DOSE-RESPONSE RELATIONSHIPS OF INHALED SALBUTAMOL IN COMPETITIVE NON-ASTHMATIC ATHLETES: EFFECTS ON PERFORMANCE AND URINE CONCENTRATIONS.

by

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ABSTRACT

Currently, the World Anti-Doping Agency (WADA) permits asthmatic athletes to use inhaled salbutamol (SAL) to help attenuate compromised lung function during exercise. Although the majority of previous research shows no benefit in non-asthmatic athletes, there lacks an examination of the dose-response effect of SAL on performance using a sport-specific evaluation. Additionally, there lacks a description of how dose affects the concentration of SAL in the urine (*cSAL*). We hypothesized that salbutamol would have no effect on performance in non-asthmatic athletes and that *cSAL* would be affected by dose and be highly variable. Three projects were completed. **Study 1** established the typical error and reliability of time-trial performance using the Velotron cycle ergometer. Highly trained, male cyclists performed three 20-km time-trials (TT) demonstrating the test to be highly reliable with low coefficients of variance for power and time (1.8-2.0% and 0.8-1.0% respectively). In **Study 2**, lung function was positively affected by SAL and urine analysis revealed a dose-response relationship with *cSAL* while at rest, up to a dose 800μg. Peak values were observed at 60min post-inhalation and *cSAL* was highly variable at each time point. Although several samples approached the WADA limit of 1000 $ng \cdot ml^{-1}$, none exceeded this value. Using doses of 200μg, 400μg, and 800μg, **Study 3** revealed no effects of SAL on time-trial performance or physiological measures over placebo. Additionally, athlete perception of leg and breathing effort was unaffected across conditions. Similar to **Study 2**, *cSAL* was related to dose and highly variable, with no samples resulting in a doping violation. SG was found to be significantly related to *cSAL* and when corrected to a dehydrated state, several samples exceeded the WADA

limit. In summary, these findings allow us to accept the hypothesis that acute inhalation of SAL lacks ergogenic properties in non-asthmatic athletes and does not affect ventilation or metabolic parameters during exercise. Additionally, inhaled SAL does not appear to alter athlete perceptions of effort. The findings further suggest that urine samples will generally fall below the WADA limit following therapeutic doses of SAL, although this may be affected by hydration.

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DEDICATION

This dissertation is dedicated to everyone that pursues their passion in life and supports others to do the same without judgement.

CHAPTER ONE – GENERAL INTRODUCTION

Optimum performance in the elite athlete can be limited by pulmonary, cardiovascular, muscular, psychological, nutritional and/or environmental factors. In asthmatic athletes and individuals suffering from exercise induced-bronchospasm, lung function is reduced, thereby possibly limiting performance capabilities [6]. Currently four β_2-agonists, salbutamol, formoterol, salmeterol, and turbutaline, have been approved by the International Olympic Committee Medical Commission (IOC-MC) and the World Anti-Doping Agency (WADA) for use by asthmatics in competition to minimize the negative effects of asthma on exercise. In order to use these medications (at the Olympic Games), the athlete must provide clinical evidence of variable airflow obstruction that is assessed by an independent medical panel [2]. Appropriate tests include bronchodilator response and bronchial provocation (eucapnic voluntary hyperpnea (EVH), lab/field exercise, or chemical challenge). At the 2002 Winter Olympics in Salt Lake City, 165 athletes (6.6% of all participants) applied to use an inhaled β_2-agonist (increased from 5.6% in Nagano in 1998). Increased applications from athletes competing in the summer games have also been observed (Los Angeles (1984) – 1.7%, Atlanta (1996) – 3.6%, and Sidney (2000) – 5.5%). At the most recent games in Athens, 4.6% of all athletes applied to use a β_2-agonist [4].

ß$_2$-Agonists in Competition

For asthmatic athletes, β_2-agonists permit them to compete at an elite level by minimizing the effects of asthma on performance. Of the four β_2-agonists allowed in competition,

salbutamol is the most commonly used and is the only one considered to have anabolic effects as well as act as a bronchodilator. The 2006 World Anti Doping Code (WADC) [82] states that salbutamol is allowable only when a therapeutic use exemption (TUE) has been obtained in advance and that it may only be administered through inhaled means. There is growing concern that non-asthmatic athletes are using inhaled salbutamol in an attempt to gain a competitive edge [2]. Furthermore, anecdotal evidence suggests that both asthmatic and non-asthmatic athletes believe in its ability to enhance performance and are using doses that substantially exceed therapeutic recommendations. This poses not only an ethical question but also raises concerns of athlete safety due to the possible negative side-effects associated with excessive doses (e.g. hyperkalemia, arrhythmia).

Salbutamol and Performance in Non-Asthmatics

Although a few studies exist demonstrate an ergogenic effect [7, 68, 78] , the current research overwhelmingly suggests that inhaled salbutamol, in therapeutic doses, does not enhance athletic performance in non-asthmatics [10, 11, 22, 24, 48, 53, 61, 74]. It has been shown that the ventilatory response to salbutamol in both non-asthmatics and asthmatics is related to dose [35, 42] however, it is not clear whether a dose-response effect exists with respect to performance in elite athletes. Additionally, the majority of studies have evaluated performance using one, or a combination of, maximal oxygen consumption ($\dot{V}_{O_2}max$), Wingate, lactate threshold, or run to exhaustion tests (3-5 min). The validity of a test to be representative of performance is an important factor when evaluating the potential ergogenic effects of a treatment [34].

Two studies have investigated the effects of inhaled salbutamol using a simulated sport-specific performance test [53, 78]. Norris and colleagues [53], showed a non-significant 12-second improvement in 20-km time-trial performance time following a dose of 400 μg. In comparison, a dose of 800 μg has been shown to decrease time to complete a specific amount of work [78]. If salbutamol has an ergogenic effect, it may be related to dose. It has been shown that ventilatory response to salbutamol in both non-asthmatics and asthmatics is enhanced as dose increases [35, 42]. However, Goubault and colleagues showed no effect of dose (placebo, 200 μg, and 800 μg) on cycling time to exhaustion even though forced expiratory volume in one second (FEV_1) was enhanced following salbutamol administration [24]. More research examining the dose-response effects of inhaled salbutamol using a sport-specific performance test is needed to determine if it has ergogenic properties.

It is suggested that an improvement of 0.7-1.5 times the coefficient of variance (CV) in performance at the elite level could be a worthwhile enhancement in performance, potentially increasing the likelihood of winning for an athlete who averages 10^{th} place [34]. Depending on the length of race, the typical CV for top performers in simulated cycling time-trials is approximately 1-1.7% [37, 58, 59, 69].

Although the majority of research to date has shown no significant improvement in performance with the use of inhaled salbutamol, the dose-response effect on performance has not been evaluated in a homogenous group of highly trained athletes with a sport specific performance test.

Salbutamol and Doping Control

WADA currently requests that laboratories report all cases in which the urine concentration of salbutamol exceeds 200 ng/mL. Regardless of whether or not the athlete has a TUE, a urine concentration of greater than 1000 ng/mL (nonsulfated) is considered a doping violation [82]. A recently published case study has questioned whether or not this cut off point is appropriate as it may result in a positive doping test and subsequent 2-year ban from competition [65]. Schweizer and colleagues [65] reported an in-competition urinary salbutamol concentration of 8000 ng/mL in a male athlete with a TUE and were able to reproduce this positive test in a controlled, non-exercising trial. This is in agreement with other reports of positive test results using therapeutic doses, all with urine concentrations between 1000 and 3000 ng/mL following exercise [45].. High inter-subject variability (~38%) has been shown in urine recovery of salbutamol [77] and this may explain the recent occurrence of false positive tests. It is possible that differences in renal function, lung absorption, and/or dehydration from exercise [45] are responsible for the high variability. Furthermore, differences in time between inhalation and sample collection may affect urine concentrations. Up to 40% of the dose may be excreted in the first 4-6 hours post inhalation [21, 83] and depending on hydration, urine concentrations may vary. Despite the wealth of research on salbutamol, there lacks a clear characterization of the dose-response effect on urine concentrations as utilized by WADA at different time intervals post-inhalation for both rest and exercise.

Statement of the Problem

Although the majority of research suggests salbutamol has no performance enhancement in non-asthmatics, the dose-response effect on performance has not been evaluated in a homogenous group of highly trained athletes with a sport specific performance test. It is important that the study is conscious of the minimal enhancement that would be capable of increasing the likelihood of improving performance in competition (~0.7-1.5x the CV) [34]. A secondary problem is that there is limited data describing the effects of dose on urine concentrations of salbutamol as used in doping control at rest and after exercise. Recovery of salbutamol in the urine has shown to be highly variable between subjects [77, 80] which may help explain reports of positive doping violations for salbutamol when using therapeutic doses [45, 65]. There lacks a clear characterization of the dose-response effect on urine concentrations post-inhalation during both rest and exercise.

Purpose

The overall purpose of this study was to determine the dose-response effects of inhaled salbutamol on exercise performance in elite non-asthmatic athletes using a sport specific test of performance. A sport-specific 20km cycling time-trial was used as the method of performance evaluation. Three projects were completed. The purpose and hypotheses of each were as follows:

Project 1

The purpose of Project 1 was to develop a test for evaluating elite cyclists in a controlled environment. A cycle ergometer was used to simulate a sport specific 20km time-trial using a flat course with no wind effect. The reliability and reproducibility of this test was evaluated. A secondary goal was to determine appropriate performance criteria for Project 3 to ensure a homogenous subject group. It was hypothesized that a 20km time-trial would be reproducible in competitive cyclists and show a low coefficient of variation between tests.

Project 2

The purpose of Project 2 was to describe the dose-response relationship of urine salbutamol concentrations at rest and at 30, 60, and 120 minutes post-inhalation from a metered dose inhaler (MDI). This provided data to compare the exercise response to in Project 3. The hypotheses stated there would be a positive effect of dose on salbutamol concentrations at all time periods and that urine salbutamol concentrations would

increase at each time interval post-inhalation. It was also hypothesized that there would be high inter-subject variability in urine concentrations across all three doses.

Project 3

Once a reliable test of cycling performance had been obtained in competitive cyclists, it was used in the evaluation of salbutamol on exercise performance. The purpose of Project 3 was to examine the dose-response relationship of salbutamol on exercise performance in a sport-specific test and to examine the effects of exercise on urine concentrations of salbutamol. There were 3 hypotheses:

1. No change would occur in 20km time-trial performance following inhaled salbutamol and this would not be affected by dose.
2. Urine concentrations of salbutamol would be affected by dose following exercise and this relationship would be linear.
3. There would be high inter-subject variability in urine concentrations of salbutamol following exercise.

CHAPTER 2 – REVIEW OF THE LITERATURE

Introduction

Applications for the use of inhaled β2-agonists in international athletic competition have been increasing for the past 20 years and there is concern that this increase may be due to attempts by non-asthmatic athletes to gain a competitive advantage [2]. β_2-agonists have potent effects on bronchodilation, myocardial contractility, glycogenolysis, and membrane excitability which may enhance exercise performance. Of the four β2-agonists approved for competition, salbutamol is the most commonly prescribed. Recent reports suggest that the therapeutic use of salbutamol may result in doping violations [45, 65] and this is of concern to avoid false positive tests. There is a significant amount of research that has contributed to the understanding of the effects of β2-agonists on exercise performance in non-asthmatics [7, 10, 11, 15, 16, 22, 44, 48, 51, 53, 61, 68, 74, 76] with the majority of it suggesting no ergogenic benefit. Some of this research is limited in its applicability to elite athletes due to experimental design limitations. The literature is also lacking an evaluation of the dose-response effects on a sports specific performance test and urinary concentrations of salbutamol following exercise, and the potential relationships between performance, urine concentration, and doping violations.

This review summarizes the current research on the effects of β_2-agonists and in particular, salbutamol, on exercise performance. It also examines the respiratory system with respect to exercise, and the relationships between salbutamol dose, urine concentration, and doping control.

The Respiratory System and Exercise

The primary respiratory structures include the nasal cavity and nostrils, the mouth, pharynx, larynx, trachea, and the right and left lung with their respective bronchi. Beyond the larynx, the airways are often divided into two different zones: the conducting zone and the respiratory zone. The conducting zone includes the trachea, bronchi, bronchioles and terminal bronchioles, while the respiratory zone contains the respiratory bronchioles, alveolar ducts, and alveolar sacs. Gas-exchange occurs in the alveolar capillary unit which has a density of capillaries to alveolus of nearly 1000:1.

Of the respiratory system's functions, two are particularly important to exercise: gas exchange (CO_2 for O_2) and regulation of blood pH [40]. For purposes of this review, only gas exchange will be discussed as it is this function that may potentially be affected by β_2-agonist use in non-asthmatics.

The typical respiratory response to exercise is a linear increase in ventilation with increases in workload up to ventilatory threshold, after which increases in ventilation accelerate non-linearly with respect to oxygen consumption. As the demand for oxygen and cardiac output increases, greater demands are placed on the respiratory system to maintain the alveolar-arterial pressure gradient in order to maintain P_aO_2. Furthermore, as oxygen metabolism increases, there is a greater need to eliminate CO_2. Increased ventilation accommodates both these needs.

Resting ventilation ($\dot{V}_E$) is approximately 5-6 $L \cdot min^{-1}$ and during strenuous exercise this can be increased to as much as 150 $L \cdot min^{-1}$ or more for a short period of time. Early on in exercise, increases in ventilation are primarily accomplished through increases in tidal volume. As exercise progresses and becomes more difficult, higher ventilations are achieved through an increased breathing rate with very little further increase in tidal volume beyond the 50-60% increase over rest [40]. As ventilation increases, the airway resistance component of the work of breathing is augmented, primarily due to dynamic compression and increases in turbulence. Normally this is somewhat reduced by an exercise-induced bronchodilation [66]. It can generally be said that the respiratory system does not limit exercise capacity at sea level and it is built with plenty of reserve to provide adequate alveolar ventilation.

Two such situations where the respiratory system may limit exercise are respiratory disease [67] or exercise-induced arterial hypoxaemia (EIAH) [20]. In both these situations, exercise capacity may be limited due to inadequacies of the respiratory system to maintain arterial oxygen pressure (P_aO_2). In a large number of elite athletes (incidences of up to 50% depending on sport) either asthma and/or an exercise-induced bronchoconstriction results in reduced airway calibre and greater resistance to breathing [85]. This bronchoconstriction may have two detrimental effects on exercise. First, reductions in airway calibre may increase the work of breathing thereby shifting oxygen delivery from the working muscles. Harms and colleagues [25] have shown that with increased inspiratory muscle work during exercise total $\dot{V}_{O_2}$ doesn't change, however, the percentage of $\dot{V}_{O_2}$ directed to the legs is reduced from 81% to 71%. This was

accompanied by a significant decrease in leg blood flow from 17.8 $L \cdot min^{-1}$ to 16.9 $L \cdot min^{-1}$. In a follow-up study, it was shown that at sustained high workloads (90% $\dot{V}_{O_2}max$) increased work of breathing decreased time trial performance by approximately 15% [27]. With a greater work of breathing, respiratory muscles require a greater amount of oxygen for energy production. This leads to a redistribution of cardiac output at the cost of the working muscles.

Secondly, increased bronchoconstriction may lead to inadequate alveolar ventilation [3]. Inability to maintain a high alveolar oxygen pressure (P_AO_2) will result in a reduced $P_{A-a}O_2$ gradient. At higher levels of exercise this may lead to a lowering of P_aO_2 as seen in EIAH [20]. EIAH has been shown to result in compromised performance both at sea level [26] and in hypoxia [13]. In individuals with asthma or exercise-induced bronchoconstriction, use of β_2-agonists is encouraged during exercise to ensure normal ventilation and help alleviate this effect. In non-asthmatics, it is doubtful that this medication would have an ergogenic benefit as bronchodilation is not likely a factor that limits exercise performance. Both salbutamol and formoterol provided no performance benefit or attenuation of EIAH during a cycle to exhaustion test in non-asthmatic male athletes [75].

β_2-Agonists and Mechanism of Action

For the asthmatic athlete, bronchodilators provide rapid relief from bronchoconstriction. Bronchodilators relax the smooth muscle of the airway thereby increasing airway calibre. This has been shown to occur both in asthmatics and non-asthmatics and can easily be

confirmed using a bronchodilator response test [43]. The primary bronchodilator prescribed is often a β_2-agonist and these can be divided into both short and long acting. Short acting β_2-agonists include salbutamol and terbutaline with salbutamol being the most commonly prescribed β_2-agonist worldwide [43]. They are characterized by a rapid onset of action with a relatively short duration of effectiveness, most effective in the first 2-3 hours and complete cessation within 5-6 hours[43]. Conversely, long acting β_2-agonists (salmeterol and formoterol) have a mechanism of action lasting approximately 12 hours [8]. Both short and long acting β_2-agonists are frequently used in conjunction with inhaled glucocorticoids in asthma management.

The mechanism of action of β_2-agonists is through the β_2-receptor that is found in high concentrations in both the bronchial epithelium and bronchial smooth muscle [41, 43]. Normally these receptors are activated by the adrenergic fibres of the sympathetic nervous system [40]. The β_2-receptor is a seven transmembrane molecule that allows for intracellular signalling via a G-protein. Binding of an agonist leads to a conformational change that triggers a cascade of effects involving cAMP. The end result is an inhibition of myosin-actin binding in the bronchial smooth muscle and subsequent relaxation [43]. It has been suggested that stimulation of large potassium channels (maxi-K) by β_2-agonists may play a major role in smooth muscle relaxation [41].

It has been shown in both asthmatics and non-asthmatics that airway function is increased with salbutamol administration [11, 61, 83]. This effect has also been shown to occur

following exercise in both asthmatic and non-asthmatic individuals,[11, 61] however, any additive effect of salbutamol to the normal bronchodilatory response during exercise is questionable [11, 24, 29, 48, 78].

β_2-Agonists and Athletic Competition

For asthmatic athletes, β_2-agonist use allows them to compete at an elite level by minimizing the effects of asthma on performance. Of the four β_2-agonists that have been approved by the IOC and World Anti Doping Agency (WADA) for use in competition, salbutamol is the only one that has been shown to have an anabolic effect [44]. The 2006 WADA code [82] states that salbutamol is allowed only when a therapeutic use exemption (TUE) has been obtained in advance and that it may only be administered through inhaled means. Oral administration has been shown to have greater systemic side effects [43] and potential anabolic responses [44] and is therefore banned. Salbutamol's peak bronchodilatory effect is seen 15-30 minutes post inhalation [54]. Its effects on airway smooth muscle relaxation and resulting bronchodilation help minimize or eliminate the limitations of asthma and/or exercise induced bronchoconstriction on breathing and alveolar ventilation.

There is growing concern that non-asthmatic athletes are using inhaled salbutamol in an attempt to gain a competitive edge [2]. It is speculated that by increasing airway caliber and reducing the work of breathing, a greater percentage of whole body $\dot{V}_{O_2}$ can be utilized by the working muscles and/or alter perception of dyspnea [25]. Also, inadequate ventilation has been suggested as a possible reason for the performance

limiting exercise-induced arterial hypoxaemia (EIAH) that is often seen in elite athletes [20]. Although recent research suggests that salbutamol use does not reduce the impact of EIAH [75], anecdotal evidence suggests that non-asthmatic athletes believe in its ability to enhance performance and are using doses that substantially exceed therapeutic recommendations. This poses not only an ethical question but also raises concerns of athlete safety.

Salbutamol and Performance in Non-Asthmatics

Interest in the performance enhancing qualities of β_2-agonists in non-asthmatics has increased in the past 10 years, likely due to the increased use of β_2-agonists in competition. An extensive review of literature examining β_2-agonists as ergogenic aids has recently been published [47] and for this reason, this review will only focus on studies examining the effects of salbutamol. In this respect, some studies report an ergogenic benefit [7, 14, 17, 68, 78, 79], while the majority of the research suggests that acute salbutamol administration does not enhance athletic performance in non-asthmatics [11, 16, 22, 24, 29, 46, 48, 53, 61, 74, 75] (Table 2.1). Of the five studies that have shown a positive effect, three [14, 17, 79] utilized oral administration of salbutamol. Oral administration is banned due to its known anabolic effects and will therefore not be discussed. Bedi and colleagues [7] utilized a sport specific test to determine whether or not 180 μg of salbutamol had any effects on performance. The test consisted of 45 minutes of cycling at 75% $\dot{V}_{O_2}$max followed by a sprint to exhaustion. Salbutamol treatment resulted in an improvement in sprint time of approximately 23%. These results

have been questioned for the use of a non-homogenous group and in particular two outliers that affected mean data [47].

Table 2.1. Summary of studies examining ergogenic effects of salbutamol. TTE = Time to Exhaustion; PP = Peak Power; MP = Mean Power; TW = Total Work; TTC = Time to Completion

Measure	Administration	Dose	Performance Effect	Reference
TTE, VO_2max	Inhaled	1200μg	No Change	Sandsun *et al.* [61]
TTE	Inhaled	360μg	No Change	Fleck *et al.* [22]
AT & VO_2max	Inhaled	200μg	No Change	McKenzie *et al.* [46]
VO_2max, PP	Inhaled	400μg	No Change	Stewart *et al.* [74]
VO_2max, PP, TW	Inhaled	200μg	No Change	Meeuwise *et al.* [48]
20km TT	Inhaled	400μg	No Change	Norris *et al.* [53]
VO_2max	Inhaled	400μg	No Change	Stewart *et al.* [75]
TTE	Inhaled	200 & 800 μg	No Change	Goubault *et al.* [24]
TTE	Inhaled	800μg	Negative	Carlsen *et al.* [11]
TTE	Inhaled	180μg	Positive	Bedi *et al.* [7]
TW	Inhaled	800μg	Positive	van Baak *et al.* [78]
PP	Inhaled	180μg	Positive	Signorile *et al.* [68]
TTE	Nebulized	0.05mg/kg	No Change	Heir *et al.* [29]
10 min MP	Oral	6mg	No Change	Collomp *et al.* [16]
TTE, Strength	Oral	4mg	Positive	Van Baak *et al.* [79]
TTE	Oral	6mg	Positive	Collomp *et al.* [14]
PP, MP	Oral	4mg	Positive	Collomp *et al.* [17]
Strength (9 weeks)	Oral	16mg/day	Positive	Caruso *et al.* [12]
TTE (3 weeks)	Oral	12mg/day	Positive	Collomp *et al.* [15]
PP (3 weeks)	Oral	12mg/da	Positive	Le Panse *et al.* [38]

Signorile and colleagues [68] examined the effects of inhaled salbutamol (180 μg) on sprint performance. Recreationally active male and female subjects performed two all-out sprints of 15 seconds on a bike separated by 10 minutes. The salbutamol trial showed significant improvements over placebo in peak power but no difference in total work. With respect to competitive performance enhancement, this data should be interpreted with caution for two reasons. Recreational athletes were used in this study and the inference to elite athletes would be unjustified. Secondly, several studies have since shown that salbutamol has no effect on peak power [48, 53, 75].

Both of the above mentioned studies that have reported a positive effect on performance have reported it in anaerobic type activities. Although Bedi and colleagues [7] performed 45 minutes of submaximal exercise, they only showed significant improvement in the sprint to exhaustion (~23%) with no effect on the submaximal exercise session. The majority of athletes that are using salbutamol participate in endurance sports with the top four at the Sydney 2000 Olympics being triathlon, swimming, modern pentathlon, and cycling [19].

Only one study has reported a performance enhancing effect of inhaled salbutamol in an endurance performance test. After a dose of 800 μg, van Baak and colleagues [78] demonstrated that time complete a set amount of work cycling was 1.9% faster than following placebo. This result is questionable though as two subjects in this study were significant outliers while the majority of subjects follow the line of identity in comparing

trials. Other than this study, research examining the effects of salbutamol on endurance performance unequivocally demonstrates no enhancement.

The validity of a test to be representative of performance is an important factor when evaluating the ergogenic effects of a treatment [34]. Majority of the research that has looked at endurance performance use one of, or a combination of, a $\dot{V}_{O_2}$max , Wingate, lactate threshold, or a run to exhaustion (3-5 min) test. No study to date has demonstrated an increased endurance performance using one of these measures. Although these are valuable in a laboratory setting to look at physiological changes, rarely are these measures indicative of performance in events [34].

Carlsen and colleagues [11] compared the effects of salbutamol (800 μg) to salmeterol (50 μg) in 18 male runners and cross-country skiers ($\dot{V}_{O_2}$max = 73.9 ml·kg^{-1}·min^{-1}). All subjects had normal lung function. Each person was required to perform a $\dot{V}_{O_2}$max test as well as run at anaerobic threshold. Results showed that although lung function (FEV_1) was increased by both drugs prior to exercise when compared to placebo, there was no effect on either $\dot{V}_{O_2}$max or anaerobic threshold. Several other studies have shown similar effects on $\dot{V}_{O_2}$max [22, 48, 53, 61, 75] following doses of 200 μg[48], 360 μg[22], 400 μg[53, 75], and 1200 μg[61]. It is clear that across a variety of doses, salbutamol does not have an effect on $\dot{V}_{O_2}$max .

In an attempt to reproduce the findings of Bedi and colleagues [7] in a more sport specific test, Meeuwise et al [48] examined the effects of a 200 μg dose of inhaled salbutamol on sprint performance. Seven highly trained male cyclists ($\dot{V}_{O_2}$max = 63.5 ml·kg^{-1}·min^{-1}) performed a sprint to exhaustion following 45 minutes of continuous cycling (~70% $\dot{V}_{O_2}$max). Wingate peak power and total work were also measured. No effect on sprint endurance time was seen nor were there any improvements in peak power or total work following salbutamol inhalation. Although this protocol is more likely to simulate endurance performance than a $\dot{V}_{O_2}$max test, it still does not replicate the ability of the athlete to pace himself.

A recent study by Goubault and colleagues [24] examined the effects of two different doses of salbutamol (200 μg and 800 μg) on exercise performance in a time to exhaustion test. Twelve competitive triathletes ($\dot{V}_{O_2}$max = 57.9 ml·kg^{-1}·min^{-1}) rode to exhaustion at 85% maximal aerobic power. No differences were noted in the time to exhaustion between placebo, 200 μg and 800μg conditions (23m31s, 21m45s, and 23m18s respectively) indicating lack of a dose-response relationship. However, the variability in these results between trials questions the reliability of the measure used for performance in these subjects and it is difficult to determine the effects of dose with only two doses. The dose-response relationship should be re-examined in a reliable and reproducible measure using three or more doses.

Only one other study has investigated the effects of inhaled salbutamol on a simulated sport-specific performance test [53]. Norris *et al.*, [53] demonstrated that a 400 µg dose had no effect on time-trial performance in competitive cyclists ($\dot{V}_{O_2}$max = 63.4 ml·kg^{-1}·min^{-1}). Although statistically not significant, salbutamol treatment resulted in a 12-second improvement during a 20km time-trial which equates to a 0.6% difference in average performance velocity. It is unlikely that this would be performance enhancing for the subjects used, however, at the elite level, athletes are a homogenous group physiologically within an individual sport. It is suggested that an improvement of 0.3 and 1.5 times the coefficient of variance (CV) in performance at the elite level could have a worthwhile effect on increasing the likelihood of winning for an athlete who averages 1st and 10th place respectively [34]. The typical CV for top performers in simulated cycling time-trials is approximately 1-1.7% [37, 58, 59, 69].

An analysis of the 2002 Tour de France prologue time-trial (7km) shows that a 0.6% difference in velocity (~ 4 seconds) is the difference between 1st and 4th place (unpublished analysis). Furthermore, the average difference in performance velocity in speed skating competitions at the 1988 Winter Olympics was 0.3% between 1st and 2nd place finishers, and 1.3% between 1st and 4th places [71]. If 30 seconds (1.5% improvement in speed) was used as a difference that would have a competitively significant effect for a 20km time-trial, retrospective analysis of the data from Norris et al, [53] would show that sample sizes utilized were inadequate to detect a difference that may have competitive significance. For statistical power of .80 and an alpha level of 0.05, 241 subjects would have been required to show an increase of 30 seconds as a significant

improvement in performance. For the sample size that was used (15), the standard deviation of the sample would need to be approximately 45 seconds, which is much more homogenous than the observed 186 seconds. Although it is extremely difficult to have sufficient statistical power with athletes to detect the smallest difference that may have a competitive effect (0.3-0.6 multiple of CV), this highlights the need to use as many subjects as possible while maintaining a low standard deviation. Future studies should be conscious of what is competitively significant and we suggest they be designed to detect at least and enhancement that would significantly increase the likelihood of winning for someone who averages 10^{th} place (1.5 multiple of CV) [34].

In summary, the research clearly shows that inhaled salbutamol has no effect on endurance performance in highly trained athletes. However, the dose-response effect of salbutamol on performance has not been adequately evaluated and this needs to be assessed in a homogenous group of highly trained athletes with a sport specific performance test.

Salbutamol and Doping Control

The WADA code currently requests that laboratories report all cases in which the urine concentration of salbutamol exceeds 200 $ng \cdot mL^{-1}$. Regardless of whether or not the athlete has a TUE, a urine concentration of greater than 1000 $ng \cdot mL^{-1}$ is considered a doping violation due to salbutamol's anabolic effects [82]. At a competition, the athlete provides a urine sample after the event, with length of time since last inhalation not being standardized. Typically, this sample is then analyzed for the non-sulphated fraction of

SAL following glucuronidase enzymatic hydrolysis. This method allows for the determination of free and glucuronized forms of the drug only, and does not account for the sulphated portion. Currently the doping regulations do not specify that corrections are made for differences in urine specific gravity when analysing urine samples [65]. This would seem imperative when considering the potentially dehydrating effects of exercise. Furthermore, differences in time between inhalation and sample may affect urinary concentrations. Between 15% and 40% of the dose may be excreted in the first 4-6 hours post inhalation [21, 30, 83] and depending on both hydration and sample time, it is likely that urinary concentrations will vary.

The pharmacokinetics of salbutamol are well researched and documented in both healthy and diseased populations [54]. The vast majority of urinary results are reported as a percentage of dosage, a percentage of dosage recovered, a ratio of free salbutamol and its metabolites, or as an absolute value [21, 30, 31, 77, 83]. However, little research actually reports values in concentrations as is used by WADA. A recent examination of the dose-response effects on urinary salbutamol following 30 minutes of rest showed that inter-subject variability was quite high (36-38%) after both a single 100 μg dose and multiple doses (5 x 100 μg) [77]. Possible reasons for such high variability were thought to be due to variations in renal function and deposition of the drug in the lung between subjects. It was also shown that the absolute amount of salbutamol that was recovered in the urine was linearly related to dose inhaled. This is important to consider when analyzing urine for possible doping infractions as it will affect concentration.

Unfortunately, this study provided only absolute values and did not include volumes of urine samples so comparison to the WADC is impossible.

A review of the literature revealed only two studies that have reported urine concentrations in their findings. In a recently published case study it was shown that inhaled salbutamol resulted in a positive doping test [65]. Schweizer and colleagues (2004) reported an in-competition measurement of 8000 $ng \cdot mL^{-1}$ in a male athlete with a TUE and were able to reproduce this positive test in a controlled non-exercising trial. Urine concentrations of non-sulphated salbutamol were found to be approximately 4000 $ng \cdot mL^{-1}$ urine up to 6 hours post inhalation. The majority of this was glucoronized salbutamol (up to 3400 $ng \cdot mL^{-1}$) with the remainder being free salbutamol. The subject in this case study was using three doses of three inhalations each (100 μg salbutamol/dose) over a period of 5 hours prior to the urine sample. This may be classified as a common treatment for asthma in sport [65] yet would appear to result in a positive doping infraction. Other similar cases have been reported in a variety of sports, all with urinary concentrations between 1000 and 3000 $ng \cdot mL^{-1}$ following exercise [45]. False positives may be a result of the previously mentioned interindividual differences in renal function and/or lung deposition [77] or in exercising cases, it may be due to dehydration from exercising in hot, humid environments [45] . Furthermore, exercise following inhalation increases lung absorption of β_2-agonists in healthy individuals [64].

In the second study, Ventura and colleagues [80] examined the effects of inhaled and oral administration of salbutamol on urine concentrations in swimmers post-training. Urine

concentrations of salbutamol (non-sulphated) following inhalation of a 200 μg dose were reported to be between 100 and 600 $ng \cdot mL^{-1}$ within one hour post-training (approximately 2-3 hours after drug administration). Similar values were found when the dose was increased to 1600 μg over the 4 hours prior to exercise. These values are much lower than those reported in the previously mentioned case study [65] and fall within the allowable limits, however, they still demonstrate high variability between subjects and would constitute a reportable doping result. Furthermore, they do not follow the linear dose-response relationship expected with increased dosage suggesting inconsistencies in urine analysis of salbutamol. Despite the wealth of research on salbutamol, there lacks a clear description of the dose-response effect on urine concentrations at different time intervals post-inhalation for both rest and exercise.

Summary and Future Directions for Research

The use of salbutamol in elite sport is on the rise and there are concerns of increased use by non-asthmatics in order to gain a competitive edge. Furthermore, there is anecdotal evidence of athletes using greater than the recommended therapeutic dose which raises both ethical and safety concerns. Although the majority of research suggests salbutamol has no performance enhancement in non-asthmatics, most studies have used non-specific laboratory measures rather than a test that effectively replicates sport performance. There is a need to re-examine the dose-response relationship using a sport-specific performance test with a homogenous group of highly trained athletes.

There is also limited data describing the effects of dose on urine concentrations of salbutamol at rest and after exercise. Recovery of salbutamol in the urine is highly variable between subjects [77, 80] which may help explain reports of positive doping violations for salbutamol when using therapeutic doses [45, 65]. There lacks a clear description of the dose-response effect on urine concentrations of salbutamol at specific time intervals post-inhalation following both rest and exercise. Future research should be directed at providing a description of these responses with respect to criteria used in doping control.

CHAPTER 3 – 20KM TIME TRIAL RELIABILITY

Introduction

Determining the effect of a treatment on exercise performance enhancement in athletes is best accomplished when two criteria have been met [32, 34, 58]. The first is to utilize a test that shows a strong relationship between competitive performance and performance in the test [34]. Laboratory based tests considered to have the highest performance validity in cyclists are simulated time trials that optimize the ability of a cyclist to best reproduce the shifting, inertia, and performance on the cycle ergometer [58]. Air braked ergometers that attach to the athletes own bike have provided the lowest typical error when comparing test results to performance [58]. The second criterion is that the test is highly reproducible to avoid large sample sizes and to detect small differences [34]. At the elite level small differences in performance can result in significant changes in placing.

Both the Kingcycle and the Cyclosimulator, ergometers that attach to the cyclists own bikes, have been shown to be highly reproducible during simulated time trials with coefficients of variation (CV) of 1.0 or less [37, 58, 69]. Mean power in indoor time trials tends to demonstrate a higher CV (1.5% - 2.3%) when measured using either a Kingcycle or a Schoberer Rad Messtechnik (SRM) powermeter [70]. A new ergometer, the Velotron Pro, which uses a fully adjustable bike frame and is electronically braked rather than air braked, avoids some of the inherent problems with attaching a bike to a roller system. These include ensuring consistent air pressure in the rear tire, differences

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in bearing friction between bikes, and controlling of movement of the cyclist during a test to avoid differences in rolling resistance from the calibration position. The reliability of the Velotron Pro has yet to be evaluated with respect to time trial performance.

Several researchers have examined the predictive ability of peak power (P_{peak}) achieved during an incremental exercise test in determining time trial performance [5, 28, 37]. In a laboratory setting, P_{peak} has been shown to be highly related to 40km time trial performance [37]. In outdoor trials however there exists discrepancy. Hawley and Noakes [28] have reported P_{peak} to be a strong predictor of 20km cycle time ($r = -0.91$) while others have shown it to be a poor predictor of performance time ($r = -0.46$) but an excellent predictor of mean power output ($r = 0.99$) [5]. Differences between indoor and outdoor predictability is not surprising as the majority of ergometers calculate speed from power output and demonstrate colinearity between the two ($r = 0.999$) [57] while other factors such as frontal area, rolling resistance, and topography are either held constant or not included. Smith and colleagues [70] showed mean power output, during lab based and outdoor 40km time trials, was not significantly different (303 W vs 312 W) even though performance time varied by more than 3 minutes. The relationship found between peak power in an incremental test and performance time in indoor time trials is likely due to a strong relationship between peak power and the mean power produced during the time trial. This has yet to be clarified in the literature.

The purposes of this study were to determine the reproducibility of a laboratory based 20km cycle time-trial performance test in competitively trained cyclists using the

Velotron Pro cycle ergometer and to examine the relationships between P_{peak} achieved in an incremental exercise test and time trial performance (time and power).

Materials and Methods

Subjects

Twenty competitive, male cyclists participated in this study (mean ± SD: age = 31 ± 8 y). Subjects were determined to be competitive based on their ability to compete at the provincial level (Category 2 or higher for road cyclists and Pro/Elite for mountain bikers) with the average number of years competing being 9 ± 5 years. All subjects were required to have a maximal aerobic power ($\dot{V}_{O_2}max$) of at least 60 $ml \cdot kg^{-1} \cdot min^{-1}$ or 5.0 $L \cdot min^{-1}$. This study was completed primarily during the off season and a period of training for local cyclists that averaged a volume of 274 ± 96 $km \cdot wk^{-1}$. Three subjects completed this study at the end of their competition phase. A medical history questionnaire and written informed consent were obtained from all subjects and procedures were approved by the University of British Columbia's Clinical Research Ethics Committee on Human Experimentation.

Study Design

This study utilized a repeated measures design. Each subject came to the lab on 4 different occasions at approximately the same time of day with a minimum of 72 hours between each visit. All trials were completed within a period of four weeks. Subjects were asked to refrain from intense exercise within 24 hours prior to each testing session and refrain from consuming food or caffeine for 3 hours prior. Cyclists were also

requested to maintain a consistent diet 24 hours prior to each testing session and instructed to prepare for each time trial as they would normally for a race. A self-selected warm-up of 30-45 minutes was used for each testing session and although this differed between subjects, the same warm-up was used prior to each test for any given subject. The first visit included medical screening and an assessment of $\dot{V}_{O_2}$max. Height and weight was collected at the start of each visit. The remaining three visits involved a 20km simulated cycle time trial with each test being performed at the same time of day.

Maximal Aerobic Power Test

A $\dot{V}_{O_2}$max test was performed on the Velotron Pro cycle ergometer (Racermate Inc, Seattle). Prior to each test, factory calibration was verified using the Accuwatt "run down" verification program (Racermate Inc, Seattle) accompanying the ergometer software. Subjects were fitted to the ergometer based on the setup of their own bicycle. All settings were recorded and used in subsequent time trials. Bike settings included both seat and handle bar height and horizontal position, as well as crank length. Subjects were instructed to remain seated throughout the test. A 30 $W \cdot min^{-1}$ ramp protocol was utilized and controlled via the Velotron Coaching Software (Version 1.5.186, RacerMate Inc, Seattle) with expired gases collected and analyzed every 15 seconds (TrueOne 2400 – Parvo Medics, Utah). Oxygen consumption ($\dot{V}_{O_2}$), minute ventilation ($\dot{V}_E$), production of carbon dioxide ($\dot{V}_{CO_2}$), and respiratory exchange ratio (RER) were recorded. Air flow and gas calibrations were performed prior to each test using a 3 L calibration syringe and gases of known concentrations respectively. Standard indicators for achieving

$\dot{V}_{O_2}$max were used including volitional fatigue, a plateau in $\dot{V}_{O_2}$ with increasing work rate, HR ≥ 90% of age predicted maximum, and a RER ≥ 1.15. $\dot{V}_{O_2}$max was recorded as the mean of the two highest consecutive 15-second samples. Heart rate (HR) was measured by telemetry (Polar Vantage XL, Kempele, Finland) and recorded. Peak power was recorded as the highest completed 15 second interval with power recorded in 7.5 watt intervals.

Simulated 20km Time Trial

All time trials were performed on the Velotron Pro cycle ergometer which was calibrated prior to each test as described previously. Subjects were required to perform 2 laps of a 10km course designed using the Velotron 3D software accompanying the ergometer (Version NB04.1.0.2101, RacerMate Inc, Seattle). The course was flat with no active wind effect. Subjects were able to change gears using the ergometer's electronic gearing system. A gearing system simulating a 53/39 front chain ring setup and 23/21/19/17/16/15/14/13/12/11 rear cog set was used. Throughout the time-trial, subjects were able to watch themselves racing the course on the computer monitor. Distance traveled and gear selected were displayed while all other feedback (speed, HR, power, and time) was blinded to the subject, although they were recorded by the ergometer software and downloaded afterwards for analysis. Subjects did not receive any information as to how well they performed until all three time trials were completed. Throughout the test, subjects were not required to remain seated and were permitted to drink water *ad libitum*.

Total time to completion (T_{tot}), time for each 10km lap (T_{L1} and T_{L2}), mean performance velocity (VEL), mean performance power in watts (P_{mean}), and mean relative performance power in watts/kg (P_{rel}) were recorded for each time trial.

Data Analysis

Mean values for all performance variables were compared using a one way repeated measures analysis of variance. CVs between trials were calculated for the log-transformations of each variable measured as described by Hopkins and colleagues [34]. Relationships between trials were calculated using Pearson's product moment correlations. The relationships between peak power and both T_{tot} and P_{abs} were examined for TT1 only as both tests were completed during the same week. A multiple linear regression was performed to determine the predictive capability of peak power and P_{abs} for T_{tot}. Reliability and reproducibility statistics were performed using an Excel (Microsoft Corporation) spreadsheet [33] with confidence intervals being set at 95%. Analysis of variance and regressions were performed using Statistica software (Version 5.0, StatSoft Inc.). For all tests, α was set at 0.05 and results shown are mean ± SD unless otherwise noted.

Results

Maximal aerobic power test

All subjects met the required minimum $\dot{V}_{O_2}$max criteria of 60 ml·kg^{-1}·min^{-1} with a mean value of 68.5 ± 3.6 ml·kg^{-1}·min^{-1} (absolute 5.25 ± 0.61 L·min^{-1}). Mean absolute and

relative P_{peak} was 469 ± 33 W and 6.16 ± 0.49 W/kg respectively while peak HR was 186 ± 9 bpm.

20km time-trial performance

Measured variables for each trial are shown in Table 3.1. There were no statistical differences in any measured variables across trials. Mean performance time was slightly faster during TT1 than both TT2 and TT3 with the mean difference equal to approximately six seconds (0.10 min) however this was not statistically significant ($p=0.33$). This difference was predominantly due to a lower T_{L1} in TT1 compared to TT2 and TT3 which was also not statistically significant ($p=0.47$). Fig. 3.1 shows no apparent trend for one trial being faster than the others.

Table 3.1. Measured Variables During each 20km Time Trial Performance: Mean ± SD for Total Time (T_{tot}), First and Second Lap Times (T_{L1} and T_{L2}), Mean Velocity (VEL), Heart Rate (HR) and Absolute and Relative Power Output (P_{mean} and P_{rel}).

	T_{tot} (min)	T_{L1} (min)	T_{L2} (min)	VEL (km/hr)	HR (bpm)	P_{mean} (W)	P_{rel} (W/kg)
TT1	30.03 ± 1.24	14.93 ± 0.71	15.10 ± 0.56	40.0 ± 1.7	171 ± 8	326 ± 35	4.27 ± 0.35
TT2	30.12 ± 1.21	15.01 ± 0.73	15.11 ± 0.55	39.9 ± 1.6	170 ± 9	323 ± 35	4.24 ± 0.42
TT3	30.14 ± 1.21	15.03 ± 0.71	15.11 ± 0.55	39.9 ± 1.6	170 ± 7	322 ± 34	4.23 ± 0.42

* - denotes statistical difference between trials, $p < 0.05$.

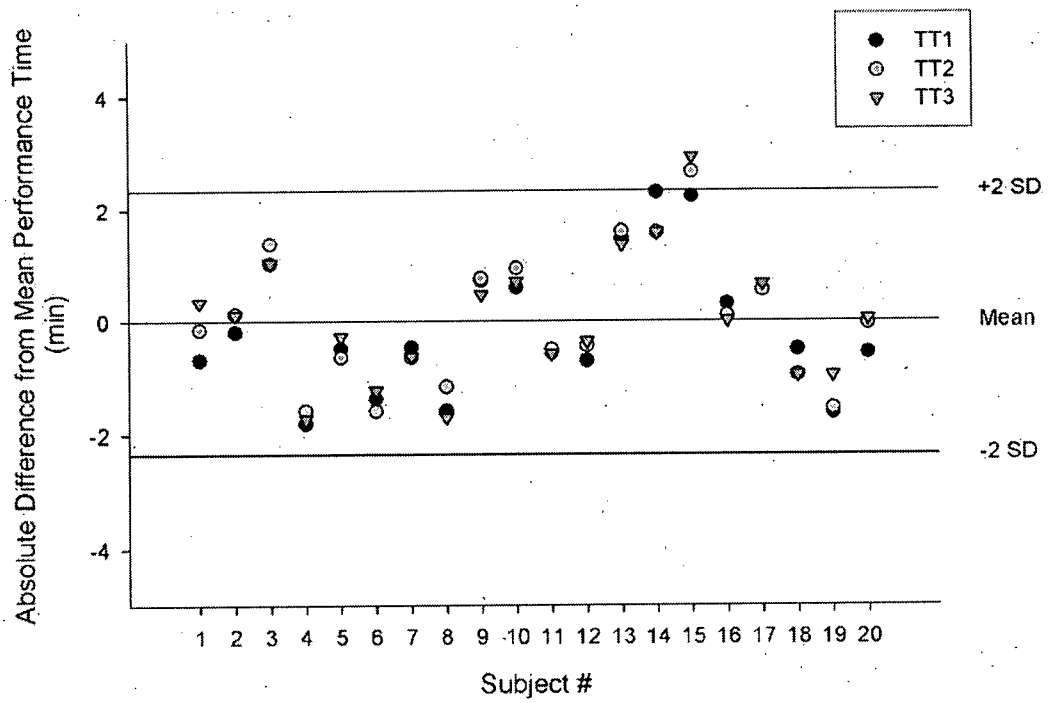

Fig. 3.1. A Bland-Altman style plot showing individual performance times for all three time trials (TT1, TT2, TT3).

All reliability measures are reported in Table 3.2. T_{tot} was highly reproducible and strongly related between TT1 and TT2 (CV= 0.8%; r = 0.96) as well as TT2 and TT3 (CV = 0.7% r = 0.97). When separated into the first and second lap (T_{L1} and T_{L2}), the CV with respect to time to complete lap one was noticeably larger between TT1 and TT2 (2.1%) than TT2 and TT3 (1.3%). Power output (P_{mean}) demonstrated a higher CV than performance time between trials as did HR and both were strongly related between trials (Table 3.2).

Table 3.2. Reproducibility Statistics Including Change in Means (Δ Means), Coefficient of Variance (CV) and Pearson Correlation Coefficients (r) along with 95% Confidence Intervals (C.I.) for TT1, TT2, and TT3.

		Δ Means (units) (95% C.I.)	CV (%) (95% C.I.)	r (95% C.I.)
T_{tot} (min)	TT1 vs TT2	0.09 (-0.06; 0.25)	0.8 (0.6; 1.1)	0.96 (0.90; 0.98)*
	TT2 vs TT3	0.02 (-0.11; 0.15)	0.7 (0.5; 1.0)	0.97 (0.92; 0.99)*
TL1 (min)	TT1 vs TT2	0.06 (-0.07; 0.20)	2.1 (1.6; 3.1)	0.79 (0.52; 0.91)*
	TT2 vs TT3	0.03 (-0.04; 0.11)	1.3 (1.0; 1.9)	0.92 (0.80; 0.97)*
TL2 (min)	TT1 vs TT2	0.03 (-0.05; 0.11)	0.8 (0.6; 1.2)	0.94 (0.88; 0.98)*
	TT2 vs TT3	0.00 (-0.10; 0.11)	1.0 (0.8; 1.5)	0.93 (0.85; 0.98)*
P_{mean} (watts)	TT1 vs TT2	-3 (-7; 2)	2.1 (1.6; 3.1)	0.96 (0.91; 0.99)*
	TT2 vs TT3	-1 (-5; 3)	1.9 (1.4; 2.8)	0.97 (0.91; 0.99)*
HR (bpm)	TT1 vs TT2	-2 (-4; 0)	2.0 (1.6; 3.0)	0.90 (0.75; 0.96)*
	TT2 vs TT3	1 (-1; 2)	1.4 (1.1; 2.1)	0.95 (0.86; 0.98)*

* - Denotes statistically significant relationship, $p<0.05$

Relationships between peak power and performance

Fig. 3.2 shows the relationships between P_{peak} and both T_{tot} and P_{mean} for TT1. Peak power was significantly correlated to T_{tot} ($r = -0.89$, $p<0.05$) and P_{mean} ($r = 0.91$, $p<0.05$), while P_{mean} demonstrated colinearity with T_{tot} ($r = 0.996$, $p<0.05$). Multiple linear regression demonstrated that P_{mean} primarily accounted for predictability of T_{tot} ($R^2 = 0.993$) by the equation T_{tot} (min) $= 40.96 - 1.1(P_{mean}) + 0.06(P_{peak})$.

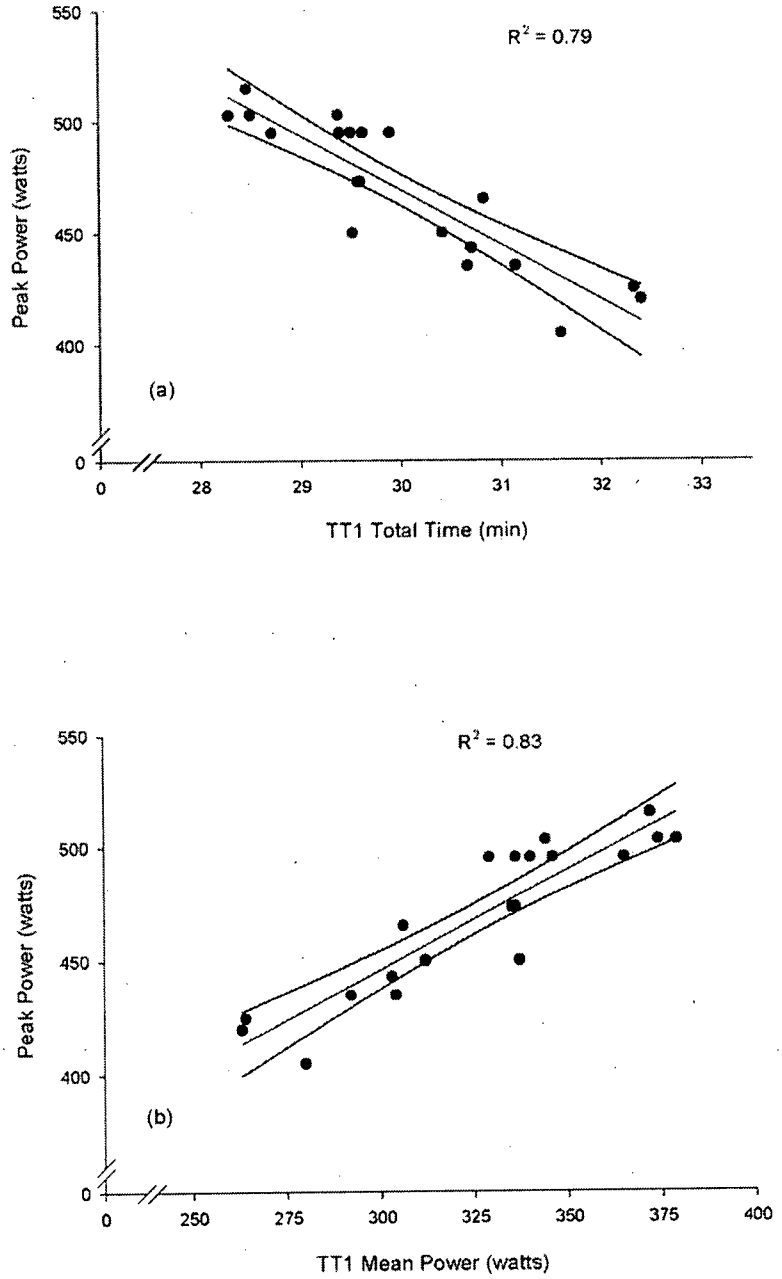

Fig. 3.2. Relationships between peak power during an incremental exercise test (P_{peak}) and (a) performance time (T_{tot}) and (b) mean power (P_{mean}) for TT1 (n=20). Lines represent 95% confidence intervals.

Discussion

The main finding of this study was that in trained, competitive cyclists, completion time in three 20km time trials performed a minimum of 72 hours apart on the Velotron Pro cycle ergometer are not significantly different from each other. It was also demonstrated that performance was highly reproducible with respect to time, power, and heart rate. Total performance time demonstrated the lowest CV between trials (0.8% or less) with power and HR being slightly higher (<2.1% and <2.0% respectively).

Often a familiarization trial is suggested when doing lab based performance tests. The T_{tot} data, and that of others [57, 70], do not suggest this is necessary in competitively trained cyclists. Although none of the subjects had used this ergometer before, all had previously used other ergometers, completed time trials, and trained with sustained of efforts of approximately 30 minutes. Furthermore, subjects reported the feeling of riding on the ergometer as being similar to riding on the road. Subjects were required to pace themselves based on perceived effort rather than heart rate, speed, or power. As an index of exercise intensity, HR did not vary between trials and demonstrated a CV similar to that previously reported when HR feedback was provided [70], suggesting trained cyclists are capable of pacing themselves without feedback. The higher CV seen for T_{LI} between the first and second trials (2.3%) is likely due to differences between the resistance produced by electronic gearing system and the equivalent gear on a bicycle over flat ground. Some subjects reported relying on gear selection initially for pacing but soon afterwards switched to perceived exertion. In competitive cyclists, a full

familiarization trial may not be necessary when using the Velotron, but we recommend an opportunity to become familiar with the resistance produced by the gearing system.

The high reproducibility of performance time across trials is comparable to that found in other reliability tests using air braked ergometers [37, 57, 70]. Over both 20 and 40km distances, performance time has been shown to have a CV of 1.1% and 1.0% respectively [57]. Following a familiarization trial, Laursen and colleagues [37] demonstrated a CV of 0.9% in time to complete a simulated 40km time trial using the Cyclosimulator (Cateye) air braked ergometer. Using a Kingcycle ergometer and over three 40km trials, Smith *et al.*[70] reported a similar CV (0.7% and 0.9%). An important aspect for evaluation of a performance test is that it is more reliable than the event itself [34]. When compared to reported values for outdoor trials (1.1% - 2.2%) [70], indoor trials appear to demonstrate higher reproducibility. This is likely due to the control of several factors that can affect speed (wind, topography, temperature, rolling resistance, and aerodynamics). Rather than time or speed, mean power is apt to be a better variable for comparisons between indoor and outdoor efforts as it represents the performance capabilities of the cyclist. Indeed, when compared between the two, mean power (SRM) does not vary over 40km, with each demonstrating similar CV (indoor = 1.9% - 2.1%; outdoor = 2.1% - 2.4%) [70]. These values are similar to the CV shown in the present study (2.1% and 1.9%) for TT1-TT2 and TT2-TT3 and suggest that the Velotron provides a reliable measure of power output over 20km. Further research is necessary to determine the validity of mean power using to the Velotron to mean power produced during an outdoor time trial.

Although power demonstrated a higher CV than performance time, it should not be assumed that power is a less reliable variable. The relationship between power and speed during cycling is non-linear and is described by the equation $P = kV^n$ where P = power, V = velocity while k and n are constants for a particular ergometer [34]. Hopkins *et al.* [34] simplify this equation to demonstrate that the percent change in power is approximately equal to the percent change in speed multiplied by a factor of n [$100\Delta P/P \approx n(100\Delta V/V)$]. Unfortunately we do not know the value of n for the Velotron but reported values for other ergometers range between 1.5 and 2.2 for speeds around 40km·hr^{-1} [34]. Assuming a similar value for the Velotron, the CV for P_{mean} would be expected in comparison to the CV for T_{tot}.

The second purpose of this study was to examine the relationships between P_{peak}, P_{mean}, and T_{tot}. It has previously been suggested that P_{peak} is a good predictor of time trial performance [28, 37]. In a laboratory setting, P_{peak} has been shown to be related to 40km time trial performance [37] (n=43) and is in agreement with our findings over 20km (r = -0.89). However, regression analysis suggests that the predictability of T_{tot} is primarily due to P_{mean} rather than P_{peak}, as it accounted for ≈ 99% of the variance. This is not unexpected as the majority of ergometers calculate speed from power output and demonstrate colinearity between the two (r = 0.999) [57]. With respect to outdoor trials, Hawley and Noakes [28] reported P_{peak} to be highly related to 20km cycle time (r = -0.91) which is surprising considering all the factors that can influence speed during cycling. Their results are likely influenced by the heterogeneity of the subjects (P_{peak} = 175 - 440

W; $T_{tot} \approx 31$ min – 45 min). With a more homogenous group of cyclists (P_{peak} = 304 - 480 W; $T_{tot} \approx 21$ min – 25 min), Balmer *et al.* [5] demonstrated a weak relationship between the two (r = -0.46) over 16.1 km, but P_{peak} was an excellent predictor of mean power output (r = 0.99) [5]. This coincides with the relationships demonstrated between P_{peak} and P_{mean} during an indoor trial in the present study. Rather than suggesting that P_{peak} is a predictor of performance time, we echo the comments of Balmer *et al.* [5] that P_{peak} is a good predictor of P_{mean}. Further, any relationships between P_{peak} and performance time are dependent on factors that affect the conversion of power output into speed.

An important aspect when evaluating reliability of a performance test is a clear distinction of the population the test is designed for [32]. The cyclists used in this study were defined as trained, competitive male cyclists based on their ability to compete at a provincial level or higher. The time taken to complete the 20km time trial averaged 30.1 minutes which equates to an average speed of 39.9 km/hr. This is slower than what would be expected for competitive cyclists during time trial events (>43km/hr) [52] and is likely due to the calculation of speed from power in the software. Input of mean power to a commonly used web-based speed calculator [18] resulted in an average speed of ~ 44 km/hr, similar to typical speeds seen in time trials reported by the cyclists in this study. On a physiological basis, they are comparable to competitive cyclists previously defined in the literature [73, 86]. Others have reported higher values in relative $\dot{V}O_2$max and relative peak power in professional male cyclists [36, 39, 52, 56], however, the number of these cyclists across the world is relatively small. We believe that the cyclists used in this

study represent the most plausible highly trained group from which sufficient sample size (>20) could be obtained for future studies examining performance enhancement. Furthermore, the CV demonstrated for T_{tot} and P_{mean} appears to be equal to or better than that demonstrated for actual performances [70] which is important when evaluating the applicability of interventions [34].

In conclusion, the present study has shown that a flat 20km time trial performed on the Velotron Pro cycle ergometer is highly reproducible over three trials in competitive cyclists and comparable to other frequently used ergometers. Although the results do not suggest a familiarization trial is necessary, we recommend cyclists become accustomed to the gearing system as it does not appear to reproduce speeds found on the road. Furthermore, there is strong relationship between P_{peak} and sustainable power during time trials and this relationship is primarily responsible for the predictability of performance time for ergometer based time trials. Predictability of outdoor performance from P_{peak} is questionable.

CHAPTER 4 – DOSE RESPONSE OF SALBUTAMOL AT REST

Introduction

For several years β_2-agonists have been approved by the World Anti Doping Agency (WADA) for use in competitive sport by athletes experiencing asthma and/or exercise induced bronchospasm (EIB). Salbutamol (SAL) is one of the approved β_2-agonists and was the most commonly used asthma medication in athletes selected for doping control at the Sydney 2000 Olympic Games [19]. The doping code specifies that administration of SAL must be through inhaled devices as oral administration may potentially have performance enhancing anabolic effects [12, 44, 79]. As applications for therapeutic use exemption (TUE) of SAL have been increasing [2], there are concerns that it may be used as an ergogenic aid by both asthmatics and non-asthmatics and is therefore monitored closely by WADA through urine sampling.

Currently WADA requests that laboratories report all cases in which the urine concentration of SAL (*cSAL*) exceeds 200 $ng \cdot ml^{-1}$. Regardless of whether or not the athlete has a TUE, a urine concentration of greater than 1000 $ng \cdot ml^{-1}$ (nonsulfated) is considered a doping violation [82]. This is likely due to previously published research indicating values over 1000 $ng \cdot ml^{-1}$ are only observed following oral administration [80]. A recently published case study has questioned whether or not this cut off point is appropriate as it may result in a false-positive doping test and subsequent 2-year ban from competition [65]. Schweizer and colleagues [65] reported an in-competition measurement of 8000 $ng \cdot ml^{-1}$ in a male athlete with a TUE and were able to reproduce

A version of this chapter has been submitted for publication. Sporer, B.C., Sheel, A.W., Taunton, J., Rupert, J.L., & McKenzie, D.C (2006). Variability in Urine Concentrations of Salbutamol: Implications for Doping Control. *International Journal of Sports Medicine.* Submitted September 2006.

this excessive test in a non-exercising trial. This is in agreement with other reports of positive test results using therapeutic doses, all with urine concentrations between 1000 and 3000 $ng \cdot ml^{-1}$ following exercise [45]. High inter-subject variability (~38%) has been shown in urine recovery of SAL [77] and this may in part explain the recent occurrence of false-positive tests. It is possible that differences in renal function, lung absorption, and/or dehydration from exercise [45] are responsible for the high variability. Furthermore, differences in time between inhalation and sample collection may affect urine concentrations. Between 15% and 40% of the dose may be excreted in the first 4-6 hours post inhalation [21, 30, 83] and depending on hydration status, *cSAL* may vary. Currently WADA does not correct for hydration and only requires that urine samples have a minimum specific gravity (SG) of 1.005. Correcting urine samples for SG may provide insight as to the effects of hydration on *cSAL*.

The pharmacokinetics of SAL are well defined and documented in both healthy and diseased populations [54]. The vast majority of urinary results are reported as a percentage of dosage, a percentage of dosage recovered, a ratio of free SAL to its metabolites, or as an absolute value [21, 30, 31, 77, 83]. However, little research actually reports values in concentrations as used by WADA. Despite the wealth of research on SAL, there lacks a description of the dose-response effect on urine concentrations at different time intervals post-inhalation. Therefore, the purpose of this study was to examine the dose-response relationship of urine SAL concentrations while resting at 30, 60, and 120 minutes post-inhalation. A secondary purpose of this study was to correct

urine samples for hydration status using a specific gravity measure and compare these values to the current WADA doping criteria.

Materials and Methods

Subjects

Healthy, male subjects (n=8) aged between 19 and 35 years were recruited for this study. All subjects were not previously diagnosed with asthma, or any other lung disease and had normal lung function with FEV_1 > 80% of the predicted value (ATS criteria [72]). Each subject was required to perform a eucapnic voluntary hyperpnea (EVH) challenge test to confirm no susceptibility to bronchospasm. This test has previously been described in detail [2] and is one of the allowable methods by the International Olympic Committee to provide evidence of need for use of asthma medication during competition. Written informed consent was obtained from all subjects and procedures were approved by the University of British Columbia's Clinical Research Ethics Committee on Human Experimentation.

Study Design

A randomized, non-blinded, repeated measures design was used with 3 different treatment protocols; 200 μg (D2), 400 μg (D4), and 800 μg (D8) of inhaled SAL. Each subject came to the lab on 3 different occasions with a minimum of 72 hours between each visit. On each day, subjects received one of the three doses of SAL and provided urine samples at 30, 60, and 120 minutes post inhalation (T30, T60, and T120 respectively). An additional pre-treatment urine sample was provided on Day 1 to act as

a baseline measure for all conditions and confirm subjects were not currently taking SAL. Spirometry manoeuvres following ATS criteria [72] were also completed prior to inhalation and at T30, T60, and T120 to confirm drug delivery and action.

Days 1-3 - Drug Administration and Urine Collection

All subjects were asked to refrain from intense exercise for 24 hours prior to each testing session. As the primary purpose of this study was to relate the findings to both in and out of competition testing, control for ingestion or food and water did not occur. Subjects were only asked to avoid alcohol or caffeine containing drinks for at least 12 hours prior to each testing session. SAL was administered using a metered dose inhaler (MDI) and spacer with each subject receiving training on proper use prior to starting the study. To avoid any potential side effects of the propellant, inhalations were done in sets of two with a period of 30 seconds used between each set. Each condition required 8 total inhalations of either 100 μg of SAL or placebo. The exact number of each was dependant on the condition with the required number of SAL inhalations administered first. Throughout the two hour period, subjects remained seated and were allowed to drink *ad libitum*. Subjects were asked to provide a urine sample of ~15 ml at T30, T60, and T120. It was requested that the sample be obtained mid-stream and that the bladder was voided of urine after each sample. Once the urine sample was obtained, specific gravity (SG) was measured using a refractometer (Pocket PAL-10S, Atago, USA). All samples were then frozen to -20° C until laboratory analysis.

Urine Analysis

Samples were analyzed by a third party laboratory for SAL concentration using a hydrolysis method, accounting for non-sulfated forms (free and glucuronized forms only) The non-sulfated portion is the value measured by WADA at the time of this study [82]. Concentrations were determined by liquid chromatography-mass spectrometry. Urine was incubated with glucuronidase (from Helix pomatia, Sigma-Aldrich Co, St. Louis, MO, USA) at 37° C for 2 hours prior to addition of the internal standard. The internal standard (propionylprocanamide) was added to 1ml of the urine specimen. The mixture was acidified with 0.5 ml 10% trichloroacetic acid and 8 ml chloroform added. The mixture was vortexed, centrifuged and the aqueous phase recovered for SAL assay. The mass spectrometry instrument (Agilent model 1100 MSD) was coupled to a liquid chromatograph (Agilent model 1090), with both instruments controlled by Agilent ChemStation software. The mobile phase used for the chromatography was 10 mmol/L aqueous ammonium acetate adjusted to pH 3.2 and acetonitrile (95:5 ramping to 75:25) and the column employed was an Eclipse XDB-C8 (4.6 mm x 30 mm x 3.5 μm) (Agilent Technologies, Wilmington, DE, USA). Primary ions used for the quantitation were 240 m/z (SAL) & 292 m/z (propionylprocanamide). Flow rate for LC MS was 0.3 $ml \cdot min^{-1}$ with a retention time for SAL of 2.30 minutes. Concentrations were determined by comparison to a standard curve of the relative intensities of the SAL ion to that of the internal standard ion for standard solutions of the drugs prepared in drug free urine.

To account for differences in SG between samples and to compare values to those that might be observed in a doping control situation, all samples were adjusted for SG using the following equation [50]:

$$\text{SG-corrected } cSAL = \text{raw } cSAL \cdot ((SG_{target} - 1.0)/(SG_{sample} - 1.0))$$

where SG_{target} refers to the SG to which values are to be adjusted, while SG_{sample} refers to the actual SG of the sample. Corrections for SG targets of 1.005 and 1.025 were calculated. The lower value represents the minimum acceptable value for a doping control sample [81] while the higher value would be considered to be representative of moderate dehydration in athletes [55] and has commonly been seen following exercise in our laboratory.

Data Analyses

Means and standard deviations were computed for all descriptive variables. Urine concentrations of SAL and spirometry measures were compared across dose and time using repeated measures ANOVA. Post-hoc analyses were performed using Tukey's test for honest significance when a significant main effect was found. All statistical procedures were performed using Statistica software (Version 5.0, StatSoft Inc.) with α set a 0.05. Data are presented as means ± standard deviation.

Results

Subjects

Eight subjects with a mean age, weight, and height of 27.9 ± 5.9 years, 77.4 ± 5.4 kg, and 179.4 ± 5.1 cm respectively completed this study. FVC (5.58 ± 0.60) and FEV_1 (4.46 ±

0.41) were 101.3% and 97.2% of predicted values. All subjects had a negative EVH test with a mean maximal drop in FEV_1 of 5.65 ± 3.84%.

Dose Response Effects

Each dose demonstrated a significant enhancement of FEV_1 over baseline values at each time point post-inhalation confirming delivery and action of the drug. All baseline urine samples returned a *cSAL* value of zero and were therefore excluded from the remainder of the analysis. There was no difference in SG across doses (D2=1.011 ± 0.011, D4=1.012 ± 0.008, D8 = 1.012 ± 0.010) however, SG did decrease across time becoming significantly lower at T120 (Table 4.1).

Table 4.1. Specific Gravity for all Urine Samples at 30, 60, and 120 Minutes (T30, T60, T120 Respectively) Post-Inhalation of Salbutamol.

	T30	T60	T120
Mean	1.015	1.011	1.009 [a]
SD	(0.009)	(0.010)	(0.009)
Min	1.002	1.001	1.002
Max	1.029	1.032	1.028

[a] – denotes significant difference from T30; $p<0.05$

As shown in Table 4.2, *cSAL* of urine samples (uncorrected for specific gravity) increased as dose increased with dose D8 being significantly greater than D2 at each time interval. No effect of time was demonstrated although the trend was for *cSAL* to peak at T60 for each dose. Large variability existed in *cSAL* across all doses with a minimum of

0 $ng \cdot ml^{-1}$ and a maximum of 904 $ng \cdot ml^{-1}$ (Table 4.2). The variability of individual samples is shown in Fig. 4.1a and of note is that no samples exceeded 1000 $ng \cdot ml^{-1}$.

Table 4.2. Urine Concentrations of Salbutamol (non-sulfated) at 30 (T30), 60 (T60), and 120 Minutes (T120) Post-Inhalation of 200μg (D2), 400μg (D4), and 800μg (D8) of Salbutamol. Mean, SD, Minimum (Min), and Maximum (Max) for Raw and Corrected for Specific Gravity (SG) Values are Reported in $ng \cdot ml^{-1}$.

		T30			T60			T120		
		D2	*D4*	*D8*	*D2*	*D4*	*D8*	*D2*	*D4*	*D8*
Raw	Mean	58	173	196 $^{+}$	66	181	272 $^{+}$	21	74	194 $^{+}$
	SD	(77)	(192)	(142)	(62)	(159)	(288)	(31)	(60)	(176)
	Min	0	29	83	0	21	47	0	0	31
	Max	189	636	519	157	529	904	74	167	562
Corrected to 1.005 SG	Mean	19	62	81 $^{+}$	41	85	151 $^{a,+,*}$	15	52	125 $^{+,*}$
	SD	(27)	(47)	(37)	(53)	(65)	(58)	(34)	(46)	(99)
	Min	0	5	36	0	31	59	0	0	35
	Max	79	141	138	157	228	230	98	143	347
Corrected to 1.025 SG	Mean	98	309	406 $^{+}$	206	423	754 $^{a,+,*}$	74	259	624 $^{+,*}$
	SD	(135)	(237)	(183)	(266)	(324)	(291)	(171)	(232)	(495)
	Min	0	24	178	0	157	294	0	0	173
	Max	394	706	692	785	1142	1150	492	713	1733

a – denotes significant difference from T30 at same dose, $p<0.05$
$^{+}$ – denotes significant difference from D2 at same time, $p<0.05$
* – denotes significant difference from D4 at same time, $p<0.05$

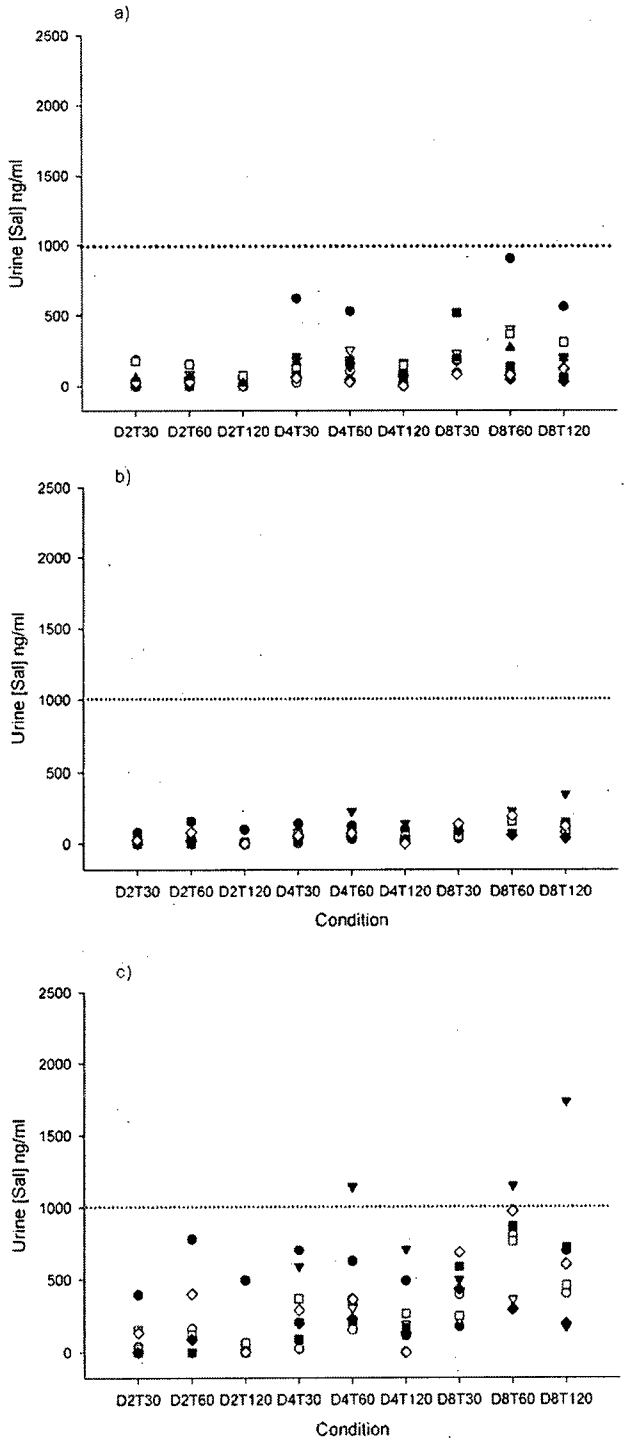

Fig. 4.1. Individual Urine Concentrations of Salbutamol (*cSAL*) for Raw Samples (a), Samples Corrected to Specific Gravity of 1.005 (b), and Samples Corrected to a Specific Gravity of 1.025 (c). Individual Samples are Shown for 30 minutes post (T30), 60 minutes post (T60), and 120 minutes post (T120) for Doses of 200μg (D2), 400μg (D4), and 800μg (D8). Dashed Line Represents Doping Control Limit of 1000 $ng \cdot ml^{-1}$.

Also noted in Table 4.2, a time effect was seen when samples were corrected for specific gravity to both the low and high targets (1.005 and 1.025 respectively) with T60 being significantly greater than T30 for dose D8 (754 ± 291 $ng \cdot ml^{-1}$ and 406 ± 183 $ng \cdot ml^{-1}$).

Individual subject plots for corrected samples are shown in Fig. 4.1b-c. Of note is the change in order of the subjects from highest to lowest when compared to the raw urine samples. Corrections to 1.005, reduced the mean values across all doses with the maximum individual sample being 347 $ng \cdot ml^{-1}$ at dose D8. When corrected to 1.025, one subject exceeded the doping limit of 1000 $ng \cdot ml^{-1}$ at doses D4 and D8 with a total of five of the eight subjects producing at least one sample that was over 750 $ng \cdot ml^{-1}$.

Discussion

The purpose of this study was to examine the dose-response effect of inhaled SAL on *cSAL* while resting at 30, 60, and 120 minutes post-inhalation. A secondary purpose of this study was to correct urine samples for hydration status using a specific gravity measure and compare these values to the current WADA doping criteria. The main findings were that urine *cSAL* values were higher with higher doses; urinary *cSAL* was highly variable between subjects, and it appeared to peak at 60 minutes post-inhalation. Although none of the uncorrected samples exceeded the WADA doping control limit of 1000 $ng \cdot ml^{-1}$, when corrected to a dehydrated state using specific gravity (1.025), the maximum value observed was 1733 $ng \cdot ml^{-1}$.

Some of the concerns regarding SAL are that supra-therapeutic doses and/or oral doses are being used in attempts to gain a competitive advantage. Currently WADA stipulates that any urine samples containing greater than 1000 $ng \cdot ml^{-1}$ of SAL is considered an adverse analytical finding unless the athlete is able to prove the result was due to an inhaled therapeutic dose [82]. The rationale for the doping control threshold of 1000 $ng \cdot ml^{-1}$ is not clear but it may in part be based on evidence from prior research [9, 80]. Previously reported values for *cSAL* rarely exceed 500 $ng \cdot ml^{-1}$ following low (200 μg) and high (1600 μg) inhaled doses [80].

To our knowledge, this is the first study reporting the dose-response effect of inhaled SAL on urine concentrations as utilized in doping control. As dose increased to 800 μg, an increase in *cSAL* was observed at each time point (Table 4.2). This is in agreement with a recent examination of the dose-response effects of inhalation on absolute SAL excretion that reported a linear relationship with inhaled doses up to 500 μg following 30 minutes of rest [77]. While the values of Tomlinson *et al.* [77] are reported in absolute values, they are comparable to the present findings as urine SG was not different between doses suggesting the increases in *cSAL* observed were due to increases in absolute excretion.

Our findings also suggest that at rest, *cSAL* concentrations peak at 60 minutes post-inhalation and begin to decrease afterwards. This was significant at higher doses when corrected for SG (Table 4.2). Hindle and Chrystyn [30] have shown that the rate of excretion of non-sulfated SAL following an inhaled dose of 400 μg is greatest in the first

hour and quickly tapers off after. They further show that the amount of drug excreted in the first 30 minutes is representative of the portion of the dose delivered to the lung. At 60 minutes, this is augmented by the portion of the dose swallowed as it becomes absorbed and available for first pass metabolism [30]. While the present data combined with excretion kinetics [30] support the idea that *cSAL* would continue to decrease beyond 120 minutes post-inhalation, this should not be assumed and requires further clarification. Peak values have been reported 2-6 hours post-inhalation in a recent-case study when 900 μg was administered over 5 hours [65]. Concentration can also be affected by hydration status, renal function, and individual variations in absorption and metabolism. Furthermore, the impact of the prior urine samples (T30 and T60) on subsequent *cSAL* cannot be discounted. While it is plausible that an athlete might pass urine post-inhalation prior to a doping control request, further work examining concentrations over longer time periods with and without repeated doses is necessary to fully characterize SAL kinetics.

Although concentrations of SAL in the urine increased with dose at each time interval, no uncorrected samples exceeded the WADA threshold of 1000 $ng \cdot ml^{-1}$. Most samples presented here fall within the range demonstrated by Ventura and colleagues [80], yet *cSAL* was highly variable and several samples from one subject exceed 500 $ng \cdot ml^{-1}$ (Fig. 4.1a). Furthermore, one of the samples from this subject approached the WADA threshold (904 $ng \cdot ml^{-1}$). Inter-subject variability of urine recovery of SAL is high (~38%) [77] and can be affected by a variety of factors, one of which is hydration. Currently, with respect to SAL, WADA does not take into consideration hydration status

other than ensuring samples are not diluted by requiring SG ≥ 1.005. Normal values for SG range between 1.005 and 1.030. To consider the impact of hydration on *cSAL* we measured SG and corrected each sample to a moderately dehydrated (1.025) and well hydrated condition (1.005) (Fig. 4.1b-c). When corrected to a moderately dehydrated state, three values from one subject exceeded 1000 $ng \cdot ml^{-1}$ with a maximum of 1733 $ng \cdot ml^{-1}$. Theoretically this subject could have produced a positive doping sample after a dose of only 400 μg, providing support for prior claims that dehydration may play a role in false-positive doping tests [45, 65]. Conversely, when corrected lower, mean values were consistently under 200 $ng \cdot ml^{-1}$ with a maximum value of 347 $ng \cdot ml^{-1}$. At the WADA minimum SG of 1.005 the potential for a false-negative test exists. It is interesting that after correcting for specific gravity, the peak value from the present study was still less than half of that reported by Schweizer and colleagues [65]. This may in part be due to the differences in the timing of the dose as well as the timing of the urine sample [45]. The subject identified in the case-study inhaled 300 μg at three different time points over 5 hours and the peak *cSAL* were from urine samples provided 2 and 6 hours after the last inhalation [65].

While SG is generally indicative of hydration and comparable to creatinine for correcting urine concentrations [50], we stress caution in applying these findings to doping control and in explaining false-positive doping violations previously reported in the literature. The relationships between hydration status and the absorption, metabolism, and excretion of SAL are complex and not well defined. It is possible that any of these rates may be altered with a change in hydration. Obtaining the volume of urine at each time point

(sample plus amount discarded) would assist evaluation of *cSAL* in doping control. Hindle and Chrystyn [30] have determined the percentage of dose recovered of both sulphated and non-sulphated forms at various time points post-inhalation. This information could be used in conjunction with *cSAL* and volume to determine the absolute values of salbutamol recovered and the likelihood that a doping sample was from inhaled administration. Future work exploring these relationships is suggested. Additionally, this study was performed at rest and although athletes can be tested out of competition, the most likely scenario is to provide a urine sample following an event. Exercise has been shown to increase lung absorption of the β_2-agonist terbutaline in healthy individuals [64]. Whether this holds true for SAL or if there are additional effects of exercise on metabolism and excretion is unclear and requires further examination.

In conclusion, urine *cSAL* increased with inhaled dose and peaked at 60 minutes post-inhalation. There is marked variability between individuals with respect to *cSAL* and this is amplified at higher doses. While no samples exceeded the 1000 $ng \cdot ml^{-1}$ limit, it was approached with a dose of 800 μg and seems plausible that it could be exceeded in some individuals. The data further suggest that hydration status should be considered when evaluating doping control samples for *cSAL* and that future work examining the relationships between timing and amount of dose inhaled, urine volume, salbutamol excretion, and individual variations in absorption, metabolism, and excretion be conducted.

CHAPTER 5 – DOSE RESPONSE OF SALBUTAMOL DURING EXERCISE

Introduction

Optimum performance in the elite athlete can be limited by pulmonary, cardiovascular, muscular, psychological, nutritional and/or environmental factors. In asthmatic athletes and individuals suffering from exercise induced-bronchospasm, lung function is reduced, thereby possibly limiting performance capabilities [6]. Currently four β_2-agonists, salbutamol (SAL), formoterol, salmeterol, and turbutaline, have been approved by the World Anti-Doping Agency (WADA) for use by asthmatics, providing the athlete obtain a therapeutic use exemption (TUE) prior. This is normally achieved by physician confirmation; however, in order to use these medications at the Olympic Games, athletes must provide objective evidence of variable airflow obstruction. This is assessed by an independent medical committee and appropriate tests include bronchodilator response and bronchial provocation (eucapnic voluntary hyperpnea (EVH), lab/field exercise, or chemical challenge) [2].

Athlete applications for use of β_2-agonists have been increasing over the past 20 years with 6.6% and 4.6% of all participants at the 2002 (Salt Lake City) [2] and 2004 (Athens) [4] Olympic games requesting their use. Of the four β_2-agonists allowed, SAL is most commonly prescribed and is only allowed to be administered through inhaled means for use in competition [82]. There is growing concern that non-asthmatic athletes are using inhaled SAL in an attempt to gain a competitive edge [2]. Anecdotal evidence suggests that both asthmatic and non-asthmatic athletes believe in its ability to enhance

performance and are using doses that substantially exceed therapeutic recommendations. This poses not only an ethical question but also raises concerns of athlete safety.

The current research overwhelmingly suggests that acute inhaled salbutamol, in therapeutic doses, does not enhance performance in non-asthmatics [10, 11, 22, 24, 48, 53, 61, 74]. The majority of studies have evaluated performance using one, or a combination of, a $\dot{V}_{O_2}max$, Wingate, lactate threshold, or work to exhaustion test. The validity of a test to be representative of athletic performance is an important factor when evaluating the ergogenic effects of a treatment [34]. Two studies have investigated the effects of inhaled salbutamol using a simulated sport-specific performance test [53, 78]. Norris and colleagues [53], showed a non-significant 12-second improvement in 20-km cycling time-trial performance time following a does of 400 μg. In comparison, a dose of 800 μg has been shown to decrease time to complete a set amount of work on a cycle ergometer (~1.9%) [78]. If salbutamol has an ergogenic effect, it may be related to dose. It has been shown that ventilatory response to salbutamol in both non-asthmatics and asthmatics is enhanced as dose increases [35, 42]. However, Goubault and colleagues showed no effect of dose (placebo, 200 μg, and 800 μg) on cycling time to exhaustion even though FEV_1 was enhanced (~5%) following salbutamol [24]. More research examining the dose-response effects of inhaled salbutamol using a sport-specific performance test is needed.

Unauthorized use of SAL is closely monitored through doping control. Even for athletes possessing a TUE, a urine concentration of non-sulphated SAL (*cSAL*) greater than 1000

$ng \cdot ml^{-1}$ is considered an adverse analytical finding resulting from oral administration and can result in a two year suspension. This cut-off point has been questioned of late with recent reports of positive test results using inhaled therapeutic doses, all with urine concentrations well over 1000 $ng \cdot ml^{-1}$ following exercise [45, 65]. Although the majority of urine samples reported in the literature rarely exceed 500 $ng \cdot ml^{-1}$ [80], it has been suggested that with variations in dose, individual differences in the ability to absorb, metabolize, and excrete salbutamol, and changes in hydration status following competition, the possibility for elevated concentrations exists [45]. Previous findings from our laboratory (Chapter 4) have shown that at rest, *cSAL* is related to dose, highly variable between subjects, and peaks at approximately 60 minutes post-inhalation. Furthermore, individual values can approach the WADA cut-off point following therapeutic doses. It is unclear whether or not similar responses would be observed following exercise. An examination of the dose-response effect of inhaled SAL on urine concentrations following exercise as used in doping control is lacking.

Although research to date has shown no significant improvement in exercise performance with the use of inhaled salbutamol, the dose-response effect on performance has not been evaluated in a homogenous group of highly trained athletes with a sport specific performance test. Therefore the purpose of this study was to examine the effects of increasing doses of SAL on 20km time-trial performance and evaluate *cSAL* following exercise in competitive athletes.

Materials and Methods

Subjects

Healthy, competitive male cyclists and triathletes (n=37) were recruited for this study. An *a priori* power calculation was performed using 1.5 times the coefficient of variance for mean power over 20km (~2% as described in Chapter 3) as the minimum improvement that will make a competitive difference. It was calculated that approximately 30 subjects were required with an estimated standard deviation of 20 W, to identify significance at 0.05 with a power of 0.80. All athletes were competing at a provincial level or higher in the elite categories for their respective sport and disciplines. Exclusion criteria included a $\dot{V}_{O_2}$max of less than 60 $ml \cdot kg^{-1} \cdot min^{-1}$ and 5 $L \cdot min^{-1}$, previous history or diagnosis of asthma, abnormal resting spirometry, or a positive eucapnic voluntary hyperpnea (EVH) test, indicative of exercise induced bronchospasm (EIB). Written informed consent was obtained from all subjects and the methods and protocol were approved by the University of British Columbia Clinical Research Ethics Board.

Study Design

A randomized, double blind, repeated measures design was utilized with 4 different treatment protocols (placebo (DP), 200 μg (D2), 400 μg (D4), and 800 μg (D8) of inhaled salbutamol). Each subject came to the lab on 5 separate occasions with a minimum of 72 hours between visits. The first visit included medical screening, measurement of height and weight, pulmonary function, and an EVH test. Qualifying subjects then performed a ramped exercise test to determine maximal oxygen consumption on the same day. The remaining four sessions involved a simulated 20km cycling time trial following one of

the four treatments. At the end of each time trial, athletes were required to provide a urine sample that was analyzed for concentration of non-sulfated salbutamol. See Figure 5.1 for a timeline of the study.

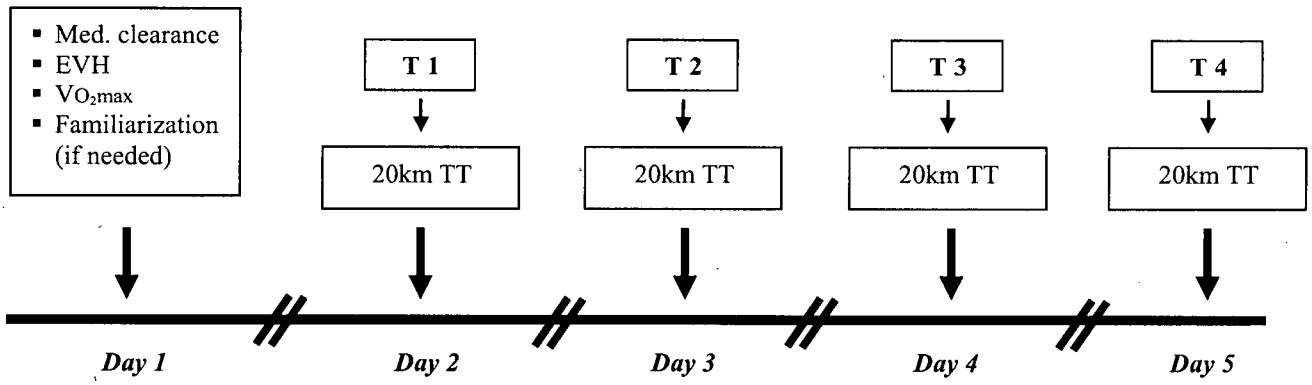

Fig. 5.1. Experimental protocol timeline.

Lung Function and Airway Hyperresponsiveness

Prior to completing the EVH test, subjects performed baseline pulmonary function measures. This was achieved via a flow-volume loop using a Medical Graphics CPX-D Metabolic cart (St. Paul, MN) with 1070 Pulmonary Function Software. Calibration was performed prior to each testing session and subjects were familiarized with the procedure prior to actual testing. Each subject performed three trials with the highest valid FEV_1 recorded. A trial was considered valid if it was greater than 80% of the predicted value and was reproducible using ATS criteria [72]. Subjects were then screened for susceptibility to bronchospasm using the EVH challenge test. This test has previously been described in detail [2] and is one of the methods approved by WADA and the IOC

Medical Committee to provide evidence for use of asthma medication during competition. Briefly, each subject was required to breathe a hypercapnic gas mixture (5% CO_2, 21% O_2, balance nitrogen) for a period of six minutes at a target ventilation which was calculated as 30 times the individuals pre-test FEV_1 (~ 85% maximal voluntary ventilation). Spirometry was measured immediately following and at 5, 10, 15, and 20 minutes post. A decrease in FEV_1 of greater than 10% from baseline measure was considered to be a positive test for bronchospasm and is usually observed in the first 10 minutes. For purposes of this study, the maximum decrease in FEV_1 at any time point of 5 minutes post or greater was identified and recorded as a percentage drop from pre-test FEV_1.

Maximal Exercise Test

A maximal exercise test was performed on the Velotron Pro cycle ergometer (Racermate Inc, Seattle, WA, USA). Prior to each test, factory calibration was verified using the Accuwatt "run down" verification program (Racermate Inc, Seattle) accompanying the ergometer software. Subjects were fitted to the ergometer based on the setup of their own bicycle. All settings were recorded and used in subsequent time trials. Bike settings included both seat and handle bar height and horizontal position, as well as crank length. Subjects were instructed to remain seated throughout the test. A 30 $W \cdot min^{-1}$ ramp protocol was utilized and controlled via the Velotron Coaching Software (Version 1.5.186, RacerMate Inc, Seattle, WA, USA) with expired gases collected and analyzed every 15 seconds (TrueOne 2400 – Parvo Medics, Utah, USA). Oxygen consumption ($\dot{V}_{O_2}$), minute ventilation ($\dot{V}_E$), production of carbon dioxide ($\dot{V}_{CO_2}$), and respiratory

exchange ratio (RER) were recorded. Flow and gas calibrations were performed prior to each test using a 3 L calibration syringe and gases of known concentrations respectively. Standard indicators for achieving $\dot{V}_{O_2}$max were used including volitional fatigue, a plateau in $\dot{V}_{O_2}$ with increasing work rate, HR ≥ 90% of age predicted maximum, and a RER ≥ 1.15. $\dot{V}_{O_2}$max was recorded as the mean of the two highest consecutive 15-second samples. Heart rate (HR) was measured by telemetry (Polar Vantage XL, Kempele, Finland) and recorded. Peak power was recorded as the highest completed 15 second interval with power recorded in 7.5 watt intervals.

Dose Response Evaluation – Exercise Protocol

A timeline of events for Days 2-5 is depicted in Figure 5.2. Subjects were encouraged to prepare for each time trial as they would a competitive event with no strenuous exercise in the previous 24 hours.

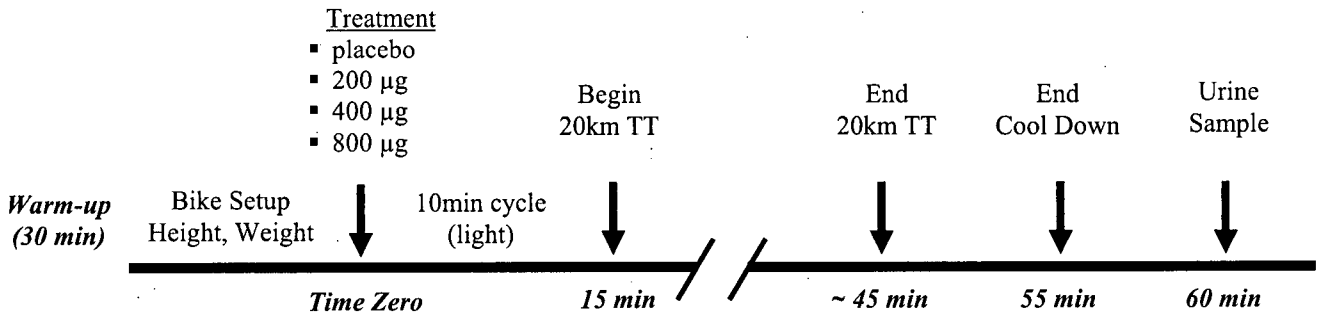

Fig. 5.2. Timeline for treatment and time trials.

Warm-up was self-selected and although this varied between individuals, it was the same for each subject for all trials. Immediately following the warm-up, subjects were weighed and began receiving a treatment. A total of 8 inhalations were administered each day from 3 different coded MDI for a dose equal to one of DP, D2, D4, or D8. Spacers were used to optimize delivery of the medication and subjects were trained in its proper use prior to participation. Following administration, bike fit was confirmed and subjects were allowed to keep loose by spinning freely. At 10 minutes post-inhalation a mask (Hans Rudolph 8930 Series, Kansas City, MO, USA) and two-way breathing valve (Hans Rudolph 2700 Series, Kansas City, MO, USA) were fitted to the subject and connected to a metabolic cart (TrueOne 2400 – Parvo Medics, Sandy, UT, USA). A complete seal of the mask was confirmed prior to testing. At 15 minutes post-inhalation, subjects began the simulated 20km time trial and were instructed to complete the distance as quickly as possible. All time trials were performed on the Velotron Pro cycle ergometer which was calibrated prior to each test. This performance test has been described previously and is highly reproducible in trained cyclists with a CV of <1% for time and <2% for mean power (Chapter 3). Approximately half of the subjects were familiar with this protocol in our laboratory and those that weren't performed a familiarization trial following a rest period at the end of Day 1. Subjects were required to perform 2 continuous laps of a 10km course designed using the Velotron 3D software accompanying the ergometer (Version NB04.1.0.2101, RacerMate Inc, Seattle, WA, USA). The course was flat with no active wind effect. Resistance was adjustable using the ergometer's electronic gearing system. A gearing system simulating a 53-39 front chain ring setup and 23-21-19-17-16-15-14-13-12-11 rear cog set was used. Throughout the time-trial, subjects were able to

watch themselves racing the course on the computer monitor. Distance traveled and gears selected were displayed while all other feedback was blinded to the subject. Power, speed, and time were recorded by the ergometer software and downloaded afterwards for analysis. The sampling rate for all ergometer variables was 1 sample·sec^{-1}. Heart rate was also recorded by the ergometer and confirmed by telemetry (Polar Vantage XL, Kempele, Finland) throughout the time trial. Subjects did not receive any information as to how well they performed until all trials were completed. Throughout the time-trial, expired gases were collected with metabolic parameters averaged every 20 seconds. Every 2km, subjects were asked to rate the perceived exertion (RPE) for leg (RPEL) and breathing (RPED) effort using a 10-point Borg RPE scale. Upon completion of the time-trial, subjects were requested to cool down and rest until the 55 minute mark post-inhalation. Subjects were allowed to rehydrate *ad libitum* during this time.

Urine Collection and Analysis

At the one hour mark post-inhalation (T60), subjects were requested to provide a urine sample of ~15 ml. It was requested that the sample be obtained mid-stream and that the bladder was voided of urine following. Once the urine sample was obtained, specific gravity (SG) was measured using a refractometer (Pocket PAL-10S, Atago, USA). All samples were then frozen to -20° C until laboratory analysis. Samples were analyzed by a third party laboratory for total *cSAL* using a hydrolysis method, accounting for free and glucuronized forms only. This is the value that is reported by the World Anti-Doping Agency at the time of the study [82]. Concentrations were determined by liquid chromatography-mass spectrometry. Urine was incubated with glucuronidase (from

Helix pomatia, Sigma-Aldrich Co, St. Louis, MO, USA) at 37° C for 2 hours prior to addition of the internal standard. The internal standard (propionylprocanamide) was added to 1ml of the urine specimen. The mixture was acidified with 0.5 ml 10% trichloroacetic acid and 8 ml chloroform added. The mixture was vortexed, centrifuged and the aqueous phase recovered for SAL assay. The instrument used was an Agilent model 1100 MSD coupled to an Agilent model 1090 liquid chromatograph, both instruments are controlled by Agilent ChemStation software. The mobile phase used for the chromatography was 10 mmol/L aqueous ammonium acetate adjusted to pH 3.2 and acetonitrile (95:5 ramping to 75:25) and the column employed was an Eclipse XDB-C8 (4.6 mm x 30 mm x 3.5 μm) (Agilent Technologies, Wilmington, DE, USA). Primary ions used for the quantitation were 240 m/z (SAL) & 292 m/z (propionylprocanamide). Flow rate for LC MS was 0.3 $ml \cdot min^{-1}$ with a retention time for SAL of 2.30 minutes. Concentrations were determined by comparison to a standard curve of the relative intensities of the SAL ion to that of the internal standard ion for standard solutions of the drugs prepared in drug free urine.

To account for differences in SG between samples and to compare values to those that might be observed in a doping control situation, all samples were adjusted for SG using the following equation [50]:

$$\text{SG-corrected } cSAL = \text{raw } cSAL \cdot ((SG_{target} - 1.0)/(SG_{sample} - 1.0))$$

where SG_{target} refers to the SG to which values are to be adjusted, while SG_{sample} refers to the actual SG of the sample. Corrections for SG targets of 1.005 and 1.025 were calculated. The lower value represents the minimum acceptable value for a doping

control sample [81] while the higher value is considered to be representative of moderate dehydration and has commonly been seen following exercise in our laboratory.

Data Analysis

Mean and standard deviations (SD) were calculated for descriptive variables. A repeated measures analysis of variance was used to determine statistical significance across treatments for all performance variables and urine concentrations measured. Post-hoc analyses were performed using Tukey's test for significance when a main effect was found. Pearson product moment correlations were used to examine relationships between urine concentrations and specific gravity. Statistical procedures were completed using Statistica Software (Version 5.0, Statsoft Inc, USA). For all tests, α was set a 0.05. Values reported are means ± SD unless otherwise noted.

Results

Subject Characteristics and Airway Hyperresponsiveness

Characteristics of subjects with negative (n=30) and positive (n=7) responses to the EVH test are shown in Tables 5.1 and 5.2. A total of seven subjects produced a positive EVH test resulting in a prevalence rate of ~19% for airway hyperresponsiveness. Maximum drop in FEV_1 following the EVH test was 27.7%. All positive responders were excluded from the remainder of the study. Baseline performance characteristics of remaining subjects (n=30) are shown in Table 5.3.

Table 5.1. Subject Characteristics for Positive and Negative Responders to a Eucapnic Voluntary Hyperpnea (EVH) Test. Values presented are Means, Standard Deviations (SD), Maximums (Max), and Minimums (Min).

Group		Age (yrs)	Height (cm)	Weight (kg)	Cycling Experience (yrs)
Negative EVH (n=30)	Mean	29	182.2	76.0	8
	(SD)	(6)	(6.7)	(7.6)	(5)
	Max	51	195.3	95	25
	Min	18	166	62.7	2
Positive EVH (n=7)	Mean	25	183.7	76.2	8
	(SD)	(5)	(6.8)	(8.62)	(5)
	Max	35	195.6	92.6	16
	Min	20	176	68.7	3

Table 5.2. Lung Function Measures Including Percent Predicted Values for Forced Vital Capacity (FVC), Forced Expiratory Volume in One Second (FEV_1), and Fraction of FVC Expired in One Seconds (FEV_1/FVC), and Decrease in FEV_1 (Max ΔFEV_1) for Positive and Negative Responders to a Eucapnic Voluntary Hyperpnea (EVH) Test. Values presented are Means, Standard Deviations (SD), Maximums (Max), and Minimums (Min).

Group		FVC (L)	% *Predicted*	FEV_1 (L)	% *Predicted*	FEV_1/FVC (%)	% *Predicted*	Max ΔFEV_1 (%)
Negative EVH (n=30)	Mean	5.86	103.8	4.86	103.0	82.8	99.4	3.9
	(SD)	(0.77)	(11.5)	(0.72)	(11.5)	(5.0)	(6.0)	(2.7)
	Max	7.01	135.8	5.98	122.1	91.5	110.0	9.8
	Min	4.56	85.0	3.55	78.9	70.4	85.0	-1.4
Positive EVH (n=7)	Mean	6.09	101.8	4.67	94.7	76.8	92.9	15.5
	(SD)	(0.80)	(6.8)	(0.63)	(11.1)	(6.2)	(7.3)	(5.9)
	Max	7.25	108.0	5.63	106.2	82.1	98.7	27.7
	Min	4.89	88.9	3.92	76.0	67.0	81.2	11.0

Table 5.3. Baseline Performance Characteristics of Negative EVH Subjects (n=30).

Subject	$\dot{V}_{O_2}$max ($mL \cdot kg^{-1} \cdot min^{-1}$)	$\dot{V}_{O_2}$max ($L \cdot min^{-1}$)	Max HR ($b \cdot min^{-1}$)	Max Power (W)	Max Power ($W \cdot kg^{-1}$)
Mean	67.1	5.08	186	457	6.06
(SD)	(4.3)	(0.54)	(10)	(31)	(0.48)

20km Time Trial Performance

Three subjects were unable to complete all conditions and therefore results of only 27 subjects are presented. Mean power (P_{mean}) over the 20km for each of the conditions ranged between 306 and 310 watts with no effect of salbutamol observed between conditions (Table 5.4). This was approximately 67% of max power (P_{max}) and equal to roughly 4.05 $W \cdot kg^{-1}$.

Table 5.4. The Effects of Salbutamol Dose (D2=200µg, D4=400µg, D8=800µg) on 20km Mean Power Output (P_{mean}), Total Time (T_{tot}), and Lap Times (T_{L1}, T_{L2}), Heart Rate (HR) and Rate of Perceived Exertion for Legs (RPEL) and Breathing (RPED). Values Reported are Means and (SD).

	Placebo	D2	D4	D8
P_{mean} (W)	306 (29)	310 (30)	307 (29)	307 (30)
T_{tot} (min)	30.72 (1.06)	30.55 (1.03)	30.67 (1.06)	30.70 (1.04)
T_{L1} (min)	15.31 (0.55)	15.25 (0.54)	15.29 (0.55)	15.35 (0.58)
T_{L2} (min)	15.40 (0.53)	15.31 (0.54)	15.38 (0.55)	15.35 (0.50)
HR (bpm)	172 (9)	173 (10)	171 (9)	171 (10)
RPEL	5.9 (1.4)	6.1 (1.5)	6.0 (1.5)	6.1 (1.4)
RPED	6.1 (1.4)	6.2 (1.5)	6.2 (1.6)	6.2 (1.4)

Similarly there was no effect of salbutamol on any of the metabolic or ventilatory parameters (Table 5.5). Mean $\dot{V}_{O_2}$ and HR throughout the time trials was approximately 55 mL·kg^{-1}·min^{-1} and 172 beats·min^{-1}. This equated to approximately 82% and 92% of the respective peak values ($\dot{V}_{O_2}$max = 67.1 ± 4.3 mL·kg^{-1}·min^{-1}; HR_{max} = 186 ± 10 beats·min^{-1}) achieved on Day 1. As shown in Table 5.5, breathing frequency was similar across conditions (~45 bpm) as was tidal volume (~2.9 L) resulting in no differences in exercise ventilation with salbutamol.

Table 5.5. The Effects of Salbutamol Dose (D2=200µg, D4=400µg, D8=800µg) on Mean Metabolic and Ventilatory Parameters over 20km. Oxygen Consumption (VO_2), Expired Carbon Dioxide (VCO_2), Ventilation Rate (V_E), Ventilatory Equivalents for Oxygen and Carbon Dioxide (V_E/VO_2, V_E/VCO_2), Respiratory Rate (RR), and Tidal Volume (V_T). Values are Reported as Means and (SD).

	Placebo	D2	D4	D8
VO_2 (mL·kg^{-1}·min^{-1})	54.5 (4.3)	55.4 (4.0)	54.6 (4.2)	55.0 (3.6)
VCO_2 (L·min^{-1})	4.03 (0.45)	4.10 (0.47)	4.02 (0.42)	4.05 (0.42)
V_E (L·min^{-1})	102.1 (15.9)	104.7 (12.8)	101.9 (16)	102.1 (13.1)
V_E/VO_2	30.5 (3.7)	30.7 (3.1)	30.2 (3.5)	30.0 (3.3)
V_E/VCO_2	30.9 (3.5)	31.3 (3.2)	30.8 (3.4)	30.7 (3.2)
RR (breaths·min^{-1})	44 (9)	45 (8)	45 (9)	44 (8)
V_T (L)	2.87 (0.45)	2.89 (0.49)	2.85 (0.45)	2.90 (0.50)

During each time trial subjects were requested to rate their rate of perceived exertion for both leg and breathing effort. Mean values over 20km were unaffected by salbutamol (Table 5.5) and there was no difference at any distance between conditions for both RPEL and RPED (Fig. 5.3).

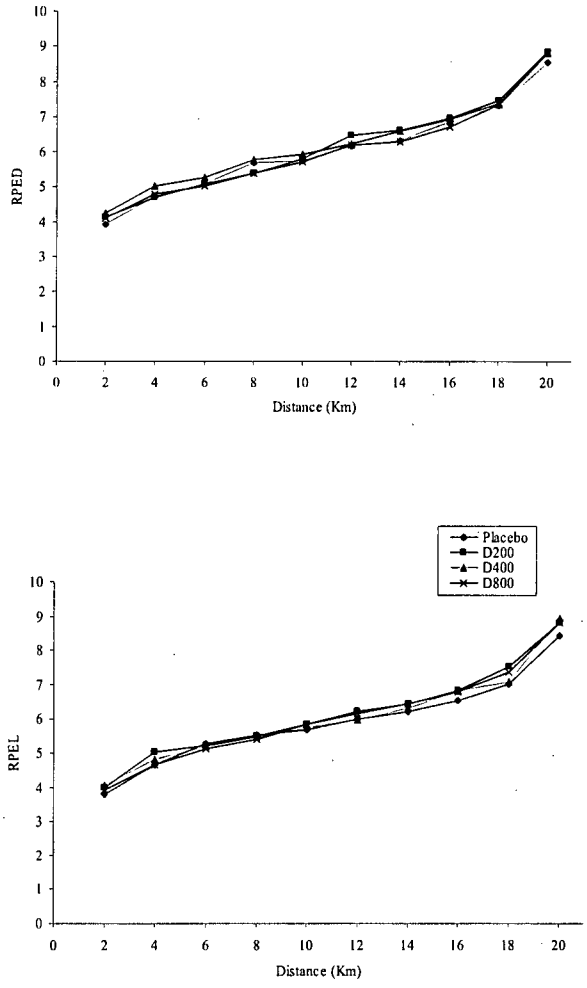

Fig. 5.3. Mean ratings of perceived exertion for breathing (RPED) and legs (RPEL) at 2km intervals. Rating of difficulty ranged from 1 (nothing at all) to 10 (maximal).

Urine Concentrations of Salbutamol

Urine concentrations are shown in Table 5.6. There was no difference in SG across conditions (DP = 1.012 ± 0.008, D2=1.013 ± 0.008, D4=1.013 ± 0.008, D8 = 1.012 ± 0.007) with the minimum and maximum values obtained across all trials being 1.002 and 1.032 respectively. As shown in Table 5.6, *cSAL* of uncorrected urine samples increased as dose increased with D4 being greater than DP, and D8 being significantly greater than all other conditions. Large variability existed in *cSAL* across all doses with a minimum of 0 $ng \cdot ml^{-1}$ and a maximum of 831 $ng \cdot ml^{-1}$ (Table 5.6).

Table 5.6. Urine Concentrations of Salbutamol (non-sulfated) at 60 Minutes (T60) Post-Inhalation of Placebo (DP), 200µg (D200), 400µg (D400), and 800µg (D800) of Salbutamol. Mean, SD, Minimum (Min), and Maximum (Max). Mean, Standard Deviation (SD), Maximum (Max), and Minimum (Min) Values are Reported Raw and Corrected for Specific Gravity (SG) Formats. Values are Reported in $ng \cdot ml^{-1}$.

		DP	*D2*	*D4*	*D8*
Raw	Mean	7	46	115 $^{+}$	210 $^{+,a,*}$
	SD	(15)	(73)	(126)	(177)
	Min	0	0	0	26
	Max	54	347	627	831
Corrected to 1.005 SG	Mean	2	19	52 $^{+}$	104 $^{+,a,*}$
	SD	(6)	(29)	(49)	(90)
	Min	0	0	0	7
	Max	25	145	210	425
Corrected to 1.025 SG	Mean	12	97	261 $^{+}$	520 $^{+,a,*}$
	SD	(30)	(147)	(245)	(451)
	Min	0	0	0	33
	Max	123	723	1050	2125

$^{+}$ – denotes significantly greater than DP, $p<0.05$
^{a} – denotes significantly greater than D2, $p<0.05$
* – denotes significantly greater than D4, $p<0.05$

Fig. 5.4a shows the variability in individual samples and of note is that no samples exceeded 1000 $ng \cdot ml^{-1}$ when uncorrected for specific gravity. A significant relationship between SG and *cSAL* was observed in conditions D4 (n=28) and D8 (n=30) ($r = 0.42$ and $r = 0.37$ respectively, $p<0.05$) (Fig. 5.5a-b). Alternatively, SG was not related to *cSAL* in either DP (n=30) or D2 (n=29) conditions ($r = 0.18$ and $r = 0.11$ respectively). Fig. 5.4b and 5.4c show the individual subject plots for corrected samples. Corrections to 1.005, reduced the mean values across all doses with the maximum individual sample being 425 $ng \cdot ml^{-1}$ at dose D8. When corrected to 1.025, three subjects exceeded the doping limit of 1000 $ng \cdot ml^{-1}$ at doses D4 and D8 (max = 2125 $ng \cdot ml^{-1}$) while four other subjects produced samples of 900 $ng \cdot ml^{-1}$ or more at dose D8.

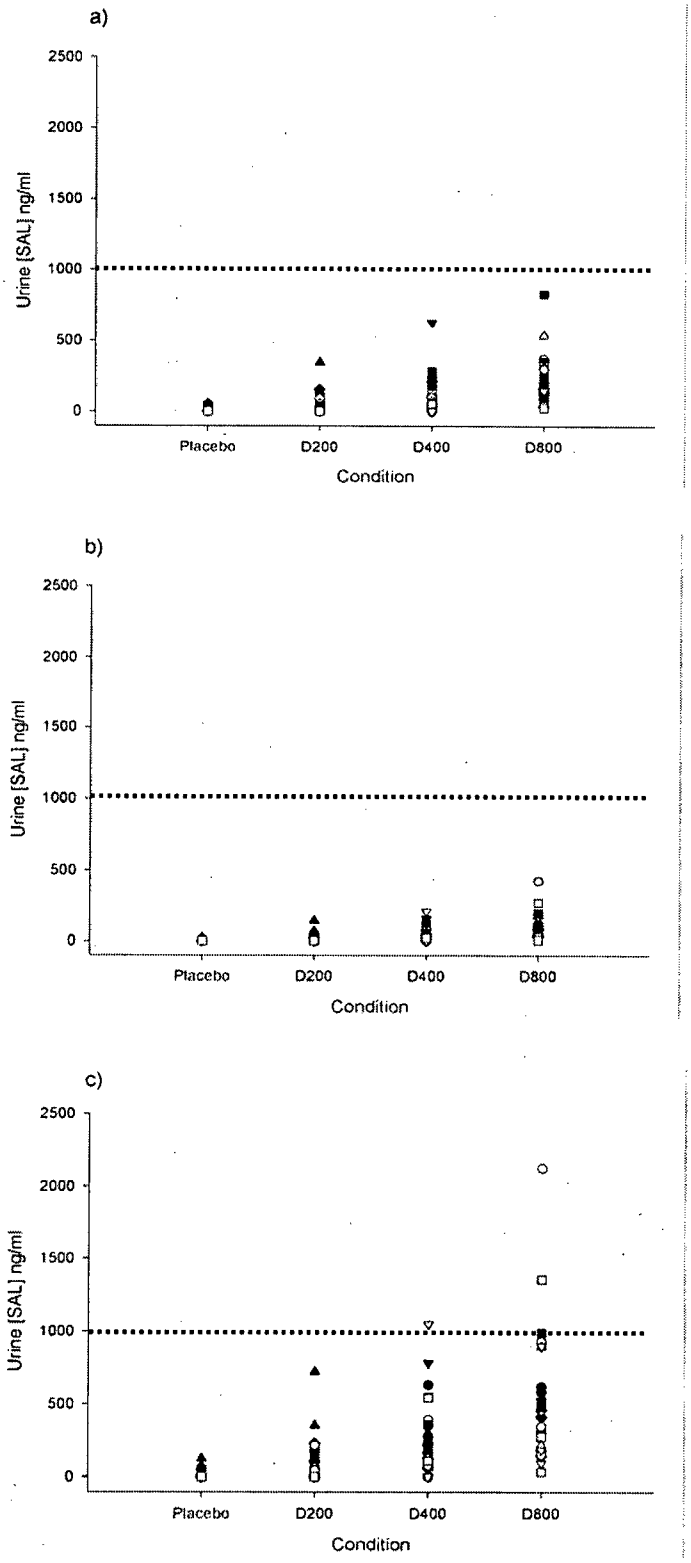

Fig. 5.4. Urine Concentrations of Salbutamol (*cSAL*) for Raw Samples (a), Samples Corrected to Specific Gravity of 1.005 (b), and Samples Corrected to a Specific Gravity of 1.025 (c). Individual Samples are Shown for Placebo, 200µg (D200), 400µg (D400), and 800µg (D800). Dashed Line Represents Doping Control Limit of 1000 ng·ml-1.

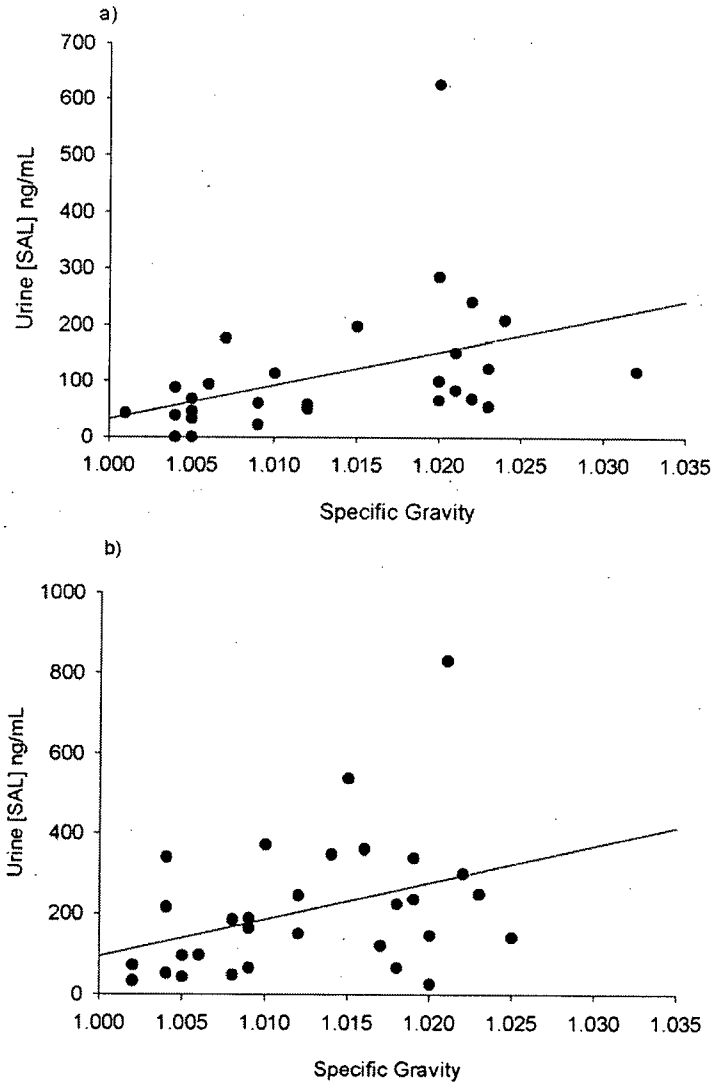

Fig. 5.5. Relationships between specific gravity and urine concentrations of salbutamol (*cSAL*) 1 hour post-inhalation of 400 μg (a) and 800 μg (b) doses.

Discussion

The main purposes of this study were to examine the dose-response effect of inhaled SAL on exercise performance and urine concentration in competitive non-asthmatic athletes. The primary findings were that SAL had no effect on 20km time trial performance as measured by mean sustainable power nor did it have effects on metabolic and ventilatory parameters during exercise. Urine concentrations of SAL following exercise at 1 hour post-inhalation increased with dose and were highly variable. No subject exceeded the WADA cut-off of 1000 $ng \cdot ml^{-1}$, however, *cSAL* was related to hydration as measured by specific gravity and the possibility exists that dehydration could lead to increased values. Additionally it was found that the prevalence of hyper-reactive airways in cyclists and triathletes not previously diagnosed with asthma is approximately 19%.

Although applications to use SAL during the Olympic Games have been increasing over the past 20 years [2], the percentage of all athletes requesting use of a β_2-agonist at the last two Olympic Games is within the range of the prevalence of asthma in the general population (~5-10%) . Nonetheless, there are certain sports where this rate is much higher; specifically cycling and triathlon where the percentage of athletes requesting a TUE for SAL at the Sydney Olympic Games (2000) was approximately 17% and 20% respectively [2]. This has prompted concerns of misuse by athletes that may be trying to gain a competitive edge [19]. The present data suggest that these numbers are not out of the ordinary for this population, however evaluations with larger samples sizes would be needed to confirm this. Of the 37 cyclists and triathletes who participated in this study, seven had a >10% reduction in FEV_1 following an EVH test equating to ~19% testing

positive for airway hyperresponsiveness. Furthermore, subjects were selected from a group of athletes that had not previously been diagnosed with asthma, suggesting the percentage of athletes that could benefit from SAL use may in fact be higher. Previous data from the 1996 US Olympic team report that prevalence of asthma in elite cyclists may be as high as 50% (10 of 20 athletes) [84]. Our findings suggest that there may be need for further education of competitive cyclists and triathletes as to the symptoms and complications of asthma and exercise-induced bronchospasm in sport. Although several factors are known to contribute to airway hyperresponsiveness, the reasons why cyclists have an increased prevalence are not clear and require further investigation.

This is the first study to utilize a sport-specific evaluation method while examining the dose-response of inhaled SAL on performance in non-asthmatic athletes. Previous research has used standard laboratory evaluations such as maximal aerobic power, anaerobic threshold, or time to exhaustion [22, 24, 46, 48, 53, 61, 75]. Overwhelmingly these studies have found inhaled SAL to have no performance enhancing effects in athletes from a variety of different sporting backgrounds [22, 24, 46, 48, 53, 61, 75]. Furthermore, Goubalt *et al.* [24] showed a lack of a dose-response with doses up to 800 μg in a time to exhaustion test at 85% of maximal oxygen consumption. Although in agreement with our current findings the applicability of non-specific test results to sport performance enhancement is questionable. The validity of a test to be representative of performance is an important factor when evaluating the ergogenic effects of a treatment [34]. Only two studies have utilized sport specific protocols and they have provided conflicting results [53, 78]. Following a dose of 400 μg, Norris and colleagues [53]

showed no effect on 20-km time trial performance in competitive cyclists. At higher doses (800 μg) however, van Baak *et al.* [78] demonstrated an improvement in time to complete a set amount of cycling work suggesting the ergogenic effects of SAL may be related to dose. Our findings do not agree with this concept and are in agreement with the majority of other investigations that have failed to show an ergogenic effect. The difference noted by van Baak and colleagues [78] may be due to the length of the protocol utilized (> 1hr) which would require different contributions from aerobic and anaerobic energy systems than ~30 minutes of intense effort. However this seems unlikely as they showed no differences in lactate measures or substrate availability during exercise. Furthermore, we observed no differences in oxygen consumption or carbon dioxide production across doses which is in agreement with previous findings [11, 22, 24, 29, 61]. We feel their significant difference is likely due to the influence of two outliers who appear to have experienced an 8-10% improvement following SAL inhalation. This plus other data begs the question - are there specific athletes who may get an ergogenic effect. Genetics variations exist in β_2-receptors which may be partially responsible for individual variability in response.

One potential mechanism for SAL to have ergogenic properties may be related to its ability to act as a potent bronchodilator. Even in non-asthmatic individuals, SAL has the ability to increase airway calibre at rest resulting in a measurable increase in airway function (Chapter 4). Theoretically, this may lead to enhanced alveolar ventilation and/or a reduced work of breathing thereby increasing available oxygen for working muscles. However, previous reports have shown that during physical activity SAL does not have

an accumulative effect to the normal bronchodilatory response to exercise [11, 24, 29, 48, 78] nor does it reduce respiratory resistance during exercise [60]. Hence, our finding that exercise ventilation was unchanged with SAL and unaffected by dose was not surprising and is similar to previous findings at both maximal and sub-maximal intensities [11, 24, 53, 61, 75]. The finding that RPED was similar and that the pattern of ventilation (tidal volume and breathing frequency) did not change between conditions further supports the notion that SAL inhalation in non-asthmatics has minimal impact on ventilation during exercise. Two other studies in which subjects subjectively rated dyspnea during exercise found similar results [22, 24]. It has also been postulated that SAL may alter substrate utilization by mobilizing fatty acids and sparing glucose. Indeed, SAL has a stimulatory effect on lipolysis at rest and leads to increased fatty acid mobilization [63]. However, evidence to support that acute SAL treatment augments any normal response to exercise is lacking [24, 78, 79]. Blood measures were not obtained in this study so additional discussion is unwarranted.

Lastly, the present study only examined the effect of acute administrations of inhaled SAL on exercise performance. Our findings cannot preclude the possibility that short term (~ 3 weeks) use by this means will not have an ergogenic effect. Continued oral administration of SAL for 3 weeks has resulted in enhanced endurance performance [15] and increases in peak and mean power during high-intensity cycling [38]. Unlike acute administrations of SAL, short term oral use has been shown to alter substrate availability and utilization during exercise [15], along with increasing strength capabilities [44]. Although oral administration is currently banned by WADA, it should not be assumed

that continued inhaled administration is non-ergogenic. The likelihood that approved athletes would be using the drug regularly in training as part of an overall management program is high. A constant presence of SAL in the plasma following inhalation may lead to some of the adaptations that have been associated with oral administration. Further examinations of short term use of inhaled SAL on performance are necessary to eliminate this possibility.

To our knowledge, this is the first study reporting the dose-response effect of inhaled SAL on *cSAL* following exercise. Previously reported post-exercise values for *cSAL* following low (200 μg) and high (1600 μg) inhaled doses show large variability between subjects with the majority of samples being less than 500 $ng \cdot ml^{-1}$ [80]. Our findings are similar in both regards and not surprising as inter-subject variability of urine recovery of SAL is high (~38%) [77] . The finding that *cSAL* is related to dose is in agreement with our previous findings at rest (Chapter 4) and previous reports of absolute SAL recovery 30 minutes post-inhalation [77]. As dose increased so did the variability between subjects, particularly at higher doses (Fig. 5.4a) which may in part explain the recent reports of urine samples resulting in positive tests for athletes with a TUE [45, 65]. Currently WADA stipulates that any urine samples containing greater than 1000 $ng \cdot ml^{-1}$ of SAL is considered an adverse analytical finding unless the athlete is able to prove the result was due to an inhaled therapeutic dose [82]. Although none of the subjects in this study exceeded the limit, there were 3 individuals who had one test over 500 $ng \cdot ml^{-1}$ with one subject approaching the limit at 831 $ng \cdot ml^{-1}$. Considering this high value, it is plausible that an individual could exceed the WADA limit with a therapeutic dose.

However, *cSAL* values in this study are significantly less than those reported in recent positive tests (upwards of 3000 $ng \cdot ml^{-1}$) [45, 65].

The high variability observed in *cSAL* between subjects may be due to several factors as the pathway SAL must pass from inhalation to excretion is complex and involves several processes which may affect the time-course of passage. Lung absorption, metabolism, renal clearance, and hydration can all affect the amount of SAL that is excreted in the urine in the non-sulphated form. Interpretation is further complicated by the fact that urine *cSAL* following inhalation is a combination of local and systemic administrations due to a significant portion of the dose being swallowed. Approximately 20% of the dose is available to the lung following inhalation from a metered-dose inhaler and this can be enhanced when using a spacer device [49]. Although representing a potential explanation for the high variability observed we feel this is unlikely as spacer devices were implemented and each subject was instructed on proper use of the device prior. A more reasonable explanation is the individual differences in absorption, metabolism and renal clearance. Using charcoal ingestion to block gastrointestinal absorption, time to peak plasma concentrations post-inhalation have been shown to vary between 8 and 18 minutes [1]. Furthermore, exercise following inhalation of terbutaline (another ß-agonist) has been shown to increase rate of lung absorption, likely due to increased blood flow to the microcirculation [64]. Damaged epithelium may further increase absorption in the lung [62] and considering the amount of time endurance athletes spend at high ventilation rates, it is plausible that variations in epithelium integrity may exist between individuals. Exercise can also adversely effect renal function as glomerular filtration rate, osmotic

clearance, and urine flow are compromised following 30 minutes of exercise at 85% $\dot{V}_{O_2}$max [23]. Considering the multiple organs and processes that are involved prior to excretion of SAL, it is difficult to isolate a single reason to explain the variability in urine concentrations observed between subjects. However, a significant positive relationship was observed between SG and *cSAL* at both D4 and D8 (Fig. 5.5a-b) suggesting it may in part be due to hydration status.

Currently, with respect to SAL, WADA does not take into consideration hydration status other than ensuring samples are not diluted by requiring SG $\geq$ 1.005. Normal values for SG range between 1.005 and 1.030 and can have a significant impact on the concentration of urine specimens. To examine the impact of hydration all samples were corrected for SG to hydrated (1.005) and dehydrated states (1.025) (Fig. 5.4b-c). When corrected to a moderately dehydrated state, values from three different subjects exceeded 1000 ng·ml^{-1} with a maximum of 2125 ng·ml^{-1}. Theoretically these subjects could have produced a positive doping sample with a dose as low as 400 μg, providing support for the role of dehydration in false-positive doping tests [45, 65]. While SG is generally indicative of hydration and comparable to creatinine for correcting urine concentrations [50], we stress caution in applying these findings to doping control and in explaining false-positive doping violations previously reported in the literature. The low correlations between SG and *cSAL* would suggest that hydration status plays only a partial role and that values exceeding the WADA limit are likely due to the interplay of this and the several factors mentioned previously. The roles of length of time between inhalation and providing the urine sample, and the effects of multiple doses over time

need to be considered. Schweizer *et al.* [65], noted peak *cSAL* between 3 and 6 hours post-inhalation following multiple inhalations over 5 hours. Additionally, urine samples were taken 60 min post-inhalation following only 30 minutes of exercise. The values reported here may not be representative of events lasting several hours that may result in significant fluid shifts and dehydration.

From a methodological standpoint, there is one other limitation to this study that is worth noting. Although the data demonstrate no impact of SAL on time-trial performance, it could be argued that the influence of the one-way valve for collection of ventilatory and metabolic parameters may have masked any benefits of bronchodilation during exercise. We expect that this impact would be minimal considering a low-resistance valve was utilized and the ventilation rates maintained during the time-trials was significantly lower than those achieved during maximal exercise and EVH tests. However, we cannot exclude this possibility and it is worth examining the effects of bronchodilators on expiratory flow resistance and work of breathing during exercise in non-asthmatics. The effect of small reductions in the work of breathing may not manifest into performance enhancement over 30 minutes of exercise, but may reduce overall fatigue in longer duration events (> 2 hours).

In conclusion, this study failed to demonstrate any effects of SAL on time trial performance and ventilatory/metabolic parameters. Furthermore the use of multiple doses up to 800 μg did not reveal trends related to dose, strengthening the consensus that acute administration of inhaled SAL to non-asthmatic athletes is not performance

enhancing in endurance sports. From a doping control standpoint, although urine *cSAL* will generally fall under 500 $ng \cdot ml^{-1}$ following inhaled therapeutic doses, the potential for exceeding the WADA limit does exist as individual responses are highly variable. This is partially related to hydration status but likely dependant more so on individual differences in absorption, metabolism and renal function. Lastly, the prevalence of asthma and airway hyperresponsiveness in cyclists and triathletes is significantly higher than that normally reported for the general population. As all athletes were previously undiagnosed with asthma, further education is suggested for athletes, coaches, and medical professionals to increase the awareness and/or education with respect to the symptoms, proper diagnosis, and consequences of airway sensitivity with respect to sport.

CHAPTER 6 – SUMMARY AND CONCLUSIONS

The intention of this dissertation was to address two questions: what are the relationships between SAL dose and exercise performance in a simulated cycling time-trial, and what are the effects of dose on *cSAL* as used in doping control?

A series of three projects was used to demonstrate that inhaled SAL does not enhance endurance performance in non-asthmatic athletes when using a highly reproducible and sport-specific test. This the first examination of the dose-response effect of inhaled salbutamol using a sport-specific performance evaluation and used a substantially larger sample size (n = 27) compared to most previous work (n = 8-16). The lack of a dose-response relationship further supports previous findings that acute SAL inhalation does not enhance exercise performance in non-asthmatics [11, 22, 24, 48, 53, 61, 74, 75].

It was also shown that *cSAL* following inhalation is highly variable both at rest and following exercise, and related to dose. At rest, *cSAL* seems to peak at approximately 60 minutes post-inhalation. These findings are unique in reporting the dose-response relationships of inhaled SAL on urine concentrations, as reported and utilized by WADA. Previous pharmacological reports are typically reported in absolute values recovered or as a percentage of total dose administered. Although observed values for *cSAL* were similar between Projects 2 and 3, suggesting minimal effects of exercise, this conclusion is limited. Each study was performed independently and fluid intake was not controlled between the two. Future studies are needed to delineate the impacts of exercise on SAL

excretion using a randomized cross-over design. Furthermore, the short duration of the time-trial may not have provided sufficient stimulus for changes in hydration status that can accompany longer duration exercise. Even though most urine samples generally fell well below the WADA limit of 1000 $ng \cdot mL^{-1}$, the possibility exists for individuals to exceed this value following inhaled administration. A significant relationship between *cSAL* and urine SG was observed at higher doses, signifying the potential impacts on hydration on values observed in doping control. As with exercise, the role of hydration and individual differences in absorption, metabolism, and excretion on *cSAL* require further investigation.

It is also noted that the finding of SAL to be non-ergogenic cannot preclude the possibility that continued, short-term (>2-3 weeks) use of inhaled SAL would not be performance enhancing. Regular use of SAL during both training and competition would be expected and it is possible that continued elevated plasma levels following inhalation may increase ergogenic properties of SAL. Future research needs to be conducted to eliminate this possibility.

Lastly, it was observed that a large portion (~19%) of the cyclists/triathletes tested were susceptible to airway hyperresponsiveness. Although a small number of cyclists and triathletes were recruited for these studies, the possibility exists that there is a significant portion of this athlete population competing with impaired airway function unbeknownst to them. Although potential mechanisms for increased airway hyperresponsiveness in

certain athletes have been postulated, longitudinal research is required to track changes in airway function with length of time in specific sports.

In conclusion, this project demonstrated a lack of a dose-response relationship with SAL and exercise performance in non-asthmatic athletes and that urine *cSAL* following both rest and exercise are highly variable and dose-dependent.

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APPENDIX A – RAW DATA FOR PROJECT 1

Table A.1. Individual Subject Characteristics Including Competitive Experience (Exp).

Subject	Age (yrs)	Height (cm)	Weight (kg)	Exp (yrs)
1	36	191.5	98.	9
2	33	186.2	85	12
3	29	180.0	71	5
4	30	194.6	92	5
7	33	183.9	71	7
8	34	181.9	80	15
9	22	190.0	85	4
10	28	192.2	86	8
11	32	178.0	84	9
12	29	171.6	62	15
13	25	180.6	78	9
14	19	193.0	81	6
15	21	172.0	63	7
16	23	180.5	66	3
17	34	176.0	71	16
18	35	172.0	70	7
19	36	181.2	68	5
20	43	188.2	80	4
21	20	185.9	74	6
22	51	179.9	73	20
Mean	31	183.0	77	9
SD	8	7.1	9.7	5

Table A.2. Individual Subject Performance Characteristics Including Peak Oxygen Consumption ($\dot{V}_{O_2}max$) in Relative (Rel) and Absolute (Abs) terms, Maximal Ventilation ($\dot{V}_{E\,max}$), Maximal Heart Rate (HR_{max}), and Peak (P_{peak}) and Relative (P_{rel}) Power Output.

Subject	Rel $\dot{V}_{O_2}max$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Abs $\dot{V}_{O_2}max$ ($L \cdot min^{-1}$)	$\dot{V}_{E\,max}$ ($L \cdot min^{-1}$)	HR_{max} ($b \cdot min^{-1}$)	P_{max} (W)	P_{rel} (W/kg)
1	63.1	6.19	160.3	180	495	5.05
2	66.9	5.66	160.8	183	495	5.85
3	68.1	4.84	136.3	190	435	6.12
4	70.3	6.49	196.4	182	503	5.45
7	72.0	5.10	156.0	183	473	6.68
8	71.9	5.74	130.3	175	495	6.20
9	65.7	5.59	162.5	183	495	5.82
10	70.4	6.04	138.1	178	503	5.86
11	66.3	5.58	220.5	187	465	5.53
12	77.9	4.80	165.1	193	443	7.19
13	70.4	5.46	157.7	188	495	6.38
14	68.2	5.50	164.9	197	503	6.24
15	71.9	4.50	131.9	207	405	6.47
16	67.1	4.43	129.0	189	420	6.36
17	65.1	4.60	147.8	190	425	6.02
18	70.1	4.89	126.6	184	450	6.45
19	67.2	4.58	126.1	190	435	6.38
20	64.0	5.14	169.0	164	473	5.89
21	68.6	5.06	130.8	207	515	6.98
22	64.9	4.71	152.6	163	450	6.20
Mean	68.5	5.25	153.1	186	469	6.16
SD	3.6	0.61	25.1	9	33	0.49

Table A.3. Individual Performance Times in Minutes for Each Time-trial (TT) Including Lap (T_{L1} and T_{L2}) and Total Times (T_{tot}).

	TT1			TT2			TT3		
Subject	T_{L1}	T_{L2}	T_{tot}	T_{L1}	T_{L2}	T_{tot}	T_{L1}	T_{L2}	T_{tot}
1	14.32	15.08	29.40	15.08	14.86	29.94	15.50	14.95	30.45
2	14.93	14.97	29.90	15.21	15.03	30.23	15.31	14.91	30.22
3	15.83	15.33	31.15	15.90	15.60	31.50	15.76	15.40	31.16
4	13.99	14.30	28.29	14.14	14.37	28.51	13.98	14.40	28.37
7	14.67	14.93	29.60	14.53	14.92	29.45	14.70	15.12	29.82
8	14.19	14.53	28.72	14.22	14.28	28.50	14.27	14.60	28.87
9	14.76	14.87	29.63	14.86	14.59	29.45	14.87	14.59	29.46
10	14.23	14.27	28.51	14.31	14.62	28.93	14.21	14.19	28.39
11	15.51	15.33	30.84	15.33	15.56	30.88	15.14	15.45	30.59
12	15.11	15.61	30.71	15.32	15.75	31.06	15.19	15.63	30.82
13	14.40	15.12	29.51	14.42	15.16	29.58	14.42	15.08	29.50
14	14.70	14.70	29.38	14.80	14.85	29.65	14.93	14.80	29.73
15	15.53	16.07	31.60	15.65	16.07	31.72	15.82	15.68	31.48
16	16.27	16.13	32.40	14.73	15.95	31.70	15.80	15.90	31.70
17	16.37	15.95	32.33	16.98	15.77	32.77	16.82	16.22	33.03
18	15.08	15.33	30.42	14.98	15.20	30.20	15.00	15.10	30.10
19	15.22	15.45	30.67	15.25	15.40	30.67	15.23	15.55	30.78
20	14.82	14.77	29.58	14.37	14.77	29.13	14.43	14.70	29.13
21	14.17	14.30	28.47	14.18	14.37	28.55	14.48	14.65	29.13
22	14.60	14.91	29.52	14.88	15.17	30.05	14.90	15.23	30.13
Mean	14.93	15.10	30.03	14.96	15.11	30.12	15.04	15.11	30.14
SD	0.71	0.56	1.24	0.70	0.55	1.21	0.71	0.55	1.21

Table A.4. Individual Mean Performance Power (P_{mean}) in Watts for Each Time-trial (TT).

Subject	TT1	TT2	TT3
1	346	328	313
2	329	317	320
3	292	284	292
4	379	377	376
7	335	340	329
8	365	372	360
9	336	340	340
10	374	362	379
11	306	303	313
12	303	294	301
13	340	337	339
14	344	335	333
15	280	278	284
16	263	278	277
17	264	257	250
18	312	318	321
19	304	304	301
20	336	351	350
21	372	369	350
22	337	321	320
Mean	326	323	322
SD	35	35	34

Table A.5. Individual Mean Heart Rate for Each Time-trial (TT).

Subject	TT1	TT2	TT3
1	175	161	166
2	169	172	169
3	167	173	168
4	167	166	166
7	171	172	169
8	170	165	170
9	168	165	165
10	166	162	163
11	167	165	171
12	174	180	176
13	182	178	178
14	182	172	171
15	195	194	192
16	175	171	172
17	162	159	164
18	169	168	169
19	179	176	179
20	149	153	155
21	191	189	189
22	153	153	152
Mean	172	170	170
SD	8	8	7

APPENDIX B – RAW DATA FOR PROJECT 2

Table B.1. Individual Subject Characteristics and Baseline Lung Function Measures with Percent of Predicted Values (% Pred). Lung Function Measures Include Forced Vital Capacity (FVC), Forced Expiratory Volume in One Second (FEV_1), and the Ratio of FEV_1 to FVC (FEV_1/FVC).

Subject	Age (y)	Height (cm)	Weight (kg)	FVC (L)	% Pred	FEV_1 (L)	% Pred	FEV_1/FVC (%)	% Pred
1	22	182.2	74.8	6.66	112.1	5.28	106.5	79.3	95.0
2	33	186.0	87.5	5.63	94.9	4.15	84.9	73.71	89.4
3	24	181.5	73.5	6.15	105.7	4.86	100.2	79.12	94.9
4	23	179.6	67.9	4.92	86.5	4.01	84.2	81.46	97.5
5	34	176.6	77.0	5.06	99.0	4.40	103.8	86.97	104.9
6	27	183.4	78.1	5.05	85.7	4.31	88.1	85.25	102.7
7	35	176.1	80.2	5.45	107.5	4.27	101.7	78.36	94.5
8	25	169.9	79.8	5.73	119.1	4.38	108.1	76.57	90.9
Mean	28	179.4	77.4	5.58	101.32	4.46	97.2	80.09	96.2
SD	5.30	5.08	5.71	0.60	11.95	0.41	9.85	4.37	5.34

Table B.2. Individual Urine Concentrations of Non-sulphated Salbutamol ($ng \cdot ml^{-1}$) at 30 (T30), 60 (T60), and 120 Minutes (T120) Post-Inhalation of 200μg (D2), 400μg (D4), and 800μg (D8) of Salbutamol. Group Means and SD for Each Condition are Included.

	T30			T60			T120	
Subject	D2	D4	D8	D2	D4	D8	D2	D4	D8
1	189	621	185	157	529	904	59	98	562
2	38	27	95	39	107	97	0	28	64
3	0	213	178	0	137	138	0	57	208
4	28	182	232	85	255	403	33	167	194
5	0	90	519	0	164	139	0	20	58
6	180	132	189	154	182	369	74	147	310
7	0	64	86	61	45	47	0	73	31
8	27	58	83	32	29	78	0	0	121
Mean	58	173	196^{+}	66	181	272^{+}	21	74	194^{+}
SD	80	192	142	62	159	288	31	60	176

a – denotes significant difference from T30 at same dose, $p<0.05$

$^{+}$ – denotes significant difference from D2 at same time, $p<0.05$

* – denotes significant difference from D4 at same time, $p<0.05$

Table B.3. Individual Urine Concentrations of Non-sulphated Salbutamol ($ng \cdot ml^{-1}$) Corrected for Specific Gravity (1.005) at 30 (T30), 60 (T60), and 120 Minutes (T120) Post-Inhalation of 200μg (D2), 400μg (D4), and 800μg (D8) of Salbutamol. Mean and SD for Each Condition are Included.

	T30			T60			T120		
Subject	D2	D4	D8	D2	D4	D8	D2	D4	D8
1	79	141	36	157	126	161	98	98	140
2	8	5	79	32	31	162	0	23	80
3	0	118	99	0	228	230	0	142	347
4	7	41	45	18	61	72	6	38	35
5	0	18	118	0	43	174	0	33	145
6	31	73	50	24	70	154	13	52	91
7	0	40	86	18	45	59	0	26	39
8	27	58	138	80	72	195	0	0	121
Mean	19	62	81^{+}	41	85	$151^{a,+,*}$	15	52	$125^{+,*}$
SD	27	47	37	53	65	58	34	46	99

a – denotes significant difference from T30 at same dose, $p<0.05$

$^{+}$ – denotes significant difference from D2 at same time, $p<0.05$

* – denotes significant difference from D4 at same time, $p<0.05$

Table B.4. Individual Urine Concentrations of Non-sulphated Salbutamol ($ng \cdot ml^{-1}$) Corrected for Specific Gravity (1.025) at 30 (T30), 60 (T60), and 120 Minutes (T120) Post-Inhalation of 200μg (D2), 400μg (D4), and 800μg (D8) of Salbutamol. Mean and SD for Each Condition are Included.

	T30			T60			T120	
Subject	D2	D4	D8	D2	D4	D8	D2	D4	D8
1	394	706	178	785	630	807	492	490	702
2	41	24	396	162	157	808	0	117	400
3	0	592	494	0	1142	1150	0	712	1733
4	35	207	223	89	304	360	32	190	173
5	0	90	590	0	216	869	0	167	725
6	155	367	249	120	350	769	66	262	456
7	0	200	430	90	225	294	0	130	194
8	135	290	692	400	362	975	0	0	605
Mean	95	309	406^{+}	206	423	$754^{a,+,*}$	74	259	$624^{+,*}$
SD	135	237	183	266	324	291	171	232	495

a – denotes significant difference from T30 at same dose, $p<0.05$

$^{+}$ – denotes significant difference from D2 at same time, $p<0.05$

* – denotes significant difference from D4 at same time, $p<0.05$

Table B.5. Specific Gravity of Individual Urine Samples at 30 (T30), 60 (T60), and 120 Minutes (T120) Post-Inhalation of 200μg (D2), 400μg (D4), and 800μg (D8) of Salbutamol. Mean and SD for Each Condition are Included.

	T30			T60			T120		
Subject	D2	D4	D8	D2	D4	D8	D2	D4	D8
1	1.012	1.022	1.026	1.005	1.021	1.028	1.003	1.005	1.020
2	1.023	1.028	1.006	1.006	1.017	1.003	1.004	1.006	1.004
3	1.004	1.009	1.009	1.002	1.003	1.003	1.002	1.002	1.003
4	1.020	1.022	1.026	1.024	1.021	1.028	1.026	1.022	1.028
5	1.002	1.025	1.022	1.001	1.019	1.004	1.003	1.003	1.002
6	1.029	1.009	1.019	1.032	1.013	1.012	1.028	1.014	1.017
7	1.012	1.008	1.005	1.017	1.005	1.004	1.006	1.014	1.004
8	1.005	1.005	1.003	1.002	1.002	1.002	1.002	1.002	1.005
Mean	1.013	1.016	1.015	1.011	1.013	1.011	1.009	1.009	1.010
SD	0.010	0.009	0.010	0.012	0.008	0.011	0.011	0.007	0.010

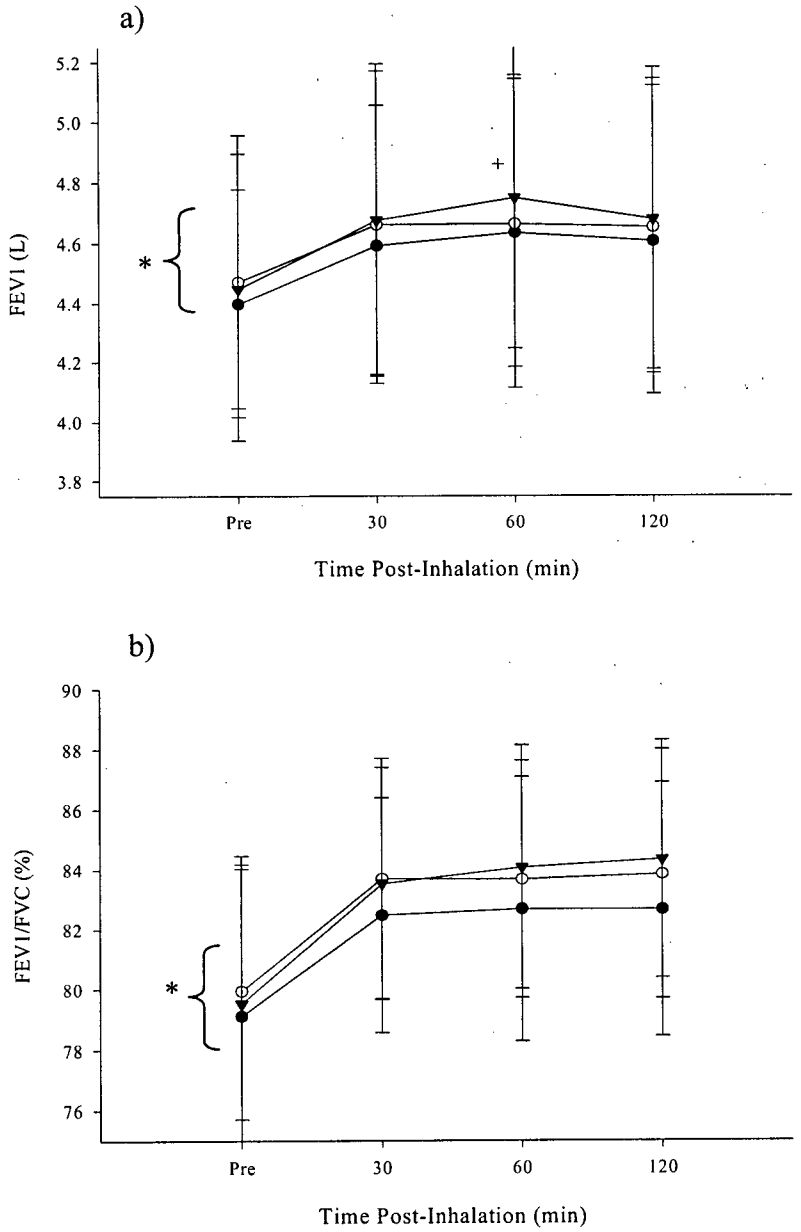

Fig. B.1. Force expiratory volume in 1 second (FEV_1) (a) and the ratio of FEV_1 to forced vital capacity (FVC) as a percentage (b) prior to (pre) and at 30, 60, and 120 minutes following inhalation of salbutamol. Values are shown for doses of 200 μg (-▼-), 400 μg (-○-), and 800 μg (-●-).

* - denotes all pre values are statistically significant from all post values for the same dose, $p<0.05$; + - denotes 800 μg at 60 min is greater than 200 μg at all time points, $p<0.05$.

APPENDIX C – RAW DATA FOR PROJECT 3

Table C.1. Individual Lung Function Measures Including % Predicted Values for Forced Vital Capacity (FVC), Forced Expiratory Volume in One Second (FEV_1), Ratio of FEV_1 to FVC (FEV_1/FVC), and Decrease in FEV_1 (Max ΔFEV_1) for Negative Responders to a Eucapnic Voluntary Hyperpnea (EVH) Test. Included are Means and SD (n=30).

Subject	FVC (L)	% Predicted	FEV_1 (L)	% Predicted	FEV_1/FVC (%)	% Predicted	Max ΔFEV_1 (%)
1	5.64	98.6	4.74	99.6	84.1	100.9	2.3
2	6.75	102.6	5.28	103.1	78.3	95.0	6.6
3	5.63	117.1	4.90	121.9	87.1	104.1	8.0
4	6.67	97.9	5.74	102.5	86.2	104.8	4.7
5	4.92	86.5	4.01	84.2	81.5	97.5	5.7
6	5.03	92.5	4.29	95.8	85.3	103.4	1.6
9	5.43	93.3	4.08	85.9	75.2	92.3	5.6
10	4.59	99.6	3.55	92.9	77.3	93.1	-1.4
13	6.84	101.6	5.96	104.6	87.1	105.5	4.5
15	6.77	112.1	5.81	115.7	85.8	103.2	1.2
16	5.96	109.6	4.97	109.5	83.4	100.0	3.4
18	5.06	85.0	3.92	78.9	77.5	92.7	6.4
19	4.97	135.8	3.76	122.1	75.7	90.0	9.8
20	4.85	94.4	4.44	103.7	91.5	110.0	-1.4
21	5.97	102.8	5.40	111.1	90.4	108.2	5.6
23	6.96	108.9	5.98	111.6	85.9	102.9	3.2
24	5.29	98.5	4.63	104.3	87.5	105.8	0.6
25	6.33	98.3	4.91	91.4	77.6	93.1	9.0
26	6.45	99.7	5.50	102.2	85.3	99.5	5.1
27	6.56	107.9	5.58	110.1	85.0	101.8	3.4
28	6.05	100.5	4.26	85.4	70.4	85.0	3.8
29	6.41	104.9	4.79	94.3	74.7	89.8	3.5
30	5.96	116.2	4.84	114.4	81.2	98.9	2.1
31	5.78	96.2	4.88	98.2	84.4	101.1	3.1
32	6.15	98.8	5.41	104.2	88.0	105.6	3.0
34	5.22	111.1	4.36	111.8	83.0	100.5	3.2
35	6.92	116.5	5.81	117.9	84.0	101.2	2.9
36	5.12	94.1	4.24	93.4	82.8	99.3	0.0
37	7.01	129.6	5.67	115.0	80.9	97.3	7.1
38	4.56	93.8	3.95	100.3	86.7	106.8	4.1
Mean	5.86	103.5	4.86	102.9	82.8	99.6	3.9
SD	0.77	11.4	0.72	11.3	5.0	6.0	2.7

Table C.2. Individual Lung Function Measures Including % Predicted Values for Forced Vital Capacity (FVC), Forced Expiratory Volume in One Second (FEV_1), Ratio of FEV_1 to FVC (FEV_1/FVC), and Decrease in FEV_1 (Max ΔFEV_1) for Negative Responders to a Eucapnic Voluntary Hyperpnea (EVH) Test. Included are Means and SD (n=7).

Subject	FVC (L)	% *Predicted*	FEV_1 (L)	% *Predicted*	FEV_1/FVC (%)	% *Predicted*	Max ΔFEV_1 (%)
7	4.89	96.6	4.01	100.0	81.9	98.7	27.7
8	7.25	103.9	4.86	84.3	67.0	81.2	17.7
11	5.52	88.9	3.92	76.0	71.0	85.5	12.2
12	6.86	108.0	5.63	106.2	82.1	98.5	12.1
14	6.38	102.6	5.16	99.6	80.9	97.2	15.3
17	5.95	107.2	4.84	104.3	81.3	97.2	11.0
33	5.81	105.4	4.26	92.4	73.3	87.7	12.4
Mean	6.09	101.8	4.67	94.7	76.8	92.3	15.5
SD	0.80	6.8	0.63	11.1	6.2	7.3	5.9

Table C.3. Individual Subject Characteristics and Training History of Negative EVH Subjects (n=30).

Subject	Age (yrs)	Height (cm)	Weight (kg)	Competitive Experience (yrs)	Training Volume ($km \cdot week^{-1}$)
1	25	180.6	79.5	9	400
2	29	192.2	90.8	9	325
3	30	171.6	62.7	15	400
4	30	195.3	95.0	4	300
5	23	181.0	66.0	3	400
6	36	181.2	67.2	6	425
9	43	188.2	81.7	4	200
10	37	171.7	67.3	12	175
13	25	194.6	79.2	5	400
15	25	184.4	77.3	12	275
16	27	178.1	73.6	6	150
18	21	182.0	70.5	6	200
19	31	166.0	67.9	10	250
20	31	175.8	66.4	3	250
21	22	180.7	73.1	8	100
23	18	186.4	73.6	5	400
24	34	179.7	73.3	6	300
25	25	187.1	87.7	5	250
26	21	188.1	77.6	7	600
27	21	183.5	74.0	6	350
28	29	185.3	75.1	14	150
29	23	184.5	78.4	3	200
30	41	179.6	77.7	2	275
31	30	185.9	73.3	10	250
32	21	185.2	83.6	5	100
34	34	172.4	69.4	12	225
35	27	184.0	82.9	8	120
36	27	178.7	81.9	10	150
37	30	178.7	76.0	10	325
38	51	179.6	73.5	25	270
Mean	29	182.1	75.9	8	274
SD	7.4	6.6	7.5	5	115

Table C.4. Baseline Performance Characteristics of Negative EVH Subjects Inclucing Peak Oxygen Consumption ($\dot{V}_{O_2}max$), Maximum Heart Rate (HR_{max}), and Peak Absolute (P_{max}) and Relative (P_{rel}) Power Outputs. Group Means and SD are Included. (n=30)

Subject	$\dot{V}_{O_2}max$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	$\dot{V}_{O_2}max$ ($L \cdot min^{-1}$)	Max HR ($b \cdot min^{-1}$)	Max Power (W)	Max Power ($W \cdot kg^{-1}$)
1	71.0	5.64	191	488	6.14
2	66.7	6.06	180	517	5.69
3	72.6	4.62	190	435	6.94
4	72.0	6.84	184	533	5.61
5	72.8	4.88	189	465	7.05
6	67.0	4.42	190	442	6.58
9	61.4	5.02	163	472	5.78
10	66.1	5.11	183	420	6.24
13	70.0	5.54	179	495	6.25
15	71.2	4.54	183	495	6.40
16	64.2	4.73	180	443	6.02
18	67.2	4.74	201	450	6.38
19	62.7	4.26	190	405	5.96
20	65.4	4.34	198	435	6.55
21	72.6	5.3	191	450	6.16
23	69.7	5.13	178	473	6.43
24	69.0	5.06	184	473	6.45
25	57.4	5.04	201	465	5.30
26	65.1	5.05	177	435	5.61
27	70.9	5.25	204	465	6.28
28	67.7	5.08	190	480	6.39
29	68.5	5.37	189	450	5.74
30	65.3	5.08	182	428	5.51
31	66.6	4.88	188	428	5.84
32	58.4	4.88	189	435	5.20
34	69.3	4.81	181	450	6.48
35	72.2	5.99	194	503	6.07
36	59.6	4.89	194	420	5.13
37	67.8	5.15	184	435	5.73
38	63.4	4.66	162	428	5.82
Mean	67.1	5.08	186	457	6.06
SD	4.3	0.54	10	31	0.48

Table C.5. Individual Mean Power Output (W) During a 20km Time-trial Post-Inhalation of Placebo (DP), 200μg (D2), 400μg (D4), and 800μg (D8) of Salbutamol. Group Means and SD for Each Condition are Included. (n=30)

Subject	DP	D2	D4	D8
1	324	n/c	315	339
2	351	365	350	360
3	288	290	295	290
4	352	373	363	369
5	293	308	289	293
6	293	287	307	287
9	340	339	345	345
10	278	269	272	277
13	345	343	343	352
15	310	314	316	311
16	298	297	300	291
18	307	305	301	310
19	262	269	270	274
20	275	275	274	270
21	332	332	330	326
23	332	333	332	313
24	312	322	299	288
25	285	n/c	n/c	281
26	297	304	290	285
27	269	293	284	267
28	321	331	n/c	343
29	314	306	316	324
30	270	287	279	279
31	293	296	302	296
32	260	280	252	287
34	306	307	299	306
35	364	379	365	374
36	307	313	309	312
37	298	307	299	305
38	310	289	307	300
Mean	306	311	307	308
SD	28	29	28	30

n/c– condition not completed

Table C.6. Individual 20km Performance Times (min) Post-Inhalation of Placebo (DP), 200μg (D2), 400μg (D4), and 800μg (D8) of Salbutamol (Including Lap (T_{L1} and T_{L2}) and Total Times (T_{tot}). Group Means and SD for Each Condition are Included. (n=30)

	DP			D2			D4			D8		
Subject	T_{L1}	T_{L2}	T_{tot}	T_{L1}	T_{L2}	T_{tot}	T_{L1}	T_{L2}	T_{tot}	T_{L1}	T_{L2}	T_{tot}
1	14.92	15.05	29.97	n/c	n/c	n/c	14.93	14.93	30.28	14.47	15.03	29.50
2	14.87	14.47	29.33	14.52	14.37	28.88	14.68	14.68	29.25	14.63	14.35	28.98
3	15.43	15.85	31.28	15.25	16.00	31.23	15.20	15.20	31.03	15.37	15.87	31.23
4	14.57	14.52	29.07	14.12	14.35	28.47	14.43	14.43	28.73	14.13	14.45	28.58
5	15.58	15.50	31.08	15.17	15.38	30.53	15.47	15.47	31.32	15.60	15.50	31.10
6	15.57	15.48	31.05	15.60	15.72	31.32	15.18	15.18	30.50	15.70	15.62	31.32
9	14.58	14.87	29.45	14.63	14.87	29.50	14.47	14.47	29.30	14.52	14.82	29.33
10	15.60	16.10	31.70	15.92	16.18	32.08	15.92	15.92	31.95	15.77	15.98	31.75
13	14.45	14.68	29.13	14.55	14.83	29.38	14.48	14.48	29.28	14.48	15.02	29.50
15	15.07	15.45	30.52	15.07	15.28	30.35	14.95	14.95	30.28	15.12	15.33	30.45
16	15.60	15.33	30.95	15.58	15.37	30.97	15.52	15.52	30.92	15.60	15.63	31.23
18	15.17	15.45	30.62	15.33	15.38	30.72	15.37	15.37	30.90	15.25	15.30	30.55
19	16.17	16.27	32.45	16.08	16.03	32.12	15.98	15.98	32.08	16.02	15.88	31.92
20	15.75	16.07	31.83	15.83	16.00	31.85	15.75	15.75	31.90	15.98	16.12	32.10
21	14.83	14.85	29.70	14.85	14.83	29.68	14.85	14.85	29.75	14.97	14.95	29.90
23	14.68	15.02	29.70	14.80	14.85	29.67	14.78	14.78	29.68	15.20	15.17	30.37
24	15.27	15.12	30.40	14.82	15.23	30.05	15.68	15.68	30.92	15.90	15.42	31.30
25	15.73	15.72	31.45	n/c	n/c	n/c	n/c	n/c	n/c	15.58	16.02	31.60
26	15.33	15.67	31.02	15.57	15.15	30.72	15.90	15.90	31.32	16.07	15.45	31.52
27	16.25	15.95	32.20	15.70	15.55	31.25	15.83	15.83	31.55	16.33	16.03	32.35
28	15.22	14.85	30.07	14.83	14.93	29.75	n/c	n/c	n/c	14.77	14.58	29.35
29	15.12	15.22	30.35	15.18	15.42	30.60	15.00	15.00	30.23	14.90	15.07	29.97
30	16.12	16.10	32.23	15.73	15.60	31.33	15.97	15.97	31.70	16.03	15.67	31.70
31	15.62	15.50	31.10	15.37	15.62	30.98	15.18	15.18	30.75	15.52	15.47	30.98
32	16.33	16.22	32.53	16.08	15.62	31.75	16.45	16.45	32.92	15.57	15.77	31.32
34	15.10	15.52	30.62	15.07	15.50	30.55	15.15	15.15	30.90	15.10	15.50	30.60
35	14.28	14.47	28.75	14.32	13.97	28.30	14.47	14.47	28.68	14.30	14.13	28.43
36	15.37	15.60	30.97	15.25	15.55	30.80	15.28	15.28	30.80	15.27	15.65	30.92
37	15.70	15.33	31.03	15.57	15.07	30.63	15.73	15.73	30.88	15.60	15.10	30.70
38	15.10	15.35	30.47	15.72	15.57	31.28	15.27	15.27	30.58	15.50	15.35	30.85
Mean	15.31	15.39	30.70	15.23	15.29	30.53	15.28	15.28	30.66	15.30	15.34	30.64
SD	0.54	0.52	1.03	0.53	0.53	1.02	0.54	0.54	1.04	0.58	0.52	1.05

n/c – condition not completed

Table C.7. Individual Mean Oxygen Consumption ($mL \cdot kg^{-1} \cdot min^{-1}$) During a 20km Time-trial Post-Inhalation of Placebo (DP), 200μg (D2), 400μg (D4), and 800μg (D8) of Salbutamol. Group Means and SD for Each Condition are Included. (n=30)

Subject	DP	D2	D4	D8
1	56.5	n/c	54.6	44.4
2	49.4	53.9	47.3	53.5
3	58.8	55.0	57.7	57.6
4	53.9	57.7	54.7	57.2
5	55.0	50.5	52.2	58.4
6	56.3	59.2	58.9	56.6
9	53.0	55.7	57.4	54.9
10	58.4	55.4	56.7	55.5
13	53.0	50.8	52.0	55.7
15	57.9	56.5	58.2	56.7
16	53.5	54.5	53.7	50.9
18	57.9	57.4	56.2	57.2
19	51.0	51.1	51.5	52.4
20	53.5	62.0	54.6	55.3
21	62.3	62.5	61.5	62.0
23	60.7	61.1	61.6	59.3
24	56.6	57.1	53.9	53.3
25	42.1	n/c	n/c	43.4
26	51.2	52.4	49.4	48.7
27	50.0	55.1	52.3	51.3
28	52.6	55.7	n/c	55.9
29	56.8	54.4	58.0	58.3
30	48.0	52.9	53.2	51.5
31	55.2	56.5	57.6	52.9
32	42.7	46.5	43.1	50.1
34	55.7	58.6	55.3	58.7
35	60.3	62.1	59.5	62.3
36	50.8	50.2	52.3	51.6
37	54.1	55.6	51.6	53.1
38	55.9	52.5	54.8	50.9
Mean	54.1	55.5	54.6	54.3
SD	4.7	3.9	4.1	4.4

n/c– condition not completed

Table C.8. Individual Mean Ventilation ($L \cdot min^{-1}$) During a 20km Time-trial Post-Inhalation of Placebo (DP), 200μg (D2), 400μg (D4), and 800μg (D8) of Salbutamol. Group Means and SD for Each Condition are Included. (n=30)

Subject	DP	D2	D4	D8
1	115.5	n/c	106.7	90.1
2	79.2	86.9	77.3	86.2
3	105.5	93.4	101.7	99.0
4	107.0	115.5	107.6	121.4
5	89.9	86.7	83.2	95.6
6	83.6	93.7	93.6	83.7
9	113.5	114.0	113.7	114.2
10	110.1	105.3	112.9	109.9
13	104.0	105.5	99.3	106.1
15	125.7	128.6	130.4	124.6
16	84.3	89.9	88.3	84.0
18	99.8	102.8	98.4	98.9
19	78.5	83.5	80.7	90.9
20	87.6	107.6	89.6	92.5
21	108.7	106.1	104.9	107.6
23	142.6	138.6	145.4	129.3
24	106.3	107.3	96.8	94.3
25	82.5	n/c	n/c	82.9
26	101.8	102.3	92.4	84.5
27	81.6	108.7	88.6	84.4
28	89.1	95.5	n/c	95.5
29	104.9	103.3	100.8	109.7
30	91.1	98.6	101.8	102.3
31	112.7	116.3	119.9	98.6
32	80.4	92.4	83.0	103.0
34	97.7	99.4	92.2	96.3
35	115.7	119.5	110.4	109.9
36	119.6	115.5	123.0	124.6
37	106.2	100.2	98.7	102.3
38	119.4	104.5	118.1	103.7
Mean	101.5	104.3	102.1	100.9
SD	15.8	12.6	15.8	13.1

n/c– condition not completed

Table C.9. Individual Mean Heart Rate (bpm) During a 20km Time-trial Post-Inhalation of Placebo (DP), 200μg (D2), 400μg (D4), and 800μg (D8) of Salbutamol. Group Means and SD for Each Condition are Included. (n=30)

Subject	DP	D2	D4	D8
1	174	n/c	178	178
2	157	166	164	162
3	173	169	169	163
4	167	171	165	174
5	172	176	172	168
6	172	177	179	174
9	155	155	158	157
10	175	170	170	169
13	166	166	168	171
15	171	167	174	170
16	165	170	173	181
18	184	187	189	188
19	171	175	177	175
20	185	186	186	186
21	180	182	181	175
23	175	173	173	166
24	177	179	166	166
25	173	n/c	n/c	178
26	172	168	161	160
27	183	194	186	188
28	173	180	n/c	178
29	176	178	173	175
30	163	163	163	163
31	171	173	174	175
32	166	170	167	175
34	171	169	174	168
35	190	184	183	184
36	185	183	176	181
37	162	156	155	155
38	154	150	155	152
Mean	172	173	172	172
SD	9	10	9	9

n/c– condition not completed

Table C.10. Individual Mean Speed ($km \cdot hr^{-1}$) During a 20km Time-trial Post-Inhalation of Placebo (DP), 200μg (D2), 400μg (D4), and 800μg (D8) of Salbutamol. Group Means and SD for Each Condition are Included. (n=30)

Subject	DP	D2	D4	D8
1	40.1	n/c	39.6	40.7
2	40.9	41.6	41.0	41.4
3	38.4	38.4	38.7	38.4
4	41.3	42.2	41.8	42.0
5	38.6	39.3	38.3	38.6
6	38.7	38.3	39.3	38.3
9	40.7	40.7	41.0	40.9
10	37.9	37.4	37.6	37.8
13	41.0	40.7	40.8	41.2
15	39.3	39.6	39.6	39.4
16	38.8	38.8	38.8	38.4
18	39.2	39.1	38.8	39.3
19	37.0	37.4	37.4	37.6
20	37.7	37.7	37.6	37.4
21	40.4	40.4	40.3	40.1
23	40.4	40.5	40.4	39.5
24	39.5	39.9	38.8	38.3
25	38.2	n/c	n/c	38.0
26	38.7	39.1	38.3	38.1
27	37.3	38.4	38.0	37.1
28	39.9	40.3	n/c	40.9
29	39.6	39.2	39.7	40.0
30	37.2	38.3	37.9	37.9
31	38.6	38.7	39.0	38.7
32	36.9	37.9	36.5	38.3
34	39.2	39.3	38.9	39.2
35	41.7	42.4	41.8	42.2
36	38.8	39.0	39.0	38.8
37	38.7	39.2	38.9	39.1
38	39.4	38.4	39.2	38.9
Mean	39.1	39.3	39.2	39.2
SD	1.3	1.3	1.3	1.4

n/c– condition not completed

Table C.11. Individual Rate of Perceived Exertion for Breathing Effort (1-10) During a 20km Time-trial Post-Inhalation of Placebo (DP), 200μg (D2), 400μg (D4), and 800μg (D8) of Salbutamol. Group Means and SD for Each Condition are Included. (n=30)

Subject	DP	D2	D4	D8
1	7.2	n/c	7.5	6.2
2	6.3	6.4	5.8	6.2
3	6.4	5.0	6.6	5.7
4	3.3	4.2	3.1	3.5
5	8.4	8.6	8.5	8.3
6	7.4	7.5	8.3	8.1
9	9.0	9.6	9.3	9.1
10	6.4	5.7	5.1	5.4
13	5.8	5.2	6.0	6.2
15	7.9	7.7	8.1	7.9
16	3.8	3.7	3.7	4.4
18	6.4	7.0	6.6	6.4
19	6.7	5.5	6.2	6.5
20	6.5	6.9	7.2	6.6
21	4.8	4.0	4.9	7.3
23	5.5	7.3	5.9	5.9
24	4.4	5.6	4.7	4.5
25	4.7	n/c	n/c	4.7
26	5.5	5.7	6.2	5.3
27	5.5	5.5	5.5	5.9
28	*	7.1	n/c	6.4
29	6.3	7.1	7.1	7.3
30	6.0	5.5	5.1	6.0
31	4.6	4.8	5.2	4.9
32	6.0	5.1	6.5	5.9
34	8.5	8.4	8.6	8.0
35	4.7	4.7	4.3	3.8
36	6.3	8.6	8.3	7.1
37	5.7	6.1	5.8	6.2
38	5.9	5.7	5.9	5.5
Mean	6.0	6.2	6.3	6.1
SD	1.4	1.5	1.5	1.3

n/c– condition not completed
* - data collection error

Table C.12. Individual Mean Rate of Perceived Exertion for Leg Effort (1-10) During a 20km Time-trial Post-Inhalation of Placebo (DP), 200μg (D2), 400μg (D4), and 800μg (D8) of Salbutamol. Group Means and SD for Each Condition are Included. (n=30)

Subject	DP	D2	D4	D8
1	7.1	n/c	7.6	5.8
2	6.8	7.2	6.5	7.4
3	5.8	7.4	6.0	6.0
4	4.4	4.4	4.5	4.4
5	8.4	8.5	8.6	8.2
6	7.5	7.5	8.0	7.8
9	8.9	9.3	9.3	9.0
10	6.2	5.4	4.9	5.3
13	4.7	4.0	4.6	4.8
15	7.8	8.0	8.1	7.7
16	4.8	5.9	4.9	4.6
18	5.6	4.9	4.6	5.0
19	5.7	5.2	5.6	6.1
20	5.9	6.5	5.4	6.5
21	5.7	5.0	5.5	6.6
23	5.0	7.0	5.9	6.7
24	2.6	4.0	3.4	3.2
25	6.4	n/c	n/c	5.2
26	5.3	5.3	5.7	5.0
27	6.1	5.1	5.6	7.0
28	*	7.0	n/c	6.4
29	6.6	6.6	7.0	6.6
30	6.5	5.7	5.5	6.1
31	5.7	6.2	6.6	6.0
32	5.8	5.0	5.4	5.8
34	8.6	8.3	8.4	8.3
35	4.6	4.6	4.2	3.8
36	5.7	8.8	8.6	7.0
37	4.4	5.8	4.9	5.1
38	5.4	4.9	5.6	4.6
Mean	6.0	6.2	6.1	6.1
SD	1.4	1.5	1.5	1.4

n/c– condition not completed
* - data collection error

Table C.13. Individual Urine Concentrations of Non-sulphated Salbutamol ($ng \cdot ml^{-1}$) at 60 Minutes (T60) Post-Inhalation of Placebo (DP), 200μg (D2), 400μg (D4), and 800μg (D8) of Salbutamol. Group Means and SD for Each Condition are Included.

Subject	DP	D2	D4	D8
1	0	n/c	118	347
2	0	84	94	372
3	0	108	627	339
4	31	0	66	67
5	0	99	286	237
6	0	25	87	217
9	0	153	210	142
10	0	0	33	33
13	54	347	123	151
15	20	46	114	539
16	48	0	83	225
18	0	41	152	66
19	26	26	177	98
20	0	34	0	340
21	0	45	198	361
23	0	0	42	72
24	0	25	60	831
25	0	n/c	n/c	250
26	0	0	55	121
27	0	90	100	165
28	0	56	n/c	52
29	0	0	22	43
30	0	0	38	247
31	0	0	58	48
32	0	29	242	186
34	0	51	68	301
35	0	0	67	189
36	0	0	0	147
37	0	38	45	96
38	0	0	51	26
Mean	7	46	115 $^{+}$	210 $^{+,a,*}$
SD	15	73	126	177

n/c– no urine sample collected

$^{+}$ – denotes significantly greater than DP, $p<0.05$

^{a} – denotes significantly greater than D2, $p<0.05$

* – denotes significantly greater than D4, $p<0.05$

Table C.14. Individual Urine Concentrations of Non-sulphated Salbutamol ($ng \cdot ml^{-1}$) Corrected for Specific Gravity (1.005) at 60 Minutes (T60) Post-Inhalation of Placebo (DP), 200μg (D2), 400μg (D4), and 800μg (D8) of Salbutamol. Mean and SD for Each Condition are Included.

Subject	DP	D2	D4	D8
1	0	n/c	18	124
2	0	38	78	186
3	0	32	157	89
4	7	0	16	19
5	0	25	71	62
6	0	42	109	271
9	0	45	44	28
10	0	0	33	82
13	25	145	27	63
15	14	15	57	180
16	11	0	20	62
18	0	15	36	37
19	9	32	126	82
20	0	42	0	425
21	0	16	66	113
23	0	0	210	180
24	0	18	33	198
25	0	n/c	n/c	54
26	0	0	12	36
27	0	21	25	92
28	0	70	n/c	65
29	0	0	12	43
30	0	0	47	103
31	0	0	24	30
32	0	6	55	116
34	0	8	15	68
35	0	0	67	105
36	0	0	0	37
37	0	24	45	96
38	0	0	21	6
Mean	2	19	52 $^{+}$	104 $^{+,a,*}$
SD	6	29	49	90

n/c – no urine sample collected

$^{+}$ – denotes significantly greater than DP, $p<0.05$

^{a} – denotes significantly greater than D2, $p<0.05$

* – denotes significantly greater than D4, $p<0.05$

Table C.15. Individual Urine Concentrations of Non-sulphated Salbutamol ($ng \cdot ml^{-1}$) Corrected for Specific Gravity (1.025) at 60 Minutes (T60) Post-Inhalation of Placebo (DP), 200μg (D2), 400μg (D4), and 800μg (D8) of Salbutamol. Mean and SD for Each Condition are Included.

Subject	DP	D2	D4	D8
1	0	n/c	92	620
2	0	191	392	930
3	0	159	784	446
4	35	0	82	93
5	0	124	357	312
6	0	208	544	1356
9	0	225	219	142
10	0	0	165	412
13	123	723	134	315
15	71	77	285	898
16	57	0	99	312
18	0	73	181	183
19	46	162	632	408
20	0	212	0	2125
21	0	80	330	564
23	0	0	1050	900
24	0	89	167	989
25	0	n/c	n/c	272
26	0	0	60	178
27	0	107	125	458
28	0	350	n/c	325
29	0	0	61	215
30	0	0	237	515
31	0	0	121	150
32	0	29	275	581
34	0	42	77	342
35	0	0	335	525
36	0	0	0	184
37	0	119	225	480
38	0	0	106	32
Mean	12	97	261 $^{+}$	520 $^{+,a,*}$
SD	30	147	245	451

n/c – no urine sample collected
$^{+}$ – denotes significantly greater than DP, $p<0.05$
^{a} – denotes significantly greater than D2, $p<0.05$
* – denotes significantly greater than D4, $p<0.05$

Table C.16. Specific Gravity of Individual Urine Samples for Placebo (DP), 200µg (D2), 400µg (D4), and 800µg (D8) Conditions. Mean and SD for Each Condition are Included.

Subject	DP	D2	D4	D8
1	1.008	n/c	1.032	1.014
2	1.006	1.011	1.006	1.010
3	1.020	1.017	1.020	1.019
4	1.022	1.010	1.020	1.018
5	1.018	1.020	1.020	1.019
6	1.004	1.003	1.004	1.004
9	1.008	1.017	1.024	1.025
10	1.007	1.007	1.005	1.002
13	1.011	1.012	1.023	1.012
15	1.007	1.015	1.010	1.015
16	1.021	1.023	1.021	1.018
18	1.011	1.014	1.021	1.009
19	1.014	1.004	1.007	1.006
20	1.003	1.004	1.005	1.004
21	1.012	1.014	1.015	1.016
23	1.002	1.002	1.001	1.002
24	1.020	1.007	1.009	1.021
25	1.021	n/c	n/c	1.023
26	1.032	1.018	1.023	1.017
27	1.029	1.021	1.020	1.009
28	1.005	1.004	n/c	1.004
29	1.003	1.004	1.009	1.005
30	1.009	1.011	1.004	1.012
31	1.013	1.008	1.012	1.008
32	1.024	1.025	1.022	1.008
34	1.008	1.030	1.022	1.022
35	1.004	1.005	1.005	1.009
36	1.003	1.023	1.004	1.020
37	1.005	1.008	1.005	1.005
38	1.021	1.028	1.012	1.020
Mean	1.012	1.013	1.014	1.013
SD	0.008	0.008	0.008	0.007

n/c – no urine sample collected

APPENDIX D – SCALE FOR MEASURING PERCEIVED EXERTION