Stimulus and mechanisms of exercise-induced bronchoconstriction

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Competing interest
S.D. Anderson is a part-time employee of the Sydney South West Area Health Service that owns the patent relating to the use of mannitol (Aridol™ and Osmohale™) for diagnosing bronchial hyperresponsiveness. S.D. Anderson owns self-funded shares and acts as a consultant to Pharmaxis Ltd and receives a 10% share of the royalties paid to SSWAHS.

Educational aims

- To describe the stimulus for exercise-induced bronchoconstriction.
- To describe the different mechanisms whereby this stimulus may act to cause the airways to narrow in asthmatics and athletes.
- To describe the relevance of the stimulus and mechanism to protocols to diagnose exercise-induced bronchoconstriction.
- To describe the inflammatory mediators involved in exercise-induced bronchoconstriction.
- To describe how and why airway injury could contribute to the pathogenesis of airway hyperresponsiveness and exercise-induced bronchoconstriction in athletes.

Summary

The stimulus for exercise-induced bronchoconstriction (EIB) is the loss of water by humidifying large volumes of air during exercise. The mechanism for EIB relates to the thermal and osmotic effects of water loss. The thermal theory proposes that EIB is a vascular event comprising vasoconstriction during exercise followed by rapid rewarming and a reactive hyperaemia at the end of exercise. The osmotic theory proposes that water loss induces an increase in osmolarity in the airways, which causes the release of mediators that cause bronchial smooth muscle to contract. Increased vascular permeability and leakage are common to both theories.

Exercise-induced bronchoconstriction (EIB) is the term used to describe the transient increase in airway resistance that follows vigorous exercise [1] that is measured as a reduction in lung function after an exercise test or natural exercise [2]. EIB is diagnosed on the basis of demonstrating a reduction in forced expiratory volume in 1 s (FEV_1) of 10–15% from the pre-exercise value within 20–30 min of exercise [3, 4]. EIB is an early manifestation of airway hyperresponsiveness (AHR), usually occurring in childhood, but it may occur for the first time in elite athletes in adulthood [5]. Exercise itself is not necessary to identify EIB and surrogates such as eucapnic voluntary hyperpnoea with dry air are now used to identify potential for EIB [6].

The stimulus for EIB

Loss of water from the airways as a result of humidifying large volumes of unconditioned air over a short period of time is believed to be the stimulus for EIB. The severity of the airway response is related to the amount of water lost from the airways [7].

Under most climatic conditions, the air needs to be heated and humidified to body conditions before it enters the alveoli. At resting ventilation, most air conditioning is provided by the nasal mucosa. During vigorous exercise, as ventilation increases above ~30 L per min, there is a switch from nose to mouth breathing and the airways below the pharynx

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are required to complete the conditioning process. Measurements of airway temperature demonstrated that during moderate hyperpnoea, air is still incompletely conditioned at the fifth generation of airways [8].

The immediate source of the water to humidify the air in the lower airways is the sol layer of airway surface liquid (ASL) lining the epithelium, also known as the periciliary fluid layer. Under resting conditions, the epithelium is an absorptive surface but during exercise, it needs to become a secretory surface to replenish the ASL [9]. The first 10 generations of airways have a cumulative surface area of ~742 cm^2. Assuming a depth of 5–10 µm (similar to the length of the cilia) for the ASL layer, the volume in the first 10 generations is 0.37–0.74 mL [9]. By generation 12, the cumulative surface area and volume doubles. The ASL contains sodium, chloride, potassium and calcium ions and has an osmolarity of ~290–320 mOsM. When ventilation exceeds 40 L per min (as occurs in an adult during exercise) water needs to be rapidly replaced on to the airway surface in order to prevent dehydration and an increase in osmolarity. Rapid replacement is achieved by condensation of water back on to the cooled airway surface during expiration. In addition, water moves by osmotic drag from the bronchial circulation through the submucosa and epithelium to the airway surface to restore normal osmolarity. However, mathematical models have estimated that ~40% of the total water loss per min comes from the lower airways, when ventilating at 60 L per min under laboratory conditions of 22–26°C and 40% humidity [10, 11].

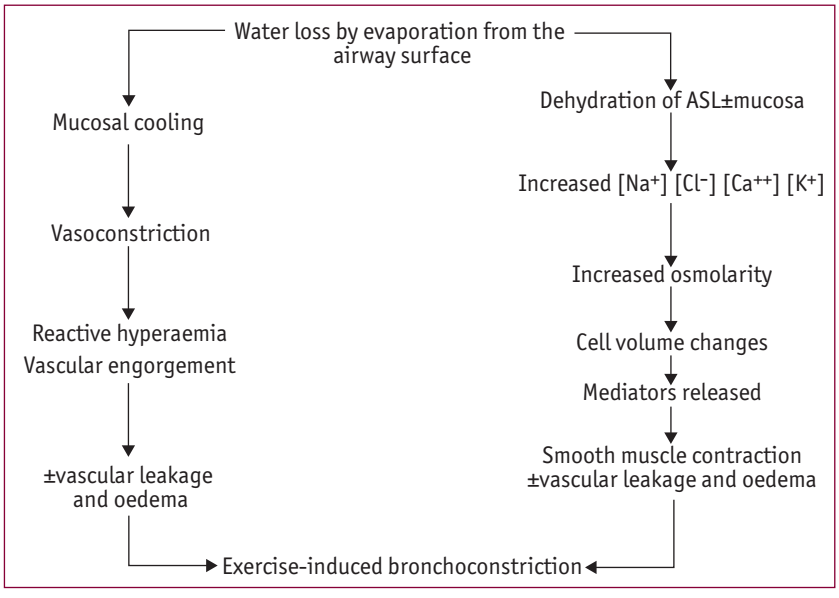

Figure 1
Diagram illustrating the thermal (left) and osmotic theory (right) of exercise-induced bronchoconstriction. ASL: airway surface fluid. Modified from [20], with permission from the publisher.

The proposed mechanisms of EIB

The thermal theory of EIB

Evaporative water loss is accompanied by heat loss and cooling of the airway surface of the lower airways. When air of subfreezing temperatures is inspired during exercise, the airway cooling is thought to be sufficient to cause a vasoconstriction of the vessels of the bronchial circulation [12]. It was this cooling of the lower airways that was first proposed as the mechanism for EIB in the 1970s.

In 1986, the airway cooling hypothesis of EIB was modified to encompass rewarming and it was proposed that EIB was a mechanical event due to a vascular engorgement and oedema of the airway wall [13]. In support of this theory, it was shown that to induce obstruction "a thermal gradient seems necessary at the end of challenge" [14]. A more rapid increase in airway temperature was shown to occur at the end of exercise in asthmatics compared with normal subjects. This finding was thought to reflect a hyperplastic bronchial vasculature in asthmatic subjects [14] and a formal hypothesis was proposed [15]. The authors considered that the development of the obstruction "appears to consist of vasoconstriction and airway cooling during exercise followed by a rapid resupply of heat when exercise ceases." Other evidence given in support of the rapid rewarming theory of EIB comes from the relationship between changes in intra-airway temperature to changes in FEV_1, measured after repetitive exercise [16], and to increases in vascular volume produced by anti-shock trousers [17] and by rapid infusion of saline [18].

While there is little argument that airway cooling is associated with vasoconstriction and a reactive hyperaemia, there is an argument as to whether these events are prerequisites for EIB to occur [19]. However, these vascular events are likely to be relevant to winter athletes exercising at high levels of ventilation under cold conditions, and they may contribute to airway narrowing and symptoms in this group. Furthermore, if the reactive hyperaemia is associated with oedema of the airway wall, this would serve to amplify the normal reduction of 4–5% in FEV_1 following exercise, causing it to appear as EIB [20]. The thermal vascular theory [14, 15] does not implicate bronchial smooth muscle (BSM) contraction and release of inflammatory mediators in the narrowing of the airways, events that are considered hallmarks of classical asthma (fig. 1) [21].

The osmotic theory of EIB

The thermal theory of EIB does not account for all observations on EIB, particularly under different climatic conditions and for the action of some drugs [19]. For example, severe EIB has been documented in asthmatics breathing hot, dry air (>36°C) during exercise [22–24]. In contrast, EIB is either markedly inhibited or completely prevented when the inspired air is at body temperature and fully saturated with water (37°C, 100% relative humidity or 44 mg H_2O per L) during exercise (fig. 2) [7, 25–27]. These findings demonstrate that abnormal airway cooling and rewarming are not prerequisites for EIB in asthmatic subjects [22–24, 28]. These and other observations led to the osmotic theory of EIB that proposed that water loss by evaporation leads to a transient increase in the concentration of ions in the ASL with a consequent increase in osmolarity [7, 29]. The osmotic theory of EIB proposed that the increase in osmolarity, though transient, stimulates the release of mast cell mediators (fig. 1). This was based on observations of histamine release *in vitro* by human mast cells following a very short (<1 min) exposure to hyperosmolar solutions [30]. Secondly, dehydration of the ASL has been demonstrated by the finding of a reduction in mucociliary clearance during dry air hyperpnoea [31]. Mathematical models of respiratory water loss based on experimental data [11, 32] would suggest that the transient dehydration during exercise also extends to the airway epithelium [33] and the submucosa [19]. Thirdly, experiments using gases of different heat capacities during exercise have confirmed that water loss is more important than heat loss in EIB [34]. Fourthly, a reduction in humidity of the expired air is reported during exercise in warm dry conditions, which is consistent with an increase in osmolarity of the ASL [35]. An increase in ion concentration and osmolarity has been demonstrated in the upper airways following dehydration in humans [36] and the lower airways in animals [37]. Unfortunately, it is technically impossible to document rapid osmotic changes in the ASL because the small volume of fluid is spread over a very large surface area. Furthermore, any measuring device inserted into the human airway would simply increase the water flux to the airway surface.

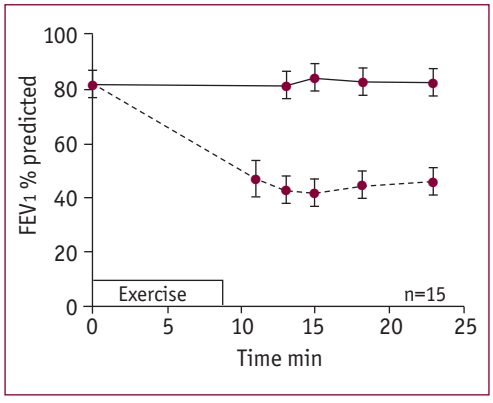

Figure 2

The forced expiratory volume in 1 s (FEV1) expressed as percentage of predicted in 15 clinically recognised asthmatic subjects (9–42 yrs of age) with exercise-induced bronchoconstriction who exercised for 8 min by running on a treadmill while breathing fully conditioned air (36.5°C, 100% relative humidity (RH); —). A second identical exercise test followed 17–20 mins later, during which the subjects inspired air of temperate conditions (20°C, 40% RH; ----). The % decrease in FEV1±SD after the first test was 6.5±5.2% and after the second, 53.9±11.5%. Data are taken from [27].

Relevance of the stimulus and mechanism to protocols to diagnose EIB

In order to bring about dehydration of the ASL and a transient increase in its osmolarity, the rate of water loss needs to exceed the rate of return in the first 10 generations of airways. This concept is important in considering the appropriateness of protocols used in the laboratory to diagnose EIB. It is generally well recognised that the drier the air inspired and the higher the ventilation sustained during exercise, the less likely a false negative test result will be achieved. However, it is less frequently appreciated that the intensity of the exercise in the first 2 min needs to be sufficient to raise ventilation to >17.5 * FEV_1 (50% of maximum voluntary ventilation) and the heart rate above 80% of predicted maximum. This intensity, and preferably a higher one, needs to be sustained for 6 min in young children, and 8 min in adolescents and adults in order to optimise the chance of a positive test [38, 39]. The target ventilation is easier to achieve during running exercise than cycling, but treadmill running at high speed can present issues of safety.

When the intensity of exercise is rapidly increased, the accompanying ventilation increases and, over a few minutes, more generations of the lower airways are recruited into the conditioning process. The lower the temperature and the drier the inspired air, the more rapidly

Educational questions

1. Which of the following are true?
a) Airway cooling is the stimulus to EIB
b) Water loss is the stimulus to EIB
c) Airway rewarming is the stimulus to EIB
d) Respiratory heat loss is the stimulus to EIB
2. Which of the following is involved in EIB?
a) The bronchial smooth muscle only
b) The vascular smooth muscle only
c) Both the bronchial and vascular smooth muscle
d) The mucous glands
3. Why are dry air and high ventilation important to provoke EIB?
a) To cause adrenalin release
b) To recruit the greatest surface area into the conditioning process
c) To increase bronchial blood flow
d) To condense more water on the airway surface
4. Which cell and mediator plays an important role in EIB?
a) The neutrophil and adenosine
b) The epithelial cell and histamine
c) The mast cell and prostaglandin D_2
d) The basophil and leukotrienes

lower generations will be recruited to condition the air. Decreasing the inspired temperature acts to increase the surface area that is dehydrated. However, increasing the duration of exercise by a few minutes appears to compensate for the effects of lower temperature. For example, the response to dry air hyperpnoea of warm air for 8 min would be expected to be similar to 4 min of cold air, provided that the ventilation exceeds 17.5xFEV1 for the warm air [40]. However, any intervention that increases water content of the air inspired, such as nasal breathing [41], use of a mask [42] or an unusually large dead space, will decrease the maximum surface area that can be dehydrated.

If the ventilation increases too slowly, refractoriness to the stimulus at higher ventilations is observed [43]. This is thought to be due to the release of 'protective' mediators that cause relaxation of the bronchial smooth muscle. For example, 20 min warm-up at submaximal intensity or 30 s repeated sprints cause refractoriness to any vigorous exercise that follows [44, 45]. There is cross-refractoriness between exercise and hyperosmolar aerosols, implicating a similar mechanism is acting for both stimuli [46].

To overcome many of the technical problems related to optimising the exercise intensity, many laboratories now use the eucapnic voluntary hyperpnoea challenge as a surrogate for exercise-induced hyperpnoea [47]. This challenge permits target ventilation levels up to 30xFEV1 to be achieved in the first 30 s and easily sustained for 6–8 min [48].

Aerosols of hyperosmolar agents, such as 4.5% saline and mannitol dry powder, have also been used to mimic the dehydrating effects of respiratory water loss on the airway surface osmolarity. In elite summer sport athletes, mannitol has been shown to be a good alternative to eucapnic voluntary hyperpnoea for identifying EIB [49]. In clinical practice, due to the dose response nature of the test and its generally wide availability, mannitol is often preferred over exercise or eucapnic hyperpnoea challenges in those summer athletes with a history of classical asthma and allergies. However, in winter sport athletes, a low prevalence of hyperresponsiveness to mannitol has recently been reported [50].

The reason that recruiting 10 generations of airways into the conditioning process is important in optimising the likelihood of identifying EIB is because that is the largest surface area of the airways that can reasonably become dehydrated and have a transient increase in osmolarity. Beyond this generation, there is an adequate amount of water, so that loss is unlikely to make a significant change in osmolarity. Also, the density of the mast cells increases as the airways get smaller [51]; thus, they are more likely to be close to the airway surface and in a position to be affected by changes in osmolarity [51]. Mast cells are also present on BSM [52] as well as in the submucosa [51].

Role of mediators in EIB

Mast cells, eosinophils, sensory nerves and epithelial cells are sources of mediators for EIB. The mast cell is considered to be the major source of mediators of bronchoconstriction, such as prostaglandin (PG) D_2, leukotriene (LT) C_4 and histamine. Eosinophils are another source of cysteinyl-LTs [53] and sputum eosinophilia is common in EIB [54]. These cell types release mediators in response to hyperosmolarity [53, 55]. The precise mechanism for this release is unclear, but the rise in intracellular calcium concentration associated with cell volume shrinkage and regulatory volume increase is likely to be important. In addition, neuropeptides are likely to be important in the cough provoked by dry air hyperpnoea in both healthy subjects and those with asthma. This type of cough can be prevented by breathing warm humid air [56].

Another potential mediator in EIB is adenosine arising from adenosine triphosphate (ATP) in the epithelium [57]. Adenosine and ATP are important regulators of the depth of ASL [58] and their concentration is likely to be increased in response to the sheer stress of hyperpnoea of dry air or changes in depth and osmolarity of the ASL [58]. Adenosine has been measured in exhaled breath condensate after exercise and the levels related to the severity of the EIB [59]. It acts on mast cells *via* A_{2b} receptors to cause release of mediators. Furthermore, many of the same mediators associated with EIB could act on the vascular smooth muscle to cause leakage and amplify the effects of BSM contraction [20].

For many years, the evidence for the importance of mast cell mediators in EIB was indirect and based on the inhibitory effects of particular drugs on EIB. Any drug that inhibits release of mast cell mediators or prevents the bronchoconstrictor effects, or drugs that reduce the production of mediators or mast cell number, have been shown to have beneficial effects in EIB. Initially, it was the efficacy of sodium cromoglycate (SCG) on EIB that led to the suggestion that mast cells were involved in EIB [60]. SCG inhibits mast cell

degranulation *in vitro* [61] and, unlike β_2-agonists, has no relaxing effect on BSM to explain its inhibitory effect in EIB. Some studies have reported that histamine receptor antagonists provide an inhibitory effect on EIB, but the findings are not universal [62, 63]. The results suggest that histamine is likely to be important in contributing to the severity of the fall in FEV1 immediately after exercise, particularly when the exercise is intense and the smaller airways are involved in the air conditioning process [64]. LT receptor antagonists and 5-lipoxygenase inhibitors reduce the severity of the fall in FEV1 and enhance recovery of FEV1 to baseline. This suggests that LTs play an important role in sustaining bronchoconstriction following exercise [65]. The failure of any one antagonist to completely inhibit EIB is a reflection of the many mediators involved.

Due to the development of sensitive assays, it is now possible to measure changes in urinary excretion of mediators in response to exercise and other provocative stimuli. The findings from studies using this technique suggest that PGD_2 is likely to be the most important mediator of EIB. An increase in the urinary excretion of 9α,11β-PGF_2, a metabolite of PGD_2, occurs for ≤90 min after exercise and eucapnic voluntary hyperpnoea in asthmatics and athletes with EIB (fig. 3) [66]. This same metabolite has been found in sputum of asthmatics after exercise [67]. There is a significant association between the change in levels of 9α,11β-PGF_2 from baseline and the percentage decrease in FEV1 after eucapnic hyperpnoea (fig. 4). There is also a sustained increase in urinary excretion of LTE_4 following eucapnic voluntary hyperpnoea (fig. 3) but the association with the decrease in FEV1 is weaker [66]. A similar time course of increase in urinary excretion of the metabolites of PGD_2 and LTE_4 has been measured following a hyperosmolar challenge with mannitol dry powder in humans [68].

Linking the mediator with the airway response

The increase in urinary excretion of 9α,11β-PGF_2 and LTE_4 is inhibited by inhalation of 40 mg SCG given 15 min before a 6-min eucapnic voluntary hyperpnoea test of dry air in athletes with EIB [69]. A similar finding for inhibition of 9α,11β-PGF_2 was made in asthmatics following inhalation of dry powder mannitol after 40 mg SCG or 12 µg of the β_2-agonist eformoterol [70]. As these drugs were delivered by inhalation shortly before the challenge, their protective effect is likely to be close to the airway surface. Furthermore, the increase in urinary excretion of 9α,11β-PGF_2 could be inhibited by a single 1,500-µg dose of the corticosteroid beclomethasone inhaled 4 h before challenge with eucapnic hyperpnoea [66]. Importantly, the inhibition of the mediators was associated with a reduction in the airway response to these provoking stimuli, supporting a role for them in the mechanism of EIB (fig. 5). When a combination of the histamine antagonist loratadine and the LT receptor antagonist montelukast was given 36 and 12 h before exercise, it reduced the concentration in induced sputum of both histamine and cysteinyl-LTs in association with a marked inhibition of the decrease in FEV1 [71]. The beneficial effects of so many drugs in EIB emphasises the importance of withholding times for medication before performing an exercise test or its surrogates.

While it can be argued that SCG and eformoterol prevented release of mast cell mediators by a direct action on mast cells, this is less likely to be the mode of action for beclomethasone. However, there are other modes of action that may explain how beclomethasone and other drugs given acutely can prevent EIB. For example, β_2-agonists may enhance delivery of water to the airway surface and retard dehydration of the ASL by stimulating epithelial chloride ion secretion. By contrast, inhaled beclomethasone may have caused fluid retention in the airway wall by stimulating ion transport to move water in the opposite

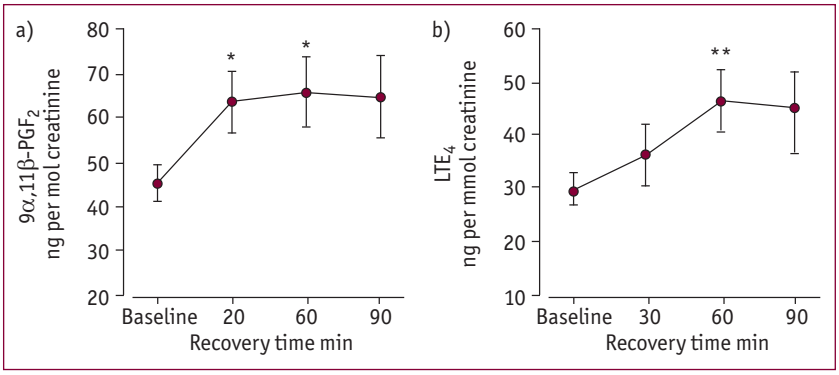

Figure 3
*The change in urinary excretion of levels of a) the PGD_2 metabolite 9α,11β-PGF_2 and b) LTE_4 over 90 min following 6 min eucapnic voluntary hyperpnoea in eight untrained asthmatics. *: p<0.05* versus *baseline; **: p<0.01* versus *baseline. Modified from [66] with the permission of the publisher.*

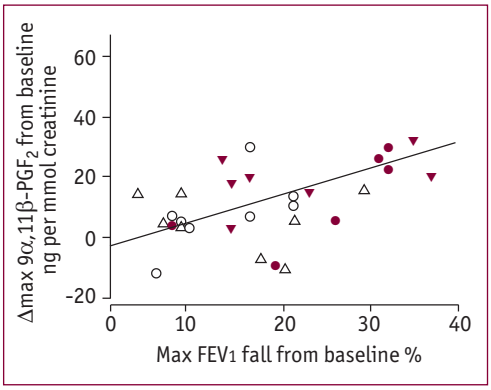

Figure 4
The maximum change in urinary excretion of 9α,11β-PGF_2 in relation to the maximum % decrease in FEV1 in asthmatics (triangles) and athletes (circles) after administration of placebo (closed symbols) and 1,500 µg beclomethasone (open symbols). r=0.544, p<0.002. Modified from [66], with the permission of the publisher.

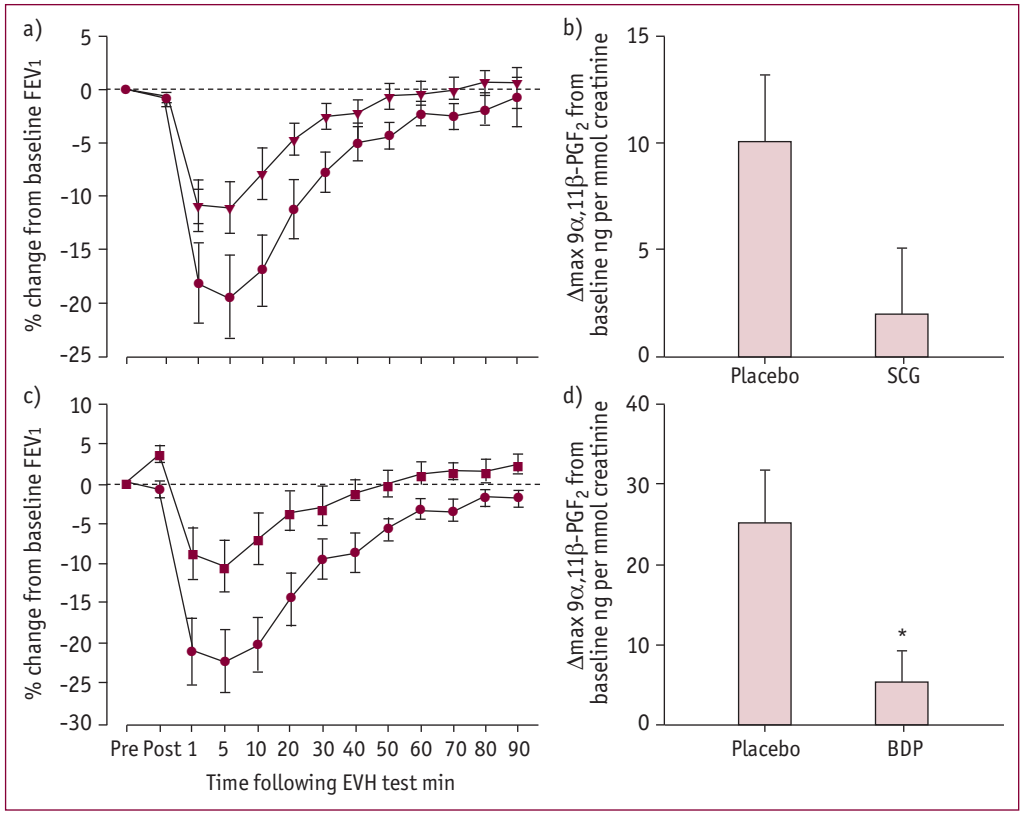

Figure 5

a, c) Per cent change in FEV1 from baseline and b, d) maximum change in 9α,11β-PGF_2 in response to 6 min eucapnic voluntary hyperpnoea of dry air a, b) in the presence of placebo (●) and after sodium cromoglycate (SCG; ▼) in 11 subjects with >10% fall in FEV1 [69] and c, d) 4.5 h after placebo (●) and 1,500 µg beclomethasone (■) in eight subjects with asthma Modified from [66], with the permission of the publisher.

direction. The effect would be to slow the rate of dehydration and change in osmotic environment of the submucosa, such as to be below the threshold for release of mediators [66]. Corticosteroids do not have any recognised inhibitory effect on human mast cell release of mediators. However, a recent study in guinea pigs proposed that steroids inhibit histamine release from mast cells by reducing the rate of the increase in intracellular calcium [72]. It is well known that regular, daily treatment with inhaled corticosteroids reduces severity of EIB [73]. One reason for this is likely to be a reduction in mast cell and eosinophil number with a consequent reduction in concentration of mediators over time of treatment [54]. Inhaled corticosteroids are highly effective for the treatment of EIB. However, for some athletes, *e.g.* cross-country skiers with "ski asthma" and asthma-like symptoms plus AHR, to methacholine, there may be no clear benefit [74]. The reason may relate to the AHR being the result of repeated injury to the airway epithelium (see below) and linked to a neutrophilic inflammation (rather than the eosinophilic inflammatory phenotype, as found in classical asthma).

For airway narrowing, the BSM needs to be hyperresponsive to the mediators released. However, a state of refractoriness often occurs after EIB and is a common feature associated with all provoking stimuli that act *via* release of endogenous mediators [75]. Refractoriness occurs in response to a repeated exercise stimulus, lasting for 2–4 h in ~50% of subjects [76]. This refractoriness is lost following ingestion of a few doses of a nonsteroidal anti-inflammatory agent *e.g.* indomethacin. Again, this emphasises the importance of medication withholding times and recent history of exercise. One of the mechanisms for refractoriness is thought to involve the bronchodilator PGE_2 [77] that is released in response to hyperpnoea.

In healthy subjects, PGD_2 and LTE_4 are 100 and 1,000 times as potent as histamine or methacholine in provoking bronchoconstriction, respectively. This difference in potency may be the reason that some children and adults are hyperresponsive to exercise but not to histamine or methacholine [4, 78, 79]. By contrast, other subjects, particularly elite athletes, may be hyperresponsive to histamine or methacholine but not to exercise, eucapnic hyperpnoea, mannitol or adenosine monophosphate [50]. The possibility that AHR may be reflecting airway injury should be considered before a diagnosis of asthma is given to subjects who are hyperresponsive to pharmacological agents but not to stimuli that cause mediator release from inflammatory cells.

Pathogenesis of AHR and EIB in elite athletes

Injury of the airways leading to AHR may arise from conditioning large volumes of dry air over long periods of training, with or without exposure to environmental irritants, allergens and viral agents [5]. Epithelial repair quickly follows epithelial injury and the process involves microvascular leak and plasma exudation [80]. Epithelial injury is associated with a reduced capacity to move water to the airway lumen in response to a hyperosmolar stimulus [81]. Thus, repetitive injury may be associated with the need to recruit a larger surface area into the conditioning process, thereby furthering the injury. It has been proposed that AHR develops in elite athletes as a result of changes in the contractile properties of BSM following repeated exposure to plasma-derived products [82]. Airway hyperresponsiveness to methacholine in winter athletes may be a result of these events, particularly in those athletes without EIB or symptoms [50, 83]. That AHR may result from repetitive airway injury in athletes is supported by documentation of normal responsiveness out of season [84] or after retirement [85]. For summer athletes with atopy and higher immunoglobulin E levels, the hypothesis proposes that passive sensitisation occurs *in vivo* as a result of the repeated exposure to plasma derived products [79, 83]. Further studies are required to confirm or refute this hypothesis. However, the hypothesis does serve to explain discordant laboratory findings in response to bronchial provocation in athletes and acts as a starting point for further investigation.

Conclusion

The stimulus to EIB is likely to be the result of an abnormal burden on the lower airways to provide water for humidification. EIB appears to be an exaggerated response to airway dehydration in the presence of inflammatory cells and mediators in a person with a responsive bronchial and vascular smooth muscle [21]. Knowledge regarding the stimulus and mechanism of EIB should help us all optimise the test conditions in the laboratory and in the field to identify this common cause of airway narrowing.

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Answers

1. b)
2. c)
3. b)
4. c)

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