University of Kent

PERFORMANCE-ENHANCING OR PURELY THERAPEUTIC? THE IMPACT OF ACUTE, SHORT-TERM, AND CHRONIC ADMINISTRATION OF ASTHMA-RELATED GLUCOCORTICOID THERAPY ON ATHLETE HEALTH, PERFORMANCE AND RECOVERY

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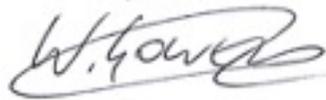
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THESIS DECLARATION

No part of this thesis has been submitted in support of an application for any degree or other qualification of the University of Kent, or any other University or Institution of learning.

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CHAPTER SPECIFIC ACKNOWLEDGEMENTS

THESIS ABSTRACT

Exercise-induced bronchoconstriction (EIB) is highly prevalent in elite athlete populations and requires pharmacological treatment such as inhaled corticosteroids (ICS) to manage the condition, or oral glucocorticoids (GC) in the event of serious emergency exacerbation. However, debate continues if access to these substances are not only therapeutic but also performance-enhancing. This thesis aimed to investigate the impact of acute, short-term, and chronic administration of asthma-related GC therapy on athlete health, performance, and recovery. The first experimental study (*Chapter 4*) examined in a population of elite swimmers the impact of diagnosing and initiating asthma therapy on airway health outcomes and real-world performance at major competitions. This study reinforced that elite athletes mandate the use of asthma therapy. However, initiating and maintaining treatment did not enhance major competition performance beyond the natural progression expected between events. *Chapter 5* gave methodological development to prospective ICS experimental work *(Chapter 6 and 7)* by modelling beclomethasone dipropionate (BDP) deposition under different conditions, including slow and fast inhalation flow rates, and with or without a valved-holding chamber (VHC). The findings showed that the inhalation flow rate can significantly impact the delivery of BDP. Additionally, the use of a VHC would reduce the reliance on BDP device actuation coordination and improve the fine particle dose, thereby theoretically improving the mechanisms of action of ICS. Following this, experimental study 3 (*Chapter 6*) investigated the ergogenic potential of acute oral GC and ICS by comparing these two administration routes on initial 40-km cycling time-trial (TT) and subsequent recovery for a further 10-km TT performed on the same day. The results suggested that supratherapeutic dose of ICS did not improve performance. However oral GC may be *"possibly beneficial"* to initial TT performance. Neither administration route impacted recovery. Finally, experimental study 4 (*Chapter 7*) investigated the effect of short-term (14-day) administration of ICS on repeated bout 10-km cycling TT performance. The findings demonstrated that there was no improvement in performance or recovery from using high-dose ICS compared to a placebo condition. Collectively, the findings of this thesis support that elite athletes require asthma therapy, and the current WADA guidelines to allow ICS in-competition is appropriate due to the lack of an ergogenic effect when assessed using ecologically valid TT assessments or when used for therapeutic purposes within an elite athlete population. Oral GC should remain controlled with a therapeutic use exemption due to uncertainties of ergogenic impact, immunosuppressive effects, and well-established long-term health implications.

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SYMBOLS AND LIST OF ABBREVIATIONS

°C	Degrees Celsius
ACP-VHC	AeroChamber Plus – Valved holding chamber
ACTH	Adrenocorticotropic hormone
ANOVA	Analysis of Variance
APSD	Aerodynamic particle size distribution
B[Glu]	Blood glucose
B[La]	Blood lactate
BDP	Beclomethasone dipropionate
bpm	Beats per minute
CHO	Carbohydrate oxidation
cm	Centimetre
cmH_2O	Centimetres of Water Column at 4°C
CO_2	Carbon dioxide
CON	Control
DEQ-5	Drug Effect Questionnaire – 5 Item
DHEA	Dehydroepiandrosterone
DPI	Dry powder inhaler
EIB	Exercise-induced bronchoconstriction
EVH	Eucapnic voluntary hyperpnoea
FeNO	Fraction of exhaled nitric oxide
FEV_1	Forced expiratory volume in one second
FEV_1/FVC	FEV_1: FVC ratio
FINA	Fédération Internationale de Natation
FO	Fat oxidation
FVC	Forced vital capacity
GC	Glucocorticoid(s)
GH	Growth Hormone
GINA	Global Institute of Asthma
GSD	Geometric standard deviation
HPA	Hypothalamic-pituitary-adrenal
HPLC	High-performance liquid chromotography
HR	Heart rate
ICS	Inhaled Corticosteroid(s)

ICS-Q	Inhaled Corticosteroid Symptom - Questionnaire
iFPM	Interpolated fine particle mass
IL-	Interleukin-
IOC-MC	International Olympic Committee – Medical Commission
kg	Kilogram
L	Litre
L/min	Litres per minute
L/s	Litres per second
LABA	Long-acting β2-agonist
LOA	Limits of agreement
m	Metre
MBD	Magnitude based decisions
MET	Minimum effects test
mg	Milligrams
min	Minute
mL	Millilitre
$ml.kg^{-1}.min^{-1}$	Millilitres per kilogram of body mass per minute
MMAD	Mass Median Aerodynamic Diameter
MVV	Maximal voluntary ventilation
n	Number of participants
N_2	Nitrogen
NGI	Next-generation impactor
NICE	National Institute for Health and Care Excellence
O_2	Oxygen
p =	p-value [level of significance]
pg/mL	Picograms per millilitre
PLA	Placebo
pMDI	Pressurised metered-dose inhaler
ppb	Particles per billion
PRL	Prolactin
RER	Respiratory exchange ratio
RH	Relative humidity
RPE	Rating of perceived exertion
RPM	Revolutions per minute
SABA	Short-acting β2-agonist

SD	Standard deviation
sec	Seconds
SPSS	Statistical Package for the Social Sciences
sRPE	Session Rating of Perceived Exertion
SRSS	Short Recovery and Stress Scale
SWC	Smallest worthwhile change
TT	Time-Trial
TTE	Time to exhaustion
TUE	Therapeutic use exemption
ULTRA-LABA	Ultra-long-acting β2-agonists
VAS	Visual analogue score
$\dot{V}CO_2$	Rate of carbon dioxide production
$\dot{V}_E$	Minute ventilation
$\dot{V}O_2$	Rate of oxygen uptake
$\dot{V}O_2max$	Maximum oxygen uptake
$\dot{V}O_2peak$	Peak oxygen uptake
W	Watt(s)
WADA	World Anti-Doping Agency
WBC	White blood cell
yr(s)	Year(s)
Δ	Delta (Change)
ηp^2	Partial eta squared [measure of effect size]
μg	Microgram

PUBLICATIONS ARISING FROM THIS THESIS

JOURNAL ARTICLES:

Chapter 4:

Gowers, W., Evans, G., Carré, J., Ashman, M., Jackson, A., Hopker, J., & Dickinson, J. (2021). Eucapnic Voluntary Hyperpnea Challenge Can Support Management of Exercise-Induced Bronchoconstriction in Elite Swimmers. *Translational Sports Medicine*, *4*(5), 657-666. (*https://doi.org/10.1002/tsm2.258*).

CONFERENCE CONTRIBUTIONS:

Chapter 4:

Dickinson, J. W., **Gowers, W**., Evans, G., Carré, J., Ashman, M., Jackson, A., & Hopker, J. (2020). Using EVH Challenges To Objectively Monitor The Long-Term Management Of Elite Swimmers With EIB: 1191 Board# 317 May 27 2:30 PM- 4:00 PM. *Medicine & Science in Sports & Exercise*, *52*(7S), 315. **ACSM Annual Meeting 2020.** (*https://doi.org/10.1249/01.mss.0000677044.69701.bb*).

Chapter 6:

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Gowers, W., Loosemore, M., Hopker, J., & Dickinson, J. W. (2022). Effect of acute oral prednisolone and inhaled Beclomethasone Dipropionate on inflammatory cytokine response following 40-km cycling time-trial. **BASEM Annual Meeting 2022.** (*https://kar.kent.ac.uk/id/eprint/95943*).

Chapter 7:

Gowers, W., Loosemore, M., Hopker, J., & Dickinson, J. W. (2022). Effect of short-term high dose inhaled beclomethasone dipropionate administration on repeated bout 10-km cycling time trial performance. **BASEM Annual Meeting 2022.** (*https://kar.kent.ac.uk/id/eprint/95942*).

COVID-19 IMPACT STATEMENT

The COVID-19 pandemic, and resultant closure of the University of Kent campus (18th March 2020), and national and international lockdowns (23rd March 2020), has directly or indirectly impacted the work contained within this thesis. The national lockdown immediately caused data collection to cease mid-project. Although some campus facilities began re-opening from mid-June 2020, research laboratories and physiological testing of human participants did not commence till October 2020 due to the complex health and safety considerations needed for maximal exercise testing and respiratory assessments. COVID-19 infections had once again begun to rise, and by November 2020 the United Kingdom was into a second national lockdown. The concurrent re-closure of research laboratories to general public volunteers impacted recruitment significantly. It would not be until March 28th 2021, that human testing of the general public could re-commence, by which time remaining data collection was scheduled for early-summer 2021 to coincide with studentship funding cessation in September 2021. Following this, full-time academic employment was sought, further impacting the writing of this thesis.

The primary limitation imposed by the COVID-19 pandemic was the lack of access to research laboratories and external participants, something vital to an applied exercise physiology research thesis. As a result, the sample sizes in *Chapter 6* and *7* are smaller than initially planned or required. Despite this, the findings still make valid contribution to existing literature.

Indirectly, the COVID-19 pandemic impacted the funding of this thesis, including retraction of an awarded research grant, and redirection of department finances. This generated additional unplanned workload in grant writing as an attempt to secure funding. Consequently, fewer biological markers were investigated than initially planned. For example, funding could not be secured to explore hormone function following glucocorticoid administration using saliva samples collected as part of *Chapter 6*.

Additionally, travel restrictions and financial implications curtailed research progression, networking opportunities and personal development, including the cancellation of international conferences where work from this thesis had been accepted to present. Nevertheless, these challenges were mitigated by disseminating findings to external scientists via online recorded presentations, geographically local conferences and focusing on manuscript generation for peer-review submission.

A significant research activity curtailed by the COVID-19 pandemic was the intended inclusion of a longitudinal study investigating the use of oral and inhaled glucocorticoid therapy combined with a training intervention. This novel research could have provided valuable insights for anti-doping regulations, especially given the World Anti-Doping Agency (WADA) allowing oral and inhaled glucocorticoids out-of-competition periods. This study was planned to be the culmination of the previous experimental chapters, so the removal shifted the thesis narrative.

However, the revised thesis allowed the review of retrospective swimming performance data from elite international major competitions in *Chapter 4*, and funding did permit the analysis of blood samples for inflammatory cytokines in *Chapters 6* and *7*, which supplemented the methodology for the better. In addition, the opportunity arose to conduct a collaborative research project with the University of Greenwich to simulate the impact of inhaler technique on Beclomethasone dipropionate deposition within the respiratory system (*Chapter 5*). This introduced me to other analytical techniques and bridged the multidisciplinary gap between pharmacology and exercise science.

Whilst the COVID-19 pandemic challenged both professional and personal environments, it provided an opportunity to adapt amid adversity.
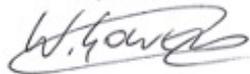
Research Student   **Primary Supervisor**

William Gowers   **Professor John Dickinson**

Date: 18/12/2023   Date: 18/12/2023

CHAPTER 1: INTRODUCTION

"I would not be able to play/train if I weren't allowed to use my asthma medicine".

[Anonymous asthmatic female team sport athlete]

"....it is too easy to cheat with a TUE [therapeutic-use exemption] certificate so as to legalize doping in individual cases. . . there has to be zero tolerance, and it is just bad luck if you are born with asthma or something like that".

[Anonymous non-asthmatic male endurance athlete]

(Overbye and Wagner, 2013)

1.1. Evolution of Anti-Doping Regulation

Otto Beckmann's 1933 'Sport-Lexikon [Dictionary]' provided one of the first documented definitions of doping, describing it as "*the use of stimulating [performance enhancing] agents, which shall push the athlete beyond his/her normal limits of performance*" (Beckmann, 1933). More recently, the terminology *'ergogenic'* can be attributed to this phenomenon (Thein, Thein and Landry, 1995). However, the use of illicit substances in sport is not a modern problem, as reports of doping can be traced back to ancient civilisations such as the Roman Empire, Ancient Greece, and even Norwegian mythology (Ljungqvist, 2017; Vlad *et al.*, 2018). Doping practices began to significantly evolve in the early 20th century, coinciding with the advent of modern sports, in which doping may have been an integral part due to a lack of regulation or the limited enforcement of rules (Dimeo, 2008). However, the proliferation of doping became more epidemic with the emergence of large multinational pharmaceutical companies, offering increased access to potentially performance-enhancing drugs (Mottram and Chester, 2022). To address this increasing issue, the International Olympic Committee (IOC) established a medical commission (IOC-MC) in 1967 to implement a more unified approach to anti-doping regulation, and to create the first list of prohibited substances for the upcoming Grenoble winter and Mexico summer Olympic games (Mazzoni, Barroso and Rabin, 2011). Subsequent prolific doping scandals in the latter 20th century led to the 'Lausanne Declaration on Doping in Sport' in 1999 and the establishment of The World Anti-Doping Agency (WADA) (Kamber and Mullis, 2010).

Today, WADA serves as a global organisation for harmonising anti-doping policies among international sporting federations and national anti-doping organisations. WADA produces '*International Standards*' for various technical and operational areas within the anti-doping movement.

One of the primary functions of WADA is to classify substances for inclusion in an annually published list of prohibited substances that is principally based on a triad of factors including: (1) they have the potential to enhance sports performance, (2) pose risk to athlete health, or (3) go against the spirit of sport (WADA, 2021c). Since 2003, WADA has published '*The Prohibited List*' for national anti-doping organisations to implement (Kamber and Mullis, 2010). Substances are classified into three categories: those that are banned at all times, those that are banned only in-competition, and those that are banned only in certain sports (WADA, 2021c).

WADA has its own definition of doping chosen for operational and legal reasons (Heuberger *et al.*, 2022), stating, *"Doping is defined as the occurrence of 1 or more of the anti-doping rule violations set forth in article 2.1 through article 2.11 of the [WADA] code"*. The eleven pre-defined violations encompass more than just the presence of a prohibited substance, but also include actions such as evasion of sample collection and acts of collusion by support personnel (WADA, 2021c).

The aetiology of doping, why some individuals dope while others stay 'clean' is considered multifactorial and perhaps a complex interplay of demographic, cultural, socioeconomic, and personality factors (Overbye, Knudsen and Pfister, 2013; Ntoumanis *et al.*, 2014). Athletes may engage in or justify doping practices as influenced by their perception of its prevalence [perhaps overestimating the commonality] within their sport, known as the 'false consensus effect' (Petróczi *et al.,* 2008), particularly evident in sports with a tumultuous history of doping, such as cycling (Henning and Dimeo, 2015). Depending on the sport and the determinants of performance it requires, an unscrupulous athlete could seek benefits from doping practices to improve recovery from an injury, increase recovery capacity after training or between competition bouts, increase muscle mass, decrease adipose tissue, and increase endurance or strength performance (Vlad *et al.*, 2018). Athletes from sports that are strength or endurance based are more likely to dope than those that are skill based, as are male athletes and those later in their career (Alaranta *et al.*, 2006; Overbye, Knudsen and Pfister, 2013). But doping is not limited to elite-level athletes, with non-elite populations also engaging in practices (Henning and Dimeo, 2018).

In the current professional and commercialised sporting landscape the deliberate use of substances and methods of doping constitute fraud against the public, sponsors, and fellow competitors (Thevis, Kuuranne and Geyer, 2021). Given the practical testing

limitations and illicit nature of doping, it is notoriously difficult to assess the exact prevalence of doping. However, based on a combination of questionnaires and statistical modelling, the prevalence of doping has previously been estimated to be between 14 - 39%, but this is a large discrepancy compared with positive doping tests of 1-2 % (de Hon, Kuipers and van Bottenburg, 2015).

Thoughts of doping often gravitate to intentional actions, but it is now acknowledged that pharmaceuticals are part of the athletes' exposome (Thevis *et al.*, 2021), and that athletes may claim they unintentionally ingest a banned substance through tampering or contamination of nutritional supplement products (Martínez-Sanz *et al.*, 2017; Chan *et al.*, 2019; Lauritzen, 2022). This could contribute in up to 40% of rule violation cases (de Hon and van Bottenburg, 2017). Despite this, strict liability in anti-doping regulation states that the athlete is solely responsible for any substance found within their provided specimen (WADA, 2021c), and as such knowledge of doping should form a part of the athletes' health literacy to reduce this risk of anti-doping rule violation (Vamos and Steinmann, 2019).

Additionally, elite athletes may experience acute exacerbation of illness or possess a legitimate diagnosis of a chronic medical condition that necessitates pharmacological intervention that is controlled by WADA. As part of its directive to maintain and promote athlete health, WADA has established threshold limits for selected substances on the '*Prohibited List'*, that are frequently used to control common chronic medical disorders, thus allowing athletes to administer specific amounts of these within predetermined time periods (WADA, 2021c). Furthermore, the '*International Standard for Therapeutic Use Exemptions'* came into effect from 2005 [replacing existing IOC-MC policy] to establish requirements that must be satisfied to enable individuals to train and compete while accessing otherwise prohibited substances (WADA, 2023d). Athletes can be granted dispensation through application of a *'Therapeutic Use Exemption'* (TUE) if; (1) their health is significantly impaired; (2) there is no evidence that the substance produces significant performance enhancement; (3) no therapeutic alternative exists, and (4) that granting the prohibited substance is not due to prior (non-therapeutic) use of a prohibited substance. A TUE can be applied prospectively, or retroactively in response to an adverse analytical finding (WADA, 2023d). Recent observations suggest that the number of athletes competing with valid TUE at Olympic games is < 1% (Vernec and Healy, 2020; Vernec *et al.*, 2024).

1.2. Asthma Therapy Within Context of Anti-Doping Research and Policy

A prominent example of a chronic disorder that requires pharmacological management is asthma, and specific to athletic populations, a common subtype of airway hyperresponsiveness known as exercise-induced bronchoconstriction (EIB). EIB is highly prevalent among elite athletes (Fitch, 2012), but the management is not dissimilar to that of asthma in the general population (Boulet and O'Byrne, 2015). In the context of asthma prophylaxis, WADA regulates two cornerstones of pharmacological management: *Beta(β)-2-Agonist*, and *Glucocorticoids*.

Inhaled β2-agonists serve a therapeutic purpose by inducing bronchodilation to alleviate respiratory symptoms in both acute [short-acting β2-agonists (SABA)] and preventative settings [long-acting β2-agonists (LABA) or ultra-long-acting β2-agonists (ULTRA-LABA)]. WADA prohibits β2-agonists substances at all times, but selected formulations can be used within threshold limits to allow therapeutic requirement. However, other selected inhaled formulations such as terbutaline, and systemic administration routes (i.e., oral) necessitate a TUE (*Prohibited List – S3 Class*; WADA, 2023$_b$). Adverse analytical findings detected above urinary thresholds serve to distinguish between permitted limits and differentiate between oral and inhaled administration routes (Hull and Pavord, 2018). This policy is attributed to the fact that the ergogenic effect of β2-agonists is considered dependant on the dose and route of administration. Systematic review and meta-analysis suggest that therapeutic doses of inhaled β2-agonists (i.e., within WADA permitted limits) do not improve endurance, or, strength and power performance (Pluim *et al.*, 2011; Riiser *et al.*, 2020, 2021). However, supra-therapeutic inhaled doses and oral administration may lead to enhancement in strength and power performance (Riiser *et al.*, 2020) derived from extrapulmonary effects by stimulation of the abundance of β2-adrenoreceptors within skeletal muscle enhancing their contractile properties (Hostrup *et al.*, 2015). Although β2-agonist therapy will not be the primary focus of this thesis, it is important to note that they have a common place within EIB management and are often used concurrently with other forms of asthma treatment.

Glucocorticoids (GC), a type of corticosteroid, are anti-inflammatory and immunosuppressive agents designed to reduce the inflammation-driven pathophysiology of asthma (GINA, 2022). Inhaled corticosteroids (ICS) are a first-line daily treatment to control asthma, while for severe exacerbations of illness a short-course of oral administration [systemic] may be required (British National Formulary, 2023a). GC are among the most common substances used at Olympic games (Vernec *et al.*, 2024), and

administered by sports medicine practitioners (Hughes *et al.*, 2020). Systemic GC are also indicated for management of chronic immune-mediated or endocrine disorders (Di Luigi *et al.*, 2020), and in elite sports are commonly used via injection to treat musculoskeletal injury (Shah *et al.*, 2019). Concerns have been raised about the prevalence of injected administration and its potential ergolytic properties [negative effects], such as an elevated risk of tissue degeneration (Nichols, 2005). In response to these concerns, recent 'no needle policies' *[i.e. the banning of routine injected substances unless for clinically justified treatment of injury, illness, or other medical condition with valid TUE]*, particularly those established by the 'Union Cycliste Internationale', have been implemented regarding injected GC (Vernec *et al.*, 2020). Although this is an important issue in sports medicine, injectable administration routes are not within the scope of this thesis.

WADA stipulate that GC are prohibited only in-competition when administered by oral, intravenous, intramuscular, or rectal routes, unless appropriate TUE provision has been granted. Outside of competition, the aforementioned systemic routes are able to be freely used but must have been cleared from the body by a pre-determined competition period (Ventura *et al.*, 2021). ICS are available to be used in-and-out of competition within the manufacturer's licensed dosage and therapeutic indications (*Prohibited List – S9 Class*; WADA, 2023$_b$). Previously, an adverse analytical finding was presumed from tested samples exceeding a GC metabolite urinary reporting threshold of 30 ng/mL designed to distinguish between systematic and local administration routes (WADA, 2019), but contemporary limits for specific substances have recently been developed (Ventura *et al.*, 2021; WADA, 2022c).

While the ergogenic action of inhaled and oral β2-agonists has been explored in great detail, GC as indicated for asthma prophylaxis have received less attention in research. Of the limited investigations conducted on oral GC, ergogenic mechanisms of action have been hypothesised to improve exercise performance from both a psychological and physiological perspective. More specifically they act on metabolism and the immune system (Heuberger and Cohen, 2019), but also may induce the perception of euphoria (Dubovsky *et al.*, 2012) and changes in other mood states (Schmidt *et al.*, 1999). To date, limited research has been conducted on ICS at therapeutic or supra-therapeutic levels, and comparisons between acute or short-term use of ICS have not been made. Moreover, of the previous investigations conducted on athletic performance following use of GC, the performance metrics used have limited external or ecological relevance, such as time-to-

exhaustion (TTE), handgrip strength, and incremental maximal tests (Hopkins, Schabort and Hawley, 2001; Laursen *et al.*, 2007). This thesis aims to contribute to this gap in the literature by using 'closed-loop' time-trials as the performance outcome.

1.3. Discourse of Asthma Management in Elite Athletes

Historically there has been considerable tension between anti-doping rules established on the evidence of potential for performance enhancement, and the acceptance of systemic GC for the treatment of medical conditions in elite athletes (Vernec *et al.*, 2020). The presence of corticosteroids on the prohibited list may strengthen the belief that asthma-related medication can enhance performance and may encourage athletes to inappropriately use inhaler therapy *[i.e., to seek a performance gain]* (Kuipers and Ruijsch van Dugteren, 2006; Allen *et al.*, 2022). When asked hypothetically, 19% of non-asthmatic responding athletes expressed interest in sampling respiratory agents if it were legal under qualified supervision (Overbye, 2018).

The ethics and legitimacy of the TUE policy has been questioned. Some concerns have arisen about its potential exploitation to enhance performance, rather than its intended purpose of solely managing acute or existing medical conditions (Cox, Bloodworth and Mcnamee, 2017; Aguilar-Navarro *et al.*, 2020). Perhaps most notably, in 2016, the Russian espionage group known as *'Fancy Bear'* exposed confidential data highlighting use of medication under TUE in elite sport (WADA, 2016). A prominent British cyclist was implicated due to a history of corticosteroid use in close proximity to Grand Tour races in order to control asthma and allergies (DCMS, 2018). This added to the scrutiny systemic GC and the TUE system were subjected to *[often ill-informed]*, and provided some suggestions for reform of the TUE system (Pitsiladis *et al.*, 2017; Fitch, 2020).

Balancing anti-doping guidelines and athlete health whilst maintaining sporting integrity is a challenge for regulatory bodies, ultimately leading to discourse between athletes using substances legitimately, those abusing them, and those who are sceptical of their need. The illustrative quotations presented at the beginning of this chapter highlight this debate. The social sciences have explored this through qualitative approaches. Notably, Overbye and Wagner (2013) observed a significant number of athletes expressing their dependence on inhaler therapy to compete effectively. Conversely, in the same study, 51% of athletes considered that teammates had received a TUE without medical need (Overbye and Wagner, 2013), and in elite cyclists, accusations of injury falsification were observed, with the aim of obtaining medication to optimise performance and/or speed

recovery [*e.g., through oral GC therapy*] (Lentillon-Kaestner and Carstairs, 2010). Moreover, athletes with and without asthma have expressed the perception that maintenance therapies, such as inhaled and oral GC, are associated with favourable physiological adaptations, including increased muscle mass, reduced body fat, and improved recovery time between training sessions (Allen *et al.,* 2022). Part of this discourse may come from the fact that asthmatic athletes outperform their non-asthmatic counterparts at major games (Fitch, 2012). Although more recent analysis presented that those with TUE at major games do not outperform those without TUE (Vernec and Healy, 2020).

Athletes might shy away from legitimate therapeutic inhaler treatments due to concerns about potential stigma, accusatory media portrayal, or the risk of breaching anti-doping regulations (Allen *et al.*, 2022). This hesitation can lead to inadequate management of their health conditions and risk exacerbation (Hull and Pavord, 2018). Appropriate management of EIB with inhaler therapy aims to restore athletes to a level akin to those without the condition, and once adequately managed, athletes should be able to train and compete without compromising their airway health, ensuring them no disadvantage compared to their non-asthmatic counterparts. However, there is still limited evidence of the effect of diagnosis and effective management on exercise performance (Price *et al.*, 2014), an area this thesis aims to explore.

Successful international competition requires the highest physiological demand and wellbeing, with illness or injury-related interruptions significantly impacting an athlete's potential to achieve performance goals (Raysmith and Drew, 2016). In a proposed performance-health coaching model, well-controlled asthma poses a low risk to both health and performance. However, if asthma is symptomatic, modified training may be necessary (Dijkstra *et al.*, 2014). As medical illness contributes significantly to the highest proportion of lost training time, elite athletes often strive to train and compete despite being unwell or injured (Dijkstra *et al.*, 2014; Trease *et al.*, 2020). But it could be argued whether an athlete with an acute exacerbation of a medical condition requiring pharmacological intervention should be competing or training at all (Henning, 2017). The severity and physiological implications of acute illness would typically demand rest from strenuous athletic activity (Hull and Pavord, 2018). Moreover, given the associated side effects of long-term use of systemic GC *[i.e. adrenal insufficiency, immunodeficiency, osteoporosis, muscle wasting, tendon/fascia failure, various electrolyte, nutrient and*

metabolic imbalances] (Vernec *et al.*, 2020), their use to enable perseverance through competition could be questioned.

It seems that Beckmann's early definition of doping has become more convoluted due to the ongoing debate whether access to asthma-related GC is purely therapeutic or has the potential for performance-enhancement.

1.4. Final Statement of Introduction

Through a series of experimental chapters, this thesis will address some of the previously outlined gaps in the current body of knowledge and provide evidence towards the implementation of anti-doping policy regarding the use of asthma-related GC therapy in athletic populations. Primarily, this thesis will explore the impact that diagnosing and initiating long-term management for EIB in an applied elite athlete setting has on respiratory function and performance at ensuing major competition. Additionally, this thesis will compare oral and inhaled GC administration routes using ecologically valid cycling time-trial performance outcomes to assess exercise performance and recovery, including evaluating these outcomes following short-term ICS administration.

"Working together, we can ensure that athletes make informed decisions on clean sport, decreasing the likelihood of intentional and inadvertent doping" (UK Anti-Doping, 2024)

CHAPTER 2: LITERATURE REVIEW

2.1. Asthma and Exercise Induced Bronchoconstriction (EIB)

2.1.1. Asthma: Definition, Epidemiology and Burden of Disease

Asthma is a chronic inflammatory disorder affecting the lower airways that commonly presents as recurrent respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough, which can vary in both time and intensity. It is a heterogeneous disease in pathophysiology and classification phenotypes; however, a central characteristic is airway hyperresponsiveness to various stimuli causing a variable expiratory airflow limitation that is reversible spontaneously or with treatment (GINA, 2022).

In 2019, the Global Burden of Diseases *[a collaborative consortium led by the World Health Organisation that is tasked to investigate the epidemiology and impact of various health conditions and risk factors]* estimated that asthma prevalence is approximately 262 million individuals worldwide (Vos *et al.*, 2020). Exact global prevalence is difficult to model due to historic unstandardised sampling and analytical methods, as such, epidemiological studies conducted over the last decade have reported large variance from the above estimate (± 109 million) (Asher *et al.*, 2020). This is perhaps magnified by evolving asthma definitions and no gold standard objective diagnosis due to the complexity and heterogeneity of the disease (Sears, 2014; Soriano *et al.*, 2017; Dierick *et al.*, 2020). Despite inconsistency in the reporting of recent global asthma prevalence, it is widely considered that rates have been increasing since the mid-20th century driven primarily by the rapid urbanisation in middle-and low-income countries. However, it is unclear if developed countries have reached a plateau in asthma prevalence (Lundbäck *et al.*, 2016).

From a UK perspective, it was suggested 6.5% of the population had clinician-diagnosed asthma in 2016 (Bloom *et al.*, 2019), yet, as with worldwide statistics, estimates can vary, with UK prevalence reported as high as 11-16% (Simpson and Sheikh, 2010; Mukherjee *et al.*, 2016).

Asthma poses a significant burden for the UK, resulting in direct economic pressure on the national healthcare service, indirect costs borne by society *[i.e. loss of productivity from work and school absenteeism],* and intangible consequences related to the human toll of increased morbidity and mortality (British Lung Foundation, 2016; Nunes, Pereira

and Morais-Almeida, 2017). A large UK epidemiological study incorporating routine administrative, health, and social care data between 2011-12 suggested asthma was responsible for at least 6.3 million primary care consultations, 93,000 hospital inpatient occurrences, 1800 intensive-care unit episodes, and 36,800 disability living allowance claims. The estimated cost to the public sector amounted to at least £1.1 billion per annum, with most of the cost (74%) for provision of primary care services *[i.e., prescribing and undertaking consultations]* and remainder for facilitating hospital care and supporting disability allowance. An additional £1.1 billion was estimated to be for medication indicated for use in asthma prophylaxis (Mukherjee *et al.*, 2016). Accounting for the indirect costs and intangible factors, estimates from 2014 suggest the total expense of asthma approaches £22 billion annually (British Lung Foundation, 2016).

Asthma hospital admissions increased by approximately 46% between the years 1999 - 2020 (Alwafi *et al.*, 2023). Amidst the COVID-19 pandemic, government-imposed lockdown measures triggered significant changes in the behaviour of asthmatics, leading to a reduction in certain risks. Patients became more proactive in managing their health, particularly in regards to preventative pharmacology (Kaye *et al.*, 2020), high-risk individuals were advised to follow stricter precautions (UK Government, 2021), air pollution reduced (Kelly *et al.*, 2022), and there was decreased transmission of community acute respiratory viruses (Chow, Uyeki and Chu, 2023). All these factors were reported to have attributed to a decrease in exacerbation episodes and dependence on general practitioner care throughout the first wave of the pandemic (Shah *et al.*, 2021; Rijpkema *et al.*, 2023). Although caution should be placed on inferring causal relationships with improved asthma management given recognised intentional primary care avoidance (Splinter *et al.*, 2021) and observational nature of many reports. In the post-COVID-19 era, pressure on primary care has increased, and asthmatics face additional challenges brought on by climate change (D'Amato *et al.*, 2020), necessitating more contemporary estimates of socio-economic cost of asthma and evaluation of patient behaviours (Khan *et al.*, 2020; Bodapati, Singh Gambhir and Kimura, 2022).

Although the economic cost to the UK public is considerable, from a patient perspective asthma continues to impose substantial and intrusive health implications resulting in reduced quality of life and premature death. The *'Global Burden of Diseases'* attributes asthma as a major cause towards years lived with disability and disability-adjusted life-years *[a metrics calculated for quantifying burden on an individual] and* responsible for

an estimated 461,000 deaths worldwide (Soriano *et al.*, 2017; Vos *et al.*, 2020; Safiri *et al.*, 2022).

2.1.2. Asthma: Pathophysiology

Asthma could be considered a diagnostic label or umbrella term, encompassing heterogeneous variants of disease with related clinical or physiological characteristics, triggering factors and inflammatory components (Wenzel, 2006; Pavord *et al.*, 2018). Phenotypes, or an evolving contemporary term 'endotypes' which refer to distinct subtypes of the condition defined by specific functional or pathophysiological mechanisms (Anderson, 2008; Lötvall *et al.*, 2011). Consequently, asthma lacks a singular unified pathophysiological mechanism (Wenzel, 2013). While asthma commonly first presents during childhood or adolescence *[referred to as early-onset asthma],* it can manifest at any stage of life. Notably, differences in phenotype emerge between individuals with early-onset and late-onset asthma, indicating corresponding disparities in underlying pathological processes (Miranda *et al.*, 2004). Most notably is the presence of allergic *[atopy]* or non-allergic features *[e.g., eosinophils, obesity-related].*

A central characteristic of most asthma phenotypes is the inflammation-driven bronchoconstriction causing a variable expiratory airflow limitation (GINA, 2022). When allergens or other irritants encounter the airway epithelium, mast cells are activated, leading to the cascade release of inflammatory mediators such as histamine, leukotriene D_4, and prostaglandin D_2 into the surrounding extra-cellular matrix. Histamine, one of the primary mediators released by mast cells, is responsible for the early-phase response of asthma, causing bronchoconstriction and increased mucus secretion. This results in airway narrowing and difficulty in breathing. Additionally, leukotriene D_4 and prostaglandin D_2 are potent mediators that contribute to prolonged bronchoconstriction and inflammation, extending the effects of the early-phase response. As the inflammatory response progresses, the infiltration of eosinophils and T-helper 2 lymphocytes into the airway epithelium play a crucial role in sustaining and amplifying the inflammation. Eosinophils are white blood cells that release cytotoxic granules, causing damage to the airway epithelium and contributing to the remodelling of the airway structure. T-helper 2 lymphocytes *[pathway]* produce cytokines that promote further inflammation and stimulate the production of immunoglobulin-E, which perpetuates the hypersensitivity to allergens (Barnes, 2008) *(Figure 2.1).*

The risk factors and aetiology of asthma are also heterogenous, driven from both a genetic predisposition and exposure to environmental factors. The activation of these inflammatory mediators and subsequent airway hyperresponsiveness can be triggered by pollutants, cold stimuli and atopic allergens (Subbarao, Mandhane and Sears, 2009; Parsons *et al.*, 2011; Beasley, Semprini and Mitchell, 2015).
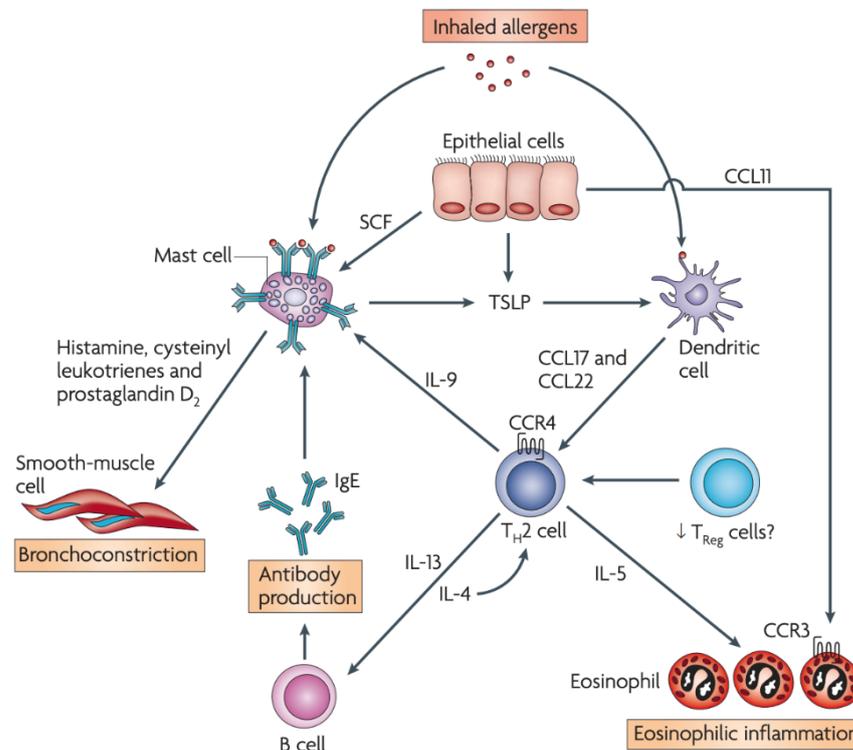
Figure 2.1. *Inflammatory and immune cells involved in processes in the airways of people with asthma (From; Barnes, 2008).*

A common trigger for this broncho-constrictive response is exercise, and for 80-90% of clinically diagnosed asthmatics intense exercise can be a stimuli that causes symptom exacerbation (McFadden and Gilbert, 1994). Breathing difficulties on exertion (such as shortness of breath, chest tightness and wheezing) are regarded as among the initial clinical signs of asthma in children and are distinctive markers of uncontrolled asthma in adults (Price, Walsted, *et al.*, 2022). Exercise-associated respiratory symptoms have been reported to impose a significant burden and diminish the quality of life for individuals. Particularly, these symptoms have been noted to restrict engagement in physical activity of the general population *[46.1% of n=1001]* (Parsons *et al.*, 2011).

2.1.3. Exercise-induced Bronchoconstriction (EIB): Definition & Pathophysiology

Exercise as a trigger for breathing difficulty has been recognised for a considerable time. As far back as the early years of the common era, Greek physician Aretaeus of Cappadocia (81–138 AD) observed and documented this phenomenon, stating: *"If from running, gymnastic exercises, or any other work, the breathing becomes difficult, it is called asthma"* (Adams, 1856). Today, this observation is recognised as exercise-induced bronchoconstriction (EIB) defined as a "*transient airway narrowing that occurs in association with physical activity in susceptible individuals*" (Parsons *et al.*, 2013; Weiler *et al.*, 2016). EIB is a form of airway hyperresponsiveness, summarised succinctly as an "*increased readiness of the bronchi to constrict in response to a variety of external factors*" (Gawlik *et al.*, 2019). A healthy physiological response to exercise would be slight bronchodilation (Crimi *et al.*, 2002). However, with EIB, a discernible functional alternation can be detected via spirometry testing, categorised by a ≥10% reduction in Forced Expiratory Volume in 1 second (FEV_1) post-exercise (Anderson *et al.*, 2001). Bronchoconstriction is typically experienced between 3 to 15 minutes following physical exertion, with a subsequent return to the initial baseline state either occurring spontaneously within 30 - 45 minutes (Godfrey and Bar-Yishay, 1993), or reversed through the use of inhaled short-acting β2-agonist (SABA) treatment.

Historically the term exercise-induced asthma has been used interchangeably with EIB to refer to this phenomenon. However, the clinical practice guidelines suggested by the '*American Thoracic Society*' (Parsons *et al.*, 2013) advises against using the term exercise-induced asthma to distinguish that exercise is the trigger for bronchoconstriction, rather than the cause for development of the condition. Moreover, exercise-induced asthma may imply that an individual has underlying features of chronic asthma, nevertheless EIB can be classified with or without signs and symptoms of asthma (Weiler *et al.*, 2010; Couto *et al.*, 2018). This means susceptible individuals may also suffer from coexisting conditions relating to the atopic triad such as hay fever *[allergic rhinitis]* or eczema *[atopic dermatitis]* (Bousquet *et al.*, 2012). Specifically to athletic populations, early review and more recent latent class analysis identified two key athlete EIB phenotypes: "*atopic or classical asthma*" marked by early-onset, allergic sensitisation, elevated exhaled nitric oxide, rhinitis, and allergic comorbidities; and "*sports asthma*" characterised by late onset of exercise-induced respiratory symptoms during sports career and airway hyperresponsiveness without allergic traits (Haahtela, Malmberg and Moreira, 2008; Couto *et al.*, 2015). Contrary to a genetic predisposition *[i.e., athletes who are*

diagnosed with EIB that have neither personal nor family history of asthma], environmental factors appear more influential in athletic populations (Couto *et al.,* 2018).

During exercise, when the minute ventilation ($\dot{V}_E$) rate surpasses the upper airway capacity of approximately 40 L/min (Niinimaa *et al.*, 1980), greater oral breathing load circumvents the body's natural humidifying and filtering mechanisms, thereby causing increased exposure of the lower airways to unconditioned air and harmful environmental stimuli (Boulet and O'Byrne, 2015; Rundell, Smoliga and Bougault, 2018). Such exposure is particularly problematic for susceptible individuals, especially when they exercise in environments with cold, dry air or other allergens and pollutants. These conditions contribute to airway desiccation, fostering airway inflammation and hyperresponsiveness (Price *et al.*, 2013; Rundell, Smoliga and Bougault, 2018).

Similar to the pathophysiological mechanisms observed in clinically recognised asthma, the exercise-induced phenotype stimulates airway hyperresponsiveness by releasing inflammatory mediators like histamine, cysteinyl leukotrienes, and prostaglandins (Hallstrand, Moody, Wurfel, *et al.*, 2005). Two primary theories have been suggested as contributors to the onset of EIB: osmolar (airway drying or water loss) and vascular (thermal – cooling) mechanisms. Both theories are based on the previously mentioned exercise-induced increased ventilation leading to water loss and mucosal cooling (Anderson and Kippelen, 2005). The airway surface liquid becomes hyperosmolar as water is evaporated, prompting mast cell induced release of mediators, such as histamine, cysteinyl leukotrienes, and prostaglandins, all known for their potent broncho-constrictive effects. Moreover, airway cooling during exercise activates cholinergic receptors, heightening the bronchial smooth muscle contraction and further increasing airway fluid secretion. Post-exercise, respiratory tract warming initiates secondary hyperaemia and heightens capillary permeability in the bronchial wall (Couto *et al.,* 2018). Emerging evidence indicates that injury to the airway epithelium caused by vigorous exercise is a key susceptibility factor for EIB (Hallstrand, Moody, Aitken, *et al.*, 2005). Airway epithelial injury in the lower airways can be assessed using clara cell secretory protein-16 *(Presented in Figure 2.2).*
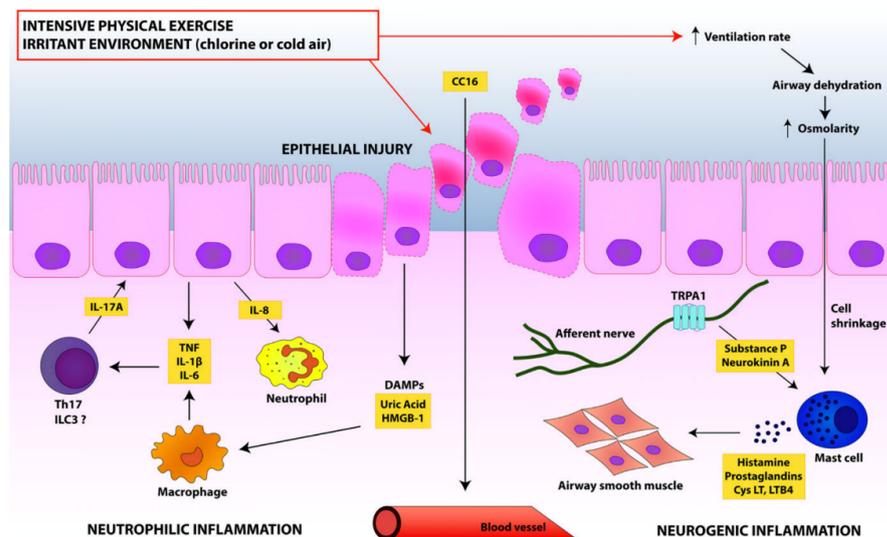
Figure 2.2*. Mechanisms of exercise-induced bronchoconstriction (From Couto et al., 2018).*

2.1.4. EIB: Diagnosis and Prevalence in Athletic Populations

One of the challenges with recognising EIB is that respiratory symptoms are non-specific and unreliable for diagnosis due to their poor predictive value (Parsons *et al.*, 2012; Simpson, Romer and Kippelen, 2015; Dickinson, Gowers, *et al.*, 2023). Previous observations have shown that elite athletes that experience respiratory symptoms can exhibit normal or enhanced lung function at rest, but conversely asymptomatic or previously undiagnosed athletes may also test positive for EIB (Rundell *et al.*, 2001; Bonini *et al.*, 2007; Dickinson, McConnell and Whyte, 2011; Simpson, Romer and Kippelen, 2015). Moreover, athletes with EIB can continue to experience respiratory issues during exercise even after initiating treatment (Jackson *et al.*, 2018). Because of this, EIB often goes unrecognised by athletes and their support network (coaches, family, teammates, primary care physicians, trainers) due to the their exceptional physical fitness, poor self-awareness of the condition, and it can be a challenge to distinguish between unconditioned state or situational fatigue (Rundell *et al.*, 2001; Parsons *et al.*, 2007, 2011). This holds clinical significance as asthma/EIB can often be under-or-over diagnosed (Aaron *et al.*, 2018) resulting in EIB-positive athletes not using respiratory medication (Burnett *et al.*, 2016), or general practitioners diagnosing based on clinical history alone (Parsons *et al.*, 2006). This is supported by more recent conference communications presenting the inconsistency in physician reported diagnosis of asthma in recreationally active individuals and their ability to provide objective evidence through bronchoprovocation (Gowers *et al.*, 2018; Dickinson, Sturridge, *et al.*, 2023). Perhaps contributing to this is the reported lack of access to bronchoprovocation tests by UK general practitioners (Hull *et al.*, 2009).

Appropriate diagnostic work-up delivered by respiratory specialists should, where possible, consider clinical history alongside physical examinations. These examinations may include spirometry, peak flow variability, fractional exhaled nitric oxide (FeNO), bronchial hyperreactivity, or bronchodilator reversibility (Anderson *et al.*, 2001; Dwyer and Abraham, 2012; NICE, 2017). Additionally, implementing other advanced assessments, such as sputum and blood sampling, impulse oscillometry, or atopic skin-prick tests, has been successfully utilised as a systematic approach to diagnosis within athletic populations (Hull *et al.*, 2021).

Comprehensive testing can support identification of differential diagnoses for other respiratory disorders that can masquerade as EIB, such as exercise-induced laryngeal obstruction *[closure of larynx during exercise]* and dysfunctional breathing *[alteration in breathing biomechanics]* (Weiler *et al.*, 2016; Hull, Burns, *et al.*, 2022). This approach can prevent the unnecessary prescription of medication and reduce the potential risk of adverse analytical finding from the excessive use of SABA above WADA threshold levels. Such misuse may occur when attempting to control respiratory symptoms that are not associated with EIB (Hull, 2015; Weiler *et al.*, 2016). Because of the reasons outlined above, some authors have advocated for the routine screening of athletes for EIB given the poor specificity of respiratory symptoms and high prevalence of asymptomatic individuals (Dickinson, McConnell and Whyte, 2011; Hull *et al.*, 2021). A visual representation of the procedure for assessing suspected EIB outlined by '*The European Academy of Allergy and Clinical Immunology'* working group (Price, Walsted, *et al.*, 2022) is presented in *Figure 2.3.*

Spirometry testing serves as an initial step in evaluating potential baseline airway obstruction. An FEV_1 <80% predicted, determined by age, sex, and height, or an FEV_1/FVC ratio <0.75 are indicative parameters of airway obstruction and may be present in individuals with EIB (Pellegrino *et al.*, 2005). This reduction in the FEV_1/FVC ratio is attributed to a decreased FEV_1 despite a relatively unchanged FVC (Louis *et al.*, 2022). Reference values provided by the *Global Lung Initiative* better incorporate factors like ethnicity and offer more specific benchmarks (Quanjer *et al.*, 2012). Moreover, reversible airflow obstruction may be observed in response to inhaled SABA if FEV_1 increases by ≥12% and 200mL, or ≥10% of the predicted value (Pellegrino *et al.*, 2005).

The adjunctive measurement of FeNO has been notably successful in asthma diagnosis as it is a cost-effective and accessible method for quantifying eosinophilic-mediated/

allergic airway inflammation *[i.e., nitric oxide upregulation is associated with the signalling activation of IL-4/IL-13 [Figure 2.1]* (Barnes *et al.*, 2010)). As such, FeNO measurement is endorsed by the *'European Respiratory Society'* and *'American Thoracic Society'* (ATS, 2005; Dweik *et al.*, 2011; Louis *et al.*, 2022). In adults, a FeNO level of > 25 ppb is considered elevated (Dweik *et al.*, 2011), with the *'National Institute for Health and Care Excellence'* (NICE) and *'European Respiratory Society'* suggesting a cut-off score of ≥ 40 ppb as high eosinophilic inflammation as this level provides an optimal compromise between sensitivity and specificity (NICE, 2017; Louis *et al.*, 2022). However, it has previously been proposed to use personalised cut-off values in an attempt to control for age, height, gender, smoking history and atopic status (Torén *et al.*, 2017). Change in FeNO can be used to assess response to ICS treatment (Dweik *et al.*, 2011). Nevertheless, FeNO should not be used in isolation since the assessment has poor predictive value in elite athlete populations (Dickinson, Gowers, *et al.*, 2023).
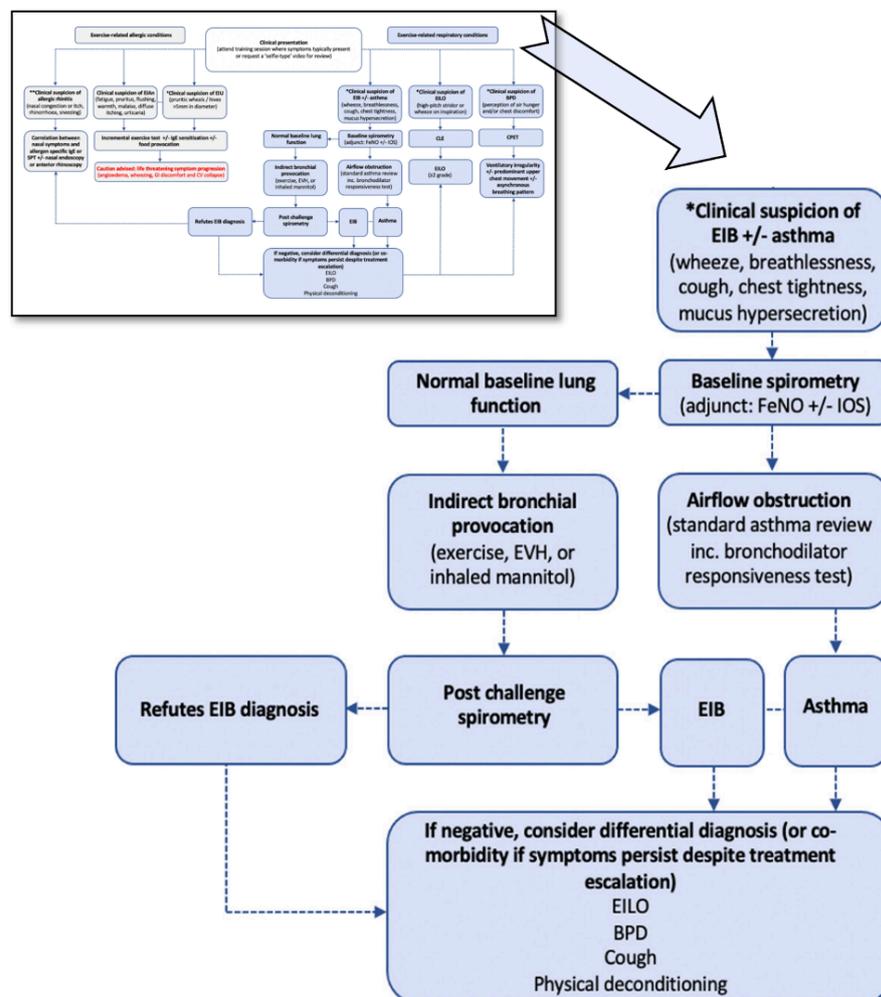
Figure 2.3. Diagnosis pathway of suspected EIB adapted from European Academy of Allergy and Clinical Immunology consensus paper (Price, Walsted, *et al.*, 2022)*. Abbreviations: **BPD**, breathing pattern disorder; **EIB**, exercise- induced bronchoconstriction; **EILO**, exercise-induced laryngeal obstruction; **EVH**, eucapnic voluntary hyperpnoea; **FeNO**, fractional exhaled nitric oxide; **IOS**, impulse oscillometry.*

Although this literature review will not critically compare bronchoprovocation diagnostic methods of EIB *[the strengths and limitations have been presented in peer-review (Rundell and Slee, 2008; Weiler et al., 2014; Hull et al., 2016)]*, it is important to note the significance of valid and reliable diagnosis methodology. Athletes must now provide objective evidence of asthma/EIB in response to potential misuse of β2-agonists, and appropriate diagnosis is mandated as medical evidence in support of TUE application (Hull *et al.*, 2009).

Indirect bronchial provocation challenges *[that indirectly promote release of inflammatory mediators associated with EIB]*, such as eucapnic voluntary hyperpnoea (EVH) and inhaled hyperosmolar aerosols *[mannitol, hypertonic saline]* offer effective means to identify EIB and are preferred to direct bronchoprovocation challenges *[that act directly on airway smooth muscle - e.g. methacholine]* (Anderson *et al.*, 2001; Dickinson *et al.*, 2006; Rundell and Slee, 2008; Holley *et al.*, 2012). The EVH is endorsed by IOC-MC (Anderson *et al.*, 2006) as it exhibits high sensitivity in inducing the pathophysiological mechanisms associated with EIB, and can be more tightly standardised than an exercise-challenge (Anderson *et al.*, 2001; Rundell *et al.*, 2004; Dickinson *et al.*, 2006; Anderson and Kippelen, 2012). EVH involves voluntary inhalation of dry-air containing 5% carbon dioxide for 6 minutes at a target ventilation of 30 times baseline FEV_1 [hyperpnea] (Hull *et al.*, 2016). However, for individuals with borderline or mild EIB diagnosis, more than one EVH test may be required to form a diagnosis *[important to minimise the chance of mis-diagnosis and subsequent mis-management]* (Price, Ansley and Hull, 2015).

EIB is prevalent among otherwise healthy recreationally active individuals without a known history of asthma. Molphy *et al.,* (2014) reported from a random sample of 136 volunteers that approximately 13% presented with EIB. But elite athletes, engaging in rigorous training and competition, exhibit even higher instances of EIB than the general population (Carlsen *et al.*, 2008a), and show the presence of epithelial injury and inflammation in the airways (Couto *et al.*, 2018). Therefore, it could be suggested that due to the high respiratory load and voluminous nature of training and competition, athletes may be more susceptible to developing airway dysfunction (Knöpfli *et al.*, 2007; Mountjoy *et al.*, 2015; Hostrup *et al.*, 2024). Airway hyperresponsiveness is among the most common chronic medical conditions within Olympic athletes (Fitch, 2012) and respiratory illness is the most common non-injury related reason for medical intervention at major athletic events (Engebretsen *et al.*, 2013; Hull and Pavord, 2018). It has been

proposed EIB could be classified as an *'occupational airway disorder'* for elite athletes (Price *et al.*, 2013).

From a UK perspective, in response to the IOC-MC requirement to provide clinical history and objective evidence for asthma (Anderson *et al.*, 2003), Dickinson *et al.*, (2005) conducted initial screening investigations within the English Institute of Sport (now the UK Sports Institute) for Summer sport athletes. They found an overall prevalence of approximately 21% among British athletes competing at the 2000 and 2004 Summer Olympic Games (Dickinson *et al.*, 2005). A recent systematic review with meta-analysis suggested prevalence of EIB in elite athlete populations has remained stable at that level between 1990 and 2020 (Price, Sewry, *et al.*, 2022). This is despite the landscape of TUE requirements for asthma-related substances changing on multiple occasions during this time *[most notably for β2-agonists].* In summary to combat concerns about the unnecessary use of β2-agonists, the WADA code was revised in 2009 to ban all forms of β2-agonists without TUE. This strategy that was suggested to be successful, as demonstrated in a cohort of Portuguese athletes whereby requests for inhaler therapy reduced by 51% (Couto *et al.*, 2013). Following this, between 2010 and 2012, specific permitted limits for inhaled salbutamol, salmeterol, and formoterol were established to no longer require TUE, unless an athlete exceeded the permitted limits in a medical emergency, in which case a retroactive TUE was still required. The decision to implement threshold values aimed to reduce the administrative burden of TUE approval (Allen *et al.*, 2019). Since then, the current regulations continue to permit the use of certain inhaled β2-agonists within specified limits without requiring a TUE (WADA, 2024).

However, while overall approximately one-in-five elite athletes may present with EIB, the prevalence is sport-specific, suggesting that the demands of the training and competition within particular sports can impact the likelihood of developing the disease (Price, Sewry, *et al.*, 2022). Environmental factors, namely the 'type' *[i.e., indoor vs outdoor]* and 'content' *[particulates]* of the ambient air can be significant risk factors in the development of EIB (Rundell *et al.*, 2015). In the observations undertaken by Dickinson *et al.*, (2005), cycling and swimming had particularly high prevalence of ~40%. Repeated high ventilation rates in potentially in asthmogenic environments seem associated. Specific to swimming sports, longitudinal inhalation of chlorination by-products such as trichloramine contribute to this effect (Bougault, Turmel and Boulet, 2011; Bougault and Boulet, 2012). Levai *et al.,* (2016) demonstrated that prevalence was

higher in elite swimmers *[who are exposed to chloramines]* than boxers *[who train in an indoor conditioned environment]* despite having similar weekly training loads. As well as the repeated high ventilation, elite road cycling exposes athletes to varying environmental conditions *[temperature and humidity]*, aeroallergens *[pollen]*, and airborne pollutants *[particles emitted from combustion engines of team support cars and media motorbikes]* leading to a higher incidence of upper respiratory symptoms and compromised lung function during major cycling events like La Vuelta and Tour de France (Allen *et al.,* 2021). Similarly, EIB prevalence is pronounced in winter sport athletes that train in cold and dry-air conditions such as cross-country skiers and biathletes (Carlsen *et al.*, 2008a), with winter sport athletes being 1.5 times more likely to develop EIB than summer athletes (Price, Sewry, *et al.*, 2022).

An exposure-response relationship may be evident, whereby athletes do not have susceptible airways when they take up competitive sport, but may develop respiratory symptoms, airway inflammation and airway hyperresponsiveness over the course of their careers (Pedersen *et al.*, 2008) or with increasing age and training volume (Stensrud *et al.*, 2007). This has been termed a "biological gradient" that may occur with exposure time in a sport or cumulative hours of training (Price *et al.*, 2013; Del Giacco *et al.*, 2015).

Table 2.1. High-risk sports for development of EIB [adapted from Del Giacco *et al.,* 2015].

Risk	Characteristics	Example of Sports
High-risk sports	Sports in which the athlete performs a >5-8 min effort and/or in a dry/cold air environment and/or in a noxious (chlorine exposure, ultrafine particles, traffic air pollution) air environment	• Swimming • Water polo • Track and field: ○ Long distance (5000 and 10000 m) ○ 3000 m steeplechase ○ Pentathlon (mixed) ○ Walks (20 and 50 km) ○ Marathon • Cycling • Cross-country skiing • Ice hockey • Ice skating • Biathlon • High-altitude sports

2.1.5. Impact Of Non-Treatment and Treatment of Athletes with EIB

Although retirement from elite sport may alleviate the severity of EIB (Helenius *et al.*, 2002), prioritising optimal respiratory health throughout an athlete's career remains paramount (Price and Hull, 2014).

It has been suggested that there is an association between uncontrolled EIB and predisposition to athletes developing upper respiratory tract infection, a common medical condition reported in an athletic population (Helenius and Haahtela, 2000; Bermon, 2007). Repeated episodes of exercise-induced inflammation are speculated to cause airway remodelling and chronic irreversible obstruction (Karjalainen *et al.*, 2000). Untreated EIB poses risks such as increased work of breathing from the presence of severe expiratory airflow limitation (Aaron *et al.*, 1992). Persistent EIB symptoms (particularly cough post-exercise) could also impede recovery, cause cognitive distress, and indirectly affect elite performance (Allen *et al.*, 2021). Respiratory symptoms have been reported to possibly disrupt sleep quality among elite athletes, potentially further impairing their performance (Fullagar *et al.*, 2015; Kennedy *et al.*, 2016). Although rare, severe episodes of EIB during training or competition could result in fatalities (Lang, 2005). Becker *et al.*, (2004) reported 61 EIB-related deaths over a 7-year period, with 81% of the fatalities occurring in athletes below 21 years of age.

Proficient use of maintenance therapy reduces the reliance on SABA, thereby minimising the risk of developing SABA/LABA tachyphylaxis [reduced effect of drug due to downregulation of β-2-adrenoreceptors] (Elers *et al.*, 2010). Regular use of ICS in the general population has been observed to reduce airway inflammation related to asthma (Boushey *et al.*, 2005). Similar results were noted in elite football athletes with EIB who were prescribed ICS, and they also demonstrated reduced EIB severity following nine weeks of individualised EIB therapy (Jackson *et al.*, 2018). However, a historical study involving cross country skiers revealed that while there was an improvement in FEV_1, there was no discernible benefit from budesonide at a daily dosage of 800 ug during three months of treatment on bronchial mucosa inflammation and asthma-like symptoms remained unchanged in 68% of the skiers (Sue-Chu *et al.*, 2000).

A key driving factor in elite sport is the impact of a medical condition or intervention on exercise performance. Successful international competition requires the highest physiological demand and wellbeing, with illness or injury-related training interruptions

significantly impacting an athlete's potential to achieve performance goals (Raysmith and Drew, 2016). Understanding the impact of uncontrolled or controlling for EIB in elite athletes on exercise performance remains challenging. Perhaps due to difficulty in accessing this calibre of athletes (Boulet and O'Byrne, 2015; Weiler *et al.*, 2016) or ethical issues associated with with-holding treatment of individuals with a known condition. At the time of its publication, a systematic review by (Price *et al.*, 2014) investigating the impact of treated versus untreated EIB on an athlete's health, wellness, and performance suggested there was no published data to suggest that EIB limits sport-specific exercise performance. As such, Price and Hull, (2014) outlined a call to research on the impact of treatment on repeated bronchoprovocation in "real-life" treatment trials e.g., in Olympic squad members. Since then, only two studies have attempted to investigate the impact of treating EIB in elite populations. Spiteri *et al.*, (2014) showed in a small cohort (n=7) of mild EIB-positive professional rugby players, 12-weeks of ICS treatment *[beclomethasone dipropionate]* did not enhance performance in a rugby specific fitness test above that seen in the placebo or control group (n=22). Jackson *et al.*, (2018) provided evidence that nine-weeks of treatment for EIB may be 'possibly beneficial' on maximum oxygen uptake in elite football players, albeit with a small sample size of three EIB positive athletes and no ecologically valid performance test completed.

2.2. Management of EIB in Athletes

Expert opinion has driven the recommendations of pharmacological management of EIB in elite athletes, due to lack of randomised clinical trials in this population (Boulet and O'Byrne, 2015). *The 'European Academy of Allergy and Clinical Immunology'* developed guidance in 2008 aimed at summarising the evidence related to EIB management in athletes (Carlsen *et al.*, 2008a, 2008b; Schwartz *et al.*, 2008). More recently, their task-force developed an up-to-date, research-informed position paper detailing the optimal approach to the diagnosis and management of common exercise-related allergic and respiratory conditions, including EIB (Price, Walsted, *et al.*, 2022). However, the recommended management of EIB is not dissimilar to that of a non-athlete (Weiler *et al.*, 2016). The overarching aim of management is to maintain symptom control, optimise pulmonary function, and minimise risk factors to reduce risk of acute exacerbations. EIB management is achieved primarily through pharmacological, but also non-pharmacological *[medical devices, nutritional, modifying risk factors]* treatment (Boulet and O'Byrne, 2015).

This section will briefly discuss non-pharmacological approaches, before introducing the mainstay management of asthma/EIB involving pharmacological interventions. *Table 2.2* presents a summary of these approaches.

Table 2.2. Summary of pharmacological and non-pharmacological management strategies for athletes with EIB (Adapted from Price et al., 2022).

Preventative	Reliever
Mild-Intermittent and Persistent EIB	**'As-needed' (i.e., symptom-driven)**
• Daily (or pre-exercise) low dose ICS, or combined with LABA	• SABA
Advanced Treatment (Add-on Therapy)	
• Leukotriene receptor antagonists	
• Anticholinergics / long-acting muscarinic antagonist	
• Mast cell stabilizing agents	
• Allergen immunotherapy in appropriate athletes (i.e., akin to the approach adopted in patients with allergic asthma).	
Non-Pharmacological	
• Avoiding Environmental and Allergen Triggers	
• Warm-Up (to induce refractory period)	
• Heat and Moisture Exchange Masks [dedicated device or face covering]	
• Nutritional Interventions [prebiotics, omega-3 polyunsaturated fatty acids]	

*Abbreviations; **ICS**: inhaled corticosteroids; **SABA**, short-acting β2-agonists; **LABA**, long-acting β2-agonists.*

2.2.1. Non-Pharmacological Interventions

Considering that environmental factors frequently serve as significant triggers for many athletes with EIB, it can be important to identify, modify or mitigate these risk factors. For instance, swimmers can minimise their exposure to the pool environment during periods of high chlorine levels and ensuring appropriate air turnover (Bougault and Boulet, 2012; Price *et al.*, 2013). Furthermore, avoiding heightened levels of exposure to pollutants and allergens during training can effectively reduce the risk of exacerbations. Winter-sport athletes could avoid exercising in extremely cold temperatures. However, it can be challenging to reduce exposure as adjustments may not always be practical due to the demands of elite sport training and competition. In situations when exercising in cold conditions cannot be avoided, using a heat and moisture exchange mask has demonstrated

efficacy in reducing the severity of EIB symptoms in recreationally active individuals (Jackson *et al.*, 2020). Additionally, heat and moisture exchange masks have been found to enhance lung function and decrease respiratory symptoms in winter sport athletes (Frischhut *et al.*, 2020). In all cases, implementing a warm-up routine can induce a refractory period, potentially reducing an individual's susceptibility to EIB symptoms during physical exertion (Stickland *et al.*, 2012). Lastly, there is some evidence that dietary interventions can mitigate the impact of EIB such as fish oil *[i.e., omega-3 polyunsaturated fatty acids]*, ascorbic acid, and reducing sodium intake (Mickleborough, Lindley and Ray, 2005; Mickleborough *et al.*, 2006; Tecklenburg *et al.*, 2007).

2.2.2. Pharmacological Methods

Current pharmacological treatments focus on reducing inflammation, bronchoconstriction, and mucus production. Inhaled or oral glucocorticoids *[corticosteroids]*, bronchodilators *[β2-agonists]*, leukotriene receptor antagonists and long-acting muscarinic antagonist are among the pharmacological agents commonly used to alleviate symptoms and prevent asthma exacerbations. The *'Global Initiative for Asthma'* (GINA) provides an annually updated, evidence-based strategy for the management and prevention of asthma. This strategy outlines a stepwise approach that involves a progressive series of treatment steps, tailored to the severity of asthma (GINA, 2022). The overarching goal is to ensure that patients receive the minimal required level of therapy necessary for effectively managing their condition. The treatment or dosage is systematically adjusted based patients perceived and objective control of their condition (NICE, 2017). In cases of uncontrolled asthma, a proactive 'step-up' approach to prophylaxis is adopted, whereas in controlled situations, a 'step-down' strategy is employed. Treatment can be classified into maintenance *[sometimes known as preventer or controller]* and reliever therapies (GINA, 2022).

Since 2019, GINA has adopted a two-track approach to asthma management (Boulet *et al.*, 2019). 'Track 1' suggests combination ICS-formoterol as the reliever therapy, introduced as result of recent evidence suggesting positive asthma-control outcomes using as-needed budesonide-formoterol. Although this approach was not as effective as daily twice daily budesonide monotherapy (Bateman *et al.*, 2018; O'Byrne *et al.*, 2018). A European-wide study observed an association between high SABA use and poor clinical outcomes (Bateman *et al.*, 2022). Specifically, from a UK context, the 2017 NICE guideline *'Asthma: Diagnosis, Monitoring and Chronic Asthma Management'* (NG80) still recommends as needed SABA, with stepping up treatment to include ICS when

"*asthma-related symptoms are experienced 3 times a week or more*" (NICE, 2017). This aligns closer to 'Track 2' of GINA strategy. However, there is an ongoing update to NG80 through a collaborative effort involving the *'British Thoracic Society'* and the *'Scottish Intercollegiate Guidelines Network'*, expected to be published in July 2024 (NICE, 2023b). This new UK-wide guidance may incorporate changes to align closer to the GINA strategy 'Track 1' (Chaplin, 2022).

First-line maintenance treatment on GINA 'Track 2' involves the use of ICS whenever SABA is used [Step 1]. From step 2, the daily use of low-dose ICS is suggested. From step 3, those experiencing moderate persistent asthma entails an increase in daily ICS dose or addition of a long-acting β2-agonists (LABA) delivered in combination. Severe persistent asthma involves medium to high-dose ICS-LABA combination if insufficiently controlled by low dose ICS/LABA treatment [Step 4]. Finally in step 5, further advanced treatment options like leukotriene receptor antagonists and anti-IL-5 agonists may also be included to target the characteristic mechanisms of the specific asthma pathophysiology (Barnes, 2011) and create individualised or personalised medicine or phenotype driven treatment (Chung, 2014). Step 5 also includes occasional use of oral glucocorticoids for serious acute exacerbation (GINA, 2022). Traditional asthma management based on disease severity may not equally benefit all patients given the varying disease endotype, sex, and age-onset (Lötvall *et al.,* 2011) and recent support for trait-based treatment (Roche *et al.*, 2024).

In athletic populations, previous advice to use SABA before exercise *[i.e. to avoid symptoms]* (Carlsen *et al.*, 2008b) is now considered outdated (Price and Hull, 2014), as multiple daily training sessions would necessitate frequent use of SABA. Although SABA can be protective against EIB [*92% of individuals using the salbutamol pMDI had effective inhibition of EIB*] (Anderson *et al.,* 2001) there is the risk of developing tachyphylaxis from excessive use (Abramson, Walters and Walters, 2003). Frequent use of SABA, even after 1 to 2 weeks, has been linked to heightened airway hyperresponsiveness, diminished bronchodilator efficacy, escalated allergic responses, and increased eosinophil counts (O'Connor, Aikman and Barnes, 1992).

Elite athletes who train multiple times per day and exhibit asthma-like symptoms *[indicative of GINA step three]* are advised be managed with daily ICS alone, or combined with LABA to prevent exacerbations and control symptoms, even with mild-intermittent disease severity (Backer, Lund and Pedersen, 2007; Hull, Burns, *et al.*, 2022;

Schwellnus *et al.*, 2022) ICS used for 4 weeks or more before exercise testing has been shown to significantly attenuated exercise-induced bronchoconstriction (Koh *et al.*, 2007.) Unlike SABA, there is no clear evidence that regular use of ICS induces glucocorticoid-receptor down-regulation (Demoly and Chung, 1998; Taheri, Cantrell and Feldman, 2013), or is noted as an adverse event by the British National Formulary (BNF, 2023b, 2023a).
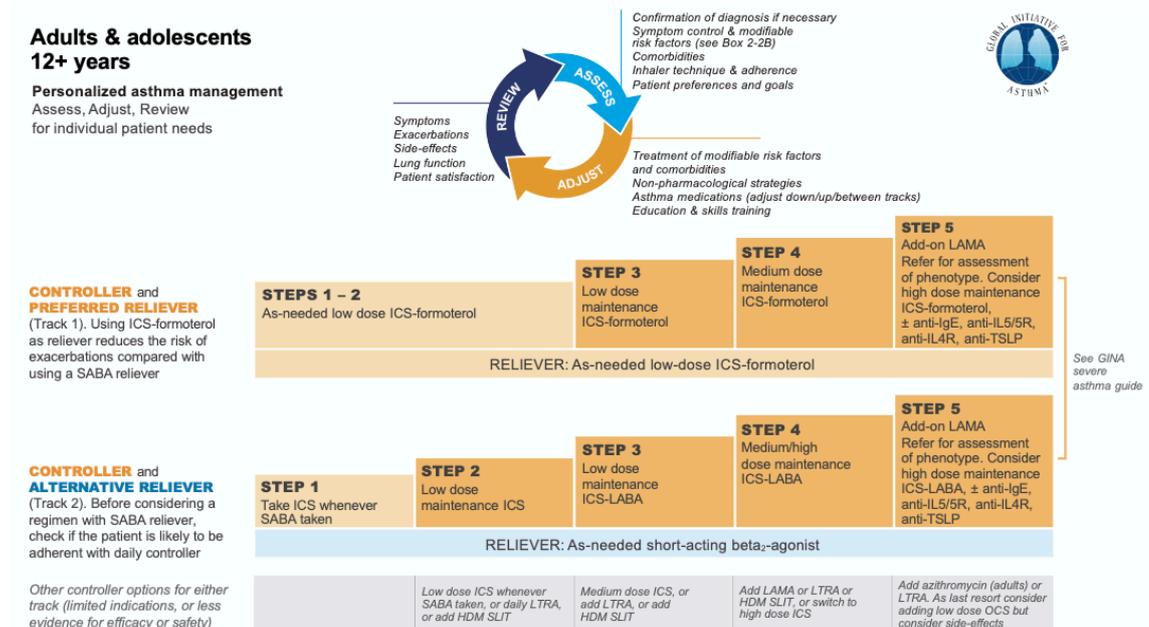
Figure 2.4. *The Global Initiative for Asthma (GINA) Stepwise Approach [Track 1 & 2] to Pharmacological Asthma Therapy in Adults and Adolescents [© Global Initiative for Asthma, www.ginasthma.org]. Abbreviations; **ICS**, inhaled corticosteroids; **SABA**, short-acting β2-agonists; **LTRA**, leukotriene receptor antagonists; **LABA**, long-acting β2-agonists; **LAMA**, long-acting muscarinic antagonists; **OCS**, oral corticosteroids; **HDM**: house dust mite; **SLIT**, sublingual immunotherapy; IL, interleukin.*

As the focus of this thesis is on asthma-related GC therapy, the pharmacological action, development, and adverse effects of this GC class of management will be discussed further.

2.2.3. Glucocorticoid Physiology and Pharmacological Therapy

Glucocorticoids (GC) *[interchangeably termed glucocorticosteroids]*, are a type of corticosteroid and the end-product of the hypothalamic-pituitary-adrenal (HPA) axis. As part of a negative feedback loop, GC are regulated by the secretion of corticotrophin-releasing hormone and adrenocorticotropic hormone (ACTH) in response to stressors (Son, Chung and Kim, 2011).

GC are adrenal steroid hormones that exert effects through both genomic and nongenomic mechanisms. Upon entering target cells, GC bind to intracellular glucocorticoid receptors, leading to their activation. The glucocorticoid receptor-alpha (α) isoform is ubiquitous in human cells, explaining their diverse pleiotropic effects.

The activated glucocorticoid receptor complex translocate into the nucleus, functioning as a transcription factor by binding to specific deoxyribonucleic acid [*DNA*] sequences known as glucocorticoid response elements within target gene promoter regions. This interaction initiates a dual regulatory process: transactivation, promoting the synthesis of anti-inflammatory proteins and metabolic enzymes; and transrepression by interacting with molecules that are activated such as CREB-binding protein associated with transcription of pro-inflammatory nuclear factor-κB, thereby reducing the expression of inflammatory mediators such as cytokines (Barnes, 2011) *(Figure 2.5)*. In addition to the genomic pathway, GC have been shown to exert non-genomic effects through membrane-associated receptors and non-genomic signalling mechanisms (Tasker and Malcher-Lopes, 2006).
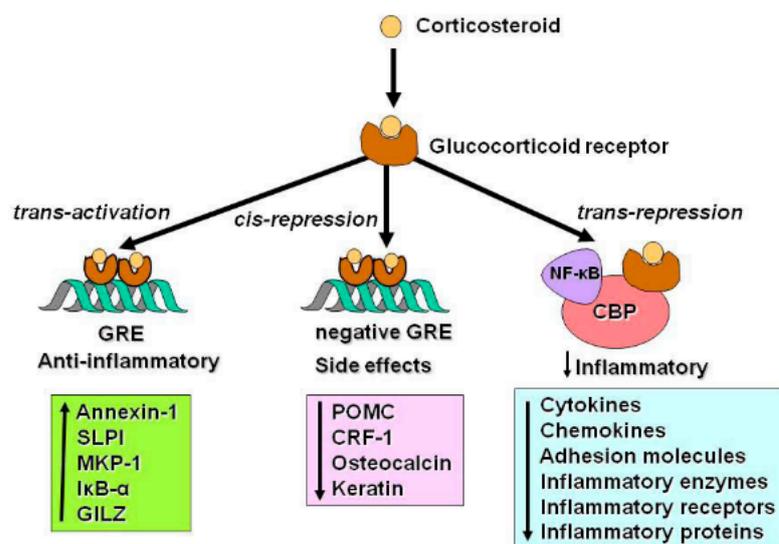
Figure 2.5. *Anti-inflammatory effects of pharmacological glucocorticoids (adapted from* (Barnes, 2010)*. Abbreviations:* ***GRE****: glucocorticoid-response elements;* ***NF-κB****; nuclear factor-κB;* ***CBP****: CREB-binding protein;* ***SLPI****: secretory leukoprotease inhibitor;* ***MKP-1****: mitogen-activated kinase phosphatase-1;* ***IκB-α****: inhibitor of NF-κB;* ***GILZ****: glucocorticoid-induced leucine zipper protein;* ***POMC****: proopiomelanocortin;* ***CRH****: corticotrophin releasing factor;* ***CRF****: corticotrophin releasing factor.*

As introduced in *Chapter 1*, synthetic GC are among the most widely used medication classes in the general population (Fuentes, Pineda and Venkata, 2018). They are potent anti-inflammatory and immunosuppressive drugs used to manage chronic diseases

including, asthma, dermatological issues, arthritis, ulcerative colitis, leukaemia, and Crohn's disease, and non-disease related factors such as acute musculoskeletal injury (Barnes, 2006; Shah *et al.*, 2019).

Inhaled GC compounds, invariably referred to as inhaled corticosteroids (ICS) were developed for the treatment of inflammatory airways diseases, including asthma, chronic obstructive pulmonary disease, and allergic rhinitis (Newton, Leigh and Giembycz, 2010). Specifically related to the inhaled formulation that will be used later in this thesis, Beclomethasone dipropionate (BDP) was the first synthetic corticosteroid asthma controller medication administered via inhalation in 1972 (Clark, 1972). ICS were developed to be a targeted treatment to locally delivery medication to target inflammatory cells in asthmatic airways including eosinophils, T-lymphocytes, mast cells and dendritic cells (Barnes, 2010) *(Figure 2.6)*.
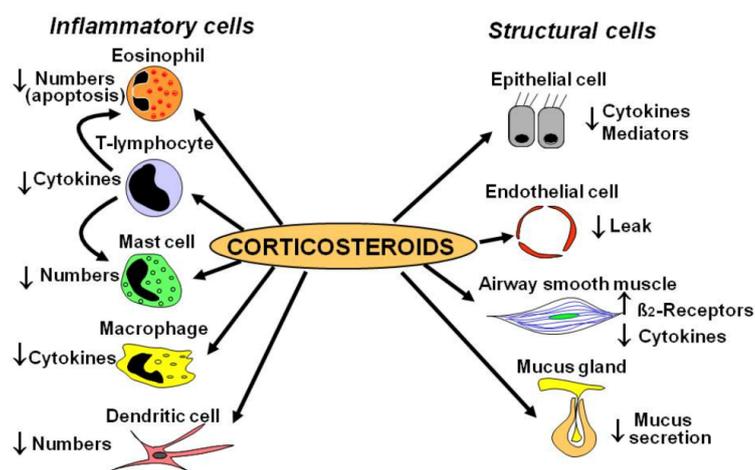
Figure 2.6. *Summary of cellular effect of inhaled corticosteroids [glucocorticoids] (Adapted from Barnes, 2010).*

Estimates suggest that in 2016, 80% of asthma patients were managed using ICS, an increase from 65% in 2006, primarily due to the recommendation of low-dose ICS as first-line treatment (Bloom *et al.*, 2019). Representative dosing for common formulations are presented in *Table 2.3*. The development of hydrofluoroalkane propelled formulations led to a significant decrease in drug dosing compared to now phased-out chlorofluorocarbons formulations (Davies, Stampone and O'Connor, 1998; Leach, Davidson and Boudreau, 1998). Hydrofluoroalkane formulations have resulted in greater pulmonary delivery, but despite this, it has been suggested that high doses of ICS can

result in deposition into systemic circulation, resulting in further extra-pulmonary effects *(Figure 2.7)*.
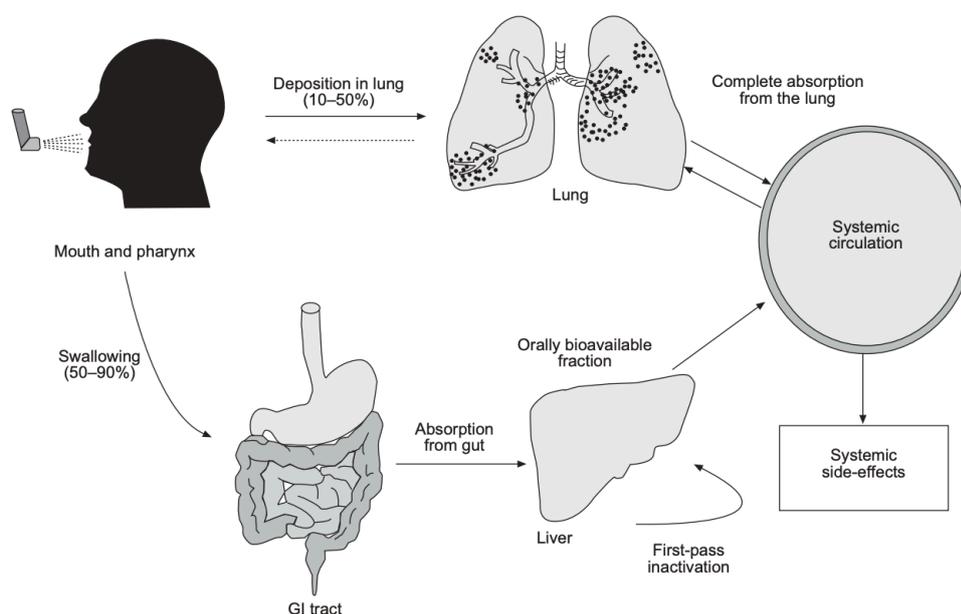
Figure 2.7. *Representation of the fate of an inhaled corticosteroid (Derendorf et al., 2006).*

Across medical conditions, oral GC are used by approximately 1% of the population (Fardet, Petersen and Nazareth, 2011). But while ICS were introduced to reduce the necessity of oral GC for asthma management, many asthmatics still require oral GC to manage the condition. A European multi-country retrospective cohort study of 702,685 patients with asthma found that 14–44% were oral GC users during the observation period, and 6–9% were high-frequency users (Tran *et al.*, 2020). Prednisolone is one of the most commonly prescribed medications (Fuentes, Pineda and Venkata, 2018). Short-course administration is an effective and fast-acting option for resolving acute asthma exacerbations and hospital admission (Rowe *et al.*, 2001; Weinberger, Hendeles and Abu-Hasan, 2018). The optimal therapeutic dose and duration of oral GC treatment to balance efficacy and adverse events are not firmly established, as demonstrated by a Cochrane systematic review (Normansell, Kew and Mansour, 2016). However, a dose of 0.5 mg/kg^{-1} for 5-10 days was reported to align with international guidelines (Hull and Pavord, 2018; Williams, 2018). More recent recommendations from the *'British National Formulary'* and GINA suggest an absolute prednisolone dose of 40–50 mg daily for at least 5-7 days to manage both mild to moderate acute asthma or severe/life-threatening exacerbation (GINA, 2022; BNF, 2023b). Proposals have called for limiting the cumulative quantity of oral GC an individual can receive within a given time period (Haughney *et al.,* 2023).

Table 2.3. Daily doses of inhaled corticosteroids (ICS) and oral glucocorticoids for asthma prophylaxis (tabulated from (NICE, 2023a) and (BTS, 2019).

Medication	Daily Dose		
	Low	**Moderate**	**High**
Beclomethasone dipropionate (HFA solution-based pMDI) [e.g., 'Qvar®, Clenil Modulite®, Combined ICS-LABA, Fostair®]	100 - 200 µg per day in 2 divided doses	300 - 400 µg per day in 2 divided doses	500 - 800 µg per day in 2 divided doses
Budesonide (DPI) [e.g., Pulmicort®]	200 - 400 µg per day as a single or in 2 divided doses	600 - 800 µg per day as a single dose or in 2 divided doses	1000 - 1600 µg per day in 2 divided doses
Ciclesonide (pMDI) [e.g., Alvesco®]	80 - 160 µg per day as a single dose	240 - 320 µg per day as a single dose or in 2 divided doses	400 - 640 µg per day in 2 divided doses
Fluticasone propionate (HFA-pMDI) [e.g., Flixotide®, Flovent®]	100 - 250 µg per day in 2 divided doses	300 - 500 µg per day in 2 divided doses	600 - 1000 µg per day in 2 divided doses
Fluticasone furoate (DPI) [e.g., Combined with ultra-LABA Vilanterol, Relvar Ellipta®]	/	100 µg per day as a single dose	200 µg per day as a single dose
Prednisolone (oral)	40 – 50 mg daily [outpatient for at least 5 days following acute exacerbation]		

Note: Specific manufacturer formulations may differ in dosage, or if delivered in combination with LABA or LAMA treatment. Abbreviations; **HFA**, *hydrofluoroalkane;* **pMDI**, *pressurised metered-dose inhaler;* **DPI**, *dry powder inhaler;* **LABA**, *long-acting β2-agonists;* **LAMA**, *long-acting muscarinic antagonist;* **µg**, microgram; ***ICS**: inhaled corticosteroids.*

a)
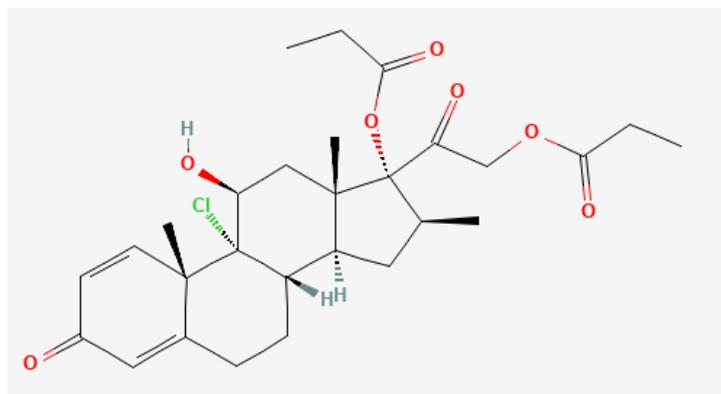
b)
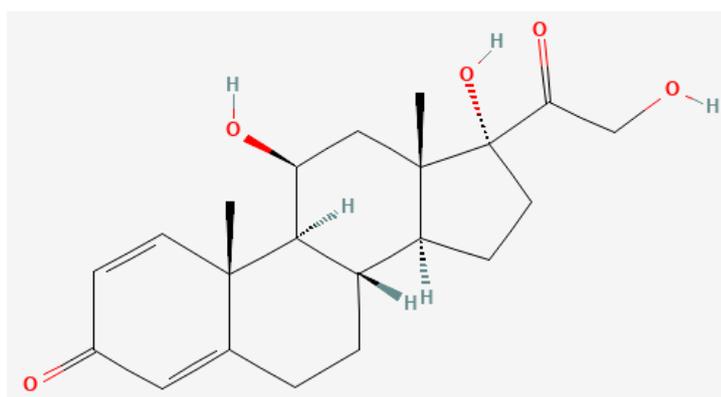
Figure 2.8. *Chemical structure depiction of Beclomethasone Dipropionate [$C_{28}H_{37}ClO_7$] (exported from PubChem, https://pubchem.ncbi.nlm.nih.gov; ID:21700) and Prednisolone [$C_{21}H_{28}O_5$] (exported from PubChem, https://pubchem.ncbi.nlm.nih.gov; ID:5755).*

<u>*Adverse Effects from Inhaled and Oral GC*</u>

While the therapeutic use of GC for asthma and other conditions is well established, a directive of WADA is to protect athlete health (WADA, 2021c), so it must be considered the health risks and implications to using such medication. Price *et al.*, (2020) presented the balance that needs to be struck between efficacy and safety of systemic GC use. Systemic GCs are associated with adrenal insufficiency, immunodeficiency, osteoporosis, muscle wasting, tendon/fascia failure, various electrolyte, nutrient and metabolic imbalances (Vernec *et al.*, 2020). Temporary suppression of the adrenal cortex (HPA axis function) warrants careful withdrawal from longer treatment periods. Chronic exposure to excess GC can result in Cushing Syndrome (Arnaldi *et al.*, 2003). Moreover, self-reported adverse effects from oral GC include insomnia, mood disturbances, hyperphagia (Morin and Fardet, 2015).

Frequent short-courses of oral GC may increase the risk of reduced bone mineral density. A 4-year longitudinal study observed that taking >2.5 short courses of oral GC per year may negatively impact on bone density. This is particularly important in the context of

elite athletes, such as cyclists, who may inherently have lower BMD compared to non-elite or sedentary individuals due to the nature of the sport (Medelli *et al.*, 2009; Mojock *et al.*, 2016; Martínez-Noguera *et al.*, 2021). While it is possible that the use of glucocorticoids for managing musculoskeletal injuries, asthma, and EIB could be a contributing factor to reduced bone mineral density due to high prevalence in this population of these clinical issues. The review by Hilkens (2021) noted caution with associating the contribution that GC has in the lower bone mineral density of the current generation of elite cyclists given the decline use and adverse analytical findings due to GC use in recent years (Vernec *et al.*, 2020).

ICS also have the potential to cause systemic side effects including the suppression of the HPA axis (Rao Bondugulapati and Rees, 2016), reduced growth velocity in children, skin thinning, cataracts, and glaucoma (Buhl, 2006). However, ICS does not appear to a risk to bone density, with no difference between higher (> 1,000 μg/d) and lower dose users (< 1,000 μg/d) (Matsumoto *et al.,* 2001). More recently a systematic review and meta-analysis suggested there is no significant association with ICS use of ≥12 months in adults or children with asthma on harmful effects on fractures or bone mineral density (Loke *et al.*, 2015). While typically less severe compared to systemic side effects, ICS are also associated with local side effects such as oropharyngeal candidiasis, dysphonia and cough (Kelly, 2003). These local adverse effects hold clinical significance as can impact patient quality of life, treatment adherence, and potentially obscuring signs of more severe illnesses. For instance, throat soreness and hoarseness are common reactions to ICS usage, also coincide with primary symptoms of throat cancer (Buhl, 2006).

2.3. Anti-Doping Position & Prevalence of Asthma-Related Glucocorticoids

Chapter 1 introduced WADA's position regarding GC (*Prohibited List – S9 Class*; WADA, 2023b) stating they are prohibited only when administered orally, intravenously, intramuscularly, or rectally during in-competition periods, unless a TUE has been granted. However, outside of competitive events, these systemic administration routes can be used, provided that the substance has been cleared from the body within the predetermined competition timeframe *["from 11:59 pm on the day before the competition to the end of such competition including the subsequent sample collection process"]*. (Ventura *et al.*, 2021; WADA, 2023c). Due to different pharmacokinetic profiles of formulations and inter-individual differences it is difficult to precisely predict this clearance period. But WADA recently created advice to athletes and support staff on clearance, suggesting the minimum washout period for oral administration is 3-days

(WADA, 2023c). This policy change were proposed following the work of Coll *et al.*, (2021) who identified that urinary concentrations of all prednisolone and prednisone metabolites investigated were below 10 ng/ml in the period 48–72 h after administration.

Conversely, inhaled GC *[and other administration methods e.g., topical, dental-intracanal, dermal, intranasal, ophthalmological, and perianal]* can be used both inside and outside of competition periods, so long as they are within the dosage and therapeutic indications specified by the manufacturer (WADA, 2023b).

To distinguish between administration routes *[namely local and systemic]*, WADA had prior established minimum performance reporting threshold levels of 30 ng/mL for GC parent compounds and their metabolites *[e.g., 16α-hydroxyprednisolone]* that would trigger an adverse analytical finding. Inhaled and oral GC were able to be distinguished using this method (Mazzarino *et al.*, 2006). However, the addition of more metabolites such as 6β-hydroxybudesonide, and more recently the minimum reporting level was amended to be substance specific (Ventura *et al.*, 2021; WADA, 2022c; Thevis, Kuuranne and Geyer, 2023) partly in response for false positive analytical finding via the inhaled administration route with budesonide formulation (Kaliszewski *et al.*, 2016; Coll *et al.*, 2020). In result to the addition of 6β-hydroxybudesonide, adverse analytical finding relating to ICS budesonide saw a significant reduction since 2014, with only a single adverse analytical finding involving budesonide observed in WADA testing figures for 2021 (Wojek, 2021, *p318*; WADA, 2023a). Now, 6β-hydroxybudesonide has an minimum reporting level of 45 ng/mL, with other ICS formulation metabolites relating to beclomethasone and fluticasone propionate-17β-carboxylic acid remaining at 30 ng/mL. Related to systemic [oral] administration relating to asthma therapy, prednisolone has a minimum reporting level of 100 ng/mL (WADA, 2022c) and has seen an increase in adverse analytical finding over the past 10 years (Wojek, 2021, *p318*; WADA, 2023a). Although a note of caution is that not all adverse analytical findings signify intentional acts of doping as include figures for athletes with valid TUE.

History of WADA's position on GC

The status of policy regarding GC has been subject to change over the past 40-years (Fitch, 2016) *(Figure 2.9)*. Most recently, a notable change occurred in 2022 when WADA discontinued GC from its out of competition *'Monitoring Program',* stating they had gathered the required prevalence data on their use (Hughes *et al.*, 2020; WADA, 2022b; Vernec *et al.*, 2024).

1986: <u>Systemic GCs prohibited</u> – Intra-articular and 'local' injections notifiable (IOC-MC)

1992: <u>TUEs required for systemic GCs</u> (IOC-MC)

1995: <u>Inhaled GCs added as a notifiable category</u> (IOC-MC)

1997: Nasal GCs permitted
1998: <u>Inhaled GCs permitted</u>
1999: Injected GCs permitted, but IF may request notification (IOC-MC)

2004: All GCs prohibited with no reporting threshold. <u>Systemic GCs required a TUE; all other methods of administration of an ATUE</u> (WADA)

2005 – 2006: Topical (aural, buccal-topical, dermatological, ophthalmological, and nasal) GCs permitted. <u>Inhaled and injected GCs unchanged. Reporting threshold of 30 ng/mL introduced for all synthetic GCs</u> (WADA)

2009: <u>ATUEs ceased—inhaled</u> and injected GCs to be <u>declared on the doping control form</u> (WADA)

2011 – 2012: 'No Needle' policy introduced to prevent GC injections in proximity to competitions. IOC implements a 'No Needle' policy for London 2012 (UCI and FISA)

2016: List Committee proposes that intra-articular and local injections are prohibited within 72 hours of a competition and require a TUE; other methods unchanged, i.e., <u>GCs prohibited only in-competition, systemic GCs need a TUE, all others are permitted</u> (WADA)

2017: <u>Glucocorticoids remain on monitoring programme</u> (WADA)

2019: <u>Low-dose ICS recommended as first-line preventer therapy</u> for all diagnosed with asthma (GINA)

2021: Change from 30 ng/mL to **substance specific AAF detection limits** (WADA)
2022: <u>Glucocorticoids removed from monitoring programme</u> as required prevalence data obtained (WADA)

*Figure 2.9. The history of glucocorticoid therapy and guidelines/status for use in athletic population (Adapted from Fitch, 2016). Underlined specific to indicated for use in asthma prophylaxis. Organisation responsible for directive in (parenthesis). Abbreviations: **AAF**, adverse analytical finding; **ATUE**, abbreviated therapeutic use exemption; **FISA**, Fédération Internationale des Sociétés d'Aviron (World Rowing Federation); **GC**, glucocorticoid; **IF**, International Federation; **IOC-MC**, International Olympic Committee Medical Commission; **TUE**, therapeutic use exemption; **UCI**, Union Cycliste Internationale (World Cycling Federation); **WADA**, World Anti-Doping Agency.*

<u>*TUE and Oral GC Prevalence*</u>

Chapter 1 introduced the TUE policy, which permits athletes to use otherwise banned oral glucocorticoids (GC) to manage conditions such as severe acute asthma exacerbation during or shortly before competition. If necessary, athletes must apply for a TUE or seek retroactive application (WADA, 2023d). WADA provides resources for physicians on guidelines regarding obtaining a TUE for asthma-related substances (WADA, 2023b).

Overall, the percentage of athletes competing at Olympic Games between 2010 – 2018 with a valid TUE for any WADA-prohibited class was 0.9%. Additionally, athletes with TUEs secured only 21 out of 2062 individual sport medals available (1%), suggesting that there was no association between being granted a TUE and the likelihood of winning a medal (Vernec and Healy, 2020). This study was extended for the Tokyo Olympic Games and, for the first time, included epidemiological data on TUE use at the Paralympic Games. The findings aligned with the previous study, with Olympic athletes' TUE use remaining below 1.0%. Additionally, it revealed that Paralympic TUE use was below 3.0%, likely higher due to the complex medical conditions common among these athletes (Vernec *et al.*, 2024). However, a limitation of these observations was the focus on individual athletes, as quantifying the impact of a single athlete with a TUE in a team sport setting is challenging (Vernec and Healy, 2020).

As previously mentioned, GC have various applications beyond respiratory medicine. Therefore, it is not surprising that athletes have a legitimate use of GC due to the prevalence of asthma and musculoskeletal injuries (Jacobsson *et al.*, 2012; Burns *et al.*, 2015). A survey of 603 sports medicine practitioners presented that 85.6% routinely utilise GC via any route. Among them, 414 (80.2%) commonly prescribed oral GC (Hughes *et al.*, 2020).

Specifically related to asthma-related therapy, β2-agonists (0.46%) and GC (0.23%) are among the most common classes of medication used by athletes holding a TUE. GC were the most frequently encountered substance for TUE at Rio de Janeiro 2016 Olympic Games with a prevalence of 0.50%, yet, this had reduced to 0.17% for the Tokyo Olympic Games (Vernec *et al.*, 2024). During the PyeongChang 2018 Winter Olympics and Paralympics, six athletes were prescribed oral GC (Stuart, Kwon and Rhie, 2019).

Among GC used, 61% were applied prospectively. However, due to the immediate need for treatment, retroactive TUEs were sought 39% of the time. This proportion of retroactive TUEs is higher compared to other substances or methods, likely due to their usage for acute exacerbations or injury management rather than planned condition control. Data from the WADAs *'Anti-Doping Administration & Management System'* (ADAMS) between 2012 - 2016 suggested that the majority of GC TUEs are granted for short-duration use, with 23% for 1 day, and 21% for less than 1 week (Vernec *et al.*, 2020).

Sex and geographical regional differences may exist in oral GC use, with recent observations suggesting females demonstrate greater usage than males, and French athletes exhibiting higher GC usage compared to their counterparts in Australia and New Zealand (Collomp *et al.*, 2022; Buisson *et al.*, 2023).

2.4. Ergogenicity of Glucocorticoids used for EIB Management

The preceding sections of this literature review have highlighted the prevalence and therapeutic necessity of ICS and oral GC in managing asthma-related conditions, but their potential ergogenic effects on athletic performance have been a topic of interest among sports medicine researchers.

An unscrupulous athlete may consider GC to be used as an ergogenic aid purely due to its inclusion in the list of banned substances and may reinforce the idea that this presence is due to performance enhancing effects, consequently encouraging their misuse (Kuipers and Ruijsch van Dugteren, 2006). Nevertheless, according to an editorial by Orchard, (2008) it was proposed that individuals seeking performance enhancement might intentionally evade substances known to be prohibited and frequently screened for to avoid detection. Consequently, an adverse analytical finding from GC could more plausibly signify unintentional or legitimate usage rather than deliberate cheating. This is supported with the evidence that an adverse analytical finding for GC is commonly from athletes who have been granted a TUE. The 'Doctrine of Double Effect', is an ethical principle suggesting that an action with a positive intent [such as therapeutic treatment] might lead to both positive [therapeutic effect] and negative outcomes [like performance enhancement or side effects harm] and has previously been discussed surrounding TUE policy (Pike, 2018).

Earlier in this chapter the HPA axis was described, whereby, endogenous GC are the end product following the secretion of CRH and ACTH from the hypothalamus and pituitary gland (Son, Chung and Kim, 2011), to then act on physiological processes within the body via activation of GC receptors. There is suggestion that GC substances might have the capacity to improve exercise performance by influencing both central and peripheral mechanisms.

Glucocorticoids, as their name suggests, have endogenous function to regulate glucose levels and also play a pivotal role in governing carbohydrate and fat metabolism (Duclos, 2010). Their impact extends to the transcriptional control of enzymes involved in hepatic

stimulation, influencing gluconeogenesis by upregulating the expression of key enzymes such as phosphoenol-pyruvate carboxykinase and glucose-6-phosphate (Magomedova and Cummins, 2015). Additionally, GC modulate lipolysis by reducing lipoprotein lipase activity (Vegiopoulos and Herzig, 2007). From an exercise physiology perspective, the metabolic effects from administration of exogenous GC have demonstrated an ability to elevate energy expenditure and promote fat oxidation to meet the increased energy demands during physical exertion (Arlettaz, Portier, *et al.*, 2008; Macfarlane, Forbes and Walker, 2008). This heightened availability of metabolic substrates holds significance in optimising exercise performance (Duclos, 2010). During competition, if an individual makes effective use of fat oxidation to support metabolism during prolonged exercise, this may reduce the requirement for endogenous carbohydrate oxidation, and therefore muscle glycogen depletion, which is linked to fatigue (Bergström et al., 1967; Ørtenblad, Westerblad and Nielsen, 2013). These metabolic changes could also potentially lead to alterations in body composition, a desirable trait for endurance-based athletes seeking an improved power-to-weight ratio (Ackland *et al.*, 2012).

Exogenous GC-induced promote anti-inflammatory cytokines *[e.g. IL-10],* and attenuation of the post-exercise systemic pro-inflammatory (IL-6) response (Arlettaz, Collomp, *et al.*, 2008). Although IL-6 is a pleiotropic cytokine that has multiple functions throughout the body, it has involvement in maintenance of glucose homeostasis (Pedersen, Steensberg and Schjerling, 2001), may contribute to development of exercise-induced fatigue (Vargas and Marino, 2014) and has been associated with worsened post-exercise recovery and muscle soreness (Robson-Ansley *et al.*, 2010). Moreover, the analgesic effects of GC could inhibit sensations of muscle pain during effort, as well as raise the fatigue threshold (Duclos, 2010). These effects are postulated to prolong exercise, or could lead to improved recovery between successive bouts of exercise *[e.g. multiple-stage cycling events]* (Allen *et al.*, 2019). However, the impact of GC administration specifically related to recovery has not yet to be investigated.

Exogenous GC may affect the central nervous system. In rodents, GC stimulates extracellular dopamine linked to reward and desire (Piazza *et al.*, 1996), but increased stimulation does not necessarily improve performance (Redon *et al.,* 2020). However, reduced serotonin activity may inhibit descending motor neurons, affecting locomotor muscle output (Meeusen *et al.,* 2006). Increased release of adrenocorticotropic hormone (ACTH), may not directly improve maximal exercise performance, but improve levels of mood and vigour (Soetens, Hueting and De Meirleir, 1995) indirectly improving

readiness for latter stages of prolonged or repeated bouts of exercise. Moreover, hormonal effects such as ACTH, dehydroepiandrosterone (DHEA) and salivary cortisol reduction signify alterations in HPA function (Collomp *et al.*, 2014), and a decrease in prolactin (PRL) has been observed following time to exhaustion trials (Arlettaz *et al.*, 2007; Le Panse *et al.*, 2009) perhaps showing that GC can delaying the perceived onset of fatigue.

The next section will present the previous studies conducted on ICS and oral GC on an acute and short-term basis with exercise or performance outcomes.

2.4.1. Inhaled Corticosteroids (ICS) in Response To Exercise and Performance Outcomes

Investigation into the effects of ICS on performance and physiological outcomes are limited. Jardim *et al.*, (2007) initiated research into ICS and their impact on exercise outcomes, primarily exploring an ergolytic perspective. They cited early case-study observations of steroid-induced myopathy in respiratory muscles and reduced quadricep force following ICS usage (Decramer and Stas, 1992). In their study, Jardim *et al.*, (2007) administered inhaled flunisolide (1000 µg/day in two boluses) or placebo inhaler for a duration of 4 weeks in a double-blinded randomised cross-over design involving thirteen sedentary male participants. The authors reported no significant change in hand-grip or respiratory muscle strength and endurance following flunisolide administration compared to the placebo condition. Additionally, there were no notable alterations in body-mass index or arm muscular circumference, marking the first observations into body composition following ICS, although the validity of these outcomes could be questioned. The authors also noted a learning and/or training effect could have had impacted the performance outcomes. Despite aiming to investigate the adverse effects of ICS on the function of respiratory and peripheral muscles, it provided initial insights into the potential effects of ICS on healthy individuals.

Subsequently, Kuipers *et al.*, (2008) completed the first study with rationale relating to anti-doping research in response to the then recent studies using systemic oral GC (Arlettaz *et al.*, 2006, 2007) *[discussed in section 2.4.2 & 2.4.3]*. The authors studied in a double-blinded, between group design the effects of 4 week of twice daily inhaled budesonide (800 ug per day) or placebo using well-trained male endurance athletes. Performance was assessed using a maximal graded exercise test after two and four weeks of administration. The authors failed to observe significant differences between treatment groups in maximal power output after two- (budesonide: 377 ± 40 W vs placebo: $374 \pm$

22 W) or four-weeks (budesonide: 378 ± 37 W vs placebo: 374 ± 26 W) of the intervention period. Moreover, there were no differences in the measures with the profile of mood states (POMS) questionnaire between groups, particularly relating to euphoria *[previously noted as a potential ergogenic mechanism]*. Despite the similar baseline characteristics between groups of participants, this study is limited by the between group nature of the study design without cross-over. The authors noted that higher doses may be required and given the transient nature of euphoria, with more frequent of assessment of POMS may be required in future research rather than the weekly recording in their study.

Adding to prior investigations, Schwindt *et al.*, (2010) evaluated the impact of a two-week inhaled fluticasone proprionate *[440 μg twice daily]* administration regimen on selected immune responses *[leukocytes, IL-6]* and HPA axis mediators *[adrenocorticotrophic hormone (ACTH), cortisol, growth hormone (GH), insulin, catecholamines]* at rest and following cycling exercise at 70% of $\dot{V}O_2$max. The exercise responses *[heart rate, work-rate, lactate]* were comparable between treated and untreated conditions, but $\dot{V}O_2$ showed a small increase in the treated group [mean change of 0.22 ml.kg^{-1}.min^{-1}]. Resting ACTH and cortisol significantly decreased with ICS-treatment, and also resulted in significantly lower magnitude of change in response to exercise. GH remained similar at rest but had a blunting response to exercise in the treated group. Insulin and catecholamines *[dopamine, norepinephrine, and epinephrine]* did not show significant differences between conditions at baseline or post-exercise. Pre-exercise, ICS treatment led to a notable rise in leukocyte and neutrophil count, yet the exercise-induced increase was similar between treated and untreated conditions. Resting IL-6 levels increased with ICS treatment and showed a blunted response to exercise. The authors expected these immune and HPA function alterations due to the ICS treatment, noting suppressed ACTH and thus inadequate stimulation of adrenal cortisol production were the cause. The authors observed that exercise significantly increased peripherally circulating fluticasone levels [median increase of ~50%], possibly attributed to a mobilising of drug stores in the lungs or pulmonary circulation. This observation might be relevant to athletes, but caution is warranted due to exercise-induced haemoconcentration that can impact on samples due to factors such as dehydration (Hill *et al.*, 2008). While participants acted as their own controls, this study lacked randomisation, blinding, or crossover, introducing a potential risk of bias. Although not directly investigating the effect of ICS on athletic performance, this study provided

evidence towards physiological changes that can occur following ICS treatment when associated with an endurance exercise task.

Most recently, Hostrup *et al.*, (2017) studied the synergistic effect of concurrent ICS and $\beta2$-agonists on exercise performance, as a glucocorticoid-induced increase in Na^+, K^+ ATPase content potentially augments the ergogenic effects of $\beta2$-agonists. Participants exercised at 90% peak incremental power output until fatigue. This was conducted before and after 2-weeks of daily inhalation of 1.6 mg budesonide. Thirty-minutes before the TTE, participants also inhaled an acute dose of 4 mg terbutaline in both trials. Expression of Na^+, K^+ ATPase in skeletal muscle was enhanced by 17% following ICS treatment. However, cycling endurance was not enhanced following the budesonide-terbutaline combination. This study had an absence of a placebo condition, so it is not possible to understand if exercise performance was enhanced without the interaction of $\beta2$-agonists.

Both Schwindt *et al.*, (2010) and Hostrup *et al.*, (2017) supported that significant proportion of inhaled drug can reach the systemic circulation (Pedersen, Steffensen and Ohlsson, 1993). The interesting observation by Hostrup *et al.*, (2017) was the systemic concentration of budesonide was correlated with the Na^+, K^+ ATPase content after the intervention. So, inducing ergogenic impact from inhaled substance could just be a question of dose. But thus far no research has investigated supratherapeutic doses of ICS on exercise performance or immune and metabolic function. However, the predicted performance enhancing inhaled dose is well above that indicated for therapeutic use (Ventura *et al.*, 2021).

For an inhaled administration route, recent meta-analysis based only on the Jardim *et al.*, (2007) and Kuipers *et al.*, (2008) studies determined that a standardised difference in mean of -0.055 (-0.507 to 0.397, p=0.812), suggesting the current consensus is that ICS at therapeutic doses have no ergogenic effect on exercise performance (Riiser, Stensrud and Andersen, 2023). However, this is only based on the participants from two studies, and performance outcomes that are not particularly valid to the performance of elite athletes.

ICS Reporting Quality of Inhaler Technique

Aside to the ergogenicity of ICS, one limitation of studies using respiratory substances is the reporting of inhaler technique. Inhaler technique can impact on the delivered mass of

drug from an inhaler device. Literature related to ergogenic action of inhaled substances do often make attempts to standardise inhaler technique and incorporate monitoring of participants. For example, a strength of a previous study by Jessen *et al.,* (2018) is the supervision of participants in person or via online monitoring during inhalation of study drugs, thus ensuring 100% drug compliance. However, studies often use vague adjectives such as *"proper inhalation technique", "correct technique", "effective"* or *"optimal"* delivery but fail to define what this means. With also no reporting of inspiratory flow rate. Specifically to the previously outlined studies on ICS, Kuipers *et al.*, (2008) *stated "Using a placebo inhaler, the subjects practised inhalation in the presence of the researcher until a proper inhalation technique had been acquired".* Moreover, (Dickinson *et al.*, 2014) suggested *"..A limitation to our study is the potential variability in actual dose inhaled. Although the use of a pocket chamber aimed to reduce this limitation, it remains possible that some participants inhaled lower doses of salbutamol compared to others"* – this may be a reason why the authors observed differences in urinary salbutamol between some individuals.

Table 2.4 summaries the current available evidence of the effect of ICS on exercise performance.

2.4.2. Acute and Short-Term Oral GC on Exercise Performance

The impact of asthma-related oral GC administration has received more attention than ICS. When combining acute and short-term trials, meta-analysis determined there was moderate-quality evidence that oral doses are performance enhancing [0.361; 0.124 to 0.598, p=0.003] (Riiser, Stensrud and Andersen, 2023). The observed difference in effect between oral and inhaled doses is likely due to the route of administration and therefore concentration within systemic circulation.

Acute dose of prednisolone (20 mg) administered approximately 2-3 hours prior to exercise was shown not to impact on TTE performance at either 70-75% (Arlettaz, Collomp, *et al.*, 2008), or 80-85% $\dot{V}O_2max$ (Arlettaz *et al.*, 2006) compared to a placebo condition. Based on these two studies, prednisolone was suggested to not significantly affect TTE compared to a placebo condition (0.31 (-0.30 to 0.92, p=0.32) and these studies had low risk of bias as assessed with GRADE (Trinh, Chen and Diep, 2022).

As one of the theories of ergogenic effect of GC is alterations in substrate usage, Arlettaz, Portier, *et al.*, (2008) examined the hypothesis that acute therapeutic glucocorticoid intake

(20 mg) prednisolone could impact the contribution of fat (FO) and carbohydrate (CHO) oxidation in energy production during submaximal exercise at 60% $\dot{V}O_2$max. The authors found significant alteration during exercise towards FO following prednisolone compared to placebo.

When separating oral administration between acute (< 24 hours) and short-term administration ($\geq$24 hrs but <14 days), the consensus is that acute doses do not enhance performance (-0.091; -0.202 to 0.392, p=0.565) (Riiser, Stensrud and Andersen, 2023). However acute doses have only used relatively low doses of prednisolone [i.e., 20 mg], so it is unknown the effect on exercise performance, substrate utilisation or inflammatory response when a higher therapeutic dose is used in acute manner (Duclos, 2010). A dosage 40 - 50 mg may be indicated for asthma exacerbations (BNF, 2023b).

However, there is evidence that short-term administration of asthma-related oral GC has the potential to enhance performance (0.428; 0.148 to 0.709, p=0.003) (Riiser, Stensrud and Andersen, 2023). Arlettaz *et al.*, (2007) observed an improvement in TTE at 70-75% $\dot{V}O_2$max compared with a placebo condition (prednisolone: 74.5 ± 9.5 vs placebo: 46.1 ± 3.3 min, p<0.01) following 7 days of prednisolone administration (oral dose 60 mg/day). These findings were supported by Le Panse *et al.*, (2009) who investigated 50 mg/day of prednisolone on TTE at 70-75% $\dot{V}O_2$max in recreationally trained females (prednisolone: 66.4 ± 8.4 vs placebo: 47.9 ± 6.7 min, p<0.01). In both studies, this improvement was accompanied by reductions in ACTH, DHEA, PRL, GH. Conversely, Zorgati *et al.*, (2014) found that oral ingestion of prednisone (60 mg/day for 7 days) had no impact on Time to Exhaustion (TTE) during hopping exercise when compared to a placebo. This absence of an ergogenic effect was observed despite the previously established notable hormonal changes in DHEA, cortisol, anti-inflammatory cytokine IL-10 and pro-inflammatory IL-6.

In a double-blind crossover study, Collomp *et al.*, (2008) incorporated prednisolone (60 mg/day) alongside a standardised period of cycling training (2 hours/day) for 7 days compared to a placebo TTE at 70–75% $\dot{V}O_2$peak was conducted before and after administration [with washout of 3 weeks between conditions]. Prednisolone resulted in a significant improvement in TTE (56.1 ± 9.1 to 107.0 ± 20.7 min) compared to placebo (50.4 ± 6.2 to 64.0 ± 9.1 min). Similar to previous studies using oral GC, prednisolone resulted in reductions in ACTH, DHEA, PRL and GH. However, the sample size was

small (n=8) and the recreational level of participants may impact on the repeatability of the TTE assessment.

Table 2.5 summarises the current available evidence of the effect of acute and short-term prednisolone and prednisone oral GC on exercise performance.

Summary of Gaps in Ergogenic Effect Research of GC

Despite the above postulated mechanisms of action, there is only limited high-quality investigations on the performance enhancing potential of GC (Heuberger *et al.*, 2022). Previous narrative review articles have cited the differing responses observed in previous studies can be linked to methodological decisions made during study design, including factors such as the dosage, and mode of administration (Collomp *et al.*, 2016; Tacey *et al.*, 2017). The recent systematic reviews summarised succinctly that route of administration may play a role in generating a potential ergogenic effect driven by the differences in systemic availability from oral vs inhaled administration. ICS used in previous studies has been within therapeutic indications, but it has yet to be investigated in supratherapeutic doses. Moreover, in the field of β2-agonists research, both oral and inhaled routes have been explored within a single-study. But this has not been investigated in GC class substances. Previous studies determined that acute administration is not performance enhancing, however as previously mentioned, the studies used relatively low dose of prednisolone – so exploring higher doses is warranted.

<u>Mode of Exercise and Performance Outcome Critique</u>

As presented, there are discrepancies in exercise performance responses among studies, which may arise not only from differences in the dosing regimen of glucocorticoids (acute vs. short-term, inhaled vs. systemic) but also from the type of exercise modality used and the specific performance outcomes measured (Tacey *et al.*, 2017).

A critique of these former investigations is the utilisation of performance outcomes that lack ecological validity. For example, while $\dot{V}O_2$max serves as a reliable predictor of aerobic exercise performance in the general population, its value in distinguishing performance within a homogeneous group of elite athletes is relatively limited (Legaz-Arrese *et al.*, 2007). Additionally, it is unclear how a TTE relates to real-life endurance performance. Endurance competitions typically conclude at a finish line rather than exhaustion, often involving a clear 'end-spurt' in the pacing profile (Heuberger and Cohen, 2019). Thus, the assumption that prolonged TTE is directly indicative of enhanced

performance may be impractical, as these testing protocols fail to replicate real-world competitive sport settings, and due to the larger inherent variability can hide meaningful performance changes. Studies are needed to better reflect practical changes in exercise performance e.g., via simulated cycling time trial (TT) which although can differ in distance and intensity, generally require athletes to perform faster rather than for longer (Tacey *et al.*, 2017). Furthermore, in elite sports, minor margins can determine success, yet previous studies have not considered what might constitute a meaningful change for an athlete. Notably, all studies conducted on GC have exclusively involved non-elite participants; none have included professional athletes (Trinh, Chen and Diep, 2022). The focus on more sedentary individuals increases the likelihood of observing an effect, as elite athletes often operate closer to their maximal capabilities due to genetic predispositions and extensive training adaptations (McKay *et al.*, 2022).

Table 2.4. *Methodological summary and direction of main outcome change in studies that have examined the effect of ashma-related inhaled glucorticoid therapy on exercise performance.*

Author(s)	Protocol	Fasted State	GC Administration				Placebo Controlled	Performance / Main Outcome	Population	Sex	Asthmatic
			Medication	Mode	Dose	Timing					
Kuipers *et al.,* 2008	POMax from incremental exercise	Not Stated	Budesonide	Short-Term	800 µg/ day	4-weeks	Yes	No change in peak power output at week 2 or week 4.	28 HT	M	No
Horstrup *et al.,* 2017	Cycling TTE @ 90% POMax.	Not Stated	Budesonide (combined with terbutaline)	Short-Term	1.6mg/day (+ 4 mg terbutaline on prior to exercise task)	2-weeks	No	No Change in TTE. Increase in Na$^+$, K$^+$ ATPase	10 RT	M	No
Jardim *et al.,* 2007	Respiratory muscle function & handgrip strength	Not stated	Flunisolide	Short-Term	Twice daily 500 µg (1000 µg / day)	4-weeks	Yes	No difference in maximal inspiratory and expiratory pressure and handgrip strength.	13 SED	M	No
Schwindt *et al.,* 2010	30 mins @ 70% POMax.	Not Stated	Fluticasone Proprionate	Short-Term	Twice daily 220 µg (total 440 µg / day)	2-weeks	No	Blunted inflammatory response + increase in V̇O2.	11 RT	M	No

Abbreviations: ***HT****; highly trained,* ***RT****; recreationally trained,* ***SED****; sedentary individuals,* ***M****; male,* ***F****; female,* ***POMax****; maximum power output during incremental exercise,* ***TTE****; time to exhaustion.*

Table 2.5. *Methodological summary and direction of main outcome change in studies that have examined the effect of asthma-related oral (per os) glucorticoid therapy on exercise performance.*

Author	Protocol	Fasted State	GC					Performance / Main Outcome Change	Population	Sex	Asthmatic
			Medication	Mode	Dose	Timing	Placebo				
(Arlettaz *et al.*, 2006)	80-85% Peak power cycling to exhaustion	500kcal meal ~1 hr pre-exercise	Prednisolone + Prednisolone/Salbutamol	Acute	20 mg	2 hours pre - exercise	Yes	No Change	7 RT	M	No
(Arlettaz, Collomp, *et al.*, 2008)	70-75% Peak power cycling to exhaustion	500kcal meal ~2 hr pre-exercise	Prednisolone	Acute	20 mg	3h pre - exercise	Yes	No Change	14 RT	M	Unspecified
(Arlettaz, Portier, *et al.*, 2008)	60min @ 60% VO$_2$max	Overnight Fast	Prednisolone	Acute	20 mg	2h pre - exercise	Yes	Altered	9 RT	M	No
(Tacey *et al.*, 2019)	High intensity interval exercise. 4 x 4 min cycling at 90-95% HR max.	Unspecified	Prednisolone	Acute	20 mg	12hrs pre-exercise	Yes	Decrease in work capacity.	9 RT	M	Unspecified

(Zorgati et al., 2014)	Hopping 30s, 3x & to exhaustion	500kcal meal ~2hr pre-exercise	Prednisone	Short Term	60 mg/day	7 days	Yes	TTE = Peak force +	10 RT	M	No
(Collomp et al., 2008)	70-75% Peak power cycling to exhaustion	500kcal meal ~2 hr pre-exercise	Prednisolone + standardised training programme.	Short Term	60 mg/day	7 days	Yes	TTE +	8 RT	M	Unspecified
(Le Panse et al., 2009)	70-75% Peak power cycling to exhaustion	500kcal meal ~2 hr pre-exercise	Prednisone	Short Term	50 mg/day	7 days	Yes	TTE +	9 RT	F	No

Abbreviations: **HT***; highly trained,* **RT***; recreationally trained,* **SED***; sedentary individuals,* **M***; male,* **F***; female,* **POMax***; maximum power output during incremental exercise,* **TTE***; time to exhaustion.*

2.5. Literature Review Summary

To conclude, *Chapter 1* and *Chapter 2* have presented the discourse surrounding asthma prophylaxis and TUE policy, the prevalence of EIB among elite athletes, and outlined the corresponding management strategies. *Chapter 2* also stated there is limited investigations on the effect of diagnosing and treating athletes with EIB on respiratory health and performance outcomes. These chapters emphasised the use of ICS as a first-line treatment to manage the condition, while noting that oral GC may be needed in serious exacerbation of illness. Furthermore, it presented WADA's stance on GC substances [class S9] that allows ICS usage within therapeutic indication at all times but prohibits oral GC administration during in-competition periods.

Chapter 2 then presented the current knowledge on the ergogenic potential of ICS and acute and short-term oral GC. The limited research on ICS suggest they are not ergogenic, but acute supratherapeutic doses have not yet been investigated. Conversely, orally administered GC exhibit potential ergogenic properties due to their heightened systemic bioavailability, however acute doses do not appear to have the same performance enhancing effect as short-term administration despite exhibiting similar changes in hormonal and metabolic blood markers. Nevertheless, previous studies on acute administration used relatively low doses, therefore the impact of higher doses of acute oral GC on exercise performance and substrate utilisation is unknown.

Furthermore, previous investigations in this field have primarily utilised performance measures with lower external or ecological validity, (i.e., TTE, handgrip strength, incremental maximal power output, and functional tasks [sprint shuttles and TTE hopping]). However, it remains uncertain whether these findings from prior studies directly relate with actual endurance performance such as time-trials (Heuberger and Cohen, 2019).

2.6. Thesis Statement of Purpose, Aims and Hypotheses

This thesis aims to address the aforementioned gaps in the literature relating to ergogenic potential of asthma-related GC treatment. Furthermore, it will align with the *'call to action'* presented in recent review articles and meta-analyses, advocating for future research priorities in asthma medication to determine the impact on sports performance, understanding the impact of maintaining respiratory health to optimise performance (Allen *et al.*, 2019), and utilising valid test protocols like closed-end tests *[e.g., time-*

trials] (Riiser, Stensrud and Andersen, 2023). Additionally, this research aims to explore the relationship between dosage and ergogenic benefits (Trinh, Chen and Diep, 2022). The experimental chapters of this thesis may reveal the abuse potential and performance-enhancing effects of currently permitted ICS, or TUE controlled oral GC in competitive sports, and may provide WADA with the scientific basis for improved drug regulation and inform the annually published list of prohibited substances.

The overall aim of this thesis was to investigate the impact of acute, short, and long-term use of asthma-related glucocorticoid therapy on athlete health, performance, and recovery. The specific aims and hypotheses of the four experimental studies are as follows:

Chapter 4 – Experimental Study 1

Title: The Impact of Long-Term Asthma Therapy on Hyperpnoea-Induced Bronchoconstriction and Real-World Major Competition Performance in Elite Swimmers.

Aim: Through a retrospective analysis, investigate the impact of diagnosing and initiating EIB management has on respiratory function [spirometry, FeNO, EIB severity], and performance in elite athletes during major competitions.

Hypothesis: Appropriate long-term management of EIB would reduce airway inflammation and EIB severity in comparison to a group of athletes who discontinued treatment during the observation period. The initiation of EIB therapy would not lead to an increase in performance beyond the expected progression between major competitions.

Chapter 5 – Experimental Study 2

Title: Assessing Inhaler Technique in Research on the Ergogenicity of Asthma Therapy: Evaluating Beclomethasone Dipropionate Deposition with-and-without an AeroChamber Plus Valved-Holding Chamber (VHC) at Increasing Simulated Inhalation Flow Rates.

Aim: To establish the impact that inhalation flow-rate and VHC use has on the performance [delivered dose, fine-particle mass] of Beclomethasone Dipropionate (BDP) pMDI [Qvar® 100 µg] device, thereby aiding the methodological development of inhaler technique for prospective experimental work *(Chapters 6 and 7)* investigating ergogenic effects in this thesis.

Hypothesis: Deposition profile would change depending on the simulated inhalation flow-rate, and use of VHC will decrease due to BDP mass entrapped in the chamber.

However, the addition of a VHC would enhance fine particle dose and practically enable prospective participants to better co-ordinate device actuation and inhalation flow-rate.

Chapter 6 – Experimental Study 3

Title: Effect of Acute Inhaled & Oral Doses of Glucocorticoids on Initial 40-km Cycling Time-Trial, and Recovery for a Subsequent 10-km Time-Trial.

Aim: Compare two administration routes of GC class substances on initial 40-km cycling time-trial (TT) and recovery for a further 10-km TT performed on the same day.

Hypothesis: Oral GC will enhance time-trial completion time, but supratherapeutic ICS will not. Furthermore, oral GC are expected to induce metabolic and immunosuppressive changes during and following exercise, whereby this will not be observed in ICS or placebo conditions.

Chapter 7 – Experimental Study 4

Title: Effect of Short-Term Daily Inhaled Corticosteroid Administration on Repeated 10-km Cycling Time-Trial Performance.

Aim: Investigate the impact of short-term daily administration (14-days) of high-dose ICS on 10-km cycling time-trial performance time, and recovery for a subsequent 10-km TT performed on the same day.

Hypothesis: Short-term use of ICS will not enhance time-trial performance time compared to a placebo. Furthermore, it is expected that there will be no observable changes in immunosuppression, as measured by IL-6 levels.

CHAPTER 3: GENERAL METHODS

The research in this thesis was conducted in accordance with the Declaration of Helsinki. Ethical documentation, risk assessments and insurance confirmation were submitted to and approved by the University of Kent School of Sport and Exercise Sciences (SSES) Research Ethics Advisory Group (REAG), or the Faculty of Sciences REAG *(Appendix A)*. Experimental studies not involving human participants (*Chapter 5*) did not require ethical approval.

Several methods were utilised to investigate the impact of acute, short-term, and chronic administration of glucocorticoid asthma therapy on athlete health, performance, and recovery. This chapter outlines the methods used throughout this thesis that were common to two or more chapters. Tests and procedures that were used only in one chapter are defined within the respective experimental chapters. A summary of the repeated procedures used in each chapter is presented in *Appendix B*.

3.1. Health Screening Questionnaire and Informed Consent

Prior to commencing research that required human subjects, all participants completed a health screening questionnaire and provided written informed consent (*Appendix C and Appendix D*). The health questionnaire was designed to screen for acute illness or infection, and chronic medical conditions. In experimental studies that required pharmaceutical intervention (*Chapter 6 and 7),* the completed health questionnaire was inspected by a collaborating physician to assess participant suitability and ensure safety from serious adverse effects.

3.2. Preparation for exercise sessions

In order to standardise the repeated measures design conducted in *Chapter 6* and *7*, participants were asked to adhere to some pre-exercise considerations, including:

- No high intensity exercise (including heavy weights) 24 hours before each visit.
- Not have taken any analgesics (painkilling) or anti-inflammatory medications [i.e., paracetamol, ibuprofen] 48 hours before each visit.
- Not be taking any glucocorticoid treatment during the study (i.e., topical cremes)
- Not have consumed alcohol 24 hours before each visit.
- Not consume sports drinks or caffeine on the day, or throughout the testing visit.
- Arrive to the session well hydrated, appropriately fed, and ready to exercise as if the performance trials were a competitive event.

3.3. Assessment of Airway Inflammation (FeNO)

Fraction of exhaled nitric oxide (FeNO) was used to determine airway inflammation and was assessed using one of two devices; NIOX VERO® (Aerocrine, Solna, Sweden) [Chapter 4] or NObreath® (Bedfont Scientific, Maidstone, UK) [Chapter 6 & 7]; *Figure 3.1a*). Both devices have shown good correlation, although NIOX VERO® is reported to produce consistently higher values (Tsuburai *et al.*, 2017; Saito *et al.*, 2020). Measurements were performed in accordance to standardised procedures recommended by American Thoracic Society and European Respiratory Society (Dweik *et al.*, 2011). Assessments of airway inflammation were always performed prior to maximal lung function manoeuvres. From a seated position, participants formed a tight seal around the mouthpiece with their lips and inhaled to total lung capacity. An on-screen animation then guided participants to exhale against the slight resistance of the machine for 10 seconds at a steady flow rate (50 mL.s^{-1} against pressure of 16 cmH$_2$O). Measurements were performed in duplicate, and the mean value recorded. Results were interpreted as: <25 ppb, 'normal, eosinophilic airway inflammation unlikely'; 25-50 ppb, 'Elevated, eosinophilic airway inflammation likely'. >50 ppb 'High, eosinophilic airway inflammation significant' (Dweik *et al.*, 2011).

3.4. Assessment of Lung Function (Spirometry)

Lung function was assessed by forced flow-volume spirometry using a turbine transducer spirometer device (MicroLab ML3500, CareFusion, Basingstoke, Hampshire, UK; *Figure 3.1b)*. Predicted values were automatically generated using reference ranges from the European Community of Coal and Steel (ECCS; (Kuster *et al.*, 2008) incorporated into the manufacturer spirometry software (Spirometry PC Software, CareFusion, Basingstoke, Hampshire, UK). Participants from non-Caucasian background had a factor (90%) applied to the predicted value. Prior to testing, participants were screened for any contraindications to spirometry and excluded from participation where necessary (Cooper, 2011).

Initially the full procedure was explained and demonstrated. In a seated upright position, participants were instructed to inhale fully to total lung capacity. Then, without pausing, seal their lips around the mouthpiece and exhale with the maximum possible force, and continued to exhale until residual volume. On reaching residual volume, participants finally inhaled to total lung capacity. Participants wore a nose clip to prevent air escaping through their nose, and verbal encouragement was provided throughout to ensure maximal effort.

In studies conducted during COVID-19 pandemic, a low resistance microbial filter was used (*Figure 3.1c*), and no inspiratory manoeuvre was performed through the spirometer In addition, further mitigations were implemented such as pre-visit rapid antigen test (lateral flow), additional personal protective equipment, fallow periods and room occupancy limits (ERS, 2020; Lombardi, Milanese and Cottini, 2020).

From each spirometry effort, all expiratory volumes were recorded but most notably; forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), and FEV_1:FVC ratio (FEV_1/FVC) were of interest. Spirometry was performed in accordance with the within-and-between manoeuvre acceptability criteria outlined in the practice guidelines published by the American Thoracic Society/European Respiratory Society joint task-force. Data collection completed pre-2019 utilised Miller *et al.*, (2005), thereafter Graham *et al.*, (2019).
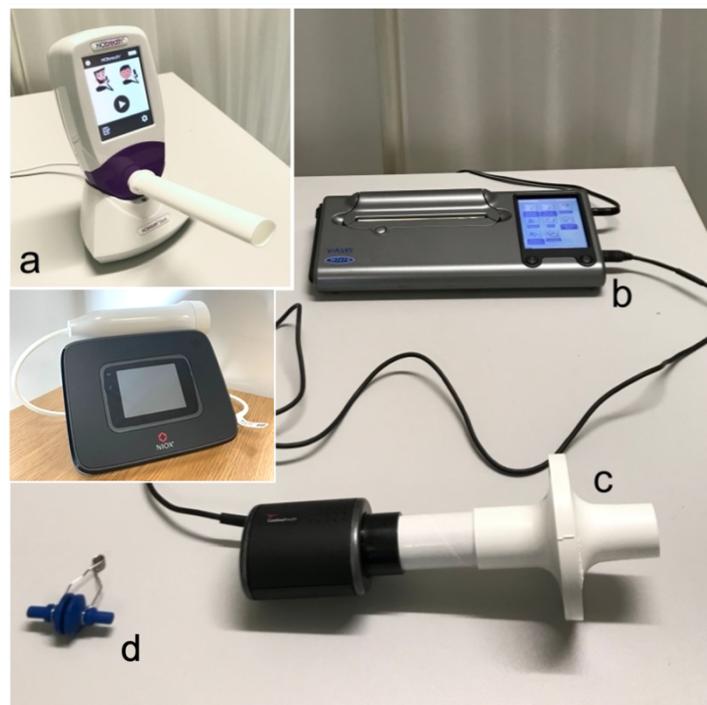
Figure 3.1. Respiratory Assessment Equipment. *(a) NObreath® (Bedfont Scientific, Maidstone, UK); (b) NIOX VERO® (Aerocrine, Solna, Sweden) (c) turbine transducer spirometer (MicroLab ML3500, CareFusion, Basingstoke, Hampshire, UK); (d) Antimicrobial filter (MicroGard®, VIASYS Respiratory Care, Yorba Linda, USA); (e) silicone nose clip (Reusable Series 9015, Hans Rudolph Inc, Kansas City, MO, USA).*

Table 3.1. Summary of within- and between-manoeuvre acceptability criteria, as outlined by Miller et al., 2005.

Within-manoeuvre criteria

Free from artefacts, including:
- 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 greatest

They show satisfactory exhalation
- Duration of 6 s or a plateau in the volume-time curve

Between-manoeuvre criteria

After three acceptable spirograms have been obtained, apply the following tests:
- The two largest values of FVC must be within 0.150 L of each other
- The two largest values of FEV_1 must be within 0.150 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

or
- A total of eight tests have been performed

or
- The patient/subject cannot or should not continue

Save, as a minimum, the three satisfactory manoeuvres

Abbreviations: ***FVC***: *forced vital capacity;* ***FEV₁***: *forced expiratory volume in one second,* ***L***: *Litre.*

3.5. Assessment of Airway Hyperresponsiveness (for EIB Screening)

For *Chapter* 6 and *7*, participants were recruited without a history of asthma-related conditions. In addition, airway hyperresponsiveness was objectively assessed by completing spirometry prior to, then 3 and 5 minutes post incremental cycling test to exhaustion *(Subheading 3.8)* in ambient conditions.

As discussed in *Chapter 2*, although not the 'gold-standard' method of assessing airway hyperresponsiveness, an exercise challenge can be used to screen for EIB, but does have limitations (Rundell and Slee, 2008). It has been suggested that an incremental work rate profile as used in cardiopulmonary exercise testing, [in which exercise intensity is progressively increased], is less likely to be effective in evaluating EIB than a brief, intense bout of exercise (Weiler *et al.*, 2016). During an incremental maximal

cardiopulmonary exercise assessment, the initial sub-maximal 'warm-up' period has the potential to induce refractoriness to EIB, and, high levels of minute ventilation are usually sustained only for a relatively short time (namely at the very end of the test) (Crapo *et al.*, 2000). Nevertheless, a cardiopulmonary exercise assessment can still provide valuable information on the diagnosis, exercise limitations and capacity of individuals with asthma-related conditions (Boutou *et al.*, 2020).

To provide some assurance that participants had a workload that was sufficiently hard, the inclusion criteria required minute ventilation to be sustained for 6 min above 60% of predicted maximum voluntary ventilation (MVV), calculated as $FEV_1 \times 35$ and/or HR above 85% predicted maximum (Weiler *et al.*, 2016). If the above condition had been met, and a fall in FEV_1 greater than 10% at two consecutive time-points was evident, this would result in the participant being withdrawn from the study.

Because of the exercise protocol used for objective screening of EIB in the present thesis, it may have provided false negative diagnostic outcomes. However, the objective evidence from post-exercise spirometry, combined with lack of asthma history, naivety to asthma therapy, and, the time / financial restraints of these experimental chapters, this method was deemed suitable to screen for airway hyperresponsiveness.
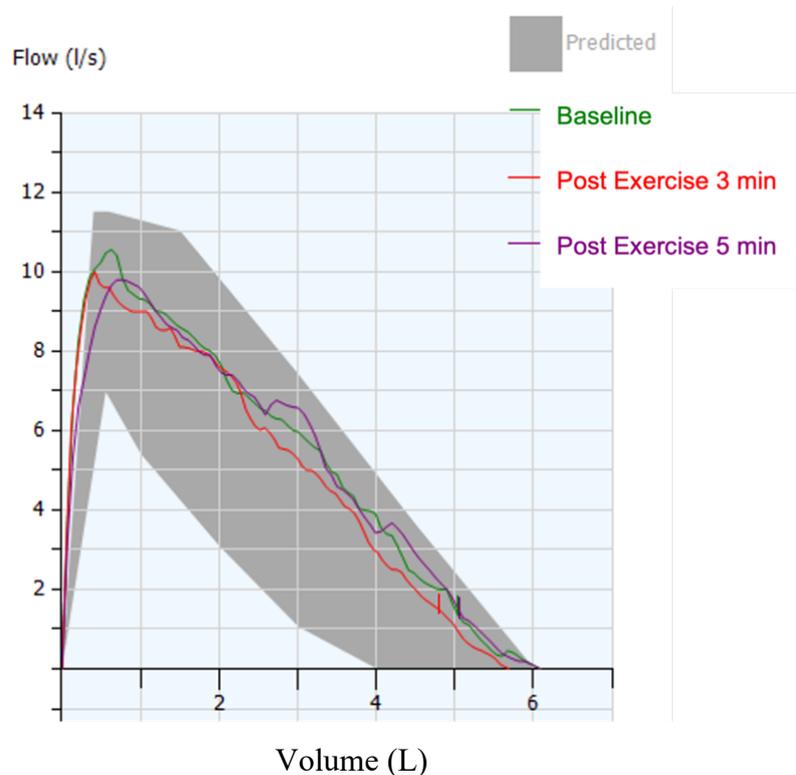
Figure 3.2. *Representative EIB negative flow-volume manoeuvre performed pre and post incremental exercise testing. Inspiratory flow omitted from testing protocol due to COVID-19 mitigations.*

3.6. Inhaler Administration Technique

Chapter 6 and *7* adopted a single slow and deep inhalation using a valved holding chamber, followed by a ten second breath hold for pMDI administration (Haidl *et al.*, 2016). This method was deemed most appropriate from methodology developed in *Chapter* 5. A standardised checklist was created for participants to learn the inhaler administration technique *(Table 3.2)*. This was adapted from a guide for the correct use of a metered-dose inhaler by the American Society of Health-System Pharmacists (https://www.safemedication.com/how-to-use-medication/metered-dose-inhalers, Last Accessed 4th March 2021).

Table 3.2. *Steps for Use of Pressured Metered-Dose Inhaler (pMDI)*

1.	Hold inhaler and volume spacer upright and shake well.
2.	Breathe out gently to residual volume.
3.	Keep head upright, put mouthpiece between teeth without biting and close lips to form a good seal.
4.	Actuate the inhaler and breathe in slowly and deeply (You will hear a whistle if you are breathing too fast).
5.	Continue to breathe in slowly and deeply until lungs are full.
6.	Hold breath for about 10 seconds.
7.	While holding breath, remove inhaler from mouth.
8.	Breathe out gently away from mouthpiece.
9.	Wait 30 seconds, then repeat the above process for remainder doses.
10.	Rinse your mouth thoroughly with water.

3.7. Blinding of Inhaler Equipment

ICS were dispensed from a similarly coloured metered dose casings *(Figure 3.4)* with canister label removed and black tape applied *(Figure 3.3a)*. Due to the taller canister of the placebo inhaler, a taller casing was sourced *(Figure 3.4d)*. The valved holding chamber (AeroChamber Plus™, Trudell Medical International, Ontario, Canada), was blacked out to blind visual differences in expelled vapour from the placebo and active inhalers *(Figure 3.4b)*. A commercially available mouthwash was provided for use before each inhalation set to disguise any differences in taste.

Figure 3.3. Inhalers and valved holding chamber provided to the participants.

Figure 3.4. Blinding of pMDI and valved holding chamber (VHC) (a) blinded and unblinded active inhaled corticosteroid beclomethasone dipropionate (Qvar ® 100 µg, Teva UK Limited, Castleford, United Kingdom) and Placebo water vapour inhaler (Vitalograph Ltd, Buckinghamshire, UK) canisters. (b) blinded and unblinded valved holding chamber (AeroChamber Plus™, Trudell Medical International, Ontario, Canada). (c) blinded and unblinded Qvar ® canister inside inhaler casing. (d) blinded placebo inhaler and casing.

3.8. Assessment of Maximum Oxygen Uptake ($\dot{V}O_2$peak Test)

In order to provide descriptive statistics and set submaximal exercise intensities, participants completed a ramp incremental cycling test to exhaustion on a cycling ergometer (Cyclus 2, Avantronic, Leipzig, Germany). Participants could use their own racing bicycle, or a suitable provided frameset. Bike geometry was adjusted as to preference of participant and repeated for any subsequent visits. Use of clipless pedals and cycling specific shoes was mandatory to ensure a constant fixed pedal/shoe interface. The ergometer was calibrated annually by the manufacturer.

Prior to participant arrival, the metabolic cart (Cortex Metalyzer 3B, Biophysik, Leipzig, Germany) was calibrated in accordance with the manufacturer's guidelines using ambient air and a known compressed gas composition (17% O_2, 5% CO_2). Following this, the volume transducer was calibrated using a 3-litre syringe (Hans Rudolph, Kansas, USA).

Initially anthropometrical measures of height (cm) and body mass (kg) were recorded to allow for relative standardisation of results. After this, a resting capillary blood lactate sample was collected prior to exercise to confirm the participant was in a rested state. Following a standardised warm-up of 100 W for 10 minutes, the work rate increased progressively at a ramp rate of 25 W per minute (1 W every 2.4 seconds). Throughout the test, participants were instructed to maintain a preferred cadence (above 70 rpm) and told that they were able to increase cadence as the intensity progressed. The test was terminated upon volitional exhaustion, or when the participant was no longer able to maintain cadence above 65 rpm for >5 seconds. Heart rate was recorded throughout using a commercially available heart rate monitor (HRM-Dual, Garmin, Olathe, USA). Rating of perceived exertion (RPE) was monitored at the end of each minute using 6–20 scale (Borg, 1982). Immediately post exercise, a further capillary blood lactate sample was collected.

Gas exchange measures were sampled breath-by-breath and subsequently averaged over a 5-sec time interval using dedicated software (Metasoft 3, Biophysik, Leipzig, Germany). The highest volume of oxygen ($\dot{V}O_2$) uptake achieved during exercise (5-sec average) was defined as the $\dot{V}O_2peak$. All tests were accepted as maximal following the attainment of at least two of the following secondary criteria of; RPE ≥ 17, RER ≥ 1.10, HR ± 10 bpm of age-predicted maximum, and end test blood lactate ≥ 8 $mmol^{-1}$ (Howley, Bassett and Welch, 1995).

Figure 3.5. Participant undertaking ramp incremental cycling test to exhaustion.

3.9. Determination of Submaximal Exercise Intensity

To provide a fixed stead-state submaximal cycling intensity for the estimation of fat and carbohydrate oxidation, the work rate associated with 50% of the $\dot{V}O_2$peak obtained during the ramp incremental cycling test was determined.

3.10. Estimation of Fat and Carbohydrate Oxidation

Indirect calorimetry was measured breath-by-breath during the exercise for determination of carbohydrate oxidation (CO) and fat oxidation (FO). Relative load and cadence were matched between experimental visits. The first 5 minutes were excluded to allow for steady state to be achieved. $\dot{V}O_2$ and $\dot{V}CO_2$ were averaged over the remaining 15 minutes. CO and FO were determined using stoichiometric equations developed by Frayn (1983), whereby it is assumed urinary nitrogen excretion is negligible *(Equation 3.1)*. This method has previously been used to investigate substrate utilisation following oral glucocorticoid intake (Arlettaz, Portier, *et al.*, 2008).

$$\dot{V}O_2\ (L.min^{-1}) = Volume\ of\ Oxygen\ Uptake.$$

$$\dot{V}CO_2\ (L.min^{-1}) = Volume\ of\ Carbon\ Dioxide\ Expired.$$

$$Fat\ Oxidation\ (FO)\ (g.min^{-1}) = \left(1.695\ \times\ \dot{V}O_2\right) - (1.701\ \times\ \dot{V}CO_2)$$

$$Carbohydrate\ Oxydation\ (CO)\ (g.min^{-1})$$
$$= \left(4.585\ \times\ \dot{V}CO_2\right) - (3.226\ \times\ \dot{V}O_2)$$

Equation 3.1. Calculation of fat and carbohydrate Oxidation using indirect calorimetry (developed by Frayn, 1983).

3.11. Assessment of Time-Trial Performance

All cycling time-trials were completed on the same electronically braked cycle ergometer (Cyclus 2, Avantronic, Leipzig, Germany), which was factory calibrated on an annual basis. Participants used their own bicycle to provide the highest level of comfort and familiarisation, or a suitably adjusted laboratory frame. Protocols were pre-programmed for the required distance on a flat profile. Intermediate splits were exported to collect the power output and HR data [10km time trial, 2-km splits; 40-km time-trial, 5-km splits]. Participants were instructed to complete the distance 'as fast as possible'. All performance data was obscured from view except distance completed *(Figure 3.6)*. No verbal encouragement was given throughout the time-trial, except a reminder of the task at each split distance. A fan was placed two meters behind the participant at an angle 45°.

Gas exchange measures were collected throughout using a metabolic cart (Cortex Metalyzer 3B, Biophysik, Leipzig, Germany). The calibration protocol was as outlined previously in *subheading 3.8.1*. Participants completed a single habituation trial before the experimental conditions commenced. Indoor 40-km time-trials with trained cyclists have a low coefficient of variation for completion time (~0.9%) and mean power (~2.1%) (Smith *et al.*, 2001). A single familiarisation is deemed sufficient to familiarise trained cyclist participants to a 40-km and 10-km time-trial distances (Laursen, Shing and Jenkins, 2003). In addition, recruited participants were accustomed to cycling and the pacing of sustained efforts. Performance times for all time-trials were disclosed only at the completion of the entire study.
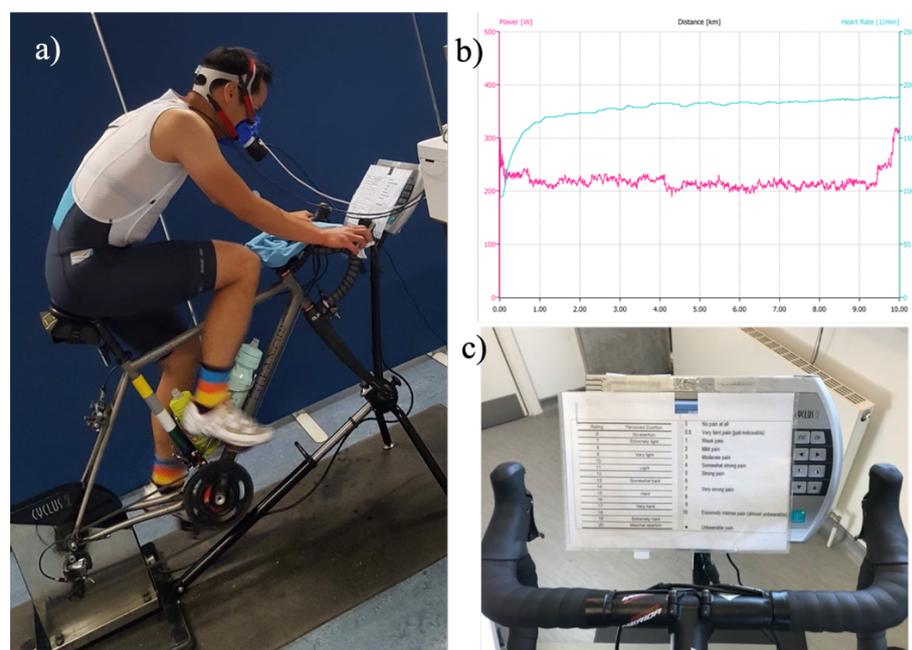
Figure 3.6. *Set-up of cycling time-trial assessments. (a) participant undertaking a time-trial protocol, (b) representative exported 10km time-trial effort, (c) visual feedback of participant during time-trials.*

3.12. Self-Report Psychometric Measures

<u>Rating of Perceived Exertion (RPE)</u>

Rating of perceived exertion (RPE) scale was used to measure how hard, heavy, and strenuous the participant rated the cycling task at a given time. The category-ratio scale consists of a fifteen point vertical list of numbers which are supplemented by a descriptor every other point, from '6- No Exertion' to '20 – Maximal Exertion' (Borg, 1982) *(Appendix E)*. Participants were provided with written instructions and verbally reinforced before each experimental session.

<u>Session RPE (sRPE)</u>

Session RPE (sRPE) measurements were recorded using a category-ratio scale of 0-10 as proposed by Foster *et al.*, (2001). sRPE measurements were asked 30 minutes after the conclusion of the final time-trial *(Appendix E)*.

<u>Muscle Pain (MP)</u>

Perception of muscle pain during exercise was investigated using a 10-point category-ratio scale for assessing pain (Cook *et al.*, 1997). The ordinal category-ratio scale ranges from 0 (No pain at all) to 10 (Extremely intense pain). There is also an additional item [*] for 'Unbearable pain'. Participants were asked to rate the sensation of pain localised to the working muscles at that time during the task (namely cycling exercise) *(Appendix E)*.

<u>Short Recovery & Stress Scale (SRSS)</u>

Participant moment perception of recovery and stress was assessed using the psychometric instrument 'Short Recovery and Stress Scale' (SRSS) (Kölling *et al.*, 2020). The two dimensions of 'Stress' and 'Recovery' are divided into eight sub-scales. These sub-scales represent Recovery in terms of Physical Performance Capability, Mental Performance Capability, Emotional Balance, and Overall Recovery. Stress is represented by Muscular Stress, Lack of Activation, Negative Emotional State, and Overall Stress. Participants rate these eight scales as single items on a 7-point Likert scale, ranging from 0 (does not apply at all) to 6 (fully applies). This rating reflects how the participant is currently feeling in comparison to their highest level of recovery or stress. (*Appendix E)*. The SRSS is a time economical version of the originally validated long-form Acute Recovery and Stress Scale (ARSS) by the same authors. The SRSS was preferred to the ARSS due to the short time of completion (~40 seconds) as to reduce questionnaire burden on the participant due to collection at multiple time-points on an experimental visit, however doing so can lose some detailed information relating to each descriptor (Kellmann and Kölling, 2019). High correlation is evident between ARSS and SRSS in both overall recovery (r_s 0.71) and overall stress (r_s 0.73) respectively. The SRSS has previously been used to assess perceived recovery between bouts of exercise after recovery interventions (Pelka *et al.*, 2017). The SRSS is more economical and previous observation shave shown corelation between other instruments such as REST-Q-Sport and Profile of Mood States (POMS) (Kellmann and Kölling, 2019).

Drug Effect Questionnaire (DEQ-5) and Reporting Side Effects

The Drug Effects Questionnaire (DEQ-5) is a validated measure, with each item reflective of pharmacologically-induced effects (Morean *et al.*, 2013). Participants are required to indicate on a visual analog scale (0 – 100 mm) the extent they are experiencing an adjective or description. Distance from 0 was measured in millimetres (mm) using a ruler. An additional open-ended question was included to allow the participant to report on any adverse events experienced following condition administration. Instructional set, item wording and response anchors are presented in *Appendix E.*

3.13. Blood sampling

All blood sampling was conducted and disposed of in accordance with the Human Tissue Act (2004).

Capillary (Sampling and Processing)

Blood sampling by capillary action was used for the measurement of metabolic function, namely for glucose (B[Glu]) and lactate (B[La]) concentrations. After wiping the index finger with an alcohol swab, a spring-loaded lancet was used to puncture the skin, and the first bleed wiped away with a tissue. Next, blood was drawn into a 20 μL (0.02 mL) capillary tube and placed into a pre-filled eppendorf containing 0.5 mL of haemolysing solution. Each specimen was analysed using an automated laboratory analyser (Biosen C-Line, EKF diagnostic, Madgeburg, Germany). The device was calibrated before each session using the manufacturer's recommended 12 mmol.L^{-1} standard (EKF diagnostic, Madgeburg, Germany), and this calibration process was then repeated automatically every 60-min. This device has whole-blood reproducibility CV of <1.5% for B[La] (Davison *et al.*, 2000) and ~1.27% for B[Glu] (Nowotny *et al.*, 2012). Values are reported values as millimoles per litre (mmol/L^{-1}).

Venous (Sampling and Processing)

A venous blood sample was collected from an anti-cubital vein using venepuncture method. 6 mL of whole blood was collected into a heparin coated anti-coagulant vacutainer (BD Vacutainer™ Plasma Tubes 367885, BD Diagnostics, Plymouth, UK) using a one inch, 21-gauge needle (PrecisionGlide™, BD Diagnostics, Plymouth, UK). As soon as feasible, the vacutainer was then centrifuged at 1500 x relative centrifugal force (*RFG*), 4°C for 10 minutes using a large benchtop centrifuge (Heraeus Megafuge 8R, Thermo Scientific™, Waltham, MA, USA). The resultant acellular supernatant was

transferred into separate 1.5 mL aliquots, and immediately stored at −80 °C for later analysis.

3.14. Enzyme-Linked Immunosorbent Assay (ELISA)

Plasma Interleukin-6 (IL-6) was analysed using a commercially available sandwich enzyme-linked-immunoassay (ELISA) (*Human Uteroglobin Immunoassay, Quantikine® ELISA; R&D Systems, kit HS600C)*. This high sensitivity assay has a range of 0.156 – 10 pg/mL and sensitivity for IL-6 of 0.09 pg/mL. Each 96-well monoclonal antibody coated plate was prepared according to the method outlined by the manufacturer (Available at: https://resources.rndsystems.com/pdfs/datasheets/hs600c.pdf?v=20210322). Below states additional technical information and equipment used in the procedure.

Previously stored plasma samples were thawed to room temperature prior to analysis. As recommended by assay manufacturer to improve precision, thawed samples were placed into a small benchtop centrifuge (accuSpin Micro 17R, Fischer Scientific, Waltham, MA, USA) at 13,000 rpm for 2 minutes prior to dilution. A 2-fold dilution (with supplied calibrator diluent) was used to ensure detected optical absorbance fell within the linear range of the assay. All samples were thoroughly mixed at 20 Hz using a vortex (TopMix FB15024, Fisher Scientific, UK) before plate loading. During incubation steps (and only if indicated to do so), the plate was gently shaken at 500 rpm using a horizontal orbital microplate shaker (Grant-Bio PMS-1000, Cambridge, UK). Wash steps were conducted using an automated plate washer (Autura 1000, Mikura Ltd., UK).

The absorbance of each well was read at 450 nm using a microplate reader (Fluostar Optima, BMG Labtech, Offenburg, Germany). Wavelength corrections were set at 530 nm and 610 nm (with highest selected to subtract from 450 nm absorbance). In addition, the average of the blank wells was subtracted to adjust for any background optical imperfections in the plate. The standard curve for each plate was generated using a four-parameter logistic curve, and final concentrations, including adjustment for dilution factor were calculated using an online data analysis tool (http://www.MyAssays.com). Values are reported in pg/mL (picograms per millilitre).

Typical IL-6 plasma concentrations are expected to range from approximately 1-5 pg/mL at healthy resting levels. Post-exercise levels can vary between 5-100 pg/mL, depending on factors such as exercise modality, intensity, and training status (Nash *et al.*, 2023).

CHAPTER 4: THE IMPACT OF LONG-TERM ASTHMA THERAPY ON HYPERPNOEA-INDUCED BRONCHOCONSTRICTION AND REAL-WORLD MAJOR COMPETITION PERFORMANCE IN ELITE SWIMMERS

BACKGROUND: Exercise-induced bronchoconstriction (EIB) is highly prevalent in aquatic sports, but there is currently limited evidence on the impact of diagnosing, initiating, and maintaining use of pharmacological asthma treatment on respiratory function and performance outcomes in elite athletes with EIB. **OBJECTIVES:** Investigate the effectiveness of long-term asthma therapy in elite swimmers with EIB, and the impact treatment initiation has on real-world major competition performance. **METHODS:** Twenty-seven elite-international swimmers were included in this retrospective analysis of comprehensive respiratory assessments and major-competition performance data. Following an initial 'withheld-therapy' assessment, athletes with EIB had been prescribed appropriate pharmacological therapy, and returned twelve-months later for a follow-up assessment to monitor EIB protection afforded by treatment (ΔFEV_1max). Athletes were retrospectively grouped into either 'Therapy Adherent Group' (n=12) or 'Repeated Test Group' (discontinued therapy at follow-up or EIB negative, n=15). Then, using each swimmers highest international point-scoring event at major competitions, log-transformed performance time were used to calculate change in performance following treatment initiation. Smallest worthwhile change in performance was estimated as 0.6% (0.5 × published between-competition progression and variability of 1.2% in elite swimming) and interpreted using p-value derived magnitude-based decisions (MBD) and minimum effects testing (MET). **RESULTS**: EIB was significantly attenuated following long-term asthma therapy use (pre ΔFEV_1max = -24.0 ± 11.3%; post = -11.8 ± 3.8%; $p<0.01$). Resting FEV_1 was significantly increased following treatment (+240 ± 356 mL; $p=0.04$). Effect of treatment on major-competition performance was estimated to be 'Very Unlikely Beneficial' (mean change ± 90% confidence limits: -0.25 ± 0.55%; pMET=0.86; MDB 13.6% beneficial, 85.5% trivial, 0.9% harmful). **CONCLUSION:** Appropriate use of pharmacological asthma therapy in elite swimmers with EIB improves resting FEV_1 and attenuated EIB severity. However, initiation of treatment did not lead to a meaningful improvement in major competition performance above the expected progression and variability between competitions.

4.1. Introduction

The basis of pharmacological treatment allowed by WADA through permitted routes, threshold substances or TUE process is to merely restore normal physiological function for an athlete with an acute or chronic medical disorder ensuring them no disadvantage compared to their healthy counterparts (WADA, 2022a). Although EIB is highly prevalent in athletic populations, and maintenance asthma inhaler devices [LABA and ICS] are widely used, there is sparse evidence on the effectiveness of diagnosing, then initiating therapeutic levels of treatment on the attenuation of EIB in elite cohorts, and the subsequent monitoring on a long-term basis. Jackson *et al.,* (2018) investigated this in elite football players, demonstrating that after nine-weeks of treatment there was a clinically meaningful attenuation of EIB induced by hyperpnoea and reduction in FeNO. Given there is a higher prevalence of EIB in endurance aquatic sports compared to other Olympic sports (Levai *et al.*, 2016), understanding the impact of long-term use of LABA and ICS treatment on respiratory outcomes is vital in this population.

In *Chapter 2*, the *'doctrine of double effect'* was presented suggesting that an action with a positive intent (such as therapeutic treatment) might lead to both positive (therapeutic effect) and negative outcomes (like performance enhancement or side effects harm) (Pike, 2018). The balance between managing therapeutic need, whilst minimising the potential for ergogenic effect is a challenge for WADA. Particularly as mistrust towards asthmatic athletes has been reported (Overbye and Wagner, 2013).

Laboratory studies have extensively investigated the effect of inhaled β2-agonists therapy (Pluim *et al.*, 2011; Riiser *et al.*, 2020, 2021), and to a lesser extent ICS therapy (Kuipers *et al.*, 2008; Hostrup *et al.*, 2017) on exercise performance in non-asthmatic sub-elite cohorts. Although considered superior in the hierarchy of research evidence, the results from such tightly controlled laboratory-based studies may not necessarily reflect the high variability evident in elite-level sport (Chung *et al.*, 2012).

However, there is currently only limited investigations on the impact of initiating and maintaining use of pharmacological asthma treatment on performance outcomes in elite athletes with EIB (Brukner *et al.*, 2007; Price *et al.*, 2014; Spiteri *et al.*, 2014; Jackson *et al.*, 2018). A pertinent limitation these studies are the utilisation of less ecologically valid laboratory-based performance outcomes, such as changes in maximum oxygen uptake, or field-based sport-specific fitness drills. Thus, any inference of change in performance from asthma treatment should also be investigated within the 'noise' of an applied

competition setting (Chung *et al.*, 2012). Investigating this in the context of EIB therapy will contribute to a previous call for further research on the impact of treatment in a *"real-life"* treatment setting *[i.e., in Olympic squad members]* (Price and Hull, 2014).

Therefore, the aims of this experimental chapter were two-fold. (1) In a cohort of elite swimmers newly diagnosed with EIB, demonstrate the impact of initiating long-term [12-month] individualised pharmacological asthma therapy on resting lung function, EIB severity [assessed via voluntary hyperpnoea], and airway inflammation [FeNO]. (2) Retrospectively assess the effect that initiating and maintaining use of therapy has on real-world major competition performance.

4.2. Methods

Study Overview

This study involved retrospective analysis of data collected from a subsection of the Great British Swimming Team at annual medical assessments between 2016-2019. Sixty-three swimmers were assessed during this period. However, twenty-seven athletes had multiple assessments during this time due to initiating pharmacological intervention or having persistent respiratory symptoms.

In brief, athletes who presented in this study initially underwent a comprehensive respiratory assessment*,* then twelve-months later returned for a follow-up assessment to monitor EIB protection afforded by therapy, or to confirm a negative test. Then, major competition performance times were extracted from publicly available resources and used to calculate change in performance pre to post treatment *(Figure 4.1)*. The study was approved by the University of Kent School of Sport and Exercise Sciences Research Ethics Committee (Prop 86_2018_19). All participants provided written informed consent to anonymised data analysis.
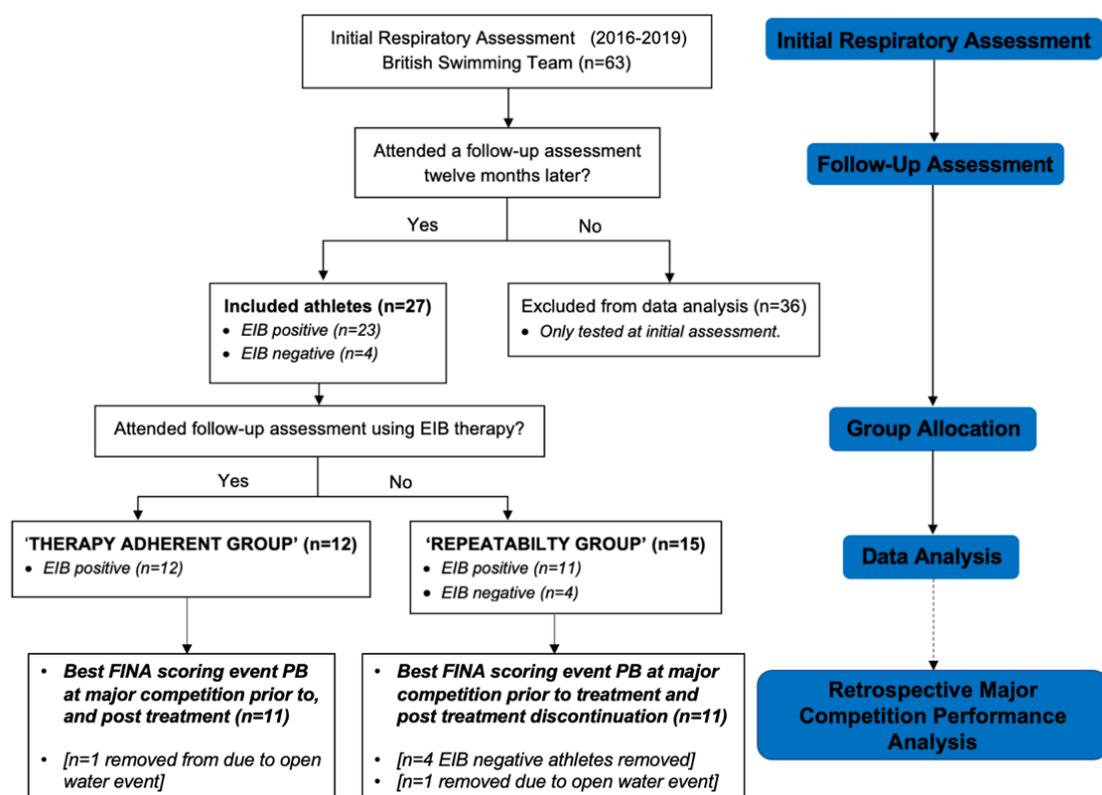
Figure 4.1. *Schematic of retrospective data analysis. Abbreviations:* ***EIB****, exercise-induced bronchoconstriction;* ***FINA****, Fédération Internationale de Natation.*

Table 4.1. *Participant Characteristics of Therapy Adherent Group, Repeated Test Group, and Combined Cohort.*

	Therapy Adherent Group (n=12)	Repeated Test Group (n=15)	Overall Cohort (n=27)
Sex (Male, Female)	M=7, F=5	M=7, F=8	M=14, F=13
Age (yrs.)	21 ± 3	20 ± 3	20 ± 2
Height (cm)	179.7 ± 7.4	180.1 ± 7.0	179.6 ± 7.1
Body Mass (kg)	72.4 ± 9.2	70.7 ± 8.4	71.3 ± 8.7
Swimming Training History (yrs.)	11 ± 3	11 ± 3	11 ± 3
Weekly Pool Training Volume (hrs)	24 ± 5	23 ± 3	23 ± 4
FINA Points Score	898.2 ± 49.8	901.0 ± 40.4	899.5 ± 43.9

Data presented as mean ± standard deviation. Abbreviations; ***FINA****, Fédération Internationale de Natation.*

Initial Respiratory Assessment

Participants attended the initial assessment to objectively assess whether they had EIB. All participants were asked to withhold use of any previously prescribed EIB medication and avoid caffeine and exercise ≥ 4 h before assessments in accordance with EVH guidelines (Anderson and Kippelen, 2013).

Upon arrival, participants completed a general health screening questionnaire *(Appendix C)*, and supplementary respiratory specific questionnaire to determine if they experienced coughing, chest tightness, dyspnoea or excess mucus during or after training or competition, and, if exposure to cold air, dry air, high pollen levels, high pollution, altitude or any other environmental conditions exacerbated these symptoms (Dickinson, McConnell and Whyte, 2011) *(Appendix F)*.

Following this, eosinophilic airway inflammation was assessed via fraction of exhaled nitric oxide (FeNO) in accordance with methods described in *Chapter 3.3*, then, resting maximal flow-volume manoeuvres were performed in triplicate using the method described in *Chapter 3.4*.

Participants then completed an EVH challenge: inhaling medical-grade dry-air at a target ventilation rate of 85% predicted maximum voluntary ventilation (MVV) (30 x baseline forced expiratory volume in 1 s (FEV_1) for six minutes. The gas was composed of 21% O_2, 5% CO_2 and 74% N to prevent syncope. Expired air passed through a dry-gas meter (Harvard Apparatus, Kent, UK), as such, minute ventilation ($\dot{V}_E$) and the achieved percentage of MVV (%MVV) could be calculated. Maximal flow volume manoeuvres were then completed in duplicate at 3, 5, 7, 10 and 15 minutes following the EVH.

An EVH challenge result was deemed positive (EIB positive) if an athlete displayed a fall in FEV_1 of $\geq 10\%$ from baseline at two consecutive time-points (Anderson, Argyros, *et al.*, 2001), with the maximum change defined as $\Delta FEV_1 max$. To reverse bronchoconstriction, EIB positive athletes inhaled between 200 ug - 400 ug salbutamol depending on EIB severity, and maximal flow volume manoeuvres were assessed 10-minutes post-inhalation *(Figure 4.2)*.
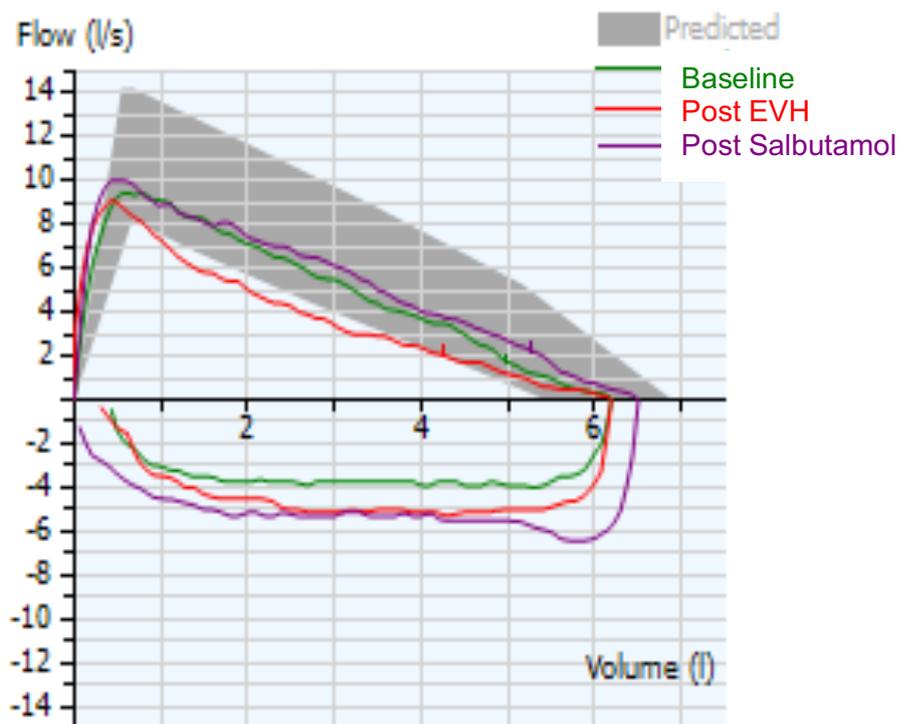
Figure 4.2. *Representative EIB positive flow-volume manoeuvre performed pre and post eucapnic voluntary hyperpnoea (EVH), and post inhalation of salbutamol. Inspiratory flow has been included as data collection for this study was conducted before the COVID-19 pandemic.*

EIB Therapy

EIB positive swimmers were prescribed EIB therapy within WADA regulations by the team physician, in a stepwise approach in accordance with EIB severity (ΔFEV_1max). Mild ($\geq 10\%$ but $<25\%$), moderate ($\geq 25\%$ but $<50\%$), severe ($\geq 50\%$) (Anderson and Kippelen, 2013; Parsons *et al.*, 2013, WADA 2021). Swimmers with mild EIB were prescribed daily inhaled corticosteroid (ICS), in addition to an inhaled short-acting $\beta 2$-agonists (SABA) as required. Those with moderate EIB were prescribed a combination inhaler containing ICS and a long-acting $\beta 2$-agonist (LABA), with SABA as needed. Finally, if appropriate, swimmers with severe EIB were given an additional daily leukotriene receptor antagonist. EIB negative swimmers were not prescribed any EIB medication.

Follow-up Assessment

Twelve months after the initial visit, athletes returned for a follow-up and completed the same respiratory assessments. Athletes diagnosed EIB positive at the initial assessment were asked to continue using therapy as prescribed to evaluate attenuation of EIB provided by pharmacological treatment. Complete protection against EIB was defined as $<10\%$ ΔFEV_1max at the follow-up assessment, or clinical attenuation if ΔFEV_1max

reduced by 50% compared to the initial test (Weiler *et al.*, 2016). Minimally important reduction in airway inflammation was defined as ≥20% reduction in FeNO (if >50 ppb), or a 10-ppb reduction (if <50 ppb) (Dweik *et al.*, 2011).

EIB negative athletes were required to adhere to the same criteria as at the initial test. EIB negative athletes were retested on the follow-up assessment as they were still reporting persistent respiratory symptoms or had a previous differential diagnosis of EIB.

Data Analysis

Respiratory Assessments

Athletes were retrospectively grouped according to whether they had arrived at the follow-up assessment using prescribed EIB therapy or not, as evaluated by a pre-assessment medical questionnaire. The groups were defined as; those who had arrived using EIB therapy (Therapy Adherent Group) and those who had discontinued therapy or were EIB negative (Repeated Test Group) *(Figure 4.1)*.

Meaningful Change in Real-world Competition Performance

To investigate the impact of treatment maintenance and discontinuation on real-world major-competition performance, all EIB swimmers from the 'Therapy Adherent' and 'Repeated Test' groups were included in a further analysis [*open water swimmers were removed due to additional event variability (Baldassarre et al., 2017)*].

All analyses were established from publicly available resources. In a first step, each swimmer's best discipline was identified by their highest '*Fédération Internationale de Natation*' (FINA) point-scoring event, considering only individual and long-course events as of September 2020. The FINA point score (ranging from 300–1,100) reflects each swim performance relative to the world record for that event at a defined annual cut-off date, with higher scores indicating performances closer to the world record (FINA, 2020).

In a second step, major competition performance data was screened and extracted from 'www.swimrankings.net'. Before treatment, each swimmers' best finals time at a major competition was selected [from; Olympic Games, Commonwealth Games, World Championships, European Championships, British National Championship] (2016-2017). Then, a further competition time was selected post-treatment (2017-2019). Finally, data was log-transformed to account for differences in event length and sex.

Statistical Analysis

Data is presented as mean ± standard deviation (SD). For all statistical analysis, the significance level was set at $P \leq 0.05$ and performed using statistical package SPSS (SPSS v25, IBM, New York, USA) unless stated. Figures were produced using GraphPad Prism V9 (GraphPad Software, California USA). Initially all data was assessed for a normal distribution through Shapiro-Wilk test ($P \geq 0.05$).

Individual differences between the initial assessment and the follow-up assessment for respiratory outcome measures (resting spirometry, FeNO, $\Delta FEV_1 max$) were analysed using paired-samples t-test.

To investigate the practical significance and likelihood that the true value of performance change effect was greater than the smallest worthwhile change, this study employed a progressive analytical approach involving Minimum Effects Testing (MET), and the more established Magnitude-Based Decisions (MBD) (Murphy and Myors, 1999; Hopkins *et al.*, 2009). Estimation of the smallest worthwhile change in swimming performance was based on 0.5 times the previously published between-competition variation for international swimming performance estimated to be ~0.8%, with an additional ~0.4% between-competition progression estimated to substantially increase a swimmer's chances of a medal (Hopkins, Hawley and Burke, 1999; Stewart and Hopkins, 2000; Pyne, Trewin and Hopkins, 2004; Trewin, Hopkins and Pyne, 2004; Fulton *et al.*, 2009). Therefore, a difference in performance equating to 0.6% (that is, 0.5 x 1.2% between-competition variation (0.8%) + progression (0.4%)) was assigned as the smallest worthwhile change in performance accounting for between competition variation and progression.

The probability that a meaningfully positive change occurred (pMET) was calculated (pMET <0.05 = significant meaningful change). In addition, the more-established MBD on effect and confidence intervals (90%) was interpreted from p-value derived calculations using excel spreadsheet by Hopkins (2007) downloaded from (http://www.sportsci.org/2007/wghinf.htm). The latter approach has been used previously to investigate effect of training interventions, illness and injury in elite swimming (Pyne *et al.*, 2005; Robertson *et al.*, 2010; Chung *et al.*, 2012). Thresholds for assigning qualitative terms for the chance of substantial effects were: <1%, almost certainly not; <5%, very unlikely; <25%, unlikely; <50%, possibly not; >50%, possibly; >75%, likely; >95%, very likely; >99%, almost certain (Hopkins, 2002).

4.3. Results

Participant Characteristics

Twenty-seven elite swimmers, competing regularly at international level were included in this retrospective data analysis of comprehensive respiratory assessments and major-competition performance, with participant characteristics presented in *Table 4.1*. Prior to the initial assessment, twelve athletes (44%) self-reported a history of asthma or EIB, and thirteen (48%) reported allergenic environments worsened their respiratory symptoms. No athlete had evidence of significant airflow obstruction at rest (i.e., FEV_1 >80% predicted & FEV_1/FVC >70%; *Table 4.1*).

From the initial assessment, twenty-three athletes (85%) were diagnosed as EIB positive. At the time of the follow-up assessment, all EIB positive athletes were prescribed SABA therapy for emergency use. All EIB positive athletes were also prescribed a form of maintenance therapy, with eleven (48%) using daily ICS monotherapy, and twelve (52%) requiring a combination of ICS and LABA therapy. In addition, two (9%) were prescribed add-on leukotriene receptor antagonist therapy. The remaining four EIB negative athletes were not prescribed any treatment for EIB.

Therapy Adherent Group

Twelve EIB positive athletes returned to the follow-up assessment having used prescribed therapy as instructed (Therapy Adherent Group). No athlete reported acute use of SABA therapy on the day of the follow-up assessment. Resting FEV_1 was significantly higher at the follow-up assessment compared to initial assessment (*P*=0.04; *Table 4.2*). The group magnitude of change in resting FEV_1 was 240 mL (± 356 mL), with individual responses presented in *Figure 4.4*. On a group level, FeNO was not significantly different between assessments (*P*=0.07; *Table 4.2)*. However, five athletes (42%) demonstrated a minimally important reduction in FeNO following use of therapy. Individual FeNO responses are presented in *Figure 4.5*. Minute ventilation (V_E) during the EVH was not significantly different between assessments (*P*=0.40)*, however, the %MVV achieved was significantly different *(P*=0.04; *Table 4.2)*. ΔFEV_1max was significantly lower at the follow-up assessment (-11.8 ± 3.8%) compared to the initial assessment (-24.0 ± 11.3%) (*P*<0.01; *Table 4.2; Figure 4.3a)*. Adherence to maintenance therapy provided complete EIB attenuation to four athletes (33%) but provided clinical attenuation to a further four athletes (33%). Thus, eight (66%) of the adherent cohort demonstrated substantial

reduction in EIB severity following long-term treatment. Athletes using ICS/LABA combined therapy demonstrated the greatest reduction in EIB severity *(Figure 4.3a)*. However, one athlete using ICS monotherapy showed a substantial reduction in EIB severity (-25% to -10% ΔFEV_1max).

Repeated Test Group

Eleven EIB positive athletes arrived at the follow-up assessment having discontinued EIB therapy (Repeated Test Group). This group also included four EIB negative athletes (total n=15). Resting pulmonary function, FeNO, $\dot{V}_E$, and %MVV achieved did not differ significantly between assessments (*P*>0.05; *Table 4.2).* There was no significant difference in ΔFEV_1max between initial assessment (-13.1 ± 4.5%) and follow-up assessment (-12.3 ± 5.6%; *P*=0.32). Individual ΔFEV_1max responses are shown in *Figure 4.3b.*

As presented in *Appendix G*, EVH has good test-retest repeatability over a 12-month period.

Table 4.2. Resting pulmonary function and EVH outcomes for 'Therapy Adherent' and 'Repeated Test' group.

Measure	Therapy Adherent Group (n=12)			Repeated Test Group (n=15)		
	Initial Assessment	Follow-up Assessment	*P*-value	Initial Assessment	Follow-up Assessment	*P-value*
FEV$_1$ (L)	4.60 ± 0.68	4.84 ± 0.77	0.04*	4.59 ± 0.60	4.57 ± 0.64	0.81
FEV$_1$ (% of predicted)	110.2 ± 12.8	115.6 ± 15.3	0.03*	111.1 ± 16.4	112.1 ± 18.4	0.57
FVC (L)	5.98 ± 1.10	6.19 ± 1.10	0.07	5.74 ± 0.86	5.85 ± 0.90	0.15
FVC (% of predicted)	121.3 ± 14.0	124.3 ± 12.8	0.17	118.0 ± 13.3	122.3 ± 15.8	0.01*
FEV$_1$/FVC (%)	77.6 ± 6.5	79.08 ± 8.3	0.17	80.0 ± 7.7	78.27 ± 8.0	0.06
Baseline FeNO	33.7 ± 23.2	22.2 ± 17.2	0.07	21.6 ± 13.7	24.5 ± 11.4	0.98
ΔFEV$_1$max (%)	-24.0 ± 11.3	-11.8 ± 3.8	<0.01*	-13.1 ± 4.5	-12.3 ± 5.6	0.32
Achieved $\dot{V}_E$ (L/min^{-1})	116.3 ± 20.6	112.9 ± 17.8	0.40	105.23 ± 29.49	108.82 ± 28.74	0.31
Achieved Ventilation (%MVV)	72.4 ± 8.1	66.7 ± 8.15	0.04*	65.26 ± 15.79	67.71 ± 13.73	0.31

*Note: Data presented as mean ± standard deviation. * Within-group statistically significant difference (P ≤0.05). Abbreviations: **FEV$_1$**, Forced expiratory volume in 1 second; **FVC**, Forced vital capacity; **FeNO**, Fraction of exhaled nitric oxide; **ΔFEV$_1$max**, Maximum fall in FEV$_1$ from baseline following eucapnic voluntary hyperpnoea **(EVH)** challenge; **V_E**, Minute ventilation; **%MVV**, Percentage of maximum voluntary ventilation.*
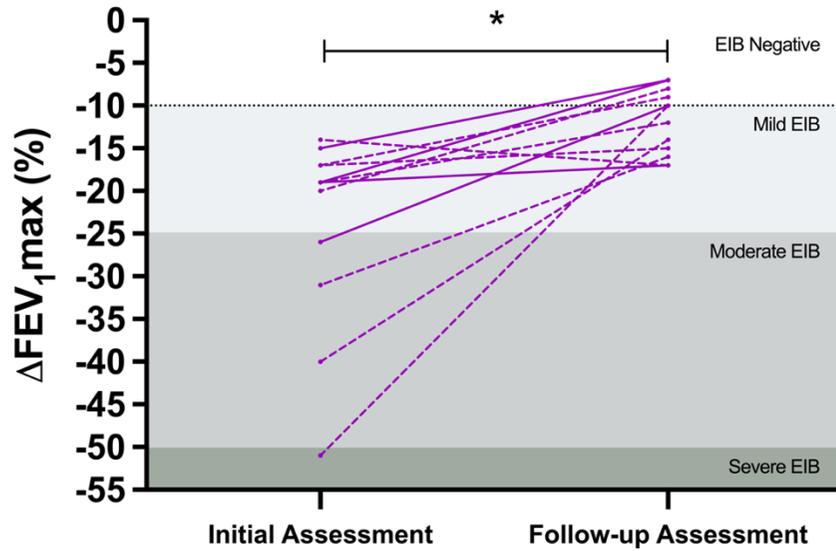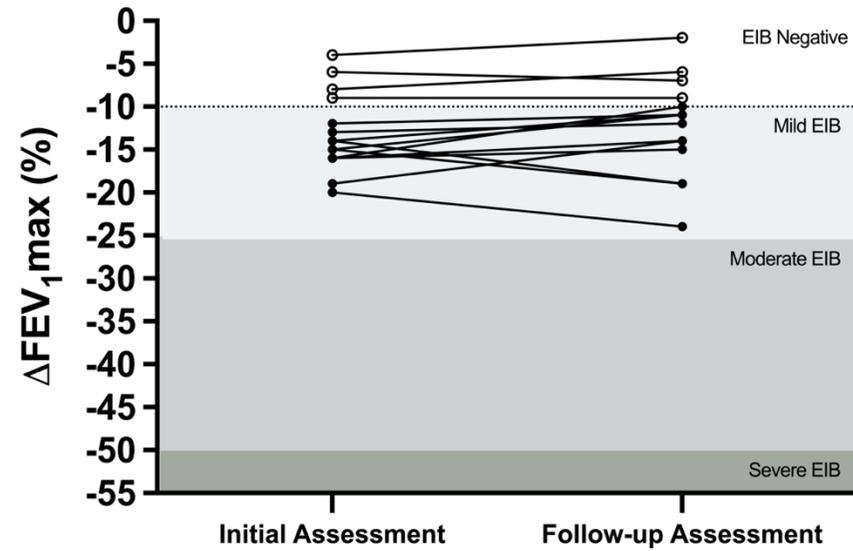
Figure 4.3. Individual $\Delta FEV_1 max$ response in 'Therapy Adherent Group' (a) and 'Repeated Test Group' (b).

*(a) Solid line denotes inhaled corticosteroid (ICS) treatment only, broken line denotes ICS combined with long-acting β2-agonist (LABA). Dotted horizontal line denotes 10% fall in FEV_1 diagnostic threshold. * Statistically significant difference between time-points P ≤ 0.05. Abbreviations: **FEV_1**, Forced expiratory volume in 1 s; **EIB**, exercise-induced bronchoconstriction.*

*(b) [○ = EIB negative ● = EIB positive]. Broken horizontal line denotes 10% fall in FEV_1 diagnostic threshold. Abbreviations: **FEV_1**, forced expiratory volume in 1 s; **EIB**, exercise-induced bronchoconstriction.*
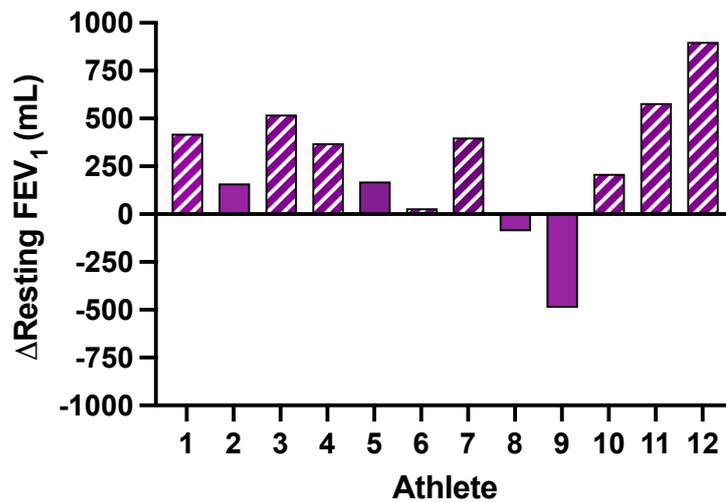
Figure 4.4. *Individual change in resting forced expiratory volume in one-second (ΔResting FEV₁)* *between initial and follow-up respiratory assessments in 'Therapy Adherent' group. Solid fill bar denotes use of inhaled corticosteroid (ICS) treatment alone, dual-colour denotes ICS combined with long-acting β2-agonist (LABA).*
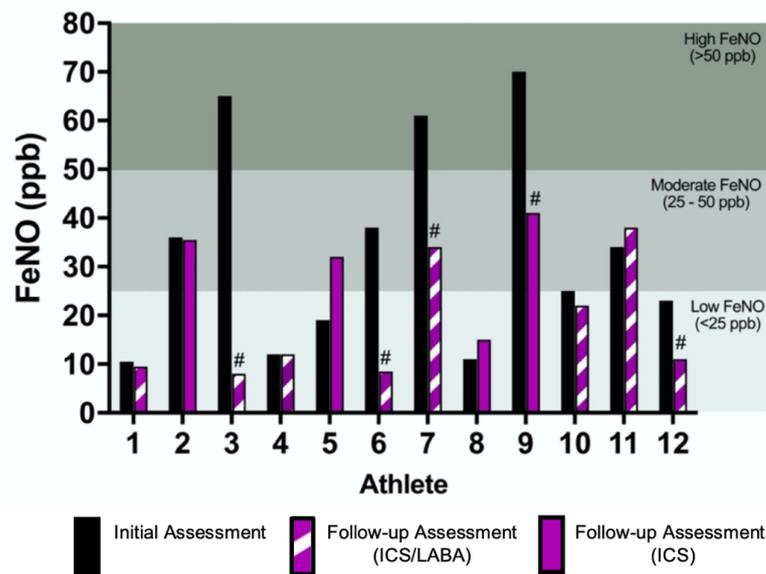
Figure 4.5. *Individual change in Fraction of Exhaled Nitric Oxide (FeNO) between initial and follow-up respiratory assessments in 'Adherent' group. Shaded areas and horizontal lines denote low, moderate, and high levels of FeNO. Solid fill bars denote inhaled corticosteroid (ICS) treatment only, dual-colour denotes ICS combined with long-acting β2-agonist (LABA). # Minimally important reduction in FeNO.*

Meaningful Change in Real-world Competition Performance.

Log-transformed competition performance times for 'Therapy Adherent Group' athletes (n=11) pre-treatment and post-treatment were 4.800 ± 0.61 and 4.798 ± 0.61 respectively. There was a mean improvement in performance of -0.25% (90% CI; -0.80 – 0.30%) pre to post treatment. However, this was shown to be *"Very Unlikely Beneficial – Likely Trivial"* to performance (*pMET* = 0.86; MDB 0.9% harmful; 85.5% trivial; 13.6% beneficial) *(Table 4.3; Figure 4.6).*

'Repeated Test Group' athletes (n=10) log-transformed performance times were 4.927 ± 0.61 and 4.931 ± 0.61 respectively. The mean effect on performance was +0.41% (90% CI; -0.04 – 0.78%) This was shown to be *"Very Unlikely Harmful – Likely Trivial"* (*pMET* = 0.81; MDB 18.6% harmful; 81.4% trivial; 0.0% beneficial) *(Table 4.3; Figure 4.6).*

Table 4.3. Group Mean (90% CI) change in log-transformed major competition performance time following adherence or discontinuation of asthma therapy.

Therapy Adherent Group (n=11)	
Δ Log Performance Time (%)	-0.25 (-0.80 – 0.30)
p_{MET}	0.86
MBD Descriptors	13.6% Beneficial, 85.5% Trivial, 0.9% Harmful
MBD Interpretation	Very Unlikely Beneficial / Likely Trivial
Repeated Test Group (n=10)	
Δ Log Performance Time (%)	0.41 (-0.04 – 0.80)
p_{MET}	0.81
MBD Descriptors	0.0% Beneficial, 81.4% Trivial, 18.6% Harmful
MBD Interpretation	Very Unlikely Harmful / Likely Trivial

*Data presented as mean ± 90% confidence intervals (CI). Abbreviations: **pMET** (probably that a meaningfully positive change occurred); **MBD**, Magnitude Based Decisions *(meaningfully positive change from baseline $p_{MET} \leq 0.05$).*
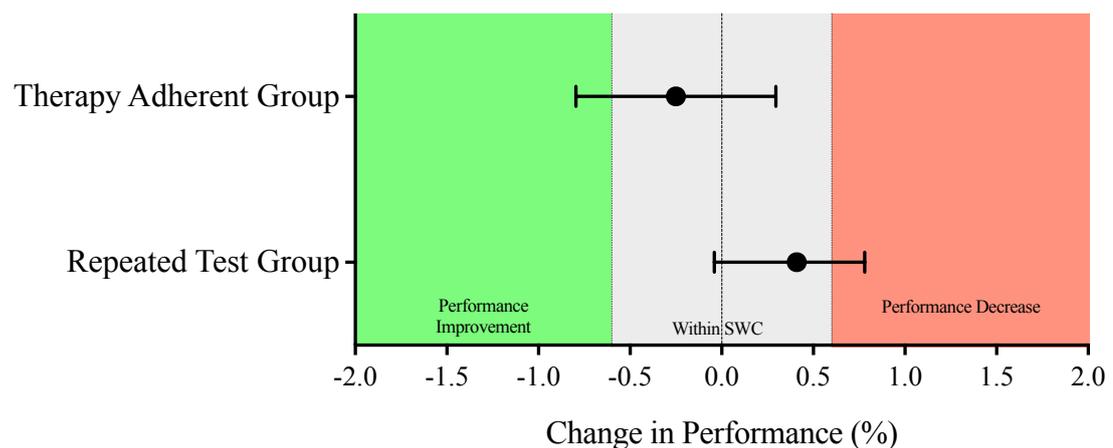
***Figure 4.6.** Group mean change in performance for 'Therapy Adherent Group' and 'Repeated Test Group'. Markers are mean change, with 90% confidence intervals shown by bars either side. Open circles represent a meaningfully different change (PMET <0.05). The grey zone represents the threshold for smallest meaningful change in competition performance time (0.5 x 1.2% between competition variation/progression in elite swimming).*

4.4. Discussion

This chapter has demonstrated that in a cohort of elite swimming athletes, diagnosis, and then subsequent long-term use of pharmacological inhaler therapy to manage EIB improved resting FEV_1, and a provided clinical attenuation to bronchoconstriction induced by hyperpnoea. Moreover, using MBD, effective treatment of EIB was shown to be *"very unlikely beneficial – likely trivial"* to major competition performance when accounting for the expected between-competition variation and progression of an elite swimming athlete.

Impact of Treatment on Respiratory Outcomes

The use of long-term individualised asthma therapy afforded complete protection against EIB to four athletes (33%) and provided clinical attenuation to a further four athletes (33%). These findings concur with the previous study by Jackson *et al.*, (2018), who demonstrated reduced EIB severity [in all but one elite footballer] following nine-weeks use of individualised pharmacological therapy.

Results of the present study demonstrated a mean increase of 240 mL (↑ of 5%) in resting FEV_1 at the follow-up respiratory assessment. A change in FEV_1 of > 200 mL would be considered minimal clinically important (Bonini *et al.*, 2020). This relative magnitude of change in baseline FEV_1 was similarly presented by Simpson *et al.*, (2013), who in a cohort of recreationally active athletes showed a smaller (but statistically significant) bronchodilator effect following acute 0.5 mg terbutaline (mean increase of 170 mL (↑ of 5%)). This finding was further enhanced to 194 mL when a larger cohort was later included in their analysis (Simpson, Romer and Kippelen, 2015). Even though athletes in the present study would have been prescribed a SABA as needed, no athlete reported acute use of this prior to the follow-up assessment, suggesting the increase in resting FEV_1 came from a form of maintenance therapy (i.e., ICS or ICS/LABA combined therapy). Eight of the adherent group were prescribed a LABA in combination with ICS. The inclusion of LABA therapy may have contributed to an increase in baseline FEV_1, as the largest magnitude of change came from those using concurrent therapy, rather than ICS alone *(Figure 4.4).* Importantly, individuals with mild EIB treated solely with ICS, may exhibit minimal or no signs of airway obstruction at rest. Conversely, those classified as having moderate or severe EIB may present evidence of obstruction at rest, thereby greater scope for reversible FEV_1 from the combined treatment incorporating LABA bronchodilator prescribed based on the severity of the disease.

Airway inflammation related to clinically recognised asthma (with allergic features) has been shown to reduce following regular use of ICS in the general population (Boushey *et al.*, 2005). One would therefore expect this to occur in athletes with EIB actively using ICS, as has previously been reported in a cohort of elite footballers (Jackson *et al.*, 2018). In the present study, despite no significant group effect in the cohort, five athletes (42%) did display a minimally important reduction in airway inflammation following adherent use of maintenance therapy. However, due to the fact that no formal diagnostic allergy test was conducted, it can only be hypothesised that this is due to differing asthma phenotypes, as evident that six of the adherent athletes (50%) may fall into the category of 'EIB without allergic features', displaying 'low' levels of FeNO at the initial assessment *(Figure 4.5)* (Couto *et al.*, 2015). Use of personalised FeNO thresholds may have been advantageous (Torén *et al.*, 2017). With inherently low levels of FeNO, these athletes are likely to have affected the statistics on a group level towards the non-significant finding. Altogether, these results provide further evidence of an individualised inflammatory profile associated with EIB, as such, FeNO is useful in selected athletes to support the diagnosis of EIB (Dweik *et al.*, 2011; Dickinson, Gowers, *et al.*, 2023) and monitor efficacy of pharmacological therapy over time.

Impact of Treatment for EIB on Real-world Competition Performance

The aim of initiating asthma inhaler therapy is to manage the condition, enable the continuation of athletic career, and ensure that athletes with EIB have equal opportunities to compete alongside their healthy counterparts, without the concern of facing doping suspicions. The present study demonstrated that despite effective treatment of EIB on respiratory outcomes, this had an *"unlikely beneficial – likely trivial"* effect on swimming performance at major competition swimming when accounting for the expected between-competition variation and progression of an elite swimming athlete. These findings add to the paucity of studies that have investigated the effect of treated and untreated EIB on exercise performance (Price *et al.*, 2014).

These findings are supported by Brukner *et al.*, (2007), who demonstrated in newly diagnosed sub-elite Australian rules football players that although 6-weeks of treatment for EIB induced a significant increase in $\dot{V}O_2max$, there was no concurrent enhancement in field-based running performance. Spiteri *et al.*, (2014) agreed with this notion, in that using a cohort of EIB-positive professional rugby players, 12-weeks of ICS treatment [beclomethasone] did not enhance performance in a rugby specific fitness test above that seen in the placebo or control group. Jackson *et al.*, (2018) also provided evidence that

nine-weeks of treatment for EIB may be 'possibly beneficial' on maximum oxygen uptake in elite football players, albeit with a small sample size of three EIB positive athletes and no ecologically valid performance test completed. Regarding these previous observations on enhancement in $\dot{V}O_2$max following treatment, this could be attributed to improved alveolar ventilation and efficiency of alveolar-to-arterial blood O_2 exchange post-EIB treatment (Haverkamp *et al.*, 2007). Although $\dot{V}O_2$max sets the upper limit for oxygen uptake in endurance events, it is not a good predictor of final sporting performance so should be used cautiously to infer performance enhancement (Bassett and Howley, 2000). Notably, when laboratory-based performance outcomes have been utilised in previous studies [e.g., a 10-km cycling TT], although therapeutic-use of SABA asthma treatment was associated with increased resting spirometry values and oxygen uptake in athletes regardless of EIB status, these observations did not result in improvements in TT performance or key ventilatory parameters during exercise [such as minute ventilation, tidal volume and respiration rate] (Koch, Karacabeyli, *et al.*, 2015; Koch, Macinnis, *et al.*, 2015).

In the present study, all athletes were treated with therapeutic doses of inhaled asthma prophylaxis within the WADA code, and no use of oral corticosteroids were reported. This is reassuring to athletes with [and fellow competitors without] that therapeutic doses of inhaler therapy administered in a world-class did not enhance performance at major-competition, and follows the consensus seen in previous laboratory-based studies on therapeutic levels of inhaler therapy on performance outcomes (Kuipers *et al.*, 2008; Hostrup *et al.*, 2017; Riiser *et al.*, 2020). However, given ICS can be used with relative freedom within competition periods, future studies should investigate the impact that supratherapeutic doses of ICS has on performance *[outside of an elite setting to maintain sporting integrity],* particularly utilising ecologically valid performance outcomes such as TT performance. Additionally, there is still debate surrounding systemic routes of GC used to manage severe exacerbation of illness [i.e., oral administration] (Trinh, Chen and Diep, 2022).

Strengths and Limitations

The present study utilised a retrospective analysis of longitudinal data, rather than a prospective experimental design. More specifically, the treatment of athletes was not studied in a randomised blinded placebo-controlled manner, and the investigation into major-competition performance was observational in nature. For respiratory assessments, this approach does have valid strengths, including avoiding environmental seasonal

variation, a consistent stage of periodisation at each assessment, and making use of publicly available open access data. An elite performance environment is inherently time restricted due to large volume of training and competition. Therefore, it can be troublesome to obtain access to this population for initial consultation, but more so for a follow-up assessment to investigate the response to a treatment or intervention.

Within literature assessing the impact of a treatment or intervention, especially those controlled by WADA, multimethodology approaches should be welcomed, as there can be other evidence or arguments for a substance to be prohibited in sports, including observations from cross-sectional studies, case reports, pharmacovigilance, surveys, and qualitative data. This abductive reasoning approach has recently been used with β_2-agonist therapy (Breenfeldt Andersen *et al.*, 2021). The present study contributes to the novel concept of modelling competition performance data to investigate change in performance after a specific intervention, an approach previously used with beta-alanine supplementation (Chung *et al.*, 2012). Additionally, this approach has previously been piloted to investigate potential doping practices with hammer throw and discus athletes (Iljukov and Schumacher, 2017), shot put athletes, 100m sprinters, 800m middle-distance runners (Hopker *et al.*, 2020), and long-distance running (Iljukov, Bermon and Schumacher, 2018). It should also be noted that a substantial improvement in performance does not necessarily suggest doping practices, and could be from restructuring fundamental pillars of a performance high-programme, such-as, well-chosen eating practices, consistent training, absence of illness and injury, and improved recovery strategies (Chung *et al.*, 2012; Iljukov, Bermon and Schumacher, 2018). Yet the observations of accelerated progression may be worthwhile for targeted anti-doping tests on specific athletes (Iljukov, Bermon and Schumacher, 2018; Hopker *et al.*, 2020).

Another limitation of this methodological design is that only a single competition was assessed, and it can only be assumed that the swimmers in this study were highly motivated in each competition to gain national team selection at British Championships, or to achieve top finishes in European, World and Olympic Championships. However, as only one 'Olympic cycle' was observed, we do not know what competitions were prioritised for each athlete's specific periodisation. Thus, an athlete may not have been at the peak of form at the championships that was selected to compare to pre-treatment. Unfortunately, the COVID-19 pandemic and consequential postponement of the Tokyo 2020 Olympic Games prevented the analysis across two Olympic cycles from being

feasible. In addition, it was also not considered if the duration of event that the swimmer competes impacts on the magnitude of the effect, i.e., sprint vs endurance events.

Consequently, the absence of detectable effect also relates to the inherent variability in real-world swimming performance *[in both training and competition]*. Namely confounding factors such as prior training, diet, illness, residual fatigue, underlying soreness or injury, changes in stroke technique, and the level of motivation of the athlete (Troup, 1999; Chung *et al.*, 2012). These confounding variables are usually controlled to a greater degree in randomised-controlled trials, and as such, are usually considered superior in the hierarchy of research evidence (Howick, Glasziou and Aronson, 2009).

Treatment Adherence and Discontinuation

A pertinent observation of the present study is that cessation of asthma therapy was high within the cohort, with eleven (48% of EIB positive athletes) returning to the follow-up assessment having ceased treatment. This is higher than the previously self-reported discontinuation rate of therapy (24%) observed in a cohort of elite Swedish athletes (Stenfors, Irewall and Lindberg, 2023). Although not statistically significant, the group of athletes in the present study that had ceased therapy showed a higher likelihood of experiencing harmful effect on swimming performance compared to those athletes who had adhered to treatment. This finding supports the importance of athletes with EIB continuing their therapy as prescribed.

However, the respiratory assessments were completed following a periodised recovery mesocycle, so it was often anecdotally reported that athletes had stopped EIB therapy due to cessation of training and competition, suggesting symptoms that normally would be present had reduced, thus negating the perceived requirement for therapy. It is likely that some EIB positive athletes deployed an 'on-off' relationship with therapy throughout the twelve-month period, but, as it was not possible to report or quantify exactly when treatment was used, it is unknown whether the presence or lack of effect is the result of acute or longitudinal inhalation *[i.e., an athlete may have only ceased or recommenced therapy in the days-weeks preceding the scheduled follow-up, and not maintained use religiously over the observation period]*. The implication of this is it can take up to four-weeks following the initiation of maintenance therapy to see maximal protection, particularly in outcome measures such as FeNO (Parsons *et al.*, 2013). Further investigation is required to understand the barriers towards the non-use of asthma medications in athletic populations.

Additionally, although athletes in this study received education on inhaler technique *[i.e., regarding appropriate inhalation flow-rate for pMDI device, importance of coordination, and breath-holding]* and were recommended to use a valved-holding chamber (VHC), it is unknown if this was maintained. Elite athletes often demonstrate critical errors in pMDI inhaler technique particularly relating to inhalation flow rate (Jackson, 2018), so using a VHC can aid in auditory feedback for flow-rate and reduce the dependence of co-ordinating device and breath actuation. A VHC can also increase the proportion of particles emitted from a typical hydrofluoroalkane-propelled pMDI device (Williams *et al.*, 2001).

The ability to quantify adherence and technique, such as using a 'smart inhaler' device to timestamp inhaler actuations, send administration reminders and measure the inhalation profile (Chrystyn *et al.*, 2019) would be advantageous in future studies with elite populations. Failure to optimise respiratory care presents an increased risk of tachyphylaxis development, respiratory condition exacerbation, and greater dependence on SABA use (Anderson, Caillaud and Brannan, 2006; Williams *et al.*, 2011). The latter is an issue that places an athlete at risk of an adverse analytical finding due to tight threshold-level restrictions implemented by WADA.

4.5. Conclusion

This experimental chapter is the first study to demonstrate that in a cohort of elite swimming athletes the identification of EIB through diagnosis, and then subsequent long-term use of pharmacological inhaler therapy improved resting FEV_1, and a provided clinical attenuation to bronchoconstriction induced by voluntary hyperpnoea. Moreover, using magnitude-based decisions, effective treatment of EIB was shown to be *"very unlikely beneficial – likely trivial"* to major competition performance when accounting for the expected between-competition variation and progression of an elite swimming athlete. Athletes who had ceased therapy showed a higher likelihood of experiencing harmful effect on swimming performance compared to those athletes who had adhered to treatment, reaffirming the importance of treatment adherence. Despite the limitations of the retrospective study design, the use of a non-laboratory-controlled competitive setting suggests that these results are presumably more indicative of the likely performance effect from real-world treatment. Future research should consider supratherapeutic dosing of medication, as an unscrupulous athlete may use the inhaled route of administration to avoid detection of an adverse analytical finding.

CHAPTER 5: INHALER TECHNIQUE IN RESEARCH ON THE ERGOGENICITY OF ASTHMA THERAPY: EVALUATING THE DEPOSITION OF BECLOMETHASONE DIPROPIONATE WITH-AND-WITHOUT AN AEROCHAMBER PLUS VALVED-HOLDING CHAMBER AT INCREASING SIMULATED INHALATION FLOW RATES

BACKGROUND: Elite athletes with asthma-related conditions commonly use pressurised metered-dose inhalers sub-optimally, with critical errors including high inhalation flow rate and limited use of valved-holding chambers (VHCs). However, inadvertent reduced pulmonary drug delivery may impact clinical outcomes, increase the risk of adverse side-effects, and promote dependence on heavily World Anti-Doping Agency (WADA) controlled emergency therapy. Moreover, the reporting of pMDI technique in research on the ergogenicity of inhaled asthma therapy is often inadequate or lack inter-intra participant standardisation. **OBJECTIVES:** The purpose of this chapter was to support the methodological development of prospective ergogenic effect experimental chapters in this thesis by investigating the impact of inhalation flow rate and VHC use on ICS device performance. **METHODS:** Using Next Generation Impaction (NGI) technique, the delivered dose, mouth-throat region deposition, and interpolated fine particle mass (iFPM) of Beclomethasone dipropionate were quantified at simulated inhalation flow rates of 30, 60, and 100 L/min. Following this, an AeroChamber Plus® VHC (ACP-VHC) was added to the 30 and 100 L/min conditions. **RESULTS:** Inhalation flow rate at simulated 30 L/min resulted in the greatest mouth-throat deposition, thus consequently the lowest delivered dose and iFPM ($p<0.05$) compared to both 60 and 100 L/min. The addition of ACP-VHC significantly reduced mouth-throat deposition at 30 and 100 L/min ($p<0.001$), with 30 L/min demonstrating increased iFPM as a result ($p<0.001$). This observation was not seen at 100 L/min ($p=0.377$). **CONCLUSION:** Despite methodological limitations with in-vitro impaction techniques for inferring in-vivo pulmonary deposition, these findings add evidence that inhalation flow rate and addition of VHC impact the aerodynamic properties of ICS delivered using a pressurised metered-dose inhaler, and therefore could impact on clinical outcomes or ergogenicity in exercise performance trials. Prospective studies in this thesis will use a VHC incorporating 30 L/min auditory feedback in an attempt to standardise inhalation flow rate and reduce inter-and-intra differences in participant inhaler technique.

5.1. Introduction

Chapter 4 demonstrated that diagnosing and initiating long-term therapeutic use of asthma inhaler therapy incorporating ICS in elite athletes with EIB provided positive outcomes to respiratory health, but this did not result in an improvement in real-world swimming performance above the variability and expected progression between major competitions. This adds to evidence that therapeutic levels of asthma treatment do not provide an ergogenic benefit to athletes.

However, *Chapter 4* also highlighted a high non-adherence rate *[i.e., ceasing treatment during observation period]*. Much like the general asthmatic population (Sanchis, Gich and Pedersen, 2016), elite athletes often demonstrate critical errors in pMDI inhaler technique. Jackson *et al.*, (2018) reported in a cohort of elite swimmers the main fault in inhaler technique was high inhalation flow rate (mean ± SD; 348 ± 49 L/min^{-1}), which was ten-fold the 30 L/min^{-1} suggested for a pMDI (Laube *et al.*, 2011). Additionally, it is not known the exact prevalence of VHC use in athletic populations, but during comprehensive respiratory assessments like those used in *Chapter 4*, it has been anecdotally reported use is low *(unpublished)*. The athletes screened by Jackson *et al.*, (2018) also reported frequent side-effects such as voice disturbance and sore throat, most likely attributed to suboptimal drug delivery. A further impact of sub-optimal inhaler delivery is the reliance on additional dosing of threshold level-controlled formulations [such as salbutamol], increasing the risk of an adverse analytical finding, or the need for advanced therapy that require a TUE, such as terbutaline, or oral β2-agonists and GC.

Whilst optimal inhaler technique is important for respiratory health and adherence to the WADA code, sub-optimal drug delivery may also impact on laboratory-based research investigating the ergogenic impact of inhaled asthma treatment. As described in *Chapter 2*, some attempts are made to standardise inhaler technique, maintain good reporting practices in describing inhaler technique, show evidence of familiarisation, and report monitoring of participant compliance within previous investigations on exercise performance following asthma prophylaxis. However, the reporting of these factors is often inconsistent, and authors fail to acknowledge or control for inter-and-intra individual differences in participant inhaler technique.

The *'Aerodynamic Particle Size Distribution' (APSD)* is a measure of the size distribution of particles in an air stream based on their aerodynamic behaviour (Sheth, Stein and Myrdal, 2014). The APSD of an inhaler formulation is a critical factor in determining the

ability of aerosols to enter various regions of the human respiratory system (Laube *et al.*, 2011). Inhaled substances <5 microns are considered within the respirable fractions, whereas larger inhalable particles may settle in extra-thoracic regions. APSD can be assessed using a cascade impactor, such as Next Generation Impactor (NGI).

Inhaler technique has previously been shown to impact on mass depositing in the respiratory system or regional portions therein, however, research into the impact of above recommended 30 L/min inhalation flow rate is limited and mostly from a historical perspective (Farr *et al.*, 1995; Smith, Chan and Brown, 1998; Feddah *et al.*, 2000; Cheng *et al.*, 2001; Rahmatalla *et al.*, 2002; Biswas, Hanania and Sabharwal, 2017; Hira *et al.*, 2020) particularly at extreme flow rates as observed in elite athlete cohorts (Jackson, 2018).

By gaining a better understanding of how critical errors in inhaler technique can impact the amount of drug delivered to regions of the respiratory system, researchers can consider its potential influence on dependant variables when designing studies on ergogenicity and attempt to standardise inter-and-intra differences in participant dosing. To knowledge, this approach has not been explored in the context of a prospective ergogenic study.

Since the formulation has already received licensing approval, this study does not aim to evaluate critical quality attributes against industry criteria, such as >75% dose uniformity or >85% label claim compliance (Thorat, Meshram and Santosh, 2015; US FDA, 2018). Instead, the objective of this study was two-fold, (1) to investigate the effect that inhalation flow rate and VHC use has on ICS device performance *[i.e., delivered dose, mouth-throat deposition and interpolated fine particle mass]*, and (2) use this data to support the methodological development for prospective experimental chapters in this thesis on the ergogenic impact of ICS by determining a practicable method to standardise inhaler technique and estimate the delivered dose to participants.

5.2. Materials and Methods

Development and validation of the High-Performance Liquid Chromatography (HPLC) method, NGI testing, and recovery of deposited ICS was conducted at the University of Greenwich Medway Centre for Pharmaceutical Sciences. This research did not require ethical approval as human participants were not involved. All procedures took place in the ambient temperature and humidity of the inhalation laboratory (21.5 ± 1.2 °C, 65.8 ±

4.9 %) as environmental conditions can impact on method performance (Shemirani *et al.*, 2013).

5.2.1. Method for Next Generation Impactor (NGI)

The Next Generation Impactor (NGI) aerodynamic particle sizer is a device that measures the size distribution of airborne particles. It operates by using a controlled, linear velocity of airflow to impact the particles onto a collection surface. The NGI consists of a mouth-throat model (also referred to as an induction port) and a series of horizontally arranged stages that decrease in particle cut-off size *(Figure 5.1; Figure 5.2)*. The particle size range is dependent on the flow rate but is between 0.24 – 11.7 microns at 30 L/min (Copley Scientific Limited, 2021). When an inhaler device is attached to the impactor and actuated, the sample-laden airflow passes through the mouth-throat model, and then horizontally across the impactor stages. The largest particles under the velocity of the airflow deposit on the first plate. Then, smaller particles with lower inertia, pass around that plate and to the next stage where the orifices are smaller. Thus, the impactor could metaphorically be thought of as a "particle sieve". The airflow is terminated once the desired volume has passed through the impactor. After particles are impacted onto the different collection surfaces, the drug mass is dissolved into a liquid solution and analysed using high-performance liquid chromatography (HPLC). The method developed for this study presented in *Appendix H.*

This principle allows the delivered dose and aerodynamic particle size distribution (APSD) to be determined, and some inference can be made in both the inhalable and thoracic fractions *(Figure 5.3)*. Particles ranging from 0.5 to 5 μm in aerodynamic diameter have the highest likelihood of depositing in the lungs. Smaller particles are more likely to penetrate deeply into the lung, while particles with aerodynamic diameters exceeding 5 μm are more likely to impact in the oropharyngeal cavity (Heyder *et al.*, 1986). However, the NGI is not considered as a direct simulation model of the respiratory system (Dunbar and Mitchell, 2005).
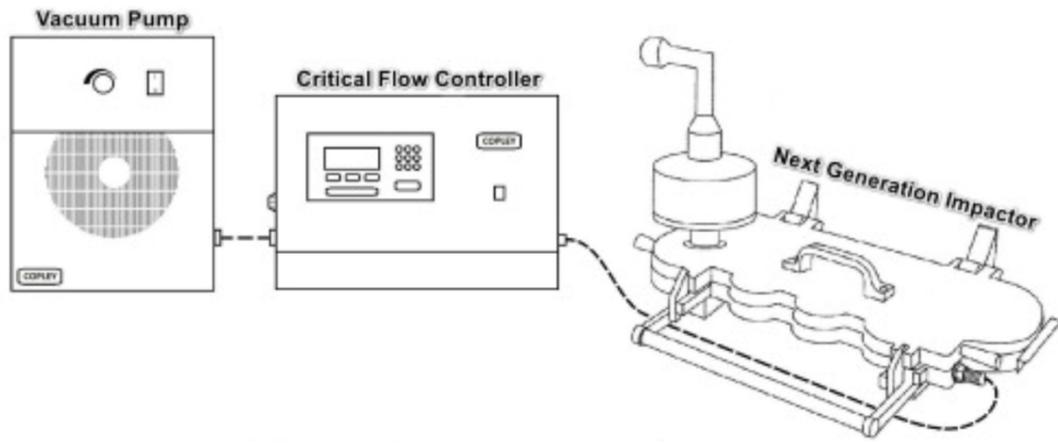
Figure 5.1. *Schematic of Next Generation Impactor (with preseparator fitted, not used in the present study), Critical Flow Controller and Vacuum Pump (Image from Ahookhosh et al., 2019).*
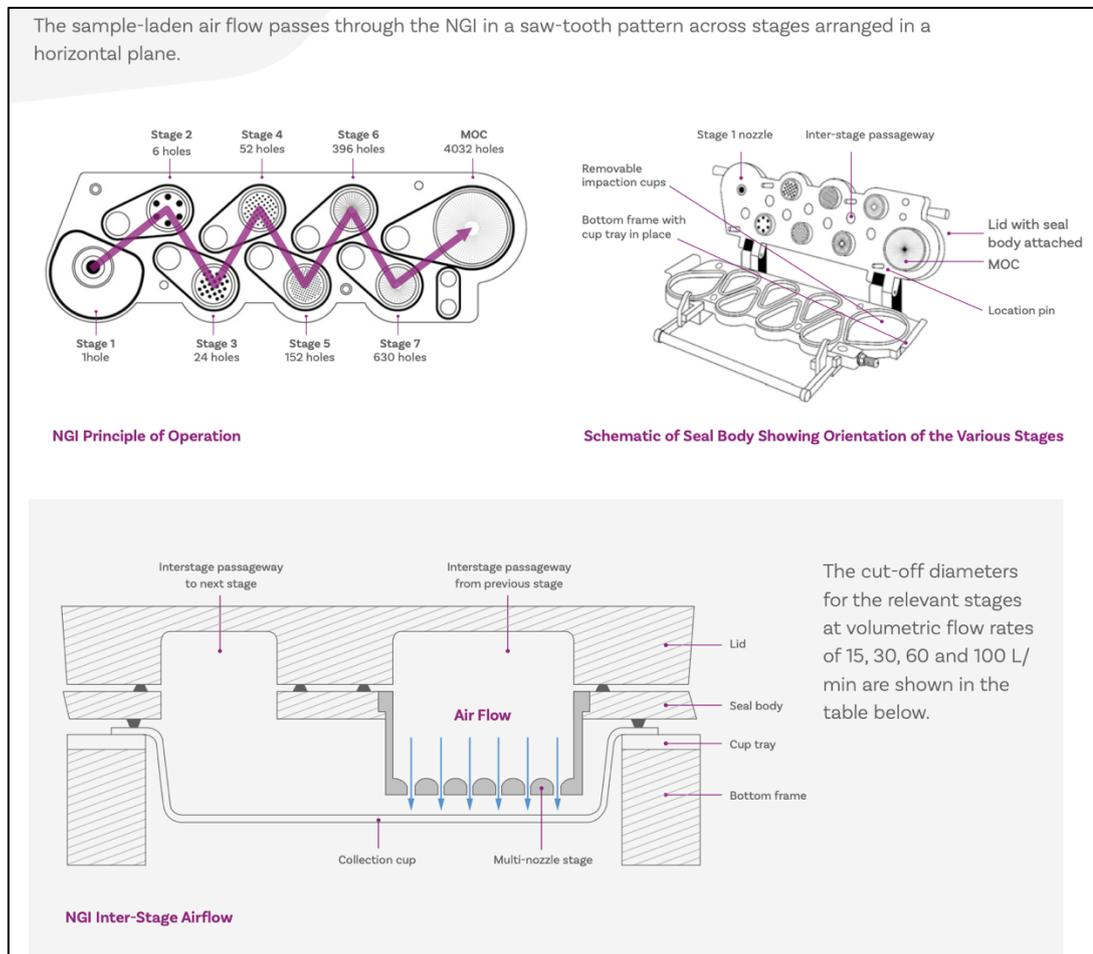
Figure 5.2. *Principle of Next Generation Cascade Impactor (Copley Scientific Limited, 2021).*
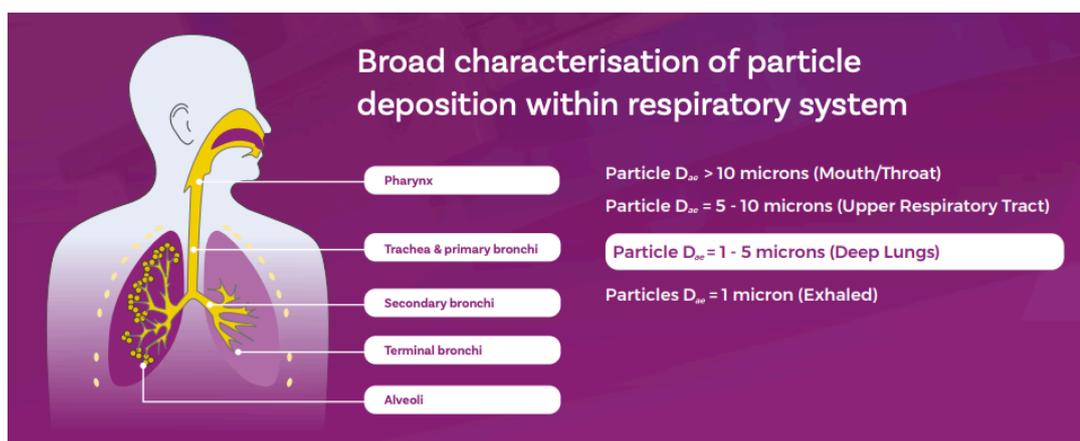
Figure 5.3. *Inference of particle size and deposition in intra and extra-thoracic regions (Copley Scientific Limited, 2021).*

5.2.2. Calculation of Inspiratory Volume and Inlet Opening Time

The calculation of the inspiratory volume was based on four times the internal dead volume of the NGI and mouth-throat components (United States Pharmacopeia 2019a). An NGI including the induction port (but without the pre-separator) has an internal dead volume of 1172 mL. When the AeroChamber Plus™, spacer device was used, the additional 149 mL of internal volume was included in the calculation (Roberts *et al.*, 2020). As such, the inspiratory volumes for the inhaler device alone and with add-on VHC equated to 4.69 L and 5.25 L respectively. This method is to ensure that the aerosol bolus emitted from pMDI devices passes through the entire volume of current sizing instruments, the duration of air drawn through these devices during in vitro testing is typically adjusted to obtain an inhaled volume of at least 4.0 L (Mohammed et al., 2012; United States Pharmacopeia 2019a). However, some researchers aiming to achieve greater clinical accuracy in evaluating inhalers in vitro have recently shown interest in matching the inhalation volume of an adult in a single breath (Mitchell, Newman, and Chan, 2007). An inspiratory volume of 4.69 and 5.25 L would closely match this.

$$Inspiratory\ Volume\ (4\ x\ NGI\ and\ induction\ port\ internal\ volume\ [plus\ spacer\ add-on\ devices]) = 4.69\ L\ or\ 5.25\ L\ respectively.$$

From this, the inlet opening time was calculated using the equation below.

$$Flow\ Rate\ [Q] = 30, 60\ or\ 100\ L/min.$$

$$Inhalation\ Time\ [seconds] = \frac{Inspiratory\ Volume\ [L] \times 60}{Flow\ Rate\ [Q; L/min]}$$

Inlet Opening Time for 30 L/Min.

$$\frac{Inspiratory\ Volume\ [L] \times 60}{Flow\ Rate\ [Q; L/min]} \quad = \quad \frac{4.69 \times 60}{30} \quad = \quad \boldsymbol{9.3\ seconds}$$

Inlet Opening Time for 30 L/Min plus add-on VHC.

$$\frac{Inspiratory\ Volume\ [L] \times 60}{Flow\ Rate\ [Q; L/min]} \quad = \quad \frac{5.25 \times 60}{30} \quad = \quad \boldsymbol{10.5\ seconds}$$

Inlet Opening Time for 60 L/Min.

$$\frac{Inspiratory\ Volume\ [L] \times 60}{Flow\ Rate\ [Q; L/min]} \quad = \quad \frac{4.69 \times 60}{60} \quad = \quad \boldsymbol{4.7\ seconds}$$

Inlet Opening Time for 100 L/Min.

$$\frac{Inspiratory\ Volume\ [L] \times 60}{Flow\ Rate\ [Q; L/min]} \quad = \quad \frac{4.69 \times 60}{100} \quad = \quad \boldsymbol{2.8\ seconds}$$

Equation 5.1. Calculation of inlet opening time from inspiratory volume and target flow rate.
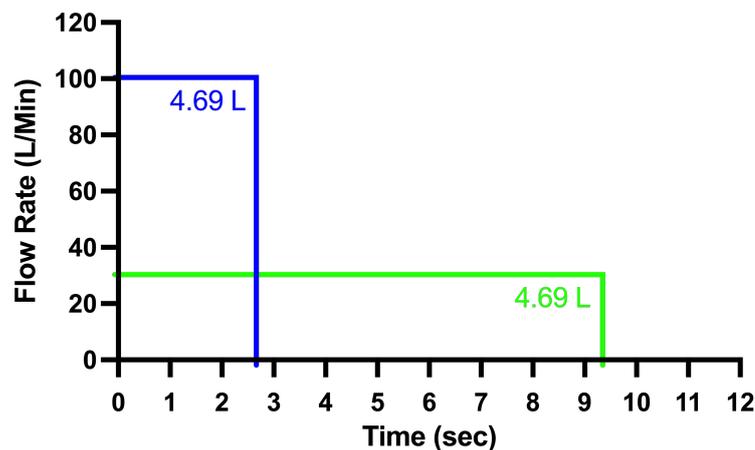
Figure 5.4. Example of the relationship between flow rate and inlet opening time for 30 L/min and 100 L/min.

5.2.3. Mouth-Throat Model

Two mouth-throat models were utilised in this experimental chapter. Firstly, an industry standard United States Pharmacopeia throat model (Copley Scientific, Nottingham,

United Kingdom) was used to validate the impaction method at the three flowrates *(Appendix I)*. Then, to more closely resemble in-vivo structures, a medium sized adult anatomical throat model (Emmace Consulting AB, Sweden) was used for data collection.

Figure 5.5. United States Pharmacopeia throat model [left]. Emmace Consulting anatomical throat model (medium) [middle]. Interior surfaces of Emmace Consulting anatomical throat model [right].

5.2.4. Mouth-Throat Coating

The United States Pharmacopeia throat model was not coated with any solution. However, surface coating of mouth-throat models may provide better in vivo deposition estimation (Kaviratna *et al.*, 2019). As such, the anatomical throat was coated in a solution of ethanolic Brij®35 and glycerol. This 'wet' coating is to capture any particles that collide with the interior mouth-throat surface during inhalation event, closer simulating the mouth-throat region coated with saliva and mucus. The anatomical throat coating solution was made using the guidance provided by Emmace Consulting AB [*method below*].

1. 15 g of Brij®35 (Millipore Sigma, USA) was dissolved into 100 ml of Ethanol (≥99%, Fisher Scientific, Loughborough, UK).
2. Then, 20 ml of 'Step 1' solution was mixed with 40 g of Glycerol (≥99.5%, Sigma Aldrich, St. Louis, MO, USA).
3. Finally, 'Step 2' solution was diluted in equal measures of Ethanol (Fisher Scientific, Loughborough, UK).

To coat the anatomical throat, it was plugged at one end, and the coating solution poured into the mouth opening. Once half-full, the anatomical throat was tilted and rotated to allow for any dead space to be coated, then the remaining volume was filled to ensure

complete coating of interior surface. Following this, the anatomical throat was suspended up-right, and the excess coating material allowed to drain for 2 minutes before commencing experiment.

Figure 5.6. *Anatomical Throat Model internally coated with 'wet' ethanolic Brij®35 and glycerol solution.*
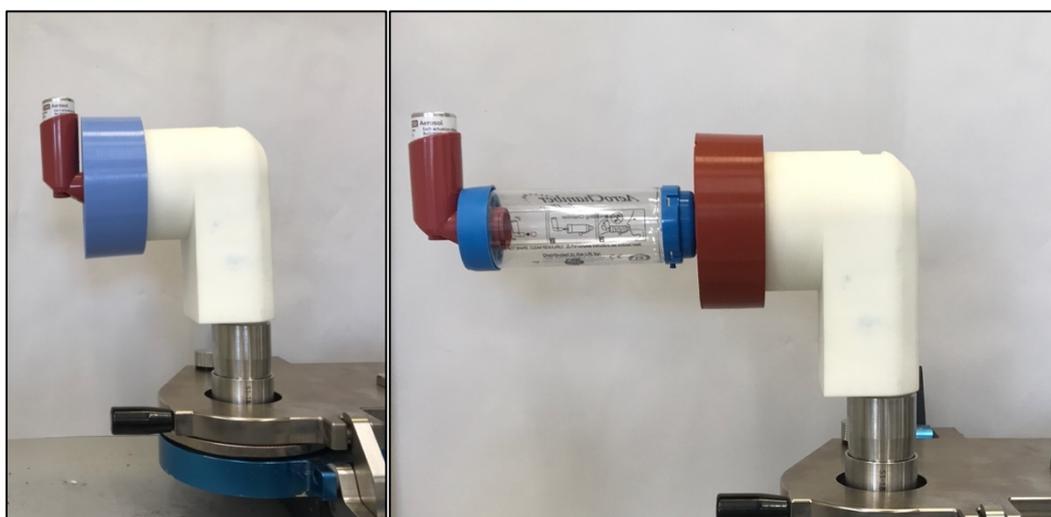
Figure 5.7. *Anatomical Throat Model with Qvar® inhaler alone, and with AeroChamber Plus™ valved holding chamber.*

5.2.5. Metered-Dose Inhaler and Valved Holding Chamber

A pressurised metered-dose inhaler (pMDI) containing beclomethasone dipropionate (BDP) (Qvar® 100 µg nominal dose, Teva UK Limited, Castleford, United Kingdom) was used throughout data collection. The formulation of Qvar® is a pMDI designed for oral inhalation, with each inhaler canister containing a solution of the corticosteroid

beclomethasone dipropionate in hydrofluoroalkane-134a (1,1,1,2 tetrafluoroethane) propellant and ethanol. A new inhaler canister was used for each experiment condition as the mass of delivered dose can vary during the lifespan of pMDI devices.

To investigate the impact of an add-on spacer device on drug delivery, an AeroChamber Plus™ valved holding chamber (ACP-VHC) (Trudell Medical International, Ontario, Canada) was used. The internal volume of this device is 149 mL and features an auditory inhalation flow-rate warning at 30 L/min (Aerochamber_Plus_Product-Monograph, 2005).

5.2.6. Delivered Dose and Aerodynamic Particle Size Deposition (APSD) Measurement using Qvar® Inhaler Alone (Part 1) and with Valved Holding Chamber (Part 2)

The first part of the present study was to investigate delivered dose and Aerodynamic Particle Size Deposition (APSD) using a Next Generation Impactor (Copley Scientific, Nottingham, United Kingdom). The following procedure was repeated six times for each experimental condition.

5.2.7. NGI Test Procedure

Firstly, the surface of each collection plate was coated with a cyclohexane silicone oil solution and left to evaporate for fifteen minutes to minimize particle bounce and re-entrainment. The collection plates and induction port were then fitted, and the system checked for leakage (Leak Tester, Copley Scientific, Nottingham, United Kingdom) [<100 pascal per second]. Following this, the impactor outlet was connected to the critical flow controller and the target constant inhalation flowrate set using a digital flowmeter (DFM-4; Copley Scientific, Nottingham, United Kingdom) and vacuum pump (LCP6, Copley Scientific, Nottingham, United Kingdom). Flowrates utilised in this study were 30 L/min, 60 L/min and 100 L/min. These flowrates targeted the common practice for pMDI use (Laube *et al.*, 2011). The pMDI device was primed by shaking for ten seconds and discharging to waste. This was repeated twice as recommended by the manufacturer (Qvar _Product-information, 2019), before being attached to the induction port using a bespoke mouthpiece adaptor to ensure an airtight seal [*see mouth-throat model section*]. When in position, the inhaler device was actuated, and vacuum pump allowed to flow for the required inhalation time *[Calculation of Inspiratory Volume and Inlet Opening Time - Section 5.2.2].*

5.2.8. Recovery of BDP

To extract deposited BDP from the impactor, the collection plates, ACP-VHC, mouth-throat model and mouthpiece adapter were washed into solution for quantification by high-performance liquid chromatography.

5.2.9. Collection Plate Stages

The collection plates were transferred to a 'Gentle Rocker' (Copley Scientific, Nottingham, United Kingdom), and the required volume of diluent (outlined below) was accurately dispensed using a repeating auto pipette (HandyStep® Touch 705200, Brandtech® Scientific, USA). The collection plates were agitated for 15 minutes, to ensure the impaction surface was completely covered with diluent, and deposited BDP dissolved into solution. When complete, an aliquot of solution from each plate was transferred into a labelled high-performance liquid chromatography vial.

Table 5.1. Next Generation Impactor (NGI) Collection Plate Dilution Volumes.

Stage	Volume Dilution	Label
ACP-VHC	100 mL	ACP-VHC
Mouthpiece adapter and throat	200 mL	MT
Stage 1	10 mL	S1
Stage 2	5 mL	S2
Stage 3	5 mL	S3
Stage 4	5 mL	S4
Stage 5	5 mL	S5
Stage 6	5 mL	S6
Stage 7	5 mL	S7
Stage 8	10 mL	S8

Abbreviations: ACP-VHC, AerochamberPlus Valved-holding Chamber

Figure 5.8*. NGI collection plate stages [four to eight] with beclomethasone dipropionate (BDP) deposited from Qvar® 100 µg at 100 L/min. (black and white image with contrast filter applied for better visualisation of the fine particles deposited).*

5.2.10. Mouthpiece adapter / throat and ACP-VHC Spacer Drug Recovery

The mouthpiece adapter and MT model were thoroughly rinsed into a 200 mL volumetric flask containing 60 mL of water [to give final 30/70 v:v ratio]. The ACP-VHC was rinsed into a separate 100 mL volumetric flask containing 30 mL of water [to give final 30/70 v:v ratio]. Once at required volume, the volumetric flasks were inverted to ensure complete mixing, and the solution allowed to reach room temperature. Once at room temperature, the remaining volume was filled with diluent and an aliquot of the solution transferred into a labelled high-performance liquid chromatography vial.

5.2.11. Data Processing and Statistical Analysis

Device performance outcomes *(Table 5.2)* for sections *5.3.2, 5.3.3 and Appendix I* were calculated using analysis software Inhalytix™ (Copley Scientific LTD, Nottingham, United Kingdom).

Table 5.2. Key definition of device performance outcomes.

Device Performance Outcome	Definition
Ex-Inhaler Dose	The mass of drug that has been recovered outside of the inhaler device output.
Delivered Dose	The mass of drug that has been recovered from an impactor and a mouth-throat model [i.e. indicative of the dose entered into the body].
Fine Particle Dose / Mass (FPD or FPM)	The mass of drug that has been recovered that is smaller than 5 μm in size.
Fine Particle Fraction (FPF)	The percentage of the FPD relative to the total mass of drug recovered. Expressed as a percentage.
Interpolated FPD <5μm	Flow-rate corrected mass of recovered drug under 5 micrometres in size, that has been recovered [i.e., NGI stage that 5μm differs depending on the flow rate used (Copley, 2021, p84)
Mass Median Aerodynamic Diameter (MMAD):	The average size of particles that have been recovered in terms of diameter.
Geometric Standard Deviation (GSD)	The variation in size of particles that have been recovered. The larger the GSD value, the greater the spread of the aerodynamic diameters of the particles (Sheth, Stein and Myrdal, 2014).

Data is presented in written form and tabulated as mean (±SD). A one-way analysis of variance (ANOVA) was used to determine any statistically significant differences in inhaler performance outcomes between the target flowrates (30, 60, 100 L/min) when using the device in isolation. Next, when comparing the addition of an ACP-VHC within the same flowrate [i.e., 30 L/min ≠ 30 L/min + ACP-VHC], the interaction between flowrate (with or without VHC] and individual impactor stage deposition was analysed using a two-way ANOVA, and device performance outcomes with an independent sample t-test. Finally, an independent sample t-test was used to compare the difference in ACP-VHC performance between 30 and 100 L/min. For all main-effect tests [one-and-two-way ANOVA], to identify the location of any significant differences, post-hoc pairwise comparisons with Bonferroni adjustment were conducted. These inferential

statistic tests were selected following assessment and confirmation of common statistical assumptions [normality, independence, equality of variance and sphericity], and where appropriate the corresponding statistics extracted from statistical software. Significance level was set at $p<0.05$. All analyses were performed using SPSS Statistics Version 28.0 (IBM Corporation, New York, USA), and data visualisation completed in GraphPad Prism Version 9 (GraphPad Software Inc, California, USA).

5.3. Results

5.3.1. Pilot Work - Aerodynamic Particle Size Distribution (APSD) with USP and AT Model

Based on the results of the pilot work comparing mouth-throat models (presented in *CHAPTER 11:Appendix I),* the remaining analysis was conducted solely with the anatomical model, as it better represents human mouth-throat physiology.

5.3.2. Part 1 - Device performance of Beclomethasone Dipropionate [BDP] Qvar® 100 μg [micrograms] inhaler at 30 L/min, 60 L/min and 100 L/min.

In all conditions tested, the delivered dose was lower than the advertised nominal dose (100 μg), with the remaining drug proposed to be within the actuator orifice. Drug recovery was not completed from the actuator due to the priming action and difficulty in rinsing between repetitions.

When using the inhaler device in isolation, there was a significant main effect of flowrate on all device performance outcomes, except for MMAD *(Table 5.3)*. Post-hoc analysis revealed 30 L/min resulted in significantly lower delivered dose than both 60 L/min and 100 L/min (p<0.001 respectively), but there was no significant difference between 60 L/min and 100 L/min (*p*=0.939; *Table 5.3, Figure 5.9a*). However, mouth-throat deposition was highest at 30 L/min and decreased significantly in an ordinal manner at 60 L/min and 100 L/min *(Table 5.3, Figure 5.9b)*. Finally, interpolated FPM was also significantly different across flow rate post-hoc comparisons *(Table 5.3, Figure 5.9c)*.

Table 5.3. Device performance of Beclomethasone Dipropionate [BDP] Qvar® 100 µg [micrograms] inhaler at 30 L/min, 60 L/min and 100 L/min (± 5%) (n=6 repetitions).

	30 L/min	60 L/min	100 L/min	Inferential Test Statistics $(df_{within}, df_{between}) = F$, p-value.
Mouth and Throat [µg]	36.32 (2.08)	20.87 (1.80)	14.05 (1.78)	$F(2,15) = 219.1$, $p < 0.001$.
Delivered Dose [µg]	80.40 (1.84)	88.94 (1.88)	88.57 (1.86)	$F(2,15) = 40.3$, $p < 0.001$.
Interpolated FPM <5µm	44.03 (1.81)	67.86 (1.64)	74.30 (2.20)	$F(2,15) = 423.0$, $p < 0.001$
MMAD	0.64 (0.03)	0.67 (0.02)	0.66 (0.01)	$F(2,15) = 0.64$, $p = 0.540$
GSD	2.53 (0.05)	2.41 (0.02)	2.39 (0.06)	$F(2,15) = 12.88$, $p = 0.001$

*Note. Data presented as Mean (SD). Abbreviations: **MMAD**, Mass Median Aerodynamic Diameter; **GSD**, Geometric Standard Deviation; **FPM**, Fine Particle Mass; **µg**, micrograms; **µm**, microns.*
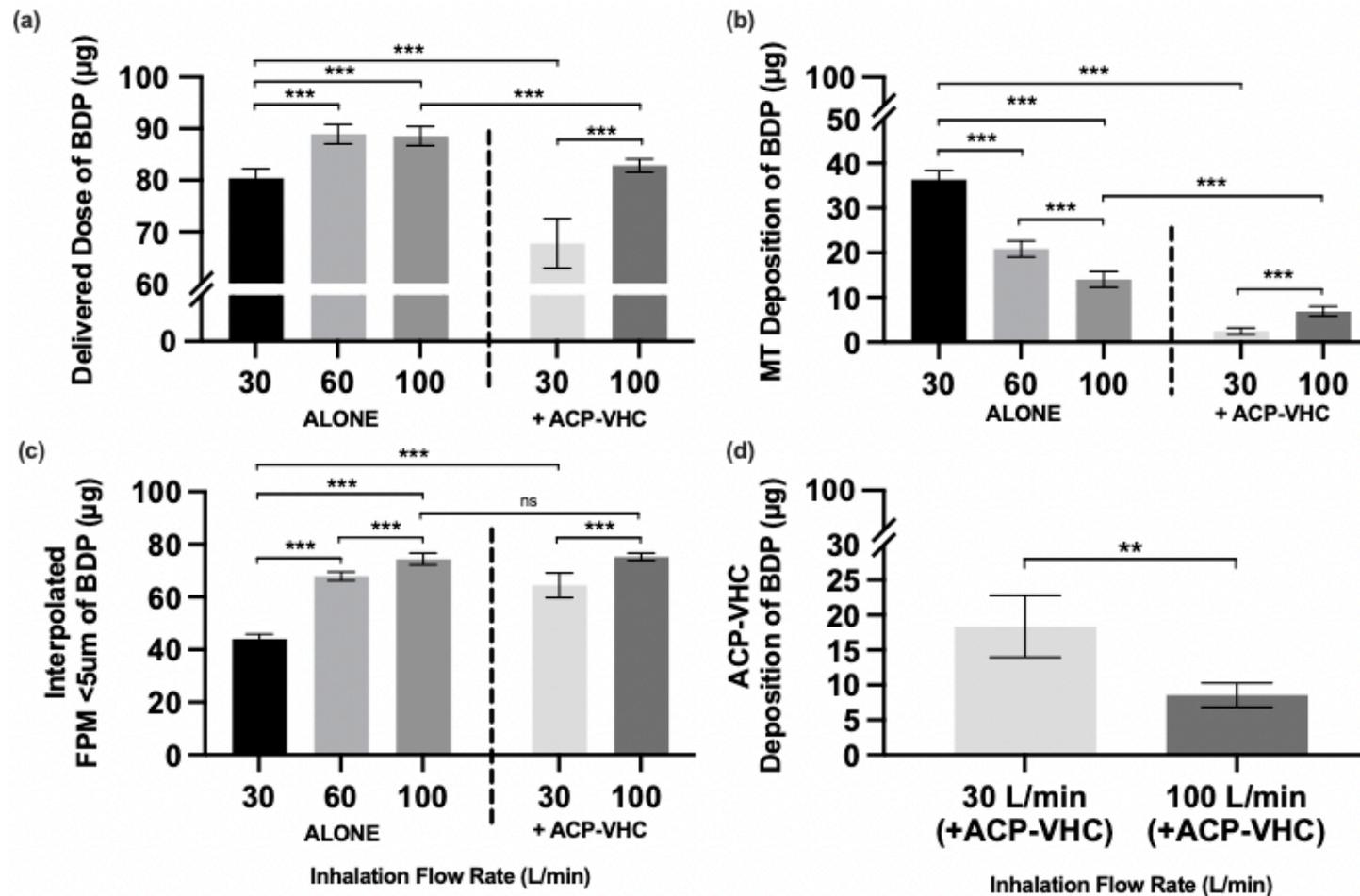
Figure 5.9. *Device performance of Beclomethasone Dipropionate [BDP] Qvar® 100 µg [micrograms] in isolation and with addition of AeroChamber Plus Valved-Holding Chamber [ACP-VHC] at 30 L/min, 60 L/min and 100 L/min. (a) Delivered Dose (b) Mouth-Throat [MT] Deposition (c) Interpolated Fine Particle Mass [FPM] <5µm (microns) (d) ACP-VHC deposition. Error bars represent standard deviation around mean. Post-hoc pairwise comparisons signified as; ns, not significant; *P<0.05; ***P<0.02; ***P<0.001.*

5.3.3. Part 2 - Aerodynamic Particle Size Distribution (APSD) and Inhaler Device Performance with Addition of Valved Holding Chamber

When an ACP-VHC was added at the simulated flowrate of 30 L/min, a significant interaction between testing condition and NGI stage deposition was observed ($F_{(9,90)} = 309.00$, $p < 0.001$). Post-hoc pairwise comparisons revealed that at all impactor stages [mouth-throat to S8], the addition of an ACP-VHC resulted in significantly greater mass of BDP deposition *(Table 5.4, Figure 5.10)*. At 100 L/min, a significant interaction effect was also observed between testing condition and impactor stage deposition ($F_{(9,90)} = 77.69$, $p < 0.001$). However, significant post-hoc pairwise comparisons were only evident in the mouth-throat model, and S3, S4, S6, S7 collection plates *(Table 5.5, Figure 5.11)*.

Regarding overall device performance comparisons within the same flowrate, the delivered dose [i.e., mass of drug simulated to enter the body] was significantly higher when using Qvar® pMDI device alone compared to addition of ACP-VHC at both 30 L/min ($p < 0.001$) and 100 L/min ($p < 0.001$). This is attributed to the mass of drug retained inside the ACP-VHC (30 L/min, $18.37 \pm 4.40\ \mu g$; 100 L/min, $8.56 \pm 1.74\ \mu g$ respectively). Consequently, the mouth-throat deposition was significantly reduced with ACP-VHC use at both 30 L/min and 100 L/min compared to using the device in isolation *(Figure 5.9a)*. Interpolated FPM was greatly increased with addition of ACP-VHC at 30 L/min ($p < 0.001$), but this was not observed at 100 L/min ($p = 0.377$).

When considering differences in ACP-VHC performance between 30 L/min and 100 L/min, the faster flowrate resulted in greater mouth-throat deposition compared the slower inhalation speed *(Table 5.6)*. As mentioned previously, some mass of drug is retained inside the ACP-VHC, with 30 L/min retaining significantly greater mass than at 100 L/min ($p = 0.002$). Interpolated FPM was significantly greater at 100 L/min than 30 L/min.

Table 5.4. Device performance and mass of Beclomethasone Dipropionate (BDP) deposited on each stage of the next-generation impactor (NGI) using anatomical throat model, from Qvar® 100 µg in isolation and with added AeroChamber Plus valved holding chamber (ACP-VHC) at 30 L/min (± 5%) (n=6 repetitions).

	Alone (30 L/min)	AeroChamber Plus (30 L/min)	Inferential Test Statistics (df) = t/f, p-value.
Spacer [µg]	N/A	18.37 (4.40)**[b]	
Mouth and Throat [µg]	36.32 (2.08)	2.48 (0.73)***[b]	
Stage 1 [µg]	0.18 (0.01)	0.13 (0.03)*[b]	*Two-way ANOVA*
Stage 2 [µg]	0.06 (0.01)	0.12 (0.02)*[b]	*Condition*Stage*
Stage 3 [µg]	0.11 (0.02)	0.25 (0.06)*[b]	*Interaction*
Stage 4 [µg]	1.05 (0.21)	3.71 (0.94)**[b]	
Stage 5 [µg]	8.34 (0.76)	17.99 (2.04)***[b]	$F(9,90) = 309.00,$
Stage 6 [µg]	15.55 (0.78)	22.35 (1.34)***[b]	$p < 0.001.$
Stage 7 [µg]	10.18 (0.20)	11.51 (0.37)***[b]	
Stage 8 [µg]	8.61 (0.11)	9.23 (0.23)**[b]	
Ex-Inhaler Dose	N/A	86.13 (2.33)	N/A
Delivered dose [µg]	80.40 (1.84)	67.76 (4.82)***	$t(10) = 6.00,$ $p < 0.001.$
Interpolated FPM <5µm	44.03 (1.81)	64.45 (4.74)***	$t(6.421) = -9.86, p < 0.001.$
MMAD	0.64 (0.03)	0.92 (0.04)***	$t(10) = -12.67,$ $p < 0.001.$
GSD	2.53 (0.05)	2.13 (0.02)***	$t(5.909) = 17.31, p < 0.001.$

*Note. Data presented as Mean (SD). Significant between-condition pairwise comparison *P<0.05; ***P<0.02; ***P<0.001. Bonferroni corrected [b]). Abbreviations: **MMAD**, Mass Median Aerodynamic Diameter; **GSD**, Geometric Standard Deviation; **FPM**, Fine Particle Mass; **µg**, micrograms; **µm**, microns.*

Table 5.5. Device performance and mass of Beclomethasone Dipropionate (BDP) deposited on each stage of the next-generation impactor (NGI) using anatomical throat model, from Qvar® 100 μg in isolation and with added AeroChamber Plus valved holding chamber (ACP-VHC) at 100 L/min (± 5%) (n=6 repetitions).

	Alone (100 L/min)	AeroChamber Plus (100 L/min)	Inferential Test Statistics *(df) = t/f, p-value.*
Spacer [μg]	N/A	8.56 (1.74)***[b]	
Mouth and Throat [μg]	14.05 (1.78)	6.90 (1.11)***[b]	
Stage 1 [μg]	0.92 (0.11)	0.85 (0.19)	*Two-way ANOVA*
Stage 2 [μg]	0.88 (0.07)	1.11 (0.39)	*Condition*Stage*
Stage 3 [μg]	1.19 (0.7)	2.17 (0.27)**[b]	*Interaction*
Stage 4 [μg]	10.15 (0.40)	14.10 (1.05)***[b]	
Stage 5 [μg]	22.35 (1.17)	22.06 (0.38)	*f(9,90) = 77.69,*
Stage 6 [μg]	22.29 (0.72)	19.76 (0.60)***[b]	*p<0.001*
Stage 7 [μg]	9.28 (0.23)	8.50 (0.18)***[b]	
Stage 8 [μg]	7.46 (0.41)	7.41 (0.47)	
Ex-Inhaler Dose	N/A	91.41 (1.91)	N/A
Delivered Dose [μg]	88.57 (1.86)	82.85 (1.26)***	*t*(10) = 6.22, *p* <0.001.
Interpolated FPM <5μm	74.30 (2.20)	75.28 (1.39)	*t*(10) = -0.92, *p* =0.377.
MMAD	0.66 (0.01)	0.73 (0.02)***	*t*(10) = -7.99, *p* <0.001.
GSD	2.39 (0.06)	2.31 (0.07)	*t(*10) = 2.04, *p* =0.069.

Note*. Data presented as Mean (SD). Significant between-condition pairwise comparison *P<0.05; ***P<0.02; ***P<0.001. Bonferroni corrected* [b]*). Abbreviations:* **MMAD***, Mass Median Aerodynamic Diameter;* **GSD***, Geometric Standard Deviation;* **FPM***, Fine Particle Mass;* **μg***, micrograms;* **μm***, microns.*
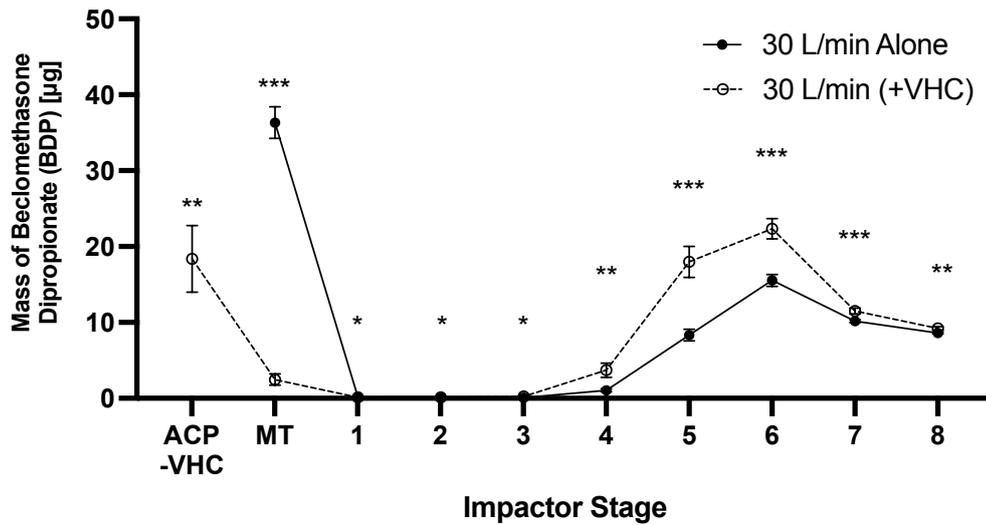
Figure 5.10. *Aerosol Particle Size Distribution (APSD) of Qvar® 100 μg alone and with AeroChamber Plus at 30 L/min. Abbreviations:* **MT***, Mouth-Throat;* **ACP-VHC***, AeroChamber Plus - Valved-holding Chamber;* **μg***, micrograms. Significant between-condition pairwise comparison (*P<0.05; P<0.02; ***P<0.01 – Bonferroni corrected).*
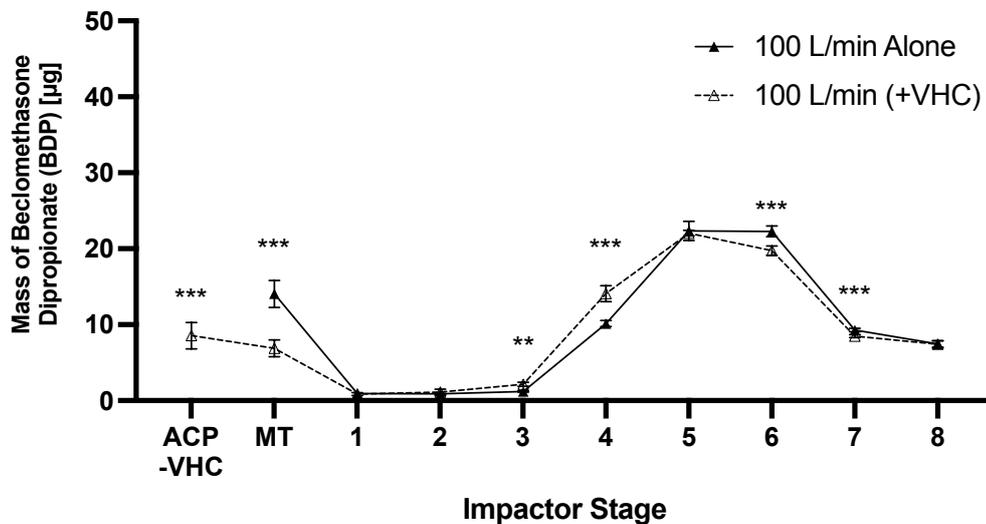
Figure 5.11. *Aerosol Particle Size Distribution (APSD) of Qvar® 100 μg alone and with AeroChamber Plus at 100 L/min. Abbreviations:* **MT***, Mouth-Throat;* **ACP-VHC***, AeroChamber Plus - Valved-holding Chamber;* **μg***, micrograms. Significant between-condition pairwise comparison (*P<0.05; **P<0.02; ***P<0.01 – Bonferroni corrected).*

Table 5.6. Device performance of Beclomethasone Dipropionate [BDP] Qvar® 100 µg [micrograms] inhaler at 30 L/min and 100 L/min (± 5%) with added AeroChamber Plus valved holding chamber (ACP-VHC) (n=6 repetitions).

	AeroChamber Plus (30 L/min)	AeroChamber Plus (100 L/min)	Inferential Test Statistics (df) = t, p-value.
ACP-VHC [µg]	18.37 (4.40)	8.56 (1.74) **	$t(6.526) = 5.07$, $p = 0.002$.
Mouth and Throat [µg]	2.48 (0.73)	6.90 (1.11) ***	$t(10) = -8.15$, $p < 0.001$.
Delivered dose [µg]	67.76 (4.82)	82.85 (1.26) ***	$t(5.712) = -7.40$, $p < 0.001$.
Interpolated FPM <5µm	64.45 (4.74)	75.28 (1.39) ***	$t(5.855) = -5.37$, $p < 0.001$.
MMAD	0.92 (0.04)	0.73 (0.02) ***	$t(10) = 9.88$, $p < 0.001$.
GSD	2.13 (0.02)	2.31 (0.07) **	$t(5.514) = -6.03$, $p = 0.001$.

*Note. Data presented as Mean (SD). Significant between-condition pairwise comparison (*P<0.05; P<0.02; ***P<0.01). Abbreviations: **ACP-VHC**, AerochamberPlus-Valved Holding Chamber; **MMAD**, Mass Median Aerodynamic Diameter; **GSD**, Geometric Standard Deviation; **FPM**, Fine Particle Mass; **µg**, micrograms; **µm**, microns.*

5.4. Discussion

This chapter aimed to better understand ICS delivery for methodological development of prospective experimental chapters on ergogenicity of ICS on cycling performance. To do so, the present chapter evaluated the device performance of a Beclomethasone dipropionate (Qvar® 100 µg) pMDI inhaler at different flow rates with-and-without a valved-holding chamber (VHC). The results demonstrated that flow rate had a significant effect on device performance, with 30 L/min resulting in lower delivered dose, lower interpolated FPM and higher mouth-throat deposition when compared to higher flow rates [60 and 100 L/min]. Use of an VHC increased interpolated FPM at 30 L/min whilst greatly decreasing mouth-throat deposition. At 100 L/min, interpolated FPM remained high with VHC use, but when compared to 30 L/min, the 100 L/min flow rate retained less drug inside the VHC, thus resulting in higher mouth-throat deposition of larger particles. Overall, the addition of a VHC was beneficial in reducing flow rate-induced

variation in ICS pMDI delivery, which may help reduce risk of adverse events, maximise pharmacological impact, and increase ergogenic potential under in-vivo conditions. As such, future research in this thesis will use a VHC with auditory feedback to standardise inhaler technique between individuals.

5.4.1. Inhaler Device Performance

The findings of the present study provide continued support for using a VHC with a pMDI device (Williams *et al.*, 2001; Asmus *et al.*, 2003). A notable issue with pMDI devices are they impart an undesirably high initial momentum to the particles during actuation (Yazdani *et al.*, 2014), meaning, the velocity of aerosol particles emitted from a pMDI is typically higher than that of the surrounding ambient air being inhaled by the individual (Liu, Doub and Guo, 2012). This observation is evident with the formulation used within the present study, as a Qvar® pMDI actuates aerosol at ~5.4 m/sec, much faster than an individual would inhale if following the recommended 30 L/min inspiratory flow of the device (Liu, Doub and Guo, 2012). The addition of a VHC facilitates an area for particles to lose their initial momentum, allowing them to more easily traverse the 'right-angle bend' between horizontal emission from the VHC and vertical passage through the larynx, thus reducing oropharyngeal deposition through impaction mechanisms (Yazdani *et al.*, 2014). The results of the present study support this notion, as mouth-throat deposition reduced with VHC use at both 30 L/min and 100 L/min. Yet, despite VHC reducing oropharyngeal inertial impaction, the present study observed significantly greater deposition within the VHC at 30 L/min compared to 100 L/min. This could be attributed to increased sedimentation and adsorption mechanisms of aerosol particles, both of which are proportional to the time particles occupy within the VHC. Additionally, a VHC increases the time between the aerosolised particles being actuated from the pMDI device and their inhalation allowing the propellant to evaporate and the particles to reduce to a size that can travel through the whole bronchial tree as far as the alveoli (i.e., with mass median diameter less than ~5 microns, particles emitted directly from a pMDI device are initially much larger) (Leach and Colice, 2010).

The present study challenged the traditional practice of inhaling a pMDI device *"as slow as possible"* (Broeders *et al.*, 2009; Laube *et al.*, 2011; Mitchell, Suggett and Nagel, 2016), therefore, this notion may not always be the most effective way to maximise drug delivery to the lower region of the lungs. Several previous studies have also shown that increasing the flow rate during inhalation can improve drug deposition in the lungs and

reduce deposition in the oropharyngeal region. For example, Cheng *et al., (2001)* demonstrated in a human airway replica that increasing the flow rate from 30 L/min to 90 L/min with a Salbutamol hydrofluoroalkane formulation pMDI resulted in lower deposition of particles in the oropharyngeal region and higher lung deposition. Similarly, Smith, Chan and Brown, (1998) reported a 40% rise in FPM of salbutamol sulphate upon increasing the flow rate from 30 L/min to 55 L/min in an in vitro investigation on β2-agonists. Feddah *et al.*, (2000) observed a similar trend in a glucocorticoid formulation, where simulated inspiratory flow rates of 30 L/min, 60 L/min and 90 L/min were compared. Closely related to the present study, Rahmatalla *et al.*, (2002) demonstrated this same observation in a Qvar® device. One mechanism behind this effect is thought to be related to the interaction between the aerosol particles and the inhalation air flow. At higher flow rates, there is more efficient momentum transfer between the expelled droplets and the inhalation air flow, allowing the droplets to easily follow the airstream and minimise deposition in the oropharyngeal region. This creates a smoother laminar flow, reducing particle loss along the oropharyngeal region and upper airways. In contrast, slower flow rates result in disturbed laminar flow due to the propellant hitting a slower air stream, allowing for more time for impaction in the oropharyngeal region (Roller *et al.*, 2007).

Whilst the above publications date back 20 years, more recently, Biswas, Hanania and Sabharwal, (2017), and, Hira *et al.*, (2020) also supported that a slow flow rate may not always be the optimal inhalation profile for a pMDI. The authors of the latter study attributed this finding to be particularly important when the formulation is dissolved in solution (such is Qvar®), because the excipients are rapidly evaporated after actuation. However, suspension-based formulations, like those used in phased-out chlorofluorocarbon formulations, may be more susceptible to reductions in drug efficiency as flow rate increases. Leach *et al.,* (2002) presented differences between BDP hydrofluoroalkane and chlorofluorocarbon formulation deposition, indicating that hydrofluoroalkane-BDP has greater lung deposition than chlorofluorocarbon-BDP due to the smaller particle size. Cheng *et al.,* (2001) noted that higher flow rates resulted in more efficient evaporation, which produced smaller droplets and generated softer plume characteristics. This, in turn, led to a reduction in oropharyngeal deposition caused by small aerosol velocities emitted from hydrofluoroalkane-pMDIs with smaller orifice diameters compared to chlorofluorocarbons. Similarly, in a study using monodisperse radiolabelled Albuterol, Usmani, Biddiscombe and Barnes (2005) demonstrated that fast

inhalation (67.1 ± 16.7 L/min) resulted in higher lung deposition than slower inhalation (30.8 ± 4.7 L/min) for particles with a diameter of 1.5 μm. However, the lung deposition of larger particles with a diameter of 3 μm slightly decreased (by 1.4%) at the higher flow rates compared to lower flow rates, with this observation more pronounced for larger particles with a diameter of 6 μm (decreasing by 24.7%). Since Qvar® has an extrafine particle size of ~1.1 microns, these findings support why the faster flow rate used in the present study did not negatively impact drug delivery (Leach, 1998; Rahmatalla *et al.*, 2002). Other formulations with larger particle sizes may be more impacted by higher flow rate.

Although it was not the aim of the present chapter to analyse the impact of device actuation and breath initiation (co-ordination), a study by Biswas, Hanania, and Sabharwal (2017) suggested the most effective way to administer medication from Ventolin® hydrofluoroalkane-pMDI was to inhale at inspiratory flow rates ranging from 60-90 L/min, and to actuate the pMDI during the first half of inspiration. An important finding of that study was that while higher inspiratory flow rates yielded higher lung deposition, the coordination of pMDI actuation was deemed a more important factor governing lung deposition. In that the effect of poor coordination was more impactful than differences in inspiratory flow rate. A VHC add-on device decreases the necessity for simultaneous inhalation and actuation. These observations add support for incorporating a VHC into the prospective chapters of this thesis to better control for poor coordination – and using an ACP-VHC provides specific feedback on flow rate at 30 L/min.

In the current study, inhalation flow rate was only possible to investigate up to 100 L/min [see limitations section]. This speed is above the standard recommended for a pMDI, although it would not be considered "extreme". Moreover, the fastest flow rate investigated in this study is lower than the pMDI peak inhalation speed reported in elite athletes (>300 L/min) (Jackson *et al.*, 2018). In clinic observations, Farr and colleagues (1995) studied radio-labelled salbutamol in asthmatic participants at slow (30 L/min), moderate (90 L/min) and fast (270 L/min) flow rates. They observed similar fractional deposition percentage (indicative of peripheral lung delivery) between 30 and 90 L/min, however, the "extreme" flow rate of 270 L/min resulted in greater mouth-throat deposition and lower lung deposition, but the chlorofluorocarbon formulation should be noted within the study. Mechanistically, a rapid inhalation is not recommended when using pMDI, since it creates a turbulent air flow (Darquenne, 2012; Ibrahim, Verma and

Garcia-Contreras, 2015) which increases the amount of impaction deposition in the upper respiratory tract. As extreme flow rates were not investigated in the present study due to methodological limitations, it is unclear if similar results would have been obtained. Thus far no study has investigated ICS formulations at such extreme flow rates suggesting an upper limit of flow rate may be evident. Therefore, a further extension of this work would be to measure particle size distribution at extreme flow rates using in-vitro and vivo models specifically in an athlete context.

5.4.2. Context In Future Experimental Chapters & Research Projects On Ergogenic Impact

A secondary aim of this chapter was methodological development for prospective experimental chapters for this thesis on the ergogenic impact of ICS on cycling performance. The findings suggest that inhaler administration technique may influence the delivered dose, and consequently could impact on the potential of ergogenic effect. *Chapter 2* highlighted that reporting of inhaler technique in published literature is often weak. So therefore, it should be considered the most appropriate inhaler technique utilised in a research study and avoid reporting vague adjective statements such as 'optimal' technique. A future systematic review on the reporting quality of inhaler technique in research on ergogenic potential of asthma therapy would be advantageous, and within this a specific quality assessment tool developed to assess the study quality relating to inhaler technique using appropriate framework (Whiting *et al.*, 2017).

Despite the current study presenting that the delivered dose of Qvar® is lower than the stated nominal dose, there are important ethical and methodological considerations that must be respected when administering inhaled formulations for research purposes. To meet requirements of institutional insurance cover, it would not be permitted to deliver more than the maximum metered dose recommended by the manufacturer and *'British National Formulary'* during a remote, unsupervised short-term administration study (Qvar®, 800 μg per day – British National Formulary, 2023; Teva UK Limited, 2023). With acute administration, due to closer monitoring of participants in a laboratory setting, the dose can be supratherapeutic. However, due consideration should be given to relevant prior studies; for example, by closely following the dosage used in previous studies utilising acute supratherapeutic doses of BDP (Qvar®, 1500 μg nominal dose) for attenuating exercise-induced bronchoconstriction (Kippelen *et al.*, 2010). Moreover, given the potential for adverse side effects associated with oropharyngeal deposition, it

is advisable to avoid administering high doses of inhaled substances medication orally. To account for some of these considerations, laboratory experiments involving inhaled asthma therapy should adopt the use of a VHC as a practicable solution to standardise administration technique and dosing regimens, in order to ensure more even dosing inter- and-intra participants, as well as providing auditory feedback to standardise flow rate. At present, commercially available VHC are limited to 30 L/min, due to the guidance to inhale as slow as possible with a pMDI (Broeders *et al.*, 2009; Laube *et al.*, 2011; Mitchell, Suggett and Nagel, 2016),

An aim of this chapter was to provide a quantification of the delivered dose to participants in prospective chapters on ergogenic impact of ICS *[Chapters 6 and 7]*. It is expected that approximately 70% of the nominal dose will be delivered into the body. For instance, in Chapter 6, the acute bolus of 1600 µg of BDP proposed is estimated to result in a delivered dose of approximately 1084 µg. Similarly, in Chapter 7, the daily bolus of BDP is estimated to deliver around 542 µg per day *(Table 5.7)*. The remaining, unaccounted-for BDP mass is likely to be deposited within the device's components, such as the actuator orifice, or retained within the AeroChamber Plus chamber.

Table 5.7. Estimation of Beclomethasone dipropionate in prospective experimental work in this thesis.

	Proposed Nominal Dose	Estimated Delivered Dose
Chapter 6	1600 µg (Acute)	1084 µg
Chapter 7	800 µg Daily (Short-term for 14 Days) (Dosed as morning and evening bolus of 400 µg)	542 µg daily

Inhalation is an attractive route of therapeutic drug delivery as it targets the local binding site of the mechanisms of treatment [e.g. downregulation of inflammatory processes or $\beta 2$ adrenoreceptors stimulation for bronchodilator], but also bypasses hepatic first-pass metabolism resulting in reduced drug metabolism and increased bioavailability (Matera *et al.*, 2019). For this reason, in an anti-doping context inhaled delivery of asthma therapy could be seen as an attractive route for an unscrupulous athlete too. Using an abductive

reasoning approach to ergogenic mechanisms of inhaled β2-agonists therapy, Breenfeldt Andersen *et al.*, (2021) presented compelling arguments for inhalation as a route for doping practices, noting *"if the oral route offers a performance-enhancing effect, then the inhaled route will too—it just becomes a matter of dose"*. Whilst that notion fails to consider the complex pulmonary pharmacokinetic processes (Borghardt, Kloft and Sharma, 2018), the ability to deliver drug to the blood/alveoli border could be an important factor that experimental studies should consider when investigating ergogenic effect.

5.4.3. Limitations

The finding of the present study should be interpreted with some methodological considerations. While in vitro methods have their advantages in predicting in vivo deposition, they are not without limitations. Methods such as Cascade Impaction are unable to perfectly replicate in vivo conditions due to factors such as the rigidity of airway models, a lack of heating and humidification of inspired air as within the respiratory system, the inability to predict exhaled doses, and, the stage size-selectivity of an NGI does not match the selectivity of the processes that govern deposition in the human respiratory tract (Dunbar and Mitchell, 2005; Byron *et al.*, 2010). Nevertheless, when carefully designed, in vitro methods can still offer valuable insights into the prediction of in vivo deposition (Ruzycki, 2022). A pertinent strength of this study was the use of an anatomical throat. But more recently, the application of a 'Split Anatomical Throat' enabling regional breakdown of throat deposition – highlighting large proportion of oropharyngeal deposition occurring on tongue and offers additional real data and insight into drug deposition location post pMDI mouthpiece (Potts *et al.*, 2021). Radio labelled drugs allow the drug deposition to be tracked in the lungs, while broncho-scintigraphy and gamma scintigraphy allow the drug deposition to be monitored in real-time and provide a more accurate picture of the drug deposition. These methods all provide valuable insight into the impact of inhaler technique on drug deposition and can help clinicians to improve inhaler technique in order to improve patient outcomes. Moreover, an NGI uses a constant flow rate to classify particle size distribution, which does not commonly reflect in vivo inhalation pattern, or the slowing of air flow through the respiratory tract. In practice, it is unlikely that a patient would perform such technique, with pauses or a progressive profile usually evident (Mitchell, Newman and Chan, 2007). An NGI cannot also simulate breath holding; an important factor with inhaler technique to reduce small particles being exhaled, allowing for greater impaction/sedimentation. To

enable closer in-vivo comparisons, future investigations may need additional equipment, such as a Copley breathing simulator, to investigate different breathing profiles during device actuation, including those from healthy and diseased airways. In addition, in the VHC condition, a time delay between actuation of the inhaler device and breath initiation was not investigated, which may be a technique utilised by patients. The present study only investigated one inhaler formulation (namely solution-based BDP branded as Qvar®).

5.5. Conclusion

In conclusion, this chapter has provided methodological development to inform the study design of prospective experimental chapters in this thesis on the ergogenic impact of ICS treatment on cycling performance. Although there are limitations with cascade impaction techniques for inferring in-vivo deposition, the findings add evidence that inhalation flow rate and VHC affects the aerodynamic properties of inhaled drugs, and therefore could impact the ergogenic effect in exercise performance trials. The main finding indicated that the addition of a VHC was beneficial to reduce oropharyngeal deposition and reduced the flow rate induced variability in fine particle mass delivery compared to using a pMDI alone. As such, using a VHC with auditory feedback can be a practicable solution to attempt standardise inhaler technique and control for inter-intra individual inhalation differences. Despite these results, experimental study design should continue to consider logistics and participant safety.

CHAPTER 6: EFFECT OF ACUTE INHALED & ORAL DOSES OF GLUCOCORTICOIDS ON INITIAL 40-KM CYCLING TIME-TRIAL, AND RECOVERY FOR A SUBSEQUENT 10-KM TIME-TRIAL

BACKGROUND: The World Anti-Doping Agency (WADA) permits inhaled corticosteroids (ICS) at all times, but oral glucocorticoids (GC) are prohibited during competition without a therapeutic-use exemption (TUE). Previous studies on ergogenic effect have used performance outcomes with low ecological validity, and no investigation has explored repeated bout whole-body exercise. **OBJECTIVES:** Compare two administration routes of GC class substances on initial 40-km cycling time-trial (TT) and recovery for a further 10-km TT performed on the same day. **METHODS:** Nine trained male cyclists ($\dot{V}O_2$peak; 58.4 ± 3.2 ml.kg.min^{-1}) completed a 40-km TT (TT$_{40km}$) four-hours after administration of oral prednisolone (0.5 mg.kg^{-1}, OR-GC), inhaled beclomethasone dipropionate (1600 μg, IN-GC), microcrystalline cellulose capsules (OR-PLA), water vapour inhaler (IN-PLA) or control (CON). Physiological (heart rate; HR, oxygen-uptake; $\dot{V}O_2$), and immunosuppressive response (Interleukin-6; IL-6) was assessed. Following one-hour recovery, participants completed a further 10-km TT (TT$_{10km}$). **RESULTS:** No statistically significant difference was seen in completion time (CT) for TT$_{40-km}$ (OR-GC: 4079 ± 252; OR-PLA: 4109 ± 202; IN-GC: 4108 ± 257; IN-PLA: 4102 ± 266; CON: 4096 ± 244 seconds; $p=0.72$, $\eta p^2=0.06$), however, when applying qualitative magnitude-based decision anchors, the improvement in CT was considered '*possibly-beneficial*' [OR-GC] and '*unlikely beneficial*' [IN-GC] compared to respective placebo conditions. No condition*time interaction was seen in physiological response (HR: $p=0.39$; $\dot{V}O_2$: $p=0.43$) during TT$_{40-km}$. OR-GC resulted in significant IL-6 reduction post-TT$_{40km}$ in all conditions, most notably compared to OR-PLA ($p=0.05$) and IN-GC ($p=0.03$). Subsequent TT$_{10-km}$ CT was not significantly different between conditions (OR-GC: 975 ± 57; OR-PLA: 979 ± 54; IN-GC: 977 ± 59; IN-PLA: 984 ± 56; CON: 984 ± 63 seconds; $p=0.67$, $\eta p^2=0.07$). **CONCLUSION:** Current WADA guidelines on ICS are appropriate given lack of ergogenic effect when assessed using an ecologically valid TT assessment. Oral GC should remain controlled with TUE due uncertainly of ergogenic impact, immunosuppressive effects, and well-established long-term health implications.

6.1. Introduction

Elite athletic populations have a high prevalence of exercise-induced bronchoconstriction (EIB) (Price, Sewry, *et al.*, 2022) and *Chapter 4* added to evidence that athletes mandate the use of pharmacological asthma treatment such as ICS to manage the condition (Price, Walsted, *et al.*, 2022), or oral GC in the event of serious emergency exacerbation (BNF, 2023b). At present [2023], the World Anti-Doping Agency (WADA) prohibited list allows the use of ICS [e.g., beclomethasone dipropionate] at all times when used within the manufacturer's licensed doses and therapeutic indications, as stipulated in the *'Glucocorticoid S9'* substance class. However, oral GC administration routes [e.g., prednisolone] are permitted outside of competition periods, but during competition they are subject to controlled usage through the TUE process (WADA, 2023e, 2023d). Prevalence data indicates that oral and inhaled GC are frequently used by athletes (Fitch, 2016), commonly prescribed by sports medicine physicians (Hughes *et al.*, 2020; Vernec *et al.*, 2020), and are dispensed by pharmacy services at major games (Stuart, Kwon and Rhie, 2019). Yet in 2022, WADA removed GC from its *'Monitoring Program'* citing it had gathered the required prevalence data on their use (WADA, 2022b).

Despite this recent change in policy, the ergogenic properties of GC treatment on athletic performance have previously been investigated in a limited capacity, and those completed subjected to recent systematic review and meta-analysis (Trinh, Chen and Diep, 2022; Riiser, Stensrud and Andersen, 2023). Although there are relatively few studies in the field, the consensus is that short-term oral administration may significantly improve endurance performance (Arlettaz *et al.*, 2007; Collomp *et al.*, 2008; Le Panse *et al.*, 2009; Casuso *et al.*, 2014). Proposed physiological and psychological mechanisms include altering substrate utilisation to increase fat oxidation during exercise (Arlettaz, Portier, *et al.*, 2008), and inducing neuro-stimulatory effects such as increasing the feeling of euphoria (Dubovsky *et al.*, 2012). By contrast, acute oral GC administration have been shown to not statistically improve exercise performance (Petrides *et al.*, 1997; Arlettaz, Collomp, *et al.*, 2008; Arlettaz, Portier, *et al.*, 2008), however, relatively low doses were used, and the smallest worthwhile improvement, which may be of interest to elite athletes, has not been explored. Moreover, only four studies have used an inhaled administration route (Jardim *et al.*, 2007; Kuipers *et al.*, 2008; Schwindt *et al.*, 2010; Hostrup *et al.*, 2017), and no study has investigated impact on exercise performance following acute supratherapeutic inhaled doses akin to those previously shown to be protective against bronchoprovocation (Kippelen *et al.*, 2010).

Previous observations suggest the varied response in the literature can be attributed to methodological choices in study design such as exercise modality, dose, administration route (Collomp *et al.*, 2016; Tacey *et al.*, 2017). Notably, the performance outcomes used in former investigations have lower external or ecological validity (i.e., time-to-exhaustion (TTE), handgrip strength, and incremental maximal tests) (Hopkins, Schabort and Hawley, 2001; Laursen *et al.*, 2007; Coakley and Passfield, 2018), and if used with untrained, non-cyclist cohorts can reduce the reliability of the performance tests (Currell and Jeukendrup, 2008). No previous study has used time-trials (TT) to explore ergogenic effect of asthma-related GC.

Another proposed ergogenic mechanism observed in GC therapy is the attenuation of the post-exercise pro-inflammatory response (Arlettaz, Collomp, *et al.*, 2008), thought to lead to improved recovery between successive bouts of exercise (Allen *et al.*, 2019). However, the latter has also yet to be explored.

Oral and inhaled routes of administration for asthma therapy result in different bioavailability and pharmacokinetics of drug metabolites, leading to distinct WADA legalities (Elers *et al.*, 2012; Dyreborg *et al.*, 2016). In the context of anti-doping literature relating to asthma therapy, only one previous study has investigated oral and inhaled administration routes on exercise performance within a single study, albeit with β2-agonist substances (Eibye *et al.*, 2021). Notably, this type of comparative analysis has not been studied with glucocorticoid class asthma treatment.

The objectives of the present study were threefold: [1] to address a key limitation of previous investigations and assess in a single study the potential ergogenic effects of acute oral and inhaled glucocorticoid administration within an ecologically valid performance protocol that closer resembles real-world conditions, specifically, a 40-km cycling TT; [2] Investigate the recovery for a subsequent bout of competitive cycling (10-km TT) performed on the same day; and [3] to examine the physiological and perceptual responses to these bouts of exercise related to theorised ergogenic mechanisms.

6.2. Materials and Methods

6.2.1. Ethics Statement

Institutional ethics approval was obtained from the University of Kent Faculty of Sciences Research Ethics Committee (Prop 73_2018_19). Before providing written consent, participants were informed of the associated risks and experimental protocols, but not the hypotheses.

6.2.2. Participants

Sample Size Calculation

An *a priori* sample size calculation based on the primary outcome (completion time of TT_{40km}), for the proposed within-subject, five repeated measures (one-way ANOVA) analysis, incorporating conventional parameters of alpha level (α) 0.05, statistical power ($1 - \beta$) of 0.80, and attempt to detect a moderate to large effect size partial-eta^2 of 0.13 (Bakeman, 2005) was undertaken. These parameters indicated that 10 participants would provide adequate power (G*Power software package, Version 3.1.9.4, Kiel University, Germany). Twelve participants aimed to be recruited to allow for possible participant attrition.

Inclusion/Exclusion Criteria

Participants were recruited through convenience sampling with snowball effect via physical posters, online advertisements, and word-of-mouth referrals. Inclusion criteria required participants to be 18–50 years of age, participate in a minimum of 3 sessions per week cycling-related endurance training (i.e., cycling, triathlon, duathlon) and have a $\dot{V}O_2peak$ of ≥55 ml.kg.min^{-1}. This criteria was based on 'Performance Level 3' representing trained subject groups as recommended by De Pauw *et al.,* (2013).

Exclusion criteria were a diagnosed asthma-related condition (clinical asthma or EIB), or objective evidence of EIB assessed through change of forced expiratory volume one in second (FEV_1) following maximal exercise (Rundell and Slee, 2008; Weiler *et al.*, 2016). Participants needed to be otherwise healthy (i.e., no chronic health conditions such as cardiovascular, neurological, or metabolic disease), and free of illness or musculoskeletal injury leading up-to, and for duration of the study. Eligible participants completed a health screening questionnaire, which was screened and approved by the collaborating physician.

Participants were naïve users of inhaled and oral glucocorticoids and were not in possession of a TUE for any substance. Moreover, to avoid possible doping violations arising from participation in this research, volunteers were asked to not compete in events where doping control was in force for the complete duration of the study (and a further seven-days following completion).

6.2.3. Experimental Overview

Data collection for the present study took place between August 2019 and March 2020. All participants attended the University of Kent Exercise Physiology laboratory on seven

occasions for: preliminary incremental exercise test [Visit 1], experimental familiarisation [Visit 2], and five experimental trials [Visit 3-7]. The experimental trials were completed in a randomised, crossover, single-blinded design. All visits were separated by 7 days to avoid carry over effects of acute glucocorticoid administration (Czock *et al.*, 2005; Mazzarino *et al.*, 2006), and conducted at the same time of day (08:00 AM $\pm$ 30 minutes) to control for circadian rhythm of cortisol (Thuma *et al.*, 1995). Participants were instructed to refrain from vigorous physical activities within 48 hours before any laboratory visits. They were also asked to avoid consuming alcohol, caffeine, and any anti-inflammatory or pain-relieving medications (such as paracetamol or ibuprofen) for a period of 24 hours prior to testing. In order to standardise the metabolic state pre-trial, participants were instructed to maintain a food diary, recording their dietary intake for the 24 hours preceding the first experimental visit, and asked to replicate this exactly for subsequent trials. Additionally, participants were able to consume breakfast following the pre-test measures, but it was mandated that they replicate the meal and timing precisely. All testing took place under conducted controlled conditions at (19.8 $\pm$ 2.1°C, relative humidity 52.1 $\pm$ 5.1%). The study design, testing protocol and timing of outcome measures are presented in *Figure 6.1.*

6.2.4. Preliminary Testing (Visit 1) and Familiarisation (Visit 2)

Before undertaking experimental trials, all subjects underwent an incremental exercise test on a cycle ergometer (Cyclus 2, Avantronic, Leipzig, Germany) to determine maximal aerobic power ($\dot{V}O_2$peak), which was used to determine participant suitability and prescribe a sub-maximal exercise intensity for the experimental sessions. In brief, following a 10-minute warm-up at 100 W, the power output increased at a ramped rate equivalent to 25 W every minute until voluntary exhaustion, with continuous cardiorespiratory responses monitored using wireless telemetry (Garmin HRM-Dual, Garmin, Olathe, USA) and indirect calorimetry (Cortex Metalyzer 3B, Biophysik, Leipzig, Germany) [Visit 1]. From this, the power output that would elicit 50% of $\dot{V}O_2$peak was determined for each participant.

Within one week of the incremental exercise test, participants underwent a separate familiarisation trial [Visit 2] of the experimental procedures to mitigate the potential confounding learning effects of repeated time trial performances (Laursen, Shing and Jenkins, 2003) and acquaint them with validated questionnaires.
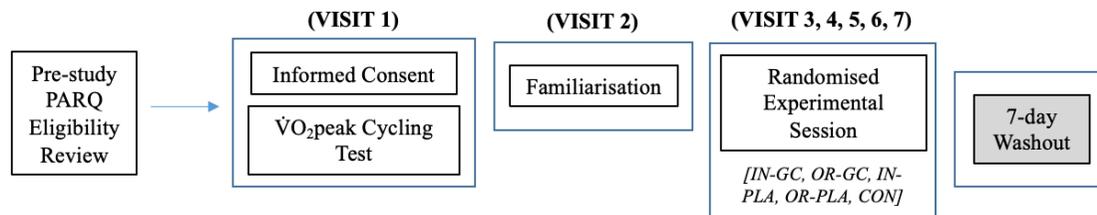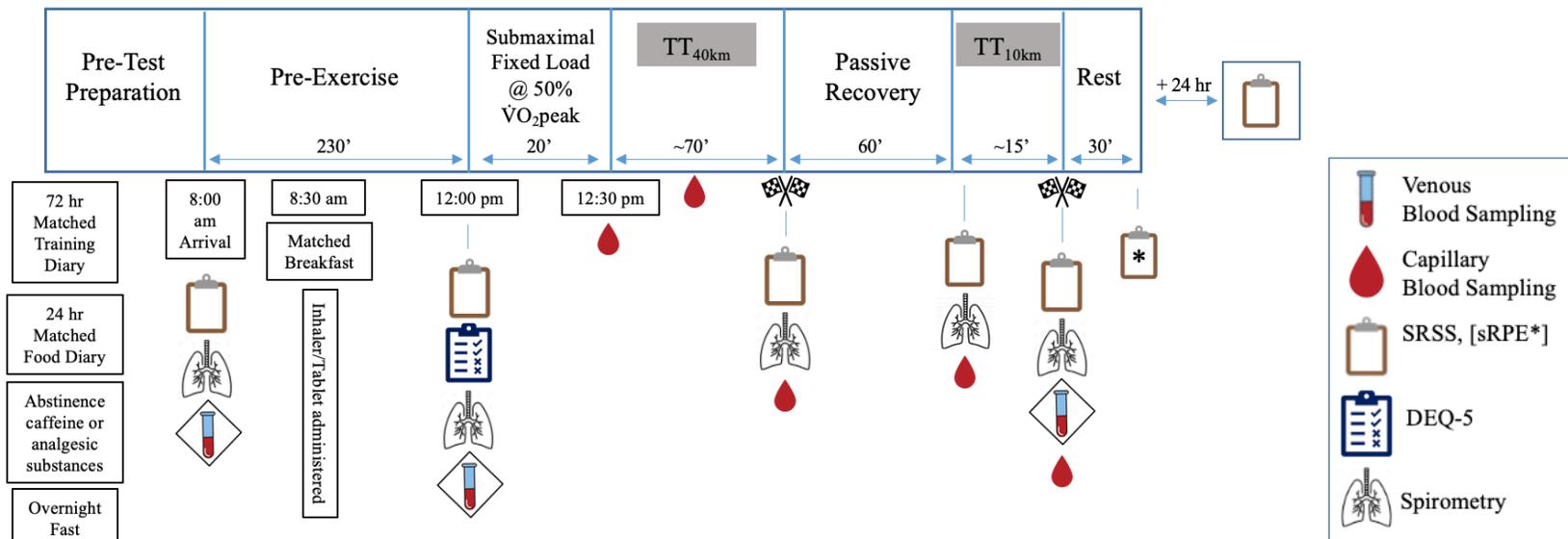
Figure 6.1. *Schematic of preliminary testing and experimental procedures. Three-and-a-half hours following administration of either 1600 μg inhaled beclomethasone dipropionate (IN-GC), water vapour inhaler (IN-PLA), 0.5 mg.kg^{-1} prednisolone (OR-GC), microcrystalline cellulose capsule (OR-PLA), or control (CON), participants completed 20-minutes of fixed intensity cycling followed by a 40-km self-paced time trial (TT$_{40km}$). After a further one-hour passive recovery, participants completed a further 10-km time trial (TT$_{10km}$).*

6.2.5. Experimental Procedure (Visit 3-7)

On the morning of the experimental visits, participants arrived at the laboratory following an overnight fast and having followed strict preparation instructions outlined above.

Glucocorticoid and Placebo Administration

Three-and-a-half hours before the initiation of exercise, participants self-administered either:

- 1600 µg inhaled beclomethasone dipropionate (IN-GC) - (16 x Qvar ® 100 µg pressured metered dose inhaler (pMDI), Teva UK Limited, Castleford, UK).

- Placebo water vapour inhaler (IN-PLA) - (16 x Vitalograph Ltd, Buckinghamshire, UK).

- 0.5 mg.kg^{-1} prednisolone (OR-GC) - (Accord Healthcare Ltd, Barnstaple, UK) [range administered 30 – 45 mg].

- Placebo microcrystalline cellulose capsule (OR-PLA) - (Redwells Creative Ltd, Berkshire, UK).

- Control condition (CON) - (where neither a drug nor placebo was administered. During the control trial, no substance was provided, however the same time duration elapsed before commencing exercise).

This administration timing was selected to match previous investigations of oral GC (Arlettaz, Collomp, *et al.*, 2008), and to elicit maximum pharmacokinetic activity during the testing period (Coll *et al.*, 2021).

Blinding and Inhaler Administration Technique

Oral administration conditions (OR-GC and OR-PLA) were encapsulated into identical gelatine capsules, and quantity matched *(Figure 6.2)*. Inhaled administration conditions (IN-GC and IN-PLA) were dispensed from similarly coloured metered dose casings. Due to the taller canister of the placebo inhaler, a taller casing was sourced. In attempt to strictly regulate the administration of IN-GC and reduce reliance on the coordination device actuation and inhalation, the inhaled conditions were administered through a valved holding chamber (AeroChamber Plus™, Trudell Medical International, Ontario, Canada) (ACP-VHC) that incorporated auditory feedback at a flow rate of ~30 L/min. The ACP-VHC was blacked out to blind visual differences in expelled vapour from the inhaler *(Figure 6.2)*. Inhalation technique adopted a single, slow and deep inhalation, followed by a ten second breath hold for pMDI administration (Haidl *et al.*, 2016), repeating this for the required dosing.

Figure 6.2. Experimental conditions used during the study.

Submaximal Preloaded Exercise – Substrate Utilisation

Prior to undertaking the initial TT_{40km} performance protocol, participants completed a preloaded 20-minute warm-up corresponding to power output which would elicit 50% of $\dot{V}O_2peak$ (153 ± 27 W) using an electromagnetically braked ergometer (Cyclus 2, Avantronic, Leipzig, Germany) aimed at providing a standardised low-intensity exercise bout for assessing substrate utilisation [Aim 3]. Respiratory gas exchange measurements were measured continuously by indirect calorimetry (Cortex Metalyzer 3B, Biophysik, Leipzig, Germany). The first 5 minutes were excluded to allow steady state to be achieved, then $\dot{V}O_2$ and $\dot{V}CO_2$ averaged between minutes 5-20 to determine carbohydrate oxidation (CO) and fat oxidation (FO) using stoichiometric equations whereby it is assumed urinary nitrogen excretion is negligible (Frayn, 1983). This set of equations has previously been used to investigate substrate utilisation following oral glucocorticoid intake (Arlettaz, Portier, *et al.*, 2008).

Initial 40-km TT Performance [Aim 1]

Following the pre-loaded exercise *(four hours post-substance administration),* participants then undertook a 40-km time trial (TT_{40km}) [Aim 1] in which they were blinded to all performance data feedback except for distance elapsed and instructed to complete the TT in the shortest possible time. The TT started at the lowest gear ratio, and participants could change virtual gearing as needed. Cardiorespiratory responses were continuously monitored using wireless telemetry (Garmin HRM-Dual, Garmin, Olathe, USA) and indirect calorimetry (Cortex Metalyzer 3B, Biophysik, Leipzig, Germany). The calorimetry mask (Hans Rudolf Inc, Kansas City, USA) was removed at 10, 20 and

30-km time-points to allow participants to drink water [with volume and timing matched across sessions]. Due to this, gas exchange measurements between 10-12 km, 20-22 km and 30-32 km were omitted from cardiorespiratory analysis. Rating of Perceived Exertion (RPE, 6 – 20 scale; (Borg, 1982)) and perceived muscle pain (MP, 10-point numerical scale; (Cook *et al.*, 1997) were verbally reported every 5-km.

Subsequent 10-km TT Performance [Aim 2]

Participants then rested passively for 1-hour in the laboratory, before completing a 5-minute self-paced warm-up, followed by a further 10-km TT (TT_{10km}) [Aim 2]. RPE and MP noted every 2-km.

Supplementary Outcomes: Spirometry and Validated Psychometric Questionnaires [Aim 3]

Upon arrival to the laboratory, immediately before and after each TT, participants underwent spirometry testing in accordance with procedure guidelines (Graham *et al.*, 2019) [protocol outlined in *Chapter 3.4*], and completed validated questionnaires relating to muscular stress and recovery (Short Recovery and Stress Score; SRSS) (Kölling *et al.*, 2020). Thirty minutes following completion of TT_{10km}, session RPE (sRPE) (Foster *et al.*, 2001) and SRSS was assessed. Additionally on the following day at 08:00 am (± 30 minutes), a final assessment of SRSS was obtained.

Reporting of Drug Effects, Adverse Events and Blinding Integrity

After the administration period, participants completed the Drug Effects Questionnaire (DEQ-5) to reflect on any pharmacologically-induced effects (Morean *et al.*, 2013). This five-item validated measure requires participants to indicate on a visual analog scale (VAS, 0 – 100 mm) the extent they "feel", "are high from", "like", "dislike", and would want "more" of the substance they have taken. The VAS is anchored from "Not at all" to "extremely".

To assess the integrity of blinding, participants were asked to report the condition they believed they had just experienced and provide a qualitative statement explaining their decision. Throughout and after each experimental visit, participants were expected to report any adverse events to the principal researcher.

Blood Sampling and Analyses

Blood sampling by capillary action was used for the measurement of glucose (B[Glu]) and lactate (B[La]) concentrations [Aim 3]. B[La] and B[Glu] concentrations was

determined upon arrival, before TT_{40km}, every 10-km of TT_{40km}, and immediately after the TT_{40km}. Further samples were taken before and immediately after TT_{10km}. Values are reported values as millimoles per litre ($mmol/L^{-1}$). To assess the immunosuppressive effect of GC administration on exercise-induced inflammation [Aim 3] plasma Interleukin 6 (IL-6) concentrations were determined from venous blood samples pre- and post-TT_{40km}. Values are reported in pg/mL (picograms per millilitre). Detailed information on the collection and analysis of blood sampling outcomes are provided in *Chapter 3.13 and 3.14.*

6.2.6. Data and Statistical Analysis

Descriptive values were obtained and are reported as mean ± standard deviation (SD) unless stated otherwise. Data analysis was performed using statistical package SPSS (SPSS v29, IBM, New York, USA) and figures created using Graph Pad Prism (v10, GraphPad Software Inc, San Diego, California, USA).

To assess the impact of OR-GC and IN-GC administration on the main outcome of TT_{40km} [Aim 1] and TT_{10km} [Aim 2] completion time, a one-way analysis of variance (ANOVA) with five 'treatment' levels (CON, IN-GC, OR-GC, IN-PLA, OR-PLA) was used. In addition to investigate whether there was a meaningful change in TT_{40km}, the smallest-worthwhile change (SWC) was estimated at approximately 0.6 to 0.7% as previously reported in high-level cyclists (Paton and Hopkins, 2006; Lamberts *et al.*, 2009), equating to an improvement of ~29 seconds based on pilot work. To estimate the likelihood that PRED and BDP treatment would have a beneficial, trivial, or negative effect on performance, magnitude-based decisions using mean difference and confidence intervals (95%) were interpreted from p-value derived calculations using excel spreadsheet by Hopkins (2007) (Hopkins, 2007) downloaded from (http://www.sportsci.org/2007/wghinf.htm). Thresholds for assigning qualitative terms for the chance of substantial effects were: <1% almost certainly not; <5% very unlikely; <25% unlikely; <50% possibly not; >50% possibly; >75% likely; >95% very likely; >99% almost certain (Batterham and Hopkins, 2006). This approach has been used previously to investigate ergogenic effect of an intervention on time-trial performance (Spence *et al.*, 2013).

For the secondary outcomes, differences in power output and physiological responses at each TT section were assessed using a two-way repeated measures ANOVA with five 'Treatment' factors (CON, IN-GC, OR-GC, IN-PLA, OR-PLA) and eight 'Time' factors

(elapsed distances of 5, 10, 15, 20, 25, 30, 35, 40-km). Furthermore, this same approach was used for 'Time' factors relating to questionnaire responses (SRSS), and blood analysis [IL-6] [Aim 3] (pre-treatment, pre-post each exercise bout, 30-mins post completion, and 24hr post-treatment). If a significant main effect or interaction was observed, post-hoc pairwise comparisons with Tukey correction was used to interpret location of effect. Significance level was set at $p < 0.05$ for all tests. Assumptions of normality and sphericity were assessed using Shapiro Wilk Test and Mauchly's Test of Sphericity. Data that was not normally distributed was log-transformed (BLa, BGlu, IL-6). Data that violated sphericity had a Greenhouse-Geisser correction applied. Partial eta-squared (η_p^2) was calculated as an estimation of effect size and interpreted as small (<0.09), medium (>0.09-0.25), or large (≥ 0.25) (Cohen, 1988).

6.3. Results

Participants

Twelve participants enrolled onto the study, however, nine participants (age; 30 ± 8 years, height; 176.3 ± 8.2 cm, body-mass; 73.4 ± 9.8 kg, $\dot{V}O_2$peak; 58.5 ± 3.6 mL.kg^{-1}.min^{-1}, power-output at $\dot{V}O_2$peak; 346 ± 45 W; cycling exercise 7.2 ± 3.6 h.wk^{-1}) fully completed all experimental sessions (n=1 excluded as could not commit to experimental visits, n=2 did not start due to COVID-19 global pandemic ceasing data collection) *(Figure 6.3).* All participants demonstrated >80% predicted baseline FEV$_1$ (103.1 $\pm$ 9.4%) and no significant decrease in FEV$_1$ was evident following maximal exercise (0.14 $\pm$ 2.7%).

Participants demonstrated high intra-class correlation coefficient (ICC) in completion time between the CON and familiarisation trials for TT$_{40km}$ (0.896, 95% CI; 0.632 – 0.975) and TT$_{10km}$ (0.978, 95% CI; 0.916 – 0.995). Across all experimental visits, the typical error of measurement (coefficient of variation) between trials was $1.08 \pm 0.46\%$ for TT$_{40km}$ and $1.23 \pm 1.01\%$ for TT$_{10km}$.
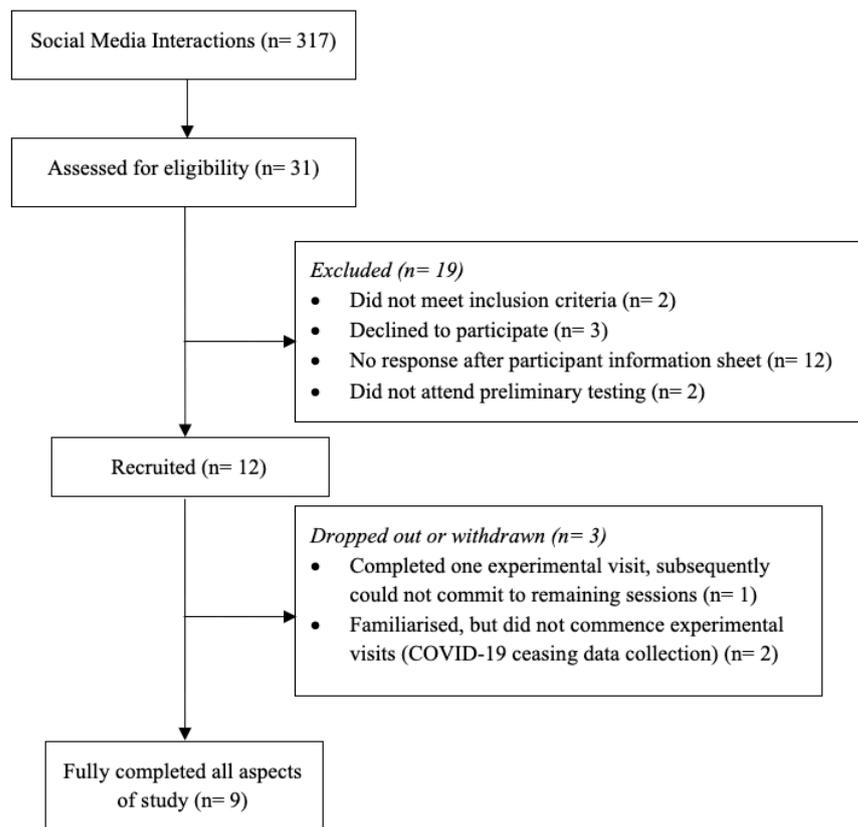
Figure 6.3. *Flow chart detailing participant recruitment and drop-out.*

Study Aim 1: Initial 40-km Time-Trial Performance

Initial 40-km Time-Trial (TT1) Performance Outcomes

There was no main effect of condition observed in TT_{40km} completion time ($F_{(4,32)}$ = 0.516, p = 0.724, ηp^2 = 0.06) between the OR-GC (4079 ± 252 sec), OR-PLA (4109 ± 202 sec), IN-GC (4108 ± 257 sec), IN-PLA (4102 ± 266 sec) or CON (4096 ± 244 sec) conditions. Overall and individual responses for completion time are presented in *Figure 6.4a.*

Compared to their respective placebo conditions (OR-PLA and IN-PLA), the meaningful change in performance (threshold of >0.6%) was considered *"possibly beneficial, possibly not trivial"* following OR-GC [-29.3, -87.0 to 28.3] (MDB; harmful 2.4%; trivial 47.1%; beneficial 50.5%), and *"unlikely beneficial, likely trivial"* from IN-GC administration [5.7, -44.3 to 55.7] (MDB: harmful 7.4%; trivial 76.9%; beneficial 15.7%). Mean change, 95% confidence intervals (CI) and boundaries of SWC are presented in *Figure 6.4b.*

There was no significant main effect of condition between OR-GC (215 ± 40 W), OR-PLA (210 ± 32 W), IN-GC (211 ± 38 W), IN-PLA (211 ± 41 W), and CON (212 ± 37 W) ($F_{(4,32)}$ = 1.025, p = 0.410, ηp^2 = 0.11). Whilst there was a significant main effect of

power output over time categorised by an 'end-spurt' at between 35 to 40-km ($F_{(1.778, 14.225)} = 8.120$, $p = 0.005$, $\eta p^2 = 0.50$), this was not dependant on the interaction between condition and time ($F_{(28,224)} = 1.123$, $p = 0.313$, $\eta p^2 = 0.12$). Distance elapsed mean power output is shown in *Figure 6.4c*.
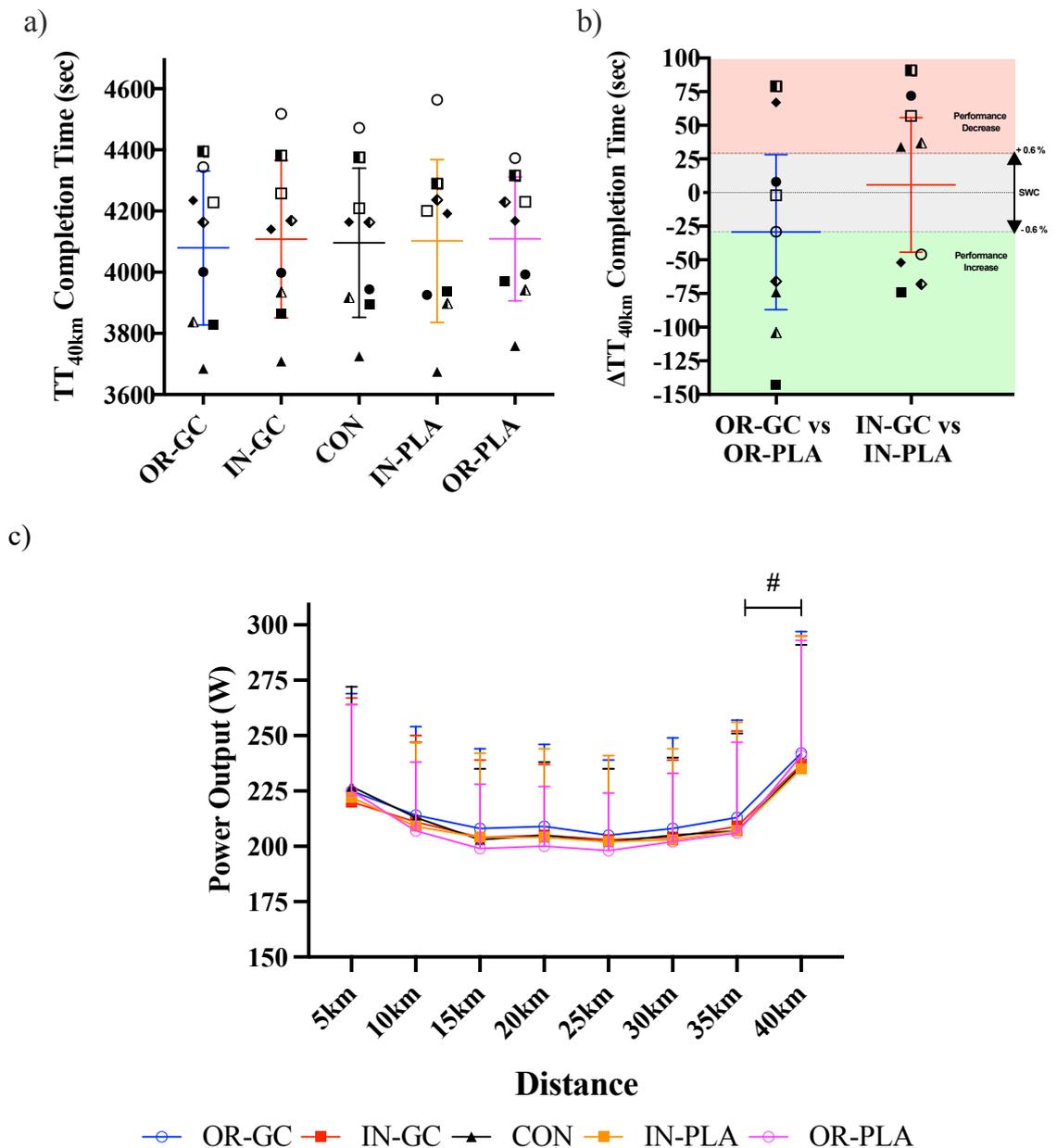
Figure 6.4. Completion time of 40-km time trial (TT) [a] presented as individual performance (shapes), the condition mean (centre line), and standard deviation (top/bottom error bars) in oral prednisolone (0.5 mg.kg-1, OR-GC), inhaled beclomethasone dipropionate (1600 µg, IN-GC), microcrystalline cellulose capsules (OR-PLA), water vapour inhaler (IN-PLA) or control (CON) conditions. [b] Mean difference ± 95% confidence intervals in TT_{40km} completion time between active [OR-GC, IN-GC] and respective placebo [OR-PLA, IN-PLA] condition. The grey zone represents the threshold for smallest meaningful change (SWC) in performance time of 0.6%. [c] Power output averaged for each 5-km section of the 40-km TT. # signifies significant within condition post-hoc pairwise comparison effect of time.

Physiological and Perceptual Outcome Measures during initial 40-km cycling time-trial (TT1) [Aim 3]

A significant main effect of time was observed in TT_{40km} for all physiological parameters ($\dot{V}O_2$, $\dot{V}_E$, RER, HR) and perceptual variables (RPE, PAIN). There were no significant main effects or interactions between time and condition for HR, RER, PAIN, and RPE during the TT_{40km}. Additionally, a significant main effect of condition was observed for $\dot{V}O_2$ and $\dot{V}_E$; however, Tukey corrected post-hoc multiple comparisons did not reveal any significant differences between conditions or interactions between time and condition for these variables. Tables, figures, and repeated measures ANOVA statistics detailing physiological and perceptual outcomes during TT_{40km} can be found in *CHAPTER 11:Appendix J: Supplementary Table 11.9.*

Study Aim 2: Effect short-term daily ICS treatment on the recovery for a subsequent 10-km time-trial performed on the same day.

Subsequent 10-km Time-Trial (TT2) Performance Outcomes

There was no significant difference observed in TT_{10km} completion time ($F(1.912, 15.295) = 0.596$, $p = 0.668$, $\eta p^2 = 0.07$) between the OR-GC (975 ± 57 sec), OR-PLA (979 ± 54 sec), IN-GC (977 ± 59 sec), IN-PLA (984 ± 56 sec) or CON (984 ± 63 sec) conditions.

Whilst there was a significant main effect of power output over time ($F(1.769, 14.155) = 5.188$, $p = 0.023$, $\eta p^2 = 0.39$), there was not a condition (OR-GC; 243 ± 44 W, OR-PLA; 235 ± 39 W, IN-GC; 242 ± 44 W, IN-PLA; 237 ± 42 W, CON; 238 ± 47 W, ($F(1.657, 13.255) = 1.067$, $p = 0.359$, $\eta p^2 = 0.12$) or interaction effect observed ($F(4.399, 35.190) = 1.204$, $p = 0.327$, $\eta p^2 = 0.13$). Individual responses for completion time and mean power output are shown in *Figure 6.5a & Figure 6.5b* respectively.

a)
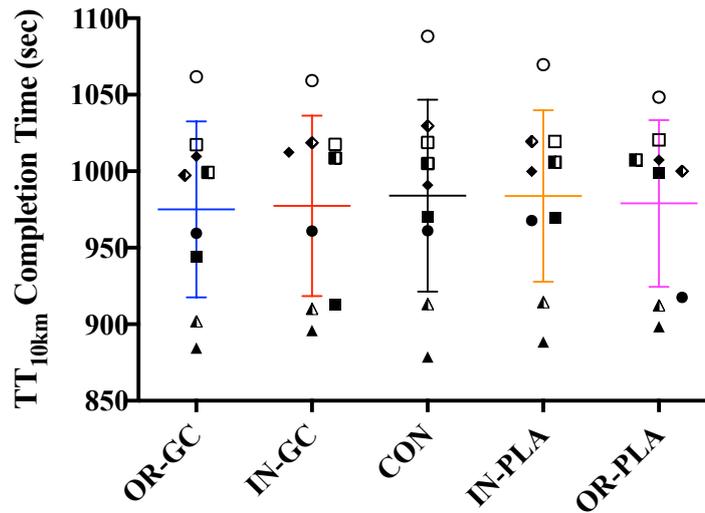
b)
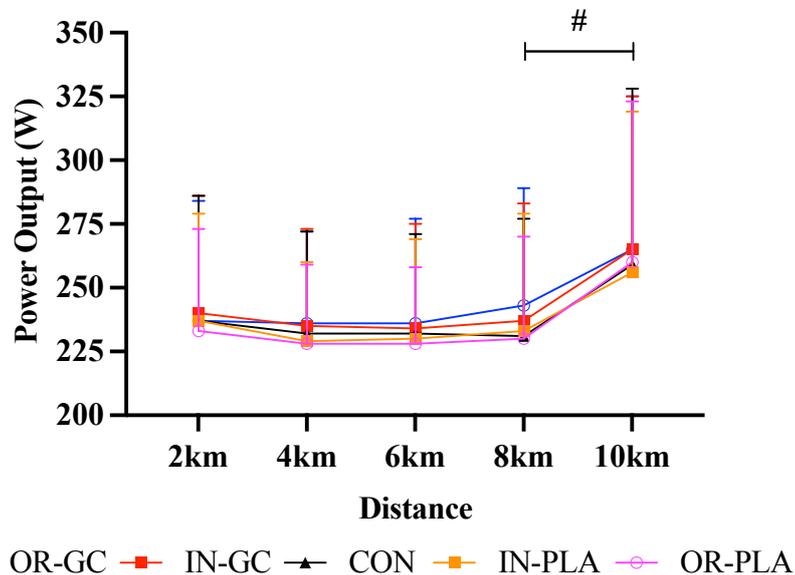
Figure 6.5. *Subsequent 10-km time trial completion time (a) presented as individual performance [shapes], mean [centre line], and standard deviation [error bars]. (b) Displays the power output averaged for each 2-km section of the 10-km TT in the oral prednisolone (0.5 mg.kg⁻¹, OR-GC), inhaled beclomethasone dipropionate (1600 μg, ICS), microcrystalline cellulose capsules (OR-PLA), water vapour inhaler (IN-PLA) or control (CON) conditions. # signifies significant within condition post-hoc pairwise comparison effect of time.*

Study Aim 3: Ergogenic Action Mechanisms

Psychometric Outcomes of Stress and Recovery (SRSS)

A significant main effect of time across the experimental visits was observed in SRSS subdomains, *Muscular Stress, Lack of Activation, Negative Emotional State, Overall Stress, Physical Performance Capability, Emotional Balance, and Overall Recovery.* However, when the Tukey pairwise correction was applied, CON did not exhibit significantly higher level of stress than IN-PLA and OR-PLA conditions. All other SRSS subdomains had no significant main condition effects or interactions between time and

condition. Mean $\pm$ SD data and repeated measures ANOVA statistics relating to SRSS can be found in *Appendix J: Supplementary Table 11.10.*

Session Rating of Perceived Exertion (sRPE)

No significant main effect of condition was seen in sRPE between OR-GC (8.72 ± 0.83 A.U.), OR-PLA (8.33 ± 0.90 A.U.), IN-GC (8.61 ± 0.82 A.U.), IN-PLA (8.33 ± 0.86 A.U.) or CON (8.44 ± 1.0 A.U.) conditions ($F_{(4, 32)} = 1.178$, $p = 0.339$, $\eta p^2 = 0.13$).

Capillary Blood Lactate and Glucose

For Lactate, there no significant main effect for condition ($F_{(4, 32)} = 2.13$, $p = 0.100$, $\eta p^2 = 0.21$), but was a significant main effect of time ($F_{(7, 56)} = 21.47$, $p < 0.001$, $\eta p^2 = 0.73$). The interaction between condition and time was not significant ($F_{(28, 224)} = 1.05$, $p = 0.398$, $\eta p^2 = 0.13$, *Figure 6.6a*).

Regarding glucose levels, a significant main effect was observed for condition ($F_{(4, 32)} = 14.39$, $p < 0.001$, $\eta p^2 = 0.64$), time ($F_{(7, 56)} = 8.44$, $p < 0.001$, $\eta p^2 = 0.51$), and the condition*time interaction ($F_{(28, 228)} = 3.51$, $p < 0.001$, $\eta p^2 = 0.30$). However, when applying the Tukey correction for post-hoc pairwise comparisons, significant interactions were solely evident for OR-GC during the TT_{40km} at 10km (OR-PLA: $t = 5.70$, $p = 0.04$), 20km (CON: $t = 6.48$, $p = 0.02$; IN-PLA: $t = 6.42$, $p = 0.02$; OR-PLA: $t = 9.12$, $p = 0.02$), and 40km (OR-PLA: $t = 5.84$, $p = 0.04$) timepoints. Additionally at the PRE-TT_{10km} timepoint OR-GC was significantly higher versus CON ($t = 6.95$, $p = 0.01$), IN-PLA ($t = 6.22$, $p = 0.02$), and OR-PLA ($t = 6.53$, $p = 0.02$) conditions, as shown in *Figure 6.6b.* All other condition*time permutations were non-significant when accounting for multiple comparisons.
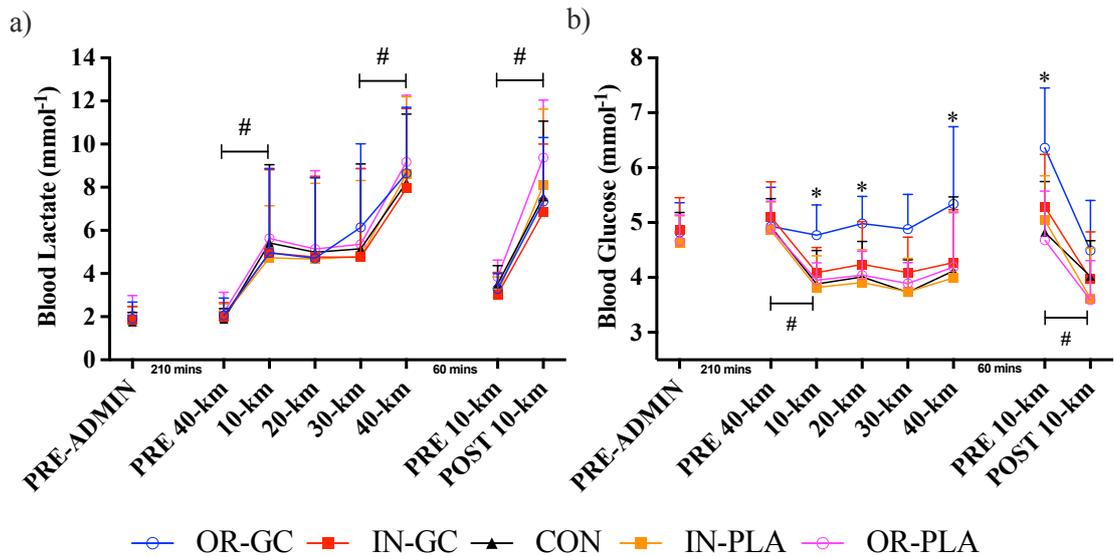
Figure 6.6. *Capillary blood lactate (a) and glucose (b) before and after the initial 40km and subsequent 10km cycling time-trials. Oral prednisolone (0.5 mg.kg-1, OR-GC), inhaled beclomethasone dipropionate (1600 µg, IN-GC), microcrystalline cellulose capsules (OR-PLA), water vapour inhaler (IN-PLA) or control (CON) conditions. * Indicates significant difference between conditions. # signifies significant within condition post-hoc pairwise comparison effect of time.*

Interleukin-6 (IL-6)

Venous blood samples were successfully obtained in all conditions and time points from eight participants (n=8). There was a significant main effect of condition ($F_{(1.80, 12.59)}$ = 15.63, $p < 0.001$, $\eta p^2 = 0.69$) and time ($F_{(1.00, 7.00)} = 36.76$, $p < 0.001$, $\eta p^2 = 0.84$). Additionally, there was a significant interaction between condition*time ($F_{(1.61, 11.27)}$ = 7.18, p = 0.01, $\eta p^2 = 0.50$). Pairwise comparisons using Tukey correction revealed that there was no significant difference between any conditions before substance administration ($p > 0.05$). The TT_{40km} induced a significant increase in IL-6 concentrations in all conditions (IN-GC: $t = -5.12$, $p = 0.02$; CON: $t = -7.24$, $p = 0.01$; IN-PLA: $t = -5.70$, $p = 0.01$; OR-PLA: $t = -7.38$, $p = 0.01$), except OR-GC ($t = -2.42$, $p = 0.42$). Moreover, OR-GC administration resulted in significantly lower IL-6 concentrations when compared to all other conditions at the post TT_{40km} timepoint (IN-GC: $t = -4.63$, $p = 0.04$; CON: $t = -4.50$, $p = 0.04$; IN-PLA: $t = -4.46$, p = 0.04; OR-PLA: $t = -4.31$, $p = 0.05$). Absolute values are presented in *Figure 6.7*.
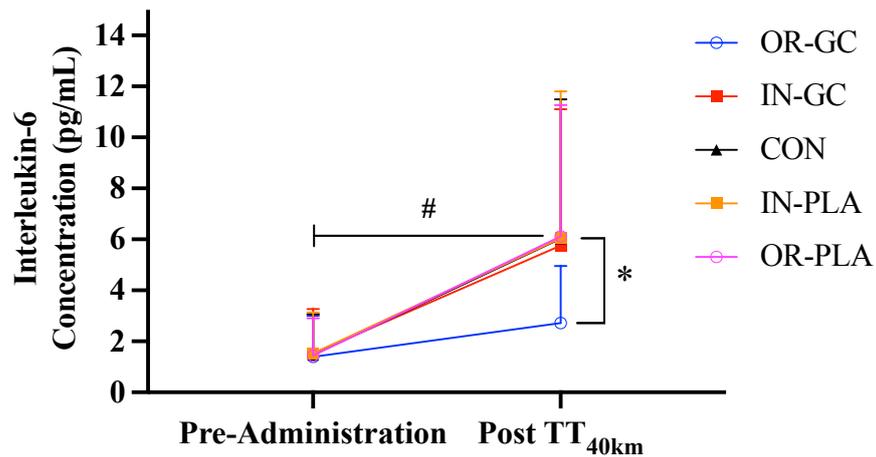
Figure 6.7. Absolute plasma interleukin-6 (IL-6) concentration pre administration and post 40km time trial exercise following acute oral prednisolone (0.5 mg.kg-1, OR-GC), inhaled beclomethasone dipropionate (1600 μg, IN-GC), microcrystalline cellulose capsules (OR-PLA), water vapour inhaler (IN-PLA) or control (CON) conditions. * Indicates significant difference between conditions. # denotes significant difference within condition from pre-administration values.

Submaximal Pre-loaded Exercise

There was no significant difference in any substrate utilisation, cardiorespiratory and perceptual response outcomes during the pre-loaded submaximal cycling at 50% of $\dot{V}O_2$peak between OR-GC, OR-PLA, IN-GC, IN-PLA or CON conditions ($p > 0.05$; *CHAPTER 11:Appendix J: Supplementary Table 11.11).*

Adverse Effects, Drug Effects and Blinding

No participants experienced serious adverse effects. Common side-effects were reported by a total of three participants (33%) during an active drug condition. From OR-GC, two participants (22%) reported a mild headache, with one of those also having feelings of nausea. Following IN-GC administration two participants (22%) experienced a mild headache.

In accordance with the validation of the DEQ-5 (Morean *et al.*, 2013), there is difficulty including participants in analysis who report low experience of a drug effect (sensation close to zero on the 100mm VAS scale). Consequently, after removal of those individuals, the sample was unsuitable for conducting any inferential statistics. Nevertheless, from a descriptive perspective, one participant experienced substantial 'feeling' (76mm) and 'dislike' (79mm) following OR-GC, with the remaining eight participants reporting any DEQ-5 effects < 5mm. After the administration of IN-GC, only one participant reported a moderate drug effect of 30mm, while all other participants reported < 3mm. There were

no indications of 'feeling high' or a desire for 'more' of any substance. OR-GC and IN-GC was correctly identified in 44.4% and 55.6% respectively. The main reason for correct identification was due to experiencing a side effect, or from random guess.

Lung Function Measures

Regarding FEV_1, there was a significant effect of time ($F_{(2,16)} = 14.915$, $p <0.001$, $\eta p^2 = 0.651$) characteristics of bronchodilation following exercise in healthy individuals, but no effect of condition ($F_{(4,16)} = 1.248$, $p = 0.311$, $\eta p^2 = 0.135$), or interaction between time*condition ($F_{(8,64)} = 0.636$, $p = 0.745$, $\eta p^2 = 0.074$) (*Figure 6.8a*). For FVC there was no significant effect of condition ($F_{(4,32)} = 0.453$, $p = 0.770$, $\eta p^2 = 0.054$), time ($F_{(2,16)} = 2.155$, $p = 0.148$, $\eta p^2 = 0.212$), or interaction ($F_{(8,64)} = 0.636$, $p = 0.744$, $\eta p^2 = 0.074$) (*Figure 6.8b*).
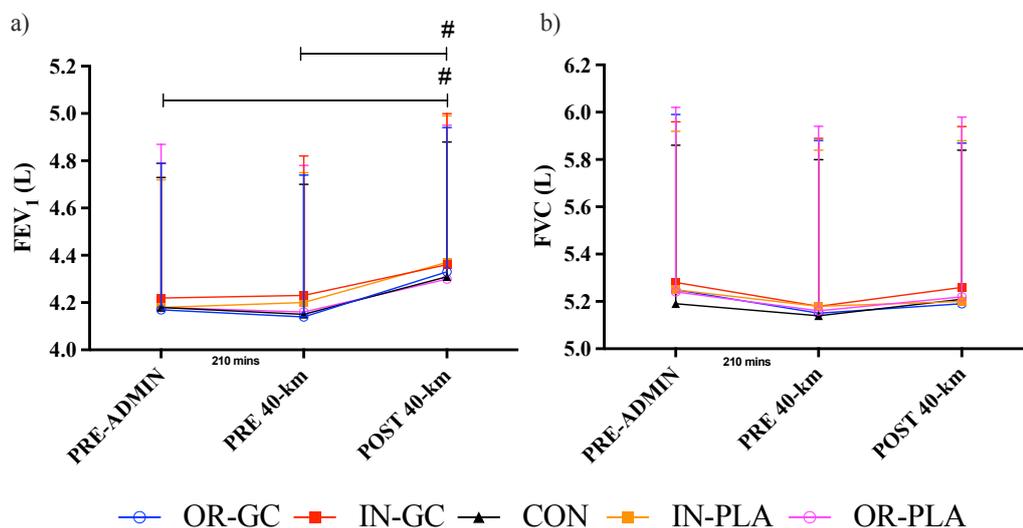
Figure 6.8. *(a) Forced Expiratory Volume in One Second (FEV_1) and (b) Forced Vital Capacity (FVC) pre-administration, after acute administration of oral prednisolone (0.5 mg.kg-1, OR-GC), inhaled beclomethasone dipropionate (1600 μg, IN-GC), microcrystalline cellulose capsules (OR-PLA), water vapour inhaler (IN-PLA) or control (CON), and immediately post-exercise. # denotes significant difference within condition.*

6.4. Discussion

Main Findings

The present study demonstrated that acute supratherapeutic dose of ICS provided no significant or meaningful change to 40-km cycling TT performance, however therapeutic-dose oral GC may be '*possibly beneficial*' when the smallest worthwhile improvement threshold is considered at 0.6%, as suggested for high-level athletes (Paton and Hopkins, 2006). Neither administration route affected any perceptual or cardiorespiratory outcomes measured in the 40-km TT. However, oral GC induced elevated blood glucose

during the 40-km TT, and significant blunting of pro-inflammatory cytokine IL-6 when assessed post-exercise. Nevertheless, performance in a subsequent 10-km TT was also unaffected by acute inhaled or oral GC administration, nor were psychometric scales relating to stress and recovery.

Initial TT Performance

To knowledge, this is the first study to investigate acute therapeutic dose oral GC and supratherapeutic dose of ICS on self-paced exercise performance. Previous literature has focused primarily on fixed-intensity exercise tasks (e.g., TTE), incremental exercise or field-based tasks.

Although no prior studies have investigated acute ICS, the findings support previous investigations that observed no effect following a short-term [2 – 4 weeks] administration period assessed using maximum power output during ramp incremental test (Kuipers *et al.*, 2008).

Regarding oral GC, although this condition did not reach statistical significance and supported previous findings regarding exercise performance following acute oral GC administration (Arlettaz *et al.*, 2006; Arlettaz, Collomp, *et al.*, 2008), the -29 second [95% CI -87.0 to 28.3] (0.6%) improvement 40-km TT compared to the oral placebo condition may be meaningful to an elite population. A recent meta-analysis attributed acute prednisolone to have a standardised mean difference of 0.09 [-0.202 to 0.392] when compared to a placebo (Riiser, Stensrud and Andersen, 2023). This is smaller than the standardised mean difference observed in the present study, suggesting the higher dosing of acute oral GC used in the present study may have had a greater effect. No previous studies on ergogenic effect of GC have considered the smallest worthwhile improvement. Previous observations have determined that the SWC in elite athletes performing a cycling TT is ~0.6-0.7% (Paton and Hopkins, 2006; Lamberts *et al.*, 2009). However, although the participants in the present study had a history of cycling and presented repeatable coefficient of variation % comparable to highly trained cyclists, they would not be considered elite, and therefore the SWC in their performance is likely higher than the threshold used in the MBD analysis. Moreover, the interpretation that acute oral GC is 'possibly beneficial' to TT performance should be approached with caution. This is because MBD may increase the risk of a type I error, leading to overly optimistic conclusions (Harrison *et al.*, 2020). However, the small sample size also increases the likelihood of committing a type II hypothesis error. Given there are so few studies

available on GC in ergogenic context, these results are still valid but should warrant further investigation.

Mechanisms of Performance Enhancement

The present study primary focus was on performance outcomes, but also attempted to investigate some of the previously postulated mechanisms of ergogenic action relating to GC. Interleukin-6 (IL-6) concentration increases during exercise locally within the working skeletal muscle and is related to the intensity and duration of the exercise being undertaken (Pedersen, Steensberg and Schjerling, 2001). IL-6 is a key function in modulating substrate utilisation (Duclos, 2010), and could be considered an 'energy sensor' (Nash *et al.*, 2023). Previous observations have shown that carbohydrate supplementation attenuates the IL-6 response (Febbraio *et al.*, 2003; Robson-Ansley, Walshe and Ward, 2011), while depletion of muscle glycogen can amplify it (Steensberg *et al.*, 2001). Moreover, an IL-6 receptor blockade reduces the mobilisation of free fatty acids in both lean and obese men at rest, during exercise, and into recovery (Trinh *et al.*, 2021). In agreement with other studies in an exercise setting, this chapter found an attenuation of IL-6 concentration from acute oral GC administration (Arlettaz, Collomp, *et al.*, 2008; Arlettaz, Portier, *et al.*, 2008). Additionally, the present chapter observed elevated blood glucose concentrations during the 40-km TT following oral GC administration, compared to the other conditions investigated. This finding is supported by previous observations (Tacey *et al.*, 2019). The maintenance of glucose homeostasis could be attributed to increased gluconeogenesis or the suppression of IL-6, but consequently may lead to less depletion of glycogen stores. These mechanisms may have contributed to the 'possibly beneficial' effect observed in the present study.

However, the acute supratherapeutic ICS condition in the present study did not observe this effect, and is in contradiction to the previous findings on short-term ICS administration that showed significant changes in IL-6 concentration in response to exercise (Schwindt *et al.*, 2010), suggesting that a longer intervention period may be required for systemic changes following ICS.

Arlettaz, Portier, *et al.*, (2008) reported an increase in FO during one hour of submaximal cycling exercise at 60% $\dot{V}O_2max$ following administration of 20 mg of acute prednisolone. Contrary to this, the present study found no impact of oral GC administration on FO at 50% $\dot{V}O_2peak$, despite the aforementioned alterations in metabolic activity during the oral-GC time-trial performance. These differences in

findings may be attributed to the unfasted state of the present study (Tacey *et al.*, 2017), with exercise commencing approximately three hours after standardised dietary intake.

Previous research has suggested that GC might indirectly influence sports performance by altering mood states and reducing feelings of fatigue (Soetens, Hueting and De Meirleir, 1995). Given that IL-6 and its trans-signalling soluble IL-6 receptor (sIL-6r) has been associated in the perception of pain (De Jongh *et al.*, 2003), fatigue, mood state changes (Vargas and Marino, 2014; Cullen *et al.*, 2017) and exercise-induced muscle soreness (Robson-Ansley *et al.*, 2010), a reduction in subjective measures related to these mechanisms might have been anticipated due to the reduction in post-exercise IL-6 levels following the oral GC condition. However, in the present study no impact was observed in SRSS sub-domains, particularly relating to lack of activation, negative emotional states, and emotional balance. This also supports previous studies that saw no change in profile of mood states following short-term use of ICS (Kuipers *et al.*, 2008).

Strengths and Limitations

The present study has pertinent strengths being the first study to explore the effects of acute oral and inhaled GC administration in trained cyclists during a closed-loop TT performance trial, improving on previous research that used TTE or other functional exercise tasks. Time-trials have lower coefficient of variation and greater external validity when compared to a TTE task (Currell and Jeukendrup, 2008), as involve decisions on pacing (Tucker *et al.*, 2006). Previously cited theories of GC ergogenic action relates to psychomotor changes such as enhancing decreased feeling of fatigue (Soetens, Hueting and De Meirleir, 1995), so investigating using this type of performance trial is warranted in studies on ergogenic aids (Close, Kasper and Morton, 2019). The 40-km TT was selected as the distance used in the cycling portion of an Olympic distance triathlon and a common road cycling time-trial distance. However, TTs have the assumption that participants attempt to perform their best at each assessment. Implementation of extrinsic motivation such a monetary incentive can be useful, however this can interfere with pacing (Skorski *et al.*, 2017).

Despite this, a postulated ergogenic mechanism of GC has been cited to be the ability to improve recovery between bouts of exercise. For the first time, this study has shown this not to be the case. The study involved a 10-km time trial (TT) following a fatiguing 40-km TT and was intended to test the hypothesis of GC impact on recovery, and this approach may simulate sporting scenarios where multiple efforts occur within a single

day, such as the track cycling multi-event omnium. However, omnium events are typically shorter, and performed at higher intensities (Craig and Norton, 2001). Although participants were encouraged to complete the initial 40-km as fast as possible, it remains unclear whether they paced their effort in anticipation of the subsequent exercise bout. The 10-km TT was selected for the subsequent performance test to provide an intense bout of endurance exercise that would require pacing decisions in the presence of fatigue. A more controlled approach could have involved using a fixed time and 'heavy' intensity initial preloaded bout of exercise to induce fatigue, allow comparison of perceptual and cardiorespiratory effects at an intensity more akin to competition, and then follow this with an ecologically valid 40-km TT. This approach has recently been used to investigate the ergogenic impact of tramadol administration (Mauger *et al.*, 2023). Another consideration is the one-hour recovery period provided between the time trials, which may not accurately reflect the typical competition schedule. In scenarios involving repeated muscle damage where muscle soreness persists, strategies to reduce inflammation and soreness could improve an athlete's perceived readiness to train or enhance their performance. GC administration may be beneficial in situations of intensified training, fixture congestion in team sports, or consecutive days of competition, such as in the Tour de France, especially when there is chronic elevation of IL-6 (Robson-Ansley, Blannin and Gleeson, 2007). Future research may consider evaluating the effectiveness of GC during periods of intense training or repeated competition, and incorporate additional haematological and muscle biopsy markers related to exercise-induced muscle damage and recovery e.g., myoglobin, creatine kinase, C-reactive protein, lactate dehydrogenase, tumoral necrosis factor-alpha to reduce reliance on subjective measures (Peake *et al.*, 2017).

The timing of substance administration was based on previous investigations, specifically for the OR-GC condition (Arlettaz, Collomp, *et al.*, 2008) and to elicit maximum GC activity during the exercise tasks. However, this may not have been suitable for the ICS condition, which has a different half-life clearance rate of approximately 2.7 hours for the active metabolite (Rao Bondugulapati and Rees, 2016). The implications of this are that the timing of the performance trials may not have been optimal for observing an ergogenic effect. Future studies should ensure that performance trials are aligned with the clearance times specific to the administration route. This issue is further compounded by the fact that the subsequent time trial was completed approximately six hours after administration, potentially placing it outside the pharmacokinetic window (Coll *et al.*, 2021).

Additionally, in the context of enhancing recovery, it is possible that an unscrupulous athlete might administer the substance after an initial bout of exercise—a scenario that the present study did not investigate.

Chapter 6 follows *Chapter 5*, which explored appropriate inhaler technique designed to provide standardised delivery and reduce risk of local adverse events. However, the use of VHC would likely have substantially reduced the delivered dose. Estimates from Chapter 5 suggest that out of the aimed 1600 μg to be administered, only approximately ~1084 μg was likely delivered to the body. The mass that would sediment within the VHC would likely have been deposited in the mouth and throat region if using pMDI alone, though it may still be metabolised and enter systemic circulation (Derendorf *et al.*, 2006; Borghardt, Kloft and Sharma, 2018). Future studies should explore supratherapeutic doses using pharmacokinetic parameters such as urine excretion to understand the impact of using a VHC on the ergogenic potential.

The present study administered a supratherapeutic dose of ICS, which is to knowledge, currently larger than any previous study on ergogenic effect. However, it is worth noting that this dosage does not align with the typical prescription or usage of ICS, which is at a lower dose and indicated to be taken multiple times per day over a prolonged period. Moreover, although the dose of oral GC is also higher than any study previously investigated, the outdated relative dosing used in this chapter was more frequently lower [in 5 out of 9 participants] than the 40–50 mg per day now recommended by the *'British National Formulary'* for acute asthma exacerbation (BNF, 2023b). To enhance the external validity of our findings, future research should explore the effects of longer-term, high-dose inhaled or oral GC administration on TT performance.

Implications for Policy

Current WADA guidelines allow ICS to be used inside and outside of competition periods. Despite the limitations outlined, the lack of ergogenic effect observed with supratherapeutic doses suggest that these regulations are likely appropriate. However, oral GC should remain controlled with TUE due uncertainties surrounding its ergogenic impact, potential immunosuppressive effects, and well-established long-term health implications. Based on this, anti-doping stakeholders may wish to commission investigations into whether even higher trained athletes experience ergogenic action when assessed with TT from oral GC as demonstrated in this thesis to add to the limited studies that have shown a performance enhancing effect from short-term use of oral GC.

6.5. Conclusion

In conclusion, the present study found that a supratherapeutic dose of ICS did not produce any significant or meaningful improvements in 40-km TT performance, however oral GC may be '*possibly beneficial*' for high level cyclists. Acute oral GC administration did lead to metabolic changes, particularly elevated glucose levels during the 40-km TT, and a significant reduction in post-exercise inflammatory cytokines. Additionally, performance in a subsequent 10-km TT remained unaffected, and psychometric scales related to stress and recovery showed no significant changes. Neither administration method had an impact on perceptual, substrate utilisation or other cardiorespiratory measures during a 50% $\dot{V}O_2$peak steady state fixed intensity cycling. As such, current WADA guidelines on ICS are appropriate given lack of ergogenic effect when assessed using an ecologically valid TT assessment. However, oral GC should remain controlled with TUE due uncertainties surrounding its ergogenic impact, potential immunosuppressive effects, and well-established long-term health implications. Future studies should continue to use ecologically valid performance outcomes, have consideration for meaningful difference in performance, and explore effect of GC on recovery from longer term use akin to how an athlete would use ICS in practice.

CHAPTER 7: EFFECT OF SHORT-TERM DAILY BECLOMETHASONE DIPROPIONATE ADMINISTRATION ON REPEATED 10-KM CYCLING TIME-TRIAL PERFORMANCE

BACKGROUND: The World-Anti Doping Agency (WADA) stipulates that athletes can use inhaled corticosteroids (ICS) for asthma-related conditions at all times, including during competition periods. It remains unclear if ICS provides a competitive advantage for single, or repeated bout exercise. **OBJECTIVES:** This study aimed to investigate the impact of short-term daily high-dose ICS administration on 10-km cycling time-trial (TT), and recovery for a subsequent 10-km TT performed on the same day. **METHODS**: In a randomised cross-over order, eight trained non-asthmatic male cyclists ($\dot{V}O_2$peak; 60.0 ± 4.8 ml.kg.min^{-1}) completed a 10-km TT (TT1) after 14 days administration of either beclomethasone dipropionate (800 μg, ICS) or water vapour inhaler (PLA). Then, after a one-hour passive recovery, participants completed a further 10-km TT (TT2). Before commencing each TT, subjective overall recovery was assessed using Short Recovery Stress Score (SRSS). Plasma Interleukin-6 (IL-6) concentration was determined from samples collected at baseline, and after completion of TT2 (difference between baseline and post-exercise are reported as ΔIL-6). Data was tested for normality, then statistically analysed using Paired Samples T-Test. **RESULTS:** No significant difference was seen in completion time of TT1 (ICS: 962.1 ± 45.3; PLA: 964.7 ± 44.1 seconds; $p=0.50$) or TT2 (ICS: 982.5 ± 48.8; PLA: 985.7 ± 54.7 seconds; $p=0.63$). Baseline SRSS was not different between conditions ($p=1.00$), nor prior to the subsequent 10-km bout ($p=0.35$). Baseline IL-6 was significantly lower in ICS than PLA (0.70 ± 0.47 pg/mL, 0.93 ± 0.54 pg/mL respectively; $p=0.05$), however ΔIL-6 was not significantly different between conditions ($p=0.64$). **CONCLUSION:** Short-term high-dose ICS medication did not enhance 10-km TT performance. Furthermore, perceived recovery prior to, or measured performance during a subsequent 10-km TT was not different between conditions. Future research should consider the applied significance of ICS related performance and recovery outcomes.

7.1. Introduction

Maintenance asthma therapy in the form of ICS is a first-line treatment and commonly prescribed to athletes with EIB to manage airway inflammation (Parsons *et al.*, 2013; Price and Hull, 2014; Barnes and Ulrik, 2015). ICS can help reduce the reliance on SABA therapy and lower the risk of developing tachyphylaxis (Weiler *et al.*, 2016). *Chapter 4* added that diagnosing and initiating EIB management at therapeutic doses *[incorporating ICS]* over 12-months enhanced resting FEV_1 and attenuated EIB, but treatment did not improve real-world performance at subsequent major competition above the expected progression of an elite athlete.

As per the latest guidelines from the WADA, the use of ICS is permitted during both competition and non-competition periods (WADA, 2023). However, evidence on the performance-enhancing effects of ICS remains limited, with only a few studies investigating inhaled administration routes (Jardim *et al.*, 2007; Kuipers *et al.*, 2008; Schwindt *et al.*, 2010; Hostrup *et al.*, 2017). Although the current consensus is that exercise performance outcomes are unaffected by ICS, the methodological designs of these previous studies have not focused on achieving optimal performance in ecologically valid closed-end exercise tasks (Riiser, Stensrud and Andersen, 2023). *Chapter 6* has contributed to this knowledge and demonstrated that acute supratherapeutic doses of ICS did not enhance time-trial exercise performance or recovery in repeated bouts of exercise. Furthermore, contrary to therapeutic dose oral GC, supratherapeutic ICS did not exert impact on inflammatory cytokine suppression in response to exercise. Nevertheless, it is worth noting that the acute nature of *Chapter 6* does not accurately represent the typical usage of ICS treatment by athletes with EIB for therapeutic requirements or by unscrupulous athletes attempting to use ICS for doping purposes.

As discussed in *Chapter 6*, the competition requirements of many athletic events typically require multiple efforts over the course of a day, with competitors usually required to perform qualifying races before the finals. Currently, it is not known whether short-term daily use of ICS can be beneficial to repeated exercise performance on the same day.

Therefore, the aim of this chapter was to investigate the effect of short-term daily ICS treatment on; (1) an initial 10-km cycling TT; (2) the recovery for a subsequent 10-km cycling TT performed on the same day, and (3) the physiological and immunoendocrine response from these bouts of exercise. The main objective was to address the limitations

of previous research and provide experimental evidence that can inform whether ICS should be subjected to stricter regulations for use during competitions in sports.

7.2. Materials and Methods

7.2.1. Ethics Statement

Institutional ethics approval was obtained from the University of Kent School of Sport and Exercise Sciences Research Ethics Advisory Group (REF No. 49_2019_20), further amendments were required to mitigate risk of COVID-19 transmission.

7.2.2. Participant Characteristics (Inclusion / Exclusion Criteria)

Sample Size Calculation

An *a priori* sample size calculation was undertaken based on the primary outcome (completion time of TT_{10km}), for the proposed paired sample analysis, incorporating conventional parameters of alpha level (α) 0.05, statistical power ($1 - \beta$) of 0.80, and attempt to detect a large effect size of 0.8 (Cohen's *d*). These parameters indicated that 15 participants would provide adequate power (G*Power software package, Version 3.1.9.4, Kiel University, Germany).

Inclusion/Exclusion Criteria

Males aged between 18 and 50 years old, who participated in a minimum of 3 cycling-related endurance training sessions per week and had a $\dot{V}O_2$peak of ≥ 55 ml.kg.min^{-1}, were recruited through posters, online advertisements, and word-of-mouth referrals. This criteria was based on 'Performance Level 3' representing a trained subject group (De Pauw *et al.*, 2013).

Exclusion criteria comprised of any objective evidence of asthma-related conditions, regular use of any medicine to control a chronic condition, or a current and recent illness or musculoskeletal injury. The study also required participants to be naive to ICS and not in possession of a TUE for any substance.

Before participating, volunteers were informed of the associated risks and experimental protocols. Eligible participants had their PARQ screened and approved by the collaborating physician. Additionally, to avoid possible doping violations, participants were asked not to compete in events where doping control is in force during the study period, and seven days after completion.

7.2.3. Pre-Experimental Trial Procedures

Pre-Test Preparation

As described in *Chapter 3.2*, participants were advised to avoid engaging in any intense physical activity for 48 hours before their laboratory visits. They were also instructed to abstain from consuming alcohol, caffeine, as well as any anti-inflammatory or analgesic medications (e.g., paracetamol, ibuprofen) for 24 hours prior to their scheduled visits.

All tests were conducted under controlled laboratory conditions ($19.5 \pm 2°C$, 42%).

Before undertaking experimental trials, all subjects underwent an incremental exercise test on a cycle ergometer (Cyclus 2, Avantronic, Leipzig, Germany) to determine maximal aerobic power ($\dot{V}O_2peak$). The methodology used for determining $\dot{V}O_2peak$ is explained in detail in *Chapter 3.8*. To summarise, after a 10-minute warm-up at 100 W, the power output increased at a ramp rate equivalent to 25 W every minute until voluntary exhaustion, with continuous cardiorespiratory responses monitored using wireless telemetry (Garmin HRM-Dual, Garmin, Olathe, USA) and indirect calorimetry (Cortex Metalyzer 3B, Biophysik, Leipzig, Germany).

Within one week of the incremental exercise test, participants underwent a familiarisation trial of the experimental protocol to mitigate the potential confounding learning effects of repeated 10-km time trials, acquaint them with validated questionnaires, and provide coaching on technique for using inhalers through an ACP-VHC.

In order to strictly regulate the administration of ICS, an ACP-VHC device (AeroChamber Plus™, Trudell Medical International, Ontario, Canada) was employed to give auditory feedback on flow rate (~30 L/min) and reduce reliance on the coordination of actuation and inhalation.

Participants were instructed to use a *"single inhalation with 10 second breath hold"* and provided with a standardised demonstration video resource for reference (Asthma UK, 2020). During the initial days of the intervention period, the participants' inhalation technique was monitored either in-person or online (for example, using Zoom, Microsoft Teams or FaceTime) to ensure that the correct technique was being followed.

7.2.4. Intervention Administration Period

The study employed a randomised, single-blinded, cross-over trial design, where the participants remotely self-administered either 800 µg Beclomethasone dipropionate

(Qvar® 100 µg, Teva UK Limited, Castleford, United Kingdom) (ICS) or a placebo water vapor inhaler (PLA) (Vitalograph Ltd, Buckinghamshire, UK) for an intervention period of 14-days. The blinding procedure is outlined in *Chapter 3.7*.

During the 14-days, the participants were instructed to take four puffs from the inhaler device twice daily (in the morning and evening). On average, the inhalation bolus required approximately four minutes (each manoeuvre lasting ~25 seconds, and a 30-second pause between each breath). The participants were responsible for logging their dosing to ensure compliance with the intervention and advised to report any serious adverse events that occurred during the study period. An *a priori* acceptable compliance criteria was set at >90%. Additionally, during the first 14-day intervention period, the participants were instructed to maintain a record of their exercise training and to replicate the same regimen as closely as possible during the subsequent arm.

Following completion of the initial intervention, participants completed a 14-day wash-out period, before proceeding to the remaining condition, i.e. (ICS → PLA) or (PLA → ICS). The familiarisation visit was repeated during the wash-out period.

7.2.5. Experimental protocol (Visits 2 & 3)

The experimental sessions were completed on the 14th day of the intervention period. Participants were instructed to maintain a food diary, recording their dietary intake for the 24 hours preceding the experimental visits, including breakfast on the morning of the testing, and asked to replicate the same diet for subsequent trials.

Participants arrived at the laboratory between 07:00 and 08:00 AM to control for circadian rhythm of cortisol (Thuma *et al.*, 1995), and the arrival time was matched for subsequent sessions (± 30 minutes).

Firstly, participants administered the final intervention inhalation in a single bolus, and then completed the DEQ-5 questionnaire to assess any moment drug effects prior to exercise. Participants were also instructed to recall any adverse effects that occurred during the intervention period using the 'Inhaled Corticosteroids Side-Effect Questionnaire (ICQ-S)'.

Primary Outcomes – Cycling Performance: Participants completed a preloaded 20-minute warm-up at 50% $\dot{V}O_2$peak to provide a standardised low-intensity exercise bout for assessing substrate utilisation (*Chapter 3.10*), before undertaking a 10-km time trial

(TT) on their own racing bicycle using an electromagnetically braked ergometer (Cyclus 2, Avantronic, Leipzig, Germany). Participants were blinded to all performance data feedback except for distance elapsed and instructed to complete the TT in the shortest possible time. The TT started at the lowest gear ratio, and participants could change virtual gearing as needed. Cardiorespiratory responses were continuously monitored using wireless telemetry (Garmin HRM-Dual, Garmin, Olathe, USA) and indirect calorimetry (Cortex Metalyzer 3B, Biophysik, Leipzig, Germany). Rating of Perceived Exertion (RPE 6 – 20 scale; Borg, 1982) and perceived muscle pain (MP 10-point numerical scale; Connor & Cook, 1999) were verbally reported every 2-km. Participants then rested for 1-hour in the laboratory, before completing a further 10-km time-trial using the same procedure. Participants were able to drink water ad libitum between the TTs. Full protocol relating to TT performance is outlined in *Chapter 3.11*.

Secondary Outcomes – Perceptual and Metabolic Response: Upon arrival at the laboratory and after the final TT, venous blood samples were taken from an antecubital vein, with 10 µL of blood immediately transferred into a disposable microcuvette to assess white blood cell (WBC) and 5-part differential counts (neutrophils, lymphocytes, monocytes, eosinophils, and basophils; WBC-diff) using an image-based haematology system (HemoCue® WBC DIFF, HemoCue AB, Ängelholm, Sweden). The remaining venous sample was processed and stored for later determination of IL-6 concentration (as described in *Chapter 3.14*). Then, validated questionnaires to assess stress and recovery (SRSS) were completed before and immediately after each TT, alongside spirometry assessments and providing capillary blood samples (B[Glu] and B[La]). Finally, session RPE (sRPE) was obtained 30 minutes after completing all exercise tasks, and the SRSS, collected the next day at 08:30 am (± 1 hour). Detailed information on the collection and analysis of the secondary outcomes is provided in the *Chapter 3.12*.

The study design, protocol and timing of outcome measures are presented in *Figure 7.1*.
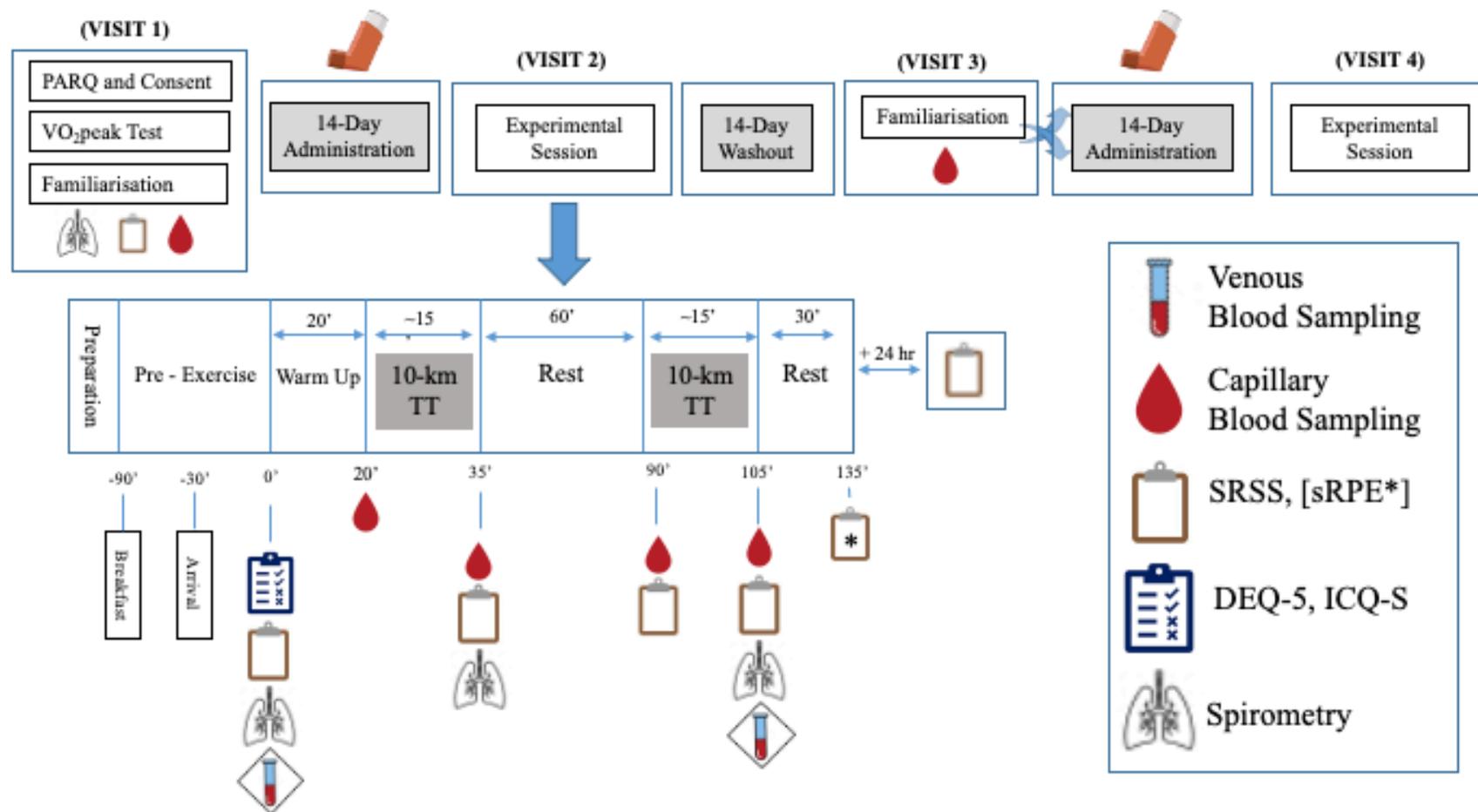
Figure 7.1. Schematic of the study design and protocol

7.2.6. Data and Statistical Analysis

To investigate the effect of short-term daily ICS use on cycling time-trial (TT) performance, several inferential and magnitude-based statistical analyses were conducted. Firstly, [Aim 1] a paired-samples t-test was used to compare the completion time and mean power output of the 10-km TT between the active drug (ICS) and placebo (PLA) conditions. In addition to investigate whether there was a meaningful change in TT_{10km}, the smallest-worthwhile change (SWC) was estimated at approximately 0.6 to 0.7% as previously reported in high-level cyclists (Paton and Hopkins, 2006; Lamberts *et al.*, 2009), equating to an improvement of ~6.25 seconds based on the previous work in *Chapter 6* [assuming ~965 second 10-km TT]. To estimate the likelihood that ICS treatment would have a beneficial, trivial, or negative effect on performance, magnitude-based decisions using mean difference and confidence intervals (95%) were interpreted from p-value derived calculations using excel spreadsheet by Hopkins (2007) (Hopkins, 2007) downloaded from (http://www.sportsci.org/2007/wghinf.htm). Thresholds for assigning qualitative terms for the chance of substantial effects were: <1% almost certainly not; <5% very unlikely; <25% unlikely; <50% possibly not; >50% possibly; >75% likely; >95% very likely; >99% almost certain (Batterham and Hopkins, 2006).

Secondly, a two-way repeated measured analysis of variance (ANOVA) was performed to examine the impact of treatment or placebo on subsequent TT performance [Aim 2], by comparing the changes in performance between the two 'Time' factors (TT1, TT2).

For the secondary outcomes, differences in power output and physiological responses at each TT section between the two conditions were assessed using a two-way repeated measures ANOVA with 'Treatment' factor (ICS vs. PLA) and a repeated measures Time factor (5 elapsed distances: 2, 4, 6, 8, 10-km). Furthermore, this same approach was used for 'Time' factors relating to questionnaire responses [SRSS], and blood analysis [IL-6, WBC_{diff}] [Aim 3] (pre-treatment, pre-exercise, and post-exercise). If a significant main effect or interaction was observed, post-hoc pairwise comparisons with Bonferroni or Tukey correction was used to interpret location of effect.

Intra-class correlation coefficient (ICC) was calculated to present the consistency in the two familiarisation trials completed during the protocol (Visit 1 and Visit 3).

All data were checked for the assumptions of the statistical tests, and a Greenhouse-Geisser correction was applied to ANOVA analyses when assumptions of sphericity were violated. Effect sizes were calculated using Cohen's d and partial eta square (η_p^2), which were interpreted as small (0.2-0.5), medium (0.5-0.8), or large (≥ 0.8) effects.

Data analysis was performed using Jamovi (v2.3.21, The Jamovi Project, Sydney, Australia), and data visualization was conducted using GraphPad Prism software (v9.5.1, GraphPad Software, San Diego, California, USA). Significance for all tests was deemed at $p < 0.05$. Data is presented as mean $\pm$ SD unless otherwise stated.

7.3. Results

Participant Characteristics

Ten participants commenced experimental sessions for this study. However, one participant was excluded due to inability to commit to further experimental sessions, and one withdrew due to the COVID-19 global pandemic interrupting and ceasing data collection mid-intervention. Therefore, eight (n=8) trained male cyclists fully completed all experimental sessions (age; 27 $\pm$ 6 years, height; 176.6 $\pm$ 4.0 cm, body-mass; 71.6 $\pm$ 9.4 kg, $\dot{V}O_2peak$; 60.0 $\pm$ 4.8 mL.kg^{-1}.min^{-1}, maximum power-output; 357 $\pm$ 43 W). Participants participated in regular endurance training at least 3 times per week (cycling training volume; 4 $\pm$ 1 days.wk^{-1}, 4.1 $\pm$ 1.5 hr.wk^{-1}). In accordance with the inclusion criteria, all participants demonstrated no meaningful decrease in FEV$_1$ (>10% fall) following maximal exercise (-0.3 $\pm$ 0.04%). Participants demonstrated high intra-class correlation coefficient (ICC) in completion time between the two familiarisation trials (0.955, 95% CI; 0.772 – 0.991).

All participants met the self-reported acceptable compliance criteria of >90% of the required doses.

Study Aim 1: Ergogenic effect of short-term daily ICS treatment on initial 10-km time-trial (TT1)

Submaximal Pre-loaded Exercise

Prior to performance testing, participants completed a submaximal fixed intensity exercise bout at power output eliciting 50% $\dot{V}O_2peak$ (135.5 $\pm$ 30.8 W). There was no significant difference in any cardiorespiratory and perceptual response outcomes between

ICS or PLA conditions (p >0.05) *(Figure 7.2; numerical data and inferential statistics presented in Supplementary Table 11.12).*
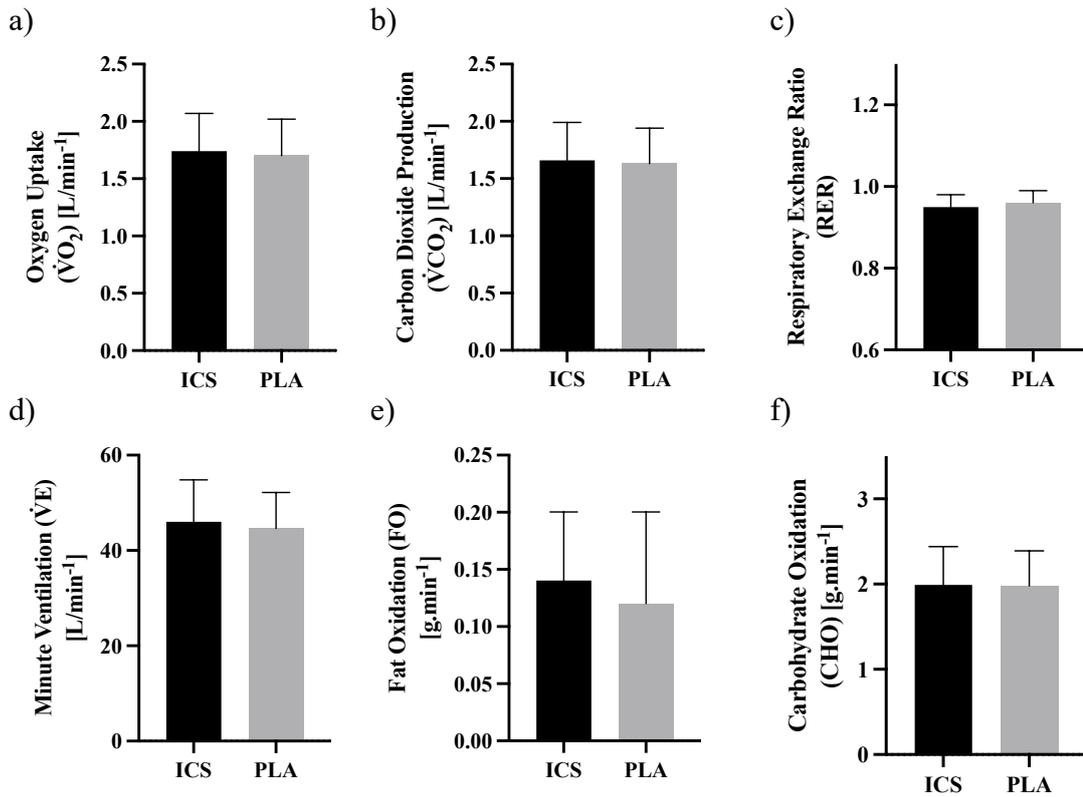
*Figure 7.2. Mean physiological response ($\dot{V}O_2$ [a], $\dot{V}CO_2$ [b], RER [c], VE [d]), fat oxidation ([FO [e]) and carbohydrate oxidation, CHO [f]) outcome measures averaged between 5 to 20 minutes of submaximal exercise at power output eliciting 50% $\dot{V}O_2peak$. Data presented as mean ± standard deviation. *p <0.05 vs. placebo. Abbreviations: **ICS**, Inhaled corticosteroids; **PLA**, Placebo.*

<u>Initial 10-km Time-Trial Performance</u>

Individual responses for completion time and mean power output are shown in *Figure 7.3a & 7.3b* respectively. There was no significant difference observed in completion time (t(7) = 0.705, p = 0.503, $\bar{x}_{diff}$= -2.6, 95% CI$_{diff}$ = -11.3 – 6.1 sec, d = -0.25) between the PLA (964.7 ± 45.3 sec) and ICS (962.1 ± 45.3 sec) conditions. Moreover, this was not shown to be a meaningful change in performance (*pMET* = 0.85; MDB 1.9% harmful; 83.4% trivial; 14.7% beneficial; *Figure 7.4*).

Whilst there was a significant main effect of power output over time (F(4,28) = 6.979, p = 0.022, ηp^2 = 0.50), there was no significant difference in mean power output (F(1,7) = 0.562, p = 0.478, $\bar{x}_{diff}$ = 2.0, 95% CI$_{diff}$ = -7.9 – 3.9, ηp^2 = 0.074) between PLA (248 ± 37 W) and ICS (250 ± 38 W) conditions or interaction effect (F(2.17,15.20) = 0.183, p = 0.851, n^2p = 0.025) *(Figure 7.3c).*
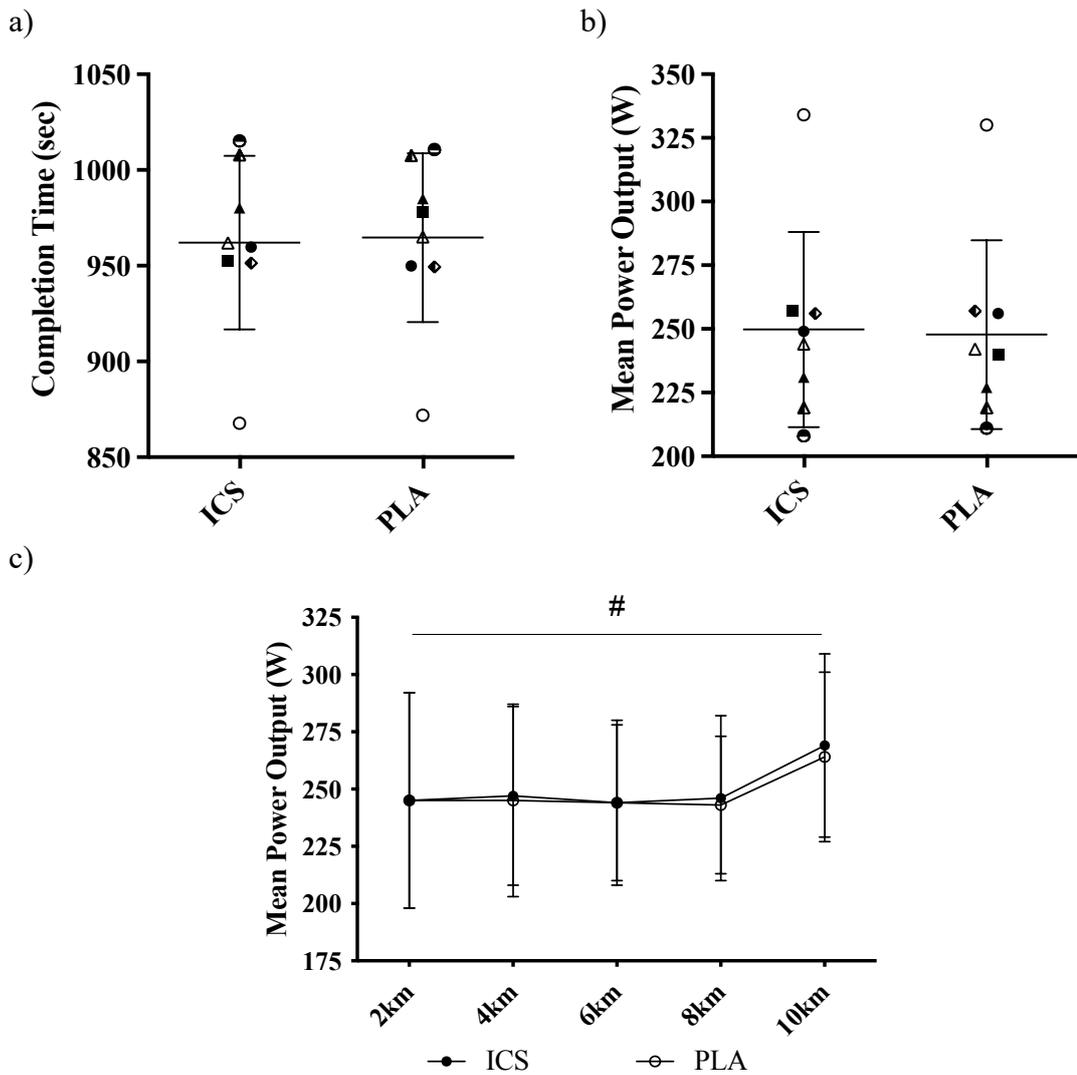
Figure 7.3. *Initial 10-km (TT1) completion time (a) and mean power output (b) presented as individual performance (shapes), the condition mean (centre line), and standard deviation (top/bottom error bars).(c) displays the mean power output averaged for each 2-km section of the 10-km time trial in the inhaled corticosteroids (ICS) and placebo (PLA) conditions. # signifies significant main effect of time.*
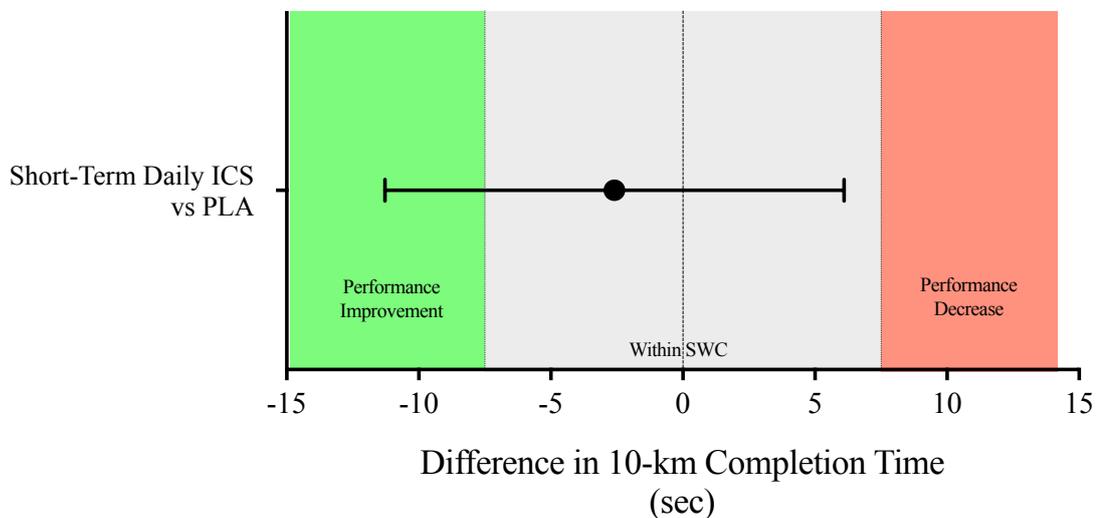
Figure 7.4. *Mean difference (95% confidence intervals) in initial 10-km TT (TT1) completion time between ICS and Placebo (PLA) condition. The grey zone represents the threshold for smallest meaningful change in performance time (SWC = 0.7% of 965 sec = 6.75 sec).*

For $\dot{V}O_2$, there was a significant main effect of time ($F(1.41, 9.85) = 61.06$, $p < 0.001$). However, no significant main effect was found for condition ($F(1.00, 7.00) = 5.32$, $p = 0.06$) or their interaction ($F(1.96, 13.74) = 1.43$, $p = 0.273$).

Similarly, for HR, a significant main effect of time was observed ($F(1.29, 9.01) = 65.756$, $p < 0.001$), but no significant main effect was detected for condition ($F(1.00, 7.00) = 0.158$, $p = 0.703$) or their interaction ($F(1.82, 12.72) = 0.781$, $p = 0.467$).

For RPE, a significant main effect of time was found ($F(1.25, 8.73) = 52.568$, $p < 0.001$), but there was no significant main effect for condition ($F(1.00, 7.00) = 0.811$, $p = 0.398$) or their interaction ($F(1.69, 11.80) = 0.668$, $p = 0.507$).

Finally, for muscle pain, a significant main effect of time was identified ($F(1.38, 9.69) = 54.291$, $p < 0.001$), while no significant main effect was found for condition ($F(1.00, 7.00) = 0.432$, $p = 0.532$) or their interaction ($F(1.61, 11.27) = 0.230$, $p = 0.752$).

Figure 7.5 presents these outcomes.
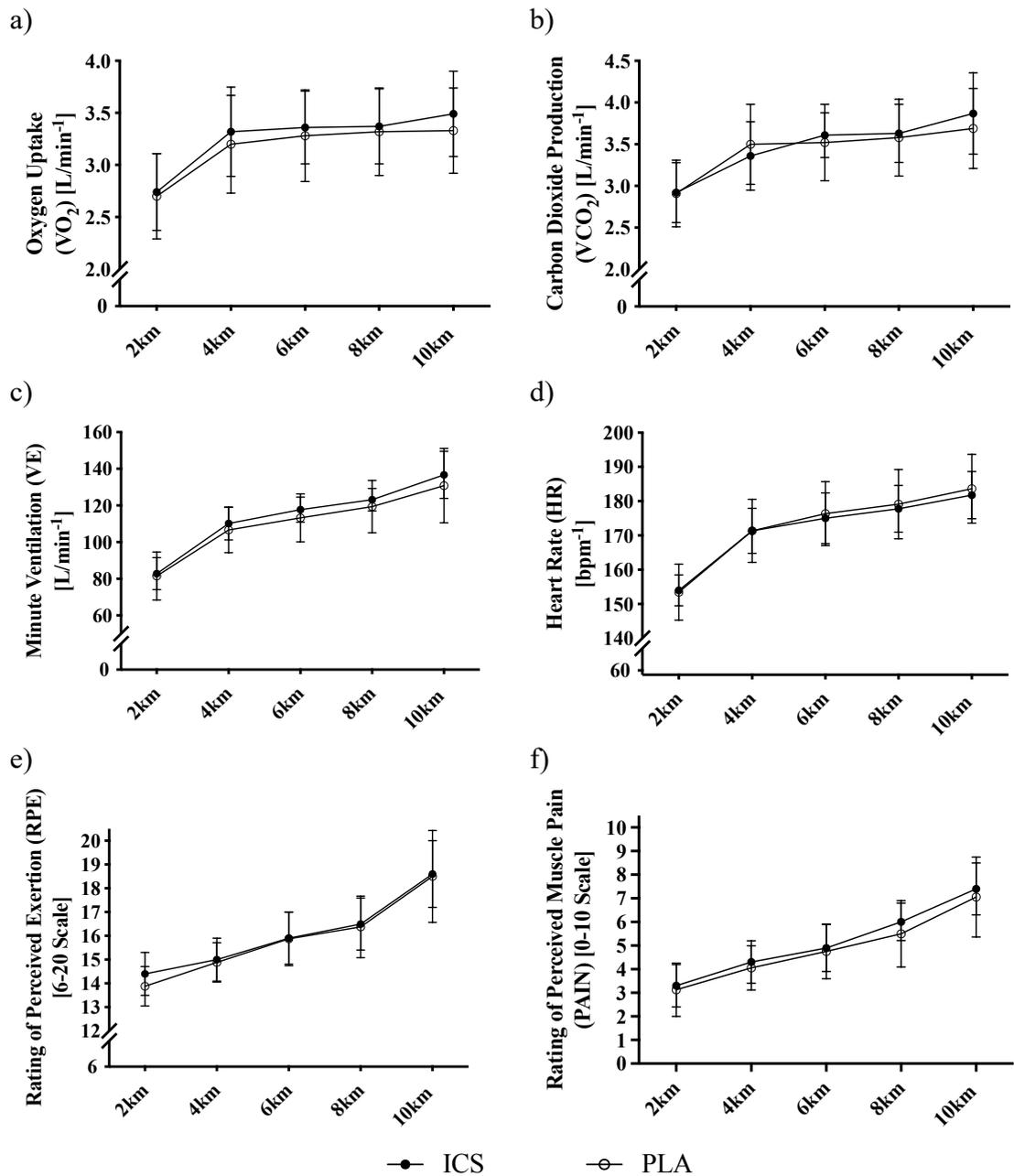
Figure 7.5. *Physiological response ($\dot{V}O_2$ [a], $\dot{V}CO_2$ [b], $\dot{V}_E$ [c], HR [d]) and Perceptual ([RPE [e], PAIN [f]) outcome measures during initial 10-km cycling time-trial (TT1). Data presented as mean ± standard deviation by 'treatment' and 'time' ANOVA factors. Abbreviations: **ICS**, Inhaled corticosteroids; **PLA**, Placebo.*

Study Aim 2: Effect Short-Term Daily ICS Treatment On The Recovery For A Subsequent 10-Km Time-Trial Performed On The Same Day

Subsequent 10-km Time-Trial (TT2) Performance Outcomes

There was no significant difference observed in completion time between the PLA (985.69 ± 54.68 sec) and ICS (982.51 ± 48.83 sec) conditions ($t(7) = 0.278$, $p = 0.789$, $\bar{x}_{diff} = 3.2$, 95% CI$_{diff} = -24.0 – 30.3$ sec, $d = 0.10$). Individual responses for completion time are shown in *Figure 7.6a*.

Whilst there was a significant main effect of power output over time ($F_{(1.57,11.01)}$ = 12.714, $p = 0.002$, *Figure 7.6c*), there was not a main effect of condition (PLA; 235 ± 42 W, ICS; 237 ± 39 W, $p = 0.804$) [Individual responses for mean power output are presented in *Figure 7.6b*] or interaction effect between condition and time ($p = 0.773$, *Figure 7.6c*).
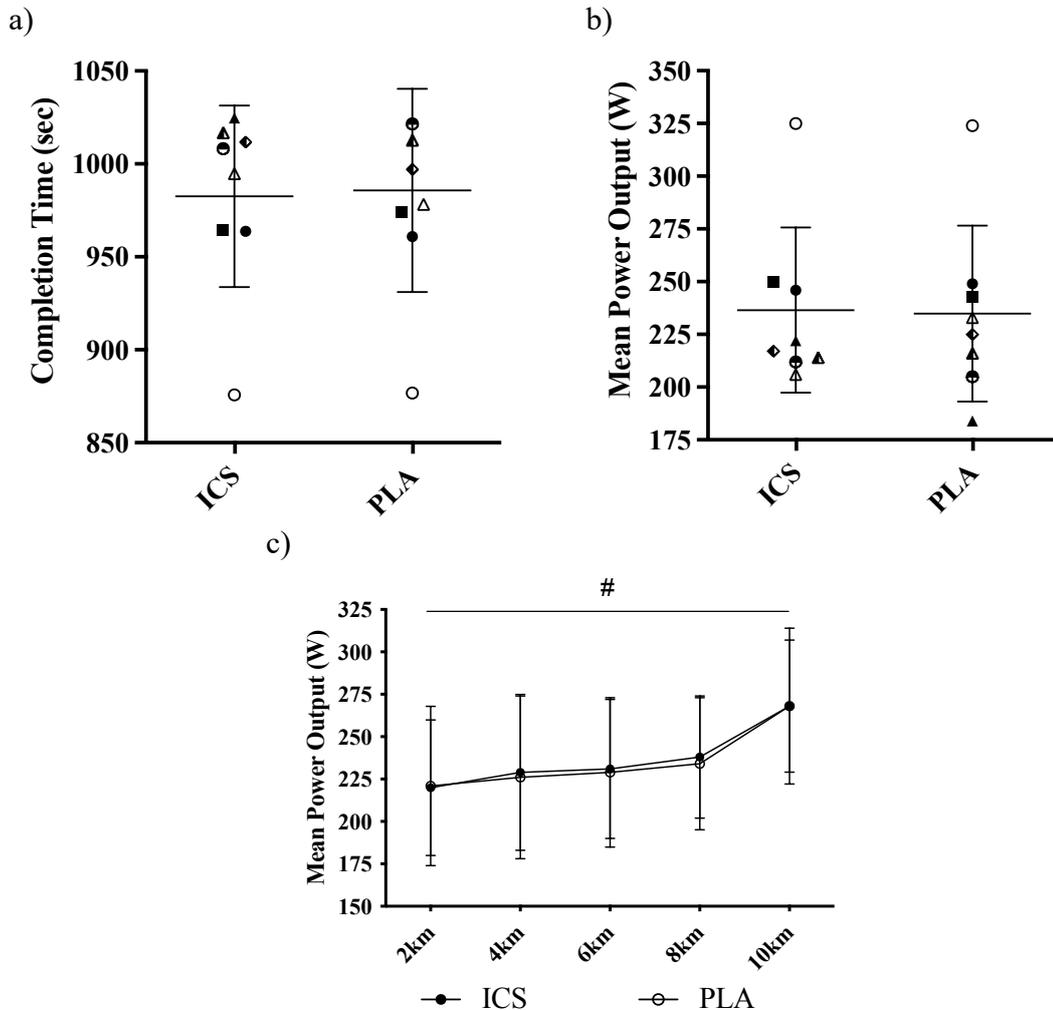
Figure 7.6. *Subsequent 10-km completion time (TT2) (a) and mean power output (b) presented as individual performance [shapes], mean [centre line], and standard deviation [error bars]. (c) displays the mean power output averaged for each 2-km section of the subsequent 10-km time trial (TT) in the inhaled corticosteroid (ICS) and placebo (PLA) conditions. # signifies significant main effect of time.*

Subsequent 10-km TT (TT2) Physiological Response

For $\dot{V}O_2$, there was a significant main effect of time ($F_{(1.74, 12.15)}$ = 26.819, $p < 0.001$). However, no significant main effect was found for condition ($F_{(1, 7)}$ = 0.259, $p = 0.627$) or their interaction ($F_{(4, 28)}$ = 0.329, $p = 0.856$). Similarly, for HR, a significant main effect of time was observed ($F_{(1.60, 11.20)}$ = 64.557, $p < 0.001$), but no significant main effect was detected for condition ($F_{(1.00, 7.00)}$ = 0.224, $p = 0.651$) or their interaction ($F_{(1.46, 10.25)}$ = 0.684, $p = 0.482$).

For RPE, a significant main effect of time was found ($F_{(4, 28)} = 48.08$, $p < 0.001$), but there was no significant main effect for condition ($F_{(1, 7)} = 2.15$, $p = 0.186$) or their interaction ($F_{(4, 28)} = 2.51$, $p = 0.064$). Finally, for muscle pain, a significant main effect of time was identified ($F_{(4, 28)} = 44.313$, $p < 0.001$), while no significant main effect was found for condition ($F_{(1, 7)} = 3.500$, $p = 0.104$) or their interaction ($F_{(4, 28)} = 0.467$, $p = 0.760$) *(Figure 7.7)*.
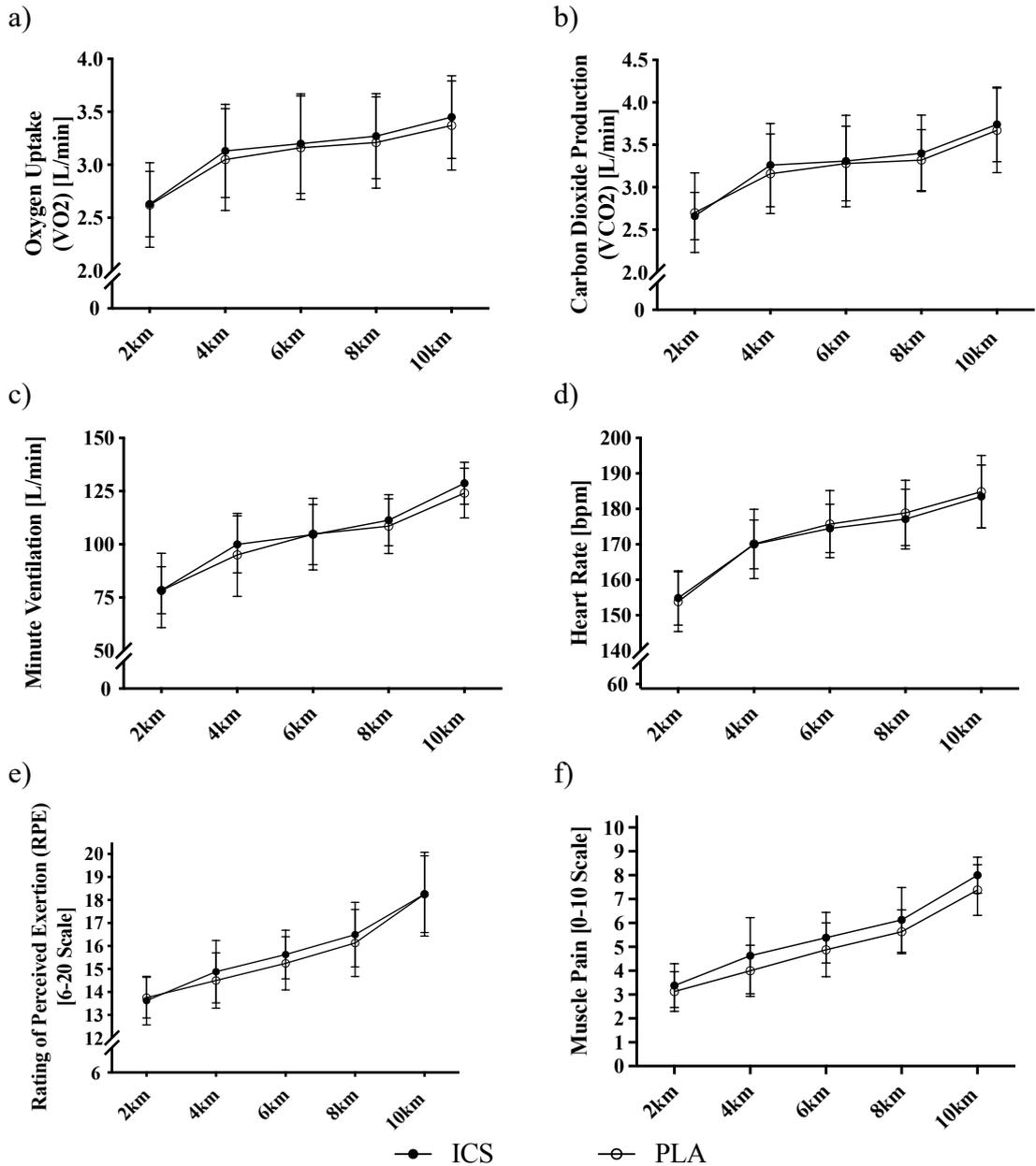
Figure 7.7. *Physiological response ($\dot{V}O_2$ [a], $\dot{V}CO_2$ [b], $\dot{V}_E$ [c], HR [d]) and Perceptual ([RPE [e], PAIN [f]) outcome measures during subsequent 10-km cycling time-trial (TT2). Data presented as mean ± standard deviation by 'treatment' and 'time' ANOVA factors. Abbreviations: **ICS**, Inhaled corticosteroids; **PLA**, Placebo.*

In terms of completion time, there was a significant overall effect of time ($F_{(1,7)} = 7.326$, $p = 0.030$) between TT1 and TT2. However, when comparing TT1 and TT2 within each treatment (ICS and PLA), there was no significant difference (-20.4, $p = 0.064$ for ICS and -21.01, $p = 0.070$ for PLA). Moreover, there was no significant main effect found for condition ($F_{(1,7)} = 0.211$, $p = 0.660$) or the time*condition interaction ($F_{(1,7)} = 0.002$, $p = 0.960$), suggesting the magnitude of change in completion time was not affected by the condition (*Figure 7.8c*). This is further supported with the median change ($\pm$ IQR) in completion time not significantly different between the ICS (10.2 ± 18.9) and PLA (10.9 ± 16.5) ($W = 17.0$, $p = 0.945$).

Likewise, this same observation was evident in mean power output between TT1 and TT2 [time ($p = 0.027$), condition ($p = 0.640$), interaction ($p = 0.955$)] *(Figure 7.8b & 7.8d)*.
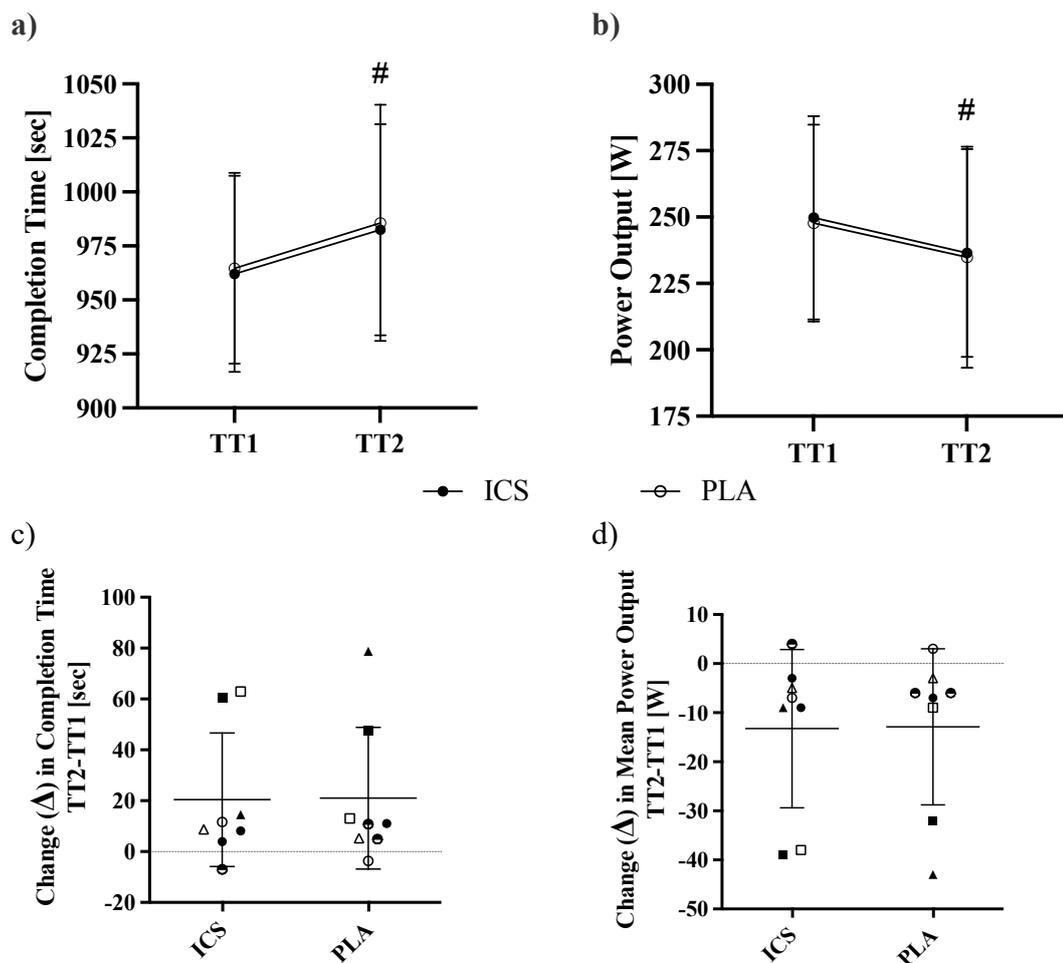
Figure 7.8. *Subsequent 10-km completion time (a) and mean power output (b) in TT1 and TT2. Magnitude of change (Δ) in completion time (c) and power output (d) between initial and subsequent TT. Individual performance [shapes], mean [centre line], and standard deviation [error bars]. Abbreviations; Inhaled corticosteroids (ICS); Placebo (PLA).*

Short Recovery Stress Scale (SRSS)

No significant differences were indicated in SRSS between ICS and PLA conditions prior to TT2 ($p > 0.05$ for all subdomains). Yet, small effect sizes ($d = 0.21–0.49$) were noted for muscular stress, negative emotional state, mental performance capability, overall stress, and overall recovery sub-domains *(Table 7.1)*.

Table 7.1. Results of Short Recovery and Stress Scale (SRSS) for inhaled corticosteroid (ICS) and placebo (PLA) conditions prior to the subsequent 10-km time-trial (TT2).

Variable	ICS	PLA	Test Statistic [t / z]	P-Value	Effect Size (Cohen's *d*)
Muscular Stress	2.56 (1.55)	2.31 (1.10)	0.798 [t]	0.451	0.28
Lack of Activation	2.13 (1.25)	2.19 (1.51)	-0.205 [t]	0.844	-0.07
Negative Emotional State	1.25 (1.17)	1.00 (0.93)	4.00 [z]	0.733	0.33
Overall Stress	2.50 (1.60)	2.13 (1.13)	1.158 [t]	0.285	0.41
Physical Performance Capability	3.13 (1.64)	3.25 (1.75)	-0.314 [t]	0.763	-0.11
Mental Performance Capability	3.88 (1.55)	4.00 (1.51)	-1.000 [t]	0.351	-0.35
Emotional Balance	3.63 (1.77)	3.75 (1.45)	9.00 [z]	0.824	-0.14
Overall Recovery	3.38 (1.41)	3.63 (1.30)	-1.00 [t]	0.351	-0.35

Note. Data presented as mean ± (standard deviation) Abbreviations: **ICS**, Inhaled corticosteroids; **PLA**, Placebo; **z**, Wilcoxon test statistic; **t**, paired samples t-test.

Session Rating of Perceived Exertion (sRPE)

No significant difference was seen in sRPE between ICS (8.13 ± 1.27 A.U.) and PLA (8.00 ± 1.41 A.U.) conditions (z = 4.50, $p = 0.586$).

Capillary Lactate and Glucose

For Lactate, there was a significant main effect of time ($F_{(1.30, 9.11)} = 50.55$, $p < 0.001$). However, no significant main effect was found for condition ($F_{(1, 7)} = 0.002$, $p = 0.969$) or their interaction ($F_{(3, 21)} = 0.316$, $p = 0.806$) *(Figure 7.9a)*.

For Glucose, there was a significant main effect of time ($F_{(3, 21)} = 3.817$, $p = 0.025$). However, no significant main effect was found for condition ($F_{(1, 7)} = 0.170$, $p = 0.693$) or their interaction ($F_{(1.58, 11.03)} = 0.262$, $p = 0.723$). *(Figure 7.9b)*.
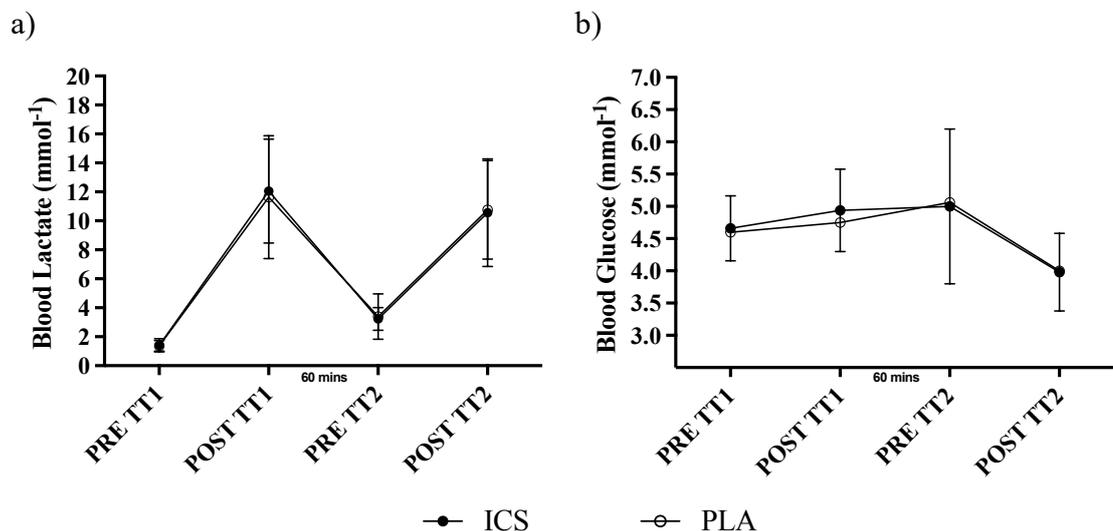
Figure 7.9. Capillary blood lactate (a) and glucose (b) before and after the initial (TT1) and subsequent (TT2) cycling time-trials.

Interleukin-6 (IL-6)

Venous blood samples were obtained from all participants and at all time points. There was a significant main effect of time ($F_{(1,7)} = 15.197$, $p = 0.006$) and condition ($F_{(1,7)} = 8.725$, $p = 0.021$). Pairwise comparisons using Tukey correction revealed that there was no significant difference between the conditions before exercise ($p = 0.171$). However, both ICS ($p = 0.031$) and PLA ($p = 0.031$) conditions showed a significant increase after exercise. Nonetheless, there was no significant difference between the ICS and PLA conditions after exercise ($p = 0.250$), indicating no significant interaction between the condition and time point ($F_{(1, 7)} = 0.243$, $p = 0.637$). *Figure 7.10* presents these findings.
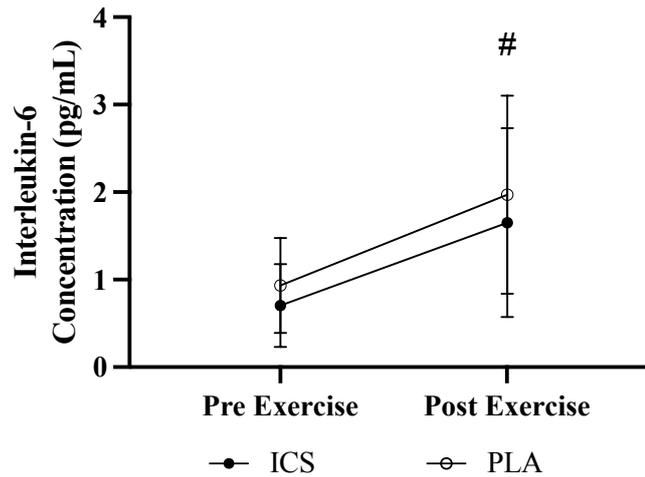
Figure 7.10. Absolute plasma interleukin-6 (IL-6) concentration pre and post exercise following short-term inhaled corticosteroid (ICS) administration or placebo (PLA). (*) denotes significant difference between condition. (#) denotes significant difference within condition from pre-exercise values.

Differential White Blood Cell Count (WBCdiff)

Regarding the WBC-diff, white blood cell (WBC), neutrophils, lymphocytes, and monocytes all significantly increased post-exercise ($p < 0.001$). However, there was no interaction between the condition and time-point ($p < 0.05$). Eosinophils did not present a main effect of time or condition, and thus no interaction between condition and time evident. Basophils were not analysed as all concentrations were measured as 0 units, suggesting the device was not sensitive to detecting the concentration in the sample.

Supplementary Outcome Measures

Drug Blinding

Three participants (37.5%) correctly identified the ICS condition. The main reason for correct identification was a difference in taste between the substances, or a random guess.

ICS-Q and Adverse Effects

No participants experienced serious adverse effects. Common side-effects were reported by a total of two participants (25%). One participant (12.5%) reported a mild headache shortly after inhalation on five occasions during active substance intake period. One participant (12.5%) experienced an 'itchy throat' during the intake period. Despite this, there was no difference in ICS-Q score between ICS (2.12 ± 3.09 a.u.) and PLA (1.88 ± 1.96 a.u.) conditions (t(7) = 0.344, $p = 0.741$).

Drug Effect Questionnaire (DEQ-5)

There was no significant difference between ICS and PLA conditions in any DEQ-5 sub-domains ($p > 0.05$).

Lung Function Measures

There was a significant effect of time ($F_{(1,7)} = 11.72$, $p = 0.011$), condition ($F_{(1,7)} = 10.34$, $p = 0.02$), and interaction ($F_{(1,7)} = 5.46$, $p = 0.05$) for FeNO. Pre-administration, there was not significant difference between ICS and PLA conditions ($p = 0.351$). FeNO significantly decreased in ICS ($p = 0.023$), but not with PLA ($p = 0.949$). As such, FENO was significantly lower at post-admin in ICS than PLA ($p = 0.023$) *(Figure 7.11a)*. Regarding FEV$_1$, there was no significant effect of time ($F_{(2,14)} = 1.190$, $p = 0.33$), condition ($F_{(1,7)} = 0.532$, $p = 0.489$), or interaction ($F_{(2,14)} = 0.854$, $p = 0.447$) *(Figure 7.11b)*. Likewise, with FVC there was no significant effect of time ($F_{(2,14)} = 3.070$, $p = 0.08$), condition ($F_{(1,7)} = 0.596$, $p = 0.465$), or interaction ($F_{(2,14)} = 0.621$, $p = 0.552$) *(Figure 7.11c)*.
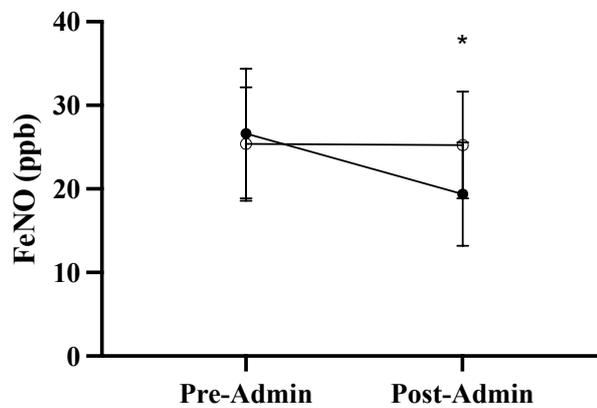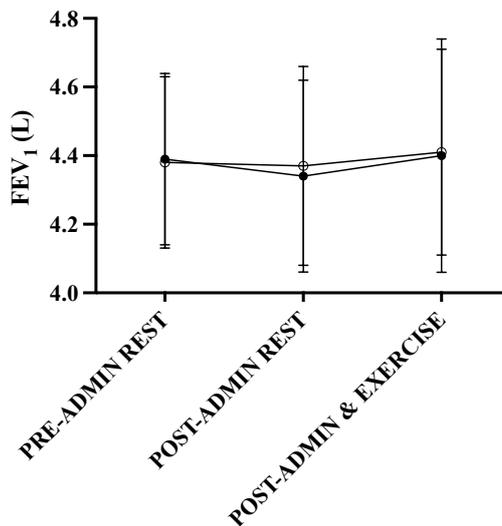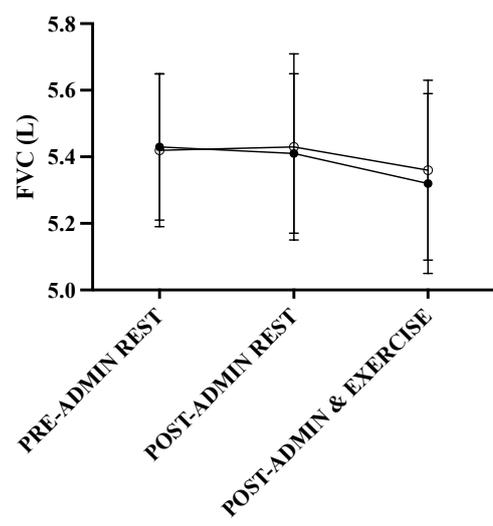
Figure 7.11. (a) Fractional Exhaled Nitric Oxide (FeNO), (b) Forced Expiratory Volume in One Second (FEV₁) and (c) Forced Vital Capacity (FVC) before and after short-term administration of inhaled corticosteroid (ICS) or placebo (PLA) at rest and immediately post-exercise.

7.4. Discussion

The principle finding of this study was that short-term daily inhalation of 800 µg ICS [Beclomethasone dipropionate] did not induce any statistically ($p = 0.50$) or meaningful (0.27%) improvement in completion time, mean power output, or impact on physiological response among trained cyclists completing a 10-km cycling time trial. Furthermore, short-term daily use of ICS did not exert impact on a subsequent 10-km cycling time-trial performance conducted on the same day, nor did it influence the associated recovery questionnaire data assessed. Finally, ICS administration did not demonstrate any notable effect on systemic inflammatory cytokine response either prior to or after exercise when compared to the PLA condition.

Initial Exercise Performance

The present study responded to a call for research identified by previous narrative reviews and a recent meta-analysis highlighting the lack of investigations into short-term administration of inhaled GC class therapy, and the need for closed-end performance outcomes (Collomp *et al.*, 2016; Tacey *et al.*, 2017; Riiser, Stensrud and Andersen, 2023).

The findings provide initial evidence indicating that daily high-dose ICS does not result in enhanced cycling performance evaluated through an ecologically valid time-trial outcome measure. The mean difference in completion time of 0.27% between the ICS and placebo condition would not be considered as a meaningful ergogenic effect, and the MBD interpreted this as 'trivial'. The findings align with the most comparable research on maximal oxygen consumption ($\dot{V}O_2max$), in which it was demonstrated no improvement following a 14- or 28-day intake period of budesonide (Kuipers *et al.*, 2008). Moreover, the small, standardised effect size calculated within meta-analysis from the data of Kuipers *et al.,* (2008) closely match that of the present study (Riiser, Stensrud and Andersen 2023). This is despite different formulations and types of inhaler (pMDI in present study, dry powder inhaler in former investigations). Moreover, a major strength of this study is the randomised crossover enabled each participant to act as their own control, minimising the potential impact subtle differences in participant characteristics and individual responses to ICS could have upon the outcome variable. This builds upon the between-subject design implemented by Kuipers *et al.,* (2008).

The daily short-term use of ICS built upon Chapter 6 investigating acute use of high-dose ICS administration, also demonstrating no impact on cycling time-trial performance. The daily dosing regimen akin to the dosage potentially used by a moderate to severe asthma

sufferer [rather than acute use], and findings of the present chapter are more indicative of the likely outcomes of use in real life practice. The 14-day administration period was designed to provide enough time for maximum therapeutic effect, bioavailability, and a feasible experimental design aligned with previous investigations (Kuipers *et al.*, 2008; Schwindt *et al.*, 2010).

IL-6

This experimental chapter further explored the hypothesis of GC treatment blunting exercise-induced pro-inflammatory responses (Arlettaz, Collomp, *et al.*, 2008). There was no change in IL-6 pre-exercise or blunting post-exercise compared to the placebo condition, signifying that short-term daily ICS treatment did not impact on systemic pro-inflammatory response, supporting and building on the findings of *Chapter 6* after acute doses of ICS. As with *Chapter 6*, the lower systemic bioavailability from an inhaled substance compared to oral administration may contribute to this observation (Trinh, Chen and Diep, 2022). This however is contradictory to a previous study utilising two-weeks administration of high-dose inhaled fluticasone proprionate with VHC, whereby significant blunting of IL-6 post exercise was demonstrated (Schwindt *et al.*, 2010). Although Schwindt and colleagues (2010) described participants completed 'heavy' intensity exercise for 30 minutes, this differs from the more physically demanding TT of the present study, as indicated by the large difference in work-rate and post-exercise lactate between the studies. The TT would cause greater levels of stress on the body due to an positive relationship between exercise intensity and cortisol response (Hill *et al.*, 2008), and would rationalise the greater increase in IL-6 observed in the present study. However, the meaningful difference in blunting of IL-6 post-exercise is unknown (Walshe *et al.*, 2010). Therefore, more research is required to determine consensus on the impact of short-term ICS treatment following intense exercise performance.

Recovery and Stress State

The present study observed no effect on perceived recovery (or any other SRSS subdomains) before, or any performance and physiological outcomes during a subsequent bout of time-trial cycling. Furthermore, no significant change in SRSS subdomains were seen 24 hrs post-experimental visit. These findings align with (Kuipers *et al.*, 2008) who observed no effect on profile of mood states between budesonide and placebo groups. These findings built on *Chapter 6* that saw this same observation from acute ICS but is novel in that recovery between multiple-bouts of exercise were performed on the same day from short-term ICS treatment.

Submaximal exercise

In this study, the pre-loaded exercise trial was conducted to establish a consistent low-intensity exercise session to evaluate whether ICS influenced the oxidation of carbohydrate and fat. This choice was motivated by the suggested notion that GC administration could indirectly influence sports performance by promoting changes in substrate utilisation (Arlettaz *et al.* 2008). The short-term daily administration of ICS did not demonstrate any impact on fat oxidation during low-intensity exercise or any other perceptual response to exercise, including ratings of perceived exertion (RPE) and muscle pain. This builds upon the findings of *Chapter 6*, which demonstrated similar results following the acute administration of ICS. Related to this, previous suggestions are that GC can induce changes in body composition from increase in free fatty acids. This has not specifically been explored within a controlled-trial, yet previous reports suggest no significant changes in body mass or index following short-term oral (Le Panse *et al.*, 2009) and inhaled GC intake (Jardim *et al.*, 2007). However, the intake period of the present study and the those mentioned would likely not be sufficient to induce changes in body fat or muscle hypertrophy. Moreover, prospective studies investigating body composition should consider high precision equipment such as dual-energy x-ray absorptiometry.

The findings of the present study indicate that short-term daily administration of high-dose ICS did not lead to improvements in FEV_1. This lack of enhancement may be attributed to the non-asthmatic status of the participants, who did not show any signs of obstruction at rest. It is possible that these effects could have been more pronounced if asthmatic participants were included. But critically, bronchodilation in healthy individuals is not considered an ergogenic mechanism (Koch, Macinnis, *et al.*, 2015). For example, Decorte *et al.*, (2008) observed in non-asthmatic individuals an increase in FEV_1 of 0.2 Litre following 800 μg salbutamol, and subsequently did not result in greater $\dot{V}_E$ or improved endurance performance. Additionally, the present study saw a reduction in FVC post exercise, an observation thought to result from exercise-induced dehydration (Simpson, Romer and Kippelen, 2017). Although ICS intake significantly reduced FeNO levels, participants with low FeNO levels (<25 ppb) did not meet the threshold for a minimally important reduction in airway inflammation [*defined as a 10-ppb decrease if the initial level is <50 ppb*] (Dweik *et al.*, 2011). It is worth also noting that FeNO can be influenced by transient factors such as allergies or respiratory illness, even in non-asthmatic populations. Taken together, the changes in lung function (FEV_1) and exercise

ventilatory parameters (e.g., $\dot{V}_E$, $\dot{V}O_2$) add evidence that ICS are not ergogenic by these mechanisms.

Limitations

In this study, the highest recommended therapeutic dose of ICS *[Beclomethasone dipropionate]* was employed with the theory that this would more likely observe any changes in performance or inflammatory process. Limitations imposed by institutional insurance and ethics committee raised concerns regarding the administration of ICS at doses exceeding the manufacturer's recommendations for unsupervised inhalation over the 14-day intake period. Consequently, we were unable to investigate the effects of supratherapeutic doses in attempt to increase the extrapulmonary effects.

Assessment of inhaler technique was only possible at the midway point of the intervention. To enhance this aspect of the study, participants could have been requested to provide video evidence demonstrating their inhaler technique, or alternatively, a smart inhaler with a time stamp could have been employed to monitor and record inhaler usage consistently throughout the intervention period.

Although all participants had high adherence to the intervention (>90%), a limitation concerns the reliance on self-reported records to evaluate drug compliance, raising uncertainty about whether participants actually administered all the doses they reported and thus impacting the validity of the findings. This self-reported approach has been used previously in most ergogenic effect inhaler studies (Dickinson *et al.*, 2014; Jessen *et al.*, 2018) *[to name a limited number]*. However, this method has exhibited limitations, as studies have revealed notably low drug compliance when relying on self-reported records (Farmer, 1999), especially *[and relevant to the present study]* when multiple inhalations per day are required (Bateman *et al.*, 2008). While periodic monitoring through online tools was implemented to ensure proper inhaler technique, closer supervision *[such as monitoring daily dosing via video]* may be advantageous in ensuring intervention compliance. Furthermore, employing objective methods to quantify adherence, like using a 'smart inhaler' device to timestamp inhaler actuations and sending administration reminders (Chrystyn *et al.*, 2019) could be beneficial in future studies to alleviate burdens on both participants and researchers.

Implications and Future Research

Current WADA guidelines regarding ICS to allow use within therapeutic indications during 'in-competition' periods are appropriate given the lack of ergogenic effect observed during an ecologically valid TT assessment or recovery between bouts of performance. Future research should explore ICS combined with LABA [notably formoterol] as this is a common treatment pathway for moderate to severe asthma/EIB and suggested by GINA. This combined action is warranted given the observations of Hostrup *et al.*, (2017) who demonstrated changes in Na+/K+-ATPase activity following two-weeks of budesonide administration. Increases in Na+/K+-ATPase may serve to reduce the impact of peripheral fatigue by counteracting exercise-induced accumulation of extracellular K^+ (Hostrup *et al.*, 2014). Moreover, it is suggested to research the interaction of ICS with training, as this has not been explored.

7.5. Conclusion

In conclusion, the present study examined the effects of daily short-term (14-day) administration of 800 µg ICS [Beclomethasone dipropionate] on the performance and physiological responses of trained cyclists during repeated 10-km cycling time trials. The findings revealed that ICS did not enhance mean power, completion time, or physiological response during the time trial, nor did it influence the subsequent 10-km cycling time-trial performed on the same day. In addition, there was no observable effect on pre- or post-exercise systemic inflammation, as measured by IL-6. These results align with previous research on short-term ICS use, but add the same null effect is observed when using an ecologically valid TT outcome. Taken together, this experimental chapter suggests that short-term daily ICS inhalation does not provide an ergogenic benefit for trained cyclists, as such can remain allowed within competition periods by the WADA for therapeutic indication. Further research should understand the effect of combined ICS/LABA treatment on hormonal response, exercise performance, and adaptations associated with training.

CHAPTER 8: GENERAL DISCUSSION

8.1. Summary of Key Findings

The overall aim of the present thesis was to investigate the impact that acute, short, and long-term administration of asthma-related glucocorticoid therapy has on respiratory outcomes, exercise performance, and recovery.

- The first experimental chapter *(Chapter 4)* was conceptualised following review of literature that demonstrated there is a paucity of studies investigating the impact that diagnosing and initiating management of EIB has on respiratory health and performance outcomes. It added evidence that diagnosis and then long-term use of asthma inhaler therapy *[12-months]* had positive effects on respiratory outcomes in an elite swimming population with EIB, but this did not lead to a meaningful improvement in performance at major competitions above the expected progression and between-competition variation. Discontinuation of therapy resulted in maintained EIB severity and had an increased likelihood of detriment in performance at major games compared to adhering to treatment.

- *Chapter 5* investigated the deposition of ICS using a simulation model. The findings showed that inhalation flow rate can significantly impact the delivery of ICS, and use of a valved-holding chamber (VHC) improved fine particle dose that would better target ICS mechanisms of action. The findings of provided methodological development for the remainder of laboratory based experimental chapters utilising ICS administration, and allowed the estimation of delivered dose to participants in these studies.

- *Chapter 6* incorporated three aims to comparing oral and inhaled glucocorticoid administration routes in relation to WADA legality. [1] to address a key limitation of previous investigations by assessing in a single study the potential ergogenic effects of acute oral and inhaled glucocorticoid administration within an ecologically valid performance protocol that closer resembles real-world conditions, specifically, a 40-km cycling TT; [2] Investigate the recovery for a subsequent bout of competitive cycling (10-km TT) performed on the same day; and [3] to examine the physiological and perceptual responses to these bouts of exercise related to theorised ergogenic mechanisms. The results suggested that supratherapeutic dose of ICS did not improve performance. However oral GC although not significant did have a 'possible' ergogenic effect on the initial TT performance.

- Lastly, the final experimental study *(Chapter 7)* built on *Chapter 6* by investigating the short-term effect of high dose ICS administration [800 μg daily for 14-days]. The findings demonstrated that there was no improvement in performance or ergogenic mechanisms from using high-dose ICS compared to placebo condition.

8.2. Performance Enhancing or Purely Therapeutic? - Implications for Anti-Doping Policy

The title of this thesis posed the rhetorical statement regarding the doctrine of double effect: Is asthma-related GC therapy 'purely therapeutic' or 'performance-enhancing'? To explore this, four experimental chapters were undertaken to investigate the role of asthma-related GC in three distinct dimensions: (1) the impact on respiratory outcomes, (2) its potential ergogenic effect on exercise performance, and (3) its role in facilitating recovery following repeated bouts of exercise.

It is clearly evident that there is a therapeutic necessity for GC in the management of asthma-related conditions (e.g., EIB), serving as either maintenance treatment (ICS) or for the acute management of serious exacerbation (Oral GC) (Barnes and Ulrik, 2015; Matera *et al.*, 2019; Price *et al.*, 2020). However, *Chapter 2* presented there is a lack of studies specifically investigating the impact of diagnosing and initiating treatment in elite athlete populations (Price and Hull, 2014; Price *et al.*, 2014). Jackson *et al.*, (2018) investigated this in elite football players, demonstrating that after nine-weeks of treatment there was a clinically meaningful attenuation of EIB induced by hyperpnoea and reduction in FeNO. *Chapter 4* added support that following twelve-months of treatment with LABA and ICS treatment provided reduction in EIB severity in elite swimmers, an important finding given the higher prevalence of EIB in endurance aquatic sports compared to other Olympic sports (Levai *et al.*, 2016). Moreover, athletes who had discontinued treatment in the observation period had no changes in respiratory health, highlighting the importance of adherence to treatment and the therapeutic need. While researching the implications of the findings from *Chapter 4*, it was noted that Jackson *et al.*, (2018) reported that elite athletes often demonstrate critical errors in pMDI inhaler technique, suggesting the main fault in inhaler technique was high inhalation flow rate, up to ten-fold the 30 L/min^{-1} suggested for a pMDI (Laube *et al.*, 2011). This in part led to the development of *Chapter 5* investigating how flow-rate and VHC may impact on

the delivered dose of ICS, and then related to *Chapter 6* and *7* if appropriate technique be implemented in studies on ergogenic effects of asthma medication.

From an athlete care perspective, *Chapter 4* demonstrated that a follow-up assessment provides an opportunity to ensure EIB therapy is adequate, reinforce inhaler technique, emphasise importance of adherence, and assist education to athletes and support staff in anti-doping matters (Parsons *et al.*, 2013). Moreover, current NICE guidelines for asthma (NICE, 2017) suggest that clinicians should take into account possible reasons for uncontrolled asthma before increasing dosage or changing medication. E.g., lack of adherence, psychosocial factors, sub-optimal inhaler technique. A systematic approach to management of EIB could help implement this approach (Gowers *et al.*, 2021; Hull *et al.*, 2021).

As part of WADAs directive is to maintain and promote athlete health, the prohibited list ensures that no harm is provided to the athlete (WADA). This is one of the arguments as to why systemic GC should remain banned (Pigozzi *et al.*, 2012), given the adverse events that may occur such as adrenal suppression, metabolic imbalances and immunodeficiencies (Vernec *et al.*, 2020). Although *Chapter 6* did not specifically investigate ergolytic effects and was only used on an acute basis, some participants did experience mild side effects such as nausea and headaches from both supratherapeutic ICS and high-dose oral GC. Interestingly, Kuipers *et al.*, (2008) also suggested the negative impacts GC could have on performance given previous observations of catabolic effect and adrenal suppressive effects – but the relatively short observation period of *Chapter 7* and retrospective nature of *Chapter 4* has not generated any evidence to comment on this.

The second facet of the rhetorical question revolved around whether substances related to asthma, such as GC, could be considered as having a *"performance-enhancing"* effect. As outlined *Chapter 1* and *2*, WADA current prohibits asthma-related GC in-competition when delivered systemically (i.e., oral GC), but local routes such as inhaled administration (i.e. ICS) are able to be used openly in-competition periods within therapeutic indications. Moreover, GC are allowed for out-of-competition use through any method of administration (WADA, 2023e), but systemically delivered substances must have *'washed out'* the bodily system by the predetermined period (Ventura *et al.*, 2021; WADA, 2023c). Historically there has been considerable tension between anti-doping rules established on the evidence of performance enhancement potential and the

accepted use of GC for the treatment of medical condition in elite athletes (Vernec *et al.*, 2020).

The experimental work within this thesis has made novel contributions that may be of specific interest to anti-doping policy and stakeholders [Aims 2 & 3]. These contributions include:

- Long-term therapeutic use of maintenance asthma therapy [incorporating ICS or LABA/ICS combined] did not enhance performance in real-world exercise performance at major competition above the progression expected between competitions in athletes with EIB *(Chapter 4)*.

- Supratherapeutic dose of ICS [outside therapeutic indications] administered on an acute basis prior to a cycling time-trial had no impact on TT performance or recovery to subsequent bout of exercise *(Chapter 6)*.

- Acute oral GC [within therapeutic indications] may be *'possibly beneficial'* in 40-km TT when considering the smallest meaningful change in performance an athlete may be interested in *(Chapter 6)*. There was also significant suppression of pro-inflammatory cytokine [IL-6] and changes in metabolic function regarding elevated blood glucose. However, there was no enhancement in recovery for a subsequent bout of exercise, or self-reported questionnaire relating to stress and recovery.

- High-dose ICS [but within therapeutic indications] taken on a short-term basis [14-days] had no impact on a cycling time-trial performance or recovery for a subsequent bout of exercise *(Chapter 7)*.

Collectively, the findings of this thesis support that elite athletes mandate the use of asthma therapy *(Chapter 4)* and vindicates the current WADA guidelines on ICS are appropriate given lack of ergogenic effect when assessed using an ecologically valid TT assessment following acute supratherapeutic *(Chapter 6),* short-term high-dose *(Chapter 7)* or when used for therapeutic purposes within an elite athlete population *(Chapter 4)*. This adds to the limited evidence (Jardim *et al.*, 2007; Kuipers *et al.*, 2008) that was previously available on ICS and can give WADA more confidence in applying this regulation.

Previous editorial review has raised questions about whether GC should be removed from WADA's list of banned substances due to the lack of evidence demonstrating performance enhancement (Orchard, 2008). However, it can be argued that oral GC

should remain controlled with a TUE due to uncertainty of ergogenic impact and immunosuppressive effects at higher acute doses [as *observed in Chapter 6*], previous evidence that short-term use may be performance enhancing at low-moderate exercise intensity (<75% $\dot{V}O_2max$) (Arlettaz *et al.*, 2007; Le Panse *et al.*, 2009) and well documented health implications from long-term use (Montalvan and Duclos, 2008). The recent refined adverse analytical detection testing limits for specific metabolites such as 6β-hydroxybudesonide contribute to distinguishing between inhaled permitted and oral prohibited administration routes, thus strengthening the enforcement of WADA policy (Ventura *et al.*, 2021; WADA, 2022c).

There are currently no dedicated studies informing athletic return-to-sport following an acute exacerbation of asthma, so little evidence to support robust prescriptive recommendations, however there should be a balance between risk versus benefit (Hull, Burns, *et al.*, 2022). In the general population, it is advised to completely avoid vigorous exercise during the period immediately following such an exacerbation (Schwellnus *et al.*, 2022). Relating to athletes' health *[Thesis Aim 1]*, Pigozzi *et al.*, (2012) reaffirmed that using GC may not be an immediate solution for a condition and that an athlete may still require a period of recuperation before continuing sport. It seems reasonable to suggest that an athlete requiring maximum treatment for an acute exacerbation of asthma *[i.e. oral GC]* should not partake in vigorous exercise (Schwellnus *et al.*, 2022). The decision to return to training and competition is unquestionably complex, multifactorial and involves a shared decision process (Dijkstra *et al.*, 2017). The return to sport following a COVID-19 infection garnered significant attention, bringing this issue to the forefront of both research and guidance to athletes and medical professionals (Wilson *et al.*, 2020; Haan *et al.*, 2021; Hull, Wootten, *et al.*, 2022). But ultimately, the judgement to compete comes down to the athlete themselves, even if this potentially jeopardises their health. Evidently, elite athletes endeavour to train and compete despite being unwell or injured (Dijkstra *et al.*, 2014), and current policy does allow athletes to do so through approval of a prospective or retroactive TUE providing required conditions have been met (WADA, 2023d). Elite athletes may use pharmacological substances as method to remain healthy through training and competing (Lentillon-Kaestner, Hagger and Hardcastle, 2012). With anti-doping policy also functioning to support health promotion by banning substances that are harmful (WADA, 2021c), various models of harm-reduction approaches have been proposed (Henning *et al.*, 2021). One approach included more rigorous and regular evaluation of an athlete's health and fitness to perform, and

exclude athletes from competing if they were deemed not healthy enough (Savulescu, Foddy and Clayton, 2004). Other reviews have proposed options to 'de-regulate' and abolish all regulatory frameworks for performance enhancing drugs (Goh, 2021), or advocate for 'medically supervised doping' to level the playing field (Kayser, Mauron and Miah, 2007).

Education emerges as a crucial facet of the appropriate use of asthma-related medications in elite sport. Athletes and their key stakeholders *[e.g., coaches, family, teammates, primary care physicians, trainers]* require comprehensive understanding of the use, misuse, and abuse of asthma-related management (Miller *et al.*, 2005). For asthma-related conditions, individuals should understand how to avoid common anti-doping rule violations. These include: the in-competition use of an orally ingested GC (e.g., prednisolone) without a prior TUE; failing to apply for a retroactive TUE if GC were used in an emergency; exceeding the decision limit for a β2-agonist in a urine sample potentially due to poor condition control; using a non-specified β2-agonist; or using any β2-agonist through a systemic route of administration (Hostrup *et al.*, 2024). Sport physicians and general practitioners often have a limited understanding of which asthma-related substances are permitted, and ability to identify the approved routes of administration for GC (Hull *et al.*, 2009; Hughes *et al.*, 2020). To address this, evidence-informed and expert opinion based clinical statements, such as those created by the *'British Thoracic Society'*, can be useful to provide concise and pragmatic guidance for clinicians on the management for athletes with respiratory issues, including the anti-doping considerations (Hull, Burns, *et al.*, 2022). WADA also provide specific guidance on asthma for physicians (WADA, 2023b). Previous observations have shown that knowledge of pharmacological substances (Mottram *et al.*, 2008) and asthma regulations (Allen *et al.*, 2022) is often poor among athletes. Regarding asthma-related therapy, the lack of knowledge can be damaging to the harmonisation of sport, and the management of athletes. Allen *et al.,* (2022) observed in a qualitative study that there was no distinction made between the types of medication or the method of administration when athletes considered their opinions on the performance-enhancing effects of asthma treatment. This lack of understanding likely leads to generalisations or misconception that ***all*** asthma medications constitute doping, which may further contribute to medication avoidance or misuse. The authors noted that targeted educational programmes should aim to address athlete knowledge and the negative stigma attached to the use of asthma therapy (Allen *et al.*, 2022).

Online eLearning courses and education initiatives like WADA's Anti-Doping Education and Learning Platform (ADEL) serve as a repository for education resources to improve awareness, mitigate the risk of adverse findings, and promote fair play (WADA, 2021b). In 2021, WADA implemented the *'International Standard for Education'*, mandating that anti-doping education cover several key areas: [1] increasing awareness of the dangers of doping, [2] providing current information on rules and procedures, [3] explaining the purpose of anti-doping to competitive athletes to ensure legitimacy and inform them of their rights and responsibilities within the system, and [4] instilling essential values to uphold the spirit of sport. Additionally, the *International Standard for Education* introduced a requirement for performance-based evaluations of anti-doping education programs, compelling organisations to assess the outcomes of the education provided to athletes and their support teams (WADA, 2021a; Blank and Petróczi, 2023). A recent systematic review determined to be effective, anti-doping interventions should use multifaceted approaches; including values development, comprise of several sessions, and be delivered by well-trained staff (Filleul *et al.*, 2024). Anti-doping education is linked to better knowledge, indicating that athletes who receive repeated education have more accurate knowledge than those with less education (Murofushi *et al.*, 2018).

8.3. Limitations

Despite the novel contributions this thesis has made through an array of research approaches, and the strengths of these studies exploring real-world and ecologically valid TT outcomes, several limitations are apparent across the four experimental chapters that may *limit the strength of conclusions and implications for policy.* These limitations pertain to particularly to methodological considerations and the participant characteristics.

Methodological considerations
Chapters 6 and *7* lacked treatment verification, primarily the assessment of metabolite concentration from a bodily sample. This limitation restricts the exploration of pharmacokinetic differences and equivalence in HPA axis stimulation between the oral and inhaled administration routes and inter-participant differences. Without this detail it also raises the question of whether the high and supra-therapeutic inhaled doses used in these studies would exceed the adverse analytical reporting threshold of 30 ng/mL for GC had the participants been subject to doping control. However, Coll *et al.*, (2021) described an unpublished model suggesting that even at the maximum licensed therapeutic doses,

inhaled routes are unlikely to achieve performance-enhancing levels. The supratherapeutic dosage used was above therapeutic indications, so was outside of the WADA code regardless.

Although this thesis utilised dosing relative to body mass for oral GC conditions, the use of fixed doses of ICS without accounting for morphological differences among participants may result in varying relative dosing. Additionally, there is a heterogeneity of response in systemic activity among individuals even receiving identical doses of corticosteroids, whether administered orally or via inhalation means (Szefler *et al.*, 2002). The absolute dosing approach was chosen because it is the standard prescription method for ICS with doses based on disease severity (GINA, 2022). However, given these factors, it is challenging to determine whether a dose-response relationship exists in the ergogenic effects of ICS from this work.

One limitation of the studies involving human participants (*Chapter 4, 6* and *7)* is the lack of direct mechanistic insight. Although *Chapter 6* and *7* analysed IL-6, glucose and lactate based on observations of previous literature, those chapters lacked other markers of HPA function or metabolism (e.g., ACTH, cortisol, GH, prolactin) that would have greater understood the impact treatment was having on health, performance, and recovery. For example, findings by Hostrup *et al.*, (2017) propose that the performance-enhancing properties of GC may operate through the mediation of metabolic induction factor 'Krüppel-like factor 15' (KLF-15), which improves lipid and amino acid metabolism (Morrison-Nozik *et al.*, 2015). The authors noted that this challenges some previous understanding that the performance benefits of GC may in part be associated with increased Na+, K+ ATPase content in skeletal muscle. It is not known if the inhalation of ICS at dosages used in *Chapter* 6 and 7 would adequately stimulate KLF-15 and could potentially explain why beclomethasone dipropionate did not demonstrate an ergogenic effect in Chapter 6 and 7, or budesonide in previous research conducted by Kuipers *et al.,* (2008) and Hostrup *et al.,* (2017). Nevertheless, further investigations are warranted on the specific function of Na^+, K^+ ATPase and KLF-15 in the performance-enhancing effects attributed to GC. Ensuring consensus with previous studies e.g., using common blood markers or validated questionnaires would be beneficial to allow direct comparison. This can be difficult due to the invasive nature of blood sampling and muscle biopsy procedure on participants, and the cost, time, and personnel implications.

Given that a key mechanism of GC administration is its impact on substrate utilisation, specifically the shift towards FO, *Chapters 6* and *7* incorporated a 20-minute steady-state cycling trial at 50% $\dot{V}O_2$peak. This intensity was chosen to ensure RER remained below 1.00 [*with familiarisation trial as confirmation*] and aligns with previous studies indicating the intensity at which maximal fat oxidation occurs is approximately $49.3\pm14.8\%$ and $48.3\pm0.9\%$ $\dot{V}O_2$max in athletic and healthy individuals, respectively (Venables, Achten and Jeukendrup, 2005; Randell *et al.*, 2017). This intensity is also closely aligned to the 60% $\dot{V}O_2$peak used by Arlettaz, Portier, *et al.*, (2008) to investigate substrate utilisation during 60 minutes of submaximal cycling following oral GC administration. To improve specificity, it might have been more appropriate to set the exercise intensity at a relative domain such as lactate or gas exchange threshold, which may lead to more metabolically homogeneous workloads between individuals. and closely align with the intensity of a TT (Padilla *et al.*, 2000). For future research, it may be valuable to assess maximum fat oxidation following GC administration during a maximum fat oxidation incremental graded cycling (FAT_{max}), or to utilise more prolonged steady-state exercise protocols (Achten, Gleeson and Jeukendrup, 2002), However, it should be noted that FAT_{max} testing may not fully represent fat oxidation during prolonged exercise, as studies have shown no significant differences in fat oxidation at FAT_{max} compared to constant intensity work rates above and below this point (Schwindling *et al.*, 2013; Takagi *et al.*, 2014; Amaro-Gahete *et al.*, 2019). While this intensity is suitable for examining low-intensity exercise and prolonged endurance efforts, it may not reflect the metabolic demands during a TT, which is typically performed at higher intensity. Changes in methodological approach could provide a clearer understanding of how GC influences FO under conditions that more closely mimic competitive scenarios.

Chapters 6 and *7* utilised the DEQ-5 questionnaire to assess perception of drug effects. Employing additional qualitative approaches such as open-ended questions or semi-structured interviews could have helped assess the nuances around potential performance enhancement of substances. For instance, using questions like, *"Did you perceive any impact on your performance due to today's experimental condition? If yes, in what way?"*, *"Did you hold any expectations regarding today's trial?"* and *"Did the experimental condition you experienced lead you to approach your trial differently today?"*.

Chapters 6 and *7* exhibited a significant strength by strongly advocating familiarisation of inhaler techniques, including repeated demonstrations. This approach has demonstrated its efficacy in enhancing patient-reported outcome measures and improving respiratory outcomes within asthmatic populations (Giraud, Allaert and Roche, 2011). *Chapter 5* introduced a novel aspect, specifically aiming to better standardise dosage delivered to participants, and mitigate against local adverse effects in *Chapter 6* and *7*. However, it is important to note that the use of a VHC reduces the delivered dose compared to not using VHC. Tomlinson *et al.*, (2005) illustrated that in an asthma medication study, utilising a time-based inhalation *[instead of specifying a particular flow rate]* could effectively provide feedback to participants, encouraging slow and deep inhalations when a VHC is not employed. Tomlinson *et al.*, (2005) also emphasised breath coordination, suggesting the training of patients to actuate the pMDI while already inhaling, rather than striving for split-second coordination between dose release and the onset of inhalation. Furthermore, contemporary tools such as *'smart inhalers'* capable of measuring inhalation technique, could significantly benefit future studies (Chrystyn *et al.*, 2019).

Chapters 6 and *7* were implemented as single-blind due to personnel constraints on experimental sessions and maintaining safety of participants. Moreover, the primary researcher had experience with the ICS used, making it possible for them to identify the placebo and active inhaler based on visual canister differences. However, this does increase the risk of bias. A double-blind design would have been more robust but was not feasible in the current setting. With hindsight, to maintain double-blinding and the safety of the participants, the primary researcher could have received sealed envelopes for each participants treatment allocation that could have been opened in the event of a serious adverse event. This approach has recently been implemented in a trial of β2-agonists treatment (Zügel *et al.*, 2021).

Chapter 4, 6 and *7* used magnitude-based decisions (MBD) to investigate practically important effects and compliment the null hypothesis significance testing commonly used within exercise physiology research. Moreover, good reporting practices were followed regarding indication of confidence intervals and standardised effect size that will help contribute to future systematic review and meta-analysis (Williams, Carson and Tóth, 2023). However, MBD may increase type-1 error and encourage over optimistic conclusions (Harrison *et al.*, 2020), so the interpretation that high-dose acute oral GC is 'possibly beneficial' to TT performance should be approached with caution.

The recruitment process for participants in Chapters 6 and 7 was confined to local convenience sampling *[supplemented by snowball effect]*. This approach posed challenges due to several factors: the extensive time commitment and invasive nature of the studies, use of WADA banned substances, and the significant impact of COVID-19, [e.g., enforcing social contact and travel restrictions]. Consequently, both *Chapter 6 and did not* meet the a priori sample size criteria significantly impacting the study's statistical power. Post-hoc analysis suggested that both studies had low statistical power to detect the small effects observed (*Chapter 6;* $\eta_p^2 = 0.06$, $1\text{-}\beta = 0.21$, $\alpha = 0.05$; *Chapter 7*, Cohen's $d = 0.25$, $1\text{-}\beta = 0.094$, $\alpha = 0.05$) thus this increases the likelihood of a type II error. This is a persistent issue within the field of ergogenic potential of GC and was underscored by recent meta-analyses (Riiser, Stensrud and Andersen, 2023) who identified 6 of the 15 studies reviewed had <10 participants. Future studies should prioritise ensuring adequate sample sizes. Despite these limitations, attempts were made to employ MBD using SWC, and presenting the individual responses to treatment, thereby rendering the findings of these experimental chapters a valuable contribution to future meta-analyses in the area.

Homogeneity of Participant Characteristics

In *Chapters 6* and *7*, only non-elite, non-asthmatic participants were recruited. From the perspective of a thesis investigating doping using therapeutic substances, the use of solely non-asthmatics may appear obscure, as WADA would likely be more interested in studying the effects of substances on athletes with EIB due to them competing whilst managing the condition [ICS] or with approved TUE for oral GC. The presence of airway disease might impact drug efficiency, as more substance may be deposited in the upper airway rather than reaching the alveoli periphery *(Figure 8.1)*. Previous observations and modelling indicate that the proportion of the dose deposited in the lung could be influenced by the severity of airway obstruction (Mortimer *et al.*, 2007; Wang *et al.*, 2014), supporting evidence suggesting that the systemic effects of ICS might vary based on underlying disease. However, this discrepancy could be formulation-dependent, evident in fluticasone but not budesonide due to differences in their formulations' oral bioavailability and water solubility (Mortimer *et al.*, 2007). Additional validation of this notion stems from prior pharmacokinetic studies that found no difference in urine concentrations of salbutamol between athletic and non-athletic subjects or between asthmatics and non-asthmatics after oral and inhaled administration (Elers *et al.*, 2012).

Hence, it remains unclear whether the presence or absence of reduced airway calibre and airway inflammation would impact the ergogenic effects of substances.

Investigating the effects of high-dose asthma therapy in competitive athletes is challenging, as banned substances would necessitate dispensation from WADA and requires athletes to refrain from competition for extended periods. *Chapter 4* involved elite athletes diagnosed with EIB using ICS and LABA in therapeutic indications, so despite the study limitations may hold greater relevance for WADA.
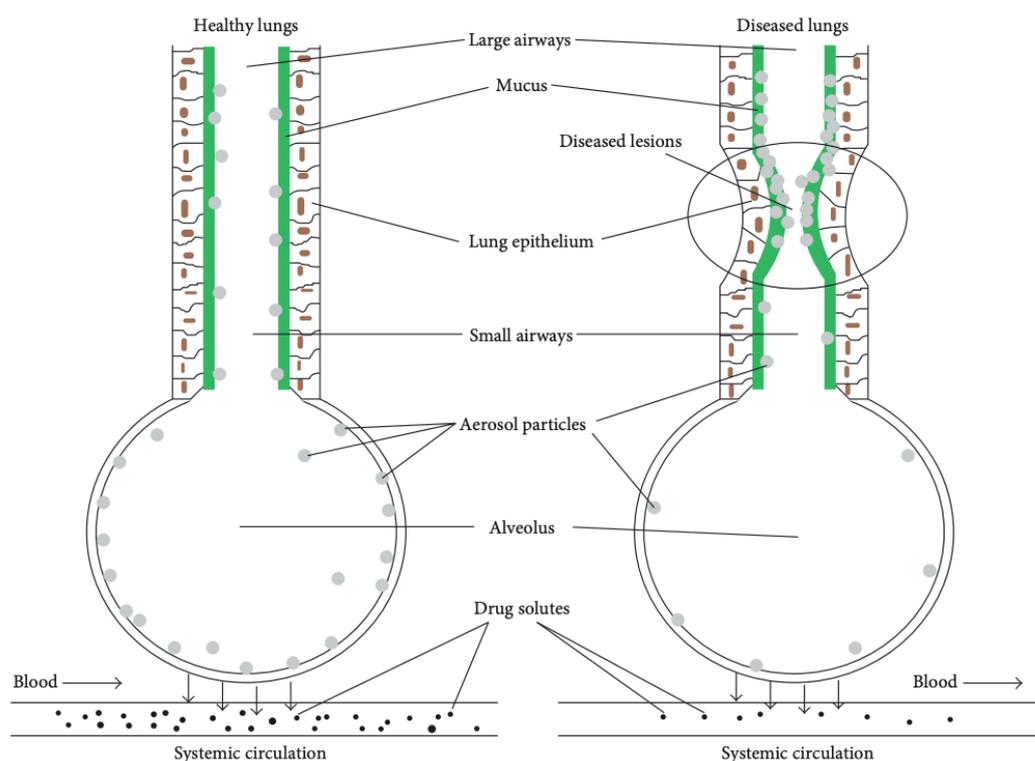
Figure 8.1. *Inhaled drug particle deposition in healthy versus diseased lungs* (Wang *et al.*, 2014)*.*

Finally, the homogeneity of the participants involved in *Chapters 6* and *7*, namely that they were exclusively male, raises questions about the generalisability of the findings to female participants given the physiological differences between sexes (Sheel, 2016). Furthermore, in *Chapter 4*, although there was an approximate equal split between male and female athletes, the analysis on major competition performance was not separated by sex. Therefore, it is unknown if this impacts the findings of the work. Le Panse *et al.,* (2009) identified similar exercise performance findings in female participants than previous observations in male participants (Arlettaz *et al.*, 2007; Collomp *et al.*, 2008). Additionally, HPA axis suppression by oral therapeutic doses of GC was found to be comparable between women and men (Jollin *et al.*, 2010; Collomp *et al.*, 2014). However,

in contrast to observations in male athletes (Arlettaz *et al.*, 2007) and *Chapter 6*, elevated blood glucose has not been observed in female athletes following short-term prednisone treatment (Le Panse *et al.*, 2009), so it may be inferred that women have a lower susceptibility than men to the insulin resistance caused by GCs. Previously observed data indicated a higher incidence of GC use among French female athletes compared to male athletes (Collomp *et al.*, 2022), but similar TUE prevalence for GC was reported in elite athletes of both genders at the Olympic Games (Vernec and Healy, 2020). As such, further research may be necessary to expand upon previous study by Le Panse et al., (2009) on exercise performance in female athletes. Regardless, the participation in research of female athletes and underrepresented groups should be encouraged to reduce inequities in sport and exercise medicine publications (Cowan *et al.*, 2023).

8.4. Future Research

The previous section *[8.3 - Limitations]* has identified concerns regarding methodological design and participant characteristics from this thesis that should be considered in future investigations. Beyond these, this thesis has identified gaps in literature that may warrant further investigation.

Current WADA policy allows inhaled and oral GC outside of competition. There has been call that systemic GC usage during training should be subject to a TUE (Pigozzi *et al.*, 2012). However, this approach may cause mis-management as athletes may avoid required medication (Hull and Pavord, 2018). Pigozzi *et al.,* (2012) cited the hypothesised mechanisms could potentially enhance their capacity to perform more work and adapt more effectively. Prospective studies should therefore explore peripheral adaptation at the muscular level by conducting muscle biopsies when oral GC and/or daily ICS is combined with endurance or strength training. This would add to the single previous study conducted by Collomp *et al.*, (2008) who incorporated a short (1-week) training intervention with prednisolone (oral GC) administration.

To knowledge, *Chapters 6* and *7* are the first studies investigating the effects of oral GC and ICS administration on outcomes associated with recovery between bouts of exercise. Further research should focus on the short-term administration of oral GC or ICS across consecutive days of testing. This approach would aim to simulate the stress endured by athletes participating in multi-day competitions, such as Grand Tour cycling events, which often span several days and necessitate high-quality recovery between stages. A

similar study design has been previously implemented in a study on the ergogenic effects of caffeine over consecutive days of simulated competition (Stadheim *et al.*, 2014).

Athletes with moderate to severe EIB will likely be prescribed with combination therapy incorporating inhaled β2-agonists and corticosteroids. Moreover, given that NICE guidelines may soon be updated to align with GINA strategy to suggest as needed or maintenance combined ICS/Formoterol (Parikh *et al.*, 2019; Chaplin, 2022), more UK-based athletes may in future be using ICS and fast-acting LABA (ICS-formoterol) to manage respiratory symptoms (often under proprietary name Symbicort®). It is widely acknowledged that these two classes of drugs exhibit molecular interactions (Barnes, 2002) to increase affinity of β2-agonist treatment (*Figure 8.2*). As discussed previously in this chapter, only one study, conducted by Hostrup *et al.*, (2017) has investigated the concurrent use of corticosteroids with β2-agonists in response to exercise. There also remains a scarcity of research on novel ultra-long-acting β2-agonists, such as Vilanterol that are delivered in combination with ICS (proprietary name Relvar Ellipta®) (Crisafulli *et al.*, 2017).
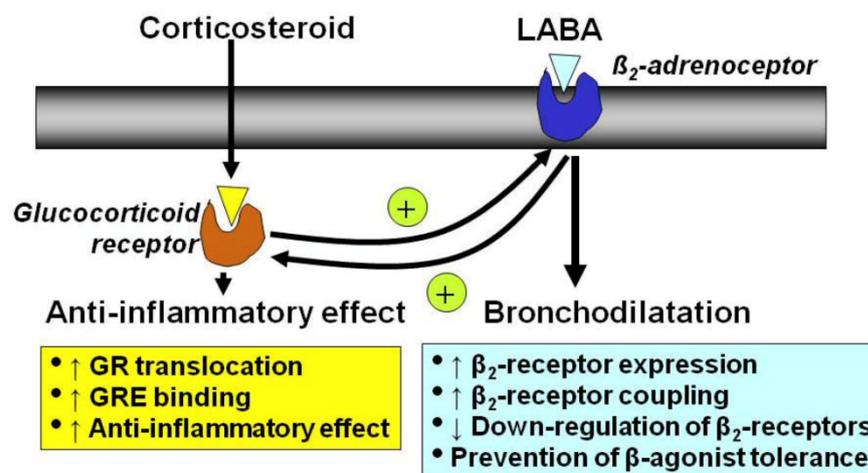
Figure 8.2. *Interaction between corticosteroids and long-acting β2-agonists (LABA). Corticosteroids have anti-inflammatory effects but also increase the numbers of β2- receptors, whereas β2-agonists, as well as inducing direct bronchodilatation, act on glucocorticoid receptors to increase the anti-inflammatory effects of corticosteroids (From Barnes, 2010).*

Another area requiring further investigation is the practice of combining low doses (micro-dosing) of different drug formulations (colloquially termed cocktail formulations) to achieve additive or synergistic effects while remaining within individual detection threshold values. This method has been observed in other doping practices involving other banned substances, such as erythropoietin (Martin *et al.*, 2016). Specifically to asthma therapy, Kalsen *et al.*, (2014) demonstrated that the combined inhalation of salbutamol, formoterol, and salmeterol [all β2-agonist] within permitted doses enhanced

swim ergometer performance and quadriceps maximal voluntary isometric force in elite swimmers, both with and without airway hyperresponsiveness. However, the same study also presented that TTE at 110% $\dot{V}O_2$max in a swimming test remained unchanged, with the authors attributing this to the larger muscle mass contribution and aerobic component of the TTE performance outcome are less affected by β2-agonist therapy. Recently, Bizjak *et al.*, (2023) investigated separate and combined acute administrations of SABA (salbutamol) and LABA (formoterol) β2-agonist therapy within permitted doses against placebo, but observed no difference in 10-min TT performance in healthy male and female athletes. However, the combined treatment revealed significant upregulation in gene expression from muscle biopsy samples relating to energy metabolism and hypertrophy. As with the Kalsen *et al.,* (2014) study, perhaps the null finding regarding exercise performance was due to the aerobic nature of the task and the dosage used, whereas consensus is that β2-agonist is ergogenic towards strength and at supratherapeutic doses (Riiser *et al.*, 2020). Further investigation is needed regarding the maximum permitted doses of LABA-ICS and SABA when combined with oral GC or ICS on strength and power exercise outcomes.

CHAPTER 9: CONCLUSIONS

In conclusion, this thesis began with an introductory chapter that addressed the contemporary issue surrounding asthma management in elite athletes *(Chapter 1)*. Following this, a literature review outlined the current knowledge of ergogenic potential of inhaled and oral GC *(Chapter 2)*. Subsequently, four experimental chapters were undertaken to investigate the impact of acute, short-term, and chronic administration of asthma-related GC therapy on athlete health, performance, and recovery *(Chapter 4 - 7)*.

Chapter 4 added evidence that there is a therapeutic need for elite athletes with EIB to access pharmacological GC treatment such as ICS to manage respiratory health outcomes. A pertinent observation was that initiating treatment did not enhance performance at major competitions beyond the expected progression of an elite swimming athlete. *Chapter 5* gave methodological development to prospective experimental work *(Chapter 6 and 7)* by modelling ICS deposition under different conditions, including slow and fast inhalation flow rates and with or without a VHC. *Chapters 6 and 7* are novel contributions as are the first known investigations on GC administration using an ecologically valid performance assessment [i.e., cycling TT]. They added to evidence that acute (supratherapeutic dose) or short-term (high dose) ICS does not enhance TT performance or recovery between bouts of exercise. *Chapter 6* was inconclusive with regards to acute high-dose oral GC, as magnitude-based decisions suggested that prednisolone may be *"possibly beneficial"* to initial TT performance. However, there was no evidence that GC conditions that were investigated improved recovery between exercise bouts. The laboratory studies *(Chapters 6 and 7)* were limited by sampling issues, nevertheless, the data presented will inform future research into the ergogenic impact of GC and contribute to future meta-analysis in the field.

Despite the methodological considerations outlined in *Chapter 8*, the evidence presented in this thesis collectively suggests that the current [as of 2023] WADA policy allowing the inhaled administration of GC at all times is likely appropriate, given the lack of ergogenic impact at therapeutic, high, and supra-therapeutic doses. However, oral GC should remain controlled with a TUE as uncertainty remains surrounding its ergogenic impact, potential immunosuppressive effects, and health risks associated with prolonged use. Due to the current guidelines allowing systemic GC outside of competition, further research should investigate the impact of oral GC and ICS on training adaptations. Additionally, the concurrent treatments used for asthma prophylaxis warrants investigation given the synergistic effect between ICS and LABA.

CHAPTER 10: REFERENCE LIST

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CHAPTER 11: APPENDICES

Appendix A. Confirmation of Ethical Approval

University of Kent

School of Sport & Exercise Sciences
Research Ethics and Advisory Group (REAG)
University of Kent at Medway
Chatham Maritime
Kent
ME4 4AG

Ethics Reference:

Prop 86_2018_19

Date: 28th May 2019

Dear Will Gowers,

Re: Dysfunctional Breathing in Swimmers

I am delighted to confirm that SSES REAG has approved your research study (REF No. Prop 86_2018_19) and you are now permitted to recruit participants and commence your research.

If you need to amend any aspect of your research, please ensure you inform SSES REAG by completing a request for amendment form and submitting all revised paperwork (e.g. participant information sheet, questionnaires).

If there should happen to be any adverse event during your study, please also ensure SSES REAG is kept informed.

I hope your study is successful.

With kind regards,

Louis Passfield

(Chair SSES REAG)

University of Kent

School of Sport & Exercise Sciences
Research Ethics and Advisory Group (REAG)
University of Kent at Medway
Chatham Maritime
Kent
ME4 4AG

Ethics Reference:

Prop 73_2018_19

Date: 29th April 2019

Dear Will Gowers,

Re: Ergogenic action of oral and inhaled Glucocorticoids on cycling performance

I am delighted to confirm that SSES REAG has approved your research study (REF No. Prop 73_2018_19) and you are now permitted to recruit participants and commence your research.

If you need to amend any aspect of your research, please ensure you inform SSES REAG by completing a request for amendment form and submitting all revised paperwork (e.g. participant information sheet, questionnaires).

If there should happen to be any adverse event during your study, please also ensure SSES REAG is kept informed.

I hope your study is successful.

With kind regards,

Louis Passfield

(Chair SSES REAG)

University of Kent

School of Sport & Exercise Sciences
Research Ethics and Advisory Group (REAG)
University of Kent at Medway
Chatham Maritime
Kent
ME4 4AG

Ethics Reference: 49_2019_20

Date: 3 August 2020

Dear Will,

Re: Prop 49_2019_20 Ergogenic action of short-term inhaled glucocorticoids on cycling performance.

I am delighted to confirm that SSES REAG has approved your research study (REF No. 49_2019_20) and you are now permitted to recruit participants and commence your research.

If you need to amend any aspect of your research, please ensure you inform SSES REAG by completing a request for amendment form and submitting all revised paperwork (e.g. participant information sheet, questionnaires).

If there should happen to be any adverse event during your study, please also ensure SSES REAG is kept informed.

I hope your study is successful.

With kind regards,

Karen Hambly

(Chair SSES REAG)

Appendix B. Summary of Repeated Procedures used in Thesis

Measure	Experimental Chapter			
	1	2	3	4
Health Screening and Informed Consent	✓		✓	✓
Pre-test Preparation	✓		✓	✓
Spirometry	✓		✓	✓
Airway Inflammation (FeNO)	✓		✓	✓
Inhaler Administration Technique			✓	✓
Inhaler Blinding			✓	✓
Assessment of Airway Hyperresponsiveness			✓	✓
Assessment of Maximal Oxygen Uptake ($\dot{V}O_2$peak)			✓	✓
Submaximal Fixed Intensity Cycling			✓	✓
Estimation of Fat and Carbohydrate Oxidation			✓	✓
Time-Trial Performance Assessments			✓	✓
Rating of Perceived Exertion (BORG)			✓	✓
Rating of Muscle Pain (MP)			✓	✓
Short Recovery Stress Score Questionnaire (SRSS)			✓	✓
Drug Effects Questionnaire (DEQ-5)			✓	✓
Capillary Blood Sampling			✓	✓
Venous Blood Sampling			✓	✓
Enzyme-Linked Immunosorbent Assay (ELISA)			✓	✓

Appendix C. Health Screening Questionnaire

PARTICIPANT HEALTH QUESTIONNAIRE

HEALTH QUESTIONNAIRE

University of **Kent**

Participant Number Code:.......................

Please ensure you have completed and signed the Informed Consent Form to show that you have read and completed this Health Questionnaire

Please answer these questions truthfully and completely. The sole purpose of this questionnaire is to ensure that you are in a fit and healthy state to complete the exercise test.

ANY INFORMATION CONTAINED HEREIN WILL BE TREATED AS CONFIDENTIAL.

SECTION 1: GENERAL HEALTH QUESTIONS

Please read the ten questions below carefully and answer each one honestly: check YES or NO.

	YES	NO
1. Has your doctor ever said that you have a heart condition or high blood pressure?	☐	☐
2. Do you feel pain in your chest at rest, during your daily activities of living, or when you do physical activity?	☐	☐
3. Do you lose balance because of dizziness or have you lost consciousness in the last 12 months? (Please answer NO if your dizziness was associated with over-breathing including vigorous exercise).	☐	☐
4. Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)?	☐	☐
If yes, please list condition(s) here:		
5. Are you currently taking prescribed medications for a chronic medical condition?	☐	☐
If yes, please list condition(s) and medications here:		
6. Do you currently have (or have you had within the past 12 months) a bone, joint or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please	☐	☐

answer NO if you had a problem in the past but it *does not limit your ability* to be physically active.		
If yes, please list condition(s) here:		
7. Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐
8. Do you, or any in your immediate family, has a history or brain or mental disorders?	☐	☐
9. Are you currently taking any medication that may affect the central nervous system?	☐	☐
10. Are you, or is there a chance you may be pregnant?	☐	☐

If you answered NO to all of the questions above, you are cleared to take part in the exercise test

✅ Go to SECTION 3 to acknowledge declaration. You do not need to complete section 2.

❌ If you answered YES to one or more of the questions in Section 1 - PLEASE GO TO SECTION 2.

SECTION 2: CHRONIC MEDICAL CONDITIONS

Please read the questions below carefully and answer each one honestly: check YES or NO.

		YES	NO
1.	**Do you have arthritis, osteoporosis, or back problems?** If YES answer questions 1a-1c. If NO go to Question 2.	☐	☐
1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	☐	☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebrae (e.g. spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	☐	☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	☐	☐
2.	**Do you have cancer of any kind?** If YES answer questions 2a-2b. If NO, go to Question 3.	☐	☐
2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head and neck?	☐	☐
2b.	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	☐	☐
3.	**Do you have heart disease or cardiovascular disease? This includes coronary artery disease, high blood pressure, heart failure, diagnosed abnormality or heart rhythm.** If YES answer questions 3a-3e. If NO go to Question 4.	☐	☐
3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	☐	☐
3b.	Do you have an irregular heartbeat that requires medical management? (e.g. atrial fibrillation, premature ventricular contraction)	☐	☐
3c.	Do you have chronic heart failure?	☐	☐
3d.	Do you have a resting blood pressure equal to or greater than 160/90mmHg with or without medication? Answer YES if you do not know your resting blood pressure.	☐	☐
3e.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	☐	☐

		YES	NO
4.	**Do you have any metabolic conditions? This includes Type 1 Diabetes, Type 2 Diabetes and Pre-Diabetes.** If YES answer questions 4a-4c. If NO, go to Question 5.	☐	☐
4a.	Is your blood sugar often above 13mmol/L? (Answer YES if you are not sure).	☐	☐
4b.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, OR the sensation in your toes and feet?	☐	☐
4c.	Do you have other metabolic conditions (such as thyroid disorders, current pregnancy related diabetes, chronic kidney disease, or liver problems)?	☐	☐
5.	**Do you have any mental health problems or learning difficulties?** This includes Alzheimer's, dementia, depression, anxiety disorder, eating disorder, psychotic disorder, intellectual disability and down syndrome. If YES answer questions 5a-5b. If NO go to Question 6.	☐	☐
5a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	☐	☐
5b.	Do you also have back problems affecting nerves or muscles?	☐	☐
6.	**Do you have a respiratory disease?** This includes chronic obstructive pulmonary disease, asthma, pulmonary high blood pressure. If YES answer questions 6a-6d. If NO, go to Question 7.	☐	☐
6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	☐	☐
6b.	Has your doctor ever said you blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	☐	☐
6c.	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?	☐	☐
6d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	☐	☐
7.	**Do you have a spinal cord injury?** This includes tetraplegia and paraplegia. If YES answer questions 7a-7c. If NO, go to Question 8.	☐	☐

	7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	☐	☐
	7b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	☐	☐
	7c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as autonomic dysreflexia)?	☐	☐

		YES	NO
8.	**Have you had a stroke?** This includes transient ischemic attack (TIA) or cerebrovascular event. If YES answer questions 8a-8c. If NO go to Question 9.	☐	☐
8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	☐	☐
8b.	Do you have any impairment in walking or mobility?	☐	☐
8c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	☐	☐
9.	**Do you have any other medical condition which is not listed above or do you have two or more medical conditions?** If you have other medical conditions, answer questions 9a-9c. If NO go to Question 10.	☐	☐
9a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months?	☐	☐
9b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, and kidney problems)?	☐	☐
9c.	Do you currently live with two or more medical conditions?	☐	☐
	Please list your medical condition(s) and any related medications here:		
10.	**Have you had a viral infection in the last 2 weeks (cough, cold, sore throat, etc.)?** If YES please provide details below:	☐	☐
11.	**Is there any other reason why you cannot take part in this exercise test?** If YES please provide details below:	☐	☐
12.	**Please provide brief details of your current weekly levels of physical activity (sport, physical fitness or conditioning activities), using the following classification for exertion level:**		

L = light (slightly breathless)

M = moderate (breathless)

V = vigorous (very breathless)

	Activity	Duration (mins.)	Level (L/M/V)
Monday			
Tuesday			
Wednesday			
Thursday			
Friday			
Saturday			
Sunday			

Please see below for recommendations for your current medical condition and sign this document:

✅ **If you answered NO to all of the follow-up questions about your medical condition, you are cleared to take part in the exercise test.**

❌ **If you answered YES to one or more of the follow-up questions about your medical condition it is strongly advised that you should seek further advice from a medical professional before taking part in the exercise test.**

SECTION 3: DECLARATION

Signing the study Consent Form signifies that you have completed this questionnaire.

This health questionnaire is based around the PAR-Q+, which was developed by the Canadian Society for Exercise Physiology www.csep.ca

Appendix D. Participant Informed Consent Forms

University of Kent

INFORMED CONSENT FORM	
Eucapnic Voluntary Hyperpnoea Challenge	
Please read and complete this form carefully.	*please tick if applicable*
I have read and understood the Information Sheet.	☐
I have had an opportunity to ask questions and discuss this protocol and I have received satisfactory answers.	☐
I understand I am free to withdraw from the testing at any time, without having to give a reason for withdrawing, and without prejudice.	☐
I agree to take part in this testing.	☐
I agree that my results can be stored and anonymised for research purposes	☐
I would like to receive feedback on the results from the testing at the email address given below. Email address………………………………………………………	☐

Signature of athlete... Date....……………..

(NAME IN BLOCK LETTERS)...……………………..

Signature of Parent / Guardian in the case of a minor

....

- *Consent form approving use of screening data for research purposes.*

CONSENT FORM

University of Kent

Title of project: Ergogenic action of short-term inhaled glucocorticoid use on cycling performance.

Name of investigator: William Gowers

Participant Identification Number for this project:

Please initial box

1. I confirm I have read and understand the information sheet dated… (Version 4 – 13/10/20) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. *(Contact William Gowers (weg6@kent.ac.uk)).* ☐

3. I understand that my responses will be anonymised before analysis. I give permission for members of the research team to have access to my anonymised responses. ☐

4. I understand that my blood will be taken during the course of my participation in this research project and used only for the purposes described in the information sheet (before being disposed of). ☐

5. I have completed the "**HEALTH & SCREENING QUESTIONNAIRE**" as honestly and completely as possible. ☐

6. I agree to take part in the above research project. ☐

7. Would you like to receive a report of the study outcomes and your individual responses once the study complete? ☐

_____ _____ _____
Name of participant   Date   Signature

_____ _____ _____
Name of person taking consent   Date   Signature
(if different from lead researcher)

To be signed and dated in presence of the participant

_____ _____ _____
Lead researcher   Date   Signature

Copies when completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in main file

CONSENT FORM

University of Kent

Title of project: Ergogenic action of oral and inhaled Glucocorticoids on cycling performance

Name of investigator: William Gowers

Participant Identification Number for this project:

Please initial box

1. I confirm I have read and understand the information sheet dated… (Version 2 – 03/04/19) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. *(Contact William Gowers (weg6@kent.ac.uk)).* ☐

3. I understand that my responses will be anonymised before analysis. I give permission for members of the research team to have access to my anonymised responses. ☐

4. I understand that my saliva and blood will be taken during the course of my participation in this research project and used only for the purposes described in the information sheet (before being disposed of). ☐

5. I have completed the "**HEALTH & SCREENING QUESTIONNAIRE**" as honestly and completely as possible. ☐

6. I agree to take part in the above research project. ☐

7. Would you like to receive a report of the study outcomes and your individual responses once the study complete? ☐

_____ _____ _____
Name of participant   Date   Signature

_____ _____ _____
Name of person taking consent   Date   Signature
(if different from lead researcher)

To be signed and dated in presence of the participant

_____ _____ _____
Lead researcher   Date   Signature

Copies when completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in main file

Appendix E. Questionnaires Used in Experimental Chapters

Psychomotor Subjective Outcomes

Rating of Perceived Exertion Scale, Borg 1983

Rating	Perceived Exertion
6	No exertion
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

Rating of Muscle Pain

0	No pain at all
0.5	Very faint pain (just noticeable)
1	Weak pain
2	Mild pain
3	Moderate pain
4	Somewhat strong pain
5	Strong pain
6	
7	Very strong pain
8	
9	
10	Extremely intense pain (almost unbearable)
●	Unbearable pain

Recovery Outcomes

Rating of Session RPE,

0	Rest
1	Very, Very Easy
2	Easy
3	Moderate
4	Somewhat hard
5	Hard
6	
7	Very Hard
8	
9	
10	Maximal

Muscle Soreness VAS

Muscle Soreness Visual Analog Scale

PARTICIPANT ID:	CONDITION:	TIME POINT:

No Soreness |_____| Extreme Soreness

Rate sensation of muscle soreness as you **feel right now.**

Short Recovery Stress Scale (SRSS),

Effects of Drug

Drug Effect Questionnaire (DEQ-5)   *ICQ-S*

DRUG EFFECTS QUESTIONNAIRE

PARTICIPANT ID:		CONDITION:	

Instructions: This questionnaire asks about how you are feeling after taking the substance that was given to you. Please draw a mark on the line to show how strongly you are feeling each of the following effects *right now*. You can mark anywhere on the line, but please draw a vertical line (one that goes straight up and down).

Let's look at an example first.

EXAMPLE: Do you feel dizzy right now?
If you do not feel dizzy, draw a line at NOT AT ALL. If you feel very dizzy, draw a line at EXTREMELY. If you feel somewhere in between, you can draw a mark anywhere along the line between NOT AT ALL and EXTREMELY to indicate how dizzy you are. For example, if you feel a little dizzy, you might draw a line that looks something like the example below.

NOT AT ALL ———————— EXTREMELY

1. Do you FEEL a drug effect right now?

NOT AT ALL ———————— EXTREMELY

2. Are you HIGH right now?

NOT AT ALL ———————— EXTREMELY

3. Do you DISLIKE any of the effects you are feeling right now?

NOT AT ALL ———————— EXTREMELY

4. Do you LIKE any of the effects you are feeling right now?

NOT AT ALL ———————— EXTREMELY

5. Would you like MORE of the drug you took, right now?

NOT AT ALL ———————— EXTREMELY

Inhaled Corticosteroids Related Health Status Questionnaire

Short Form

Participant ID: Condition:

During the last 2 weeks how much have you been affected by the following side-effects of your regularly inhaled medication?

	Not at all	A very little	A little	A moderate amount	Quite a lot	A great deal	A very great deal
1. Hoarseness of the voice?	0	1	2	3	4	5	6
2. A need to clear your throat ?	0	1	2	3	4	5	6
3. An itchy feeling in the back of your throat.	0	1	2	3	4	5	6
4. Oral thrush (sore throat covered with pustules, and difficulty swallowing)?	0	1	2	3	4	5	6
5. A terrible taste in your mouth?	0	1	2	3	4	5	6
6. A change in your ability to taste?	0	1	2	3	4	5	6
7. Wanting to drink liquid? (because of a dry mouth)	0	1	2	3	4	5	6
8. A swollen face or fluid around the face?	0	1	2	3	4	5	6
9. Bruising easily?	0	1	2	3	4	5	6
10. Mood swings ?	0	1	2	3	4	5	6
11 Some kind of affect to your vision	0	1	2	3	4	5	6
12.Sweating?	0	1	2	3	4	5	6
13. Any form of dental decline(tooth decay,tooth staining, etc.)?	0	1	2	3	4	5	6
14. Feeling tired?	0	1	2	3	4	5	6
15. Dry Eyes?	0	1	2	3	4	5	6

(Please circle one number on each line)

Effects of Drug (cont.)

Open question on side effects   *Blinding Check*

SIDE EFFECTS: Please report any other adverse side-effects you may have/be experiencing during the intake period (E.g. Headaches, Nausea). Also use this space to make general comments (i.e. if certain effects came and went, frequency of effects etc.)

Experimental Chapter 3

Oral and Inhaled GC - University of Kent

In order to assess the blinding of the medication, we ask you to consider what condition you have been using during each trial. Please place the corresponding letter into your choices below.

PARTICIPANT ID : ……………………………………

Condition	CHOICE (A, B, C, D, or E)
INHALED GC	
INHALED PLACEBO	
ORAL GC	
ORAL PLACEBO	
CONTROL	

Experimental Chapter 4

Short-Term Inhaled GC - University of Kent

In order for us to assess the blinding of the medication, we ask you to consider what condition you have been using during each trial. Please place the letter in your choices below.

PARTICIPANT ID : ……………………………………

Condition	CHOICE (A, B)
INHALED GC	
INHALED PLACEBO	

Comments:

Participant Monitoring / Standardisation

Training Diary

Medication Administration Checklist and Technique Prompt

TRAINING DIARY

INSTRUCTIONS FOR USE

PARTICIPANT ID:………………..

CONDITION:…………

Please record all training sessions performed during the intake period. The more information you can provide, the easier it will be to replicate. Please try to keep your training as normal as possible. However, the 3-days leading to the experimental session MUST be replicated as identical as possible.

Day	Training Session (Type, Duration, Intensity, Indoors/ Outdoors)
Day 1	
Day 2	
Day 3	
Day 4	
Day 5	
Day 6	
Day 7	
Day 8	
Day 9	
Day 10	
Day 11	
Day 12	(Remember, no heavy exercise)
Day 13	(Remember, no heavy exercise)
Day 14	**TESTING DAY**

Medication Administration Checklist

PARTICIPANT ID: CONDITION:

Instructions

1. Use mouthwash for 10 seconds.
2. Remove the cap.
3. Shake the inhaler well.
4. Attach the spacer and hold inhaler upright.
5. Breathe out gently until lungs are empty.
6. Keep your head upright and place mouthpiece between lips.
7. Press the inhaler, and breathe in slowly and deeply until your lungs are full (You will hear a whistle if you are breathing too fast).
8. Hold your breath for 10 seconds, then breathe out.
9. Wait approximately 30 seconds, then repeat the above process for remainder doses (from step 5).

Rinse your mouth with water to minimise risk of throat irritation due to deposits of medication in mouth.

DAY	AM				PM			
1	1	2	3	4	5	6	7	8
2	1	2	3	4	5	6	7	8
3	1	2	3	4	5	6	7	8
4	1	2	3	4	5	6	7	8
5	1	2	3	4	5	6	7	8
6	1	2	3	4	5	6	7	8
7	1	2	3	4	5	6	7	8
8	1	2	3	4	5	6	7	8
9	1	2	3	4	5	6	7	8
10	1	2	3	4	5	6	7	8
11	1	2	3	4	5	6	7	8
12	1	2	3	4	5	6	7	8
13	1	2	3	4	5	6	7	8
14	1	2	3	4	5	6	7	8

Participant Monitoring / Standardisation (cont.)
Food Diary

Food Diary:

PARTICIPANT ID:

Instructions for use

Please record everything you eat and drink on the ____ days before your first test (**Visit ___**). Try to repeat it as close as possible on the day before all subsequent testing sessions.

Please record/report the cooked/served weight and **NOT** the pre-cooked weight (e.g. dry pasta). Where possible, all food and drink should be weighed and recorded using scales (i.e. place your plate on the scales and zero them before adding each individual item, allowing you to weigh even small additions such as ketchup or butter….e.g. place plate on scales and zero, place toast on plate and read scales [i.e. 60g] then zero scales again, with toast on, before buttering toast and replacing on scales to read weight of butter [i.e. 15g]). Please remember to record each food or drink item individually, for example for cornflakes remember to include the **individual weights of the cereal, the milk and any sugar etc, that you may add**… or for diluted squash drinks please include the weight of the concentrate and water separately. **Any leftovers (e.g. apple cores or food not eaten) should also be weighed and recorded in the 'amount leftover' column.**

- If it is inconvenient for you to weigh all of your food (i.e. when eating out) please record the food eaten and give an approximate portion size (small portion, medium portion etc.)

- Don't forget to include **ALL** food eaten, including second helpings and **snacks** between meals. Please eat as you **NORMALLY** would.

- Please provide as much information as possible about each food, including the brand name and how it was cooked etc.
  - e.g.   stir fry (chicken and veg), **is insufficient information**
    - stir fried veg. (yellow peppers, 20g; onions, 40g; mushrooms, 30g) and chicken breast (150g), fried in 10g extra virgin olive oil, **is sufficient**

A short example is provided overleaf for your reference.

Example:

Day and Time	Food (include brand name)	Method of preparation (e.g. Boil, fry, grill, etc.)	Amount served (grams)	Amount Left over (grams)
Mon. 08:30	Tesco orange squash (concentrate)	--------	75	0
Mon. 08:30	Water	--------	325	0
Mon. 08:30	Kelloggs Cornflakes	--------	50	0
Mon. 08:30	Dairygate semi-skimmed milk	--------	250	0
Mon. 08:30	Banana	--------	150	30
Mon. 10:45	Pear (conference)	--------	120	20
Mon. 10:45	Water	--------	200	0
Mon. 12:30	Bread (Hovis, white), 2 slices	Toasted	60	0
Mon. 12:30	Margarine (Flora original)	--------	15	0
Mon. 12:30	Heinz Baked Beans (can)	Reheated in pan on the hob	410	0
Mon. 18:00	White rice (Sainsbury's own brand)	Boiled	400	0
Mon. 18:00	Diced onion	Lightly pan fried in 10g of olive oil	40	0
Mon. 18:00	Chopped peppers (red)	Lightly pan fried with onions (above)	40	0
Mon. 18:00	Diced Chicken Thigh	Pan fried with vegetables (above)	150	0
Mon. 18:00	Uncle Ben's sweet and sour sauce (jar)	Simmer on hob with above chicken and veg, after they had been pan fried	200	0

24 HOURS PRIOR TESTING SESSION

Day and Time	Food (include brand name)	Method of preparation (e.g. Boil, fry, grill, etc.)	Amount served (grams)	Amount Left over (grams)

Day and Time	Food (include brand name)	Method of preparation (e.g. Boil, fry, grill, etc.)	Amount served (grams)	Amount Left over (grams)
TESTING MORNING BREAKFAST				

Appendix F. Supplementary Respiratory Specific Screening Questionnaire

SECTION A. – Sport Information

1. **How many hours of exercise do you do per week?** _____
2. **What is your main sport?** _____
3. **How long have you been taking part in your main sport?** _____
4. **How often do you train for your main sport?** _____
5. **How often do you compete in your main sport?** _____
6. **At what level of competition do you compete?**

   ☐ Recreational ☐ Local ☐ County ☐ National ☐ International

7. **During or after training or competition do you experience any of the following?**

   Please select as many as appropriate.

   ☐ Coughing ☐ Excess Mucus Production ☐ Chest Tightness

   ☐ Wheezing ☐ Difficulty Breathing (Dyspnoea) ☐ **NONE** of above

   Other: _____

8. **During training or competition what environmental conditions seem to make your breathing worse?**

   Please select as many as appropriate.

   ☐ Cold Climate ☐ Dry Air ☐ High Pollen Content

   ☐ High Pollution ☐ Altitude ☐ **NONE** of above

   Other: _____

9. **In addition to medication do you use any other form of therapy/training to aid your breathing?**

   _____

SECTION B. – Respiratory Health

	Yes	No
1. **Have you ever suffered from asthma?**	☐	☐
2. Did you use asthma medication in the past?	☐	☐
3. **Are you currently diagnosed asthmatic?**	☐	☐
4. Are you currently using medication for your asthma?	☐	☐
5. Have you been hospitalised in the last 6 months due to your asthma?	☐	☐
6. **Do you suffer from exercise-induced asthma (EIA)?**	☐	☐
7. Are you currently using medication for your exercise-induced asthma (EIA)?	☐	☐
8. **Have you ever had a Eucapnic Voluntary Hyperventilation Challenge (EVH) test?**	☐	☐

If yes, the test was ☐ Positive ☐ Negative ☐ Don't know/Can't remember

If you have answered **YES** to either or all of questions 2, 4 and 6, please complete table below.

Asthma Medication Table Example:

Type of Drug	Drug Name	Dose	Dose Frequency	Year Started
Reliever	Ventolin	200 mcg	3 times a day	1998
Preventer	Pulmicort	250mcg	Twice a day	1998
Other	Serevent	150 mcg	2 times a week	1998

For further guidance, please go to Page 7.

Type of Drug	Drug Name	Dose	Dose Frequency	Year Started
Reliever	1.			
	2.			
Preventer	1.			
	2.			
Other	1.			
	2.			

2

Glossary of Asthma Medications   1

Reliever (Blue inhaler)	Preventer (Brown, red or orange inhaler)		Combination (Purple inhaler)	Other
SALBUTAMOL	**BECLOMETASONE**	**SALMETEROL**	**FORMOTEROL + BECLOMETASONE**	Montelukast
Airomir®	Asmabec®	Serevent®	Fostair®	Pranlukast
Asmasal®	Beclazone®	**FORMOTEROL**	**SALMETEROL + FLUTICASONE**	Zafirlukast
Salamol®	Becodisks®	Atimos®	Seretide®	
Salbulin®	Clenil Modulite®	Foradil®	**FORMOTEROL + BUDENOSIDE**	
Salbutamol®	Pulvinal Beclomethasone®	Oxis®	Symbicort®	
Pulvinal®	Qvar®	**CROMOGLYCATE**		
Ventolin®	**BUDENOSIDE**	Intal®		
TERBUTALINE	Easyhaler Budenoside®	**NEDOCROMIL**		
Bricanyl®	Novolizer Budenoside®	Tilade®		
	Pulmicort®			
	CICLESONIDE			
	Alvesco®			
	FLUTICASONE			
	Flixotide®			
	MOMETASONE			
	Asmanex Twisthaler®			

3

Appendix G. Supplementary Materials from Chapter 4 - Repeatability of EVH over 12-months in Elite Swimmers who discontinue asthma therapy

Introduction

The eucapnic voluntary hyperpnoea (EVH) challenge has previously been shown to have good short (≤7 days) to medium term (≤70 days) test-retest repeatability (Anderson, Argyros, *et al.*, 2001; Dickinson *et al.*, 2006; Stadelmann, Stensrud and Carlsen, 2011; Anderson and Kippelen, 2012; Williams *et al.*, 2015). However, the long-term repeatability of the EVH after treatment discontinuation has not yet been reported in an athletic population.

Study Aims

To evaluate the long-term test-retest repeatability of the EVH in elite swimmers who had discontinued therapy, or without EIB diagnosis but persistent symptoms.

Methods and Statistics

Participants included in the 'Repeatability Group' in Chapter 4 formed this analysis. The level of test-retest repeatability between 'Repeatability Group' assessments was expressed as mean bias with 95% limits of agreement (LOA) and interpreted by Bland–Altman plot. Proportional bias was analysed using linear regression. Correlation between 'Repeatability Group' assessments was calculated using Pearson's correlation coefficient (r_p).

Results

Bland-Altman analysis indicated acceptable test-retest repeatability. The mean bias between assessments was 0.6% (95% LOA = -5.9, 7.1), with no data points outside the LOA (*Supplementary Figure* **11.1**). Linear regression analysis determined there was no proportional bias, as the distribution of agreement was not dependent on FEV_1max (P=0.61). There was a statistically significant strong correlation in ΔFEV_1max between assessments (r_p = 0.81, P<0.01; *Supplementary Figure* **11.2**).
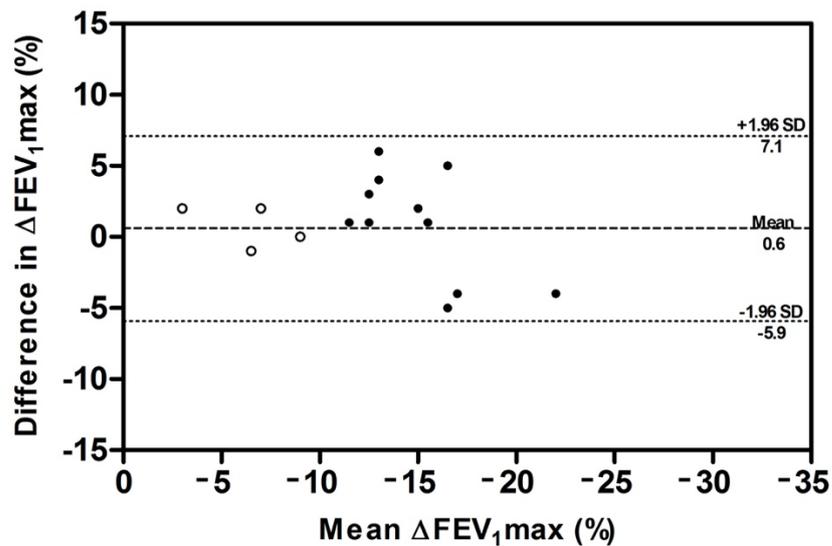
Supplementary Figure 11.1. *Bland-Altman plot for test-retest repeatability of maximum reduction in FEV₁ (ΔFEV₁max) between EVH assessments.[○ = EIB negative ● = EIB positive]. Broken horizontal line denotes mean bias, dotted lines indicate 95% upper and lower limits of agreement. FEV₁, forced expiratory volume in 1 s; EVH, Eucapnic voluntary hyperpnoea; EIB, exercise-induced bronchoconstriction.*
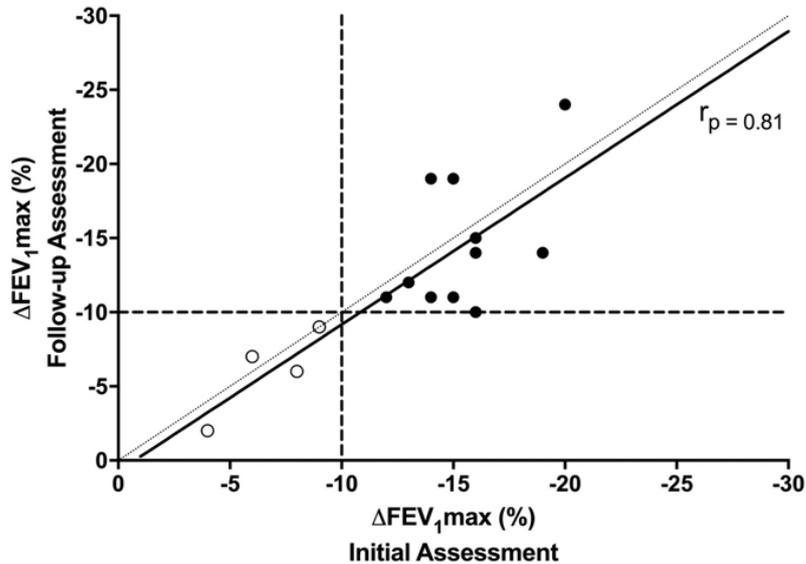
Supplementary Figure 11.2. *Correlation of maximum reduction in FEV₁ (ΔFEV₁max) between EVH assessment visits. [○ = EIB negative ● = EIB positive]. Broken horizontal and vertical lines denote 10% fall in FEV₁ diagnostic threshold. Solid line indicates line of equality, dotted line denotes dataset line of best fit. FEV₁, forced expiratory volume in 1 s; EVH, Eucapnic voluntary hyperpnoea; EIB, exercise-induced bronchoconstriction.*

Discussion

For the EVH to be suitable clinical utility for monitoring efficacy of EIB therapy, it requires good test-retest repeatability (Berchtold, 2016). The present study showed good long-term test-retest repeatability of EVH in a cohort of elite swimmers who discontinued inhaler therapy. These findings support previous research demonstrating that EVH produces repeatable results on a short (Argyros *et al.*, 1996; Stadelmann, Stensrud and Carlsen, 2011; Williams *et al.*, 2015) and medium-term (Williams *et al.*, 2015) basis in elite and recreational athletes. The findings of the present study are consistent with a previous investigation on elite swimmers, where the authors demonstrated strong correlation between repeated EVH challenges, and good test-retest repeatability, albeit over a short period (~1 day) (Stadelmann, Stensrud and Carlsen, 2011). Moreover, the mean bias (0.7%) and LOA (~6%) reported by Stadelmann, Stensrud and Carlsen, (2011) were similar to our study. Medium-term ($\leq$70 days) repeatability has been demonstrated previously in physically active individuals with EIB (Williams *et al.*, 2015).

In the present study, EIB positive elite swimmers produced repeatable results, including those with mild EIB severity. Our results support findings by Williams and colleagues, who reported reproducible ΔFEV_1max irrespective of EIB severity. However, Price, Ansley and Hull, (2015) demonstrated wider limits of agreement in a cohort of recreationally active individuals, particularly those with a mild or borderline response. This inconsistency in literature may be due to the population investigated, and the severity of EIB within the group. The comparable low mean ΔFEV_1max ($10 \pm 8\%$), small cohort of physician diagnosed asthmatics, and a non-elite athletic population may have contributed to wider limits of agreement seen by Price, Ansley and Hull, (2015). It has previously been suggested that using EVH with elite athletes is more suitable than with recreationally active individuals, due to the ability to maintain high-ventilation rates and the stimulus closer mimics the demands of high-intensity exercise, at which elite athletes are more accustomed (Hull *et al.*, 2016). Moreover, as previously discussed, the heterogenous phenotypes of EIB may impact how EIB develops in recreational compared to elite athletes.

Conclusion

Elite swimmers who discontinue regular use of EIB therapy have a repeatable EVH challenge twelve-months later.

Appendix H. Supplementary Materials from Chapter 5 - Validation of the High-Performance Liquid Chromatography (HPLC) method for the quantification of Beclomethasone Dipropionate (BDP)

Principle

A HPLC system with UV detection (Agilent 1100, Agilent Technology, Palo Alto, CA, USA) was used for the detection and separation of BDP. Quantification of this compound was performed by assay of absorbance against a reference material shown to be linear across a concentration gradient (*see Linearity*). The HPLC analytical method development and validation was guided by previous literature of Almeziny (2009), and refined by technical expertise of laboratory staff and industry consultant.

Reagents / Solvents

- Acetonitrile, HPLC Grade (Fisher Scientific, Loughborough, UK)
- Water, HPLC Grade (Fisher Scientific, Loughborough, UK)
- Silicon Oil (Sigma Aldrich, UK)
- Cyclohexane, HPLC Grade (Fisher Scientific, Loughborough, UK)
- Methanol, HPLC grade (Fisher Scientific, Loughborough, UK)
- Reference Material: 99% pure Beclomethasone (Sigma–Aldrich, St. Louis, MO, USA).

Chromatographic Conditions

Supplementary Table 11.1. Chromatographic conditions for Beclomethasone Dipropionate (BDP) High-Performance Liquid Chromatography (HPLC) analysis.

Parameter	Material or Setting
Column	C18 ODS Hypersil, 4.6 mm × 250 mm, 5-µm (30105-254630, Thermo scientific, Waltham, UK)
Mobile Phase (organic/aqueous, v:v)	Acetonitrile : Water (70:30), Isocratic.
Flow Rate (mL/min)	1.0 mL/min
Detection / Wavelength (nm)	UV absorbance at 240 nm
Column Temperature	20 °C
Injection Volume	100 µL
Run Time	15 minutes
Needle Wash	Acetonitrile
Blank and Diluent	Acetonitrile : Water (v:v, 70:30)

Standard Preparation

Standard solutions were pre-prepared for use in each HPLC run and stored in amber glassware at room temperature in for up to 14-days [*see solution stability*]. The required mass of reference material was weighed and transferred into a volumetric flask, then filled part-way to the neck of the flask with diluent. To ensure reference material was completely dissolved, the stock standard solutions were sonicated for one minute, allowed to rise to room temperature then filled to volume with diluent.

Supplementary Table 11.2. High-Performance Liquid Chromatography (HPLC) Stock Standard Dilution Solutions.

Compound	Mass	Volumetric Flask	Diluent	Nominal Concentration	Solution Label
BDP	10 mg	250 mL	Acetonitrile : Water (70:30)	40 µg/mL	RSS1
BDP	10 mg	250 mL	Acetonitrile : Water (70:30)	40 µg/mL	RSS2

Input Standard	Volume	Volumetric Flask	Diluent	Nominal Concentration	Solution Label	Dilution Factor
BDP S1	10 mL RSS1 90 mL Diluent	100 mL	Acetonitrile : Water (70:30)	4 µg/mL	S1	2500
BDP S2	10 mL RSS2 90 mL Diluent	100 mL	Acetonitrile : Water (70:30)	4 µg/mL	S2	2500

Specificity

The HPLC method demonstrated specificity and selectivity for BDP. The blank sample (Acetonitrile: water 70:30 v/v) and coating for the NGI cups (silicon/cyclohexane) or mouth-throat did not produce any detection peaks that interfered with BDP as shown in *Supplementary Figure 11.3.*

(a)

(b)
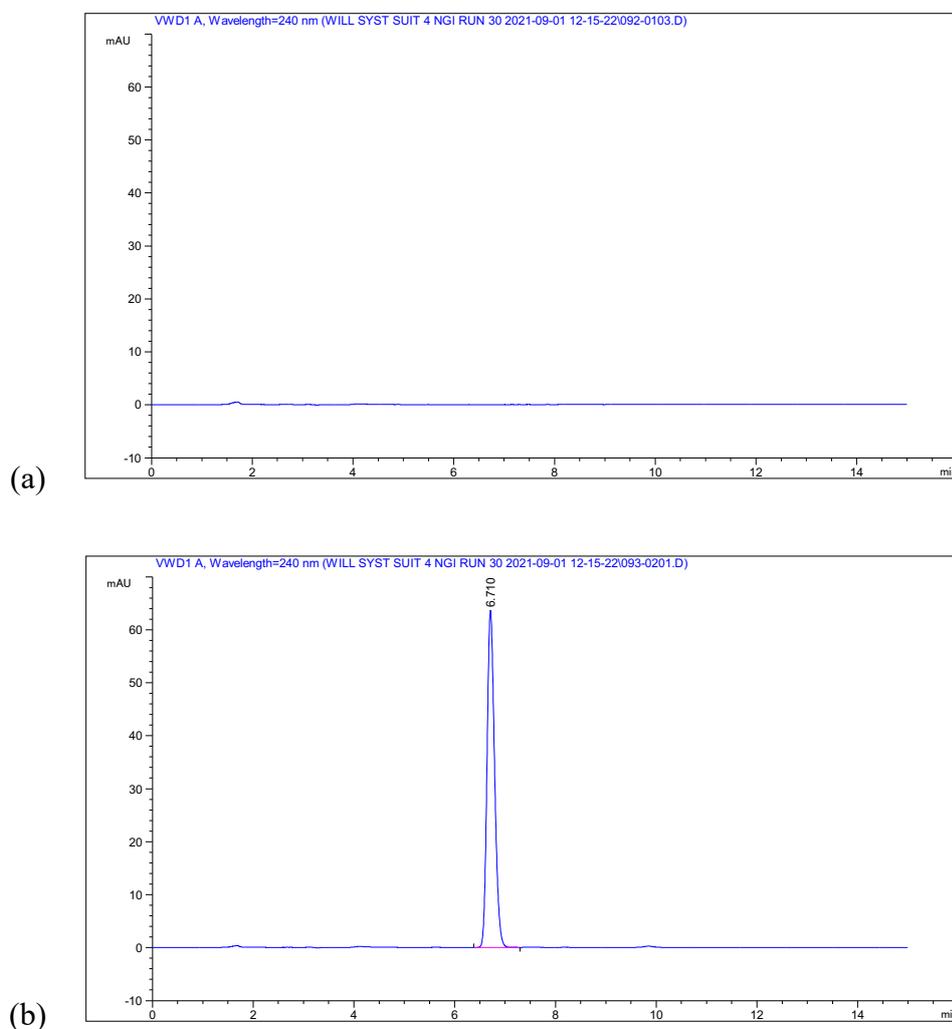
Supplementary Figure 11.3*. High-Performance Liquid Chromatography (HPLC) specificity detection profiles. (a) Chromatogram of blank (Acetonitrile: Water 70:30 v/v); (b) Chromatographic profile of beclomethasone Dipropionate.*

Precision

The intraday variation in retention time was determined by running six replicate analysis of one concentration. Mean retention time was 6.84 minutes.

Linearity

Dilutions from a stock solution (15.00 µg/mL) were prepared to assess the linearity of detected concentration. Linear responses were obtained for BDP over the concentration

range 0.03 μg/mL – 15 μg/mL (0.015, 0.15, 3.00, 6.00, 9.00, 12.00, 15.00), with a regression equation of y = 155.12x – 0.8538), where Y denotes peak area and X the concentration of BDP (μg/mL). The correlation coefficient was R^2 = 0.9998. For verifying linearity of calibration in the proposed working range, residuals of the regression line are plotted against the concentration. The residuals are distributed around the zero-line at random and without any trend (Ferenczi-Fodor *et al.*, 2001).

Supplementary Table 11.3. Beclomethasone Dipropionate (BDP) dilutions.

Compound	Amount	Final Volume	Concentration	Sample Label
Beclomethasone dipropionate	7.5 mg	500 mL	15.00 μg/mL	BC0
	8.0 mL	10 mL	12.00 μg/mL	BC1
	6.0 mL	10 mL	9.00 μg/mL	BC2
	4.0 mL	10 mL	6.00 μg/mL	BC3
	2.0 mL	10 mL	3.00 μg/mL	BC4
	1.0 mL	10 mL	1.50 μg/mL	BC5
	0.5 mL	500 mL	0.015 μg/mL	BC6

Supplementary Table 11.4. Linearity results for Beclomethasone dipropionate (BDP).

Concentration of BDP (μg/mL)	Peak area
15.0	2339.9
12.0	1862.3
9.0	1375.5
6.0	917.8
3.0	463.2
1.5	248.3
0.015	2.5
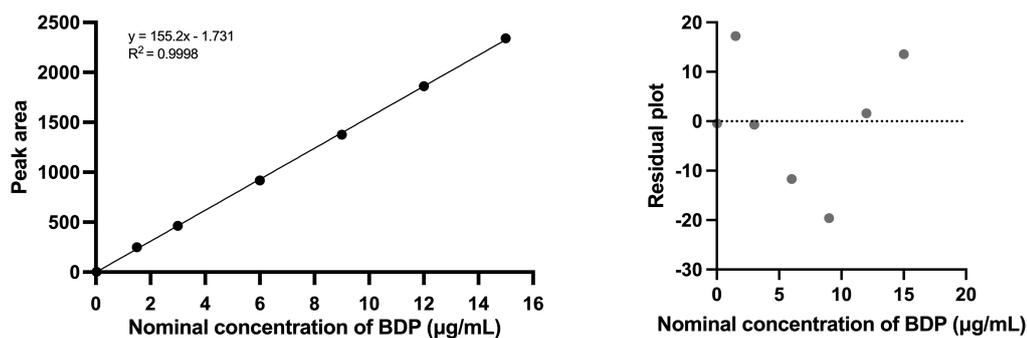
Supplementary Figure 11.4. *Linearity plot for Beclomethasone dipropionate (BDP) (a) and Residual plot from linear regression (b).*

Range

Specified range was derived from previous validation studies and laboratory experience.

Solution Stability and Chromatographic Robustness

The stability of BDP solution when stored at fridge and room temperature was assessed. Standards were prepared using the previously defined HPLC method, then concentration was determined on day 0 and stored at both room temperature and fridge in clear and amber volumetric flasks. Standards were then assessed at +24h, +72h, + 6 days and +14 days. Standard concentration should be 98 - 102% of the Day 0 concentration. Beclomethasone in solution was deemed to be stable for up to 14 days.

Supplementary Table 11.5. Stability of Beclomethasone Dipropionate (BDP) Concentration (µg/mL) assessed via High-Performance Liquid Chromatography (HPLC)

	BDP	BDP	BDP	BDP
	(Fridge °C, Clear)	*(Fridge °C, Amber)*	*(Ambient °C, Clear)*	*(Ambient °C Amber)*
Day 0		3.996		
Day +1	3.951	3.939	3.951	3.934
Day +3	3.963	3.945	3.929	3.955
Day +6	3.984	3.955	3.944	3.942
Day +14	3.962	3.949	3.934	3.950
% Initial	99.17	98.44	98.32	98.45

Following this, the robustness of the chromatography was assessed by making small changes to method parameters and investigating the effect on retention time and trailing factor. Standards were run to look at column temperature (+5°C) and mobile phase (± 10% organic compound). Based on these results, it suggests that the organic/aqueous ratio of the mobile phase is a sensitive aspect of the detection method, and as such should be prepared with care. On the other hand, column temperature does not seem to significantly effect chromatographic profile of BDP.

Supplementary Table 11.6. Robustness of Beclomethasone dipropionate (BDP) Chromatographic Profile.

Column Temp	Mobile Phase	BDP Retention Time	Area	Width	Height	Symmetry
20°C	Normal	6.854	659.628	0.1511	67.092	0.829
25°C	Normal	6.716	659.543	0.1487	68.545	0.814
20°C	+ 10% Organic	5.284	653.715	0.1179	85.904	0.799
20°C	(-) 10% Organic	9.610	661.133	0.2210	46.557	0.843

HPLC System Clean Down Procedure

Supplementary Table 11.7. High-Performance Liquid Chromatography (HPLC) System Clean Down Procedure

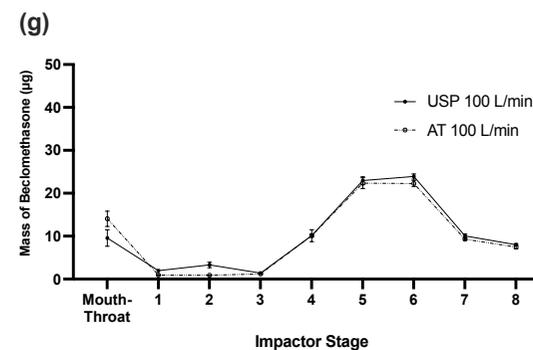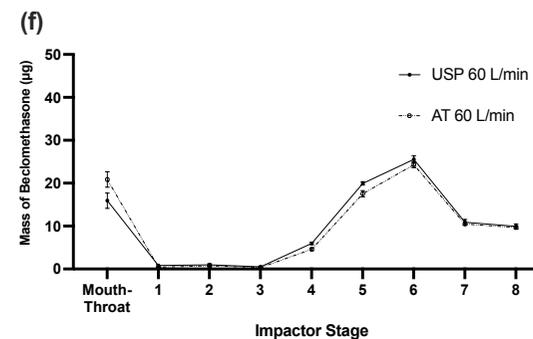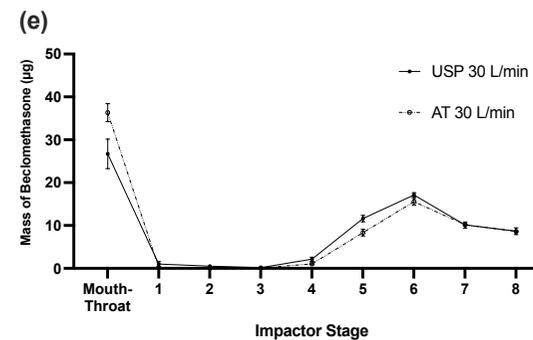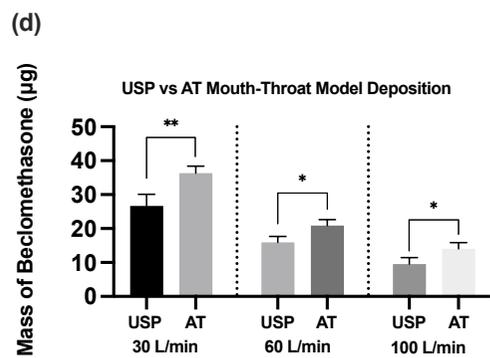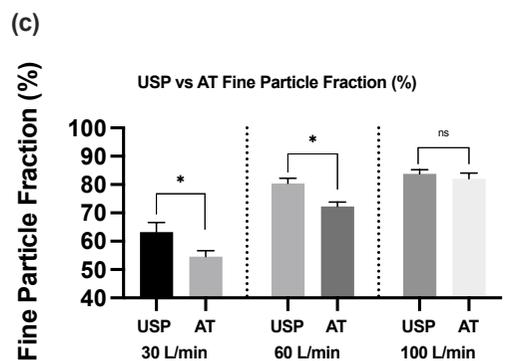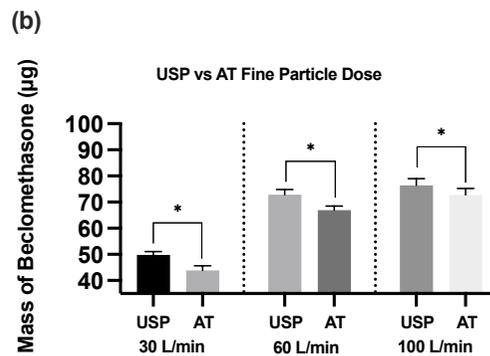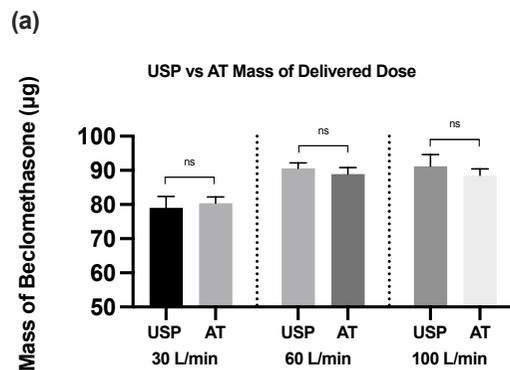|	
Mobile Phase (organic/aqueous, v:v)	Acetonitrile : Water (70:30)
Flow Rate (mL/min)	1.0 mL/min
Time	4 runs (15 minutes each)

Appendix I. Supplementary Materials from Chapter 5 - Pilot Work: United States Pharmacopeia (USP) verses Anatomical Throat (AT) Induction Port

There was no significant difference in delivered dose between USP and AT induction ports at any flow-rate ($p>0.05$; Supplementary Figure 11.5). However, the AT resulted in greater deposition in the MT region at 30 L/Min ($p<0.001$), 60 L/min ($p=0.001$) and 100 L/min ($p=0.002$; Supplementary Table *11.8*, Supplementary Figure 11.5). Thus, compared to the USP model, the anatomical throat displayed lower FPD and FPF% at all flow-rates ($p<0.05$; Supplementary Table *11.8*, Supplementary Figure 11.5), with the exception of 100 L/min for FPF% ($p=0.127$).

Supplementary Table 11.8. Concentration of BDP deposited on each stage of the NGI using USP throat model, from Qvar® 100 mg alone at 30, 60 and 100 L/min inhalation flow rate (n=6).

	30 L/min	**60 L/min**	**100 L/min**
Mouth and Throat [µg]	26.69 (3.44)	15.92 (1.78)	9.56 (1.92)
Stage 1 [µg]	1.01 (0.57)	0.83 (0.18)	1.96 (0.15)
Stage 2 [µg]	0.51 (0.24)	0.99 (0.25)	3.29 (0.62)
Stage 3 [µg]	0.18 (0.04)	0.51 (0.04)	1.39 (0.14)
Stage 4 [µg]	2.14 (0.48)	5.96 (0.28)	10.12 (1.39)
Stage 5 [µg]	11.61 (0.75)	19.96 (0.41)	22.94 (0.91)
Stage 6 [µg]	17.12 (0.55)	25.56 (0.80)	23.90 (0.59)
Stage 7 [µg]	10.12 (0.71)	10.91 (0.63)	10.07 (0.42)
Stage 8 [µg]	8.67 (0.78)	9.95 (0.57)	8.00 (0.28)
Delivered dose [µg]	**79.00 (3.35)**	**90.60 (1.59)**	**91.23 (3.43)**
Fine Particle Dose [µg]	**49.84 (1.28)**	**72.86 (1.95)**	**76.42 (2.55)**
Fine Particle Fraction [%]	**63.20 (3.42)**	**80.42 (1.86)**	**83.79 (1.50)**
Interpolated FPD <5µm	51.13 (1.15)	74.50 (2.03)	81.29 (2.16)
MMAD [µm]	**0.77 (0.06)**	**0.71 (0.01)**	**0.70 (0.01)**
GSD	**3.04 (0.23)**	**2.53 (0.05)**	**2.71 (0.07)**

*Data presented as Mean (SD). Abbreviations: **MMAD**, Mass Median Aerodynamic Diameter; **GSD**, Geometric Standard Deviation; **FPM**, Fine Particle Mass; **µg**, micrograms; **µm**, microns.*

(a)

USP vs AT Mass of Delivered Dose

(b)

USP vs AT Fine Particle Dose

(c)

USP vs AT Fine Particle Fraction (%)

(d)

USP vs AT Mouth-Throat Model Deposition

(e)

(f)

(g)

Supplementary Figure 11.5. *(a) Estimated Delivered Dose (b) Fine Particle Dose (c), Fine Particle Fraction, (d) mouth-throat deposition and Aerodynamic Particle Size Distribution (APSD) [e, f, g] of Qvar® 100 μg at 30 L/min, 60 L/min and 100 L/min using United States Pharmacopeia (USP) and anatomical (AT) mouth-throat (MT) model. Error bars represent SD about the mean. *p< 0.05, **p<0.001.*
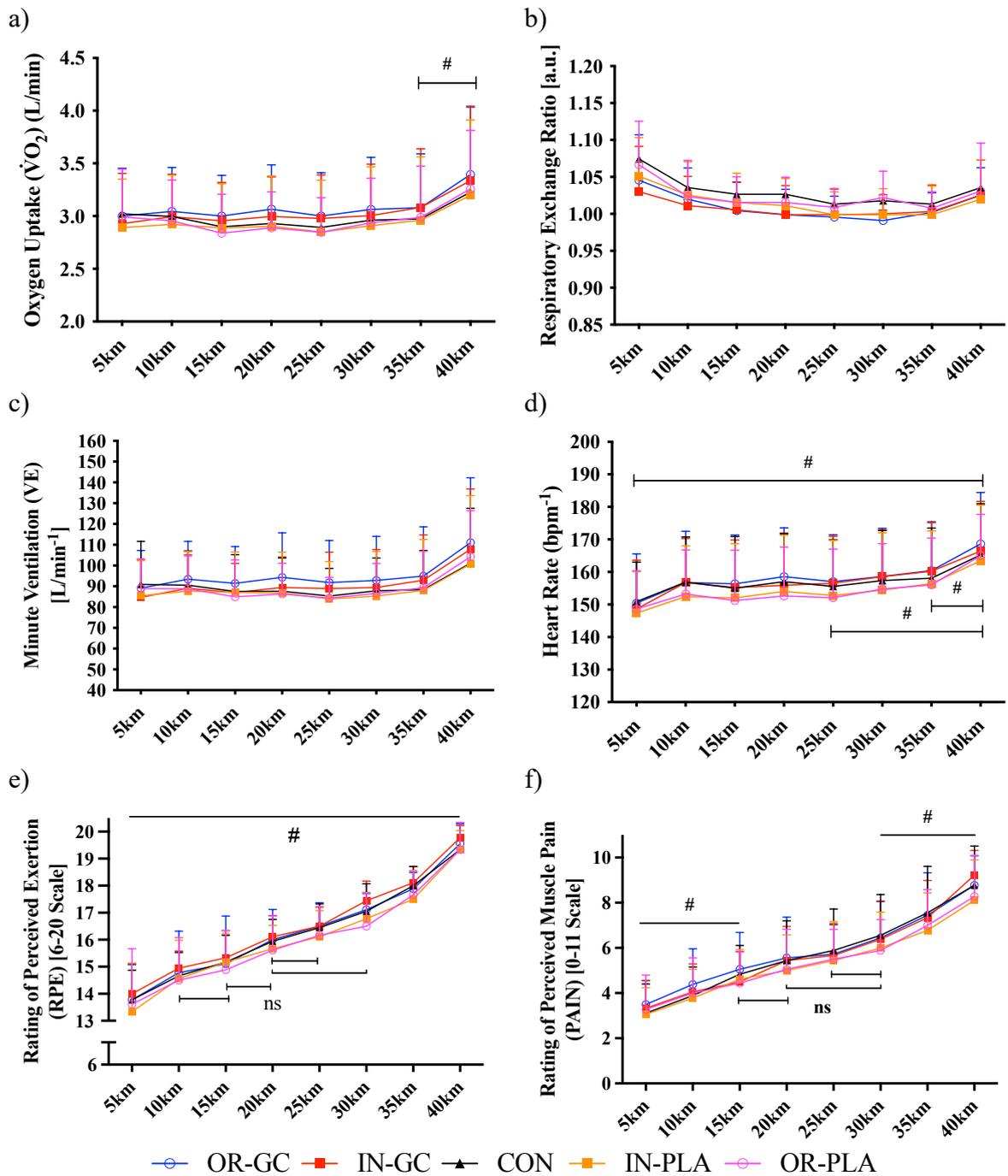
Appendix J. Supplementary Materials from Chapter 6.

Supplementary Table 11.9. TT_{40km} Physiological and Perceptual Response Repeated Measures ANOVA statistics.

Variable	Main / Simple Effect	d.f, d.f ^{er}	F Value	P Value	η_p^2
$\dot{V}O_2$ [L/min^{-1}]	Condition	4, 32	3.825	0.01*[b]	0.32
	Time	2.196, 17.565$^\$$	4.526	0.02[#]	0.36
	Condition x time	4.726, 37.806$^\$$	1.008	0.43	0.11
RER [a.u.]	Condition	4, 32	1.884	0.14	0.19
	Time	2.071, 16.569$^\$$	5.172	0.02[#b]	0.39
	Condition x time	3.903, 31.223$^\$$	1.399	0.26	0.15
Minute Ventilation [L/min^{-1}]	Condition	2.805, 22.440$^\$$	2.933	0.04*[b]	0.27
	Time	1.889, 15.113$^\$$	3.208	0.07	0.29
	Condition x time	4.189, 33.509$^\$$	1.200	0.33	0.13
Heart Rate [beats/min^{-1}]	Condition	1.718, 13.747$^\$$	1.666	0.23	0.17
	Time	2.214, 17.710$^\$$	9.719	0.001[#]	0.55
	Condition x time	5.144, 41.150$^\$$	1.072	0.39	0.12
RPE [a.u.]	Condition	4, 32	1.814	0.15	0.19
	Time	1.770, 14.159$^\$$	103.575	<0.001[#]	0.93
	Condition x time	4.508, 36.065$^\$$	0.699	0.61	0.08
PAIN [a.u.]	Condition	4, 32	1.658	0.18	0.17
	Time	2.183, 17.466$^\$$	89.902	<0.001[#]	0.92
	Condition x time	4.638, 37.104$^\$$	1.095	0.38	0.12

Abbreviations. **d.f.,** *degrees of freedom,* **d.f.** ^{er}*, degrees of freedom error;* η_p^2**,** *partial eta squared effect size.* * $p < 0.05$. $^\$$*Greenhouse-Geisser correction applied for violation of sphericity.*

[b]*signifies non-significant between conditions when the Bonferroni correction was applied.*

Supplementary Figure 11.6. *Physiological response ($\dot{V}O_2$ [a], RER [b], $\dot{V}_E$ [c], HR [d]) and Perceptual ([RPE [e], PAIN [f]) outcome measures during initial 40-km cycling time-trial. Data presented as mean ± standard deviation by 'treatment' and 'time' ANOVA factors. #significant main effect of time. ns, non-significant. **OR-GC**, oral prednisolone (0.5 mg.kg^{-1}); **IN-GC**, inhaled beclomethasone dipropionate (1600 µg); **OR-PLA**, microcrystalline cellulose capsules; **IN-PLA**, water vapour inhaler; **CON**, control. D.f., degrees of freedom. η_p^2, partial eta squared.*

Supplementary Table 11.10. Descriptive statistics [mean ± standard deviation] and Repeated Measures ANOVA main/interaction effect on Short Recovery Stress Scale (SRSS) sub-domains during experimental trials.

Muscular Stress [a.u.]	Baseline	Pre-40km TT	Post-40km TT	Pre-10km TT	Post-10km TT	Post-30 mins	Post-24hr	F-Value, P-Value, Effect Size (η_p^2)
OR-GC	1.22 ± 1.20	1.11 ± 1.45	4.78 ± 0.83	3.56 ± 0.88	5.22 ± 0.97	4.33 ± 0.87	2.11 ± 1.27	*Condition: F(4,32), 0.470, P = 0.758, $\eta_p^2 = 0.06$).*
OR-PLA	1.11 ± 1.27	1.11 ± 1.17	4.78 ± 0.67	3.67 ± 0.71	5.00 ± 0.71	4.00 ± 0.87	2.11 ± 2.03	*Time: F(6,48), 64.829, P <0.001, η_p^2 =0.89).*
IN-GC	1.00 ± 0.87	1.00 ± 1.32	5.22 ± 0.83	3.78 ± 0.87	5.33 ± 0.87	4.22 ± 0.83	2.11 ± 0.93	
IN-PLA	1.00 ± 0.87	0.78 ± 0.67	5.00 ± 0.50	3.44 ± 0.88	5.22 ± 0.67	4.22 ± 1.09	2.11 ± 0.93	*Condition x Time: F(24,192), 0.743, P = 0.802, $\eta_p^2 = 0.09$).*
CON	0.78 ± 0.67	1.00 ± 1.00	5.11 ± 0.60	3.89 ± 0.78	5.56 ± 0.73	4.67 ± 0.71	2.00 ± 1.00	
Lack of Activation [a.u.]	**Baseline**	**Pre-40km TT**	**Post-40km TT**	**Pre-10km TT**	**Post-10km TT**	**Post-30 mins**	**Post-24hr**	**F-Value, P-Value, Effect Size (η_p^2)**
OR-GC	1.78 ± 1.48	1.44 ± 1.33	2.89 ± 1.76	2.11 ± 1.45	2.67 ± 1.58	2.56 ± 1.59	1.89 ± 1.36	*Condition: F(4,32), 1.76, P = 0.16, $\eta_p^2 = 0.18$).*
OR-PLA	1.22 ± 0.97	1.00 ± 0.87	2.78 ± 1.39	2.11 ± 1.62	2.78 ± 1.57	1.56 ± 0.88	1.44 ± 1.13	*Time: F(6,48), 9.04, P <0.001, η_p^2 =0.53).*
IN-GC	1.44 ± 1.13	1.33 ± 1.23	2.56 ± 1.42	2.44 ± 1.24	2.67 ± 1.12	2.67 ± 1.12	1.56 ± 1.42	
IN-PLA	1.33 ± 1.50	1.11 ± 1.05	3.00 ± 1.32	2.22 ± 1.39	2.56 ± 1.24	2.33 ± 1.32	1.67 ± 1.80	*Condition x Time: F(24,192), 1.11, P = 0.339, $\eta_p^2 = 0.122$).*
CON	1.78 ± 1.20	1.22 ± 1.48	3.22 ± 1.56	2.00 ± 1.12	3.11 ± 1.27	2.11 ± 0.78	2.22 ± 1.48	

Negative Emotional State [a.u.]	Baseline	Pre-40km TT	Post-40km TT	Pre-10km TT	Post-10km TT	Post-30 mins	Post-24hr	F-Value, P-Value, Effect Size (η_p^2)
OR-GC	0.78 ± 0.97	0.44 ± 0.73	1.56 ± 1.67	0.89 ± 1.36	1.44 ± 1.67	1.22 ± 1.64	1.11 ± 1.17	*Condition: F(4,32), 0.867, P = 0.494, η_p^2 = 0.098).*
OR-PLA	0.44 ± 0.53	0.44 ± 0.73	1.44 ± 0.73	0.67 ± 1.00	1.11 ± 1.12	0.78 ± 0.83	1.11 ± 1.05	*Time: F(6,48), 2.683, P =0.025, η_p^2 =0.251).*
IN-GC	0.78 ± 1.10	0.67 ± 0.87	1.67 ± 1.80	1.00 ± 1.23	1.44 ± 1.51	0.89 ± 1.27	1.33 ± 1.23	
IN-PLA	0.67 ± 1.00	0.22 ± 0.67	1.33 ± 1.66	1.00 ± 1.23	1.00 ± 1.32	0.89 ± 1.10	1.11 ± 1.62	*Condition x Time: F(24,192), 0.477, P = 0.983, η_p^2 = 0.056).*
CON	0.56 ± 1.01	0.56 ± 1.01	1.67 ± 1.73	0.67 ± 1.00	1.56 ± 1.67	1.00 ± 1.32	1.22 ± 0.67	
Overall Stress [a.u.]	**Baseline**	**Pre-40km TT**	**Post-40km TT**	**Pre-10km TT**	**Post-10km TT**	**Post-30 mins**	**Post-24hr**	**F-Value, P-Value, Effect Size (η_p^2)**
OR-GC	1.33 ± 1.32	1.22 ± 1.39	4.67 ± 1.00	3.11 ± 1.27	4.00 ± 1.23	1.44 ± 1.67	1.33 ± 1.32	*Condition: F(4,32), 2.722, P = 0.04, η_p^2 = 0.257).*
OR-PLA	1.11 ± 0.93	1.00 ± 1.00	4.44 ± 0.73	3.11 ± 1.05	3.67 ± 0.87	1.11 ± 1.17	1.00 ± 1.23	*Time: F(6,48), 49.157, P <0.001, η_p^2 =0.860).*
IN-GC	1.22 ± 1.39	1.22 ± 1.39	5.00 ± 0.87	3.00 ± 0.71	4.22 ± 0.83	1.44 ± 1.51	1.22 ± 1.39	
IN-PLA	0.90 ± 1.05	0.98 ± 0.92	4.78 ± 0.67	3.33 ± 0.50	3.78 ± 0.67	1.00 ± 1.32	1.11 ± 0.93	*Condition x Time: F(24,192), 0.541, P = 0.961, η_p^2 = 0.063).*
CON[§]	1.44 ± 1.13	1.11 ± 1.62	4.78 ± 0.83	3.67 ± 1.00	4.22 ± 0.67	1.56 ± 1.67	1.44 ± 1.13	

Physical Performance Capability [a.u.]	Baseline	Pre-40km TT	Post-40km TT	Pre-10km TT	Post-10km TT	Post-30 mins	Post-24hr	F-Value, P-Value, Effect Size (η_p^2)
OR-GC	4.11 ± 0.93	4.22 ± 1.09	2.00 ± 1.50	2.78 ± 1.20	1.78 ± 1.20	2.11 ± 1.05	3.67 ± 0.71	*Condition: F(4,32), 2.120, P = 0.101, η_p^2 = 0.21).*
OR-PLA	3.78 ± 0.67	4.11 ± 0.78	1.78 ± 1.64	2.56 ± 1.13	2.00 ± 1.66	2.44 ± 1.24	3.33 ± 0.71	*Time: F(6,48), 15.856, P <0.001, η_p^2 =0.67).*
IN-GC	3.89 ± 1.17	4.00 ± 1.00	2.00 ± 1.66	2.56 ± 1.24	1.44 ± 1.51	2.22 ± 1.20	3.56 ± 1.01	
IN-PLA	4.22 ± 0.83	4.00 ± 1.00	2.00 ± 1.87	2.78 ± 1.20	1.78 ± 1.56	2.33 ± 1.12	4.00 ± 0.87	*Condition x Time: F(24,192), 0.842, P = 0.680, η_p^2 = 0.10).*
CON	3.78 ± 0.97	4.00 ± 1.41	1.44 ± 1.51	2.33 ± 1.32	1.78 ± 1.20	2.33 ± 1.00	3.67 ± 1.00	

Mental Performance Capability [a.u.]	Baseline	Pre-40km TT	Post-40km TT	Pre-10km TT	Post-10km TT	Post-30 mins	Post-24hr	F-Value, P-Value, Effect Size (η_p^2)
OR-GC	3.44 ± 1.24	3.33 ± 1.41	2.33 ± 1.32	2.78 ± 1.48	2.33 ± 1.50	2.44 ± 1.51	3.44 ± 1.01	*Condition: F(4,32), 0.151, P = 0.961, η_p^2 = 0.019).*
OR-PLA	3.44 ± 1.24	3.33 ± 1.41	2.22 ± 1.92	2.56 ± 1.88	2.22 ± 2.05	2.89 ± 1.45	3.11 ± 1.36	*Time: F(6,48), 2.150, P =0.065, η_p^2 =0.212).*
IN-GC	3.33 ± 1.32	3.22 ± 1.09	2.56 ± 1.88	2.89 ± 1.17	2.44 ± 1.74	2.67 ± 1.73	3.11 ± 1.45	
IN-PLA	3.11 ± 1.54	3.44 ± 1.13	2.67 ± 1.87	2.67 ± 1.50	2.56 ± 2.19	2.78 ± 1.64	3.11 ± 1.24	*Condition x Time: F(24,192), 0.664, P = 0.881, η_p^2 = 0.077).*
CON	3.56 ± 1.26	3.22 ± 1.30	2.56 ± 2.01	3.00 ± 1.25	2.22 ± 1.92	2.78 ± 1.56	3.33 ± 1.12	

Emotional Balance [a.u.]	Baseline	Pre-40km TT	Post-40km TT	Pre-10km TT	Post-10km TT	Post-30 mins	Post-24hr	F-Value, P-Value, Effect Size (η_p^2)
OR-GC	3.33 ± 1.73	3.78 ± 0.09	2.67 ± 1.66	3.11 ± 1.17	2.78 ± 1.30	3.22 ± 1.30	3.44 ± 1.51	*Condition: F(4,32), 1.37, P = 0.268, η_p^2 = 0.146).*
OR-PLA	3.78 ± 1.39	3.79 ± 1.20	2.56 ± 1.42	2.78 ± 1.72	2.67 ± 1.50	3.44 ± 1.33	3.44 ± 1.24	*Time: F(6,48), 4.33, P =0.001, η_p^2 =0.35).*
IN-GC	3.56 ± 1.33	4.00 ± 1.00	3.00 ± 1.32	3.00 ± 1.58	2.56 ± 1.01	3.33 ± 1.23	3.78 ± 1.09	
IN-PLA	4.22 ± 1.20	4.11 ± 0.93	2.89 ± 1.54	3.00 ± 1.32	2.89 ± 1.45	3.56 ± 1.51	4.00 ± 1.23	*Condition x Time: F(24,192), 1.27, P = 0.193, η_p^2 = 0.137).*
CON	4.33 ± 1.00	3.89 ± 1.54	2.56 ± 1.33	2.67 ± 1.32	2.44 ± 1.33	3.44 ± 1.01	3.56 ± 1.01	

Overall Recovery [a.u.]	Baseline	Pre-40km TT	Post-40km TT	Pre-10km TT	Post-10km TT	Post-30 mins	Post-24hr	F-Value, P-Value, Effect Size (η_p^2)
OR-GC	4.00 ± 1.87	4.22 ± 1.20	0.67 ± 0.87	2.11 ± 0.78	0.67 ± 0.71	1.89 ± 0.93	3.78 ± 1.20	*Condition: F(4,32), 0.809, P = 0.528, η_p^2 = 0.09).*
OR-PLA	4.00 ± 1.00	3.89 ± 1.36	0.67 ± 0.71	2.22 ± 0.83	0.89 ± 0.93	1.89 ± 1.27	3.44 ± 1.13	*Time: F(6,48), 36.509, P <0.001, η_p^2 =0.82).*
IN-GC	3.67 ± 1.41	3.89 ± 0.93	0.78 ± 0.83	2.22 ± 0.97	0.44 ± 0.73	1.89 ± 1.05	3.56 ± 1.33	
IN-PLA	4.22 ± 1.20	3.67 ± 1.23	0.56 ± 1.01	2.44 ± 1.33	0.67 ± 0.87	2.22 ± 0.83	4.00 ± 1.00	*Condition x Time: F(24,192), 0.803, P = 0.730, η_p^2 = 0.09).*
CON	3.67 ± 1.23	4.22 ± 1.20	0.44 ± 0.53	2.00 ± 0.50	0.33 ± 0.71	1.78 ± 0.83	3.67 ± 1.23	

*Note. Data presented as mean ± standard deviation. Abbreviations; **OR-GC**, oral prednisolone (0.5 mg.kg^{-1}); **IN-GC**, inhaled beclomethasone dipropionate (1600 µg); **OR-PLA**, microcrystalline cellulose capsules; **IN-PLA**, water vapour inhaler; **CON**, control. **D.f.,** degrees of freedom. η_p^2, partial eta squared.* [§] Significant main effect for condition, yet, non-significant when Tukey pairwise correction applied.

Supplementary Table 11.11. Descriptive statistics [mean (standard deviation)] and Repeated Measures ANOVA completed on cardiorespiratory and perceptual response during submaximal preloaded cycling [50% $\dot{V}O_2$peak]

Variable	OR-GC	OR-PLA	ICS	IN-PLA	CON	D.f.	Test Statistic [F Value / X^2]	P-Value	Effect Size (η_p^2 / ε^2)
$\dot{V}O_2$ [L/min^{-1}]	1.98 (0.20)	1.99 (0.20)	1.98 (0.22)	1.95 (0.19)	2.00 (0.19)	4	1.09	0.376	0.12
$\dot{V}CO_2$ [L/min^{-1}]	1.85 (0.21)	1.89 (0.18)	1.86 (0.23)	1.85 (0.21)	1.91 (0.19)	4	1.27	0.302	0.14
Respiratory-Exchange Ratio (RER) [a.u.]	0.93 (0.03)	0.95 (0.02)	0.93 (0.04)	0.95 (0.03)	0.95 (0.02)	4	2.03	0.113	0.20
Carbohydrate Oxidation (CHO) [g.min^{-1}]	2.10 (0.39)	2.22 (0.27)	2.12 (0.44)	2.19 (0.39)	2.30 (0.29)	4	1.77	0.159	0.18
Fat Oxidation (FO) [g.min^{-1}]	0.22 (0.11)	0.17 (0.17)	0.21 (0.13)	0.17 (0.08)	0.16 (0.08)	4	2.12	0.101	0.21
Heart Rate (HR) [beats/min]	125 (12)	123 (10)	125 (8)	122 (12)	124 (12)	4	0.816	0.524	0.09
RPE [a.u.]	9.3 (1.7)	9.7 (1.4)	9.7 (1.4)	9.7 (1.1)	9.6 (1.3)	4	0.200	0.995$^\$$	0.005 ε^2
PAIN [a.u.]	1.22 (0.1)	1.11 (1.2)	1.33 (1.1)	1.22 (1.2)	1.22 (1.1)	4	0.612	0.962$^\$$	0.014 ε^2

*Note. Data averaged between 5-to-20-minute time-points. * signifies p < 0.05 between conditions. $^\$$ signifies Kruskal-Wallis non-parametric data analysis. Data presented as mean ± (standard deviation). Abbreviations; $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, carbon dioxide production; **RPE**, rating of perceived exertion; **PAIN**, rating of perceived pain; **FO**, fat oxidation; **CHO**, carbohydrate oxidation; **OR-GC**, oral prednisolone (0.5 mg.kg^{-1}); **IN-GC**, inhaled beclomethasone dipropionate (1600 μg); **OR-PLA**, microcrystalline cellulose capsules; **IN-PLA**, water vapour inhaler; **CON**, control. **D.f.,** degrees of freedom. η_p^2, partial eta squared. ε^2, epsilon squared.*

Appendix K. Supplementary Materials from Chapter 7.

Supplementary Table 11.12. Cardiorespiratory and Perceptual Response During Submaximal Preloaded Exercise [50% $\dot{V}O_2$peak]

Variable	ICS	PLA	P-Value	Effect Size (Cohen's *d*)
$\dot{V}O_2$ [L/min^{-1}]	1.74 (0.33)	1.70 (0.32)	0.158	0.56
$\dot{V}CO_2$ [L/min^{-1}]	1.66 (0.33)	1.63 (0.31)	0.315	0.38
Respiratory-Exchange Ratio (RER) [a.u.]	0.95 (0.03)	0.96 (0.03)	0.415	-0.28
Carbohydrate Oxidation (CHO) [g.min^{-1}]	1.99 (0.45)	1.98 (0.42)	0.866	0.06
Fat Oxidation (FO) [g.min^{-1}]	0.14 (0.06)	0.12 (0.08)	0.460	0.28
Heart Rate (HR) [beats/min]	124 (10)	123 (9)	0.537	0.23
RPE [a.u.]	10.3 (1.75)	10.4 (1.77)	0.732	-0.13
PAIN [a.u.]	0.69 (0.26)	0.56 (0.32)	0.170	0.54

Note. Data averaged between 5-to-20-minute time-points, (* signifies $p < 0.05$ between conditions), data presented as mean ± (standard deviation). Abbreviations: $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, carbon dioxide production; **RPE**, rating of perceived exertion; **PAIN**, rating of perceived pain; **FO**, fat oxidation; **CHO**, carbohydrate oxidation; **ICS**, Inhaled corticosteroids; **PLA**, Placebo.

END