% (Green et al., 2007). One-legged knee extensor exercise increased the vesicular content of α_2 by 70 % and β_1 by 26% (Juel et al., 2000a). Prolonged submaximal exercise elevated α_3 and β_2 mRNA by 2.2- and 1.9-fold, respectively but did not elevate pump isoform protein abundance (Murphy et al., 2006). Training increases the Na^+, K^+-pump content in skeletal muscle. Sprint training led to elevated muscle [^{3}H] ouabain binding site content (McKenna et al., 1993), whilst endurance training also increased Na^+, K^+-pump concentration by 15% (Madsen et al., 1994). Sprint training increased the muscle Na^+, K^+-pump α_1 subunit by ~ 30% (Iaia et al.,

2008). Intense exercise decreased maximal in-vitro Na^+, K^+-pump (3-O-MFPase) activity and this was suggested to contribute to muscular fatigue (Fraser et al., 2002; Petersen et al., 2005). The Na^+, K^+-pump contributes to the control of K^+ homeostasis thus may be an important determinant of fatigue during short term exhaustive and repeated high-intensity exercise (Iaia et al., 2008).

2.2 K^+ dynamics during exercise and muscle contraction

2.2.1 Effects of exercise on muscle intracellular [K^+]

Intracellular [K^+] ([K^+]i) decreases rapidly during muscular activity. In humans muscle $[K^+]_i$ fell from 161 to 141 mM during cycling exercise at 120% $\dot{V}O_{2max}$ on a cycle ergometer (Sjøgaard, 1983) and from 165 at rest to 129 mM during one-legged dynamic knee-extension exercise to exhaustion (Sjøgaard et al., 1985). In animal models, $[K^+]_i$ decreased from 168 mM to 32 mM in mouse soleus muscle and from 182 mM to 48 mM in extensor digitorum longus (EDL) muscle during 40 Hz stimulation (Juel, 1986), whilst in frog muscle fibres, $[K^+]_i$ declined with stimulation from ~ 142 to ~ 97 mM (Balog and Fitts, 1996).

2.2.2 Effects of exercise on muscle interstitial [K^+]

Muscle interstitial [K^+]($[K^+]_I$) increases markedly with increasing exercise intensity (Nielsen et al., 2004) and the [K^+] gradient between skeletal muscle interstitial and plasma spaces rose from 1 to 6 mM as exercise intensity increased (Green et al., 2000). During one-legged knee extension exercise, muscle $[K^+]_I$ was higher at 60% compared to 30% of maximal work capacity (Lott et al., 2001). During one-legged knee-extensor exercise, muscle $[K^+]_I$ rose to 9 mM with increasing power outputs (Figure 2.3) (Juel et

al., 2000b) and in another study increased up to ~11 mM (Mohr et al., 2004). Muscle $[K^+]_I$ was found to increase from 5.3 to 10 mM (Green et al., 2000a) and reached ~11 mM during higher intensity exhaustive exercise (Nordsborg et al., 2003, Mohr et al., 2004). Increases in $[K^+]_I$ to as high as 15 mM have been reported during intense exhaustive exercise (Nielsen et al., 2004, Green et al., 2000a, Juel et al., 2000b). This increased $[K^+]_I$, together with lowered $[K^+]_i$, mean that the $[K^+]_i$ / $[K^+]_I$ ratio must be drastically reduced during exercise.

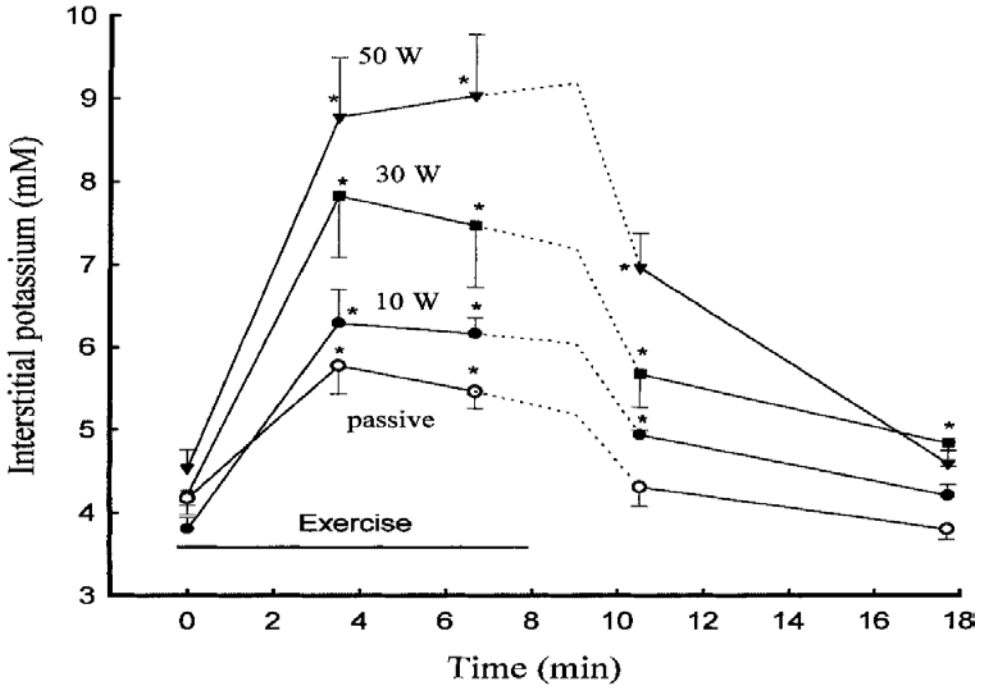

Figure 2.3 Muscle interstitial K_I during passive movements, prior to exercise and during exercise at power outputs of 10, 30 and 50 W. From (Juel et al., 2000b).

2.2.3 Effects of exercise on plasma [K^+]

Plasma potassium is substantially elevated during exercise, depending on the type, duration and intensity of exercise; typically, exercise utilising a large muscle mass and high intensity induce a large rise in circulating potassium. Arterial plasma [K^+] increases rapidly with increasing exercise intensity (Vollestad et al., 1994; Tenan et al., 2010). During an isometric contraction of quadriceps muscle sustained for 3 min, arterial plasma [K^+] ($[K^+]_a$) increased from 4.1 to 5 mM whilst femoral venous plasma [K^+] increased to 5.9 mM (Figure 2.4) (West et al., 1996). During a set of four 30 s sprints on a cycle ergometer, arterialised forearm venous [K^+] increased from 4.7 to 6.5 mM (McKenna *et al.*, 1993). During high-intensity, intermittent exercise venous plasma [K^+] reached peak values of 6.2 mM (Hargreaves et al., 1998). During long duration exercise such as a 100 km run, venous plasma [K^+] increased to a lesser extent from 4 to 5 mM (Overgaard et al., 2002).

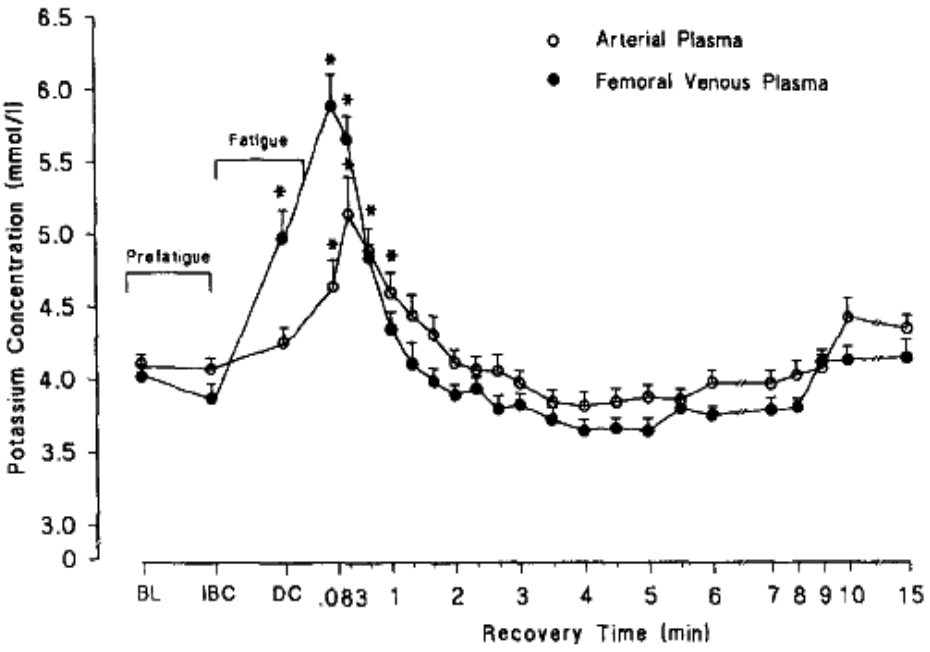

Figure 2.4 Arterial and femoral venous plasma during 3 min isometric exercise. From (West et al., 1996).

Some studies have been shown plasma [K^+] increases up to 8 mM during exercise. During 3 min of high intensity knee extensor exhaustive exercise, plasma $[K^+]_a$ rose to 8 mM (Juel et al., 1990). During high intensity, 1 min exhaustive treadmill exercise plasma $[K^+]_a$ increased to around 8 mM in arterial and femoral venous blood (Figure 2.5) (Medbo and Sejersted, 1990). Arterial plasma [K^+] increased to 8.5 mM during high intensity cycling exercise at 120 % $\dot{V}O_{2max}$ (Vøllestad et al., 1994).

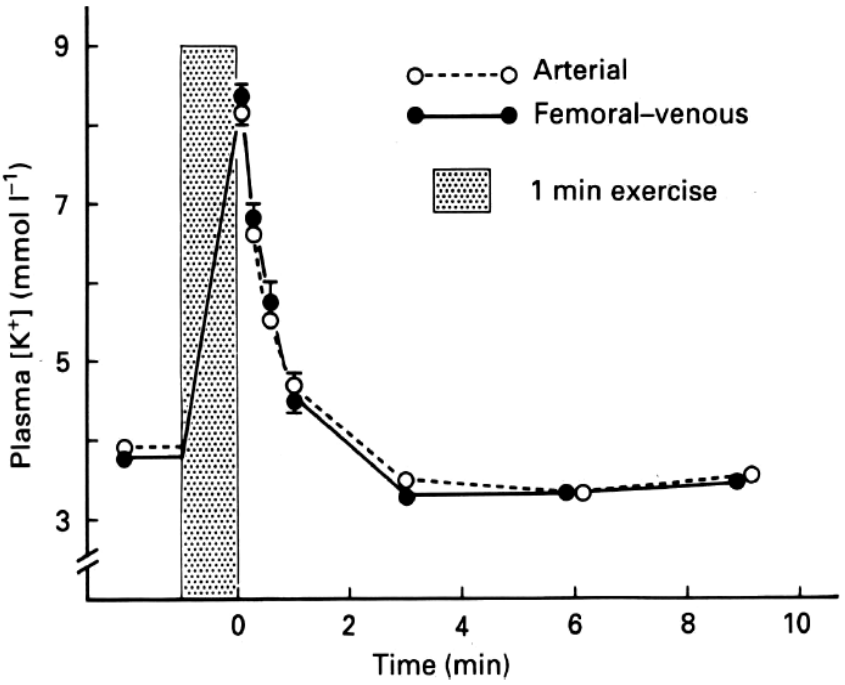

Figure 2.5 Arterial and femoral-venous plasma potassium concentration before and after 1 min exhausting treadmill exercise (Medbo and Sejersted, 1990).

Further, (Zoladz et al., 2002) found that venous plasma [K^+] was higher during incremental cycling exercise at 120 rev.min^1 compared to cycling at 60 rev.min^1 (Figure 2.6). During submaximal cycling exercise, arterialised venous [K^+] rose from 3.9 to 4.3 mM during knee extensor exercise at 40 % maximal work output (Petersen et al., 2005).

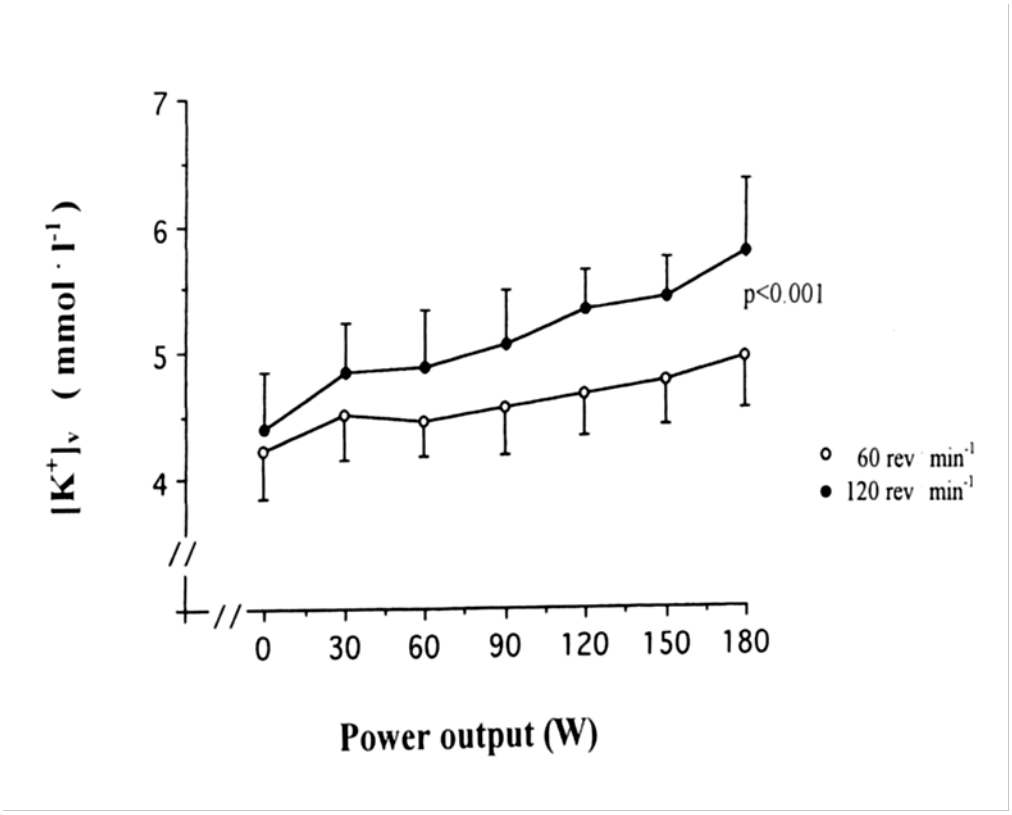

Figure 2.6 Venous plasma [K^+] during cycling exercise at moderate and high cadence (Zoladz et al., 2002).

2.3 Role of [K^+] in muscle fatigue

2.3.1 Overview of mechanisms of fatigue

Muscle fatigue has been defined as a transient and recoverable decline in muscle force and/or power with repeated or continuous muscle contractions (McKenna et al., 2008, Kent-Braun et al., 2002). The mechanisms of muscle fatigue are complex and

controversial. Causes of muscle fatigue are likely multifactorial and may depend on muscle fibre type, size of muscle, type of exercise, exercise intensity and environmental factors. Furthermore, fatigue can be defined as having two components, one associated with a failure within the central nervous system (CNS), where the CNS is unable to sufficiently activate all of the motor neurons during exercise, even when muscles are still able to perform; and fatigue due to failure within the active muscles themselves (Fitts, 1994a; Hargreaves, 2008; Ferreira and Reid, 2008). Fatigue may arise during muscular contractions due to failure at one or more sites along the pathway of force production from the central nervous system to the contractile apparatus (Kent-Braun et al., 2002).

The vast literature on muscle fatigue is beyond the scope of this thesis. Rather this review will focus briefly on the potential aspect of muscle excitability and the potential roles of K^+ and the Na^+, K^+- pump in muscle fatigue.

2.3.2 Potassium [K^+] and relationship to fatigue

K^+ shifts from the intracellular space during high intensity exercise lead to K^+ accumulating in muscle interstitium, which likely plays a role in the development of muscle fatigue (Mohr et al., 2004, Sejersted and Sjogaard, 2000). The rise of extracellular [K^+] close to the sarcolemma and within t-tubules during muscle contraction may cause fatigue. The proposed link between the [K^+] and fatigue is that the rise in $[K^+]_I$ caused by muscle contraction as a consequence of membrane depolarisation inactivates Na^+ channels and impairs action potential (AP) development (Fitts, 1994b). Increased extracellular [K^+] to 8-10 mM has been reported to reduce muscle excitability due to membrane depolarisation (Cairns et al., 1995). Mouse soleus

muscle exposed to 12.5 mM [K^+] was associated with a 95% loss of contractile force (Clausen et al., 1993). In isolated frog sartorius muscle, muscle twitch and tetanic force were reduced when extracellular K^+ was elevated to 11 mM (Bouclin et al., 1995).

Some support for the concept that K^+ is involved in fatigue is the improvement in K^+ regulation and fatigue with training. High intensity training reduced $[K^+]_I$ in human skeletal muscle, and this was suggested to be linked with delayed fatigue during intense exercise (Nielsen et al., 2004). Endurance, sprint and strength training each improved muscle and blood K^+ regulation and enhanced muscle performance (McKenna et al., 1996, McKenna et al., 1993a).

2.3.3 K^+_{ATP} channels and fatigue

K^+ can also move across cell membranes through proteins referred to as K^+ channels (Doyle et al., 1998). One such protein is referred to as a K_{ATP} channel, and is expressed in heart, brain, skeletal and smooth muscle (Rodrigo and Standen, 2005; Miki et al., 2002). In muscle, the K_{ATP} channels play a roles in maintaining electrical activity of the cell membrane potential as a result of a perturbed metabolic state (Miki et al., 2002) and are thought to contribute to regulation of cellular excitability (Manley, 2001). The channels become activated in response to metabolic inhibition (low ATP, increased ADP) (Rodrigo and Standen, 2005). The K_{ATP} channels play an important role in protecting muscle fatigue and improve force recovery after fatigue and may also contribute to determining resting tension (Miki et al., 2002) and repolarization of the action potential during fatigue (Light et al., 1994).

2.4 β-adrenergic agonists and K^+ regulation

2.4.1 Background of β-adrenergic agonists

The β_2 adrenergic agonists are drugs that bind to the β_2-adrenergic receptor (β_2AR), a heptahelical receptor that couples predominantly to the stimulatory G protein, Gs (Figure 2.7) (Shore and Drazen, 2003). The β_2 adrenergic agonists are prescribed medicines for the treatment of asthma (Sears and Lötvall, 2005, Hanania and Cazzola, 2011). The primary function of β_2 adrenergic agonists is relaxation of bronchial (airway) smooth muscle (Hanania and Cazzola, 2011) causing bronchodilation (improvement in lung mechanics) and bronchoprotection (reduced responsiveness to nonspecific contractile stimuli).

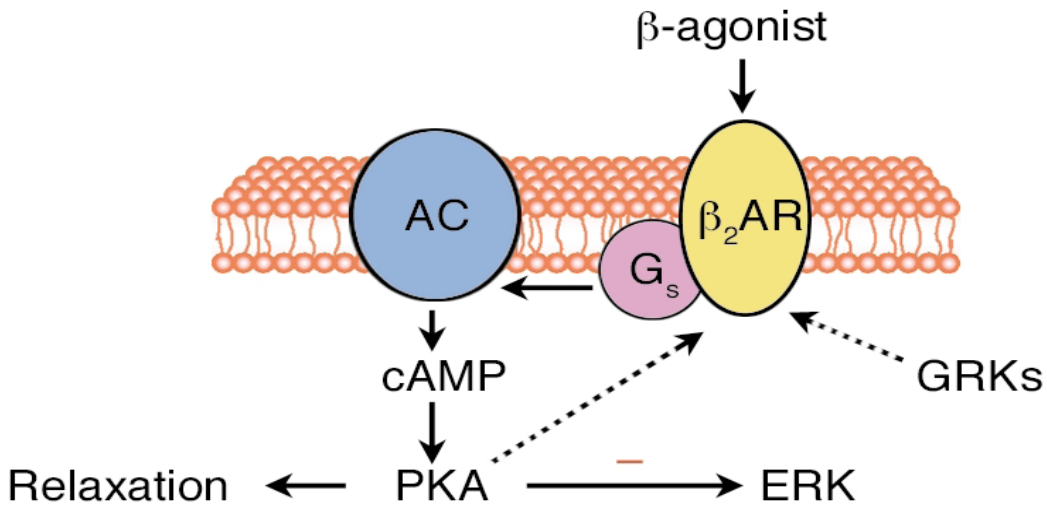

Figure 2.7 Mechanism of β-agonist–induced airway smooth muscle relaxation. Ligand binding to the β2AR activates Gs, leading to adenylyl cyclase (AC) activation, cAMP formation, and subsequent protein kinase A (PKA) activation. PKA phosphorylation of target proteins leads to smooth muscle relaxation and may also inhibit ERK activation. PKA also phosphorylates the β2AR, leading to increased Gs coupling. In addition, ligand binding causes G protein receptor

kinase (GRK) phosphorylation of the β2AR, recruiting β-arrestin (ARR). From (Shore and Drazen, 2003).

The β_2 adrenergic agonists are classified into two groups depending on their duration of action. Short-acting β_2 agonists comprise salbutamol, fenoterol and terbutaline, which have approximate duration of action of 4 to 6 hours and isoprenaline, which has less than 20 minutes of duration. Long acting β_2 agonists such as salmeterol and formoterol have approximate duration of action of 12 hours (Figure 2.8) (Hanania and Cazzola, 2011, Sears and Lötvall, 2005).

This review focuses on salbutamol as this is a commonly used drug for the treatment of asthma. Salbutamol has an approximate onset of action from 2–3 minutes, with bronchodilation usually starting within 3 – 5 minutes and peaking after 15 – 20 minutes; the duration of effect is approximately 4 hours (Hanania and Cazzola, 2011, Sears and Lötvall, 2005).

2.4.2.1 Effects of salbutamol on K^+ during rest

Salbutamol has a well-defined systemic K^+ lowering effect. In baboons, the decline of arterial serum potassium [K^+] was ~0.48 mM after injection of 100 µg kg^{-1} of salbutamol and 0.58 mM following injection of 500 µg kg^{-1} (Du Plooy et al., 1994). In eight healthy men, venous [K^+] during rest decreased incrementally following oral doses of 2 mg, 4 mg and 8 mg of salbutamol (Figure 2.9) (Grove et al., 1995). Resting venous [K^+] fell by 0.39 mM after ingestion of a dose of 6 mg salbutamol in nine healthy, moderately trained males (Collomp et al., 2000a). Injection of salbutamol induced a decrease in plasma [K^+], by 0.39 mM within 30 minutes following a dose of 1 mg and by 1.15 mM after a 4 mg dose, in fourteen healthy participants (Newnham et al., 1993).

A dose of 1200 µg of salbutamol delivered via inhalation reduced venous serum [K^+] by ~0.32 mM at 30 min and by ~0.4 mM at 60 min in 17 renal failure patients (Mandelbereg el al, 1999). Inhalation of a similar dose induced a 0.20 mM decline in venous plasma [K^+] in 14 healthy participants (Bennett and Tattersfield, 1997) and by 0.31 mM in 10 healthy males (Clark and Lipworth, 1996).

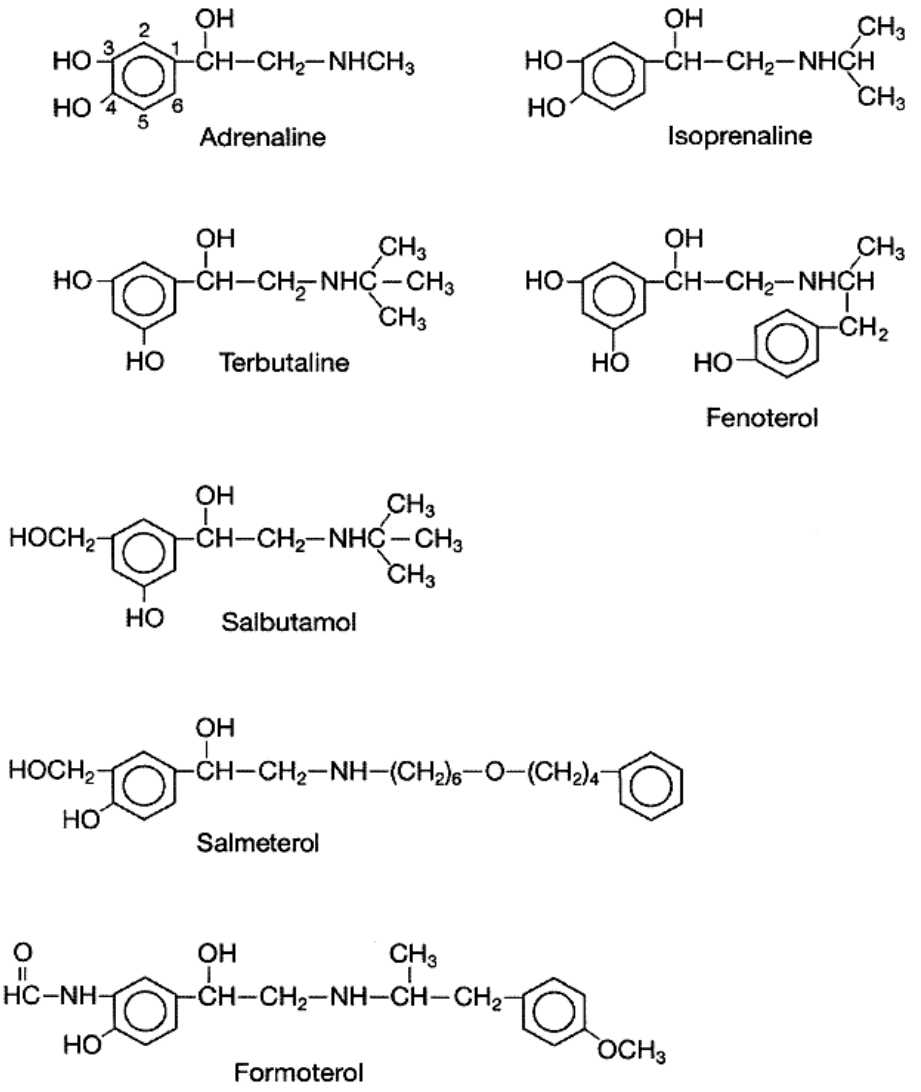

Figure 2.8 Chemical structure of selected β-agonists **(**Sears and Lötvall, 2005).

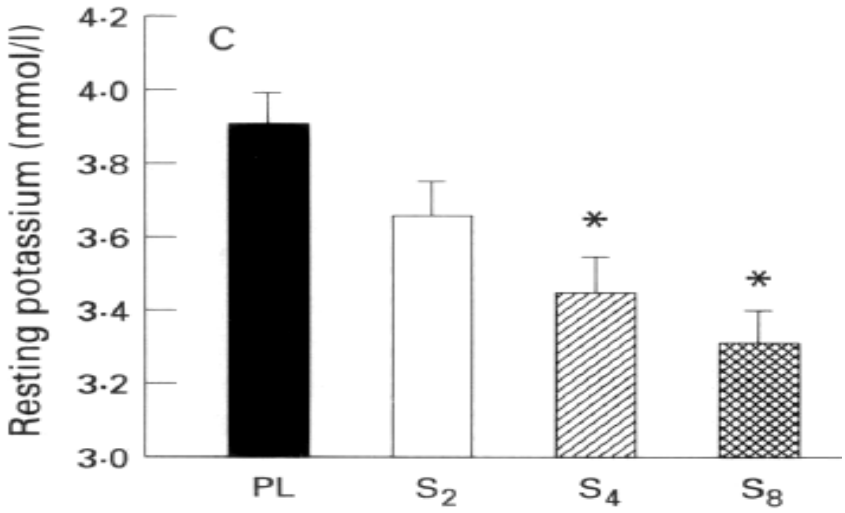

Figure 2.9 Effect of single oral dose of salbutamol on venous [K^+] during rest compared to placebo (PL). Salbutamol was taken at a dose of 2 mg (S2), 4 mg (S4) and 8 mg (S8). From (Grove et al., 1995).

2.4.2.2 Effects of salbutamol on plasma K^+ during exercise

The effects of salbutamol on [K^+] during exercise in athletes are not fully understood. Several studies have investigated these effects using salbutamol delivered either orally or via inhalation. In nine healthy, moderately trained participants, ingestion of 6 mg salbutamol lowered plasma [K^+] by 0.38 mM compared to placebo during submaximal cycling exercise at 80-85% $\dot{V}O_{2max}$ (Collomp et al., 2000a). During a 3 min exercise step test an 8 mg dose of salbutamol significantly increased the change in venous [K^+] compared to placebo, whereas 2 mg and 4 mg did not (Figure 2.10) (Grove et al., 1995,) Harrington et al.(2000) reported no effect of an oral dose 8 mg of salbutamol in heart failure patients during a symptom-limited treadmill exercise. Whereas in healthy participants, an oral dose of 4 mg salbutamol decreased plasma [K^+] from 5.0 to 4.4 mM during exercise to exhaustion on a cycle ergometer (Mayer et al., 2000). In contrast, inhalation of 800 μg of salbutamol did not affect plasma venous [K^+] during cycling exercise at 85% $\dot{V}O_{2max}$ in thirteen healthy participants (Goubault et al., 2001), or in

sixteen male cyclists during exercise on a cycle ergometer (Van Baak et al., 2004). It would appear that the K^+-lowering effects of salbutamol during exercise are more likely across a range of exercise types when an oral mode of delivery is used, rather than by inhalation, where two studies have found no change. However, inhalation is the typical means in which salbutamol is taken in the general asthmatic population and a more carefully controlled study is required.

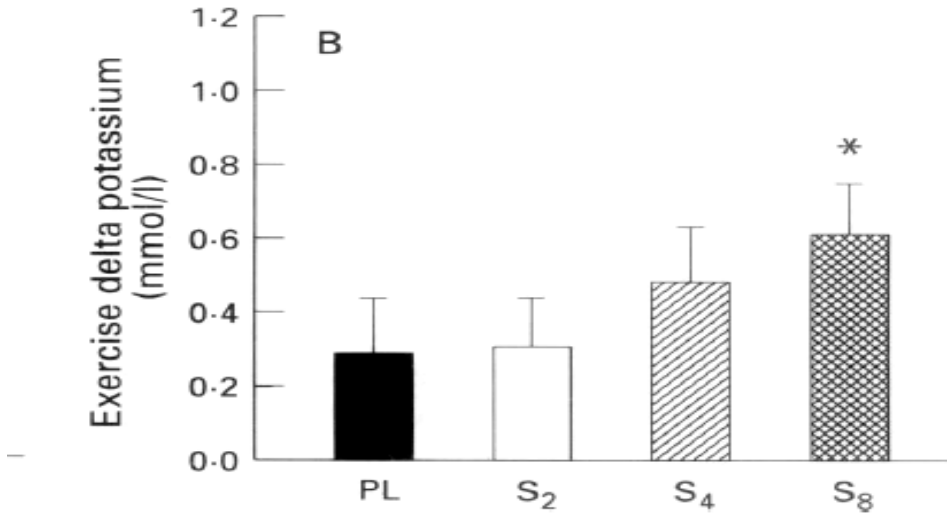

Figure 2.10 Effect of single oral dose of salbutamol at 2mg (S2), 4mg (S4) and 8mg (S8) on exercise delta [K^+] compared to placebo (PL). From (Grove et al., 1995).

2.4.2.3 Effects of salbutamol on muscle K^+ regulation

The mechanisms of salbutamol-induced decreased plasma [K^+] are suggested to be linked with the stimulation of the Na^+, K^+-pump in muscles (Smith and Kendall, 1984, Cairns et al., 1995, Clausen and Nielsen, 2007; Collomp et al., 2000a, Hanna et al., 1998). Salbutamol may play an active role in maintaining muscle membrane excitability via its direct role on the Na^+, K^+-pumps (Clausen, 2003). In animal models, stimulation of the Na^+, K^+-pump via salbutamol accelerated the recovery of force output and

attenuated fatigue development. In wistar rats, stimulation of the Na^+, K^+-pump via 10^{-5} M of salbutamol improved membrane excitability and reduced muscle fatigue via an effect on the transmembrane electrochemical gradients for Na^+ and K^+ (Overgaard et al., 1999). In rat soleus muscles, salbutamol stimulation of the Na^+, K^+-pump in K^+-depressed muscle restored tetanic force by 55% in fibre bundles (Cairns et al., 1995) and in EDL rats by 77% (Mikkelsen et al., 2006) . Further, stimulation of the Na^+,K^+-pump by 10^{-6} M salbutamol restored tetanic force at 11 mM $[K^+]_o$ in rat whole muscle and reduced the myoplasmic Na^+ content and increased K^+ content (Cairns et al., 1995).

2.4.2.4 Terbutaline and other β-adrenergic agonist effects on $[K^+]$

Terbutaline is a short acting β_2 agonist similar to salbutamol. It also stimulates the Na^+, K^+ -pump and increased uptake of K^+ in the human forearm (Clausen, 1996). In six healthy males, injection of a dose of 500 µg terbutaline decreased plasma $[K^+]_a$ by 0.83 mM at rest (Hallen et al., 1996). Injection of a 0.25 mg dose of terbutaline in a forearm vein decreased serum $[K^+]_v$ from 4.17 to 3.32 mM, but with no change detected in muscle $[K^+]$ in ten healthy participants (Tveskov et al., 1994). The Na^+, K^+-pump content increased from 1,104.1 nmol/kg dry weight to 1,273.3 nmol/kg dry weight (Tveskov et al., 1994). During recovery after maximal running exercise on a treadmill in 20 elite athletes, serum $[K^+]$ declined from 3.8 to 3.4 mM following inhalation of 3 mg of terbutaline (Larsson et al., 1997).

A similar effect is induced by other β-$_2$ adrenergic agonists. Plasma $[K^+]$ decreased by 0.45 mM following 400 µg of salmeterol in 10 healthy males (Maconochie and Forster, 1992). At rest, serum $[K^+]$ decreased by 0.36 mM after inhalation of 300 µg of salmeterol and by 0.28 mM following 72 µg dose of formoterol in eight healthy subjects (Grove and Lipworth, 1996). In six healthy males a higher dose of 1200 µg fenoterol via

inhalation reduced plasma [K^+] by 0.80 mM (Scheinin et al., 1987). In 14 healthy participants plasma [K^+] decreased by 0.59 mM and by 1.26 mM following 1 mg and 4 mg of inhaled fenoterol respectively during a standardised exercise step test (Newnham et al., 1993).

2.4.3.1 Effects of salbutamol on performance

Numerous studies have investigated the effects of salbutamol via inhalation or oral administration on exercise performance; a summary of performance effects of salbutamol is shown in Table 1.

Endurance, submaximal and maximal exercise have not usually improved after inhalation dose of salbutamol (Meeuwisse et al., 1992, Norris et al., 1996, Goulbault et.,al 2001, Sporor et al., 2008). The one exception was an improvement in endurance cycling performance by 1.9% after inhalation 800 μg of salbutamol during cycling exercise (Van Baak et al., 2004).

Most of the studies that examined effect of an oral administration of salbutamol on exercise performance improved performance. During cycling exercise at 80-85 %$\dot{V}O_{2peak}$, an oral dose of salbutamol improved time to exhaustion (Collomp et al., 2000a, Collomp et al., 2000b). An oral dose of salbutamol also improved peak power and mean power during a 30 sec Wingate test (Collomp et al, 2005, Le Panse et al., 2007, Le Panse et al., 2006, Le Panse et al., 2005). No effect was found on mean power during cycling exercise at 90 % $\dot{V}O_{2max}$ (Collomp et al., 2002). During sprint exercise power output increased after oral dose of salbutamol (Sanchez et al, 2012).

2.4.3.2 Other β-adrenergic agonist effects on exercise performance

Some researchers have examined the effects of other β_2 adrenergic agonists on exercise performance. An inhaled dose of 50 µg of salmeterol reduced running time until exhaustion to 18 seconds compared to placebo in 18 healthy well-trained athletes during treadmill running on (Carlsen et al., 1997). In 24 healthy, well-trained males, an inhaled dose of 9 µg formoterol did not improve endurance performance as measured by VO_{2max} or time to exhaustion (Carlsen et al., 2001).

Neither inhalation nor oral studies have investigated the effects of salbutamol on exercise performance during repeat sprint exercise. Also, in all these studies the potential mechanism of an increased performance (or not) was not explained in relation to Na^+, K^+-pump and the possible role of K^+. Hence the effects of salbutamol on performance linked to K^+ homeostasis remains to be determined. To my knowledge no study has investigated the effects of salbutamol on K^+ regulation and potential to improve exercise performance. Therefore, this thesis examined whether salbutamol modulated K^+ dynamics at rest, during exercise, during recovery after exercise and how these might improve performance during repeat sprint exercise.

Table 1 Summary of studies that examined the effects of salbutamol on performance

Reference	number	Subjects	Salbutamol dose and delivery	Protocol	Exercise mode	Key findings
(Meeuwisse et al., 1992)	7	highly trained male athletes	200 µg inhalation	Incremental exercise 45 min cycling at 70% VOmax then sprint until exhaustion 30-s Wingate test	CE	↔ VO2max ↔ Endurance print time ↔Total work / ↔Peak power
(Norris et al., 1996)	15	well-trained male cyclist	400 µg inhalation	Incremental exercise 20 km time trial 60 s modified Wingate test	CE	↔ VO2max ↔Endurance time trial↔ Total work /↔Peak Power
(Van Baak et al., 2004)	16	male cyclist and triathletes	800 µg inhalation	75% Wmax	CE	↓Time-trial cycling by 82 s↑ cycling performance by .9%
(Sporor et al., 2008)	72	trained male cyclist and triathletes	200,400 and 800 µg inhalation	20 km time trial	CE	↔ time-trial / ↔ peak power
Goulbault et.,al 2001)	13	healthy male	200 and 800µg inhalation	85% VO2 max	CE	↔ Endurance performance
(Sandsund., 1998)	8	male cross-country skiers	1.2 mg inhalation	60 -95% VO2 max	Treadmill	↔ VO2max ↔ Time to exhaustion
(Arlettaz et al., 2009	8	healthy trained men	6 mg oral	1 h at 60% VO2 peak	CE	↔ emerge expenditure ↔ substrate oxidation
(Collomp et al., 2000b	8	athletes male	(12 mg/day for 3weeks) oral	80-85% VO2peak	CE	↑ time to exhaustion by ~7 min

Collomp et al., 2000)	9	moderately trained male	6 mg oral	80-85% VO2peak	CE	↑ Time of cycling by 19%
Collomp et al., 2002)	8	moderately trained male	6 mg oral	90 % VO2 max	CE	↑ Mean power
Collomp et al, 2005	13	healthy male	6 mg oral	30 sec Wingate test	CE	↑ Mean power / ↑Peak power
(Le Panse et al., 2007	11	recreationally trained women	4 mg oral	30 sec Wingate test	CE	↑ Mean power / ↑Peak power ↔ fatigue index
Le Panse et al, 2006)	14	healthy women	12 mg daily for 4 weeks	30 sec Wingate test	CE	↑ Mean power / ↑Peak power ↔ fatigue index
(Le Panse et al, 2005)	8 7	trained male athletes untrained male	13 mg daily during 3 weeks	30 sec Wingate test	CE	↑ Peak power by ~ 12% for trained and ~ 8% for untrained ↔ fatigue index
(Sanchez et al., 2012)	8	athletes male	12 mg daily for 4 weeks/oral 6 mg oral	Sprint exercise	CE	↔Power output Force-velocity

(**↔**) no effect / (**↑**) improved **(CE)** cycling exercise

2.5 Aims and hypotheses:

2.5.1 Aims

The aim of this thesis was to comprehensively investigate the effects of acute inhalation of salbutamol (1000µg) on potassium regulation at rest, during and following exercise, as well as on exercise performance during repeat sprint exercise in healthy, young, non-asthmatic adults.

2.5.2 Hypotheses

This thesis tested the hypotheses that inhalation of 1000 µg salbutamol would:

1. Decrease arterial and venous plasma potassium concentration at rest, during continuous incremental exercise and during repeat sprint exercise, as well as in recovery, when compared to a placebo.
2. Enhance work output and thus improve performance during repeat sprint exercise, when compared to a placebo.
3. Increase arterio – venous plasma K^+ differences at rest, during continuous incremental exercise and during repeat sprint exercise, as well as in recovery, when compared to a placebo.

CHAPTER 3. METHODOLOGY

3.1 Participants

Seven healthy, recreationally-active but not well-trained male participants volunteered for the study after giving written informed consent. Subject physical characteristics were (mean ± SD) age 23 ± 6. yr, height 175 ± 13 cm, body mass 71.6 ± 10.4 kg. All experiments and procedures were approved by the Victoria University Human Research Ethics committee.

3.2 Study design

The participants visited the laboratory on three occasions. The first was for screening, pre-testing and familiarisation purposes. In visits two and three, each participant performed an exercise trial after inhaling either a placebo or salbutamol. These trials were performed in a double-blind, randomized, cross-over design and were separated by two weeks to ensure a complete washout of salbutamol.

3.3 Salbutamol and placebo administration

Subjects were given salbutamol (1000 µg) using a standard metered dose inhaler used for asthma treatment (Asmol inhaler, alphapharm, Queensland, Australia). The inhaler delivers 100 µg of salbutamol with each actuation (i.e. actuations 10 x 100 µg =1000 µg); the placebo was delivered by a similar metered dose inhaler containing the propellant only (Propellant, Allen and Hanburys, Melbourne, Australia). In both cases a standard spacer device (Volumatic spacer, Allen and Hanburys Melbourne, Australia) was used to allow optimal delivery of the drug to the lung. The drug inhalation time averaged approximately 2 min (10 breaths, each ~12 seconds). Complete uptake of

salbutamol cannot be concluded as any residual salbutamol in the spacer, in the mouth or remaining in the lung was not measured. However, the spacer technique is utilised exactly for this purpose, to maximise the oral drug delivery. (Mandelbereg el al, 1999). Administration was performed 30 minutes prior to the test, allowing for maximum pharmacological activity of salbutamol (Hopkins, 1999).

3.4 Experimental and Procedures Overview

3.4.1 Screening and pre – tests

Each participant was familiarised with all testing procedures. Participants had preliminary screening to verify normal plasma electrolytes, respiratory function and heart rhythm**.** An antecubital venous blood sample was taken for determination of resting plasma potassium concentration ($[K^+]$) and for other electrolytes. To exclude any participant with any lung disease or asthma, the forced expiratory volume in 1 second (FEV_1) as percentage of forced vital capacity (FVC) was measured using a flow turbine (Medical Graphics). All participants had an FEV_1/FVC ratio exceeding 80% (mean ± SD 84 ± 7%). Participants then performed a familiarisation comprising four, 5-second "all-out" sprints on a cycle ergometer. After 30 min of rest, participants then performed an incremental exercise test on a cycle ergometer to determine peak oxygen uptake ($\dot{V}O_{2\ peak}$) using a custom-built metabolic cart. This $\dot{V}O_2$ data was used for calculation of work rates corresponding to 40, 60 and 130 % $\dot{V}O_{2\ peak}$. Heart rate and rhythm were monitored at rest and during incremental exercise using a 12-lead ECG (Model X-Scribe Stress Test System, Mortara Instrument Inc, Milwaukee,WI, USA).

3.4.2 Incremental Test

3.4.2.1 $\dot{V}O_2$ peak

Participants undertook an incremental test on an electronically braked cycle ergometer (Lode, Groningen, Netherlands), commencing with three minutes cycling performed at submaximal workrates of 60, 90, 120 and 150 W, with cadence of at 65-80 rpm. The workrate was then increased by 25 W each minute, until volitional fatigue was reached, defined as an inability to maintain pedal cadence above 60 rpm.

3.4.2.2 Experimental Exercise Trials

Each experimental trial comprised an initial placebo/salbutamol inhalation, 30 min rest then continuous exercise, a short recovery, repeat sprint exercise and a final 30 min recovery (Figure 3.1). The continuous exercise test comprised cycling for 5 min at 40% $\dot{V}O_{2\ peak}$, followed by 5 min at 60% $\dot{V}O_{2\ peak}$ and then 90 s at 130% $\dot{V}O_{2\ peak}$ on an electronically braked cycle ergometer (Lode, Groningen, Netherlands). A regression equation of $\dot{V}O_2$ versus power output was derived from the submaximal workloads and was used to determine the power output corresponding to 40%, 60% and130% $\dot{V}O_2$ peak. After 20 min of passive recovery on couch, subjects performed repeat sprint exercise on a custom air-braked cycle ergometer (Repco, Melbourne, Australia). Subjects pedalled against air resistance caused by air vanes attached perpendicularly to the axis of rotation of the flywheel. Power output and work output were calculated for each sprint bout from the flywheel velocity using optical sensors and custom software. The repeat sprint exercise test comprised three sets of five 4-s “all out” sprints, each separated with 20 s of passive recovery, and a 4.5-min passive recovery between sets,

all remaining seated on the cycle ergometer. This was then followed by a 30 min supine recovery period. Reliability repeat sprint performance calculated as typical error expressed as a CV during treadmill running was 4.7 and 10.8% for mean and peak power, respectively (Serpiello et al 2011) and for cycle ergometry was 9.6 (Bishop et al 2011). Due to technical difficulties with one trial mechanical data is reported for 6 subjects.

3.4.2.3 Respiratory Measurements

Respiratory data was collected during the $\dot{V}O_{2peak}$ and during continuous exercise tests. Subjects breathed through a Hans-Rudolph two-way non-rebreathing valve, with the expired gas passing through low-resistance plastic tubing into a 4 l mixing chamber. The nasal passage was blocked by a nose clip. Mixed expired oxygen (O_2) and carbon dioxide (CO_2) fractions were analysed by rapidly responding O_2 and CO_2 analysers (Ametek S-3A/II and Ametek CD-3A, Pittsburgh, USA). Expired volumes were determined from a flow transducer (KL Engineering K520, California, USA). Oxygen consumption (VO_2), CO_2 output, (VCO_2) and respiratory exchange ratio (RER) were calculated every 15 s on a PC (Turbo fit, California, USA). The ventilometer and gas analysers were calibrated before each test with a standard 3 L syringe and precision references gases, respectively.

3.5 Blood Samples

A cannula was inserted into the radial artery of one arm (Arrow Quick Flash, Radial Artery 20 gauge, USA), and another into the antecubital vein of the other arm (Optiva, I.V. Catheter 20 gauge, Italy). Participants then rested supine for 20 min, to allow for

stabilisation of any fluctuations in fluid shifts, as well as in potassium that might have occurred due to adrenergic responses to the invasive procedures. Arterial and venous blood samples were obtained simultaneously during all phases of the study, to allow calculation of arterio-venous differences across the forearm musculature (Figure 3.1). The blood sampling times and posture for each phase comprised sampling: (i) after 20 min supine rest and prior to salbutamol or placebo inhalation, referred to as the baseline sample. (ii) at 5, 10, 20 and 30 minutes following salbutamol or placebo inhalation supine rest (iii) seated on the cycle ergometer during the last 30 s of each workload of continuous cycling exercise; (iv) during the fourth bout of each set of sprints; and (vi) during supine rest at 1, 2, 5, 10 and 30 min in recovery after repeat sprint exercise. A total of 19 blood samples were collected for each of the arterial and venous measures. Therefore the total volume of blood sampled in each trial was approximately 200 ml (19 samples x 2 [arterial, venous] x 5 ml). Any effect of this level of blood withdrawal on the subsequent trial was minimised through the randomised order of salbutamol/placebo trials.

3.5.1 Blood Analyses

A 2 ml blood sample was drawn for immediate analyses of plasma pH, gases and electrolyte concentrations including sodium (Na^+), chloride (Cl^-), calcium (Ca^{++}) and potassium concentrations ($[K^+]$) using an automated blood – gas analyser (Rapid Point 405, Siemens Medical Solutions Pty Ltd, Bayswater, Australia). A 3 ml sample of blood was also collected in a plain syringe, then ejected into a tube containing lithium heparin and after mixing immediately separated into two eppendorf tubes. Then approximately 1 ml was separated and used for analysis of haematocrit (Hct) and haemoglobin

concentration ([Hb]) using an automated analyser (Sysmex K- 800 TOA Medical Electronics Kobe, Japan), and blood glucose and lactate concentrations ([Lac^-]) were measured using automated glucose/lactate analyser (2300 STAT plus, YSI Inc. Yellow Spring, ON, USA). The remaining 2 ml was immediately spun down for 1.5 min at 4500 rpm in a non-refrigerated centrifuge (Eppendorf Centrifuge, model 5415C, Englesdorf, Germany). Plasma was then separated and stored in an eppendorf tube at -20° C for later analysis of plasma [K^+].

3.6 Calculations

The decline in plasma volume (ΔPV) from baseline levels was calculated from [Hb] and [Hct] during continuous and repeat sprint exercise, as well as in recovery, as indicated in Equation 1 (Harrison, 1985).

Equation 1**:**

$$\% \Delta PV = \left(\left[\frac{[Hb_1] \times (1 - Hct_2)}{[Hb_2] \times (1 - Hct_1} \right] - 1 \right) \times 100$$

Plasma [K^+] during and after exercise was corrected for ΔPV to represent haemoconcentration changes, according to Equation 2.

Equation 2: $[K^+]_{corrected} = [K^+]_{measured} \times [1 + (\Delta PV)/100]$, where [$K^+$] is given in millimoles per litre and ΔPV is given as a negative percentage (McKenna *et al.*, 1993b). The change in plasma [K^+] from baseline ($\Delta[K^+]$) was calculated according to Equation3.

Equation 3: $\Delta [K^+] = [K^+] - \text{baseline } [K^+]$, in $mmol.L^{-1}$.

3.7 Statistical Analyses

Two-way analyses of variance (ANOVA) with repeated measures (drug treatment, time) were used to compare physiological and performance variables. The Newman–Keuls post hoc test was used to locate differences when ANOVAs revealed a significant F ratio for main effects or for the treatment x time interactions. Statistical significance was accepted at $P<0.05$. Data are presented as mean ± standard deviation (SD). “With an expected within subject variation of 0.2 mM and a minimum expected difference of 0.35 mM for arterial and venous potassium concentration with salbutamol, the required sample size to achieve a statistical signifcance at $P<0.05$ was n=8). Data checked for normality using Shapiro Wilk test, and the result showed that 16 out of 19 data points were normally distributed.

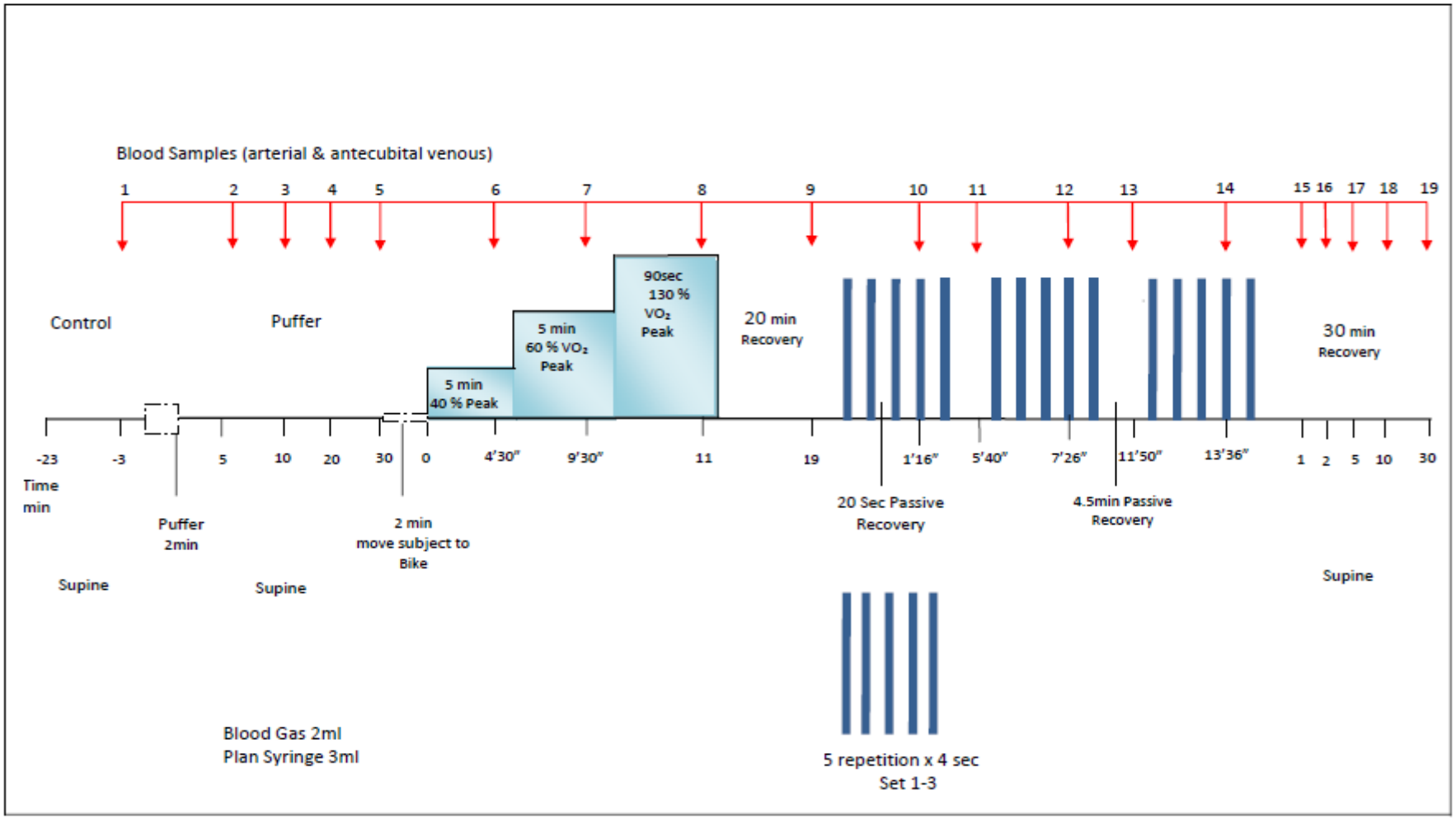

Figure 3.1 Experimental design overview showing rest, exercise, and recovery and blood sampling interval

CHAPTER 4. RESULTS

4.1 Oxygen consumption

A significant time main effect was seen with $\dot{V}O_2$ increased during continuous exercise at 40%, 60% and at 130% $\dot{V}O_{2peak}$ ($P < 0.01$), but no significant salbutamol main effect or time x salbutamol interaction was found, indicating no significant differences in $\dot{V}O_2$ between salbutamol and placebo (Figure 4.1).

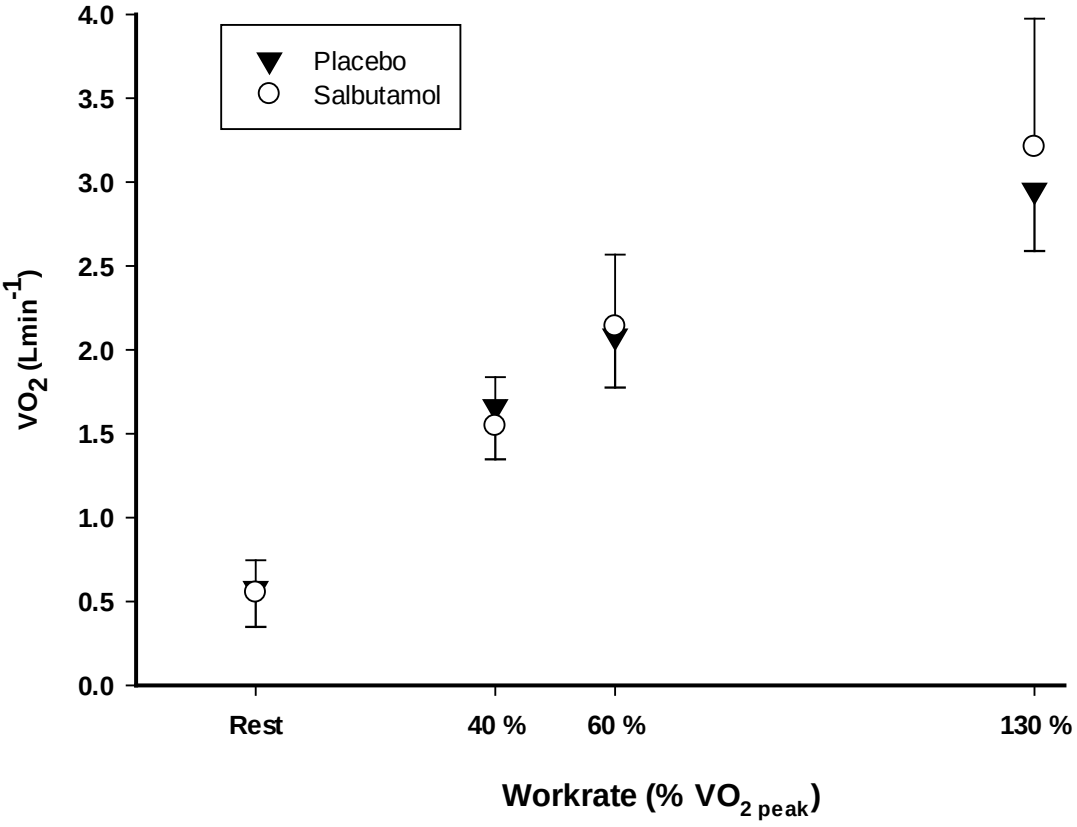

Figure 4.11 Effects of salbutamol and placebo inhalation on $\dot{V}O_2$ during continuous exercise at 40%, 60% and at 130% $\dot{V}O_{2peak}$. Values are mean ± SD, n = 7.

4.2 Mechanical work during repeat sprint exercise

4.2.1 Work output in individual sprints.

No significant time main effect, salbutamol main effects or time x salbutamol interaction were found. No significant differences in mechanical work were found between salbutamol and placebo during the three sets of repeat sprint exercise (5 x 4 s) (Figure 4.2).

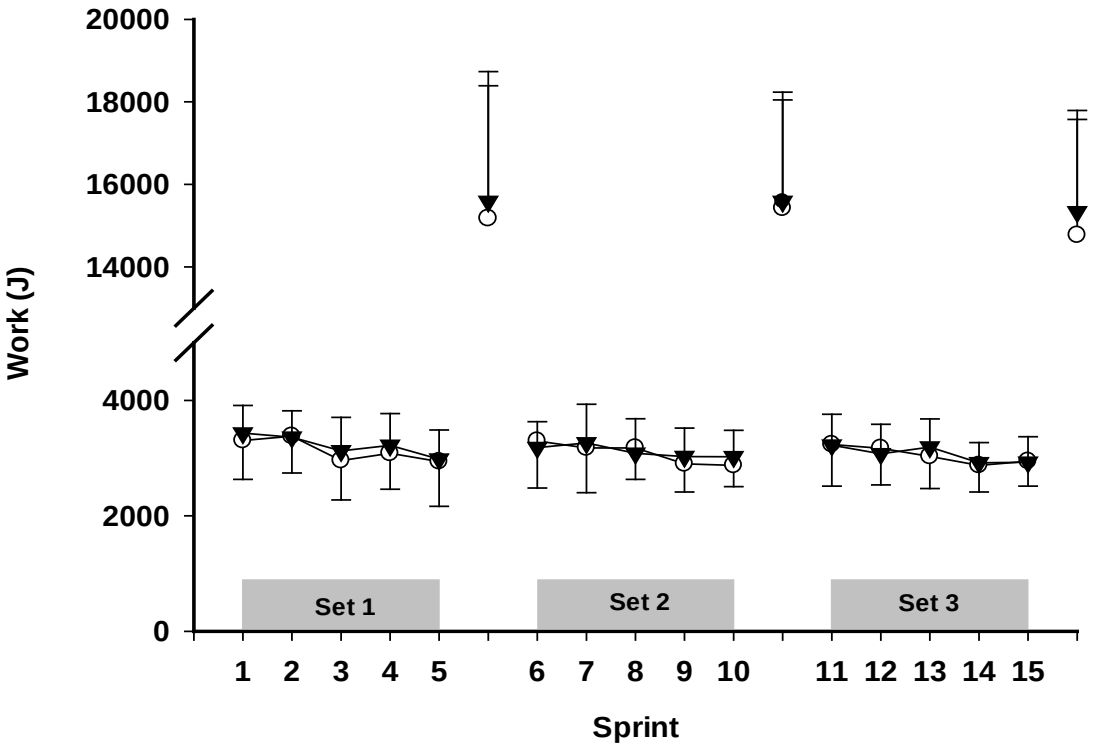

Figure 4.2 Effects of salbutamol and placebo inhalation on mechanical work output during repeat sprint exercise (3 sets x 5 repetitions x 4 s). Values are mean ± SD, n = 6. Figure indicates work output during each sprint within each set and cumulative work for each set.

4.2.2 Total work

No significant difference in cumulative total work was found between salbutamol and placebo trials during repeat sprint exercise (Figure 4.3).

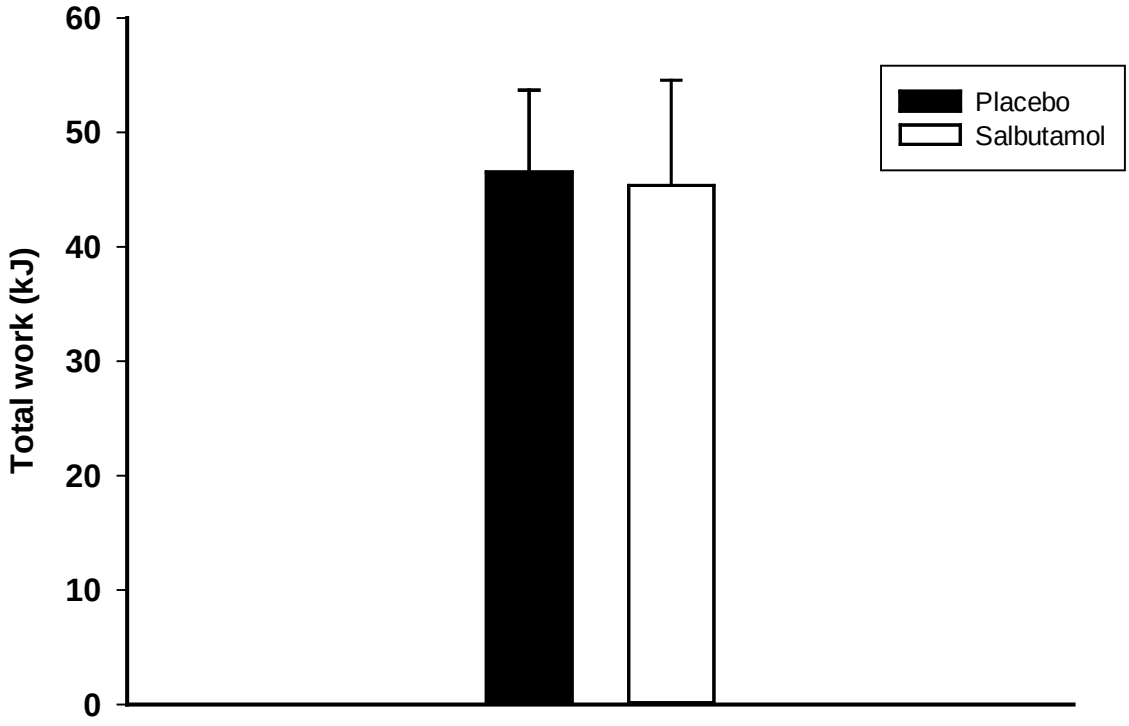

Figure 4.3 Effects of salbutamol and placebo inhalation on total cumulative work during repeat sprint exercise (3 sets x 5 repetitions x 4 s). Values are mean ± SD, n = 6.

4.3 Arterial Blood Lactate Concentration

4.3.1 Main effects and interactions

A significant time main effect was seen for arterial blood [Lac^-] ($P < 0.01$), but no significant salbutamol main effect, or time x salbutamol interaction was found (Figure 4.4).

4.3.2 Time main effect

Blood lactate was unchanged during the 30 min rest, increased progressively during continuous exercise at 40, 60 and 130% $\dot{V}O_{2\ peak}$ and remained elevated at 19 min of recovery ($P < 0.01$). Blood [Lac^-] was elevated above baseline value during each set of repeat sprint exercise, peaked at 1 min of recovery after repeat sprint exercise, subsequently declining but remained above baseline value at 30 min of recovery ($P<0.01$).

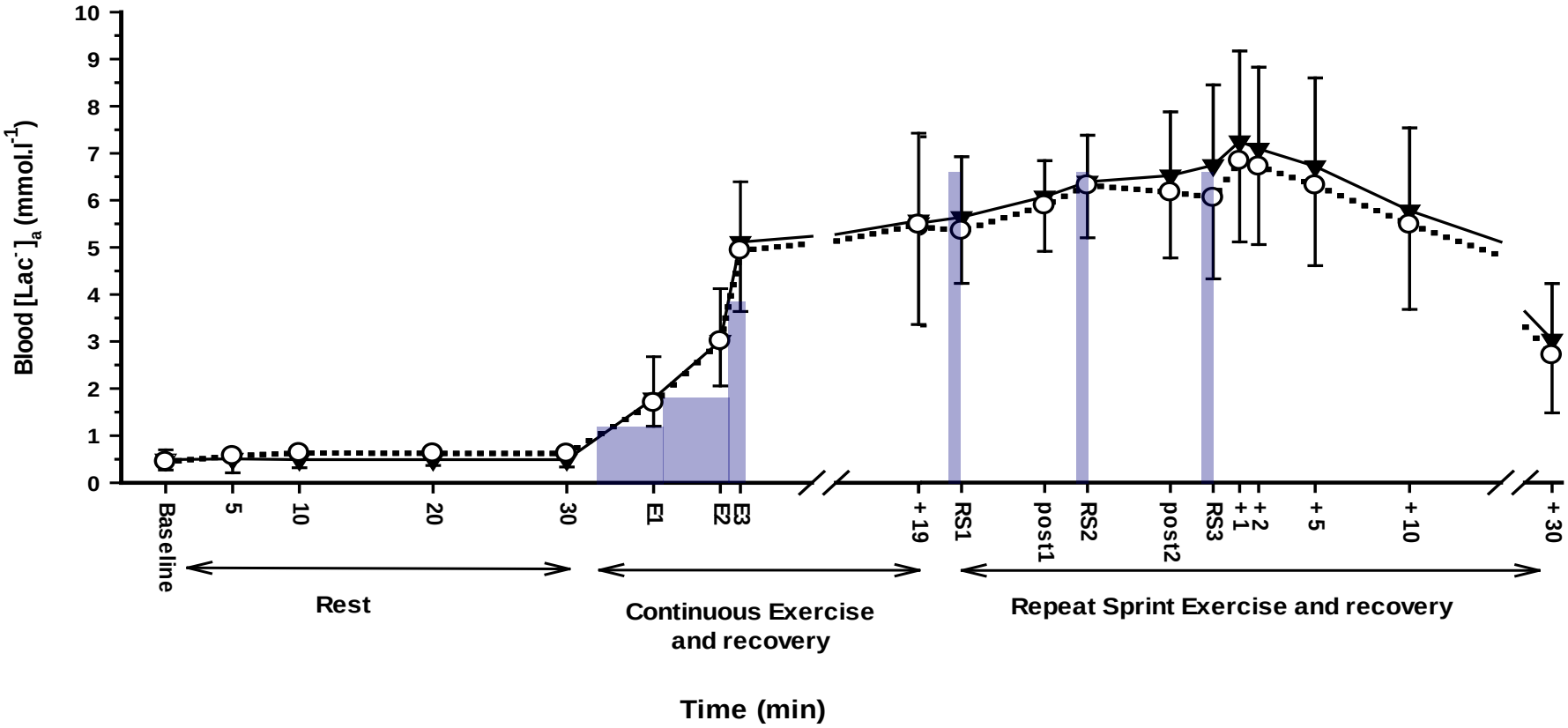

Figure 4.4 Effects of salbutamol (○) and placebo (▼) inhalation on arterial blood lactate concentration ($[Lac^-]_a$) at rest, during continuous exercise at 40%, 60% and 130% $\dot{V}O_{2peak}$, during repeat sprint exercise (3 sets x 5 repetitions x 4 s) and 30 min recovery. Values are mean ± SD, n = 7. Exercise, (E1) exercise at 40% $\dot{V}O_{2peak}$, (E2) exercise at 60% $\dot{V}O_{2peak}$, (E2) exercise at 130% $\dot{V}O_{2peak}$, (+19) recovery after continuous exercise, (RS1) first set of repeat sprint exercise, (Post1) recovery after first set, (RS2) second set of repeat sprint exercise, (Post 2) recovery after second set, (RS3) third set of repeat sprint exercise.

4.4 Arterial Plasma pH

4.4.1 Main effects and interactions

A significant time main effect was seen for pH ($P < 0.01$) but no significant salbutamol main effect or time x salbutamol interaction was observed (Figure 4.5).

4.4.2 Time main effects

Arterial plasma pH was unchanged during the initial rest period, declined during continuous exercise and repeat sprint exercise ($P < 0.01$), remained below baseline at 1, 2, 5 and 10 min of recovery and returned to baseline at 30 min of recovery.

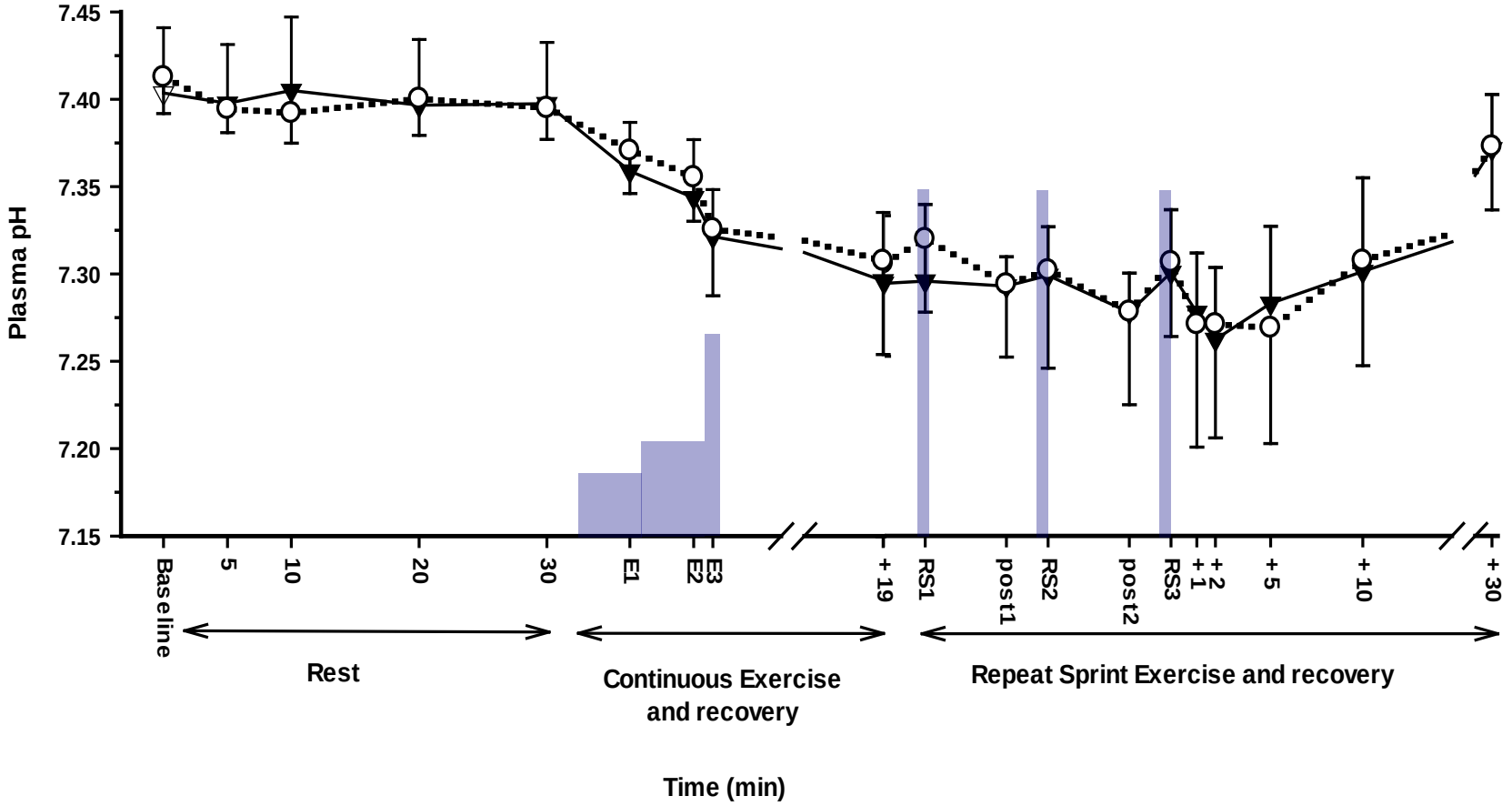

Figure 4.5 Effects of salbutamol (○) and placebo (▼) inhalation on arterial plasma pH at rest, during continuous exercise at 40%, 60% and 130% $\dot{V}O_{2peak}$, during repeat sprint exercise (3 sets x 5 repetitions x 4 s) and 30 min recovery. Values are mean ± SD, n = 7. Symbols and abbreviations as defined in figure 4.4.

4.5 Change in plasma volume from baseline

4.5.1 Main effects and interactions

A significant time main effect was seen for change in plasma volume ($P< 0.01$) but no salbutamol main effect was found. The time x salbutamol interaction was significant ($P< 0.01$ Figure 4.6) and therefore comparisons were performed between treatments and within treatments over time.

4.5.2 Salbutamol versus Placebo Comparisons

No significant differences in ΔPV_a were found between salbutamol and placebo at any specific time point, except ΔPV remained a lower (more negative) in the salbutamol trial at 30 min of recovery ($P < 0.05$).

4.5.3 Within Placebo Trial Comparisons

Rest. No changes were found in ΔPV during the initial 30 min of rest.

Continuous Exercise. The ΔPV declined during continuous exercise, falling by 9.7 ± 2.0 % during exercise at 130% $\dot{V}O_{2peak}$.

Repeat Sprint Exercise. The ΔPV remained negative during repeat sprint exercise and until 10 min post-exercise ($P < 0.05$), then returned to baseline at 30 min of recovery.

4.5.4 Within Salbutamol Trial Comparisons

Rest. No significant differences were found in ΔPV during the initial 30 min of rest.

Continuous Exercise. The ΔPV fell by 10.22 ± 4.8% during cycling at 130% $\dot{V}O_{2peak}$ ($P < 0.05$), with partial recovery at 19 min post-exercise, but with ΔPV remaining negative ($P < 0.05$).

Repeat Sprint Exercise. The ΔPV oscillated at ~ 8-10% below baseline during repeat sprint exercise ($P < 0.05$) and remained less than baseline at 1, 2, 5 and 10 min of recovery ($P < 0.05$).

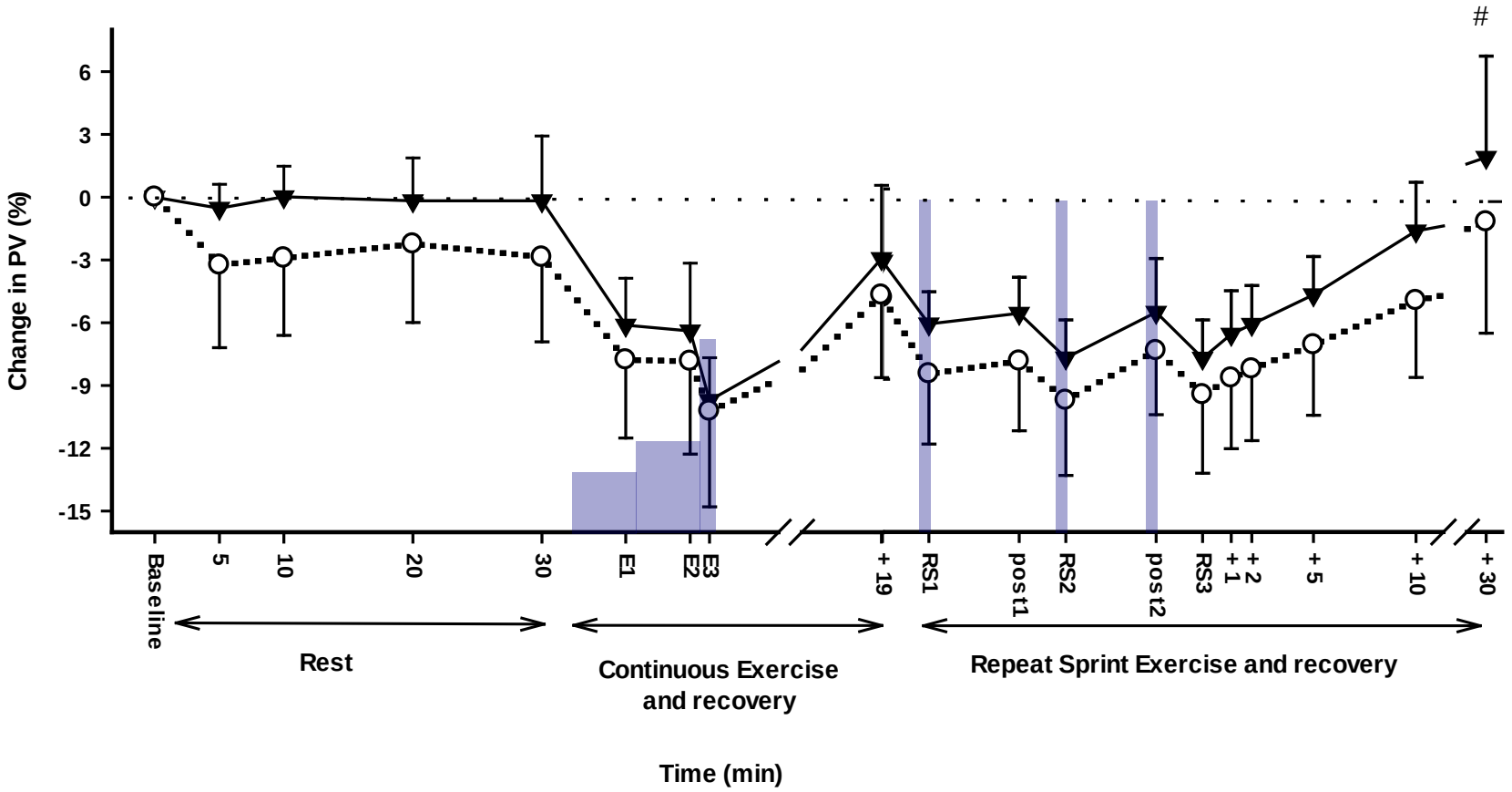

Figure 4.6 Effects of salbutamol (○) and placebo (▼) inhalation on change in plasma volume from baseline (ΔPV) at rest, during continuous exercise at 40%, 60% and 130% $\dot{V}O_{2peak}$, during repeat sprint exercise (3 sets x 5 repetitions x 4 s) and 30 min recovery. $^{\#}$Salbutamol < than placebo (P< 0.01). Values are mean ± SE, n = 7. Symbols and abbreviations as defined in figure 4.4.

4.6 Arterial plasma [K^+]

4.6.1 Main effects and interactions.

A significant time main effect was seen plasma $[K^+]_a$ ($P< 0.01$), but no significant salbutamol main effect was found (Figure 4.7). The time x salbutamol interaction was significant ($P< 0.01$) and therefore comparisons were performed between treatments and within treatments over time.

4.6.2 Salbutamol versus placebo Comparisons

Comparisons between salbutamol and placebo trials revealed no significant differences in $[K^+]_a$ at any time.

4.6.3 Within Placebo Trial Comparisons

Rest. After placebo, plasma $[K^+]_a$ was unchanged from baseline values.

Continuous exercise. Plasma $[K^+]_a$ increased above baseline at 40%, 60% and 130% $\dot{V}O_{2\ peak}$ ($P< 0.01$) and had returned to baseline at 19 min of recovery.

Repeat Sprint Exercise. Plasma $[K^+]_a$ increased above baseline during sets 1-3 ($P < 0.05$) and decreased in each intervening recovery period, falling to below baseline during recovery only after sprint set 2 ($P < 0.05$). Plasma $[K^+]_a$ did not differ from baseline during 30 min of recovery after repeat sprint exercise.

4.6.4 Within Salbutamol Trial Comparisons

Rest. In contrast to the placebo trial, salbutamol decreased plasma $[K^+]_a$ below baseline at 20 min ($P < 0.05$).

Continuous Exercise. Similar to the placebo tria,l plasma $[K^+]_a$ increased during cycling at 40%, 60% and 130% $\dot{V}O_{2\ peak}$ and returned to baseline at 19 min of recovery.

Repeat Sprint Exercise. Similar to the placebo trial, plasma $[K^+]_a$ increased above baseline during sets 1-3, decreased after each set and had fallen to below baseline during the 4.5 min of recovery after both sets 1 and 2 ($P < 0.05$). In contrast to the placebo trial, $[K^+]_a$ declined to below baseline following set 3 at each of 2, 5 and 10 min of recovery ($P < 0.05$).

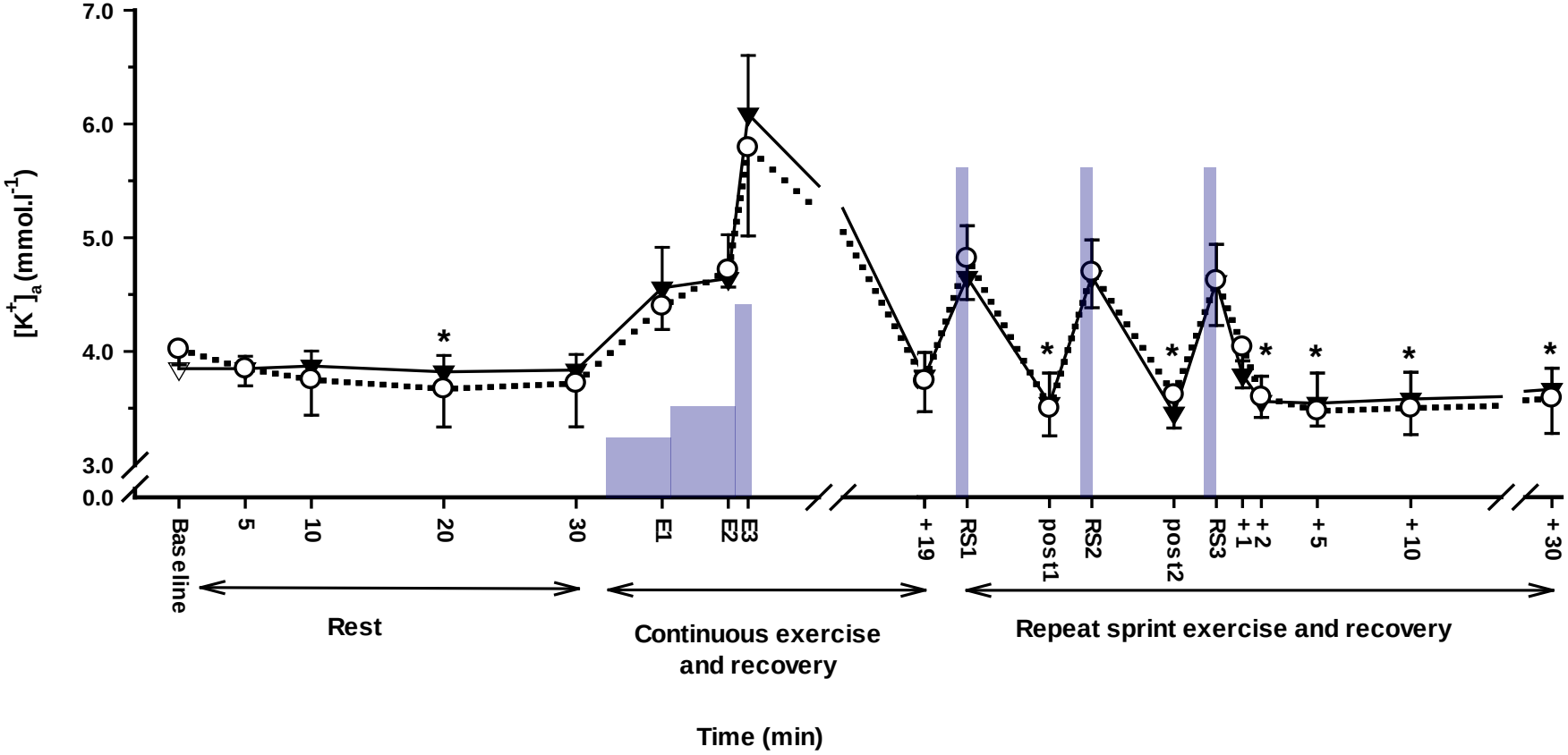

Figure 4.7 Effects of salbutamol (○) and placebo (▼) inhalation on arterial plasma $[K^+]_a$ at rest, during continuous exercise at 40%, 60% and 130% $\dot{V}O_{2peak}$, during repeat sprint exercise (3 sets x 5 repetitions x 4 s) and 30 min recovery. *Salbutamol trial $[K^+]$ less than baseline ($P< 0.01$). Values are mean ± SD, n = 7. Shaded bars denote exercise period. Differences from baseline in placebo trial are not indicated for clarity. Symbols and abbreviations as defined in figure 4.4.

4.7 Change in arterial plasma [K^+] from baseline

4.7.1 Main effects and interactions

Due to the small differences in baseline $[K^+]_a$ between salbutamol and placebo trials, where the salbutamol trial baseline $[K^+]_a$ was ~ 0.2 mM higher than placebo $[K^+]_{a'}$ the change in plasma $[K^+]_a$ from baseline ($\Delta[K^+]_a$) was calculated for each of the salbutamol and placebo trials.Significant main effects were seen for time ($P< 0.01$) and salbutamol for $\Delta[K^+]_a$ ($P< 0.01$), with significant time x salbutamol interactions ($P< 0.01$, Figure 4.8). $\Delta[K^+]$ was less in salbutamol trial than placebo trial ($P< 0.01$).

4.7.2 Salbutamol versus placebo Comparisons

Rest. The $\Delta[K^+]_a$ at 20 min was greater (more negative) after salbutamol compared with placebo (-0.35 ± 0.32 vs -0.03 ± 0.06 mM, respectively, $P < 0.05$).

Continuous Exercise. The $\Delta[K^+]_a$ during exercise at 40% and at 130% $\dot{V}O_{2peak}$ was lower during salbutamol (less positive) compared with placebo (0.38 ± 0.17 vs 0.71 ± 0.39 mM and 1.77 ± 0.77 vs 2.24 ± 0. mM, respectively $P < 0.05$).

Repeat Sprint Exercise. The $\Delta[K^+]_a$ did not differ between salbutamol and placebo during the repeat sprint exercise bouts or during the subsequent 30 min recovery.

4.7.3 Within Placebo Trial Comparisons

Rest. The $\Delta[K^+]_a$ did not differ from baseline during 30 min of rest.

Continuous exercise. The $\Delta[K^+]_a$ rose during continuous exercise and reached ~ 2.3 mM at 130% $\dot{V}O_{2peak}$ ($P < 0.05$), and returned to baseline values at 19 min of recovery.

Repeat Sprint Exercise. The $\Delta[K^+]_a$ increased during each set of sprints to ~ 0.7 mM then fell to below baseline during recovery after set 2 ($P < 0.05$), and then did not differ from baseline during 30 min of recovery.

4.7.4 Within Salbutamol Trial Comparisons

Rest. In contrast to the placebo trial, the $\Delta[K^+]_a$ decreased (become more negative) at 20 min after salbutamol inhalation, declining by ~ 0.4 mM ($P < 0.05$).

Continuous Exercise. The $\Delta[K^+]_a$ rose during continuous exercise at 40, 60 and 130% $\dot{V}O_{2peak}$ ($P < 0.05$) and had returned to baseline at 19 min of recovery.

Repeat Sprint Exercise. Similar to the placebo, trial $\Delta[K^+]_a$ increased above baseline with each set of repeat sprint exercise to ~ 0.7 mM. In contrast to placebo trial, $\Delta[K^+]_a$ declined to below baseline during recovery after set 2 ($P < 0.05$), and following set 3 at each of 2, 5, 10 and 30 min of recovery ($P < 0.05$).

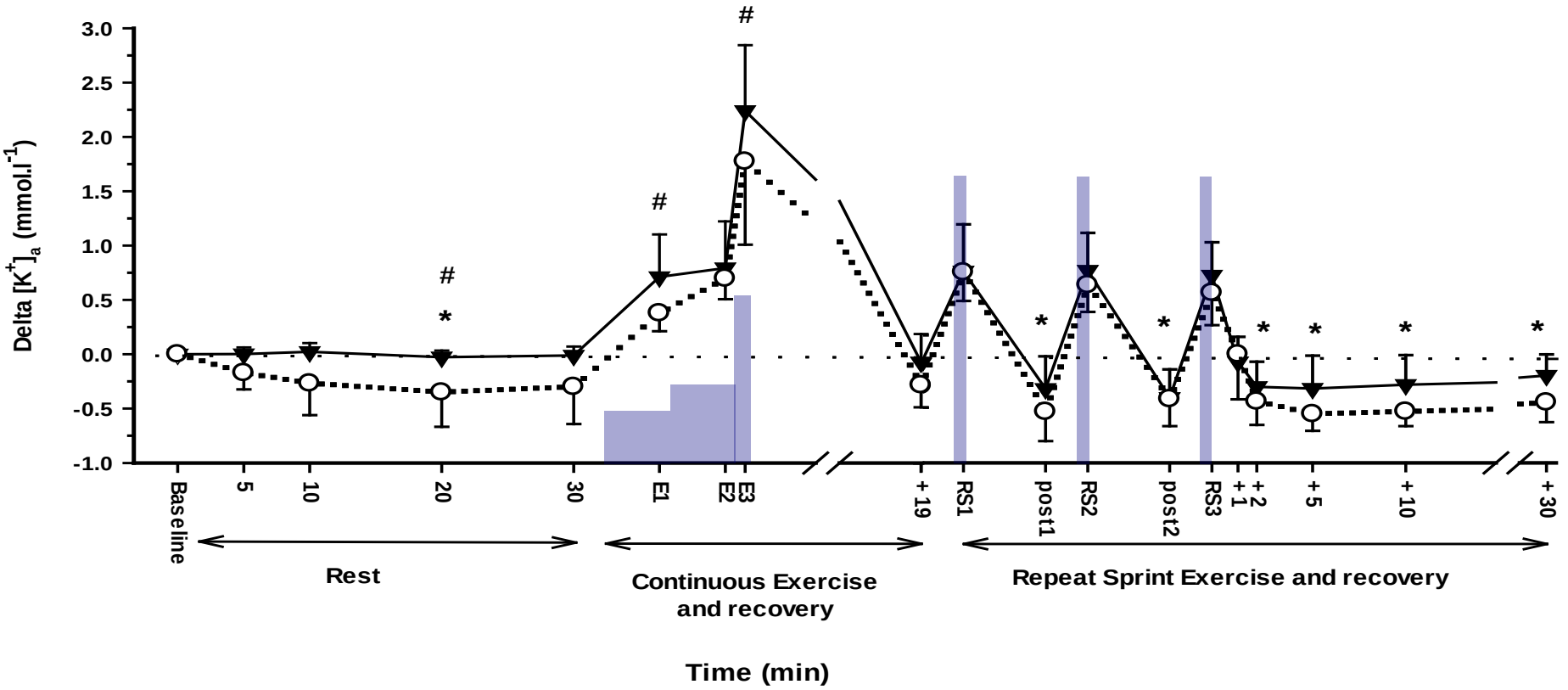

Figure 4.8 Effects of salbutamol (○) and placebo (▼) inhalation on the change in plasma $[K^+]_a$ from baseline ($\Delta[K^+]_a$) at rest, during continuous exercise at 40%, 60% and 130% $\dot{V}O_{2peak}$, during repeat sprint exercise (3 sets 5 repetitions x 4 s) and 30 min recovery. $^{\#}$Salbutamol < than placebo ($P< 0.01$). *Salbutamol less than baseline values ($P< 0.01$). Values are mean ± SD, n =7. Shaded bars denote exercise period. Differences for baseline in placebo trial are not indicated for clarity. Symbols and abbreviations as defined in figure 4.4.

4.8. Change in arterial plasma [K^+] from baseline corrected for change in plasma volume

4.8.1 Main effects and interaction

Significant time and salbutamol main effects for $\Delta[K^+]_{a,corr}$ was less in salbutamol vs placebo were seen ($P< 0.01$), and the time x salbutamol interaction was also significant ($P< 0.01$, Figure 4.9).

4.8.2 Salbutamol versus placebo trials Comparisons

Rest. The change in arterial plasma [K^+] from baseline corrected for change in plasma volume from baseline ($\Delta[K^+]_{a,corr}$) was more negative ($P < 0.05$) after salbutamol compared with placebo at each of 10 (-0.38 ± 0.27 vs 0.02 ± 0.08 mM), 20 min (-0.43 ± 0.30 vs -0.03 ± 0.07 mM) and 30 min of the initial period rest (-0.42 ± 0.27 vs -0.02 ± 0.07 mM), respectively.

Continuous Exercise. The $\Delta[K^+]_{a,corr}$ was less during salbutamol compared with placebo ($P < 0.05$) during exercise at 40% $\dot{V}O_{2\ peak}$ (0.04 ± 0.26 vs 0.43 ± 0.33 mM) and at 130% $\dot{V}O_{2\ peak}$ (1.18 ± 0.73 vs 1.64 ± 0.53 mM).

Repeat Sprint Exercise. The $\Delta[K^+]_{a,corr}$ did not differ between salbutamol and placebo during repeat sprint exercise but was more negative after salbutamol compared with placebo at both 10 (-0.70 ± 0.18 vs -0.32 ± 0.30 mM) and 30 (-0.48 ± 0.26 vs -0.11 ± 0.19) min of the subsequent recovery ($P < 0.05$).

4.8.3 Within Placebo Trial Comparisons

Rest. No change was found from baseline in $\Delta[K^+]_{a,corr}$ during the 30 min of rest.

Continuous Exercise. The $\Delta[K^+]_{a\ corr}$ increased during exercise at 40%, 60% and 130% $\dot{V}O_{2\ peak}$ and had returned to baseline at 19 min of recovery ($P < 0.01$).

Repeat Sprint Exercise. The $\Delta[K^+]_{a,corr}$ increased above baseline within each set of repeat sprint exercise and then fell to below baseline during recovery after set 2 ($P < 0.05$) and after set 3 at 1, 2, 5 and 10 min of recovery ($P < 0.05$).

4.8.4 Within Salbutamol Trial Comparisons

Rest. In contrast to the placebo trial, the $\Delta[K^+]_{a,corr}$ become more negative at 10, 20 and 30 min after salbutamol inhalation ($P < 0.05$).

Continuous Exercise. The $\Delta[K^+]_{a,corr}$ rose from negative to positive values during exercise at 60 and 130% $\dot{V}O_{2peak}$ ($P < 0.05$), and had declined to baseline after 19 min of recovery.

Repeat Sprints Exercise. $\Delta[K^+]_{a,corr}$ increased above baseline within each set of repeat sprint exercise and then fell below baseline during recovery after set 1, set 2 ($P < 0.05$), and after set 3 at 1, 2, 5,10 and 30 min of recovery ($P < 0.05$).

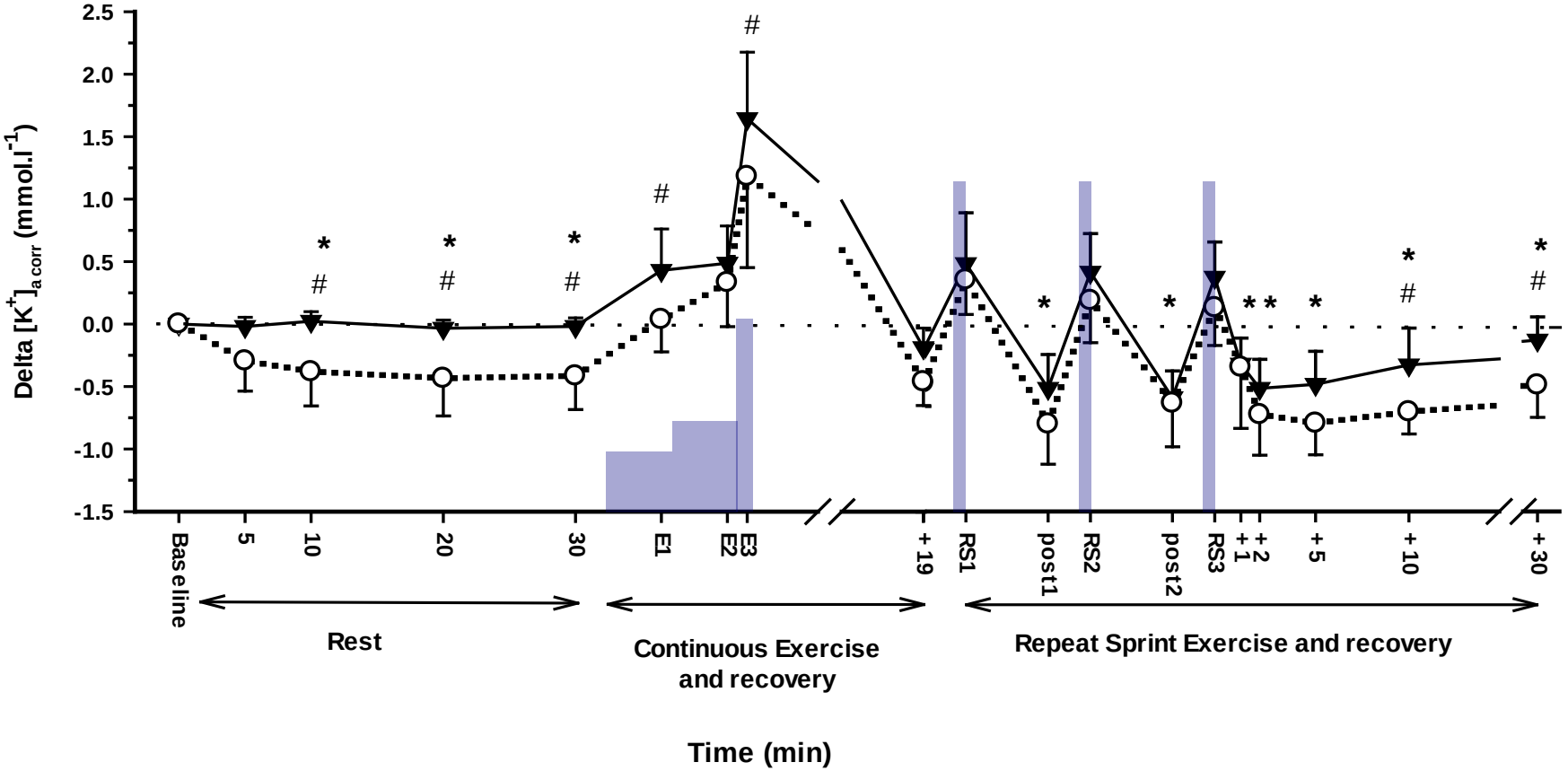

Figure 4.9 Effects of salbutamol (○) and placebo (▼) inhalation on change plasma $[K^+]_a$ corrected from baseline ($\Delta[K^+]_a$) at rest, during continuous exercise at 40%, 60% and 130% $\dot{V}O_{2peak}$, during repeat sprint exercise (3 sets 5 repetitions x 4 s) and 30 min recovery). #Salbutamol < than placebo (P< 0.01).*Salbutamol less from baseline (P< 0.01). Values are mean ± SD, n =7. Shaded bars denote exercise period. Differences for baseline in placebo trial are not indicated for clarity. Symbols and abbreviations as defined in figure 4.4.

4.9 Venous plasma potassum concentration

4.9.1 Main effects and interactions

A significant time main effect ($P< 0.01$), but no significant salbutamol main effect or time x salbutamol interactions were found for venous plasma $[K^+]$ ($[K^+]_v$) (Figure 4.10).

4.9.2 Time main effects

Rest. Plasma $[K^+]_v$ was unchanged during the initial 30 min rest.

Continuous Exercise. The $[K^+]_v$ was increased above baseline value during at 40, 60 and 130% $\dot{V}O_{2peak}$ and then returned to baseline at 19 min of recovery.

Repeat Sprint Exercise. Plasma $[K^+]_v$ was increased during repeat sprint exercise then declined in each intervening recovery, falling to below baseline values at 5, 10 and 30 min of recovery ($P < 0.05$).

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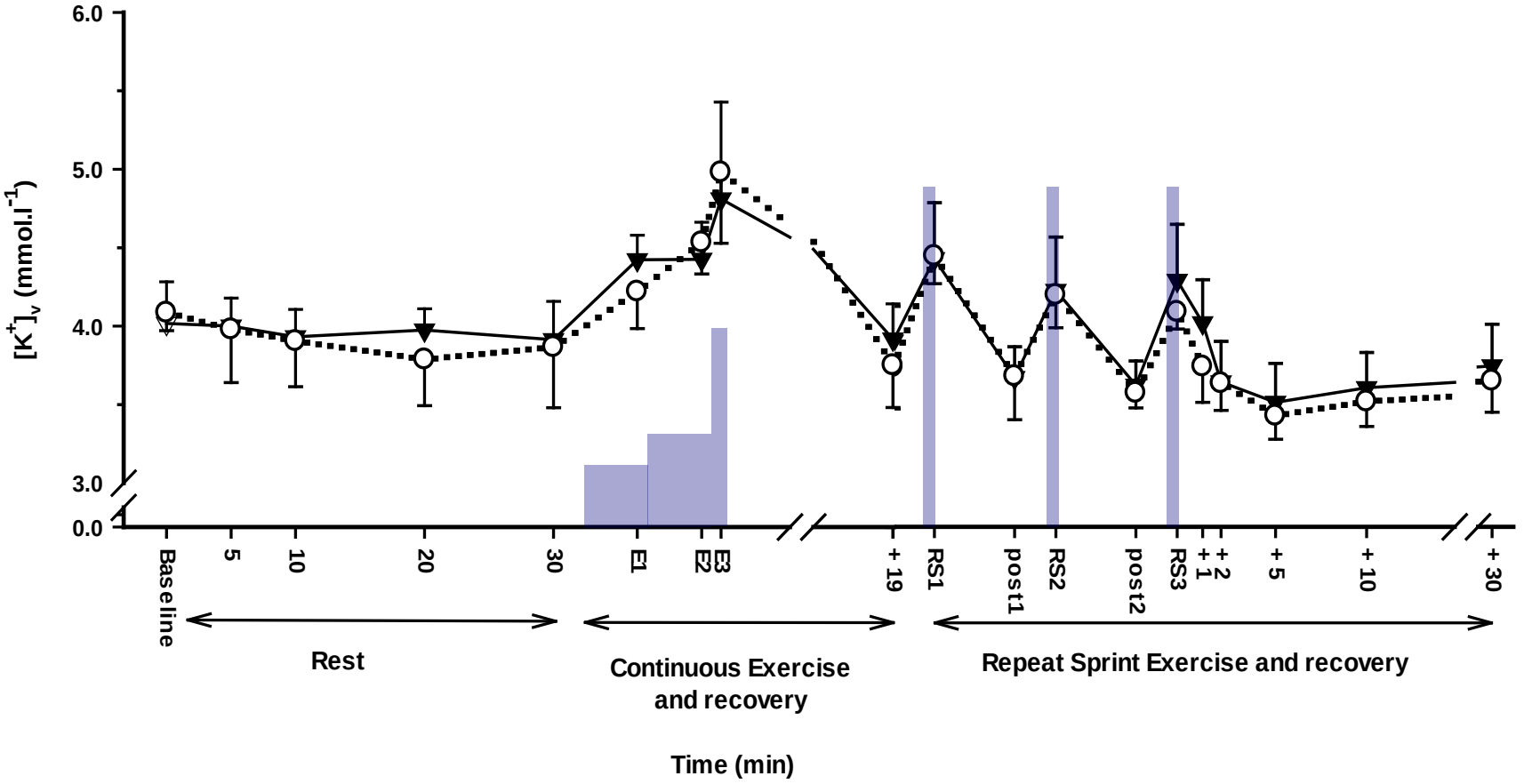

Figure 4.10 Effects of salbutamol (○) and placebo (▼) inhalation on $[K^+]_v$ at rest, during continuous exercise at 40%, 60% and 130% $\dot{V}O_{2peak}$, during repeat sprint exercise (3 sets 5 repetitions x 4 s) and 30 min recovery. Values are mean ± SD, n = 7. Symbols and abbreviations as defined in figure 4.4.

4.10 Change in venous plasma [K^+] from baseline

4.10.1 Main effects and interactions

A significant time main effect was seen ($P< 0.01$) but no significant salbutamol main effect or time x salbutamol interactions were found for the change in $[K^+]_v$ from baseline ($\Delta[K^+]_v$) (Figure 4.11).

4.10.2 Time main effects

Rest. Plasma $[K^+]_v$ was unchanged after placebo at the 30 min rest.

Continuous Exercise. The $[K^+]_v$ was increased above baseline at 40, increased further at 60 and at130% $\dot{V}O_{2peak}$ and then returned to baseline at 19 min of recovery.

Repeat Sprint Exercise. Plasma $[K^+]_v$ was increased during repeat sprint exercise then declined in each intervening recovery, falling to below baseline values at 2, 5, 10 and 30 min of recovery ($P < 0.05$).

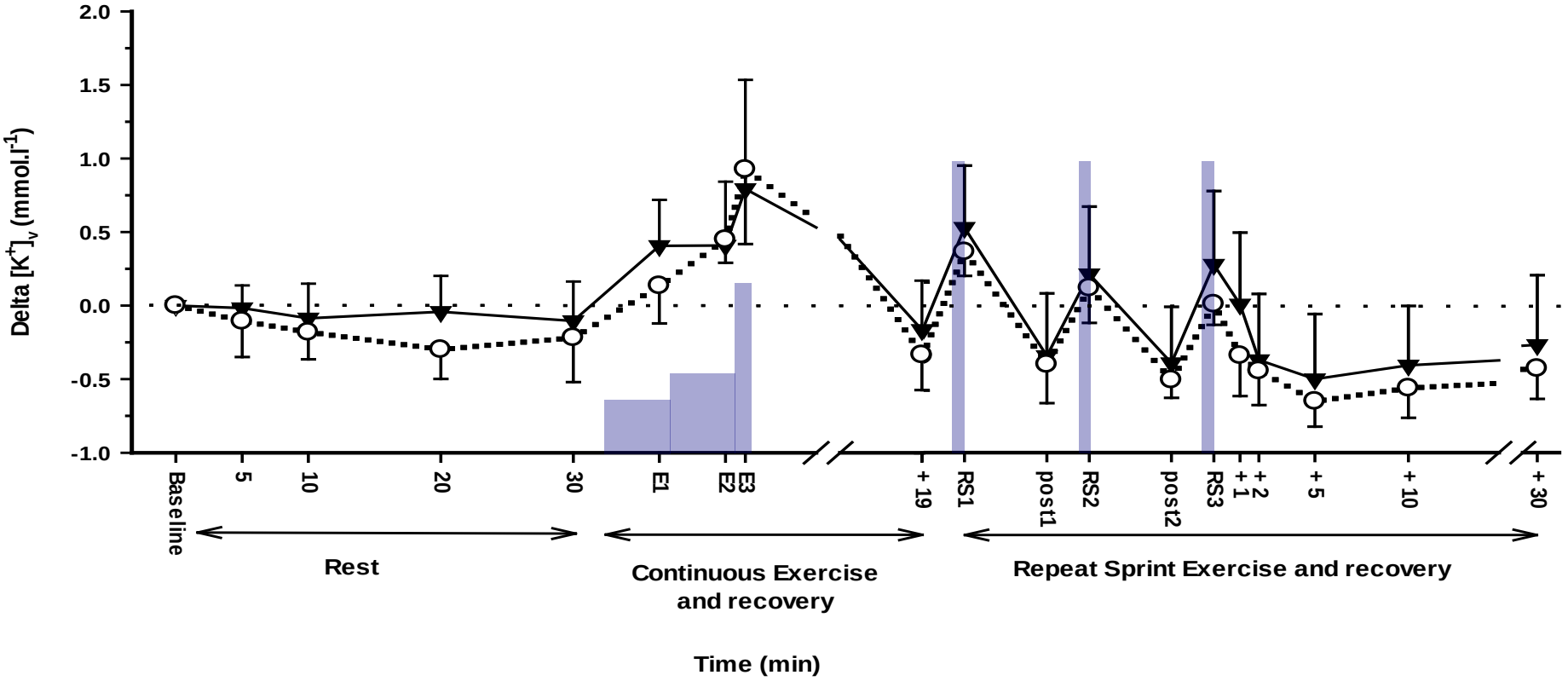

Figure 4.11 Effects of salbutamol (○) and placebo (▼) inhalation on change in venous plasma from rest $\Delta[K^+]_v$ at rest, during continuous exercise at 40%, 60% and 130% $\dot{V}O_{2peak}$, during repeat sprint exercise (3 sets x 5 repetitions x 4 s) and 30 min recovery. Values are mean ± SD, n = 7. Symbols and abbreviations as defined in figure 4.4.

4.11 Arterio – Venous Plasma K^+ Differences

4.11.1 Main effects and interactions

A significant time main effect ($P< 0.01$) but no significant salbutamol main effect or time x salbutamol interactions were seen for the arterio-venous plasma $[K^+]$ differences ($[K^+]_{a-v}$) (Figure 4.12).

4.11.2 Time main effects

The plasma $[K^+]_{a-v}$ was unchanged (slightly negative) during the 30 min rest, increased above baseline during continuous exercise at 40%, 60% and 130% $\dot{V}O_{2peak}$ ($P < 0.05$) then had returned to baseline value at 19 min of recovery. The $[K^+]_{a-v}$ increased above baseline value during each set of repeat sprint exercise, and then returned to baseline value at 2, 5, 10 and 30 min of recovery.

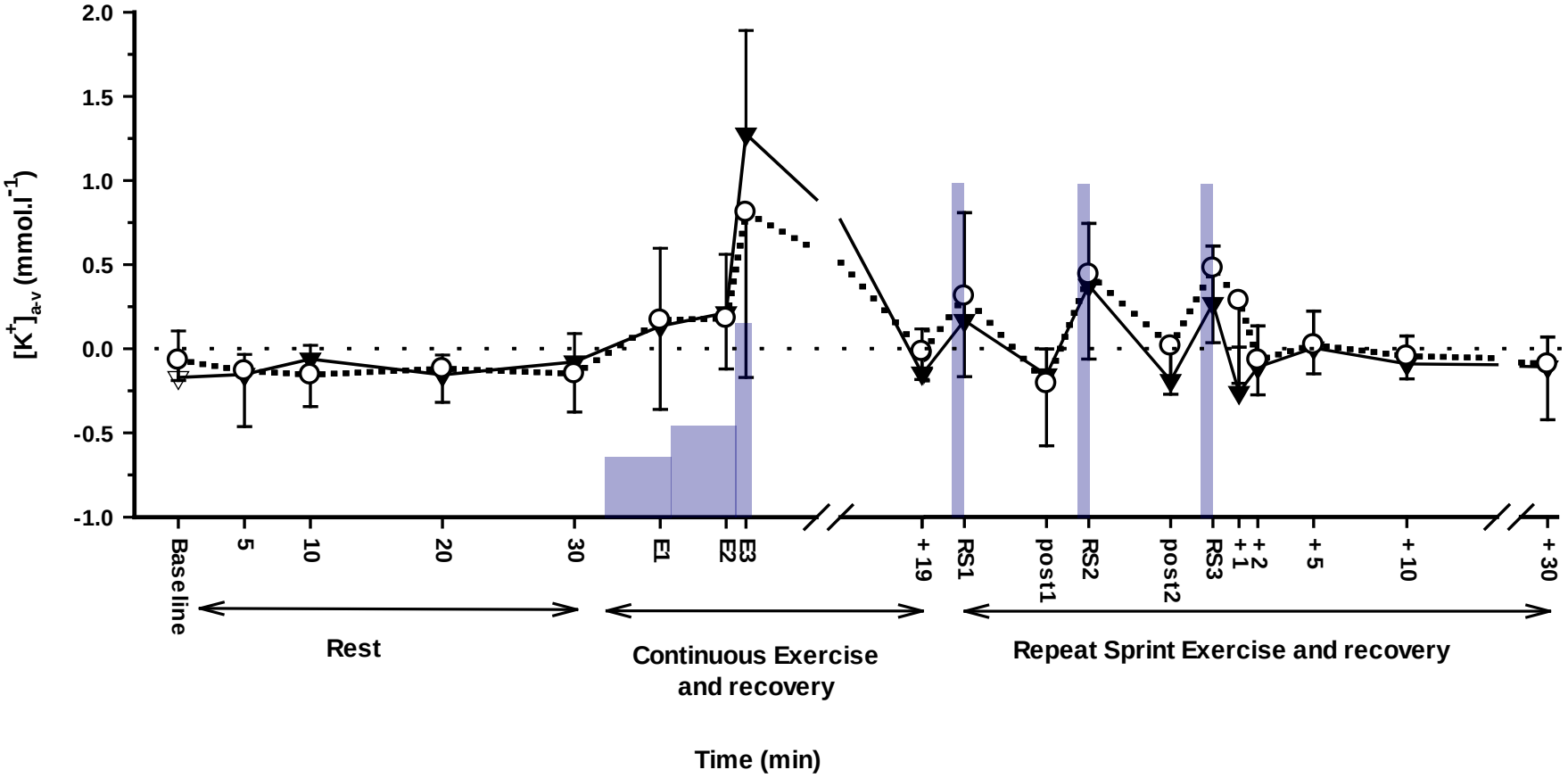

Figure 4.12 Effects of salbutamol (○) and placebo (▼) inhalation on plasma potassium arterial – venous difference at rest, during continuous exerciseat 40%, 60% and 130% $\dot{V}O_{2peak}$, during repeat sprint exercise (3 sets 5 repetitions x 4 s) and 30 min recovery. Values are mean ± SD, n = 7. Symbols and abbreviations as defined in figure 4.4.

CHAPTER 5. DISCUSSION

This thesis investigated the effects of salbutamol (1000 μg) administered acutely via inhalation on systemic potassium regulation at rest, during continuous and high intensity intermittent exercise (repeat-sprint exercise) and recovery and on performance during repeat-sprint exercise in healthy adult males. Whilst salbutamol lowered K^+ at rest, during continuous exercise and in recovery, salbutamol had no effect on performance during repeat-sprint exercise.

5.1 Salbutamol inhalation lowered $[K^+]_a$ during rest

Salbutamol lowered plasma $[K^+]_a$ at 20 min of rest by ~ 0.35 mM compared to the baseline. Due to small differences in the baseline $[K^+]$ between the two trials, the $\Delta[K^+]_a$ from baseline was calculated for individuals in each trial to provide a more sensitive marker of $[K^+]$ change. The $\Delta[K^+]_a$ was 0.32 mM more negative in salbutamol than placebo at 20 min of rest, indicating a K^+ lowering effect of salbutamol, that is also consistent with findings from other studies. Infusion or injection of salbutamol produced a greater K^+ lowing effect than inhalation. Salbutamol infusion at 20 μg/min for 30 min decreased $[K^+]_a$ by as much as 1 mM (Tobin et al., 2006). Similarly, injection of the β_2-adrenergic agonist terbutaline at a dose of 500 μg decreased $[K^+]_a$ by 0.83 mM (Hallen et al., 1996). Smaller K^+ declines were found after salbutamol inhalation. Plasma $[K^+]_v$ decreased by 0.31 mM at 20 min after inhalation of 1200 μg salbutamol (Clark and Lipworth, 1996). Mandelberg et al (1999) reported a similar decline in serum $[K^+]_v$ by ~ 0.28 mM at 10 min and by 0.32 mM at 30 min after inhalation of 1200 μg of salbutamol. In contrast to these studies, no significant effects of salbutamol on $[K^+]_v$ at rest was found in this thesis and the reason for this is not clear.

The $\Delta[K^+]_a$ was also corrected for fluid shifts, since a tendency to a more negative ΔPV (greater decline) was observed in the salbutamol trial. Salbutamol is a potent vasodilator in skeletal muscle (Chang 1997) and this may have caused the small initial drop in PV after 5 minutes evident at rest, at a time when no change in lactate was evident. The $\Delta[K^+]_{a,corr}$ was less (greater decline) after salbutamol compared to placebo, at each of 10 min (~0.36 mM), 20 min (-0.40 mM) and 30 min of rest (-0.40 mM), respectively which most likely due to stimulated uptake of K^+ into skeletal muscle. . Hence the $\Delta[K^+]_{a,corr}$ clearly demonstrated an early K^+ shift out of plasma following salbutamol inhalation, that persisted the 30 min prior to exercise.

This rapid time course of salbutamol action, with lowered $\Delta[K^+]_{a,corr}$ within 5 min of salbutamol inhalation, is consistent with previous reports of salbutamol onset of action occurring within 2-3 min and peaking within ~ 20 min (Buch, 2010; Sears and Lötvall, 2005). In other studies, after inhalation of a large 1200 μg dose of salbutamol, a reduction in serum $[K^+]_v$ was detected within 3 min, with significant declines at 5, 10 and 30 min following delivery (Mandelberg et al., 1999). Another study has also reported K^+ lowering effects of salbutamol persist for the same time. The K^+ lowering was evident at 30 min following injection of 4 μg/kg of salbutamol in children with renal failure (McClure et al., 1994). Furthermore, studies in healthy adults have shown that decreased $[K^+]$ persisted at 60 min (Rey et al., 1989) and at 120 min (Mandelberg et al., 1999) after salbutamol. The results from this thesis also showed that plasma $[K^+]$ remained significantly lower than baseline at ~114 min after inhalation of salbutamol (time includes exercise). This finding is consistent with previous reports that the duration of action of salbutamol is 2-4 hours (Pearlman et al., 1992).

The likely mechanism for the salbutamol-induced decline in $[K^+]$ is via stimulation of the Na^+,K^+-pump, most likely mediating uptake of K^+ into skeletal muscle (Clausen, 2003).

However, no difference in the plasma $[K^+]_{a-v}$ was detected between the salbutamol and placebo trials. Therefore the systemic K^+-lowering detected with salbutamol at rest could not be confirmed as being due to K^+ entering the forearm muscle. Hence K^+ clearance may have occurred in other tissues. This does not exclude the possibility that K^+ entered the skeletal muscle, as a positive $[K^+]_{a-v}$ reflecting net K^+ uptake may have been more easily detected across a large muscle mass such as legs.

5.2 Salbutamol lowered K^+ during continuous exercise

During continuous cycling exercise $[K^+]_a$ increased with increasing exercise intensity, reaching ~ 6 mM during exercise at 130% $\dot{V}O_{2peak}$. This acute hyperkalaemia results from K^+ efflux from the muscle intracellular space to the muscle extracellular space, which then accumulate in plasma (Clausen and Nielsen, 2007; Clausen, 2008; Mohr et al., 2004). This finding is consistent with other studies that show elevated arterial plasma $[K^+]$ as being proportional to exercise intensity (Vøllestad et al., 1994; Fraser et al., 2002;Medbo and Sejersted, 1990). This increase in $[K^+]_a$ occurs via K^+ release from contracting muscle, resulting from K^+ efflux across the sarcolemmal and t-tubular plasma membranes during the repolarisation phase of each action potential (Clausen, 2003). The plasma $[K^+]$ reflects the balance between K^+ released into plasma from active muscles, and K^+ cleared by inactive muscle and other tissues where the amount of K^+ released from muscle is dependent on the balance between cellular K^+ efflux and K^+ reuptake rates (Sejersted and Sjøgaard, 2000). Accumulation of K^+ can be cleared from the interstitial space via diffusion toward areas in the interstitium with lower $[K^+]$ or into the capillaries, to be transported in the blood to other tissues; and via active transport by the Na^+, K^+-pump back into the intracellular space, which is also activated by increased intracellular $[Na^+]$ (Sejersted and Sjøgaard, 2000; Glitsch, 2001). Following continuous exercise the plasma $[K^+]_a$ had returned to baseline values at 19

min of recovery, probably as a result of K^+ reuptake via the Na^+, K^+- pump. Importantly, at this time no difference in $[K^+]_a$ was found between salbutamol and placebo trials.

This continuous exercise model allowed comparison of K^+ dynamics during an identical exercise intensity and duration in the salbutamol and placebo trials. Whilst differences in $[K^+]_a$ during continuous exercise were not detected with salbutamol compared to placebo, the calculated $\Delta[K^+]_a$ exhibited a lesser rise following salbutamol during exercise at both 40% $\dot{V}O_{2peak}$ (~0.33 mM) and 130% $\dot{V}O_{2peak}$ (~0.47 mM), compared to placebo. This is the first study to investigate the effects of salbutamol on K^+ dynamics during exercise measured in arterial blood. Grove et al (1995) reported that $\Delta[K^+]_v$ was greater, with lower values by 0.33 mM after 8 mg an oral dose of salbutamol compared to placebo during a 3 min exercise step test. However, blood samples were drawn after exercise when $\Delta[K^+]$ is known to be declining rapidly and this also explains why no actual increase in $\Delta[K^+]$ was seen with exercise. Thus this result cannot be accepted as exercise $[K^+]$ data and should be rejected.

Previous studies have also reported lower serum $[K^+]$ during submaximal exercise at 80-85% $\dot{V}O_{2max}$ was following a 6 mg oral dose of salbutamol (Collomp et al., 2000a) and by 0.62 mM following a 4 mg oral dose of salbutamol during cycling exercise until exhaustion at 70% of maximal workload (Van Baak et al., 2000). This decrease in $[K^+]$ is most likely because salbutamol stimulates the Na^+, K^+-pump (Clausen, 2003). After correcting $\Delta[K^+]$ for fluid shifts, the $\Delta[K^+]_{a,corr}$ was less after salbutamol compared to placebo during exercise at 40% $\dot{V}O_{2peak}$ (~0.39mM) and at 130% $\dot{V}O_{2peak}$ (~0.46 mM). This clearly indicates that K^+ loss from plasma was greater and/or K^+ release from achieve muscles into plasma during exercise with salbutamol than placebo. It is not clear why no K^+- lowering was seen during exercise at 60% $\dot{V}O_{2peak}$.

5.3 Salbutamol had no effects on K^+ during Repeat Sprint Exercise

During repeat sprint exercise plasma $[K^+]_a$ increased above baseline to ~ 5 mM, although the rise was quite small (~0.5mM) compared with continuous exercise. This is probably because the very short duration of sprints caused limited K^+ release from muscles, plus the intervening recovery between each sprint and after each set allowed Na^+, K^+-pump-mediated K^+ uptake. Together, both likely constrained the rise in $[K^+]$ within each set. In comparison, $[K^+]_a$ was increased to 7.0 mM during a 30 s cycle ergometer sprint bout (McKenna *et al.*, 1997).

Arterial $[K^+]_a$ just prior to repeat sprint exercise did not differ between salbutamol and placebo. Consequently, it is not surprising that salbutamol did not induce any differences in $[K^+]_a$ during repeat sprint exercise compared with placebo. Furthermore, starting the exercise components of the trial with continuous exercise may have effectively served as a "warm up" for the Na^+, K^+- pump, increasing the in-vivo activity prior to commencement of the repeat sprint exercise. This and the subsequent recovery could be an explanation for not finding any effects on $[K^+]$ just prior to and during repeat sprint exercise, and thus the precluded detection of any performance enhancement.

5.4 Effects of salbutamol on exercise performance

To my knowledge this is the first study to examine the potential performance-enhancing effects of salbutamol during repeat sprint exercise. Administration of 1000 µg of salbutamol via inhalation did not improve work output during repeat sprint exercise measured either for individual sprint or for cumulative work. This is similar to findings in previous studies that investigated inhalation of salbutamol. Salbutamol inhalation at three different doses (200, 400 and 800 µg) did not improve 20 km time trial performance in cyclists or triathletes (Sporer et al., 2008). Similarly, inhalation of 800 µg salbutamol had no effect on endurance time during

cycle exercise at 85% $\dot{V}O_{2max}$ until exhaustion (Goubault et.al, 2001). In contrast, using a high oral dose (12 mg) salbutamol improved performance during cycling at 80-85% $\dot{V}O_{2\ peak}$ (Collomp et al., 2000b). A 6 mg oral salbutamol dose improved exercise performance by ~6 min during cycle ergometer exercise at 80-85% $\dot{V}O_{2max}$ until exhaustion (Collomp et al., 2000a), but no improvement was evident when cycling at 90% $\dot{V}O_{2max}$ (Collomp et al., 2002). Inhalation of 400 µg of salbutamol also had no effect on peak power, total work during cycling exercise at 70% $\dot{V}O_{2max}$ or on subsequent sprint time (Meeuwisse et al., 1992). During a 30 second Wingate test after a 4 mg oral dose of salbutamol both peak power and mean power were improved (Le Panse et al., 2007). However, a 12 mg oral dose of salbutamol had no effect on fatigue index during a Wingate test (Le Panse et al., 2006). Salbutamol did not appear to influence metabolism during exercise, with no effect on $\dot{V}O_2$ during exercise at 40%, 60% and 130% $\dot{V}O_{2peak}$, similar to lack of change found during cycling exercise at 70 % $\dot{V}O_{2max}$ following inhalation of 4 µg salbutamol (Meeuwisse *et al.*, 1992). Salbutamol also had no effect on blood lactate, which is similar to findings in previous studies (Van Baak et al., 2004, Collomp et al, 2005).

In consequence of all these results, it seems that the effects of salbutamol on exercise performance may depend on the dosage, delivery (inhalation or oral) and type of the exercise. Most studies that investigated inhalation of salbutamol failed to detect an improvement in exercise performance during either prolonged or sprint exercise. In contrast oral salbutamol administration improved performance during either submaximal or maximal exercise intensity. Salbutamol delivery via inhalation was used in this study since this is the means of administration most likely to be used in a non-clinical setting.

5.5 Salbutamol lowered K^+ during 30 min of recovery after repeat sprint exercise

The plasma $[K^+]_a$ during the first two minutes of the final 30 min recovery after repeat sprint exercise was not significantly different from baseline values following placebo. However, after salbutamol, $[K^+]_a$ declined to values as low as 3.47 mM at 5 min and 3.49 mM at 10 min during final recovery. This indicates that a combination of acute repeat sprint exercise and salbutamol caused acute hypokalaemia (i.e. < 3.5 mM). The decreased K^+ after exercise is likely related to K^+ re-uptake by previously exercised muscle due to increased muscle Na^+, K^+ ATPase activity. This finding is consistent with significantly lower (more negative) $\Delta[K^+]_{corr}$ after salbutamol. It is likely that relatively more K^+ shifts out of plasma after salbutamol. Also, Na^+, K^+ ATPase activity in muscle would likely be increased due to higher $[Na^+]$ in the working muscle exultation itself (Clausen, 2003) and the large increase in plasma catecholamines caused by high intensity exercise. When the activating effects of the catecholamines on K^+ uptake in muscles are suppressed by propranolol, the undershoot in plasma $[K^+]$ during recovery was prevented (Nielsen and Harrison, 1998; Medbo and Sejersted, 1990).

5.6 Limitations

This thesis has several limitations. Firstly, the results are based on data from a small number of subjects for most measures (n=7); hence limited statistical power may have resulted in failing to detect an existing effect (Type II error). The plasma salbutamol concentration was not measured due to technical difficulties as I could not find any clinical laboratory that measured salbutamol concentration in the blood, nor an established analytical method to do so. This would have been valuable in relating the $\Delta[K^+]_a$ to the actual plasma [salbutamol] and in understanding K^+ dynamics related to [salbutamol]. It is likely that an increased muscle Na^+, K^+-ATPase activity contributed to the lowering effects of salbutamol on $[K^+]$ at

rest, during exercise and in recovery, but this cannot be confirmed since no muscle analyses were undertaken in this thesis. Furthermore, the K^+_{a-v} differences across the forearm at rest were not significantly different from baseline so it is not clear that increased K^+ uptake occurred at least in forearm muscle. Another limitation was that forearm blood flow was not measured in this study; this would have been beneficial in calculating arterial K^+ uptake into or release from forearm musculature. Finally, the effects of salbutamol on heart rate at rest and during exercise could not be ascertained as the ECG data was not backed up on the PC hard drive at the time of trials and unfortunately was subsequently written over during later laboratory tests and could not be recovered.

5.7 Conclusions

In conclusion, inhalation of 1000 µg salbutamol reduced plasma $[K^+]_a$ by ~ 0.35 mM during rest. This salbutamol induced K^+- lowering effect was more clearly evident after calculating the $\Delta[K^+]_a$ and correcting for fluid shifts from baseline. This revealed K^+ lowering as early as 5 min after salbutamol inhalation As the plasma $[K^+]_{a-v}$ differences across the forearm were small and negative, an increased K^+ uptake into forearm muscle at rest could not be confirmed. These findings supports the hypothesis that salbutamol lowered systemic $[K^+]$, but does not confirm a skeletal muscle site of action. During continuous exercise of increasing intensity, the $\Delta[K^+]_a$ (rise in $[K^+]_a$) and $\Delta[K^+]_{a\ corr}$ were less after salbutamol during exercise at 40% and 130% $\dot{V}O_{2peak}$ compared with placebo. However, no difference in the $[K^+]_{a-v}$ was found with salbutamol, No differences in $[K^+]_a$, $\Delta[K^+]_a$ or $\Delta[K^+]_{a\ corr}$ were found between salbutamol and placebo during repeat sprint exercise and hence no performance change was evident. Therefore salbutamol altered plasma K^+ dynamics, decreasing arterial plasma $[K^+]$ at rest, during continuous exercise and during post-exercise recovery, but had no effect on performance during repeat sprint exercise. In summary, some of non-asthmatic athletes use b2-agonists salbutamol believing this could potentially improve their performance. However,

the results from this study showed that inhaled b2-agonists salbutamol do not have a relevant performance-enhancing effect in non-asthmatic athletes. Therefore recommended that inhaled salbutamol continue to be permitted in sport competition

5. 8 Recommendation for further research

There are many possibilities for further research into beta$_2$ agonists, potassium and exercise performance.

It would be valuable to investigate the effects of salbutamol on arterio-venous [K^+] differences across a large contracting muscle mass, such as the thigh muscle during cycling/kicking, to better understand impacts on muscle K^+ release during contraction and re-uptake during recovery. To gain more insight into the effects of salbutamol on muscle K^+ homeostasis, muscle biopsy studies would allow investigation of muscle K^+ contents and Na^+, K^+- pump activity.

This study investigated the effects of salbutamol on performance during repeat sprint exercise. Further research could use a different type of exercise, particularly a single bout of high intensity exercise where circulating [K^+] reach very high levels.

Finally, further studies are required to identify the effects of longer acting beta$_2$ agonists such as formoterol, salmeterol and fenoterol on K^+ regulation and exercise performance.

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APPENDICEs

Appendix 1

INFORMATION TO PARTICIPANTS INVOLVED IN RESEARCH

You are invited to participate in a research project entitled "The effects of salbutamol on potassium and exercise performance".
This project is being conducted by a student researcher Muath Altarawneh as part of a Master of Applied Science degree at Victoria University under the supervision of Prof. Michael J McKenna, Dr . Aaron Petersen and Dr. Francoise Billaut from the Institute of Sport, Exercise and Active Living, and the School of Sport and Exercise Science.

Project explanation

The project investigates the effects of inhaling salbutamol on potassium levels in the blood and on exercise performance. Salbutamol is a drug commonly used to treat symptoms of asthma attacks (i.e. it is the drug in Ventolin, or in this study, Asmol), due to its ability to relax smooth muscle and open airways. Salbutamol lowers potassium in the blood. We are testing whether salbutamol can reduce the large increase in potassium in the blood that normally occurs with exercise; and which is believed to be related to muscle fatigue.
We will measure potassium in the blood during rest, cycling exercise with increasing work rates, and during cycling exercise with repeated fatiguing sprint exercise bouts.
The study will enhance understanding of the relationships between salbutamol and potassium and whether potassium is important in muscle fatigue during exercise.

What will I be asked to do?

We will ask you to fill in several short questionnaires of about your family medical history and your exercise habits. You will be requested to attend the exercise physiology Laboratory at Victoria University, Footscray Park Campus (Room L305, building L) on three separate occasions for exercise testing. This will firstly involve doing a "maximal exercise test" where the work rate is increased progressively until you reach your highest level of using oxygen. The two experimental trials will involve doing cycling tests for a set time and load, followed by short "sprints" on the cycle ergometer. We will request your permission to take repeated blood samples from an artery and vein during these trials. We will ask you to take a dose of salbutamol or placebo, using a standard asthma "puffer" and a spacer. We will monitor your heart rhythm continuously during these tests. The exercise and salbutamol are described more fully below.

What will I gain from participating?

From participating to this study you can expect to gain information on your aerobic fitness levels (VO2max), understanding of fitness tests and of research.

How will the information I give be used?

Your samples will be stored under alphanumeric codes (i.e. without your name or personal details) and only the researchers will be able to connect the samples to you. The data that will be collected during the study will be used/published in peer-reviewed journals and conference presentations. No personal details will be revealed without your written consent.

What are the potential risks of participating in this project?

All exercises tests involve risks of sudden death due to myocardial infarct (heart attack the blockage of blood supply to part of the heart) as well as vasovagal episodes (slowing of heart rate fainting due to drop in blood pressure), muscle soreness and stiffness. Taking salbutamol can cause potassium levels in the blood to drop, which could cause muscle weakness and heart irregularities. We will monitor potassium levels and your heart rhythm closely throughout the tests to minimise this risk. You may experience some anxiety about participating.

How will this project be conducted?

You will be requested to attend the Exercise Physiology Laboratory at Victoria University, Footscray Park Campus (Room L305, building L) on three separate occasions for exercise testing trials.

Visit 1. Screening and Pre-Test Procedures:

You will be screened prior to entry into the study. You will be requested to complete the standard VU Cardiovascular Risk Questionnaire, the Catheterisation Questionnaire. You will be pre- screened by recording the electrical activity of the heart (ECG , electrocardiography), resting blood sample for potassium levels, baseline lung function test and incremental cycling exercise test to determine aerobic fitness (VO_2 peak). After a 30 min rest you will be familiarised with the exercise protocol for the later visits. You will exercise for 30 s at 130% VO_2 peak and undertake one set of 5 x 4 s "all – out" sprints on a cycle ergometer.

Visit 2 and 3. Experimental Trials

Salbutamol

You will be asked to inhale salbutamol (1000 ug, Asmol) or a placebo using a standard asthma puffer and a disposable spacer. You will be asked to take 10 "puffs" of the inhaler through the spacer. We will show you how to do this. In between each puff you will be asked to take 2-4 breaths through the spacer to ensure all of the salbutamol or placebo is inhaled. This will be done 30 minutes before starting the exercise tests. We anticipate that this dose of salbutamol will lower your blood potassium levels slightly. This will be monitored closely. If they drop too much we will give you some tablets with potassium and stop the test. If you experience any symptoms associated with low blood potassium levels, such as muscle weakness, muscle aches and cramps, or electrocardiography (ECG irregularities), then the trial will be stopped. We will monitor your heart rhythm throughout the test.

Venous Catheterisation

Blood samples will be taken during rest, exercise and recovery via a catheter placed in the arm. The catheter consists of a needle and teflon tubing. The tubing is fed over the top of the needle on entering the vein. The needle is then withdrawn, leaving only the teflon tubing in your vein for the remainder of the experiment. A tap (stopcock) is placed into the tubing so the flow of blood along the tubing can be altered at will. This procedure allows the taking of multiple blood samples without the need for multiple venepuncture (puncturing of the vein). Each time a blood sample is taken, a small volume of fluid will be injected to keep the catheter from clotting. Catheterisation is slightly uncomfortable, with minimal possibility of bruising and infection. The use of sterile, disposable catheters, syringes, single dose vials and aseptic techniques will markedly reduce the possibility of infection. Only staff qualified and experienced in venepuncture will be used in order to prevent complications. Although the possibility of infection, bleeding, local blood clots, local swelling and redness, and bruising are remote, should any one of these conditions eventuate, please inform us immediately and then consult your doctor.

Arterial Catheterisation

A similar catheter will be used as above, but will be inserted into the radial artery (wrist) of the other arm after the hand has been pre-warmed by holding the hand in warm water or under a warm-air hand dryer for approximately 5 minutes. Arterial puncture and catheterisation is more difficult and may involve more discomfort and bruising formation than with venous punctures. Pain is minimised by use of a local

anaesthetic in the skin and near the artery, whilst bleeding and bruising are minimised through use of appropriate pressure techniques for an adequate amount of time after arterial puncture or removal of the catheter. Infection is unlikely as only sterile, unused disposable instruments; single dose vials and aseptic techniques will be used. An experienced medical practitioner, who will remain throughout the entire testing and recovery procedures, will perform all arterial catheterisations.

Blood Samples

We will take blood samples from an artery and a vein whilst you are lying on a couch, during the exercise tests and in recovery.

Exercise Tests

For experimental trials you will exercise on a cycle ergometer for 5 minutes at 40% peak oxygen consumption (VO_2 peak), then 5 minutes at 60% peak oxygen consumption (VO_2 peak) and 90 seconds at 130% peak oxygen consumption (VO_2 peak). After 10 minutes passive recovery, you will perform a repeated-sprint exercise test comprising three sets of 5 x 4 second "all out" sprint with 20 seconds of passive recovery between sprints and 4.5minutes passive recovery between sets, followed by 30 minutes recovery. Fatigue during repeated-sprint exercise will be determined by relative decline in power and work.

Each exercise test is completed when you finish the protocol, or become too tired to continue (wish to stop), or if we have to stop the test due to you having an abnormal response to exercise, such as unusual heart rhythm, inappropriate heart rate or sweating responses, chest pain or severe shortness of breath. We will closely monitor you and your heart electrical activity electrocardiography (ECG) during exercise to minimise the risks of these. The most common event associated with maximal exercise testing is fainting. This risk will be minimised using our standard laboratory procedures. In the unlikely event of emergency situations, a medical practitioner will be in attendance, two members of the research team have current CPR (cardio pulmonary resuscitation) qualifications and the Western Hospital is minutes away by ambulance.

Leg Muscle Activity

We will place electrodes with gel on the skin over your leg muscles to measure how active they are by measuring the amount of electrical activity the muscle produce (EMG). During cycling test we will measure your leg muscle activity using electromyography (EMG). The skin over your leg muscles will be shaved, abraded and cleaned with alcohol swabs before placing the electrodes onto the skin. The EMG transmitter will be attached to you via a leather belt around the waist. This is powered by a low voltage battery (12 volts) and is not connected to any external electrical device. There is no discomfort associated with the application, wearing, or removal of the EMG electrodes. This is a safe and painless procedure.

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Who is conducting the study?

The study is conducted by the Institute of Sport, Exercise and Active Living and by the School of Sport and Exercise Science, Victoria University. The Main Investigators:

Prof. Michael J. McKenna, Telephone number 9919 4499, Mobile 0432 757 859, email michael.mckenna@vu.edu.au

Mr. Mauth Altarawneh, Mobile 0411806819, email muath.altarawneh@live.vu.edu.au

Any queries about your participation in this project may be directed to the Principal Researcher listed above. If you have any queries or complaints about the way you have been treated, you may contact the Secretary, Victoria University Human Research Ethics Committee, Victoria University, PO Box 14428, Melbourne, VIC, 8001 phone (03) 9919 4781.

Appendix 2

CONSENT FORM FOR PARTICIPANTS INVOLVED IN RESEARCH

I
NFORMATION TO PARTICIPANTS:
We would like to invite you to be a part of a study investigating the effects of salbutamol on plasma potassium and exercise performance.

INVESTIGATORS:
Professor Michael McKenna
Dr. AaronPetersen and
Dr. Francoise Billaut
Mr. Muath Altarawneh

AIMS OF THE STUDY - The major aim of this project is to investigate the effect of acute administration of salbutamol (1000 ug) by inhalation on potassium concentration, fatigue, and exercise performance during cycle exercise, in non-asthmatic participants.

PARTICIPANT INVOLVEMENT AND OVERVIEW OF TESTING - Participants will be requested to attend the Exercise Physiology Laboratory at Victoria University, Footscray Park Campus (Room L305, building L) on three separate occasions for exercise testing trials,

EXERCISE TESTING PROCEDURES – Participants will be asked to undertake high-intensity cycling exercise tests over several visits.

SALBUTAMOL DOSE - Each subject will be given an inhaled dose of salbutamol 30 minutes prior to the test.

VENOUS CATHETERISATION - Blood samples will be taken during rest, exercise and recovery via a catheter placed in the arm. This procedure allows the taking of multiple blood samples without the need for multiple venepuncture (puncturing of the vein). Catheterisation is slightly uncomfortable, with minimal possibility of bruising and infection.

ARTERIAL CATHETERISATION - After pre-warming the hand, a similar catheter will be used as above. Arterial puncture and catheterisation is more difficult and may involve more discomfort and bruising formation than with venous punctures. An experienced medical practitioner, who will remain throughout the entire testing and recovery procedures, will perform all arterial catheterisations.

MUSCLE ACTIVITY – Electrodes will be placed on the skin above your muscles in your legs to determine how active your muscles are. This uses an EMG which is a battery operated system that is non-invasive and poses no inconvenience.

CERTIFICATION BY SUBJECT

I, (participant name) …….

of (suburb) ……..

certify that I am at least 18* years old and that I am voluntarily giving my consent to participate in the study: ***Effects of salbutamol on potassium and exercise performance,*** being conducted at Victoria University by: Professor Michael McKenna.

I certify that the objectives of the study, together with any risks and safeguards associated with the procedures listed hereunder to be carried out in the research, have been fully explained to me by:

Mr Muath Altarawneh

and that I freely consent to participation involving the use on me of these procedures:

Preliminary screening
Maximal incremental cycling test
High-intensity exercise on cycle ergometer
salbutamol and placebo administration under experimental conditions
Arterial catheterisation and blood sampling during rest, exercise and recovery
Antecubital venous catheterisation and blood sampling during rest, exercise and recovery
Measurement of muscle activity by Electromyographic (EMG)

I certify that I have had the opportunity to have any questions answered and that I understand that I can withdraw from this study at any time and that this withdrawal will not jeopardise me in any way.

I have been informed that the information I provide will be kept confidential.

Signed: …….

Witness other than the researcher: …….

Date: ……

Any queries about your participation in this project may be directed to the researcher Professor Michael McKenna, 9919 4499. If you have any queries or complaints about the way you have been treated, you may contact the Secretary, Victoria University Human Research Ethics Committee, Victoria University, PO Box 14428, Melbourne, VIC, 8001 phone (03) 9919 4710
[*please note: Where the participant/s are aged under 18, separate parental consent is required; where the participant/s are unable to answer for themselves due to mental illness or disability, parental or guardian consent may be required.]

Appendix 3

VICTORIA UNIVERSITY | A NEW SCHOOL OF THOUGHT

CARDIOVASCULAR AND OTHER RISK FACTORS QUESTIONNAIRE

In order to be eligible to participate in the experiment investigating: "*Effects of salbutamol on potassium and exercise performance*" you are required to complete the following questionnaire which is designed to assess the risk of you having a cardiovascular event occurring during an exhaustive exercise bout.

Name: _____ Date: _____

Age: _____ years Weight: _____ kg Height: _____ cms Gender:
M F

Give a brief description of your average activity pattern in the past 2 months:

_____
_____

_____
_____

_____
_____

Circle the appropriate response to the following questions.

Are you overweight?	Yes	No	Don't know
Do you smoke?	Yes	No	Social
Are you an asthmatic?	Yes	No	Don't Know
Are you a diabetic?	Yes	No	Don't Know
Does your family have a history of diabetes?	Yes	No	Don't Know
Do you have a thyroid disorder?	Yes	No	Don't Know
Does your family have a history of thyroid disorders?	Yes	No	Don't Know
Do you have a pituitary disorder?	Yes	No	Don't Know

Does your family have a history of pituitary disorders? Yes No

Don't Know

Do you have a heart rhythm disturbance? Yes No Don't Know

Do you have a high blood cholesterol level? Yes No Don't Know

Do you have elevated blood pressure? Yes No Don't Know

Are you being treated with diuretics? Yes No

Are you on any other medications? Yes No

List all medications? (including oral contraceptives)

Do you think you have any medical complaint or any other reason which you know of which you think may prevent you

from participating in strenuous exercise? Yes No

If Yes, please elaborate

_____

Have you had any musculoskeletal problems that have required medical treatment (eg, broken bones, joint reconstruction etc)? Yes No

If Yes, please provide details (including dates)

_____

Are you currently pregnant or expect to become pregnant during the time in which this experiment is conducted? Yes No

Does your family have a history of premature cardiovascular problems

(e.g. heart attack, stroke)? Yes No Don't Know

I, _____, believe that the answers to these questions are true and correct.

Signed: _____ Date: _____

Appendix 4

VICTORIA UNIVERSITY | A NEW SCHOOL OF THOUGHT

ARTERIAL & VENOUS CATHETERISATION QUESTIONNAIRE

Effects of salbutamol on potassium and exercise performance.

NAME: _____

ADDRESS: _____

_____

DATE: _____ AGE: _____ years

1. Have you or your family suffered from any tendency to bleed excessively? (e.g. Haemophilia) or bruise very easily? Yes No Don't Know

   If yes, please elaborate

   _____

2. Are you allergic to local anaesthetic? Yes No Don't Know

   If yes, please elaborate

   _____

3. Do you have any skin allergies? Yes No Don't Know

   If yes, please elaborate

   _____

4. Have you any other allergies? Yes No Don't Know

   If yes, please elaborate

   _____

5. Are you currently on any medication? Yes No

   If yes, what is the medication?

   _____

6. Do you have any other medical problems? Yes No

   If yes, please elaborate

   _____

7. Have you ever fainted when you had an injection or blood sample taken? Yes No Don't know

   If yes, please elaborate

   _____

8. Have you previously had heparin infused or injected? Yes No Don't know

If yes, please elaborate

   _____

9. Do you or other members of your family have Raynauds disease, or suffer from very poor circulation in the fingers,

leading to painful fingers that turn white/blue? Yes No Don't know

   If yes, please elaborate

   _____

To the best of my knowledge, the above questionnaire has been completely accurately and truthfully.

Signature: _____ Date: _____

Appendix 5. Subject physical characteristics

Subjects	Age (years)	Body mass (kg)	Height (cm)
1	35	87.63	191.4
2	19	73.11	179.6
3	18	60.79	172.5
4	23	71.8	189.6
5	27	76.76	171
6	21	68.5	170.7
7	20	67	171.4
n	7	7	7
Mean	23.29	72.23	178.03
SD	5.96	8.47	9.06

Appendix 6. Mechanical work (J) during Repeat Sprint Exercise in salbutamol and placebo

Salbutamol																						
	Set1					**Set1**	**Set2**					**Set2**	**Set3**					**Set3**				
Subjects	**sp1**	**sp2**	**sp3**	**sp4**	**sp5**	**Sum 5sprints**	**sp1**	**sp2**	**sp3**	**sp4**	**sp5**	**Sum 5sprints**	**sp1**	**sp2**	**sp3**	**sp4**	**sp5**	**Sum 5sprints**				
																					TOTAL WORK	
1	3673.86	3966.742	2641.83	3669.381	3526.457	17478.27	3968.743	3788.783	3603.71	2738.387	3256.46	17356.08	3790.025	3582.161	3244.411	2735.623	3148.724	16500.94			51335.3	
2	3609.929	3686.202	3463.448	3305.615	3281.879	17347.07	3400.717	3294.182	3213.722	3239.347	3119.405	16267.37	3548.501	3357.198	3214.332	3090.337	3081.48	16291.85			49906.3	
3	2849.257	2584.763	2165.479	2205.67	1700.357	11505.53	2259.482	2109.098	2406.503	2351.691	2451.783	11578.56	2260.764	2334.886	2432.792	2364.898	2514.117	11907.46			34991.54	
4	4290.767	4146.036	3955.812	3800.399	3516.825	19709.84	4343.474	4219.848	3932.497	3704.738	3228.988	19429.54	4172.951	4081.12	3909.03	3594.364	3486.215	19243.68			58383.06	
5	2440.781	2949.805	3121.564	2908.803	2674.834	14095.79	2472.097	3072.98	3058.542	2692.728	2631.044	13927.39	2960.433	3019.816	2934.76	3029.708		11944.72			39967.89	
6	2962.632	2944.896	2396.403	2611.628		10915.56	3330.797	2567.965	2838.622	2670.552	2554.109	13962.05	2688.849	2655.161	2449.937	2431.609	2492.868	12718.43			37596.03	
Mean	3304.537	3379.741	2957.423	3083.583	2940.07	15175.34	3295.885	3175.476	3175.599	2899.574	2873.632	15420.17	3236.92	3171.724	3030.877	2874.423	2944.681	14767.84			45363.35	
SD	674.1761	637.436	681.3666	621.3377	774.6884	3559.784	814.2663	774.2979	543.3952	486.853	366.6441	2816.136	722.339	635.6211	557.6451	461.3052	431.0241	3023.617			9196	
	Placebo																					
	Set1					**Set1**	**Set2**					**Set2**	**Set3**					**Set3**				
Subjects	**sp1**	**sp2**	**sp3**	**sp4**	**sp5**	**Sum 5sprints**	**sp1**	**sp2**	**sp3**	**sp4**	**sp5**	**Sum 5sprints**	**sp1**	**sp2**	**sp3**	**sp4**	**sp5**	**Sum 5sprints**				
																					TOTAL WORK	
1	3654.794	3626.725	3522.216	3329.605	2785.106	16918.45	3621.495	3661.171	3400.171	3196.711	3149.538	17029.09	2950.049	2946.27	3446.655	3239.026	3045.637	15627.64			49575.17	
2	3950.408	3662.765	3112.521	3418.715	3173.914	17318.32	3351.241	3391.575	3294.469	3282.103	3313.86	16633.25	3573.399	3299.471	3376.784	2934.529	3012.104	16196.29			50147.86	
3	2938.312	2816.521	2675.794	2654.35	2459.625	13544.6	2763.002	2686.343	2507.198	2514.423	2476.575	12947.54	2723.705	2550.523	2624.805	2440.339	2471.586	12810.96			39303.1	
4	3951.852	3913.837	3970.354	3987.396	3846.348	19669.79	3130.685	4235.509	3944.964	3808.472	3676.941	18796.57	4119.219	3948.397	3922.226	3381.482	3712.135	19083.46			57549.82	
5	3168.125	2819.227	2348.058	2711.435	2572.126	13618.97	2565.043	2370.005	2330.24	2619.623	2563.928	12448.84	2802.597	2623.162	2742.408	2661.938	2621.967	13452.07			39519.88	
6	2937.732	3323.042	3128.167		3056.149	12445.09	3662.787	3237.741	3030.743	2746.65	2967.749	15645.67	3174.781	3079.448	3043.221	2849.487	2697.185	14844.12			42934.88	
Mean	3433.537	3360.353	3126.185	3220.3	2982.211	15585.87	3182.375	3263.724	3084.631	3027.997	3024.765	15583.49	3223.958	3074.545	3192.683	2917.8	2926.769	15335.76			46505.12	
SD	478.806	460.1551	579.3077	552.0406	503.7989	2805.549	449.8404	671.0371	598.1989	492.3127	456.204	2461.453	534.5735	511.6705	485.4275	351.1603	445.2003	2237.658			7184.557	

Appendix 7. Oxygen consumption (L.min-1) during cycling exercise at 40%, 60%, and 130% VO_{2peak} in salbutamol and placebo

	Salbutamol				placebo			
Subjects	**Rest**	**40%**	**60%**	**130%**	**rest**	**40%**	**60%**	**130%**
		(L.min-1)				(L.min-1)		
1	0.51	1.62	2.70	4.26	1.00	1.50	2.53	2.76
2	0.74	1.62	2.22	3.26	0.48	1.58	2.31	3.30
3	0.55	1.85	2.39	3.51	0.48	1.90	2.08	3.11
4	0.37	1.43	1.94	2.62	0.36	1.32	1.69	2.59
5	0.43	1.06	1.55	2.39	0.64	1.39	1.86	2.90
6	0.37	1.22	1.61	2.17	0.39	1.51	1.81	2.51
7	0.93	1.88	2.62	4.10	0.44	2.26	2.42	3.58
8	0.52	1.69	2.09	3.36	0.83	1.83	1.96	2.86
n	8	8	8	8	8	8	8	8
mean	0.55	1.55	2.14	3.21	0.58	1.66	2.08	2.95
SD	0.19	0.29	0.43	0.77	0.23	0.31	0.31	0.36
SEM	0.07	0.10	0.15	0.27	0.08	0.11	0.11	0.13

Appendix 8. Arterial plasma [K^+] (mmol.l^{-1}) in salbutamol (uncorrected)

Subject	PreS	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3	1	2	5	10	30
1	3.94	3.67	3.38	3.29	3.26	4.32	4.62	6.38	3.74	4.73	3.31	4.75	3.45	4.65	3.81	3.57	3.38	3.39	3.59
2	4.20	3.78	3.65	3.62	3.92	4.40	4.67	6.04	4.03	5.07	3.85	4.98	4.12	4.84	3.85	3.68	3.68	3.95	4.03
3	4.17	4.10	4.20	4.09	4.14	4.68	4.79	5.45	3.99	5.00	3.38	4.73	3.78	4.80	3.97	3.61	3.43	3.61	3.85
4	3.90	3.94	3.39	3.24	3.29	4.56	4.89	6.40	3.21	4.90	3.55	4.87	3.74	4.58	4.82	3.80	3.62	3.27	3.35
5	3.87	3.70	4.04	4.07	4.18	4.07	4.47	4.19	3.74	4.16	3.72	4.05	3.52	3.85	3.90	3.35	3.33	3.32	3.12
6	4.1	3.89	3.87	3.75	3.77	4.50	4.70	5.97	3.66	5.17	3.17	4.79	3.27	5.05	3.93	3.37	3.39	3.43	3.66
7	3.95	3.86	3.73	3.63	3.49	4.25	4.88	6.12	3.77	4.52	3.48	4.55	3.41	4.43	3.90	3.75	3.49	3.49	3.47
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
mean	4.02	3.85	3.75	3.67	3.72	4.40	4.72	5.79	3.73	4.79	3.49	4.67	3.61	4.60	4.02	3.59	3.47	3.49	3.58
SD	0.13	0.15	0.31	0.33	0.38	0.20	0.15	0.78	0.27	0.35	0.24	0.31	0.29	0.39	0.35	0.17	0.13	0.23	0.30
SEM	0.05	0.06	0.12	0.13	0.14	0.08	0.06	0.29	0.10	0.13	0.09	0.12	0.11	0.15	0.13	0.07	0.05	0.09	0.11

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Appendix 9. Arterial plasma potassium concentration $[K+]_a$ (mmol.l-1) in placebo (uncorrected)

Subject	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post S1	RS2	Post S2	RS3	1	2	5	10	30
1	3.74	3.82	3.77	3.66	3.76	4.33	4.53	6.55	3.80	4.85	3.35	4.61	3.37	4.68	3.68	3.44	3.36	3.46	3.55
2	3.72	3.74	3.79	3.76	3.85	4.57	4.98	6.05	4.07	3.96	3.70	5.21	3.75	4.72	3.93	3.53	3.86	3.76	3.85
3	3.84	3.87	3.98	3.83	3.88	4.28	4.30	5.55	3.55	4.39	3.37	4.44	3.21	4.25	3.76	3.41	3.41	3.17	3.46
4	3.75	3.78	3.76	3.70	3.65	4.61	4.66	6.45	3.54	5.17	3.29	4.59	3.25	4.79	3.81	3.47	3.40	3.57	3.47
5	3.91	3.90	3.97	3.96	3.90	4.20	4.05	5.38	3.65	4.21	3.43	4.27	3.27	4.17	3.63	3.34	3.20	3.49	3.60
6	4.18	4.06	4.07	4.06	4.08	4.68	4.82	5.90	3.87	5.01	3.59	4.89	3.63	5.09	3.74	3.75	3.67	3.70	3.85
7	3.80	3.78	3.76	3.79	3.76	5.26	5.16	6.74	3.95	4.82	4.04	4.46	3.70	4.45	3.95	3.95	3.87	3.87	3.84
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
mean	3.85	3.85	3.87	3.82	3.84	4.56	4.64	6.09	3.77	4.63	3.54	4.64	3.45	4.59	3.78	3.55	3.54	3.57	3.66
SD	0.16	0.11	0.13	0.14	0.14	0.36	0.38	0.52	0.20	0.45	0.26	0.32	0.23	0.32	0.12	0.22	0.26	0.23	0.18
SEM	0.06	0.04	0.05	0.05	0.05	0.13	0.15	0.19	0.08	0.17	0.10	0.12	0.09	0.12	0.05	0.08	0.10	0.09	0.07

Appendix 10. Arterial plasma [K^+] ($mmol.l^{-1}$) in salbutamol (corrected for fluid shifts)

Subject	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3	1	2	5	10	30
1	3.94	3.62	3.36	3.29	3.28	4.10	4.35	5.77	3.61	4.46	3.12	4.42	3.32	4.35	3.60	3.38	3.27	3.30	3.62
2	4.20	3.69	3.54	3.54	3.70	4.09	4.30	5.40	3.81	4.62	3.59	4.54	4.12	4.35	3.47	3.34	3.36	3.68	3.97
3	4.17	4.06	4.19	4.13	4.03	4.36	4.45	5.31	3.92	4.68	3.13	4.28	3.41	4.23	3.57	3.25	3.13	3.38	3.78
4	3.90	3.93	3.41	3.28	3.37	4.34	4.60	5.83	3.23	4.64	3.41	4.59	3.57	4.40	4.61	3.67	3.54	3.29	3.53
5	3.87	3.51	3.80	3.85	3.92	3.62	3.97	3.67	3.34	3.74	3.37	3.57	3.19	3.42	3.49	3.01	3.04	3.11	2.99
6	4.10	3.44	3.49	3.40	3.44	3.84	3.97	4.91	3.35	4.40	2.71	3.99	2.91	4.28	3.39	2.92	2.98	3.07	3.28
7	3.95	3.81	3.67	3.58	3.47	4.03	4.81	5.50	3.63	4.18	3.24	4.17	3.22	4.12	3.65	3.53	3.29	3.42	3.62
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
mean	4.02	3.72	3.64	3.58	3.60	4.05	4.35	5.20	3.56	4.39	3.22	4.22	3.39	4.16	3.68	3.30	3.23	3.32	3.54
SD	0.13	0.22	0.29	0.31	0.29	0.26	0.31	0.74	0.26	0.34	0.28	0.36	0.38	0.34	0.42	0.27	0.19	0.20	0.32
SEM	0.05	0.08	0.11	0.12	0.11	0.10	0.12	0.28	0.10	0.13	0.11	0.13	0.14	0.13	0.16	0.10	0.07	0.08	0.12

Appendix 11. Arterial plasma [K^+] (mmol.l^{-1}) in placebo (corrected for fluid shifts)

Subject	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3	1	2	5	10	30
1	3.74	3.79	3.80	3.63	3.76	4.10	4.20	5.96	3.68	4.55	3.15	4.27	3.16	4.34	3.46	3.23	3.21	3.36	3.71
2	3.72	3.62	3.68	3.63	3.59	4.15	4.48	5.33	3.70	3.71	3.41	4.70	3.41	4.18	3.53	3.21	3.54		3.56
3	3.84	3.88	3.99	3.79	3.91	4.18	4.12	5.21	3.59	4.28	3.28	4.24	3.08	4.00	3.58	3.24	3.30	3.10	3.57
4	3.98	3.92	3.95	4.13	3.88	4.21	3.99	5.55	3.70	4.15	3.57	4.30	3.47	4.35	3.68	3.44	3.10	3.89	3.64
5	3.91	3.90	3.97	3.96	3.90	4.20	4.05	5.38	3.65	4.21	3.43	4.27	3.27	4.17	3.63	3.34	3.20	3.49	3.60
6	4.18	4.06	4.07	4.06	4.08	4.68	4.82	5.90	3.87	5.01	3.59	4.89	3.63	5.09	3.74	3.75	3.67	3.70	3.85
7	3.80	3.78	3.76	3.79	3.76	5.26	5.16	6.74		4.82	4.04	4.46	3.70	4.45	3.95	3.95	3.87	3.87	3.84
n	7	7	7	7	7	7	7	7	6	7	7	7	7	7	7	7	7	6	7
mean	3.88	3.85	3.89	3.85	3.84	4.40	4.40	5.72	3.70	4.39	3.49	4.45	3.39	4.37	3.65	3.45	3.41	3.57	3.68
SD	0.16	0.14	0.14	0.20	0.15	0.42	0.44	0.53	0.09	0.44	0.28	0.26	0.23	0.35	0.16	0.29	0.28	0.31	0.12
SEM	0.06	0.05	0.05	0.08	0.06	0.16	0.17	0.20	0.04	0.17	0.11	0.10	0.09	0.13	0.06	0.11	0.11	0.13	0.05

Appendix 12. Venous plasma [K^+](mmol.l^{-1}) in salbutamol (uncorrected)

subject	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3	1	2	5	10	30
1	3.95	3.64	3.44	3.34	3.35	4.10	4.40	**4.37**	3.66	4.32	3.45	4.11	3.61	4.08	3.92	3.70	3.42	3.43	3.60
2	4.08	3.71	3.87	3.59	3.85	4.16	4.44	4.84	3.93	4.36	3.84	4.13	3.58	3.97		3.99	3.70	3.88	3.82
3	4.17	3.99	4.23	4.10	4.45	4.53	4.52	4.47	4.27	4.29	4.21	4.04	3.75	3.97	4.13	3.70	3.56	3.47	3.84
4	3.95	3.71	3.66	3.56	3.39	4.06	4.22	5.48	3.43	4.34	3.76	4.11	3.55	4.11	3.61	3.58	3.24	3.48	3.34
5	4.06	3.98	3.88	3.91	4.03	4.55	4.80	5.38	3.63	4.71	3.39	4.63	3.44	4.21	3.74	3.59	3.43	3.55	3.89
6	4.25	4.56	4.22	4.05	4.00	3.92	4.64	4.96	3.69	4.59	3.68	4.13	3.63	4.21	3.51	3.46	3.34	3.42	3.48
7	4.16	4.27	4.05	3.98	4.01	4.23	4.72	5.38	3.66	4.65	3.53	4.38	3.53	4.21	3.62	3.52	3.38	3.48	3.69
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	6	7	7	7	7
mean	4.09	3.98	3.90	3.79	3.87	4.22	4.53	4.98	3.75	4.47	3.69	4.22	3.58	4.11	3.75	3.65	3.44	3.53	3.66
SD	0.11	0.34	0.29	0.29	0.39	0.24	0.20	0.45	0.27	0.18	0.28	0.21	0.10	0.11	0.23	0.17	0.15	0.16	0.20
SEM	0.04	0.13	0.11	0.11	0.15	0.09	0.08	0.17	0.10	0.07	0.11	0.08	0.04	0.04	0.09	0.07	0.06	0.06	0.08

Appendix 13. Venous plasma potassium concentration $[K^+]_a$ ($mmol.l^{-1}$) in placebo (uncorrected)

Subject	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3	1	2	5	10	30
1	4.46	4.17	3.87	3.89	3.80	4.34	4.18	4.39	3.92	4.56	3.44	4.23	3.40	4.29	3.75	3.51	3.36	3.42	3.56
2	3.75	3.71	3.68	3.81	3.70	4.37	4.31	4.77	3.93	5.03	3.67	4.44	3.62	4.68	4.34	3.76	3.57	3.58	4.12
3	4.06	4.11	4.02	4.13	4.13	4.50	4.31	4.35	3.63	3.80	3.48	3.53	3.46	3.60	3.78	3.17	3.30	3.25	3.55
4	3.98	3.91	3.89	4.00	3.77	4.44	4.25	6.15	3.72	4.40	3.71	4.61	3.66	4.68	3.89	3.61	3.21	3.92	3.42
5	4.05	4.04	4.07	4.07	4.18	4.73	4.47	4.53	4.34	4.50	3.66	4.32	3.77	4.31	4.42	3.89	3.60	3.68	3.98
6	4.17	4.20	4.20	4.12	4.20	4.26	4.64	4.64	3.95	4.42	3.80	4.36	3.71	4.29	3.88	3.71	3.70	3.74	3.72
7	3.66	3.89	3.80	3.83	3.63	4.34	4.84	4.86	3.94	4.44	4.03	4.24	3.83	4.33	4.21	3.95	3.93	3.75	3.98
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
mean	4.02	4.00	3.93	3.98	3.91	4.42	4.43	4.81	3.92	4.45	3.68	4.24	3.63	4.31	4.04	3.65	3.52	3.62	3.76
SD	0.27	0.18	0.17	0.13	0.24	0.16	0.24	0.62	0.22	0.36	0.20	0.34	0.16	0.36	0.28	0.26	0.25	0.23	0.27
SEM	0.10	0.07	0.07	0.05	0.09	0.06	0.09	0.23	0.09	0.14	0.08	0.13	0.06	0.14	0.10	0.10	0.09	0.09	0.10

Appendix 14. Venous plasma [K^+](mmol.l^{-1}) in salbutamol (corrected for fluid shifts)

Subject	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3	1	2	5	10	30
1	3.95	3.57	3.39	3.34	3.37	3.91	4.12	4.19	3.55	4.09	3.24	3.89	3.43	3.86		3.50	3.25	3.30	3.57
2	4.08	3.59	3.72	3.50	3.63	3.85	4.07	4.45	3.66	4.00	3.54	3.76	3.27	3.61		3.99	3.35	3.85	3.82
3	4.17	4.03	4.20	4.06	4.34	4.20	4.18	4.13	4.18	4.32	3.81	3.80	3.37	3.61	3.73	3.36	3.22	3.22	3.74
4	3.95	3.68	3.66	3.54	3.57	3.77	3.90	4.97	3.40	4.32	3.53	3.82	3.55	3.76	3.42	3.35	3.11	3.39	3.40
5	4.06	3.94	3.56	3.54	3.76	4.57	4.20	4.84	3.15	4.23	2.95	4.16	3.44	3.79	3.27	3.13	3.06	3.20	3.57
6	4.25	4.20	3.95	3.85	3.80	4.03	4.25	0.00	3.58	4.30	3.29	3.73	3.63	3.79	3.18	3.13	3.10	3.22	3.41
7	4.16	4.24	4.12	3.96	4.07	4.15	4.44	5.11	3.55	4.36	3.31	4.11	3.53	3.95	3.39	3.31	3.24	3.47	3.86
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	5	7	7	7	7
mean	4.09	3.89	3.80	3.68	3.79	4.07	4.17	3.96	3.58	4.23	3.38	3.89	3.46	3.77	3.40	3.40	3.19	3.38	3.62
SD	0.11	0.28	0.30	0.27	0.32	0.27	0.17	1.79	0.31	0.14	0.27	0.17	0.12	0.12	0.21	0.29	0.10	0.23	0.19
SEM	0.04	0.11	0.11	0.10	0.12	0.10	0.06	0.67	0.12	0.05	0.10	0.06	0.05	0.05	0.09	0.11	0.04	0.09	0.07

Appendix 15. Venous plasma $[K^+]_v$ (mmol.l^{-1}) in placebo (corrected for fluid shifts)

Subject	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3	1	2	5	10	30
1	4.46	4.11	3.87	3.86	3.83	4.07	4.01	4.17	3.76	4.29	3.26	3.92	3.18	3.96	3.52	3.29	3.19	3.32	3.69
2	3.75	3.65	3.61	3.73	3.51	4.05	3.90	4.28	3.56	4.42	3.35	4.06	3.32	4.24	3.96	3.44	3.33	3.36	3.86
3	4.06	3.99	3.89	3.97	4.05	4.20	4.02	4.07	3.58	3.62	3.27	3.42	3.22	3.37	3.47	3.18	3.18	3.07	3.54
4	3.98	4.10	3.98	4.11	3.87	4.24	4.03	5.66	3.74	4.16	3.48	4.30	3.44	4.32	3.65	3.39	3.39	3.93	3.64
5	4.05	4.01	4.04	4.07	4.19	4.59	4.27	4.28	4.23	4.38	3.42	4.12	3.54	4.13	4.10	3.63	3.39	3.53	3.98
6	4.17	4.18	4.22	4.16	4.21	4.46	4.23	4.31	3.92	4.23	3.59	4.12	3.65	4.16	3.68	3.56	3.64	3.77	4.09
7	3.66	3.76	3.68	3.70	3.65	3.99	4.29	4.38	0.00	4.04	3.69	3.92	3.50	4.02	3.87	3.62	3.67	3.59	4.02
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
mean	4.0	4.0	3.9	3.9	3.9	4.2	4.1	4.4	3.3	4.2	3.4	4.0	3.4	4.0	3.8	3.4	3.4	3.5	3.8
SD	0.27	0.19	0.21	0.18	0.27	0.22	0.15	0.54	1.45	0.27	0.16	0.28	0.17	0.31	0.23	0.17	0.20	0.29	0.21
SEM	0.10	0.07	0.08	0.07	0.10	0.08	0.06	0.20	0.55	0.10	0.06	0.11	0.07	0.12	0.09	0.06	0.07	0.11	0.08

Appendix 16. Arterio -Venous difference plasma $[K^+]_{a-v}$ ($mmol.l^{-1}$) in salbutamol (uncorrected)

Subject	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3	1	2	5	10	30
1	-0.01	0.03	-0.06	-0.04	-0.09	0.22	0.23	2.02	0.08	0.40	-0.15	0.64	-0.17	0.58	-0.12	-0.13	-0.04	-0.04	0.00
2	0.13	0.06	-0.22	0.04	0.06	0.24	0.23	1.20	0.11	0.71	0.01	0.86	0.54	0.88		-0.31	-0.03	0.07	0.22
3	0.00	0.12	-0.03	-0.01	-0.31	0.15	0.27	0.98	-0.28	0.70	-0.83	0.69	0.03	0.84	-0.16	-0.10	-0.14	0.14	0.01
4	-0.05	0.24	-0.27	-0.32	-0.10	0.49	0.67	0.93	-0.22	0.57	-0.21	0.76	0.19	0.48	1.21	0.22	0.38	-0.21	0.01
5	-0.19	-0.29	0.17	0.15	0.15	-0.48	-0.34	-1.20	0.12	-0.55	0.33	-0.59	0.08	-0.37	0.16	-0.24	-0.11	-0.23	-0.77
6	-0.15	-0.68	-0.36	-0.30	-0.24	0.58	0.06	1.01	-0.03	0.58	-0.51	0.66	-0.37	0.84	0.43	-0.09	0.05	0.01	0.18
7	-0.20	-0.41	-0.32	-0.35	-0.52	0.02	0.16	0.74	0.11	-0.14	-0.05	0.17	-0.12	0.22	0.27	0.23	0.10	0.01	-0.22
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	6	7	7	7	7
mean	-0.07	-0.13	-0.16	-0.12	-0.15	0.17	0.18	.0.81	-0.02	0.33	-0.20	0.46	0.03	0.49	0.30	-0.06	0.03	-0.04	-0.08
SD	0.12	0.33	0.19	0.20	0.23	0.21	0.19	0.41	0.17	0.48	0.37	0.51	0.29	0.45	0.50	0.21	0.17	0.14	0.33
SEM	0.05	0.13	0.07	0.08	0.09	0.13	0.11	0.37	0.06	0.18	0.14	0.19	0.11	0.17	0.20	0.08	0.07	0.05	0.13

Subjects	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3	1	2	5	10	30
1	-0.73	-0.36	-0.10	-0.23	-0.04	0.00	0.35	2.16	-0.12	0.29	-0.09	0.38	-0.04	0.39	-0.07	-0.06	-0.01	0.04	0.005
2	-0.03	0.03	0.11	-0.05	0.15	0.20	0.67	1.28	0.13	-1.07	0.03	0.77	0.14	0.04	-0.41	-0.23	0.29		-0.27
3	-0.22	-0.24	-0.04	-0.31	-0.26	-0.22	-0.01	1.20	-0.09	0.60	-0.11	0.91	-0.25	0.66	-0.02	0.24	0.11	-0.08	-0.09
4	-0.23	-0.13	-0.13	-0.30	-0.13	0.17	0.41	0.31	-0.18	0.77	-0.43	-0.03	-0.42	0.11	-0.08	-0.15	0.20	-0.35	0.05
5	-0.14	-0.13	-0.10	-0.11	-0.28	-0.53	-0.42	0.85	-0.69	-0.28	-0.23	-0.05	-0.49	-0.14	-0.79	-0.55	-0.40	-0.18	-0.38
6	0.01	-0.14	-0.13	-0.06	-0.12	0.42	0.19	1.26	-0.08	0.59	-0.21	0.53	-0.08	0.81	-0.14	0.04	-0.03	-0.04	0.14
7	0.14	-0.11	-0.04	-0.04	0.13	0.92	0.32	1.88	0.01	0.38	0.01	0.23	-0.14	0.12	-0.26	0.00	-0.05	0.12	-0.14
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	6	7
mean	-0.17	-0.15	-0.06	-0.16	-0.08	0.14	0.21	1.28	-0.14	0.18	-0.15	0.39	-0.18	0.28	-0.25	-0.10	0.01	-0.08	-0.10
SD	0.28	0.12	0.08	0.12	0.17	0.46	0.35	0.62	0.26	0.65	0.16	0.37	0.22	0.35	0.27	0.25	0.22	0.17	0.18
SEM	0.10	0.04	0.03	0.04	0.06	0.17	0.13	0.23	0.10	0.24	0.06	0.14	0.08	0.13	0.10	0.09	0.08	0.07	0.07

Appendix 17. Arterio -Venous difference plasma $[K^+]_{a-v}$ ($mmol.l^{-1}$) in placebo (uncorrected)

Appendix 18. Arterio -Venous difference plasma $[K^+]_{a-v}$ ($mmol.l^{-1}$) in salbutamol (corrected for fluid shifts)

Subject	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3	1	2	5	10	30
1	-0.02	0.07	-0.02	-0.06	-0.10	0.17	0.23	1.24	0.04	0.34	-0.13	0.45	-0.08	0.42		-0.13	0.04	0.02	0.08
2	0.11	0.13	-0.15	0.05	0.07	0.24	0.23	0.77	0.19	0.58	0.08	0.77		0.67		-0.65	0.03	-0.41	0.16
3	-0.05	-0.10	-0.04	0.12	-0.37	0.13	0.24	1.42	-0.28	-0.01	-0.65	0.28	0.04	0.46	-0.22	-0.18	-0.09	0.17	0.02
4	-0.11	0.20	-0.27	-0.27	-0.34	0.62	0.72	0.81	-0.17	0.03	-0.10	0.77	0.02	0.78	1.18	0.39	0.44	-0.03	0.20
5	-0.32	-0.68	0.21	0.35	0.05	-1.49	-0.30	-1.40	0.15	-0.62	0.42	-0.77	-0.24	-0.53	0.17	-0.14	-0.05	-0.10	-0.57
6	0.06	-0.73	-0.42	-0.44	-0.31	-0.68	-0.39	4.56	-0.25	-0.10	-0.56	0.16	-0.72	0.45	0.21	-0.19	-0.13	-0.15	-0.25
7	-0.19	-0.45	-0.56	-0.41	-0.66	-0.24	0.61	0.12	0.07	-0.22	-0.08	-0.01	-0.31	0.14	0.29	0.25	0.02	-0.10	-0.24
n	7	7	7	7	7	7	7	7	7	7	7	7	6	7	5	7	7	7	7
mean	-0.07	-0.22	-0.18	-0.09	-0.24	-0.18	0.19	1.07	-0.04	0.00	-0.15	0.23	-0.22	0.34	0.33	-0.09	0.04	-0.09	-0.08
SD	0.15	0.39	0.26	0.29	0.26	0.71	0.42	1.80	0.20	0.39	0.37	0.53	0.29	0.43	0.51	0.34	0.19	0.18	0.28
SEM	0.06	0.15	0.10	0.11	0.10	0.27	0.16	0.68	0.07	0.15	0.14	0.20	0.12	0.16	0.23	0.13	0.07	0.07	0.10

Appendix 19. Arterio -Venous difference plasma $[K^+]_{a-v}$(mmol.l^{-1}) in placebo (corrected for fluid shifts)

Subject	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3	1	2	5	10	30
1	-0.75	-0.32	-0.07	-0.25	-0.12	0.02	0.03	1.50	-0.06	0.22	-0.16	0.33	-0.03	0.36	-0.09	-0.08	0.02	0.02	0.02
2	-0.04	-0.10	0.04	-0.16	0.01	0.01	0.54	0.94	0.14	-0.49	0.10	0.58	0.05	-0.16	-0.49	-0.26	0.14		-0.35
3	-0.35	-0.13	0.10	-0.19	-0.15	0.02	0.07	1.00	-0.01	0.60	0.03	0.62	-0.15	0.50	0.10	-0.22	0.02	0.03	0.03
4	0.00	-0.35	-0.06	0.03	0.03	-0.06	-0.08	-0.22	-0.08	-0.03	0.17	0.00	0.07	0.06	0.05	0.09	-0.54	-0.08	0.00
5	-0.09	-0.05	-0.05	-0.09	-0.29	-0.46	0.01	0.97	-0.60	-0.30	0.01	-0.04	-0.27	-0.11	-0.48	-0.30	-0.15	-0.03	-0.40
6	-0.02	-0.15	-0.16	-0.13	-0.15	-0.37	0.55	1.25	-0.06	0.61	-0.03	0.56	-0.02	0.72	0.06	0.18	-0.05	-0.05	-0.44
7	0.00	0.01	0.09	0.09	0.00	1.09	0.85	2.04	0.00	0.69	0.31	0.41	0.20	0.30	0.03	0.32	0.15	0.28	-0.20
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	6	7
mean	-0.18	-0.16	-0.02	-0.10	-0.10	0.04	0.28	1.07	-0.10	0.19	0.06	0.35	-0.02	0.24	-0.12	-0.04	-0.06	0.03	-0.19
SD	0.28	0.13	0.10	0.12	0.12	0.51	0.36	0.69	0.23	0.47	0.15	0.27	0.15	0.32	0.26	0.24	0.24	0.13	0.21
SEM	0.11	0.05	0.04	0.05	0.04	0.19	0.14	0.26	0.09	0.18	0.06	0.10	0.06	0.12	0.10	0.09	0.09	0.05	0.08

Appendix .20 Change in plasma [K^+] volume from baseline (ΔVP) % in salbutamol

Subject	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3
1	0	-1.38	-0.69	0.00	0.71	-4.98	-5.93	-9.50	-3.38	-5.62	-5.62	-6.85	-3.72	-6.54
2	0	-2.12	-2.81	-2.12	-5.45	-7.04	-7.97	-10.64	-5.46	-8.88	-6.73	-8.89		-10.07
3	0	-1.04	-0.35	1.06	-2.74	-6.89	-7.20	-2.43	-1.73	-6.27	-7.50	-9.56	-9.57	-11.81
4	0	-0.37	0.75	1.13	2.68	-4.62	-5.94	-8.84	0.75	-5.30	-3.94	-5.64	-4.29	-3.94
5	0	-4.92	-5.83	-5.22	-6.14	-11.03	-11.03	-12.38	-10.76	-10.21	-9.36	-11.84	-9.37	-11.04
6	0	-11.55	-9.77	-9.15	-8.52	-14.65	-15.46	-17.80	-8.53	-14.93	-14.38	-16.52	-10.98	-15.19
7	0	-1.36	-1.70	-1.36	-0.68	-5.25	-1.39	-9.98	-3.66	-7.38	-6.78	-8.27	-5.56	-7.08
n	7	7	7	7	7	7	7	7	7	7	7	7	6	7
mean	0.00	-3.25	-2.91	-2.24	-2.88	-7.78	-7.85	-10.22	-4.68	-8.37	-7.76	-9.65	-7.25	-9.38
SD	0.00	3.94	3.70	3.76	4.04	3.73	4.43	4.57	3.95	3.39	3.36	3.62	3.09	3.78
SEM	0.00	1.49	1.40	1.42	1.53	1.41	1.67	1.73	1.49	1.28	1.27	1.37	1.26	1.43

(continued)

1	**2**	**5**	**10**	**30**
-5.30	-5.30	-3.39	-2.72	0.71
-9.77	-9.18	-8.58	-6.73	-1.42
-10.14	-9.86	-8.69	-6.29	-1.73
- 4.29	-3.25	-2.19	0.75	5.50
-10.49	-10.21	-8.51	-6.44	-4.29
-13.83	-13.27	-11.85	-10.37	-10.36
-6.18	-5.87	-5.56	-2.03	4.34
7	7	7	7	7
-8.57	-8.13	-6.97	-4.83	-1.03
3.42	3.46	3.40	3.71	5.37
1.29	1.31	1.29	1.40	2.03

Subject	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3
1	0	-0.70	0.72	-0.71	0.00	-5.38	-7.25	-9.07	-3.11	-6.02	-6.01	-7.27	-6.03	-7.27
2	0	-2.98	-2.66	-3.31	-6.70	-9.03	-9.88	-11.79	-9.03	-6.41	-7.60	-9.61	-9.05	-11.26
3	0	0.32	0.32	-1.00	1.00	-2.30	-4.17	-5.98	1.33	-2.61	-2.62	-4.49	-4.17	-5.99
4	0	0.36	1.46	3.34	2.96	-5.12	-6.09	-9.75	-0.37	-5.77	-3.82	-6.72	-5.13	-7.04
5	0	-0.34	-1.36	-0.68	-0.68	-5.51	-0.38	-8.77	-4.27	-6.72	-7.03	-9.62	-7.32	-8.77
6	0	-0.38	0.76	1.14	0.76	-7.67	-8.63	-11.67	-0.39	-7.03	-5.37	-8.63	-0.76	-6.37
7	0	0.00	0.85	0.01	1.43	-7.78	-8.37	-10.95	-4.69	-7.17	-5.64	-6.87	-5.32	-6.56
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7
mean	0	-0.53	0.01	-0.17	-0.18	-6.11	-6.40	-9.71	-2.93	-5.96	-5.44	-7.60	-5.40	-7.61
SD	0	1.15	1.47	2.05	3.10	2.24	3.24	2.04	3.50	1.56	1.74	1.84	2.60	1.84
SEM	0	0.43	0.56	0.77	1.17	0.85	1.22	0.77	1.32	0.59	0.66	0.69	0.98	0.70

Appendix 21. Change in plasma volume from baseline (ΔVP) % in placebo

(continued)

1	2	5	10	30
-6.03	-6.03	-4.43	-2.78	4.46
-10.16	-9.04	-8.19		-7.30
-4.79	-5.09	-3.25	-2.31	3.08
-5.46	-4.81	-3.14	-0.72	6.50
-8.48	-7.91	-5.52	-4.59	-1.69
-4.34	-3.65	-2.93	2.30	5.15
-5.94	-5.32	-4.37	-0.70	4.44
7	7	7	6	7
-6.46	-5.98	-4.55	-1.47	2.09
2.10	1.88	1.85	2.35	4.89
0.79	0.71	0.70	0.96	1.85

Appendix 22. The change in plasma [K^+] (mmol.l^{-1}) from baseline ([ΔK^+]$_a$) in salbutamol

Subject	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3	1	2	5	10	30
1	0.00	-0.27	-0.56	-0.65	-0.68	0.38	0.68	2.44	-0.20	0.79	-0.63	0.81	-0.49	0.71	-0.14	-0.37	-0.56	-0.55	-0.35
2	0.00	-0.43	-0.56	-0.58	-0.29	0.20	0.47	1.84	-0.17	0.87	-0.35	0.78	-0.08	0.64	-0.35	-0.52	-0.53	-0.26	-0.17
3	0.00	-0.07	0.03	-0.08	-0.03	0.52	0.63	1.28	-0.18	0.83	-0.79	0.57	-0.39	0.64	-0.20	-0.56	-0.74	-0.56	-0.32
4	0.00	0.04	-0.51	-0.66	-0.61	0.66	1.00	2.51	-0.69	1.01	-0.35	0.97	-0.16	0.69	0.92	-0.10	-0.28	-0.63	-0.55
5	0.00	-0.18	0.17	0.19	0.31	0.20	0.60	0.32	-0.13	0.29	-0.16	0.18	-0.35	-0.03	0.03	-0.52	-0.55	-0.55	-0.75
6	0.00	-0.22	-0.23	-0.36	-0.34	0.40	0.60	1.87	-0.44	1.07	-0.94	0.69	-0.84	0.95	-0.17	-0.73	-0.72	-0.67	-0.44
7	0.00	-0.09	-0.22	-0.32	-0.46	0.30	0.93	2.17	-0.18	0.57	-0.47	0.60	-0.54	0.48	-0.06	-0.20	-0.47	-0.46	-0.48
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
mean	0.00	-0.17	-0.27	-0.35	-0.30	0.38	0.70	1.77	-0.28	0.77	-0.53	0.65	-0.41	0.58	0.01	-0.43	-0.55	-0.52	-0.44
SD	0.00	0.15	0.29	0.32	0.34	0.17	0.19	0.77	0.20	0.27	0.27	0.25	0.25	0.30	0.42	0.22	0.16	0.14	0.18
SEM	0.00	0.06	0.11	0.12	0.13	0.06	0.07	0.29	0.08	0.10	0.10	0.10	0.10	0.11	0.16	0.08	0.06	0.05	0.07

Appendix 23. The change in arterial plasma $[K^+]_a$ (mmol.l^{-1}) from baseline ($[\Delta K^+]_a$) in placebo

Subject	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3	1	2	5	10	30
1	0.00	0.08	0.04	-0.08	0.02	0.60	0.79	2.82	0.06	1.11	-0.39	0.87	-0.37	0.94	-0.06	-0.30	-0.38	-0.28	-0.19
2	0.00	0.02	0.07	0.04	0.13	0.85	1.26	2.33	0.35	0.24	-0.02	1.49	0.03	1.00	0.21	-0.20	0.14	0.04	0.13
3	0.00	0.03	0.14	-0.01	0.04	0.44	0.46	1.71	-0.30	0.55	-0.47	0.60	-0.63	0.41	-0.09	-0.43	-0.43	-0.67	-0.38
4	0.00	0.03	0.01	-0.05	-0.11	0.86	0.91	2.70	-0.21	1.42	-0.47	0.84	-0.51	1.04	0.06	-0.29	-0.35	-0.18	-0.29
5	0.00	-0.01	0.06	0.05	-0.01	0.29	0.14	1.47	-0.26	0.30	-0.48	0.36	-0.64	0.26	-0.28	-0.57	-0.71	-0.42	-0.31
6	0.00	-0.12	-0.11	-0.12	-0.11	0.50	0.64	1.72	-0.32	0.83	-0.59	0.71	-0.55	0.91	-0.44	-0.43	-0.51	-0.48	-0.33
7	0.00	-0.01	-0.04	0.00	-0.04	1.46	1.36	2.94	0.16	1.02	0.24	0.67	-0.10	0.66	0.16	0.16	0.08	0.08	0.04
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
mean	0.00	0.00	0.02	-0.03	-0.01	0.71	0.79	2.24	-0.07	0.78	-0.31	0.79	-0.39	0.74	-0.06	-0.29	-0.31	-0.27	-0.19
SD	0.00	0.06	0.08	0.06	0.08	0.39	0.43	0.60	0.26	0.44	0.30	0.35	0.26	0.31	0.24	0.23	0.31	0.27	0.20
SEM	0.00	0.02	0.03	0.02	0.03	0.15	0.16	0.23	0.10	0.17	0.11	0.13	0.10	0.12	0.09	0.09	0.12	0.10	0.07

Appendix 24. The change in arterial plasma $[K^+](mmol.l^{-1})$ from baseline ($[\Delta K^+]_{a\ corr}$) corrected from change in plasmas volume from baseline in salbutamol

Subject	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3	1	2	5	10	30
1	0.00	-0.32	-0.58	-0.65	-0.66	0.16	0.41	1.83	-0.33	0.52	-0.82	0.48	-0.62	0.41	-0.34	-0.56	-0.67	-0.64	-0.32
2	0.00	-0.51	-0.66	-0.66	-0.50	-0.11	0.10	1.20	-0.39	0.42	-0.61	0.34	-0.08	0.15	-0.73	-0.86	-0.84	-0.52	-0.23
3	0.00	-0.11	0.02	-0.03	-0.14	0.19	0.28	1.15	-0.24	0.52	-1.04	0.11	-0.75	0.07	-0.60	-0.92	-1.04	-0.78	-0.39
4	0.00	0.03	-0.48	-0.62	-0.52	0.45	0.70	1.94	-0.66	0.75	-0.48	0.70	-0.32	0.50	0.71	-0.22	-0.35	-0.60	-0.36
5	0.00	-0.36	-0.07	-0.02	0.05	-0.25	0.10	-0.20	-0.53	-0.13	-0.50	-0.30	-0.68	-0.45	-0.38	-0.86	-0.83	-0.76	-0.88
6	0.00	-0.66	-0.61	-0.70	-0.66	-0.26	-0.13	0.81	-0.75	0.30	-1.39	-0.11	-1.19	0.18	-0.71	-1.18	-1.12	-1.03	-0.82
7	0.00	-0.14	-0.28	-0.37	-0.48	0.08	0.86	1.55	-0.32	0.23	-0.71	0.22	-0.73	0.17	-0.30	-0.42	-0.66	-0.53	-0.33
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
mean	0.00	-0.30	-0.38	-0.43	-0.42	0.04	0.33	1.18	-0.46	0.37	-0.79	0.21	-0.63	0.15	-0.33	-0.72	-0.79	-0.70	-0.48
SD	0.00	0.24	0.27	0.30	0.27	0.26	0.35	0.73	0.19	0.28	0.33	0.34	0.35	0.31	0.49	0.33	0.26	0.18	0.26
SEM	0.00	0.09	0.10	0.11	0.10	0.10	0.13	0.28	0.07	0.11	0.12	0.13	0.13	0.12	0.19	0.12	0.10	0.07	0.10

Appendix 25. The change in arterial plasma $[K^+]_a$ (mmol.l^{-1}) from baseline ($[\Delta K^+]_{a\ corr}$) corrected from change in plasmas volume from baseline in placebo

Subject	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3	1	2	5	10	30
1	0.00	0.05	0.06	-0.11	0.03	0.36	0.47	2.22	-0.05	0.82	-0.59	0.54	-0.57	0.60	-0.28	-0.50	-0.53	-0.37	-0.03
2	0.00	-0.10	-0.04	-0.09	-0.13	0.43	0.76	1.61	-0.02	-0.01	-0.31	0.98	-0.31	0.46	-0.19	-0.51	-0.18	0.04	-0.16
3	0.00	0.04	0.15	-0.05	0.07	0.34	0.28	1.37	-0.25	0.44	-0.56	0.40	-0.76	0.16	-0.26	-0.60	-0.54	-0.74	-0.27
4	0.00	0.04	0.06	0.07	0.00	0.62	0.62	2.07	-0.22	1.12	-0.59	0.53	-0.67	0.70	-0.15	-0.45	-0.46	-0.21	-0.06
5	0.00	-0.02	0.01	0.02	-0.04	0.06	0.13	1.00	-0.42	0.02	-0.73	-0.05	-0.88	-0.11	-0.59	-0.84	-0.89	-0.58	-0.37
6	0.00	-0.14	-0.08	-0.07	-0.07	0.14	0.22	1.03	-0.33	0.48	-0.78	0.29	-0.58	0.59	-0.61	-0.57	-0.62	-0.40	-0.13
7	0.00	-0.01	-0.01	0.00	0.01	1.05	0.93	2.20	-0.03	0.67	0.01	0.36	-0.30	0.36	-0.08	-0.06	-0.09	0.05	0.22
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
mean	0.00	-0.02	0.02	-0.03	-0.02	0.43	0.49	1.64	-0.19	0.50	-0.51	0.43	-0.58	0.39	-0.31	-0.50	-0.47	-0.32	-0.11
SD	0.00	0.07	0.08	0.07	0.07	0.33	0.30	0.53	0.16	0.41	0.27	0.31	0.22	0.28	0.21	0.23	0.27	0.30	0.19
SEM	0.00	0.03	0.03	0.02	0.03	0.13	0.11	0.20	0.06	0.16	0.10	0.12	0.08	0.11	0.08	0.09	0.10	0.11	0.07

Appendix 26. The change in venous plasma $[K^+]_v$ from baseline ($[\Delta K^+]_v$) in salbutamol

subjects	PostS	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post S1	RS2	Post S2	RS3	1	2	5	10	30
1	0.00	-0.31	-0.51	-0.61	-0.60	0.16	0.45	0.42	-0.29	0.38	-0.50	0.16	-0.34	0.13	-0.02	-0.25	-0.53	-0.52	-0.35
2	0.00	-0.37	-0.21	-0.49	-0.23	0.09	0.36	0.77	-0.15	0.28	-0.24	0.05	-0.50	-0.11		-0.09	-0.38	-0.20	-0.26
3	0.00	-0.19	0.06	-0.07	0.28	0.36	0.35	0.30	0.10	0.12	0.04	-0.13	-0.42	-0.21	-0.04	-0.47	-0.61	-0.70	-0.34
4	0.00	-0.24	-0.29	-0.39	-0.56	0.12	0.27	1.53	-0.52	0.39	-0.19	0.16	-0.40	0.16	-0.34	-0.37	-0.71	-0.47	-0.61
5	0.00	-0.08	-0.19	-0.15	-0.03	0.49	0.74	1.32	-0.44	0.65	-0.68	0.57	-0.63	0.15	-0.32	-0.47	-0.63	-0.52	-0.17
6	0.00	0.31	-0.03	-0.21	-0.25	-0.33	0.39		-0.56	0.34	-0.58	-0.13	-0.62	-0.04	-0.75	-0.80	-0.92	-0.83	-0.77
7	0.00	0.12	-0.11	-0.18	-0.14	0.08	0.57	1.23	-0.50	0.50	-0.63	0.22	-0.62	0.06	-0.53	-0.63	-0.77	-0.67	-0.47
n	7	7	7	7	7	7	7	6	7	7	7	7	7	7	6	7	7	7	7
mean	0.00	-0.11	-0.18	-0.30	-0.22	0.14	0.45	0.93	-0.34	0.38	-0.39	0.13	-0.50	0.02	-0.33	-0.44	-0.65	-0.56	-0.42
SD	0.00	0.24	0.18	0.20	0.30	0.26	0.16	0.51	0.24	0.17	0.27	0.24	0.12	0.14	0.28	0.24	0.17	0.20	0.21
SEM	0.00	0.09	0.07	0.08	0.11	0.10	0.06	0.21	0.09	0.06	0.10	0.09	0.05	0.05	0.11	0.09	0.07	0.08	0.08

Appendix 27. The change in venous plasma $[K^+]_v$ (mmol.l^{-1}) from baseline ($[\Delta K^+]_v$) in placebo

subjects	Post S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3	1	2	5	10	30
1	0.00	-0.29	-0.59	-0.58	-0.66	-0.13	-0.28	-0.07	-0.54	0.09	-1.03	-0.24	-1.06	-0.17	-0.71	-0.96	-1.10	-1.05	-0.91
2	0.00	-0.04	-0.07	0.06	-0.05	0.62	0.56	1.02	0.18	1.28	-0.08	0.69	-0.14	0.93	0.59	0.00	-0.18	-0.17	0.37
3	0.00	0.05	-0.04	0.07	0.07	0.44	0.25	0.29	-0.43		-0.58	-0.54	-0.60	-0.47	-0.29	-0.89	-0.76	-0.82	-0.51
4	0.00	-0.08	-0.09	0.01	-0.21	0.46	0.27	2.17	-0.27	0.42	-0.27	0.63	-0.32	0.70	-0.09	-0.37	-0.78	-0.06	-0.57
5	0.00	-0.01	0.02	0.02	0.13	0.69	0.43	0.48	0.30	0.45	-0.39	0.28	-0.28	0.27	0.37	-0.16	-0.45	-0.37	-0.06
6	0.00	0.03	0.03	-0.05	0.03	0.09	0.47	0.47	-0.23	0.25	-0.38	0.19	-0.47	0.12	-0.30	-0.46	-0.47	-0.43	-0.46
7	0.00	0.23	0.14	0.17	-0.04	0.68	1.18	1.20		0.78	0.37	0.57	0.17	0.67	0.55	0.29	0.27	0.09	0.32
n	7	7	7	7	7	7	7	7	6	6	7	7	7	7	7	7	7	7	7
mean	0.00	-0.02	-0.09	-0.04	-0.10	0.41	0.41	0.79	-0.16	0.55	-0.34	0.23	-0.38	0.29	0.02	-0.36	-0.50	-0.40	-0.26
SD	0.00	0.15	0.24	0.24	0.27	0.31	0.43	0.74	0.33	0.43	0.43	0.46	0.39	0.51	0.49	0.45	0.45	0.41	0.48
SEM	0.00	0.06	0.09	0.09	0.10	0.12	0.16	0.28	0.14	0.17	0.16	0.18	0.15	0.19	0.19	0.17	0.17	0.15	0.18

Appendix 28. Arterial Haemoglobin concentration ([Hb], g dl^{-1}) in salbutamol

subjects	Post S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post RS1	RS2	Post RS2	RS3	1	2	5	10	30
1	14.35	14.55	14.45	14.35	14.25	15.10	15.25	15.85	14.85	15.20	15.20	15.40	14.90	15.35	15.15	15.15	14.85	14.75	14.25
2	13.90	14.20	14.30	14.20	14.70	14.95	15.10	15.55	14.70	15.25	14.90	15.25		15.45	15.40	15.30	15.20	14.90	14.10
3	14.25	14.40	14.30	14.10	14.65	15.30	15.35	14.60	14.50	15.20	15.40	15.75	15.75	16.15	15.85	15.80	15.60	15.20	14.50
4	13.45	13.50	13.35	13.30	13.10	14.10	14.30	14.75	13.35	14.20	14.00	14.25	14.05	14.00	14.05	13.90	13.75	13.35	12.75
5	14.60	15.35	15.50	15.40	15.55	16.40	16.40	16.65	16.35	16.25	16.10	16.55	16.10	16.40	16.30	16.25	15.95	15.60	15.25
6	13.45	15.20	14.90	14.80	14.70	15.75	15.90	16.35	14.70	15.80	15.70	16.10	15.10	15.85	15.60	15.50	15.25	15.00	15.00
7	14.50	14.70	14.75	14.70	14.60	15.30	14.70	16.10	15.05	15.65	15.55	15.80	15.35	15.60	15.45	15.40	15.35	14.80	13.90
n	7	7	7	7	7	7	7		7	7	7	7	6	7	7	7	7	7	7
mean	14.07	14.56	14.51	14.41	14.51	15.27	15.29	15.69	14.79	15.36	15.26	15.59	15.21	15.54	15.40	15.33	15.14	14.80	14.25
SD	0.48	0.62	0.66	0.66	0.73	0.71	0.70	0.78	0.88	0.64	0.67	0.73	0.72	0.78	0.70	0.73	0.70	0.70	0.82
SEM	0.18	0.24	0.25	0.25	0.28	0.27	0.27	0.29	0.33	0.24	0.25	0.28	0.29	0.29	0.26	0.28	0.26	0.27	0.31

Appendix 29. Arterials plasma Haemoglobin concentration ([Hb], g dl^{-1}) in placebo

subjects	Post S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post R2	RS2	Post R2	RS3	1	2	5	10	30
1	14.10	14.20	14.00	14.20	14.10	14.90	15.20	15.50	14.55	15.00	15.00	15.20	15.00	15.20	15.00	15.00	14.75	14.50	13.50
2	14.65	15.10	15.05	15.15	15.70	16.10	16.25	16.60	16.10	15.65	15.85	16.20	16.10	16.50	16.30	16.10	15.95		15.80
3	15.05	15.00	15.00	15.20	14.90	15.40	15.70	16.00	14.85	15.45	15.45	15.75	15.70	16.00	15.80	15.85	15.55	15.40	14.60
4	13.95	13.90	13.75	13.50	13.55	14.70	14.85	15.45	14.00	14.80	14.50	14.95	14.70	15.00	14.75	14.65	14.40	14.05	13.10
5	14.65	14.70	14.85	14.75	14.75	15.50	14.70	16.05	15.30	15.70	15.75	16.20	15.80	16.05	16.00	15.90	15.50	15.35	14.90
6	13.30	13.35	13.20	13.15	13.20	14.40	14.55	15.05	13.35	14.30	14.05	14.55	13.40	14.20	13.90	13.80	13.70	13.00	12.65
7	14.30	14.30	14.20	14.30	14.10	15.50	15.60	16.05	15.00	15.40	15.15	15.35	15.10	15.30	15.20	15.10	14.95	14.40	13.75
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	6	7
mean	14.29	14.36	14.29	14.32	14.33	15.21	15.26	15.81	14.74	15.19	15.11	15.46	15.11	15.46	15.28	15.20	14.97	14.45	14.04
SD	0.57	0.62	0.70	0.79	0.85	0.58	0.62	0.51	0.89	0.51	0.66	0.63	0.90	0.78	0.83	0.82	0.77	0.89	1.11
SEM	0.22	0.24	0.27	0.30	0.32	0.22	0.23	0.19	0.34	0.19	0.25	0.24	0.34	0.29	0.31	0.31	0.29	0.36	0.42

Appendix 30. Arterial Haematocrit (Hct, %) in salbutamol

subjects	Post S	R5	R10	R20	R30	E1	E2	E3	Rec	S1	Post R2	S2	Post R3	S3	1	2	5	10	30
1	0.39	0.39	0.39	0.39	0.38	0.40	0.41	0.42	0.40	0.41	0.41	0.42	0.41	0.41	0.41	0.41	0.41	0.39	0.38
2	0.37	0.39	0.39	0.38	0.39	0.40	0.40	0.41	0.40	0.40	0.40	0.41		0.41	0.41	0.41	0.40	0.39	0.37
3	0.39	0.39	0.39	0.39	0.40	0.42	0.42	0.43	0.40	0.41	0.42	0.43	0.44	0.44	0.44	0.44	0.43	0.43	0.40
4	0.37	0.37	0.36	0.37	0.36	0.38	0.36	0.40	0.36	0.39	0.38	0.39	0.39	0.38	0.39	0.38	0.38	0.37	0.35
5	0.40	0.43	0.43	0.43	0.43	0.46	0.46	0.47	0.47	0.46	0.45	0.47	0.46	0.47	0.46	0.46	0.45	0.43	0.43
6	0.38	0.43	0.42	0.41	0.40	0.43	0.44	0.45	0.41	0.44	0.44	0.46	0.44	0.44	0.44	0.44	0.43	0.42	0.41
7	0.43	0.43	0.43	0.43	0.43	0.45	0.47	0.48	0.44	0.46	0.46	0.48	0.46	0.46	0.46	0.46	0.46	0.44	0.41
n	7	7	7	7	7	7	7	7	7	7	7	7	6	7	7	7	7	7	7
mean	0.39	0.40	0.40	0.40	0.40	0.42	0.42	0.44	0.41	0.43	0.42	0.44	0.43	0.43	0.43	0.43	0.42	0.41	0.39
SD	0.02	0.03	0.03	0.02	0.03	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
SEM	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Appendix 31. Arterial Haematocrit (Hct, %) in placebo

subjects	Post S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post R2	RS2	Post R3	RS3	1	2	5	10	30
1	0.38	0.38	0.38	0.38	0.38	0.39	0.40	0.42	0.40	0.41	0.40	0.42	0.41	0.41	0.41	0.42	0.40	0.40	0.37
2	0.39	0.39	0.39	0.40	0.40	0.42	0.43	0.44	0.42	0.41	0.42	0.44	0.44	0.44	0.43	0.44	0.43		0.41
3	0.39	0.40	0.40	0.41	0.40	0.42	0.43	0.44	0.41	0.42	0.43	0.44	0.43	0.45	0.44	0.44	0.43	0.43	0.40
4	0.38	0.38	0.37	0.37	0.37	0.40	0.41	0.42	0.39	0.40	0.40	0.41	0.41	0.42	0.41	0.41	0.40	0.39	0.37
5	0.41	0.41	0.42	0.41	0.42	0.44	0.45	0.46	0.43	0.45	0.46	0.47	0.46	0.47	0.46	0.46	0.45	0.44	0.43
6	0.37	0.37	0.37	0.37	0.37	0.40	0.41	0.42	0.38	0.40	0.40	0.41	0.38	0.40	0.39	0.39	0.38	0.37	0.36
7	0.42	0.42	0.28	0.42	0.41	0.46	0.46	0.48	0.44	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.43	
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	6	7
mean	0.39	0.39	0.37	0.39	0.39	0.42	0.43	0.44	0.41	0.42	0.42	0.43	0.42	0.43	0.43	0.43	0.42	0.41	0.39
SD	0.02	0.02	0.05	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.03	0.02	0.03	0.02	0.02	0.02	0.03	0.03	0.03
SEM	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Appendix 32. Venous Haemoglobin concentration ([Hb], g dl^{-1}) in salbutamol

subjects	Post S	R5	R10	R20	R30	E1	E2	E3	Rec	S1	Post R2	S2	Post R3	S3	1	2	5	10	30
1	14.30	14.60	14.5	14.3	14.2	15	15.25	14.9	14.75	15.1	15.2	15.1	15.05	15.1	15	15.1	15	14.85	14.4
2	13.85	14.30	14.4	14.2	14.7	14.95	15.1	15.05	14.85	15.1	15	15.2	15.15	15.2			15.3	14	
3	14.1	13.95	14.2	14.25	14.45	15.2	15.25	15.25	14.4	14	15.55	15	15.7	15.5	15.6	15.5	15.6	15.2	14.45
4	13.25	13.35	13.25	13.3	12.55	14.25	14.35	14.6	13.35	13.3	14.1	14.25		14.45	14	14.15	13.8	13.6	13
5	14.1	14.25	15.35	15.55	15.10	14.00	16.10	15.65	16.20	15.70	16.15	15.70	16.20	15.65	16.10	16.15	15.80	15.60	15.35
6	14.15	15.35	15.10	14.85	14.90	13.75	15.45	15.20	14.60	15.10	15.80	15.65	15.60	15.70	15.60	15.60	15.20	15.00	14.45
7	14.55	14.65	14.30	14.60	14.35	14.85	15.45	15.30	15.00	15.50	15.50	15.50	15.35	15.50	15.55	15.50	15.20	14.60	13.90
n	7	7	7	7	7	7	7	7	7	7	7	7	6	7	6	6	7	7	6
mean	14.04	14.35	14.44	14.44	14.32	14.57	15.28	15.14	14.74	14.83	15.33	15.20	15.51	15.30	15.31	15.33	15.13	14.69	14.26
SD	0.41	0.62	0.68	0.69	0.84	0.56	0.52	0.33	0.84	0.86	0.66	0.50	0.42	0.43	0.73	0.67	0.64	0.69	0.77
SEM	0.15	0.24	0.26	0.26	0.32	0.21	0.20	0.13	0.32	0.33	0.25	0.19	0.17	0.16	0.30	0.27	0.24	0.26	0.32

Appendix 33. Venous plasma Haemoglobin concentration ([Hb], g dl^{-1}) in placebo

subjects	Post S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post R2	RS2	Post R3	RS3	1	2	5	10	30
1	14.00	14.20	14.00	14.10	13.90	14.90	14.60	14.75	14.60	14.85	14.75	15.10	14.95	15.15	14.90	14.90	14.75	14.40	13.50
2	14.60	14.80	14.90	14.90	15.40	15.75	16.10	16.25	16.10	16.60	16.00	15.96	15.90	16.10	16.00	15.95	15.65	15.55	15.55
3	14.55	14.95	15.00	15.15	14.85	15.55	15.60	15.55	14.75	15.25	15.50	15.00	15.65	15.50	15.80	14.50	15.10	15.40	14.60
4	13.95	13.30	13.65	13.55	13.60	14.60	14.70	15.15	13.85	14.75	14.85	14.95	14.85	15.10	14.85	14.85	13.20	13.90	13.10
5	14.8	14.9	14.9	14.8	14.75	15.25	15.5	15.65	15.2	15.2	15.8	15.5	15.75	15.45	15.95	15.85	15.7	15.4	14.8
6	13.2	13.25	13.15	13.05	13.15	12.6	14.45	14.2	13.3	13.8	13.95	13.95	13.4	13.6	13.9	13.75	13.4	13.1	12
7	13.80	14.25	14.25	14.30	13.70	15.00	15.55	15.30	15.00	15.15	15.05	14.90	15.10	14.85	15.00	15.05	14.75	14.40	13.65
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
mean	14.13	14.24	14.26	14.26	14.19	14.81	15.21	15.26	14.69	15.09	15.13	15.05	15.09	15.11	15.20	14.98	14.65	14.59	13.89
SD	0.56	0.72	0.71	0.76	0.81	1.05	0.63	0.66	0.91	0.83	0.70	0.62	0.85	0.77	0.76	0.76	1.00	0.91	1.19
SEM	0.21	0.27	0.27	0.29	0.31	0.40	0.24	0.25	0.34	0.31	0.27	0.23	0.32	0.29	0.29	0.29	0.38	0.35	0.45

Appendix 34. Venous Haematocrit (Hct, %) in salbutamol

subjects	Post S	R5	R10	R20	R30	E1	E2	E3	Rec	S1	Post RS1	S2	Post RS2	S3	1	2	5	10	30
1	0.39	0.38	0.39	0.39	0.39	0.40	0.41	0.40	0.40	0.41	0.41	0.41	0.41	0.42	0.41	0.42	0.41	0.40	0.39
2	0.39	0.39	0.39	0.40	0.40	0.42	0.43	0.44	0.42	0.41	0.42	0.44	0.44	0.44	0.43	0.44	0.43		0.41
3	0.39	0.38	0.39	0.39	0.40	0.42	0.42	0.43	0.40	0.39	0.42	0.41	0.44	0.43	0.43	0.44	0.43	0.42	0.40
4	0.36	0.36	0.36	0.36	0.34	0.38	0.39	0.41	0.37	0.40	0.39	0.39		0.40	0.39	0.40	0.38	0.37	0.35
5	0.40	0.40	0.43	0.44	0.43	0.39	0.44	0.44	0.46	0.45	0.45	0.45	0.46	0.44	0.46	0.46	0.45	0.44	0.43
6	0.41	0.44	0.42	0.41	0.41	0.38	0.43	0.41	0.42	0.43	0.45	0.44	0.45	0.44	0.43	0.44	0.43	0.42	0.40
7	0.46	0.43	0.42	0.43	0.42	0.44	0.46	0.45	0.44	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.44	0.41
n	7	7	7	7	7	7	7	7	7	7	7	7	6	7	7	7	7	6	7
mean	0.40	0.40	0.40	0.40	0.40	0.40	0.43	0.42	0.42	0.42	0.43	0.43	0.44	0.43	0.43	0.43	0.43	0.42	0.40
SD	0.03	0.03	0.02	0.03	0.03	0.02	0.02	0.02	0.03	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.03	0.03	0.02
SEM	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Appendix 35. Venous Haematocrit (Hct, %) in placebo

subjects	PostS	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post R1	RS2	Post R3	RS3	1	2	5	10	30
1	0.38	0.38	0.39	0.39	0.38	0.40	0.40	0.41	0.39	0.45	0.40	0.43	0.41	0.41	0.41	0.42	0.41	0.39	0.36
2	0.39	0.39	0.39	0.39	0.41	0.41	0.44	0.43	0.43	0.43	0.42	0.43	0.43	0.43	0.42	0.43	0.42	0.41	0.42
3	0.40	0.40	0.40	0.41	0.41	0.43	0.42	0.42	0.41	0.42	0.43	0.42	0.44	0.43	0.44	0.41	0.42	0.42	0.41
4	0.39	0.36	0.36	0.37	0.37	0.39	0.41	0.42	0.39	0.40	0.41	0.41	0.41	0.42	0.41	0.40	0.37	0.39	0.36
5	0.42	0.43	0.42	0.42	0.42	0.44	0.44	0.44	0.44	0.44	0.45	0.45	0.46	0.44	0.46	0.46	0.45	0.45	0.42
6	0.37	0.37	0.37	0.36	0.37	0.35	0.41	0.40	0.37	0.39	0.39	0.39	0.38	0.38	0.40	0.39	0.38	0.37	0.34
7	0.41	0.42	0.42	0.42	0.40	0.45	0.46	0.46	0.44	0.45	0.45	0.44	0.44	0.44	0.45	0.44	0.44	0.42	0.40
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
mean	0.39	0.39	0.39	0.39	0.39	0.41	0.42	0.42	0.41	0.43	0.42	0.43	0.42	0.42	0.43	0.42	0.41	0.41	0.39
SD	0.02	0.03	0.02	0.02	0.02	0.03	0.02	0.02	0.03	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.03	0.03	0.03
SEM	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Appendix 36. Arterial plasma pH in salbutamol

subjects	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post R1	RS2	Post R3	RS3	1	2	5	10	30
1	7.46	7.42	7.43	7.44	7.43	7.41	7.36	7.31	7.32	7.33	7.32	7.32	7.33	7.34	7.37	7.32	7.30	7.34	7.43
2	7.39	7.39	7.39	7.40	7.38	7.36	7.34	7.30	7.25	7.30	7.30	7.29	7.26	7.29	7.22	7.22	7.23	7.29	7.37
3	7.40	7.39	7.39	7.38	7.39	7.39	7.37	7.35	7.36	7.35	7.27	7.29	7.20	7.25	7.18	7.18	7.17	7.21	7.31
4	7.43	7.40	7.39	7.41	7.40	7.38	7.39	7.37	7.38	7.39	7.37	7.39	7.36	7.36	7.33	7.34	7.36	7.38	7.40
5	7.41	7.39	7.39	7.38	7.40	7.35	7.35	7.32	7.23	7.28	7.27	7.29	7.28	7.30	7.29	7.30	7.29	7.33	7.38
6		7.38	7.37	7.38	7.38	7.35	7.31	7.26	7.32	7.27	7.24	7.20	7.24	7.27	7.21	7.22	7.22	7.26	7.35
7	7.36	7.32	7.33	7.33	7.33	7.32	7.29	7.30	7.22	7.28	7.26	7.26	7.26	7.26	7.27	7.26	7.27	7.29	7.32
n	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
mean	7.41	7.39	7.38	7.39	7.39	7.37	7.34	7.32	7.30	7.31	7.29	7.29	7.27	7.30	7.27	7.26	7.26	7.30	7.37
SD	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.06	0.04	0.04	0.06	0.05	0.04	0.07	0.06	0.06	0.06	0.04
SEM	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02

Appendix 37. Arterial plasma pH in placebo

subjects	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post R1	RS2	Post R2	RS3	1	2	5	10	30
1	7.45	7.43	7.42	7.43	7.44	7.38	7.36	7.32	7.30	7.33	7.31	7.33	7.28	7.31	7.28	7.25	7.28	7.35	7.41
2	7.43	7.44	7.47	7.46	7.43	7.35	7.34	7.29	7.24	7.22	7.27	7.28	7.26	7.25	7.24	7.19	7.23	7.23	7.40
3	7.43	7.42	7.42	7.41	7.43	7.40	7.40	7.36	7.34	7.35	7.31	7.32	7.26	7.30	7.26	7.27	7.28	7.26	7.36
4	7.41	7.40	7.41	7.38	7.39	7.38	7.36	7.33	7.33	7.32	7.30	7.33	7.31	7.34	7.29	7.29	7.31	7.35	7.37
5	7.35	7.35	7.35	7.35	7.35	7.32	7.30	7.31	7.31	7.30	7.30	7.28	7.27	7.27	7.26	7.26	7.25	7.25	7.33
6	7.38	7.37	7.37	7.38	7.37	7.34	7.31	7.29	7.31	7.26	7.27	7.26	7.28	7.33	7.28	7.26	7.29	7.32	7.36
7	7.37	7.38	7.39	7.37	7.37	7.35	7.34	7.34	7.24	7.30	7.31	7.31	7.31	7.34	7.35	7.33	7.36	7.36	7.40
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
mean	7.40	7.40	7.41	7.40	7.40	7.36	7.34	7.32	7.30	7.30	7.29	7.30	7.28	7.30	7.28	7.26	7.28	7.30	7.37
SD	0.04	0.03	0.04	0.04	0.04	0.03	0.03	0.03	0.04	0.04	0.02	0.03	0.02	0.04	0.03	0.04	0.04	0.05	0.03
SEM	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.01

Appendix 38. Arterial plasma lactate concentration [Lac^-] $mmol.L^{-1}$ in salbutamol

subjects	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post R2	RS2	Post R2	RS3	1	2	5	10	30
1	0.30	0.42	0.48	0.65	0.60	1.71	4.11	6.65	7.51	6.97	7.30	7.51	7.30	7.92	8.40	7.90	7.24	5.88	7.41
2	0.72	1.31	1.30	1.16	1.23	2.01	3.41	5.73	7.36	7.00	5.41	6.53		6.64	7.76	7.66	7.17	6.41	7.40
3	0.52	0.54	0.53	0.48	0.46	0.78	1.45	2.96	2.55	4.30	6.58	7.09	7.55	8.42	9.27	9.24	8.99	8.63	5.17
4	0.44	0.43	2.54	0.52	0.52	1.88	2.85	5.46	4.32	4.50	4.25	4.31	4.07	4.23	4.29	4.26	3.78	2.97	1.40
5	0.35	0.34	33.20	0.37	0.35	2.02	3.04	3.90	7.17	5.40	6.09	6.37	6.28	4.02	6.22	6.27	5.78	4.77	1.76
6		0.25	0.55	0.52	0.51	1.29	2.22	4.14	3.08	4.96	6.51	7.30	7.83	6.58	7.06	6.92	6.87	6.00	3.04
7	0.56	0.68	0.70	0.68	0.68	2.20	4.01	5.71	6.42	4.73	5.55	5.58	5.40	5.02	5.37	5.29	4.86	4.12	2.29
n	6	7	7	7	7	7	7	7	7	7	7	7	6	7	7	7	7	7	7
mean	0.48	0.57	5.61	0.62	0.62	1.70	3.01	4.93	5.49	5.41	5.96	6.38	6.40	6.12	6.91	6.79	6.38	5.54	4.07
SD	0.15	0.35	12.19	0.26	0.29	0.50	0.95	1.29	2.13	1.13	0.99	1.12	1.46	1.74	1.74	1.68	1.72	1.82	2.59
SEM	0.06	0.13	4.61	0.10	0.11	0.19	0.36	0.49	0.80	0.43	0.37	0.42	0.60	0.66	0.66	0.63	0.65	0.69	0.98

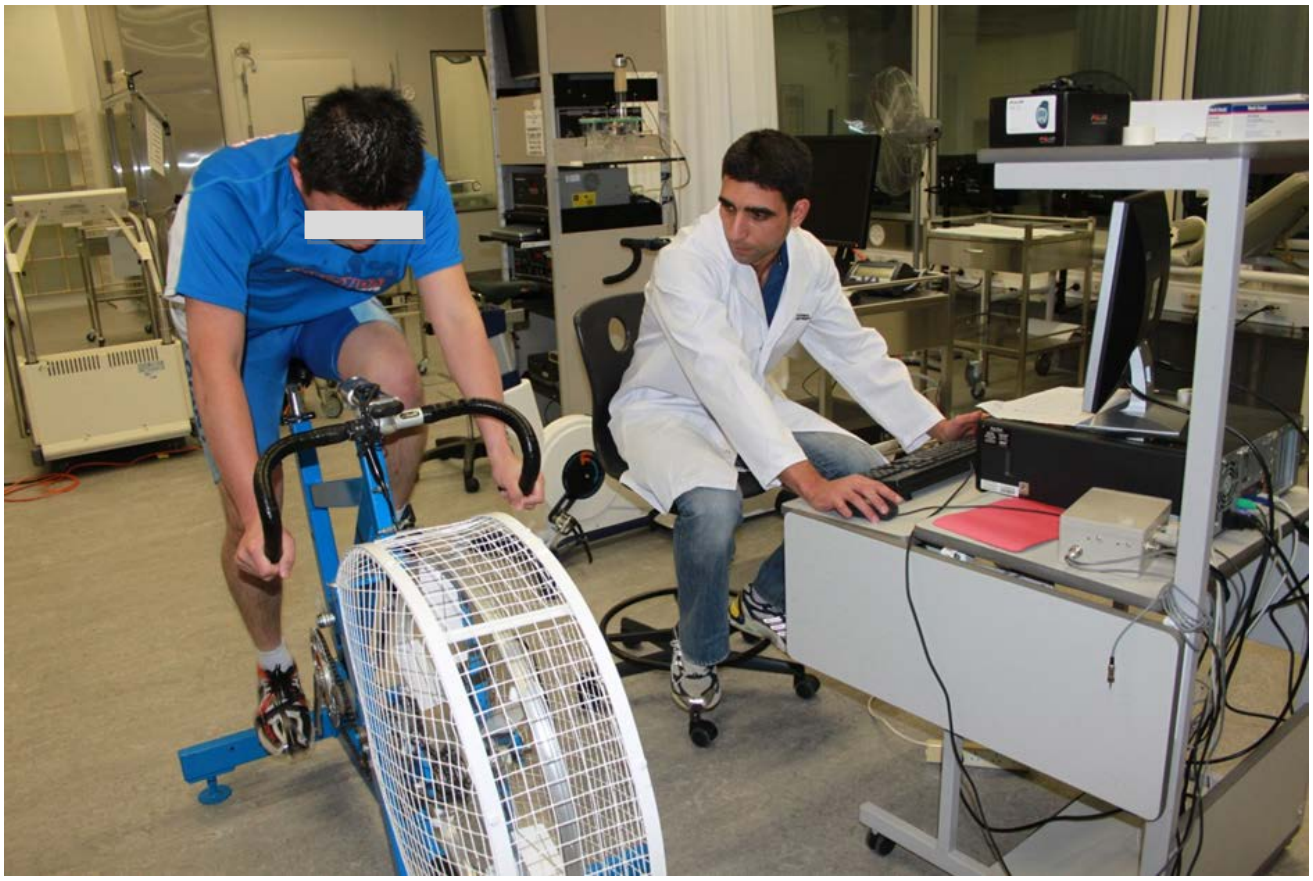
Appendix 39. Arterial plasma lactate concentration [Lac^-] $mmol.L^{-1}$ in placebo

subjects	Pre S	R5	R10	R20	R30	E1	E2	E3	Rec	RS1	Post R1	RS2	Post R2	RS3	1	2	5	10	30
1	0.37	0.43	0.42	0.40	0.41	1.46	3.79	6.64	6.84	6.76	7.15	7.89	8.05	8.81	9.82	8.78	7.96	6.72	7.41
2	0.93	0.80	0.78	0.73	0.74	1.77	3.88	5.52	8.02	7.65	7.13	7.29	7.47	8.17	8.29	8.24	7.79		4.42
3	0.33	0.38	0.40	0.54	0.48	1.01	1.31	3.69	3.72	4.10	5.92	6.15	7.57	7.83	8.42	8.31	8.07	7.47	4.31
4	0.43	0.47	0.45	0.42	0.47	2.17	3.07	6.39	5.37	6.43	6.35	6.72	6.36	6.66	6.86	6.67	6.08	4.97	2.66
5	0.56	0.66	0.61	0.57	0.53	1.40	2.56	3.88	4.25	4.82	5.82	6.62	7.20	6.93	8.25	8.44	8.85	7.99	3.99
6	0.43	0.42	0.43	0.41	0.41	1.13	2.08	3.90	3.41	4.61	5.32	5.72	5.20	5.30	5.83	5.85	5.26	4.33	1.81
7	0.37	0.38	0.35	0.35	0.33	3.60	4.43	5.84	7.38	5.62	5.32	4.86	4.35	3.87	3.96	3.99	3.55	3.07	1.42
n	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	6.00	7.00
mean	0.49	0.51	0.49	0.49	0.48	1.79	3.02	5.12	5.57	5.71	6.14	6.46	6.60	6.79	7.35	7.18	6.79	5.76	3.72
SD	0.21	0.16	0.15	0.13	0.13	0.89	1.11	1.27	1.86	1.29	0.77	1.00	1.37	1.72	1.96	1.77	1.90	1.94	2.02
SEM	0.08	0.06	0.06	0.05	0.05	0.34	0.42	0.48	0.70	0.49	0.29	0.38	0.52	0.65	0.74	0.67	0.72	0.79	0.77

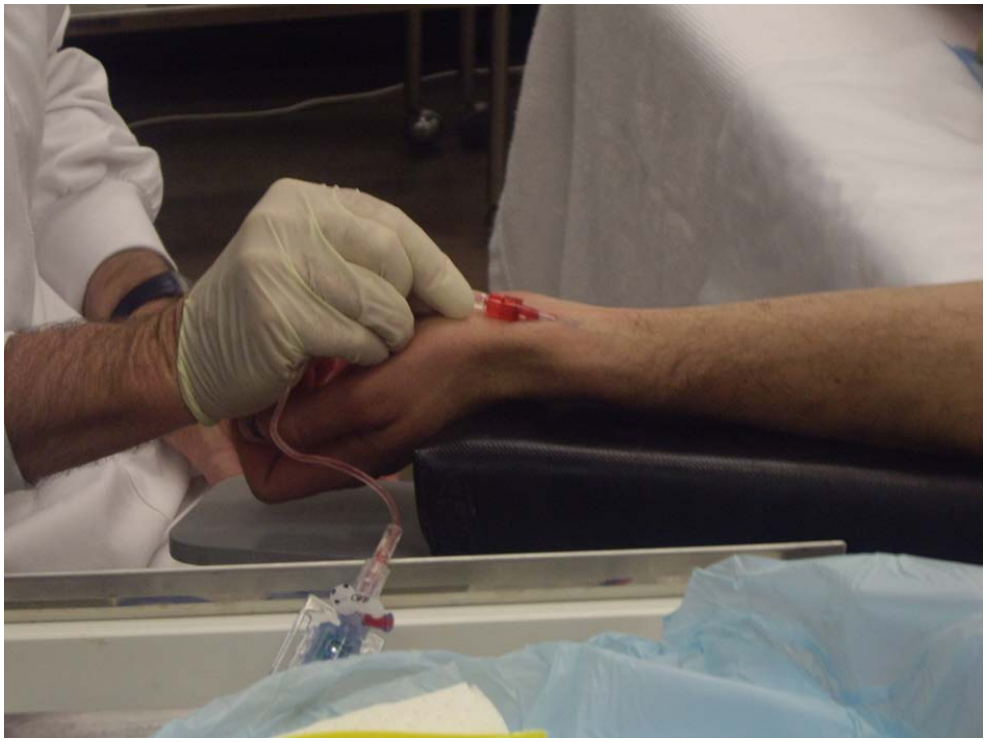
Appendix 40 Photos from testing days

Volumatic Spacer

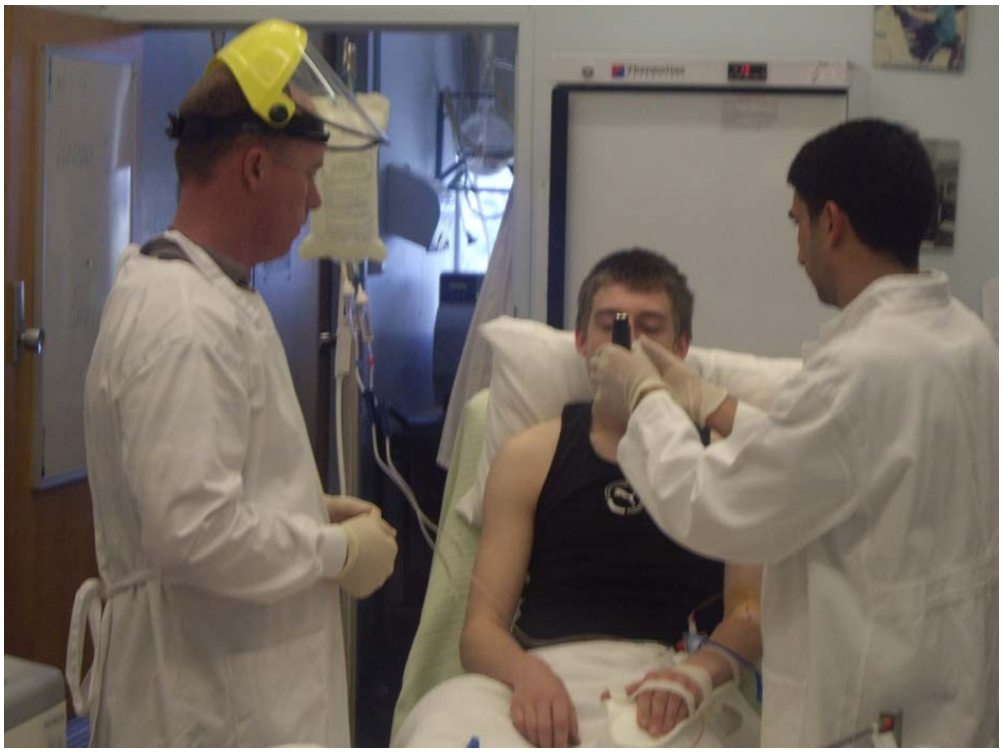
Salbutamol and placebo inhalers

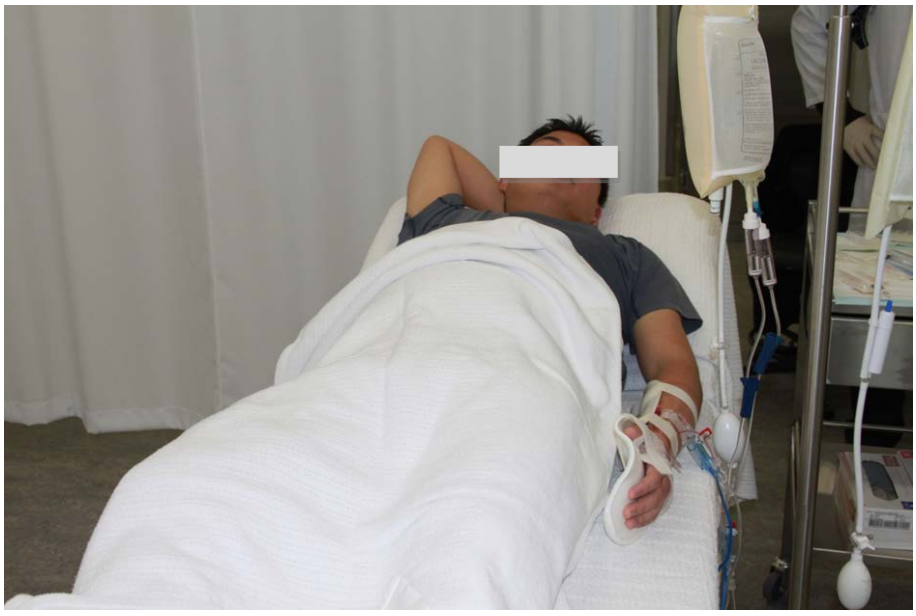


Subject performing repeat sprint exercise during screening day pre-testing



Radial arterial canulation





Subject inhling salbutamol and then during 30 min rest before exercise

Continuous Exercise

Repeat sprint exercise

Some of member of the research, from lift, Dr David Rouffet, Mr Mu’ath Altarawneh, Professer Michael McKenna, Dr Antone Tobin and Dr Aaron Petersen.