
Asthma and Sports

Asma e Desporto

Mechanisms and effects of airway damage in elite athletes

Mecanismos e efeitos de lesão das vias aéreas em atletas de elite

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“The deepest sin against the human mind is to believe things without evidence.”

Aldous Huxley

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Abstract

Elite athletes have an increased risk for asthma, especially those who take part in endurance sports, such as swimming or running, and in winter sports. Asthma is a significant problem for both recreational and competitive athletes and is the most common chronic condition among Olympic athletes, with obvious implications for their competing performance, health and quality of life. Some hypotheses explain how intensive physical activity may cause exercise-induced asthma. Classical postulated mechanisms include the osmotic and the cooling hypothesis, resulting in the release of inflammatory mediators that cause airway smooth muscle contraction. However, the inflammatory pathway explanation does not seem to be entirely satisfactory to justify the increased prevalence of exercise-induced asthma in athletes. Studies are needed to better define the etiologic factors and mechanisms involved in development of asthma in elite swimmers, to ultimately propose relevant preventive and therapeutic measures.

Therefore this thesis aims to investigate phenotypes and mechanisms of asthma development on elite athletes.

This thesis is based on three types of studies: 1. *Cross-sectional studies*: a) analysis of asthmatic elite athletes to assess different phenotypes of asthma; b) analysis of asthmatic and healthy elite athletes to investigate the association between bronchial hyperresponsiveness (BHR) and parasympathetic activity measured by pupillometry; 2. *Observational studies*: a) prospective evaluation of swimmers to assess the effect of a swimming training session on the exhaled breath temperature in the airways of both asthmatic and non-asthmatic elite athletes; b) investigation of long-term swimming effect on airway inflammation in competitive swimmers; and c) retrospective analysis of asthma medication requests submitted by elite athletes to the Anti-Doping Authority of Portugal between 2008 and 2010 to explore the impact of asthma and anti-doping regulations in elite athletes' health; 3) A *randomized cross-over* trial to compare the reversibility to inhaled ipratropium bromide with the reversibility to inhaled salbutamol and to investigate correlation between PD₂₀ obtained with methacholine bronchial challenge and the reversibility to each of the drugs. A total of 127 swimmers participated in the observational prospective studies, 22 elite winter athletes participated in the randomized cross-over trial and 27 elite swimmers in one of the cross-sectional studies. For the other cross-sectional study, data from clinical files of elite athletes at the Portuguese database of Olympic athletes and at database of Norwegian School of Sport Sciences were analyzed. Data from asthma medication requests submitted to the Anti-Doping Authority of Portugal referring to 326 elite athletes were reviewed for the retrospective study.

Since objective evidence of asthma based on the International Olympic Committee definition became mandatory for the diagnosis of asthma in athletes, the number of applications for the use of anti-asthmatic inhalers has been reduced to approximately half. By requiring additionally objective evidence to validate asthma diagnosis, guidelines improved athlete's health care. Using latent class analysis and the IOC definition of asthma, two patterns of asthma aggregation features were identified: "atopic asthma",

defined by the presence of allergic sensitization, rhinitis and other allergic co-morbidities, and increased exhaled nitric oxide levels; and “sports asthma”, defined by the presence of exercise-induced respiratory symptoms and BHR in the absence of allergic features. Exposure to particular environmental conditions of training and competition was associated with increased risk to develop the “sports asthma” phenotype: water sports increased the risk by almost three times whereas in winter sports the risk increased by almost nine times. In both swimmers and winter athletes, an imbalance of the autonomic nervous system towards an increased parasympathetic activity was demonstrated and related to BHR. Among swimmers with BHR, an association was observed between methacholine PD₂₀ and four parasympathetic parameters measured by pupillometry; no significant differences were observed between asthmatic and non-asthmatic elite swimmers, suggesting that in these athletes the increased parasympathetic tone is associated with BHR particularly through the contraction of the bronchial smooth muscle rather than related with other features of asthma. In winter athletes, methacholine PD₂₀ showed a highly significant inverse correlation to the cholinergic antagonism of inhaled ipratropium bromide, but not to the bronchodilating effect of inhaled salbutamol. This suggests that BHR in winter endurance athletes is associated with increased parasympathetic bronchial tone which may represent an important role for the cholinergic system in the development of asthma in athletes. In swimmers, although exhaled breath temperature significantly increased after training, supporting the hypothesis of heat loss from airways during exercise, no differences were observed between asthmatic and healthy swimmers after controlling for confounders; a relationship between airway’s inflammation and the increase in exhaled breath temperature during exercise could not be confirmed, suggesting that the respiratory heat loss is a physiological rather than a pathological response to exercise. Besides, long-term follow-up of competitive swimmers demonstrated that eosinophilic airway inflammation measured by exhaled nitric oxide decreases after finishing competitive swimming, despite the increase observed in the prevalence of asthma and use of asthma medication in both active and past adolescent swimmers. This suggests a relative independence of the two conditions.

All together our study provides further support to the hypothesis that swimmers and winter sport athletes present a different phenotype of asthma, related to a specific mechanism linked to an increased parasympathetic tone and consequent susceptibility to bronchospasm and BHR. Our results lead to hypothesize that in these specific groups of athletes, inflammation occurs due to environmental conditions and hyperpnea but not so much as an underlying etiopathogenic mechanism of asthma. Guidelines have an impact on the care of athletes with documented asthma and influence how respiratory symptoms are managed and treated in these patients and should be taken into account for long-term planning of screening and medical programs for athletes. The way forward is to continue investigating the neurogenic features of asthma in athletes, in order to clearly define the etiologic factors and mechanisms involved in the development of asthma in elite athletes, and understand the complex relation between the different components of this puzzle: epithelial damage, airways inflammation, exposure to polluted air or cold temperatures, and increased parasympathetic tone leading to increased BHR and asthma symptoms, ultimately enabling to propose relevant and specific therapeutic measures.

Resumo

Os atletas têm um risco acrescido de asma, especialmente aqueles que praticam desportos de resistência, como a natação ou a corrida, e desportos de inverno. A asma é um importante problema de saúde tanto para atletas que praticam desporto de forma recreativa, como de competição, e é a doença crónica mais frequente em atletas Olímpicos, com óbvias implicações na sua performance competitiva, saúde e qualidade de vida. Algumas hipóteses explicam de que forma a atividade física pode provocar asma induzida pelo exercício. Os mecanismos classicamente postulados incluem as hipóteses osmótica e térmica, ambas resultando na libertação de mediadores inflamatórios com consequente contração do músculo liso brônquico. No entanto, a explicação inflamatória não parece ser inteiramente satisfatória para esclarecer a elevada prevalência de asma induzida pelo exercício nos atletas.

São necessários mais estudos para melhor definir os fatores etiológicos e mecanismos envolvidos no desenvolvimento de asma nos atletas de alta competição, de forma a propor medidas preventivas e terapêuticas.

O objetivo da presente tese é investigar **os fenótipos e mecanismos de desenvolvimento de asma em atletas de elite**.

Esta tese baseia-se em três tipos de estudos: 1. a) *Estudos transversais* incluindo atletas de elite asmáticos para identificar diferentes fenótipos de asma; b) análise de atletas asmáticos e saudáveis para investigar associação entre hiperreactividade brônquica (HRB) e atividade parassimpática avaliada através de pupilometria; 2) *Estudos observacionais*: a) avaliação prospetiva de nadadores para analisar o efeito de uma sessão de treino de natação na temperatura do ar exalado nas vias aéreas de atletas asmáticos e saudáveis; b) investigação do efeito da prática continuada e de longa duração de natação sobre a inflamação das vias aéreas em nadadores de competição; e c) análise retrospectiva dos pedidos de autorização de utilização de medicação anti-asmática submetidos pelos atletas de alta competição à Autoridade Antidopagem de Portugal entre 2008 e 2010 para explorar o impacto da regulamentação antidoping e relativa a asma sobre a saúde dos atletas; 3) *Estudo randomizado* para comparar a reversibilidade ao brometo de ipratrópio inalado com a reversibilidade ao salbutamol e investigar a correlação entre o PD₂₀ na prova de provocação brônquica com metacolina e a reversibilidade com cada fármaco. Um total de 127 nadadores participaram nos estudos observacionais prospetivos, 22 atletas de desportos de inverno participaram no estudo randomizado e 27 nadadores de elite no em um dos estudos transversais. No outro estudo transversal foram recolhidos dados dos ficheiros clínicos nas bases de dados dos atletas olímpicos portugueses e nas bases de dados da Norwegian School of Sport Sciences. Foram revistos dados de 326 pedidos de autorização de utilização de medicação anti-asmática para o estudo retrospectivo.

Desde a implementação da obrigatoriedade de apresentar testes objetivos nos atletas que confirmem o diagnóstico de asma baseado na definição do Comité Olímpico Internacional, o número de atletas a requisitar a utilização de terapêutica anti-asmática

diminuiu para cerca de metade. Ao requerer a evidência objetiva para validar o diagnóstico de asma, as diretrizes melhoraram os cuidados de saúde prestados aos atletas. Através da análise de clusters usando classes latentes, foram identificados dois fenótipos de asma: o fenótipo de “asma atópica”, definido pela presença de sensibilização alérgica, rinite e outras co-morbilidades alérgicas, e aumento dos níveis de óxido nítrico exalado; e o fenótipo “asma do desporto”, definido pela presença de sintomas respiratórios induzidos pelo exercício e HRB na ausência de características alérgicas. A exposição a condições ambientais específicas durante o treino e competição associou-se a um risco aumentado de desenvolver este fenótipo de “asma do desporto”: os desportos aquáticos aumentam o risco cerca de 3 vezes, enquanto nos desportos de inverno o risco é quase 9 vezes superior. Tanto em nadadores como em atletas que praticam desportos de inverno foi demonstrado um desequilíbrio no sentido de uma maior atividade parassimpática e que se relaciona com a HRB. Nos nadadores com HRB observou-se uma correlação significativa entre o PD₂₀ na prova de metacolina e 4 parâmetros parassimpáticos avaliados através de pupilometria; as diferenças entre nadadores asmáticos e não-asmáticos não foram significativas, o que sugere que nestes atletas o aumento do tónus parassimpático se associa particularmente à HRB através da contração do músculo liso brônquico, em vez de outras características de asma nesta população. Em atletas que praticam desportos de inverno observou-se uma correlação negativa significativa entre o PD₂₀ na prova de metacolina e o antagonismo colinérgico exercido pela inalação de brometo de ipratrópio, mas não pela inalação de salbutamol; observou-se um número superior de atletas com reversibilidade ao brometo de ipratrópio comparativamente ao salbutamol. Estes factos sugerem que a HRB em atletas que praticam desportos de inverno associa-se a um aumento do tónus parassimpático brônquico e que este pode representar um papel importante no desenvolvimento de asma. Em nadadores, embora a temperatura do ar exalado aumente significativamente após o treino, o que suporta a hipótese de ocorrer perda de calor das vias aéreas durante o exercício, não foram observadas diferenças entre asmáticos e indivíduos saudáveis após controlar o efeito de variáveis confundidoras; não se confirmou uma possível relação entre inflamação e o aumento da temperatura do ar exalado durante o exercício, o que sugere que a perda de calor das vias aéreas ocorre por um mecanismo fisiológico e não patológico. Além disso, o seguimento a longo prazo de nadadores de competição demonstrou que a inflamação eosinofílica das vias aéreas avaliada através da medição dos níveis de óxido nítrico exalado diminui após terminar a natação, apesar do aumento na prevalência de asma e utilização de terapêutica anti-asmática tanto nos nadadores que mantêm como naqueles que cessam a prática de natação. Este facto sugere uma relativa independência das duas condições.

No seu conjunto, estes estudos permitem suportar a hipótese de nadadores e atletas de desportos de inverno apresentarem um fenótipo diferente de asma, em relação com um mecanismo específico ligado a um aumento do tónus parassimpático com consequente susceptibilidade ao broncospasmo e HRB. Os nossos resultados sugerem que nestes grupos específicos de atletas a inflamação ocorre devido às condições ambientais e em resposta à hiperpneia, mas não tanto como um mecanismo etiopatogénico de asma. As diretrizes têm impacto nos cuidados prestados aos atletas com asma e influenciam a forma como os sintomas respiratórios são abordados e tratados nestes doentes, e devem ser tidos

em conta no planeamento de programas médicos e de rastreio para atletas. O caminho futuro passa provavelmente por continuar a investigar as características neurogénicas da asma em atletas, de forma a definir claramente os fatores etiológicos e mecanismos envolvidos no desenvolvimento de asma em atletas de elite, e compreender a relação complexa entre os diferentes componentes deste puzzle: dano epitelial, inflamação das vias aéreas, exposição a poluentes ou baixas temperaturas, e aumento do tónus parassimpático conduzindo a aumento da reactividade brônquica e sintomas de asma, de forma a permitir propor medidas terapêuticas relevantes e específicas.

Abbreviations

ACV	Average constriction velocity
ADOP	Anti-Doping Authority of Portugal
ADV	Average dilation velocity
AQUA [®]	Allergy Questionnaire for Athletes (protected by an international copyright)
ATS	American Thoracic Society
aTUE	abbreviated Therapeutic Use Exemption
BD	Bronchodilation
BHR	Bronchial hyperresponsiveness
DoU	Declaration of Use
EBT	Exhaled breath temperature
EIA	Exercise-induced asthma
EIB	Exercise-induced bronchoconstriction
EVH	Eucapnic voluntary hyperpnoea
FCP	Futebol Clube do Porto
FeNO	Fraction of exhaled nitric oxide
FEF ₂₅₋₇₅	forced expiratory flow in the middle portion of FVC
FEF ₅₀	forced expiratory flow rate at 50% of FVC
FEV ₁	Forced expiratory volume in the first second
FVC	Forced vital capacity
GINA	Global Initiative for Asthma
IBAs	Inhaled β 2-agonists
ICS	Inhaled corticosteroids
IOC-MC	International Olympic Committee – Medical Commission
IPAQ	International Physical Activity Questionnaire
ISAAC	International Study of Asthma and Allergies in Childhood
LCA	Latent class analysis
MCV	Maximum constriction velocity
PA	Physical activity
PC ₂₀	Provocative concentration of methacholine causing a 20% decrease in FEV ₁
PD ₂₀	Provocative dose of methacholine causing a 20% decrease in FEV ₁
T75	Total time taken by the pupil to recover 75% of the initial resting pupil size after it reached the peak of constriction
TUE	Therapeutic Use Exemption
WADA	World Anti-Doping Agency

List of original publications

This thesis is based on the following publications, which are referred to in the text by their roman numerals:

- I Couto M, Horta L, Delgado L, Capão-Filipe M, Moreira A. Impact of changes in anti-doping regulations (WADA Guidelines) on asthma care in athletes. Clin J Sport Med. 2013;23(1):74-6.
 doi: 10.1097/JSM.0b013e31826869ef

- II Couto M, Stang J, Horta L, Stensrud T, Severo M, Mowinckel P, Silva D, Delgado L, Moreira A, Carlsen KH. Two distinct phenotypes of asthma in elite athletes identified by latent class analysis. J Asthma. 2015;52(9):897-904
 doi: 10.3109/02770903.2015.1067321

- III Couto M, Silva D, Santos P, Queirós S, Delgado L, Moreira A. Exploratory study comparing dysautonomy between asthmatic and non-asthmatic elite swimmers. Rev Port Pneumol. 2015;21(1):22-9.
 doi: 10.1016/j.rppnen.2014.05.004.

- IV Stang J, Couto M, Carlsen KH, Stensrud T. Increased bronchial parasympathetic tone in elite cross-country and biathlon skiers: a randomised crossover study. Br J Sports Med. 2015;49(1):56-61
 doi: 10.1136/bjsports-2014-094053.

- V Couto M, Santos P, Silva D, Delgado L, Moreira A. Exhaled breath temperature in elite swimmers: the effects of a training session in adolescents with or without asthma. Pediatr Allergy Immunol. 2015;26(6):564–570
 doi: 10.1111/pai.12426

- VI Couto M, Andrade P, Pereira M, Araújo J, Moreira P, Delgado L, Moreira A. Effect of competitive swimming on airway inflammation: a 3-yr longitudinal study. Pediatr Allergy Immunol. 2014;25(2):193-5.
 doi: 10.1111/pai.12172

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1. INTRODUCTION

Physical exertion is one of the many stimuli that can produce episodes of airway obstruction in asthmatic patients, so-called exercise-induced asthma (EIA). This is particularly relevant for subjects practicing competitive sports. It is well known that elite athletes have an increased risk for asthma, especially those who take part in endurance sports, such as swimming or running, and in winter sports (Carlsen, Anderson et al. 2008, Schwartz, Delgado et al. 2008, Fitch 2012).

Two different clinical phenotypes of asthma in elite athletes were previously suggested (Haahtela, Malmberg et al. 2008), but these phenotypes have not been further confirmed, and the contribution of risk factors, such as environmental exposures, for the occurrence of particular phenotypes was not explored. Some hypotheses explain how intensive physical activity may cause EIA. Classical postulated mechanisms include the osmotic hypothesis (Anderson and Kippelen 2005), the disruption of the airway epithelium (Anderson and Kippelen 2008) and cooling of the airways (Carlsen, Anderson et al. 2008, Schwartz, Delgado et al. 2008), causing heat loss from the respiratory mucosa. These hypotheses consider inflammation and mast cells activation as being crucial for the development of EIA (Ali, Norsk et al. 2012). Recently, it has been debated whether the exercise-induced bronchoconstriction (EIB) that occurs in athletes during their sports career without other features of clinical asthma is identical to what is usually considered to be asthma in clinical practice, or rather has peculiar clinical and pathologic features.

Some answers in the explanatory model of EIB in athletes are still lacking. It has been proposed that intensive training can have effects on autonomic regulation. In fact, autonomic nervous system activity assessed by pupillometry in endurance athletes showed increased parasympathetic activity of the pupillary light reflex (Filipe, Falcão-Reis et al. 2003). Increased parasympathetic (cholinergic) tone could predispose to an increase in bronchomotor tone and therefore susceptibility to bronchospasm. However, the impact of dysautonomy in asthma and bronchial hyperresponsiveness (BHR) has not been fully investigated. As a proof of concept of this theory, it should be expected to observe a therapeutic effect of anticholinergic drugs in these athletes. On the other hand, evidence of the cooling hypothesis acting as mechanism of EIA would require asthmatic elite athletes to present a higher exhaled breath temperature during exercise, an increase that would also be expected in the presence of airway inflammation.

Development of asthma generally occurs in young adults rather than in adolescent competitive swimmers, suggesting that airway inflammation and hyperresponsiveness develop during the training career. While the latter seems to be a transient phenomenon (Bougault, Turmel et al. 2011), whether airway inflammation persists is still debated (Helenius, Ryttilä et al. 2002, Bougault, Turmel et al. 2009, Bougault, Loubaki et al. 2012).

Studies are needed to better define the factors and mechanisms involved in the complex etiology and pathogenesis of asthma in athletes, ultimately leading to propose relevant therapeutic measures. Also, the long term effects of swimming need to be fully established in order to find new strategies to prevent their negative impact.

2. REVIEW OF THE LITERATURE

2.1 Asthma, physical activity and exercise

Regular physical exercise and participation in sports are considered to be important components of a healthy life and are recommended for all individuals (Physical Activity Guidelines Advisory Committee 2009). There is unquestionable evidence that regular physical activity contributes to the primary and secondary prevention of cardiovascular diseases and several other chronic conditions (Bauman 2004). International guidelines recommend children above 2 years and youth to participate in at least 60 minutes of enjoyable, moderate-intensity physical activities every day (Physical Activity Guidelines Advisory Committee report 2009). Compared to inactive young people, physically active children and youth have higher levels of cardiorespiratory endurance (Physical Activity Guidelines Advisory Committee report 2009). Regarding asthma, evidence has shown that physical training improves cardiopulmonary fitness (Chandratilleke, Carson et al. 2012) and may even improve quality of life of asthmatics (Eichenberger, Diener et al. 2013) both in children and their caregivers (Silva, Couto et al. 2013). It has been suggested that moderate intensity physical training may decrease both total and allergen specific IgE levels (Moreira, Delgado et al. 2008). Also, it has been previously shown that intense swimming activity causes a lung growth greater than normal in children and adolescents (Silvestri, Crimi et al. 2013) and teaches airway control (Whinder 2013); also, the hot and humid conditions of swimming training have been pointed out as less asthmogenic. So, physical activity should be recommended as a supplementary therapy to medication in asthmatic subjects (Craig and Dispenza 2013, Eichenberger, Diener et al. 2013) and swimming has turned out to become a very popular sport, both among children with and without asthma (Vahlkvist and Pedersen 2009, Vahlkvist, Inman et al. 2010).

But physical exertion is a powerful trigger of bronchoconstriction and symptoms in patients with asthma. Symptoms of asthma during exercise may result in avoidance of physical activity leading to detrimental consequences to physical and social well-being of patients with asthma. It is an even more relevant and important problem when considering patients practicing sports. Exercise is a frequent trigger of asthma symptoms, which impairs athlete's performance.

2.2 Asthma in athletes

Although structured exercise on a recreational level has been shown to be beneficial, repeated high-intensity exercise performed by elite athletes contributes to the development of asthma and BHR (Weiler, Anderson et al. 2010).

In recent years there has been a special focus on the increased occurrence of asthma and BHR among top athletes within endurance sports (Carlsen 2009). As early as 1989, an increase in nonspecific bronchial responsiveness after heavy endurance training was found in young competitive swimmers (Carlsen, Oseid et al. 1989). Later, reports were published

concerning increased prevalence of asthma and BHR to methacholine among top cross-country skiers (Larsson, Ohlsén et al. 1993, Heir and Oseid 1994). These and other studies confirmed that both BHR and airway inflammation increased through heavy endurance training (Carlsen, Oseid et al. 1989, Larsson, Ohlsén et al. 1993, Heir and Oseid 1994, Sue-Chu, Karjalainen et al. 1998, Karjalainen, Laitinen et al. 2000). In the Olympic arena, such reports were confirmed by the observed prevalence of EIA of 11% among the American 1984 summer Olympic athletes (Voy 1986); this prevalence increased to >20% among the American participants in the 1996 summer Olympic Games, and was especially high among cyclists and mountain bikers (Weiler, Layton et al. 1998). The use of asthma drugs and, in particular, inhaled β_2 -agonists, was shown to be highest in cross-country skiing and speed-skating followed by cycling, Nordic combined (the combination of both cross-country skiing and ski jumping) and swimming during the last three summer Olympic and the last three winter Olympic Games (Fitch 2012).

So, it is nowadays well established that elite athletes have an increased risk for asthma, especially those who take part in endurance sports, such as swimming or running, and in winter sports (Carlsen, Anderson et al. 2008, Schwartz, Delgado et al. 2008, Fitch 2012). Asthma is a significant problem for both recreational and competitive athletes and is more prevalent than in the general population (Schwartz, Delgado et al. 2008). EIA is actually the most common chronic condition among Olympic athletes (Fitch 2012), with obvious implications for their health, competing performance and quality of life. There are substantial data showing that EIB occurs very commonly in athletes at all levels. Many studies have been performed in Olympic or elite-level athletes that have documented prevalence of EIB varying between 30 and 70%, depending on the population studied and methods implemented (Weiler, Bonini et al. 2007, Parsons, Hallstrand et al. 2013).

2.3 Exercise-induced asthma: definition and heterogeneity

”If from running, gymnastic exercises, or any other work, the breathing become difficult, it is called ASTHMA (αμθσα)”.

The extant of Arataeus, the Cappadocian (100A.D.)

It has long been recognized, even during biblical times, that physical exercise may induce asthma symptoms in susceptible individuals (Chan-Yeung, Malo et al. 2003, Weiler, Bonini et al. 2007). In fact, exercise has been implicated as the most common trigger of an acute asthma attack among elite athletes who have been clinically diagnosed with asthma and it has been estimated that up to 90% of all individuals with asthma are hyperresponsive to exercise. Nevertheless, the term exercise-induced asthma (EIA) only became popular in the 1960s and 1970s when several reports addressed the pattern of airway response to exercise and the influence of drugs on EIA, particularly in children (Jones, Buston et al. 1962, Godfrey 1974, Weiler, Bonini et al. 2007). But asthma induced by sport practicing has not always been easy to describe and recognize, and the concept that exercise may induce bronchial obstruction only in asthmatic patients has been questioned (Bonini 2008). For this reason, in 2008, a Joint Task Force was set up and

defined EIA as exercise-induced symptoms and signs of asthma occurring after intensive physical exercise (Carlsen, Anderson et al. 2008); exercise-induced bronchoconstriction (EIB) was defined as the reduction in lung function occurring after a standardized exercise. This has been a controversial item. The American Academy of Allergy, Asthma & Immunology Work Group Report defined EIA as the condition in which exercise induces symptoms of asthma in patients who have asthma, while the term EIB is used to describe the airway obstruction that occurs in association with exercise without regard to the presence of chronic asthma (Weiler, Bonini et al. 2007). More recently, an American Thoracic Society Clinical Practice Guideline (Parsons, Hallstrand et al. 2013) recommended to abandon the term of EIA (because exercise is not the cause, but only a trigger of asthma) and to name EIB with asthma (EIB_A), the occurrence of bronchial obstruction after exercise in asthmatic patients, and EIB without asthma (EIBw_A), the occurrence of bronchial obstruction in subjects without other symptoms and signs of clinical asthma. The International Olympic Committee (IOC) Independent Asthma Panel defines asthma and validates its diagnosis by the presence of either a positive bronchodilator or bronchoprovocation test. These different positions reflect the difficulties not only in defining asthma, but probably also in understanding distinct mechanisms possible occurring in relationship to asthma heterogeneity. Certainly, EIB that occurs in athletes without other features of clinical asthma has peculiar clinical and pathologic features (Parsons and Mastronarde 2005).

Although some authors consider EIA as a distinct asthma phenotype (Wenzel 2006), there is considerable controversy regarding the heterogeneity of the disorder that we call asthma (Bonini, Rasi et al. 2001, Wenzel 2004, Weiler, Bonini et al. 2007). Defining phenotypes of asthma has been a major objective in recent years, as it would facilitate research into etiology and pathophysiology, targeted treatment and preventive measures, and improve prediction of long-term outcomes (Spycher, Silverman et al. 2010). For athletes with asthma, although it is generally recognized that it is highly unlikely that the asthmatic condition which develops during their sports career is identical to what is usually considered to be asthma in clinical practice (Larsson, Carlsen et al. 2005), there is no evidence until now to support clusters of grouping characteristics. Several elite athletes who are diagnosed with EIA have neither personal or family history of asthma (Rupp, Guill et al. 1992, Rupp, Brudno et al. 1993, Hammerman, Becker et al. 2002, Ali, Norsk et al. 2012), suggesting that environmental factors are more important than genetic inheritance. At rest, they seldom experience asthma symptoms (Lund, Pedersen et al. 2009), but rather they occur during high-intensity exercise.

It has been claimed that athletes' care needs further attention and that more studies are needed to further investigate if and how the asthma phenotype of elite athletes differs from that of classical asthma (Lund, Pedersen et al. 2009). The hypothesis of different phenotypes of asthma occurring in athletes was only approached once in literature, in a review article. Haahtela *et al.* suggested the possibility of two different clinical phenotypes of asthma occurring in elite athletes: the pattern of "classical asthma" characterized by early onset of asthma in childhood, BHR diagnosed by methacholine challenge, atopy and signs of eosinophilic airway inflammation; and another distinct phenotype with onset of symptoms during sports career, bronchial responsiveness to eucapnic hyperventilation test and a variable association with atopic markers and eosinophilic airway inflammation

(Haahtela, Malmberg et al. 2008). However, these phenotypes reflected the experience only with Finnish athletes, and have so far not been fully established. Furthermore, these phenotypes were defined by logistic regression analysis, which is a method centered on the variables employed. Most recent attempts to describe different disease phenotypes have been based on cluster analysis, but these methods have so far not been applied to the population of athletes. Also, no attempt was made to investigate whether the different phenotypes were related to the practice of different sports. As suggested in a recent report, the development of EIA and EIB in athletes is possibly caused by different mechanisms according to three different training environments: 1) those training in cold air; 2) swimmers training in indoor-pools; and 3) those training mainly in ambient dry air (Langdeau, Turcotte et al. 2000, Langdeau and Boulet 2001). Being able to define such distinct phenotypes would give further knowledge and understanding of the underlying mechanisms of asthma in elite athletes, and also would improve diagnosis and treatment; different therapeutic modalities could be specifically applied for the targeted phenotypes, rather than for asthmatic athletes in general, which is the current management approach.

2.4 Mechanisms of asthma in athletes

EIB was initially thought to be secondary to mediator's release from mast cells (Godfrey 1977). This hypothesis was supported by the refractory period observed after a positive exercise challenge, interpreted as the time needed for mast cell recharge, and by the preventive effect offered by mast cell stabilizing agents, such as sodium cromoglycate. Although mediator's release does contribute to cause EIB, pathophysiologic changes induced by intense exercising are definitely more complex (Bonini and Palange 2015). Though the pathogenesis of EIA is not fully elucidated, at present it is widely accepted that it is likely multifactorial and is probably caused by exercise-induced increased ventilation and related changes in airway physiology. An increased ventilatory rate is needed to meet the higher muscular oxygen requirements during exercise. This increased ventilatory rate challenges the ability of the airways to condition the inhaled air to the correct moisture and heat levels before the air reaches the alveoli. Vigorous exercise results in the inhalation of increased volumes of relatively cold and dry air with resulting heat loss from the respiratory mucosa.

One of the classical postulated mechanisms includes the osmotic, or airway-drying, hypothesis. Accordingly, the exercise-induced increased ventilation seems to be the most important factor inducing water loss and mucosal cooling and dehydration (Anderson and Kippelen 2005). At the heart rate of 140 bpm, the amount of exhaled water is approximately four times higher than during rest and equals about 60-70 mL/h. When the temperature of the inspired air and its humidity is 35°C and 75%, respectively, the water loss is 7 mL/h; whereas when these parameters are changed to -10°C and 25%, lung excretion of H₂O increases up to 20 mL/h (Zielinski and Przybylski 2012). The airway surface liquid becomes hyperosmolar as water is evaporated due to the increased ventilation, providing an osmotic stimulus for water to move from any cell nearby, which results in cell shrinkage and release of inflammatory mediators that cause airway smooth

muscle contraction and airway obstruction (Anderson and Kippelen 2008, Carlsen, Anderson et al. 2008, Couto, Silva et al. 2013).

In addition to inflammatory mediators triggered by the osmotic change, the increased ventilation during exercise cools the surface epithelium of the airways. Airway cooling stimulates cholinergic receptors in the airways, increasing airway smooth muscle tone and airway secretions. According to the cooling hypothesis, cold air inhalation results in heat loss from the respiratory mucosa and induces pulmonary vasoconstriction. During and, primarily, post-exercise, a rewarming process begins, which is a physiological consequence of the previous cooling of the airways. The rewarming process would cause secondary hyperemia, and by that increased permeability in capillaries, which contributes to a leakage of fluid from the capillaries to the submucosa. This would result in airway edema in predisposed subjects whereby mast cells are stimulated to release inflammatory mediators, leading to airway inflammation and bronchoconstriction (McFadden 1987, Makker and Holgate 1994, Anderson and Daviskas 2000, Weiler, Bonini et al. 2007, Ali, Norsk et al. 2012).

2.4.1 The inflammatory hypothesis

Studies of airway pathology are scarce, but injury to the airway epithelium, over expression of cysteinyl leukotrienes, relative underprotection of prostaglandin E2 and greater airway eosinophilia have been found to be distinctive immunopathologic features of asthma with EIB, demonstrating an inflammatory basis of EIB (Hallstrand, Moody et al. 2005). Hallstrand *et al.* verified a relationship between columnar epithelial cells in induced sputum and severity of EIB, and an association between the concentration of columnar epithelial cells with the levels of histamine and cysteinyl leukotrienes in the airways, confirming the role of mediators' release (Hallstrand, Moody et al. 2005). Various other investigators have documented that athletes have increased levels of chemical mediators such as histamine, cysteinyl leukotrienes and chemokines, and airway cellular inflammatory markers (Helenius, Ryttilä et al. 1998, Sue-Chu, Larsson et al. 1999, Karjalainen, Laitinen et al. 2000, Lumme, Haahtela et al. 2003). The cellular markers include increased eosinophils (Helenius and Haahtela 2000), neutrophils (Sue-Chu, Karjalainen et al. 1998, Sue-Chu, Larsson et al. 1999), and/or epithelial cells (Hallstrand, Moody et al. 2005, Bougault, Turmel et al. 2009). Recently, it was demonstrated an increased presence of damage-associated molecular patterns (DAMPs) in the sputum of athletes (Seys, Hox et al. 2015), which may feature as early inducers of the proinflammatory cytokines.

Interestingly, the inflammatory markers observed in athletes airways are not consistently related to lung function, BHR or disease exacerbations (Karjalainen, Laitinen et al. 2000, Helenius, Ryttilä et al. 2002, Vergès, Devouassoux et al. 2005, Schwartz, Delgado et al. 2008). Studies on the effects of asthma medication, such as the anti-inflammatory drugs montelukast (Helenius, Lumme et al. 2004) and inhaled corticosteroids (Sue-Chu, Karjalainen et al. 2000, Hoshino, Koya et al. 2015) in elite ice hockey players and cross-country skiers have reported no beneficial or only partial effect on asthma-like symptoms, BHR or airway cellular inflammation. Sue-Chu *et al.* showed

that during one competitive winter season, adolescent cross-country skiers developed signs of inflammation in their bronchial biopsies whether they were asthmatic or not (Sue-Chu, Karjalainen et al. 1998). Therefore, it has recently been proposed that the increased airway inflammatory cells in athletes represent physical injury secondary to rigorous hyperpnoea that heals with rest and may not be the initial factor implying detrimental effects on respiratory health (Sue-Chu, Larsson et al. 1999, Helenius, Ryttilä et al. 2002, Bonsignore, Morici et al. 2003, Hallstrand, Moody et al. 2005, Carlsen, Anderson et al. 2008, Schwartz, Delgado et al. 2008, Moreira, Palmares et al. 2011).

In recent years, exhaled breath temperature (EBT) has been investigated based on the assumption that inflammation of the airways would influence the temperature of the air coming from the alveoli (Paredi, Kharitonov et al. 2002) and would be related to the degree of airway inflammation. In fact, it was shown that in asthmatic adults and children EBT correlates with the bronchial blood flow, fraction of exhaled nitric oxide (FeNO), and number of sputum eosinophils (Piacentini, Bodini et al. 2002, Paredi, Kharitonov et al. 2005, Piacentini, Peroni et al. 2007, Popov 2011). It was recently observed a significant increase in EBT after exercise in a group of allergic asthmatic children (Peroni, Chinellato et al. 2012) and in asthmatic patients compared to healthy controls (Svensson, Nilsson et al. 2012). Evidence of both the cooling and inflammatory hypotheses acting as mechanisms of EIA would determine asthmatic athletes to present higher EBT, during or just after exercise, but this has not been previously investigated.

2.4.2 The airway epithelial damage hypothesis

Vigorous exercise affects the airway epithelium (Kippelen and Anderson 2012), which has likewise been linked to EIA and bronchoconstriction in elite athletes. Injury of the distal airway epithelium was originally demonstrated after hyperpnea with dry air in animals (Omori, Schofield et al. 1995, Freed, Wang et al. 1999, Davis and Freed 2001) and after endurance training in mice and half marathon runners (Chimenti, Morici et al. 2007, Chimenti, Morici et al. 2010). Strenuous exercise characteristic of endurance sports is believed to have a detrimental effect on the respiratory epithelium structure integrity, which would turn it more prone to asthma (Anderson and Kippelen 2008). The evidence to support this proposal comes from markers of injury. It has also been shown experimentally that epithelial damage heals slower in cells from asthmatic airways, but that this process is speeded up by steroids (Freishtat, Watson et al. 2011).

Clara cell secretory protein (CC16) has been used as a peripheral marker for assessing the epithelial barrier disruption in the lower airways (Broeckaert and Bernard 2000, Bernard, Carbonnelle et al. 2007). It has been previously shown that exercise induces epithelial stress by demonstrating that CC16 is increased after exercise in urine (Bolger, Tufvesson et al. 2011, Romberg, Bjermer et al. 2011) and in serum (Nanson, Burgess et al. 2001, Carbonnelle, Francaux et al. 2002, Chimenti, Morici et al. 2010), as well as after eucapnic voluntary hyperventilation in athletes (Bolger, Tufvesson et al. 2011). In a recent study, CC16 in plasma was shown to be correlated to EBT after exercise challenge, suggesting an overall epithelial involvement (Tufvesson, Svensson et al. 2013). However, no difference was found between asthmatics and healthy controls (Tufvesson, Svensson et

al. 2013), which may indicate a physiological rather than a pathological response to exercise. Also, it may only be related to exercise and hyperpnea, since previous studies have shown that urinary CC16 levels are increased after a swimming exercise challenge, but not after mannitol challenge (Romberg, Bjermer et al. 2011).

Besides the repeated training activity with high ventilation, also the environmental conditions in which training is performed could further contribute both to increased airways epithelial damage and inflammation. During performance of their sport, swimmers are exposed to high levels of chlorine and chlorination by-products, such as trichloramines, that may cause further damage to the airways (Catto, Sabrina et al. 2012). In accordance, it has been observed that acute exposure to these lung irritants produces an increase in CC16 (Broeckaert and Bernard 2000). On the other hand, the same group has later reported reduced serum levels of CC16 in children exposed to chlorinated swimming pools compared to nonswimming children both before and after outdoor exercise (Lagerkvist, Bernard et al. 2004); these results led to speculate that repeated exposure to chlorination by-products may have adverse effects on the Clara cell function in children.

Also, it has been shown that bronchial epithelial cells are released in higher amounts into sputum (*epithelial shedding*) of cold air athletes and swimmers (Bougault, Turmel et al. 2009) highlighting the impact on epithelial layer. The high degree of persistent epithelial cell shedding is not associated with airway inflammation but suggested significant underlying airway damage (Bougault, Turmel et al. 2009). It should be noted, however, that the statistical significance of this finding seemed to be due to some outliers rather than due to the majority of the athletes. Moreover, a damaged airway epithelium might result in release of DAMPs in the airways of athletes (Seys, Hox et al. 2015) since both bronchial epithelial cells as well as alveolar macrophages release DAMPs (Ferhani, Letuve et al. 2010).

Contradictory results have recently been obtained regarding the occurrence of bronchial epithelial damage in athletes. Although signs of injury to the airway epithelium have been observed at rest in swimmers (Bougault, Turmel et al. 2009) and after half-marathon races in nonasthmatic runners (Chimenti, Morici et al. 2010), no such evidence was obtained at rest in cold air athletes (Bougault, Turmel et al. 2009) or after a marathon in nonasthmatic runners (Bonsignore, Morici et al. 2003). Moreover, in swimmers and cold weather athletes, no association was found between baseline epithelial cell count in sputum and BHR (Bougault, Turmel et al. 2009). Further studies conducted in athletes suggest that increased ventilation related to exercise causes transient disruption of the airway epithelium *independently* of bronchoconstriction (Morici, Bonsignore et al. 2004, Chimenti, Morici et al. 2010, Kippelen, Larsson et al. 2010, Bolger, Tufvesson et al. 2011). It therefore remains to be determined whether damage of the airway epithelium contributes to EIB in athletes. As previously shown in animal (Freed, Wang et al. 1999) and human (Bolger, Tufvesson et al. 2011) studies, the distresses to the airway epithelial barrier induced by hyperpnea seem to be only transient. The restorative process after injury involves plasma exudation and movement of cells into the airways, a process repeated many times during a season of training (Ali, Norsk et al. 2012). It is possible that periods with heavy training and periods with frequent and repeated competitions may maintain the epithelial damage as a stimulus for inflammation, but in periods with less heavy training the epithelial barrier is restituted reducing the inflammatory stimulus.

2.4.3 The neurogenic hypothesis

The observation that the exaggerated bronchoconstriction to non-immunological stimuli could be inhibited by atropine, which blocks post-ganglionic vagal pathways, suggested the involvement of a parasympathetic neural reflex in the constrictor response of asthma (Barnes 1986, Goyal, Jaseja et al. 2010). With the identification of non-adrenergic, non-cholinergic (NANC) system and neuropeptides, the focus has shifted from asthma as a pure immunological disease to a complex interaction and/or disbalance in immunological and neurogenic mechanisms (Goyal, Jaseja et al. 2010). In fact, the parasympathetic system plays an important role in the regulation of airway tone and bronchial secretory activity (Langdeau and Boulet 2001). The cholinergic-parasympathetic nerves stimulate bronchoconstriction, whereas β_2 -adrenergic sympathetic and/or non-cholinergic parasympathetic nerves bronchodilate (Canning and Fischer 2001, Carlsen, Anderson et al. 2008, Schwartz, Delgado et al. 2008). In humans, the predominant innervation at the bronchial level is parasympathetic in nature (Barnes 1987) while the sympathetic (adrenergic) innervation is rather sparse (Langdeau and Boulet 2001). Mechanisms of modulation of bronchial tone and their possible role in the development of BHR need to be further investigated but with respect to EIB, cooling of the airways caused by hyperpnea of cold air induces reflex parasympathetic nerve stimulation leading to bronchoconstriction through stimulation of the vagal nerve (Deal, McFadden et al. 1978, Carlsen 2011).

An individual and exercise-specific etiologic factor for EIB may be the autonomic dysregulation associated with high-intensity and prolonged physical training (Langdeau and Boulet 2001). Cross-sectional studies show that endurance trained subjects have higher parasympathetic activity than untrained subjects (Knöpfli and Bar-Or 1999, Carter, Banister et al. 2003, Filipe, Falcão-Reis et al. 2003, Kaltsatou, Kouidi et al. 2011). Intensive endurance training is believed to have an effect upon autonomic regulation promoting *vagal activity predominance* (Goldsmith, Bigger et al. 1997) as a compensatory response to the sympathetic stimulation associated with frequent and intense training. The vagal hegemony induces not only the well-known resting bradycardia of athletes (Carter, Banister et al. 2003) but given that the parasympathetic system modulates airway tone, it is logical to suppose that hyperactivity of that system would produce an *increase in basal bronchomotor tone and contribute to a greater likelihood of developing BHR* (Langdeau and Boulet 2001). A therapeutic *effect of anticholinergic drugs*, such as ipratropium bromide, would be expected in these athletes, since the action depends on vagal activity (Knöpfli, Bar-Or et al. 2005).

The dysfunctional neuroendocrine-immune interface may also play a role in the pathogenesis of EIB due to the release and *action of neuropeptides* from primary sensory nerve terminals, in a so called *neurogenic inflammation pathway* (Moreira, Delgado et al. 2011, Couto, de Diego et al. 2013). Tachykinins are potent neurogenic mediators of a number of functions in the airways (Groneberg, Harrison et al. 2006). Activation of substance P and receptors of neurokinins in the airways induces pronounced and readily quantifiable effects in the lungs, such as bronchospasm, vasodilatation, vascular leakage and mucus secretion (Ramalho, Soares et al. 2011). Increased circulating levels of substance P, one of the major initiators of neurogenic inflammation, have been found after

strenuous exercise (Lind, Brudin et al. 1996). Also, intense and prolonged physical exercise is associated with increased nerve growth factor serum levels in athletes (Bonini, Fioretti et al. 2013).

2.5 Risk factors for asthma in athletes

Exercise-induced asthma may be modulated by the baseline condition of the patient or by sport-specific characteristics. For instance, with pre-existent airway inflammation or BHR, such as in a patient with allergic asthma, an amplification of these mechanisms should be expected. Athletes practicing regular strenuous exercise may be at increased risk of upper respiratory tract infection during periods of heavy exercise and for a couple of weeks following competition events (Peters and Bateman 1983, Peters 1990). The quality of inspired air is dependent on the type of exercise (e.g., indoor/outdoor) (Katelaris, Carrozzi et al. 2000) and sport-specific conditions (e.g., winter and water sports) (Weiler, Bonini et al. 2007). Also, it has long been recognized that *atopy* is positively associated with asthma and increased BHR in athletes (Helenius, Tikkanen et al. 1998-a, Helenius, Tikkanen et al. 1998-b). It is known that extrinsic factors, together with intrinsic factors, can affect both the athlete's quality of life and athletic performance (Sacha and Quinn 2011). However, uncertainty remains regarding the relative contributions of these factors in explaining the high degree of bronchospasm seen in various populations of athletes (Sacha and Quinn 2011).

Both *training in cold air* (Helenius, Tikkanen et al. 1998-a) *and swimming* (Helenius, Tikkanen et al. 1998-b) were identified as risk factors for asthma. The prevalence of asthma and EIB is highest in athletes who are exposed to environments with high concentrations of particulate matter and/or gaseous irritants (Rundell 2004, Fisk, Steigerwald et al. 2010) such as indoor swimming pools (Bernard 2007). The deposition of pollutants is greater during high-ventilation exercise than at rest, allowing more irritants to reach the distal airways (Daigle, Chalupa et al. 2003) and athletes participating in high-ventilation sports, such as cross-country skiing, long-distance running, and swimming are at greater risk of developing EIB compared with athletes in low-ventilation sports (Parsons and Mastronarde 2005). In fact, it has been shown that swimming has the highest prevalence of asthma/BHR in comparison with the other aquatic disciplines; and that endurance aquatic disciplines have a higher prevalence of asthma/BHR than the aquatic non-endurance disciplines (Mountjoy, Fitch et al. 2015). The same study found that in comparison with other Olympic sports, swimming, synchronized swimming, and open water swimming were among the top 5 sports for asthma/BHR prevalence (Mountjoy, Fitch et al. 2015). Also, other recent study evidenced that asthma was present in 60% of swimmers and 29% of cold-weather athletes compared to only 17% of non-athlete controls (Bougault, Turmel et al. 2010). For athletes practicing water and winter sports, in addition to frequent episodes of prolonged hyperpnoea, their sports performance demands exposure to potentially sport-specific environmental noxious stimuli (Sacha and Quinn 2011, Price, Ansley et al. 2013). Increased neutrophil counts in induced sputum from both swimmers

and winter sports athletes have been observed, and interestingly they were correlated with the number of training hours per week in both groups (Bougault, Turmel et al. 2009).

Hyperpnoea with cold dry air represents a significant environmental stress to airways and it has been previously reported that exposure to cold air cause parasympathetic stimulation of airways, contributing to EIB (McFadden and Ingram 1979). It has been observed that during one competitive winter season, adolescent cross-country skiers develop signs of inflammation (lymphoid follicles and deposition of tenascin) in their bronchial biopsies (Sue-Chu, Karjalainen et al. 1998).

The association between asthma in relation to indoor swimming facilities (Bernard, Carbonnelle et al. 2003, Bernard 2007, Bernard, Carbonnelle et al. 2007) is likely the result of the chemical disinfectants used in pools and the poor air circulation in these indoor facilities, often referred to as the “chlorine hypothesis”. Competitive swimmers with allergic asthma show a mixed type of airway inflammation (Moreira, Delgado et al. 2008), a probable consequence of both endurance training and chronic exposure to a chlorine-rich atmosphere and microaspiration of water droplets. It has been argued that an increase in neutrophils is a consequence of endurance training and that increased eosinophils and lymphocytes result from exposure to swimming environmental factors related to the sport (Bonsignore, Morici et al. 2003, Pedersen, Lund et al. 2008). In recent years, it was observed that regular pool attendance, especially in young children, was associated with lung hyperpermeability and increased risk of developing asthma later in life (Bernard 2007, Voisin, Sardella et al. 2010). Children who participated in an infant swimming program were found to have a $\geq 15\%$ decrease in FEV₁ and changes in biomarkers (CC16/surfactant-associated protein D ratio) associated with lung epithelial damage by the age of 13 years (Bernard, Carbonnelle et al. 2007). Teenagers who participated in infant swimming also had more frequent symptoms of asthma and were 3 times as likely to test positive for EIB, have physician-diagnosed asthma, and suffer from recurrent bronchitis (Bernard, Carbonnelle et al. 2007). In two studies, the presence of chloramines in the air of swimming pools was associated with an increased prevalence of allergic symptoms (conjunctivitis, rhinitis, laryngitis) and asthma in elite swimmers (Thickett, McCoach et al. 2002, Goodman and Hays 2008). However, a meta-analysis examining the relationship between swimming and asthma revealed no clear evidence that childhood exposure to swimming is associated with an increased risk of asthma (Goodman and Hays 2008). Despite this evidence, controversy still surrounds the issue of childhood swimming. Childhood swimming and new-onset childhood asthma have clear implications for public health. If attendance at indoor pools increases the risk of childhood asthma, then concerns are warranted and action is necessary. If there is no such relationship, these concerns could unnecessarily discourage children from indoor swimming and/or compromise water disinfection (Weisel, Richardson et al. 2009).

The high prevalence of asthma among swimmers compared to the other aquatic disciplines points to other etiologic factor than only environmental exposure, such as training intensity, type, and/or duration (Mountjoy, Fitch et al. 2015). This finding emphasizes the need for further research to determine the etiologic mechanism of asthma in swimmers including long term follow-up studies. Until now, the different impact of the environmental exposures as risk factors for asthma and their contribution for the complex etiopathogenesis has not been fully understood.

2.6 Long-term effects of sports on the airways of athletes

The development of asthma in previously healthy endurance athletes is characterized by exacerbations caused by repetitive exhaustive physical exercises, and occurs after years of intensive training within endurance sports, most often in cross-country skiers and swimmers. Not only the prevalence of asthma is higher among swimmers and winter athletes compared to other sports, but also the development of BHR is known to increase with age (Stensrud, Mykland et al. 2007, Pedersen, Lund et al. 2008, Bougault and Boulet 2012). Of the 193 athletes competing at the 2006 Winter Olympics who met the IOC's criteria for approval of use of asthma drugs in sports, only 32.1% had childhood asthma and 48.7% of athletes reported onset at age 20 years or older (Fitch 2006). These findings lead to speculation that the relatively late onset of asthma/BHR in many older athletes is related to the years of endurance training (Fitch 2012). However, our current knowledge of the natural history of asthma in elite athletes is incomplete, and further studies are clearly needed.

Concerns have been raised about long-term effects of sport practice. In a recent study, Knöpfli et al. studied seven athletes from the Swiss national triathlon team, who at baseline were characterized as nonasthmatic, not treated with anti-asthmatic medication, and who had performed at international level for at least three consecutive years (Knöpfli, Luke-Zeitoun et al. 2007). Running tests were conducted on a 400 meters track for 8 minutes at intensities equal to the anaerobic threshold; and the study revealed a five times greater prevalence of EIA, defined as a post-exercise fall in FEV₁ of more than 10%, in elite athletes compared to the prevalence of asthma in the general population. After extrapolation of the decrease in FEV₁ in all seven athletes, the limit of 10% was determined to occur within 1.77–4.81 years, resulting in 21–57% of athletes with newly developed BHR per year (Knöpfli, Luke-Zeitoun et al. 2007).

For a long time it was not known if athletes with asthma had simply chosen swimming based on recommendations that swimming is less likely to induce asthma attacks compared to other sports or if the increased prevalence of asthma in elite swimmers was a direct consequence of swimming. The possibility of swimming increasing the risk of asthma has been regarded with concern and there is a clear need of follow-up studies that assess the temporal relationship between asthma and swimming taking into account exposure assessment, volume and intensity of training and potential confounders.

What happens after stopping swimming remains largely unknown. Increased numbers of both eosinophils and mast cells were observed in bronchial biopsies of competitive adult swimmers, leading to believe that airway inflammation and hyperresponsiveness develop during the training career. While the later seems to be a transient phenomenon (Bougault, Turmel et al. 2011), whether airway inflammation persists is still debated (Helenius, Ryttilä et al. 2002, Bougault, Turmel et al. 2009, Bougault, Loubaki et al. 2012). Research is necessary to determine how many athletes will continue to experience asthma/BHR in the years after they cease intensive endurance training (Fitch 2012).

Only one study prospectively assessed this issue in swimmers: in 42 Finnish elite swimmers, after a 5 year follow up, BHR and asthma attenuated or even disappeared in those who stopped high-level training, while mild eosinophilic airway inflammation was aggravated among those who remained active (Helenius, Ryttilä et al. 2002). A detailed

knowledge of remission as assessed by objective testing and natural course of the disease in former elite athletes is still missing and should be investigated (Ali, Norsk et al. 2012).

2.7 The importance of appropriate management

It has been claimed that athletes' care needs further attention (Lund, Pedersen et al. 2009). The diagnosis of asthma in this population is crucial because of potential implications not only on their general health, but also on their competing performance. Recent data indicate that asthma is often underdiagnosed and very often undertreated (Bonini, Gramiccioni et al. 2015). Asthma in elite athletes needs adequate attention and management considering that 23.1% of the 263 sudden deaths in athletes reported by Becker *et al.* occurred in asthmatic athletes (Becker, Rogers et al. 2004).

However, setting a correct diagnosis is often challenging and poses several issues unique to this population (Couto, Moreira et al. 2012). The symptoms are often mild to moderate in severity and may cause impairment of athletic performance, but frequently are not severe enough to cause significant respiratory distress (Parsons, Hallstrand et al. 2013). Actually, symptoms have been shown to be poor predictors of asthma in athletes (Rupp, Guill et al. 1992, Rupp, Brudno et al. 1993, Rundell, Im et al. 2001, Bonini, Gramiccioni et al. 2015). The heavy training with the extremely high level of physical fitness and maximal oxygen uptake ($\dot{V}O_{2max}$) makes it difficult to discriminate between physiological and pathological limitations to maximum exercise (Carlsen, Anderson et al. 2008). On the other hand, to avoid asthmatic stigma and doping concerns by fellow athletes, some athletes may be poorly motivated to complain of respiratory symptoms (Couillard, Bougault et al. 2014); in fact, it has recently been evidenced that compliance to treatment in Olympic athletes was very poor (Bonini, Gramiccioni et al. 2015). There is a lack of education in athletes and coaches regarding the recognition of exercise-induced respiratory symptoms, the high EIB/BHR prevalence in the athlete population, and the effects of asthma medication, which could contribute to inadequate symptom perception, especially in young athletes (Couillard, Bougault et al. 2014). Interestingly, a previous study underlined that the elite swimmer population is characterized by a high prevalence of symptomatic and asymptomatic BHR and/or EIB, whereas winter sport athletes often report exercise-induced cough but with a prevalence of BHR and/or EIB similar to controls (Bougault, Turmel et al. 2010). This was recently confirmed in other study on cross-country skiers (Bordeleau, Turmel et al. 2014). In a review article devoted to this issue, it was highlighted that prevalence of physician-diagnosed asthma, EIA and BHR to methacholine or other agents are homogeneous for various athletes such as sprinters, long-distance runners, football and basketball players and other track and field athletes, but for athletes training in cold air environment and for swimmers the prevalence of reported BHR is quite high; surprisingly, the prevalence of physician-diagnosed asthma is low (Langdeau and Boulet 2001). Taken together these observations raised the question: Should a systematic screening for EIB be done in athletes, especially swimmers? (Bougault, Turmel et al. 2010).

A number of organizations and investigators advocate screening for asthma in athletes (Holzer and Brukner 2004, Dickinson, Whyte et al. 2005, Dickinson, Whyte et al. 2006). This recommendation is pertinent and, indeed, some sporting organizations have established EIB screening programs for their internationally competitive athletes (Wilber, Rundell et al. 2000, Dickinson, Whyte et al. 2005). Yet, to date, expert working groups have not directly addressed EIB screening policy (Weiler, Bonini et al. 2007, Carlsen, Anderson et al. 2008, Schwartz, Delgado et al. 2008), and so in Portugal there aren't any screening programmes.

Since the 2002 Salt Lake City Games, the International Olympic Committee's Independent Asthma Panel required testing to validate asthma diagnosis and justify the use of inhaled β 2-agonists in Olympic athletes - either a positive bronchodilator or bronchoprovocation test (Medical Commission of the International Olympic Committee 2002, Fitch, Sue-Chu et al. 2008). This strategy has provided valuable guidelines to the practicing physician. This program was educational and documented the variability in the prevalence of asthma and/or BHR and inhaled β 2-agonist's use between different sports and different countries. It provided a standard of care for the athlete with respiratory symptoms. In 2009, the World Anti-Doping Agency (WADA) followed the IOC approach, extending these guidelines to all other athletes, requiring objective evidence of asthma diagnosis to allow the use of inhaled β 2-agonists (IBAs). In Portugal, changes to the WADA 2009 Prohibited List permitted rigorous screening of asthmatic athletes due to the implementation of objective criteria for inhalers use. The WADA guidelines on asthma, however, have changed in recent years: for instance, since 2012, IBAs are prohibited and require a therapeutic use exemption (TUE), except for salbutamol, formoterol and salmeterol when taken according to manufacturer's instructions and not exceeding the published doses. Data are lacking regarding the impact of these changes in athlete's health and medical care.

3. AIMS OF THE THESIS

The general aim of the present study was to investigate **phenotypes of asthma, and mechanisms and effects of airway damage in elite athletes.**

Specific questions for the study programme were:

- How the definition of asthma in elite athletes impacts on its management (**study I**) and are there different phenotypes of asthma under this definition (**study II**)?
- What is the relation between parasympathetic activity with asthma and bronchial hyperresponsiveness in elite swimmers? (**study III**), and how inhaled anti-cholinergic drugs perform compared with β 2-agonists in elite athletes? (**study IV**)
- Is the heat loss hypothesis supported by acute changes after vigorous exercise and does it relate to airway's inflammation? (**study V**)
- What are the long-term effects of competitive swimming on airway's inflammation? (**study VI**)

4. MATERIAL AND METHODS

4.1 Participants and study design

This thesis is based on three types of studies:

1) *Observational studies*

- a. Retrospective analysis of asthma medication requests submitted by elite athletes older than 16 years to the Anti-Doping Authority of Portugal (ADOP) between 2008 and 2010 to analyze the impact of asthma and anti-doping regulations in elite athletes' health (**study I**).
 - b. Prospective studies: evaluation of elite swimmers to assess the effect of a swimming training session on EBT from the airways of both asthmatic and non-asthmatic elite athletes (**study V**); investigation if long-term swimming is associated with airway injury in swimmers (**study VI**).
- 2) *Cross-sectional study* of asthmatic elite athletes to assess different phenotypes of asthma (**study II**); examination of elite swimmers to investigate the association between asthma, bronchial hyperresponsiveness and parasympathetic activity measured by pupillometry (**study III**).
- 3) *Randomized cross-over trial* to compare reversibility to inhaled ipratropium bromide with reversibility to inhaled salbutamol in elite winter athletes and to explore the relation between PD₂₀ obtained with methacholine challenge and reversibility to each drug (**study IV**).

A total of 127 swimmers participated in observational prospective studies, 22 elite winter athletes participated in the randomized cross-over trial and 27 elite swimmers in one of the cross-sectional studies (**Figure 1**). Data from asthma medication requests submitted to the ADOP referring to 326 elite athletes, as well as data from clinical files of elite athletes at the Portuguese database of Olympic athletes and at database of Norwegian School of Sport Sciences were also analyzed (n=324). A summary of studies design and subjects is presented in **Table 1**.

Figure 1 Swimmers from Futebol Clube do Porto (FCP) main swimming team, included in studies III and V.



Table 1. Summary of subjects and studies designs.

Study	Study design and subjects	Asthma	Gender (M/F)	Age	Duration	Intervention
I	Observational, retrospective 326 requests for asthma medication	326 (100%)	254/72	27±9	2 years	n.a.
II	Cross-sectional 324 elite athletes	150 (46)	107/43	25±7	n.a.	n.a.
III	Cross-sectional 27 elite swimmers	11 (41)	14/13	17±3	n.a.	n.a.
IV	Randomized, cross-over 22 elite winter athletes	16 (72)	14/8	26±5	2 weeks	BD with inhaled ipratropium bromide and inhaled salbutamol
V	Observational 22 elite swimmers	10 (45)	10/12	17±3	1 swimming training session	n.a.
VI	Observational, prospective 105 competitive non-elite swimmers	12 (11)	61/44	14±6	3 years	n.a.

Data are presented as counts (%) or mean±SD. **BD**: Bronchodilation; **n.a.**: non-applicable.

4.1.1 Diagnosis of asthma and healthcare of asthmatic athletes (Study I)

A retrospective analysis of asthma medication requests submitted to the Anti-Doping Authority of Portugal between 2008 and 2010 was carried out. The study sample included all athletes older than 16 years who requested permission to use ICS and/or inhaled β_2 -agonists for over 3 months between 2008 and 2010. Those requesting the use for shorter periods of time (e.g. less than three months) were excluded.

The study protocol included collection of data on respiratory symptoms, medication requested, results from spirometry, bronchodilation tests, bronchial challenges, FeNO levels and allergic sensitization defined by the presence of at least one positive skin prick test and/or positive specific IgE. Asthma diagnosis was established according to criteria set by IOC to document asthma in athletes (Medical Commission of the International Olympic Committee 2002, Carlsen, Anderson et al. 2008). The years of 2008, 2009 and 2010 were compared to assess the impact of changes in guidelines throughout the years.

4.1.2 Phenotypes of asthma in elite athletes (Study II)

An analysis of elite athletes records kept in databases files was performed. In Portugal, registries available at the Anti-doping Authority of Portugal and the Portuguese database of Olympic athletes were used; in Norway, database of medical files of Norwegian School of Sport Sciences was analyzed, including Olympic athletes participating in the 2008 summer and the 2010 winter Olympic Games. Both Portuguese and Norwegian athletes training at high competitive levels (national, international or Olympic teams) were identified through institution databases and those with available information on symptoms,

lung function and airway inflammation, BHR, and atopy were selected. Healthy athletes and those with other conditions rather than asthma were excluded.

Study protocol included the collection of data about demographics (age, gender, height, weight and sport practiced), presence of respiratory symptoms, current use of asthma medication, presence of rhinitis or other allergic diseases (conjunctivitis, urticaria, eczema, anaphylaxis and drug, food and venom allergies) previously identified through AQUA[®] questionnaire (Bonini, Braido et al. 2009), lung function and reversibility, airway inflammation, BHR, and allergic sensitization. The first ever performed spirometry and the first ever performed bronchial provocation challenge, respectively, were used.

From all reviewed files, 324 files had complete information available and informed consent for data use (**Table 2**).

Table 2. Features of athletes screened at the Anti-Doping Authority of Portugal and at the Norwegian School of Sports Sciences databases.

	Asthmatic athletes (n=150)	Non-asthmatic athletes (n=174)	P
Male, n (%)	107 (71)	89 (51)	<0.001^e
Age, years	25 (14 – 40)	26 (16 - 38)	0.251 ^d
BMI, Kg/m²	23 [23;24]	23 [22;23]	0.06 ^c
Physician reported rhinitis, n (%)	54 (36)	33 (19)	0.003^e
Other allergic disease, n (%)	20 (13)	26 (15)	0.750 ^e
Allergic sensitization, n (%)	89 (59)	58 (33)	<0.001^e
Respiratory symptoms, n (%)[*]	138 (92)	89 (51)	<0.001^e
Dyspnea/ heavy breathing	48 (32)	20 (11)	<0.001^e
Chest tightness	12 (8)	11 (6)	0.379 ^e
Wheezing	42 (28)	15 (9)	<0.001^e
Cough	44 (29)	33 (19)	0.002^e
Tiredness	1 (0.7)	1 (0.6)	0.427 ^f
Phlegm	18 (12)	15 (9)	<0.001^e
Asthma treatment, n (%)			<0.001^f
Inhaled steroids alone	9 (6)	1 (0.6)	
Beta-2-agonists alone	13 (9)	2 (1)	
Inhaled steroids + beta-2-agonists	96 (64)	13 (8)	
Airway obstruction^a, n (%)	43 (29)	21 (12)	<0.001^e
FVC			
L	5.4 [5.1;5.7]	5.2 [5.0;5.4]	0.41 ^c
% of predicted	114 [110;117]	112 [109;116]	0.60 ^c
FEV₁			
L	4.1 [3.9;4.4]	4.3 [4.1;4.4]	0.06 ^c
% of predicted	101 [96;106]	109 [106;111]	0.001^c
FEV₁/FVC	69 [65;74]	76 [72;80]	0.012^c
Reversibility^b, n (%)	26 (17)	1 (0.6)	0.037^f
Airway hyperresponsiveness, n (%)	126 (84)	51 (29)	<0.001^e
FeNO, ppb	33 (6 – 213)	19 (4 – 70)	0.01^d

Data presented as counts (%) and mean [95% Confidence interval] except for age and FeNO which are presented as median (min-max); **BMI**: Body mass index; **FeNO**: fraction of exhaled nitric oxide; **L**: liters; **FVC**: forced vital capacity; **FEV₁**: forced expiratory volume in one second. ^a Defined as a FEV₁/FVC ratio <0.70; ^b Defined as an increase in FEV₁ ≥200 mL and ≥12% from baseline; ^c Independent samples t-test; ^d Independent samples Mann-Whitney U-test; ^e Chi-square test; ^f Fisher's exact test. *Some athletes presented more than one symptom.

The type of sport practiced was classified according to environmental training conditions given the previous suggestion that three different training environments could be related to different mechanisms of asthma (Langdeau, Turcotte et al. 2000, Langdeau and Boulet 2001):

- *water* sports (swimming and water polo);
- *winter* sports (cross-country skiing, biathlon, skeleton, alpine skiing, and ski cross);
- *other* sports (handball, judo, triathlon, football, cycling, beach volley, rowing, athletics, sailing, badminton, canoeing, curling, equestrian, taekwondo, auto-racing, billiards, paragliding, rugby, tennis, roller hockey, kickboxing, fencing, basketball and golf).

Asthma diagnosis was established by a medical doctor according to criteria set by the IOC to document asthma in athletes (Medical Commission of the International Olympic Committee 2002, Carlsen, Anderson et al. 2008), using objective evidence of either reversibility after bronchodilator administration or evidence of BHR after a bronchial provocation challenge. Of these 324 athletes, 150 athletes fulfilled asthma criteria and were included for Latent Class Analysis to define phenotypes.

4.1.3 Parasympathetic activity and bronchial hyperresponsiveness (Study III)

Elite swimmers of the Futebol Clube do Porto (FCP) main swimming team were invited to participate. Recruitment was made through an invitation letter sent to all swimmers. Athletes aged above 14 year-old, who agreed to take part in the study, were enrolled. To be included, participants had to be competitive swimmers, free from any respiratory infection in the 2 weeks before testing, not to drink coffee or smoke or perform exercise on the testing day, not to wear contact lenses and withdraw their asthma medication 48 hours before (except for inhaled corticosteroids, which were asked to be suspended for at least 2 weeks prior to the study). Subjects who met any of the following criteria were excluded from the study: under any systemic medication with central nervous system effects; any topical eye treatment; systemic conditions with known ocular involvement; orbit structure damage or surrounding soft tissue with open lesion or edema at the day of testing; past history of ocular abnormalities or trauma; pregnancy; recent episode of hemoptysis; forced expiratory volume in the first second (FEV₁) lower than 60% of the predicted value or 1.5 L; orally administered corticosteroids in the last month; neurological or psychiatric illness; lack of collaboration or presence of diseases that limit the patient's ability to carry out the tests; recent stroke or heart attack or malignant diseases.

The study was developed in two visits. The first visit was performed between 8 to 11 am due to the circadian rhythm of pupil's sizes (Fountas, Kapsalaki et al. 2006). Medical history and potential medication were reviewed to determine eligibility. The eligible subjects answered a structured questionnaire, and performed pupillometry, skin prick tests, spirometry and reversibility to salbutamol. The second visit took place on a different day and a bronchial challenge with methacholine was performed to assess BHR. In both visits,

swimmers were asked to withhold anti-asthmatic and anti-allergic medication, according to guidelines (American Thoracic Society 2000).

Asthma diagnosis was established according to criteria set by IOC to document asthma in athletes (Medical Commission of the International Olympic Committee 2002, Carlsen, Anderson et al. 2008). Swimmers who fulfilled asthma diagnostic criteria were compared to healthy swimmers regarding parasympathetic activity and relation to BHR.

4.1.4 Anti-cholinergic drugs and BHR in elite athletes (Study IV)

Twenty cross-country skiers and two biathlon skiers, all members of the Norwegian national teams, were included. All subjects were high-level athletes competing at a top international level. All athletes had been free from any respiratory disease for the last three weeks before the first study day, and refrained from exercise and any food or drink containing nitrate on the testing day. Subjects were asked to withhold medication, according to guidelines (American Thoracic Society 2000).

The athletes attended one visit at the laboratory at the Norwegian School of Sport Sciences in Oslo for assessment of lung function, BHR to methacholine and FeNO. Prior diagnoses of asthma, EIB, allergic rhinitis and current symptoms of dyspnea, phlegm and cough during or after exercise, and use of asthma medication during the previous year were recorded with the AQUA[®] questionnaire (Bonini, Braido et al. 2009) and clinical interview. Later, two reversibility tests were obtained during a training camp in Val Senales, Italy, 2000 meters above sea level. The reversibility tests with inhaled ipratropium bromide and with inhaled salbutamol were performed in a randomized order on two separate days, with 24 hours between each test.

Results from reversibility to inhaled ipratropium bromide and reversibility to inhaled salbutamol were compared for each athlete as well as the relation between PD₂₀ obtained with methacholine challenge and reversibility to each drug.

4.1.5 Exhaled breath temperature, exercise and asthma (Study V)

Elite swimmers of the FCP main swimming team who had been screened for asthma and atopy at the beginning of the training season (annual screening) were invited to the present study. In order to be eligible to participate in this study, a subject had to meet all the following criteria: competitive level swimmer; aged ≥ 14 year-old; training ≥ 10 hours per week; free from respiratory infection in the last 3 weeks; provided signed and dated informed consent. A potential subject who met any of the following criteria was excluded: pregnancy; recent episode of hemoptysis; forced expiratory volume in the first second (FEV₁) lower than 60% of the predicted value or 1.5 L; orally administered corticosteroids in the last month; neurological or psychiatric illness; lack of collaboration or presence of diseases that limit the patient's ability to carry out the tests; recent stroke or heart attack or malignant diseases.

Data from the screening visits performed at the beginning of the training season were retrospectively collected to assess eligibility and diagnosis of asthma, including

information on skin prick tests, lung volumes before and after salbutamol inhalation, airway responsiveness to methacholine, induced sputum cell counts and the use of asthma medication. In those screening visits, swimmers were asked to withhold anti-asthmatic and anti-allergic medication, according to guidelines (American Thoracic Society 2000).

Swimmers enrolled in study V had a specific evaluation where EBT and axillary temperature were collected before and after a training session at their open-air chlorine disinfected swimming-pool. EBT was measured 5 minutes before and 5 minutes after the swimming training session according to previously validated methods (Popov, Dunev et al. 2007)

In this study, the swimmers performed their regular training session and no changes were imposed by the investigators. However, the intensity of the training session was recorded (coded as: *aerobic training* [bouts of 30-60 minutes achieving heart rate between 120-160 bpm]; *anaerobic mild training* [bouts of exercise achieving a heart rate >180bpm, until 10 minutes of duration]; and *anaerobic moderate/intense training* [bouts of exercise achieving a heart rate >180bpm, from 10 to 30 minutes of duration]) in order to identify a possible confounding effect.

Changes in EBT after the swimming training sessions were compared between swimmers who fulfilled asthma diagnostic criteria and healthy swimmers. Linear regression models were used to determine the effect of asthma and other possible explanatory variables.

4.1.6 Airway inflammation and long-term competitive swimming (Study VI)

Competitive non-elite young swimmers from two main Portuguese swimming teams were invited (n=120) to participate. Recruitment was made through an invitation letter sent to all swimmers.

The study protocol included two visits. The baseline visit was performed at the swimming pool where swimmers trained and included: two self-administered questionnaires, one with questions from the ISAAC questionnaire reporting physician diagnosis of asthma and allergic rhinitis and use of asthma medication, and the other was the International Physical Activity Questionnaire (Craig, Marshall et al. 2003) to assess physical activity; measurement of FeNO to assess eosinophilic airway inflammation; and skin prick testing to common aeroallergens to assess atopy.

The follow-up visit was 3-years later, at the swimming pool for those who remained active in swimming, and at the laboratory for past swimmers. Both questionnaires were repeated, and FeNO was again measured to assess eosinophilic airway inflammation. At the 3-years follow-up visit, swimmers were categorized as “active swimmers” if remaining at high level of competitive swimming; and “past swimmers” if quitted swimming at least 6 months before the follow-up visit.

4.2 Measurements

A summary of the studies outcomes and instruments is shown in **Table 3**.

Table 3. Summary of the studies outcomes and instruments.

Outcome	Instrument	Study				Reference
		III	IV	V	VI	
Lung function						
Spirometry	SpiroBank USB (MIR®)	x		x		Miller et al. 2005
	MasterScreen (Jaeger, Germany)		x			Quanjer et al. 1993
Reversibility	Holding chamber	x		x		Pelegrino et al. 2005
	Nebulizing Chamber (Respironics Respiratory Ltd, UK) and CR60 compressor (Medic-Aid Ltd, UK)		x			
BHR						
PD ₂₀	MEFAR MB3 (Mefar, Brescia, Italy)	x		x		ATS. 2000
	APS (Jaeger, Germany)		x			ATS. 2000
Allergic sensitization						
Skin prick tests	Leti® (Madrid, Spain)	x		x	x	Heinzerling et al. 2009
Body temperature						
Axillary temperature	MPTI 020 (MediCare, UK)			x		Svensson et al. 2012
Airway inflammation						
EBT	X-halo® (Delmedica, Singapore)			x		Popov et al. 2007
Induced sputum	OMRON NE-U17 (Omron Healthcare Europe, Netherlands)			x		Araújo et al. 2011
FeNO	NIOX® (Aerocrine, Sweden)				x	ATS, 2005
	CLD 88sp (Eco Medics, Switzerland)		x			ATS, 2005
Parasympathetic activity						
Pupillometry	PLR-200™ (NeuroOptics Inc, USA)	x				Capão-Filipe et al. 2003
Physical Activity						
IPAQ	International Physical Activity Questionnaire				x	Craig et al. 2003
Asthma diagnosis						
	International Olympic Committee criteria	x		x		IOC, 2002
	ISAAC questionnaire				x	Rosado-Pinto J. 2011
	Allergy Questionnaire for Athletes – AQUA®		x			Bonini et al. 2009

4.2.1 Lung function and reversibility (Study III, IV and V)

Spirometry was performed with the subject in the sitting position breathing room air, with the nose occluded by a clip, according to ATS criteria (Miller, Hankinson et al. 2005).

Results of spirometry are reported as forced expiratory volume in the first second (FEV₁), forced vital capacity (FVC), forced expiratory flow rate at the 50% of FVC (in study IV) or forced expiratory flow in the middle portion of FVC (FEF₂₅₋₇₅) (in study V); all are presented as both absolute and predicted values, according to published reference algorithms (Stanojevic, Wade et al. 2008). Airflow obstruction was defined as a FEV₁/FVC ratio < 0.70 (Pellegrino, Viegi et al. 2005).

In **studies III and V** a SpiroBank USB Spirometer (Medical International Research®) was used and lung function measurements were repeated 15 minutes after 400 µg of salbutamol administered by holding chamber to assess reversibility, which was defined as an increase on FEV₁ ≥ 200mL and 12% from baseline (Pellegrino, Viegi et al. 2005).

In **study IV**, lung function was measured by maximum expiratory flow-volume loops using a MasterScreen Pneumo spirometer (Jaeger GmbH, Würzburg, Germany) according to guidelines (Quanjer, Tammeling et al. 1993). Salbutamol (0.1mg/mL×10kg/body mass) and ipratropium bromide (0.500 mg/mL) were mixed in 1 mL isotonic NaCl and delivered through a Sidestream nebulizing chamber (Respironics Respiratory Ltd, Chichester, UK) connected to a CR60 compressor (Medic-Aid Ltd, West Sussex, UK) at a flow rate of >6L/min. Lung function was repeated 15 min and 45 min after inhalation for salbutamol and ipratropium bromide, respectively, to assess reversibility.

4.2.2 Bronchial hyperresponsiveness (Study III, IV and V)

Bronchial hyperresponsiveness was measured using methacholine bronchial challenge, performed according to ATS guidelines; anti-asthmatic medication was withheld as recommended (American Thoracic Society 2000).

In **studies III and V**, a 100mg vial of methacholine chloride (Provocholine methapharm) was prepared using a saline solution (NaCl 0.9%) with a dilution scheme recommended by the ATS, in order to obtain the different dilutions of 0.0625 mg/mL, 0.25 mg/mL, 1.0 mg/mL, 4.0 mg/mL, and 16.0 mg/mL. After prepared, the different dilutions were stored at 4°C according to the drug labeling, and 30 minutes before the challenge the necessary amount of each dilution was collected and placed in the test location to reach the room temperature. Methacholine was delivered by inspiration triggered by an automatic dosimeter MEFAR MB3 (Mefar, Brescia, Italy) that delivers a single dose soon after the onset of a deep breath. The five-breath dosimeter protocol, with quadrupling doses was used (American Thoracic Society 2000). During tidal breathing, the subject was instructed to inhale slowly and deeply from the nebulizer. Soon after the inhalation began the dosimeter was triggered. Participants were encouraged to inhale slowly and deeply over 5 seconds to total lung capacity and to hold the breath for another 5 seconds. This was repeated for a total of five inhalations. FEV₁ was measured at 30 and 90 seconds after each step. PD₂₀ (provocative dose causing a 20% fall in FEV₁ from baseline) was calculated by linear interpolation on the dose-response curve.

In **study IV**, methacholine bronchial challenge was performed with an inspiration-triggered nebulizer (Aerosol Provocation System, Jaeger, Würzburg, Germany). Methacholine chloride was inhaled in doubling doses from a starting dose of 0.51 µmol (0.1 mg). Lung function measurements were performed 60 seconds after every delivered dose. PD₂₀ was calculated by linear interpolation on the dose-response curve.

4.2.3 Allergic sensitization (Study III, V, and VI)

Allergic sensitization was assessed by performing skin prick tests, which were carried out according to international guidelines (Heinzerling, Burbach et al. 2009) with a standard battery of commercial extracts for common aeroallergens (Leti®, Madrid, Spain). Histamine dihydrochloride 10mg/ml and diluent were used as positive and negative controls, respectively. Testing solutions are stored at +2 to +8°C when not in use. A small

drop of each testing solution was placed on the volar forearm surface of the patient arm and then pressed against the skin for at least 1 second without causing bleeding.

Results were recorded after 15 minutes. The mean wheal diameter for each of the allergens was measured, and a subject was defined as having allergic sensitization by the presence of at least one positive (≥ 3 mm) result (Heinzerling, Burbach et al. 2009).

4.2.4 Body temperature (Study V)

Axillary temperature was chosen to evaluate systemic body temperature and was measured with an axillary thermometer (MediCare®) before collecting baseline EBT. The decision of choosing axillar rather than oral temperature was based on previous studies suggesting that oral temperature is related to the airways and/or the oral cavity and thus may be affected differently than systemic temperature during exercise (Svensson, Nilsson et al. 2012).

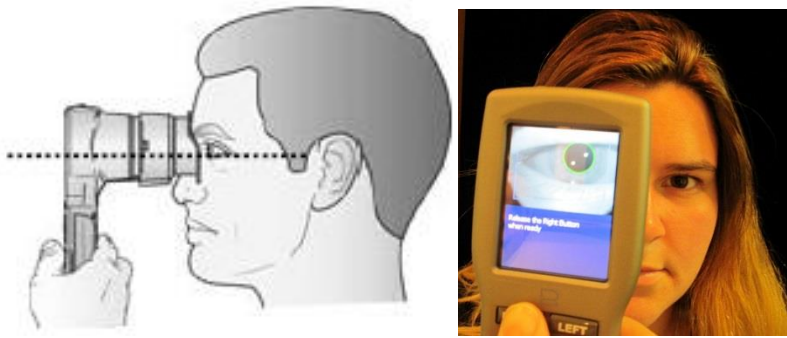
4.2.5 Autonomic nervous system activity (Study III)

Autonomic nervous system activity was assessed using the portable infrared PLR-200™ Pupillometer (NeuroOptics Inc, CA, USA) for pupillary measurements. Subjects were asked to spend at least 15 minutes in a semi-dark and quiet room as previously described (Filipe, Falcão-Reis et al. 2003) to accommodate their eyes to the low lighting level; after, they were instructed to focus with the eye that was not being tested on a small target object standing at least three meters away, keeping head straight and eyes wide open during targeting and measurement (**Figure 2A and 2B**). The measurement took about three seconds. In case of blinking, the measurement was repeated.

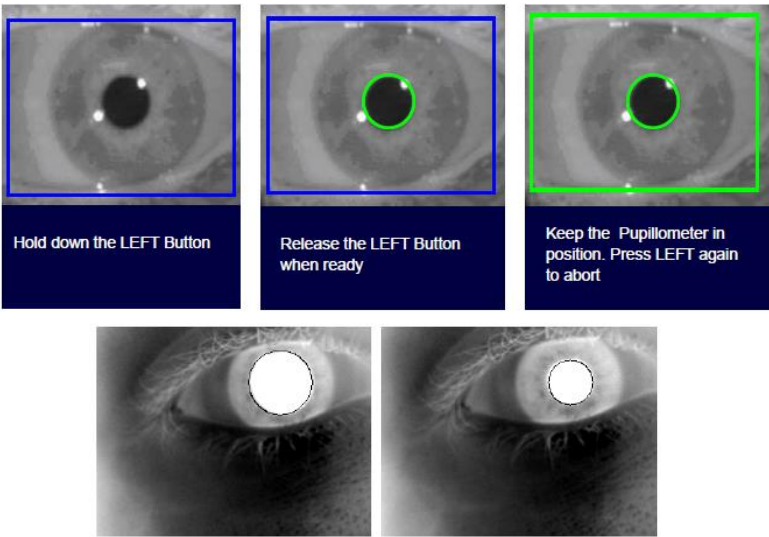
One stimulus of light emitting diodes briefly illuminated the eye with 180 nm peak wavelength. At the end of the measurement cycle, a graph of the pupil's diameters as a function of time appeared on the screen and was recorded (**Figure 2C**). One pupil curve to each eye, starting with the left, was recorded for each subject, and the mean values of both eyes were used for statistical analysis. The following parameters were collected: the diameter of the pupil before (initial) and at constriction peak (minimal), in millimeters; the percentage of constriction; the time of the onset of the constriction (latency), in seconds; the average and the maximum constriction velocities (ACV and MCV respectively), and the dilation velocity (ADV), all given in millimeters/second; and the total time taken by the pupil to recover 75% of the initial resting pupil size after it reached the peak of constriction (T75), in seconds.

Pupil diameters, latency, ACV, MCV, and the constriction amplitude are related to *parasympathetic activity*, while ADV and T75 are measures of *sympathetic activity*.

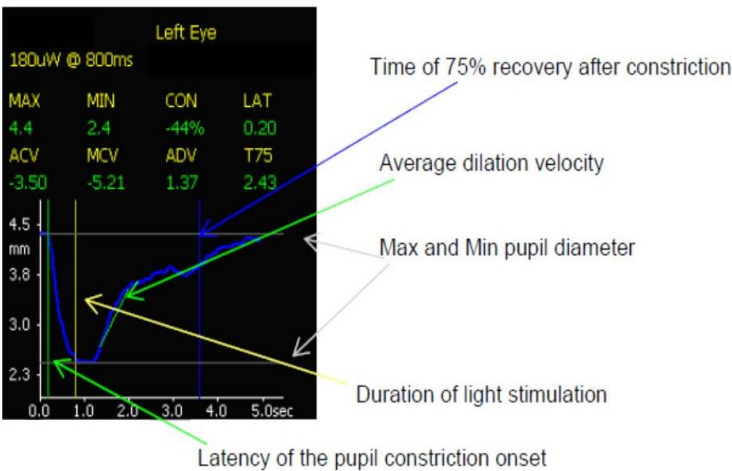
Figure 2 Procedure for measurement of pupillary light reflexes and pupil sizes and pupillometer displaying results.



A- The sequence of figures represent the adequate position to perform the scan: at the right angle to the patient’s axis of vision, in a good alignment, closely adapted to the face and the pupil in the center of LCD screen.



B- The sequence of figures presents the pupil measurement phases: targeting phase (1), ready phase (2) and measurement phase.



C- Pupillometer display of one measurement results.

4.2.6 Airway inflammation (Study IV, V and VI)

Three different methods were used to assess airway inflammation:

4.2.6.1 FeNO (Study IV and VI)

Measurement of FeNO was performed using the online technique according to guidelines (American Thoracic Society and European Respiratory Society 2005). Through a mouthpiece, participants inhaled NO-free air to total lung capacity and exhaled with a standardized flow of 50 ml/s (American Thoracic Society and European Respiratory Society 2005). Repeated exhalations were performed up to a maximum of eight, until obtaining three reproducible measurements that agree within 10%. The mean FeNO of three acceptable exhalations was calculated and expressed in parts per billion (ppb). In **study IV**, FeNO was measured by Eco Medics CLD 88 sp Exhalyzer (Eco Medics AG, 8635 Duernten, Switzerland). In **study VI**, a portable device, NIOX MINO® (Aerocrine AB, Sweden), was used.

4.2.6.2 Induced sputum (Study V)

Cell counts of induced sputum provide a relatively noninvasive method to evaluate the presence, type, and degree of inflammation in the airways (Belda, Leigh et al. 2000). The method is safe and the cell count results are repeatable (Pizzichini, Pizzichini et al. 1996).

Sputum collection and analysis were performed as previously described (Helenius, Ryttilä et al. 1998, Araújo, Moreira et al. 2011). After induction with a 4.5% sodium chloride solution as recommended by guidelines (Paggiaro, Chanez et al. 2002) through an ultrasonic nebulizer (OMRON NE-U17; Omron Healthcare Europe, Netherlands), sputum was sampled and treated with dithiothreitol (Sputolysin; Calbiochem Corporation, San Diego, CA, USA). The suspension was centrifuged and the cell pellet resuspended. Cytospins were prepared and stained using May-Grünwald/Giemsa. Differential cell counts were made by counting a minimum of 500 nonsquamous cells. The procedure was classified as unsuccessful in the following cases: when sputum induction was tolerated for less than 4 minutes, when the volume of the sample was less than 1 mL, or when the percentage of squamous cells exceeded 80% as this would have prevented the performance of differential cell counts of intact bronchial epithelial cells and leukocytes up to a total of 500 nonsquamous cells. The lower respiratory origin was confirmed by the presence of macrophages and bronchial epithelial cells. Normal values for sputum differential cell percentages were based on induced sputum collection in healthy subjects previously published (Belda, Leigh et al. 2000).

4.2.6.3 Exhaled Breath Temperature (Study V)

EBT has been widely investigated based on the assumption that inflammation of the airways and increased vascularization of airway mucosa would influence the temperature of the air coming from the alveoli (Paredi, Kharitonov et al. 2002). Previous studies have reported increased EBT in patients with asthma compared to healthy controls (Piacentini, Peroni et al. 2007). It has been shown that, in asthmatic adults and children, EBT correlates with bronchial blood flow, FeNO levels and sputum eosinophils (Piacentini, Bodini et al. 2002, Paredi, Kharitonov et al. 2005, Piacentini, Peroni et al. 2007, Popov 2011).

EBT was measured using an X-halo device (Delmedica Investments®, Singapore), five minutes before (baseline EBT) and five minutes after the swimming training session (post-exercise EBT), according to previously validated methods (Popov, Dunev et al. 2007). Briefly, the swimmers were requested to inhale freely through the nose and to exhale into the device at a rate and depth typical of their normal tidal breathing rhythm. The maneuver was continued until the built-in software of the instrument indicated that the measured value was stable (**Figure 3**).

The decision of collecting EBT five minutes after the exercise was based upon previous studies that have shown that this was the time point in which EBT reaches the highest values, and thereafter decreases (Svensson, Nilsson et al. 2012). Measurement of EBT has been shown to be highly reproducible (Vermeulen, Barreto et al. 2014), which supports its use before and after the swimming session. Before and between measurements, the device was kept at room temperature in order to maintain a stable starting temperature.

Figure 3 Collection of Exhaled Breath Temperature.



4.2.7 Physical activity (Study VI)

Physical activity (PA) was assessed using the short seven days International Physical Activity Questionnaire (IPAQ) that provides information about frequency and duration of four domains of physical activity (sedentary activity, time spent walking and moderate- and vigorous- intensity physical activity) (Craig, Marshall et al. 2003). A combined total physical activity was computed as the sum of the activity domains scores (Total PA = Walking + Moderate-intensity PA + Vigorous-intensity PA) and reported as a continuous measure (Total PA score = total MET-min/week).

4.2.8 Assessment and diagnosis of asthma (Study I, II, III, IV, V and VI)

For **studies I, II, III, and V**, asthma diagnosis was established according to criteria set by IOC to document asthma in athletes (Medical Commission of the International Olympic Committee 2002, Carlsen, Anderson et al. 2008), using objective evidence of either reversibility after bronchodilator administration or evidence of BHR after a bronchial provocation challenge.

For studies **IV and VI**, self-administered questionnaires were used to assess previous diagnosis of asthma, as described below:

4.2.8.1 AQUA[®] (Study IV)

Allergy Questionnaire for Athletes (AQUA[®]) is a validated, simple, easy-to-use, self-administered tool that permits identification of allergy and asthma with a high positive predictive value (Bonini, Braidò et al. 2009). It was developed from the European Community Respiratory Health Survey Questionnaire. On the basis of open interviews with team doctors, coaches, and athletes, questions were added about: the type, duration, and intensity of training; exercise-related allergic and infectious symptoms; social habits (smoking); drug and food supplements intake; antidoping regulations.

4.2.8.2 ISAAC questionnaire (Study VI)

The International Study of Asthma and Allergies in Childhood (ISAAC) is a unique worldwide epidemiological research programme established in 1991 to investigate asthma, rhinitis and eczema in children.

The ISAAC questionnaire on asthma is a validated one-page self-administered questionnaire with a specific version developed for teenagers aged 13-14 years-old (Asher, Keil et al. 1995). The questions were designed as a minimum set for inclusion in self-completed or interview-administered questionnaires used in population surveys of respiratory disease in children. These questions (self-complete version) were included in a pilot study conducted among 8,000 13-14 year olds in four centers during 1991 (Asher, Keil et al. 1995). The questionnaire has been translated into several languages using

standardized procedures, and the Portuguese version (Rosado-Pinto 2011) for 13-14 years respondents was used.

4.3 Statistical analysis (Studies I-VI)

Data analysis was performed using Statistical Package for Social Sciences version 20.0 for Windows (SPSS, Chicago, IL, USA), considering a significance level of 0.05. In **Study II**, LCA models were fitted using MPlus (V.5.2; Muthen & Muthen, Los Angeles, California, USA). Besides, MedCalc Statistical System (version 10.4.6.0, Mariakerke, Belgium) was also used in **study IV**.

Demographic data and results are given as means with 95% confidence interval (CI), or means with standard deviation (SD), or medians with interquartile range (IQR) in case of skewed distribution; categorical data are presented as counts and proportions (n, %).

T-test was used for comparison of normally distributed independent continuous data, and Mann–Whitney test in case of skewed distribution. In **study V**, Wilcoxon test was used to compare the differences between baseline and post-exercise EBTs. In **study VI**, differences between groups were assessed with 1-way ANOVA for normally distributed data, or Kruskal-Wallis for non-normally distributed data. Categorical variables were compared by Chi-square or Fisher's exact tests. Correlation analyses were performed using Pearson or Spearman's tests as appropriate with respect to distribution of data. In **study V**, given the small sample size, only non-parametric tests were used.

In **study I**, exhaled nitric oxide results were converted into personal predicted values using the FeNO Interpretation Aid tool (<http://www.enovis.org>), and considered increased if above 150% of predicted.

Logarithmic transformation was applied to continuous data whenever a skewed distribution was observed: in **studies IV and V**, PD₂₀ was log-transformed for correlation testing; in **study VI**, levels of FeNO and of physical activity were log-transformed.

Latent Class analysis

In **Study II**, latent class analysis (LCA) was used to uncover from a sample distinct groups of individuals homogeneous within the group (patterns), considering that the performance of an individual in a set of items is explained by a categorical latent variable with K classes, commonly called 'latent classes'. Model interpretation was based on item profiles in each category and obtained from probabilities of endorsing each item response, conditional on class membership. The number of latent classes was defined according to Bayesian Information Criterion (BIC). Starting from one single class and increasing one class at each step, the best solution was identified when the increase of number of classes did not lead to a decrease in BIC. LCA used nine variables of importance for asthma definition or relevant for differential diagnosis (**Table 4**). The selection of variables was based on the assumption of their clinical relevance. The Lo-Mendell-Rubin likelihood

ratio test of model fit was used to quantify the likelihood that the data could be described by a model with one-less class.

Table 4. Definitions of variables set for Latent Class Analysis.

Variable	Definition
Airflow obstruction	FEV ₁ /FVC ratio lower than 0.70
Reversibility	Increase of FEV ₁ of at least 200 mL and 12% from baseline
Rhinitis ^a	Positive answer to the question “Did any doctor diagnose you an allergic disease?” AND “Rhinitis”; OR Positive answer to the question “Do you frequently sneeze, have a running, itchy nose (apart from colds)?”
Any other allergic disease ^a	Positive answer to the question “Did any doctor diagnose you an allergic disease?” (except rhinitis); OR Positive answer to the question “Have you frequently red eyes with tearing and itching?”; OR Positive answer to the question “Have you ever had severe allergic or anaphylactic reactions?”; OR Positive answer to the question “Have you ever had allergic reactions to foods?”; OR Positive answer to the question “Have you ever had allergic reactions to drugs?”
Respiratory symptoms ^a	Self-reported recurrent breathlessness, cough, wheezing, chest tightness and/or phlegm production; OR Positive answer to the question “Did any doctor diagnose you an allergic disease?” AND “Asthma”; OR Positive answer to the question “Have you ever had shortness of breath, cough and/or itching of the throat following exercise?”
Asthma treatment	Current or recent treatment with ICS and/or β_2 -agonists
Airway hyperresponsiveness	A fall in FEV ₁ $\geq 10\%$ from baseline with exercise or EVH; OR a fall in FEV ₁ $\geq 15\%$ from baseline after inhaling 22.5 ml of 4.5g% NaCl or ≤ 635 mg of mannitol; OR a fall in FEV ₁ $\geq 20\%$ from baseline with methacholine: PC ₂₀ ≤ 4 mg/ml, or PD ₂₀ ≤ 400 μ g (cumulative dose) or ≤ 200 μ g (noncumulative dose) in those not taking ICS, and PC ₂₀ ≤ 16 mg/ml or PD ₂₀ ≤ 1600 μ g (cumulative dose) or ≤ 800 μ g (noncumulative dose) in those taking ICS for at least 1 month
Eosinophilic inflammation	FeNO levels above 25 ppb.
Allergic sensitization	Presence of at least one positive (mean of largest and perpendicular diameter of the wheal ≥ 3 mm for each allergen) skin prick test or presence of positive specific IgE (≥ 0.35 kU/L) for at least one common aeroallergen in the local geographic area

^a Considering AQUA[®]. **EVH**: eucapnic voluntary hyperpnea; **FVC**: forced vital capacity; **FEV₁**: forced expiratory volume in one second; **PC₂₀**: provocative concentration inducing a 20% decrease in FEV₁; **PD₂₀**: provocative dose inducing a 20% decrease in FEV₁; **FeNO**: fraction of exhaled nitric oxide.

Multiple factor analysis

Study of associations was performed using linear regression and general linear models.

In **study II**, among asthmatic athletes, the risk associated with the sport training environment was estimated by using regression analysis to predict the odds of having a specific asthma pattern (phenotype), having “other sports” as reference.

In **study V**, linear regression models were used to determine the effect of asthma and other possible explanatory variables (age, gender, height, weight, PD₂₀, baseline EBT, intensity of the training session, axillary temperature, and the number of hours trained in the week previous to measurements) in the outcome Δ EBT. These variables were selected based on the authors’ a priori hypothesis that they may influence EBT. A univariate linear regression analysis was performed to assess the individual effect of each of the selected variables; those that were significant at the 0.25 level were included in a multiple linear regression model. A stepwise method was used to select the variables to include in the final model, taking into account their significance and effect in the adjusted r². The effect of asthma, being a major outcome of the present study, was included in the multiple regression analysis and was kept in the final model independently of the significance level and adjusted r² change with its inclusion.

In **study VI**, differences in changes in FeNO after the three year follow up were assessed by general linear model adjusting on confounding factors: gender, age, allergic sensitization, physician-diagnosed asthma, and use of asthma medication.

Power and sample size calculations

Sample size was only calculated for **study IV**, the randomized cross-over trial. To achieve a high-grade correlation of ≥ 0.7 with power of 80%, 13 subjects were calculated to be required based upon previous measurements in top athletes.

In the remaining studies, all athletes that were able to participate and were eligible were included. In **study V**, only 22 swimmers (of which ten with asthma) accepted to participate, retrieving a power of 49% to detect a Δ EBT of 0.43°C (Peroni, Chinellato et al. 2012). In **study III**, no power calculation has been made because this was an exploratory study and the needed a priori information was not available.

4.4 Ethics

All studies were conducted in accordance to the Declaration of Helsinki for Medical Research Involving Human Subjects.

Protocols were approved by the Ethics Committee of Hospital São João / Faculdade de Medicina da Universidade do Porto (n_id_cic 174/12 ; n_id_ces 58/12) for studies developed in Portugal (Studies I, II, III, V, VI) and the Committee for Regional Medical Research Ethics and the Norwegian data inspectorate in Norway (Studies II and IV).

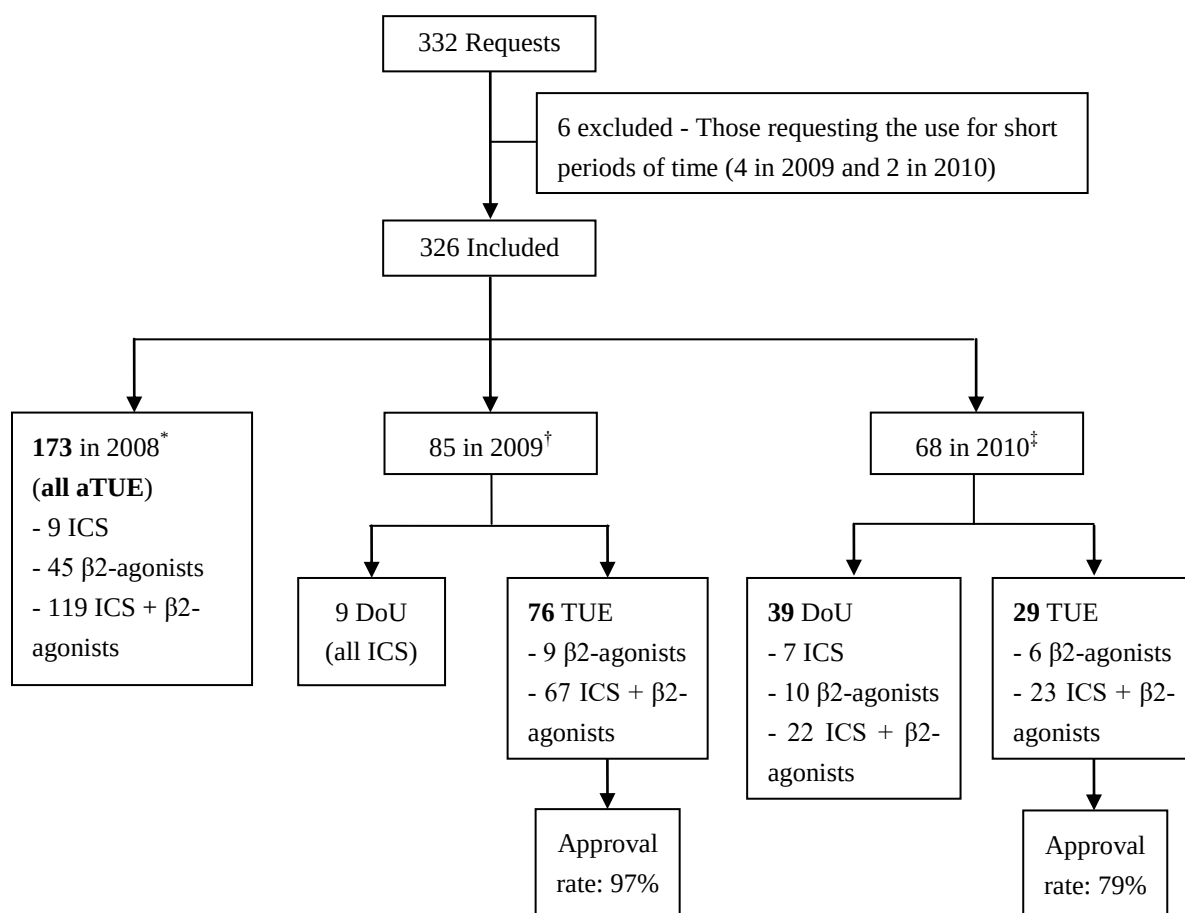
All participants or their legal guardians/parents (in case of participants under 18 years-old) signed an informed consent.

5. RESULTS

5.1 Diagnosis of asthma and healthcare of asthmatic athletes (study I)

We analyzed requests from 326 athletes [254 males; median age 24 years (range 16-62 years)] (**Figure 4**). The requests were as follows: in 2008, 173 abbreviated Therapeutic use exemptions (TUEs) were submitted; in 2009 and 2010, 9 and 39 Declarations of Use (DoU) were submitted, respectively; regarding TUEs, the approval rate was 97% (74/76) in 2009 and 79% (23/29) in 2010 ($p=0.005$).

Figure 4 Flow chart showing requests for asthma medication submitted by athletes to the Anti-Doping Authority of Portugal between 2008 and 2010.



* In 2008 all β2-agonists, as well as inhaled corticosteroids required only an aTUE. Approval for aTUEs was not necessary. † In 2009, salbutamol, salmeterol, terbutaline and formoterol required a TUE; use of inhaled corticosteroids only required a DoU. ‡ In 2010, salbutamol, salmeterol and inhaled steroids required DoU; for using terbutaline or formoterol use a TUE was required.

aTUE: abbreviated Therapeutic use exemption; **DoU:** Declaration of use; **ICS:** inhaled corticosteroids; **TUE:** Therapeutic use exemption.

The most frequent requests were for using ICS combined with inhaled β 2-agonists. Applications for inhaler use have decreased by approximately half from 2008 to 2009 (173 to 85) since objective asthma testing became mandatory. This more rigorous screening allowed withdrawal of unnecessary medication. Requests for isolated inhaled β 2-agonists increased significantly from 2009 to 2010 ($p=0.03$), highlighting safety issues stemming from the unsupervised use of β 2-agonists.

The clinical and diagnostic tests performed are shown in **Table 5** and the corresponding results in **Table 6**. No tests were reported in 2008 because all the requests submitted in that year were aTUEs. Changes to the WADA guidelines on β 2-agonists in 2010 led to a dramatic decrease in the number of tests performed in Portuguese athletes with asthma (**Table 5**). The proportion of positive tests in 2009 and 2010 (**Table 6**) was similar, supporting the strategy of objective diagnosis.

Table 5. Tests performed in athletes whose request to use asthma medication was approved by the Anti-doping Authority of Portugal.

Tests performed	2009 (n = 83)	2010 (n = 62)	P
Spirometry	72 (87)	23 (37)	<0.001
Bronchodilation test	37 (45)	15 (24)	0.011
Bronchoprovocation challenge	49 (59)	10 (16)	<0.001
Methacholine	46 (55)	9 (15)	-
Mannitol	0	1 (2)	-
Exercise	3 (4)	0	-
FeNO	15 (18)	6 (10)	0.155
SPT or sIgE	56 (67)	18 (29)	<0.001

No tests were reported in 2008 as it was not necessary to provide objective evidence of asthma at that time. Data reported as n (%). **SPT**, skin prick tests; **sIgE**, specific immunoglobulin E; **FeNO**, fraction of exhaled nitric oxide.

Table 6. Reported symptoms and positive test results in athletes with asthma who submitted a Therapeutic Use Exemption application to the Anti-Doping Authority of Portugal.

	2009 n = 83	2010 n = 62
Respiratory symptoms	81 (98)	62 (100)
Airflow limitation on spirometry	12/72 (17)	4/23 (17)
Positive bronchodilation	20/37 (54)	8/15 (53)
Positive bronchoprovocation	46/49 (94)	10/10 (100)
Airway allergic inflammation ^a	10/15 (67)	2/6 (33)
Allergic sensitization	50/56 (89)	15/18 (83)

Data reported as positive/performed (%). ^a Defined as exhaled nitric oxide >150% of predicted value for age and height, calculated using the FeNO Interpretation Aid tool (<http://www.enovis.org>).

5.2 Phenotypes of asthma in elite athletes (study II)

Among the 150 asthmatic athletes, 45 were diagnosed based on positive bronchodilation (the mean $\pm$ SD of FEV₁ increase was 450mL $\pm$ 292 and 13% $\pm$ 9.4), and 105 by presenting BHR after a provocation challenge: 1 positive challenge to mannitol, 3 positive challenges with exercise and the remaining 101 positive challenges with methacholine (7 reporting PC₂₀: mean 3.9 mg/ml; 94 reporting PD₂₀: mean 6.8 μ g). LCA retrieved two clusters (**Table 7**): “atopic asthma” defined by allergic sensitization, increased FeNO, rhinitis and allergic co-morbidities; and “sports asthma” defined by exercise-induced respiratory-symptoms and BHR without allergic features (**Figure 5 and Table 8**).

Table 7. Latent class analysis for asthma features in the elite athletes' population.

Number of classes ¹	Asthma			
	Log L	Number of parameters	BIC	p ²
1	-503.304	9	1051	
2	-472.636	19	1040	<0.001
3	-457.683	29	1060	0.0614

BIC, Bayesian information criteria; Log L, log likelihood; ¹ The bold font denotes the best models according to lowest BIC; ² Lo-Mendell-Rubin likelihood ratio test of model fit to quantify the likelihood that the data can be described by a model with one-less class.

Figure 5 Percent of athletes presenting each of the features included for LCA.

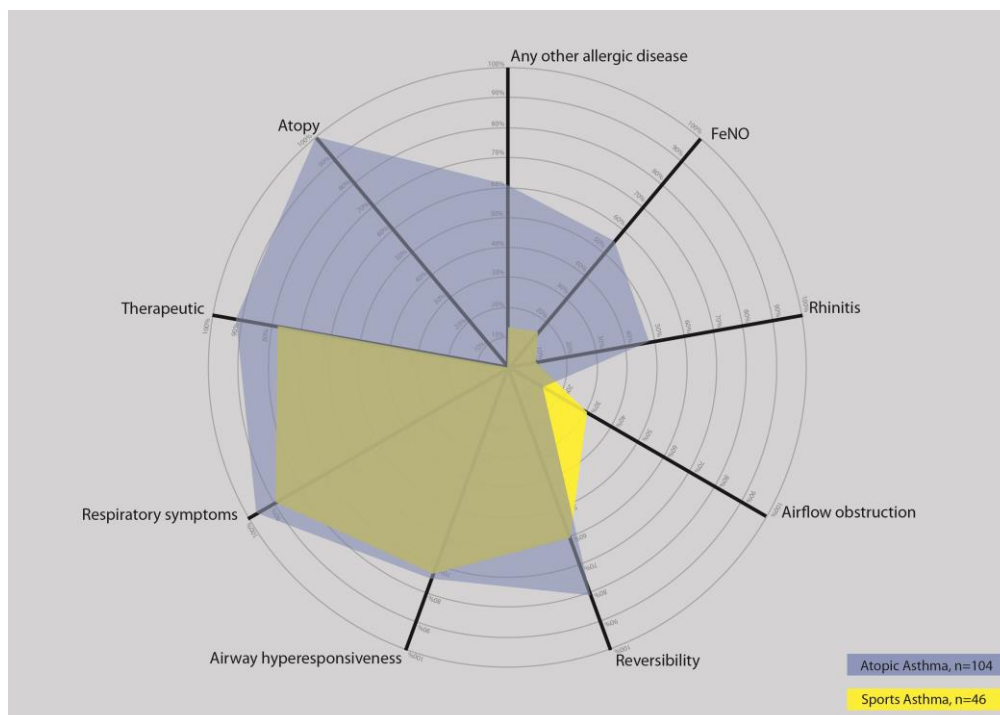


Table 8. Characteristics of asthmatic athletes according to their asthma phenotype and variables in each assigned latent class.

	Total	Atopic Asthma, n=104	Sports asthma, n=46	p
Males, n (%)	107	81 (78)	26 (57)	0.008[#]
Age, median±IQR in years	-	23.0±12	24.5±8	0.522 [§]
Height, mean±SD in cm	-	175.4±8.7	176.5±8.7	0.530 [‡]
Weight, mean±SD in Kg	-	70.9±11.5	71.4±10.3	0.815 [‡]
BMI, mean±SD in Kg/m ²	-	23.0±2.6	22.8±1.9	0.741 [‡]
FEV ₁ , mean±SD in L	-	4.0±0.9	4.1±0.7	0.221 [‡]
FEV ₁ , mean±SD in % predicted	-	98.1±20.4	99.7±21.1	0.640 [‡]
FVC, mean±SD in L	-	5.1±1.0	5.3±1.1	0.413 [‡]
FVC, mean±SD in % predicted	-	108.0±15.4	113.4±15.0	0.084 [‡]
FEV ₁ /FVC, mean±SD	-	77.9±8.9	78.7±11.1	0.649 [‡]
Variables used in Latent Class Analysis				
Airflow obstruction*				0.036
No	80.5	85.3	69.4	
Yes	19.5	14.7	30.6	
Reversibility**				0.023
No	23.4	19.0	39.7	
Yes	76.6	81.0	60.3	
Rhinitis				<0.001
No	64.0	51.5	90.9	
Yes	36.0	48.5	9.1	
Any other allergic disease				<0.001
No	61.5	39.3	87.5	
Yes	38.5	60.7	12.5	
Respiratory symptoms				0.133
No	6.1	4.0	10.7	
Yes	93.9	96.0	89.3	
Asthma treatment				0.017
No	11.9	7.5	22.0	
Yes	88.1	92.5	78.0	
Bronchial hyperresponsiveness				0.834
No	25.7	25.0	26.9	
Yes	74.3	75.0	73.1	
FeNO [†]				<0.001
Normal	62.8	44.8	84.5	
Increased	37.2	55.2	15.5	
Allergic sensitization				<0.001
No	31.0	0	100	
Yes	69.0	100	0	

Data presented as percent of total, except otherwise stated; **BMI**: Body mass index; **FeNO**: fraction of exhaled nitric oxide; **L**: liters; **FVC**: forced vital capacity; **FEV₁**: forced expiratory volume in one second; * Defined as a FEV₁/FVC ratio <0.70; ** Defined as an increase in FEV₁ ≥200mL and ≥12% from baseline; † Defined as increased if above 25ppb; ‡ Independent samples t-test; § Independent samples Mann-Whitney U-test; # Qui-square test.

Water (OR=2.87; 95%CI [1.82-4.51]) and winter (OR=8.65; 95%CI [2.67-28.03]) sport athletes had increased risk of “sports asthma” compared with other sport athletes.

5.3 Dysautonomia, asthma and BHR in elite swimmers (study III)

Twenty-seven swimmers were included. No differences on demographic and personal characteristics were observed between groups, except for the expected significantly lower PD₂₀ among asthmatic swimmers (**Table 9**).

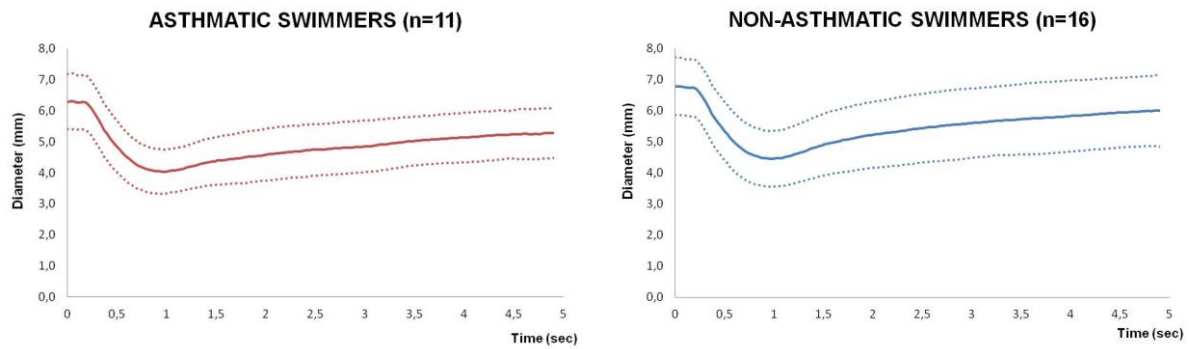
Pupillometry measurements in asthmatics compared with non-asthmatic swimmers are presented in **Table 9** and **Figure 6**. Although lower pupil diameters and a higher percentage of constriction were observed in asthmatics, differences were not significant.

Table 9. Characteristics of asthmatic and non-asthmatic swimmers included.

	Asthmatic swimmers (n=11)	Non-asthmatic swimmers (n=16)	<i>p</i>
Males, n (%)	8 (73)	6 (38)	0.072
Age, years	17 [15-19]	18 [16-20]	0.479
BMI, kg/m²	21.5 [20.2-22.9]	21.3 [20.2-22.3]	0.725
Atopy, n (%)	5 (46)	6 (38)	0.679*
Years of competition	8.9 [7.0-10.8]	9.6 [7.6-11.7]	0.602
Training hours per week	16.3 [13.1-19.4]	17.5 [16.1-18.9]	0.393
Previous diagnosis of asthma, n (%)	4 (36)	1 (6)	0.113*
Previous diagnosis of rhinitis, n (%)	1 (9)	3 (18)	0.488*
PD₂₀ methacholine, µmol	0.8 [0.4-1.2]	4.6 [3.3-5.8]	0.001
Lung function			
FEV ₁ /FVC	83.5 [78.3-88.8]	88.0 [84.6-91.4]	0.236
FVC			
Liters	5.1 [4.4-5.8]	4.9 [4.1-5.7]	0.612
% of predicted	114.5 [107.0-122.0]	114.8 [108.5-121.0]	0.957
FEV ₁			
Liters	4.3 [3.7-4.8]	4.3 [3.7-4.9]	0.863
% of predicted	111.1 [100.7-121.5]	115.6 [109.8-121.3]	0.379
FEF ₂₅₋₇₅			
Liters	4.2 [3.4-5.1]	4.7 [4.0-5.4]	0.570
% of predicted	97.3 [78.2-116.4]	109.3 [97.3-121.4]	0.230
Airway obstruction, n (%)	1 (9)	0	0.219*
Increase in FEV₁ after salbutamol			
%	5.0 [2.0-8.0]	3.4 [1.7-5.4]	0.840
Milliliters	197.0 [87.2-306.8]	149.4 [61.5-237.3]	0.922
Parasympathetic parameters			
Maximal diameter (in millimeters)	5.9 [4.6-7.3]	7.0 [6.4-7.6]	0.180
Minimum diameter (in millimeters)	3.9 [2.9-4.9]	4.7 [4.0-5.4]	0.708
Percent of constriction	35.1 [31.9-38.3]	33.8 [29.3-38.4]	0.295
Latency (in seconds)	0.2 [0.2-0.2]	0.2 [0.2-0.2]	0.183 [#]
ACV (in millimeters/second)	4.0 [3.5-4.6]	4.1 [3.6-4.5]	0.664
MCV (in millimeters/second)	5.7 [4.7-6.8]	5.5 [4.9-6.0]	0.592
Sympathetic parameters			
ADV (in millimeters/second)	0.9 [0.7-1.2]	0.9 [0.8-1.0]	0.440
T75 (in seconds)	2.4 [1.3-3.5]	3.1 [2.8-3.4]	0.154

Data reported as mean [95% Confidence interval] unless otherwise stated. * Fisher Exact test; # Mann-Whitney U test. **ACV**: Average constriction velocity; **ADV**: Average dilation velocity; **BMI**: body mass index; **FEV₁**: forced expiratory volume in the first second of FVC; **FVC**: forced vital capacity; **FEF₂₅₋₇₅**: forced expiratory flow middle portion of FVC; **MCV**: Maximum constriction velocity; **PD₂₀**: provocative dose determining a 20% fall in FEV₁; **T75**: the total time taken by the pupil to recover 75% of the initial resting pupil size after it reached the peak of constriction.

Figure 6 Results of pupillary response variables: Mean (bold line) $\pm$ SD (traced lines) values of pupillary diameters among asthmatic and non-asthmatic swimmers.



When stratified by BHR severity, pupil diameters before (maximal) and at constriction peak (minimum), as well as the percent of constriction, were significantly lower among those with severe BHR (**Table 10**).

Table 10. Autonomic nervous system parameters across BHR degrees of severity.

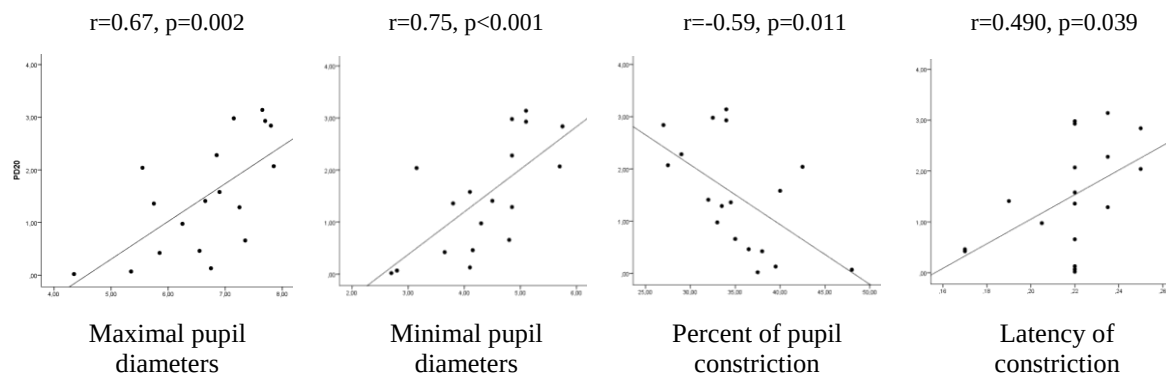
	No BHR (n=3)	Borderline BHR (n=6)	Mild BHR (n=12)	Moderate BHR (n=4)	Severe BHR (n=2)	p
Parasympathetic parameters						
Maximal diameter (mm)	6.4 $\pm$ 1.0	6.4 $\pm$ 0.9	6.9 $\pm$ 0.8	6.6 $\pm$ 0.6	4.9 $\pm$ 0.7	0.04
Minimum diameter (mm)	4.3 $\pm$ 1.1	3.9 $\pm$ 0.7	4.7 $\pm$ 0.8	4.2 $\pm$ 0.5	2.8 $\pm$ 0.7	0.03
Percent of constriction	34.3 $\pm$ 7.8	38.3 $\pm$ 2.2	33.3 $\pm$ 4.5	37.3 $\pm$ 1.9	42.8 $\pm$ 7.4	0.05
Latency (sec)	2.8 (2.6-3.0)	3.2 (2.7-3.5)	3.1 (1.7-3.8)	3.3 (3.3-3.3)	1.7 (1.7-1.7)	0.08*
ACV (mm/sec)	3.6 $\pm$ 0.9	4.3 $\pm$ 0.4	4.0 $\pm$ 0.3	3.8 $\pm$ 0.4	3.5 $\pm$ 0.4	0.13
MCV (mm/sec)	5.1 $\pm$ 0.9	5.8 $\pm$ 0.4	5.5 $\pm$ 0.6	5.6 $\pm$ 0.4	5.1 $\pm$ 1.1	0.52
Sympathetic parameters						
ADV (mm/sec)	0.9 $\pm$ 0.1	0.8 $\pm$ 0.2	0.9 $\pm$ 0.2	0.5 $\pm$ 0.3	0.9 $\pm$ 0.1	0.08
T75 (sec)	2.8 $\pm$ 0.3	3.1 $\pm$ 0.4	2.8 $\pm$ 0.8	3.3 $\pm$ u.d.	1.7 $\pm$ u.d.	0.42

Data reported as mean $\pm$ SD, except for latency - median (min-max). u.d: unavailable data. * Kruskal-Wallis test.

ACV: Average constriction velocity; **ADV:** Average dilation velocity; **BMI:** body mass index; **FEV₁:** forced expiratory volume in the first second of FVC; **FVC:** forced vital capacity; **FEF₂₅₋₇₅:** forced expiratory flow middle portion of FVC; **MCV:** Maximum constriction velocity; **PD₂₀:** provocative dose determining a 20% fall in FEV₁; **T75:** the total time taken by the pupil to recover 75% of the initial resting pupil size after it reached the peak of constriction

Among swimmers with clinically relevant BHR (n=18), a significant correlation was found between PD₂₀ and maximal and minimal pupil's diameters, percent of constriction and latency (**Figure 7**), but not with ACV (r=0.243, p=0.332), MCV (r=0.061, p=0.810), ADV (r=0.075, p=0.776), and T75 (r=0.373, p=0.351).

Figure 7 Correlation between PD_{20} and maximal and minimal diameter, percentage of constriction and latency of constriction among swimmers with clinically relevant BHR.



5.4 Anti-cholinergic drugs for BHR in elite athletes (study IV)

The characteristics of the participants are presented in **Table 11**. Sixteen athletes (73%) had a doctor's diagnosis of asthma and 17 (77%) used inhaled corticosteroids. Seven (32%) reported doctor-diagnosed allergic rhinitis and four (18%) used antihistamines regularly. Lung function was within normal range in all athletes (**Table 11**).

Table 11. Lung function and characteristics of subjects included in Study IV.

Characteristics	
Males, n (%)	14 (63.7)
Age (years)	26.0 (20.0-37.0, ± 4.7)
Height (cm)	175.5 (162.0-190.0, ± 8.8)
Weight (kg)	69.7 (52.0-85.0, ± 10.5)
Asthma diagnosis, n (%)	16 (72.7)
Allergy, n (%)	7 (31.8)
BHR, n (%)	12 (54.4)
Drug use, n (%)	
Short-acting beta2-agonists	8 (36.4)
Long-acting beta2-agonists	10 (45.5)
Inhaled Corticosteroids	17 (77.3)
Ipratropium bromide	15 (68.2)
Leukotriene antagonists	0 (0)
Antihistamines	4 (18.2)
FEV₁ (L)	4.3 (3.0-5.5, ± 0.7)
% of predicted values	103 % (91-130, ± 9.5)
FVC (L)	5.5 (3-6-7.3, ± 1.2)
% of predicted values	112 % (93-131, ± 11)
FEF₅₀ (L/s)	4.5 (2.5-8.6, ± 1.34)
% of predicted values	85.6% (51-159, ± 27.38)
FeNO (ppb)	24.7 (9.0-113.0, ± 25.6)

Data reported as mean (range, $\pm$ SD) unless otherwise stated. **BHR**: Bronchial hyperresponsiveness; **FEV₁**: forced expiratory volume in 1 s; **FVC**: forced vital capacity. **FEF₅₀**: forced expiratory flow at 50% of vital capacity; **FeNO**: fraction of exhaled nitric oxide.

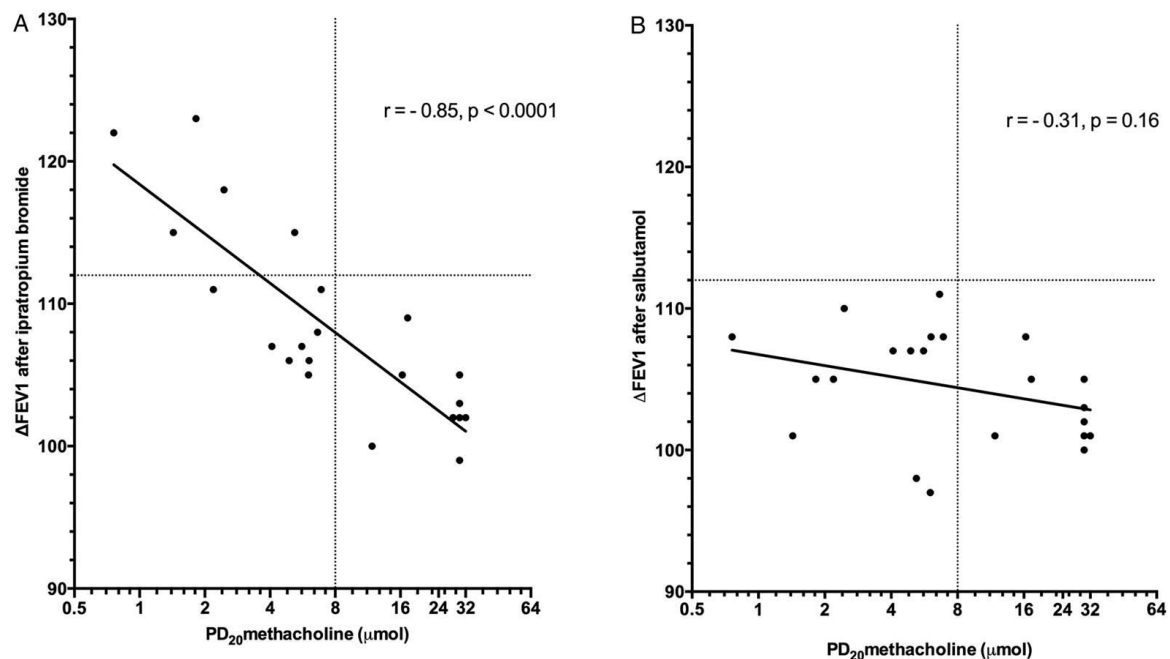
Five athletes presented reversibility (Δ FEV₁ $\geq$ 12%) to inhaled ipratropium bromide (with mean $\pm$ SD Δ FEV₁ of 7.8% $\pm$ 6.9 and 330.5 $\pm$ 289.8 ml) and none to inhaled salbutamol (mean Δ FEV₁ after inhaled salbutamol of 4.3% $\pm$ 3.8 or 185.9 $\pm$ 166.9 ml). A significant difference was observed between Δ FEV₁ after inhaled ipratropium bromide and inhaled salbutamol of 3.4% (p=0.002).

PD₂₀ correlated negatively with Δ FEV₁ after inhaled ipratropium bromide ($r=-0.85$, $p<0.0001$) (**Figure 8A**) but not after inhaled salbutamol ($r=-0.31$, $p=0.16$) (**Figure 8B**). All athletes with positive reversibility to ipratropium bromide had PD₂₀ $\leq 8\mu\text{mol}$ (**Figure 8A**), all had doctor diagnosed asthma and used inhaled corticosteroids ($n=12$).

Athletes that used inhaled corticosteroids had greater bronchodilator response to inhaled ipratropium bromide (mean 109.9% [95% CI 106.4, 113.3]), as compared to the athletes that did not use corticosteroids (101.8% [98.8, 104.7], $p=0.01$). No differences were observed in bronchodilator effect after inhalation of salbutamol in athletes that used short-acting or long-acting β_2 -agonists, nor inhaled corticosteroids as compared to athletes that did not use these medications.

Mean age of the athletes with positive reversibility to inhaled ipratropium bromide was higher compared to the athletes with no reversibility (28.8 years [± 6.6] vs. 25.0 years [± 3.6] (non-significant).

Figure 8 Relationship between PD₂₀ methacholine and Δ FEV₁ (%) 15 and 45 min after inhalation of salbutamol (B) and Ipratropium Bromide (A), respectively, in elite cross-country and biathlon skiers.



Sixteen athletes reported respiratory symptoms during or after exercise. Only three athletes were non-symptomatic. Cough (73%) was the most prevalent self-reported symptom, followed by phlegm (68%) and dyspnea (41%). Self-reported symptoms were not associated with BHR ($\log_{10}$ PD₂₀), FeNO or Δ FEV₁ after inhalation of ipratropium bromide or salbutamol. We observed no association between FeNO and PD₂₀.

5.5 Exhaled breath temperature, exercise and asthma (Study V)

Twenty-two elite swimmers training for an average period of 9 years of competition, of which ten with asthma, accepted to participate in this study. Swimmers diagnosed with asthma were all based on hyperresponsiveness, none presented complete criteria for reversibility. No differences were observed between asthmatic and non-asthmatic swimmers for demographic and personal characteristics, except for the expected lower PD₂₀ among those with asthma (**Table 12**).

Table 12. Personal and demographic characteristics of swimmers included in the study.

	Asthmatics (n=10)	Controls (n=12)	p*
Males, n (%)	7 (70)	3 (23)	0.084
Age, years	17 ± 2.8	17 ± 2.9	0.872
BMI, Kg/m ²	21.7 ± 2.1	21.1 ± 1.9	0.456
Allergic sensitization, n (%)	4 (40)	4 (33)	1.000
PD ₂₀ methacholine	0.71 ± 0.6	4.39 ± 2.4	<0.001
FEV ₁ , Liters	4.26 ± 0.75	4.07 ± 0.92	0.582
FEV ₁ , % of predicted	111.10 ± 14.54	115.50 ± 8.89	0.722
FVC, Liters	5.13 ± 0.94	4.60 ± 1.20	0.093
FVC, % of predicted	114.50 ± 10.49	114.08 ± 11.48	0.923
FEV ₁ /FVC	83.52 ± 7.32	89.36 ± 7.18	0.123
FEF ₂₅₋₇₅ , Liters	4.22 ± 1.20	4.67 ± 1.17	0.314
FEF ₂₅₋₇₅ , % of predicted	97.3 ± 26.67	113.42 ± 22.54	0.140
FEV ₁ increase after BD in mL, median (IQR)	185 (325)	145 (210)	0.771
FEV ₁ increase after BD in %, median (IQR)	5 (8)	4.5 (5)	0.582
IS percentage of eosinophils, median (IQR)	3.3 (16.4)	1.1 (8.13)	0.696
IS percentage of neutrophils, median (IQR)	7.8 (19.9)	12.3 (29.1)	0.573
IS percentage of epithelial cells, median (IQR)	30.1 (37.8)	25.2 (10.6)	0.133

Data presented as mean±SD, unless otherwise stated. * Mann-Whitney was used for comparisons or Chi-square tests in case of categorical variables. **BD**: bronchodilation; **FEF₂₅₋₇₅**: forced expiratory flow middle portion of FVC; **FEV₁**: forced expiratory volume in one second; **FVC**: forced vital capacity; **PD₂₀**: provocative dose inducing a 20% decrease in FEV₁.

EBT significantly increased after training (Δ EBT=0.32±0.57; p=0.016) (**Figure 9A**) without differences between asthmatic and healthy swimmers (0.15±0.39 vs 0.45±0.68; p=0.254) (**Table 13, Figure 9B**). Baseline and post-exercise EBT were similar between both groups (**Table 13**). Also, no differences were observed between those with (n=6) or without inhaled corticosteroids (p=0.853).

Figure 9 Exhaled breath temperature (EBT) before and after a swimming training session considering all participants (A) and asthmatics vs healthy swimmers (B).

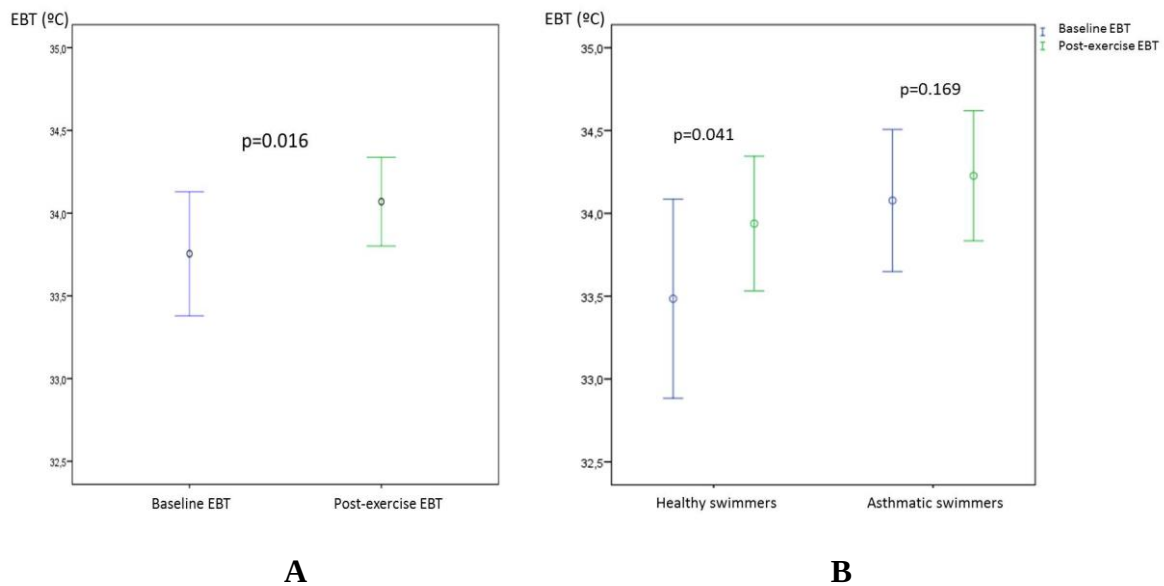


Table 13. Results of measurements performed and training characteristics of asthmatic vs healthy swimmers.

	Asthmatic swimmers (n=10)	Healthy swimmers (n=12)	p [#]
Baseline EBT, °C	34.08 ± 0.60	33.49 ± 0.95	0.180
Post-exercise EBT, °C	34.23 ± 0.55	33.94 ± 0.64	0.283
Body Temperature*, °C	35.97 ± 0.42	36.02 ± 0.31	0.771
Hours of training in previous week	9.4 ± 2.6	9.3 ± 3.4	0.974
Intensity of the session, n			0.063
Aerobic	3	9	
Mild anaerobic	5	1	
Moderate/severe anaerobic	2	2	

Data presented as mean±SD, except otherwise stated. *Axillary temperature was used as a measure for body temperature. [#]Mann-Whitney test was used for comparisons, or Chi-square test in case of categorical variables.

A significant correlation was observed between baseline and post-exercise EBTs ($r=0.827$, $p<0.001$). In multiple linear regression analysis, after controlling for baseline EBT and axillary temperature, asthma was not a significant predictor of Δ EBT (**Table 14**). In univariate regression analysis, baseline EBT was the variable most strongly associated with Δ EBT ($r^2=0.464$).

Correlations between BHR (LnPD_{20}) and baseline EBT ($r=0.224$, $p=0.533$) and Δ EBT ($r=0.044$, $p=0.845$) for asthmatic swimmers were not significant.

Table 14. Multiple linear regression model for predicting Δ EBT.

Variable	Standardized Coefficient B	P-value	95% CI
Baseline EBT	-0.675	0.001	[-0.685;-0.229]
Asthma	-0.008	0.961	[-0.388;0.370]
Axillary temperature	0.327	0.043	[0.018;1.027]

$R^2=0.596$, $\text{adj}R^2=0.529$

p-values of the univariate analysis (including all the tested variables): sex – 0.547, age – 0.336, height – 0.025, weight – 0.196, baseline EBT – <0.001, axillary temperature – 0.089, training intensity – 0.415, asthma – 0.222, PD_{20} – 0.600, number of training hours in the previous week – 0.583. The following explanatory variables were initially entered into the multiple regression model, but were excluded in the final model as they did not reach significance at the 0.05 level: height, weight. Variable asthma, being a major outcome of this study, was kept in the final model independently of the significance level and adjusted r^2 change with its inclusion; is coded as “0”=without asthma; “1”=with asthma.

Both groups presented median values of eosinophils and epithelial cells higher and neutrophils lower than reference values for healthy populations (Belda, Leigh et al. 2000). When compared to healthy swimmers, asthmatics presented higher eosinophil and epithelial cell counts and lower neutrophils, but differences were not statistically significant (**Table 12**).

Correlations between sputum eosinophils with baseline EBT ($r=0.064$, $p=0.800$) and Δ EBT ($r=-0.210$, $p=0.404$) were not significant, even if considering only asthmatics ($r=-0.286$, $p=0.493$ and $r=0.048$, $p=0.911$, for baseline EBT and Δ EBT, respectively). Regarding neutrophils, correlations were also non-significant for the global sample for baseline EBT ($r=0.194$, $p=0.441$) and Δ EBT ($r=0.101$, $p=0.689$) and also for the asthmatic group ($r=0.500$, $p=0.207$ and $r=-0.048$, $p=0.911$, for baseline and Δ EBT, respectively).

5.6 Effect of long-term swimming on airways (study VI)

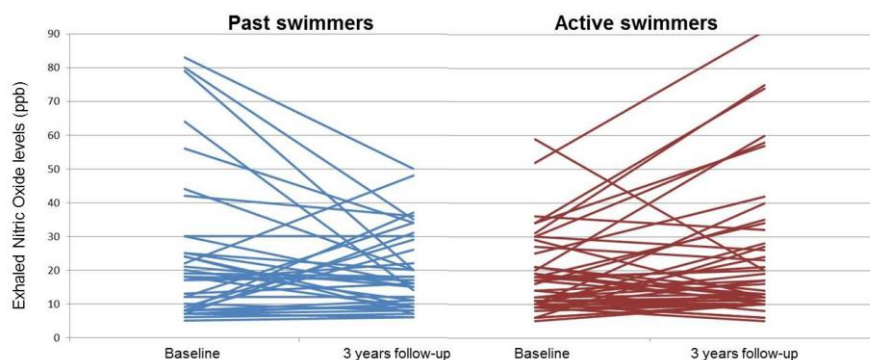
From the 105 swimmers assessed at baseline visit, 86 attended the 3-years follow-up visit and were considered for final analysis. The 19 lost to follow-up subjects did not differ from the others at baseline evaluation. We observed a significant difference in changes in FeNO; those who remained active significantly increased their levels of eosinophilic airway inflammation independently of their gender, age, allergic sensitization, or asthma status (**Table 15, Figure 10**). After the 3-year follow-up, the prevalence of asthma, allergic rhinitis and use of asthma medication increased significantly in both groups. All subjects increased their overall physical activity levels; however, significant increases in moderate and vigorous physical activity levels were only observed in active swimmers.

Table 15. Changes in airway inflammation, prevalence of asthma and rhinitis and physical activity levels in athletes according with their swimming status at baseline visit and after the 3 y follow up.

	Past swimmers, n=39		Active swimmers, n=47		Past vs Active swimmers
	Baseline	Follow up	Baseline	Follow up	
FeNO[†], ppb	18 (19.5)	16 (20)	14 (11)	14 (18)	.008[#]
Asthma, n (%)	6 (15)	9 (23)*	6 (13)	9 (19) *	.656
Allergic rhinitis, n (%)	13 (33)	16 (41)*	11 (23)	13 (28) *	.305
Asthma drugs, n (%)	5 (13)	12 (31)*	9 (19)	12 (26) *	.382
Physical Activity[†]					
Walking	462 (685)	693 (2402)	396 (1188)	739 (1148)	.016
Moderate	720 (2010)	1320 (1440)	120 (960)	960 (3120)**	.065
Vigorous	5760 (1920)	3840 (6120)	5760 (7040)	13440 (11520)*	<.001
Total	7173 (3864)	7242 (5718)*	6222 (9159)	17196 (8544)*	.001

Data presented as median (IQR) unless otherwise stated; Physical activity expressed as MET-min/week; bold figures represent statistically significant differences between comparing groups; Paired samples or chi-square tests were used as appropriate and changes between groups after the follow-up were assessed by GLM adjusting on confounders for FeNO: gender, age, allergic sensitization, physician-diagnosed asthma, use of asthma medication; and on gender and age for physical activity levels, with baseline values as covariable; *p<0.001; **p=0.002; p=0.005. †FeNO and physical activity are presented as absolute values, although they were log-transformed for comparison analysis. # Comparison of ΔFeNO variation from baseline to 3 y follow-up among active and past swimmers.

Figure 10 Variation of FeNO at baseline and at 3y follow-up among past and active swimmers.



6. DISCUSSION

6.1 Main findings and relation to previous studies

6.1.1 Diagnosis of asthma and healthcare of asthmatic athletes (Study I)

The introduction of mandatory objective asthma diagnosis for inhaler's use in 2009 decreased the requests submitted to the Anti-Doping Authority of Portugal by approximately half, suggesting that a large number of athletes were receiving medication based on symptoms only. The relative similarity between the proportion of positive tests in 2009 and 2010 suggests that the IOC more rigorous testing criteria strategy is reliable. This study also revealed that changes to the WADA guidelines on inhaled β 2-agonists in 2010 led to a dramatic decrease in the number of tests performed in Portuguese athletes with asthma and increased the number of athletes taking inhaled β 2-agonists without ICS.

These findings clearly show that guidelines for asthma diagnosis have an impact on the care of athletes with asthma and influence how respiratory symptoms are managed and treated in these patients. These results are in line with a previous study that evaluated the impact of IOC-MC recommendations. Dickinson *et al* found that 21% of British Olympic athletes were receiving asthma medication for which there was no clinical indication (Dickinson, Whyte et al. 2005). In Portugal, changes to the WADA 2009 Prohibited List also permitted more rigorous screening of asthmatic athletes due to the implementation of objective criteria for inhaler use; the new requirements also led to the withdrawal of unnecessary medication. It improved athlete's care by investigation of alternative diagnoses. Constant changes to WADA guidelines, however, jeopardize the achievements made to date and adversely affect the health of asthmatic athletes. Diagnosis of asthma is complex, and results of lung function, airway inflammation, and BHR provide important, complementary information that can aid asthma control.

Currently, unrestricted use of inhaled salbutamol, salmeterol, or formoterol is permitted as long as specified doses are not exceeded. Such a change, however, might lead to an increased use of long-acting β 2-agonists, without ICS, as recently was evidenced to occur in Olympic athletes (Bonini, Gramiccioni et al. 2015). This is a matter of concern since inhaled β 2-agonists may mask worsening of airway inflammation; furthermore, airway inflammation might contribute to the downregulation of β 2-agonists receptors (Bonini, Permaul et al. 2013). Therefore, although the current guidelines may seem fairer and seem to improve access to treatment among asthmatic athletes, they introduce safety issues stemming from the unsupervised use of inhaled β 2-agonists. As shown by our study, in the absence of mandatory objective testing for certain asthma medications, athletes may skip lung function tests.

6.1.2 Phenotypes of asthma in elite athletes (Study II)

This study identified two distinct phenotypes of asthma in athletes: “*atopic asthma*” defined by the occurrence of allergic sensitization, increased levels of FeNO, rhinitis and other allergic co-morbidities; and “*sports asthma*”, defined by the existence of exercise-induced respiratory symptoms and BHR in the absence of allergic features. Moreover, specific training and environment conditions were associated with increased risk of developing “*sports asthma*”: athletes practicing water and winter sports present a three and a nine fold increase in their risk of “*sports asthma*” respectively, compared with others.

The two patterns of asthma obtained in this present study are remarkably in accordance with the only previous report of phenotypes for athletes, a Finnish study relying on different study design and an a priori list of selected variables for statistical analysis (Haahtela, Malmberg et al. 2008).

Our study contributes to confirm that different risk factors, such as atopy and environmental training conditions, result in different patterns of asthma. For athletes practicing water and winter sports, their “occupation” demands exposure to potentially noxious stimuli, such as sport-specific environment, in addition to frequent episodes of prolonged hyperpnoea (Price, Ansley et al. 2013). The possibility that these exposures determine different underlying mechanisms of asthma has been previously raised (Langdeau, Turcotte et al. 2000, Langdeau and Boulet 2001) and should be emphasized.

The natural course of asthma in athletes has been difficult to change by anti-inflammatory treatment (Helenius, Lumme et al. 2005). This highlights a possible need of a different therapeutic approach for these subjects. To define these distinct phenotypes could lead not only to further understanding underlying mechanisms of asthma in elite athletes, but, and most important from a clinical point of view, also to recognize that potentially different treatments specifically targeted for the defined phenotypic groups in relation to the specific underlying mechanism are needed.

6.1.3 Dysautonomia, asthma and BHR in elite swimmers (Study III)

In this exploratory study, no significant differences were observed in parasympathetic parameters between asthmatic and non-asthmatic elite swimmers. However, in those with severe BHR, a significant difference became clear. Also, in those with clinical relevant BHR, significant correlations were observed between parasympathetic parameters and PD₂₀ methacholine. This suggests that in these athletes the increased parasympathetic tonus is associated with BHR particularly through the contraction of the bronchial smooth muscle rather than related with other features of asthma.

Previous studies supporting this hypothesis of dysautonomy associated with training are in accordance with our findings. Pichon *et al.* demonstrated that subjects with an increased BHR had a higher vagal tone (Pichon, de Bisschop et al. 2005), which was corroborated by Park *et al.* findings of a relationship between BHR and a diminished sweat secretion, tearing and salivary flow rate in healthy athletes, indicating autonomic dysfunction (Park, Stafford et al. 2008). All these studies have used BHR as an outcome measure, rather than asthma diagnosis, which is also the probable reason for the lack of

significant differences in parasympathetic outcomes between asthmatic and non-asthmatic swimmers in our study. In fact, while bronchoconstriction is largely dependent on airway smooth muscle cells, asthma is far more complex as a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, including airway epithelium, eosinophils, neutrophils, lymphocytes and mast cells (Holgate, Lemanske et al. 2008, Holgate 2008, Global Strategy for Asthma Management and Prevention 2012).

In elite competitive adolescent swimmers, an increase in BHR correlated with the exercise intensity after 3000 meters swimming in an indoor swimming pool was demonstrated, for both asthmatic and healthy subjects (Carlsen, Oseid et al. 1989). Together with our results, this seems to point out that dysautonomia might contribute to the severity of bronchial reactivity in swimmers.

6.1.4 Anti-cholinergic drugs for BHR in elite athletes (Study IV)

In this study, 23% of winter athletes had significant reversibility to inhaled ipratropium bromide and none to inhaled salbutamol. The main finding of this study was a highly significant correlation between BHR and reversibility to inhaled ipratropium bromide, but not so between BHR and reversibility to salbutamol. The significant high-grade negative correlation between a cholinergic bronchoconstrictor and an anticholinergic bronchodilator stimulus, and the lack of correlation with a β 2-stimulating bronchodilator, suggest an important role of an increased parasympathetic tone and sensitivity in the development of BHR and asthma in these top endurance-trained athletes.

Increased parasympathetic activity has previously been reported in endurance-trained athletes measured both by the parasympathetic activity of the eye by pupillometry in long distance runners (Filipe, Falcão-Reis et al. 2003, Kaltsatou, Kouidi et al. 2011) and of the cardiovascular system by the variation in the heart rate induced by an exercise test (Knöpfli and Bar-Or 1999). Furthermore, a significant correlation between heart rate variability indices and $\dot{V}O_2\text{max}$ was shown (Goldsmith, Bigger et al. 1997), which suggests that endurance training and increased aerobic capacity are followed by increased parasympathetic activity. These findings are supported by the present study in cross-country skiers who, in addition to the endurance training, are also exposed to cold air, an exposure previously reported to cause parasympathetic stimulation of the airways and contribute to EIB (McFadden and Ingram 1979).

Anticholinergic agents, such as ipratropium bromide, inhibit parasympathetic nerve impulses through competitive inhibition on the muscarinic acetylcholine receptors in smooth muscle and respiratory glands (de Jongste, Jongejan et al. 1991). It has been suggested that differences in the parasympathetic bronchial tone may explain why some patients are responders and others non-responders to anticholinergic treatment (Knöpfli, Bar-Or et al. 2005, Knöpfli, Luke-Zeitoun et al. 2007). As methacholine acts as a non-selective muscarinic receptor agonist, the bronchial responsiveness to methacholine may itself be considered to reflect the parasympathetic bronchial tone. In a recent study, ipratropium did not significantly influence the number and the perception of cough following exercise in cross-country skiers (Bordeleau, Turmel et al. 2014). Moreover, that study suggested that exercise-induced cough in these athletes is not mainly associated with

EIB, except for a subgroup of athletes which seemed to show a beneficial response to ipratropium, proposing different cough responses in this population (Bordeleau, Turmel et al. 2014). When activated, muscarinic receptors promote bronchoconstriction, glandular secretion, and blood vessel vasodilation (Lee, Jacoby et al. 2001, Bateman, Rennard et al. 2009). As there are no muscarinic receptors on airway sensory afferent nerves, it is unlikely that anticholinergic agents act through a direct effect on the cough reflex (Bateman, Rennard et al. 2009).

Although respiratory symptoms are common among cross-country skiers (Larsson, Ohlsén et al. 1993, Heir and Oseid 1994), our results showed no associations between self-reported symptoms and BHR, reversibility to inhaled ipratropium bromide or inhaled salbutamol, or to FeNO. The incidence of asthma symptoms found by self-reports is higher compared with objectively measured BHR and reversibility tests. This finding confirms that objective tests are a necessity in athletes in addition to symptoms to confirm the asthma diagnosis, as previously reported (Rundell, Im et al. 2001).

6.1.5 Exhaled breath temperature, exercise and asthma (Study V)

Elite swimmers presented increased EBT after a training session, supporting the hypothesis of heat loss during exercise. Interestingly, asthmatic swimmers did not experience a higher increase in EBT when compared to healthy swimmers after controlling for baseline EBT and body temperature. These findings may suggest that heat loss occurs as a physiological rather than a pathological response to exercise. Furthermore, no relation was observed between EBT and inflammatory cells in induced sputum, neither with the degree of BHR. Therefore, it is tempting to speculate that an inflammatory response to the heat loss of exercise might not be a key etiopathogenic mechanism of swimmers asthma.

Our results are in accordance with the two previous studies investigating the relation of EBT to EIA (Svensson, Nilsson et al. 2012, Tufvesson, Svensson et al. 2013). Both studies showed an increase in EBT after a standardized exercise challenge at the laboratory, but no differences were observed between asthmatic and healthy controls (Svensson, Nilsson et al. 2012, Tufvesson, Svensson et al. 2013), in agreement with our results obtained with the regular exercise training of competitive swimmers. During exercise, unperfused alveoli become perfused through recruitment of pulmonary capillaries, and underperfused units receive an increased blood supply. It is therefore conceivable to expect a physiologic increase in airways temperature related to this capillary recruitment instead of a pathological mechanism related to vasodilation resulting from inflammation. That is consistent with the lack of correlation previously found between nitric oxide levels with EBT after exercise (Tufvesson, Svensson et al. 2013), as well as the lack of correlation with sputum inflammatory cell counts observed in our study.

Vigorous exercise causes epithelial damage, which has likewise been linked to EIA/EIB in elite athletes (Bougault, Turmel et al. 2009). Clara cell secretory protein (CC16) a peripheral marker for assessing the epithelial barrier disruption in the lower airways (Broeckaert and Bernard 2000, Bernard, Carbonnelle et al. 2007) was shown to correlate

to EBT after an exercise challenge, reflecting an overall epithelial involvement (Tufvesson, Svensson et al. 2013); no differences were found between asthmatics and healthy controls (Tufvesson, Svensson et al. 2013), which again leads to the concept of a physiological rather than a pathological response to exercise.

Bronchial epithelial cells are released in higher amounts into sputum (epithelial shedding) of cold air athletes and swimmers (Bougault, Turmel et al. 2009), highlighting the role of environmental exposure impact on epithelial layer. These results are in agreement with our findings. However, we observed no significant differences between asthmatic and healthy swimmers, which support that exercise and irritant exposures contribute to a detrimental effect on the athletes' airways but probably not the hypothesis of these two acting as etiopathogenic mechanisms of EIA.

So, in the light of these results, along with previous published findings, we would like to underline that: 1) Exercise causes EBT to increase, probably due to a physiological increase in blood flow in the respiratory mucosa, rather than due to a pathological mechanism since no differences were observed between asthmatic subjects and healthy controls; 2) Mechanic noxious stimulus of repeated bouts of increased ventilation during training cause epithelial damage of the airways and susceptibility to irritative stimuli, supported by observed increased numbers of bronchial epithelial cells in athletes and high levels of CC16, regardless of their asthmatic status; 3) Due to the daily repeated training, epithelial repair is delayed and a "frustrated" inflammatory response occurs to heal the damage of physical injury in both asthmatic and healthy swimmers. No differences were observed between asthmatic and healthy subjects with regards to the above mentioned observations, suggesting the explanatory model of EIA and bronchoconstriction in athletes will probable include the interplay between environmental training factors and athlete's personal and genetic risk factors (Moreira, Delgado et al. 2011).

6.1.6 Effect of long-term swimming on airways (Study VI)

This prospective study of competitive swimmers shows that those who remained active after 3-years significantly increased their levels of airway inflammation measured by exhaled nitric oxide compared to those who quitted swimming, independently of their gender, age, allergic sensitization, or asthma status. After the 3-year follow-up the prevalence of asthma, allergic rhinitis and use of asthma medication increased significantly in both groups. All subjects increased their overall physical activity levels; however, significant increases in moderate and vigorous physical activity levels were only observed in active swimmers.

Two studies prospectively assessed BHR and airway inflammation in swimmers. Bougault *et al* conducted a study comparing the same swimmers during intensive training period and after at least 15 days without intense swimming (Bougault, Turmel et al. 2011), while Helenius *et al* followed 42 Finnish swimmers during 5 years and compared those who kept versus those who retired from swimming (Helenius, Ryttilä et al. 2002). Both studies suggest that BHR is reduced when intense training is stopped.

Conflicting results, however, occurred with respect to airway inflammation. In accordance with our results, Helenius *et al* found that after a 5-year follow-up, mild

eosinophilic airway inflammation was aggravated among those swimmers who remained at high-level training and tended to attenuate in swimmers who finished their sports careers. On the contrary, no significant change was observed between intense training and resting periods for swimmers' airway inflammatory cell counts (Bougault, Turmel et al. 2011). However, in Bougault *et al.* study, only 7 swimmers and 2 controls were able to produce sputum in the two visits and therefore only swimmers' data are presented (Bougault, Turmel et al. 2011). Such small numbers, together with the short time of recovery, might be responsible for the results found.

During the 5 year follow-up period, Helenius *et al* found that the occurrence of exercise-induced bronchial symptoms and asthma tended to decrease in former swimmers, a result that differs from our findings. However, in our study, subjects remained physically active despite stopping swimming, which may responsible for keeping asthma symptoms and need of asthma medication. Also, asthma in our study was defined by doctor diagnosis, rather than by objective evidence, which might also be related to this discrepancy.

The diagnosis of asthma in athletes dramatically impacts on the disease management and the definition of asthma in this population includes two specific different phenotypes. Given that both swimmers and winter sport athletes have shown a higher risk of presenting the “sports asthma” phenotype in study II, a different mechanism of asthma within these athletes may be hypothesized. Both studies III and IV support that this mechanism is most probably related to an imbalance towards an increased parasympathetic activity that predisposes to EIB. Study V further supports that other mechanism rather than the inflammation and thermal loss is associated with EIA in swimmers and study VI confirms a relative independence of the two conditions given that eosinophilic airway inflammation decreases in swimmers who finished their career while the prevalence of asthma and use of asthma medication increases significantly in both active and past adolescent swimmers.

6.2 Methodological considerations – limitations and strengths

Our findings in **Study I** are limited by the retrospective nature of the study and the anonymity of the data collected. However, the decrease in requests observed in 2009 is not due to athletes being already covered by a previous submission, as renewal was yearly at that time. TUEs approval lasted for 4 years and we can therefore be sure that no repetitions occurred in 2010. Also, our study contributes to overcoming the paucity of data regarding asthma in Portuguese athletes. Moreover, we have evaluated how changes in WADA guidelines have impacted the clinical management of asthma in this setting.

Establishing different phenotypes of asthma in elite athletes (**Study II**) was limited by the cross-sectional design of the study, which does not permit to identify causality; but the statistical models used to pool and characterize different clusters make this study especially useful by retrieving a clear view on asthma phenotypes in athletes. Replication

of results in other datasets is important when using these exploratory statistical techniques, and the two patterns of asthma obtained in this present study are remarkably in accordance with the only previously published report, a study relying on different study design and an a priori list of selected variables for statistical analysis (Haahtela, Malmberg et al. 2008). The use of different methods (both direct and indirect challenges) to assess BHR in athletes must be also pointed out as a limitation. In **Study II**, information was collected from medical files, so there was no possibility to homogenize tests performed by athletes in two centers. In any case, the final diagnosis was made according to IOC criteria using standardized methods. Moreover, the absence of information about age of asthma onset limits the extent of conclusion because we cannot be aware if previous presence of asthma would influence the type of sport chosen. It does not seem to be the case, based on previous literature, as the prevalence of asthma is known to increase with age both in swimmers and skiers (Stensrud, Mykland et al. 2007, Pedersen, Lund et al. 2008, Bougault and Boulet 2012). However, this study should be succeeded by new prospective studies following young athletes from adolescence until adulthood. Besides, although motivating, results provided by this exploratory analysis have to be interpreted in context of future work addressing whether the two phenotypes are relevant from a clinical perspective. Potential phenotypes require prospective validation with clinical interventional trials. Our **study IV** has shown that Norwegian competitive endurance winter athletes respond with a higher reversibility to ipratropium bromide than to inhaled β 2-agonists, supporting that different phenotypes may reflect different mechanisms of EIA, and therefore imply different treatment modalities. The fact that only winter athletes were included in **Study IV** makes it difficult to generalize data for elite athletes practicing other sports. However, as shown in **Study II**, these athletes have a higher risk of developing a distinct asthma phenotype. Furthermore, in **Study IV** respiratory symptoms and asthma diagnosis were assessed only by questionnaire. However, this is unlikely to affect objective BHR and reversibility assessments. Also, the bronchodilation procedures were performed 2000 meters above the sea level, which not only does not reproduce the real-life situation of most athletes, but may induce altitude changes to some pulmonary ventilatory parameters (FVC, FEV₁, and FEF_{25-75%}) (Hashimoto, McWilliams et al. 1997). Anyway, the bronchodilator effect has not been found to increase at high altitude (Hashimoto, McWilliams et al. 1997); moreover, the high altitude is unlikely to affect differently the two bronchodilator procedures. However, in this study, eight athletes used short-acting β 2-agonists, ten used long-acting β 2-agonists and ten athletes used ICS. A potential influence from the use of these medications on the absence of any significant bronchodilator effect after inhaled salbutamol cannot be excluded. Collection of data for this study was carried out before the competitive season, but during an intensive training period, which may have influenced the degree of BHR in the athletes. An earlier study including young competitive skiers has shown that BHR in cross-country skiers varied according to seasons and exercise training intensity (Heir and Larsen 1995). In any case, **Study IV** is the first randomized cross-over study comparing the bronchodilator efficacy of inhaled ipratropium bromide versus inhaled salbutamol in winter athletes, and the relationship to bronchial methacholine responsiveness. It benefits from including the Norwegian national cross-country skiing team, and thus only athletes at the elite level. Also, as self-reported

respiratory symptoms are known to be poor predictors of asthma in elite athletes, an objective tool was employed in this study to avoid this potential bias.

The main limitation in both **studies III and V** is the sample size too small to draw solid conclusions; this small number was due to a strong effort we made to include only elite swimmers from the same team, in order to homogenize the characteristics of training and environment. But as a consequence, we cannot be certain that lack of differences between asthmatic and healthy swimmers was not due to low power. It is remarkable that even so some results are statistically significant.

Study III is also limited by the cross-sectional nature of data, which does not permit to identify causality. Though, it must be highlighted as strengths of this study, that a highly controlled evaluation was performed regarding all variables known to possibly interfere with the pupillometry (Fountas, Kapsalaki et al. 2006). Also, this was the first study to address the question of dysautonomia assessed by pupillometry related with swimmers' asthma and BHR. Swimmers with clinically relevant BHR, showed a significant correlation between PD₂₀ and four of the parasympathetic parameters, reflecting the reliability of pupillometry, since methacholine acts as a non-selective muscarinic receptor agonist in the parasympathetic nervous system. We have previously also shown that this is a very reproducible method (Stang, Couto et al. 2015).

We have used methacholine bronchial challenge to diagnose EIA. This method is used as a test for asthma (Bougault, Turmel et al. 2010), and seems more sensitive in confirming the diagnosis of BHR at least in cross-country skiers (Stensrud, Mykland et al. 2007). About 30% of the requests for β 2-agonist use at the 2004 Athens Olympic Games were based on the methacholine bronchial challenge (International Olympic Committee , Anderson, Sue-Chu et al. 2006). Although EVH is an indirect challenge acting as a surrogate of exercise inducing the release of inflammatory mediators considered responsible for EIB, in a cohort of recreational athletes, EVH demonstrated poor clinical reproducibility for the diagnosis of EIB (Price, Ansley et al. 2015). Methacholine challenge acts directly on the airway smooth muscle and mainly reflects bronchial responsiveness related to airway structural elements. Previous studies using indirect and direct challenges to characterize asthma in athletes had similar results (Stadelmann, Stensrud et al. 2011), and they can be used interchangeably for asthma diagnosis accordingly to IOC guidelines. Other point to bear in mind concerning the methacholine challenge in our studies is the fact that we have used a device in **study IV** (APS) different from the device used in most of the other studies (Mefar MB3), and evidence exists that the biological dose may vary between these two nebulizer systems, causing incomparable outcomes for subjects tested with different systems (Praml, Scharrer et al. 2005). More recent studies, however, have contradicted such findings (Praml, Scharrer et al. 2005, Schulze, Rosewich et al. 2009) and have also shown a close relationship between categories of BHR of APS single concentration method compared to the ATS five-breath dosimeter method. Nevertheless, we have not compared in any case athletes included in study IV to those enrolled in the remaining studies so it should not impact our conclusions.

Other question relates to the methacholine cut-off used to diagnose asthma. We have used different cut-offs to define clinically relevant BHR in **Study III** (PD₂₀ $\leq$ 3.2 μ mol) and **Study IV** (PD₂₀ $\leq$ 8 μ mol). This mostly reflects the divergent ideas concerning this

issue: it has been claimed that a methacholine challenge cut-off for BHR as suggested by the WADA and IOC-MC for athletes may underestimate the prevalence of EIB in symptomatic athletes not taking ICS with a PC₂₀ between 4 and 16 mg/mL (Bougault, Turmel et al. 2010). Previous reports suggested that a methacholine PC₂₀ ≤ 16 mg/mL indicates the presence of very mild or borderline BHR in the general population (Malo, Pineau et al. 1983, Crapo, Casaburi et al. 2000). Thus, respiratory symptoms in subjects with a PC₂₀ between 4 and 16 mg/mL may be due to asthma. Such differences mostly reflect the difficulties arising from asthma definition itself. The IOC-MC criteria to diagnose asthma in athletes mostly reflect the occurrence of bronchoconstriction and do not fully take into account the inflammatory characteristics of asthma as defined by GINA. In fact, while bronchoconstriction is largely dependent on airway smooth muscle cells, asthma is far more complex and is defined as a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, including airway epithelium, eosinophils, neutrophils, lymphocytes and mast cells. But IOC criteria are the current standards for asthma diagnosis in elite athletes and using such criteria homogenizes publications in this field. The recent American Thoracic Society Clinical Practice Guidelines overcome these discrepancies by using the terms *EIB with asthma* (EIB_A), for the occurrence of bronchial obstruction after exercise in asthmatic patients, and *EIB without asthma* (EIB_{W_A}), for the occurrence of bronchial obstruction in subjects without other symptoms and signs of clinical asthma.

Study V was not performed at the same time as the usual screening, which led some of the swimmers to be under inhaled corticosteroid therapy at the time of EBT collection. Nevertheless, no differences were observed between those with and without therapy, as previously reported in other studies (Peroni, Chinellato et al. 2012). This was the first time, to our knowledge, that EBT was evaluated in elite athletes, and also before and after a real-life training session, in order to assess if changes in EBT could support the hypothesis of heat loss due to physical exercise and inflammation occurring in the airways as mechanisms of EIA. Our study has other strengths: we have used a regression analysis model to adjust for baseline EBT, which is of particular relevance given the high dependence of both post-exercise and ΔEBT with baseline EBT, and for axillary temperature as a marker of body temperature. Also, recent studies have highlighted several factors influencing EBT (Vermeulen, Barreto et al. 2014), and these factors were addressed in our study. We showed that the central temperature significantly influences ΔEBT in elite swimmers; and we corrected for the possible confounding effect of several demographic variables (sex, age, height, weight) and training characteristics (intensity and number of hours of training). Measurement of EBT has been shown to be highly reproducible by the method employed in the present study (Vermeulen, Barreto et al. 2014), which supports the comparison before and after the swimming session. Furthermore, cell counts in induced sputum were used to evaluate the presence, type, and degree of inflammation in the airways, which gives more complete and reliable information than FeNO levels only.

The external validation of our findings in **studies III, IV, V and VI** may be questioned; our results may be difficult to extrapolate for athletes practicing other types of sports since, as previously discussed, swimmers and winter athletes are a special

population among elite athletes due to a potential detrimental effect of the swimming pool environmental exposure and by cold air exposure.

Study VI was partly limited by the number of subjects lost during follow-up (18%); however these subjects were similar to the others at the baseline evaluation. Second, we have only evaluated eosinophilic inflammation, although it has been previously reported that swimmers present a mixed type of airway inflammation (Moreira, Delgado et al. 2008). However, it has been argued that increased eosinophils and lymphocytes result from exposure to environmental factors related to different types of sport, while the increase in neutrophils is a consequence of endurance training (Bonsignore, Morici et al. 2003, Pedersen, Lund et al. 2008). The relationship between airways inflammation and swimming was our main focus in the present study and the reason for choosing FeNO as a marker. Mild eosinophilic airway inflammation has been shown to affect swimmers (Helenius, Rytälä et al. 1998, Helenius, Rytälä et al. 2002), and it has been suggested that for each 100 hours spent in the swimming pool for recreational swimmers, the risk of asthma in atopics and of elevated FeNO in non-atopics increases by 30% (Bernard, Carbonnelle et al. 2006). Even athletes who had finished swimming continued to be physically active and would thus possibly have neutrophilic inflammation and misperceive the results. Finally, asthma in **study VI** was defined as a positive response to the question "Has a doctor ever said that you have asthma?". Therefore, misclassification of disease status is possible. However, misclassification is unlikely to affect differently any of the groups and was independent of airway inflammation assessment. This study has important strengths: this is the first prospective study assessing the risk of airway inflammation in competitive non-elite swimmers. Moreover, we have adjusted for confounders and known risk factors that affect FeNO levels; and finally we have used a validated tool to monitor physical activity levels during the study. The follow-up study was performed through use of exactly the same methods and definitions that were used for the baseline measurements.

6.3 Implications for practice and future research

The different prevalence of asthma/BHR between sports disciplines raised the possibility that exercise *per se*, or even repeated increased ventilation, may not be the primary mechanism involved in the development of these conditions in athletes. Environmental factors involving the 'type' and 'content' of the ambient inhaled air could play an important role. In fact, we have shown that there are two phenotypes of asthma in elite athletes. The mechanisms involved in the development of asthma/BHR for these categories may be different, and we have demonstrated that athletes practicing water and winter sports are at increased risk of presenting the phenotype of "sports asthma". The content and physical characteristics of the inhaled air seem to be important factors, while immune and neurohumoral influences could play a modulatory role. Based on our findings, the inflammatory hypothesis may not completely justify the increased prevalence of EIA. We could not confirm a relationship between airway's inflammation and respiratory heat loss during exercise, and despite decrease of airway inflammation after finishing competitive swimming, the prevalence of asthma and use of asthma medication

increased significantly in both active and past adolescent swimmers. But hyperpnea of large volumes of air inflicts damage to the airways, and inflammation is a nonspecific response of tissues to injury, which usually leads to repair and restoration of the normal structure and function. The restorative process after injury involves plasma exudation and movement of cells into the airways, a process repeated many times during a season of training (Ali, Norsk et al. 2012). This process has the potential to expose smooth muscle to a wide variety of plasma- and cell-derived substances. The exposure to these substances over time can lead to an alteration in the contractile properties of the smooth muscle and turn it more prone to bronchoconstriction. BHR is likely a result of both those structural airway changes, also called airway remodeling, and increased bronchial tonus related to higher parasympathetic activity. We have shown that elite swimmers with higher BHR present higher parasympathetic activity. Also, we have shown that drugs inhibiting bronchial parasympathetic activity, such as anti-cholinergics, are more effective for bronchodilation in elite winter athletes. With the recent approval of tiotropium bromide for asthma treatment, and the advents of new long-acting anti-cholinergics, research about the use of such drugs in EIA should be pursued in a near future.

Genetic susceptibility, neurogenic mechanisms and epithelial sensitivity should also be taken in account when explaining the athlete's individual risk for bronchoconstriction, and recent research is heading towards with these hypotheses. Epithelial damage exposes nerve endings and can cause tachykinin secretion, such as substance P (Naline, Devillier et al. 1989). Substance P is one of the major initiators of neurogenic inflammation and increased circulating levels have been found after strenuous exercise (Lind, Brudin et al. 1996). Crimi *et al* demonstrated that bronchoconstriction induced by substance P may be due to cholinergic activation (Crimi, Palermo et al. 1990). Why some athletes further develop asthma, could be the result of upregulation of PPT-1 gene, from which substance P is derived from, and NK-1 receptors (Moreira, Delgado et al. 2011). Furthermore, it has been shown that acetylcholine released from the respiratory epithelium may have a stimulant effect upon airways inflammation (Wessler and Kirkpatrick 2008).

Also, some of the genetic factors which predispose to asthma do so via a tendency towards increased epithelial damage (Loxham, Davies et al. 2014), and increased susceptibility to oxidative stress (Bucchieri, Puddicombe et al. 2002). It has been claimed that acute physical exercise performed at high intensity may result in oxidative stress, due to reactive oxygen species being generated excessively by enhanced oxygen consumption (Vezzoli, Pugliese et al. 2014). This phenomenon, usually defined as *exercise-induced oxidative stress*, has been implicated in the damage of cellular membranes, increased cellular swelling, and decreased cell membrane fluidity (Tappel 1973, Esterbauer and Zollner 1995). It is noteworthy that CC16 is produced in order to protect the respiratory tract against inflammation, but also against oxidative stress (Broeckaert and Bernard 2000). Interestingly, data show association of CC16 gene polymorphism with BHR and asthma (Sengler, Heinzmann et al. 2003). Thus, there are still many open questions and further studies are needed within this field.

In the specific case of elite swimmers, the presence of asthma seems to be multifactorial. Besides the high levels of exercise with hyperpnea, exposure to chlorine derivatives likely results in even higher levels of oxidative stress through reaction with substrates in the epithelial lining, which decreases bronchial intrinsic antioxidants

(Krishna, Madden et al. 1998, Stenfors, Pourazar et al. 2002) and produces subsequent inflammation (Li, Gauderman et al. 2006), leading to progression of BHR (Southam, Ellis et al. 2007, Fisk, Steigerwald et al. 2010) and a vicious cycle of continued airway oxidative stress and inflammation (Fisk, Steigerwald et al. 2010). In the meanwhile, it was recently showed, in a mouse model, that BHR can be induced by a single hypochlorite-ovalbumin instillation, independently of bronchial influx of inflammatory cell (Hox, Vanoirbeek et al. 2013). In that study, Hox *et al.* highlighted the role of transient receptor potential ankyrin 1 channel on nociceptive airway sensory nerves in nonallergic BHR (Hox, Vanoirbeek et al. 2013). These chemoreceptors are expressed on substance P-producing airway sensory nerve fibers and are involved in irritant-induced airway disease. Substance P, as mentioned, is one of the major initiators of neurogenic inflammation, and the activation of substance P receptor leads to asthma-related features such as bronchoconstriction (Barnes 1991). It seems, therefore, that induction of BHR by exposure to hypochlorite depends on a neuroimmune interaction (Hox, Vanoirbeek et al. 2013). However, if trichloramines themselves cause swimmers to have a high prevalence of asthma/asthma symptoms, then all swimmers should theoretically develop the disease; however, this is not the case (Fisk, Steigerwald et al. 2010). Genetic co-factors may promote or prevent the development of asthma in response to chloramines and other environmental irritants (Xu, Rijcken et al. 1997). Genetic variations that account for intrinsic antioxidants and inflammatory mediators in bronchial tissue may indicate whether inhalant irritants will initiate the development of asthma (Imboden, Rochat et al. 2008, Melén, Nyberg et al. 2008). Currently, there is no published research relating genotype to asthma specifically in regards to swimmers and chloramine exposure and that is a pertinent hypothesis to pursue.

Given the high prevalence of asthma/BHR in this population, it is important to ascertain the potential health burden. Knowledge of the long-term health repercussions for the aquatic athlete after retirement is still scarce, and further studies in this area are required, especially considering some discrepancies found between ours and the two previous studies (Helenius, Ryttilä et al. 2002, Bougault, Turmel et al. 2011). Understanding the health risks of the practice of aquatic sports is necessary in order to guide team physicians in their screening programs to optimize health and performance at the elite level, as well as the recreational level.

It has been highlighted that preventive strategies to protect airways, as recommended for other types of relevant occupational exposures, may be a useful therapeutic option for highlevel athletes (Boulet and O'Byrne 2015). Future research should evaluate athletes' subjective awareness, understanding, and attitudes toward respiratory symptoms during training, based on objective measurements of associated physiologic changes. Educational programs on the recognition of abnormal respiratory symptoms in athletes could help them optimize their health and training. The high prevalence of asthma in sports underscores the need for authorities to develop educational initiatives and strategies for prevention, screening, identification, and treatment in this population. Further research is required to better delineate the pathophysiologic mechanisms for the development of asthma/BHR in the athletes, and to understand the natural history of the disease after retirement to assess long-term health consequences for athletes who have asthma as a result of endurance training, especially for aquatic and winter sports. Despite the increased

risk of asthma in elite swimming, the physical, mental health, and lifestyle benefits of participation in the sport are numerous (Romberg, Tufvesson et al. 2012). With attention to the findings and recommendations resulting from this study, healthy participation in sports at the elite level can be improved and enjoyed.

7. CONCLUSIONS

In the present study, phenotypes and mechanisms of asthma development in elite athletes were investigated. Based on the results presented in this thesis, the following conclusions can be drawn:

- 1) Defining asthma in athletes implies a correct diagnosis which is crucial for management. Strict guidelines were shown to impact asthmatic athletes' care; by starting a more rigorous screening withdrawal of unnecessary medication was possible in approximately half of the athletes.
- 2) Two asthma phenotypes in elite athletes were identified: "atopic asthma" and "sports asthma". The type of sport practiced was associated with different phenotypes: water and winter sport athletes had 3 and 9-fold increased risk of "sports asthma", respectively.
- 3) Differences in parasympathetic parameters between asthmatic and non-asthmatic swimmers were not significant, but among those with clinically relevant bronchial hyperresponsiveness an association was evident, supporting the relation between dysautonomia and bronchial hyperresponsiveness.
- 4) In elite skiers, a significantly higher reversibility was observed after inhaled ipratropium bromide compared to inhaled salbutamol. Methacholine bronchial reactivity had a highly significant inverse correlation to the cholinergic antagonism of inhaled ipratropium bromide, but not to the bronchodilating inhaled salbutamol. This markedly increased bronchial parasympathetic tone may represent an important mechanism in the development of skier's asthma.
- 5) A relationship between airway's inflammation and respiratory heat loss after vigorous exercise could not be confirmed, suggesting that the heat loss, and consequent increase in exhaled breath temperature, occur as a physiological rather than a pathological response to exercise.
- 6) Competitive swimmers who remained active at a 3-year follow-up significantly increased their levels of airway inflammation measured by exhaled nitric oxide levels, independently of their gender, age, allergic sensitization, or asthma status, while in those who finished swimming airway inflammation decreases. The prevalence of asthma and use of asthma medication increased significantly in both active and past adolescent swimmers, suggesting a relative independence of the two conditions.

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Antoine de Saint-Exupéry

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ORIGINAL PUBLICATIONS

Study I

Impact of changes in anti-doping regulations (WADA Guidelines) on asthma care in athletes

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Impact of Changes in Anti-doping Regulations (WADA Guidelines) on Asthma Care in Athletes

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Objective: To investigate how changes to the World Anti-Doping Agency (WADA) guidelines on asthma medication requests have impacted the management of asthmatic athletes in Portugal.

Design: Retrospective analysis of asthma medication requests submitted in 2008 to 2010.

Setting: Portuguese Anti-Doping Authority database.

Participants: Athletes requesting the use of inhaled corticosteroids and/or β_2 -agonists.

Independent Variables: Demographic, therapeutic, and diagnostic test data.

Main Outcome Measures: Yearly changes in number of asthma medication requests and diagnostic procedures.

Results: We analyzed 326 requests: 173 abbreviated Therapeutic Use Exemptions (TUEs) in 2008 (objective tests not required), 9 Declaration of Use (DoU) and 76 TUEs in 2009, and 39 DoU and 29 TUEs in 2010. Spirometry was performed in 87% and 37% of athletes in 2009 and 2010, respectively; the corresponding figures for bronchoprovocation were 59% and 16%, almost all positive in both years.

Conclusions: Applications for inhaler use have decreased by approximately half since objective asthma testing became mandatory. Our findings show that WADA guidelines have an impact on asthmatic athletes care: In 2009 a more rigorous screening was possible, leading to withdrawal of unnecessary medication. Constant changes, however, jeopardize this achievement and nowadays introduce safety issues stemming from the unsupervised use of inhaled β_2 -agonists.

Key Words: asthma, airway hyperresponsiveness, anti-doping, bronchoconstriction, exercise, inhaled beta-2 agonists, sports, WADA

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INTRODUCTION

Diagnosing asthma in athletes is challenging. Multiple phenotypes of asthma exist, and different underlying mechanisms contribute to etiopathogenesis.^{1,2} Also, alternative diagnoses must be considered,³ and in athletes, symptoms are poor predictors of this condition.⁴ Objective evidence (eg, positive bronchodilator or bronchoprovocation test), thus, is needed to confirm a diagnosis in this setting.⁵

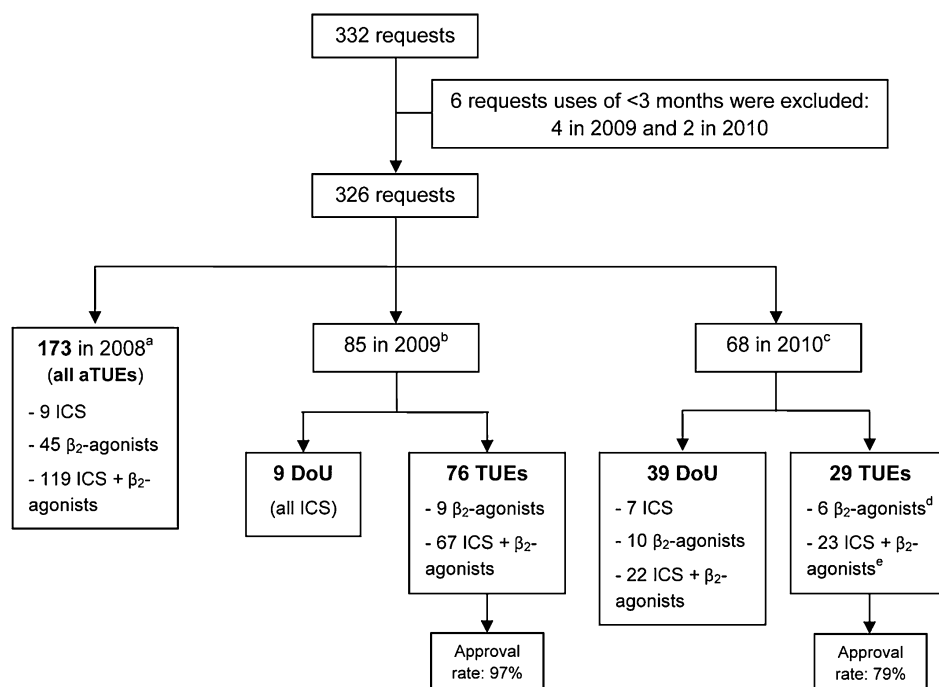
The recommendation of the International Olympic Committee Medical Commission (IOC-MC) for Olympic athletes to present objective evidence of asthma before allowing the use of inhaled β_2 -agonists (IBAs), in place since 2002, has facilitated the study of how asthma impacts different sports and has benefited athletes by ensuring better care. In 2009, the World Anti-Doping Agency (WADA) followed the IOC approach, extending it to all other athletes.⁶ The WADA guidelines on asthma, however, have changed in recent years. Before 2009, an abbreviated Therapeutic Use Exemption (aTUE), which did not require objective evidence of asthma, accompanied by a physician's report of asthma, was sufficient for requesting permission to use inhaled corticosteroids (ICS) and inhaled formoterol, salbutamol, salmeterol, and terbutaline. In 2009, however, aTUEs were withdrawn and replaced by a Declaration of Use (DoU) for ICS and a full Therapeutic Use Exemption (TUE) requiring objective evidence for the same 4 IBAs. In 2010, the DoU was extended to salbutamol and salmeterol, but the other IBAs still required a TUE.

The aim of this study was to assess the impact of WADA guideline changes on asthma medication requests by Portuguese athletes.

METHODS

We retrospectively analyzed asthma medication requests submitted to the Portuguese Anti-Doping Authority between 2008 and 2010. Athletes older than 16 years who requested permission to use ICS and/or IBAs for more than 3 months were included. Data on respiratory symptoms, medication requested, spirometry, and atopy (at least 1 positive skin prick test or positive specific IgE) were collected. A diagnosis of asthma was based on a positive bronchodilator test or bronchoprovocation test.⁵ Exhaled nitric oxide results were converted to personal predicted values using the FeNO Interpretation Aid tool (<http://www.enovis.org>) and considered increased if above 150% of predicted. Data were expressed as median and range, and categorical variables were compared using the χ^2 or Fisher

FIGURE 1. Flow chart showing requests for asthma medication submitted by athletes to the Anti-Doping Authority of Portugal between 2008 and 2010. Notes: ^aIn 2008, only an abbreviated TUE was required for ICS and IBAs salbutamol, salmeterol, formoterol, and terbutaline (ie, no objective testing or approval was required). ^bIn 2009, salbutamol, salmeterol, terbutaline, and formoterol required a full TUE and ICS required a DoU. ^cIn 2010, salbutamol, salmeterol, and ICS required a DoU while terbutaline and formoterol required a full TUE. ^dThree requests for formoterol (of which 2 were not approved), 2 for terbutaline (of which 1 was not approved), and 1 for indacaterol. ^eTwenty-one requests for formoterol (of which 3 were not approved) and 2 requests for salbutamol/terbutaline use.



exact test (statistical significance, $P < 0.05$). Analyses were performed using SPSS version 18.0 (SPSS Inc, Chicago, Illinois).

RESULTS

We analyzed requests from 326 athletes [254 males; median age, 24 years (range, 16-62 years)] (Figure 1). The requests were as follows: in 2008, 173 aTUEs were submitted; in 2009 and 2010, 9 and 39 DoU were submitted, respectively; regarding TUEs, the approval rate was 97% (74 of 76) in 2009 and 79% (23/29) in 2010 ($P = 0.005$). Requests for ICS combined with IBAs were the most frequent. Requests for isolated IBAs increased significantly from 2009 to 2010 (from 9 to 16, $P = 0.03$). The clinical and diagnostic tests performed are shown in Table 1 and the corresponding results in Table 2. No tests were reported in 2008 because all the requests submitted in that year were aTUEs.

DISCUSSION

The introduction of mandatory objective criteria for inhaler use (2009) decreased the requests submitted to the Portuguese Anti-Doping Authority by approximately half, suggesting that a large number of athletes were receiving medication based on symptoms only. The relative similarity between the proportion of positive tests in 2009 and 2010 suggests that the more rigorous testing criteria strategy is reliable. We also saw that changes to the WADA guidelines on IBAs in 2010 led to a dramatic decrease in the number of tests performed in Portuguese athletes with asthma.

Our findings clearly show that WADA guidelines have an impact on the care of athletes with documented asthma and influence how respiratory symptoms are managed and treated in these patients. A study that evaluated the impact of IOC-MC rules found that 21% of British Olympic athletes

were receiving asthma medication for which there was no clinical indication.⁷ In Portugal, changes to the WADA 2009 Prohibited List permitted more rigorous screening of asthmatic athletes, thanks to the implementation of objective criteria for inhaler use. The new requirements also led to the withdrawal of unnecessary medication. It improved athlete's care by investigation of alternative diagnoses.

Constant changes to WADA guidelines, however, jeopardize the achievements made to date and adversely affect the health of asthmatic athletes. Diagnosis of asthma is complex, and lung function, airway inflammation, and hyper-responsiveness tests provide important complementary information that can aid asthma control.⁸ Also, the fact that a TUE is necessary for some IBAs while a DoU is sufficient for

TABLE 1. Tests Performed in Athletes Whose Request to Use Asthma Medication Was Approved by the Portuguese Anti-doping Authority

	2009 (n = 83)	2010 (n = 62)	P
Spirometry	72 (87)	23 (37)	<0.001*
Bronchodilation test	37 (45)	15 (24)	0.011*
Bronchoprovocation challenge	49 (59)	10 (16)	<0.001*
Methacholine	46 (55)	9 (15)	—
Mannitol	0	1 (2)	—
Exercise	3 (4)	0	—
Exhaled nitric oxide	15 (18)	6 (10)	0.155
SPT or sIgE	56 (67)	18 (29)	<0.001*

No tests were reported in 2008 because it was not necessary to provide objective evidence of asthma at this time. Data are reported as n (%).

*Statistically significant.

SPT, skin prick tests; sIgE, specific immunoglobulin E.

TABLE 2. Reported Symptoms and Positive Test Results in Athletes With Asthma Who Submitted a Therapeutic Use Exemption Application to the Anti-doping Authority of Portugal

	2009 (n = 83)	2010 (n = 62)
Respiratory symptoms	81 (98)	62 (100)
Airflow limitation on spirometry	12/72 (17)	4/23 (17)
Positive bronchodilation	20/37 (54)	8/15 (53)
Positive bronchoprovocation	46/49 (94)	10/10 (100)
Airway allergic inflammation*	10/15 (67)	2/6 (33)
Atopy	50/56 (89)	15/18 (83)

Data are reported as positive/performed (%).

*Defined as exhaled nitric oxide >150% of predicted value for age and height, calculated using the FeNO Interpretation Aid tool (<http://www.enovis.org>).

others has generated intense debate⁹ and led to different management strategies being used in this setting, as evidenced by our study. In 2010, for example, athletes could avoid objective testing by simply applying to use an IBA that required a DoU.

Our findings are limited by the retrospective nature of the study and the fact that the data we collected were anonymous. However, we are certain that the decrease in requests observed in 2009 is not due to the fact that athletes were already covered by a previous submission as renewal was yearly at that time. TUEs now last for 4 years and we can therefore be sure that no repetitions occurred in 2010.

Our study contributes to overcoming the paucity of data regarding asthma in Portuguese athletes. Moreover, we have evaluated how changes in WADA guidelines have impacted the clinical management of asthma in this setting. In the 2012 WADA guidelines, unrestricted use of inhaled salbutamol, salmeterol, or formoterol is permitted as long as specified doses are not exceeded. Such a change, however, might lead to an increased use of long-acting IBAs, without ICS. This is a matter of concern as IBAs may mask worsening of airway inflammation; furthermore, airway inflammation might contribute to the downregulation of IBA receptors.¹⁰ Therefore, although the 2012

guidelines may seem fairer and improve access to treatment among asthmatic athletes, they introduce safety issues stemming from the unsupervised use of IBAs. As shown by our study, in the absence of mandatory objective testing for certain asthma medications, athletes may choose not to undergo lung function tests. The risks associated with such a decision should be investigated in new prospective studies.

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Study II

Two distinct phenotypes of
asthma in elite athletes
identified by latent class
analysis

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ORIGINAL ARTICLE

Two distinct phenotypes of asthma in elite athletes identified by latent class analysis

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Abstract

Introduction: Clusters of asthma in athletes have been insufficiently studied. Therefore, the present study aimed to characterize asthma phenotypes in elite athletes using latent class analysis (LCA) and to evaluate its association with the type of sport practiced. **Methods:** In the present cross-sectional study, an analysis of athletes' records was carried out in databases of the Portuguese National Anti-Doping Committee and the Norwegian School of Sport Sciences. Athletes with asthma, diagnosed according to criteria given by the International Olympic Committee, were included for LCA. Sports practiced were categorized into *water*, *winter* and *other* sports. **Results:** Of 324 files screened, 150 files belonged to asthmatic athletes (91 Portuguese; 59 Norwegian). LCA retrieved two clusters: "*atopic asthma*" defined by allergic sensitization, rhinitis and allergic co-morbidities and increased exhaled nitric oxide levels; and "*sports asthma*", defined by exercise-induced respiratory symptoms and airway hyperresponsiveness without allergic features. The risk of developing the phenotype "*sports asthma*" was significantly increased in athletes practicing water (OR = 2.87; 95%CI [1.82–4.51]) and winter (OR = 8.65; 95%CI [2.67–28.03]) sports, when compared with other athletes. **Conclusion:** Two asthma phenotypes were identified in elite athletes: "*atopic asthma*" and "*sports asthma*". The type of sport practiced was associated with different phenotypes: water and winter sport athletes had three- and ninefold increased risk of "*sports asthma*". Recognizing different phenotypes is clinically relevant as it would lead to distinct targeted treatments.

Keywords

Asthma, athletes, clusters, exercise-induced bronchoconstriction, latent class analysis, phenotypes, sports, training environment

History

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Introduction

Exercise training improves asthma symptoms, quality of life, exercise capacity, bronchial hyperresponsiveness (BHR) and lung function in asthmatics [1,2]. Thus, physical activity should be recommended as a supplementary therapy to medication in asthmatic subjects [1]. However, although moderate exercise has proven to be beneficial, repeated high-intensity exercise performed by elite athletes seems to contribute to the development of asthma and BHR. In fact, it has been recognized that elite athletes have increased risk of developing asthma, especially those who practice endurance sports, such as swimming and running, or winter sports [3,4]. Nevertheless, asthma is a complex syndrome with variable clinical presentation, and different physiologic and pathologic

parameters. Characterization of this heterogeneity has promoted the concept of asthma consisting in multiple phenotypes or consistent groupings of characteristics [5].

Defining phenotypes of asthma has been a major objective in recent years, as it would facilitate research into etiology and pathophysiology, targeted treatment and preventive measures, and improve prediction of long-term outcomes [6]. Up to this moment, in what concerns athletes with asthma, there is no evidence to support clusters of grouping characteristics, although it is generally recognized that the asthmatic condition which develops in athletes during their sports career is not likely to be similar to what is usually considered to be asthma in clinical practice [7]. The hypothesis of different phenotypes of asthma occurring in athletes has only been approached once in the literature, in a review article. Haahtela et al. [8] suggested that there may be two different clinical phenotypes of asthma in elite athletes: "classical asthma" characterized by early onset childhood asthma, methacholine hyperresponsiveness, atopy and signs of

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eosinophilic airway inflammation reflected by increased exhaled nitric oxide levels (FE_{NO}); and another distinct phenotype with late onset of symptoms during sports career, airway responsiveness to eucapnic voluntary hyperpnoea (EVH) and a variable association with atopic markers and FE_{NO}. These phenotypes were described only in Finnish athletes, and have not been fully established so far.

Most recent efforts to describe phenotypes are based on cluster analysis. These multivariate statistical methods allow splitting the differences between patient group data into disease categories and clinically meaningful groups, therefore being less dependent on *a priori* assumptions. These methods have already been successfully applied within respiratory medicine [6,9–11] to identify asthma phenotypes that exhibited differences in clinical, physiological and inflammatory parameters as well as response to treatment [10,11]. However, such methods have not been applied to athletes with asthma.

The objectives of the present study were to identify and characterize asthma phenotypes in elite athletes using latent class analysis (LCA) and to assess a possible association with the type of sport practiced.

Methods

Design and participants

In the present cross-sectional study, an analysis of elite athlete records kept in database files of two different countries was performed. Portuguese and Norwegian athletes training at high competitive levels (national, international or Olympic teams) were identified through existing institution databases. In Portugal, we used registries of elite athletes available at the Portuguese Anti-doping Authority and the Portuguese database of Olympic athletes; in Norway, we analyzed medical files from the respiratory medical team of the Norwegian School of Sport Sciences, including Olympic athletes participating in the 2008 summer and 2010 winter Olympic Games. Athletes were selected according with available information on symptoms, lung function and airway inflammation, BHR, and allergic sensitization. Healthy athletes and those with other conditions rather than asthma were excluded. From all reviewed files, 324 files had complete information available and informed consent for data use. Of these 324 athletes, 150 athletes fulfilled asthma criteria and were included for LCA.

The present study was conducted in accordance with Declaration of Helsinki for Medical Research Involving Human Subjects and was approved by Regional Medical Ethics Committees and Norwegian Data Inspectorate. All included subjects signed an informed consent for data usage.

Definitions

Asthma diagnosis was established by a medical doctor according to criteria set by the International Olympic Committee to document asthma in athletes [4,12], with objective evidence of either reversibility after bronchodilator administration or BHR after a bronchial provocation challenge. The demographic data obtained included age, gender, height, weight and sport practiced. The type of sport was

classified according to environmental training conditions into *water* sports (swimming and water polo), *winter* sports (cross-country skiing, biathlon, skeleton, alpine skiing and ski cross) and *other* sports (speed skating, curling, handball, judo, triathlon, football, cycling, beach volley, rowing, athletics, sailing, badminton, canoeing, curling, equestrian, taekwondo, auto-racing, billiards, paragliding, rugby, tennis, roller hockey, kickboxing, fencing, basketball or golf). Medical data collected included presence of respiratory symptoms, current use of asthma medication and presence of rhinitis or other allergic diseases (conjunctivitis, urticaria, eczema, anaphylaxis and drug, food and venom allergies). These data were sampled through allergy questionnaire for athletes (AQUA) questionnaire [13] at the time of the medical consultation. For statistical purposes, variables were categorized according to the definitions presented in Table 1. Spirometry was performed in agreement with the European Respiratory Society guidelines [14] and results (forced expiratory volume in first second – FEV₁ and forced vital capacity – FVC) were presented as both absolute and predicted values, according to published reference algorithms [15]. For both airflow obstruction and BHR, the first ever performed spirometry and the first ever performed bronchial provocation challenge, respectively, were considered.

Statistical analysis

Results are presented as mean values [95% confidence interval (CI)], mean $\pm$ standard deviation (SD), or medians $\pm$ interquartile range (IQR) in case of skewed distribution, or counts (*n*, %). Independent samples *t*-test was used for comparison of normally distributed continuous data, and Mann–Whitney test was used on data with skewed distribution. Categorical variables were compared by Chi-square or Fisher's exact tests. These analyses were performed using SPSS (IBM SPSS Statistics for Windows, Version 20.0, IBM Corp., Armonk, NY), considering a significance level of 0.05.

LCA was used to uncover distinct groups of individuals from a sample (patterns) homogeneous within the group, considering that the performance of an individual in a set of items is explained by a categorical latent variable with *K* classes, commonly called “latent classes”. Model interpretation was based on item profiles in each category and obtained from probabilities of endorsing each item response, conditional on class membership. In the present study, the number of latent classes was defined according to Bayesian Information Criterion (BIC). Starting from one single class and increasing one class at each step, the best solution was identified when the increase of number of classes did not lead to a decrease in BIC. LCA used nine variables important for asthma definition or relevant for differential diagnosis (Table 1). The selection of variables was based on the assumption of their clinical relevance for asthma definition. The Lo–Mendell–Rubin likelihood ratio test of model fit was used to quantify the likelihood that the data could be described by a model with one-less class. All LCA models were fitted using MPlus (V.5.2; Muthen & Muthen, Los Angeles, CA). Later, among asthmatic athletes, we estimated the risk associated with the sport training environment, by using regression

Table 1. Definitions of variables set for LCA.

Variable	Definition
Airflow obstruction	FEV ₁ /FVC ratio lower than 0.70
Reversibility	Increase of at least 200 mL and 12% in FEV ₁
Rhinitis ^a	Positive answer to the question “Did any doctor diagnose you an allergic disease?” AND “Rhinitis” OR Positive answer to the question “Do you frequently sneeze, have a running, itchy nose (apart from colds)?”
Any other allergic disease ^a	Positive answer to the question “Did any doctor diagnose you an allergic disease?” (except rhinitis) OR Positive answer to the question “Have you frequently red eyes with tearing and itching?” OR Positive answer to the question “Have you ever had severe allergic or anaphylactic reactions?” OR Positive answer to the question “Have you ever had allergic reactions to foods?” OR Positive answer to the question “Have you ever had allergic reactions to drugs?”
Respiratory symptoms ^a	Self-reported recurrent breathlessness, cough, wheezing, chest tightness and/or phlegm production OR Positive answer to the question “Did any doctor diagnose you an allergic disease?” AND “Asthma” OR Positive answer to the question “Have you ever had shortness of breath, cough and/or itching of the throat following exercise?”
Asthma treatment	Current or recent treatment with ICS and/or Beta ₂ -agonists
Airway hyperresponsiveness ^b	A fall in FEV ₁ ≥10% from baseline with exercise or EVH OR A fall in FEV ₁ ≥15% from baseline after inhaling 22.5 ml of 4.5 g% NaCl or ≤635 mg of mannitol OR A fall in FEV ₁ ≥20% from baseline with methacholine: PC ₂₀ ≤4 mg/ml, or PD ₂₀ ≤400 µg (cumulative dose) or ≤200 µg (noncumulative dose) in those not taking ICS, and PC ₂₀ ≤16 mg/ml or PD ₂₀ ≤1600 µg (cumulative dose) or ≤800 µg (noncumulative dose) in those taking ICS for at least 1 month
Eosinophilic inflammation	The presence of FE _{NO} levels above 25 ppb
Allergic sensitization	The presence of at least one positive (mean of largest and perpendicular diameter of the wheal ≥3 mm for each allergen and controls showing adequate reactions) skin prick test or the presence of positive specific IgE (≥0.35 kU/L) for at least one common aeroallergen in the local geographic area

EVH, eucapnic voluntary hyperpnoea; FEV₁, forced expiratory volume in the first second; FVC, forced vital capacity; ICS, inhaled corticosteroids; FE_{NO}, exhaled nitric oxide; PD₂₀, provocative dose of methacholine causing a 20% decrease in FEV₁; PC₂₀, provocative concentration of methacholine causing a 20% decrease in FEV₁.

^aConsidering the AQUA questionnaire.
^bAccording to International Olympic Committee Medical Commission to diagnose asthma in athletes.

analysis to predict the odds of having a specific asthma pattern (phenotype), having “other sports” as reference.

Results

Included subjects

From 324 files reviewed, 150 files belonged to athletes who fulfilled asthma criteria (91 Portuguese; 59 Norwegian). Forty-five athletes were diagnosed with asthma based on positive bronchodilation (the mean ± SD of FEV₁ increase was 450 mL ± 292 and 13% ± 9.4), and 105 by presenting airway responsiveness after a provocation challenge: 1 positive challenge to mannitol, 3 positive challenges with exercise and the remaining 101 positive challenges with methacholine (7 reporting PC₂₀: mean 3.9 mg/mL; 94 reporting PD₂₀: mean 6.8 µg). The remaining athletes were healthy (n = 129) or had other pathologic conditions (n = 45). Asthmatic subjects included in the present study presented airflow limitation, more reversibility to salbutamol, more BHR, atopy, rhinitis and airway inflammation assessed by FE_{NO} (Table 2).

LCA model

Relying on asthma defining variables, the increase in likelihood values leveled off when increasing from one to

two classes, and BIC reached its optimum value at two classes (Online Table). This result was confirmed by Lo–Mendell–Rubin likelihood ratio test.

Class 1 was characterized by allergic sensitization, rhinitis and other allergic co-morbidities, and increased FE_{NO} levels (“Atopic asthma”); while class 2 was characterized by the occurrence of respiratory symptoms and BHR, in the absence of atopic features (“Sports asthma”) (Table 3 and Figure 1).

Subject’s differences between classes

The athletes which were assigned to “atopic asthma” presented higher values of FE_{NO} than those in “sports asthma” (32.2 vs. 15.7, p = 0.002). In “atopic asthma”, 28 athletes presented increased values of FE_{NO}, compared to only 7 among those in “sports asthma”.

Allergic diseases were evident in 60.7% of athletes in “atopic asthma”, and in 12.5% of those assigned to “sports asthma”, namely: conjunctivitis (48% of athletes in “atopic asthma” and none in “sports asthma”), atopic eczema (12% of athletes in “atopic asthma” and none in “sports asthma”), and food allergy (31% of athletes in “atopic asthma” and none in “sports asthma”). Hymenoptera venom allergy, drug allergy and anaphylaxis had a similar prevalence in both

Table 2. Features of athletes screened at Portuguese National Anti-Doping Organization and at Norwegian School of Sports Sciences databases.

	Asthmatic athletes (n = 150)	Non-asthmatic athletes (n = 174)	p
Male, n (%)	107 (71)	89 (51)	<0.001 ^e
Age, years	25 (14–40)	26 (16–38)	0.251 ^d
BMI, kg/m ²	23 [23;24]	23 [22;23]	0.06 ^c
Physician reported rhinitis, n (%)	54 (36)	33 (19)	0.003^e
Other allergic disease, n (%)	20 (13)	26 (15)	0.750 ^e
Atopy, n (%)	89 (59)	58 (33)	<0.001 ^e
Respiratory symptoms, n (%) [*]	138 (92)	89 (51)	<0.001 ^e
Dyspnea/heavy breathing	48 (32)	20 (11)	<0.001 ^e
Chest tightness	12 (8)	11 (6)	0.379 ^e
Wheezing	42 (28)	15 (9)	<0.001 ^e
Cough	44 (29)	33 (19)	0.002^e
Tiredness	1 (0.7)	1 (0.6)	0.427 ^f
Phlegm	18 (12)	15 (9)	<0.001 ^e
Asthma treatment, n (%)			<0.001 ^f
Inhaled steroids alone	9 (6)	1 (0.6)	
Beta-2-agonists alone	13 (9)	2 (1)	
Inhaled steroids + β 2-agonists	96 (64)	13 (8)	
Airway obstruction ^a , n (%)	43 (29)	21 (12)	<0.001 ^e
FVC			
L	5.4 [5.1;5.7]	5.2 [5.0;5.4]	0.41 ^c
% of predicted	114 [110;117]	112 [109;116]	0.60 ^c
FEV ₁			
L	4.1 [3.9;4.4]	4.3 [4.1;4.4]	0.06 ^c
% of predicted	101 [96;106]	109 [106;111]	0.001^c
FEV ₁ /FVC	69 [65;74]	76 [72;80]	0.012^c
Reversibility ^b , n (%)	26 (17)	1 (0.6)	0.037^f
Airway hyperresponsiveness, n (%)	126 (84)	51 (29)	<0.001 ^e
FE _{NO} , ppb	33 (6–213)	19 (4–70)	0.01^d

Bold values indicate $p < 0.05$.

Data presented as mean (95% confidence interval) except for age and FE_{NO} which are presented as median (min–max).

BMI, body mass index; FE_{NO}, exhaled fraction of nitric oxide; L, liters; FVC, forced vital capacity; FEV₁, forced expiratory volume in one second.

^aDefined as a FEV₁/FVC ratio <0.70.

^bDefined as an increase in FEV₁ ≥ 200 mL and $\geq 12\%$.

^cIndependent samples *t*-test.

^dIndependent samples Mann–Whitney *U* test.

^eChi-square test.

^fFisher's exact test.

classes (4% of athletes for both diseases). Male gender was predominant in “sports asthma”.

Regarding therapeutic, 92.5% of those athletes with “atopic asthma” and 78% of those with “sports asthma” were under anti-asthmatic drugs. Thirteen asthmatic athletes were using only short-acting β 2-agonists as therapeutic – 9 (8%) among the “atopic asthma” and 4 (8%) among the “sports asthma” phenotype; the remaining athletes were on inhaled corticosteroids (ICS) alone or combined with long-acting β 2-agonists.

Risk factors for each class

A 2.87 (95%CI: 1.82–4.51) and 8.65 (95%CI: 2.67–28.03) fold increase for risk of “sports asthma” was observed in athletes practicing water sports and winter sports, respectively, when compared to other sports (Figure 2).

Discussion

Using LCA, this present study identifies two distinct phenotypes of asthma in athletes: “atopic asthma” defined by the occurrence of atopy, increased levels of FE_{NO}, rhinitis and other allergic co-morbidities; and “sports asthma”, defined by the presence of exercise-induced respiratory symptoms and

BHR in the absence of allergic features. Moreover, specific training and environmental conditions are associated with an increased risk of developing “sports asthma”, as athletes practicing water and winter sports had, respectively, a three- and ninefold increase in their risk of “sports asthma”, when compared with others.

This study allows for hypothesis generation and has several strengths. Its major strength is the new type of statistical models used to pool and characterize different clusters. This methodological approach makes this study especially useful by retrieving a clear view on asthma phenotypes in athletes. Replication of results in other datasets is important when using these exploratory statistical techniques; and the two asthma patterns obtained in this study are remarkably in accordance with the only previous report, a study relying on different study design and an *a priori* list of selected variables for statistical analysis [8]. Another strength of the present study is its multicentric nature, allowing the inclusion of a large sample of elite athletes, all competing at top levels, some of which are among the world's best in their discipline with several winners of Olympic Gold medals.

Athletes in this study are all competing in an elite level and, therefore, all are more prone to negative consequences of exercise “injuring” airways due to prolonged and repeated

Table 3. Characteristics of asthmatic athletes according with their asthma phenotype and variables in each assigned latent class.

	Total	Atopic asthma, n = 104	Sports asthma, n = 46	p
Male, n (%)	107	81 (78)	26 (57)	0.008^c
Age, median ± IQR in years	–	23.0 ± 12	24.5 ± 8	0.522 ^f
Height, mean ± SD in cm	–	175.4 ± 8.7	176.5 ± 8.7	0.530 ^g
Weight, mean ± SD in kg	–	70.9 ± 11.5	71.4 ± 10.3	0.815 ^g
BMI, mean ± SD in kg/m ²	–	23.0 ± 2.6	22.8 ± 1.9	0.741 ^g
FEV ₁ , mean ± SD in L	–	4.0 ± 0.9	4.1 ± 0.7	0.221 ^g
FEV ₁ , mean ± SD in % predicted	–	98.1 ± 20.4	99.7 ± 21.1	0.640 ^g
FVC, mean ± SD in L	–	5.1 ± 1.0	5.3 ± 1.1	0.413 ^g
FVC, mean ± SD in % predicted	–	108.0 ± 15.4	113.4 ± 15.0	0.084 ^g
FEV ₁ /FVC, mean ± SD	–	77.9 ± 8.9	78.7 ± 11.1	0.649 ^g
<i>Variables used in LCA</i>				
Airflow obstruction ^a				0.036
No	80.5	85.3	69.4	
Yes	19.5	14.7	30.6	
Reversibility ^b				0.023
No	23.4	19.0	39.7	
Yes	76.6	81.0	60.3	
Rhinitis				<0.001
No	64.0	51.5	90.9	
Yes	36.0	48.5	9.1	
Any other allergic disease ^c				<0.001
No	61.5	39.3	87.5	
Yes	38.5	60.7	12.5	
Respiratory symptoms				0.133
No	6.1	4.0	10.7	
Yes	93.9	96.0	89.3	
Asthma treatment				0.017
No	11.9	7.5	22.0	
Yes	88.1	92.5	78.0	
Airway hyperresponsiveness				0.834
No	25.7	25.0	26.9	
Yes	74.3	75.0	73.1	
FE _{NO} ^d				<0.001
Normal	62.8	44.8	84.5	
Increased	37.2	55.2	15.5	
Atopy				<0.001
No	31.0	0	100	
Yes	69.0	100	0	

Bold values indicate $p < 0.05$.
Data presented as percentage of total, except otherwise stated. BMI, body mass index; FE_{NO}, exhaled fraction of nitric oxide; L, liters; FVC, forced vital capacity; FEV₁, forced expiratory volume in one second.
^aDefined as a FEV₁/FVC ratio <0.70.
^bDefined as an increase in FEV₁ ≥200 mL and ≥12%.
^cOther allergic diseases include conjunctivitis, urticaria, eczema, anaphylaxis and drug, food and venom allergies, sampled through AQUA questionnaire.
^dDefined as increased if above 25 ppb.
+Chi-square test.
^fMann–Whitney *U* test.

hyperpnoea. For athletes practicing water and winter sports, in addition to frequent episodes of prolonged hyperpnoea, their “occupation” demands exposure to potentially noxious stimuli, such as sport-specific environmental exposures [16]. Keeping in mind the close relation to environmental conditions, one could speculate whether “*sports asthma*” should be classified as a variant of occupational asthma, as recently suggested [16]. This designation could help improve the general idea of this concept of asthma dependent upon environmental factors which are part of an athlete’s occupation. The “sports asthma” phenotype is similar to the late-onset phenotype identified among “normal” asthmatics. In many cases, the late-onset phenotype appears to be more severe, less responsive to standard therapy and more related to environmental risk factors [17]. However, “sports asthma” tends to improve after cessation of sport participation, in what

concerns airway inflammation and hyperresponsiveness [18,19].
In athletes, atopy has been long recognized to be positively associated with asthma and BHR [20,21]. Moreover, training in cold air [21] and swimming [20] were identified as risk factors for asthma. In both swimmers and cross-country skiers, the prevalence of asthma is known to increase with age [22–24], which is consistent with the hypothesis of “sports asthma” occurring throughout the sport career and being induced by cumulative years of exposure to environmental training conditions. The results of our study contribute to confirm that different risk factors, such as atopy and environmental training conditions, result in different patterns of asthma. The effect of these risk factors on determining different underlying mechanisms of asthma should be considered.

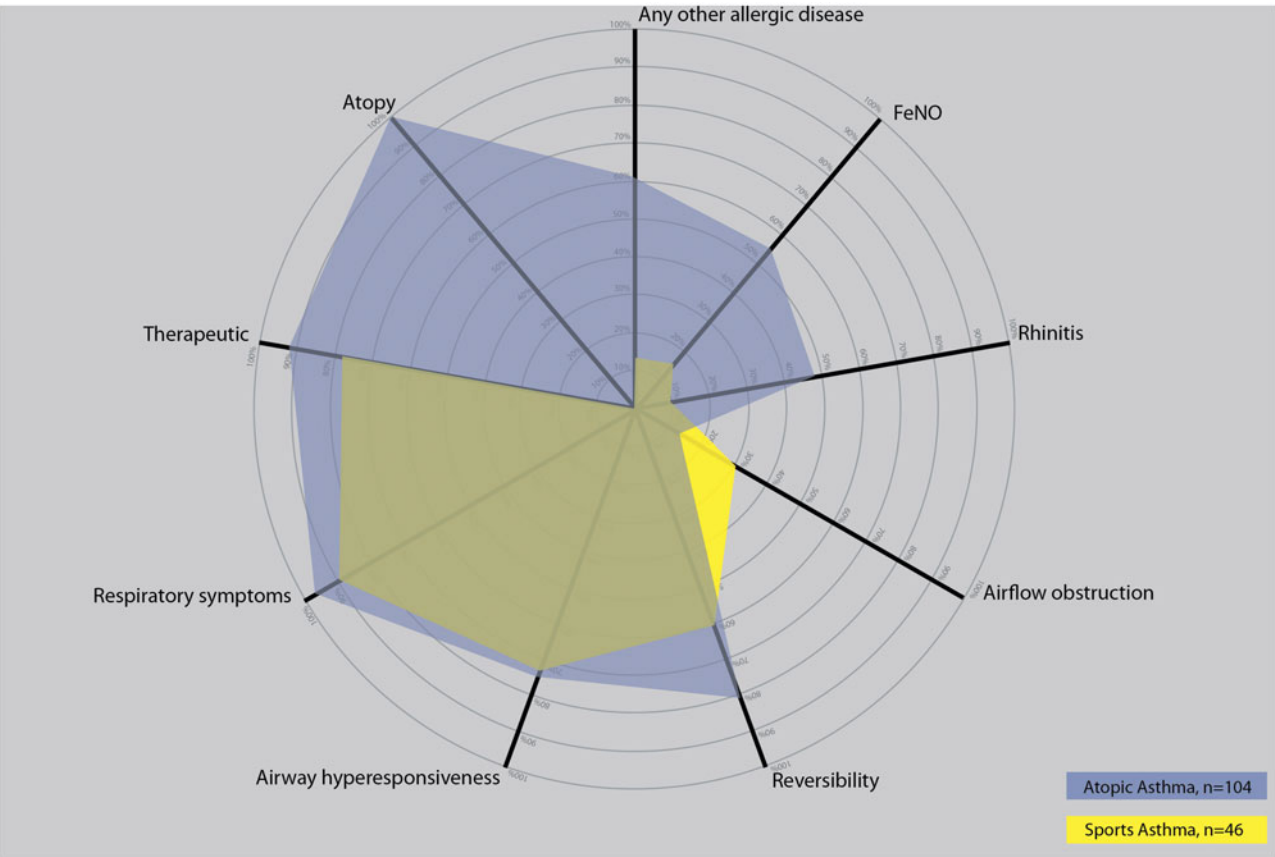


Figure 1. Percent of athletes presenting each of the variables included for LCA.

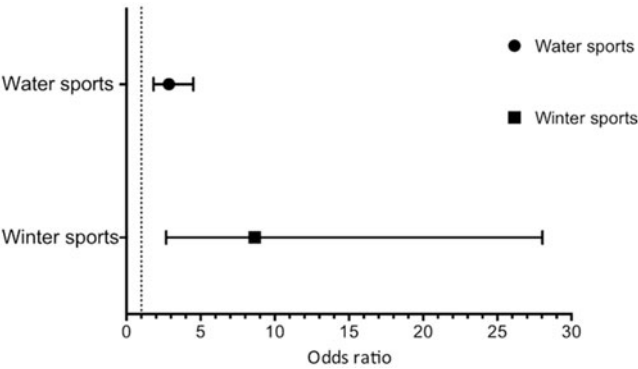


Figure 2. Risk of presenting the “sports asthma” phenotype of athletes practicing water and winter sports, considering other sports as reference.

Defining these distinct phenotypes could lead not only to further understanding the underlying mechanisms of asthma in elite athletes, but also, and most important from a practical point of view, to recognizing that potentially different treatments specifically targeted for defined phenotypic groups are needed. Optimal asthma treatment is a prerequisite for asthmatic athletes because of potential implications in performance, since airway narrowing during exercise could compromise ventilatory capacity and efficiency. However, it has been recognized that the natural course of asthma in athletes is difficult to change by “normal” anti-inflammatory treatment [25]. This highlights the need for a different therapeutic approach in these subjects, which leads us to the

clinical implications of our study. Differences in airway response to bronchodilating drugs have been reported in the literature, and whether athletes with asthma occurring during sports career respond to anti-asthmatic drugs similarly to subjects with classic allergic or with nonallergic asthma has not been extensively studied [7] and needs further research. Most recent guidelines for treatment of exercise-induced bronchoconstriction (EIB) state a strong recommendation for using a short-acting β_2 -agonist before exercise in *all* patients with EIB [26]. However, we have recently shown that elite skiers with asthma respond better to anticholinergic treatment as compared with β_2 -agonists [27]. Differences in parasympathetic bronchial tone were suggested as a possible explanation to why some subjects are responders and other non-responders to anticholinergic drugs [28,29]. It seems, therefore, that the approach of “one treatment fits all” is *insufficient* to comply with the needs of asthmatic athletes.

Despite its several strengths, our study also has some limitations that must be pointed out. The first is the use of different methods (both direct and indirect challenges) to assess BHR in athletes. In the present study, information was collected from medical files, so there was no possibility to homogenize tests performed by athletes in two centers. In any case, final diagnosis was made according to IOC criteria. Another weakness to be noted is the absence of information about age of asthma onset; this limits the extent of our conclusions as we cannot be aware of whether the previous presence of asthma would influence the type of sport chosen. However, based on previous literature, it does not seem to be

the case as the prevalence of asthma is known to increase with age both in swimmers and skiers [22–24]. The interpretation of our results is also limited by the cross-sectional design, which is not able to identify causality; however, it is suitable for hypothesis generation. Thus, the present study should be succeeded by new prospective studies following youth athletes from adolescence until adulthood. Moreover, although motivating, results provided by this exploratory analysis have to be interpreted in context of future work, addressing whether the two phenotypes are relevant from a clinical perspective. Potential phenotypes require prospective validation with clinical interventional trials. A recent trial showed that Norwegian competitive endurance winter athletes respond with a higher reversibility to ipratropium bromide than to inhaled β_2 -agonists [27], helping research in this field to move forward and toward a new direction.

Conclusion

Using LCA on a large sample of top elite athletes from two national databases we were able to identify two patterns of asthma aggregation features based on findings routinely collected in clinical practice: “*atopic asthma*”, defined by the presence of allergic sensitization, rhinitis and other allergic co-morbidities and increased FE_{NO} ; and “*sports asthma*”, defined by the presence of exercise-induced respiratory symptoms and BHR in the absence of allergic features. Moreover, exposure to particular environmental conditions of training and competition was associated with increased risk to develop “*sports asthma*” phenotype: water sports increased the risk by almost three times, whereas in winter sports the risk increased by almost nine times. Recognizing different phenotypes as a result of probable different underlying mechanisms related to environmental exposures highlights the need for distinct targeted treatments. These potential phenotypes require prospective validation by larger clinical interventional trials. If confirmed by other studies, such a model could be useful for the standardization of clinical diagnosis and future treatment of asthmatic athletes.

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Declaration of interest

The authors report no conflicts of interest. To European Academy of Allergy and Clinical Immunology for the 2011 Exchange Research Fellowship award allowing the first author to work in Oslo and therefore turned this project possible.

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Supplementary material available online

Study III

Exploratory study
comparing dysautonomy
between asthmatic and non-
asthmatic elite swimmers

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ORIGINAL ARTICLE

Exploratory study comparing dysautonomia between asthmatic and non-asthmatic elite swimmers



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Parasympathetic
activity;
Swimmers

Abstract

Background: Dysautonomia has been independently associated with training and exercise-induced bronchoconstriction. In addition, neurogenic airway inflammation was recently associated with swimmers-asthma. We aimed to assess the relation between autonomic nervous system and airway responsiveness of asthmatic elite swimmers.

Methods: Twenty-seven elite swimmers, 11 of whom had asthma, were enrolled in this exploratory cross-sectional study. All performed spirometry with bronchodilation, skin prick tests and methacholine challenge according to the guidelines. Pupillometry was performed using PLR-200™ Pupillometer. One pupil light response curve for each eye was recorded and the mean values of pupil's maximal and minimal diameters, percentage of constriction, average and maximum constriction velocities (parasympathetic parameters), dilation velocity, and total time to recover 75% of the initial size (sympathetic parameters) were used for analysis. Asthma was defined using IOC-MC criteria; subjects were divided into airway hyperresponsiveness (AHR) severity according to methacholine PD₂₀ in: no AHR, borderline, mild, moderate and severe AHR. Differences for pupillary parameters between groups and after categorization by AHR severity were assessed using SPSS 20.0 ($p \leq 0.05$). In individuals with clinically relevant AHR, correlation between PD₂₀ and pupillary parameters was investigated with Spearman's correlation test.

Results: No statistically significant differences were observed between asthmatic and non-asthmatic swimmers regarding parasympathetic parameters. When stratified by AHR, maximal and minimal diameters and percentage of constriction were significantly lower among those with severe AHR. Among swimmers with clinically relevant AHR ($n=18$), PD₂₀ correlated with *parasympathetic activity*: maximal ($r=0.67$, $p=0.002$) and minimal diameters ($r=0.75$, $p<0.001$), percentage of constriction ($r=-0.59$, $p=0.011$) and latency ($r=0.490$, $p=0.039$).

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Conclusions: No significant differences were observed between asthmatic and non-asthmatic swimmers regarding parasympathetic parameters, but among those with relevant AHR an association was found. Although limited by the sample size, these findings support the relation between dysautonomia and AHR in asthmatic swimmers.

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Introduction

An increased risk for asthma has been recognized in elite athletes who take part in endurance sports, such as swimming.¹ Classical postulated mechanisms behind exercise-induced asthma (EIA) include the osmotic, or airway-drying, hypothesis.² As water is evaporated from the airway surface liquid, it becomes hyperosmolar and provides an osmotic stimulus for water to move from any cell nearby, resulting in cell shrinkage and release of inflammatory mediators that cause airway smooth muscle contraction. In fact, the airways of athletes present increased inflammatory cells and levels of histamine, cysteinyl leukotrienes and chemokines; however these inflammatory changes are not consistently related to lung function or disease exacerbations and it has been thought that they represent physical injury secondary to rigorous hyperpnoea that will heal with rest.^{3,4} Alternative hypotheses to explain EIA have been pursued.

Besides inflammatory mediators, the autonomic system also mediates the contraction and relaxation of bronchial smooth muscle. Cholinergic-parasympathetic nerves stimulate bronchoconstriction, whereas β_2 -adrenergic sympathetic and/or noncholinergic parasympathetic nerves cause bronchodilation.^{3,4} Intensive training can have affect autonomic regulation by promoting the predominance of vagal activity, as a compensatory response to the sympathetic stimulation associated with frequent and intense training.² It has been hypothesized that repeated intensive training could provoke vagal hegemony, which induces not only the well-known resting bradycardia of athletes, but could also lead to a predisposition for increased bronchomotor tone and therefore susceptibility to bronchospasm.² This autonomic nervous system imbalance is known as *dysautonomia* and it has been previously shown, using pupilometry, that pupillary light reflex of endurance runners reveals an increased parasympathetic activity and reduced sympathetic activity.⁵ But the relationship between these observations and asthma is not established.

Research into the hypothesis of dysautonomia in the pathogenesis of asthma in athletes is urgently needed because definite answers would allow for better targeted treatment of this specific asthmatic population. In the particular case of elite swimmers, in both asthmatic and healthy ones, an increase in bronchial responsiveness correlating with exercise intensity was demonstrated after 3000 m swimming in an indoor swimming pool.⁶ Moreover, neurogenic airway inflammation was recently associated with swimmers-asthma.⁷ Therefore, we aimed to assess the relationship between autonomic nervous system and airway responsiveness of elite swimmers with asthma. It was

hypothesized that airway hyperresponsiveness in asthmatic swimmers is related to increased parasympathetic activity.

Methods

Participants

Swimmers of the FCPorto main swimming team were invited to participate. Athletes of over 14 years-old, who agreed to take part in the study, were enrolled. To be included, participants had to be elite swimmers, free from any respiratory infection in the 2 weeks before testing, not having drunk coffee or smoked in the 2 h prior to testing, not having taken exercise on the testing day, not using contact lenses and not having taken their asthma medication for 48 h (except for inhaled corticosteroids, which they had been asked to stop taking for at least 2 weeks prior to the study).

Subjects who met any of the following criteria would have been excluded from the study: under any systemic medication which could affect the central nervous system; any topical eye treatment; systemic conditions with known ocular involvement; orbit structure damage or surrounding soft tissue with open lesion or edema on the day of testing; a past history of ocular abnormalities or trauma.

None of the subjects was excluded based on the above mentioned criteria.

Study design

This is an exploratory cross-sectional study, developed in two visits. The first visit was in the morning (from 8 to 11 am) because of the circadian rhythm of the size of the pupil.⁸ Medical history and potential medication were reviewed to determine eligibility. The eligible ones answered a structured questionnaire, and performed pupilometry, spirometry and skin prick testing. Subsequently, reversibility to salbutamol was evaluated. The second visit took place on a different day and a bronchial challenge with methacholine was performed to assess airway hyperresponsiveness (AHR). Asthma diagnosis was based on the typical clinical features in conjunction with objective documentation of airway dysfunction, either presenting reversibility or AHR, according to the criteria set by the International Olympic Committee to document asthma in athletes.^{4,9}

The study was conducted according to the Declaration of Helsinki and approved by the Ethical Commission. All participants or their legal guardians/parents (in the case of participants under 18 years-old) signed an informed consent form.

Procedures

Portable infrared PLR-200™ Pupillometer (NeuroOptics Inc, CA, USA) was used for pupillary measurements. Subjects spent at least 15 min in a semi-dark and quiet room to allow their eyes to adjust to the low lighting levels, after which they were instructed to focus with the eye that was not being tested on a small target object standing at least 3 m away, keeping their head straight and eyes wide open during targeting and measurement (Fig. 1A). If they blinked, the measurement was repeated. One stimulus of light-emitting diodes briefly illuminated the eye with 180 nm peak wave light (Fig. 1B). At the end of the measurement cycle, a graph of the pupil diameters as a function of time appeared on the screen (Fig. 1C). One pupil curve to each eye, starting with the left, was recorded for each subject, and the mean values of both eyes were used for statistical analysis. The following parameters were collected: the diameter of the pupil before (initial) and at constriction peak (minimal), in millimeters; the percentage of the constriction; the time of the onset of the constriction (latency), in seconds; the average and the maximum constriction velocities (ACV and MCV respectively), and the dilation velocity (ADV), all given in mm/s; and the total time taken by the pupil to recover 75% of the initial resting pupil size after it reached the peak of constriction (T75), in seconds. Pupil diameters, latency, ACV, MCV, and the constriction amplitude are related to *parasympathetic activity*, while ADV and T75 are measures of *sympathetic activity*.

Subjects underwent spirometry, which was carried out according to the American Thoracic Society criteria (ATS).¹⁰ Results of spirometry are reported as forced expiratory volume in the first second (FEV₁), forced vital capacity (FVC) and forced expiratory flow in the middle portion of FVC (FEF_{25–75}); all are presented as both absolute and predicted values, according to published reference algorithms.¹¹ Airflow obstruction was defined as a FEV₁/FVC ratio lower than 0.70.¹² Lung function measurements were repeated 15 min after salbutamol inhalation (400 µg) in aerochamber to assess reversibility, which was defined as an increase on FEV₁ ≥ 200 mL or 12% from baseline.¹² Bronchial challenge with methacholine was performed as recommended by ATS guidelines and accordingly medication was withheld.¹³ Criteria for a positive challenge were, according to the International Olympic Committee, set to a provocative dose determining a 20% fall in FEV₁ (PD₂₀) ≤ 3.2 µmol in steroid naïve athletes and to a PD₂₀ ≤ 0.8 µmol in athletes on inhaled steroids for at least 1 month.⁹

Subjects were divided by AHR severity according to PD₂₀ in: no AHR (>7.8 µmol), borderline (3.2–7.8 µmol), mild (0.8–3.2 µmol), moderate (0.1–0.8 µmol) and severe (≤0.1 µmol) AHR. A PD₂₀ ≤ 3.2 µmol was considered as clinically relevant AHR.

A structured questionnaire was applied that addressed demographic data, medications and medical conditions.

Atopy was defined as the presence of at least one positive skin prick test to common aeroallergen extracts (Leti®, Madrid, Spain); positive (histamine 10 mg/mL) and negative controls were performed.

Statistical analysis

Statistical analyses were performed with SPSS version 20.0. *p*-Values <0.05 were considered statistically significant. Continuous results are expressed as mean (95% confidence interval, CI) or, if not normally distributed, as median (minimum and maximum); categorical data are expressed as counts (%). Differences between groups were assessed with Student's *t*-test or Mann-Whitney in cases of non-normally distributed data, and Chi-Square or Fisher's exact tests for categorical variables.

Subjects were then categorized by AHR severity and differences between groups for pupillary parameters were assessed with 1-way ANOVA or Kruskal-Wallis if non-normally distributed data.

In individuals with clinically relevant AHR, Spearman's correlation test was used to assess the relation between PD₂₀ and pupillary parameters.

Results

Twenty-seven elite swimmers were enrolled, of which 11 (41%) had asthma. Demographic, personal and clinical features are presented in Table 1. Regarding their normal medication, 2 swimmers were under inhaled corticosteroids, 4 with a combination of long-acting β₂-agonists plus inhaled corticosteroids and 2 were treated with anti-leukotrienes. All refrained from taking their regular medication prior to the study.

Pupillary response curves were easily recorded in all subjects (Fig. 2). Pupillometry was repeated if blink artifacts occurred, but all participants were able to complete the measurements, except for T75 which was not retrieved by the device for all athletes. None reported discomfort.

Pupillometry measurements in asthmatics compared with non-asthmatic swimmers are presented in Table 1 and Fig. 2. Although lower pupil diameters and a higher percentage of constriction were observed in asthmatics, differences were not statistically significant.

When stratified by AHR severity, pupil diameters before (maximal) and at constriction peak (minimum) as well as the percentage of constriction were significantly lower among those with severe AHR (Table 2).

In the 18 swimmers with clinically relevant AHR, a significant correlation was found between PD₂₀ and maximal ($r=0.67$, $p=0.002$), and minimal pupil's diameters ($r=0.75$, $p<0.001$), percentage of constriction ($r=-0.59$, $p=0.011$) and latency ($r=0.490$, $p=0.039$), but not with ACV ($r=0.243$, $p=0.332$), MCV ($r=0.061$, $p=0.810$), ADV ($r=0.075$, $p=0.776$), and T75 ($r=0.373$, $p=0.351$).

Discussion

In our exploratory study, no significant differences were observed in parasympathetic parameters between asthmatics and non-asthmatic elite swimmers. However, for those with severe AHR a significant difference became clear, suggesting that the increased parasympathetic tone is particularly associated with the contraction of the bronchial smooth muscle.

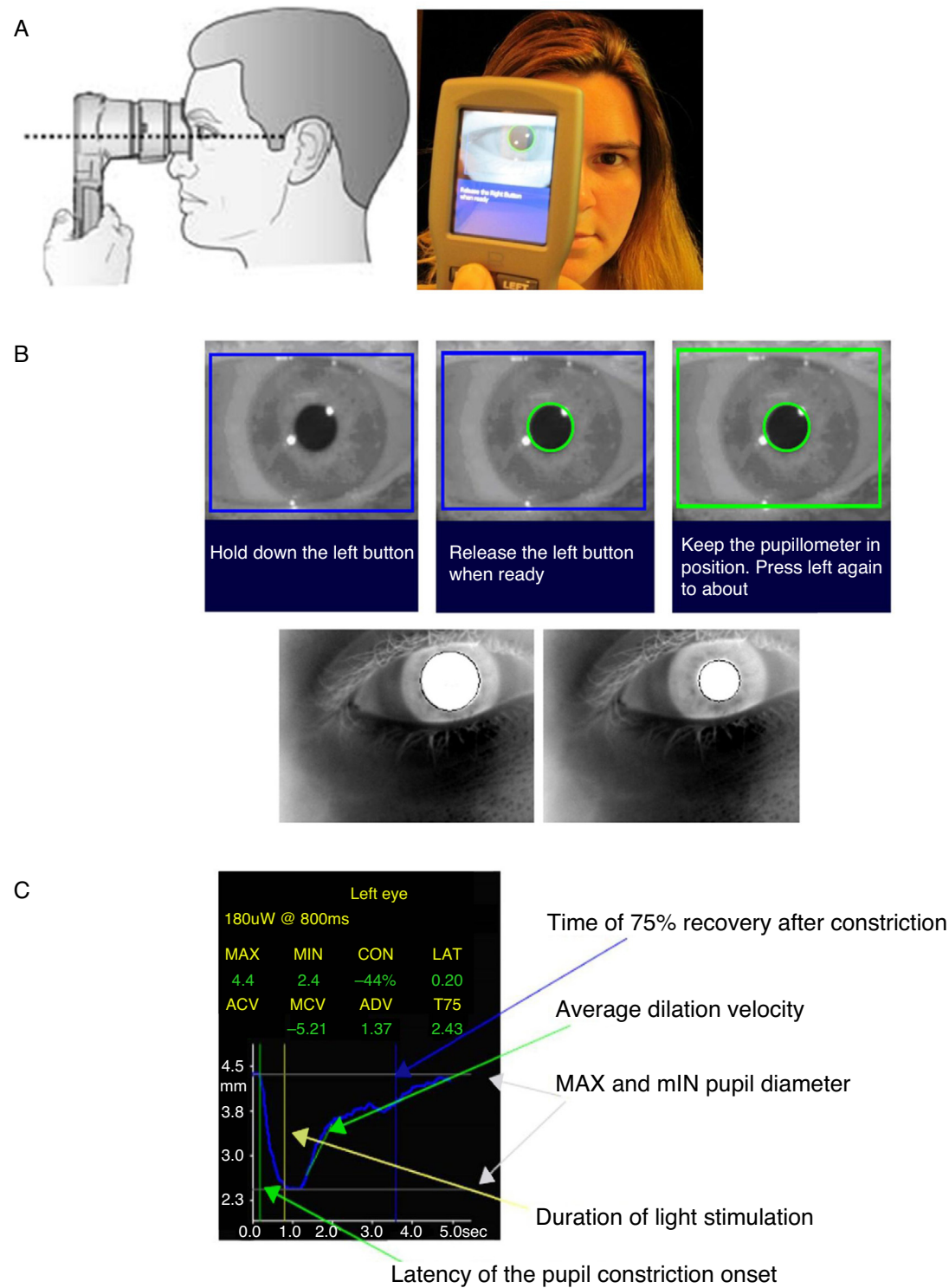


Figure 1 Procedure for measurement of pupillary light reflexes and pupil sizes and pupillometer displaying results. (A) The sequence of figures represent the adequate position to perform the scan: at the right angle to the patient’s axis of vision, in a good alignment, closely adapted to the face and the pupil in the center of LCD screen. (B) The sequence of figures presents the pupil measurement phases: targeting phase (1), ready phase (2) and measurement phase. (C) Pupillometer display of one measurement results.

Previous studies supporting this hypothesis of dysautonomy associated with training match our findings. Pichon et al. demonstrated that subjects with an increased AHR had a higher vagal tone,¹⁴ which was corroborated by Park et al.

findings of a relationship between AHR to methacholine and a diminished sweat secretion, tearing and salivary flow rate in healthy athletes.¹⁵ All these studies have used AHR as an outcome measure, rather than asthma status. This also

Table 1 Characteristics of asthmatic and non-asthmatic swimmers.

	Asthmatic swimmers (n = 11)	Non-asthmatic swimmers (n = 16)	p
Males, n (%)	8 (73)	6 (38)	0.072
Age (years)	17 [15–19]	18 [16–20]	0.479
BMI (kg/m ²)	21.5 [20.2–22.9]	21.3 [20.2–22.3]	0.725
Atopy, n (%)	5 (46)	6 (38)	0.679 ^a
Years of competition	8.9 [7.0–10.8]	9.6 [7.6–11.7]	0.602
Training hours per week	16.3 [13.1–19.4]	17.5 [16.1–18.9]	0.393
Previous diagnosis of asthma, n (%)	4 (36)	1 (6)	0.113 ^a
Previous diagnosis of rhinitis, n (%)	1 (9)	3 (18)	0.488 ^a
PD ₂₀ methacholine (μmol)	0.8 [0.4–1.2]	4.6 [3.3–5.8]	0.001
<i>Lung function</i>			
FEV ₁ /FVC	83.5 [78.3–88.8]	88.0 [84.6–91.4]	0.236
<i>FVC</i>			
Liters	5.1 [4.4–5.8]	4.9 [4.1–5.7]	0.612
% of predicted	114.5 [107.0–122.0]	114.8 [108.5–121.0]	0.957
<i>FEV₁</i>			
Liters	4.3 [3.7–4.8]	4.3 [3.7–4.9]	0.863
% of predicted	111.1 [100.7–121.5]	115.6 [109.8–121.3]	0.379
<i>FEF_{25–75}</i>			
Liters	4.2 [3.4–5.1]	4.7 [4.0–5.4]	0.570
% of predicted	97.3 [78.2–116.4]	109.3 [97.3–121.4]	0.230
Airway obstruction, n (%)	1 (9)	0	0.219 ^a
<i>Increase in FEV₁ after salbutamol</i>			
%	5.0 [2.0–8.0]	3.4 [1.7–5.4]	0.840
Milliliters	197.0 [87.2–306.8]	149.4 [61.5–237.3]	0.922
<i>Parasympathetic parameters</i>			
Maximal diameter (mm)	5.9 [4.6–7.3]	7.0 [6.4–7.6]	0.180
Minimum diameter (mm)	3.9 [2.9–4.9]	4.7 [4.0–5.4]	0.708
Percent of constriction	35.1 [31.9–38.3]	33.8 [29.3–38.4]	0.295
Latency (s)	0.2 [0.2–0.2]	0.2 [0.2–0.2]	0.183 ^b
ACV (mm/s)	4.0 [3.5–4.6]	4.1 [3.6–4.5]	0.664
MCV (mm/s)	5.7 [4.7–6.8]	5.5 [4.9–6.0]	0.592
<i>Sympathetic parameters</i>			
ADV (mm/s)	0.9 [0.7–1.2]	0.9 [0.8–1.0]	0.440
T75 (s)	2.4 [1.3–3.5]	3.1 [2.8–3.4]	0.154

Data reported as mean [95% confidence interval] unless otherwise stated.

ACV: average constriction velocity; ADV: average dilation velocity; BMI: body mass index; FEV₁: forced expiratory volume in the first second of FVC; FVC: forced vital capacity; FEF_{25–75}: forced expiratory flow middle portion of FVC; MCV: Maximum constriction velocity; PD₂₀: provocative dose determining a 20% fall in FEV₁; T75: the total time taken by the pupil to recover 75% of the initial resting pupil size after it reached the peak of constriction.

In bold, p ≤ 0.05.

^a Fisher Exact test.

^b Mann–Whitney U test.

seems to be the case with swimmers. Among elite competitive adolescent swimmers, for both asthmatic and healthy ones, an increase in bronchial responsiveness correlating with the exercise intensity was demonstrated after 3000 m swimming in an indoor swimming pool.⁶ Together with our results, this seems to point out that dysautonomia might contribute to the severity of airway reactivity in swimmers.

The lack of significant differences in parasympathetic outcomes between asthmatic and non-asthmatic swimmers is probably related to the fact that asthma a complex disease to which, besides AHR, there are many more

parameters contributing. Asthma is defined as a clinical syndrome of intermittent respiratory symptoms triggered by viral infections, environmental allergens, or other stimuli, and is characterized by nonspecific airway hyperresponsiveness and inflammation.¹⁶ While bronchoconstriction is largely dependent on airway smooth muscle cells and might be related to increased parasympathetic tonus, inflammation is a multi-cellular process involving airway epithelium, eosinophils, neutrophils, lymphocytes and mast cells.¹⁷ Although in the particular case of athletes it has been proposed that inflammatory changes represent physical

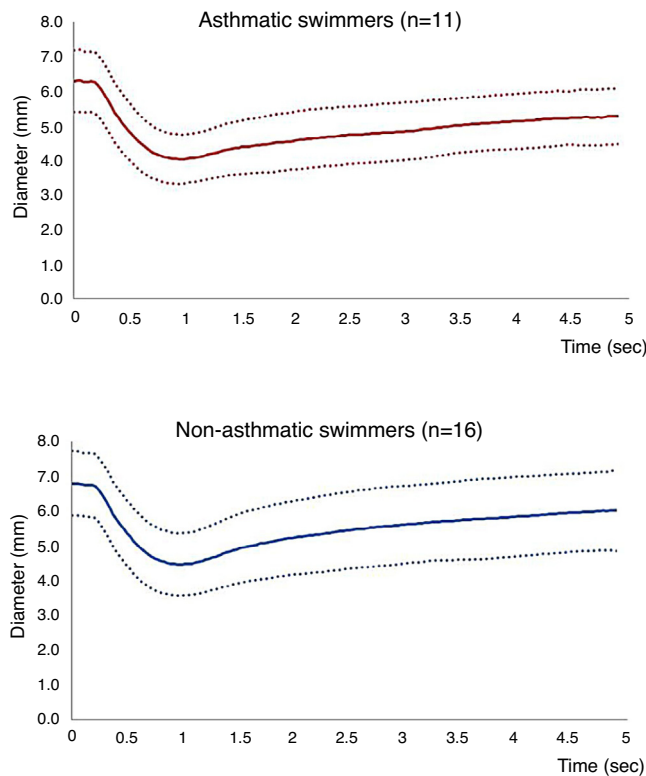


Figure 2 Results of pupillary response variables: Mean (bold line) $\pm$ SD (traced lines) values of pupillary diameters among asthmatic and non-asthmatic swimmers.

injury secondary to rigorous exercise,^{3,4} there are several issues that are unique to this population. In athletes, two different clinical phenotypes of asthma have been suggested by Hahtela et al. The pattern of "classical asthma" characterized by early onset childhood asthma, methacholine responsiveness, atopy and signs of eosinophilic airway inflammation; and another distinct phenotype with onset of symptoms during sports career, bronchial responsiveness

to eucapnic hyperventilation test and a variable association with atopic markers and eosinophilic airway inflammation.¹⁸ Our group has recently observed that athletes involved in water sports have a 3-fold increased risk of presenting the later phenotype of asthma – called "sports asthma", not related to atopy but rather developed through their career (unpublished data). This suggests different predominant pathophysiological mechanisms of EIA in athletes and therefore a different role of the parasympathetic tonus.

In the particular case of swimming, EIA has been mainly thought to be associated with epithelial damage resulting from exposure to chloramines.¹⁹ In recent years, the observation that regular pool attendance, especially by young children, was associated with lung hyperpermeability and increased risk of developing asthma led to the "pool chlorine hypothesis".^{20,21} According to this, the increasing and largely uncontrolled exposure of young children to chlorination by-products contaminating the air of indoor swimming pools could have contributed to the childhood asthma rise in industrialized countries.^{20,21} In their studies, Bernard et al. described an association between asthma prevalence and cumulated pool attendance, as well as lung hyperpermeability and total IgE levels.²² In fact, also in elite swimmers it has been shown that the endothelial cell layer, through vascular adhesion and permeability control, determines the infiltration of immune cells and leads to edema in the lungs.²³ It has been hypothesized that regular attendance at chlorinated swimming pools might have a role in the development of asthma by causing an increased lung permeability, which in turn would facilitate allergen sensitization.²³ We have previously demonstrated that swimmers that remain active at a 3-year follow-up significantly increase their levels of airway inflammation compared to those who quit swimming, but asthma incidence remained similar.²⁴ Taken together all these studies suggest that the EIA explanatory model in swimmers is not only related to inflammation and allergy, but will probable include the interplay between environmental training factors including allergens and ambient conditions and the athlete's personal risk factors such as genetic and neuro-immune-endocrine determinants.²

Table 2 Autonomic nervous system parameters across airway hyperresponsiveness (AHR) degrees of severity.

	No AHR (n = 3)	Borderline AHR (n = 6)	Mild AHR (n = 12)	Moderate AHR (n = 4)	Severe AHR (n = 2)	p
<i>Parasympathetic parameters</i>						
Maximal diameter (mm)	6.4 $\pm$ 1.0	6.4 $\pm$ 0.9	6.9 $\pm$ 0.8	6.6 $\pm$ 0.6	4.9 $\pm$ 0.7	0.04
Minimum diameter (mm)	4.3 $\pm$ 1.1	3.9 $\pm$ 0.7	4.7 $\pm$ 0.8	4.2 $\pm$ 0.5	2.8 $\pm$ 0.7	0.03
Percent of constriction	34.3 $\pm$ 7.8	38.3 $\pm$ 2.2	33.3 $\pm$ 4.5	37.3 $\pm$ 1.9	42.8 $\pm$ 7.4	0.05
Latency (s)	2.8 (2.6–3.0)	3.2 (2.7–3.5)	3.1 (1.7–3.8)	3.3 (3.3–3.3)	1.7 (1.7–1.7)	0.08 ^a
ACV (mm/s)	3.6 $\pm$ 0.9	4.3 $\pm$ 0.4	4.0 $\pm$ 0.3	3.8 $\pm$ 0.4	3.5 $\pm$ 0.4	0.13
MCV (mm/s)	5.1 $\pm$ 0.9	5.8 $\pm$ 0.4	5.5 $\pm$ 0.6	5.6 $\pm$ 0.4	5.1 $\pm$ 1.1	0.52
<i>Sympathetic parameters</i>						
ADV (mm/s)	0.9 $\pm$ 0.1	0.8 $\pm$ 0.2	0.9 $\pm$ 0.2	0.5 $\pm$ 0.3	0.9 $\pm$ 0.1	0.08
T75 (s)	2.8 $\pm$ 0.3	3.1 $\pm$ 0.4	2.8 $\pm$ 0.8	3.3 $\pm$ u.d.	1.7 $\pm$ u.d.	0.42

Data reported as mean $\pm$ SD, except for latency which is expressed as median (min-max).

ACV: average constriction velocity; ADV: average dilation velocity; MCV: maximum constriction velocity; T75: the total time taken by the pupil to recover 75% of the initial resting pupil size after it reached the peak of constriction; u.d.: unavailable data.

In bold, $p \leq 0.05$.

^a Kruskal–Wallis test.

As a major ambient factor, chloramines, and trichloramine in particular, are quite volatile and they are very easily inhaled and therefore act as potent irritants in the airways. Chemical stimulation of vagal sensory fibers by irritants reaching the lower airways can trigger tracheal and bronchial constriction, bronchospasm, mucus secretion, and neurogenic inflammation.²⁵ This neurogenic pathway is connected to release and action of neuropeptides, such as tachykinins, including substance P, or calcitonin gene related peptide (CGRP) from primary sensory nerve terminals, by activation of transient receptor potential (TRP) channels as a response of sensory neurons to noxious stimuli.^{26–28} In a recent study, the role of TRP-ankyrin 1 channel on nociceptive airway sensory nerves in nonallergic AHR in relation with chloramines exposure was highlighted.⁷ It was demonstrated in a mouse model, that AHR can be induced by a single hypochlorite-ovalbumin instillation, independently of bronchial influx of inflammatory cells.⁷ These chemoreceptors are expressed on substance P-producing airway sensory nerve fibers and are involved in irritant-induced airway disease. The activation of substance P receptor leads to bronchoconstriction.²⁸ Our results help to support the influence of the nervous system in this hypothesis of an interaction between exposure to hypochlorite and AHR independent of bronchial inflammation.

A proof of concept of this hypothesis of a higher parasympathetic activity as an etiological mechanism for bronchoconstriction in athletes would require a better responsive effect to inhaled anticholinergics, than to other drugs. It has been confirmed by practical experiments arising from Norwegian competitive endurance athletes that revealed that they responded particularly well and with a higher reversibility to inhaled ipratropium bromide than to inhaled beta2-agonists.²⁹

Our study has important limitations. First, the reduced number of subjects included, which may prevent the study from reaching statistical significance; however, they were all elite swimmers. Secondly, the cross-sectional nature of our data. Though, it must be highlighted as a strength of our study, that a highly controlled evaluation was performed for all variables known to possibly interfere with the pupillometry.⁸ Also, this is the first study to address the question of dysautonomia assessed by pupillometry related with swimmers asthma and AHR. Previous studies using this method to evaluate autonomic nervous system have not addressed the relationship with bronchial outcomes. An evaluation of Portuguese Olympic athletes showed that they have higher parasympathetic control, with concomitant reduction of the sympathetic tone.³⁰ Capão-Filipe et al. reported an increased parasympathetic activity and a reduced sympathetic activity of endurance runners when compared to other sports.⁵ In our study, swimmers with clinically relevant AHR, showed a significant correlation between PD₂₀ and 4 of the parasympathetic parameters, reflecting the reliability of pupillometry, since methacholine acts as a non-selective muscarinic receptor agonist in the parasympathetic nervous system. The recent development of a portable, user-friendly, and reliable infrared pupillometer, which allows for accurate, easy, and reproducible quantitative pupillary measurements, has revived the initial enthusiasm about the clinical usage of pupillometry.⁷ It has been employed to evaluate a large number of conditions

as it allows quantitative pupillary measurement of several parameters,⁷ and now has revealed itself as a reliable tool to assess parasympathetic activity in elite swimmers.

To conclude, no significant differences were observed between asthmatic and non-asthmatic swimmers regarding parasympathetic parameters, but among those with clinically relevant airway hyperresponsiveness an association was seen. Although limited by the sample size, these findings provide further support for the role of autonomic nervous system in airway responsiveness development in elite swimmers and might be a new target for future therapeutic options in this particular population.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Study IV

Increased bronchial
parasympathetic tone in elite
cross-country and biathlon
skiers: a randomised
crossover study

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Increased bronchial parasympathetic tone in elite cross-country and biathlon skiers: a randomised crossover study

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ABSTRACT

Background Increased parasympathetic activity in endurance-trained athletes has been reported by heart rate variability and pupillometry. Our primary objective was to assess parasympathetic activity and tone in the lower respiratory tract by investigating the effect of cholinergic antagonism by inhaled ipratropium bromide compared to the β_2 -receptor stimulating effect of inhaled salbutamol in elite cross-country and biathlon skiers. We also examined the medications' relationship to cholinergic sensitivity as measured by bronchial responsiveness to methacholine (PD_{20met}).

Methods In a randomised crossover study, 20 cross-country and two biathlon skiers (14♂/8♀) aged 20–37 years from the Norwegian national teams measured reversibility to inhaled ipratropium bromide and inhaled salbutamol and PD_{20met} on three separate days. A positive reversibility test was defined as an increase in forced expiratory volume in 1 s (FEV₁) of $\geq 12\%$. Spirometry was performed before and 45 and 15 min after inhaled ipratropium bromide and inhaled salbutamol, respectively. Bronchodilating medication was withheld according to the European Respiratory Society (ERS) guidelines. Correlations were assessed by Pearson's correlation coefficient (r).

Results Five athletes had significant reversibility after inhaled ipratropium bromide, and none after inhaled salbutamol. Twelve athletes (54.5%) had PD_{20met} $< 8 \mu\text{mol}$ (1.57 mg). PD_{20met} correlated negatively with ΔFEV_1 after inhaled ipratropium bromide ($r = -0.85$, $p < 0.0001$), but not after inhaled salbutamol ($r = -0.308$, $p = 0.16$).

Conclusions In elite skiers, cholinergic sensitivity (PD_{20met}) had a highly significant inverse correlation to the cholinergic antagonism of inhaled ipratropium bromide, but not at all to the bronchodilating inhaled salbutamol. This markedly increased bronchial parasympathetic tone may represent an important cholinergic role in the development of skier's asthma.

INTRODUCTION

Elite endurance athletes have an increased risk of developing bronchial hyper-responsiveness (BHR) and exercise-induced asthma (EIA).¹ Reports show that cross-country and biathlon skiers, who train and compete in cold air, are among those athletes with the highest prevalence of self-reported respiratory symptoms and objectively measured BHR.^{2–4} However, the mechanisms of these pathophysiological airway changes in athletes are currently not completely understood.

Since the bronchoconstrictor effect of a variety of cholinergic stimulants in the airways was abolished

after blocking cholinergic efferent pathways with an intravenous injection of atropine sulfate, increased cholinergic reflexes have long been proposed to contribute to BHR.⁵ Cooling of the airways during exercise may cause exercise-induced bronchoconstriction (EIB) through an increase in vagal efferent tone.⁶ Knöpfli and coworkers found an association between changes in heart rate variability (HRV) at the onset of exercise, thought to reflect the effect of parasympathetic stimulation on the chronotropic activity of the heart, and the bronchodilating effect of inhaled ipratropium bromide, blocking efferent cholinergic pathways in the airways, in endurance-trained cross-country runners exercising at -5°C ,⁷ and in children with EIB.⁸

Parasympathetic activity is higher in endurance-trained athletes compared to non-athletes as assessed by HRV⁷ and pupillometry.^{9–10} Participants with BHR to methacholine (PD_{20met}) had an increased HRV, reflecting a higher parasympathetic tone after a methacholine bronchial challenge, as compared to participants without BHR.¹¹ However, the relationship between HRV and BHR was weak, and the increased parasympathetic tone could not alone explain the increased prevalence of asthma and BHR among athletes.^{12–13} Nevertheless, the heart is continuously under sympathetic and parasympathetic influence, and does not necessarily reflect a parasympathetic tone in the bronchi. Horvath *et al*¹⁴ assessed airway resistance and heart rate period (inter-beat interval, milliseconds) after cholinergic blockade by atropine sulfate administration and found that vagal control of bronchial tone and heart rate were not related in resting healthy participants.¹⁴

The primary objective of the present study was to assess a possible relationship between increased bronchial responsiveness to methacholine, reflecting the sensitivity of a cholinergic stimulation on the bronchial smooth muscle and mucous glands, and the bronchodilating effect of inhaled ipratropium bromide, blocking the effect of increased parasympathetic (cholinergic) tone on the bronchial smooth muscle in top endurance-trained elite competitive cross-country skiers. Second, we aimed to compare the bronchodilating effect of inhaled ipratropium bromide blocking parasympathetic tone with that of inhaled salbutamol as a general bronchodilating agent not affecting cholinergic receptors in this group of athletes.

METHODS

Subjects and design

The Norwegian National team of cross-country skiing, including 20 cross-country skiers, as well as



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Original article

two biathlon skiers (14♂/8♀), aged 20–37 years, who were present on a training camp in Val Senales, Italy, were included in a randomised crossover study. All participants were elite athletes competing at the top international level. All were non-smokers and did not consume snuff. Subject characteristics are described in table 1. All athletes had been free from any respiratory disease for the past 3 weeks before the first study day, and refrained from exercise and any food or drink containing nitrate on the same day of testing. Antiasthmatic medication was withheld according to the European Respiratory Society (ERS) guidelines.¹⁵ Inhaled short-acting β_2 -agonists were withheld for 8 h before testing; inhaled long-acting β_2 -agonists, theophylline, and leukotriene antagonists were withheld for the past 72 h; antihistamines were withheld for the past 7 days; and orally administered glucocorticosteroids were withheld for the last month. Inhaled corticosteroids were not to be used on the day of testing.

The athletes attended one visit at the laboratory at the Norwegian School of Sport Sciences, Oslo, Norway for assessment of lung function, BHR to methacholine (PD_{20met}) and fractional exhaled nitric oxide (FE_{NO}). Prior diagnoses of asthma, allergic rhinitis and EIB, use of asthma medication in the last year and current symptoms of dyspnoea, phlegm and cough during or after exercise were recorded with the AQUA-questionnaire¹⁶ and clinical interview. Two reversibility tests were obtained during a training camp in Val Senales, Italy, 2000 m above sea level. The reversibility tests were performed in a randomised order on two separate days, with 24 h between each test. All tests were performed according to current guidelines from the American Thoracic Society (ATS).¹⁷ Data collection was conducted during September–October 2011. The study was approved by the regional medical ethics committee and carried out according to the principles stated in the Declaration of Helsinki. Signed informed consent was obtained from each participant.

Procedures

Measurement of lung function was performed by maximum expiratory flow-volume loops using a MasterScreen Pneumo spirometer (Jaeger GmbH, Würzburg, Germany). The predicted values used are according to Quanjer *et al.*¹⁸ The following variables were recorded: forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), and forced expiratory flow at 50% of vital capacity (FEF_{50}).

Methacholine bronchial challenge was performed with an inspiration-triggered nebuliser (Aerosol Provocation System, Jaeger, Würzburg, Germany). The nebuliser output was

controlled and calibrated before the start of the study and weekly during the study period. After measuring baseline lung function, lung function was measured after inhaling nebulised isotonic saline (0.9%). Methacholine chloride, 32 mg/mL, was inhaled in doubling doses from a starting dose of 0.51 μ mol (0.1 mg). Lung function measurements were performed 1 min after every delivered dose until FEV_1 decreased 20% from the measurement after inhaled saline. The maximum cumulative dose was 24.48 μ mol (4.8 mg). A positive response to methacholine was defined as a 20% reduction in FEV_1 and the methacholine provocation dose causing a $\geq 20\%$ decrease in FEV_1 was calculated by linear interpolation on the dose–response curve and recorded as PD_{20met} . Clinical significant BHR was defined as $PD_{20met} \leq 8 \mu$ mol. After the methacholine provocation, all athletes received salbutamol inhalation (0.1 mg/mL $\times$ 10 kg/body mass) by nebulisation to reverse bronchial obstruction.

Measurement of FE_{NO} was performed before lung function measurement by the Eco Medics CLD 88 sp Exhalyzer (Eco Medics AG, 8635 Duernten, Switzerland). Participants inhaled NO-free air to total lung capacity and exhaled with a standardised flow of 50 mL/s according to the ATS/ERS recommendations.¹⁹ Mean values were used after measurements performed in triplicates.

Reversibility tests for inhaled ipratropium bromide and salbutamol were performed using identical procedures on two separate days. Salbutamol (0.1 mg/mL $\times$ 10 kg/body mass) and ipratropium bromide (0.500 mg/mL), respectively, were mixed in 1 mL isotonic NaCl and delivered through a Sidestream nebulising chamber (Respironics Respiratory Ltd, Chichester, UK) connected to a CR60 compressor (Medic-Aid Ltd, West Sussex, UK) at a flow rate of >6 L/min. Lung function by maximal expiratory flow volume loops was measured, as previously described, before and 15 and 45 min after inhaled salbutamol and inhaled ipratropium bromide, respectively. Clinical significant reversibility was defined as a $\geq 12\%$ increase in FEV_1 from before to after inhalation.

Statistical analysis

Demographic data and results are expressed as mean values with 95% CIs, unless otherwise stated. Correlations were assessed by Pearson's correlation coefficient (r_p) for normally distributed data on $\log_{10}$ -transformed values of PD_{20met} ($\log_{10}PD_{20met}$). To achieve a high-grade correlation of more than or equal to 0.7 with a power of 80%, 13 participants were calculated to be required based on previous measurements in top athletes. Differences between two measurements were analysed by Student *t* tests and differences in categorical data were analysed by χ^2 and Fisher's exact tests. Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS, V21.0; Chicago, Illinois, USA) and MedCalc Statistical System (V10.4.6.0, Mariakerke, Belgium). A *p* value less than or equal to 0.05 was considered statistically significant.

RESULTS

Subject characteristics

The characteristics of the participants included in this study are presented in table 1. Sixteen athletes (73%) had a doctor's diagnosis of asthma. Seven (32%) reported they had doctor-diagnosed allergic rhinitis. Lung function was within normal range in all athletes (table 1).

Reversibility and BHR

Mean ΔFEV_1 after inhaled salbutamol was 4.3% (± 3.8 (mean $\pm$ SD)) or 185.9 mL ($\pm$ (SD)166.9), and mean ΔFEV_1 after

Table 1 Subject characteristics with lung function (FEV_1 , FVC, FEF_{50}) and fractional exhaled nitric oxide (FE_{NO})

	Mean (range, $\pm$ SD)	Percentage of predicted values (range, $\pm$ SD)
Height (cm)	175.5 (162.0–190.0, ± 8.8)	NA
Weight (kg)	69.7 (52.0–85.0, ± 10.5)	NA
Age (years)	26 (20–37, ± 4.7)	NA
FEV_1 (L)	4.3 (3.0–5.5, ± 0.7)	103 (91–130, ± 9.5)
FVC (L)	5.5 (3.6–7.3, ± 1.2)	112 (93–131, ± 11.0)
FEF_{50} (L/s)	4.5 (2.5–8.6, ± 1.3)	86 (51–159, ± 27.4)
FE_{NO} (ppb)	24.7 (9.0–113.0, ± 25.6)	NA

FEV_1 , forced expiratory volume in 1 s; FVC, forced vital capacity; FEF_{50} , forced expiratory flow at 50% of vital capacity; FE_{NO} , fractional exhaled nitric oxide; NA, not applicable.

inhalation of ipratropium bromide was 7.8% ($\pm$ (SD)6.9) or 330.5 mL ($\pm$ (SD)289.8). A significant difference between Δ FEV₁ after inhaled ipratropium bromide and inhaled salbutamol of 3.4% (0.55, 6.31) was observed ($p=0.002$). Five athletes (4♂) had a positive reversibility to inhaled ipratropium bromide (Δ FEV₁ $\geq 12\%$), and none had a positive reversibility to inhaled salbutamol (figure 1A). Mean PD_{20met} was 8.14 (4.86 to 13.64)

(geometric mean with 95% CI). Twelve athletes (8♂) had PD_{20met} ≤ 8 μ mol (1.57 mg; figure 2) and six athletes (5♂) had PD_{20met} ≤ 4 μ mol (0.78 mg).

Correlations

Log₁₀PD_{20met} correlated significantly with Δ FEV₁ after inhalation of ipratropium bromide ($r=-0.85$, $p<0.0001$; figure 1A), but not with Δ FEV₁ after inhalation of salbutamol ($r=-0.308$, $p=0.16$; figure 1B). Similarly, the mean Δ FEF₅₀ after inhaled ipratropium bromide of 29.2% ($\pm$ (SD)20.2) was negatively associated with log₁₀PD_{20met} ($p=0.017$). Mean Δ FEF₅₀ after inhaled salbutamol was 18.3% ($\pm$ 16.2), and no significant relationship with log₁₀PD_{20met} was found.

Exhaled nitric oxide

Mean FE_{NO} was increased (>20 ppb) and ranged from 9 to 113 ppb (table 1). FE_{NO} was ≥ 30 ppb in five athletes (3♂), of whom four had doctor-diagnosed asthma and respiratory symptoms and used asthma medication, and one had allergic rhinitis and used antihistamines regularly. There was no difference in FE_{NO} between athletes with allergic rhinitis (mean 26.1 ppb (3.7–48.6), $n=7$) and non-allergic athletes (mean 25.8 ppb (10.9–40.8), $n=15$). We observed no association between FE_{NO} and PD_{20met}.

Use of medication

Seventeen athletes (77%) used inhaled corticosteroids and four (18%) used antihistamines regularly (table 2). One athlete had doctor-diagnosed allergic rhinitis and used antihistamines, inhaled corticosteroids and ipratropium bromide, but did not have doctor-diagnosed asthma. All athletes with BHR (PD_{20met} ≤ 8) used inhaled corticosteroids ($n=12$). There were 10 athletes who had a PD_{20met} >8 μ mol, and of these 50% used inhaled corticosteroids. Athletes with inhaled corticosteroids had PD_{20met}: 6.08 (3.36 to 10.99) (geometric mean with 95% CI); those without: 22.05 (12.82 to 37.92) (geometric mean with 95% CI) ($p=0.025$). Athletes who used inhaled corticosteroids had greater bronchodilator response to inhaled ipratropium bromide: 109.9% (106.4% to 113.3%), as compared to the athletes who did not use corticosteroids: 101.8% (98.8% to 104.7%; $p=0.01$). No differences were observed either in bronchodilator effects after inhaled salbutamol in athletes who used short-acting or long-acting β_2 -agonists, or in inhaled corticosteroids, respectively, as compared to athletes who did not use these medications.

All athletes with positive reversibility to ipratropium bromide had PD_{20met} ≤ 8 μ mol (figure 1A) and doctor-diagnosed asthma and used asthma medication. Of all who used inhaled corticosteroids, four of five used short-acting β_2 -agonists and ipratropium bromide, and two of five used long-acting β_2 -agonists. Two of the athletes had doctor diagnosed allergic rhinitis, of whom one used antihistamines regularly.

Presence of respiratory symptoms

Nineteen athletes reported that they had respiratory symptoms during or after exercise, and three athletes were non-symptomatic (figure 3). Cough (73%) was the most prevalent self-reported symptom, followed by phlegm (68%) and dyspnoea (41%). BHR (PD_{20met} ≤ 8 μ mol) was more prevalent in athletes with respiratory symptoms than in non-symptomatic athletes (figure 3). Self-reported symptoms were not associated with FE_{NO} or Δ FEV₁ after inhalation of neither ipratropium bromide nor salbutamol.

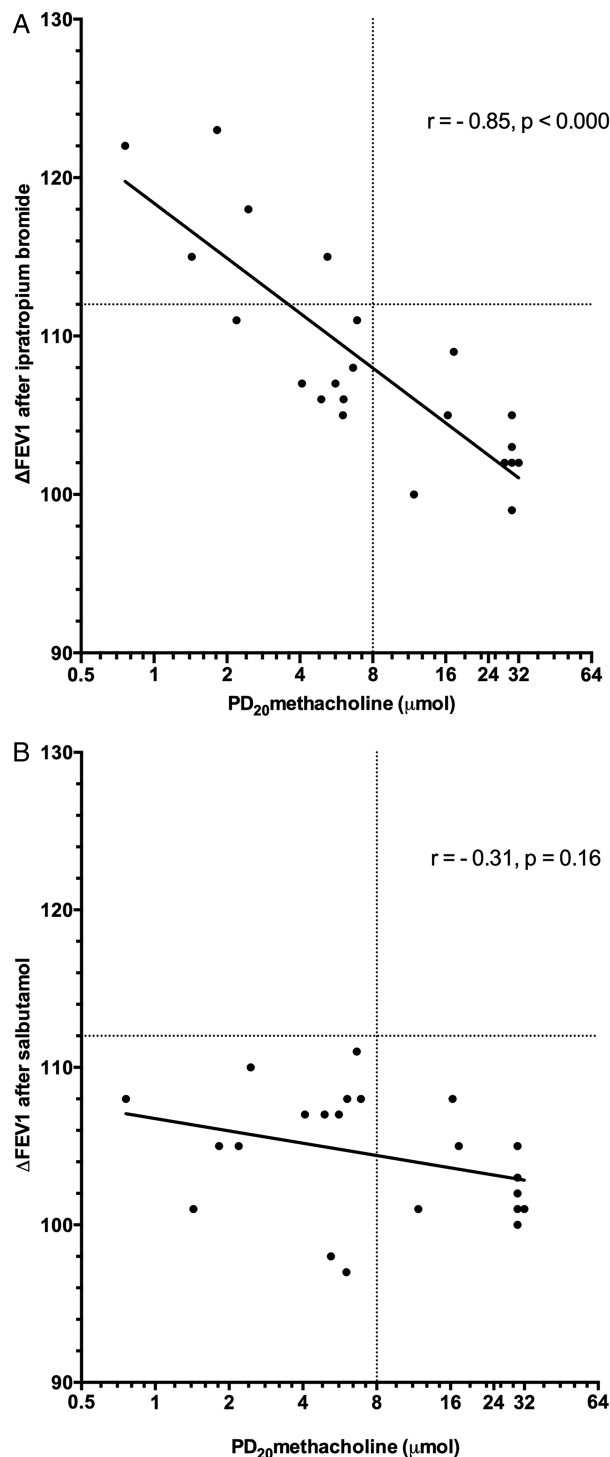


Figure 1 Relationship between the methacholine dose causing a 20% reduction in forced expiratory volume in 1 s (FEV₁) (PD_{20met}) and (A) Δ FEV₁ (%) 45 min after inhaled ipratropium bromide and (B) Δ FEV₁ (%) 15 min after inhaled salbutamol, in elite cross-country and biathlon skiers.

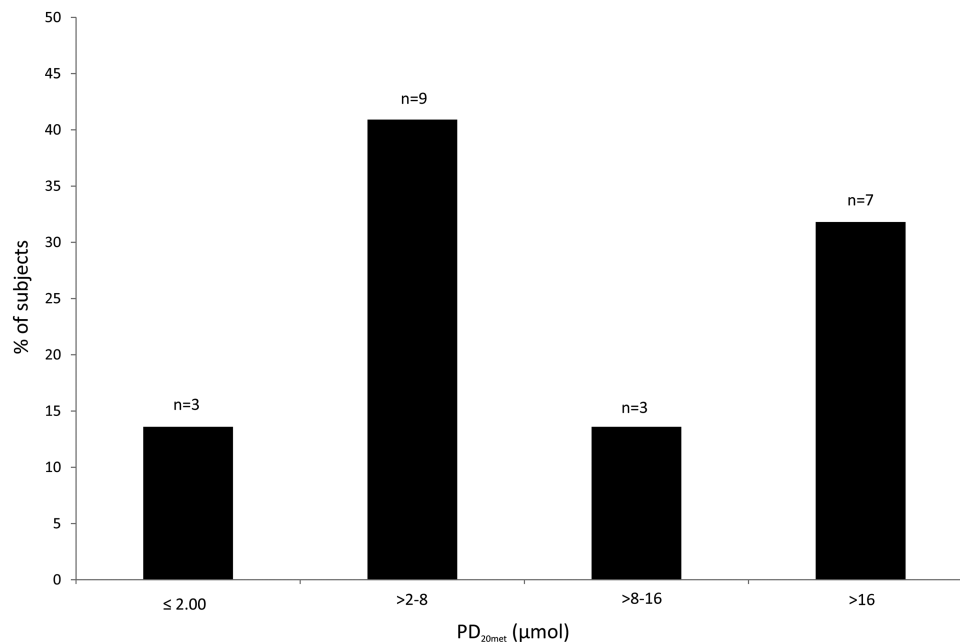


Figure 2 Distribution of the degree of bronchial hyper-responsiveness (BHR), measured as the methacholine dose (0.05–30 μmol) causing a 20% reduction in forced expiratory volume in 1 s (FEV₁) (PD_{20met}), in elite Norwegian cross-country and biathlon skiers. A PD_{20met} less than 2 μmol is considered as severe BHR; greater than 2 μmol and less than 8 μmol is mild to moderate BHR; and greater than 8 μmol and less than 16 μmol is borderline BHR.

DISCUSSION

In this study, 54% of the athletes (national team cross-country and biathlon skiers) had increased BHR (PD_{20met} ≤ 8 μmol), 23% had significant reversibility to inhaled ipratropium bromide (ΔFEV₁ ≥ 12%) and none to inhaled salbutamol. The main finding of this study was a highly significant correlation between BHR (log₁₀PD_{20met}) and reversibility to inhaled ipratropium bromide ($r = -0.85$, $p < 0.0001$), but not so between BHR and reversibility to salbutamol ($r = -0.31$, $p = 0.16$).

Role of the parasympathetic system in asthma symptoms

BHR to inhaled methacholine may be regarded as increased sensitivity to a cholinergic stimulus, whereas increased reversibility to inhaled ipratropium bromide, which blocks acetylcholine, can be regarded as a sign of increased bronchial cholinergic

(parasympathetic) tone. The high correlation between the two (increased sensitivity to cholinergic stimulation and increased cholinergic tonus), together with the bronchodilator response to the ipratropium bromide observed, suggests an important role of the parasympathetic nervous system in causing bronchial obstruction and asthma symptoms in these elite endurance trained athletes of winter sports.

The significant high-grade negative correlation between a cholinergic bronchoconstrictor and an anticholinergic bronchodilator stimulus, and lack of correlation with a β₂-stimulating bronchodilator, suggest an important role of an increased parasympathetic tone and sensitivity in the development of BHR and asthma in these top endurance-trained athletes. Increased parasympathetic activity has previously been reported in endurance-trained athletes measured both by the parasympathetic activity of the eye by pupillometry in long distance runners^{9 10} and of the cardiovascular system by the variation in the heart rate induced by an exercise test.⁷ These findings are supported by this study in cross-country skiers who, in addition to the endurance training, are also exposed to cold air during their training and competitions, an exposure previously reported to cause parasympathetic stimulation of the airways and contribute to EIB.⁶ Furthermore, Goldsmith *et al*²⁰ showed a correlation of $r = 0.75$ ($p = 0.0001$) between HRV indices reflecting parasympathetic activity and maximal oxygen uptake (VO_{2max}), which suggests that endurance training and increased aerobic capacity are followed by increased parasympathetic activity.

In addition, Park *et al*²¹ found a relationship between PD_{20met} and diminished sweat secretion, and between tearing rate and salivary flow rate, indicating autonomic dysfunction, in healthy athletes suspected of having EIB. These findings indicate that exercise-induced alterations of the parasympathetic branch influence bronchial tone, and may be involved in the development of BHR and asthma in susceptible elite athletes.^{22 23}

Table 2 Prevalence of doctor-diagnosed asthma and allergic rhinitis, bronchial hyper-responsiveness (BHR), defined as a methacholine dose causing a 20% reduction in forced expiratory volume in 1 s (FEV₁) of ≤ 8 μmol (PD_{20met}), and use of medications in elite Norwegian cross-country and biathlon skiers

	n	Total (%)
Asthma	16	72.7
Allergic rhinitis	7	31.8
BHR	12	54.4
Use of medication		
Short-acting β ₂ -agonists	8	36.4
Long-acting β ₂ -agonists	10	45.5
Inhaled corticosteroids	17	77.3
Ipratropium bromide	15	68.2
Leukotriene antagonists	0	0
Antihistamines	4	18.2

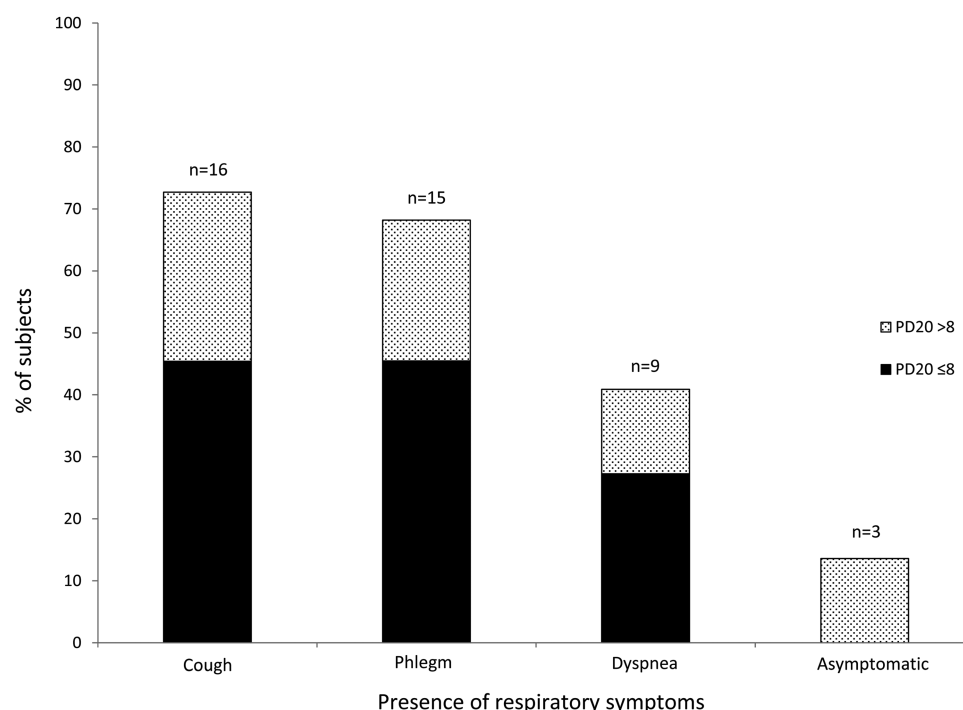


Figure 3 Presence of respiratory symptoms, and type of symptom in relation to bronchial hyper-responsiveness (BHR) (methacholine dose causing a 20% reduction in forced expiratory volume in 1 s (FEV₁) [PD_{20met}] less than or equal to 8 µmol) in elite cross-country and biathlon skiers.

What influences the prevalence of bronchial hyper-reactivity?

The prevalence of BHR and asthma is reported to be higher in endurance athletes than in strength and power athletes and non-athletes.²⁴ The high prevalence of asthma in endurance athletes is reportedly related to the repeated daily training activity with high ventilation rates resulting in epithelial damage of the airways, delayed repair due to the daily repetition of the training and increased airway mucosal inflammation.^{22–25} In addition, the ambient training environments may contribute to the high prevalence of EIB in endurance athletes through increased exposure with high ventilation rates during exercise. This includes swimmers, triathletes, cyclists and speed skaters.^{26–29} Cross-country skiing is one of the most demanding sports in regard to the aerobic metabolic system. These are highly conditioned athletes with mean peak oxygen consumption (VO_{2peak}) levels of more than 6.0 L/min or approximately 80 mL/kg/min for males.³⁰ The athletes included in this study are among the world's best in their discipline with several winners of Olympic Gold medals and of the World Cup in cross-country skiing.

Mechanisms—Why does this occur?

Cold and dry air will influence participants with EIA through increased respiratory heat and water loss during exercise,³¹ and exposure to cold air combined with strenuous exercise is shown to be associated with the development of airway inflammation and epithelial damage in animal and human studies.^{32–34} In this study, the prevalence of asthma diagnosis, use of asthma medication and presence of respiratory symptoms were all above 70% (table 2 and figure 3), whereas more than half of the athletes had objectively measured BHR. In regard to the high performance level of these athletes, and considering the type of sport, the asthma prevalence found in this study can be considered to be in line with previous studies. The incidence of self-reported respiratory symptoms or BHR in Swedish cross-country skiers

was as high as 80%.⁴ In a recent study, the evidence of asthma, defined as at least one positive objective provocation test to either methacholine or exercise, was 60% in swimmers and 29% in cold-weather athletes compared to 17% of non-athlete controls.²⁹ In that particular study, winter sport athletes included speed skaters in addition to cross-country and biathlon skiers, and the 25% prevalence of BHR to methacholine, defined as PD_{20met} ≤ 4 µmol, was similar to that in this study.

Our results are based on bronchial responsiveness assessed by a methacholine bronchial challenge and reversibility of the athletes at their current asthma treatment and asthma control during data collection. In a study by Stadelmann *et al*,³⁵ the direct assessment of BHR by PD_{20met} corresponded well with the indirect stimuli of an EVH-challenge in swimmers. Further studies using other bronchial challenges should be conducted in order to support our results. Testing was performed in the fall, prior to the competitive season. Heir *et al*³⁶ have found that BHR in cross-country skiers varied according to seasonal changes and exercise intensity. Their study showed that BHR, expressed as the methacholine concentration causing a fall in FEV₁ by 10% (PC₁₀), was lower at the end of winter and the competitive season and that it was highly affected by the amount of high-intensity training. The data collection of this study was carried out before the competitive season, but during an intensive training period, which may have influenced the degree of BHR in the athletes.

The use of β₂-agonists among athletes is increasing.³⁷ Interestingly, none of the athletes in this study showed a positive reversibility to salbutamol (ΔFEV₁ ≥ 12%), despite the fact that 73% reported that they had asthma and 55% had clinical BHR (PD_{20met} ≤ 8 µmol). Regular treatment with short-acting and long-acting β₂-agonists may result in tolerance development to the effect of β₂-agonists.³⁸ In this study, 8 athletes used short-acting β₂-agonists and 10 used long-acting β₂-agonists. In addition, 10 athletes used ICS. The potential influence from the use

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of these medications on the absence of any significant bronchodilator effect after inhaled salbutamol in this study cannot be excluded. Optimal asthma treatment is a pre-requisite for the asthmatic athlete, both from a competitive and health perspective. Haahtela *et al*²⁵ have described different phenotypes of asthma in athletes and individual differences in the airway response to bronchodilators are reported in the literature, especially among the athletes who develop asthma symptoms and BHR later in life and during their sport careers.³⁹ This is somewhat in agreement with this study where we found that athletes with a positive reversibility test to ipratropium bromide were characterised by BHR (PD_{20met} of <8 µmol), as well as the use of inhaled corticosteroids and self-reported exercise-induced respiratory symptoms. There was no difference observed in bronchodilator response to inhaled salbutamol between athletes using inhaled corticosteroids, short-acting or long-acting β₂-agonists, or no asthma treatment.

The high prevalence of anticholinergic drug use in this study can be explained by that previous experience with athletes of the National teams of cross-country and biathlon skiing, which has demonstrated the improved reversibility to inhaled ipratropium among this group of athletes. Therefore, reversibility tests of both salbutamol and ipratropium bromide have been performed on these elite athletes. Treatment is then based on the best effect of the reversibility tests. Another reason is that ipratropium bromide has not been listed on Wada's World Anti-doping prohibited list of drugs.

Anticholinergic agents, such as ipratropium bromide, inhibit parasympathetic nerve impulses through competitive inhibition on the muscarinic acetylcholine receptors in smooth muscle and respiratory glands.⁴⁰ It has been suggested that differences in the parasympathetic bronchial tone may explain why some patients are responders and others non-responders to anticholinergic treatment.^{8 27} Pichon *et al*¹¹ have shown that non-athletes with BHR to methacholine also seem to have a high parasympathetic tone, measured as increased HRV. As methacholine is a synthetic choline ester that acts as a non-selective muscarinic receptor agonist, the bronchial responsiveness to methacholine may itself be considered to reflect the parasympathetic bronchial tone.

Although respiratory symptoms are common among cross-country skiers,^{3 4} the results from this study showed no associations between self-reported symptoms and BHR, reversibility to inhaled ipratropium bromide or inhaled salbutamol, or to FE_{NO}. The incidence of asthma symptoms found by self-reports is higher compared with objectively measured BHR to methacholine and reversibility tests. This finding extends to other studies and suggests that objective tests are a necessary addition to self-reported symptoms to ascertain the diagnosis of asthma in athletes.⁴¹

CONCLUSION

This study reported a high prevalence of BHR and asthma symptoms among top cross-country skiers. The high correlation between reversibility to ipratropium bromide and methacholine BHR in this study provides evidence that increased parasympathetic activity in the airways contributes to asthma development in elite cross-country and biathlon skiers.

What are the new findings?

- ▶ The present study demonstrated a marked increased bronchial parasympathetic tone in highly trained athletes.
- ▶ It showed differences in responses to two different bronchodilators in elite cross-country skiers.
- ▶ It also confirms that self-reported symptoms are not related to objective tests for asthma in athletes.

How might it impact on clinical practice in the near future?

- ▶ This highly increased bronchial parasympathetic tone may represent an important cholinergic role in the development of skier's asthma.
- ▶ The results from this study suggest that elite skiers with asthma respond better to anticholinergic treatment as compared with β₂-agonists.
- ▶ This study suggests that BHR to methacholine may be a measure of bronchial parasympathetic tone in elite cross-country and biathlon skiers.

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Study V

Exhaled breath temperature
in elite swimmers: the
effects of a training session
in adolescents with or
without asthma

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Exhaled breath temperature in elite swimmers: The effects of a training session in adolescents with or without asthma

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Keywords

airway inflammation; asthma; athletes; exhaled breath temperature; training; swimmers

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Abstract

Background: Cooling of the airways and inflammation have been pointed as possible mechanisms for exercise-induced asthma (EIA). We aimed to investigate the effect of training and asthma on exhaled breath temperature (EBT) of elite swimmers.

Methods: Elite swimmers annually screened (skin prick tests, spirometry before and after salbutamol inhalation, induced sputum cell counts, and methacholine bronchial challenge) at our department ($n = 27$) were invited to this prospective study. Swimmers who agreed to participate in the present study ($n = 22$, 10 with asthma) had axillary temperature and EBT measured (X-halo[®]) before and after a swimming training session (aerobic/non-aerobic). Linear regression models were used to assess the effect of asthma and other possible explanatory variables (demographics, PD₂₀, baseline EBT, training intensity, axillary temperature, and the number of hours trained in that week) on EBT change.

Results: EBT significantly increased after training independently of lung function, airway responsiveness, and inflammation in all swimmers (mean $\pm$ SD: 0.32 ± 0.57 ; $p = 0.016$). No differences were observed between asthmatic swimmers and others. A significant correlation was observed between baseline and post-exercise EBTs ($r = 0.827$, $p < 0.001$). Asthma was not a predictor of Δ EBT after adjusting for confounders; baseline EBT was the variable most strongly associated with Δ EBT, explaining by itself alone 46% of the outcome ($r^2 = 0.464$).

Conclusion: Although these are preliminary data, a relationship between airway's inflammation and respiratory heat loss during exercise could not be confirmed, suggesting that the increase in exhaled breath temperature is a physiologic rather than a pathological response to exercise.

Asthma is characterized by bronchial hyperresponsiveness (BHR) and inflammation. Vasodilatation is a critical feature of inflammation and is induced in asthmatic airways by the release of several mediators, such as nitric oxide (NO). Moreover, angiogenesis and vascular and bronchial remodeling are features of asthma (1, 2). Increased vascularization of airway mucosa occurring in asthmatics leads to increased heat exchange during expiration (3) and previous studies reported increased exhaled breath temperature (EBT) in asthmatic compared to healthy subjects (4). Increased EBT correlates with exhaled NO and sputum eosinophilia in asthmatic children (4).

Although the association between sports and airway injury is not fully elucidated, airways inflammation has been suggested as a cause of exercise-induced asthma (EIA).

Vigorous exercise results in inhalation of a large volume of cold, dry air, warmed to 37°C during its passage through the airways with resulting heat loss from the respiratory mucosa, causing airway cooling. Furthermore, the markedly increased ventilation may cause mechanical damage to the respiratory tract epithelial lining, thereby permitting influx of inflammatory cells (5, 6). Increased numbers of inflammatory cells are found in induced sputum of athletes from different sports (7, 8). Swimmers, in particular, show a marked sputum eosinophilia (7).

Evidence of these hypotheses acting as mechanisms of EIA suggests that asthmatic athletes have higher respiratory heat loss during exercise, which may cause a higher EBT after exercise. A significant increase in EBT in asthmatic children after exercise has been reported (9), but similar to controls (10,

11). To our knowledge, EBT has not been investigated in athletes after performance of their sports activity and they present increased risk for asthma, especially those who take part in endurance sports such as swimming (5). Thus, the main objective of the present study was to investigate whether exercise induces a change in EBT and, secondly, whether this was affected by asthma.

Methods

Study design and participants

Elite swimmers annually screened in our Department ($n = 27$) were invited to participate in this prospective study. Competitive swimmers, aged ≥ 14 years old, training ≥ 10 h per week for an average period of 9 years of competition and free from respiratory infection in the last 3 weeks were included. International Olympic Committee criteria (5) were used to diagnose asthma, that is, the presence of respiratory symptoms together with a positive bronchodilator or a bronchial provocation test (with regard to methacholine provocation, a $PC_{20} \leq 4$ mg/ml, or $PD_{20} \leq 400$ μ g [cumulative dose] or ≤ 200 μ g [non-cumulative dose] in those not taking inhaled corticosteroids, and $PC_{20} \leq 16$ mg/ml or $PD_{20} \leq 1600$ μ g [cumulative dose] or ≤ 800 μ g [non-cumulative dose] in those taking inhaled corticosteroids for at least 1 month). No differences were observed between asthmatic ($n = 10$) and non-asthmatic swimmers for demographic and personal characteristics, except for the expected lower PD_{20} among those with asthma (Table 1). Swimmers who agreed to participate in the present study had a specific evaluation with EBT and axillary temperature measured before and after a training session in the swimming pool during summer months.

Subjects (or caregivers) signed informed consent. The study was approved by the Ethical Commission.

Data collection and study procedures

Data collected from screening visits

Data from screening visits previously performed were retrospectively collected. In these visits, swimmers were asked to withhold medication according to guidelines (12): inhaled short-acting β_2 -agonists were withheld for 8 h before testing; inhaled long-acting β_2 -agonists for the last 48 h; leukotriene antagonists were withheld for the last 24 h; antihistamines were withheld for the last 3 days; orally administered corticosteroids were withheld for the last month; and inhaled corticosteroids were not to be used on the day of testing.

Methacholine bronchial challenge. BHR was measured according to guidelines (12) (Mefar, Brescia, Italy) starting with a 0.0625 mg solution, and the provocative dose causing a 20% fall in FEV_1 (PD_{20}) was determined.

Lung function. Spirometry was performed according to European Respiratory Society guidelines (13), and reversibility was assessed 15 min after 400 μ g of inhaled salbutamol. Clinical significant reversibility was defined as a $\geq 12\%$ and 200 mL increase in FEV_1 after inhalation (14).

Skin prick tests. Atopy was defined by the presence of ≥ 1 positive test with standardized aeroallergens extracts (Leti[®], Madrid, Spain).

Induced sputum. Sputum collection and analysis were performed as previously described (15). Briefly, after induction with a commercial 4.5% sodium chloride solution as recommended by guidelines (16) through an ultrasonic nebulizer

Table 1 Personal and demographic characteristics of swimmers included in the study

	Asthmatics ($n = 10$)	Controls ($n = 12$)	p*
Men, n (%)	7 (70)	3 (23)	0.084
Age, years	17 ± 2.8	17 ± 2.9	0.872
BMI, Kg/m ²	21.7 ± 2.1	21.1 ± 1.9	0.456
Atopy, n (%)	4 (40)	4 (33)	1.000
PD_{20} methacholine	0.71 ± 0.6	4.39 ± 2.4	<0.001
FEV_1 , Liters	4.26 ± 0.75	4.07 ± 0.92	0.582
FEV_1 , % of predicted	111.10 ± 14.54	115.50 ± 8.89	0.722
FVC, Liters	5.13 ± 0.94	4.60 ± 1.20	0.093
FVC, % of predicted	114.50 ± 10.49	114.08 ± 11.48	0.923
FEV_1/FVC	83.52 ± 7.32	89.36 ± 7.18	0.123
FEF_{25-75} , Liters	4.22 ± 1.20	4.67 ± 1.17	0.314
FEF_{25-75} , % of predicted	97.3 ± 26.67	113.42 ± 22.54	0.140
FEV_1 increase after BD in mL, median (IQR)	185 (325)	145 (210)	0.771
FEV_1 increase after BD in %, median (IQR)	5 (8)	4.5 (5)	0.582
IS percentage of eosinophils, median (IQR)	3.3 (16.4)	1.1 (8.13)	0.696
IS percentage of neutrophils, median (IQR)	7.8 (19.9)	12.3 (29.1)	0.573
IS percentage of epithelial cells, median (IQR)	30.1 (37.8)	25.2 (10.6)	0.133

Data presented as mean $\pm$ SD, unless otherwise stated.

BD, bronchodilation; FEF_{25-75} , forced expiratory flow middle portion of FVC; FEV_1 , forced expiratory volume in 1-s; FVC, forced vital capacity; PD_{20} , provocative dose inducing a 20% decrease in FEV_1 .

*Mann-Whitney *U*-test was used for comparisons or chi-square tests in case of categorical variables.

(OMRON NE-U17; Omron Healthcare Europe, Netherlands), sputum was sampled and treated with dithiothreitol (Sputolysin; Calbiochem Corporation, USA). The suspension was centrifuged and the cell pellet resuspended. Cytospins were prepared and stained using May-Grünwald/Giemsa. Differential cell counts were made by counting a minimum of 500 non-squamous cells. The lower respiratory origin was confirmed by the presence of macrophages and bronchial epithelial cells. Normal values for sputum differential cell percentages were based on published healthy subjects counts (17).

Study procedures

Exhaled breath temperature. EBT was measured using X-halo breath thermometer (Delmedica Investments®, Singapore), 5 min before (baseline EBT) and 5 min after swimming (post-exercise EBT), according to validated methods (18). Briefly, swimmers were requested to inhale through the nose and exhale by the mouth into the device at a rate and depth typical of their normal tidal breathing rhythm (Fig 1) until indication of the software. The decision of collecting EBT 5 min after the exercise was based on findings that this is the time point in which EBT reaches the highest value (10).

Body temperature. Axillary temperature was measured before collecting baseline EBT with an axillary thermometer (MediCare®). Choosing axillary rather than oral temperature was based on previous suggestions that oral temperature is related to the airways and/or the oral cavity and so could possibly be affected differently during exercise (10).

Swimmers performed their regular training in a chlorine-treated open-air swimming pool. The session intensity was recorded and categorized as 1: *aerobic training* (bouts of 30–60 min achieving heart rate between 120 and 160 bpm), 2: *anaerobic training* (bouts of exercise achieving a heart rate

>180 bpm, until 10 min of duration), and 3: *anaerobic moderate/intense training* (bouts of exercise achieving a heart rate >180 bpm, 10–30 min of duration).

Statistical analysis

To achieve the 0.43°C increase in EBT with exercise (9), assessed by paired *t*-test with a power (1- β) of 70%, a total target of 36 subjects would be needed for a level of significance of 0.05. However, only 27 elite swimmers have been previously screened and, among these, only 22 (of which ten with asthma) accepted to participate in the present study, retrieving a power of 49% to detect a Δ EBT of 0.43°C.

Categorical variables are expressed as counts (%) and continuous variables as mean $\pm$ SD or as median $\pm$ IQR. Given the sample size, only nonparametric tests were used: Wilcoxon test to compare differences between baseline and post-exercise EBTs, Mann–Whitney *U*-test to assess differences between asthmatic and non-asthmatic swimmers, and Spearman's test for correlations. Chi-square test was used for categorical variables. PD₂₀ was log-transformed for correlation testing (LnPD₂₀). The change in EBT was computed (Δ EBT = post-exercise EBT—baseline EBT).

Linear regression models were used to determine the effect of asthma and other possible explanatory variables (age, gender, height, weight, PD₂₀, baseline EBT, training session intensity, axillary temperature, and hours trained in the previous week) in the outcome Δ EBT. These variables were selected based on the authors' *a priori* hypothesis that they may influence EBT. A univariate linear regression analysis was performed to assess the individual effect of each variable; those significant at the 0.25 level were included in a multiple linear regression model. A stepwise method was used to select

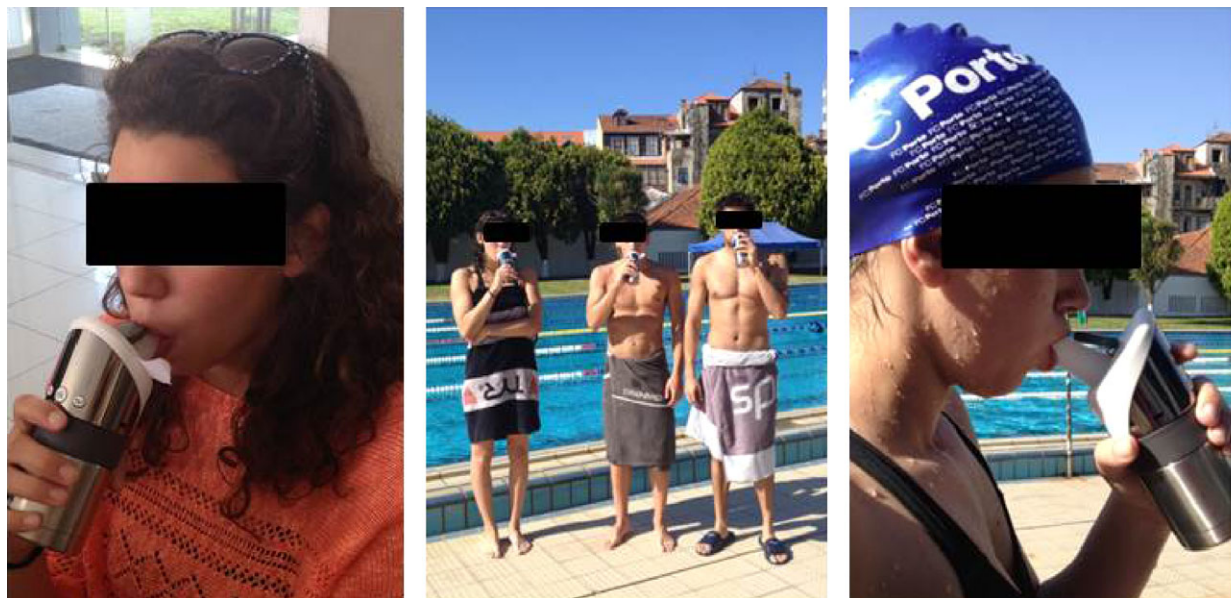


Figure 1 Exhaled breath temperature collection before and after a real-life swimming training session.

variables to include in the final model, taking into account their significance and effect in the adjusted r^2 . The effect of asthma, a major outcome of the present study, was included in the multiple regression analysis and kept in the final model independently of the significance level and adjusted r^2 change with its inclusion.

All analyses were performed using SPSS 20.0 (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp) and considering a $p < 0.05$ for statistical significance.

Results

EBT significantly increased after training ($\Delta\text{EBT} = 0.32 \pm 0.57$; $p = 0.016$) (Fig 2a) without differences between asthmatic and healthy swimmers (0.15 ± 0.39 vs. 0.45 ± 0.68 ; $p = 0.254$) (Table 2, Fig 2b). Baseline and post-exercise EBT were similar between both groups (Table 2). Also, no differences were observed between those with ($n = 6$) or without inhaled corticosteroids ($p = 0.853$).

A significant correlation was observed between baseline and post-exercise EBTs ($r = 0.827$, $p < 0.001$). In multiple linear regression analysis, after controlling for baseline EBT and axillary temperature, asthma was not a significant predictor of ΔEBT (Table 3). In univariate regression analysis, baseline EBT was the variable most strongly associated with ΔEBT ($r^2 = 0.464$).

Correlations between BHR (LnPD_{20}) and baseline EBT ($r = 0.224$, $p = 0.533$) and ΔEBT ($r = 0.044$, $p = 0.845$) for asthmatic swimmers were not significant.

Both groups presented median values of eosinophils and epithelial cells higher and neutrophils lower than normal, comparatively to reference values for healthy populations (17). When compared to healthy swimmers, asthmatics presented

higher eosinophil and epithelial cell counts and lower neutrophils, but with no significant differences (Table 1). Correlations between sputum eosinophils with baseline EBT ($r = 0.064$, $p = 0.800$) and ΔEBT ($r = -0.210$, $p = 0.404$) were not significant, even if considering only asthmatics ($r = -0.286$, $p = 0.493$ and $r = 0.048$, $p = 0.911$, for baseline EBT and ΔEBT , respectively). Regarding neutrophils, correlations were also non-significant for the global sample for baseline EBT ($r = 0.194$, $p = 0.441$) and ΔEBT ($r = 0.101$, $p = 0.689$) and also for the asthmatic group ($r = 0.500$, $p = 0.207$ and $r = -0.048$, $p = 0.911$, for baseline and ΔEBT , respectively).

Discussion

An increase in EBT after a training session was observed, supporting the hypothesis of heat loss during exercise. Interestingly, asthmatics did not experience a higher increase when compared to healthy swimmers after controlling for baseline EBT and body temperature. These results support the previous findings suggesting heat loss occurs as a physiologic rather than pathological response to exercise (10, 11). Furthermore, no relation was found between EBT and sputum inflammatory cells, neither with the degree of BHR. It is therefore tempting to speculate that an inflammatory response to the heat loss of exercise might not be a key ethiopathogenic mechanism of swimmers asthma.

Our results are in accordance with previous studies exploring the relationship of EBT to EIA (10, 11). Although EBT has been widely investigated based on the assumption that airways inflammation would influence the temperature of the air coming from the alveoli (19), and has been shown to correlate

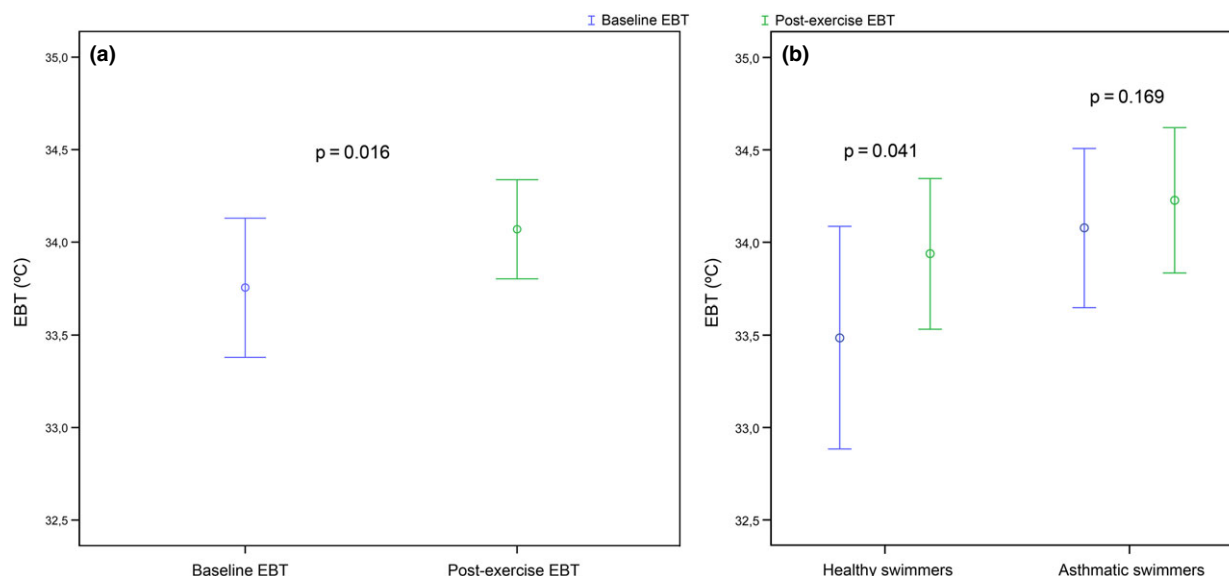


Figure 2 Exhaled breath temperature (EBT) before and after a swimming training session considering all participants (a) and asthmatics vs. others (b).

Table 2 Results of measurements performed in the present study, and training characteristics of asthmatic vs healthy swimmers

	Asthmatic swimmers (n = 10)	Healthy swimmers (n = 12)	p*
Baseline EBT, °C	34.08 ± 0.60	33.49 ± 0.95	0.180
Post-exercise EBT, °C	34.23 ± 0.55	33.94 ± 0.64	0.283
Body temperature†, °C	35.97 ± 0.42	36.02 ± 0.31	0.771
Hours of training in previous week	9.4 ± 2.6	9.3 ± 3.4	0.974
Intensity of the session, n			
Aerobic	3	9	0.063
Mild anaerobic	5	1	
Moderate/severe anaerobic	2	2	

Data presented as mean ± SD, except otherwise stated.

*Mann–Whitney *U*-test was used for comparisons or chi-square test in case of categorical variables.

†Axillary temperature was used as a measure for body temperature.

with bronchial blood flow, exhaled NO, and sputum eosinophils in asthmatics (3, 4, 20, 21), only two studies explored the effect of exercise (10, 11). Both studies showed an EBT increase after a laboratory standardized exercise challenge, but no differences were observed between asthmatics and controls (10, 11), in agreement with our results obtained with the regular exercise training of competitive swimmers.

We have introduced several novelties. This is the first time, to our knowledge, that EBT has been evaluated in elite athletes, and also before and after a real-life training session, in order to assess whether EBT changes could support the hypothesis of heat loss due to physical exercise and inflammation occurring in the airways as mechanisms of EIA. Our study has other strengths: we have adjusted our analysis for baseline EBT, which is of particular relevance given the high dependence to Δ EBT. Also, recent studies have highlighted several factors influence EBT (22) and we have addressed that question also. We have shown that central temperature significantly influences Δ EBT in elite swimmers, and we have analyzed the possible confounding effect of several demographic variables and training characteristics. Furthermore, sputum cell counts were used to evaluate the presence, type, and degree of inflammation in the airways.

During exercise, unperfused alveoli become perfused and underperfused units receive an increased blood supply. It is therefore conceivable to expect a physiologic increase in airways temperature related to capillary recruitment rather than a pathological mechanism of vasodilation resulting from inflammation, that is, consistent with the lack of correlation previously found between NO levels and EBT after exercise (11), as well as the lack of correlation with sputum inflammatory cells observed in our study. In fact, it has been proposed that airway inflammation in athletes may represent a training adaptation to injury secondary to rigorous hyperpnoea, and not necessarily related to EIA or implying detrimental effects (5, 23–25). The restorative process after injury involves plasma exudation and movement of cells into the airways, a process repeated many times during a season of training (26). This process has the potential to expose smooth muscle to a wide variety of plasma- and cell-derived substances. The exposure to these substances over time can lead

Table 3 Multiple linear regression model for predicting Δ EBT

Variable	Standardized Coefficient B	p-value	95% CI
Baseline EBT	−0.675	0.001	[−0.685; −0.229]
Asthma	−0.008	0.961	[−0.388; 0.370]
Axillary temperature	0.327	0.043	[0.018; 1.027]

$R^2 = 0.596$, adj $R^2 = 0.529$.

p-values of the univariate analysis (including all the tested variables): sex—0.547, age—0.336, height—0.025, weight—0.196, baseline EBT—<0.001, axillary temperature—0.089, training intensity—0.415, asthma—0.222, PD20—0.600, number of training hours in the previous week—0.583. The following explanatory variables were initially entered into the multiple regression model, but were excluded in the final model as they did not reach significance at the 0.05 level: height, weight. Variable asthma, being a major outcome of this study, was kept in the final model independently of the significance level and adjusted R^2 change with its inclusion; it is coded as '0' = without asthma and '1' = with asthma.

to an alteration in the contractile properties of the smooth muscle, turning it more prone to bronchoconstriction. Accordingly, we have shown that although airway inflammation decreases after finishing competitive swimming, the prevalence of asthma and use of asthma medication increases significantly in both active and past adolescent swimmers, suggesting a relative independence of the two conditions (25). In the meanwhile, the IOC criteria for asthma diagnosis in athletes mostly reflect the occurrence of bronchoconstriction, while asthma in common patients is defined by a chronic course and inflammatory changes (27).

Vigorous exercise causes epithelial damage, which has likewise been linked to EIA in elite athletes (28). Mechanical stress of sustained extreme breathing is believed to result in epithelial injury and turn it more prone to asthma. Clara cell secretory protein (CC16), a peripheral marker of lower airways epithelial barrier disruption, is increased after exercise in urine (29, 30) and in serum (31–33), as well as after eucapnic voluntary hyperventilation in athletes (34). It was recently shown plasmatic CC16 correlates to EBT after exercise,

reflecting an overall epithelial involvement (11), but no differences were observed between asthmatic and healthy subjects (11), which again leads to the concept of a physiologic rather than a pathological response to exercise. And in fact, it seems to be only related to exercise and hyperpnea, as urinary levels of CC16 are increased after an exercise but not a mannitol challenge (30).

Also the environmental conditions may increase airway epithelial damage. Bronchial epithelial cells are released in higher amounts into sputum (epithelial shedding) of cold air athletes and swimmers (28), a result also found in our study. We observed no significant differences between asthmatic and healthy swimmers, which support that exercise and irritant exposures contribute to a detrimental effect on the athletes' airways but not as ethiopathogenic mechanisms of EIA. Age is a crucial point to bear in mind in our study. Sports asthma has been claimed to result from cumulative effects of years of training. Subjects in our sample are adolescents, so it could be argued that lack of differences could be due to their relatively young age. However, in the specific case of competitive swimmers, the environmental exposure is considered to be so strong that very frequently they have already developed BHR during their teens (7). We found a 45% prevalence of asthma diagnosis, and they are all elite swimmers with mean of 9 years of competition.

There are some limitations in our study. First, the small sample size limits solid conclusions. Due to a strong effort to homogenize training characteristics and environment, only elite swimmers from the same team were included. We cannot assure that lack of differences is not related to low power; nevertheless, some results are statistically significant. Second, some swimmers were under inhaled steroids at the time of EBT collection. However, no differences were observed between those with and without therapy, a result that has also been previously reported (9). To finalize, external validation is compromised; our results are not possible to extrapolate for other sports as swimmers are a special population among elite

athletes due to a potential detrimental effect of environmental exposure.

So, in light of these results, along with previous published findings, the authors remark 3 points: 1) exercise increases EBT, probably due to a physiologic increase in lungs blood flow, rather than pathological mechanism, as no differences are observed between asthmatic and healthy subjects; 2) mechanic noxious stimulus of high ventilation may lead to airways epithelial damage and susceptibility to irritative stimuli, supported by increased numbers of bronchial epithelial cells in swimmers and high levels of CC16, regardless of their asthmatic status; and 3) due to daily recurrence of training, repair is delayed and a 'frustrated' inflammatory response occurs to heal the damage of physical injury in both asthmatic and healthy swimmers. How this relates to ethiopathogenic mechanisms of EIA is not clear and thus calls for further studies. The explanatory model of EIA in athletes will probably include the interplay between environmental training factors and athlete's personal and genetic risk factors. Our study supports the previous hypothesis of a physiologic rather than a pathological significance of airways heat loss and inflammation in elite swimmers, but further studies are needed.

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Study VI

Effect of competitive
swimming on airway
inflammation: a 3-yr
longitudinal study

Pediatr Allergy Immunol. 2014; 25:193-5

CORRESPONDENCE

Effect of competitive swimming on airway inflammation: A 3-yr longitudinal study

DOI:10.1111/pai.12172

To the Editor,

In recent years, the observation that regular pool attendance, especially by young children, was associated with lung hyper-permeability and increased risk of developing asthma led to the 'pool chlorine hypothesis' (1). Accordingly, the increasing and largely uncontrolled exposure of young children to chlorination byproducts contaminating the air of indoor swimming pools could contribute to the childhood asthma rise in industrialized countries (1). Moreover, an increasing body of literature suggests an association between competitive swimming and asthma (2).

A higher prevalence of asthma and asthma-like symptoms has been identified in elite swimmers. Environmental exposures, mechanical stress to the airways, increased prevalence of respiratory infections and dysautonomia, have been recognized as asthma contributory factors (2). Increased numbers of both eosinophils and mast cells were observed in bronchial biopsies of competitive adult swimmers, leading to believe that airway inflammation and hyper-responsiveness develop during the training career (3). While the latter seems to be a transient phenomenon (4), whether airway inflammation persists is still debated (5–7). Therefore, we aimed to assess changes in airway inflammation of swimmers during a 3-yr follow-up.

Competitive non-elite young swimmers from the two main Portuguese swimming teams were invited ($n = 120$) to participate in this cohort prospective study. Informed consent was obtained from 105, which were assessed at the baseline visit. From these, 86 attended the 3-yr follow-up visit and were included in the final analysis. No significant differences were observed between the 19 lost to follow-up subjects and the remaining (Table 1). 'Active swimmers' were defined as those remaining at high level of competitive swimming; those who quit at least 6 months before the follow-up visit were considered 'past swimmers'. None of the subjects smoked. The local hospital ethical committee approved the study.

Subjects completed a self-administered questionnaire, including questions from the ISAAC questionnaire, reporting physician diagnosis of asthma and allergic rhinitis, and use of asthma medication. Physical activity (PA) was measured using the short 7 days International Physical Activity Questionnaire (IPAQ) (8). A combined total physical activity was computed as the sum of the activity domains scores (total PA = walking + moderate-intensity PA + vigorous-intensity PA) and reported as a continuous measure (total PA score = total MET-min/wk). Eosinophilic airway inflammation was assessed measuring lower airway's exhaled nitric oxide (NO) levels according to guidelines, before a training session, using NIOX MINO (Aerocrine AB, Solna, Sweden), and expressed in parts per billion (p.p.b.). Atopy was defined by positive skin prick testing to common aeroallergens (Laboratorios LETI S.L.,

Spain). The study was performed at the swimming pool in which swimmers trained, except for the follow-up visit of past swimmers which occurred at the laboratory. Results were expressed as mean (SD) or, if not normally distributed, as median (interquartile range). Levels of exhaled NO and of physical activity were log-transformed because of skewed distribution. Differences between groups were assessed with one-way ANOVA or Kruskal–Wallis for normally or non-normally distributed data or chi-Square for categorical variables. Differences in changes in exhaled NO after the 3-yr follow-up were assessed by general linear model adjusting on confounding factors: gender, age, atopy, physician-diagnosed asthma, and use of asthma medication.

We observed a significant difference in changes in exhaled NO; those who remained active significantly increased their levels of eosinophilic airway inflammation independently of their gender, age, atopy, or asthma status (Table 2, Fig. 1). After the 3-yr follow-up, the prevalence of asthma, allergic rhinitis, and use of asthma medication increased significantly in both groups. All subjects increased their overall physical activity levels; however, significant increases in moderate and vigorous physical activity level were only observed in active swimmers.

Table 1 Baseline characteristics of participants according with their swimming status at the three-year follow-up

	Past swimmers ($n = 39$)	Active swimmers ($n = 47$)	Lost to follow-up ($n = 19$)	p
Demographics				
Age in yr, median $\pm$ IQR	14 $\pm$ 6.3	13 $\pm$ 3.2	15 $\pm$ 2.0	0.080*
Males	23 (59)	27 (57)	11 (42)	0.990
Swimming h/wk, median (IQR)	12 (9)	12 (7)	14 (4)	0.113*
Years of competition, median (IQR)	5 (5)	4 (4)	6 (3)	0.109*
Clinical features				
Atopy	21 (54)	15 (32)	11 (58)	0.056
Allergic rhinitis	13 (33)	11 (23)	3 (16)	0.317
Asthma	6 (15)	6 (13)	0 (0)	0.208
Asthma drugs	5 (13)	9 (19)	0 (0)	0.116

Data presented as n (%) unless otherwise stated. The p value relates to chi-square tests to assess differences between past swimmers, active swimmers, and 'lost to follow-up', except otherwise marked. *Kruskal–Wallis test results to assess the differences between past swimmers, active swimmers, and 'lost to follow-up'.

Table 2 Changes in airway inflammation, prevalence of asthma and rhinitis, and physical activity levels in athletes according with their swimming status after the 3-yr follow-up

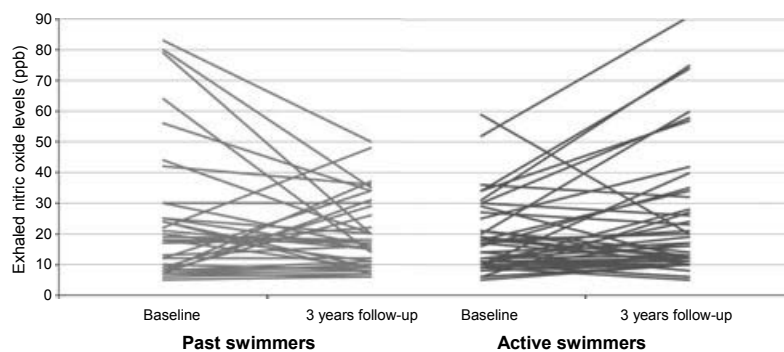
	Past swimmers, n = 39		Active swimmers, n = 47		Past vs. active swimmers†
	Baseline	Follow-up	Baseline	Follow-up	
Exhaled NO, p.p.b.‡	18 (19.5)	16 (20)	14 (11)	14 (18)	0.008
Asthma, n (%)	6 (15)	9 (23)*	6 (13)	9 (19)*	0.656
Allergic rhinitis, n (%)	13 (33)	16 (41)*	11 (23)	13 (28)*	0.305
Asthma drugs, n (%)	5 (13)	12 (31)*	9 (19)	12 (26)*	0.382
Physical activity‡					
Walking	462 (685)	693 (2402)	396 (1188)	739 (1148)	0.016
Moderate	720 (2010)	1320 (1440)	120 (960)	960 (3120) **	0.065
Vigorous	5760 (1920)	3840 (6120)	5760 (7040)	13440 (11520)*	<0.001
Total	7173 (3864)	7242 (5718)*	6222 (9159)	17196 (8544)*	0.001

Data presented as median (IQR) unless otherwise stated; Physical activity expressed as MET-min/wk; bold figures represent statistically significant differences between comparing groups; Paired sample *t*-tests or chi-square tests were used as appropriate, and differences in changes between groups after the follow-up were assessed by general linear model adjusting on the following confounders: gender, age, atopy, physician-diagnosed asthma, use of asthma medication for exhaled NO and on gender and age for physical activity levels, with baseline values as covariable.

p* < 0.001; *p* = 0.002; *p* = 0.005.

†Comparison of exhaled NO variation (Δ) from baseline to 3-yr follow-up among active and past swimmers.

‡Data presented on exhaled NO and physical activity are absolute values, although they were log-transformed for comparison analysis.

**Figure 1** Variation of exhaled nitric oxide at baseline and at 3 yr follow-up among past and active swimmers.

Our study has some limitations. First, we had an 18% of losses to follow-up; however, these subjects were similar to others at baseline evaluation. Second, asthma was defined as a positive response to the question, ‘Has a doctor ever said that you have asthma?’ Therefore, misclassification of disease status is possible, although would unlikely differently affect any of the groups and being furthermore independent of airway inflammation assessment. Our study has also important strengths: For the first time, it is reported a prospectively assessment of eosinophilic airway inflammation in non-elite swimmers; it was extensively adjusted for confounders and known risk factors that affect exhaled NO levels; and finally, we used a validated tool to monitor physical activity levels.

Only one study prospectively assessed the effect on asthma in those who kept vs. those who retired from swimming (7). In 42 Finnish elite swimmers, after a 5-yr follow-up, airway responsiveness and asthma attenuated or even disappeared in swimmers who stopped high-level training, while mild eosinophilic airway inflammation was aggravated among those who

remained active. A detailed knowledge of inflammation remission as assessed by objective testing and natural course of the disease in former elite athletes is also missing and should be investigated (9).

Although several studies report an association, the relationship between asthma and swimming remains controversial. Previous data have shown that elite training in chlorinated pools affects airway structure; also, asthma is more commonly found in swimmers than among other high-level athletes (10). Adolescent competitive swimmers with allergic asthma show a mixed type of airway inflammation (11), and the development of airway hyper-responsiveness generally occurs in young adults rather than in adolescent competitive swimmers (3), which supports that it may be the result of the cumulative effects of years of exposure as they develop respiratory disorders during their athletic career. However, a causal relationship between swimming and asthma could not be established as most studies are cross-sectional (3) and the association has never been confirmed among non-competitive swimmers (10).

In conclusion, our prospective study of competitive swimmers shows that those who remained active at a 3-yr follow-up significantly increased their levels of airway inflammation measured by exhaled NO independently of their gender, age, atopy, or asthma status. Nevertheless, asthma incidence did not increase in active swimmers. In fact, it has been previously shown that intense swimming activity causes a lung growth greater than normal in children and adolescents (12), and that physical training does not increase allergic inflammation in children with asthma (13). Our study supports the recommendation to engage in physical activity to all asthmatics as long as the disease is controlled.

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