

**Salbutamol effects on systemic potassium dynamics during and following intense continuous and intermittent exercise.**

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Running head: Salbutamol modulates K<sup>+</sup> during intense exercise and recovery

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## ABSTRACT

### Purpose.

Salbutamol inhalation is permissible by WADA in athletic competition for asthma management and affects potassium regulation, which is vital for muscle function. Salbutamol effects on arterial potassium concentration ( $[K^+]_a$ ) during and after high-intensity continuous exercise ( $HI_{cont}$ ) and intermittent exercise comprising repeated, brief sprints ( $HI_{int}$ ), and on performance during  $HI_{int}$  are unknown and were investigated.

### Methods.

Seven recreationally-active men participated in a double-blind, randomised, crossover design, inhaling 1000  $\mu$ g salbutamol or placebo. Participants cycled continuously for 5 min at 40% $\dot{V}O_{2peak}$  and 60% $\dot{V}O_{2peak}$ , then  $HI_{cont}$  (90 s at 130% $\dot{V}O_{2peak}$ ), 20 min recovery, then  $HI_{int}$  (3 sets, 5x4 s sprints), with 30 min recovery.

### Results.

Plasma  $[K^+]_a$  increased throughout exercise and subsequently declined below baseline ( $P<0.001$ ). Plasma  $[K^+]_a$  was greater during  $HI_{cont}$  than  $HI_{int}$  ( $P<0.001$ ,  $HI_{cont}$   $5.94\pm 0.65$  vs  $HI_{int}$  set 1,  $4.71\pm 0.40$  mM); the change in  $[K^+]_a$  from baseline ( $\Delta[K^+]_a$ ) was 2.6-fold greater during  $HI_{cont}$  than  $HI_{int}$  ( $P<0.001$ ). The  $\Delta[K^+]_a$  throughout the trial was less with salbutamol than placebo ( $P<0.001$ , treatment main effect,  $0.03\pm 0.67$  vs  $0.22\pm 0.69$  mM, respectively); and remained less after correction for fluid shifts ( $P<0.001$ ). The  $\Delta[K^+]_a$  during  $HI_{cont}$  was less after salbutamol ( $P<0.05$ ), but not during  $HI_{int}$ . Blood lactate, plasma pH, and the work output during  $HI_{int}$  did not differ between trials.

### Conclusions.

Inhaled salbutamol modulated the  $[K^+]_a$  rise across the trial, comprising intense continuous and intermittent exercise and recovery, lowering  $\Delta[K^+]_a$  during  $HI_{cont}$ . The limited  $[K^+]_a$  changes during  $HI_{int}$  suggest salbutamol is unlikely to influence systemic  $[K^+]_a$  during periods of intense effort in intermittent sports.

### Keywords

$K^+$ , repeat sprint exercise, fatigue,  $\beta_2$ -adrenergic agonist, hypokalemia,  $Na^+$ ,  $K^+$ -ATPase



## INTRODUCTION

Skeletal muscle plays a key role in potassium ( $K^+$ ) homeostasis during exercise. Marked  $K^+$  shifts occur from intracellular to extracellular fluid in contracting muscles and then into plasma; rapid and precise control of these  $K^+$  shifts is essential for maintaining muscle excitability and is regulated by the  $Na^+,K^+$ -ATPase ( $Na^+,K^+$ -pump, NKA) and other  $K^+$  transport proteins in skeletal muscle (Clausen 2013b; McKenna et al. 2008; Sejersted and Sjøgaard 2000; McDonough and Youn 2005; Clausen 2013a). Pronounced increases in circulating catecholamines occur during intense exercise (Kjaer 1989), which activate NKA in skeletal muscle (Clausen 2003). Hence there is considerable interest in  $\beta_2$ -agonist effects on  $K^+$  concentration ( $[K^+]$ ) and exercise performance in humans, with some evidence reported for both performance-enhancing and  $K^+$ -lowering effects.

Terbutaline infused intravenously reduced the arterial and femoral venous  $[K^+]$  by 0.8mM at rest and during 8 minutes intense knee extensor exercise; and also reduced the initial  $[K^+]$  rise during intense contractions (0.45 mM) and the post-exercise decline in femoral venous  $[K^+]$  (Hallen et al. 1996). Terbutaline infusion during prolonged (1 h) knee extensor exercise reduced arterial and femoral venous  $[K^+]$ , but actually increased the muscle  $K^+$  loss (40%) during exercise due to augmented leg blood flow (Rolett et al. 1990). When administered by inhalation, the effects of terbutaline during intense exercise were less clear, but appears to reduce post-exercise venous  $[K^+]$ . Terbutaline inhalation (3 mg) did not affect incremental treadmill exercise performance, but lowered post-exercise venous  $[K^+]$  (-0.2-0.3 mM) (Larsson et al. 1997), whilst inhalation (450  $\mu$ g) enhanced 30 s sprint cycling performance and reduced the post-exercise antecubital venous  $[K^+]$  by up to 0.6 mM (Hostrup et al. 2014b). However, terbutaline is prohibited by the World Anti-Doping Agency (WADA), whereas salbutamol is permissible for athlete treatment, by inhalation only, up to 1600  $\mu$ g.d<sup>-1</sup> for the purposes of asthma management (WADA 2016).

Numerous studies have investigated salbutamol effects on intense exercise performance and  $K^+$  dynamics at rest, during and after exercise. Salbutamol lowers resting plasma  $[K^+]$ , whether administered intravenously (Leitch et al. 1976; Whyte et al. 1987; Tobin et al. 2006), by ingestion (Collomp et al. 2000; Edner and Jogestrand 1990; Hostrup et al. 2014a; Grove et al. 1995) or inhalation (Lipworth et al. 1989; Bennett and Tattersfield 1997; Clark and Lipworth 1996). However, salbutamol effects on  $[K^+]$  during intense exercise remain unclear, due to previous methodological limitations. Firstly, interpretation is difficult from studies measuring only antecubital venous  $[K^+]$

(Hostrup et al. 2014a; Collomp et al. 2000; Grove et al. 1995; Newnham et al. 1993; Van Baak et al. 2000), as this can underestimate arterial  $[K^+]$  by as much as 2 mM during intense exercise, due to substantial  $K^+$  clearance by forearm muscles (Lindinger 1995; McKenna et al. 1997; Lindinger et al. 1995; Kowalchuk et al. 1988). Secondly, numerous studies measured  $[K^+]$  in blood drawn only after completion of exercise (Hostrup et al. 2014a; Kalsen et al. 2014; Larsson et al. 1997; Grove et al. 1995; Hostrup et al. 2014b), when plasma  $[K^+]$  is falling precipitously (McKenna et al. 1997; Sejersted and Sjøgaard 2000). Nonetheless, despite these limitations, there is evidence suggesting salbutamol may affect both  $K^+$  dynamics and performance under certain conditions.

Salbutamol typically does not enhance performance during endurance exercise (Koch et al. 2015; Pluim et al. 2011), when  $K^+$  disturbances are modest (Sejersted and Sjøgaard 2000), with some exceptions, where venous  $[K^+]$  was also reduced by ~0.2-0.4 mM (Van Baak et al. 2000; Collomp et al. 2000). Performance during a 30 s cycle sprint was enhanced by oral salbutamol (4-8 mg) (Le Panse et al. 2007; Collomp et al. 2005; Hostrup et al. 2014a), with the post-exercise antecubital venous  $[K^+]$  reduced (Hostrup et al. 2014a). However, salbutamol inhalation is more relevant for athletes than ingestion. Salbutamol inhalation (200, 800  $\mu$ g) failed to affect either time to fatigue cycling at 85% $VO_{2peak}$  (~21-23 min) or the end-exercise antecubital venous  $[K^+]$  (Goubault et al. 2001), possibly indicating the exercise intensity and salbutamol dose were too low. No studies have investigated inhaled salbutamol effects on sprint exercise performance, but inhalation of other  $\beta_2$ -agonists have shown beneficial effects. A combination of inhaled  $\beta_2$ -agonists (1600  $\mu$ g salbutamol, 36  $\mu$ g formoterol, 200  $\mu$ g salmeterol) improved performance during a 200 m swim sprint lasting ~57-58 s and reduced post-exercise antecubital venous  $[K^+]$  (Kalsen et al. 2014). Inhalation of the  $\beta_2$ -agonist albuterol (180  $\mu$ g) increased peak power during a 15 s cycle sprint (Signorile et al. 1992), whilst terbutaline inhalation (450  $\mu$ g) enhanced 30 s cycle sprint power and reduced post-exercise venous  $[K^+]$  (Hostrup et al. 2014b). Investigation of the effects of inhaled salbutamol at a higher dose within WADA approved limits, on arterial  $[K^+]$  during and in recovery from intense exercise is warranted.

Numerous sports with high-participation rates feature intermittent high intensity exercise, comprising repeated brief sprints and short recovery intervals, including various football codes, basketball, netball and field hockey (Bishop et al. 2011). The antecubital venous  $[K^+]$  reported during intense intermittent exercise varies, reaching 4.3 mM during squash (Struthers et al. 1988), 5.1 mM during soccer (Krustrup et al. 2006), and 5.5-6 mM during repeated intermittent sprint testing (Wylie et al. 2013;

Mohr et al. 2011; Mohr et al. 2007; Duffield and Marino 2007; Mohr et al. 2006). However, the effects of salbutamol inhalation on arterial  $K^+$  regulation and on performance during repeated brief sprints are unknown, and are of interest since  $[K^+]$  lowering during intense exercise might be associated with enhanced performance. We therefore investigated the effects of inhaled salbutamol (1000  $\mu$ g) on  $K^+$  dynamics in arterial plasma, during and in recovery from both high intensity continuous exercise and intermittent exercise. We hypothesised that salbutamol inhalation will decrease arterial plasma  $[K^+]$  at rest, during intense continuous and intermittent exercise and in recovery, and lessen the changes in  $[K^+]$  from baseline, as well as enhance intermittent sprint performance.

## METHODS

### *Participants*

Seven healthy, recreationally-active but not well-trained males (age  $23 \pm 6$  yr, height  $175 \pm 13$  cm, body mass  $71.6 \pm 10.4$  kg,  $57.7 \pm 12.9$  ml.kg<sup>-1</sup>.min<sup>-1</sup> mean  $\pm$  SD) volunteered for the study after giving written informed consent. Participants were typically involved in community level sporting and recreational activities comprising football, gymnasium, swimming and/or other aerobic activities on around 4 d/week. Participants were asked not to undertake any heavy training and to maintain their normal diet during the period of the trials. Participants refrained from caffeine, alcohol and intense exercise in the 24 hr prior to the experimental trials. All experiments and procedures were approved by the Victoria University Human Research Ethics committee.

### *Pre-screening and familiarisation*

Participants initially visited the laboratory for pre-screening of plasma electrolytes, respiratory function and cardiac rhythm; and undertook pre-testing and familiarisation with all procedures. To exclude participants with electrolyte abnormalities, an antecubital venous blood sample was taken for determination of resting plasma [K<sup>+</sup>] and for other electrolytes; all participants displayed electrolytes within normal limits. To exclude any participant with existing lung disease or asthma, the forced expiratory volume in 1 second (FEV<sub>1</sub>) and forced vital capacity (FVC) were measured using a flow turbine (Medical Graphics Corporation, St. Paul, Min, USA); all participants had an FEV<sub>1</sub>/FVC ratio exceeding 80% ( $84 \pm 7\%$ , mean  $\pm$  SD). No participants exhibited ECG abnormalities under resting or exercise conditions.

For familiarisation purposes, participants performed a maximal intensity, sprint exercise test comprising four, 5-second "all-out" sprints, performed on a custom air-braked cycle ergometer (RepcO, Melbourne, Australia) (McKenna et al. 1993). This ergometer was also used for the intermittent exercise trials. After 30 min of rest, subjects then performed an incremental exercise test on an electronically braked cycle ergometer (Lode, Groningen, Netherlands) to determine peak oxygen uptake ( $\dot{V}O_{2peak}$ ) and allow calculation of exercise workrates for the experimental trials. Subjects cycled at 65-80 rpm for three min at each of 60, 90, 120 and 150 W, with workrate then increased by 25 W each min, until volitional fatigue, defined as an inability to maintain pedal cadence

above 60 rpm. The regression of  $\dot{V}O_2$  versus power output was then used to determine the power output corresponding to 40%, 60% and 130%  $\dot{V}O_{2\text{ peak}}$  for use on the same cycle ergometer in the subsequent experimental trials. All respiratory measurements were as previously described (Atanasovska et al. 2014). 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).

### *Experimental Exercise Trials*

In two subsequent visits, participants inhaled either salbutamol or a placebo and then performed an exercise trial with invasive measures. These trials were performed in a double-blind, randomized, cross-over design, separated by two weeks to ensure complete washout of salbutamol. Each experimental trial comprised the initial salbutamol or placebo inhalation, 30 min of rest, then on the Lode electronically braked cycle ergometer, submaximal cycling exercise for 5 min at both 40%  $\dot{V}O_{2\text{ peak}}$  and 60%  $\dot{V}O_{2\text{ peak}}$ , followed by continuous high intensity cycling for 90 s at 130%  $\dot{V}O_{2\text{ peak}}$  ( $HI_{\text{cont}}$ ). The  $\dot{V}O_2$  during 40%, 60% and 130%  $\dot{V}O_{2\text{ peak}}$  bouts did not differ between salbutamol and placebo trials, being  $1.55 \pm 0.29$  vs  $1.66 \pm 0.31$ ;  $2.14 \pm 0.43$  vs  $2.08 \pm 0.31$ ; and  $3.19 \pm 0.83$  vs  $3.17 \pm 0.38$  L.min<sup>-1</sup>, respectively. After 20 min passive supine recovery on an adjacent couch, subjects then performed high intensity intermittent exercise ( $HI_{\text{int}}$ ) on the custom air-braked cycle ergometer. The  $HI_{\text{int}}$  test comprised three sets of five, 4-s “all out” sprints whilst seated on the cycle ergometer, with each sprint separated by 20 s passive recovery; each set had an intervening 4.5 min passive recovery where participants remained quietly seated on the cycle ergometer. Due to technical difficulties during one trial, mechanical data is reported for 6 subjects. Participants then transferred to an adjacent couch for a final 30 min supine recovery period.

### *Salbutamol and placebo administration*

Subjects inhaled 1000  $\mu\text{g}$  salbutamol using a standard metered dose inhaler used for asthma treatment (Asmol inhaler, Alphapharm, Queensland, Australia) and a standard spacer device (Volumatic spacer, Allen and Hanburys Melbourne, Australia) was used to allow optimal delivery of the drug to the lung. The inhaler delivers 100  $\mu\text{g}$  of salbutamol with each actuation (i.e. 10 actuations gives 1000  $\mu\text{g}$ ) and inhalation time was ~2 min (10 breaths). This spacer technique was utilised to



maximise respiratory drug delivery, although complete uptake of salbutamol cannot be concluded as any residual salbutamol in the spacer, mouth or remaining in the lung was not measured (Mandelberg et al. 1999). Salbutamol administration was performed 30 min prior to exercise commencement, allowing for maximum pharmacological activity (Hopkins 1999). The placebo was delivered by a similar metered dose inhaler containing the propellant only (Allen and Hanburys, Melbourne, Australia). The 1000 µg salbutamol dose was utilised to modulate K<sup>+</sup> homeostasis and enhance intense intermittent exercise performance, as this fell comfortably within the WADA limit of 1600 µg.d<sup>-1</sup> salbutamol by inhalation (WADA 2016) exceeded the 600 µg.d<sup>-1</sup> typically recommended for asthmatic athletes and the 800 µg previously used for submaximal exercise (Goubault et al. 2001).

#### *Blood sampling and analyses*

A cannula was inserted into the radial artery (Arrow Quick Flash, Radial Artery 20G, USA), for arterial (a) blood sampling during all phases of the experimental trials. Participants then rested supine for 20 min to allow for stabilisation of fluid shifts and of [K<sup>+</sup>]. Blood sampling times and posture for each phase comprised: (i) baseline sample taken after 20 min rest and prior to salbutamol or placebo inhalation during supine rest; (ii) at 5, 10, 20 and 30 min after salbutamol or placebo inhalation during supine rest; (iii) during the final 30 s of each workrate during submaximal and HI<sub>cont</sub> whilst seated on the cycle ergometer; (iv) during the fourth bout of each set of sprints during HI<sub>int</sub> whilst seated on the ergometer; and (vi) at 1, 2, 5, 10 and 30 min in supine recovery after HI<sub>int</sub>. A second cannula was inserted into the antecubital vein (v) of the other arm (Optiva, I.V. Catheter 20 gauge, Italy), with samples taken at similar time points, for determination of [K<sup>+</sup>]<sub>v</sub> for comparison with the literature.

Two samples were taken at each time point. First a 2 ml blood sample was drawn into a heparinised blood gas syringe for immediate analyses of plasma pH and [K<sup>+</sup>] using an automated analyser (Rapid Point 405, Siemens Medical Solutions Pty Ltd, Bayswater, Australia). A 3 ml blood sample was then collected in a plain syringe, ejected into a tube containing lithium heparin and after mixing immediately separated into two eppendorf tubes. Approximately 1 ml was then 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 of blood glucose ([glucose]) and lactate concentrations ([Lac]) using an automated analyser (2300 STAT plus, YSI Inc. Yellow Spring, ON, USA). The remaining 2 ml was immediately centrifuged for 1.5 min at 4500 rpm in a non-refrigerated

centrifuge (Eppendorf Centrifuge, model 5415C, Englesdorf, Germany), and plasma separated and stored in an eppendorf tube at  $-20^{\circ}\text{C}$  for later analysis of plasma  $[\text{K}^+]$ .

#### *Calculations*

The change in plasma  $[\text{K}^+]_a$  from baseline ( $\Delta[\text{K}^+]_a$ ) was calculated at each time for each trial to control for any small intra-individual  $[\text{K}^+]_a$  differences between salbutamol and placebo trials. As  $[\text{K}^+]_a$  is affected by fluid losses from plasma during exercise, the decline in plasma volume ( $\Delta\text{PV}$ ) from baseline was also calculated from  $[\text{Hb}]$  and  $[\text{Hct}]$ . The plasma  $[\text{K}^+]$  during and after exercise was corrected for  $\Delta\text{PV}$  to represent hemoconcentration changes, as earlier described (McKenna et al. 1997; McKenna et al. 1993).

#### *Statistical Analyses*

A linear mixed model (treatment\*time) was used to assess the effects of exercise and salbutamol on electrolytes and fluid shifts. Time-by-treatment interactions are indicated only when significant. Effect size for potassium variables was calculated using Cohen's  $d$ . Data are presented as mean  $\pm$  standard deviation (SD). Statistical significance was accepted at  $P < 0.05$ . Statistical analyses were conducted using SPSS version 22.

## RESULTS

### *Arterial plasma $[K^+]_a$ ( $[K^+]_a$ )*

Plasma  $[K^+]_a$  was increased above baseline during each of submaximal exercise, HI<sub>cont</sub>, and HI<sub>int</sub> sets 1-3 ( $P < 0.001$ , time main effect, Figure 1). The peak  $[K^+]_a$  during HI<sub>cont</sub> was also greater than during each set of HI<sub>int</sub> (HI<sub>cont</sub>  $5.94 \pm 0.65$  vs  $4.71 \pm 0.40$  mM for RS1, time main effect,  $P < 0.001$ ). In the recovery periods,  $[K^+]_a$  declined to baseline after HI<sub>cont</sub>, but after HI<sub>int</sub> fell below baseline after sets 1 and 2 ( $P < 0.05$ ), and after set 3 at 5 min ( $P < 0.01$ ) and 10 min recovery ( $P < 0.05$ , Figure 1). Plasma  $[K^+]_a$  did not, however, differ significantly between salbutamol and placebo trials, with a very low effect size also found ( $d = 0.13$ ).

### *Change in arterial plasma $[K^+]_a$ from baseline ( $\Delta[K^+]_a$ )*

The  $\Delta[K^+]_a$  was calculated to account for small, non-significant variations in resting  $[K^+]_a$  within an individual between trials. The  $\Delta[K^+]_a$  was increased above baseline during each of submaximal exercise, HI<sub>cont</sub>, and HI<sub>int</sub> sets 1-3 ( $P < 0.001$ , time main effect, Figure 1). The  $\Delta[K^+]_a$  was considerably higher during HI<sub>cont</sub> than during each set of HI<sub>int</sub> ( $P < 0.001$ ); during HI<sub>cont</sub>  $\Delta[K^+]_a$  was  $2.01 \pm 0.71$  mM, 2.6-fold greater than during HI<sub>int</sub> set 1,  $0.78 \pm 0.35$  mM ( $P < 0.001$ ). During recovery, the  $\Delta[K^+]_a$  became negative relative to baseline after HI<sub>int</sub> set 1 and 2 ( $P < 0.05$ ) and after set 3 at 5 min ( $P < 0.01$ ) and 10 min recovery ( $P < 0.05$ , Figure 1). The  $\Delta[K^+]_a$  was less in salbutamol than the placebo trial ( $0.03 \pm 0.67$  vs  $0.22 \pm 0.69$ , respectively,  $P < 0.001$ , treatment main effect, Figure 1), consistent with a large effect size found ( $d = 1.56$ ). The  $\Delta[K^+]_a$  was less in salbutamol than placebo during HI<sub>cont</sub> but not during HI<sub>int</sub> (HI<sub>cont</sub>  $1.77 \pm 0.77$  vs  $2.24 \pm 0.60$  mM,  $P < 0.05$ ; HI<sub>int</sub> set 1  $0.77 \pm 0.27$  vs  $0.78 \pm 0.44$  mM, respectively, paired t-test).

### *Change in plasma volume ( $\Delta PV_a$ ) and in $\Delta[K^+]_a$ after correction for $\Delta PV_a$*

As hemoconcentration with exercise affects  $[K^+]_a$ , the change in arterial plasma volume from rest ( $\Delta PV_a$ ) was also calculated. The  $\Delta PV_a$  fell throughout the trial and remained negative at 10 min recovery after HI<sub>int</sub> ( $P < 0.05$  time main effect);  $\Delta PV_a$  was slightly less during HI<sub>cont</sub> than during set 1 of HI<sub>int</sub> ( $-10.0 \pm 3.4$  % vs  $-7.2 \pm 2.8$  %, respectively,  $P < 0.05$ , time main effect). The  $\Delta PV_a$  was lower (more negative) with salbutamol ( $-6.0 \pm 0.6$  vs  $-4.0 \pm 0.6$  %,  $P < 0.05$ , treatment main effect).

The  $\Delta PV_a$  was then used to correct  $\Delta[K^+]_a$  ( $\Delta[K^+]_{a(\text{corr})}$ ), to determine whether the salbutamol effects observed for  $\Delta[K^+]_a$  remained after accounting for fluid shift differences. The  $\Delta[K^+]_{a(\text{corr})}$  was similarly

(to  $\Delta[K^+]_a$ ) increased during exercise, being higher than baseline during  $60\%\dot{V}O_{2peak}$ ,  $HI_{cont}$  and  $HI_{int}$  set 1 and higher than other pre-exercise rest times (i.e. 5-30 min rest) for  $40\%\dot{V}O_{2peak}$  and  $HI_{int}$  sets 2 and 3; the  $\Delta[K^+]_{a(corr)}$  during  $HI_{cont}$  exceeded that during  $HI_{int}$  ( $P<0.05$ , time main effect, data not shown). The  $\Delta[K^+]_{a(corr)}$  was similarly depressed in recovery, after  $HI_{int}$  set 1 and 2 ( $P<0.05$ ) and after set 3 at 2, 5 and 10 min ( $P<0.001$ , time main effect). The  $\Delta[K^+]_{a(corr)}$  was less in salbutamol than placebo ( $3.80\pm 0.58$  vs  $3.97\pm 0.61$  mM, respectively,  $P<0.005$ , treatment main effect); the time-by-treatment interaction for  $\Delta[K^+]_{a(corr)}$  was close to significance ( $P=0.06$ ).

#### *Venous plasma $[K^+] ([K^+]_v)$*

The plasma  $[K^+]_v$  was increased during exercise above baseline at  $60\%\dot{V}O_{2peak}$  ( $P<0.005$ ),  $HI_{cont}$  ( $P<0.001$ ), set 1 of  $HI_{int}$  ( $P<0.005$ ), and above other pre-exercise times during  $40\%\dot{V}O_{2peak}$  (above 10-30 min rest) and set 2 of  $HI_{int}$  (above rest 20-30 min) ( $P<0.05$ , time main effect, Table 1). The  $[K^+]_v$  fell below baseline after  $HI_{int}$  sets 1 ( $P<0.05$ ) and 2 ( $P<0.001$ ) (data not in Table) and after set 3 at each of 2 ( $P<0.005$ ), 5, 10 ( $P<0.001$ ) and 30 min recovery ( $P<0.05$ ). The  $[K^+]_v$  during  $HI_{cont}$  was greater than during each set of  $HI_{int}$  ( $P<0.001$ , Table 1). No differences were found between salbutamol and placebo trials for  $[K^+]_v$  (Table 1), although a large effect size was found ( $d=0.60$ ).

#### *Arterial blood lactate and plasma pH*

Blood  $[Lac]_a$  was increased above baseline, during  $HI_{cont}$  and each set of  $HI_{int}$  ( $5.03\pm 1.24$  vs  $5.56\pm 1.18$  mM for  $HI_{cont}$  and set 1, respectively) and all recovery periods to 10 min ( $P<0.001$ ) and at 30 min ( $P<0.01$ ) (time main effect, data not shown). Plasma  $pH_a$  fell below baseline during exercise at  $60\%\dot{V}O_{2peak}$ ,  $HI_{cont}$ , each set of  $HI_{int}$  and throughout the trial to 10 min recovery ( $P<0.001$ , time main effect, data not shown). No effects of salbutamol were found on either blood  $[Lac]_a$  or plasma  $pH_a$ .

#### *Work output during $HI_{int}$ and $VO_2$*

No significant differences were found between trials for work output during each set of  $HI_{int}$  (salbutamol,  $15.18\pm 3.56$ ,  $15.42\pm 2.89$ ,  $14.77\pm 3.02$  kJ vs placebo,  $15.59\pm 2.81$ ,  $15.58\pm 2.46$ ,  $15.34\pm 2.24$  kJ, for sets 1, 2 and 3, respectively), for total cumulative work (salbutamol  $45.36\pm 9.20$  vs placebo  $46.51\pm 7.18$  kJ), or for pulmonary  $VO_2$  (data not shown).

## DISCUSSION

We investigated whether salbutamol inhalation affected arterial plasma  $K^+$  regulation during and following intense continuous and intermittent exercise in healthy adult males. We utilised salbutamol intake by inhalation, as this is a commonly used practice and a dose of 1000  $\mu\text{g}$  that is also WADA-permissible for competitions. Intermittent repeated sprints were utilised as an exercise model, due to the prevalence of participation in sports requiring intermittent efforts, with intense continuous exercise also examined. We measured for the first time salbutamol inhalation effects on each of arterial  $[K^+]$ , the changes in  $[K^+]_a$  from baseline ( $\Delta[K^+]_a$ , i.e. rise during exercise, decline during recovery), as well as the  $\Delta[K^+]_a$  after correction for fluid shifts ( $\Delta[K^+]_a \text{ corr}$ ), with three important findings. First, salbutamol inhalation modulated  $K^+$  homeostasis, evidenced by the smaller mean rise in  $[K^+]_a$  above baseline of 0.17 mM across all rest, exercise and recovery time periods (treatment main effect). This effect remained after accounting for fluid shifts, indicating that this was due to actions of salbutamol and not simply to a hemoconcentration effect. Second, whilst the  $\Delta[K^+]_a$  during  $HI_{\text{cont}}$  was reduced by salbutamol, the  $\Delta[K^+]_a$  during  $HI_{\text{int}}$  was unaffected. Third, the  $\Delta[K^+]_a$  during  $HI_{\text{cont}}$  intense was 2.6-fold larger than during  $HI_{\text{int}}$ , most likely due to the differing duration and recovery characteristics of these two exercise modalities. This is also the first time  $[K^+]$  changes have been compared across these two exercise modalities within the same individual. Despite salbutamol inhalation modulating systemic  $K^+$ , the small rise in  $[K^+]_a$  during  $HI_{\text{int}}$ , together with the lack of salbutamol effect on both the  $\Delta[K^+]_a$  and work output during  $HI_{\text{int}}$ , suggest that salbutamol is unlikely to be of performance enhancing benefit during sports utilising repeated, high-intensity, intermittent sprints.

### ***Salbutamol modulated systemic $K^+$ homeostasis***

Salbutamol inhalation resulted in a smaller rise in  $[K^+]_a$  above baseline (mean -0.17 mM) across all exercise and recovery time periods. Whilst plasma  $[K^+]_a$  itself was not significantly lowered across all times with salbutamol, this most likely reflects minor (non-significant) variations in the resting  $[K^+]_a$  between trials, as well as the apparent different effects that occurred during  $HI_{\text{cont}}$  and  $HI_{\text{int}}$ , with a lesser rise in  $[K^+]_a$  after salbutamol found during exercise at 130%  $\dot{V}O_{2\text{peak}}$  (-0.47 mM, -21%), but not during  $HI_{\text{int}}$ . Together these suggest that the actions of salbutamol in lowering  $\Delta[K^+]_a$  were dominant during rest, continuous exercise and during recovery, but not during intermittent exercise. These salbutamol effects also appeared to persist over the entire time frame of the experiment, incorporating

all exercise and recovery periods up to 114 min after inhalation; with the  $\Delta[K^+]_a$  being  $\sim 0.3$  mM less after salbutamol, both after 20 min rest pre-exercise and at 30 min recovery, relative to placebo. Since the effect size for salbutamol effects on  $[K^+]_a$  was very low ( $d=0.13$ ), it is unlikely that a lowering of plasma  $[K^+]_a$  would have been detected after salbutamol with a larger sample size. In contrast, the large effect size seen for  $\Delta[K^+]_a$  ( $d=1.56$ ), is consistent with the significant salbutamol treatment main effect detected. This suggests the lack of salbutamol effect on  $[K^+]_a$  was due to variations in the resting  $[K^+]_a$  and differences in response to  $HI_{cont}$  and  $HI_{int}$ , as noted above, rather than sample size limitations, whereas the significant lowering of  $\Delta[K^+]_a$  found with salbutamol likely reflected real effects.

The smaller  $\Delta[K^+]_a$  during  $HI_{cont}$  after salbutamol inhalation does indicate improved  $K^+$  regulation, which could be due to reduced  $K^+$  released into and/or greater  $K^+$  clearance from plasma. Substantial  $K^+$  release occurs from contracting muscles during exercise and is primarily responsible for the increased  $[K^+]_a$  (Sostaric et al. 2006; Hallen et al. 1996; Medbø and Sejersted 1990; Juel et al. 1999), suggesting the salbutamol  $K^+$ -lowering effect could occur via direct effects on contracting muscles. Salbutamol stimulates NKA in isolated skeletal muscle from rats (Clausen 2003), an effect that was not synergistic with muscle stimulation (Clausen and Flatman 1980). Terbutaline was recently shown to protect against an exercise-induced decline in NKA activity (Hostrup et al. 2014b) and other  $\beta$ -adrenergic agonists also induced  $K^+$ -lowering effects during continuous exercise in humans (Hallen et al. 1996; Rolett et al. 1990); this effect was reversed with  $\beta$ -blockers, resulting in greater elevations in  $K^+$  (McKelvie et al. 1997; Katz et al. 1985). However, whilst terbutaline lowered  $[K^+]_a$  it also enhanced  $K^+$  loss from contracting muscles due to a greater leg blood flow (Rolett et al. 1990), suggesting the  $K^+$ -lowering effect of  $\beta_2$ -agonists occurred in non-contracting muscles and/or other tissues. It is unclear whether a greater muscle  $K^+$  loss occurs similarly with salbutamol and also whether this effect occurs during two-legged cycling rather than single leg knee extension where blood flows can differ considerably. We cannot resolve this here, as the  $K^+$  release from the active leg was not measured. Potassium clearance during exercise also occurs via uptake by other non-contracting or relatively inactive muscles, and also splanchnic  $K^+$  uptake (Lindinger 1995; Sejersted and Sjøgaard 2000; Clausen 2003). It is likely that salbutamol inhalation increased NKA activity in inactive muscles and possibly in contracting muscles, which lowered the rise in  $[K^+]_a$  during intense continuous exercise.

A novel finding was the lack of effect of inhaled salbutamol on  $\Delta[K^+]_a$  during  $HI_{int}$ . This might in part be explained by the similar  $\Delta[K^+]_a$  immediately prior to  $HI_{int}$ , suggesting that muscle NKA activation and  $K^+$  reuptake into contracting muscle had already been stimulated by prior continuous exercise, without further effects of the  $\beta_2$ -adrenergic agonist. Both the low peak  $[K^+]_a$  attained and the lack of salbutamol effect on  $\Delta[K^+]_a$  during  $HI_{int}$  are consistent with the lack of effect of salbutamol on work output during individual sprints or cumulative work during  $HI_{int}$ . Since a similar practical effect of warm up and early exercise bouts on stimulating muscle NKA activation would also likely occur during competitive field-based team sports, it is therefore likely that salbutamol inhaled at this dose would exert little influence on systemic  $K^+$  during intense periods of effort in such sports.

These findings further advance earlier studies investigating salbutamol effects on  $[K^+]$ , that measured  $[K^+]$  only in antecubital venous blood during leg exercise (Hostrup et al. 2014a; Collomp et al. 2000; Grove et al. 1995; Newnham et al. 1993; Van Baak et al. 2000), or in samples drawn only after completion of, rather than during exercise (Hostrup et al. 2014a; Kalsen et al. 2014; Hostrup et al. 2014b; Larsson et al. 1997; Grove et al. 1995); and/or utilised oral salbutamol (Collomp et al. 2000; Van Baak et al. 2000; Hostrup et al. 2014a; Goubault et al. 2001). The magnitude of the sampling site effect is substantial, with the venous  $[K^+]$  being 1.28 mM less than arterial  $[K^+]$  during  $HI_{cont}$ , further indicating the importance of arterial sampling, or sampling directly from the vein draining the contracting musculature for interpreting  $K^+$  regulation. Although no significant reduction in venous  $[K^+]$  was found with salbutamol, the large effect size ( $d=0.6$ ) suggests that a venous  $[K^+]$ -lowering effect may not have been detected with the sample size utilised.

The salbutamol-induced reduction in  $\Delta[K^+]_a$  across all times strongly suggests that the  $K^+$ -lowering effects of inhaled salbutamol persisted in recovery after  $HI_{int}$ . This is consistent with the contributory effects of muscle excitation, increased cellular  $[Na^+]$ , neuro-humoral changes combined with salbutamol stimulation of muscle NKA (Clausen 2003). The  $[K^+]$  undershoot during recovery from exercise was prevented when the activating effects of catecholamines on muscle  $K^+$  uptake were suppressed by propranolol (Gullestad et al. 1995). Hence salbutamol-stimulated  $K^+$  uptake into skeletal muscles via NKA is the likely mechanism for the lesser  $\Delta[K^+]$ . The salbutamol lowering effect on  $\Delta[K^+]_a$  incorporates recovery from  $HI_{int}$  and presumably indicates ongoing effects of salbutamol and possibly diminished or ceased contraction-mediated NKA stimulation. We cannot confirm the tissues responsible for this systemic  $\Delta[K^+]$ -lowering with salbutamol, but it is likely to be a combination of  $K^+$

uptake by the previously contracting leg musculature and inactive muscle; as well as increased  $K^+$  clearance in the splanchnic bed and other tissues. The salbutamol-lowering of  $\Delta[K^+]$  effect remained after accounting for fluid shifts, indicating that this was due to actions of salbutamol and not simply to a hemoconcentration effect.

The lowering of arterial  $[K^+]$  post-exercise to around 3.5 mM suggests that risks of hypokalaemia should be considered in susceptible individuals, particularly after taking salbutamol. There is, however, considerable inconsistency in the literature on the post-exercise effects of  $\beta_2$ -agonists on  $[K^+]$ . After oral salbutamol, there was no additional post-exercise decline in antecubital venous  $[K^+]$  after prolonged cycling to exhaustion (Van Baak et al. 2000), or at 5-10 min recovery after repeated 30 s sprints or repeated bouts of high intensity cycling, although greater reductions were evident at 1 or 2 min after some individual bouts (Hostrup et al. 2014a). Similarly, after salbutamol inhalation (800  $\mu$ g), there was no further change in antecubital venous  $[K^+]$  at 2-10 min after exhaustive cycling at 85%  $\dot{V}O_{2peak}$  (Goubault et al. 2001). Alternately, inhaled terbutaline reduced antecubital venous  $[K^+]$  at 10 min after an incremental TM test (Larsson et al. 1997) and immediately after repeated 30 s cycle ergometer sprints through to 60 min in recovery (Hostrup et al. 2014b). After a combination of inhaled salbutamol, formoterol and salmeterol, antecubital venous  $[K^+]$  was decreased at 5 and 10 min after a 200 m swim ergometer sprint test (Kalsen et al. 2014). Finally, after intravenous terbutaline infusion, arterial  $[K^+]$  was decreased compared to control at 3.5 min after knee extensor exercise, but in contrast, for femoral venous  $[K^+]$ , the rate of decline post-exercise was less and the post-exercise  $[K^+]$  were higher after terbutaline (Hallen et al. 1996). Further research is required to more fully understand salbutamol effects on post-exercise  $K^+$  regulation.

### ***Modest arterial $K^+$ disturbances during high intensity intermittent exercise***

This is the first report of arterial  $[K^+]$  during  $HI_{int}$ , comprising brief sprints repeated in bursts to simulate intense activity undertaken in many team sports (Bishop et al. 2011). The small  $\Delta[K^+]_a$  during  $HI_{int}$  of only  $\sim 0.7$  mM above baseline to  $[K^+]_a$  around 4.7 mM sharply contrasts the 2.6-fold greater peak rise of  $\sim 2$  mM in  $[K^+]_a$  to  $\sim 6$  mM during 90 s  $HI_{cont}$ . The contrast in  $[K^+]_a$  between modalities is further indicated by the similar  $\Delta[K^+]_a$  ( $\sim 0.7$  mM) during both  $HI_{int}$  and continuous exercise at a moderate exercise intensity of only 60%  $\dot{V}O_{2peak}$ . The cumulated sprint exercise duration during  $HI_{int}$  was 60 s (3 sets x 5x4 s sprints), allowing  $[K^+]_a$  of  $\sim 4.7$  mM to be contrasted against the far higher  $[K^+]_a$  attained



during continuous sprint exercise, of 7 mM during a 30 s cycle sprint (McKenna et al. 1997; Lindinger et al. 1995; McKelvie et al. 1997), 8 mM after 60 s intense treadmill running (Medbø and Sejersted 1990) and 7 mM after 90s intense rowing exercise (Atanasovska et al. 2014). Whilst work duration and intensity were not matched between the HI<sub>cont</sub> and HI<sub>int</sub> exercise bouts, these  $[K^+]_a$  differences are likely explained by several other factors. A lesser  $K^+$  release from contracting muscles would be anticipated due to the lesser contraction time with short sprint durations, with less time for  $K^+$  overflow from the muscle interstitium into the venous circulation before rapid  $K^+$  re-accumulation into the muscle cells during recovery. This  $K^+$  release might also have been attenuated by already increased muscle NKA activation due to the preceding exercise bouts, as muscle NKA is highly activated by even brief intense contractions (McKenna et al. 2003). The recovery periods with intermittent exercise, with 20 s recovery between sprints within each set and 2.5 min recovery between sets, would also each have facilitated subsequent muscle cellular  $K^+$  reuptake. These effects might be partially offset by a smaller endogenous catecholamine response anticipated during intermittent compared to continuous exercise. The low  $[K^+]_a$  and  $[K^+]_v$  during HI<sub>int</sub> explains the broadly similar venous  $[K^+]$  measured in intermittent sports or repeated short sprints (Krustrup et al. 2006; Wylie et al. 2013; Mohr et al. 2011; Mohr et al. 2007; Duffield and Marino 2007; Mohr et al. 2006; Struthers et al. 1988).

### **Conclusions**

Inhalation of 1000 µg salbutamol modulated  $K^+$  dynamics, with lesser changes from baseline in arterial plasma  $[K^+]$  during rest, exercise and recovery periods. However, the rise in  $[K^+]$  above baseline was reduced with salbutamol during high intensity continuous exercise, but not during intense intermittent repeated sprint exercise. Thus, when exercise bouts are brief and interspersed with recovery periods, even with a cumulative total of sixty seconds of all-out effort, salbutamol does not appear to alter plasma  $K^+$  regulation, likely in part due to the low arterial  $[K^+]$  attained. This was consistent with a lack of performance benefit in HI<sub>int</sub> and suggests that inhaled salbutamol is unlikely to confer an advantage during sporting competitions that comprise repeated bouts of brief sprints.

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**Table legends.**

**Table 1.** Antecubital venous plasma  $[K^+]$  at rest, during continuous exercise at 40%, 60% and 130%  $\dot{V}O_{2peak}$  (HI<sub>cont</sub>) and recovery, during high intensity intermittent exercise (HI<sub>int</sub>) comprising repeated sprints (3 sets x 5 repetitions x 4 s) and 30 min recovery, with salbutamol and placebo inhalation. Values are mean  $\pm$  SD, n = 7.

### Figure legends.

**Figure 1.** Effects of salbutamol ( $\circ$ ) and placebo ( $\blacktriangledown$ ) inhalation on (A) arterial plasma  $[K^+]_a$  and (B) changes in  $[K^+]_a$  from baseline ( $\Delta[K^+]_a$ ). Plasma  $[K^+]_a$  measured at rest (Baseline – 30 min); during continuous exercise for 5 min at each of  $40\% \dot{V}O_{2peak}$  and  $60\% \dot{V}O_{2peak}$  and for 90 s high intensity at  $130\% \dot{V}O_{2peak}$  ( $HI_{cont}$ ), then 19 min recovery (+19 min); during high intensity intermittent exercise ( $HI_{int}$ ) comprising 3 sets of repeated sprint exercise (RSE1- RSE3, each set with 5 repetitions x 4 s); and in corresponding recovery after Sets 1, 2 (Post1, Post 2) and 3 from 1 to 30 min recovery (+30). Shaded bars denote exercise periods.

Values are mean  $\pm$  SD, n = 7.

\*  $P < 0.05$ ; \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , different to baseline (time main effect)

†  $HI_{cont}$  greater than  $HI_{int}$ ,  $P < 0.001$  (time main effect)

‡ salbutamol less than placebo,  $P < 0.01$  (treatment main effect).

&  $\Delta[K^+]_a$  during  $HI_{cont}$  less after salbutamol,  $P < 0.05$

**Figure 2.** Effects of salbutamol ( $\circ$ ) and placebo ( $\blacktriangledown$ ) inhalation on A) change in plasma volume from baseline ( $\Delta PV$ ), (B) plasma  $pH_a$  and (C) blood  $[Lac^-]_a$  at rest, during continuous exercise at 40%, 60% and  $130\% \dot{V}O_{2peak}$  ( $HI_{cont}$ ) and recovery, during high intensity intermittent exercise ( $HI_{int}$ ) comprising repeated sprints (3 sets x 5 repetitions x 4 s) and 30 min recovery.

Values are mean  $\pm$  SD, n = 7.

\*  $P < 0.05$ ; \*\*  $P < 0.01$ , \*\*\*  $P < 0.01$  different to baseline (time main effect)

†  $HI_{cont}$  greater than  $HI_{int}$   $P < 0.01$  (time main effect)

‡ salbutamol less than placebo ( $P < 0.01$ , treatment main effect).

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### List of Abbreviations:

HI<sub>cont</sub> high-intensity continuous exercise

HI<sub>int</sub> high-intensity intermittent exercise

VO<sub>2peak</sub> peak oxygen consumption

[K<sup>+</sup>]<sub>a</sub> arterial potassium concentration

Δ[K<sup>+</sup>]<sub>a</sub> change in [K<sup>+</sup>]<sub>a</sub> from baseline

[K<sup>+</sup>]<sub>v</sub> Venous plasma [K<sup>+</sup>]

ΔPV<sub>a</sub> change in arterial plasma volume from rest

NKA Na<sup>+</sup>,K<sup>+</sup>-ATPase

WADA World Anti-Doping Agency