University of Kent | School of Sport and Exercise Sciences

"THE EFFECT OF INHALED β_2-AGONISTS ADMINISTRATION ON PHYSICAL PERFORMANCE"

The thesis is presented for the degree of Doctor of Philosophy at the University of Kent

by

Mr Michele Merlini

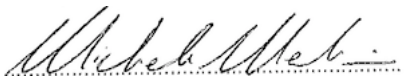
School of Sport and Exercise Sciences, University of Kent

June 2019

DECLARATION

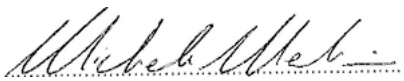
STATEMENT 1

This work has not previously been accepted in substance for any degree and is not being concurrently submitted for any degree, diploma or other qualification.

Signature:  Date: 30/06/2019

STATEMENT 2

This thesis is the product of my own investigation, except where otherwise stated. Other sources are acknowledged in brackets by giving explicit references. A reference list is included at the end of this thesis.

Signature:  Date: 30/06/2019

STATEMENT 3

I hereby consent for my thesis, if accepted, to be available for photocopying, for interlibrary loan and for digital repositories, and for the title and summary to be made available to outside organisations.

Signature:  Date: 30/06/2019

This thesis was supervised by *Dr John Dickinson* (School of Sport and Exercise Sciences, University of Kent) and *Dr Samuele Maria Marcora* (School of Sport and Exercise Sciences, University of Kent).

Acknowledgment

I remember the day I came in the UK with the intent to live an experience at the University of Kent observing how to conduct sport researches.

I've been completely fascinated by the facilities, the equipment, the technicians, the PhD students, the Master students, the lecturers and most important by the numerous volunteers that took part in those experiments… (brave people ☺).

For me was a kind of playground, all I have read inside books was there, amazing!!! Since I came in Kent I though that it would have been a long and difficult ride due to cultural barriers that I faced from the first day I went at the University as "Visitors".

The "English" language I studied at High School was not the English language I faced at the University. Thanks God I was in a good companion of foreigner students and the communication was not such a difficult thing as with English people.

While I was Visitors I used to work early in the morning of the week-end in a fast-food as kitchen closer and I remember my long conversations with vegetables when I did the washing-up. My daily questions were: "Shall I begin to understand English people talking? Shall I talk to people more fluently? Shall….Shall…Shall… After 3 weeks from my arrive in the Uk I decided, even encouraged by my girlfriend Suher A.K.A. "Bimbi", to participate to English teaching classes in London… bad idea, there were more Italians and Spaniards than English people in London and I though to be destined to speak "English Maccaronico" until the ends of my days.

Notably, my Spanish language became better and better during that time…

I found in Walter and Luca my anchor points at that time, they've been living in Kent from 2 years and they were running research studies under Sam Marcora supervision.
Prof. Sam Marcora was the reason why I decided to leave Italy, I was tremendously curios about the research conducted by Sam and the good speeches about his methodologies that in Italy was something of unusual… the typical "CERVELLO IN FUGA".

From the first day I step at the University of Kent, Sam made my self welcome, I really thank you Sam for your hospitality even if we did not pass so a lot of time together due to his travels around the world. Every time I had a problem Sam suggested to ask to Walter.

Therefore, I knew Walter A.K.A. Walteruzzo, Staianz. At the beginning he was submitting his PhD Thesis and was between England and Wales but after a while I came permanently in Kent.
What a guy… Try to pick-up a country in Europe … ok, Walter probably has lived there at least 6 months of his life, what a Super-trump guy.

It was probably his energy, his will to be curious and the way he was helping people to solved their problem; "Walter do you remember Akhram and Ali ambushes for blocking you? Ahahahah". I've moved my first steps in the academic world with him, yes because I forgot to say I had 9 months contract as research assistant in a project funded by the Ministry of Defense where Walter was ahead.

Could you imagine that? 3 months ago I was cutting bread and making sandwiches and now I was a member of the staff at the University of Kent?!?...I should remind this more often when I'm feeling sad, depress and unhappy!!!! There's always a chance, we have just to wait our moment and be prepared to start a new adventure.

Yes, this was probably the best working experience of my time and I used to recount every single episode to Bimbi when we had dinner in that time. We basically spent 2 hours during the day together because she woke up very early in the morning (4.00 am) and I back home late in the night (21/22). Bimbi waited me terribly tired every single day for sharing dinner and talking about the day. I will never forget it, you are wonderful life companion, you are Mother Nature force, I LOVE YOU!!

Life went well, work was good and I never felt the research we were working at as a real job. We used to work between 10 to 12 hours/day, our salvation was the coffee machine that Walter brought from Napoli, the authentic "caffettiera napoletana". It was a kind of talisman for us, the brain-stimulation we needed when training session began at 7 am…what a training time-table.

We had a special collaborator, Walter's brother A.K.A. "Pinuccio Santamaria" helping us to deal busy moment of the day when there were all three cycle-ergometers occupied by the participants. Thank you Ale for helping us to deal it and thank you for preparing "pasta e patane", "pasta e fasule" during that night we finished testing late in the night.

At that time I had not intention to begin a PhD career, I'm still thinking to be a "field-man" than a "desk-man". I'm feeling peaceful to coach athletes, living on the pitch during the cold winters and the hot summers, but life not always goes as you planned.
I felt football nostalgia, I grew up in football changing rooms with "any bloody Sunday" movie as the emblem of last 4 years in Italy. I felt empty, no more happy as I was before… Homesickness!!!

When you leave your birthplace is never easy, for none. I remember the very first period I was in London I was a mix of feelings… I was happy, sad, euphoric, depressed, happy again.
Fortunately, my girlfriend was always closer to me and I remember she said "It's not easy to me neither, but we are together and together is less difficult". It's true I have been never alone, probably I could not survived 3 years alone without her, thank you again for you "Gypsy state of mind!!!".
She is a traveller, she does not care about where to go, I think she loves much more the journey than the destination…what a GIRL!!!

When my contract was expiring I had no ideas about my future. Fortunately, here it comes another important person of my last 5-years, John Dickinson. A research project funded by WADA needed a research assistant and John gave me the chance to be the main investigator.

At the beginning I was a bit scared by this responsibility because this time, contrary to the previous project, I was alone and I had a lot of stuff to do.
Basically, I lived for one year in the gym of the University to assist the participants in those it would have been lately the first study of my PhD.

During this time I was thinking that it would have been a great opportunity to keep going with this research topic because was anti-doping fight was closer to my moral principles and to the sport I would see.
This probably was the biggest reason of may choice to jump in a new adventure called doctorate of research.

Such as big word for a strength & conditioning coach, I am not sure I will never get used to hear my name closer to this title…… I talked to John about my idea to start this long path and he was at that

time really happy, I'm sure he will never forget that day when he said "Why not Michele?!"… yes, because from that day I choked his email box.

I really have a really good working relationship with John, he was quite available during throughout my PhD especially because I run two of the three studies abroad. Whether this could seems a problem, in my opinion was a good experience that make me feel proud of my career progresses. He supported me especially during the draft of the papers and I learnt from him how to write a paper and the way to do research, I'm happy of his supervision and I'm thankful to him!!!

I think that when you begin PhD you don't realise exactly how challenging and frustrating it will become especially the attempt to publish a study extracted from your PhD thesis. I spent hours and hours during my spare time to work on my thesis and on my studies and after a while I found my self lost. I deleted and re-wrote many many times same documents until I've got the chance to get a paper published!!! This represents for me a great achievement and the right acknowledgment for the hard work made by me from the first day I began this adventure.

I can't forget the night of the week-end seated on a chair with my "English teacher Bimbi" in the attempt to write as best I could a scientific paper. I have indelible in my eyes Suher's reaction to the comments made by John in manuscript because she did not understand what particular grammar rule had scientific papers.
Don't worry my love, you will have time during the writing of other papers to understand it….. LOL
I won't forget the innumerous files and grammar books attached in the emails by you (Suher), it helped me to understand a little bit more how to create a paper.
I still remember your "mantra": "TESI, ANTITESI, SINTESI". I had bad dreams with this jingle as soundtrack.

I have to thank you, again, for the motivation and your abnegation in keeping me into my mission, getting my bad days less bitter. I have to confess that when I was in difficult you have been my extra energy and I finish this also to show you that hard work pay off!!!! I think that one part of this thesis is your, truly, and I really hope that the future will give you the opportunity to prosecute your studies and maybe obtaining a doctorate of research in foreign literature!!!
You are a GEEK, similar to Sheldon Cooper ☺.

In conclusion, I thank you one more time to have always put my PhD before everything, before Saturday Nights out, before week-ends out, before YOU!
You are such an incredible person, a pure soul!!!

I have to thanks Luca, A.K.A. “caghino” because his strictness and his professional way to work make me feel improved as researcher and more patient as a man…we lived 1 week together in Swiss for a data collection and I really risked to kill him!!! (I would have kicked your bottom many times for your negative way to see the life… COME ON MATE don’t be so sad).

Thank you Irisz, Andrea Nicolò, Chiara and “Kurdish gang”, I miss you guys.

A special thanks to Jose A.K.A. o “maestro”, the Brazilian more Italian than me, our friendship is something special. You taught me a lot of in physiology of exercise and training tips but the most important life-lesson you taught me is represented by your simplicity, a person with noble principles and a big heart. I wish you the best as cyclist coach because you deserve it. I miss you my friend and I miss Marylin too.

I have to thanks all the University staff for helping me in booking accommodations and flights for the conferences I participated during this marvelous years. How could I forget the good time spent in pubs and discos with John, Walter, Lex, Mark and others… You are great guys!!!

I want thank you Mum and thank you Dad for the big opportunity you gave me to have an education; I hope to be good as you are with my sons in the future. Thank you Daddy for supporting me financially at the end of the PhD because I finished all my savers in the attempt to pay PhD fees.

Thank you Sergio, A.K.A. Gigio for visiting me during my permanence in Brixton and in Bromley, you only missed Verona but it has been better for your liver ☺, we had funny time together with Walter in London!!! … If I accomplished this result you will graduate soon as well, you are always been the smart one!! so hurry UP!!

Last but not least a special thanks to ME.
You did it Michele, you fought as you never did before, you wanted and you get it, this is the results of great efforts and determination because honestly this was out you’re your comfort zone.

How can we measure our strength and limits if we don't go over what we perceive as easy? The answer is probably unknown but in my case was step by step, day by day and…. I'm still not realising it.

"Challenges are what make life interesting, and overcoming them is what makes life meaningful"

Joshua J. Marine

ABSTRACT

The prevalence of asthma related condition in athletes is higher than the general population. The use of inhaled β_2-agonists (IBA) by athletes with an asthma related condition is common, however some question whether the therapy has ergogenic action.

The purpose of this thesis was to investigate the ergogenic action of short and long acting IBA on athletic performance.

According to this, the aim of the first study (Chapter 4) was to investigate whether long-term (5 weeks) daily administration of LABAs (12 μg formoterol and 100 μg salmeterol) during a resistance programme aimed to increase strength and power performance. Thirty-eight recreational males and females took part in this program developed in order to increase daily the training load in order to simulate training habits. Although no changes in strength and body mass were observed, running sprint performance was significantly improved in formoterol and salmeterol groups. The outcomes coming from this study suggest a probable enhancement of β_2-adrenoceptors in the systemic concentration following this practice able to speed-up physiological adaptation showed to occur during resistance training.

The second study (Chapter 5) investigated the physiological and performance effects of short acting IBA on 35-mt sprint performed both in "fatigued" and "non-fatigued" state by football players. In this study football players completed 35 m sprints prior to and immediately following a progressive intermittent running shuttle test to exhaustion (Yo-Yo intermittent recovery test). Prior to the trial football players inhaled either a placebo of 1600 μg salbutamol. No sprint enhancement was observed in sprints performed before and after the Yo-Yo intermittent recovery test following IBA administration when compared against placebo.

The third study (Chapter 6) investigated the potential effect of long-acting β_2-agonists inhalation on "non-fatigued" and "fatigued" conditions and was able to show increased 12-s sprint power output (PLA = 831 ± 112 W vs. SAL 915 ± 135 W) after one-hour cycling time-trial designed to induce fatigue in the cyclists. These findings suggest athletes using 100 µg long-acting IBA who engage in prolonged endurance events may become more resistant to fatigue.

In conclusion, it seems that high therapeutic doses of long-acting β_2-agonists may lead to enhanced sprinting performance and offset the onset of fatigue.

Future investigations should focus on the use and dose of inhaled long-acting β_2-agonists in and out competition by athletes with asthma related condition to ensure they are not likely to experience ergogenic action in addition to maintain airway health.

TABLE OF CONTENTS

Declaration	i
Acknowledgements	ii - iv
Abstract	v - vii
List of Figures	xii - xiii
List of Tables	xiv - xv
Abbreviations	xvi - xx
Chapter 1 – INTRODUCTION	**1-8**
Chapter 2 -LITERATURE REVIEW	**9-45**
2.1 Background	10-12
2.2 Asthma and Exercise Induced Bronchoconstriction (EIB)	12-21
2.2.1 Pathophysiology of asthma and EIB	13-14
2.2.2 Diagnosis of asthma	14-15
2.2.3 Assessment to support diagnosis of EIB	15-19
2.2.4 Asthma Treatment & Management	19-21
2.3 Asthma, EIB and the elite athlete	21-22
2.4 β_2-adrenergic receptors	23-25
2.4.1 Short and Long acting β_2-agonists action model	23-25
2.5 β_2-agonists and the WADA Prohibited List of Substances and Methods	26-30
2.6 Potential ergogenic action of β_2-agonists in athletes	30-33
2.6.1 SABA and endurance performance	30-31
2.6.2 LABA and endurance performance	32
2.6.3 Long-term SABAs and LABAs effect on endurance performance	32-33
2.7 The effect of β_2-agonists administration on body composition	34-37
2.7.1 Anabolic effect induced by SABAs administration	34-35
2.7.2 Anabolic effect induced by LABAs administration	36
2.7.3 Anabolic mechanism induced by SABAs and LABAs	36-37
2.8 Strength and Power performance induced by β_2-agonists administration	37-38
2.9 Sprint performance induced by IBAs	39-41

2.9.1 Sprint performance induced by SABAs 39-40
2.9.2 Sprint performance induced by LABAs 40-41

2.10 Overall Summary 41-43

2.11 AIM of the Thesis 43-44

2.12 HYPHOTHESIS of the Thesis 44-45

Chapter 3 – General Methods **46-52**

3.1 "Spirometry test" 47-49
3.2 "Eucapnic Voluntary Hyperpnoea Challenge" 49-52
3.3 "Peak Oxygen Consumption (VO2peak)" 52

Chapter 4 – "Improved Sprint Performance With Inhaled Long-Acting β_2-agonists Combined With Resistance Training" **53-68**

4.1 Background 54-56

4.2 Methods 56-61
4.2.1 Study Participants 56
4.2.2 Broncho-provocation Challenge 57
4.2.3 Treatment Groups 57
4.2.4 Assessments 57
4.2.5 30 Meters Sprint 58
4.2.6 Isokinetic Dynamometry 58
4.2.7 Maximal One Repetition Bench Press and incline Leg Press 58
4.2.8 Power – Counter Movement Vertical Jump (CMJ) 59
4.2.9 Body Composition 59
4.2.10 Recovery, Sleep and Mood Questionnaires 59
4.2.11 Strength and Power Training Program 60
4.2.12 Statistical Analysis 60-61

4.3 Results 62-65
4.3.1 Baseline Groups Comparison 62
4.3.2 30 Meters Sprint 62
4.3.3 Strength and Power Assessments 63
4.3.4 Anthropometric measures 64
4.3.5 Recovery, Sleep and Mood Questionnaires 64

4.4 Discussion 65-67
4.4.1 Limitations 67
4.4.2 Conclusions 68

Chapter 5 – "The Effect of 1600 µg Inhaled Salbutamol Administration On 30-Meters Sprint Performance Pre and Post Yo-Yo Intermittent Running Challenge In Football Players" 69-84

5.1 Background 70

5.2 Methods 71-75
5.2.1 Study participants 71
5.2.2 Experimental protocol 72
5.2.3 Sprint test 73
5.2.4 Yo-Yo test (Yo-Yo IRT) 74
5.2.5 Motivation and Mood questionnaires 74-75
5.2.6 Statistical Analysis 75

5.3 Results 76-80
5.3.1 Participants characteristics 76
5.3.2 30 m Sprint Pre and Post Yo-Yo IRT 76-78
5.3.3 Yo-Yo Intermittent Recovery Test 79
5.3.4 Mood and Motivation questionnaire 80

5.4 Discussion 81-84
5.4.1 Conclusion 84

CHAPTER 6 – "Acute Administration of Salmeterol Improves Sprint Performance Following Variable Cycling Trial" 85-99

6.1 Background 86-87

6.2 Methods 87-91
6.2.1 Study participants 87
6.2.2 Experimental protocol 87-88
6.2.3 Broncho-provocation Challenge 88
6.2.4 VO2Max Test 88

6.2.5 Sprint Familiarisation 88
6.2.6 “Non-fatigued” Sprint test 88-89
6.2.7 Variable Cycling Trial 89
6.2.8 “Fatigued” Sprint test 89-90
6.2.9 Motivation Questionnaire 90
6.2.10 Statistical Analysis 90-91

6.3 Results 91-94
6.3.1 Participants Characteristics 91
6.3.2 Motivation Questionnaire 91
6.3.3 Sprint Power Analysis 91-92
6.3.4 Sprint RMS Analysis 92-93
6.3.5 Variable Cycling Trial Perceptual and Physiological Analysis 93-94

6.4 Discussion 95-98
6.4.1 Technical Consideration and Study Limitations 99
6.4.2 Conclusion 99

Chapter 7 – General Discussion **100-116**

References 118 - 139

Appendices **140 - 152**

Ethical approvals 141 - 148
Studies Published 149 - 151

LIST OF FIGURES

CHAPTER 2 - LITERATURE REVIEW

Figure 2.1 "ANATOMY OF AIRWAY DURING ASTHMA SYMTOMS" pag 12

CHAPTER 3 - General Methods

Figure 3.1 "MAXIMAL FLOW-VOLUME LOOP ACCEPTANCE CRITERIA" pag 49
Figure 3.2 "EQUIPMENT USED TO PERFORM EVH TEST" pag 51

CHAPTER 4 – "Improved Sprint Performance With Inhaled Long-Acting β_2-agonists Combined With Resistance Training"

Figure 4.1 "30 METERS SPRINT IN SAL, FOR AND PLA" pag 62

CHAPTER 5 – "The Effect of 1600 µg Inhaled Salbutamol Administration on 30-Meters Sprint Performance Pre and Post Yo-Yo Intermittent Running Challenge In Football Players"

Figure 5.1A "Best Sprint: at pre and post Yo-Yo IRT in SAL and PLA" pag 77
Figure 5.1B "Mean Sprint: at pre and post Yo-Yo IRT in SAL and PLA" pag 77
Figure 5.1C "Maximal Speed at pre and post Yo-Yo IRT in SAL and PLA" pag 78
Figure 5.2 "Lactate values collected after sprint pre Yo-Yo IRT and after sprint post Yo-Yo IRT in SAL and PLA" pag 78
Figure 5.3 "Distance covered by SAL and PLA during the Yo-Yo IRT" pag 79
Figure 5.4A "Heart rate response at the end of each Yo-Yo IRT level completed by the participants" pag 79
Figure 5.4B "RPE response at the end of each Yo-Yo IRT level completed by the participants" pag 80

CHAPTER 6 – "Acute Administration of Salmeterol Improves Sprint Performance Following Variable Cycling Trial"

Figure 6.1 "Effect of treatment on Peak Power Output (A) and Mean Power Output (B)" pag 92

Figure 6.2 "Effect of treatment on physiological and psychological responses during Variable Cycling Trial pag 93

Figure 6.3 "Effect of salmeterol on blood lactate after 12-s sprint and during Variable Cycling Trial in SAL and PLA" pag 94

LIST OF TABLES

CHAPTER 2 - LITERATURE REVIEW

Table 2.1 "DIRECT AND INDIRECT STIMULI " pag 19

Table 2.2 "ASTHMA THERAPIES" pag 20-21

Table 2.3 "PREVALENCE OF EIB IN ELITE BRITISH ATHLETES" pag 22

Table 2.4 "THE HISTORY OF THE β_2-AGONISTS AND THEIR GUIDELINES FOR USE IN THE ATHLETIC POPULATION" pag 28-30

CHAPTER 4 – "Improved Sprint Performance With Inhaled Long-Acting β_2-agonists Combined With Resistance Training"

Table 4.1 "30 METERS SPRINT, POWER & STRENGTH PERFORMANCE IN SAL, FOR & PLA" pag 63

Table 4.2 "SKINFOLDS & BODY MASS in SAL, FOR AND PLA" pag 64

Table 4.3 "REST Q RECOVERY & STRESS INDEX IN SAL, FOR AND PLA" pag 65

CHAPTER 5 – "The Effect of 1600 µg Inhaled Salbutamol Administration on 30-Meters Sprint Performance Pre and Post Yo-Yo Intermittent Running Challenge In Football Players"

Table 5.1 "Descriptive features of participants (n=13)" pag 72

Table 5.2 "Vigour at pre and post-test and Motivation at pre- test in SAL and PLA" pag 81

ABBREVIATIONS

AB	Abdominal
ANOVA	Analysis of variance
ARTP	Association for Respiratory Technology and Physiology
ASL	Airway surface liquid
AAF	Adverse analytical finding
AC	Adenylate cyclase
AHR	Airway hyper-responsiveness
ATP	Adenosine tri-phosphat
ATS	American Thoracic Society
B	B-lymphocyte
BbB	Breath-by-breath
BF	Breathing frequency
BHT	Breath holding time
BPD	Breathing pattern disorder
BTS	British Thoracic Society
°C	Celsius degree
Ca2+	Calcium
cAMP	Cyclic adenosine monophosphate
cm	Centimetre
CMJ	Counter movement jump
CNS	Central nervous system
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease

DB	Dysfunctional breathing
DEXA	dual-energy x-ray absorptiometry
DoU	Declaration of use
EFL	Expiratory flow limitation
EIA	Exercise-induced asthma
EIB	Exercise-induced bronchoconstriction
EIIS	Exercise-induced inspiratory symptoms
EMG	Electromyography
EVH	Eucapnic voluntary hyperpnoea
EVH+ve	Eucapnic voluntary hyperpnoea positive
EVH-ve	Eucapnic voluntary hyperpnoea negative
ERS	European Respiratory Society
FeNO	Fraction of exhaled nitric oxide
FET	Forced expiratory time
FEV_1	Forced expiratory volume in one second
FEV_1/FVC	FEV_1: FVC ratio
FI	Fatigue Index
FOR	Formoterol
FVC	Forced vital capacity
GB	Great British
Gi	G-protein i
GPCRs	G-protein coupled receptors
HR	Heart rate
HR_{peak}	Peak heart rate
HR_{max}	Maximum heart rate

HVS	Hyperventilation syndrome
ICS	Inhaled corticosteroid
IgE	Immunoglobulin E
IOC	International Olympic Committee
IOC-MC	InternationalOlympicCommittee –Medical Commission
IT	Italy
kg	Kilogram
Km	Kilometres
L/min	Litre per minute
La	Lactate
LABA	Long acting beta agonist
LT	leukotriene
m	Metre
Mc	mast Cell
mg	milligrams
ml	millilitres
MLCK	myosin light-chain kinase
mm	millimetres
M	macrophage
min	Minutes
mg/l	Milligram per litre
MIP	Maximal inspiratory pressure
MEP	Maximal expiratory pressure
ml/min	Millilitres per minute
ml/kg/min	Millilitres per kilogram of body weight per minute

mmol/L	Millimoles per litre
MPO	Mean Power Output
MVV	Maximal voluntary ventilation
n	Nano
N	Number
NA	Noradrenaline
N_2	Nitrogen
O_2	Oxygen
p	Significance level
PAP	Peak aerobic power
PEF	Peak expiratory flow
PKA	Protein kinase A
PLA	Placebo
PPO	Peak power output
r	Reliability coefficient
RER	Respiratory exchange ratio
RIP	Repetitions
RM	Repetition maximum
RMS	Root mean Square
RPE	Perceived exertion
RPE_L	Leg perceived exertion
RPM	Revolutions per minute
RSA	Repeated Sprint Ability
RyR	Ryanodine receptor
s	Seconds

SABA	Short acting beta agonist
SAL	Salmeterol
SBM	Subepithelial basement membrane
SD	Standard deviation
SE	Standard error
SEM	Mean standard error
sEMG	Surface Electromyography
SPSS	Statistical package for social sciences
SR	Sarcoplasmic reticulum
T	T-lymphoycte
TT	Time trial
TTE	Time to exhaustion
TUE	Therapeutic use exemption
TER	Terbutaline
UK	United Kingdom
USA	United States of America
$\dot{V}_E$	Minute ventilation
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2\ peak}$	Peak oxygen consumption
vs.	Versus
VT	Tidal volume
W	Watts
WADA	World Anti-Doping Agency
WR	Work rate

YO-YO IRT	Yo-Yo intermittent recovery test
yr	Year (s)
β	Beta
β_2	Beta-2
β2R	β2-Receptor
ε	Degrees of freedom
μ	Micro
μg	Micrograms
μl	Microlitre
μm	Micrometre
=	Equal
Δ	Change

Chapter 1. Introduction

Asthma is an obstructive airway disease that is characterised by a reversible bronchoconstriction driven by lower airway inflammation. The airway inflammation and bronchoconstriction related to asthma is only activated in the presence of a trigger (e.g. dust, pollen pollution). Exercise is a trigger for up to 90% of individuals with asthma (Mc Fadden & Gilbert, 1994). When exercise is the only trigger the condition is termed exercise-induced bronchoconstriction (EIB) (Mc Fadden & Gilbert, 1994).

The prevalence of EIB among elite athletes is relatively high and can range between 10–70% (Levai et al., 2016; Dickinson et al., 2005). The prevalence is EIB between sports is variable and is largely dependent on the environment in which the sport is performed, the ventilatory demands of the sport and the method used to record the diagnosis (Weiler et al., 2010). Typically sports with the highest prevalence of EIB are endurance-based sports that require athletes to sustain a high minute ventilation that take place in challenging environments responsible of air-way dehydration. This temporary modification in the airways brings to water lost from the airway surface that terminates with bronchial smooth muscle contraction and consequence EIB.

In most cases asthma and EIB can be managed appropriately using inhaled therapy in the form of corticosteroids and β_2-agonists. The aim of the therapy is to prevent the occurrence of asthma/EIB and reduce the severity. Inhaled corticosteroid therapy is seen as the therapy to prevent the development of airway inflammation and bronchoconstriction, whereas β_2-agonists provide immediate relief from bronchoconstriction by relaxing the constricted airway smooth muscle almost instantly following inhalation. Despite guidance to suggest athlete therapy should include both inhaled corticosteroid and β_2-agonists therapy there is evidence of an increase in the number of athletes only using inhaled β_2-agonists to control asthma/EIB (Fitch, 2016).

Once an athlete with asthma/EIB is adequately managed they should be able to train and compete without compromising their airway health and at no disadvantage to non-asthmatic athletes. To the extent that athletes with documented asthma/EIB tend to out-perform non-asthmatic athletes at the Olympic Games (Fitch, 2012). However, these findings have led some to suggest the therapy used by athletes with asthma/EIB provides them with an ergogenic advantage. A possible ergogenic effect provoked by these drugs, shown by increased glycolytic and glycogenolysis activity and a faster ATP resynthesis, has become a research topic since Torino 2006 Olympic Winter Games approved for IBA where 131 out of 193 athletes were recorded without previous asthma history (Fitch, 2006). 14.4% of them won a medal. Two years later, during Summer Olympic Games in Beijing (2008) this percentage became even larger with a fifth of the swimmers and cyclists (19.3% and 17.3%) were asthmatic; however, of these one out of three (32.9 % and 28.9%) was a medal winner.

Instinctively, the potential ergogenic effect produced by IBAs administration could be presumable as supported by the data presented above; however, results collected in literature seem not to support this theory especially when aerobic performance is evaluated (Pluim et al., 2011).

In 2001, the International Olympic Committee (IOC) established the necessity for athletes to present verification of current asthma, exercise induced asthma (EIA), exercise induced bronchospasm (EIB) or airway hyper-responsiveness (AHR) through the Therapeutic Use Exception (TUE) certificate process. More specifically, to be granted a TUE the user must prove that the drug is necessary to treat an acute or chronic symptom, the drug is not going to produce any performance benefit and there is no permitted therapeutic alternative to the use of the prohibited drug.

Therefore, the requirement of demonstrable evidence through the TUE process improves the quality of attention to athletes. WADA's List of Prohibited Substances and Methods (reviewed

annually) declares all β_2-agonists are prohibited in and out competition except inhaled salbutamol, salmeterol and formoterol. Oral administration of any β_2-agonists is banned.

Salbutamol represents the most used short acting IBA (SABA) used by asthmatic people. As suggested by WADA (2019), therapeutic dose of inhaled salbutamol ranges from 200-400 µg, athletes are permitted to inhale up to 800 µg in any 12 hours period and 1600 µg in a 24-hour period (WADA Prohibited List, 2019).

Studies present in literature seem not to show an ergogenic effect provoked by inhaled salbutamol administration (Pluim et al., 2011). More specifically, when endurance performance was investigated in therapeutic dose (Molphy et al., 2018; Goubault et al., 2001; Sporer et al., 2008; Sandsund et al., 1998; Norris et al., 1996; Elers et al., 2010) no ergogenic effect was noted. Similar results have been observed after supra-therapeutic regime (Stewart et al., 2002; Dickinson et al., 2014; Koch et al., 2015a, 2015b; McKenzie et al., 1983; Goubault et al., 2001; Sporer et al., 2008). A study by Elers et al. (2012a) suggests that inhaling an extremely supra-therapeutic salbutamol dose of 4000 µg results in no improvement in cycling time to exhaustion or oxygen kinetics. This effect has been observed also at lower doses of salbutamol (800 µg) that was able to reduce time to exhaustion performance (Carlsen et al., 1997). Notably, despite no mechanisms were able to explain the results obtained by Carlsen (1997) a possible mechanism could be the premature fatigue experienced under salbutamol condition expressed by higher muscle perturbation compared to placebo condition.

In contrast, after 800 µg salbutamol inhalation improved cycling time-trial performance was recorded by Van Baak et al., (2004). Notably, this change in performance was not explained by any measures taken such as β_2-agonists concentration in plasma, glycerol and potassium levels or ventilatory levels collected at rest and after exercise. Moreover, the big inter-variability between the two tests performed between 4 and 14 days could have been affected

by changes in subject fitness level. Similarly, work by Kalsen et al., (2013) has highlighted performance improvement following combined short (1600 μg salbutamol) and long-acting β_2-agonists inhalation (200 μg salmeterol; 36 μg formoterol). In addition to this, the amount of drug inhaled was maximal in both short and long-acting β_2-agonists with ergogenic effects possibly explained by the high systemic level of drug reached after these doses.

Contrasting evidence has been shown from studies that looked at the impact of high-doses of inhaled short acting β_2-agonists on sprint and power performance

No changes in performance were observed after therapeutic and supra-therapeutic salbutamol inhalation (Stewart et al., 2002; Norris et al., 1996; Morton et al., 1993; Meeuwisse et al., 1992). Conversely, some studies reported improvements in peak power anaerobic performance after low albuterol 180 μg dose (Signorile et al., 1992), after supra-therapeutic 800 μg dose (Decorte et al., 2013) and 1600 μg dose (Kalsen et al., 2013;).

Mechanisms potentially responsible for improved performance following inhalation of short and/or long acting β_2-agonists could be attributed to higher systemic concentration reached after supra-therapeutic dose regime (e.g. > 400 μg inhaled salbutamol). In detail, skeletal muscle hypertrophy has been associated to increased power production during sprint exercise (Kalsen et al., 2013) with slow-twitch fibers presenting shorter half-relaxation time after β_2-agonists administration (Crivelli et al., 2011). The variation in half-relaxation time could be attributed to an increased rate of Ca^{2+} release and re-uptake within the skeletal muscles that has been indicated to increase force by an increase in intracellular Ca^{2+} transient amplitude (Cairns & Dulhunty, 1994). This effect may be produced by increased ryanodine receptor channel release of Ca^{2+} and sarcoplasmic reticulum Ca^{2+}-ATPase reuptake of Ca^{2+} (Ha et al., 1999).

Another mechanism observed after β_2-agonists administration is the increased activity of the Na+/K+-ATPase leading to enhanced re-uptake of K^+ into the muscle cell, potentially

preventing loss of membrane excitability and development of fatigue (Sejersted & Sjøgaard, 2000). Studies adopting oral salbutamol administration found amplified glycogenolysis and glycolysis in skeletal muscles, which is also supported by elevated venous lactate (Van Baak et al., 2004; Collomp et al., 2005). We could speculate that supra-therapeutic dose of β_2-agonists could mimic the effect brought by different route of administration considered as an AAF (Elers et al., 2012).

Other IBAs such as terbutaline have been largely investigated since the early 80', it possesses analogous pharmacological actions to salbutamol in 2-4 mg recommended dosage (Sweetman, 2011). The ergogenic effect showed by supra-therapeutic inhaled terbutaline administration has been recognised in sprint (Hostrup et al., 2014) and endurance performance (Kalsen et al., 2014), but due to the difficulty in distinguishing between oral and inhaled administration this substance is presently on the list of prohibited substances redacted by WADA (2019) and banned for use by athletes. Athletes requesting to use terbutaline, via a TUE, can only be approved as long as no other permitted β_2-agonists could represent an alternative therapeutic cure with a special treatment for those athletes used to inhaled terbutaline for a long time that kept this practice unchanged.

Asthmatic athletes are responsible for the administration of medications and must follow the Anti-Doping rules as laid out by the World-Anti-Doping-Agency. They can be tested at any time of day on any day of the year during competitions as well during training and are obliged to communicate to authorities their domicile to be always available to test. Despite severe penalties (e.g. disqualified from competition for two years), recent sport history reports a few cases of athletes tested positive to β_2-agonists drug. Former Italian cyclist Alessandro Petacchi, during a stage of 2017 "Giro di Italia" was tested positive to salbutamol after being found with

1320 ng.ml-1 in urine. He was disqualified for one year from all competitions. In 2014, Diego Ulissi reported almost double the urine cut-off limits (1900 ng.ml-1) and stopped from professional cycling season for 9 months with the accusation of negligence. Four Tour de France Winner Chris Froome, during 2017 “la Vuelta” was 19% over the decision limit (1040 ng/ml) in urine sample collected. He was not suspended from competition due to the nature of the substance for which he was under investigation and because salbutamol was not considered a “specified substance” (WADA, 2017).

The investigation on the British cyclist continued for several months until he was judged not guilty based on scientific paper results that showed a very large range of salbutamol concentrations, with a significant portion of virtual subjects (15.4%) exceeding the WADA threshold limit of 1000 ng ml-1 at 1 h post-dose (Heuberger et al., 2018). In addition, loss of body mass experienced by endurance athletes during long-stage (up to 5% of bodyweight) can lead to individuals presenting a urinary salbutamol concentration above 1200 ng.ml-1 (Haase et al., 2016); thus, dehydration can cover an important role in anti-doping analysis and significantly affect the concentration of salbutamol in urine samples.

Most of the studies investigating inhaled β_2-agonists have primarily focused on acute administration of therapeutic and supra-therapeutic dose. According to this, Dickinson et al., (2014) after 6 weeks daily inhaled administration of supra-therapeutic salbutamol dosage (1600 μg) did not result in significant improvements in endurance, strength and power performances. However, due to difference in pharmacokinetic profile long-term daily administration of long-acting β_2-agonists at the maximum WADA permitted dose should be investigated deeply.

The current World Anti-Doping Agency (2020) list of prohibited substances and methods permits athletes to use inhaled therapeutic doses of formoterol (54 μg per day) and salmeterol (200 μg per day) without the requirement of objective evidence of an asthma-related condition.

Chapter 2. Literature Review

2.1 Background

Breathing signifies both the start and the end of our lives, and it is the most fundamental of physiological process. This process of respiratory ventilation occurs automatically but it is possible to come under partial conscious control (e.g. during times of stress).

At rest, the average adults takes 10 to 15 breaths per minute, with a tidal volume of approximately 0.5 L, producing a minute ventilation (V_E) between 5 to 7.5 L.min^{-1} (Leonard et al., 2004). V_E depends on body size and metabolic rate (Gillooly et al., 2001). During high-intensity exercise the rate and depth of breathing are increased, which requires the breathing muscles to contract more forcefully and quickly in order to raise tidal volume and expiratory airflow rate. Breathing frequency during high intensity exercise rises to around 40 to 50 breaths per minute; in a physically active young male, tidal volume rises approximately 3 to 4 L (minute ventilation is 120 to 160 L.min^{-1}; Dempsey, 2019). Failure to do so, results in changes that lead to breathless, increased perception of effort, earlier on set of fatigue and earlier end point of exercise (when completing time trial to exhaustion or ramped incremental exercise efforts). At all intensities of exercise, the majority of the work of breathing is undertaken by the inspiratory muscles; expiration is always assisted to some extend by elastic energy that is stored in the expanded lungs and rib cage from the preceding inhalation.

In susceptible individuals, it is possible that exercise can trigger bronchoconstriction. This is brought about by a switch mouth breathing and increased V_E during exercise exposing the lower airways to “unconditioned” relatively dry, cold and unfiltered air. The action of the lower airways warming and humidifying the unconditioned air can lead to dehydration of airway

epithelial cells, which leads to water movement from airway cells to restore osmolarity balance to airway epithelial cells. This leads to cell shrinkage of airway inflammatory cells, release of airway inflammatory mediators and airway smooth contraction (Andersoon & Kippelen, 2008). The bronchoconstriction typically occurs following a bout of continuous exercise peaking five to ten minutes post exercise. The above process is known as exercise induced bronchoconstriction (EIB) and is either reversible either spontaneously or through the use of asthma therapy such as inhaled β_2-agonists (e.g. salbutamol).

Asthma related conditions, such as EIB, may limit an athletes ability to appropriately ventilate their airways and therefore develop a respiratory limitation to exercise performance (Dempsey et al., 2008). However, when athletes appropriately use asthma inhaler therapy to protect against asthma and EIB, they experience an improvement in airway health, reduction in asthma/EIB severity. Therefore, appropriate inhaler therapy enables athletes to perform on a level playing field with their non-asthmatic counter parts (Jackson et al., 2018).

Well controlled Asthma/EIB should not be seen as a limitation to athletic performance, in fact, Fitch (2012) reported that athletes with asthma who compete at Olympic Games out-perform their non-asthmatic counterparts and are more successful at winning Olympic medals. This report is good news for athletes with asthma who should feel they can protect their airway health whilst competing on a level playing field against non-asthmatic athletes. However, reports such as those by Fitch (2012), have led to some questioning whether the therapy athletes use to attenuate asthma/EIB has an ergogenic action providing athletes with asthma an advantage over their non-asthmatic counterparts.

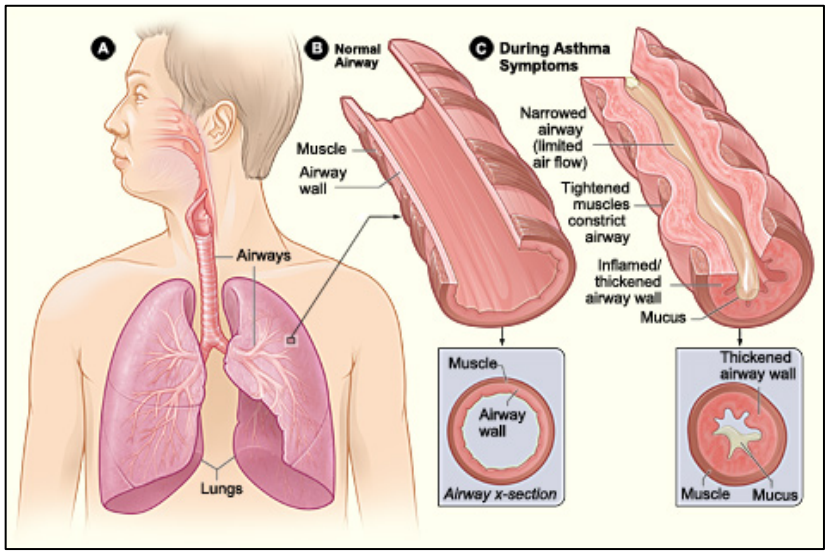
In this literature review I will cover the action of how asthma and EIB develops and how athletes may use various therapy to attenuate their asthma/EIB. I will then discuss how asthma

therapy has the potential to provide an ergogenic action. I will discuss the World Anti-Doping Agency (WADA) List of Prohibited Substances (WADA, 2020) and debate whether the code allows athletes with asthma to compete, protecting their airway health, and prevents unscrupulous athletes abusing asthma therapy for performance gain.

2.2 Asthma and Exercise Induced Bronchoconstriction (EIB)

Asthma is a reversible obstructive airway disease that, starting from normal airway status (FIGURE 2.1/B), is triggered when encounters a specific asthma trigger that leads to an inflammation process causing the bronchi to swell and airway smooth muscle to constrict creating airway resistance and reduction in expiratory airflow (FIGURE 2.1/C). The airway obstruction is reversible either spontaneously or through inhalation of bronchodilator therapy (e.g. inhaled β_2-agonist).

FIGURE 2.1 ANATOMY OF AIRWAY DURING ASTHMA SYMTOMS



Retrieved from https://www.nhlbi.nih.gov/health/health topics/topics/asthma/

The severity of airway obstruction can differ between individual and only occurs in the presence of asthma triggers such as allergies, exposure to air pollution (cold and dries air), medications, emotional anxiety and stress (Reche et al., 2020). Many patients with asthma develop asthma symptoms when exercising, this is called exercise-induced bronchoconstriction (EIB). However, it possible that people only experience EIB in the absence of asthma, which is likely due to the elevated ventilatory volume of air inhaled and exhaled during their sport discipline (Molis & Molis, 2010).

Asthma is one of the most prevalent respiratory diseases affecting more than 300 million people worldwide with a trend to increase the number of patients affected over the next 15–20 years (Masoli et al., 2004). EIB is an asthma related condition and it is estimated 80% - 90% of people with asthma experience EIB (Parsons & Mastronarde, 2005). The prevalence of EIB in athletic populations is greater than the prevalence of asthma in the UK general population (9-12%; British Lung Foundation, asthma, Uk). The prevalence of asthma in the Great British Summer Olympic Team has been reported to be 21% (Dickinson et al., 2005) but the prevalence can range between sports with athletes in sports such as swimming (68%) being more susceptible to EIB than others (e.g. boxing 8%; Levai et al., 2016).

2.2.1 Pathophysiology of asthma and EIB

The phenotypes of asthma and triggers (e.g. exercise, pollen, pollution) that initiate an inflammatory process differ between individuals (Froidure et al., 2016). However, once the trigger has activated the mechanism towards bronchoconstriction the process is similar in both asthma and EIB. Once the process has been triggered activation of mast cells, infiltration of eosinophils and infiltration of T helper 2 (T_H2) lymphocytes into the airway epithelium is

initiated (Barnes, 2008). When these airway inflammatory cells (e.g. mast cells) are stimulated they release inflammatory mediators into the extra-cellular matrix including histamine, leukotriene (LT) D_4 and prostaglandin (PG) D_2 ending in a bronchoconstriction effect.

In the case of individuals susceptible to EIB, sustained high minute ventilation during exercise leads to an increased exposure of the lower airway to relatively cold, dry air, which is required to be warmed and humidified by the lower airways. This results in dehydration of the airway surface liquid (ASL; Reinhard-Groebli & Nicod, 2017). More specifically the airway dehydration leads to (1) an increase in intracellular osmolarity, (2) initial water movement from the surrounding cells toward the airway lumen, (3) the shrinkage of sub-epithelial cells and (4) the release of broncho-constrictive inflammatory mediators, including histamines, cytokines and leukotrienes; this causes a cascade of inflammatory process leading to airway smooth muscle constriction (Anderson & Kippelen, 2012; Hallstrand et al., 2013).

2.2.2 Diagnosis of asthma

Due to the various phenotypes of asthma there is not one gold standard test to diagnose the condition. Diagnosis of asthma is made by a combination of past history, physical examination and objective airway assessments (Quirt et al., 2018).

The major feature of asthma is airway smooth muscle contraction, causing in obstructed airways during expiration phases and reduced forced expiratory volume in 1 second (FEV_1) due to reduced flow rate through the airways. The most adopted test for lung function is spirometry (Dwyer, 2012), where measurements of FEV_1, forced vital capacity (FVC) and their ratio (FEV1/FVC) are measured. If the FVC and the FEV_1 are within 80% of predicted values, the results are considered normal (NICE, 2017). The normal value for the FEV_1/FVC ratio is

usually between 80-85%, with a ratio < 70% (and 65% in persons older than age 65) considered to be characteristic of obstructive airway disease (NICE, 2017). Individuals with an abnormal resting lung function a bronchodilation challenge is an appropriate assessment of airway reversibility. A bronchodilation challenge involves measurement of baseline spirometry, inhalation of 200 mcg salbutamol, then spirometry measured 15 minutes post inhalation. If FEV_1 improved by 12% or more from baseline this was considered significant reversibility and used to support the diagnosis of asthma. Notably, when bronchoconstriction occurs there is a noticeable fall in FEV_1, a greater time to FVC, and an FVC value that remains similar, bringing to an imbalance between FEV_1/FVC from resting values (Hansen et al., 2007).

2.2.3 Assessment to support diagnosis of EIB

The American Thoracic Society states that EIB diagnosis is made based upon changes in lung function post-exercise and not based upon the presence of symptoms (Parsons et al., 2013). There are two recognised methods of challenge testing dependent upon whether the bronchial smooth muscle is elicited *directly* or *indirectly* (TABLE 2.1).

Direct challenge tests such as methacholine are sensitive to detect asthma but are not entirely specific for EIB as subjects with other airway disorders may have direct AHR. However, a negative methacholine challenge test in a currently symptomatic patient is highly suggestive of an alternative diagnosis (Coates et al., 2017).

Indirect airway challenges are the most appropriate to use and support a diagnosis of EIB. Indirect airway challenges are considered to be more appropriate as they indirectly trigger airway smooth muscle contraction by initiating the inflammatory pathways described above. Indirect airway challenge can either be lab or field based depending on the challenge used.

Indirect airway challenge includes laboratory-based exercise challenges, sport-specific exercise challenge, eucapnic voluntary hyperpnoea (EVH) and dry powder mannitol.

Direct airway challenges, because of the high sensitivity, work best to exclude asthma; a positive test result is consistent with but not diagnostic of asthma related conditions. On the contrary, indirect challenges are more specific for asthma but are insensitive, particularly for mild and/or well-controlled asthma. The lower sensitivity may relate to the fact that many indirect challenges (e.g. exercise, eucapnic voluntary hyperpnea, adenosine monophosphate) are dose limited (e.g. the dose of stimulus cannot be increased above a level based on physiology or solubility; Cockroft et al., 2009). In support of this, indirect challenges would be suggested for monitoring of asthma control and used successively to help diagnose occupational asthma.

Exercise Challenges

The exercise challenge involves a participant completing continuous exercise lasting between 8-10 minutes in total. The exercise should incorporate progressive increases in exercise intensity over about 2 to 4 minutes to achieve a high level of ventilation (for athletes $\dot{V}_E$ must be > 25 times the FEV_1), which then sustain for a further 4 to 6 minutes. The target HR during the final 4 to 6 minutes of the exercise should be > 80% HRmax for non-trained individuals and >90% HR max for trained individuals (Eggleston et al., 1979). Measurements of FEV_1 are made prior to exercise and then in duplicate at 3, 5, 7 10, 15 minutes post exercise, and the higher of the two values is recorded at each time point. The criterion to diagnose EIB using this assessment method is a post exercise fall in FEV_1 of ≥ 10% from baseline (Parson et al., 2013).

Due to the release of bronchodilation agents in the body (e.g. lysophosphatidic acid, phospholipase A2 and leukotriene C4), exercise that starts with a very low workload or uses a continuous increase in intensity for assessing maximum capacity may lead to false negative results (Anderson, 2011). Although a sports-specific exercise field test may simulate the exercise environment and demonstrate a reduction in airflow, due to its poor sensitivity for diagnosis and difficulty to perform reliably, this method of assessment may not be appropriate as the initial investigation for EIB (Dryden et al., 2010).

Thus, conducting field based exercises challenge requires to monitoring the environmental conditions (e.g. temperature, humidity) and having appropriate equipment to assess lung function (e.g. spirometer, heart rate monitor). In contrast, a laboratory-based exercise challenge is limited due to the controlled environment and less sport specific exercise. Therefore, the use of both forms exercise challenge may not be as sensitive to detect EIB in athletes. Hence, surrogate indirect airway challenges maybe more appropriate to assist in the diagnosis of EIB.

Eucapnic Voluntary Hyperpnoea Challenges

Eucapnic voluntary hyperpnoea (EVH) challenge is a surrogate for exercise. The EVH challenge requires a participant to sustain a high minute ventilation (85% of maximum voluntary ventilation) for six minutes inhaling a relatively dry (2% relative humidity) gas containing 21% O_2, 5% CO_2 and 74% nitrogen (N_2). After the test lung function measurements are made at the same time interval as for exercise and a sustained $\geq$ 10% fall in FEV_1 is considered a positive test (Anderson et al., 2001).

EVH is more sensitive to detect the EIB compared with exercise. This is thought to be due to the high minute ventilation and dry air (2% humidity) that the athlete is exposed to during the

assessment (Rundell et al., 2004; Dickinson et al., 2005) and methocholine (Holzer et al., 2003).

Mannitol

Dry powder mannitol inhalation increases the osmolarity of the airway surface and causes release of the same inflammatory mediators (Anderson & Kippelen, 2012). Mannitol has similar sensitivity to EVH when performed in summer athletes but poor sensitivity in detecting EIB in winter athletes (Holzer et al., 2003). The commercially available test kit contains pre-packed capsules containing 5, 10, 20 or 40 mg of mannitol and an inhaler device. FEV_1 is measured in duplicate 60 seconds after each 40 mg dose. A 15% fall in FEV_1 at ≤ 635 mg is considered a positive test result (Anderson & Kippelen, 2012).

Hyper-responsiveness to mannitol has been shown to have high specificity for a clinical diagnosis of asthma (95%), with a sensitivity of approximately 60% (Sverrild et al., 2009; 2010). This sensitivity increases to 89% when the use of inhaled corticosteroids (ICS) is taken into account (Brannan et al., 2005). This finding means that like exercise, a positive mannitol test is consistent with the diagnosis of asthma, but a negative test does not exclude asthma (Anderson & Kippelen, 2012).

In summary, an accurate diagnosis of asthma, should include a full symptoms history, physical examination and objective measurements of lung function (e.g. spirometry). The objective evidence of lung function should include evidence of airway reversibility if baseline lung function appears abnormal or evidence of bronchoconstriction following either a direct or indirect airway challenge (TABLE 2.1; NICE, 2017).

TABLE 2.1: DIRECT AND INDIRECT STIMULI

Direct Stimuli	Cholinergic agonist (methacoline, etc) Histamine
Indirect Stimuli	*Exercise* *Anisotonic spray (mannitol, distilled water)* *Eucapnic Voluntary Hyperventilation (EVH)*

2.2.4 Asthma Treatment & Management

While there is no cure for asthma, symptoms can be controlled with effective asthma therapy and management. A number of therapeutic strategies have been adopted in an attempt to control the symptoms associated with asthma, including β_2-agonists, inhaled corticosteroids (ICS), and other anti-inflammatory agents (TABLE 2.2).

The degree of airway hyperresponsiveness is aided by the administration of corticosteroids to reduce airway inflammation and the β_2-agonists allow for airway smooth muscle relaxation, therefore bronchodilator therapies and inhaled corticosteroids are the preferred first choice of therapy for symptom relief in asthmatic individuals (McFadden & Gilbert ,1994). Among the β_2-agonists, the individual agents vary in the rapidity of onset and duration of action. Inhaled, short-acting, selective β_2-adrenergic agonists are the mainstay of acute asthma therapy, while long-acting, selective β_2-adrenergic agonists play a role in long-term control of moderate to severe asthma (Cazzola et al., 2013).

TABLE 2.2: ASTHMA THERAPIES

Therapy	**Example of drug**	**Mode of Action**
β_2-Agonists (Short & Long-Acting)	Salbutamol Salmeterol Terbutaline Formoterol Fenoterol Reproterol Pirbuterol Albuterol	• Increase levels of cAMP to relax bronchial smooth muscle through the inhibition of myosin light chain kinase. • Relaxation of smooth muscles • Increase voluntary muscle strength & peak twitch force • Increase lean mass • Increase rates of glycolysis • Increase rates of glycogenolysis
Inhaled & Oral Corticosteroids	Beclomethasone (BDP) Budesonide Flunisolide Fluticasone Mometasone Prednisolone	• Reduction of prostaglandin synthesis (COX-2 inhibition) • Interference with synthesis of interleukin 1 and 2. • Inhibition of the synthesis of other cytokines. • Reduction of airway inflammatory cells.
Leukotriene receptor antagonists	Monteluekast	• Inhibit antigen-induced contraction of bronchial smooth muscle.

Mast cell stabilizers	Methylxanthines, ketotifen, olopatadine, rupatadine, mepolizumab	• Prevents the release of the mediators of type I allergic reactions, such histamine, from sensitized mast cells.
PDE_4 Inhibitors	Diazepam, ibudilast, luteolin, cilomilast, rolipram, piclamilast	• Prevent the hydrolysis of cAMP, leading to bronchodilation and reduced inflammation • Inhibition of cell trafficking

2.3 Asthma, EIB and the elite athlete

Elite athletes are more likely to have asthma related conditions, such as EIB, than the general population. It has been reported in that 21% of the 2004 Great British Summer Olympic Team had an asthma related condition (Dickinson et al., 2005) which is more than double the prevalence of asthma in the UK general population (9%; Asthma UK).

Athletes engaged in endurance-based sports are more susceptible to asthma related conditions such as EIB because they sustain relatively high V_E over a prolonged period of training with significant volume of air entering the lower airways. Therefore, elite athletes who take part in certain sports such as swimming, cross-country skiing and rowing are more likely to experience EIB compare to other disciplines due to multiple physiological aspects such as a increment of ventilatory demand during physical activity and environmental conditions (TABLE 2.3; Parsons et al., 2007). Mountjoy et al., (2015) indicates that about 25% of competitive

swimmers have asthma, and that this problem occurs due to extensive exposure to chlorine that swimmers experience.

The inhalation of β_2-agonists targets β_2-adrenergic receptors present in the body stimulating the sympathetic nervous system (SNS) and increasing momentarily heart rate, energy mobilisation and skeletal muscle blood flow in order to get the body ready for physical performance.

TABLE 2.3: PREVALENCE OF EIB IN ELITE BRITISH ATHLETES

Sport	EIB prevalence
Badminton	9%
Boxing	8%
Judo	13%
Athletics	16%
Cycling	39%
Field Hockey	31%
Rowing	19% - 31%
Swimming	44% - 68%
Football	25% - 29%

2.4 β_2-adrenergic receptors

In the airways of individuals experiencing smooth muscle contraction, activation of β_2-adrenergic receptors leads to relaxation of airway smooth muscle, dilation and opening of the airways. In particular, the combination of decreased intracellular calcium and protein kinase A, provoked by cyclic adenosine monophosphate (cAMP), activates myosin light-chain phosphatase that in addition with to calcium and potassium channel exert smooth muscle bronchodilation (Johnson, 2006). This is why inhaled β_2-agonists therapy plays a significant role in the management of asthma and EIB for most individuals.

In addition to the bronchodilation action, β_2-agonists therapy may be used in a clinical setting to promote skeletal muscle hypertrophy (Westfall, 2006). The effect brought by β_2-agonists administration has been identified with therapeutic applications for muscle wasting conditions such as sarcopenia, cancer cachexia, denervation and neuromuscular disease with the intent to reduce muscle weakness and enhance muscle growth (Lynch & Ryall, 2008). Despite the clear therapeutic application for β_2-agonists use by athletes with asthma related conditions, there is a clear potential for asthma therapy to have an ergogenic action that may tempt unscrupulous athletes to seek the use of β_2-agonists to better sport performance.

2.4.1 Short and Long acting β_2-agonists action model

There are three types of β_2-agonists: short-acting (SABA), long-acting (LABA) and ultra-long-acting (ULABA). The principal difference in the clinical pharmacology of SABA, LABA and ULABA is represented by the duration of action on airway smooth muscle (SABA ~ 4 hours, LABA ~ 12 hours, ULABA ~ 24 hours; Ullman et al., 1990; Cazzola et al., 2005).

The β_2-agonists are similar in structure to adrenaline with a single benzene ring and an amino group side-chain. The LABAs such as formoterol and salmeterol have an additional benzene ring attached to the amino-group, which likely accounts for their longer duration of action (Anderson et al., 1994). The physicochemical nature of the interactions of formoterol or salmeterol with the membrane lipid bilayer that may determine the duration of action of these molecules; moreover, the side-chain affects the lipophilicity of salmeterol that is more lipid soluble than the moderately lipophilic formoterol molecule (Jeppsson et al., 1989).

An important feature possessed only by LABAs is the capacity to assert relaxation of airway smooth muscle; this retention of salmeterol in the tissue is very persistent even after 10 wash-out cycles or numerous hours of continuous superfusion of the tissue with drug-free medium (Ball et al., 1991). The apparent binding affinity of salmeterol for the β_2-adrenoceptor is actually lower than formoterol, and formoterol can be slowly washed from airway smooth muscle in vitro (Löfdahl et al., 1990), whereas the biological activity of salmeterol persists for periods in excess of 10h (Brittain, 1990; Johnson et al., 1993).

Athletes that regularly use SABAs daily may benefit from using LABAs such as formoterol and salmeterol. This will reduce the number of time they use therapy which will improved response to rescue inhaler and extend the bronchodilation effect each time they used the LABAs.

The duration of a β_2-agonists action can be explained by the microkinetic model where plasmalemma lipid bilayer of airway smooth muscle acts as a store for β_2-adrenoceptor agonists with moderate to high lipophilicity. In fact, the balance of evidence currently indicates that the membrane itself is a main determinant of the nature of agonist at the β_2-adrenoceptor for lipophilic mixtures (Anderson et al., 1994). In the case of salbutamol (very low lipid

solubility), the partition equilibrium is powerfully in favour of the extracellular aqueous compartment. As such, the onset of effect is rapid, since the molecule can quickly diffuse to the active site of the receptor, but no persistent relaxation of airway smooth muscle occurs (Katzung, 2004).

In the case of formoterol, (moderately lipophilic), the partition equilibrium allows the molecule to enter the plasmalemma and to be retained. Concurrently, adequate drug is available in the aqueous bio-phase to permit immediate interaction with the active site of the receptor, accounting for the rapid onset of bronchodilation observed clinically. Subsequently, small amounts of formoterol leach out from the plasmalemma and become available for activation of the β_2-adrenoceptor with a persistent relaxation of airway smooth muscle after wash-out (Katzung, 2004).

For highly lipophilic drugs, such as salmeterol, the interaction with the β-adrenoceptor is suggested to be energetically unfavourable because the lack of effect of removing the epithelium on relaxation time precludes retarded penetration through the epithelium alone as a determinant of slow onset (Ullman et al., 1992).

2.5 β_2-agonists and the WADA Prohibited List of Substances and Methods

Athletes are allowed only to administer β_2-agonists salbutamol, formoterol and salmeterol by inhalation. All other routes of administration and forms of BAs are not permitted for use by athletes (WADA, 2020). The current WADA restriction on β_2-agonists use by athletes has been reached over the past four decades. Along with the International Olympic Committee (IOC), WADA have continuously modified their rules regarding the use of β_2-agonists by athletes in and out of competition. In part, this is due to the findings from scientific literature investigating the potential ergogenic action of β_2-agonists via various routes of administration including inhaled, oral and intravenous (Martineau et al., 1992; Collomp et al., 2010; Pluim et al., 2011; Decorte et al., 2008; Larsson et al., 1997; Meeuwisse et al., 1992; Le Panse et al., 2006; Caruso et al., 2005; McKenzie et al., 1983; Van Baak et al., 2000).

In concomitance with the results coming from the scientific literature, increased use of inhaled β_2-agonists has been highlighted in the last decades, more specifically between the 1984 Los Angeles Olympic Games and the 1996 Atlanta Olympic Games with a 212% increase in the use of β_2-agonists by athletes (Fitch, 2006). A further increase by 151% was observed between the Atlanta games and the 2000 Sydney Olympic Games, leading ultimately to the IOC-Medical Commission (IOC-MC) decision to change from the requirement for declared use to the requirement for objective evidence (indirect or direct airway challenge) of bronchoconstriction in 2001, with the health and wellbeing of the athlete being the main influencing factor for the decision (TABLE 2.4). The regulations imposed by the IOC were largely due to the risk to down-regulate adrenoceptors following repeated supra-therapeutic administration of β_2-agonists with the risk to become less effective (Fitch, 2006; 2012; 2019; Lynch et al., 2008). Seven years later, in 2009, the WADA adopted these guidelines in their

Prohibited List of Substances and Methods where the use of inhaled β_2-agonists (IBA; salbutamol, terbutaline, salmeterol, formoterol) required athletes to provide objective evidence of an asthma related condition (e.g. indirect or direct airway challenge) and obtain a therapeutic use exemption (TUE; WADA, 2009). This decision was moderately overturned in 2010 when salbutamol (1600 µg) and salmeterol were the only permitted IBAs via the declaration of use (DoU; WADA, 2010). The percentage of athletes who received a TUE for IBA from the three Winter Olympic games (2002, 2006 and 2010) was around 5.2%, 7.8%, 7.1%, respectively (Fitch, 2012). The 2010 WADA policy to require athletes wishing to use IBAs to undertake a broncho-provocation challenge helped to improve the respiratory care athletes received (Dickinson et al., 2005; Dickinson et al., 2011).

In 2011, WADA changed the status of inhaled salbutamol, salmeterol and formoterol; the inhaled forms of these drugs became permitted within recommended dosages (salbutamol 1600 µg/day; formoterol 54 µg/day; salmeterol without the requirement for a DoU; WADA, 2011). In addition to this, requirement existing urinary thresholds for salbutamol (1000 ng.mL-1) and formoterol (30 ng.mL-1) were delineated with the aim of distinguishing between therapeutic inhaled use and prohibited oral use (Ventura et al., 2000; Elers et al., 2011). No threshold levels were outlined for salmeterol mainly due to the absence of any oral equivalent. Terbutaline use was permitted only through the athlete obtaining a TUE certificate, which had to be obtained via the presentation of objective proof of asthma or EIB and demonstrating permitted asthma therapy was not providing adequate control of the athletes asthma related condition (WADA, 2015).

In 2019, WADA made small modifications in salbutamol doses not allowed to exceed 800 micrograms over 12 hours starting from any dose; in addition to this, requirement existing urinary thresholds for salbutamol (1200 ng.mL-1) salmeterol maximal doses were established

at 200 micrograms over 24 hours and urinary thresholds for formoterol enlarged to 40 ng.mL-1 (WADA, 2020).

TABLE 2.4: THE HISTORY OF THE β_2-AGONISTS AND THEIR GUIDELINES FOR USE IN THE ATHLETIC POPULATION

YEAR	History of β2-agonists Guidelines for use in the athletes	
1972	Permission to administer inhaled salbutamol by IOC-MC	X
1975	Inhaled salbutamol and terbutaline with prior notification	X
1976	Olympic team doctors notified IOC-MC of intended use of salbutamol or terbutaline	X
1980	Permission to use fenoterol by inhalation granted prior to the Moscow Olympics	X
1984	Fenoterol prohibited at the Sarajevo winter games because of metabolism to p-hydroxyamphetamine	X
1984	Because of concerns of the effect of air pollution on bronchial airways in Los Angeles, team doctors are permitted to notify β_2-agonists post-administration	X
1985	Biltolterol, orciprenaline (isoprotenerol) and rimiterol added as permitted β_2-agonists.	X
1986	Notification of administration of β_2-agonists to IOC-MC lo longer required; oral β_2-agonists reconfirmed to be prohibited.	X

1992	Clenbuterol prohibited. Two athletes disqualified in Barcellona for using clenbuterol, β_2-agonists listed as anabolic agents when administered systemically (orally or by injection)	X
1993	Biltolterol, orciprenaline, and rimiterol no longer permitted β_2-agonists. Notification of administration of permitted inhaled β_2-agonists reintroduced.	X
1994	Permission to administer inhaled salmeterol refused by IOC-MC	X
1996	Salmeterol permitted to provide prolonged protection from exercise-induced asthma	X
2001	Formoterol permitted	X
2001	Because of concerns at the large and increasing number of athletes inhaling β_2-agonists, as a health measure, the IOC-MC introduces the necessity for demonstrating that an athlete has asthma and/or EIB and is given a therapeutic use exemption certificate (TUE)	X
2009	The World Anti-Doping Agency establishes regulations for all athletes to obtain a TUE for the use of β_2-agonists	X
2010	Salbutamol and Salmeterol permitted via inhalation with a declaration of use.	X
2012	Salbutamol, Salmeterol and Formoterol permitted via inhalation without declaration of use. Monitoring of salbutamol and formoterol in the urine with threshold limits	X

2012	Terbutaline remains prohibited except when used with a TUE certificate	X
2017	Dosing parameters of salbutamol were revised; divided doses of salbutamol may not exceed 800 micrograms over any 12 hours. Inhaled salbutamol – max. 1600 mcg over 24 hours.	X
2020	Dosing parameters of salmeterol were added; salmeterol may not exceed 200 micrograms over hours. Formoterol urinary thresholds was enlarged to 40 ng.mL-1.	X
	IOC-MC – International Olympic Committee Medical Commision; EIB – Exercise-Induced Bronchoconstriction X relaxation of guidelines for β_2-agonists use X stricter guidelines for β_2-agonists use	

2.6 Potential ergogenic action of β_2-agonists in athletes

2.6.1 SABA and endurance performance

Research investigations into the ergogenic action of SABAs have been mainly developed on salbutamol and terbutaline administration. The inhalation of therapeutic doses of salbutamol has not shown to improve endurance performance in either time trial and time to exhaustion (Pluim et al., 2011). Improved V_E is a current mechanism observed after the inhalation of salbutamol that could be connected with increased maximal oxygen uptake at supra-maximal doses (Uren et al., 1993). However, the SABAs inhalation does not appear to significant alter V_E or seem not to induce an improvement in aerobic performance when supra-therapeutic doses of salbutamol (800 and 1600 μg) are inhaled before running (Dickinson et al., 2014) and

cycling performance (Koch et al., 2005a; 2005b). Furthermore, a dose of 4000 μg salbutamol was not able to rise incremental time to exhaustion or increase the oxygen kinetics in support of the absence of a dose-effect relationship (Elers et al., 2012a).

Together these findings suggest that even at supra-therapeutic doses inhaled salbutamol does not induce ergogenic action leading to no enhancement of aerobic performance. Moreover, high doses of salbutamol could bring side effects (e.g. increased resting heart rate, nausea, tremor, dizziness) and the risk to overcome WADA cut-off limits (>1000 ng/mL; Elers et al., 2012a).

Similarly, inhaling under 15 mg of terbutaline has been shown to not change cycling time trial performance (300-Kcal) was recorded when compared to placebo condition (Kalsen et al., 2014). Despite terbutaline not enhancing time trial performance, it has been shown to increase carbohydrate metabolism in skeletal muscles during submaximal exercise regardless AMPK and ACC phosphorylation (Kalsen et al., 2014). Moreover, this effect seems to diminish as drug exposure time, exercise duration, and intensity increase (Kalsen et al., 2014). Hence, increases in carbohydrate metabolism in skeletal muscles observed during submaximal exercise could be helpful to improve subsequent high intensity activity as the result of a larger amount of glycogen phosphorylase. The incapacity to observe an enhancement in the aerobic performance following SABAs administration could be mainly the result of premature depletion of muscle glycogen in long-lasting endurance events.

2.6.2 LABA and endurance performance

Human studies developed to evaluate the ergogenic effect exerted by LABAs on aerobic performance are limited; nonetheless studies that have investigated inhaled salmeterol and formoterol have reported differing performance outcomes when compared to SABAs (Pluim et al., 2011). In support of this, a reduced time to exhaustion performance (3.92 vs 4.11 minutes) was recorded after inhalation of 54 μg salmeterol when compared to placebo. This may be due to an over stimulation of β_2-receptors present in skeletal muscle causing an earlier onset of muscle fatigue (Carlsen et al., 1997). The limited evidence on LABAs suggest they do not provide an ergogenic action on aerobic exercise performance. Indeed, they may actually lead to speed onset of muscles fatigue. However, the ergogenic effect of these drugs has been previously assessed only to a very limited extents, further investigation should be conducted in order to understand the mechanisms induced by their administration with special attention to fatigue development observed during aerobic trials.

2.6.3 Long-term SABAs and LABAs effect on endurance performance

Daily inhalation of 1600 μg salbutamol doses during a 6-week strength and endurance program did not improve 3-km running time trial when compared to placebo (Dickinson et al., 2015). The nature of the training made of both strength and aerobic training, makes difficult to analyse the aerobic performance alone without taking into account the potential overall effects induced by the combination of strength and endurance training.

Especially, long-term administration of terbutaline has been observed to blunt training-induced improvements in VO2max when high intensity interval training (HIIT) was performed in healthy men (Hostrup et al., 2018). As matter of fact, the blunted effect detected in HIIT +

terbutaline might be the result of the inhibitory effect induced by β_2-agonists in cardiovascular adaptations, oxidative capacity of mitochondria present in muscles (Holloszy & Coyle, 1984), or associated to the high systemic bioavailability induced by continuous adrenergic fight-or-flight response (Hostrup et al., 2014). Furthermore, long term inhalation of β_2-agonists seems to attenuate training-induced upregulation of NADH dehydrogenase of the oxidative phosphorylation that causes a repression of carbohydrate metabolic pathways such as downregulation of glycogen phosphorylase. This effect seems to happen only when repeated doses are administered for prolonged time contrary to what observed during acute administration (Kalsen et al., 2014).

In summary, endurance performance seems not to be improved by β_2-agonists administration as shown by the absence of change in maximal oxygen uptake regardless the type of individuals (habitual active athletes or resistance trainer athletes (Lemminger et al., 2019). When endurance training was performed habitually, β_2-agonists blunt upregulation in skeletal muscle mitochondrial proteins (Hostrup et al., 2018); conversely, when β_2-agonists are administered in subjects training for resistance a decrease in mitochondrial proteins in skeletal muscle was observed (Lemminger et al., 2019).

Despite endurance performance not to improving after inhaled β_2-agonists administration there is very limited data on acute use of LABAs and long-term use of LABAs and SABAs that need more research to confirm these initial reports.

2.7 The effect of β_2-agonists administration on body composition

Several studies in the past have demonstrated that β_2-agonists injection elicits muscle hypertrophy, increases muscle strength, and reduces fat mass in a variety of animals (Emery et al., 1984). As a consequence of this, β_2-agonists have been applied in hypertrophic model systems to examine cellular signalling pathways significant for muscle growth during drug administration (Ryall et al., 2006).

Generally, it seems that clenbuterol is the only β_2-agonists exerting anabolic and lipolytic actions, whereas inhaled β_2-agonists prescribed for respiratory disease (i.e. SABAs and LABAs) do not show similar responses in terms of fat mass reduction and lean mass augmentation (Mottram & Chester, 2018). Clenbuterol is the most used β_2-agonists drug among bodybuilders (Lynch & Ryall, 2008) because it promotes the production of adrenaline hormone in the organism causing an increase in overall body temperature; this increment causes thermogenesis and fast lipolysis (Rothwell & Stock, 1987). Therefore, bodybuilders experience high perspiration due to the dramatic increase in organism metabolic rate. Although LABAs and SABAs are inserted in WADA list of selective and non-selective β_2-agonists both in and out of competition (S3-category), the anabolic property of clenbuterol places this drug in a different category: S1-catergory (WADA, 2020-b).

2.7.1 Anabolic effect induced by SABAs administration

Studies present in literature demonstrate the anabolic effect of short-acting β_2-agonists in muscle growth when administered chronically as an increase in protein turnover rates in skeletal muscle during long-term resistance exercise program and oral salbutamol

administration (Hostrup et al., 2019). Furthermore, it seems that the increment in protein turnover rates is concomitant with increased cAMP/PKA and Akt2, signalling and modulating mRNA response of growth-regulating proteins. According to this, the anabolic effect induced by β_2-agonists administration seems to be dose-dependent with protein synthesis augmentation with consecutive days of treatment (Koopman et al., 2010). With regard to this, high doses of oral salbutamol (16 μg) have been shown to augment muscle hypertrophic response when administered for 14 days during a resistance training program (Caruso et al., 2005). Together these results explain observations by Hostrup et al., (2019), demonstrating oral salbutamol administration (6 × 4 mg oral) augmented remodelling and recycling myocellular proteins process during post exercise recovery period; SABAs administration seems to double the rate of protein breakdown and synthesis when compared to placebo.

When β_2-agonists are administered in concomitance with resistance training, four weeks terbutaline administration has shown to increase lean mass and reducing fat mass in young trained men after oral (Hostrup et al., 2015) and inhaled administration (Jessen et al., 2018). These observations contrast with outcomes of studies conducted on similar drugs (e.g. salbutamol) where no effect on lean body mass was detected after three weeks of oral salbutamol (8 mg/twice daily) despite increased muscle strength (Martineau et al., 1992). Similarly, after three weeks salbutamol ingestion, no changes in body composition were collected in several studies carried out by Le Panse et al., (2005; 2006). The discrepancy in the results could be explained by anabolic features that are strongly associated with the class-specific of the drug. However, there are not studies comparing the pharmacodynamic and the bioequivalence between terbutaline and salbutamol helpful to understand the main differences between these two drugs.

2.7.2 Anabolic effect induced by LABAs administration

When administered chronically formoterol has been shown to increase resting energy expenditure and fat oxidation; this effect seems more marked in women than in men by an augmented level of leucine turnover (Lee et al., 2015). Notably, not only after oral administration but also after inhalation, formoterol increases resting energy expenditure (+38%) through free fatty acids mobilisation from white adipose tissue (Hoeks et al., 2003) and activates hormone-sensitive lipase in skeletal muscle (Jocken et al., 2008).

Moreover, when subjects performed exercise at submaximal intensity, formoterol-induced increase in energy expenditure was reduced and fat oxidation increased at rest independent by subject fat composition and fitness levels (Onslev et al., 2017).

Currently, the anabolic effect provoked by long-term salmeterol administration has not been investigated in literature. Therefore, future investigations into the long-term use of salmeterol in combination with resistance training would provide useful data that could inform future WADA policy.

2.7.3 Anabolic mechanism induced by SABAs and LABAs

SABAs and LABAs elicit anabolic actions binding β_2-adrenoceptors with the activation of the main signal (cAMP/protein kinase A (PKA)-dependent pathway) that leads to cell proliferation and increases protein synthesis. Not only does this signal lead to metabolism regulation and gene modifications, but also it leads to cell proliferation, adaption and decreased proteolysis (Chen et al., 2000). Therefore, the change in lean mass observed after β_2-agonists administration is mainly due by a repression in the growth inhibiting protein myostatin and upregulation of follistatin as reported also using terbutaline (Hostrup et al., 2015).

Similar to anabolic synthesis, lipolysis involves cAMP/PKA-dependent activation of hormone-sensitive lipase (HSL) and perilipin A causing catalyses the hydrolysis of triglycerides (Hoeks et al., 2003).

2.8 STRENGTH and POWER performance induced by β_2-agonists administration

Change in lean mass observed above is likely to lead to the improvement of strength and power performance due to an increment in size (hypertrophy) and in the number of muscle fibres (hyperplasia; Taylor & Wilkinson, 1986). The majority of the studies conducted investigating β_2-agonists administration on strength and power are focused on observations of long-term use. The effect of SABAs (e.g. albuterol, salbutamol, terbutaline) and LABAs (e.g. fenoterol, clenbuterol and formoterol) administration were observed in several studies developed around humans and animals (Pluim et al., 2011; McCormick et al., 2010).

Findings coming from scientific literature are sometimes in contrast with each other, for instance maximal voluntary contraction (MVC) of quadriceps has increased after 3 weeks administration of 8 mg/day oral salbutamol (Martineau et al., 1992) while similar doses did not enhance MVC in endurance athletes (Hostrup et al., 2016). However, Martineau et al. (1992) could not explain this change through body weight and lean body mass variations, speculating a potential impact on the central nervous system following β_2-agonists administration. Noteworthy, the discrepancy between these studies could be attributed also to differences in subjects training status and in β_2-adrenoceptors content that has been shown to be lower in athletes organs compared to non-athlete people (Butler et al., 1982).

Route of administration seems also to contribute to an ergogenic effect following this practice; in support of this, similar salbutamol doses reported different results when oral administration was compared to the inhaled route. Specifically, 5-week daily inhalation of 1600 μg salbutamol

did not increase strength and power performance in lower and upper body exercise (Dickinson et al., 2014). In contrast, the same amount of drug administered orally has shown to increase concentric and eccentric power during 9-week resistance training program (Caruso et al., 1995; 2008).

Similar to the results reported by Caruso et al., (2005; 2008), increased MVC and peak twitch force were reported after oral terbutaline (15 μg) by Hostrup et al., (2015). The author explained the improvement in 30 s sprint with an increase in lean body mass and muscle hypertrophy in the area of myosin heavy chain (MHC) I and (MHC) II fibres. Together these results would suggest that the ergogenic features of SABAs on strength and power performance seems to act mainly after chronic oral acute administration without observing changes in the number of muscle proteins (Hostrup et al., 2016; Sanchez et al., 2012). Similarly, when oral salbutamol has been administered in concomitance with strength training, significant maximal anaerobic power was recorded in the group undertaking oral salbutamol on daily bases regardless subjects' training status. A significant increment in peak power and strength was observed in strength-trained subjects (+11.9 %) and sedentary subjects (+8.3 %) without reporting change in lean body mass and fat mass (Le Panse et al., 2005).

In conclusion, there is potential for ergogenic action on strength and power performance following long-term administration of β_2-agonists as observed with SABAs oral administration. Future investigations should incorporate the impact of LABAs administration on peripheral muscle adaptation in athletes.

2.9 SPRINT performance induced by IBAs

2.9.1 Sprint performance induced by SABAs

Sprint improvements following the administration of β_2-agonists have been mainly observed following the administration of prohibited substances and methods present in the list of WADA (WADA, 2020). Increased sprint performance was reported after inhaled terbutaline (15 mg) administration (Kalsen et al., 2016; Hostrup et al., 2015) and oral salbutamol administration (Hostrup et al., 2016; Sanchez et al., 2012; Collomp et al., 2010; Le Panse et al., 2005; Le Panse et al., 2006). An increased rate of Ca^{2+} release and re-uptake from the SR in skeletal muscle seems to have improved power output in the above studies. Moreover, augmented Na^+_K^+ATP_ase activity may have also contributed to the higher power output recorded by counteracting loss of membrane excitability via an increased re-uptake of K^+ from the interstitium (Sejersted & Sjøgaard, 2000). In addition to this, accelerated rate of ATP resynthesise has been observed to be significantly augmented after the administration of β_2-agonists (Allen et al., 2008). Notably, even after supra-therapeutic salbutamol doses ATP resynthesise is not increased probably because low doses are not able to stimulate β_2-adrenoceptors sites present in the body needed to induce an increase in glycolytic mechanism (Lemmer et al., 1995; Meeuwisse et al., 1992; Morton et al., 1993).

When inhaled for 6 weeks, salbutamol did not show an increase in power performance when inhaled in parallel with strength and aerobic training program (Dickinson et al., 2014); this could be linked with β_2-adrenoceptors down-regulation that decrease both the density and sensitivity of β_2-adrenoceptors (Butler et al., 1983). In support of this, the reduced effect observed on sprint performance following 3-weeks administration compared with acute administration observed by Sanchez et al., (2012) could be explained as dampening of the

effect of salbutamol provoked by repeated β_2-agonists administration. Interestingly, when low oral doses (8 μg) were administered for 2-weeks β_2-adrenoceptors down-regulation seems not to take place as shown by a significant enhancement on sprint performance (Hostrup et al., 2016).

In conclusion, increased sprint performance has been observed after terbutaline and salbutamol oral administration in both acute and chronic; however, this effect does not happen after the inhalation of supra-therapeutic doses of SABAs.

2.9.2 Sprint performance induced by LABAs

The effect provoked by LABAs administration on sprint and power performance is limited in scientific literature. It seems that acute formoterol inhalation (54 μg) is able to enhance maximal voluntary contraction and power output during maximal sprint activity (Kalsen et al., 2016); however, this effect seems to be strongly correlated with the amount of drug inhaled because the same sprint activity did not record a change in performance after 27 μg of formoterol (Stewart et al., 2002) or after 42 μg of salmeterol (McDowell et al., 1997). McDowell (1997) did not report significant difference changes in blood lactate concentration between salmeterol and to placebo condition suggesting insufficient stimulation of β_2-adrenoceptors.

After high doses of LABAs, increased release of Ca^{2+} from the ryanodine receptor 1 (RYR1) of the sarcoplasmic reticulum (SR) has been documented to enhance force production (Hostrup et al., 2014) and prevent loss of membrane excitability through the stimulation of Na^+/K^+_ATP_ase and to neutralise a reduction in release of Ca^{2+} from RYR1 (Sejersted et al., 2000).

In conclusion, it seems that even at supra-therapeutic doses allowed by WADA, SABAs inhalation is not able to induce an ergogenic effect on strength and power activities. In addition to this, chronic inhalation of salbutamol is associated with side effects and seem to provoke down-regulation in β_2-adrenoceptors. Therefore, the attempt to increase the systemic concentration of β_2-agonists in the body through repeated inhalation of SABAs in consecutive days seems not able to induce change in strength and power performance. Conversely, formoterol has been observed to enhance sprint power output and maximal voluntary contraction during 30-s sprint. Whether the cause of augmented performance could be mainly associated with increased glycolytic activity, the smaller drop observed in MVC after the sprint could be linked to fatigue resistance features of LABAs. In support of this, LABAs administration increase the rate of Ca^{2+} release and $Na^{+}\_K^{+}ATP\_ase$ that counteract the muscle fatigue experienced by repeated sprint activities.

2.10 Overall Summary

Athletes are lightly to experience EIB due to the high ventilatory demands required by the practice; it is not uncommon for these athletes to be diagnosed with exercise-induced asthma or exercise-induced bronchoconstriction. Therefore, prescription of IBAs represents the first choice to reverse these symptoms. However, some SABAs and LABAs may provide an ergogenic action when administered in “non-asthmatic” subjects even at doses allowed by WADA where a therapeutic use exemption is not mandatory.

The administration of SABA seems not improve endurance performance in either inhalation or oral form when time-trial and time to exhaustion is performed. The small number of studies

designed around LABAs administration have been mainly developed using salmeterol without finding an ergogenic effect when endurance performance was developed. Furthermore, the stimulation of β_2-receptors present in skeletal muscle following LABAs could induce a prolonged effect compared to SABAs without provoking β_2-adreceptors down-regulation. Moreover, from the inhalation of supra-therapeutic doses of SABAs and LABAs an increment of the glycolytic metabolism has been recorded with high level of blood lactate collected.
Strength performance performed after SABAs administration seems to be increased only after chronic oral salbutamol administration without changes in muscle proteins and maximal voluntary contraction were recorded. SABA administration regardless the amount of drug inhaled does not appear to increase strength performance even at supra-maximal doses. Due to the absence of studies observing the effect of LABAs administration on strength performance, future studies need to be developed around this field even in concomitance with long-term LABAs administration. According to this, the observation of LABAs administration and training would mimic real-life strategies experienced by athletes training for strength improvements.

The enhancement in strength following SABAs oral administration could be explained through the increase in body composition; in fact, change in hypertrophy and lipolysis has been observed after daily oral salbutamol administration when the drug was administered at least for 2-weeks. However, this effect seems not to happen after supra-therapeutic inhalation of SABAs. Similarly, LABAs formoterol has shown to increase fat oxidation and fat mobilisation expressed by increased energy expenditure that was more marked in women than in men. Currently, there are not studies looking at anabolic effect of salmeterol and formoterol when combined with resistance training.

An increase in sprint performance following SABAs administration has been observed only after oral terbutaline and salbutamol administration but not after others way of administration mainly due to the lower amount of β_2-agonists present in the systemic circulation after inhalation. The inhalation of LABAs increased power output during maximal sprint activity after high dose of formoterol (54 μg); contrarywise, low doses were not able to record similar change of performance even when after the inhalation of salmeterol.

Notably, no studies present in literature have looked at the effect of LABAs administration on sprint performance during fatigued condition. Therefore, the need to test the ergogenic effect of LABAs administration in acute fatigue and in chronic fatigue could add extra info helping to understand long-acting β_2-agonists mechanisms.

2.11 AIM of the Thesis

I. To investigate the effect of supra-maximal dose of inhaled salbutamol (1600 μg) on running sprint ability in "non-fatigued" and "fatigued" state performed before and after the Yo-Yo IRT. Moreover, total distance cover and physiological measures collected during Yo-Yo IRT will be analyse in order to observe any ergogenic effect induced by SABAs.

II. To investigate the effect of high-dose inhaled salmeterol (100 μg) on 12-s maximal sprint in high-level cyclists. Immediately after the sprint, one-hour Variable cycling trial will be performed in order to simulate the intensities experienced by cyclists

during the Gran Tour stage. The increment in power output throughout the Variable cycling trial will increase participants levels of fatigue before 12-s sprint performance will be repeated at the end of the session. Power output recorded during the sprints and physiological measure collected during the aerobic trial will be then analysed and compared with placebo condition.

III. To investigate the effect of daily administration of inhaled LABAs salmeterol (100 μg) and formoterol (12 μg) during 5-week of strength and power training program performed by recreational active individuals 3-times a week. Maximal strength, sprint performance, jump height and body composition will be assessed at the beginning and at the end of the programme in order to observe any performance and physiological changes provoked by daily LABAs when compared to placebo group.

2.12 HYPHOTHESIS of the Thesis

I. **Chapter 4** hypothesis is that daily doses of LABAs could increase strength and power performance when developed in concomitance with a resistance training program. This will allow subjects to lift heavier weights during training sessions with a possible increment also in sprint performance as consequence of better muscle synchronisation and lean mass augmentation.

II. **Chapter 5** hypothesis is that after supra-maximal salbutamol dose will increase both maximal sprint ability in "non-fatigue" condition (before the Yo-Yo IRT) and in "fatigue" condition (after the Yo-Yo IRT). Furthermore, I will not aspect to find

significant difference in the distance covered during the intermittent aerobic trial due to the observations present in literature that did not show SABAs effectiveness on aerobic performance.

III. **Chapter 6** hypothesis is that salmeterol inhalation will record a significant increment in both the 12-s sprint performed in "non-fatigued" and "fatigued" conditions performed before and after the Variable cycling trial when compared with placebo conditions. In addition to this, I'm expecting to find a marked difference between conditions especially during the final sprint performed at the end of the Variable cycling trial where the effect of LABAs could be more consistent.

Chapter 3. General Methods

This chapter will explain the assessment tests that were adopted for every experimental study in this thesis and will be discussed. The current, extended method for each experiment can be read in the methodology section of each study chapter.

3.1 "Spirometry test"

Spirometry is a medical test used to measure the volume of air that an individual inhales or exhales as a function of time (Miller et al., 2005). In the following collection of studies forced vital capacity (FVC) manoeuvres were performed exclusively. FVC is the maximal volume of air exhaled with maximally forced effort from a maximal inspiration (Miller et al., 2005). The American Thoracic Society (ATS) and the European Respiratory Society (ERS) has published guidelines for the measurement of lung function (Miller et al., 2005), which were respected when spirometry measurements were performed.

Throughout the studies maximal flow volume loops were collected using either of the following turbine spirometers: SpiroUSB™ (Carefusion 234 Germany GmbH) or MicroLab® Spirometer (Carefusion 234 Germany GmbH).

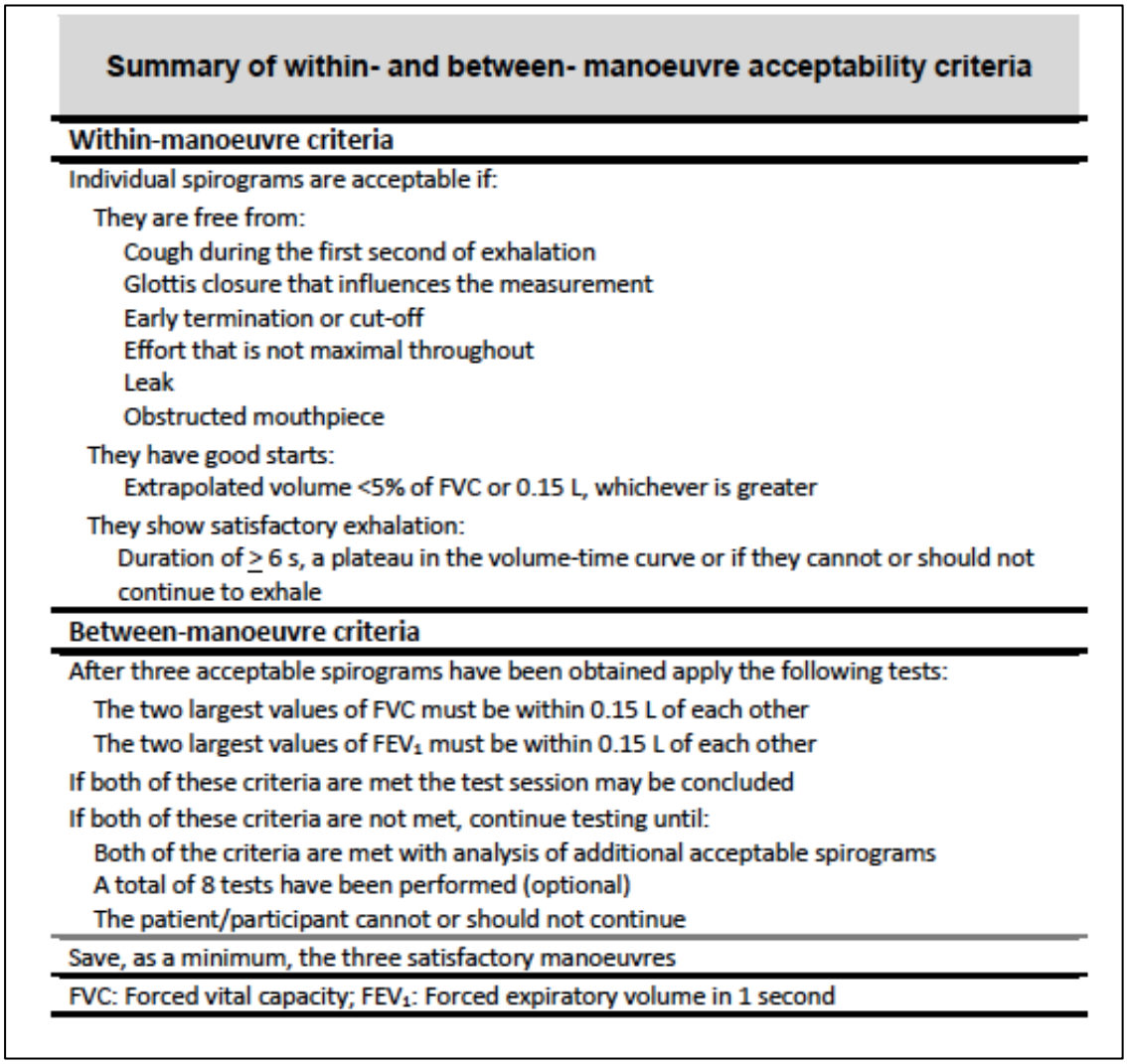
Participants were asked to assume the correct posture (FIGURE 3.1) and sat upright in a chair, wore a nose clip to prevent nasal breathing and were instructed to reach maximum inhalation before placing the spirometer mouthpiece in the mouth and forcefully exhaling as hard as they could.

Breathing out continued until they had completely emptied their lungs; this point was indicated by a plateau in the volume/time graph on the spirometer, at which point the experimenter would encourage the participant to breathe in as fast as possible to maximum inspiration, completing the maximal flow-volume maneuver.

A minimum of three maximum attempts were required for baseline measurements, flow-volume measurements were rejected if: the participant was considered not to have reached maximum inspiration prior to the maneuver; the participant manifested obstructions such as cough during the maneuver; the participant was believed to have performed a slow start or to have not maintained the pressure of expiration for the duration of exhalation and also if the values were not coherent (i.e. large variability among the three baseline values).

Flow-volume measures recorded from each maximal flow-volume loop were;
Forced Expiratory Volume in one second (FEV1), Forced Vital Capacity (FVC), FEV1 to FVC ratio (FEV1/FVC %), Peak Expiratory Flow (PEF) and forced expiratory flow between 25% and 75% of FVC (FEF25–75). Individual maximal flow-volume loops were accepted in accordance with European Respiratory Society American Thoracic Society criteria (Miller et al., 2005).

FIGURE 3.1 MAXIMAL FLOW-VOLUME LOOP ACCEPTANCE CRITERIA



Summary of within- and between- manoeuvre acceptability criteria

Within-manoeuvre criteria

Individual spirograms are acceptable if:

- They are free from:
  - Cough during the first second of exhalation
  - Glottis closure that influences the measurement
  - Early termination or cut-off
  - Effort that is not maximal throughout
  - Leak
  - Obstructed mouthpiece
- They have good starts:
  - Extrapolated volume <5% of FVC or 0.15 L, whichever is greater
- They show satisfactory exhalation:
  - Duration of ≥ 6 s, a plateau in the volume-time curve or if they cannot or should not continue to exhale

Between-manoeuvre criteria

After three acceptable spirograms have been obtained apply the following tests:

- The two largest values of FVC must be within 0.15 L of each other
- The two largest values of FEV_1 must be within 0.15 L of each other

If both of these criteria are met the test session may be concluded

If both of these criteria are not met, continue testing until:

- Both of the criteria are met with analysis of additional acceptable spirograms
- A total of 8 tests have been performed (optional)
- The patient/participant cannot or should not continue

Save, as a minimum, the three satisfactory manoeuvres

FVC: Forced vital capacity; FEV_1: Forced expiratory volume in 1 second

Adapted from Miller et al., (2005)

3.2 "Eucapnic Voluntary Hyperpnoea Challenge"

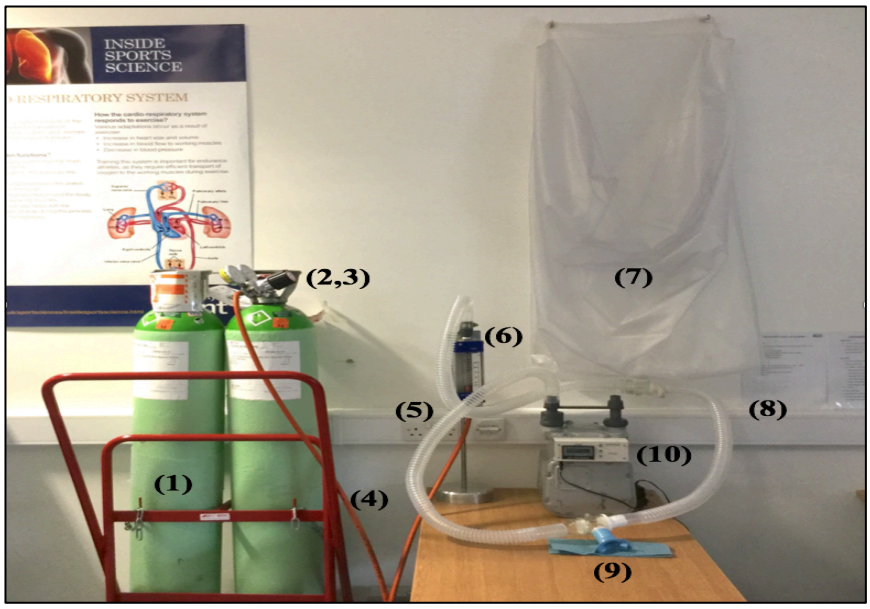
All participants underwent a eucapnic voluntary hyperpnoea (EVH) challenge in accordance with the methods described by Anderson et al., (2001). Participants were instructed to avoid intense exercise the day of the test. Due to the bronchodilator effects of caffeine (Duffy & Phillips, 1991), participants were inquired to avoid consumption of caffeine-containing beverages and foods such as tea, coffee, cola, energy drinks and chocolate 4 hours before testing.

If an athlete was suffering from an illness that may limit the results of the test, they were asked to return when they were well and fit to complete the test. Baseline FEV1 was recorded from three maximal voluntary flow-volume maneuver. Whether the participant had an FEV1 less than 80% of the predicted value or if they had an FEV1/FVC ratio less than 70% they were excluded from participation in any studies.

Participants were asked to attain target minute ventilation ($\dot{V}_E$) of 85% (FEV1 x 30) of their predicted maximal voluntary ventilation rate (MVV) for 6 minutes (Anderson et al., 2001). During the 6 minutes, participants breathed air from a compressed gas cylinder containing 21% Oxygen (O_2), 5% Carbon Dioxide (CO_2) and 74% Nitrogen (N_2) with inspired air temperature 19°C, and humidity < 2%. Annotation the 5% CO_2 concentration in gas to maintain eucapnia and prevent participant from the adverse effect of prolonged hyperventilation, such as dizziness and nausea.

The gas was delivered to each participant via a gas cylinder, reservoir and a two-way valve; V_E was recorded by calculating the volume of air passing through a dry gas meter every minute (FIGURE 3.2).

FIGURE 3.2 "EQUIPMENT USED TO PERFORM EVH TEST"



Gas cylinder (1), regulator (2), demand resuscitator 30-150 L/min (3) high pressure tubing (4), rotameter (5), three-way tap (6), Douglas bag (7), wide bore tubing (8), large low resistance low dead space two-way valve (9), gas meter (10).

During the EVH challenge verbal feedback was provided to the participant in order to encourage them to meet their target $\dot{V}_E$.

After the 6-minute EVH challenge, two maximal voluntary flow-volume loops were measured at 3, 5, 7, 10 and 15-minute time-points, with the highest of the two FEV1 values recorded. A fall in FEV1 greater than 10% on two occasions post-challenge resulted in a positive test. The grade of a positive EVH-challenge was graded as mild, moderate or severe if the percent fall in FEV_1 from baseline values were $\geq 10\%$ but $< 25\%$, $\geq 25\%$ but $< 50\%$ and $\geq 50\%$, respectively (Parsons et al., 2013).

Following the test participants that did not reach FEV_1 baseline levels within 15 minutes after stopping the activity were offered 400μg inhaled Salbutamol to assess their reversibility to bronchoconstriction. Spirometry was performed 15 minutes post-inhalation of Salbutamol (Miller et al., 2005). A positive bronchodilator response (reversibility) was defined as an increase of ≥ 12% and 200 ml in post-EVH FEV_1 and/or FVC (Miller et al., 2005).

3.3 "Peak Oxygen Consumption (VO_{2peak})"

Participants completed an incremental cycling exercise test (2 min at 50 W + 50W/ 2 min) until voluntary exhaustion or until pedal frequency fell below 60 revolutions/min (RPM) for more than 5 s to establish peak oxygen consumption (VO_{2peak}; Withers et al*.,* 2000). This test was performed on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). Prior to starting, the participants were fitted with a heart rate monitor (Polar RS400; Polar Electro Oy, Kempele, Finland) and connected to a breath-by-breath online gas analyser via a face-mask (MetaLyzer 3B, Cortex Biophysik, Leipzig, Germany). At the end of every stage and upon trial cessation the following were measured: time (s), heart rate (HR), oxygen consumption (VO_2), carbon dioxide production (VCO_2), minute ventilation (V_E), respiratory exchange ratio (RER) and rating of perceived exertion (RPE). Participants were instructed to avoid intense exercise the day before the test.

Chapter 4. Improved Sprint Performance With Inhaled Long-Acting β_2-agonists Combined With Resistance Exercise

4.1 Background

The 2019 World Anti-Doping Agency (WADA) permits athletes to use inhaled therapeutic doses of β_2-agonists salbutamol (1600 μg/d, no more than 800 μg in a 12-h period), formoterol (54 μg/d), and salmeterol (200 μg/d). However, there is some debate as to whether the current rules allow unscrupulous athletes, with and without asthma-related conditions, to use inhaled β_2-agonists for the purpose of benefitting from a potential ergogenic action.

Previous research investigating the acute and short-term use (e.g. 2 weeks) of inhaled β_2-agonists suggests they do not have an ergogenic action on endurance performance (Pluim et al., 2011). Furthermore, endurance performance is not improved from acute doses of inhaled formoterol (Tjørhom et al., 2007) and salmeterol (Sue-Chu et al., 1999). However, moderately and highly trained individuals may experience enhanced strength and power performance from the acute use of short acting (Hostrup et al., 2014) and long acting β_2-agonists (Kalsen et al., 2016).

The mechanisms behind the ergogenic action from acute doses that have been observed in skeletal muscle include: β_2-adrenergic stimulation that counteracts exercise-induced reductions in Na+-K^+ ATPase maximum rate of activity, elevated glycolytic activity during high intensity exercise and enhanced rates of Ca^{2+} release and uptake from the sarcoplasmic reticulum (Hostrup et al., 2014). Furthermore, increased anaerobic ATP utilisation has been suggested as a potential mechanism (Kalsen et al., 2016). However, others have failed to demonstrate changes in peak velocity and have shown maximal strength deteriorates following acute oral terbutaline administration (Sanchez et al., 2013). Long-term use of β_2-agonists also has the potential to produce an ergogenic action. Data from animal models suggests long-term β_2-adrenergic stimulation results in muscle hypertrophy (Burniston et al., 2006). Studies investigating the long-term β_2-adrenergic stimulation in humans suggest increases in skeletal muscle mass (Jessen et al., 2018) and portioning of amino acids from oxidative loss toward

protein synthesis (Lee et al., 2013) may occur. Furthermore, salbutamol has been shown to counteract a negative net protein balance following resistance training in males (Hostrup et al., 2018). These changes to skeletal muscle from long-term use of β_2-Agonists (8mg/d oral salbutamol for 2 week) has been shown to increase peak muscle strength (Hostrup et al., 2016) and power output, (Hostrup et al., 2015) whilst also inducing a slow-to-fast twitch muscle phenotype transition in humans (Hostrup et al., 2018).

Long-term use of terbutaline (4mg/d for 4 week) may also decrease body fat due to increased fat mobilization from adipose tissue (Hostrup et al., 2015), decreased fat synthesis in adipose tissue and liver (Onslev et al., 2017), or a combination of both (Yang et al., 1989). Although there is clear potential for ergogenic action with oral or supra-therapeutic inhaled doses of β_2-agonists, we do not know whether long-term stimulation of β_2-adrenoreceptors via therapeutic doses of long acting inhaled β_2-agonists has a similar effect.

Endurance training has been shown to confound the ergogenic action of inhaled short acting β_2-agonists (Dickinson et al., 2014). However, the ergogenic action of inhaled short acting β_2-agonists is augmented with resistance training (Jessen et al., 2018). It is not known whether there is a similar interaction when long acting β_2-agonists are inhaled whilst engaging in strength training. This is a realistic consideration as athletes using long acting inhaled β_2-agonists (salmeterol or formoterol) are prescribed to do so on a daily basis, which may modify their response to strength and power training.

Therefore, the hypothesis behind this study is that repeated doses of β_2-agonists could mimic the effect provoked by oral route when developed in concomitance with a resistance training program that could induce an increased rate of force development and power performance.

4.2 Methods

4.2.1 Study Participants

I initially recruited twenty-four male participants however one male participant withdrew from the study due to an injury not related to the study in week four.

I therefore had twenty-three healthy recreationally active males (mean ± SD: age 27.9 ± 5.5 years; height 179.8 ± 7.3 cm; weight, 78.8 ± 10.3 kg) and fifteen recreational active females (age 24.1 ± 4.1 years; weight 65.4 ± 9.5 kg; height 168 ± 4.3 cm) who volunteered for the study, provided informed consent and completed the study.

All participants have been involved in strength and power activities over the past year during their weekly training habits. The heterogeneous nature of the male participants taking part in the study was characterised by their involvement in a variety of sports at an amateur competitive level including: football (n = 9); basketball (n = 4); track and field (n = 2); martial arts (n = 3); swimming (n = 1); running (n = 2); and cycling (n = 3). Female participants were characterised by their involvement in a variety of sports at an amateur competitive level including: basketball (n = 8); football (n = 4); boxing (n = 2); running (n = 1).

Prior to participation in the study none of the participants completed in strength and power lifting sports. Participants engaged in strength and power training sessions at least three times per week completing a diary to record their training engagement and progress.

The participants completed all assessments at the same time of the day (within 1 h), separated by a minimum of 48 hours. They were instructed to sleep for at least 7 hours, drink at least 35 mL/kg/day of water, refrain from consumption of alcohol for 24 hours prior to the visit and avoid vigorous exercise on the day before each of the following visits.

4.2.2 Broncho-provocation Challenge

All participants were free from asthma and EIB, which was confirmed by the presentation of a negative EVH challenge (Anderson et al., 2001).

4.2.3 Treatment Groups

Participants were randomly assigned to one of three groups using a minimisation method (Scott et al., 2002). As part of this randomisation we factored in gender balance between groups so that they were balanced eight males to five females in each group. In a single blinded randomised design each group was allocated to use either:

- Placebo inhaler (containing water vapor) twice daily (PLA)
- Inhaled 100μg salmeterol twice daily (Serevant, Accuhaler 50 μg/dose, GSK, UK)
- Inhaled 12μg formoterol twice daily (Oxis Turbohaler 6 μg/dose, Astra Zeneka, UK)

These doses were chosen as they are high therapeutic doses permitted for use by athletes (WADA, 2019). Participants were instructed about their inhaler technique. At each training session researchers checked the participants were using their inhalers as instructed by reading the inhalation counter on their device to confirm they were adhering to the protocol.

4.2.4 Assessments

Participants completed each of the following assessments at baseline and one week after the final inhaler dose and training session. Prior to the start of the study participants attended two familiarization of sprint sessions performed on non-motorised treadmill.

4.2.5 30 Meters Sprint

Participants were asked to complete a maximal 30 m sprint on a non-motorised calibrated treadmill (Force Treadmill System, Woodway, SA). The peak speed data collected from the non-motorised treadmill has been described in literature to be approximately 80% of that achieved in free-sprint track performance (Morin et al., 2011). Each participant completed three 30 m sprints separated by 5 minutes. The fastest 30 m sprint was recorded.

4.2.6 Isokinetic Dynamometry

Participants performed three maximal voluntary contractions of the knee extensors at 120°.s-1 and three maximal voluntary contractions of the knee flexors at 120°.s-1 (Biodex 830-210, Biodex Medical System, Shirley, New York, USA). The highest peak torque measurement was taken as a measurement of maximal strength in the knee extensors and knee flexors.

4.2.7 Maximal One Repetition Bench Press and incline Leg Press

Participants progressively worked toward a maximum one repetition for both incline leg press and bench press. The incline leg press (CF800 Leg Press/Hack Squat Machine, Bodymax, UK) was performed at 45° by first completing a six-repetitions maximum. This was followed by a four-repetition maximum and a two-repetition maximum at increasing weights. The bench press was performed using a 20 Kg Olympic bar with weights added to it accordingly. The participants continued to complete one-repetition efforts at increasing weight until they reached failure. Each effort was separated by four minutes (Willardson et al., 2006). The weight lifted during the last complete repetition was taken as their maximal one repetition (Baechle et al., 2008).

4.2.8 Power – Counter Movement Vertical Jump (CMJ)

A counter movement jump was performed on a jump mat (Probiotics Inc., Huntsville, AL, USA). The participants were instructed to jump as high as they could by performing a CMJ with an arm swing. Coaching of technique was only provided if participants consistently landed off the jump mat demonstrating poor technique. Participants performed three counter movement jumps and the greatest vertical jump height achieved was recorded.

4.2.9 Body Composition

Skin-fold thickness was taken at the following recognised sites on the right-hand side of the body: triceps, biceps, subscapular and supraspinale. All measurements were taken by the same technician using a single set of Harpenden skinfold callipers (Baty International, Sussex, UK). Skin-fold thickness measurements were taken from each site consecutively a total of two times with the mean of the two measurements taken as the skin fold thickness for each specific site. The criterion for a valid measurement was a difference of less than 1 mm between the two totals. If this was not the case the measurements were repeated until the criterion was met. The sum of four mean skin-folds thickness measurement was calculated.

4.2.10 Recovery, Sleep and Mood Questionnaires

Participants completed questionnaires in week three and five to measure recovery and stress from training and mood. Recovery and stress from training were assessed via the Recovery-Stress Questionnaire for athletes (Kellmann et al., 2010). Mood was assessed using the Brunel Mood Scale (Terry et al., 2003).

4.2.11 Strength and Power Training Program

Following the completion of the above baseline assessments participants began a strength and power training program. The training program focused on developing strength, power and sprinting. Participants training was individualised and supervised by a strength and conditioning specialist.

Participants training incorporated lower body exercise such as lunges, squat, leg press and leg curl; upper body exercises included chest and shoulder press, shoulder dumbbell raise and arm exercises using both barbell and dumbbells. Each training session consisted of twelve exercises. Each exercise was completed with a target of completing three sets of eight repetitions, with each set separated by two minutes. When participants were able to complete all three sets they increased the load. Sprint training included exercises involving quickness and coordination with a set of 5 to 10 m sprint accelerations performed five times at the end of the training session. Participants recorded total work done during each session.

Participants were asked not to engage in strength, power or sprint training outside of the program. Aerobic training outside of the study was restricted to two sessions per week. It was not feasible to accurately record or control for the intensity and duration of any additional endurance-based training.

4.2.12 Statistical Analysis

Changes in sprint performance, strength, power, mood, recovery, sleep and skinfold thickness from baseline to week five between PLA, SAL and FOR were analysed using repeated measures ANOVA (3 group x 2 time).

Assumptions for this analysis was checked and corrected for according to the methods described by Atkinson & Nevill (2001).

A $p < 0.05$ was deemed significant. Effect size was calculated according to Cohen's statistical power analysis used to indicate the standardised difference between two means measuring small, medium and large effect sizes (d= 0.20, 0.50, 0.80; Cohen et al., 1992).

4.3 Results

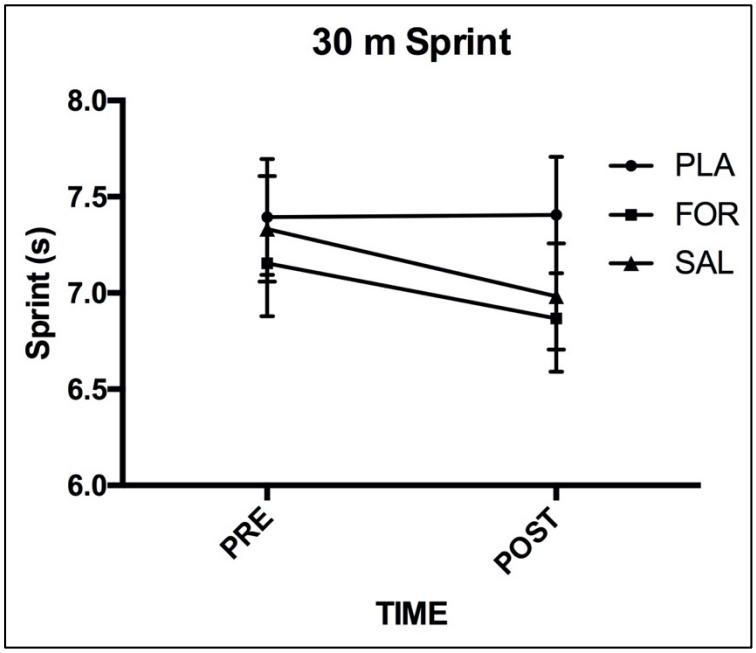
4.3.1 Baseline Groups Comparison

There were no differences at baseline between groups for any of the sprint (p=0.670), strength and power (p=0.226), anthropometric (p=0.438) and skinfolds (p=0.762). Psychological variables were different at baseline (p=0.001) but not at week 3 and week 5 between groups (p=0.234).

4.3.2 30 Meters Sprint

Between baseline and week five 30 m sprint time improved in both the FOR (–0.29 ± 0.11 s; p=0.049; ES= 0.50) and SAL (–0.35 ± 0.05 s; p=0.040; ES= 0.41) groups when compared to the placebo group (+0.01 ± 0.11 s); (FIGURE 4.1).

FIGURE 4.1 "30 METERS SPRINT IN SAL, FOR AND PLA"



Data are presented at week 0 (PRE) and week 5 (POST); (Mean ± SD).

4.3.3 Strength and Power Assessments

Over the five weeks all groups improved markers of strength and power (TABLE 4.1). There was no difference in the rate of improvement between groups.

TABLE 4.1 "30 METERS SPRINT, POWER & STRENGTH PERFORMANCE IN SAL, FOR & PLA"

	SAL			**FOR**			**PLA**		
	Week 0	**Week 5**	**Mean Change**	**Week 0**	**Week 5**	**Mean Change**	**Week 0**	**Week 5**	**Mean change**
1RM Inc. Leg Press (Kg)	102 ± 59	135 ± 92	33 ± 41	111 ± 59	135 ± 92	24 ± 44	102 ± 58	131 ± 83	29 ± 41
1RM Hack Squat (Kg)	135 ± 57	201 ± 57	66 ± 40	123 ± 33	190 ± 34	67 ± 15	108 ± 34	166 ± 52	58 ± 21
1RM Bench Press (Kg)	55 ± 20	64 ± 27	9 ± 26	51 ± 20	57 ± 17	6 ± 18	53 ± 27	60 ± 31	7 ± 44
Leg Extension (N•m)	226 ± 48	228 ± 22	1 ± 41	199 ± 24	212 ± 23	13 ± 33	186 ± 19	189 ± 36	3 ± 27
Leg Flexion (N•m)	123 ± 23	137 ± 10	14 ± 25	121 ± 18	128 ± 16	7 ± 9	110 ± 12	121 ± 10	11 ± 14
Vertical Jump (cm)	49.6 ± 10.7	53.5 ± 9.4	3.9 ± 5.2	49.7 ± 8.8	54 ± 10.4	4.3 ± 5.9	49.4 ± 8.1	52.3 ± 8.4	2.9 ± 7.3
30 m Sprint (s)	7.38 ± 0.70	7.03 ± 0.72*	- 0.35 ± 0.05	7.10 ± 0.70	6.81 ± 0.74*	- 0.29 ± 0.11	7.40 ± 1.33	7.41 ± 1.23	0.01 ± 0.11

Abbreviations: 1RM = one maximal repetition; SAL = salmeterol; FOR = formoterol; PLA = placebo * = Significantly different from PLA ($p < 0.05$). Incline Leg press 45°, Hack Squat and Bench Press measured is reported at 1RM; Leg Extension, Leg Flexion is reported as Peak Torque measured at 120o.s-.a. Data are presented at week 0 and week 5 (Mean ± SD).

4.3.4 Anthropometric Measures

Over the five weeks of training the sum of skinfold thickness across four sites did not change significantly (p=0.762; TABLE 4.2). No significant changes in body mass between groups were observed over the five weeks of training (p=0.915; TABLE 4.2).

TABLE 4.2 "SKINFOLDS & BODY MASS in SAL, FOR AND PLA"

	SAL			**FOR**			**PLA**		
	Week 0	**Week 5**	**Mean Change**	**Week 0**	**Week 5**	**Mean Change**	**Week 0**	**Week 5**	**Mean change**
Skinfolds Σ4 (mm)	46 ±18	41 ± 12	- 5 ± 17	47±16	46±15	-1 ± 28	44 ± 10	42 ±7	2 ± 12
Body Mass (Kg)	80.8± 12	81.6± 10.6	- 0.8± 2.1	76.6± 7.1	76.9± 5.8	0.3 ± 4.1	80.4± 13.3	80.1± 13.5	0.3±7.4

Abbreviations: Σ4 = Sum of the four skinfold sites (triceps, biceps, subscapular and supra-spinale); RM = repetition maximum; SAL = salmeterol; FOR = formoterol; PLA = placebo. DATA Aare presented at week 0 to week 5. Mean ± SD

4.3.5 Recovery, Sleep and Mood Questionnaires

Recovery-Stress Questionnaire index did not change (p=0.395) across the five-week training period in PLA, SAL or FOR groups (TABLE 4.3).

TABLE 4.3 "REST Q RECOVERY & STRESS INDEX IN SAL, FOR AND PLA"

	SAL			**FOR**			**PLA**		
	Week 3	**Week 5**	**Mean Change**	**Week 3**	**Week 5**	**Mean Change**	**Week 3**	**Week 5**	**Mean change**
Rest Q Recovery Index (A.U.)	2.5 ± 0.1	2.7 ± 0.4	0.2 ± 0.5	2.4 ± 0.2	2.5 ± 0.4	0.1 ± 0.5	2.6 ± 0.3	2.8 ± 0.1	- 0.2 ± 0.1
Rest Q Stress Index (A.U.)	1.9 ± 0.1	1.8 ± 0.2	- 0.1 ± 0.7	1.7 ± 0.1	1.8 ± 0.2	0.1 ± 0.7	1.9 ± 0.1	1.8 ± 0.2	- 0.1 ± 0.4

Date are presented at weeks 3 and 5 and as mean change (Mean ± SD)"

4.4 Discussion

My study suggests that 30 m sprint performance is improved when daily doses of inhaled formoterol or salmeterol are combined with strength, power and sprint training over a five-week period. However, I did not observe significant changes in strength, power, mood, recovery or skinfold thickness between formoterol, salmeterol and placebo over the five-week period. The improved 30 m sprint performance following five weeks of inhaled formoterol or salmeterol administration in our study was similar to previous reports examining acute and long-term use of β_2-agonists. Likewise, administration of oral β_2-agonists enhances muscle strength and peak power output during maximal cycling (Martineau et al., 1992; Le Panse et al., 2005; 2006). Improvements in sprint and power performance from short-term use of β_2-Agonists has been suggested to be as a result of increased skeletal muscle mass and maximal muscle force production, leading to greater initial peak power (Hostrup et al., 2015). Furthermore, high doses of formoterol can augment resting energy expenditure and fat utilization in active males (Lee et al., 2013). However, in some cases where authors report changes in peak power this does not correspond to significant improvements in the mean power produced during a Wingate test (Hostrup et al., 2016). Although Wingate performance is related to sprint performance, the duration of our 30 m sprint was approximately seven seconds compared to the 30-s Wingate challenge. It may be long-term use of formoterol and salmeterol have a greater potential for ergogenic action for explosive sprints lasting under 10 s, compared to longer sprinting activities. Further research is required to confirm this hypothesis.

In my study although I observed sprint performance improvement not supported by changes in strength, power or skinfold thickness. This may due to the smaller therapeutic doses of salmeterol and formoterol use in our study, compared to other studies using supra-therapeutic doses (Hostrup et al., 2014). Although, using skin folds to assess changes in body composition

may not have been sensitive enough to detect changes in muscle mass. By using other means of measuring changes in body composition (e.g. DEXA) I may have detected changes in muscle mass. In a recent study by Jessen et al., (2018) a significant increase in lean body mass of 1.03 – 1.04 kg was observed with DEXA.

Gender differences in pulmonary anatomy may influence the potential ergogenic action of formoterol and salmeterol. Although previous studies that have suggested this may be the case for salbutamol, the hypothesis has not been rigorously investigated (Koch et al., 2015a). Due to the relatively small number of females in my study, my data was underpowered to conduct meaningful sub analyses.

Future studies should investigate the relationship between long acting β_2-agonists administration in male and female athletes and the bio-availability required to stimulate an increase in muscle protein turnover and synthesis between sexes. Previous studies suggest formoterol induces opposing effects between oxidation and synthesis but ultimately results in net anabolic gain because of a greater anti-catabolic effect (oxidation) over reduced synthesis (Lee et al., 2013). In females, these anti-catabolic and synthesis effects were three-fold larger when compared to men (Lee et al., 2013).

Previous studies have demonstrated that the potential ergogenic action of long-term use of β_2-agonists can be confounded by endurance training (Jessen et al., 2018). In my study I specifically focused on strength and power training that has previously been shown not to confound increases in lean mass. We have not investigated whether endurance training would confound the improvements in 30 m sprint performance, which has been previously reported when short acting β_2-agonists have been used over a four to six-week period incorporating endurance training (Dickinson et al., 2014).

Athletes use formoterol and salmeterol to protect against bronchoconstriction. Both drugs have side effects including increased heart rate, headaches, tremors, muscle cramps and palpitations.

It is not known whether athletes using either inhaled formoterol or salmeterol increase the risk of these side effects. However, in our study we did not observe athletes reporting these symptoms throughout the study. Furthermore, we did not see any significant differences between the recovery, sleep and mood between groups parameters to see if salmeterol or formoterol may have influenced perception of recovery between training days. As we have not detected any differences between groups, we can exclude this as a potential mechanism to explain improvements in 30 m sprint performance.

4.4.1 Limitations

A limitation of this study is that I cannot assume the observations I have seen in my participants who take part in recreational sport translate to elite athletes. However, if elite athletes did take part in my study they may have been subject to a doping test, in which they may have provided a urine sample with a concentration of formoterol that is above the permitted level and therefore committed an anti-doping violation. For this reason, I excluded elite athletes from participating. Future studies may incorporate highly trained individuals to investigate whether they experience a similar response to sprint performance following five weeks of inhaling either formoterol or salmeterol.

4.4.2 Conclusions

This study was the first to demonstrate five weeks of therapeutic doses of either inhaled salmeterol or formoterol in combination with strength, power and sprint training may improve 30 m sprint performance. At this stage I am not able to conclude that similar effects will occur in highly trained athletes using similar doses. Therefore, anti-doping stake-holders may wish to commission investigations into whether highly trained athletes experience a similar ergogenic action from inhaled formoterol or salmeterol. These studies should be conducted before changes to the WADA Prohibited List are recommended. However, these findings suggest that consideration should be given to closer monitoring of inhaled long acting β_2-agonists use by athletes in and out of competition. Future research is required to investigate the mechanism behind the potential improvement in sprint performance in both males and females.

Chapter 5. The Effect of 1600 µg Inhaled Salbutamol Administration On 30 Meter Sprint Performance Pre and Post a Yo-Yo Intermittent Running Challenge in football players.

5.1 Background

Athletes with asthma related conditions are permitted to use inhaled salbutamol in accordance with current WADA regulations (WADA, 2019). Although a therapeutic dose of inhaled salbutamol ranges from 200-400 µg, football players are permitted to inhale up to 800 µg in any 12 hours period and 1600 µg in a 24 hour period without a TUE. Limited data is available to explain whether football players may gain an ergogenic advantage from doses of inhaled salbutamol up to 1600 µg.

Recent research by Dickinson et al. (2015) suggests that doses of up to 1600 µg of inhaled salbutamol do not improve repeated sprint performance following a 52-minute football specific treadmill running protocol. However, others have observed improvement in sprint cycling after the inhalation of salbutamol 180 µg (Signorile et al., 1992) and 15 mg terbutaline (Kalsen et al., 2016). The latter authors suggested the improved 10 s sprint cycling performance was associated with a greater fatigue resistance in type II fibres due to increased rates of glycogenolysis and glycolysis. Furthermore, it has been shown when well-trained swimmers inhale a combination of Salbutamol (1600 µg), Formoterol (36 µg) and Salmeterol (200 µg), their 100 m sprint performance is enhanced maybe due to high systemic concentration reach through repeated doses of β_2-agonists (Kalsen et al., 2013). Recently, Hostrup et al., (2016) demonstrated that initial Wingate sprint performance was enhanced after 8 mg oral salbutamol administration but subsequent sprints were not enhanced in the same way. However, in the same study after two weeks daily administration of 8 mg oral salbutamol, a significant effect in sprint performance has been recorded in the first two performances.

Therefore, the ergogenic action demonstrated in the previous studies after acute use of short acting β_2-agonists may improve peak power and one-off sprint performance but there are

limited data to assess whether sprint performance is enhanced with prior inhaled salbutamol after a fatiguing football protocol. The purpose of this study is to investigate the potential ergogenic action of inhaled 1600 μg salbutamol on 30-m sprint performance in football players before and after the Yo-Yo test (Yo-Yo IRT Lev1).

5.2 Methods

5.2.1 Study Participants

Thirteen male (mean ± SD; age 18.1 ± 0.9 years; weight 69.5 ± 8.3 kg; height 178.0 ± 6.5 cm; VO_{2max} 51.2 ± 3.4 mL min-^{1} kg^{-1}) football players volunteered and provided written and verbal informed consent. All players took part in amateur football league, competing once a week and trained specifically for football at least three times a week (mean ± SD; h.week^{-1} = 8 ± 1.4). All participants were free from respiratory disease including asthma related conditions, cardiopulmonary disease, metabolic disease and musculoskeletal injury (TABLE 5.1).

The participants completed all assessments at the same time of the day (within 1 h), separated by a minimum of 48 hours. They were instructed to sleep for at least 7 hours, drink at least 35 mL/kg/day of water, refrain from consumption of alcohol for 24 hours prior to the visit and avoid vigorous exercise on the day before each of the following visits.

Table 5.1**:** Descriptive features of participants (n=13)

Parameter	**Mean ± St.Dev**
Age (years)	18.1 ± 0.9
Height (cm)	178 ± 6.5
Weight (kg)	69.5 ± 8.3
BMI ($kg*m^{-2}$)	22 ± 1.7
VO_{2Max} (mL/min/kg)	51.2 ± 3.4
HR_{Max} (bpm)	198 ± 4.0
Training (h/week)	8 ± 1.4
Rpe_{Max} (u.a.)	9 ± 1.0

Abbreviations: BMI, body mass index; VO_{2Max}, maximal oxygen uptake;

HRmax, maximal heart rate; RPEmax, maximal rating of perceived exertion.

* Training data are presented for 13 male participants.

Data are presented as mean and standard deviation (Mean ± SD)

5.2.2 Experimental Protocol

Participants visited the respective testing facilities on three separate occasions, the first of which was a familiarization session. The final two visits were completed in a single blind random order and separated by two days. On each of these separate occasions players were required to complete three maximal “30-m” sprints followed by the Yo-Yo IRT and followed by a further three maximal “30-m” sprints, performed 5 minutes after the cessation of the Yo-Yo IRT.

Fifteen minutes prior to the initiation of the first three 30 m sprints players inhaled one of the following treatments, via a pocket chamber:

Treatment 1: 16 inhalations of placebo (containing water vapor) (PLA)

Treatment 2: 16 inhalations of 100µg salbutamol (Ventolin Evohaler- GlaxoSmithKline, UK) (SAL)

The order of PLA and SAL was randomized and counterbalanced by an order generated by online software (randomization.com).

5.2.3 Sprint Test

Before the assessments all participants completed the 11+ warm-up (Bizzini and Dvorak 2015) concluding with standardised mobility and flexibility exercises. After the warm up football players completed three 30 m maximal sprint with 60 s passive recovery time between each sprint (Taskin, 2008). Five minutes following the end of the Yo-Yo IRT, participants completed a further three 30 m sprints with a 60 s active recovery between each one.

Consistent verbal encouragement was given to the players during each sprint. Players were encouraged to complete the sprints as fast as they could. In order to record the time to cover the distance, a system of light gates (Brower Timing, Fairlee, Vermont, USA) were utilised. Heart rate (HR) was recorded throughout the sprints with the heart rate monitor (Polar m400 HR, Polar Oy Finland); peak HR being noted for each condition during the intermittent football test. The mean time of the three efforts and the best sprint time were recorded. At the end of each set of three sprints a drop of blood (0.5 µL) was collected from the ear to obtain a capillary blood lactate sample at the end of the third sprint for the analysis of blood lactate concentration (Lactate Pro, Arkray KDK, Japan).

5.2.4 Yo-Yo test (Yo-Yo IRT)

The Yo-Yo IRT intermittent recovery test consisted of repeated 2 x 20 m shuttle runs at a progressively increasing speed controlled by audio bleeps (Krustrup et al. 2003). Between each running bout the players had a 10 s active rest period, in a space of 5 m where they are encouraged to walk or jog. Following the completion of each stage players were asked to provide a rating of perceived exertion on a scale between 6 and 20 (Borg, 1982). When the players failed on two occasions to complete the 2 x 20 m within the required time the test was concluded and the distance covered was recorded.

The reproducibility of the Yo-Yo IRT was tested and repeated within a week with a coefficient of variation (CV) around 4.9% (Krustrup et al., 2003). Heart rate was recorded during the entire fitness trial to monitor cardiovascular response induced by test.

5.2.5 Motivation and Mood Questionnaires

Before each visit participants completed questionnaires to measure motivation to train and mood via the Brunel Mood Scale and Matthews questionnaire (Matthews et al., 2001; Terry et al., 2003). The Brunel Mood Scale (BRUMS) was used to quantify current mood ("How do you feel right now?") at baseline, before the 30 m sprint (pre-sprint) and post-sprint. The questionnaire included 24 items (e.g. "angry, uncertain, miserable, tired, nervous, energetic") divided into six subscales: anger, confusion, depression, fatigue, tension, and vigour. Each item was answered on a five point scale (0 = not at all, 1 = a little, 2 = moderately, 3 = quite a bit, 4 = extremely), and each subscales, with the respective relevant items, was calculated with a raw score in the range of 0–16. For this experiment, only scores for the fatigue and vigour subscales were considered as subjective ratings of fatigue.

Motivation related to Sprint and Yo-Yo test was measured using the success motivation and intrinsic motivation scales (Matthews et al., 2001). Each scale consisted of 7 items scored from 0 to 4 points (0 = not at all, 1 = a little bit, 2 = somewhat, 3 = very much, 4 = extremely). Motivation questionnaire was administered immediately before the 30 m sprint test.

5.2.6 Statistical Analysis

All data are presented as mean ± SD unless otherwise stated. Assumptions of statistical tests for normal distribution and sphericity of data were checked as appropriate and Greenhouse-Geisser correction to the degrees of freedom was applied when violations to sphericity were found. Repeated measures ANOVAs were used to determine the effect of time (Sprint Pre vs Sprint Post) and condition (SAL vs. PLA) on peak power, mean power and highest speed reached during 30-m sprint. This same analysis was adopted to investigate vigor (pre-treatment vs. post-treatment), motivation (pre-treatment) and each physiological and perceptual parameter (Heart rate, Blood Lactate and RPE) at iso-time [end of warm-up (0 min) and each Yo-Yo IRT level completed].

Data from the Yo-Yo IRT1, heart rate and RPE were analysed from the start of the test (level 5) up to the end of level 16; values recorded beyond this point were considered as the highest point reached at exhaustion, as football players began to drop out of the test from level 16 onwards. Paired T-tests were used to compare motivation scales between the two conditions. Significant interactions were followed up with Bonferroni tests as appropriate. Significance was set at 0.05 (2-tailed) for all analyses and effect sizes for the repeated measures ANOVAs were calculated as partial eta squared (η^2p), using the small = 0.02, medium = 0.13 and large = 0.26 interpretation for effect size. All data analysis was conducted using the statistical packages for social science (SPSS, version 21, IBM, U.S.).

5.3 Results

5.3.1 Subjects

All thirteen football players completed the Yo-Yo IRT test and the sprint assessment in both conditions, PLA and SAL. None of the participants reported unpleasant symptoms such as tachycardia, nausea, headache or sleep disturbance after inhaling 1600 µg salbutamol; none of the participants could correctly distinguish the difference between the PLA and SAL conditions.

5.3.2 30 m Sprint Pre and Post Yo-Yo IRT

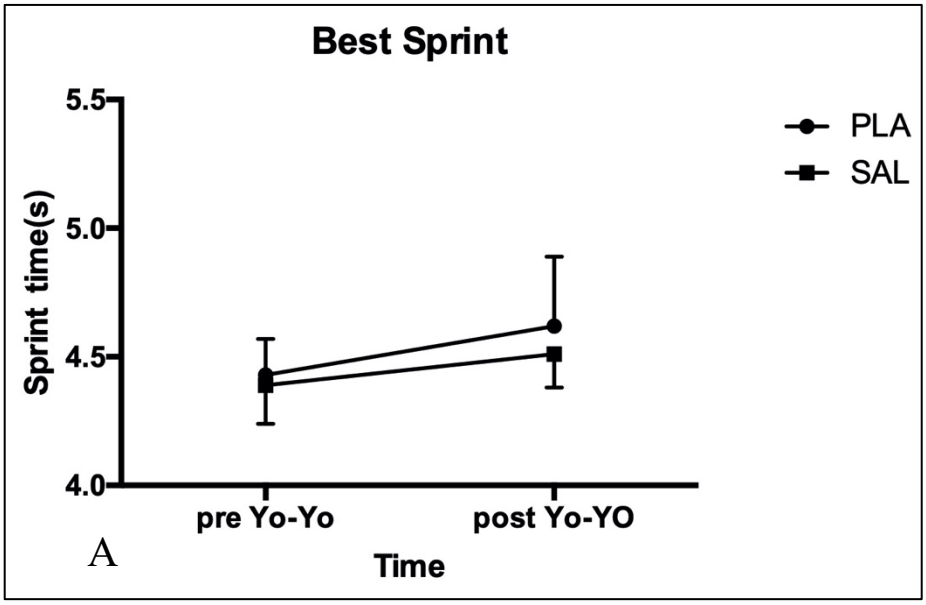
30 m sprints conducted pre and post Yo-Yo IRT demonstrated no difference between PLA and SAL conditions for best sprint time pre (PLA 4.43 $\pm$ 0.14 s; SAL 4.44 $\pm$ 0.15 s);post (PLA 4.57 $\pm$ 0.12 s; SAL 4.52 $\pm$ 0.14 s); p=0.753 $\eta^2p = 0.008$; FIGURE 5.1/A). Mean sprint time pre (PLA 4.46 $\pm$ 0.12 s; SAL 4.45 $\pm$ 0.15 s);post (PLA 4.55 $\pm$ 0.16 s; SAL 4.52 $\pm$ 0.13 s); p=0.152, η^2p = 0.163; FIGURE 5.1/B), highest speed reached pre (PLA 24.47 $\pm$ 0.65 s; SAL 24.66 $\pm$ 0.76 s);post (PLA 24.56 $\pm$ 0.34 s; SAL 24.81 $\pm$ 0.49 s); p=0.859, η^2p= 0.003; FIGURE 5.1/C).

There is no interaction for best sprint time (p = 0.754; η^2p = 0.008). Follow-up test revealed that best sprint time increased significantly from pre to post Yo-Yo IRT (p = 0.000; η^2p = 0.583) without difference in condition (p = 0.233; η^2p = 0.193).

There is no interaction for mean time (p = 0.152; η^2p = 0.163). Follow-up test revealed that mean sprint increased significantly from pre to post Yo-Yo IRT (p = 0.000 η^2p = 0.668) without difference in condition (p = 0.180; η^2p = 0.145).

There is no interaction for highest speed reached ($p = 0.859$ $\eta^2p = 0.003$). Follow-up test revealed that highest speed reached decreased significantly from pre to post Yo-Yo IRT ($p = 0.000$ $\eta^2p = 0.673$) without difference in condition ($p = 0.250$ $\eta^2p = 0.108$).

FIGURE 5.1 (A) "Best Sprint at pre and post Yo-Yo IRT in SAL and PLA"



Data is presented by Mean and SD

FIGURE 5.1 (B) "Mean Sprint at pre and post Yo-Yo IRT in SAL and PLA"

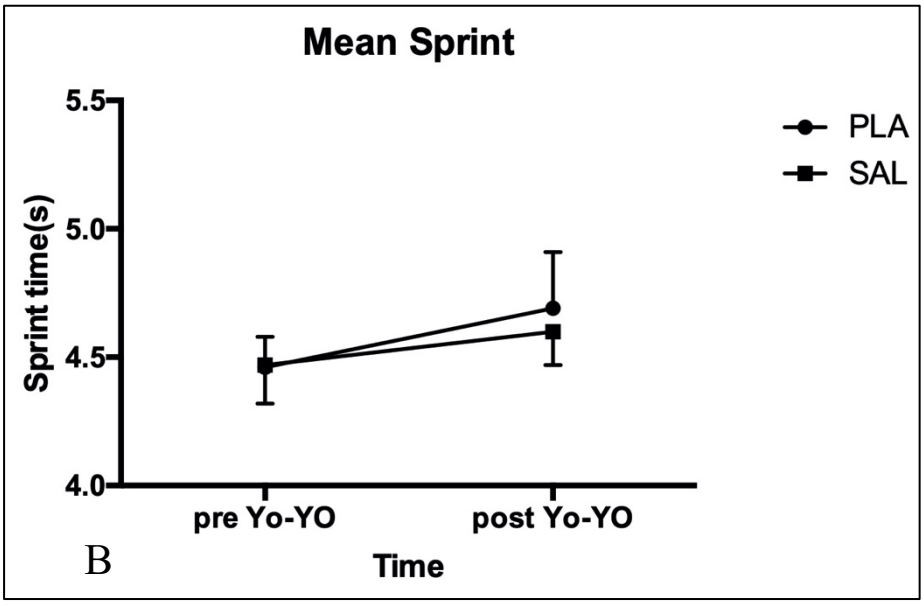
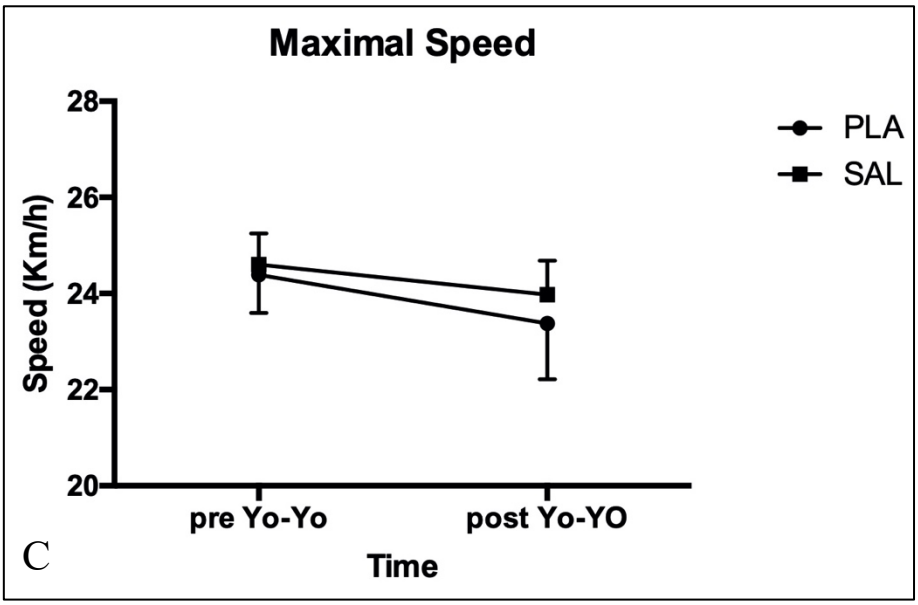


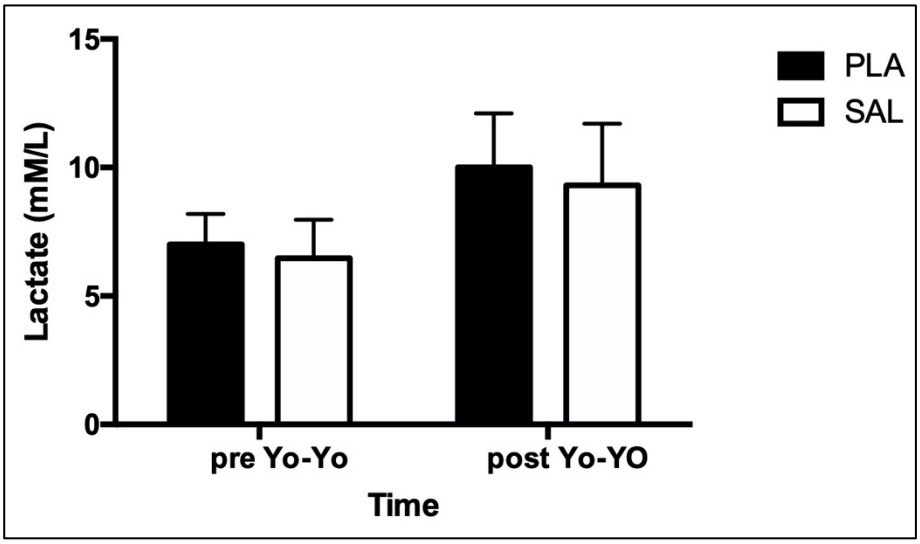
FIGURE 5.1 (C) “Maximal Sprint at pre and post Yo-Yo IRT in SAL and PLA”



Data is presented by Mean and SD.

Blood lactate following the pre yo-yo test sprints was not different between SAL and PLA (6.47 ± 1.5 mM/L vs.7.0 ± 1.2 mM/L; p=0.450, $\eta^2p = 0.021$). The post Yo-Yo test sprint lactate values were not different between SAL and PLA conditions (9.3 ± 2.4 mM/L vs.10.0 ± 2.1 mM/L; p=0.882, $\eta^2p = 0.007$; FIGURE 5.2).

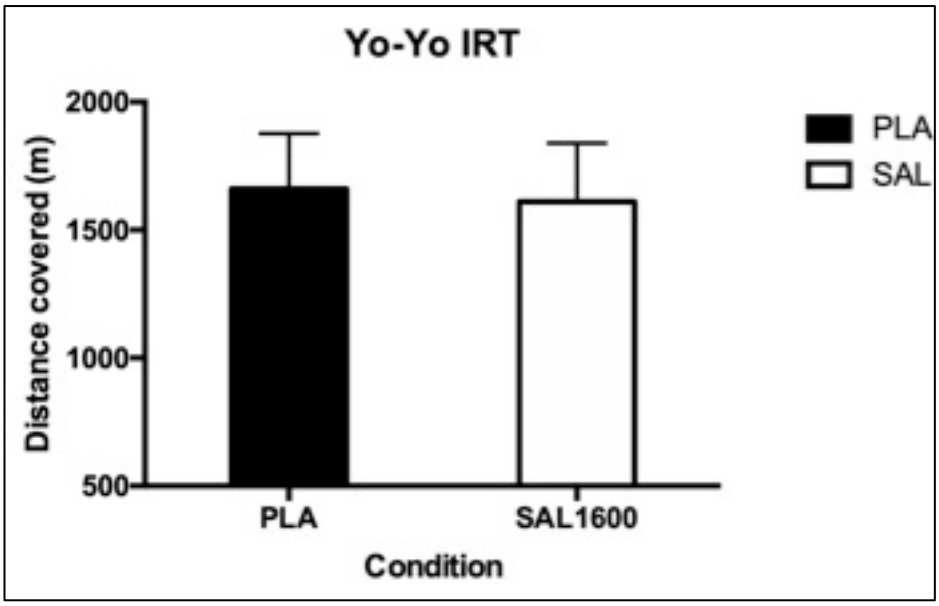
FIGURE 5.2. “Lactate values collected after sprint pre Yo-Yo IRT and after sprint post Yo-Yo IRT in SAL and PLA”



5.3.3 Yo-Yo Intermittent Recovery Test

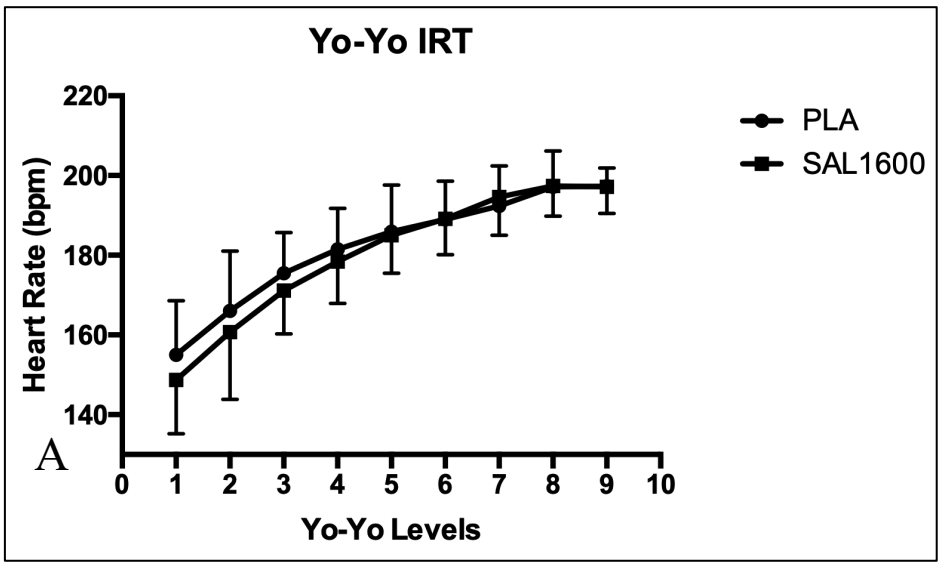
The distance covered during the Yo-Yo IRT did not differ between PLA (1660 ± 217 m) and SAL (1610 ± 229 m), p=0.153, η^2p =0.224; (FIGURE 5.3).

FIGURE 5.3 "Distance covered by SAL and PLA during the Yo-Yo IRT"



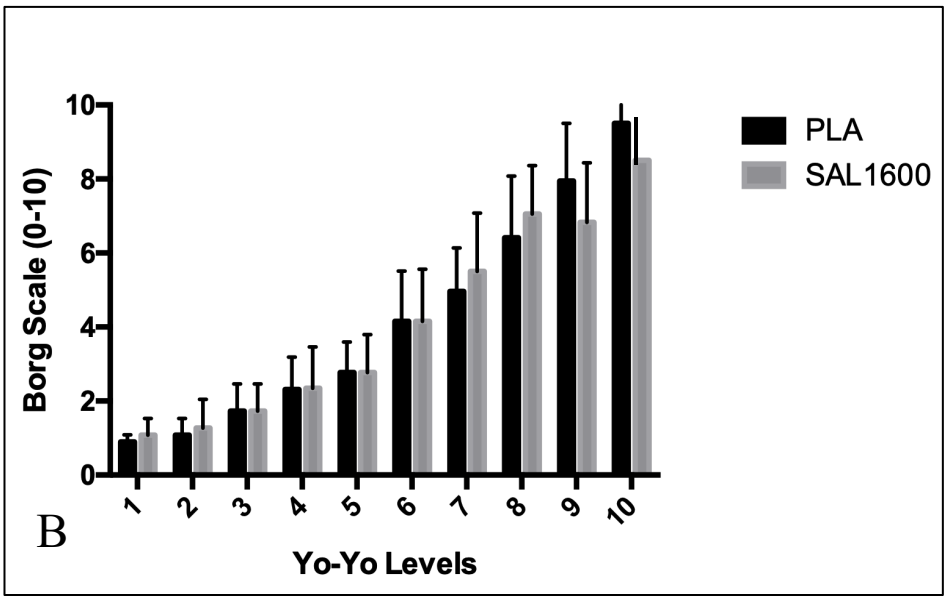
No significant changes were observed between PLA and SAL during the Yo-Yo IRT for HR (p=0.102; η^2p =0.095) or RPE (p=0.195; η^2p =0.134). HR was not different between PLA and SAL at any level of the Yo-Yo IRT (FIGURE 5.4).

FIGURE 5.4 (A) "Heart rate response at the end of each Yo-Yo IRT level completed by all participants of SAL and PLA"



RPE was not different between PLA and SAL at any level of the Yo-Yo (FIGURE 5.4/B).

FIGURE 5.4 (B) “RPE response at the end of each Yo-Yo IRT level completed by PLA and SAL”



Data is presented by Mean and SD

5.3.4 Mood and Motivation Questionnaire

The mood questionnaire revealed a significant decrease in vigour over time in both conditions (PLA pre 5.7 ± 0.6 UA, PLA post 3.6 ± 0.4 UA; SAL pre 5.4 ± 0.3 AU, SAL post 3.3 ± 0.5 AU); ($p = 0.01$) with no main effect of condition ($p = 0.49$, $\eta^2p = 0.01$) or condition × time interaction ($p = 0.29$, $\eta^2p = 0.17$; TABLE 5.2). No differences were found for intrinsic motivation ($p = 0.62$, $\eta^2p = 0.09$) and motivation to succeed the task (PLA 5.1 ± 0.4 AU; SAL 5.3 ± 0.5 AU); ($p = 0.78$; $\eta^2p = 0.02$) (TABLE 5.2).

TABLE 5.2 “Vigour at pre and post-test and Motivation at pre- test in SAL and PLA”

	SAL		**PLA**	
	Pre	**Post**	**Pre**	**Post**
BRUMS vigour (A.U.)	5.4 ± 0.3	3.3 ± 0.5	5.7 ± 0.6	3.6 ± 0.4
Motivation (A.U.)	5.3 ± 0.5		5.1 ± 0.4	

Abbreviations: A.U. = arbitrary units; SAL = salbutamol; PLA = placebo;

Data are presented as (Mean ± SD)

5.4 Discussion

My study suggests that non-asthmatic football players who inhale 1600 μg of salbutamol do not experience improvements in 30 m sprint performance pre or post the Yo-Yo IRT test. Furthermore, Yo-Yo IRT performance, HR, lactate or RPE are not altered as a result of prior inhalation of 1600 μg of salbutamol.

My results are similar to previous findings from my research group (Dickinson et al., 2015), where we did not observe any significant change in repeated sprints following a running protocol that simulated one half of a football match. The available data from previous investigations has been analysed in a systematic review and meta-analysis (Pluim et al., 2011), in which the authors concluded that endurance performance was not influenced by supra-therapeutic doses of inhaled salbutamol (up to 800 μg). Additionally, the inhalation of β_2-agonists may blunt the enhancing effects induced by high intensity interval training on VO2Max following repeated adrenergic fight-or-flight response provoked by high systemic

levels of β_2-agonists as showed by several studies (Hostrup et al., 2018; Hostrup et al., 2014; Sanchez et al., 2012; Dickinson et al., 2014; Molphy et al., 2018).

Furthermore, high intensity training (HIT) seems to blunt hypertrophy and changes in expression of sarcoplasmic reticulum Ca^{2+} ATPase isoforms (Hostrup et al., 2018). This mechanism remains to be interpreted but may be related to the fact that endurance training impedes with the growth-promoting signaling (Coffey & Hawley, 2017) induced upon β_2-adrenergic prompt (Koopman et al., 2010).

The improvements seen in other studies with regard to sprint and power performance could be due to drug ability to stimulate a receptor rather than the amount of drug administered (Baker, 2010). In regard to this, inhaled terbutaline may lead to same systemic concentration of doses administered orally with less side effects and prolonged drug persistence (Elers et al., 2012).

Therefore, the absence of ergogenic action found in this study could be a summation of factors: the type of drug and the dosage used in our studies compared to other studies. While acute doses of inhaled salbutamol up to 1600 μg seem not to enhance 30-m sprint and Yo-Yo IRT performance, 15 mg of inhaled terbutaline increases 30-s sprint peak and mean power by 3% in recreational athletes (Hostrup et al., 2014). High doses of inhaled terbutaline (15 mg) have also been shown to improve 10 s cycling sprint performance in moderately trained cyclists with mean (8.3% ± 1.1%) and peak (7.8% ± 2.5%) power greater with terbutaline than with placebo (Kalsen et al., 2016). This suggests that supra-therapeutic doses of some inhaled short acting β_2-agonists (e.g. 15 mg of inhaled terbutaline) lead to significantly greater systemic availability and therefore greater potential for ergogenic effects. However, adverse analytical finding (AAF) may be dependent on the form of short acting β_2-agonists inhaled and the dose administered.

Blood lactate is an indirect marker of muscle glycolysis; in our study I did not find a difference between PLA and SAL conditions. It has previously been reported that 800 μg dose of inhaled salbutamol can lead to detectable systemic concentrations of 10.95 ng/mL in healthy subjects (Elers et al., 2012; Elers et al., 2012a). Notwithstanding, in my study I was not able to direct measure the concentration of salbutamol in the blood or in urine as I did not have the resources available during the study. I can therefore not assume that a dose of 1600 μg inhaled salbutamol was a sufficient dose to lead to high levels of salbutamol in the systemic circulation.

In my study I advised participants how to use inhaler and observed them using the inhaler but I did not train. Future studies may also benefit from thoroughly train inhaler technique with their participants to improve the delivery of salbutamol into the lower airways.

During this study I did not have the resources available to collect urine samples from the footballers to evaluate urinary levels of salbutamol, this would have allowed us to examine any inter-subject variation that could have brought urinary levels above 1000 $ng.ml^{-1}$ threshold for an AAF, cut-off limit allowed by WADA (WADA, 2019).

However, some of my group previous findings (Dickinson et al., 2015) suggest the acute inhalation of supra-maximal doses of salbutamol (up to 1600 μg) along-side acute loss of body mass of up to 5% can lead to individuals presenting with a urinary salbutamol concentration above 1200 $ng.ml^{-1}$. Haase et al., (2016) confirmed that dehydration can significantly affect the concentration of salbutamol in urine samples. Based upon Dickinson et al., (2015) and Haase et al., (2016) results, WADA now has adjusted its methods to analyse the concentration of salbutamol with dehydration state of the athletes (WADA, 2019).

The trained status of the athlete may impact on the availability β_2-adrenoceptor activity. It could be speculated that β_2-adrenoceptors content might be lower in athletes being translated in a more prominent β_2-adrenoceptors stimulation that would bring to improved endurance

performance (Van Baak et al., 2004). In addition, it was observed that muscle mass was a confounder of the response to salbutamol administration because the more trained the participants were the lower the response (Hostrup et al., 2018). My participants were trained amateur athletes, and I may have seen different result if I had recruited less well training recreational football players.

5.4.1 Conclusion

This study demonstrates inhalation of 1600 µg salbutamol does not improve 30 m sprint performance in trained amateur football players. Furthermore, my findings suggest that acute inhalation of 1600 µg salbutamol will not lead to improvements in Yo-Yo IRT performance in football players. Although 1600 µg of inhaled salbutamol is greater than the current permitted dose (800 µg) by WADA regulations (WADA, 2019), my study suggests footballers using inhaled salbutamol for asthma therapy within the doses permitted WADA regulations will not experience an ergogenic action in either their sprint or endurance performance.

Chapter 6. Acute Administration of Salmeterol Improves Sprint Performance Following Variable Cycling Trial.

6.1 Background

Several studies suggest no ergogenic action is provoked by the inhalation of short-acting β_2-agonists (SABA) in endurance performance (Pluim et al., 2011; Koch et al., 2015a; Dickinson et al., 2014). However, studies using long-acting β_2-agonists (LABA) showed controversial results when time to exhaustion was investigated in athletes inhaling formoterol (Stewart et al., 2002) and salmeterol (Carlsen et al., 1997) when compared to the placebo.

When sprint performance was tested after the inhalation of high doses of LABA, several studies showed an ergogenic effect on performance (Kalsen et al., 2013; 2016). However, at low doses, LABA seems to not be effective to increase sprint performance (Stewart et al., 2002). Contrary to previous results, salmeterol inhaled before performing 30-s all-out sprint showed no changes in performance (McDowell et al., 1997). SABAs daily administration in concomitance with strength and endurance training did not show performance improvements (Dickinson et al., 2014). Previous studies were performed mainly in a non-fatigued state. Dickinson et al., (2015), instead, investigated the effect of 1600 μg salbutamol inhalation on repeated sprints in fresh and fatigued conditions. The authors did not find any difference in the sprint performance probably due to the effect of the fatiguing protocol that negatively impacted the potential benefit of salbutamol on sprint and repeated sprint performance.

The importance to test the effect of any ergogenic aids not only in fresh states but also in fatigued ones is prominent in elite sport. To cite some examples, cycling race is made of high-power efforts occurring during the start, steep climbing and at the final part of the stage in preparation of the way for the final sprint. Thus, the capacity to deal with increased levels of fatigue accumulated during the race is an important aspect to be considered (Menaspà et al., 2015). Due to lack of studies in the literature miming the condition experienced by cyclist at the end of a stage characterised by high levels of fatigue, the aim of this study was to observe

the effect of salmeterol administration during sprints performed before and after a "race simulation" incremental test with attention on muscle activity responses. The hypothesis of this study is that salmeterol administration could increase the power produced during the first 12-s sprint and limiting the effect of fatigue development occurring during variable cycling test and during the subsequent 12-s sprint.

6.2 Methods

6.2.1 Study Participants

Sixteen male road cyclists (mean ± SD, age 25 ± 1.7 y, height 180 ± 6 cm, body mass 75.7 ± 8.4 kg, VO_{2Max} 64.6 ± 6.9 mL• kg^{-1} min^{-1}, peak power output (PPO) 419 ± 38 W, > 5 training sessions per week, > 400 km per week, > 5 years of cycling experience) were recruited. Taking into account PPO and training history cyclists were classified as level 4 (De Pauw et al., 2013). Participants were free form medication and demonstrated the absence of asthma and exercise induced bronchoconstriction (EIB) through a negative eucapnic voluntary hyperventilation (EVH) challenge. The study procedures were approved by the local research ethics committees between the University of Kent (UK) and the university of Verona (IT) and follow the ethical principles for medical research involving human subjects set by the World Medical Association Declaration of Helsinki. Participants signed the informed consent and were provided with written instructions outlining the study`s procedures but were not informed of its aims.

6.2.2 Experimental Protocol

A single-blind, crossover, counterbalanced and randomised, using web-based computer program (www.randomization.com), design was used in which participants visited the laboratory on four separate occasions (EIB test, incremental cycling test and two visits with change in treatment administration).

Before starting to warm-up participants inhaled 100 µg salmeterol (2 x 50 µg Serevant, Accuhaler, GSK, Uk) (SAL condition) or placebo (PLA) containing water vapor.

6.2.3 Broncho-provocation Challenge

First visit aimed to investigate the presence of EIB using forced expiratory volume per second (FEV_1) measurement.

6.2.4 $VO2_{Max}$ Test

During the second visit participants completed an incremental cycling exercise test (2 min at 50 W + 50W/ 2 min) until voluntary exhaustion or until pedal frequency fell below 60 revolutions/min (RPM) for more than 5 s. This test was performed on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). PPO and maximal oxygen consumption (MetaLyzer 3B, Cortex Biophysik, Leipzig, Germany) were recorded.

6.2.5 Sprint Familiarisation

Five minutes after completion of the incremental test, the ergometer was switched to isokinetic mode constrained to 110 rpm and participants (after a short warm-up) performed a 12-s all-out sprint to familiarize with the one performed during visit 3 and 4. The instantaneous power generated during the sprints was corrected for changes in kinetic energy and averaged over 1-s intervals, taking into account the work executed in accelerating the flywheel.

6.2.6 "Non-fatigued" Sprint Test

One hour following the inhalation, subject began to warm up performing five minutes free-cadence 100 watts warm-up succeeded by three 12-s warm-up sprints performed at 25%, 50%

and 75% of the maximal perceived effort with 0.5 to 5 min of active recovery between each sprint in accordance to Coelho et al., (2015). Afterwards, they performed a 12-s all out maximal sprint (Pre). After the maximal sprint a drop of blood was collected from the right earlobe for the analysis (Lactate Pro LT-1710, Arkray, Shiga, Japan) of lactate and participants began an hour variable cycling trial (VCT) executed on individual power based on the previous incremental test.

6.2.7 Variable Cycling Test

Variable cycling trial power was structured to mimic a cycling race competition of similar duration as proposed by Menaspà and colleagues (2015). However, we modified the power to allow cyclist to match the cycling intensities according with their levels. Variable cycling test (VCT) was as follow: 20 minutes at 40% of personal PPO, 15 minutes at 45%, 15 minutes at 55%, 5 minutes at 65%, 4 minutes at 75% and one minute (59^{th}) performed at 95% of personal PPO. At end of min 35^{th} and 59^{th} blood lactate was collected. Pedal frequency (freely chosen between 80 and 100 RPM), heart rate (Polar S610i, Polar Electro Oy, Kempele, Finland) and RPE (6-20 Borg`s scale) were measured at end of min 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and at completion of the VCT.

6.2.8 "Fatigued" Sprint Test

Immediately after completion of the VCT, bike was switched in isokinetic mode and participants were asked to produce an all-out maximal 12-s sprint (Post) simulating the final sprint of a cycling race. Verbal encouragements were given (in both Pre and Post sprints) from a researcher blind to testing conditions. At completion of the sprint, blood lactate was collected. Peak power and mean power were measured at the end of each sprint. Surface EMG was measured during the sprints in the vastus lateralis of the left limb. Skin preparation,

position and orientation of the array were obtained following criteria described by Lenti et al., (2010). The root-mean squared (RMS) EMG values from the muscle were filtered analyzed according to Coelho et al., (2015). Peak RMS (highest RMS value during the sprint) and mean RMS (the average value over the whole sprint) were computed and used for the statistical analysis. A normalization procedure was adopted to compare experimental EMG results collected from different subjects or days within subjects allowing to access the relative level of activation of a given muscle by expressing the entire amplitude of the signal measured during the task as a percentage of a important reference EMG rate (Hsu et al, 2016).

6.2.9 Motivation Questionnaire

Motivation related to Sprint and Time-Trial test was measured using the success motivation and intrinsic motivation scales (Matthews et al., 2001). Each scale consisted of 7 items scored from 0 to 4 points (0 = not at all, 1 = a little bit, 2 = somewhat, 3 = very much, 4 = extremely). Motivation questionnaire was administered immediately before the inhalation undergone by the subjects before the warm-up.

6.2.10 Statistical Analysis

All data are presented as mean ± SD unless otherwise stated. Assumptions of statistical tests for normal distribution and sphericity of data were checked as appropriate and Greenhouse-Geisser correction to the degrees of freedom was applied when violations to sphericity were found. Fully repeated 2 x 2 ANOVAs were used to determine the effect of time (Pre vs Post sprint) and condition (SAL vs. PLA) on peak power and mean power during cycling sprint. Similarly, assessment was completed for peak-RMS and mean-RMS. Fully repeated 2 X 13 ANOVAs were used to determine effect of condition (SAL vs. PLA) on RPE, HR and RPM during the time-trial. Fully repeated 2 X 4 ANOVAs were employed to assess effect of

condition on blood lactate concentration at different time points. Paired T-tests were used to compare motivation scales between the two conditions. Significant interactions for ANOVA's was followed up with Bonferroni tests as appropriate. If significant interactions were not found, most relevant main effects are reported. Significance was set at 0.05 (2-tailed) for all analyses. The effect sizes for the repeated measures ANOVAs were calculated as partial eta squared (η^2p), using the small = 0.02, medium = 0.13 and large = 0.26 interpretation for effect size. All data analysis was conducted using the statistical packages for social science (SPSS version 21).

6.3 Results

6.3.1 Participants Characteristics

All sixteen cyclists completed the sprint test and the time-trial assessment in both conditions, PLA and SAL. None of the participants reported unpleasant symptoms such as tachycardia, nausea, headache or sleep disturbance after inhaling 100 μg salmeterol; none of the participants could correctly distinguish the difference between the PLA and SAL conditions.

6.3.2 Motivation questionnaire

Motivation did not change between the two conditions in PLA (3.27 ± 0.25 A.U.) and SAL condition (3.41 ± 0.32 A.U.); ($p = 0.425$, $\eta^2p = 0.049$).

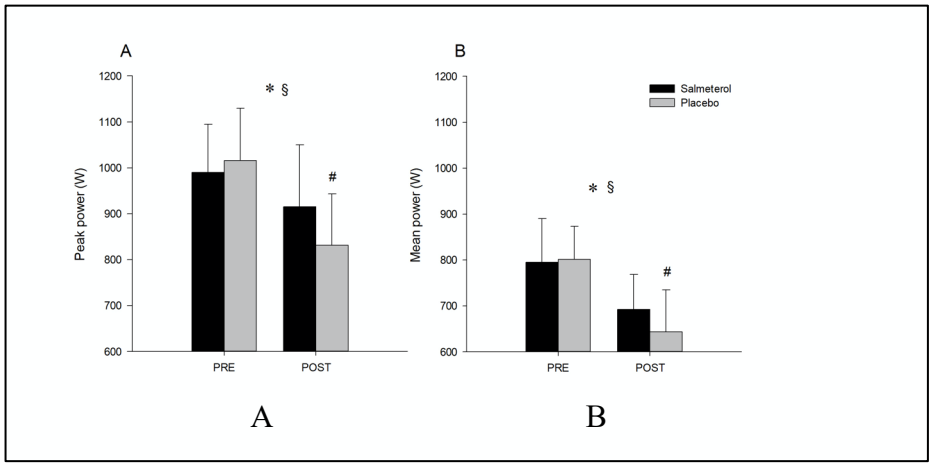
6.3.3 Sprint Power Analysis

There was a significant time x condition interaction for peak power during the sprint cycling ($p = 0.016$, $\eta^2p = 0.424$). Follow-up tests revealed that peak power decreased significantly overtime from Pre to Post Variable cycling trial in both conditions (FIGURE 6.1A); ($p < 0.001$, $\eta^2p = 0.709$), however the decrease was significantly higher ($p = 0.040$, $\eta^2p = 0.330$) in the PLA condition (Pre =1016 ± 114 W – Post = 831 ± 112 W) compared to the SAL condition

(Pre = 990 ± 105 W– Post = 915 ± 135 W).

Similarly, a significant interaction time x condition was found for mean power (FIGURE 6.1B); ($p = 0.010$, $\eta^2p = 0.470$). Decrease in mean power from Pre to Post Variable cycling trial was significant in both conditions ($p < 0.001$, $\eta^2p = 0.766$). However, mean power was reduced significantly more ($p = 0.047$, $\eta^2p = 0.313$) in in the PLA condition (Pre = 801 ± 72 W – Post = 643 ± 92 W) compared to the SAL condition (Pre =795 ± 95 W – Post = 692 ± 76 W);(FIGURE 6.1A; FIGURE 6.1B).

FIGURE 6.1 "Effect of treatment on Peak Power Output (A) and Mean Power Output (B)"



(#) Significant main effect of time ($p < 0.05$); (*) Significant condition X time interaction ($p < 0.05$); ($) and simple main effect of condition according to Holm-Bonferroni method.

6.3.4 Sprint RMS Analysis

No significant condition x time interaction was found for peak-RMS during the cycling sprint tests ($p = 0.566$, $\eta^2p = 0.021$). No significant main effect of condition was found ($p = 0.090$, $\eta^2p = 0.170$) while a significant main effect of time was detected as peak-RMS decreased overtime ($p = 0.015$, $\eta^2p = 0.319$). No significant condition x time interaction was found for mean-RMSs during the cycling sprint tests ($p = 0.601$, $\eta^2p = 0.280$). No significant main effect

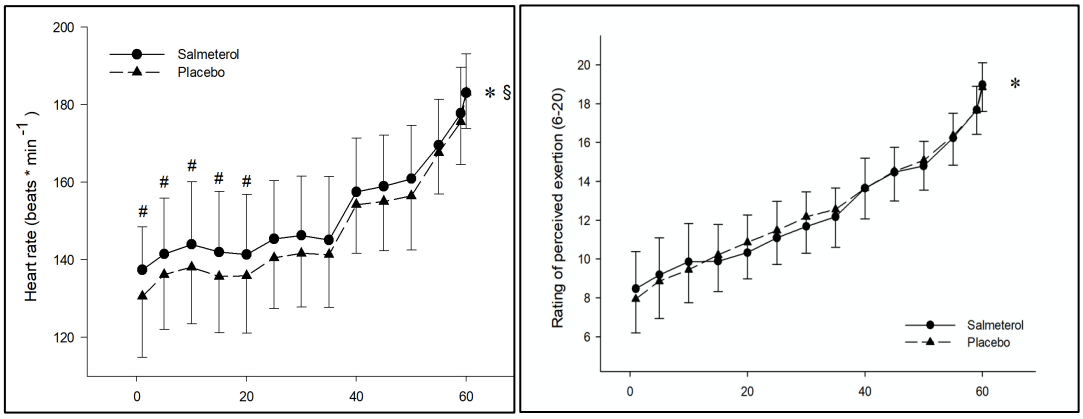
of condition was found ($p = 0.364$, $\eta^2p = 0.83$) while a significant main effect of time was detected as mean-RMS decreased overtime ($p = 0.035$, $\eta^2p = 0.372$).

6.3.5 Variable Cycling Trial Perceptual and Physiological Analysis

There was a significant condition x time interaction for heart rate during the variable cycling trial ($p = 0.004$; $\eta^2p = 0.211$); (FIGURE 6.2A);

Follow-up tests revealed that heart rate increased significantly overtime during time trial in both conditions ($p < 0.001$, $\eta^2p = 0.929$), however the increase was significantly higher in the salmeterol condition at min 1 ($p = 0.041$), 5 ($p = 0.049$), 10 ($p = 0.043$) 15 ($p = 0.038$) and 20 ($p = 0.026$) compared to the control condition. No significant condition x time interaction was found for RPE during the variable cycling trial (FIGURE 6.2B); ($p = 0.323$, $\eta^2p = 0.069$). No significant main effect of condition was found ($p = 0.802$, $\eta^2p = 0.004$) while a significant main effect of time was detected as RPE increased overtime ($p < 0.001$, $\eta^2p = 0.933$; FIGURE 6.2).

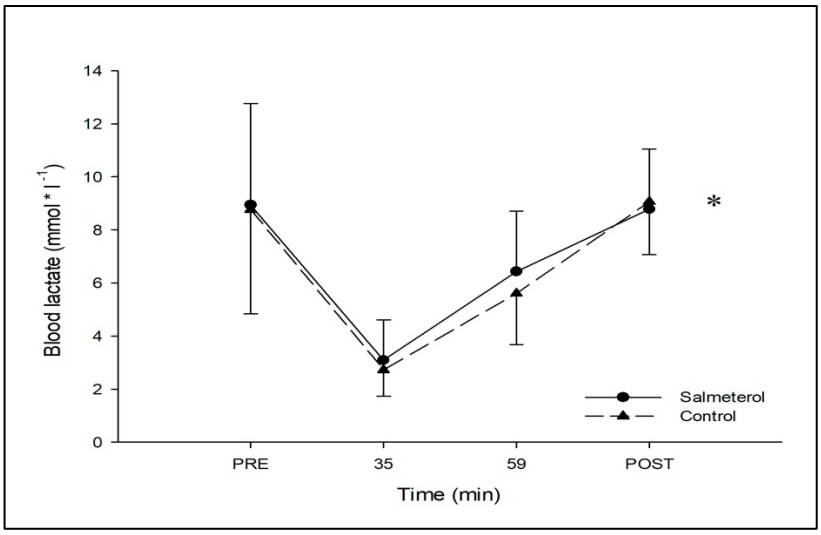
FIGURE 6.2 "Effect of treatment on physiological and psychological responses during Variable Cycling Trial". Heart rate (left) and RPE (right).



(#) Significant main effect of time ($p < 0.05$); (*) Significant condition X time interaction ($p < 0.05$) ($) and simple main effect of condition according to Holm-Bonferroni method. Data presented as means ± SD.

No significant condition x time interaction was found for Blood Lactate during the sprints and the time trial tests ($p = 0.48$, $\eta^2p = 0.049$). No significant main effect of condition was found ($p = 0.54$, $\eta^2p = 0.028$) while a significant main effect of time was detected as blood lactate changed overtime ($p < 0.001$, $\eta^2p = 0.724$; FIGURE 6.3). No significant interaction ($p = 0.538$; $\eta^2p = 0.048$) or main effect of condition ($p = 0.653$; $\eta^2p = 0.022$) or time ($p = 0.712$, $\eta^2p = 0.038$) was found for pedal frequency.

FIGURE 6.3 “Effect of salmeterol on blood lactate after 12-s sprint and during Variable Cycling Trial in SAL and PLA”.



(*) Significant main effect of time ($p < 0.05$). Data presented as means ± SD.

6.4 Discussion

My data suggests an acute 100 µg dose of inhaled salmeterol off-sets fatigue by improving all out 12-s sprint performance after a one-hour variable cycling trial through changes in power output. Similar sprint improvements were obtained in Chapter 4 aimed to investigate the effect of chronic β_2-agonists administration in conjunction with strength training programme on running performance in recreational athletes. The authors showed a significant reduction in time needed to cover 30-m running distance in the group undertaking LABAs formoterol and salmeterol when compared with placebo group.

Inhaled β_2-agonists seems to counteract fatigue development as power output recorded during the final sprint at post was significantly higher (PPO + 11.4 % and MPO + 6.8%) in salmeterol condition than in placebo condition. The only study aimed to evaluate the effect of salmeterol inhalation on sprint performance was conducted by McDowell (McDowell et al., 1997). According to the authors, after 42µg salmeterol administration participants did not record performance improvement during 30-s maximal sprint. Contrary to these results, my study showed a higher power output during a 12-s sprint post one-hour variable cycling trial under salmeterol condition. One explanation can be that the effect of salmeterol is more visible during fatigue states similarly to the results coming from animal studies where an augmented Ca^{2+} release from the SR was observed after continuous tetanic stimulation (Cairns & Dulhunty, 1994). Alternatively, it could be explained by the doses used by McDowell (1997) that were lower compared to the current study (42 µg and 100 µg respectively).

Moreover, the study designs are different as in the previous study after the inhalation participants were requested to perform 12-s sprint before and after one-hour variable cycling test, whereas McDowell (1997) only performed a 30-s sprint 3 hours after the inhalation of 42 µg salmeterol dose.

Both studies did not show any improvement of salmeterol inhalation in sprint performed in a rested condition. In contrast to McDowell et al., (1997), Kalsen et al., (2016) reported a positive effect in the power output when LABA formoterol was administered to recreational participants during 30-s cycle-ergometer sprint test. Although measured in fatigued state, our findings are similar to previous investigations that have demonstrated higher force or power production following acute and chronic supplementation of β_2-agonists (Caruso et al., 1995; Martineau et al., 1992; Van Baak et al., 2000).

It has been proposed that the administration of β_2-agonist has the potential to stimulate the central nervous system function by increasing voluntary activation of the muscle (Martineau et al., 1992). However, my results seem not to support this hypothesis as no changes in sEMG parameters were found. Similarly, previous investigations did not show changes in sEMG parameters, corticospinal response and voluntary activation (Decorte et al., 2008, 2013; Hostrup et al., 2015; Laurent et al., 2018).

Therefore, I suggest that the ergogenic effect of acute β_2-agonist seems to be mediated by improvement of peripheral muscle function (Kalsen et al., 2014; Hostrup et al., 2015).

Twitch interpolation technique could help to detect muscle fatigue combining maximal voluntary contraction (MVC) in addition to an electrical stimulus applied to the nerve in order to localise exercise and contractile fatigue.

In support of the latter, shorter half relaxation time after salbutamol administration was observed by Crivelli et al., (2011); higher ATP, G-6-P and unchanged PCr after the sprint observed by Kalsen et al., (2016) suggests a smaller accumulation of Pi, AMP and free Mg^{2+} that is identified to decrease force by impairing cross-bridge function and by reducing release of Ca^{2+} from SR (Allen et al., 2008). Furthermore, enhancement in peak power output has been

associated with increased rates of glycogenolysis and glycolysis that may contrast the development of fatigue through an accelerated rate of ATP resynthesis (Allen et al., 2008).

During the variable cycling trial, heart rate was significantly higher in SAL condition compared to placebo heart rate response collected in the early phase (min 1 to min 20) was slightly higher following the inhalation of salmeterol probably responsible of the increased resting heart rate. Unfortunately, we could not record heart rate at rest due to logistic problems, however I would have expected higher resting rate under β_2-agonists (Onslev et al., 2017). Heart rate did not reveal significant differences between conditions at higher intensities (from 55% to 95% PPO). These changes are similar to findings collected during the early phase of 10 km time-trial performed in a previous study (Koch et al. 2015). This could be the result of an overstimulation in the adrenergic nervous system mediated by both β_1 and β_2-receptors (Brodde, 1991) or by greater systemic bioavailability. The increased heart rate response observed at low intensities could be explained as β_2-adrenergic free fatty acids mobilisation from adipose tissue (Hoeks et al., 2003) and subsequence hormone-sensitive lipase activation in skeletal muscle (Jocken et al., 2008). Conversely, at higher intensities when carbohydrate oxidation is maximised I did not observed difference in heart rate and in blood lactate between conditions probably due to exercise-induced release of adrenaline and noradrenaline (Kalsen et al., 2016) thus acting as competitor for salmeterol that reduced the difference in energy expenditure between placebo and salmeterol.

However, our findings are not in agreement with Carlsen et al., (1997) who reported similar heart rate response during running time to exhaustion in placebo, salbutamol (0.8 mg) and salmeterol (50 µg) conditions. Notably, a comparison between my results and Carlsen (1997) is difficult; firstly, the type of test proposed by Carlsen's study was shorter than mine (four minutes vs one-hour) and secondly, the running intensity in Carlsen (1997) study was closer to the ventilatory threshold throughout the test (90% of HR_{Max}) whereas in the current study only

the last part of the incremental test (from minute 35 to 59) was conducted at similar intensity (91% of HR_{Max}).

In the current study perception of effort (RPE) did not reveal differences in both conditions. Previous studies measured leg discomfort (RPE_L) in 10-km cycling time trial and 5-km running time-trial with a significant enhanced RPE_L in salbutamol condition during a time trial (Dickinson et al., 2014; Koch 2015). It could be expected that increased rating of (RPE_L) might be ergolytic for the athletes due to elevations in serum creatine kinase levels observed with higher doses of salbutamol (Lisi, 1989); however, in neither study the increase in RPE_L lead to a significant effect on athletic performance (Pluim et al., 2011). Blood lactate increased significantly after the 12-s sprint in both conditions. The observation of lactate variation seems to be caused by power output changes during the time-trial. These results are in line with studies presenting muscle lactate increment from the start of supra-maximal exercise after acute salbutamol intake (Collomp et al., 2005).

Overall, salmeterol administration has an ergogenic effect expressed by a reduced power output loss in sprinting activity performed after a fatigue-induced cycling protocol. We suggest that the ergogenic effect of LABAs can be due to an improvement in peripheral muscle function. However, due to the impossibility to measure it, my conclusion is only supported by previous studies present in literature reporting similar outcomes.

6.4.1 Technical consideration and study limitations

In the previous section I speculated that the higher force production in SAL condition was mediated by an improvement in peripheral muscle function. A limitation of this experiment is that central activation and peripheral muscle function were not assessed; therefore, our conclusion is mainly based on previous investigations. As such, additional research involving established protocols are needed to quantify central activation and peripheral muscle function following SAL supplementation. Similarly, urine collection will need to establish the exact amount of drug able to reach the systemic circulation in order to create a dose-response relationship. According to this, the time waited between the inhalation and the beginning of warm-up represents a limitation of this study because did not allow us to be sure about the concentration of salmeterol present in the systemic concentration after the inhalation.

6.4.2 Conclusion

This study was the first to demonstrate that 100 μg salmeterol inhalation improves 12-s sprint performance after a fatiguing protocol. It could be speculated that β_2-adrenoceptors content might be lower in athletes being translated in a more prominent β_2-adrenoceptors stimulation that would bring to improved sprint performance (Van Baak et al., 2004). Therefore, anti-doping stake-holders may wish to commission investigations into whether asthmatic athletes experience a similar ergogenic action from inhaled long-acting β_2-agonists. Future research should explore the effect of long-term administration to detect any potential doping effect following daily β_2-agonists administration. More interestingly, whether daily β_2-agonists administration could enhance β_2-adrenergic endogenous levels even through the inhalation of similar long-acting β_2-agonists. Our results suggest that consideration should be given to closer monitoring of inhaled long acting β_2-agonists used by athletes in and out of competition.

Chapter 7. General Discussion

This thesis demonstrates that both inhaled SABAs and LABAs do not induce an ergogenic action on aerobic performance in cycling and running. Conversely, there appears to be a significant improvement in sprint performance in a fatigue state following acute inhalation of salmeterol. Furthermore, 5-week inhalation of either 100 μg salmeterol or 12 μg formoterol improved 30-m maximal running sprint in athletes involved in a training program based on strength and power exercises.

The general discussion will be structured according to the different performance parameters analysed within my studies, discussing the main mechanisms exerted by IBAs administration in footballers, cyclists and recreational active individuals.

Strength

Daily administration of either inhaled salmeterol or formoterol did not enhance strength parameters measured in subjects training for power and resistance as reported in the Chapter 4. Similarly, Dickinson et al., (2014) observed that long-term daily doses of 1600 μg inhaled salbutamol administration in parallel with a full body training program did not alter strength and power performance. My findings are also similar to studies investigating oral doses of salbutamol, which have reported improvements in sprint performance in the absence of changes in maximal voluntary contraction (Hostrup et al., 2016). These findings could result from the combination of various effects induced by the drug and reinforced by the cumulative effect provoked by the training. As a consequence of this training, increased excitation-contraction process with consequent accelerated rate of relaxation is observed. Likewise, increased concentric and eccentric strength in leg muscles was reported after 9 weeks of oral albuterol administration (Caruso et al., 2008). In Chapter 4, the lack of improvement in strength

and power assessments maybe because participants performed extra aerobic training in addition to the prescribed training program in the study. Hence, future studies should look to control exercise completed by participants outside of that prescribed within the study.

There are a limited number of studies investigating LABAs administration and its influence on strength and power in athletes. From the few that have been conducted, increases in maximal voluntary contraction (MVC) have been observed following acute high (54 μg) doses of formoterol (Kalsen et al., 2016) but not low doses (12 μg; Stewart et al., 2002). However, these studies used athletes of different training status between high level athletes (Stewart et al., 2002) and recreational athletes (Kalsen et al., 2016). This is relevant as athletes with greater training/fitness level appear to have lower number of β_2-adrenoceptors compared to lesser trained individuals (Butler et al., 1982). Overall, it seems that high doses of formoterol are able to increase strength values in recreational athletes while lower doses are not able to induce similar effect to high level athletes. However, these previous studies recruited participants with varying degrees of fitness and they did not specifically individualised the dose of drug inhaled. The limitations of these studies make it difficult to establish which of these variables mostly effected the performance.

In summary, strength values are not increased after 5-weeks daily administration of LABAs despite a significant change in sprint performance. However, due to the difficulty to monitor the amount of drug present in the body throughout the entire program a dose-response relationship has not been established. This would be helpful to obtain important information in terms of levels of β_2-agonists present in the systemic concentrations after daily doses administration, besides the time needed before a peak of drug is reached in the body avoiding unpleasant side effects and β_2-adrenoceptors down-regulation.

Sprint and Power

The effect of β_2-agonists inhalation on sprint and power performance has been tested in all the three studies presented in this thesis without bringing significant improvements when SABAs (Chapter 5) and LABAs (Chapter 4 and 6) were administered.

Despite asking participants to inhale twice the WADA permitted dose of salbutamol (WADA 2020), I was unable to replicate the improvements in sprint performance observed by Hostrup et al., (2016) after oral salbutamol administration. Inhaling up to 1600 μg salbutamol may not sufficiently increase the systemic concentration of salbutamol to a significant level. It appears for salbutamol to increase sprint performance a significant systemic concentration is required to initiate elevated Ca^{2+} release, accelerated rate of relaxation (Crivelli et al., 2011) and elevate rates of glycogenolysis and glycolysis. However, since I did not measure the exact amount of β_2-agonists present in the systemic concentration, I can only limit my observation on performance results that were unchanged when compared to placebo. The findings in my thesis fully agree with the results raised in literature where inhaled salbutamol does not induce ergogenic effects due to a weaker stimulation of β_2-adrenoceptors when compared to oral administration (Elers et al., 2012).

Similarly to inhaled salbutamol, I did not observe different peak and mean power during the 12-s sprint between acute salmeterol and placebo condition. This could be affected by salmeterol onset of action that occurs twenty minutes following the inhalation with maximum improvement happening within three hours. However, I did not measure the amount of salmeterol present in the body when subjects began to warm-up and at the beginning of the variable cycling trial; notwithstanding, I observed that the time waited from the inhalation to

the beginning of the warm-up (one hour) was possibly not long enough to induce significant drug concentration in the systemic circulation. This is an important point that should be investigated in the future to establish a dose-effect relationship through urine collection necessary to obtain the actual amount of drug concentration present in the body at the beginning of the warm-up. In addition to this, I have to consider that the type of warm-up developed in my study could have influenced the systemic concentration of LABAs. The criteria behind the decision of a warm-up made of repeated sprints is based on world-tour cyclists' warm-up routine characterised by short-duration high-intensity intervals (Stickland et al., 2012); this warm-up strategy might reduce muscle glycogen and PCr storage through the stimulation of both the anaerobic lactic and alactacid system with partial energy depletion (Gastin, 2001).

From blood lactate values collected after the sprints in Chapter 5 & Chapter 6, experimental conditions did not differ from placebo and this could be interpreted by insufficient doses administered in order to elicit adrenergic response; notwithstanding, the higher heart rate recorded during the first thirty minutes of the variable cycling trial in salmeterol condition could be the effect of a raise in the adrenergic system induced by long-acting β_2-agonists administration.

Conversely to acute IBA doses, after 5 weeks daily inhalation of either salmeterol or formoterol as reported in Chapter 4 a sprint improvement was observed under these conditions. This is a novel finding in this research area that suggests the ergogenic action of long-acting when administered in concomitance with strength and power training that could increase significantly the sprint performance in those athletes training for speed and power discipline.

The improvement in sprint performance following 5-weeks salmeterol or formoterol could have been the result of several proposed mechanisms including: transition from slow to fast twitch fires increasing contractile properties of muscle (Caruso et al., 1995), central nervous systems alteration leading to improved motor unit synchronisation (Milner-Brown et al., 1975), or from an improved synergy between agonists and antagonist muscles during whole body training (Howard et al., 2018). According to Hostrup et al., (2015), the accelerated relaxation in excitation-contraction mechanism mediated by augmented Ca^{2+} handling function and larger ion shift of K^+ and Na^+, could be the primary cause of the increased sprint recorded in my study. Furthermore, the higher content of Na^+/K^+_ATPase collected after IBAs would counteract K^+ loss and facilitating force development (Clausen, 2003).
Future research may look to investigate each of these proposed mechanisms to fully explain the observations from my thesis.

Overall, the improvement in sprint performance observed in Chapter 4 could be the result of a better synergy between muscles and an increased neurological adaptation similar to what reported after chronic salbutamol administration (Martineau et al., 1992); in fact, the change in sprint performance but not in strength and lean body mass could be driven by central nervous system observed to be involved especially during the beginning of resistance training (Kramer et al., 1996). However, in my study I could not explain whether these results come from muscle fibres composition or from the central nervous system due to limited resources; in future studies I recommend to collect muscle biopsies in order to analyse whether change in muscle fibres could be provoked also by the administration of LABAs and resistance training.

In conclusion, after acute SABAs administration no change in sprint and power performance seems to occur probably because of the incapacity to induce high systemic concentration in the

body (Hostrup et al., 2018). Similarly, after acute LABAs administration, no change in sprint performed in “non-fatigue” condition was observed.

Body composition

Five weeks of daily administration of inhaled salmeterol (100 μg) and formoterol (12 μg) daily administration did not to reduce body fat composition as when recorded by skinfolds measurements. These findings are in contrast to previous investigations utilising oral administration, where significant hypertrophic changes were recorded after oral salbutamol (Martineau et al., 1992; Le Panse et al., 2005; Le Panse et al., 2006) and reduced fat mass was detected after oral formoterol administration (Lee et al., 2015).

Due to the lack of studies to compare inhaled and oral forms of LABAs, I do not know whether the absence of anabolic effect observed in my study is due to the weak dose inhaled or by the concurrent effect of the resistance program. Importantly, the use of skinfolds to assess body composition has an increased potential for error when compare with more sophisticated tools such as dual-energy X- ray absorptiometry (DEXA) (Pineau & Frey, 2016).

When terbutaline was administered by inhalation, in concomitance with 4-week resistance training, a change in lean mass (+1.03 kg) was observed (Jessen et al., 2018). Notably, this effect seems even bigger after oral administration (+1.95 kg) in trained men (Hostrup et al., 2015). I think that the difference between my study and the outcomes coming from the above studies (Jessen et al., 2018; Hostrup et al., 2015) is due by different pharmacodynamics and bioequivalence possessed by terbutaline that seems to have higher potency, efficacy, lipophilicity and half-life compare to the others SABAs and LABAs. Therefore, anabolic and

lipolytic terbutaline feature could be manifested by elevated β_2-adrenoceptor affinities capable to modify body composition.

Muscle Fatigue

Sprint activity performed after Yo-Yo IRT (Chapter 5) did not differ with or without prior inhalation of salbutamol, however acute doses of salmeterol did improve sprint performance following a Variable cycling trial (Chapter 6). The sprint performed at the end of aerobic trials is mainly ascribable to muscle fatigue condition experienced by subjects before performing maximal 30-m running sprint and 12-s cycling sprint. With regard to this, it seems that only LABAs administration has an ergogenic action when muscles are in a relatively fatigued state, as reported by a decrement in power output compared to the cycling 12-s sprint performed under placebo condition and sprint performed in "non-fatigue" condition.

The results from Chapter 5 seem to testify that inhaled SABAs (1600 μg salbutamol) do not increase power output during sprint performance, this may be due to reduced K^+ levels as a consequence of high-intensity intermittent activity performed before. Similarly, high-dose inhaled terbutaline did not show increased MVC recording a significant lower peak twitch force after exhaustive exercise compared to placebo (Hostrup et al., 2014). Interestingly, the higher decrease in peak twitch force measured in terbutaline matched with a slower half-relaxation time collected only during the second sprint suggesting the impact of this drug in "non-fatigued" condition, possibly ascribable to enhanced SR Ca^{2+} and K^+ levels after the inhalation but not when exercise is prolonged with increased levels of fatigue.

Similarly, the mechanisms behind the increased 12-s sprint showed in Chapter 6 have not been fully identified. Lactate values collected during the sprint were not different between

conditions, thus I would not attribute the higher power output collected in salmeterol condition to enhanced glycolytic metabolism. I think that the greater power output recorded during 12-s sprint by salmeterol could be determined by a small reduction of ATP content and by increased release of Ca^{2+} from sarcoplasmic reticulum compared to placebo (Kalsen et al., 2016). An increase in contractile force compared with placebo was probably responsible of the lower sprint reduction registered in salmeterol condition after the Variable cycling trial. However, since I could not collect further data, my considerations are mainly based on previous studies detecting larger Ca^{2+} release and faster rate of force relaxation after β_2-agonists administration (Cairns & Dulhunty, 1994). Overall, the lower reduction of power output observed in salmeterol compared to placebo would be the result of muscle fatigue resilience effect of long-acting β_2-agonists in maintaining high levels of strength for prolonged period of time (Cairns & Dulhunty, 1994). However, it was not possible to confirm this mechanism within my study and future studies should investigate whether this is the case.

My thesis brings important novel findings in terms of reduced loss of power output following 100 µg salmeterol during 12-s sprint. Previous research may explain these findings by suggesting an increased rate of glycogenolysis and glycolysis along with great phosphorylation of CaMKII Thr and FXYD1 (Kalsen et al., 2016). The rationale behind the improvement observed in my study could be found in an acceleration of the accumulation of G-6-P with consequent counteraction in the reduction of ATP content in addition to reduced Pi accumulation, free Mg2+ and AMP with significant effects on fatigue development also showed by an increment in Na^+/K^+_ATPase. In future studies I would seek to confirm these mechanisms occur in 12-s sprint in "fatigue state". Conversely, the results obtained in Chapter 5 could be interpreted by the inability of salbutamol to counteract loss of force. This is possibly

related to a deprivation of Ca^{2+} release and re-uptake within the skeletal muscles and reduced K^{+} concentration manifested during the previous Yo-Yo IRT.

Endurance

The dose of salbutamol inhaled in Chapter 5 was the same used by Dickinson et al., (2015) that collected concentration of drug below the WADA urinary threshold without noticing significant improvement in performance. I expect that a similar amount of drug was reached by my participants despite I could not measure urine values. Heart rate did not significantly increase compared to placebo conditions throughout the Yo-Yo IRT test, which suggests that supra-maximal doses of SABAs (1600 μg salbutamol) did not increase systemic β_2-adrenergic levels significantly. This finding has also been reported by previous studies (Koch et al., 2015). Heart rate values were not different during the early phases of the Variable cycling trial while these were significantly higher after salmeterol inhalation. Contrary to what observed in Chapter 5, if no changes in heart rate were recorded, a possible higher adrenergic nervous system stimulation seems to occur with salmeterol compared to placebo. Notably, IBAs seem to stimulate β_2-receptors present in skeletal muscle with consequent increased muscle metabolism activation (Carlsen et al., 1997).

A limitation of this study is represented by the Variable cycling trial, as it was not performed until physical exhaustion, thus it was difficult to establish whether aerobic performance could have been augmented after LABAs administration. According to this, the choice to perform a time to exhaustion test could be suggested in order to assess the effect of LABAs in fatigue when the anaerobic metabolism is largely stressed with lactate production coming from glycolytic fibres. Based on this methodological limitation, I can only compare my results with

few studies present in literature showing how SABAs do not enhance aerobic trials increasing heart rate as the results of an increased adrenergic activity (Koch et al., 2015a; 2015b).

Due to similar RPE values in the two conditions and to the high correlation between RPE and the physiological response to exercise (Pereira et al., 2011) I would not expect a longer time to exhaustion following LABAs administration. Notably, this supposition is based on the results I collected in my studies and it is also reinforced by few studies using RPE as measure of perceived leg fatigue (Koch et al., 2015a; 2015b). Despite Dickinson et al., (2014) reported greater RPE values in salbutamol condition during the first part of 5-km running time trial, no change in performance was recorded. Therefore, RPE maybe more of a surrogate for overall performance measurement rather than a measure to assess peripheral discomfort as described by Borg (1982). Thereafter, increased perception of effort reported in the above studies did not represent overall perception of effort but a measure of peripheral discomfort that is likely related to muscle cramping feeling when SABAs are administered (Lisi, 1989). With regard to this, RPE values collected during the aerobic exercise performed in this thesis seem to describe partially the results present in literature where the administration of β_2-agonists does not improve aerobic performance even at supra-maximal regime.

In summary, this thesis shows how SABAs administration do not increase the distance covered in the Yo-Yo IRT; these findings are reinforced by unchanged values of heart rate and perception of effort reported in Chapter 5. Conversely, the higher heart rate collected in Chapter 6 during the early stages of the Variable cycling trial in salmeterol condition could be associated to a significant adrenergic activity incremented by the drug. The aim of Chapter 6 was not to directly assess aerobic performance, I propose future studies to measure aerobic performance with a test such as time trial or time to exhaustion. Moreover, a warm-up

standardisation would ensure similar levels of β_2-agonists in the body following the inhalation, avoiding low β_2-agonists levels in the systemic circulation caused by high-intensity warm-up.

Limitations

Despite a familiarising participant to good inhaler technique and using a spacer device when possible, it was not feasible to assess what proportion of the inhaled dose was delivered into the lungs and how much was deposited on the upper airway. This could be partially due to the fact that familiarisation process was undergone for a short period of time (< 5 days) and supported by the data present in literature that recognise a small percentage of subjects with optimal inhaler technique (Giraud et al., 2016). A rigorous familiarisation over at minimum of 4-weeks may improve the inhalation technique thus increasing the amount of IBAs reaching the small lower airways.

All the studies present in this thesis and others in literature (Kalsen et al., 2016; Jessen et al., 2018; Hostrup et al., 2018; Dickinson et al., 2014) administered drug to participants regardless a body-weight adjustment making difficult to establish a dose-response relationship provoked by SABAs and LABAs administration. Therefore, heavier participants may have effectively received a relatively small dose of β_2-agonists when compared to lighter subjects. Prescribing appropriate doses of drugs requiring weight-based dosing is still challenging due to poor awareness and adherence; however, in population pharmacokinetic modelling, covariate models are built to describe between-subject variability in pharmacokinetic parameters (e.g. anabolic effects, clearance, onset of action) based on patient information (e.g. weight; Mould & Upton, 2012). For this reason, one of the most identified covariates in population

pharmacokinetic models is the body weight of the patient that need to describe its relationship with pharmacokinetic parameters (Anderson et al., 2006).

Using doses of inhaled asthma therapy in relation to body mass will help to better understand the levels of β_2-agonists present in systemic concentrations and the evaluation of drug-response relationship. The above limitation is likely to persist in studies conducted in the foreseeable future, as asthma inhalers are produced delivering metered doses making it impossible to individualise the dose directly in relation to body mass.

A third limitation to report in this thesis is represented by the time waited between the inhalation of salmeterol and the beginning of Chapter 6 warm-up; this could have limited the amount of LABAs present in the systemic circulation explaining the absence of effect showed in "12-s non-fatigue" sprint. The time of absorption of salmeterol in the lungs is about 5 minutes and within 15 minutes it is distributed in the bronchiolar regions (Backstrom et al., 2018) reaching a body peak concentration in 45 to 90 minutes after the inhalation (Cazzola et al., 2002). Importantly, the drug concentration in the body seems to be affected by physical exercise with a reduced absorption rate from the GI tract at high exercise intensity (Kirjavainen et al., 2018). After the inhalation of terbutaline, blood levels and concentration curve (AUC) during exercise were greater than at rest with higher drug bioavailability (Schmekel et al., 1992). According to this, drug availability in the body could have been increased at the very beginning of the warm-up through an increment of muscle blood flow in muscle; however, the duration and intensity of the type of warm-up performed in Chapter 6 could have reduced the amount of drug present in the body without benefit the sprint activity recorded. According to this, I cannot exclude that with a different type of warm up a higher amount of drug would have been preserved in the body before performing "12-s non-fatigue" sprint test.

Notably, when looking at endurance results collected in Chapter 5, internal variability of Yo-Yo IRT test should be taken in account in behalf of day-to-day athletes' variability especially during pre-season period where footballers fitness levels change more rapidly compared to "in-season" (Castagna et al., 2013). Likewise, I tried to maintain the same application circumstances in test-retest (e.g. day-time, recovery, fatigue, quality of sleep) in order to reduce bias and limiting the observation on the effect of salbutamol administration. Furthermore, motivation and mood were measured in order to maintain the same engagement between sessions.

A limitation present in Chapter 4 was the difficulty to control external factors (e.g. training performed outside the gym) that should have been maintained identical among all the three groups to limit the observation to the effect provoked by LABAs administration. In addition to the randomisation method I adopted to randomly assigned drug treatment to the experimental groups, I could have controlled external variables matching the different groups of confounding variables (e.g. type of sport performed, volume and intensity of the training) in order to be distributed equally amongst the group (Street, 1995). Unfortunately, due to the small number of subjects, I found difficult to extend matching within all the groups tested. Future studies may consider limiting activities outside the prescribed training.

The above point links with a further limitation with regards the fitness and activity level of the participants. This is not just a limitation to my work but also most other studies on SABA and LABA. One potential solution is to recruit sedentary individuals as this may enable investigation into the ergogenic action of IBAs in the absence of physical activity. However, this strategy would not be directly applicable to athletes, partly due to reported differences in β_2-adrenoreceptors between sedentary and athletic population (Butler et al., 1982). Ideally

research investigating IBAs in relation to anti-doping regulations would recruit elite athletes to make the findings directly applicable. However, it is highly unlikely a true randomised control trial would be possible within an elite athlete population, as it would increase the risk of athletes contravening anti-doping rules. Furthermore, researchers are likely to find an inability to disrupt athletes training and competition schedule.

Practical application of thesis finding

This thesis suggests that WADA guidelines are appropriate for SABAs (salbutamol) but it should reconsider its position in regard of LABAs (SAL and FOR) guidelines to closer monitoring of athletes' use of long-acting forms in and out of competition. Before changes are made to the WADA antidoping code, further investigations need to be done to better understand the ergogenic mechanisms behind the improvement showed in sprint performance. The next mandatory step is to include professional males and female athletes in similar studies so as to observe whether β_2-agonists administration produce similar responses to what observed in recreational subjects.

Based on my findings anti-doping stakeholders may wish to commission investigations into whether highly trained athletes experience similar ergogenic action from inhaled FOR or SAL as demonstrated in this thesis. These studies should be conducted before changes to the WADA Prohibited List are recommended. In particular, WADA may focus on the impact of chronic IBAs administration on strength and power activities when athletes are in fatigue states. Measuring change in maximal voluntary contraction between the beginning and the end of repeated sprint sessions would implement the observations coming from my thesis; this would help to detect those mechanisms responsible of the increment in sprint power output recorded

after salmeterol administration. Moreover, future studies should look at the effect of acute LABAs administration in "non-fatigue" sprint ability controlling those aspects such as warm-up that could have influenced the data collected at the beginning of the session.

Further investigations on daily LABAs administration should be developed during consecutive days of cycling trial with the aim to simulate the effect of fatigue experienced by cyclists during congested period of races. This will mimic the stress accumulated by athletes involved in multi-days competition, such as Grand Tour events, where the need to high quality recovery between stages is an important criterion for maintaining a good ranking position throughout the 21-days competition.

Conclusion

In conclusion this thesis shows that supra-therapeutic SABAs doses do not increase sprint performance and aerobic performance in footballers confirming the results already present in the literature. The novel finding of this thesis is represented by the ergogenic effect provoked by the inhalation of long-acting β_2-agonists (FOR and SAL) in both acute and chronic administration. More specifically, this thesis shows that the ergogenic feature possesses by salmeterol is significantly relevant on 12-s cycling sprint performed in a state of fatigue. However, the absence of effect showed in "non-fatigue" condition could be the results of methodological limitations that should be addressed in future studies.

The combination of strength and power training and daily inhalation of LABAs showed a significant improvement in 30-m sprint performance in FOR and SAL groups but not in PLA. Future studies should look at peripheral changes through muscle biopsies and fibres analysis to detect any structural modifications provoked by daily β_2-agonists administration in concomitance with strength training.

References

Allen, DG, Lamb, GD, Westerblad H. (2008) “Skeletal muscle fatigue: cellular mechanisms”. Physiol Rev. Jan;88 (1):287-332.

Anderson, SD, Lindén A, Rabe KF. (1994) “Why are long-acting beta-adrenoceptor agonists long-acting?” Eur Respir J. Mar;7(3):569-78.

Anderson, SD, Argyros GJ, Magnussen H, Holzer K. (2001) “Provocation by eucapnic voluntary hyperpnoea to identify exercise induced bronchoconstriction”. Br J Sports Med. 35(5):344-347.

Anderson, BJ, Allegaert, K, Holford, NH. (2006). ”Population clinical pharmacology of children: modelling covariate effects”. Eur J Pediatr. 2006;165(12):819–29.

Anderson, SD and Kippelen, P. (2008) “Airway injury as a mechanism for exercise-induced bronchoconstriction in elite athletes”. Clinical reviews in allergy and immunology. 122(2):225-235.

Anderson, SD, (2011) “Bronchial challenge tests: Usefulness, availability and limitations”. Breathe, 8, 53-60.

Anderson, SD and Kippelen, P. (2012) “Assessment and Prevention of Exercise-Induced Bronchoconstriction” January, Br J Sports Med

Atkinson, G, Nevill, AM. Selected issues in the design and analysis of sport performance research. J Sports Sci. 2001;19(10):811-827.

Bäckström, A, Hamm, G, Nilsson, A, Fihn, BM, Strittmatter, N, Per Andrén, R, Goodwin, B and Fridéna, M (2018) “Uncovering the regional localization of inhaled salmeterol retention in the lung” Drug Deliv. 2018; 25(1): 838–845

Baechle, TR, Earle, RW. (2008) “Essentials of Strength Training and Conditioning”. Human Kinetics.

Baker, JG (2010) “The selectivity of beta-adrenoceptor agonists at human beta1-, beta2- and beta3-adrenoceptors.” Br J Pharmacol. Jul;160(5)

Ball, DI, Brittain, RT, Coleman, RA, Denyer, LH, Jack, D, Johnson, M, Lunts, LH, Nials, AT, Sheldrick, KE, Skidmore, IF. (1991) “Salmeterol, a novel, long-acting beta 2-adrenoceptor agonist: characterization of pharmacological activity in vitro and in vivo”. Br J Pharmacol. Nov;104(3):665-71.

Barnes, PJ. (2008) “Immunology of asthma and chronic obstructive pulmonary disease”. Nat Immunol Rev. 8:183-192.

Bizzini, M, & Dvorak, J (2015) “FIFA 11+: An Effective Programme to Prevent Football Injuries in Various Player Groups Worldwide-a Narrative Review.” British Journal of Sports Medicine 49 (9): 577–79.

Borg, GA, (1982) “Psychophysical Bases of Perceived Exertion.” Medicine and Science in Sports and Exercise 14 (5): 377–81.

Brannan, JD, Anderson, SD, Perry, CP, Freed-Martens, R, Lassig, AR, Charlton, B (2005) “The safety and efficacy of inhaled dry powder mannitol as a bronchial provocation test for airway hyper-responsiveness: a phase 3 comparison study with hypertonic (4.5%) saline”. Respir Res. Dec 9;6:144.

British lung foundation, asthma Uk; https://www.blf.org.uk/support-for-you/asthma.

Brittain, RT. (1990) “Approaches to a long-acting, selective beta 2-adrenoceptor stimulant”. Lung. 168 Suppl:111-4.

Brodde, OE. (1991) “Beta 1- and beta 2-adrenoceptors in the human heart: properties, function, and alterations in chronic heart failure.” Pharmacological Reviews June, 43 (2) 203-242.

Burniston, JG, Clark, WA, Tan, L-B, Goldspink, DF. (2006) “Dose-dependent separation of the hypertrophic and myotoxic effects of the β2-adrenergic receptor agonist clenbuterol in rat striated muscles”. Muscle Nerve. 33(5):655-663.

Butler J, O’Brien M, O’Malley K, Kelly JG. (1982) “Relationship of beta-adrenoreceptor density to fitness in athletes”. Nature, 298: 60–62.

Butler, J, Kelly, JC, Malley, K, Pidgeon (1983) “Beta-adrenoceptor adaptation to acute exercise”. J Physiol. Nov; 344: 113–117.

Cairns, SP, Dulhunty, AF. (1994) “Beta-adrenoceptor activation shows high-frequency fatigue in skeletal muscle fibers of the rat.” Am J Physiol. 266, 1204-9.

Carlsen, KH, Ingjer, F, Kirkegaard, H, Thyness, B. (1997) “The effect of inhaled salbutamol and salmeterol on lung function and endurance performance in healthy well-trained athletes”. Scand J Med Sci Sports. Jun;7(3):160-5.

Caruso, J, Signorile, J, Perry, A, Leblanc, B, Williams, R, Clark, M, (1995) “The effects of albuterol and isokinetic exercise on the quadriceps muscle group”. Med Sci Sports Exerc. 27:1471-6.

Caruso, J, Hamill, J, De Garmo, N (2005) “Oral albuterol dosing during the latter stages of a resistance exercise programme”. J Strength Cond Res. 19(1):102-107.

Caruso, J, Hamill, JL, Yamauchi, M, Saito, K, Cook, TD, Mercado, DR. (2008) “Temporal strength changes from resistance exercise and albuterol on unloaded muscle”. J Strength Cond Res. Jul;22(4):1156-63.

Castagna, C, Impellizzeri, F, Chauachi, A, Manzo, V (2013) “Pre-Season Variations in Aerobic Fitness and Performance in Elite Standard Soccer Players: a Team-Study”. The Journal of Strength and Conditioning Research 27(11) · February 2013

Cazzola, M, Page, CP, Rogliani, P, Matera, MG (2013) “β2-agonist therapy in lung disease”. Am J Respir Crit Care Med. Apr;187(7):690-6.

Cazzola, M, Testi, R, Matera MG. (2002) “Clinical pharmacokinetics of salmeterol”. Clin Pharmacokinet. 2002;41(1):19-30.

Cazzola, M, Matera, MG, Lötvall, J. (2005) “Ultra long-acting beta 2-agonists in development for asthma and chronic obstructive pulmonary disease”. Expert Opin Investig Drugs. Jul;14(7):775-83.

Chen-Izu, Y, Xiao, RP, Izu, LT, Cheng, H, Kuschel, M, Spurgeon, H, Lakatta, EG. (2000) “G(i)-dependent localization of beta (2)-adrenergic receptor signaling to L-type Ca(2+) channels”. Biophys J. Nov;79(5):2547-56.

Clausen, T. (2003) “Na-K pump regulation and skeletal muscle contractility”. Physiol Rev 83: 1269–1324.

Coates, AL, Wanger, J, Cockcroft, DW, Culver, BH. (2017) “ERS technical standard on bronchial challenge testing: general considerations and performance of methacholine challenge tests”. Eur Respir J. May 1;49(5).

Cockcroft, DW, Davis, BE, Blais, CM. (2019) “Direct bronchoprovocation test methods: history 1945-2018”. Expert Rev Respir Med. Mar;13(3):279-289

Coelho, AC, Cannon, D, Cao, R, Porszasz, J, Casaburi, Knorst, M, Rossiter HB (2015) “Instantaneous quantification of skeletal muscle activation, power production, and fatigue during cycle ergometry” Journal of applied physiology. J Physiol. June 1;395(6)

Coffey, VG, Hawley, JA. (2017) “Concurrent exercise training: do opposites distract?” J Physiol. May 1;595(9):2883-2896.

Cohen, J. (1992) “Statistical Power Analysis”. Curr Dir Psychol Sci;1(3):98-101.

Collomp, K, Le Panse, B, Portier, H, Lecoq, AM, Jaffre, C, Beaupied, H, Richard, O, Benhamou, L, Courteix, D, De Ceaurriz, J (2005) “Effects of acute salbutamol intake during a Wingate test.” Int J Sports Med. Sep;26 (7):513-7.

Collomp, K, Le Panse, B, Candau, R, Lecoq, A, Ceaurriz (2010) “Beta-2 agonists and exercise performance in humans”. Science and Sports. 25:281-290.

Crivelli, G, Millet, P, Gremion, G, Borrani, F (2011) “Effects of salbutamol on the contractile properties of human skeletal muscle before and after fatigue”. Acta Physiologica Volume 203, Issue2 Oct:311-320

Decorte, N, Verges, S, Flore, P, Guinot, M, Wuyam, B (2008) “Effects of acute salbutamol inhalation on quadriceps force and fatigability” Med Sci Sports Exerc 40, 1220–1227.

Decorte, N, Bachasson, D, Guinot, M, Flore, P, Levy, P, Verges, S, Wuyam, B (2013) “Effect of salbutamol on neuromuscular function in endurance athletes”. Med Sci Sports Exerc 45, 1925–1932.

Dempsey, JA, McKenzie, DC, Haverkamp, HC, Eldridge, MW, (2008). “Update in the understanding of respiratory limitations to exercise performance in fit, active adults”. Chest Journal, 134, 613-622.

Dempsey JA (2019) “Respiratory Determinants of Exercise Limitation: Focus on Phrenic Afferents and the Lung Vasculature”. Clin Chest Med. Jun;40(2):331-342.

De Pauw, K, Roelands, B, Cheung, S, De Geus, B, Rietjens, G, Meeusen, R. (2013) “Guidelines to Classify Subject Groups in Sport-Science Research.” International Journal of Sports Physiology and Performance 8 (2): 111–22.

Dickinson, J, Whyte, G, McConnell, AK, Harries, MG (2005) “Impact of changes in the IOC-MC asthma criteria: a British perspective” Thorax.

Dickinson, J, McConnell, A, Whyte, G. (2011) “Diagnosis of exercise-induced bronchoconstriction: eucapnic voluntary hyperpnoea challenges identify previously undiagnosed elite athletes with exercise-induced bronchoconstriction”. Br J Sports Med. Nov;45(14):1126-31.

Dickinson, J, Hu, J, Chester, N, Loosemore, M, Whyte, G. (2014) “Acute Impact of Inhaled Short Acting B2-Agonists on 5 Km Running Performance”. J Sports Sci Med.13(2):271-279.

Dickinson, J, Molphy, J, Chester, N, Loosemore, M, Whyte, G. (2014) “The ergogenic effect of long-term use of high dose salbutamol”. Clin J Sport Med. Nov;24(6):474-81.

Dickinson, J, Hu, J, Chester, N, Loosemore, M, Whyte, G. (2015). “The Impact of Inhaled Salbutamol on Repeated Sprint Ability in Pre- Fatigued Soccer Players”. Vol. 05. Journal Sports Med Doping Stud, 5:82.

Dryden, DM, Spooner, CH, Stickland, MK, Vandermeer, B, Tjosvold, L, Bialy, L, Wong, K, Rowe, BH. (2010) “Exercise-induced bronchoconstriction and asthma”. Evid Rep Technol Assess, Jan;(189):1-154

Duffy, P, Phillips, YY (1991) “Caffeine consumption decreases the response to bronchoprovocation challenge with dry gas hyperventilation”. Chest, 99, 1374-1377.

Dwyer, A. (2012) “Spirometry: How Should We Order This Bedrock of Diagnosis and Management for Asthma and COPD?”. Respiratory Care October, 57 (10) 1692-1695;

Eggleston, PA, Rosenthal, RR, Anderson, SA, Anderton, R, Bierman, CW, Bleecker, ER, Chai, H, Cropp, GJ, Johnson, JD, Konig, P, Morse, J, Smith, LJ, Summers, RJ, Trautlein, JJ. (1979) “Guidelines for the methodology of exercise challenge testing of asthmatics. Study Group on Exercise Challenge, Bronchoprovocation Committee, American Academy of Allergy”. J Allergy Clin Immunol. Dec;64 6:642-5.

Elers, J, Mørkeberg, J, Jansen, T, Belhage, B, Backer, V. (2010) “High-dose inhaled salbutamol has no acute effects on aerobic capacity or oxygen uptake kinetics in healthy trained men”. Scand J Med Sci Sports. Apr;22(2):232-9.

Elers, J, Pedersen, L, Henninge, J, Hemmersbach, P, Dalhoff, K, Backer, V (2011) "Urine concentrations of repetitive doses of inhaled salbutamol", Int J Sports Med. 32(8):574-579.

Elers, J, Pedersen, L, Henninge, J, Hemmersbach, P, Dalhoff, K, Backer, V. (2012a) "The pharmacokinetic profile of inhaled and oral salbutamol in elite athletes with asthma and non-asthmatic subjects". Clin J Sport Med. Mar;22(2):140-5.

Elers, J, Hostrup, M, Pedersen, L, et al. (2012) "Urine and serum concentrations of inhaled and oral terbutaline". Int J Sports Med;33(12):1026–33.

Emery, PW, Rothwell, NJ, Stock, MJ, Winter, PD (1984) "Chronic effects of beta 2-adrenergic agonists on body composition and protein synthesis in the rat". Biosci Rep.1984;4(1):83-91.

Fitch, K. (2006) "Beta-2 agonists at the Olympic games". Clin Rev Allergy Immunol 31:259-268.

Fitch, K. (2012) "An overview of asthma and airway hyper-responsiveness in Olympic athletes". Br J Sports Med. May;46(6):413-6.

Fitch, K. (2016) "World Anti-Doping Code: can you have asthma and still be an elite athlete?" Breathe. 2016 Jun;12(2):148-58.

Fitch, K. (2019) "Therapeutic Use Exemptions (TUEs) are essential in sport: but there is room for improvement". Br J Sports Med.

Froidure, A, Mouthuy, J, Durham, SR, Chanez, P, Sibille, Y, Pilette, C, (2016). "Asthma phenotypes and IgE responses". European Respiratory Journal; 47: 304-319;

Gastin, PB. (2001) "Energy system interaction and relative contribution during maximal exercise". Sports Med;31(10):725-41.

Gillooly, JF, Brown, JH, West, GB, Savage, VM, Charnov, EL (2001). "Effects of Size and Temperature on Metabolic Rate". Science 21 Vol. 293, Issue 5538, 2248-2251

Giraud V, Allaert FA, Roche N. (2011) “Inhaler technique and asthma: feasability and acceptability of training by pharmacists”. Respir Med. Dec;105(12):1815-22.

Goubault, C, Perault, MC, Leleu, E, Bouquet, S, Legros, P, Vandel, B, Denjean, A. (2001) “Effects of inhaled salbutamol in exercising non-asthmatic athletes”, Thorax. Sep;56(9):675-9.

Ha, TN, Posterino, GS, Fryer, MW. (1999) “Effects of terbutaline on force and intracellular calcium in slow-twitch skeletal muscle fibres of the rat”. Br J Pharmacol. Apr;126(8):1717-24.

Haase, CB, Backer, V, Kalsen , Rzeppa, S, Hemmersbach, P, Hostrup, M, Hostrup, M. (2016) “The influence of exercise and dehydration on the urine concentrations of salbutamol after inhaled administration of 1600 µg salbutamol as a single dose in relation to doping analysis”. Drug Test Anal. Jul;8(7):613-20.

Hallstrand, TS, Altemeier, WA, Aitken, ML, Henderson, WR (2013). “Role of cells and mediators in exercise-induced bronchoconstriction”. Immunology and Allergy Clinics of North America, 33, 313-28.

Hansen, JE, Sun, XG, Wasserman, K. (2007). “Spirometric criteria for airway obstruction: Use percentage of FEV1/FVC ratio below the fifth percentile, not < 70%”. Chest. Feb;131(2):349-55.

Heuberger JAAC, van Dijkman, SC, Cohen, AF. (2018) “Futility of current urine salbutamol doping control”. Br J Clin Pharmacol. Aug;84(8):1830-1838.

Hoeks, J, Van Baak, MA, Hesselink, MK, Hul, GB, Vidal, H, Saris, WH, Schrauwen, P. (2003) “Effect of beta1- and beta2-adrenergic stimulation on energy expenditure, substrate oxidation, and UCP3 expression in humans”. Am J Physiol Endocrinol Metab 285:775–782

Holloszy, JO, Coyle, EF (1984). “Adaptations of skeletal muscle to endurance exercise and their metabolic consequences”. J Appl Physiol Respir Environ Exerc Physiol 56, 831–838.

Holzer, K, Anderson, SD, Chan, HK, Douglass, J. (2003) “Mannitol as a challenge test to identify exercise-induced bronchoconstriction in elite athletes”. Am J Respir Crit Care Med. Feb 15;167(4):534-7.

Hostrup, M, Kalsen, A, Ortenblad, N. (2014) “β2-adrenergic stimulation enhances Ca2+ release and contractile properties of skeletal muscles and counteracts exercise-induced reductions in Na+-K+-ATPase Vmax in trained men”. J Physiol.;592(24):5445-5459.

Hostrup, M, Kalsen, A, Bangsbo, J, Hemmersbach, P, Karlsson, S, Backer, V. (2014). “High-Dose Inhaled Terbutaline Increases Muscle Strength and Enhances Maximal Sprint Performance in Trained Men.” European Journal of Applied Physiology 114 (12): 2499–2508.

Hostrup, M, Kalsen, A, Onslev, J, Jessen, S, Haase, C, Habib, S, Ørtenblad, N, Backer, V, Bangsbo, J. (2015) “Mechanisms underlying enhancements in muscle force and power output during maximal cycle ergometer exercise induced by chronic β2-adrenergic stimulation in men”. J Appl Physiol;119: 475–486.

Hostrup, M, Kalsen, A, Auchenberg, M, Bangsbo, J, Backer, V. (2016) “Effects of acute and 2-week administration of oral salbutamol on exercise performance and muscle strength in athletes”. Scand J Med Sci Sports. Jan;26(1):8-16.

Hostrup, M, Onslev, J, Jacobson, GA, Wilson, R, Bangsbo, J. (2018) “Chronic β2 -adrenoceptor agonist treatment alters muscle proteome and functional adaptations induced by high intensity training in young men”. J Physiol. Jan 15;596(2):231-252.

Hostrup, M, Reitelseder, S, Jessen, S, Kalsen, A, Nyberg, M, Egelund, J, Kreiberg, M, Maag, C, Kristensen, Thomassen, M, Pilegaard, H, Backer, V, Jacobson, GA, Holm, Bangsbo,

J. (2019) “Beta2‐adrenoceptor agonist salbutamol increases protein turnover rates and alters signalling in skeletal muscle after resistance exercise in young men”. J Physiol. Sep 1; 596(17): 4121–4139.

Howard, RM, Conway, R, Harrison, AJ. (2018) “Muscle activity in sprinting: a review”. Sports Biomech. Mar;17(1):1-17.

Hsu, Wei-Li, Vijaya, Krishnamoorthy, Scholz JP. (2006) “An Alternative Test of Electromyographic Normalization in Patients.” Muscle & Nerve 33 (2): 232–41.

Jackson, A, Hull, JH, Hopker, JC, Dickinson, JW. (2018) “Impact of detecting and treating exercise-induced bronchoconstriction in elite footballers”. ERJ Open Research 4: 00122-2017

Jeppsson, AB, Löfdahl, CG, Waldeck, B, Widmark, E. (1989) “On the predictive value of experiments in vitro in the evaluation of the effect duration of bronchodilator drugs for local administration”. Pulm Pharmacol;2(2):81-5.

Jessen, S, Onslev, J, Lemminger, A, Backer, V, Bangsbo, J, Hostrup, M. (2018) “Hypertrophic effect of inhaled beta-2-agonist with and without concurrent exercise training: A randomized controlled trial”. Scand J Med Sci Sports;28(10):2114-2122

Jocken, JW, Roepstorff, C, Goossens, GH, Van der Baan, P, Van Baak, M, Saris, WH, Kiens, B, Blaak, EE (2008) “Hormone-sensitive lipase serine phosphorylation and glycerol exchange across skeletal muscle in lean and obese subjects: effect of beta-adrenergic stimulation”. Diabetes 57:1834–1841

Johnson, M. (2006) “Molecular mechanisms of beta (2)-adrenergic receptor function, response, and regulation”. J Allergy Clin Immunol.Jan;117(1):18-24.

Johnson, M, Butchers, PR, Coleman, RA, Nials, AT, Strong, P, Sumner, MJ, Vardey, CJ, Whelan, CJ. (1993) “The pharmacology of salmeterol”. Life Sci;52(26):2131-43.

Kalsen, A, Hostrup, M, Bangsbo, J, Backer V. (2013) “Combined Inhalation of beta2‐agonists Improves Swim Ergometer Sprint Performance but Not High intensity Swim Performance”. Scandinavian Journal of Medicine & Science in Sports 24 (5): 814‐22.

Kalsen, A, Hostrup, M, Karlsson, S, Hemmersbach, P, Bangsbo, J, Backer, V (2014) “Effect of inhaled terbutaline on substrate utilization and 300-kcal time trial performance”. J Appl Physiol Bethesda Md;117, 1180–1187.

Kalsen, A, Anders, Hostrup, M, Söderlund, K, Karlsson, S, Backer, V, Bangsbo, J. (2016) “Inhaled Beta2-Agonist Increases Power Output and Glycolysis during Sprinting in Men.” Medicine and Science in Sports and Exercise 48 (1): 39–48.

Kalsen, A, Anders, Hostrup, M, Backer, V and Bangsbo, J. (2016) “Effect of formoterol, a long-acting Beta2-adrenergic agonist, on muscle strength and power output, metabolism, and fatigue during maximal sprinting in men.” Am J Physiol Regul Integr Comp Physiol 310: R1312–R1321.

Katzung, BG, (2004) “Basic & clinical pharmacology”. New York: Lange Medical Books/McGraw Hill.

Kellmann, M. (2010) “Preventing overtraining in athletes in high-intensity sports and stress/recovery monitoring”. Scand J Med Sci Sports;20 Suppl 2:95-102.

Kirjavainen, M, Mattila, L, Vahteristo, M, Korhonen, J, Lähelmä, S (2018) “Pharmacokinetics of Salmeterol and Fluticasone Propionate Delivered in Combination via Easyhaler and Diskus Dry Powder Inhalers in Healthy Subjects” (J Aerosol Med Pulm Drug Deliv. Oct 1; 31(5): 290–297.

Koch, S, Karacabeyli, D, Galts, C, MacInnis, MJ, Sporer, BC, Koehle, MS. (2015a) “Effects of inhaled bronchodilators on lung function and cycling performance in female athletes with and without exercise-induced bronchoconstriction”. J Sci Med Sport.;18(5):607-612.

Koch, S, Joeungyoen, RA, Koehle, MS. (2015b). “High-Dose Inhaled Salbutamol Does Not Improve 10-Km Cycling Time Trial Performance.” Medicine and Science in Sports and Exercise 47 (11): 2373–79.

Krustrup, P, Magni, M, Tommas, A, Torben, R, Johnny, J, Aadam, S, Preben, K, P, Bangsbo, J. (2003) “The Yo-Yo Intermittent Recovery Test: Physiological Response, Reliability, and Validity.” Medicine and Science in Sports and Exercise 35 (4): 697–705.

Larsson, K, Ghaved, D, Larsson, L, Holmer, I, Jorfelt, I, Ohlsen, P. (1997) “Influence of a beta2-agonist on physical performance at low temperature in elite athletes”. Med Sci Sports Med. 29(12):1631-1636.

Laurent, J, Guinot, M, LE Roux, Mallouf T, Marillier, M, Larribaut, J, Verges, S. (2018) “Effects of Acute Salbutamol Intake on Peripheral and Central Fatigue in Trained Men.” Med Sci Sports Exerc 50, 1267–1276.

Lee, P, Day, RO, Greenfield, JR, Ho, KKY. (2013) “Formoterol, a highly β2-selective agonist, increases energy expenditure and fat utilisation in men”. Int J Obes. 37(4):593-597

Lee, P, Birzniece, V, Umpleby, AM, Poljak, A, Ho. (2015) “Formoterol, a Highly B2-Selective Agonist, Induces Gender-Dimorphic Whole-Body Leucine Metabolism in Humans” Metabolism Volume 64, Issue 4, Pages 506-512

Lemmer, JT, Fleck, SJ, Wallach, JM, Fox, S, Burke, ER, Kearney, JT, Storms, WW (1995) “The effects of albuterol on power output in non-asthmatic athletes”. Int J Sports Med 16:243–249

Lemminger, AK, Jessen, S, Habib, S. (2019) “Effect of beta2 -adrenergic agonist and resistance training on maximal oxygen uptake and muscle oxidative enzymes in men”. Scand J Med Sci Sports.

Lenti, M, De Vito, G, Sbriccoli, P, Scotto di Palumbo, A, Sacchetti, M. (2010). “Muscle Fibre Conduction Velocity and Cardiorespiratory Response during Incremental Cycling

Exercise in Young and Older Individuals with Different Training Status." Official Journal of the International Society of Electrophysiological Kinesiology 20 (4): 566–71.

Leonard, P, Grubb, NR, Addison, PS, Clifton, D, Watson, JN. (2004) "An algorithm for the detection of individual breaths from the pulse oximeter waveform". J Clin Monit Comput. Dec;18(5-6):309-12

Le Panse, B, Collomp, K, Portier, H. (2005) "Effects of short-term salbutamol ingestion during a Wingate test". Int J Sports Med.;26(7):518-523.

Le Panse, B, Arlettaz, A, Portier, H, Lecoq, A-M, De Ceaurriz, J, Collomp, K. (2006) "Short term salbutamol ingestion and supramaximal exercise in healthy women". Br J Sports Med.40(7):627-631.

Levai, IK, Hull, JH, Loosemore, M, Greenwell, J, Whyte, G, Dickinson, JW. (2016) "Environmental influence on the prevalence and pattern of airway dysfunction in elite athletes." Respirology. Nov;21(8):1391-1396.

Lisi, DM. (1989) "Muscle spasms and creatine phosphokinase elevation following salbutamol administration". Eur Respir J.;2(1):98.

Löfdahl, CG. (1990) "Basic pharmacology of new long-acting sympathomimetics". Lung;168 Suppl:18-21.

Lynch, G, Ryall, J (2008) "Role of β-adrenoceptor signalling in skeletal muscle: Implications for muscle wasting and disease". Physiol Rev. 88: 729-767

Martineau, L, Horan, MA, Rothwell, NJ, Little, RA. (1992) "Salbutamol, a beta 2-adrenoceptor agonist, increases skeletal muscle strength in young men". Clin Sci. ;83(5):615-621.

Masoli, M, Fabian, D, Holt, S, Beasley, R. (2004) "The global burden of asthma: executive summary of the GINA Dissemination Committee Report". Allergy. 59:469-478.

Matthews, G, Campbell, SE, Falconer, S. (2001). “Assessment of Motivational States in Performance Environments.” Proceedings of the Human Factors and Ergonomics Society Annual Meeting 45 (13): 906–10.

McCormick, C, Alexandre, L, Thompson, J, Mutungi, G. (2010) “Clenbuterol and formoterol decrease force production in isolated intact mouse skeletal muscle fiber bundles through a beta2-adrenoceptor-independent mechanism”. J Appl Physiol, Dec;109(6):1716-27.

McDowell, S, Fleck, SJ, Storms, W. (1997) “The effects of salmeterol on power output in non-asthmatic athletes”. Journal of Allergy and Clinical Immunology. Volume 99, Issue 4, April, Pages 443-449

McFadden, E, Gilbert, I. (1994) “Exercise-induced asthma”. N Eng J Med 330:1362-1367.

McKenzie, D, Rhodes, E, Stirling, D, Wiley, J, Dunwoody, D, Filsinger, I. (1983) Salbutamol and treadmill performance in non-atopic athletes. Med Sci Sports Exerc. 15(6):520-522.

Menaspà, P, Quod, M, Martin, DT, Peiffer, JJ, Abbiss, JR. (2015) “Physical Demands of Sprinting in Professional Road Cycling.” International Journal of Sports Medicine 36 (13): 1058–62.

Meeuwisse, W, McKenzie, D, Hopkins, S, Road, J. (1992) “The effect of salbutamol on performance in elite non-asthmatic athletes”. Med Sci Sports Exerc. 24(10):1161-1166.

Miller, M, Hankinson, J, Brusasco, V. (2005) “Standardisation of spirometry”. Eur Resp J. 26:319-338.

Milner-Brown, HS, Lee, RG. (1975) “Synchronization of human motor units: Possible roles of exercise and supraspinal reflexesSynchronisation des unites motrices chez l'h homme: Roles possibles de l'exercise et des reflexes supra-spinaux” Volume 38, Issue 3, March, Pages 245-254

Molis, M, and Molis W. (2010) “Exercise-Induced Bronchospasm” Sports Health. Jul; 2(4): 311–317.

Molphy, J, Dickinson, JW, Chester, NJ, Loosemore, M, Whyte, G. (2018) “The Effects of Inhaled Terbutaline on 3-km Running Time-Trial Performance”. Int J Sports Physiol Perform. Dec 20:1-26.

Morin, J-B, Sève P. (2011) “Sprint running performance: comparison between treadmill and field conditions”. Eur J Appl Physiol;111(8):1695-1703.

Morton, AR, Papalia, SM, Fitch, KD. (1993) “Changes in anaerobic power and strength performance after inhalation of Salbutamol in nonasthmatic athletes”. Clin J Sport Med 3:14–19

Mottram, DR, Chester, N. (2018) “Drugs in sport” In: Seventh edition. ed. Milton Park, Abingdon; New York, NY: Routledge.

Mould, DR, Upton, RN. (2012) “Basic concepts in population modeling, simulation, and model-based drug development”. Pharmacometrics Syst Pharmacol;1:6.

Mountjoy, M, Fitch, K, Boulet, LP, Bougault, V, Van Mechelen, W, Verhagen, E (2015) “Prevalence and characteristics of asthma in the aquatic disciplines”. Journal of Allergy and Clinical Immunology, Vol. 136, Issue 3, p588–594

Nice (2017) “Asthma: diagnosis, monitoring and chronic asthma management” www.nice.org.uk/guidance

Norris, SR, Petersen, SR, Jones, RL. (1996) “The effect of salbutamol on performance in endurance cyclists”. Eur J Appl Physiol Occup Physiol.73(3-4):364-8.

Onslev, J, Jacobson, G, Narkowicz, C. (2017) “Beta-2-adrenergic stimulation increases energy expenditure at rest, but not during submaximal exercise in active overweight men”. Eur J Appl Physiol.;117(9):1907-1915.

Parsons, JP, Mastronarde, JG. (2005) “Exercise-induced bronchoconstriction in athletes”. Chest. 2005 Dec;128(6):3966-74.

Parsons, JP, Kaeding, C, Phillips, G, Jarjoura, D, Wadley, G, Mastronarde, JG. (2007) “Prevalence of exercise-induced bronchospasm in a cohort of varsity college athletes”. Medicine and Science in Sports and Exercise, 39, 1487-1492.

Parsons, JP, Hallstrand, T, Mastronarde, J, Kaminsky, D, Rundell, K, Hull, J. (2013) “An official American thoracic society clinical practice guideline: Exercise-Induced Bronchoconstriction”. Am J Respir Crit Care Med. 187(9):1016-1027.

Pereira, G, Correia, R, Ugrinowitsch, C, Nakamura, F, Rodacki, A, Fowler, N, Kokubun E. (2011) “The rating of perceived exertion predicts intermittent vertical jump demand and performance.” J Sports Sci. Jun;29(9):927-32.

Pineau, JC, Frey A. (2016) “Comparison of skinfold thickness models with DEXA: impact of visceral adipose tissue”. J Sports Med Phys Fitness. May;56(5):541-5.

Pluim, BM, de Hon, O, Staal, JB, Limpens, J, Kuipers, H, Shelley E, Overbeek, Aeilko H, Zwinderman, Scholten. (2011) “β2-Agonists and Physical Performance.” Sports Medicine 41 (1): 39–57.

Quirt, J, Hildebrand, KJ, Mazza, J, Noya, F and Kim, H. (2018). “Asthma” Allergy Asthma Clin Immunol. 14(Suppl 2): 50.

Reche, C, Viana, M, Van Drooge, BL, Fernández, F, Escribano, M, Castaño-Vinyals, 4, Nieuwenhuijsen, M, Adami, PE, Bermon, S. (2020) “Athletes' exposure to air pollution during World Athletics Relays: A pilot study”. Sci Total Environ. May 15; 717:137-161.

Reinhard-Groebli, F, Nicod, LP. (2017) “Exercise-induced bronchoconstriction: epidemiology, physiopathology and management” Rev Med Suisse. Nov 15;13 (583):1985-1989.

Rothwell, NJ, Stock, MJ (1987). “Influence of clenbuterol on energy balance, thermogenesis and body composition in lean and genetically obese Zucker rats”. Int J Obes. 1987;11(6):641-7.

Ryall, JG, Sillence, MN, Lynch, GS. (2006) “Systemic administration of beta2-adrenoceptor agonists, formoterol and salmeterol, elicit skeletal muscle hypertrophy in rats at micromolar doses”. Br J Pharmacol. Mar;147(6):587-95.

Rundell, K, Anderson, S, Spiering, B, Judelson, D. (2004) “Field exercise vs. Laboratory eucapnic voluntary hyperventilation to identify airway hyper-responsiveness in elite cold weather athletes”. Chest. 125:909-915.

Sanchez, AM, Collomp, K, Carra, J, Borrani, F, Coste, O, Préfaut, C, Candau, R. (2012) “Effect of acute and short-term oral salbutamol treatments on maximal power output in non-asthmatic athletes” Eur J Appl Physiol.Sep;112(9)"

Sanchez, AM, Borrani, F, Le Fur, MA, Le Mieux, A, Lecoultre, V, Py, G, Gernigon, C, Collomp, R, Candau. (2013) “Acute supra-therapeutic oral terbutaline administration has no ergogenic effect in non-asthmatic athletes”. European Journal of Applied Physiol. ;113(2):411–418

Sandsund, M, Sue-Chu, M, Helgerud, J, Reinertsen, RE, Bjermer, L. (1998) “Effect of cold exposure (-15 degrees C) and salbutamol treatment on physical performance in elite non-asthmatic cross-country skiers”. Eur J Appl Physiol Occup Physiol. Mar;77(4):297-304.

Schmekel, B, Borgstrom, L, Wollmer, P. (1992) “Exercise increases the rate of pulmonary absorption of inhaled terbutaline”. Chest, 101: 742-745, 1992.

Scott, NW, McPherson, GC, Ramsay, CR, Campbell, MK. (2002) “The method of minimization for allocation to clinical trials. a review”. Control Clin Trials. ;23(6):662-674.

Sejersted, OM, Sjøgaard, G. (2000) “Dynamics and consequences of potassium shifts in skeletal muscle and heart during exercise”. Physiol Rev. Oct;80(4):1411-81.

Signorile, JF, Kaplan, TA, Applegate, B, Perry, AC. (1992). “Effects of Acute Inhalation of the Bronchodilator, Albuterol, on Power Output.” Medicine and Science in Sports and Exercise 24 (6): 638–42.

Sporer, B, Sheel, W, Mckenzie, D. (2008) “Dose response of inhaled salbutamol on exercise performance and urine concentrations”. Med Sci Sports Exerc. 40(1) 149-157.

Stewart, I, La Breche, B, McKenzie, Donald, C. (2002) “Acute formoterol administration has no ergogenic effect in non-asthmatic athletes.” Medicine and Science in Sports and Exercise, 34(2), pp. 213-217.

Stickland, MK, Rowe, BH, Spooner, CH, Vandermeer, B, Dryden, DM. (2012) “Effect of warm-up exercise on exercise-induced bronchoconstriction”. Med Sci Sports Exerc;44(3):383–91

Street, L (1995) “Controlling extraneous variables in experimental research: A research note” Accounting Education 4(2):169-188

Sue-Chu, M, Sandsund, M, Helgerud, J, Reinertsen, RE, Bjermer, L. (1999) “Salmeterol and physical performance at -15 degrees C in highly trained nonasthmatic cross-country skiers.”. Scand J Med Sci Sports. Feb;9(1):48-52.

Sverrild, A, Porsbjerg, C, Thomsen, SF, Backer, V. (2009) “Diagnostic properties of inhaled mannitol in the diagnosis of asthma: a population study”. J Allergy Clin Immunol. Nov;124(5):928-32.

Sverrild, A, Porsbjerg, C, Thomsen, SF, Backer, V. (2010) “Airway hyperresponsiveness to mannitol and methacholine and exhaled nitric oxide: a random-sample population study”. J Allergy Clin Immunol. Nov;126(5):952-8.

Sweetman, SC. (2011) “The Complete Drug Reference”. 37th Edn. London, Pharmaceutical Press; p. 1254

Taylor, NA, Wilkinson, JG (1986) “Exercise-induced skeletal muscle growth. Hypertrophy or hyperplasia?” Sports Med. May-Jun;3(3):190-200.

Taskin, H. (2008) “Evaluating Sprinting Ability, Density of Acceleration, and Speed Dribbling Ability of Professional Soccer Players with Respect to Their Positions.” Journal of Strength and Conditioning Research 22 (5): 1481–86.

Terry, PC, Lane, AM, Fogarty, GJ. (2003) “Construct Validity of the Profile of Mood States — Adolescents for Use with Adults.” Psychology of Sport and Exercise 4 (2): 125–39.

Tjørhom, A, Riiser, A, Carlsen, KH. (2007) “Effects of formoterol on endurance performance in athletes at an ambient temperature of -20 degrees”. Scand J Med Sci Sports;17(6):628-635.

Uk asthma Journal (2001) “A true picture of asthma in the UK today” Out in the open: a true picture of asthma in the UK today. UK Asthma Asthma J 6:Suppl 3Suppl 3, 1-16.

Ullman, A, Hedner, J, Svedmyr, N. (1990) “Inhaled salmeterol and salbutamol in asthmatic patients. An evaluation of asthma symptoms and the possible development of tachyphylaxis”. Am Rev Respir Dis. Sep;142(3):571-5.

Ullman, A, Bergendal, A, Lindén, A, Waldeck, B, Skoogh, BE, Löfdahl, CG. (1992) “Onset of action and duration of effect of formoterol and salmeterol compared to salbutamol in isolated guinea-pig trachea with and without epithelium”. Allergy. Aug;47(4 Pt 2):384-7.

Uren, NG, Davies, SW, Jordan, SL, Lipkin, DP. (1993) “Inhaled bronchodilators increase maximum oxygen consumption in chronic left ventricular failure”. Eur Heart J. 1993 Jun;14(6):744-50.

Van Baak, MA, Mayer, LH, Kempinski, RE, Hartgens, F. (2000) “Effect of salbutamol on muscle strength and endurance performance in non-asthmatic men”. Med Sci Sports Exerc 32, 1300–1306.

Van Baak, MA, De Hon, OM, Hartgens, F, Kuipers, H. (2004) “Inhaled salbutamol and endurance cycling performance in non-asthmatic athletes”. Int J Sports Med. Oct;25(7):533-8.

Ventura, R, Segura, J, Berges, R, Fitch, K, Morton, A, Berruezo, S. (2000). “Distinction of Inhaled and Oral Salbutamol by Urine Analysis Using Conventional Screening Procedures for Doping Control”. Ther Drug Monit. 22(3):277-282.

WADA (2009) Prohibited List of Substances and Methods. The 2009 Prohibited List. World Anti-Doping Agency. http://www.olympic.org/Documents/Fight_against_doping/Rules_and_regulations/WADA_Prohibited_List_2009.pdf

WADA (2010) Prohibited List of Substances and Methods. The 2010 Prohibited List. World Anti-Doping Agency. http://www.olympic.org/Documents/Fight_against_doping/Rules_and_regulations/WADA_Prohibited_List_2010.pdf

WADA (2012) Prohibited List of Substances and Methods. The 2012 Prohibited List. World Anti-Doping Agency. http://www.olympic.org/Documents/Fight_against_doping/Rules_and_regulations/WADA_Prohibited_List_2012.pdf

WADA (2015) Prohibited List of Substances and Methods. The 2015 Prohibited List. World Anti-Doping Agency. http://list.wada-ama.org/wp-content/uploads/2013/11/2015-WADA_Prohibited_List_2015.pdf

WADA (2019) "Prohibited List. World Anti-Doping Agency". https://www.wada-ama.org/sites/default/files/prohibited_list_2019_en.pdf . Accessed January 1, 2019.

WADA (2020) "Prohibited List. World Anti-Doping Agency". https://www.wada-ama.org/sites/default/files/prohibited_list_2020_en.pdf . Accessed January 1, 2020.

WADA (2020-b) "Prohibited List. World Anti-Doping Agency". https://www.wada-ama.org/en/content/what-is-prohibited/prohibited-at-all-times/anabolic-agents

Weiler, R. (2010) “What about physical activity and exercise medicine?” Clin Med 2010 Oct;10(5):522

Westfall, MV. (2006) “A switch that lowers the beta AR: insights from a troponin I mutation linked to hypertrophic cardiomyopathy”. J Mol Cell Cardiol. Jan;40(1):10-2.

Willardson, JM. (2006). “A brief review: factors affecting the length of the rest interval between resistance exercise sets”. J Strength Cond Res.20(4):978-984.

Withers, R, Gore, C, Gass, G, Hahn, A. (2000) “Determination of Maximal Oxygen Consumption (VO2max) or Maximal Aerobic Power”. In: Gore C, editor. Physiological Test for Elite Athletes. Champaign, IL: Human Kinetics; p. 114-127

Yang, YT, Mc Elligott, MA. (1989) “Multiple actions of beta-adrenergic agonists on skeletal muscle and adipose tissue”. Biochem J.;261(1):1-10.

.

Appendices

Ethical Approvals

FULL ETHICS APPLICATION FOR RESEARCH WITH HUMAN PARTICIPANTS – FACULTY OF SCIENCES

University of Kent

If any of the questions in Section IV B is answered 'yes', a full ethics application must be made to the REAG. This also applies for studies not defined as 'research' in the narrow sense, i.e. evaluations/audits, etc. Complete this form and send it to the Faculties Support Office along with supporting documentation: a copy of the full research proposal; any participant information sheets and consent forms; any surveys, interview schedules; any advertising material or proposed website wording.

Overview

Name of Applicant(s)

Mr Michele Merlini Dr John Dickinson,

Contact Details (Please include your UoK address, email and telephone number)

Mr Michele Merlini: m.merlini553@kent.ac.uk, +44 (0)1634 208457

Dr John Dickinson: j.w.dickinson@kent.ac.uk, +44 (0)1634 202998

Title of Project

The impact of short acting β_2 Agonists on repeated sprint ability and Yo-Yo IRT Lev1 test in professional soccer players.

Lay Summary (Please provide a brief summary of the study)

The use of inhaled salbutamol is common amongst athletes (Fitch 2012). Inhaled salbutamol is a fast acting medication used by athletes to reverse and protect against airway narrowing during and following exercise. Currently the World Anti-doping Agency (WADA) permits athletes to use inhaled salbutamol (up to a maximum of 1600 micrograms over 24 hours) without the need for evidence of asthma or a Therapeutic Use Exemption certificate. It has been noted that athletes using inhaled salbutamol at summer and winter Olympics win more gold medals than those that do not (Fitch 2012). This statistic begs the question as to whether inhaled β2-agonists are ergogenic.

Research investigating the potential for inhaled salbutamol to improve performance in athletes is currently inconclusive. Few studies have demonstrated any performance improvement following the inhalation of acute doses of up to 800 µg salbutamol on running time to exhaustion, VO2max, peak power, 20 km cycling time-trial and total work during a 30 s Wingate test (Pluim et al. 2011). Dickinson et al. (2014a) reported no improvement in 5 km running time trial performance following inhalation of 1600 µg salbutamol. Elers et al. (2012) demonstrated inhaling an acute dose of up to 4000 µg salbutamol resulted in no increase in cycling time to exhaustion or oxygen kinetics. There also appears to be no improvement in strength, power or endurance when none asthmatic athletes inhale 1600 µg salbutamol on a daily basis for 6 weeks (Dickinson et al., 2014b). However, oral salbutamol taken acutely and over a two week period has been shown to improve peak power output on repeated cycling sprint performance (Hostrup et al. 2014). A recent study by Kalsen et al., (2013) has also suggested there may be an improvement in maximal swimming sprint performance and maximal contraction of the quadriceps femoris if an acute dose of 1600 µg inhaled salbutamol is taken in combination with inhaled long acting β2-agonists.

There seems to be the potential for inhaled salbutamol to improve individual sprint and repeated sprint

1/10/15

04.02.2016

University of Kent

Faculties Support Office

FIRST CLASS

Dr John Dickinson
Lecturer
School of Sport and Exercise Sciences
Room Medway M1-28
Medway Building
University of Kent
Chatham Maritime
Kent ME4 4AG

Ref: RS/ARC/023S12/13
Date: 11 October 2013

By Email to J.W.Dickinson@kent.ac.uk

Dear Dr Dickinson

I am pleased to tell you that your project "The Long-Term Ergogenic Effect of Long Acting β2-Agonists" (023S12/13) received ethical approval from the Sciences Research Ethics Advisory Group for Human Participants on 11/10/13.

Please note that it is essential that you:

1. Comply with the Data Protection Act of 1998
2. Comply throughout the conduct of the study with good research practice standards
3. Refer any amendment to the protocol of the Sciences Research Ethics Advisory Group

If you are intending to work unaccompanied with children or with vulnerable adults, you will need to apply for a CRB check.

Please do not hesitate to get in touch if you have any questions.

Yours sincerely

Alison Chapman
Administrator

University of Kent
Marlowe Building
Canterbury
Kent CT2 7NR
United Kingdom

ETHICS REVIEW CHECKLIST FOR RESEARCH WITH HUMAN PARTICIPANTS – FACULTY OF SCIENCES

University of Kent

Section IV: Research Checklist

Please answer all questions by ticking the appropriate box:

A) **Research that may need to be reviewed by an NHS Research Ethics Committee, the Social Care Research Ethics Committee (SCREC) or other external ethics committee (if *yes*, please give brief details as an annex)**	YES	NO
Will the study involve recruitment of patients through the NHS or the use of NHS patient data or samples?	☐	X
Will the study involve the collection of tissue samples (including blood, saliva, urine, etc.) from participants or the use of existing samples?	X	☐
Will the study involve participants, or their data, from adult social care, including home care, or residents from a residential or nursing care home?	☐	X
Will the study involve research participants identified because of their status as relatives or carers of past or present users of these services?	☐	X
Does the study involve participants aged 16 or over who are unable to give informed consent (e.g. people with learning disabilities or dementia)?	☐	X
Is the research a social care study funded by the Department of Health?	☐	X
Is the research a health-related study involving prisoners?	☐	X

If the answer to any questions in Section IV A is 'yes', please contact the Research Ethics & Governance Officer for further advice and assistance.

B) **Research that may need full review by the Sciences REAG**	YES	NO
Does the research involve other vulnerable groups: children; those with cognitive impairment; or those in unequal relationships, e.g. your own students?	☐	X
Does the project involve the collection of material that could be considered of a sensitive, personal, biographical, medical, psychological, social or physiological nature, other than one that is covered by existing block approval?	☐	X
Will the study require the cooperation of a gatekeeper for initial access to the groups or individuals to be recruited (e.g. students at school; members of a self-help group)?	☐	X
Will it be necessary for participants to take part in the study without their knowledge and consent at the time? (e.g. covert observation of people in non-public places?)	☐	X
Will the study involve discussion of sensitive topics (e.g. sexual activity; drug use; criminal activity)?	☐	X
Are drugs, placebos or other substances (e.g. food substances, vitamins) to be administered to the study participants or will the study involve invasive, intrusive or potentially harmful procedures of any kind?	X	
Is pain or more than mild discomfort likely to result from the study?	☐	X
Could the study induce psychological stress or anxiety or cause harm or negative consequences beyond the risks encountered in normal life?	☐	X
Will the study involve prolonged or repetitive testing?	X	☐
Will the research involve administrative or secure data that requires permission from the appropriate authorities before use?	☐	X
Is there a possibility that the safety of the researcher may be in question (e.g. international research; locally employed research assistants)?	☐	X
Does the research involve members of the public in a research capacity (participant research)?	☐	X
Will the research take place outside the UK?	☒	
Will the outcome of the research allow respondents to be identified either directly or indirectly (e.g. through aggregating separate data sources gathered from the internet)?	☐	X
Will research involve the sharing of data or confidential information beyond the initial consent given?	☐	X
Will financial inducements (other than reasonable expenses and compensation for time) be offered to participants?	☐	X
Will the proposed findings be controversial or are there any conflicts of interest?	☐	X

Study ID: Date:

SECTION 2. – Chronic Medical Conditions

	Yes	No
1. Do you have cancer of any kind?		
a. Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head and neck?	☐	☐
b. Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	☐	☐
2. Do you have Heart Disease or Cardiovascular Disease? *This includes coronary Artery Disease, High Blood Pressure, Heart Failure, Diagnosed Abnormality of Heart Rhythm.*	**Yes**	**No**
a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments.)	☐	☐
b. Do you have an irregular heartbeat that requires medical management? (e.g. atrial fibrillation, premature ventricular contraction)	☐	☐
c. Do you have chronic heart failure?	☐	☐
d. Do you have a resting blood pressure equal to or greater than 140/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure.)	☐	☐
e. Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	☐	☐
3. Have you had a Stroke? *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event.*	**Yes**	**No**
a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments.)	☐	☐
b. Do you have any impairment of walking or mobility?	☐	☐
c. Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	☐	☐

Study ID: Date:

	Yes	No
4. **Do you have any Metabolic Conditions?** *This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes.*		
a. Is your blood sugar often above 13.0 mmol/L? (Answer YES if you are not sure.)	☐	☐
b. Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys and the sensation in your toes and feet?	☐	☐
5. **Do you have other Metabolic Conditions** such as thyroid disorders, pregnancy-related diabetes, chronic kidney disease or liver problems?	☐	☐
6. **Do you have a Respiratory Disease?** *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure.*		
a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments.)	☐	☐
b. Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	☐	☐
c. Has your doctor ever said you have high blood pressure in the blood vessels of your lungs.	☐	☐
d. If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week) or have you used your rescue medication more than twice in the last week?	☐	☐
	If asthmatic, please go to Page 6.	
7. **Do you have any Mental Health Problems or Learning Difficulties?** *This includes Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome etc.*		
Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments.)	☐	☐

Study ID: Date:

	Yes	No
8. Do you have Arthritis, Osteoporosis or Back Problems?		
a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments.)	☐	☐
b. Do you have joint problems causing pain, a recent fracture or facture caused by osteoporosis or cancer, displaced vertebra (e.g. spondyolisthesis) and/or spondylosis/pars defect (a crack in the bony ring on the back of the spinal column)?	☐	☐
c. Have you had steroid injections or taken tablets regularly for more than 3 months?	☐	☐
9. Do you have any other medical condition not listed above or do you live with two chronic conditions?	**Yes**	**No**
a. Have you experienced blackout, fainted or lost consciousness as a result of a head injury within the last 12 months?	☐	☐
b. Do you have a medical condition that is not listed, such as epilepsy, neurological conditions, kidney problems etc.?	☐	☐
c. Do you currently live with two chronic conditions?	☐	☐
10. Are there any other relevant conditions/injuries/illnesses that you are aware that you have which have not been covered in this questionnaire?	**Yes**	**No**
	☐	☐

If yes, please provide details:

_____

Date: _____ Study ID: DB_____

University of Kent

EVH - PROVOCATION CHALLENGE

Fractioned exhaled Nitric Oxide (FeNO) **Average FeNo:** ☐

Test 1 (ppb)	
Test 2 (ppb)	

Baseline Spirometry

Spirometry	FEV_1 (litres)	FVC (litres)	PEF (l/min)	FEV_1/FVC%
1st Flow Loop				
2nd Flow Loop				
3rd Flow Loop				
Best				
% Predicted				

**Predicted value must be 80% or above*

Best Baseline FEV_1 _____ **must be within 5% of second best FEV_1*

10% of Best Baseline FEV_1 _____

Target Minute Ventilation (Best FEV_1x30) _____

EVH Challenge

Time	Total volume of air expired (litres)	Expected	Correction
1 min			
2 min			
3 min			
4 min			
5 min			
6 min			

Post EVH Spirometry

Post EVH	Effort 1			Comments	Effort 2			Comments	Best FEV_1 (at minute)
	FEV_1	FVC	PEF		FEV_1	FVC	PEF		
3 mins (9 mins)									
5 mins (11 mins)									
7 mins (13 mins)									
10 mins (16 mins)									
15 mins (21 mins)									

POST BRONCHODILATOR

Bronchodilator _____ **Dose** _____

	Effort 1			Effort 2			Effort 3		
	FEV_1	FVC	PEF	FEV_1	FVC	PEF	FEV_1	FVC	PEF
% Predicted									

COMMENTS:

Study ID: Date:

University of Kent

$VO_{2\ max}$ test – Visit ____

Stage	(time)	Workload	Borg 10	RPE	HR	RER
0.	0 – 2 mins	0W	_____	_____	_____	_____
1.	2 – 5 mins	0W	_____	_____	_____	_____
2.	5 – 6 mins	25W	_____	_____	_____	_____
3.	6 – 7 mins	50W	_____	_____	_____	_____
4.	7 – 8 mins	75W	_____	_____	_____	_____
5.	8 – 9 mins	100W	_____	_____	_____	_____
6.	9 – 10 mins	125W	_____	_____	_____	_____
7.	10 – 11 mins	150W	_____	_____	_____	_____
8.	11 – 12 mins	175W	_____	_____	_____	_____
9.	12 – 13 mins	200W	_____	_____	_____	_____
10.	13 – 14 mins	225W	_____	_____	_____	_____
11.	14 – 15 mins	250W	_____	_____	_____	_____
12.	15 – 16 mins	275W	_____	_____	_____	_____
13.	16 – 17 mins	300W	_____	_____	_____	_____
14.	17 – 18 mins	325W	_____	_____	_____	_____
15.	18 – 19 mins	350W	_____	_____	_____	_____

Papers Published

International Journal of Sports Physiology and Performance, (Ahead of Print)
https://doi.org/10.1123/ijspp.2018-0921

Human Kinetics
ORIGINAL INVESTIGATION

Improved Sprint Performance With Inhaled Long-Acting β_2-Agonists Combined With Resistance Exercise

Michele Merlini, Greg Whyte, Sam Marcora, Mike Loosemore, Neil Chester, and John Dickinson

Purpose: To investigate the impact of twice-daily inhalation of 100 µg of salmeterol (SAL) or 12 µg of formoterol (FOR) in addition to a strength- and power-training program over a 5-wk period on a 30-m sprint, strength, power, mood, stress, and skinfold thickness. ***Methods:*** In a randomized, single-blind study, 23 male and 15 female nonasthmatic, recreationally active individuals were recruited (mean [SD] age 26.3 [5.4] y, weight 76.2 [11.5] kg, height 176.9 [8.5] cm). Participants completed 3 standardized whole-body strength- and power-training sessions per week for 5 wk during which they were assigned to an SAL, FOR, or placebo group. Participants used their inhaler twice per day as instructed and completed assessments of sprint, strength, and power at baseline and 1 wk after cessation of the training program. The assessments included a 30-m sprint, vertical jump, 1-repetition-maximum (1RM) bench press, 1RM leg press, peak torque flexion and extension, anthropometric evaluation, and Rest-Q questionnaires. ***Results:*** After 5 wk of strength and power training, 30-m sprint time reduced in the FOR (0.29 [0.11] s, $P = .049$) and SAL (0.35 [0.05] s, $P = .040$) groups compared with placebo (+0.01 [0.11] s). No significant change was found in other assessments of strength, mood, or skinfold thickness. ***Conclusions:*** When strength and power training are combined with the inhalation of FOR or SAL over a 5-wk period, moderately trained individuals experience an improvement in 30-m sprint performance.

Keywords: asthma, doping, ergogenic, training

As of 2019 the World Anti-Doping Agency (WADA)[1] permits athletes to use inhaled therapeutic doses of β_2-agonists salbutamol (1600 µg/d, no more than 800 µg in a 12-h period), formoterol (FOR; 54 µg/d), and salmeterol (SAL; 200 µg/d). However, there is some debate as to whether the current rules allow unscrupulous athletes, with and without asthma-related conditions, to use inhaled β_2-agonists for the purpose of benefiting from a potential ergogenic action.

Previous research investigating the acute and short-term use (eg, 2 wk) of inhaled β_2-agonists suggests that they do not have an ergogenic action on endurance performance.[2] Furthermore, endurance performance is not improved from acute doses of inhaled FOR[3] and SAL.[4] However, moderately and highly trained individuals may experience enhanced strength and power performance from the acute use of short-acting[5] and long-acting β_2-agonists.[6]

The mechanisms behind the ergogenic action from acute doses that have been observed in skeletal muscle include β_2-adrenergic stimulation, which counteracts exercise-induced reductions in Na^+–K^+ ATPase maximum rate of activity, elevated glycolytic activity during high-intensity exercise, and enhanced rates of Ca^{2+} release and uptake from the sarcoplasmic reticulum.[5] Furthermore, increased anaerobic ATP utilization has been suggested as a potential mechanism.[6] However, others have failed to demonstrate changes in peak force velocity and have shown that maximal strength deteriorates following acute oral terbutaline administration.[7]

Long-term use of β_2-agonists also has the potential to produce an ergogenic action. Data from animal models suggest long-term β_2-adrenergic stimulation results in muscle hypertrophy.[8] Studies investigating the long-term β_2-adrenergic stimulation in humans suggest increases in skeletal muscle mass[9] and portioning of amino acids from oxidative loss toward protein synthesis[10] may occur. Furthermore, salbutamol has been shown to counteract a negative net protein balance following resistance training in males.[11] These changes to skeletal muscle from long-term use of β_2-agonists has been shown to increase peak muscle strength[12] and power output,[13] while also inducing a slow- to fast-twitch muscle phenotype transition in humans.[14]

Long-term use of β_2-agonists may also decrease body fat due to increased fat mobilization from adipose tissue,[13] decreased fat synthesis in adipose tissue and liver,[15] or a combination of both.[16] Although there is clear potential for ergogenic action with oral or supra-therapeutic-inhaled doses of β_2-agonists, we do not know whether long-term stimulation of β_2-adrenoreceptors through therapeutic doses of long-acting inhaled β_2-agonists has a similar effect.

Endurance training has been shown to confound the ergogenic action of inhaled short-acting β_2-agonists.[17] However, the ergogenic action of inhaled short-acting β_2-agonists is augmented with resistance training.[9] It is not known whether there is a similar interaction when long-acting β_2-agonists are inhaled while engaging in strength training. This is a realistic consideration, as athletes using long-acting inhaled β_2-agonists (SAL or FOR) are prescribed to do so on a daily basis, which may modify their response to strength and power training.

Accordingly, the purpose of this study was to investigate the impact of therapeutic doses of inhaled SAL or FOR combined with a resistance exercise training program on 30-m sprint, strength, power, mood, stress, and skinfold thickness.

Methods

The study procedure was approved by the Faculty of Science research ethics committee at the University of Kent, and followed

Merlini, Marcora, and Dickinson are with the School of Sport and Exercise Sciences, University of Kent, Chatham Maritime, United Kingdom. Whyte and Chester are with the Research Inst of Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, United Kingdom. Loosemore is with the Inst of Sport, Exercise and Health, University College London, London, United Kingdom. Merlini (michele.merlini1986@gmail.com) is corresponding author.

©Journal of Sports Science and Medicine (2019) 18, 716-721
http://www.jssm.org

Research article

The Effect of 1600 µg Inhaled Salbutamol Administration on 30 m Sprint Performance Pre and Post a Yo-Yo Intermittent Running Test in Football Players

Michele Merlini [1]✉, Marco Beato [2], Samuele Marcora [1] and John Dickinson [1]
[1] School of Sport and Exercise Sciences, University of Kent, Chatham Maritime, UK
[2] School of Science, Technology and Engineering, University of Suffolk, Ipswich, UK

Abstract
The purpose of the study was to investigate the effect of inhaling 1600 µg of salbutamol (SAL) on 30 m sprint before and after the Yo-Yo Intermittent Recovery test. In a randomised cross over single blind study 13 male non-asthmatic, football players volunteered (mean ± SD; age 18.1 ± 0.9 years; weight 69.5 ± 8.3 kg; height 1.78 ± 0.07 m). Participants completed two visits and were randomly assigned to either (SAL) or (PLA) treatment and performed a set of three sprints of 30 m before and after the Yo-Yo Intermittent Recovery Test (Yo-Yo IRT). Best sprint and mean sprint were analysed in addition to the distance covered during the Yo-Yo IRT; rating of perceived exertion and heart rate were collected at the end of each level completed. Repeated measures ANOVA were performed to investigate changes in performance between groups. Following the inhalation of supra-therapeutic salbutamol dose (1600 µg) neither 30 m sprint time (PLA 4.43 ± 0.14 s; SAL 4.44 ± 0.15 s, p = 0.76) nor distance covered in the Yo-Yo IRT test reported significant variation between PLA conditions (1660 ± 217 m) and SAL (1610 ± 229 m, p = 0.16). Moreover, lactate values, heart rate and RPE did not differ significantly between groups. The inhalation of 1600 µg salbutamol does not enhance 30 m sprint performance in non-fatigued and fatigue conditions. Our findings suggest when football players acutely inhale double the permitted dose of salbutamol, as indicated in the World Anti-Doping Agency List of Prohibited Substances and Methods, they will not experience improvements in sprint or endurance performance.

Key words: Football, salbutamol, sprint, asthma, doping.

Introduction

Athletes with asthma related conditions are permitted to use inhaled salbutamol in accordance with current WADA regulations (WADA, 2019). Although a therapeutic dose of inhaled salbutamol ranges from 200-400 µg, football players are permitted to inhale up to 800 µg in any 12 hours period and 1600 µg in a 24 hours period. Limited data is available to explain whether football players may gain an ergogenic advantage from doses of inhaled salbutamol up to 1600 µg.

Recent research by Dickinson et al. (2015) suggests that doses of up to 1600 µg of inhaled salbutamol do not improve repeated sprint performance following a 52 minute football specific treadmill running protocol. However, others have observed improvements in sprint cycling after the inhalation of salbutamol 180 µg (Signorile et al., 1992) and 15 mg Terbutaline (Kalsen et al., 2016). The latter authors suggested the 8% improvement in power output over a 10 s sprint cycling performance was associated with a greater fatigue resistance in type II fibers due to increased rates of glycogenolysis and glycolysis. Furthermore, when well trained swimmers inhale a combination of salbutamol (1600 µg), formoterol (36 µg) and salmeterol (200 µg), their 100 m sprint performance is enhanced by 1 s (Kalsen et al., 2013). Recently, Hostrup et al. (2016) demonstrated that initial Wingate sprint performance was enhanced after 8 mg oral salbutamol administration but subsequent sprints were not enhanced in the same way.

Acute use of short acting β_2-agonists may result in ergogenic action leading to improvements in peak power and one-off sprint performance. However, limited data suggest repeated sprint performance, with and without pre fatigue, is not enhanced. The purpose of this study is to investigate the potential ergogenic action of inhaled 1600 µg salbutamol on 30 m sprint performance in football players before and after the Yo-Yo intermittent recovery test (Yo-Yo IRT).

Methods

Participants

Thirteen male football players volunteered and provided written and verbal informed consent. All players took part in amateur football league, competing once a week and trained specifically for football at least three times a week. All participants were free from respiratory disease including asthma related conditions, cardiopulmonary disease, metabolic disease and musculoskeletal injury (Table 1).

Table 1. Descriptive features of participants (n = 13) expressed as mean and standard deviation (Mean ± SD)

Parameters	
Age (years)	18.1 ± 0.9
Height (m)	1.78 ± 0.07
Weight (kg)	69.5 ± 8.3
BMI (kg·m^{-2})	22 ± 1.7
VO_{2Max} (mL/min/kg)	51.2 ± 3.4
HR_{Max} (bpm)	198 ± 4.0
Training (h/week	8 ± 1.4
RPE_{Max} (u.a.)	9 ± 1.0

BMI, body mass index; VO_{2Max}, maximal oxygen uptake

The participants completed all assessments at the same time of the day (within 1 h), separated by a minimum of 48 hours. They were instructed to sleep for at least 7 hours, drink at least 35 mL/kg/day of water, refrain from consumption of alcohol for 24 hours prior to the visit and

Received: 12 April 2019 / Accepted: 11 September 2019 / Published (online): 19 November 2019