PHARMACOGENETICS OF INHALED BETA-2-AGONISTS AND ATHLETIC PERFORMANCE

by

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Abstract

The A46G single nucleotide polymorphism (SNP) and the C79G SNP of the adrenergic β_2-receptor gene (*ADRB2*) are associated with the regulation of cardiorespiratory responses, to the inhalation of salbutamol, such as bronchodilation, heart rate and ventilation.

PURPOSE: To determine (1) the effect of susceptibility to exercise-induced bronchoconstriction (EIB) and (2) the effect of genetic variation at the *ADRB2* A46G and the C79G SNPs on athletic performance after the inhalation of salbutamol.

METHODS: Genetic variation for the A46G SNP (AA: 4; AG: 15; GG: 21; unidentified: 2) and the C79G SNP (CC: 14; CG: 19; GG: 7; unidentified: 2) were genotyped in male cyclists with EIB (EIB+: 10) and without EIB (EIB-: 32), aged 19 – 40 years. Athletes performed two simulated 10-km time trials (TTs) on a cycle ergometer 60-min after the inhalation of either 400µg of salbutamol or placebo. FEV_1 was assessed immediately before and 30-min after inhalation. Performance was measured by mean power output relative to body weight. Mixed between-within subject ANOVAs were conducted to assess differences in lung function and cycling performance, respectively, between the two treatments based on an individual's susceptibility to EIB and based on genetic variation at the *ADRB2* A46G and C79G SNPs.

RESULTS: Change in FEV_1 after the inhalation of salbutamol (M = 6.6%, SD = 6.3%) was greater compared to placebo (M = 1.1%, SD = 3.0%), $p < 0.001$. The improvement in FEV_1 was greater in EIB+ athletes (M = 10.9 %; SD = 10.9%) compared to EIB- athletes (M = 5.3%; SD = 3.0%, p = 0.009). Performance was not altered regardless of the athletes' susceptibility to EIB and genetic variation at the *ADRB2* A46G and C79G SNPs. On average, athletes maintained

4.0W·kg^{-1} (*SD* = 0.3W·kg^{-1}) after the inhalation of salbutamol and 4.0W·kg^{-1} (*SD* = 0.4W·kg^{-1}) after the inhalation of a placebo.

CONCLUSIONS: In male EIB+ and EIB- cyclists, FEV_1 is improved after the inhalation of salbutamol (400µg). Despite this improvement in lung function, athletic performance during a 10-km TT was not altered regardless of susceptibility to EIB and genetic variation at the *ADRB2* A46G and the C79G SNPs.

Preface

Collaborators and co-authors of the present study are the following:

- Dr. Michael Koehle, MD, PhD had the research idea for this project, assisted in developing the research design, secured funding for the project through the World Anti-Doping Agency (WADA), provided advice and guidance in the data collection and writing process, wrote a substantial part of the ethics protocol and helped coordinate committee members for meetings, thesis proposal and defence.
- Dr. Benjamin Sporer, PhD assisted in the development of the research design, helped with the application for funding, provided feedback regarding questions on the physiology of cycling, on test procedures during the data collection and on the data analysis, suggested new ideas for further research in this field.
- Dr. James Rupert, PhD assisted in the development of the research design, helped with the application for funding, provided feedback in questions regarding the genetic analysis, the correct use of genetic language and provided the GRIP laboratory for the entire analysis of the genetic variants of the *ADRB2* gene.
- Martin MacInnis, BSc taught and assisted Sarah Koch with the DNA isolation and determination of the *ADRB2* variants, provided feedback on questions regarding the data collection and analysis.
- Sarah Koch assisted in the development of the research design, conducted a literature review and completed the application for ethics approval, recruited subjects, coordinated and performed the data collection, performed the DNA isolation and determination of the *ADRB2* SNPs of interest, analysed and interpreted the data and wrote the thesis document.

No publications arising from the work presented in this thesis have been published to-date. The abstract attached in the appendix H has been accepted for a poster presentation at the International Conference of Human Genetics (ICHG) organized by the American Society of Human Genetics (ASHG) in October 2011 in Montreal, Canada.

The study involved human subjects and received full board approval from the University of British Columbia Clinical Research Ethics Board (H10-02028).

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ADRB2 gene	Adrenergic β_2-receptor gene
AHR	Airway hyperresponsiveness
ATS	American Thoracic Society
β_2-agonist	Beta-2-agonist
COPD	Chronic obstructive pulmonary disease
EIA	Exercise-induced asthma
EIB	Exercise-induced bronchoconstriction
EVH test	Eucapnic voluntary hyperpnea test
FEV_1	Forced expiratory volume
IBA	Inhaled β_2-agonist
ICS	Inhaled corticosteroid
IOC	International Olympic Committee
LABA	Long-acting β_2-agonist
RPEB	Rating of perceived exertion for breathing
RPEL	Rating of perceived exertion for legs
SABA	Short-acting β_2-agonist
TUE	Therapeutic use exemption
VO_2	Oxygen consumption
WADA	World Anti-Doping Agency

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1 Introduction

1.1 Asthma

Asthma is a chronic inflammatory airway disorder, which is characterized by variable and recurring airway obstruction, bronchial hyperresponsiveness and symptoms such as coughing, wheezing and chest tightness.[1] Approximately 240 million individuals worldwide are affected by asthma.[2] The prevalence of asthma is increasing as further proportions of the world's population adapt to a modern lifestyle and become urbanized: an additional 100 million asthmatics are expected by 2025.[3] The underlying pathophysiology of asthma is still undergoing intensive study. Variable phenotypic patterns in terms of disease onset, symptom spectrum and treatment response, as well as triggering factors and type of inflammation pose a challenge in the understanding of this disorder.[4, 5] A complex interplay between genetics and environmental exposures seems to be responsible for the expression of asthma.[6] A key feature in the pathogenesis and pathophysiology of all asthma-types is the underlying airway inflammation. Its intensity and mediator patterns influence other central features of asthma like airway obstruction and bronchial hyperresponsiveness.[7] Airflow limitation and bronchial inflammation can result in recurring episodes of coughing, wheezing, shortness of breath and chest tightness. The main interacting inflammatory cells are neutrophils, eosinophils, T-lymphocytes, macrophages and mast cells. It is known that some patients undergo persistent changes in airway structure including fibrosis, mucus hypersecretion, injury to epithelial cells and smooth muscle hypertrophy. Ultimately, asthma represents a vicious cycle of pulmonary function impairment.

1.1.1 Exercise-induced asthma

Classifications of asthma based on its triggers are commonly used due to the cause-effect relationship between exposure and the development of symptoms.[5] Along with environmental allergens (e.g. dust, animal hair, chemical substances), intake of certain drugs (for example, non-steroidal anti-inflammatory drugs or aspirin) and viral infections, exercise is known to be a stimulus for acute asthma attacks.[5, 8-12] Exercise-induced asthma (EIA) and exercise-induced bronchoconstriction (EIB) are often used synonymously to describe asthma symptoms triggered by exercise. Both terms describe the transient narrowing of the airways that follows vigorous exercise.[13] While EIA refers to bronchoconstriction including symptoms such as cough, wheezing or dyspnea triggered by exercise in patients with underlying asthma, EIB refers to an identical clinical presentation in individuals without asthma.[14]

1.1.1.1 Prevalence and risk factors of exercise-induced asthma

Approximately 10 - 20 % of competitive athletes suffer from EIA.[8, 9] The prevalence of EIA appears to depend on type of sport, weekly training volume, gender of the athlete and country of residence. [9] Endurance athletes tend to show a higher prevalence of EIA than strength or power athletes.[8, 9, 13, 15] When looking at the number of athletes requesting a therapeutic use exemption (TUE) of β_2-agonists (an agent commonly used to treat asthma) over the three summer Olympic Games in Atlanta (1996), Sydney (2000) and Athens (2004), it appears that cyclists (15.4 % of all competitors) followed by triathletes (no complete data-set, as there was no participation in Atlanta where notifications of β_2-agonist use were much lower) and swimmers (11.3 %) are most prone to EIA.[16] Even higher is the prevalence of EIA in winter endurance athletes. The mean prevalence of EIA over the winter Olympic Games in Nagano (1998), Salt Lake City (2002) and Torino (2006) was highest in cross-country skiers (17.6 %), followed by speed skaters (16.2 %)

and Nordic combined athletes (13.8 %).[16] Interestingly, only 8.1 % of the biathletes requested a TUE for β_2-agonists. This is surprising, because biathletes undergo high training-volumes of cross-country skiing; therefore, a similarly high prevalence of EIA as in Nordic skiers would be expected. A possible explanation for this observation could be known side effects of β_2-agonists such as tremor and tachycardia, which might be disadvantageous for the shooting component of biathlon. In contrast to endurance disciplines, sports that demand relatively high strength and power abilities have a remarkably lower EIA prevalence (weightlifting: 1.6 %; gymnastics: 1.1%; ski jumping; 3.2 %, luge: 2.8 %).[13]

According to Nystad *et al.*[17], training volume has an impact on the prevalence of EIA. A volume greater than 20 hours per week seems to put athletes at a higher risk for EIA compared to training volumes of less than 10 hours per week.[17] Women may be more prone to develop EIA than men; however, these findings have not been uniformly accepted. [17, 18]

When looking at the prevalence of EIA among Olympic athletes from a geographical point of view, the nationality of asthmatic athletes reflects the asthma prevalence of the general population in the according country.[10] Western countries such as New Zealand (21.1 %), Australia (20.7 %) and Great Britain (19.9 %) had the highest percentages of asthmatic athletes in the Summer Games in Sydney. Only 45 (or 1.2 %) of athletes from Asia, Africa, Central and South America required asthma treatment. These trends are similar for athletes participating in the Winter Games. In Nagano (1998), the Netherlands (33.3 %), Australia (20.0 %) and the United States (16.9 %) had the highest prevalence of asthmatic athletes. Besides a generally higher prevalence of asthma in western countries, cultural, ethnic and socioeconomic factors could be a cause for these observations. Additionally, over- and under-diagnosing of EIA might be a factor for a country-dependent prevalence of EIA among athletes.[10]

1.1.1.2 Pathophysiology of exercise-induced asthma

Several mechanisms are currently discussed as possible causes for EIA. The heat loss-theory describes the cooling and drying of the airways following exercise-induced hyperventilation as the primary cause of the high prevalence of EIA among endurance athletes.[12, 15, 19] Respiratory heat loss with a consecutive re-warming of the bronchial vasculature due to an increase in blood flow may act as a stimulus for vasoconstriction and EIB.[20, 21] Another cause for EIA could be the mechanical stress within the respiratory organs due to high ventilation rates and large inhaled volumes resulting in epithelial injuries in the airways.[9, 15, 19, 22] Prolonged hyperventilation on an injured epithelium is believed to cause dehydration and a degranulation of airway cells with a release of inflammatory mediators.[23] The main cause for the mediator release is thought to be a change in the osmolarity of the fluid lining the surface of the respiratory mucosal membranes. These mediators induce constriction of smooth muscles, stimulate mucus-producing glands and promote microvascular leakage, resulting in airway edema.[19, 23] A third theory explains EIA with an altered autonomic nerve regulation.[19, 22] Both contractions and relaxations of the smooth muscles in the airways are regulated by autonomic nerves: parasympathetic nerves mediate contractions; sympathetic nerves mediate relaxations. An increased level of parasympathetic activity could be developed as a counterbalance to sympathetic stimulations associated with frequent and intense training in athletes. Resting bradycardia but also an increased broncho-motor tone, responsible for an increased susceptibility to the development of EIA, may result.

Additionally, environmental exposures, such as repeated inhalations of highly concentrated chlorine of the water surface in swimmers, can cause EIA.[24] The chronic contact with chlorine gas may promote an increase of the epithelial permeability in the lung.[25] Another cause for the relatively high prevalence of asthmatics in swimmers is the referral of physicians to engage in

this type of sport.[16] As an activity that can be conducted indoors in an environment with warm, humid air, swimming is thought to be less likely to trigger acute asthma attacks than other sports.

1.1.2 Asthma diagnosis and treatment

1.1.2.1 Asthma diagnosis

The relationship between asthma symptoms and documented airway hyperresponsiveness (AHR) is poor in many athletes.[11, 22, 26] Among others, Lund *et al.*[27] showed that asthma symptoms alone do not serve as a reliable diagnosis. Of 42 Danish elite athletes with asthma-like symptoms, only 12 were confirmed by a positive mannitol challenge. At the same time, many elite athletes fail to associate respiratory symptoms with EIB. In a study on Olympic athletes from the UK, who underwent EIB screening with a eucapnic voluntary hyperpnea (EVH) test, 78 out of 228 athletes tested positive and of those, 57 (73 %) had no previous diagnosis of EIB.[26] Therefore, besides a clinical history and physical examination, the diagnosis of EIA and EIB should be confirmed by objective tests. The International Olympic Committee (IOC) recommends a sequence of tests:

1. Spirometry: to assess airway obstruction.
2. Inhalation of a bronchodilator: to assess reversibility of airway obstruction.
3. Bronchial provocation test: to establish presence of AHR.[28]

Bronchial provocation tests include direct and indirect testing protocols. Exercise, eucapnic voluntary hyperpnea (EVH) and hyperosmolar aerosols such as mannitol act as indirect stimuli. By triggering airway smooth muscle contraction and airway constriction, they cause the release of inflammatory mediators in the airways.[11] Methacholine chloride is the most commonly used direct stimulus to assess AHR. It stimulates acetylcholine receptors directly to cause smooth muscle contraction. Usually, the dose needed to induce a fall in forced expiratory volume in one

second (FEV_1) of 20 % (PD_{20}) is indicative for the sensitivity of the airway smooth muscle cells to methacholine; thus, response to methacholine is used to describe the severity of AHR.

1.1.2.2 Asthma treatment

The goal of asthma treatment is to control symptoms and optimize pulmonary function.[29] The treatment of an asthmatic athlete does not differ from the treatment of an asthmatic non-athlete.[30, 31] One factor that has to be considered when treating an athlete's asthma is to prevent the progression of the airway disorder.[31] In athletes with EIA, it is impossible to remove the trigger for acute asthma attacks due to the nature of their profession. A tight control of symptoms is necessary to prevent irreversible, inflammatory-induced airway remodeling.[7] Additionally, one has to keep in mind when prescribing treatment, that not only the disease itself but also the side effects of drugs can impact an athlete's performance.[31]

Asthma medications can be classified as 'controllers' and 'relievers'.[29, 31] Controllers are taken on a daily long-term basis with the main goal to control the airway inflammation. Relievers are used on an as-needed basis. They act quickly to reverse bronchoconstriction and to relieve its symptoms. Routes of administration for asthma medications are either systemic or local.[7] Systemic routes include oral (ingested) or peritoneal (subcutaneous, intramuscular, or intravenous injections) administrations. Local administration, for example inhaling asthma drugs, has the advantage of delivering highly concentrated agents directly to the airways, the location of designated action. Systemic side-effects are reduced by targeting local receptors only.[32] Some drugs, like most of the inhaled corticosteroids (ICS), are therapeutically active only when inhaled.[7]

Controller treatment of exercise-induced asthma

Currently, the most effective anti-inflammatory medications for asthma are inhaled corticosteroids (ICS) and leukotriene antagonists (LA).[8, 29] They reduce symptoms, improve lung function, decrease airway hyperresponsiveness and reduce the frequency and severity of exacerbations.[29] Additionally, ICS enhance the protective effect of inhaled β_2-agonists (IBA), which are used as reliever agents (see following chapter).[31] At the Olympic Games in Athens (2004), 66.3 % of all athletes applying for permission to use IBAs advised that they were also using ICS.[16] Known side-effects of ICS are both systemic and local. Adrenal suppression, growth retardation in children and adolescence, and reduction in bone density have to be particularly monitored in the treatment of athletes.[33-35]

Reliever treatment of exercise-induced asthma

Typically, athletes use relievers prophylactically before exercising to prevent EIA attacks or to treat acute symptoms.[31] The most studied drugs in this field are β_2-agonists. It has been demonstrated that they are very effective in EIB. Short-acting β_2-agonists (SABAs) as well as long-acting β_2-agonists (LABAs) administered immediately before exercise have been shown to decrease the reduction in FEV_1 by 70 - 80 %.[36, 37] SABAs induce a relief of symptoms immediately and are therefore especially helpful in treating and preventing acute asthma attacks.[7] Examples for SABAs are salbutamol, terbutaline and fenoterol.[29] LABAs such as formoterol (5-min) and salmeterol (15-30-min) have a slower onset of action and a longer duration (greater than 12 hours).[7] They are used for long-term prevention of symptoms and can be added to a SABA- or ICS-therapy. During the Olympic Games in Athens (2004), only a small percentage of athletes attempted to control their asthma with the use of LABAs only.[16] Salbutamol was chosen as a SABA in 94.5 % of the athletes. Similarly, 92.1 % used salbutamol as reliever treatment at

the Olympic Games in Salt Lake City (2002) and 30 % inhaled both, SABAs and LABAs. The monotherapy with LABAs is contraindicated. It is associated with an increase in mortality for reasons that are unknown yet.

The most frequent adverse effects of β_2-agonists are tachycardia and tremor, headaches and irritability.[38] Their severities are dose-related and more pronounced in SABAs than in LABAs. Additionally, a small population of asthmatics is resistant to β_2-agonist treatment when administered in therapeutic dosages.[37, 39] Furthermore, daily treatment with β_2-agonists can enhance the severity of EIA.[40] A decreased duration of protection from EIA has been found.[41] This so-called "development of tolerance" has been linked to desensitization (decrease in responsiveness with repeated or chronic exposure of membrane receptors) and a net loss of β_2-receptors (downregulation).[11, 42] As a result, an increased number of inhalations is needed per day to control AHR and a slower recovery from EIA after a standard dose of β_2-agonists has been observed.[40, 43] The receptor downregulation and desensitization represents a dilemma in the therapy-management of asthmatic athletes.[11] Ideally athletes should use β_2-agonists as infrequently and in as low dosage as possible; however, this may not be doable in individuals who train daily.

1.2 Effects of asthma treatment on performance

1.2.1 Are asthmatic athletes more successful than non-asthmatic athletes?

Asthmatic athletes depend on optimal treatment not only to stop the progression of their airway disorder but also to be able to perform successfully.[31] Nevertheless, asthmatics should not be given an advantage over their non-asthmatic peers, because it is possible that asthma drugs might improve performance and have an ergogenic effect. The medical committee of the International Olympic Committee (IOC) and the World Anti-Doping Agency (WADA) supervise and regulate

the drugs used to treat asthmatic elite athletes.[28, 44] In 2001, WADA was founded and assigned by the IOC to prepare and publish the list of prohibited substances as a part of the anti-doping code.[45, 46] The anti-doping code was created by WADA to protect the health of athletes and to ensure fairness among all competitors.[46] According to the code, an agent is added to the list of banned substances if it meets at least two of the following criteria:

1. The substance has the potential to enhance sport performance
2. The use of the substance represents a health risk to the athletes
3. The use of the substance violates the spirit of sport

In the past 25 years, there has been a trend for an increase in applications for permission to use β_2-agonists by athletes competing in Olympic Games.[10, 11, 13, 16] The analysis of the TUE requests and notifications of the use of IBAs leads to speculations among exercise physiologists, whether the prevalence of β_2-agonist use among Olympic athletes is related to the mode of control and the regulations of its use given by WADA. The more liberal the regulation of salbutamol use, the greater the number of notifications of use. This on its own is a reason to wonder about potential ergogenic effects due to the utilization of IBA. Adding to the controversy, in the last five Olympic Games, IBA users won a disproportionate number of individual Olympic medals.[11, 47] For example, in Sydney (2000) and Athens (2004) 5.7 % and 4.2 % of the athletes being IBA users won 7.2 % and 5.4 % of individual medals, respectively. Even bigger was the overrepresentation of IBA users in medal counts in Winter Games: 5.2 %, 7.7 % and 7.1 % of all athletes in Salt Lake City, Torino and Vancouver won 15.6 %, 14.5 % and 11.8 % of all individual medals, respectively.

1.2.2 IOC and WADA regulations regarding asthma-drug management

Studies investigating the effect of systemically administered β_2-agonists on performance showed ergogenic effects.[48-52] Infusion and oral uptake of β_2-agonists have a hypertrophic effect on skeletal and heart muscle fibres.[53, 54] Therefore, this method of administration was banned by the IOC and WADA.[16] Most of the changes regarding the β_2-agonists use in Olympic athletes since 1975 dealt with different types of IBAs and the notification system.[16] In 2001 WADA introduced the TUE-regulation. A TUE allows athletes with documented medical conditions the use of a substance on the prohibited list.[46] When applying for a TUE, a specific form must be completed by the physician and the athlete. Additionally, a clinical history of the athlete and documented results of lung function tests are mandatory.[13] Interestingly, the number of approved TUE applications in summer Olympians dropped from 5.7 % in Sydney (2000) to 4.6 % in Athens (2004).[16] This raises the question, did the prevalence of asthma drop during those four years due to natural causes or did the tighter control due to TUEs cause this decrease. No drop in the percentage of athletes requiring IBA during the Winter Olympics in Nagano (1998) and Salt Lake City (2002) was found. As of January 01, 2010, all β_2-agonists are prohibited with the exception of salbutamol and salmeterol when taken by inhalation and in therapeutic doses.[55] No TUE is required for their use, but a declaration is still requested. To discriminate between the oral and inhaled use of salbutamol, as well as to control the dose, a urine presence of 1000ng•ml^{-1} was determined as the cut-off value. Urine salbutamol concentrations above this value are considered a positive finding. Currently, ICS are permitted with a declaration of use. Systemic administration of corticosteroids is prohibited and requires a TUE.

1.2.3 Mechanisms of action of β_2-agonists

By mimicking adrenaline and noradrenaline, β_2-agonists act on the adrenergic system.[56] Natural catecholamines, as well as β_2-agonists, activate adrenergic β_2-receptors which are expressed in many cells throughout the body.[57, 58] Besides their general function as a bronchodilator by inducing a relaxation of smooth muscle cells, β_2-agonists have various metabolic effects due to their association with cAMP production.[56] In humans, up to 40 % of total adrenergic β_2-receptors are located in the heart, which is more than was found in animals.[59] β_2-agonists influence chronotropic and inotropic effects, meaning that they increase heart rate and the contractility, respectively.[56] In the lung, β_2-agonists not only induce a bronchodilation but also decrease the release of airway constricting mediators.[60] As a vasodilator, β_2-agonists induce an increase in blood flow in the coronary vessels and in skeletal muscles.[61, 62] Additionally, β_2-agonists have anabolic effects by stimulating muscle growth when administered orally. They also stimulate speed of skeletal muscle contractions, glycogenolysis and tremor.[63] On a metabolic level, the exposure to β_2-agonists induces an increase in insulin and glucagon in the pancreas as well as an increase in hepatic glycogenolysis.[60]

1.2.4 Effects of inhaled β_2-agonists on performance

In the past decades, most studies interested in ergogenic effects after the use of IBA were designed in a randomized, double-blind, crossover fashion, including a placebo-control.[8, 64] Usually, non-asthmatic endurance athletes (cyclists, runners and swimmers) were recruited.[65-67] Studies by Signorile *et al.*[68] and Bedi *et al.*[69] are the only two studies that showed a performance enhancement after the administration of a therapeutic dose of IBA. Signorile *et al.*[68] measured an increase in peak power output in repeated 15-s Wingate tests 10-min after the inhalation of 180 µg of salbutamol. Kindermann *et al.*[9] questioned whether these results were applicable to elite

athletes since recruited subjects were not competitive but recreational athletes. Their lower fitness level might have been related to different performance limiting factors in anaerobic exercise bouts that could have been overcome by bronchodilation. Bedi *et al.*[69] tested 15 non-asthmatic individuals after the inhalation of 180 μg salbutamol. Participants completed a 1-hour continuous cycling test including an exhaustive final sprint. Because they observed an increased time to exhaustion in the salbutamol group, the authors concluded that salbutamol may provide an advantage in non-asthmatic athletes. This study has been criticized because of the inclusion of two recreational cyclists.[8, 9] Additionally, a subsequent study was not able to confirm their findings.[70] A study conducted by van Baak *et al.*[66] looked at the effect of a supra-therapeutic dose of inhaled salbutamol (800 μg) on cycling time trails. A 2 % improvement in cycling time was reported. Again, the largest improvements were found in subjects with the lowest initial performance.[9] Eleven out of 16 subjects showed an improved performance; however, the effect was very limited in five of the 11.

In contrast to studies that reported an ergogenic effect, two studies found a performance-impairment after the inhalation of salbutamol and salmeterol.[71, 72] Carlsen *et al.*[71] tested 18 healthy athletes in a cross-over, placebo controlled study with either 800 μg salbutamol or 50 μg salmeterol. Interestingly, running time until exhaustion was lower after the two IBA conditions than after placebo treatment. It is speculated that an increased adrenergic β_2-receptor stimulation of skeletal muscle might have increased muscle metabolism and therefore caused earlier muscle fatigue. No significant differences in peak power output, speed and fatigue between trials after IBAs and placebo use were found in several studies using LABAs and SABAs.[70, 73, 74]

Most studies reported an improved lung function after IBA use compared to placebo, but no changes in performance.[71, 75-78] An explanation might be an additional bronchodilating effect due

to exercise, responsible for equal lung functions post-exercise.[71, 75-78] However, Verroken points out, that the improvement in lung function due to IBA in swimmers, when used immediately prior to racing, might allow an increased time under the water after a dive.[79] This might save precious milliseconds before surfacing the water.

Three studies tested athletes under extreme environmental conditions. Cold temperatures of -15 to -20 °C, as well as hypobaric conditions corresponding to an altitude of 2000 m above sea level did not have an effect on performance after the use of IBA.[76, 77, 78] The Joint Task Force of European Respiratory Society (ERS) and European Academy of Allergy and Clinical Immunology (EAACI) summarized that IBAs do not improve athletic performance in non-asthmatic athletes.[31]

Surprisingly few studies have looked at ergogenic effects after IBA-use in asthmatic athletes. Ienna *et al.*[80] compared physiological responses to exercise with and without pre-exercise medication based on training status in asthmatic athletes. No difference was found in heart rate, oxygen consumption, ventilation, oxygen-saturation and respiratory exchange ratio between the treatment and placebo trial.[80] Inhaled salbutamol did lower airway resistance, but asthmatic athletes did not present with altered metabolic or ventilator responses during exercise.

1.3 Genetics, inhaled β_2-agonists and performance

Large inter-individual variations in the response to IBA treatment have been demonstrated and linked to genetic variations.[1, 4, 80] Pharmacogenetics is the investigation of the inter-individual variability of responses to medications due to heredity.[1] Several studies investigated whether variations in the *ADRB2* gene lead to heterogeneous responses in the pharmacological treatment of asthma.[4, 81, 82] New is the question whether these heterogeneous effects due to genetic

differences of the *ADRB2* gene divide athletes into "high-responders" and "low-responders" based on performance-enhancing reactions to IBA. In other words, based on the genetic code of the *ADRB2* gene, only athletes with the high-responder-genotype would experience an ergogenic effect after the use of IBA, while individuals with the low-responder genotype would not. Studies interested in ergogenic effects after the use of IBA might not have found statistically significant results because data were not analyzed based on athletes' genotypes. This theory could explain why asthmatic athletes tend to be more successful than non-asthmatic athletes, even though most studies investigating ergogenic effects after IBA use do not show any performance enhancing results.

1.3.1 Polymorphisms of the β_2-agonist receptor gene

The *ADRB2* gene is located on chromosome 5q31, a region consistently linked to asthma and bronchial airway hyperresponsiveness.[83, 84] It is a small, intron-less gene that encodes a 413-amino acid G-protein coupled receptor.[85] Forty-nine single nucleotide polymorphisms (SNPs) of the *ADRB2* have been identified.[86] These SNPs differ from each other by only one nucleotide. In some SNPs, a substitution of a single nucleotide results in the translation of a different amino acid. They are referred to as non-synonymous SNPs.[4] Two of the most commonly studied non-synonymous SNPs of the *ADRB2* gene include the A46G SNP (rs 1042713) and the C79G SNP (rs 1042714).[87] At the 46th base pair of the *ADRB2* gene, the substitution of an A base to an G base leads to the substitution of an arginine for a glycine amino acid. This polymorphism is also abbreviated as Arg^{16}Gly, indicating that the A-G-base pair substitution can lead to a substitution of a glycine for an arginine at the position of the 16th amino acid. Similarly, at the 79th base pair, the substitution of a C base for a G base leads to the substitution of a glutamic acid for a glutamine amino acid at the 27th amino acid position (Glu^{27}Gln). The allele distribution of SNPs

varies between ethnic groups as can be seen in Table 1. In synonymous SNPs, the substitution of a single nucleotide does not lead to a translation of a different amino acid. The amino acid sequence remains unaltered, thus the protein structure and function are identical between the genotypes of synonymous SNPs.

Table 1: Allele distributions across ethnic groups of the *ADRB2* A46G and the C79G SNP.

SNP	Frequency in Caucasians		Frequency in African-Americans		Frequency in Asians		Frequency in Hispanic-Latinos	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
A49G†	G: 64.9%	A: 35.1%	G: 39.1%	A: 61.0%	G: 42.5 %	A: 57.5%	G: 46.6%	A: 53.3%
C79G†	C: 50.7%	G: 48.3%	C: 93.9 %	G:6.3%	C: 90.0 %	G: 10.0%	C: 73.2%	G: 26.7%

Modified after Drysdale *et al.*[88] and Hawkins *et al.*[86]

† The one-letter code represents the two possible base pairs, and the specific location in the base-pair sequence is represented by the number in between.

Abbreviations: G = guanine; A = adenine; C = cytosine.

Clusters of alleles that tend to be inherited together are termed haplotypes.[87] For example, individuals who are homozygous for the G-allele at the G79G SNP tend to also be homozygous for the G-allele at the A46G SNP. The combination of the GG genotype at the A46G and the GG genotype at the C79G SNP is therefore relatively common compared to the AA genotype at the A46G SNP and the GG genotype at the C79G SNP (less than 1 % of the population). Those two SNPs are said to be in linkage disequilibrium (LD).[87] As a result of the strong LDs in the *ADRB2* gene, Drysdale *et al.*[88] investigated 13 linked SNPs in the coding and non-coding region of the *ADRB2* gene in 23 Caucasians, 19 African-Americans, 20 Asians, and 15 Hispanic-Latinos. They found that those 13 SNPs were organized into only 12 haplotypes, but only four of these haplotypes were relatively common (greater than 6 %). Differences between the frequencies of the 12 haplotypes exist between ethnic groups. These findings were confirmed by several

subsequent studies. [89-92] Jensen *et al.* [86] observed that haplotype 1 was present with a frequency of approximately 10 % among Danish Caucasians, while Hawkins *et al.*[90] did not identify any US-American Caucasians with haplotype 1.

1.3.2 Pharmacogenetics of β_2-agonist receptors

1.3.2.1 Impact of *ADRB2* SNPs

Initially, studies concerned with the response to pharmacological agents based on the genotypes of the *ADRB2* gene were mainly based on SNPs. When measuring reactions to β_2-agonists in the heart, such as contractility and heart rate, no differences among the genotypes of the A46G and the C79G SNPs were found.[93-95] Vascular responses based on *ADRB2* polymorphisms after the stimulation of *ADRB2* were more diverse.[87] Studies on Chinese hamster fibroblasts showed, that neither the *ADRB2* A46G nor the C79G SNP affected the function of the adrenergic β_2-receptor in terms of ligand binding and adenylyl (also known as adenylate) cyclase activation.[96] In studies on humans, the A46G and the C79G SNPs showed different responses after systemic infusions of salbutamol, terbutaline or adrenaline.[97-99] Individuals with the AA genotype at the A46G SNP showed greater vasodilation than subjects carrying with the AG or GG genotypes. When β_2-agonists were administered in local infusions into the brachial artery of the hand vein, greater vasodilation was measured in subjects with the GG genotype at the A46G SNP and the GG genotype at the C79G SNP.[100, 101] This finding shows that the type of administration of an agent affects the physiological response based on genetic variations among individuals.

1.3.2.2 Impact of *ADRB2* haplotypes

When analyzing phenotypic features based on findings of isolated SNPs, without considering potential interactions with other SNPS in the promoter or coding region, conflicting results can be found.[82] The analysis of polymorphisms of a gene based on its haplotypes can lead to new findings, and may reduce the costs and time required to conduct a pharmacogenetic study.

Receptor downregulation and desensitization

In asthmatics, which are regularly exposed to SABAs and/or LABAs, it appears that at least in vascular and bronchial smooth muscles, the A-allele at the A46G SNP and the C-allele at the C79G SNP seem to be more susceptible to agonist-induced desensitization.[87, 102] This is in contrast to the original findings in recombinant cell systems in Chinese hamster fibroblasts.[87, 96] The authors reported that the G-allele at the A46G SNP is more susceptible to receptor desensitization than the A-allele.[96] An explanation for these contrary findings could be the "dynamic model of receptor regulation" by Liggett *et al.*[103]: endogenous catecholamines dynamically desensitize *ADRB2*s in their basal state. This occurs to a greater extent for the G-allele than for the A-allele at the *ADRB2* A46G SNP. Accordingly, exogenous agonist-induced desensitization should then be greater for the A-allele than for G-allele at the *ADRB2* A46G SNP, because that is already endogenously desensitized.

Systemic response

Lee *et al.*[102] found that Caucasians with the AA genotype at the A46G SNP and the GG genotype at the C79G SNP undergo greater systemic responses to inhaled salbutamol compared to individuals with the GG genotype for both SNPs. Serum potassium change and diastolic blood pressure change, both measured from baseline over 20-min were significantly greater individuals with the AA genotype at the A49G SNP and the CC genotype at the C79G SNP than for

individuals with the GG genotype at the A46G and the C79G SNPs. No differences were found in heart rate changes.

Bronchodilator response

Findings by Drysdale *et al.*[88] indicate that salbutamol-induced FEV_1 reversibility is related to *ADRB2* haplotypes, but not to any of the individual SNPs. The percent change in FEV_1 was almost twice as high in individuals with the GG genotypes at the A46G and the C79G SNPs (haplotype 2) than in individuals with the AA genotype at the A46G SNP and the CC genotype at the C79G SNP (haplotype 4). Overall, haplotype 6 (AG genotype at the C46G SNP and CC genotype at the C79G SNP) showed the highest responsiveness to salbutamol. Choudhry *et al.*[104] demonstrated that both the analysis of individual SNPs and haplotypes show differences in the response to bronchodilators. In their family study, haplotype 1 (which is equal to haplotype 4 in Drysdale *et al.*[88]) showed the highest responsiveness. Subjects with haplotype 2 (haplotype 6 in Drysdale *et al.*[88]) presented with the lowest bronchodilator responsiveness. These contrary findings could be due to different study-populations. Drysdale *et al.*[88] studied 121 unrelated Caucasian subjects with asthma, whereas Choudhry *et al.*[104] tested asthmatic families (667 family trios with a total $n = 2001$) from two different populations (Puerto Ricans and Mexicans). Similarly to Choudhry *et al.*[104], Silverman and colleagues[91] found a relationship between bronchodilator responsiveness and individual SNPs and haplotypes. Their level of bronchodilator responsiveness was also lower for the haplotype with the G-allele at the A46G SNP and the C-allele at the C79G SNP (haplotype 6 in Drysdale *et al.*[88]). A dose-response relationship may exist between the haplotype pairs.[88, 91] However, findings are conflicting and further research is necessary. For example, neither Hawkins *et al.*[85] nor Taylor *et al.*[106] were able to confirm a

relationship between haplotype pairs of the *ADRB2* gene and acute bronchodilator responses in asthmatic Caucasians and African-Americans.

1.3.2.3 Impact of the *ADRB2* A46G and the C79G SNPs on athletic performance

Sarpeshkar and Bentley[105] summarized studies of the of *ADRB2* gene polymorphisms and linked SNPs and haplotypes of the *ADRB2* gene to athletic performance, especially aerobic endurance. A primary focus was set on the A46G and the C79G SNPs and haplotypes. Findings were categorized on influences of the *ADRB2* polymorphisms on the cardiovascular, pulmonary, metabolic and musculoskeletal system. According to their review the haplotype with the G-allele at the A46G SNP and the G-allele at the C79G SNP is associated with beneficial responses on all four systems in regards to exercise: increased heart rate, greater bronchodilation and increased epinephrine secretion, stimulating lipolysis (see Table 2).

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Table 2: Associations of genetic variants of the *ADRB2* gene and physiological systems.

SNP	Cardiovascular System	Pulmonary System	Metabolic System	Musculoskeletal System
General Function	Approximately 30% of B2-Adrenoceptors found in atria of the heart, allowing or calcium influx to stimulate ventricular contraction.	Regulates airway tone by maintaining homeostasis of bronchial smooth muscles, aid in maintenance of gas exchange, promote bronchodilation during exercise and enhance ventilation with minimal airway resistance.	Stimulation of sympathetic nervous system activity inhibits insulin, promotes release of catecholamines (epinephrine).	Epinephrine activity is increased by B2-receptor activity, which influences cAMP signaling, increased Cor Cycle activity, Na1K1-ATPase activity.
G46A rs 1042713	G-allele: Associated with an increase in: - receptor density, heart rate, cardiac output, mean arterial pressure, stroke volume, vasodilation resulting in improved exercise capacity over 2h. A-allele: - Greater potential for vascular desensitization.	G-allele: - Increased bronchodilation above baseline. - Prolonged bronchodilation compared to A-allele. A- allele: - Greater fluid accumulation, decreased alveolar-capillary exchange. - Quicker receptor desensitization.	G-allele: - In obese individuals: 50% enlargement of adipocytes and 5-fold increase in lipolytic sensitivity to $\beta_2$agonists. A-allele: - Favorable catecholamine stimulation resulting in lower body weight due to the regulation of fat mobilization →optimal weight-to-strength ratio - Efficient mobilization of substrates to maximize endurance performance.	A-allele: - lower nicotinic receptor function, low stimulation at the neuromuscular junction. - Decrease in muscle force.
C79G, rs 1042714	$Gly^{16}Glu^{27}$ haplotype†: increased receptor number and resistance to desensitization, enhanced SV and CO.	G-allele: delayed receptor downregulation of β_2-receptors.	$Gly^{16}Glu^{27}$ haplotype: may benefit from enhanced lipolysis, thus allowing for improvement in aerobic phenotypes.	G-Allele: In a clinical population: associated with increased muscle force during endurance activities.

†$Gly^{16}Glu^{27}$ haplotype: individuals with a combination of the G-allele at the A46G SNP and the G-allele at the C79G SNP.

1.4 Conclusion

Exercise-induced asthma is a chronic inflammatory airway disorder. In the past decades, an increasing prevalence of EIA among elite athletes has been noticed. Endurance athletes are especially affected, most likely because of a multitude of interacting mechanisms such as chronic exposures to high ventilation rates, which might not only lead to a cooling and drying of the airways, but also damage the epithelium and induce inflammatory reactions. The treatment of EIA usually includes corticosteroids to treat the underlying inflammation, and IBAs, such as salbutamol, to relieve acute symptoms. Studies have investigated the effect of inhaled β_2-agonists on performance in non-asthmatic athletes: highly trained individuals, using therapeutic dosages of IBA did not demonstrate improved performance. Conversely, van Baak *et al.*[66] exposed their athletes to supratherapeutic dosages of IBA and found a performance-enhancing effect in 11 of 16 athletes. Overall, it is surprising that most studies did not show an ergogenic effect, because when studying the medal counts of asthmatic and non-asthmatic Olympic athletes at Summer and Winter Olympic Games, athletes allowed to use IBAs tend to be more successful than athletes who do not undergo asthma treatment. A potential reason for the absence of performance-enhancing findings in the past could be the role of pharmacogenetics. The *ADRB2* receptor is polymorphic. Drysdale *et al.*[88] found 13 haplotypes that were inherited in varying frequencies among different ethnic groups. Asthmatic individuals carrying the GG genotype at the A46G and the C79G SNPs showed twice the percent change in FEV_1 compared to individuals carrying the AA genotype at the C46G SNP and the GG genotype at the C79G SNP.[88] Different responses to IBA exposures based on the genotype of the *ADRB2* gene might lead to ergogenic effects of a subgroup of athletes. Only individuals with a "responder" genotype of the *ADRB2* gene might

benefit from an enhanced performance after IBA use and thus explain the disproportionate number of medal-winning asthmatic athletes at Olympic Games.

2 Pharmacogenetics of inhaled β_2-agonists and athletic performance

2.1 Introduction

Beta$_2$-agonists, such as salbutamol, are frequently used in the treatment of asthma.[1] These agents decrease airflow obstruction, a characteristic of acute asthma attacks.[56] By mimicking adrenaline and noradrenaline, β_2-agonists induce a relaxation of smooth muscle cells in the airways that leads to bronchodilation. Beta$_2$-agonists act on β_2-adrenergic receptors. They are located in the lung, heart and skeletal muscles.[56] Due to the omnipresence of β_2-adrenergic receptors in the body, β_2-agonists may act on organs other than the airways. Therefore, IBAs may enhance performance in athletes. Specifically, IBA-induced actions may include enhanced muscle anabolism, increased heart rate and improved contractility of the heart, improved bronchodilation and metabolic processes.[56, 105]

In the past 25 years, there has been an increase in the number of applications for permission to use β_2-agonists by Olympic athletes.[10, 11, 16] Analyses of the numbers of TUE requests and notifications of the use of IBAs lead to speculations among exercise physiologists of whether the prevalence of IBA users among Olympic athletes is related to the mode of control and the regulations of its use given by WADA.[10, 16] The more liberal the regulation of salbutamol use, the higher the prevalence of IBA users. Adding to the controversy, in the last four Olympics Games, IBA users have won a disproportionate number of Olympic medals.[11] Many studies have been performed in an attempt to answer if there is an ergogenic effect after the use of IBAs.[9, 65, 66, 106] The majority of studies indicate that IBAs do not improve performance in athletes, whereas there is evidence that orally-administered β_2-agonists do have a performance enhancing effect.[48, 51] The oral uptake of β_2-agonists is therefore prohibited by WADA.[45]

With no proven performance benefit for IBA in an unselected population of athletes, there are insufficient explanations for the overrepresentation of IBA users in the medal counts. One possible unexplored cause could be that individual genetic variation mediates the effect of IBA on performance. The study of genetic determinants in the variable interindividual responses to medications is known as pharmacogenetics.[1] Only a subgroup of athletes might derive an ergogenic benefit from IBAs, and this might depend on their *ADRB2* genotype.

Forty-nine SNPs are known of the *ADRB2* gene.[4, 82, 86, 87] Drysdale *et al.*[88] studied 13 SNPs and observed that they were inherited in 13 haplotypes with varying frequencies among different ethnicities. Furthermore, Drysdale *et al.*[88] examined the effect of varying *ADRB2* haplotypes on spirometry following a dose of IBA. Certain haplotypes (high-responders) had almost twice the increase in FEV_1 in response to IBA as other haplotypes (low-responders). The genotype-dependent responsiveness of the adrenergic β_2-receptors to IBA was confirmed by subsequent studies.[91, 104] Silverman *et al.*[91] and Choudhry *et al.*[104] observed the highest bronchodilator responses in the *ADRB2* haplotype with the A-allele at the A46G SNP and the C-allele at the C79G SNP. Interestingly, Drysdale *et al.*[88] measured the lowest percent change in FEV_1 in this haplotype (A-allele at the A46G SNP and the C-allele at the C79G SNP). Furthermore, Drysdale *et al.*[88] observed the highest percent change in FEV_1 in the haplotype with the G-allele at the A46G SNP and the G-allele at the C79G SNP. Choudhry *et al.*[104] measured the lowest bronchodilator response for this haplotype (G-allele at the A46G SNP and the G-allele at the C79G SNP).

2.2 Objectives and hypotheses

The primary purpose of this study is to assess competitive cyclists for genotypic variation at the *ADRB2* gene and to examine whether the A46G and the C79G SNPs of the *ADRB2* gene confer an ergogenic benefit to IBA. To our knowledge, the pharmacogenetic approach in the discrepancy between asthmatic and non-asthmatic athletes in performance is novel. With the present study we aim to investigate the following three objectives and hypotheses:

Objective 1: To determine if there is a difference in mean power output measured over the duration of a 10-km time trial in an unselected group of competitive male cyclists after the inhalation of salbutamol.

Hypothesis 1: We hypothesize that there will be no significant increase in mean power output measured in an unselected group of competitive cyclists during a 10-km time trial after the inhalation of salbutamol.

Objective 2: To determine if there is a difference between athletes with EIB (EIB+) and athletes without EIB (EIB-) in regards to athletic performance after the inhalation of salbutamol and placebo.

Hypothesis 2: We hypothesize that EIB+ athletes will show a greater response to salbutamol and will thus present with a greater increase in mean power output over a 10-km time trial compared to EIB- athletes.

Objective 3: To determine if there is a difference in mean power output based on polymorphic variations at the *ADRB2* gene in competitive cyclists during a 10-km time trial after the exposure to IBA.

Hypothesis 3: We hypothesize that cyclists with the high-responder genotypes at the A46G and the C79G SNPs, individually would have a greater increase in mean power output after the inhalation of salbutamol than cyclists with the low-responder genotype. Due to contradictory findings regarding the bronchodilator responsiveness between the genotypes at the A46G and the C79G SNPs of the *ADRB2* gene, classification of the high- and low-responder genotypes cannot be performed *a priori*.

2.3 Methods

2.3.1 Participant information

Between December 2010 and July 2011, 68 competitive male athletes aged between 19 and 40 years were screened. All participants were Caucasian except for one athlete who was Hispanic-Caucasian. Athletes were competing at a provincial, national or international level. Exclusion criteria included a maximal oxygen consumption (VO_{2max}) of less than 60 $mL \bullet kg^{-1} \bullet min^{-1}$ (relative) or 5 $L \bullet min^{-1}$ (absolute) and a previous history of cardiac or pulmonary disease excluding asthma (see appendix G, Table 18) Of the 68 screened athletes, 18 were excluded, because they did not meet the VO_{2max} requirements. Another 8 athletes met all inclusion criteria but were unable to complete the study due to injuries or a lack of time. Among the remaining 42 athletes, who completed the entire study, there were 21 cyclists and 23 triathletes. Due to technical issues with the pneumotach mean oxygen consumption, minute ventilation and tidal volume could not be assessed in all 42 athletes. Therefore, these parameters are reported for fewer subjects in the results section.

Subjects were recruited through advertisements at race venues and online posts in classified sections of websites frequently visited by cyclists and triathletes. For each of the three visits, subjects were reimbursed $50. Prior to subject recruitment, ethics approval was received from the University of British Columbia Clinical Research Ethics Board and written informed consent was obtained from all subjects.

2.3.2 Experimental design

A randomized, double-blind study design with placebo-controlled repeated measures was used for this experiment. Data collection took place at the Environmental Physiology Laboratory of the University of British Columbia, Vancouver, Canada. Each athlete visited the laboratory on three

different occasions. The initial visit served as a general screening appointment, whereas appointments 2 and 3 were the test days with two randomly assigned treatments: the inhalation of 400 µg salbutamol and the inhalation of a placebo being pressurized air.

Prior to all visits, asthmatic subjects on medications were asked to withhold from short and long acting β_2-agonists for at least 12 hours. Continuing use of other asthma treatment such as inhaled corticosteroids was permitted but recorded. All subjects were instructed to refrain from alcoholic and caffeinated drinks 12 hours prior to testing and to be hydrated on test days. To avoid fatigue and an exercise-induced bronchodilating effect prior to testing, subjects were asked not to exercise strenuously on days prior to testing and to completely avoid major physical activity on test days.

2.3.2.1 Appointment I: Medical screening

The first appointment included anthropometric measurements, pulmonary function screening and the assessment of VO_{2max} on a cycle ergometer. A eucapnic voluntary hyperpnea (EVH) test was performed to assess bronchial hyperresponsiveness and to classify athletes with exercise-induced bronchial hyperresponsiveness (EIB). A maximal exercise (VO_{2max}) test was performed to assure adequate fitness levels and to increase the likelihood of highly repeatable time trials necessary to answer the research questions. Additionally, appointment I served as a familiarization day with the laboratory environment and the equipment used on actual test days.

2.3.2.2 Appointments II & III: Testing

On test days II and III, subjects completed a 10-km time trial following the inhalation of a single dose of either 400 µg salbutamol or placebo. The order of the two treatments was randomly assigned in a double-blind fashion. The salbutamol dosage equaled twice the therapeutic dosage of salbutamol and was chosen to allow comparisons with results of other studies. A metered dose

inhaler (MDI) was connected to a spacer to administer all treatments. Subjects were asked to perform two FEV_1 tests prior to and 30-min after the inhalation to allow for comparisons in lung function after the salbutamol and the placebo treatments. After a 20-min self-selected warm-up, the time trial was started (see Figure 1). The proposed timing was chosen to allow comparisons of performance-parameters[65, 66] as well as comparisons to previously conducted studies of bronchial responsiveness to IBA treatment based on *ADRB2* polymorphisms.[88, 91, 104]

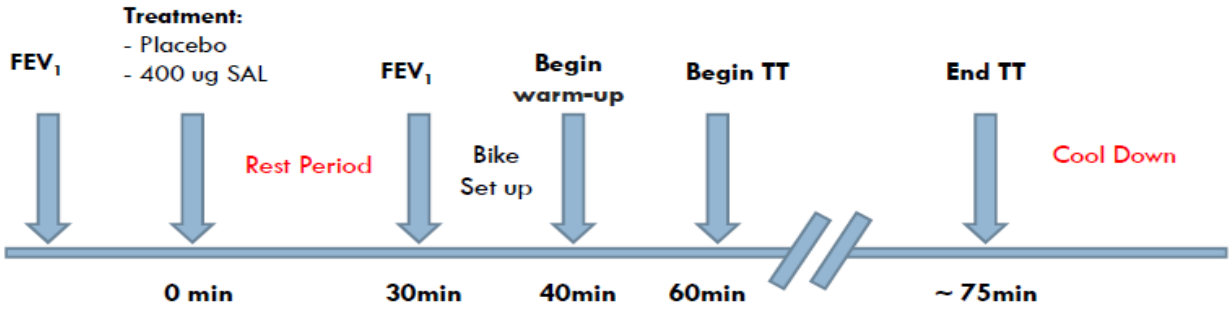

Figure 1: Timeline for Appointments II & III.

Modified after Sporer *et al.* [65]
Double-blind, repeated-measures design with two randomly assigned treatment protocols (placebo and 400 ug SAL)
Abbreviations: SAL = salbutamol; TT = time trial

2.3.3 Procedures on appointment I: Screening

2.3.3.1 Eucapnic voluntary hyperpnea (EVH) test

A eucapnic voluntary hyperpnea (EVH) test was performed to screen for EIB.[65] Firstly, subjects were asked to perform baseline spirometry.[107] Three FEV_1 measurements were taken in a standing position with a nose-clip on. The highest value served as a baseline value to calculate the percent fall index. Secondly, subjects breathed dry air with added CO_2 (5 %) at a high frequency that equaled a target minute ventilation of 30 times their baseline FEV_1 over 6-min.[11] Deep inhalations (hyperpnea) were required in order to provoke EIB.[108, 109] Following the hyperventilation phase,

subjects repeated spirometry twice each at 3-min, 5-min, 15-min and 20-min. A percent fall index greater than 10 % for two consecutive measurements post-hyperventilation were considered a positive EVH test and classified athletes with EIB (EIB+).

2.3.3.2 Ramped maximal exercise test

Following the EVH test, a graded exercise test on a cycling ergometer (Velotron Dynafit Pro, RacerMate Inc., Seattle, WA, USA) with a ramp-protocol was performed to measure VO_{2max}. A true plateau of oxygen consumption with increasing workload was not reached in all athletes; therefore $VO_{2\ peak}$ was measured in a maximal exercise test to confirm our inclusion criteria. A test was considered maximal if one of the following four conditions was reached: the subjects' heart rate exceeded 90 % of the age-predicted maximal heart rate, the respiratory exchange ratio (RER) was greater than 1.15, VO_2 reached a plateau with an increase in workload, or volitional exhaustion was reached. Subjects conducted a self-selected warm-up on the cycle ergometer. The test protocol began at 0 Watts, and resistance was continuously increased by 1 Watt every 2 seconds. Following test termination, subjects conducted a self-chosen cool-down. Athletes who tested positive on the EVH test inhaled 200 µg of salbutamol prior to the maximal exercise test. Lung function parameters were assessed 15-min, and if necessary 30-min post treatment to assure that FEV_1 and FVC (forced vital capacity) were back to baseline.

2.3.4 Procedures on appointments II-III: Testing

2.3.4.1 Forced expiratory volume in one second (FEV_1)

For baseline spirometry, subjects were asked to wear a nose clip. After 2 - 3 regular breaths, subjects breathed in maximally and then exhaled forcefully. The goal was to breathe as much air out in one second as possible. A spirometer was used to measure FEV_1 and FVC. Lung function

was assessed shortly before the inhalation of salbutamol and placebo, and 30-min after the drug treatment. The degree of bronchodilation due to each treatment was assessed by calculating percent change in FEV_1 pre- and post-drug treatment.

Bronchodilatory treatment response to salbutamol based on genetic variation at the *ADRB2* A46G and C79G SNPs was calculated by deducting percent change in FEV_1 after the inhalation of placebo from the percent change in FEV_1 after the inhalation of salbutamol.

2.3.4.2 Time trial

Exercise on test days II and III consisted of simulated cycling 10-km time trials. Following two FEV_1 tests 30-min post treatment, subjects were allowed a 20-min self-selected warm up. Athletes wore a heart rate monitor. Additionally, an elastic headset that included a facemask (Hans Rudolph, Oro-Nasal 7450 V2 Mask, Shawnee, KS, USA.) and a 2700 2-way T-shape non-rebreathing valve (Hans Rudolph), was attached to the metabolic cart (Parvo Medics, Sandy, UT, USA) via a hose and adjusted to the subjects' heads. Athletes were facing a computer screen with an uploaded 3D time trial course (RacerMate Interactive 3D software, Seattle WA, USA). The course was programmed to be all flat and straight. Besides distance (m), athletes were able to see their real time cadence (RPMs), gear and gearing ratio. Every two kilometers, athletes were asked for their rating of perceived exertion for legs (RPEL) and breathing (RPEB). Throughout the time trials, subjects were able to change gears. Except for the start, athletes were asked to remain seated during the time trial. The main outcome variable was mean power relative to body weight ($power_m$). Secondary variables measured during the time trials are listed in Table 3.

Table 3: Primary and secondary variables measured during time trial.

Variable (Abbreviation)	Units & SR	Variable (Abbreviation)	Units & SR
Mean power output ($Power_m$)	$W \bullet kg^{-1}$	Mean tidal volume (V_{Tm})	$L \bullet kg^{-1}$
Mean heart rate (HR_m)	$Beats \bullet min^{-1}$	Mean respiratory rate (RR_m)	$Breaths \bullet min^{-1}$
Mean oxygen consumption (VO_{2m})	$mL \bullet kg^{-1} \bullet min^{-1}$, SR: every 20s.	Rate of perceived exertion legs (RPEL)/ breathing (RPEB)	SR: every 2-km on a 10-point Borg scale [110]
Mean minute-ventilation (V_m)	$L \bullet min^{-1} \bullet kg^{-1}$		

Abbreviations: SR: Sampling rate; TT: Time trial; O_2: Oxygen; CO_2: Carbon dioxide

2.3.5 DNA extraction and genotyping

The A49G (rs1042713) and the G79C (rs1042714) SNPs of the *ADRB2* gene were genotyped for each athlete using a standard technique. Buccal swabs were obtained from each athlete on the second appointment using an endocervical sampling cytobrush (CooperSurgical Inc., Trumbull, CT, USA). Brushes were stored for drying in envelopes and stored at – 4° C until DNA extraction. The DNA was purified from buccal cells collected in saliva and subsequently amplified in a process known as polymerase chain reaction (PCR). Commercially available primers were used for the PCR procedure (see Table 4). Primer conditions were as follows: DNA (1 µl, unknown concentration) was amplified in a 25 µl reaction buffer containing 0.2mM dNTPs, 1.0 mM $MgCl_2$, 20 mM tris/Cl pH 8.4, 50 mM KCl, 0.033nmoles of each primer, and 0.625 units Taq polymerase (Invitrogen Corporation, Carlsbad, CA, USA) for 40 cycles of 1-min at 94° C, 1-min at 54° - 58°C

(depending on primers; see Table 4), and 1-min at 72° C. The 40 cycles were followed by a 5-min incubation at 72° C to allow DNA synthesis to be competed.

Amplified DNA was then digested with diagnostic restriction endonuclease enzymes. Gel electrophoresis, which involves the migration of negatively-charged DNA fragments through a polyacrylamide gel, was used to sort the fragments according to size. Upon staining with SYBR safe (Invitrogen Corporation, Carlsbad, CA, USA), the pattern of DNA fragments on the gel indicated the *ADRB2* genotype of each subject for one polymorphism. The genotyping of the *ADRB2* gene was performed at the GRIP Laboratory of the University of British Columbia, Vancouver, Canada.

Table 4: *ADRB2* SNPs, primers and genotype assays.

ADRB2* SNP	**Primers****	**Assay*****
A46G	5'CCT TCT TGC TGG CAC CC**C** AT 3'	A allele: 135 bp
($Arg^{16}Gly$)	5'CCAGCA CAT TGC CAA ACA CG 3'	G allele: 117bp; 18bp (*Nco*I; 56°C)
C79G	5'CCT TCT TGC TGG CAC CC**C** AT 3'	C allele: 181 bp; 55 bp; 6 pb
($Glu^{27}Gln$)	5'CCAGCA CAT TGC CAA ACA CG 3'	G allele: 236bp; 6 bp (*Fnu*4HI; 37°C)

* SNP database (dbSNP) number, base pair change and sequence-region, amino acid change and region.
** Degenerate bases changes to generate diagnostic recognition sequences are in bold and underlined.
*** Cut and uncut fragment sizes in base pairs; diagnostic restriction enzyme and PCR annealing temperature are shown in parentheses.

2.3.6 Statistical analyses

The descriptive analysis of the performance variables included mean and standard deviation. For the lung function and performance parameters assessed on the screening day, minimum and maximum values were also reported. To test if the bronchodilator response to salbutamol had an effect on power output, a correlation was run between the maximal drop in FEV_1 on the EVH test

(Δ FEV_1) and the difference in percent change in power between the two time trials (Δ Power). The two values were calculated as follows:

$$\Delta\ FEV_1 = FEV_{1\text{pre-hyperventilation}} - FEV_{1\ \text{post-hyperventilation}}$$

$$\Delta\ \text{Power} = (\text{Power}_{\text{salbutamol}} - \text{Power}_{\text{placebo}})\cdot(\text{Power}_{\text{placebo}})^{-1}$$

Mixed between-within subjects analysis of variance (ANOVA) tests were used to determine statistical significance between genotypes at the *ADRB2* gene and lung function status, respectively in regards to all dependent variables. A Shapiro-Wilks W test for normality and Levene's test for equal variances were used to confirm that the assumptions for ANOVA were met. If a main effect was found, post-hoc analyses were performed, using the Tukey's HSD test for significance.

The effect of the genotypes at the *ADRB2* A46G and the C79G SNPs on performance was investigated. Both SNPs were analyzed twice: once for all three possible genotypes of each SNP (additive model) and once for two groups, there the homozygous variant with the lower prevalence was combined with the heterozygote variant to increase the sample size and thus statistical power. For the A46G SNP the AA and the AG genotypes were combined and then compared to the GG genotype. For the C79G SNP, the CC genotype was combined with the GC genotype and combined to the GG genotype. Due to a low sample size number, haplotype analyses were not performed. Hardy-Weinberg equilibrium was tested with the Fisher's exact test.

For all tests, the significance level was set at 0.05. Nearly significant results were further described by their partial eta squared (ηp^2) indicating the effect size of the non-significant finding. According to Cohen[111] a ηp^2 = 0.01 equals a small effect, a ηp^2 = 0.06 equals a moderate effect, and a ηp^2 = 0.14 equals a large effect.

2.4 Results

2.4.1 Subject characteristics and airway hyperresponsiveness

Based on a positive EVH test, 10 athletes were diagnosed with EIB and 32 athletes did not have EIB. The 6-min hyperventilation of dry air induced a significantly greater decrease in FEV_1 in EIB+ athletes (*M* = 8.4 %, *SD* = 3.5 %) compared to EIB- athletes (*M* = 18.9 %, *SD* = 11.1 %; *p* = 0.015). Additionally, baseline FEV_1 in EIB+ athletes (*M* = 4.67 L; *SD* = 0.65 L) was significantly lower than in EIB- athletes (*M* = 5.40 L, *SD* = 0.75 L; *p* = 0.008).

Table 5: Anthropometric and lung function parameters in EIB+ and EIB- athletes.

Statistic	Age (yr)	Height (cm)	Weight (kg)	Cyc. Exp. (yr)	FVC (L)	Perc. pred. (%)	FEV_1 (L)	Perc. pred. (%)	FEV_1/ FVC (%)	Perc. pred. (%)
EIB - (*n* = 32)										
Mean	**29**	**183**	**76.8**	**6**	**6.74**	**115.7**	**5.40***	**114.1**	**80.2**	**97.4**
SD	5	7	18.0	6	0.90	10.5	0.75	11.7	4.8	5.7
Max	39	198	105.0	17	8.54	137.8	6.90	138.6	89.7	109.0
Min	19	165	61.0	2	5.20	93.06	3.94	92.0	69.7	86.0
EIB + (*n* = 10)										
Mean	**27**	**182**	**73.2**	**7**	**6.20**	**106.5**	**4.67***	**101.0**	**75.9**	**91.7**
SD	6	6	8.3	2	0.96	17.6	0.65	11.2	9.4	12.1
Max	40	196	85.0	25	8.52	131.2	6.04	115.6	86.9	105.0
Min	19	172	63.4	2	5.27	78.16	3.88	80.76	61.4	73.0

Abbreviations: Cyc.Exp: cycling experience; Perc.Pred.: percent predicted; FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; FEV_1/FVC: fraction of FVC expired in 1s; Δ Max FEV_1: decrease in FEV_1 to a eucapnic voluntary hyperpnea test.

*statistically significant: baseline FEV_1 was greater in EIB- athletes compared to EIB+ athletes; *p* = 0.015.

None of the anthropometric parameters was significantly different between EBI+ and EIB- athletes. Despite not reaching statistical significance, EIB+ athletes were on average 5 % lighter than EIB-athletes while being of similar height (see Table 5). The maximal drop in FEV_1 after the 6-min hyperventilation period of the EVH test was 8.4 % (SD = 3.5 %) in EIB- athletes compared to 18.9 % (SD = 11.1 %) in EIB+ athletes.

Susceptibility to EIB did not affect relative VO_{2max} or the absolute maximal power output achieved on the maximal exercise test (see Table 6); however, absolute VO_{2max} was significantly lower in EIB+ athletes compared to EIB- athletes, $p = 0.017$.

Table 6: Maximal oxygen consumption and power output in EIB+ and EIB- athletes.

Statistic	**VO_{2max} ($mL \cdot kg^{-1} \cdot ml^{-1}$)**	**$VO_{2\ max}$ ($L \cdot min^{-1}$)**	**Max RQ**	**Max HR ($b \cdot min^{-1}$)**	**Max Power (W)**	**Max Power ($W \cdot kg^{-1}$)**
EIB - (*n* = 32)						
Mean	**65.9**	**5.0***	**1.21**	**182**	**438**	**5.6**
SD	7.2	0.5	0.06	8	40	0.7
EIB + (*n* = 10)						
Mean	**65.1**	**4.2**	**1.22**	**185**	**426**	**5.9**
SD	5.1	1.5	0.06	11	18	0.5

Abbreviations: VO_{2max}: maximal oxygen consumption; Max RQ: maximal respiratory quotient; Max HR: maximal heart rate; EVH: eucapnic voluntary hyperpnea test; SD: standard deviation.
* statistically significant: EIB- athletes had a greater absolute VO_{2max} compared to EIB+ athletes; $p = 0.017$.

2.4.2 The effect of salbutamol on lung function and athletic performance

As shown in Figure 2, change in FEV_1 measured in an unselected group of competitive cyclists 30-min after the inhalation of salbutamol (M = 6.6 %, SD = 6.3 %) was significantly greater than the change in FEV_1 after the inhalation of placebo (M = 1.1 %, SD = 3.0 %), $p < 0.001$.

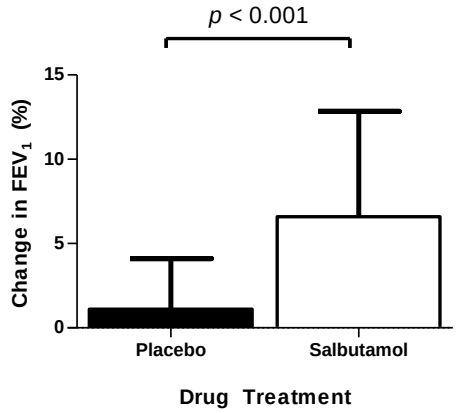

Figure 2: Change in FEV_1 30-min post drug treatment.

* statistically significant: FEV_1 was greater after the inhalation of salbutamol compared to placebo in all athletes; $p < 0.001$.

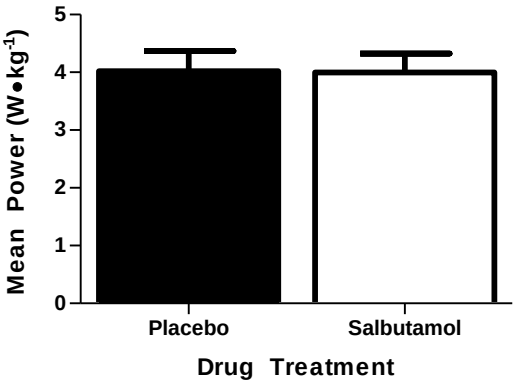

Figure 3: Power output during a 10-km time trial.

Performances, assessed by mean power output relative to body weight, were not different after the inhalation of salbutamol (M = 4.0 W•kg^{-1}, SD = 0.3 W•kg^{-1}) compared to the inhalation of placebo (M = 4.0 W•kg^{-1}, SD = 0.4 W•kg^{-1}; see Figure 3). None of the additionally assessed cardiovascular parameters describing performance was altered by the drug treatment (Table 7).

There was no statistical difference in the athletes' perception of exertion for legs (RPEL) and breathing (RPEB) was similar for the two time trials, despite the improvement in lung function after the inhalation of salbutamol (see Figures 4 and 5).

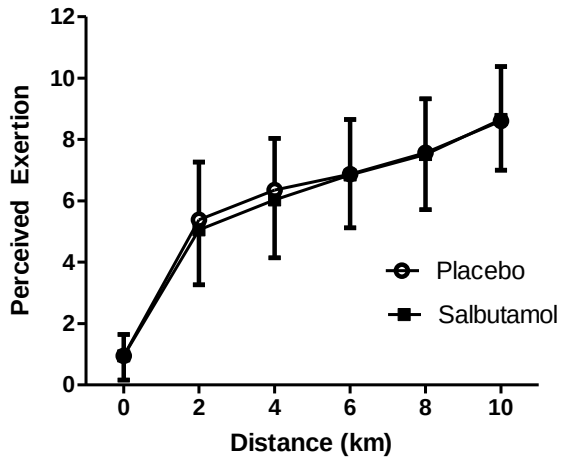

Figure 4: Rating of perceived exertion for legs.

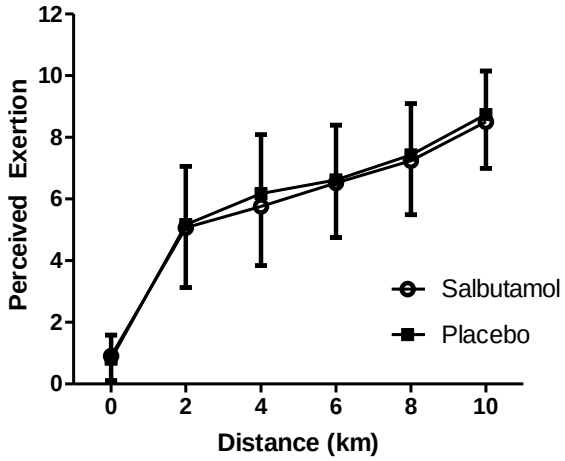

Figure 5: Rating of perceived exertion for breathing.

2.4.3 The effect of EIB-susceptibility on lung function and athletic performance after the inhalation of salbutamol

Both, EIB+ and EIB- athletes showed an improvement in FEV_1 after the exposure to salbutamol compared to placebo, $p < 0.001$. However, after the inhalation of salbutamol, lung function improved to a greater extent in EIB+ athletes (M = 10.9 % W, SD = 10.9 %) compared to EIB- athletes (M = 5.3 %, SD = 3.0 %; p = 0.009). Similarly, the exposure to placebo induced a greater change in FEV_1 in EIB+ athletes (M = 2.0 %, SD = 2.5 %) compared to EIB- athletes (M = 0.8 %, SD = 3.1 %; see Figure 6).

There were no interaction or main effects for drug treatment and susceptibility to EIB in regards to power output (see Figure 7). Furthermore, the difference in mean power produced during the two time trials was not influenced by the maximal drop in FEV_1 on the EVH test (p = 0.54). Therefore, the degree of EIB assessed by the EVH test did not affect the change in mean power output between the time trials.

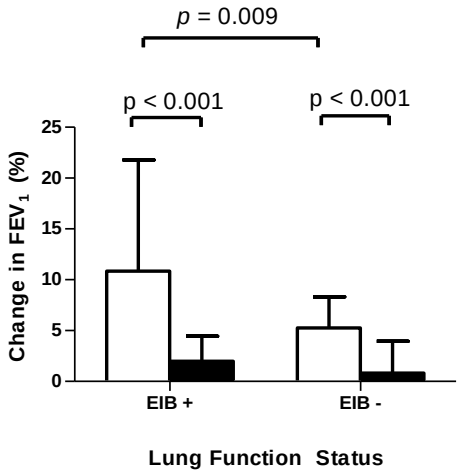

Figure 6: Change in FEV_1 in EIB+ athletes and EIB- athletes.

* Compared to EIB- athletes, EIB+ athletes presented with a significantly greater change in FEV_1 after the inhalation of salbutamol and placebo; $p < 0.001$.

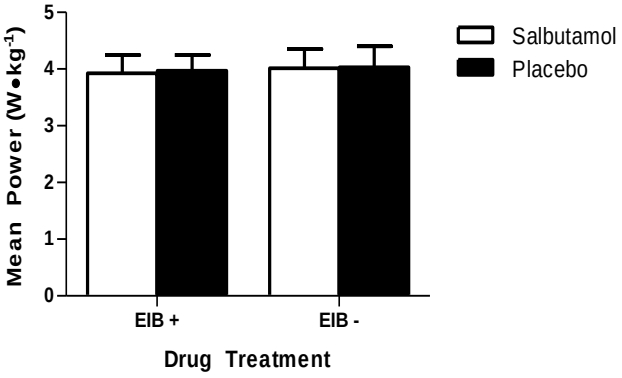

Figure 7: Mean power output in EIB+ athletes and EIB- athletes.

There was no significant interaction effect for susceptibility to EIB and cardiovascular parameters assessed to describe performance. After the exposure to salbutamol and placebo, mean ventilation was lower in EIB+ athletes compared to EIB- athletes (p = 0.005). Mean and standard deviation of each parameter describing cardiovascular function during the two time trials is summarized for EIB+ and EIB- athletes in Table 7.

Table 7: Performance parameters during 10-km time trials in EIB+ and EIB- athletes.

Parameter	**EIB**	***n***	**Salbutamol**		**Placebo**	
(units)	**susceptibility**		**Mean**	**(SD)**	**Mean**	**(SD)**
Power	**Total**	**42**	**4.0**	**(0.3)**	**4.0**	**(0.4)**
($W \cdot kg^{-1}$)	EIB +	10	3.9	(0.3)	4.0	(0.3)
	EIB -	32	4.0	(0.3)	4.0	(0.4)
Oxygen Consumption	**Total**	**39**	**56.1**	**(6.6)**	**56.7**	**(6.6)**
($L \bullet kg^{-1} \bullet min^{-1}$)	EIB +	9	52.7	(4.1)	53.0	(5.6)
	EIB -	30	57.1	(6.9)	56.5	(6.9)
Heart Rate	**Total**	**41**	**166**	**(12)**	**168**	**(10)**
($b \bullet min^{-1}$)	EIB +	10	162	(18)	166	(14)
	EIB -	31	167	(9)	168	(9)
Ventilation	**Total**	**37**	**1.59**	**(0.66)**	**1.57**	**(0.66)**
($L \bullet min^{-1} \bullet kg^{-1}$)	EIB +	9	1.46*	(0.54)	1.40*	(0.51)
	EIB -	28	1.64	(0.69)	1.62	(0.69)
Respiratory Rate	**Total**	**42**	**42**	**(8)**	**42**	**(7)**
($b \bullet min^{-1}$)	EIB +	10	40	(6)	39	(5)
	EIB -	32	43	(8)	43	(7)
Tidal Volume	**Total**	**37**	**0.043**	**(0.007)**	**0.042**	**(0.006)**
($L \bullet kg^{-1}$)	EIB +	9	0.042	(0.007)	0.040	(0.007)
	EIB -	30	0.043	(0.007)	0.042	(0.006)

* statistically significant: EIB+ athletes had an increased minute ventilation after the inhalation of salbutamol and placebo compared to EIB- athletes; $p = 0.005$.

The exertions for breathing and the lower limbs were not perceived differently in EIB+ athletes compared to EIB- athletes after either treatment (see Figures 8-11). At each assessment point, the ratings of perceived exertion for legs (RPEL) and breathing (RPEB) were at similar levels of the Borg scale, indicating that there were no differences in central and peripheral exertion over the duration of the 10-km time trials.

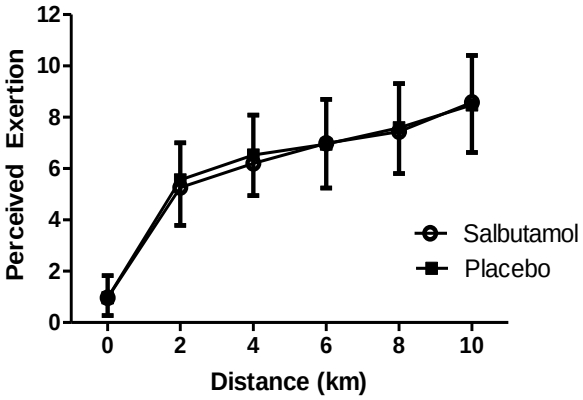

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Figure 8: Perceived exertion for legs in EIB- athletes.

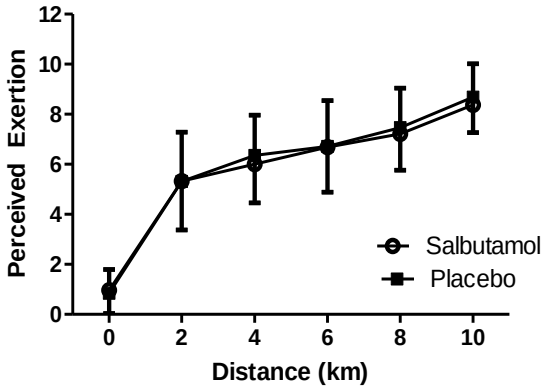

Figure 9: Perceived exertion for breathing in EIB- athletes.

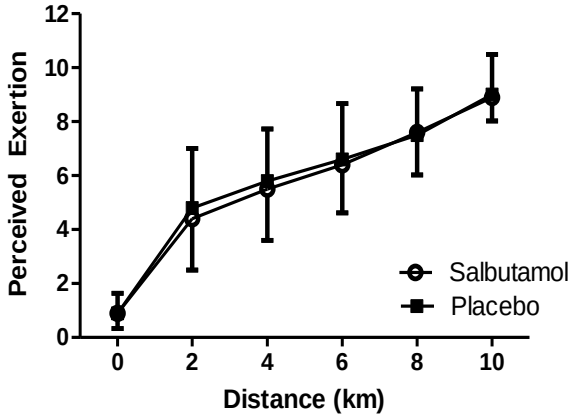

Figure 10: Perceived exertion for legs in EIB+ athletes.

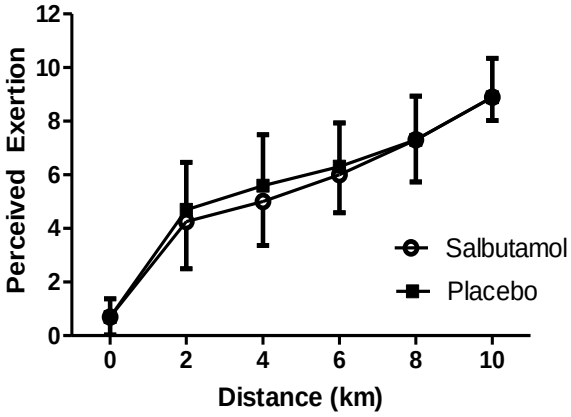

Figure 11: Perceived exertion for breathing in EIB+ athletes.

2.4.4 The effect of genetic variation at the *ADRB2* gene on lung function and athletic performance

2.4.4.1 The A46G single nucleotide polymorphism

The A46G SNP was genotyped in 40 athletes, with the GG genotype being the most common and the AA genotype being the rarest (AA = 4; AG = 15; GG = 21; unidentified = 1). Hardy-Weinberg equilibrium was met (p = 0.59). The genotype distribution did not differ between EIB+ athletes (AA = 0; AG = 3, GG = 6, unidentified = 1) and EIB- athletes (AA = 4; AG = 12; GG = 15, unidentified = 1; p = 0.75). Anthropometric parameters (height and weight), lung function parameters (baseline FEV_1, baseline FVC, percent drop in FEV_1 post EVH test), and performance parameters assessed on the maximal exercise test (VO_{2max} and maximal power output) were not affected by the genetic variation at the A46G SNP (see appendix A, Tables 12 and 13).

Lung function and time trial performance

There was no interaction effect for drug treatment and genetic variation at the A46G SNP of the *ADRB2* gene in regards to lung function (see Table 8).

Table 8: Percent change in FEV_1 based on genetic variation at the *ADRB2* A46G SNP.

Genotype	*n*	Salbutamol		Placebo	
		Mean	(SD)	Mean	(SD)
Total	**40**	**6.44**	**(6.05)**	**1.10**	**(3.07)**
AA	4	4.70	(2.37)	2.61	(3.91)
AG	15	7.00	(8.69)	1.28	(2.12)
GG	21	6.37	(4.12)	0.69	(3.50)

The change in FEV_1 between the inhalations of salbutamol and placebo were not statistically different between the three genotypes (Figure 12). Despite not reaching statistical significance, the AA genotype showed the smallest response to salbutamol (Δ FEV_1 = 7.14 %), compared to similar responses in the AG (Δ FEV_1 = 5.72 %) and GG genotypes (Δ FEV_1 = 5.68 %).

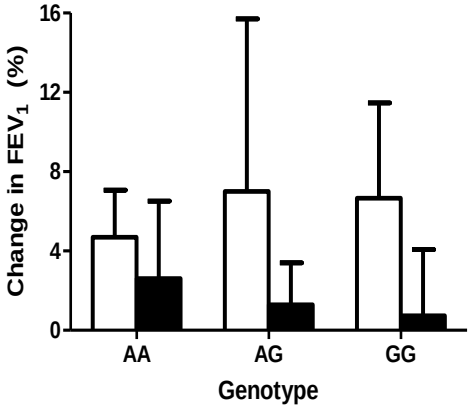

Figure 12: Change in FEV_1 based on genetic variation at the *ADRB2* A46G SNP.

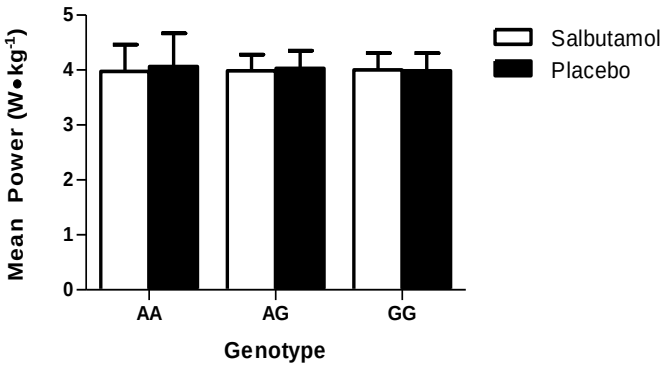

Figure 13: Mean power output based on genetic variation at the *ADRB2* A46G SNP.

Performance during the two time trials was not affected by the genotypes of the A46G SNP. No interaction and no main effects were found for power and the additionally assessed cardiovascular parameters (see Table 9).

Table 9: Athletic performance based on genetic variation at the *ADRB2* A46G SNP.

Parameter	**Genotypes**	***n***	**Salbutamol**		**Placebo**	
(units)			**Mean**	**(SD)**	**Mean**	**(SD)**
Power	**Total**	**40**	**4.0**	**(0.3)**	**4.0**	**(0.4)**
($W \cdot kg^{-1}$)	AA	4	4.0	(0.5)	4.1	(0.6)
	AG	15	4.0	(0.3)	4.1	(0.3)
	GG	21	4.0	(0.3)	4.0	(0.3)
Oxygen	**Total**	**38**	**56.0**	**(6.7)**	**55.6**	**(6.6)**
Consumption	AA	4	57.0	(12.3)	57.1	(11.8)
($L \bullet kg^{-1} \bullet min^{-1}$)	AG	14	56.3	(5.1)	57.2	(5.2)
	GG	20	55.5	(6.6)	54.2	(6.4)
Heart Rate	**Total**	**39**	**166**	**(11)**	**170**	**(6)**
($b \bullet min^{-1}$)	AA	3	168	(3)	170	(6)
	AG	15	166	(8)	169	(8)
	GG	21	165	(14)	166	(12)
Ventilation	**Total**	**36**	**1.67**	**(0.57)**	**1.65**	**(0.57)**
($L \bullet min^{-1} \bullet kg^{-1}$)	AA	3	1.52	(0.06)	1.54	(0.12)
	AG	13	1.87	(0.31)	1.87	(0.32)
	GG	20	1.82	(0.31)	1.76	(0.34)
Respiratory rate	**Total**	**40**	**42**	**(8)**	**42**	**(7)**
($b \bullet min^{-1}$)	AA	4	42	(13)	42	(13)
	AG	15	42	(8)	43	(4)
	GG	21	43	(7)	42	(7)
Tidal volume	**Total**	**37**	**0.043**	**(0.007)**	**0.041**	**(0.006)**
($L \bullet kg^{-1}$)	AA	3	0.043	(0.006)	0.043	(0.006)
	AG	14	0.041	(0.008)	0.041	(0.008)
	GG	20	0.043	(0.006)	0.042	(0.005)

Effect of genetic variation at the *ADRB2* A46G SNP, with the AA and AG genotypes combined, on lung function and athletic performance

When analyzing the effect of the genotypes of the A46G SNP with the AA and the AG genotypes combined (n = 19) versus the GG genotype (n = 21), neither change in lung function nor mean power output differed between the two groups after either treatment (see Tables 14 and 15 in the appendix B); however, there was a nearly significant interaction effect for genetic variation and drug treatment on VO_2 with a moderate to large effect size (p = 0.053, ηp^2 = 0.1). Carriers of the A-allele showed a decrease in mean VO_2 after the exposure to salbutamol (M = 56.5 mL·kg^{1}·min^{1}, SD = 6.9 mL·kg^{-1}·min^{-1}) compared to placebo (M = 57.1 mL·kg^{-1}·min^{-1}, SD = 6.7 mL·kg^{-1}·min^{1}). In contrast, athletes with the GG genotype showed an increase in mean VO_2 after the exposure to salbutamol (M = 55.5 mL·kg^{-1}·min^{1}, SD = 6.6 mL·kg^{-1}·min^{-1}) compared to placebo (M = 54.1 mL·kg^{1}·min^{1}, SD = 6.4 mL·kg^{-1}·min^{-1}).

2.4.4.2 The C79G single nucleotide polymorphism

The C79G SNP was identified in 40 athletes (GG = 14; GC = 19; GG = 7, unidentified = 2). The distribution of the polymorphisms was in Hardy-Weinberg equilibrium (p = 0.90) and did not differ between EIB+ athletes (GG = 2; GC = 5; CC = 2, unidentified = 1) and EIB- athletes (GG = 12; GC = 14; CC = 5; unidentified = 1; p = 0.68). Similarly to the A46G SNP, anthropometric parameters (height and weight), lung function parameters (baseline FEV_1, baseline FVC, percent drop in FEV_1 post EVH test), and performance parameters assessed on the maximal exercise test (VO_{2max} and maximal power) were not affected by the genetic variation at the C79G SNP (see appendix A, Tables 12 and 13).

Lung function and time trial performance

The significant increase in FEV_1 after the exposure to salbutamol did not depend on the genetic variation at the C79G SNP (Table 10). Despite not reaching statistical significance, the GC genotype showed the greatest response to salbutamol (Δ FEV_1 = 7.14 %), followed by the CC (4.74 %) and GG genotypes (3.23 %, see Figure 14)

Table 10: Percent change in FEV_1 based on genetic variation at the *ADRB2* C79G SNP.

Genotype	***n***	**Salbutamol**		**Placebo**	
		Mean	**(SD)**	**Mean**	**(SD)**
Total	**40**	**6.43**	**(6.07)**	**1.07**	**(3.07)**
GG	14	4.70	(2.51)	1.46	(2.96)
GC	19	7.88	(8.23)	0.74	(3.51)
CC	7	5.94	(2.31)	1.20	(2.12)

Mean power output between the two time trials was not affected by the genotypes of the C79G SNP (see Figure 15). The interaction between drug treatment and genetic variance at the C79G SNP nearly reached statistical significance for VO_2 with a large effect size (p = 0.058, ηp^2 = 0.15). Athletes with the GG and GC genotypes did not show a meaningful difference in VO_2 after the two exposures (ΔVO_2 = - 0.2 % and - 0.4 %, respectively), while athletes with the CC genotype sustained a greater VO_2 after the exposure to salbutamol (ΔVO_2 = 2.92 %).

Table 11: Athletic performance based on genetic variation at the *ADRB2* C79G SNP.

Parameter	**Genotype**	***n***	**Salbutamol**		**Placebo**	
(units)			**Mean**	**(SD)**	**Mean**	**(SD)**
Power	**Total**	**40**	**4.0**	**(0.3)**	**4.0**	**(0.4)**
($W \bullet kg^{-1}$)	GG	14	4.1	(0.4)	4.2	(0.4)
	GC	19	3.9	(0.3)	3.9	(0.3)
	CC	7	4.0	(0.2)	3.9	(0.2)
Oxygen Consumption	**Total**	**37**	**56.1**	**(6.7)**	**55.8**	**(6.6)**
($L \bullet kg^{-1} \bullet min^{-1}$)	GG	12	58.5	(8.5)	58.7	(7.4)
	GC	18	54.6	(5.1)	55.5	(5.7)
	CC	7	55.9	(6.7)	53.0	(6.0)
Heart Rate	**Total**	**39**	**165**	**(11)**	**167**	**(10)**
($b \bullet min^{-1}$)	GG	13	166	(11)	168	(10)
	GC	19	165	(11)	166	(11)
	CC	7	162	(15)	169	(8)
Ventilation	**Total**	**35**	**1.79**	**(0.32)**	**1.82**	**(0.31)**
($L \bullet min^{-1} \bullet kg^{-1}$)	GG	11	1.81	(0.34)	1.84	(0.29)
	GC	17	1.82	(0.27)	1.83	(0.32)
	CC	7	1.82*	(0.35)	1.64*	(0.38)
Respiratory Rate ($b \bullet min^{-1}$)	**Total**	**40**	**42**	**(8)**	**43**	**(7)**
	GG	14	43	(8)	43	(7)
	GC	19	42	(8)	43	(6)
	CC	7	45*	(8)	42	(9)
Tidal Volume	**Total**	**36**	**0.043**	**(0.007)**	**0.041**	**(0.006)**
($L \bullet kg^{-1}$)	GG	11	0.044	(0.005)	0.040	(0.013)
	GC	18	0.042	(0.007)	0.041	(0.006)
	CC	7	0.043	(0.007)	0.043	(0.008)

* statistically significant: athletes with the CC genotype presented with a significantly greater minute ventilation ($p = 0.03$) after the inhalation of salbutamol. The increase in minute ventilation ($p = 0.04$). and respiratory rate ($p = 0.045$) to salbutamol in athletes with the CC genotype was different from to athletes with the GC and GG genotypes.

Athletes with the CC genotype showed an increased mean ventilation (p = 0.003) and respiratory rate (p = 0.045) after the inhalation of salbutamol compared placebo. In contrast, ventilation and breathing frequencies remained unchanged in athletes with the GG and the GC genotypes (see Figures 16 and 17 and Table 11).

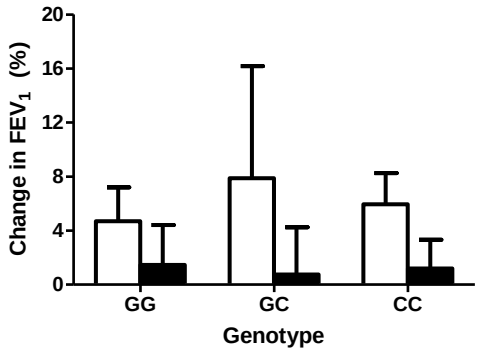

Figure 14: Change in FEV_1 based on genetic variation at the *ADRB2* C79G SNP.

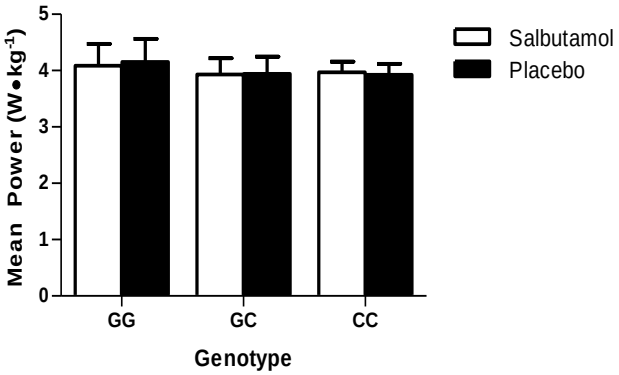

Figure 15: Mean power output based on genetic variation at the *ADRB2* C79G SNP.

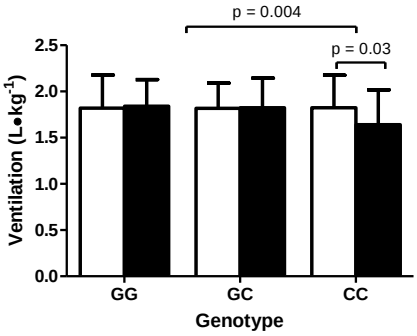

Figure 16: Mean minute ventilation based on genetic variation at the *ADRB2* C79G SNP.

*Athletes with the CC genotype had a significantly greater minute ventilation after the inhalation of salbutamol compared to placebo; p = 0.030. This reaction was significantly different from athletes with the GG and GC genotypes (p = 0.004).

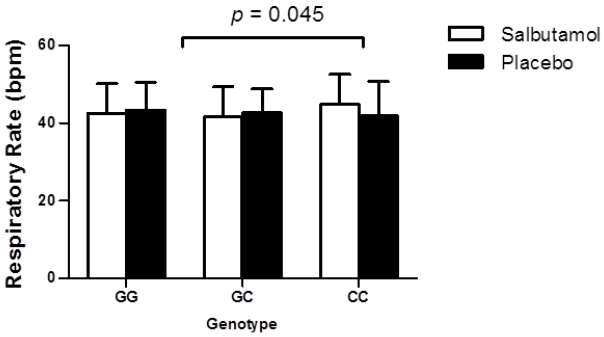

Figure 17: Mean respiratory rate based on genetic variation at the *ADRB2* C79G SNP.

*The increase in respiratory rate after the inhalation of salbutamol in athletes with the CC genotype was significantly different to the response to salbutamol in athletes with the GG and GC genotypes; p = 0.045.

The effect of genetic variation at the *ADRB2* C79G SNP, with the CC and GC polymorphisms combined, on lung function and athletic performance

When analyzing the effect of the genotypes of the C79G SNP with the CC and the GC genotypes combined (n = 26) versus the GG polymorphism (n = 14), change in lung function did not differ between the two groups after the exposures to salbutamol and placebo. No interaction or main effects were found for the additionally assessed cardiovascular parameters describing athletic performance (see appendix C, Tables 16 and 17).

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2.5 Discussion

The main purpose of this study was to assess the effect of IBA use on athletic performance based on genetic variation at the *ADRB2* gene. The structure of the discussion is divided into the three previously described levels: (1) the effect of inhaled salbutamol on lung function and athletic performance; (2) the effect of susceptibility to EIB on lung function and athletic performance after the inhalation of salbutamol; (3) the effect of genetic variation at the *ADRB2* A46G and the C79G SNPs on lung function and athletic performance after the inhalation of salbutamol.

2.5.1 Lung function and athletic performance after the inhalation of salbutamol

Our findings of an unchanged mean power output over the duration of a 10-km time trial, despite a significant change in FEV_1 after the inhalation of salbutamol, is in accordance with previous studies investigating the effect of IBA on athletic performance.[76, 112, 113] As suggested by Sue-Chu *et al.*[76], an exercise protocol with high intensity and short duration workloads was chosen to maximally challenge the athletes' respiratory capabilities. If the respiratory system was a performance-limiting factor, potential ergogenic effects of IBA due to bronchodilation could have reached its maximal effect. The 10-km time trials in this study lasted between 14- and 16-min, and the athletes' ratings of perceived exertion for breathing and legs indicate maximal efforts. Thus, despite an improved lung function after the inhalation of salbutamol, work of breathing was not perceived as being significantly easier compared to the placebo treatment. Additionally, none of the respiratory parameters (VO_2, minute ventilation, tidal volume and respiratory rate) measured during the time trials differed between the two treatments, suggesting that they were not affected by the IBA-induced bronchodilation responsible for the increase in FEV_1. Even though our exercise protocol was designed to maximally challenge the respiratory system, it did not seem to be the performance-limiting factor. This assumption is supported by the slope of the ratings of

perceived exertion for work of breathing and legs, which did not differ from each other between the two treatments.

The change in lung function after the inhalation of salbutamol is slightly greater in this study compared to previous studies investigating the change in FEV_1 after the exposure to similar dosages (360 – 400 µg) of inhaled salbutamol in non-asthmatic athletes.[70, 73, 112, 114] Changes in FEV_1 after IBA use between 2.6 % and 5.3 % have been reported. One explanation could be the inclusion of 10 EIB+ athletes in our study. All studies used for comparison investigated EIB- athletes only. In the present study, EIB+ athletes showed a greater increase in FEV_1 to salbutamol (10.9 %) compared to EIB- athletes (5.3 %). Meeuwisse *et al.*[70] reported an identical, also statistically significant, change in lung function (5.3 %) in their non-asthmatic study population, which was described as clinically non-relevant.[70] The American Thoracic Society (ATS) considers an increase in FEV_1 after the inhalation of a SABA of less than 8 % within measurement variability and defines a clinically relevant reversibility of bronchoconstriction as an increase in FEV_1 greater than 12 %.[115] The change in lung function in our EIB+ athletes nearly reached 12 %; however, even an improvement in lung function of 10.9 % did not induce an improvement in performance after IBA use. On the contrary, minute ventilation and tidal volume measured during the time trials were lower in EIB+ athletes compared to EIB- athletes after both treatments. This supports the previously discussed thought, that respiratory parameters of athletic performance are not influenced by an IBA-induced bronchodilation, even in EIB+ athletes.

2.5.2 Lung function and athletic performance after the inhalation of salbutamol in EIB+ and EIB- athletes

The prevalence of EIB+ athletes recruited for this study is comparable with other studies on summer sports.[13] Interestingly, two of the 10 EIB+ athletes were not aware of their airway hyperresponsiveness to exercise. It has been shown previously, that even Olympic athletes fail to associate their respiratory symptoms with EIB. Instead they believe their level of dyspnea to be a normal reaction to intense training.[26] Therefore, Dickinson *et al.*[26] suggested systematic screenings for EIB in elite athletes to prevent adverse health outcomes due to undiagnosed and untreated EIB while training at high intensities and volumes.

EIB+ athletes presented with lower absolute VO_{2max} compared to EIB- athletes. Since there are no differences in relative VO_{2max} achieved on the ramped exercise test, an explanation can be found in the comparatively lower body weight in the recruited group of EIB+ athletes. This also explains, why absolute power achieved on the maximal exercise test is 2.7 % lower in EIB+ athletes compared to EIB- athletes but slightly greater when presented relative to body weight.

Seven out of the 10 EIB+ athletes reported to treat their respiratory symptoms with ICS and IBA on an as needed regimen. For the past few years, the use of IBAs on an as needed basis has been recommended to decrease the risk of a β_2-repector downregulation and a reduction in the broncho-protective effect of IBA.[116] Even though FEV_1 in EIB+ athletes was not measured over a prolonged period of time, there was no evidence for a potential β_2-receptor downregulation in EIB+ athletes in the present study, since EIB+ athletes responded to salbutamol to a greater extent than EIB-athletes. An explanation for the greater response to salbutamol could be the level of inflammation, which has been reported to be responsible for the heterogeneity in the response to drug treatment in asthmatics.[117]

Despite the greater increase in FEV_1 after the exposure to salbutamol, EIB+ athletes did not show an improvement in performance due to IBA. Sandsund *et al.*[112] speculated that salbutamol may increase the ventilation-circulation imbalance which may counteract the positive effect of bronchodilation in athletes. This has been suggested in a previous study looking at gas exchange in patients suffering from severe chronic asthma after the exposure to 300 µg of inhaled salbutamol.[118] Our findings do not support this hypothesis, because oxygen uptake and minute ventilation did not differ between the two treatments in EIB+ and EIB- athletes. Further studies that include the assessment of arterial gases in EIB+ and EIB- athletes during exercise bouts performed after the exposure to salbutamol and placebo are necessary to further investigate this hypothesis.

Minute ventilation was reduced in EIB+ athletes compared to EIB- athletes after both treatments. This is interesting because EIB+ athletes rated their perceived exertion for breathing and legs slightly (but not significantly) lower at kilometers 2, 4 and 6 after the inhalation of salbutamol, which might be due to the IBA-induced bronchodilation. The decreased minute ventilation could be related to a decreased body surface area of EIB+ athletes, indicating a smaller lung.

2.5.3 Lung function and athletic performance after the inhalation of salbutamol based on genetic variation at the *ADRB2* A46G and C79G SNPs

2.5.3.1 Subject characteristics

Due to a small sample size of EIB+ athletes, conclusions on an increased prevalence of a certain polymorphic site of either of the investigated *ADRB2* SNPs is not possible. However, our data does show trends. Similar to previous studies, none of the genotypes of the A46G and the C79G SNPs in our project suggested a trend for an increased prevalence of EIB.[119-121] These findings are

in agreement with the results reported in one of two recent meta-analyses on several thousand individuals from different ethnic groups investigating asthma prevalence in dependence of genetic variation at the *ADRB2* gene.[122, 123] In the first meta-analysis, the G-allele of the A46G SNP was concluded not to be a risk factor for asthma susceptibility or bronchial hyperresponsiveness.[123] Additionally, the C79G SNP was not associated with any asthmatic phenotype. In a second meta-analysis, the GG genotype of the C79G SNP was protective against asthma, reducing the risk of asthma by approximately 27 %.[124] Also, in this second meta-analysis the A46G SNP was not associated with any of the investigated phenotypes of asthma, rather it was associated with the role of a modifier: the protective effect of the G-allele of the C79G SNP was accentuated with the A-allele of the A46G SNP compared with the G-allele.

2.5.3.2 Change in lung function after the inhalation of salbutamol

ADRB2 A49G SNP and bronchodilator response

A large inter-individual variation in the treatment response to asthma medications has been described.[1, 122, 125] By assessing its repeatability, Drazen *et al.*[117] attributed up to 60.6 % of the variation in the treatment response to salbutamol to genetics. The present study did not show any significant differences in the bronchodilator response to salbutamol between the investigated *ADRB2* SNPs. Individuals with the AA genotype of the A46G SNP showed the smallest bronchodilator response compared to similar responses in individuals with the AG and GG genotypes. This result partially supports findings by Israel *et al.*,[126] who report that patients with the AA genotype show the smallest treatment response to salbutamol. Israel *et al.*[126] matched asthmatic patients with the AA genotype to patients with the GG genotype by their level of FEV_1. In a double-blind cross-over study, patients of both groups were exposed to a regularly scheduled

salbutamol- (4 x inhalation of 180 µg) and placebo-therapy over 16 weeks. Individuals with the GG genotype of the A46G SNP benefited from salbutamol therapy. Patients with the AA genotype improved morning peak expiratory flow rate and FEV_1 only when salbutamol was withdrawn and replaced with ipratropium bromide, an anticholinergic drug used to treat asthma and chronic obstructive pulmonary disease (COPD).[126] Our findings and those by Israel *et al.* [126] conflict with the results of Lima *et al.*[127]. Lima *et al.*[127] reported a greater improvement in FEV_1 in moderate asthmatics with the AA genotype (18 %) of the A46G SNP compared to patients with the AG and GG genotypes (4.9 %) for up to 8 hours after the oral administration of 8 mg of salbutamol. The AA genotype was thought to be more responsive to IBA-induced bronchodilation than the GG or AG genotypes because they have been shown to undergo less agonist-promoted receptor downregulation.[96] Similar findings were demonstrated by Martinez *et al.,*[120] who studied the treatment response 15-min after the inhalation of salbutamol (180 µg) in asthmatic children. Asthmatics with the AA genotype showed a 5.3 times greater reversibility of bronchoconstriction to salbutamol than individuals with the GG genotype, while the AG genotype showed an intermediated treatment response to salbutamol.

ADRB2 C79G SNP and bronchodilator response

In the present study, genetic variation at the C79G SNP of the *ADRB2* gene did not influence the salbutamol-induced change in FEV_1. This is in accordance with the previously mentioned study by Martinez *et al.*[120] who did not find a significant effect on bronchodilator response in asthmatic children based on genetic variation at the C79G SNP; however, Hawkins *et al.*[86] found a significant association between the genotypes of the C79G SNP and bronchoconstriction reversibility in African-Americans. Tantisira *et al.*[1] concluded in their meta-analysis, that the treatment response to β_2-agonists appears to be influenced by genetic variation in the *ADRB2*

gene, but the precise relationships between genotype, haplotype and acute treatment response remain poorly understood.

2.5.3.3 Athletic performance after the inhalation of salbutamol

ADRB2 A49G SNP and athletic performance

Athletic performance did not vary significantly among the polymorphisms of the *ADRB2* A49G SNP after either treatment. This is contradictory to findings by Wolfarth *et al.*[8], who found a higher prevalence of AA-carriers in their elite endurance athlete group (17 %) compared to their sedentary control group (9 %). They concluded that elite endurance athletes with the AA genotype may benefit from the following two characteristics associated with the AA genotype at the A46G SNP: a lower body weight resulting in a superior weight-to-strength ratio, and an upregulation of the *ADRB2*-induced cardiovascular responses. In a study on the effect of the A46G SNP on changes in obesity from childhood through young adulthood, carriers of the G-allele showed a significantly greater increase in body mass index over the follow-up period compared to individuals with the AA genotype.[128] A different study on patients with heart failure showed, that patients with the A-allele presented with higher peak VO_2 values compared to patient carrying one or two G-alleles.[129]. This is in contrast to findings by Snyder *et al.*[130], who recruited 42 healthy adults to investigate the effect of the A46G SNP on airway function during exercise.[130] They did not find differences in VO_2 or any other measured lung function parameters based on genetic differences at the A46G SNP prior to and during exercise. Post-exercise, the airway tone in carriers of the AA genotype returned to baseline quicker than in carriers of the GG genotype, suggesting that individuals homozygous for the A-allele undergo an enhanced desensitization of the β_2-receptor. This was also suggested by Dishy *et al.*, [101] who found a smaller venodilation at

the dorsal hand vein after the infusion of isoproterol over 2 hours. In an additional study, Snyder *et al.*[131] found a decreased ratio of β_2-receptors per lymphocyte in individuals with the AA genotype compared to individuals with the GG genotype. Furthermore, a lower cardiac output due to a reduced stroke volume was recorded for participants with the AA genotype. In contrast to our findings, Snyder *et al.*[130, 131]and Dishy *et al.*[101] propose that the GG genotype of the *ADRB2* gene may be associated with a greater potential for endurance performance.

ADRB2 C79G SNP and athletic performance

Our data did not show a significant effect of genetic variation at the C79G SNP on athletic performance. Athletes with the CC genotype showed the greatest treatment responses to salbutamol with significant increases in minute ventilation and respiratory rate. This may suggest that the CC genotype at the C79G SNP favours aerobic endurance capacity after IBA use. These findings are in agreement with results from Moore *et al.*[132] who reported lower maximal oxygen consumption levels in Caucasian post-menopausal women with the GG genotype at the C79G SNP. Additionally, women with the GG genotype were found to have a higher body weight and BMI than women with the CC genotype. Thus, they concluded that the G-allele may dissociate from athletic endurance performance while the C-allele may be associated with aerobic endurance. This is in contrast with findings by Dishy *et al.*[101], who suggested that the haplotype homozygous for the GG genotypes at both, A46G and the C79G *ADRB2* SNPs should be used as markers for talent identification of athletes. Athletes with the GG genotypes at the A46G SNP and the GG genotype at the C79G SNP presented with optimal responses during short-term exercise. Increased receptor numbers, resistance to desensitization, enhanced stroke volume, and increased cardiac output were associated with this haplotype.[101]

2.6 Summary

The primary finding of this study was that athletic performance was not altered after the inhalation of salbutamol, regardless of susceptibility to EIB or genetic variation at the *ADRB2* A46G and the C79G SNPs. Despite a significant increase in lung function in EIB+ and in EIB- athletes after the exposure to salbutamol, mean power output of competitive cyclists remained unchanged over a 10-km time trial. However, EIB+ athletes had an improved minute ventilation after inhaling salbutamol, which may suggest a greater responsiveness of EIB+ athletes to salbutamol compared to EIB- athletes, possibly due to increased levels of inflammation markers in EIB+ athletes. Genetic variation at the *ADRB2* A46G SNP and the C79G SNP affected neither lung function improvement nor athletic performance after IBA use. Athletes with the CC genotype at the C79G SNP showed a greater responsiveness to salbutamol for minute ventilation and respiratory rate.

3 Conclusion

In the past 20 years, many studies tried to explain the overrepresentation of athletes using IBA in the medal counts of Olympic games by investigating the effect of IBAs on several physiological systems that are relevant for athletic performance.[9, 31, 66] None of the randomized, cross-over designed studies on highly trained, non-asthmatic, male athletes presented enough evidence to explain a potential ergogenic effect of IBA on athletic performance. Pharmacogenetic studies on the treatment response to inhaled salbutamol in asthmatics showed differences in lung function improvement based on genetic variation at the *ADRB2* gene.[86, 88, 91, 104] Additionally, the *ADRB2* gene has been linked to the regulation of obesity, blood pressure and musculoskeletal function.[101, 133, 134] The regulation of cardiovascular, pulmonary, metabolic and musculoskeletal processes makes the *ADRB2* gene a candidate gene of particular interest for the variation in endurance performance.[8, 105] Thus, it was the purpose of this study to investigate the effect of genetic variation at the *ADRB2* A46G SNP and the C79G SNP on athletic performance after the inhalation of salbutamol.

Forty-two competitive male cyclists (EIB+: n = 10; EIB-: n = 32), aged 19 – 40 years, performed two 10-km time trials: one after the inhalation of 400 µg salbutamol, one after the inhalation of a placebo. Lung function was significantly improved in EIB+ (10.9 %) and EIB- (5.3 %) athletes after the inhalation of salbutamol. Despite this salbutamol-induced bronchodilation athletic performance, assessed by mean power output relative to body weight, remained unaffected. Mean minute ventilation was lower in EIB+ athletes compared to EIB- athletes after both drug treatments. However, athletic performance was not affected by the athletes' susceptibility to EIB. The genetic variation at the *ADRB2* A46G and the C79G SNPs did not affect performance after the inhalation of salbutamol. Athletes with the CC genotype at the C79G SNP showed significant

increases in minute ventilation after the inhalation of salbutamol. The increase in mean minute ventilation and mean respiratory rate to inhaled salbutamol is significantly different to the responses to salbutamol in athletes with the GC and GG genotypes at the C79G SNP.

3.1 Conclusions regarding thesis hypotheses

3.1.1 The effect of IBA use on athletic performance

As hypothesized we did not find an improvement in athletic performance in an unselected group of competitive athletes after the inhalation of 400 µg salbutamol despite a significant increase in lung function. This is in accordance with previous studies that investigated the effect of IBA on athletic performance in competitive, non-asthmatic male athletes.[70, 71, 135]

3.1.2 The effect of susceptibility to EIB on athletic performance after IBA use

Since EIB+ athletes showed a greater treatment response to salbutamol in regards to change in lung function compared to EIB- athletes, we partially accept hypothesis 2. However, despite this greater increase in FEV_1 after IBA use, EIB+ athletes did not improve mean power output or other assessed cardiorespiratory parameters after the exposure to salbutamol.

3.1.3 The effect of genetic variation at the *ADRB2* gene on athletic performance after IBA use

We did not find statistically significant differences in mean power output over the 10-km time trials based on genetic variation at the A46G and the C79G SNPs. Athletes with the CC genotype at the C79G SNP presented with the greatest response to salbutamol with an increase in minute ventilation and respiratory rate.

3.2 Strengths, limitations and future directions

To our knowledge, this is the first study that investigated the effect of IBA in competitive EIB+ athletes and compared them to EIB- athletes. Furthermore, this is the first study analyzing the effect of genetic variation at the *ADRB2* A46G SNP and the C79G SNP on athletic performance after the inhalation of salbutamol. Only competitive male athletes with a high fitness level were included in this study to avoid potential improvements in performance due to insufficient fitness levels. Kindermann[9] pointed out, that performance improvements after IBA use in studies by Bedi *et al.*[69], Signorile *et al.*[68] and van Baak *et al.*[52] were due to performance increases in those athletes with the lowest initial fitness levels. Therefore, their findings may not be applicable to elite athletes with a highly trained cardiorespiratory system. Another strength of this study is the use of an exercise protocol of a 10-km time trial to maximally challenge the respiratory system. Sue-Chu *et al.*[76] suggested, that a short-duration, high-intensity exercise protocol may increase the magnitude of potentially ergogenic effects due to IBA-induced bronchodilation.

We compared performance-related parameters of 10 EIB+ athletes to 32 EIB- athletes. Since the effect of IBA on athletic performance based on EIB-susceptibility has not been investigated before, comparisons of our findings were limited to studies with asthmatic populations that do not exercise on a competitive level. Our study design did not allow the assessment of exercise-induced bronchodilation during the warm-up. To differentiate between exercise-induced bronchodilation generated by the 20-min warm-up and the bronchodilation due to IBA exposure, a third lung function assessment immediately prior to the time trial start should have been conducted on test days II and III. This would allow further insight in the effects of EIB-susceptibility on lung function improvements due to IBA and due to exercise-induced bronchodilation.

It is unknown how the severity of asthma and also the level of physical fitness affect the treatment response to IBA. A matched-pair study design may lead to a better understanding of potential differences in the effect of IBA on athletic performance in EIB+ and EIB- athletes. Especially in cycling body weight plays an important role in regards to power output. Analyzing performance after IBA-use in EIB+ and EIB- athletes matched for body weight and height may lead new findings in this matter.

Even though the sample size of 42 is greater than in most other studies that investigated the effect of IBA-use on performance, it is small for pharmacogenetic studies. A greater sample size for the AA genotype of the A46G SNP and the CC genotype of the C79G SNP would increase the statistical power of the study. Furthermore, the effect of the A46G and the C79G haplotype on athletic performance after IBA use would be possible with and increased sample size.

The multitude of functions in which the *ADRB2* gene is involved, makes the association of genotypes of the *ADRB2* SNPs with aerobic performance difficult. For example, one variant of a chosen SNP may beneficially influence one component of athletic performance, such as bronchodilator response, but adversely affect another component, such as metabolic function. Furthermore, many associations of the *ADRB2* SNPs with parameters that are related to athletic performance have been done on a clinical population only. In the future, studies on highly trained athletes are necessary to allow a better understanding of the impact on genetic variation at the *ADRB2* gene on athletic performance.

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Appendices

A Subject characteristics based on genetic variation at the *ADRB2* A46G and C79G SNPs

Table 12: Anthropometric and lung function parameters based on genetic variation at the *ADRB2* A46G and C79G SNPs.

Genetic variation	*n*	Height (cm) Mean (SD)	Weight (kg) Mean (SD)	FVC (L) Mean (SD)	FEV_1 (L) Mean (SD)	FEV_1/FVC (%) Mean (SD)
Total	**40**	**183 (8)**	**76.0 (8.80)**	**6.67 (0.92)**	**5.27 (0.77)**	**79.3 (6.1)**
A46G SNP						
AA	4	187 (10)	82.5 (15.5)	6.94 (0.72)	5.63 (0.71)	81.0 (4.7)
AG	15	182 (6)	74.9 (7.9)	6.69 (1.00)	5.38 (0.76)	80.7 (7.1)
GG	21	182 (8)	75.6 (7.9)	6.60 (0.92)	5.13 (0.78)	77.9 (5.4)
C79G SNP						
GG	14	185 (7)	77.6 (9.2)	6.77 (0.78)	5.44 (0.85)	80.3 (7.7)
GC	19	183 (7)	74.4 (8.9)	6.70 (1.05)	5.23 (0.76)	78.3 (5.8)
CC	7	180 (9)	77.9 (8.9)	6.20 (0.95)	4.95 (0.62)	80.2 (3.1)

Abbreviations: FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; FEV_1/FVC: fraction of FVC expired in 1s; Δ Max FEV_1: decrease in FEV_1 to a eucapnic voluntary hyperpnea test.

Table 13: Maximal oxygen consumption and power output based on genetic variation at the *ADRB2* A46G and C79G SNPs.

Genetic variation	*n*	VO_{2max} ($mL \cdot kg^{-1} \cdot mL^{-1}$) Mean (SD)	VO_{2max} ($L \cdot min^{-1}$) Mean (SD)	Max RQ Mean (SD)	Max HR ($b \cdot min^{-1}$) Mean (SD)	Max Power (W) Mean (SD)	Max Power ($W \cdot kg^{-1}$) Mean (SD)
Total	40	65.8 (6.8)	4.80 (0.94)	1.20 (0.06)	183 (10)	436 (36)	5.69 (0.68)
A46G SNP							
AA	4	63.3 (7.5)	5.15 (0.36)	1.23 (0.04)	180 (8)	467 (54)	5.75 (0.81)
AG	15	67.1 (7.0)	4.95 (0.72)	1.21 (0.05)	183 (10)	430 (37)	5.77 (0.45)
GG	21	65.3 (6.8)	4.61 (1.11)	1.20 (0.06)	181 (10)	431 (29)	5.59 (0.82)
C79G SNP							
GG	14	66.9 (7.8)	5.16 (0.52)	1.20 (0.04)	181 (10)	450 (39)	5.83 (0.55)
GC	19	65.6 (6.1)	4.47 (1.25)	1.22 (0.05)	183 (9)	426 (34)	5.76 (0.38)
CC	7	63.8 (7.1)	4.89 (0.20)	1.20 (0.08)	180 (6)	438 (29)	5.22 (1.26)

Abbreviations: VO_{2max}: maximal oxygen consumption; Max RQ: maximal respiratory quotient; Max HR: maximal heart rate

B Lung function and athletic performance of the *ADRB2* A46G SNP

Table 14: Percent change in FEV_1 based on *ADRB2* A46G SNP with combined genotypes.

Parameter	***n***	**Salbutamol**		**Placebo**	
		Mean	**(SD)**	**Mean**	**(SD)**
Total	**40**	**6.44**	**(6.05)**	**1.10**	**(3.07)**
AA & AG	19	6.37	(4.10)	1.56	(2.52)
GG	21	6.37	(4.12)	0.69	(3.50)

Table 15: Athletic performance based on *ADRB2* A46G SNP with combined genotypes.

Parameter	**Genotype**	***n***	**Salbutamol**		**Placebo**	
(units)			**Mean**	**(SD)**	**Mean**	**(SD)**
Power	**Total**	**40**	**4.0**	**(0.3)**	**4.0**	**(0.3)**
($W \cdot kg^{-1}$)	AA & AG	19	4.0	(0.3)	4.0	(0.4)
	GG	21	4.0	(0.3)	4.0	(0.3)
Oxygen	**Total**	**38**	**56.0**	**(6.7)**	**55.6**	**(6.6)**
consumption	AA & AG	18	56.5	(6.8)	57.2	(6.7)
($L \cdot kg^{-1} \cdot min^{-1}$)	GG	20	55.55	(6.6)	54.2	(6.4)
Heart Rate	**Total**	**39**	**166**	**(11)**	**170**	**(6)**
($b \cdot min^{-1}$)	AA & AG	18	167	(7)	169	(8)
	GG	21	165	(14)	166	(12)
Ventilation	**Total**	**36**	**1.67**	**(0.57)**	**1.65**	**(0.57)**
($L \cdot min^{-1} \cdot kg^{-1}$)	AA & AG	16	1.70	(0.53)	1.70	(0.53)
	GG	20	1.82	(0.31)	1.76	(0.34)
Respiratory Rate	**Total**	**40**	**42**	**(8)**	**42**	**(7)**
($b \cdot min^{-1}$)	AA & AG	19	42	(9)	43	(6)
	GG	21	43	(7)	42	(7)
Tidal Volume	**Total**	**37**	**0.043**	**(0.007)**	**0.041**	**(0.006)**
($L \cdot kg^{-1}$)	AA & AG	17	0.042	(0.008)	0.041	(0.007)
	GG	20	0.043	(0.006)	0.042	(0.005)

C Lung function and athletic performance of the *ADRB2* C79G SNP

Table 16: Percent change in FEV_1 based on the *ADRB2* C79G SNP with combined genotypes.

Genotype	*n*	Salbutamol		Placebo	
		Mean	(SD)	Mean	(SD)
Total	**40**	**6.43**	**(6.07)**	**1.07**	**(3.07)**
GG	14	4.70	(2.51)	1.46	(2.96)
GC & CC	26	7.36	(7.13)	0.87	(3.16)

Table 17: Athletic performance based on the *ADRB2* C79G SNP with combined genotypes.

Parameter	Genotype	*n*	Salbutamol		Placebo	
(units)			Mean	(SD)	Mean	(SD)
Power	**Total**	**40**	**4.0**	**(0.3)**	**4.0**	**(0.4)**
($W \cdot kg^{-1}$)	GG	14	4.1	(0.4)	4.2	(0.4)
	GC & CC	26	3.9	(0.3)	3.9	(0.3)
Oxygen	**Total**	**37**	**56.1**	**(6.7)**	**55.8**	**(6.6)**
Consumption	GG	12	58.5	(8.5)	58.7	(7.4)
($L \bullet kg^{-1} \bullet min^{-1}$)	GC & CC	25	55.0	(5.5)	54.4	(5.7)
Heart Rate	**Total**	**39**	**165**	**(11)**	**167**	**(10)**
($b \bullet min^{-1}$)	GG	13	166	(11)	168	(10)
	GC & CC	26	164	(12)	167	(10)
Ventilation	**Total**	**35**	**1.79**	**(0.32)**	**1.82**	**(0.31)**
($L \bullet min^{-1} \bullet kg^{-1}$)	GG	11	1.82	(0.29)	1.84	(0.29)
	GC & CC	24	1.82	(0.34)	1.77	(0.34)
Respiratory Rate	**Total**	**40**	**42**	**(8)**	**43**	**(7)**
($b \bullet min^{-1}$)	GG	14	43	(8)	43	(7)
	GC & CC	26	43	(8)	42	(7)
Tidal Volume	**Total**	**36**	**0.043**	**(0.007)**	**0.041**	**(0.006)**
($L \bullet kg^{-1}$)	GG	11	0.039	(0.005)	0.042	(0.006)
	GC & CC	25	0.043	(0.011)	0.039	(0.011)

D Assessment day – Data sheet

Subject Number: ..... Date:..... Time:.....

Subject information/anthropometric data

Sex:	
Age/Date of Birth:	
Number of years as active athlete:	
Training hours/week:	
Body weight:	
Body height:	
Resting HR:	
Resting BP:	
Last intake of SABA/LABA/ICS:	
Intake of other medications:	
Last strenuous exercise:	
Any history of serious illnesses	

EVH test

Room Temperature (°C)..... Humidity (%):..... Room Pressure (mmHg):.....

Time started: ..... Time ended: .....

Trials	FEV_1 /FVC PRE-testing		FEV_1 /FVC POST-testing at 3min		FEV_1 /FVC POST-testing at 5min		FEV_1/ FVC POST-testing at 10min		FEV_1 / FVC POST-testing at 15min		FEV_1 / FVC POST-testing at 20min	
Time	FEV_1	FVC	FEV_1	FVC	FEV_1	FVC	FEV_1	FVC	FEV_1	FVC	FEV_1	FVC
1												
2												
3												
4												

Highest FEV_1 pre challenge: .....FEV_1 (pre-chall.) X 30 =..... Lowest FEV_1 post challenge:.....

EIB: ☐ Yes ☐ NO

Maximal exercise test

Own bike equipment used:

.....

Bike settings: seat height: ..... handle bar height:.....

horizontal position:..... horizontal position: .....

Notes regarding warm-up:

.....

.....

.....

If asthmatic: time of salbutamol administration: .....

Time of Max. Test start: .....Time of Max Test ending: .....

Age-predicted HRmax.: .....

Measured Parameters:

VO_{2max} ($ml\text{-}kg^{-1}*min^{-1}$)	VO_{2max} ($L*min^{-1}$)	Max RER	HR_{max} (bpm)	Max Power (W)	Max Power/BW ($W*kg^{-1}$)

E Test days II and III – Data sheet

Subject Number:..... Date/Time:..... Day 1 or Day 2

Room Temperature: ..... Room Pressure: ..... Humidity: .....

☐ Take genotype sample: toothbrush

Subject information/anthropometric data

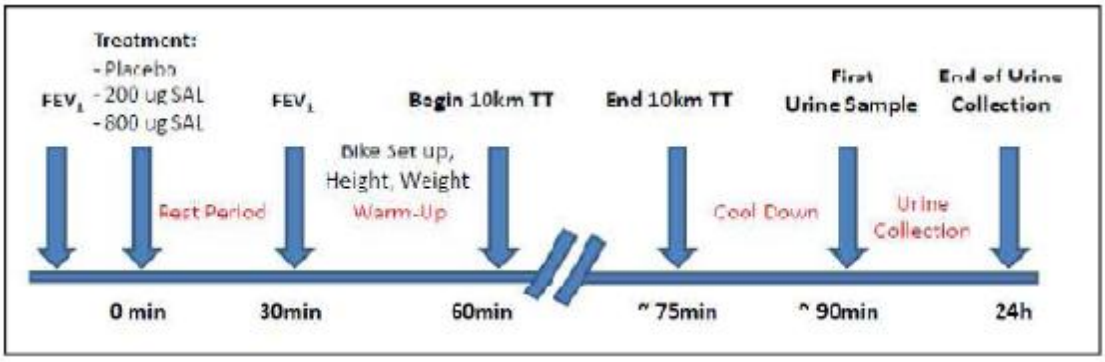
Sex:	
Age:	
Body weight	
Body height	
Last strenuous exercise:	
Last intake for SABA/LABA/ICS:	
Last intake of other medications:	
Last caffeine intake:	

Bike settings:

Bike settings: seat height: ..... handle bar height:.....

horizontal position:..... horizontal position: .....

Timing:



Time of Treatment:..... Time of Warm-Up start …….. Anticipated start of TT:……

FEV1/FVC pre treatment (time:) FEV1/FVC post treatment (time:)	Trial 1: ..... Trial 2: ..... Trial 1:..... Trial 2: .....

Variables measured:

Mean Power (W)	Mean VO_2 ($mL*kg^{-1}*min^{-1}$)	Mean HR (bpm)	Ventilation ($l*min^{-1}$)	Respir. Rate (breaths $*min^{-1}$)	Tidal Volume (L)

Rating of perceived exertion:

	0km	2km	4km	6km	8km	10km
RPE Breathing						
RPE Legs						

Time needed to complete TT: .....

Comments:.....
.....
.....
.....
.....
.....

F Participant information sheet

Please find a list of things below that you should keep in mind before you visit our lab for the assessment day and the two consecutive test days.

Test Location: Our lab is in the Wesbrook Building, 6174 University Boulevard, Vancouver V6T 1Z3, room 329 on the third floor.

Clothing: Please bring your cycling clothes including shoes and pedals if you like. Our velotron ergometer does have pedals with toeclips on one side and an SPD clipless mechanism on the other side. Bring some warm layers (sweater, warm up jacket) as there are some 20-30min breaks in between certain tests. There are showers in the Wesbrook building that you can use after the tests, so feel free to bring a towel, etc.

Exercising before assessment and test days: Do not schedule a strenuous workout for the day before the test day. Please do not exercise at all the day of the test. This also means that you should not run or bike to campus.

Medication: If you are on asthma medications, please withhold from taking Beta-2-Agonists (Ventolin, Salbutamol) over a period of 12h prior to the test. However, you are allowed to continue your glucocorticostereoid treatment.

Nutrition/hydration: Make sure you arrive well hydrated. Please avoid alcoholic beverages the evening before and caffeine on test days. Try to eat a similar meal prior to each testing day to reduce variability.

If anything comes up and you like to reschedule you test days, please send me an email or give me a call as soon as possible.

Thank you,

Sarah

G Inclusion criteria

Table 18: Inclusion criteria.

Inclusion Criteria	Exclusion Criteria
- Age 19-40 years - Cyclist or triathlete competing in class 1-3 races - Healthy adults, with or without history of asthma - Normal or abnormal eucapnic voluntary hyperpnea test or spirometry - Maximal oxygen consumption (VO_{2max}) > 60ml•kg^{-1}•min^{-1} (relative) or VO_{2max} > 5 l•min^{-1} (absolute) - English-speaking	- Females - History of atopy, pulmonary or cardiac disease - Any recent (past 6 months) respiratory or musculoskeletal injury, infection or disease that might affect athletic performance - Respiratory tract infection 3 weeks prior to test - History of Smoking

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Pharmacogenetics and cycling: the interactive effects of the ADRB2 A46G SNP and salbutamol on elite cycling performance

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Polymorphisms of the adrenergic β2-receptor gene (ADRB2) are associated with key components of cardiorespiratory function during exercise. Specifically, the G-allele of the A46G SNP (Arg16Gly; rs1042713) results in a substitution of a glycine for an arginine at amino acid 16, which is associated with increased bronchodilation, heart rate, and cardiac output. Asthmatic and non-asthmatic individuals with the Gly16 variant have a greater increase in forced expiratory volume in one second (FEV1) after the inhalation of a short-acting β2-agonist such as salbutamol (SAL). It is unclear if the influence of the Gly16 variant on change in FEV1 after the inhalation of SAL affects performance in highly trained athletes.

PURPOSE: 1. To determine if common variants of the adrenergic β2-receptor influence percent change in FEV1 after the inhalation of SAL in male cyclists. 2. To assess the influence of the A46G SNP on 10-km time trial performance in cyclists after inhaling SAL.

METHODS: The A46G SNP of the ADRB2 gene was genotyped (AA: 4; AG: 14; GG: 17) in 36 unrelated competitive male cyclists aged 19 - 40 years. Athletes performed two simulated 10-km

time trial rides on a cycle ergometer 60 min after the inhalation of either 400 µg of SAL or placebo. Medication administration was double-blinded and randomly assigned. The change in FEV1 was assessed immediately before and 30 min after inhalation. Performance was assessed by the time needed to complete the ride. Mixed between-within subject ANOVAs were conducted to assess differences between percent change in FEV1 and cycling performance after the inhalation of SAL or placebo based on an individual's A46G SNP.

RESULTS: The percent change in FEV1 after the inhalation of SAL was significantly greater than placebo, $F(1, 33)= 4.4$, $p = 0.043$, $\eta p2 = 0.118$). This is independent of the A46G SNP, $F(2,33)= 0.26$, $p = 0.77$, $\eta p2 = 0.016$. Furthermore, there was no interaction effect between the A46G SNP and the time needed to complete a time trial after the inhalation of SAL or placebo, $F(2,32) = 0.68$, $p = 0.51$, $\eta p2 = 0.01$. No main effect was found between the SAL and the placebo condition, $F(1,32)= 1.4$, $p = 0.71$, $\eta p2 = 0.004$).

CONCLUSION: In competitive male cyclists, FEV1 is improved after the inhalation of SAL (400 µg) regardless of genotype at the ADRB2 A46G SNP. In addition, the A46G SNP did not influence a 10-km time trial performance after the inhalation of SAL in male cyclists.

K Subject information and consent form

Pharmacogenomics of inhaled beta2-agonists and athletic performance in athletes

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School of Human Kinetics
University of British Columbia

Sponsors: World Anti-Doping Agency (WADA)

INTRODUCTION

You are being invited to take part in this research study with 50 subjects because you are a male elite competitive cyclist or triathlete. You are between the age of 19 and 40 with no significant heart and lung health problems.

YOUR PARTICIPATION IS VOLUNTARY

Your participation is entirely voluntary, so it is up to you to decide whether or not to take part in this study. Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts.

If you wish to participate, you will be asked to sign this form. If you do decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision.

If you do not wish to participate, you do not have to provide any reason for your decision not to participate nor will you lose the benefit of any medical care to which you are entitled or are presently receiving.

Please take time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide.

WHO IS CONDUCTING THE STUDY?

The study is being conducted by the University of British Columbia

There are no conflicts of interest between the investigators and the World Anti-Doping Agency (WADA) funding the research.

BACKGROUND

Beta2-agonists are a type of medication that is commonly used in the treatment of asthma. They can have other actions other than treating asthma that may have the potential to improve exercise performance.

In the past 25 years, there has been a trend for an increase in applications for permission to use Beta2-agonists from athletes competing in Olympic Games. In fact athletes that use these agents win a disproportionately high number of medals. Previous research has looked at unselected groups, and found no doping benefit from these agents. Recent research has shown that there is a large variety in the genes that affect how individuals respond to these Beta2-agonists. We will look at variations in the genetic response to these medications. Specifically we will divide athletes into those with a genetically high response to these drugs and those with a lower response. We will then compare their exercise performance following the administration of a Beta2-agonist. We hypothesize that a subgroup of athletes with certain genetic variations will benefit from Beta2-agonists while the rest will not. If some athletes are achieving enhanced performance from asthma medication, then the rules surrounding their use in sport will need to be reviewed.

WHAT IS THE PURPOSE OF THE STUDY?

The purpose of the proposed study would be to assess a series of elite cyclists for genetic variation in the gene that controls the response to a type of asthma medication, and then examine whether variations at certain genes provide a performance benefit to people who take this type of medication.

WHO CAN PARTICIPATE IN THE STUDY?

To participate, you must be a 19 to 40 year old, male category 1 or 2 cyclist (or equivalent) or a triathlete capable of performing a 40 km time trial in less than 60 minutes. Your maximal oxygen consumption (VO_{2max}) must equal or be higher than 60mL/kg/min (relative) or 5L/min (absolute). You must be free of heart or lung disease and you must be a nonsmoker.

WHO SHOULD NOT PARTICIPATE IN THE STUDY?

Individuals with a medical history or a current medical condition affecting their heart or lungs must not participate in the study. If for any reason you are unable to perform a maximal cycling efforts, you should not participate. If your maximal oxygen consumption (VO_{2max}) is less than 60mL/kg/min (relative) or less than 5L/min (absolute) you must not participate in the study.

WHAT DOES THE STUDY INVOLVE?

This study will take place in the Environmental Physiology Laboratory in the Wesbrook Building at the University of British Columbia in Vancouver and at the Canadian Sport Centre Pacific Performance Laboratory in Victoria, British Columbia.

Overview of the study

You will be requested to come to the laboratory on four occasions. The first visit will consist of a graded exercise test on a stationary bicycle to determine your maximal power output. Additionally a sample of your DNA (Deoxyribonucleic acid) will be collected. The final three visits will consist of a single bout of bicycle simulating a 10-kilometer time trial. Prior to the simulated time trial, you will take a dose of asthma medication (called salbutamol) or a placebo. The order of placebo and a single dose of 800mcg of Salbutamol, will be randomized. This means that the order of the inhaled medication dosage will be determined completely by chance. Similarly to flipping a coin, a computer program will choose the order of the inhaled dosage of the medication.

If you decide to join this study: specific procedures

If you agree to take part in this study, the procedures and visits you can expect will include the following:
This study is double-blind, meaning that neither you nor the investigators will know which dose of salbutamol you will inhale on which day. However this information is available in case of an emergency.

Study visits

Day 1 will consist of some baseline measurements and a graded exercise test on a stationary bicycle.

Additionally you will be asked to give a sample of your DNA. This is done by collecting some skin cells from the inside of your cheek by scraping with a wired brush. DNA will be separated from your cells and your genetic code for the Beta2-receptors will be determined. Once the study is over, your DNA sample will be stored for 5 years. In case that an additional gene of interest is discovered after the completion of this study, additional testing of your DNA might take place.

The baseline measurements involve using a machine (called a spirometer) to measure your breathing function. This will involve taking a series of breaths (at rest) as instructed to by the

investigators. You will breathe out into a machine that measures the amount of air that you breathe out. You will do this lung function measurement again after breathing a gas mixture for 6 minutes. While you are breathing this gas mixture you will be encouraged to breathe at a relatively fast rate (the same as you would during heavy exercise).

The graded exercise test starts with a comfortable warm-up. Then you will begin cycling at 0 watts. This resistance will increase by 1 watt every 2 seconds. You will cycle as long as you can until you feel you can ride no harder. At that point the test is complete, and you can do warm down however you like.

Day 2 and 3 will be very similar to each other in that you will perform the same procedures. Exercise will consist of simulated cycling on a specially designed bicycle connected to a computer. You will be able to change gears, and sit and stand as you wish. You will start with your own self-selected warm-up. Once you are ready you will perform a simulated 10-kilometer time trial, trying to ride the simulated distance in as short a time as possible. Prior to the simulated time trial, you will take a dose of asthma medication (called salbutamol) or a placebo. The order of placebo and medication will be randomized. You will not know which dose of medication or placebo you will be taking on each day until the end of the study. Right before and 30 minutes after the inhalation of the asthma medication you will be asked to perform a lung function test called “forced expiratory volume in 1 second”. You will be familiarized with this test on day one, the screening day.

During exercise you will wear your own exercise clothing, and a heart rate monitor. This is a strap that goes around your chest and monitors your heartbeat. During exercise, you will breathe out into a machine that measures the amount and contents of air that you breathe out.

Your name and all information provided throughout this study will be linked with a special code to protect your privacy.

WHAT ARE MY RESPONSIBILITIES?

On days prior to your appointments you will be asked to drink 3 liters of clear fluids within 16 hours prior to the testing. Of those 3 liters, 500 milliliters should be consumed 2 hours prior to the time trial and 250 milliliters should be consumed 30 minutes prior to the time trial on the bike. You will also refrain from eating a large meal 2 hours prior to visiting the laboratory. Additionally you should not exercise in the mornings before the treatment. This is necessary to make sure that our measurements are not influenced by any physical activity prior to testing. If you use asthma medications on a regular basis, you are asked to withhold from them 12 hours prior to testing.

WHAT ARE THE POSSIBLE HARMS AND SIDE EFFECTS OF PARTICIPATING?

The main risks of the exercise are those of stationary cycling. To participate you must be an elite cyclist/triathlete, so will likely be used to stationary cycling, and this exercise should not introduce any appreciable risk. You will be cycling as hard as you would in a real race, but the exercise bouts in this study are generally much shorter than in a real race.

The breathing test where you are breathing as fast as you would during exercise, can feel odd. It feels odd, because you are not used to breathing so much while at rest, not because anything untoward is happening. The gas that you breathe is actually mixed so that you will not get lightheaded (as you would if you were just breathing room air that fast).

The DNA sampling involves a very low risk of bleeding. The wired brush used for collecting your skin cells is a bit rougher than a toothbrush. It is designed to remove cells from the surface of the inside of your cheek.

Known side effects of salbutamol, the asthma medication you will be asked to inhale, are temporary involuntary muscle movements, dizziness, sensations of irregular heartbeats, dry mouth and nausea. Additional possible side effects are an unusual taste and a feeling of nervous unease. If you experience those side effects, they will be temporary and will resolve without further treatment.

For the event of an emergency during exercise, all personnel involved will be trained in basic cardiopulmonary resuscitation (CPR) and automated external defibrillator (AED) use. Emergency equipment, including an AED, oxygen masks, blood pressure cuff and stethoscope, will be readily available and working properly. UBC Hospital is approximately 250 meters from the laboratory. If a problem occurs during exercise testing, the supervising physician will be summoned immediately. The physician will decide whether to call for evacuation to the nearest hospital. If a physician is not available and any questions exist as to the status of the patient, then emergency transportation to the closest hospital will be summoned immediately.

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

No one knows whether or not you will benefit from this study. There may or may not be direct benefits to you from taking part in this study. You will get a complementary graded exercise test which will give you an objective measure of your aerobic fitness. We hope that the information learned from this study can be used in the future to benefit competitive athletes by increasing our knowledge of potential doping methods in sport. You will be given an honorarium of $50 for each visit to partially compensate for your time.

WHAT HAPPENS IF I DECIDE TO WITHDRAW MY CONSENT TO PARTICIPATE?

Your participation in this research is entirely voluntary. You may withdraw from this study at any time without providing any reason for your decision. If you decide to enter the study and to withdraw at any time in the future, there will be no penalty or loss of benefits to which you are otherwise entitled, and your future medical care will not be affected. The study investigators may decide to discontinue the study at any time, or withdraw you from the study at any time, if they feel that it is in your best interests.

If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment in the study will be retained for analysis. By law, this data cannot be destroyed.

WHAT HAPPENS IF SOMETHING GOES WRONG?

Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

In the event you become injured or unexpectedly ill while participating in this study, necessary medical treatment will be available at no additional cost to you. If you become injured or unexpectedly ill as a consequence of participation in the study due to study procedures, your medical condition will be evaluated and medical care will be provided by one of the investigators or you will be referred for appropriate treatment.

There will be no costs to the subject for participation in this study. Signing this consent form in no way limits the subject's legal rights against the sponsor, investigators, or anyone else. The subject will not be charged for the research procedures.

CAN I BE ASKED TO LEAVE THE STUDY?

If you are not complying with the requirements of the study or for any other reason, the investigators may withdraw you from the study.

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate the UBC Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices

WHOM DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY DURING MY PARTICIPATION?

If you have any questions or desire further information about this study before or during participation, you can contact Sarah Koch at kochsh@interchange.ubc.ca

WHOM DO I CONTACT IF I HAVE ANY QUESTIONS OR CONCERNS ABOUT MY RIGHTS AS A SUBJECT DURING THE STUDY?

If you have any concerns about your rights as a research subject and/or your experiences while participating in this study, contact the Research Subject Information Line in the University of British Columbia Office of Research Services at 604-822-8598.

SUBJECT CONSENT TO PARTICIPATE

The consent form is not a contract and as such that the subject does not give up any legal rights by signing it.

By signing the form I indicate that you have read, understood and appreciate the information concerning the study.

- I have read and understood the subject information and consent form.
- I have had sufficient time to consider the information provided and to ask for advice if necessary.
- I have had the opportunity to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.
- I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I understand that there is no guarantee that this study will provide any benefits to me (if applicable).
- I have read this form and I freely consent to participate in this study.
- **I have been told that I will receive a dated and signed copy of this form.**

SIGNATURES

Printed name of subject Signature Date

Printed name of witness Signature Date

Printed name of principal investigator/
designated representative Signature Date