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Airway injury as a mechanism for exercise-induced bronchoconstriction in elite athletes

Sandra D. Anderson, PhD, DSc, and Pascale Kippelen, PhD   *Camperdown, Australia, and Aberdeen, United Kingdom*

Exercise-induced bronchoconstriction (EIB) is a consequence of evaporative water loss in conditioning the inspired air. The water loss causes cooling and dehydration of the airway surface. One acute effect of dehydration is the release of mediators, such as prostaglandins, leukotrienes, and histamine, that can stimulate smooth muscle, causing contraction and a change in vascular permeability. Inspiring cold air increases dehydration of the surface area and causes changes in bronchial blood flow. This article proposes that the pathogenesis of EIB in elite athletes relates to the epithelial injury arising from breathing poorly conditioned air at high flows for long periods of time or high volumes of irritant particles or gases. The evidence to support this proposal comes from many markers of injury. 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. 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, making it more sensitive to mediators of bronchoconstriction. It is proposed that cold-weather athletes have airway hyperresponsiveness (AHR) to pharmacologic agents as a result of epithelial injury. In those who are allergic, AHR can also be expressed as EIB. The role of β_2-receptor agonists in inhibiting and enhancing the development of AHR and EIB is discussed. (J Allergy Clin Immunol 2008;122:225-35.)

Key words: Epithelial injury, airway smooth muscle, mast cells, microvascular permeability, eicosanoids, β_2-agonists

In 1999, the year before the Summer Olympic Games in Sydney, there was still debate about the mechanism whereby exercise provokes airway narrowing.[1] The initiating stimulus had been identified 20 years earlier as evaporative water loss after it was shown that exercise-induced bronchoconstriction (EIB) was markedly inhibited or even completely abolished by preventing that loss.[2-4] The relative importance of the thermal (cooling) and osmotic (drying) consequences of the water loss, however, remained unclear. It was clear that abnormal cooling of the airways was not necessary for them to narrow because severe EIB could occur when hot dry air was inspired.[5-7]

Both release of inflammatory mediators and contraction of airway smooth muscle (ASM) were central to the osmotic theory of EIB.[1,8] In 2000, it was proposed that EIB, as described in asthmatic subjects, is an exaggerated response to airway dehydration in the presence of inflammatory cells and their mediators.[1] By contrast, the thermal

Abbreviations used

AHR:	Airway hyperresponsiveness
ASL:	Airway surface liquid
ASM:	Airway smooth muscle
CC16:	16-kd Clara cell protein
EIB:	Exercise-induced bronchoconstriction
EVH:	Eucapnic voluntary hyperpnea
15 HETE:	15 Hydroxyeicosatetraenoic acid
5-LO:	5-Lipoxygenase
LT:	Leukotriene
LTRA:	Leukotriene receptor antagonist
MVP:	Microvascular permeability
PG:	Prostaglandin

theory proposed a purely mechanical cause of airway narrowing involving vasoconstriction of the bronchial vasculature in response to airway cooling, followed by a reactive hyperemia accompanied by vascular leakage and airway edema.[9,10] The cooling and osmotic aspects of water loss were brought together by the suggestion that cold dry air be considered not only for its capacity to cool the airways but also for its potential to increase the area of the airway surface becoming dehydrated and hyperosmotic during exercise.[1]

Everyone has to condition inspired air, and therefore respiratory water loss is common to all during exercise. The ability to return water to the airway surface, however, is likely to be different for the healthy and the inflamed lower airway, just as it is in the upper airway.[11] In healthy persons the upper limit of the decrease in FEV_1 is less than 10% in response to breathing dry air at high flows, and the response can be enhanced by breathing cold air.[12,13]

WATER RETURN IN RESPONSE TO DEHYDRATION AND INCREASED OSMOLARITY

It appears that the return of water to the airway surface is not sufficiently fast to prevent the progressive recruitment of generations of airways into the humidifying process over 6 to 8 minutes of intense exercise while breathing dry air.[1,14] That water is not replaced almost instantaneously in response to an osmotic force seems surprising. It can be explained in part by the increase in concentrations of Na^+ and Cl^- ions delaying the water flux caused by the osmotic gradient because the restoration of a normal balance of Cl^- and Na^+ ions on the airway surface takes precedence over restoration of normal osmolarity.[15,16] The water flux in response to ion concentration occurs through activation of ion channels,[16] whereas the water flux in response to osmotic changes is through activation of aquaporins.[17] The release of adenosine triphosphate at the airway surface in response to sheer stress, a change in osmolarity of the airway surface liquid (ASL), or both is also likely to be an important mechanism for restoration of normal ASL volume during exercise.[18]

In persons with clinically recognized asthma, airway hyperresponsiveness (AHR) to water loss appears early in the disease as EIB and is related to airway inflammation, particularly the presence of eosinophils.[19] Inflammation is implied by the significant reduction in severity of EIB in most asthmatic subjects when they are treated with inhaled steroids daily for 3 to 12 weeks.[20-22] The reduction in severity of EIB increases with the dose of steroid and is usually associated with a reduction in the number of eosinophils.[23]

ATHLETES WITH AHR

By the late 1990s, EIB was being reported quite frequently in cold-weather athletes.[24-26] The possibility was raised that edema and excessive mucus could amplify the small decrease in FEV_1 that normally occurs and account for this increase in prevalence of EIB in cold-weather athletes.[27] It was proposed that the amplifying effect would be sufficient for a mild responder to achieve the 10% decrease required for the diagnosis of EIB.

There also appeared to be a difference in symptoms and AHR when the ambient environment for training varied, as it did between Norway at $-5°C$ and Sweden at $-20°C$. Thus Swedish cross-country skiers reported more cough on winter training (64% vs 42%, $P < .01$) and had a higher prevalence of respiratory symptoms on exposure to cold (45% vs 14%, $P < .001$), AHR to methacholine (43% vs 14%, $P < .001$), current asthma (28% vs 9%, $P < .01$), and clinically diagnosed asthma (42% vs 12%, $P < .001$).[28] The Swedish skiers were also more likely to consult their doctor, to be given a diagnosis of asthma, and to take medication. Self-reported allergy was not associated with asthma symptoms, AHR, or current asthma. One in 3 skiers with a positive response to methacholine had no symptoms at all.[28]

It was also considered at the time that the "asthma" symptoms of breathlessness, cough, and increased mucus production (on exercise), reported frequently by cold-weather athletes, could be accounted for by the cooling and osmotic effects of airway dehydration.[27] For example, stimulation of sensory nerves[29] and mucous glands[30] could cause cough, breathlessness, and the excessive mucus production reported in cold-weather athletes.[31,32]

Athletes performing endurance sports were also reported to have a higher prevalence of nonspecific AHR than those performing other sports,[33,34] and the reason for this required an explanation.

UNEXPECTED FINDINGS ON AHR AND EIB IN ATHLETES

It was common at that time to use pharmacologic agents, such as methacholine, to demonstrate AHR in athletes with possible asthma rather than to use exercise or a surrogate of exercise (eucapnic voluntary hyperpnea [EVH] or hyperosmolar aerosols). The new insights into the pathogenesis of EIB came from unexpected findings in athletes when investigators compared responses to pharmacologic challenge with responses to exercise and its surrogates in the same subjects. In 2002, it was reported that elite summer athletes were less sensitive to provocation with methacholine than they were to provocation with EVH or inhaled dry powder mannitol.[35,36] These findings were not accounted for by the higher (20%) decrease in FEV_1 from baseline required to identify AHR to methacholine compared with EVH (10%) or mannitol (15%). There are at least 2 possible explanations for these findings. First, the mediators involved in EIB and hyperosmolar aerosols (prostaglandin [PG] D_2 and leukotriene [LT] E_4[37-40]) are 100 and 1000 times as potent as methacholine and histamine in healthy persons.[41,42] Second, finding a negative response to a pharmacologic challenge in a person with a positive response to exercise has usually been made in persons with very good lung function.[43,44] Both these observations might explain why EIB precedes AHR to pharmacologic agents by many years in children.[45,46]

In contrast to summer athletes, winter athletes with good lung function have a high prevalence of AHR to methacholine, and yet the same athletes have a low prevalence of positive responses to
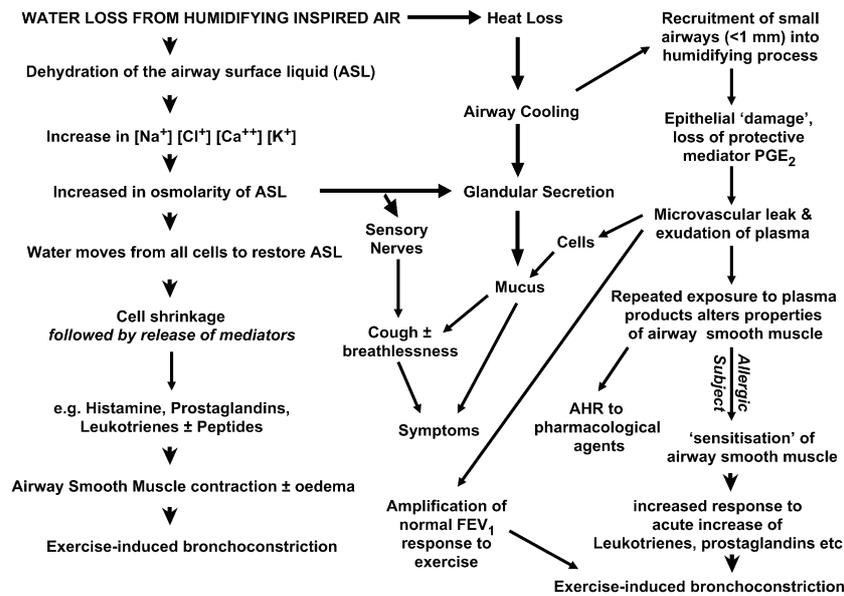
FIG 1. Flow chart describing the acute events leading to EIB in the subject with classic asthma *(left)* and the events leading to the development of EIB in the athlete *(right)*. Reproduced with permission from Anderson SD, Kippelen P. Exercise-induced bronchoconstriction: Pathogenesis. Curr Allergy Asthma Rep 2005;5:116-22.[58]

EVH, mannitol, AMP,[47] or exercise.[48] This raises the question as to whether the AHR in cold-weather/dry-weather athletes is a reflection of airway injury rather than a sign of classical asthma. The AHR in these winter athletes is also much milder than would be expected in subjects with classical asthma.[49] There are some summer athletes who also appear to have airway injury. Thus swimmers who train for long hours in irritant environments might also have a high prevalence of reported AHR to methacholine.[50,51]

There are differences in the mode of action of the provoking stimuli used to assess athletes. For example, the pharmacologic agent methacholine acts directly on acetylcholine receptors to cause bronchial smooth muscle contraction. The transient hyperosmotic effects of evaporative water loss or the inhalation of hyperosmolar aerosols are not a direct stimulus to the smooth muscle. Rather the hyperosmolar stimulus acts indirectly through release of mediators from inflammatory cells (mast cells and eosinophils) situated in or close to the airway surface. These mediators, including PGD_2, LTE_4, and histamine, then act on receptors to cause contraction of the smooth muscle and narrowing of the airways. The same mediators can increase vascular permeability. Hyperosmolarity is a stimulus for epithelial cells to produce PGE_2 and 15 hydroxyeicosatetraenoic acid (15 HETE) and for neuronal cells to release tachykinins.[52,53] Adenosine is also released in response to hyperosmolarity.[54] All these outcomes help to restore the ASL toward normal ion concentration, volume, and osmolarity.

Another unexpected finding was that the AHR in cross-country skiers with symptoms of asthma was not improved by treatment with inhaled steroids, a benefit well-described in asthmatic subjects.[55] Respiratory symptoms and airway responses only improved after a reduction in workload during training, a finding in keeping with less injury.[55] An important and unexpected finding was that montelukast provided greater protection against EIB (90%) in a high-particulate-matter environment compared with that seen in a low-particulate-matter environment (35%), suggesting the response to the particulate matter is predominantly LT

mediated.[56] Finally, an unexpected finding came from the Winter Olympics in Turin. In those games only 32.1% of the athletes applying to use a β_2-adrenoceptor agonist (β_2-agonist) reported asthma in childhood, and for 48.7% of them, the onset of asthma or EIB occurred after 20 years of age, which is very unusual.[57]

PATHOGENESIS OF AHR AND EIB IN ATHLETES

In 2005, an hypothesis for the pathogenesis of EIB and AHR in elite athletes was put forward (Fig 1).[58] In brief, the hypothesis proposed that when cold air was inspired at high flow, the epithelia of the small airways recruited into the conditioning process would become susceptible to dehydration injury. The response to this epithelial injury would involve exudation of bulk plasma as part of the restorative process.[59,60] In elite athletes performing winter sports, this process of epithelial injury and repair would be repeated many times during the season (Fig 2).[61] It was proposed that AHR and EIB could develop for the first time[58] as a result of changes in the contractile properties of ASM after repeated exposure to plasma-derived products.[61,62] Thus cold-weather athletes could experience AHR to pharmacologic agents simply as a result of epithelial injury and repair. This concept was supported by the return to the normal range of airway responsiveness out of season[63] or after retirement.[64] Furthermore, this reversal of AHR suggests that airway remodeling does not occur in response to this type of acute injury and is likely not a predictor of chronic disease.[65]

For summer athletes, who are more likely to be atopic,[66] have rhinitis, and have higher than normal levels of circulating IgE, the ASM would become "passively sensitized" *in vivo* as a result of transient but repeated exposure to bulk plasma.[58,67] The degree to which this could happen would likely be related to circulating levels of IgE.[67,68]

It had been known for several years that some athletes have an increase in circulating levels of inflammatory mediators, such as LTs and PGs, in response to exercise.[69,70] Furthermore, mast cells close to the ASM had been described in the small airways of healthy
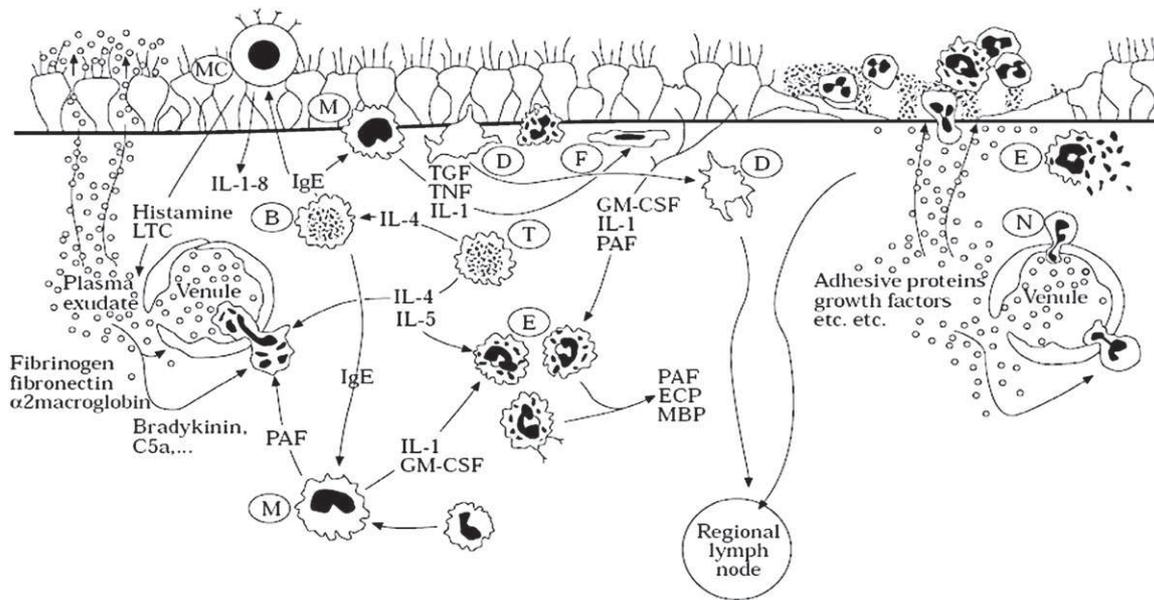
FIG 2. A diagram of the airway mucosa in which exudative inflammation goes on either with the epithelium intact *(left)* or with shedding and restitution as a prominent feature *(right)*. In both conditions plasma-derived adhesive proteins and other plasma-derived effector solutes contribute significantly to the molecular milieu of the lamina propria, epithelium, and mucosal surface. *PAF*, Platelet-activating factor; *ECP*, eosinophil cationic protein; *MBP*, major basic protein. Reproduced with permission from Persson CG, Erjefalt JS, Andersson M, Greiff L, Svensson C. Extravasation, lamina propria flooding and lumenal entry of bulk plasma exudate in mucosal defence, inflammation and repair. Pulm Pharmacol 1996;9:129-39.[61]
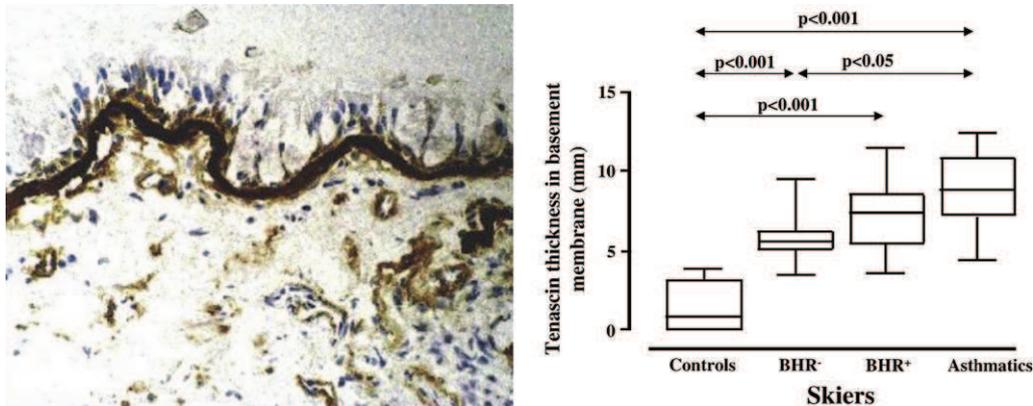
FIG 3. Thickness of the tenascin immunoreactive band in the subepithelial basement membrane zone in control subjects, skiers with and without bronchial hyperresponsiveness to methacholine *(BHR)*, and asthmatic subjects. The *horizontal bar* indicates median value. The graph is reproduced with permission from Karjalainen E-M, Laitinen A, Sue-Chu M, Altraja A, Bjermer L, Laitinen LA. Evidence of airway inflammation and remodeling in ski athletes with and without bronchial hyperresponsiveness to methacholine. Am J Respir Crit Care Med 2000;161:2086-91.[72] The histomicrograph of the tenascin band for one skier is shown in *brown* and was provided by Malcolm Sue-Chu.

subjects.[71] It was proposed that EIB could be the end result of the ASM becoming more sensitive to these mediators through changes in its contractile properties brought about by sensitization.[58]

EVIDENCE FOR AIRWAY INJURY

In the last 10 years, attention has been focused on cellular responses in the airways associated with airway injury and the potential for these responses to explain AHR. First, there is evidence, consistent with wound healing and injury, from a study reporting an increase in tenascin in the basement membrane of skiers (Fig 3).[72] Second, in keeping with airway injury, many athletes have increased numbers of neutrophils in their sputum.[72-75] Third, the severity of EIB in asthmatic subjects is related to epithelial cell numbers in the sputum at baseline, presumably a marker of epithelial injury.[76] Fourth, epithelial cells are a source of the protective PGE_2, and a reduction in PGE_2 levels in relation to cysteinyl LT levels was shown in the same asthmatic subjects and is also in keeping with injury.[77] Fifth, there is increased expression of the gene *MUC5*, which is important in the production of mucus, a key defense mechanism of the airways to cope with dehydration injury.[78] Finally, direct evidence of damage and

repair of the bronchiolar epithelium has been shown both after endurance training in mice[79] and after dry air challenges in dogs.[80]

Stimulation of epithelial cells by means of hyperosmolarity and airway cooling leads to an increased production of IL-8.[81] The increased levels of IL-8 might account for the neutrophilia observed in athletes performing heavy exercise.[72,74,82,83] Further exposure to particulate matter, such as diesel fuel, is a stimulus to attract neutrophils.[84] Elite endurance-trained athletes seem more prone to viral infections, and therefore neutrophils might be present in the airways for a longer duration than in nonexercising subjects.[85,86] For athletes exposed to particulate matter, irritants, and viral infections, there might be activation of innate immune or other mechanisms rather than IgE-based mechanisms.[87] These athletes might more resemble persons with neutrophilic rather than eosinophilic asthma.[88] Neutrophils are known to increase the responsiveness of ASM.[89] The airway inflammation caused by the presence of neutrophils can increase the sensitivity of the ASM, accounting for the increased prevalence of AHR in elite athletes. Thus the approach to treatment might need to be different between a subject with classical asthma and sputum eosinophilia and an athlete with mild EIB and sputum neutrophilia.

ASM AND MAST CELLS

Fernandes et al[90] have stated that "smooth muscle dysfunction may be one of the first mechanisms whereby inflammation leads to AHR." Studies in ASM *in vitro* have provided important supportive information. For example, sensitized ASM has increased levels of myosin light chain kinase, the significance of which is to increase the shortening velocity of the ASM.[91] Other studies have demonstrated that ASM is a very active organ, secreting cytokines, chemokines, and growth factors, which contribute to ASM hyperplasia and act as chemoattractants for mast cell and enhance their coexistence.[92,93]

It has been suggested that infiltration of the ASM by mast cells (mast cell myositis) is the key event that makes the person asthmatic.[93] Mast cells are also found in the bronchial epithelium, mucous glands,[94] and peripheral airways,[71] supporting the concept that these cells "function as sensors of the microenvironment."[95] Mast cell numbers are increased in sensitized human ASM[96] in those with asthma, and they are even present in the ASM of healthy subjects.[97] ASM responsiveness increases after incubation with mast cell products, such as TNF-α[98] and tryptase.[99] Sensitized ASM becomes more responsive to mediators such as histamine[100] and LTD$_4$.[101]

The requirement for mast cells to be present on the smooth muscle to be a case of asthma might help explain "why atopic patients do not necessarily have asthma." Although mast cell infiltration of ASM is more likely in those who are atopic, atopy *per se* is not a reflection of the presence of mast cells on ASM.

MICROVASCULAR AND EPITHELIAL PERMEABILITY

Another potential factor contributing to injury and recovery is alteration in microvascular permeability (MVP), usually identified through markers of microvascular leakage. An increased MVP after exercise has been demonstrated by an increase in the sputum/serum ratio of albumin and is related to the severity of EIB (Fig 4).[102] Many mediators increase MVP, and these include histamine, PGD$_2$, LTC$_4$, and Substance P.[103] Vascular endothelial
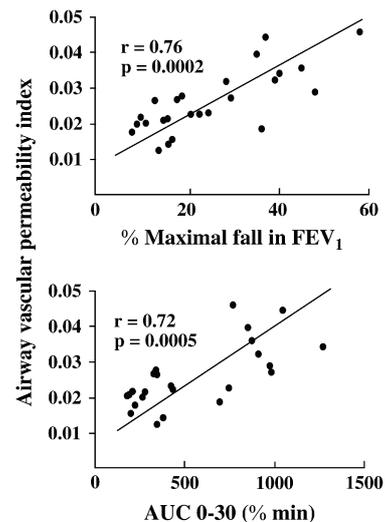
FIG 4. Relationship between the airway vascular permeability index (ie, the ratio of albumin concentration in induced sputum and serum) and the severity of EIB in asthmatic patients expressed as a maximal decrease from baseline and as the area under the FEV$_1$ time curve *(AUC)* up to 30 minutes after exercise. Reproduced with permission from Kanazawa H, Asai K, Hirata K, Yoshikawa J. Vascular involvement in exercise-induced airway narrowing in patients with bronchial asthma. Chest 2002;122:166-70.[102]

growth factor and angiopoietin 2 also stimulate MVP, and levels after exercise are related to the severity of EIB.[104,105] Furthermore, the angiopoietin 2 levels in asthmatic subjects were reduced with montelukast, and this might contribute to their mode of action in enhancing recovery from bronchoconstriction.[106]

Recently, a new approach based on the assay in serum or urine of lung-derived proteins[107] gave support to the hypothesis of altered pulmonary epithelial permeability after intense exercise. A transient increase in serum of one of the major proteins secreted by Clara cells in the airways (ie, the 16-kd Clara cell protein [CC16]) was shown in firefighters both after submaximal fire tasks and near-maximal treadmill exercise.[108] A peak of CC16 levels in serum was also identified immediately after strenuous exercise in trained swimmers, although not in recreational swimmers.[109] This could suggest a link between training status, exercise intensity, or both and the degree of "leakiness" of CC16 from the airways into the bloodstream. The higher the fitness level of the individual, the intensity of the exercise, or both, the more likely the epithelial barrier will be disrupted by hyperpnea-induced osmotic changes of the airway lining fluid during exercise. Interestingly, the postexercise peak of CC16 was present in trained athletes both in the copper/silver pool and in the chlorinated pool.[109] This is in contradiction with some earlier studies, which identified an increase of CC16 levels in serum only after exercise in an air-polluted environment.[110]

Several human exposure and laboratory animal studies have shown transient changes in serum CC16 concentrations after exposure to pulmonary irritants.[111] Recent studies performed on children attending indoor chlorinated swimming pools suggest that trichloramine, together probably with aerosolized hypochlorous acid and chloramines, could damage the lung epithelium[109] and promote the development of asthma, especially in atopic children.[112-115] This concept is in keeping with the high prevalence of asthma and AHR in elite swimmers.[83] Moreover, recent serum CC16 results obtained in cyclists before and after exercise

FIG 5. Model of transcription response and activation changes in exercise-induced asthma– relevant mediators focusing on the LT pathway. *ERK,* Extracellular signal-regulated kinase; *FLAP-5,* lipoxygenase-activating protein; *JNK,* c-Jun NH2–terminal kinases; *MAPKAP,* mitogen-activated protein kinase–activated protein; *MEKK,* MAPK/ERK kinase; *MEK,* MAPK/ERK; *NF-κB,* nuclear factor κB; *ROS,* reactive oxygen species; *STAT6,* signal transducer and activator of transcription. Reproduced with permission from Hilb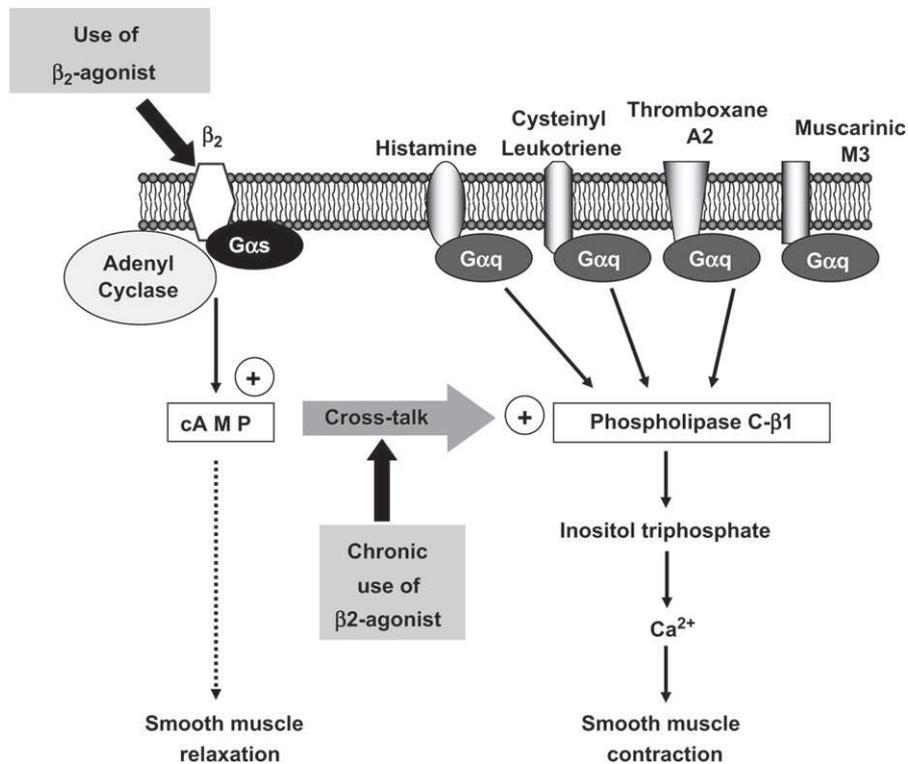erg T. Etiology of exercise-induced asthma: physical stress-induced transcription. Curr Allergy Asthma Rep 2007;7:27-32.[128]

confirm that the lung epithelial barrier is particularly sensitive to ozone-oxidative stress.[110]

It is possible, however, that an increase in MVP might be a normal adaptation to excessive dehydration in an attempt to restore normal ionic and osmotic environment.[80,116-118] Furthermore, an increase in MVP and leakage might be an epiphenomenon of exercise rather than a cause of EIB.[119,120]

NEUROGENIC INFLAMMATION

Injury to the epithelium exposes sensory nerve endings to exogenous foreign particles and to endogenous inflammatory mediators. Removal of the epithelium from human isolated bronchi enhances the contractile response to tachykinins[121] by removing functional neutral endopeptidase and slowing tachykinin degradation.[122]

In patients with asthma, a tachykinin neurokinin 1 (NK1)/NK2 receptor antagonist has been shown to block neurokinin A–induced bronchoconstriction.[123] It seems reasonable to speculate that in cold-weather athletes EIB could be partly mediated by neuropeptide from airway sensory nerves. This hypothesis is attractive because sensory neuropeptides are well known for enhancing tracheobronchial MVP, respiratory secretion, and mucociliary transport.[124] Moreover, the neutrophil infiltration that follows sensory nerve stimulation is consistent with the neutrophilia observed in skiers with "ski asthma."[72] Finally, experimental evidence suggest that the calcitonin gene-related peptide could modulate sulfidopeptide LT-induced responses[125] and be involved in the pathophysiology of EIB.[126]

EICOSANOIDS

Key enzymes in the biosynthesis of eicosanoids, such as secreted phospholipase A2–X are overexpressed in epithelial cells and macrophages in response to exercise in asthmatic subjects.[127] The events summarized in Fig 5 emphasize that physical activity in healthy subjects can trigger transcription of the genes *ALOX5* and *ALOX5AP* encoding 5-lipoxygenase (5-LO) and 5-LO activating protein (FLAP).[128] Furthermore, it shows that 5-LO activity is influenced through changes in p38 mitogen-activated protein kinase. The p38 mitogen-activated protein kinase pathway is activated by a wide variety of stimuli that include osmotic shock. Hallstrand et al[127] have suggested "dysregulation" of eicosanoid synthesis in asthmatic subjects with EIB. This is supported by the finding that pharmacologic interventions that reduce the number of cells producing these mediators,[69,129] blocking their release,[38] or blocking the responses[130,131] all have a favorable effect on EIB.

LTs have long been recognized as playing an important role in EIB. The levels of LTE$_4$ are increased after exercise in asthmatic and healthy subjects who exercise at the same intensity as elite athletes.[69,70] Studies with leukotriene receptor antagonists (LTRA) show that although other mediators initiate the decrease in lung function, LTs sustain the airway narrowing after exercise. Thus recovery from EIB is markedly enhanced in the presence of LTRAs. Agents that block the 5-LO pathway, such as zileuton, also have a modifying effect on the severity of EIB and the response to cold air.[132,133]

Another recent finding is the expression of 15-lipoxygenase (15-LO)-1 by mast cells treated with IL-4.[134] An increase in osmolarity has recently been reported to activate the 15-LO-1 pathway in human cord blood–derived mast cells, which suggests a role for mast cells in the generation of 15 HETE. The significance of this for "asthma" symptoms is that 15 HETE might be important in generating the cough in response to hyperosmolarity of the ASL. 15-HETE is an endogenous ligand of the vanilloid receptor that is stimulated by capsaicin and results in cough.[135] Cough can occur

FIG 6. β_2-Agonists stimulate β_2-receptors to activate adenyl cyclase through Gαs protein. This increases cyclic AMP levels and leads to relaxation. Chronic use can lead to cross-talk between the pathways when contraction is mediated through Gαq protein–coupled receptors, such as histamine, cysteinyl LT, thromboxane A$_2$, or muscarinic M3 receptors. The figure is based on McGraw et al[159] and reproduced with permission from Anderson SD, Caillaud C, Brannan JD. β_2-agonists and exercise-induced asthma. Clin Rev Allergy Immunol 2006;31:163-80.[141]

in healthy subjects exercising in dry environments, and the frequency of cough is reduced as the water content of the inspired air increases.[136]

Research into the role of inflammatory mediators has been facilitated by the development of sensitive assays. For example, the immunoassay developed for measurement of 9α,11β-PGF$_2$, the active metabolite of PGD$_2$, is more widely used now and has highlighted the importance of the mast cell not only in EIB but also in asthma.[95] Both sodium cromoglycate and the β_2-agonist eformoterol administered acutely through inhalation inhibited the release of PGD$_2$, and this might be one important mechanism of their action in preventing EIB.[38] In addition to being potent mediators of bronchoconstriction, these PGs affect vasomotor tone and cell recruitment.

ROLE OF β_2-AGONISTS

Considerable attention has been given in recent years to the subject of β_2-agonists.[57,137-143] Daily use of this class of drug induces tolerance that is manifested in several ways. First, there is a reduction in the duration of the protective effect of both short- and long-acting β_2-agonists against stimuli mediated through the mast cell, such as exercise, allergen, and hyperosmolar aerosols. The tolerance is in part attributed to the sequestration and degradation of mast cell β-receptors, making the cells more susceptible to osmotic and allergic stimuli, and in part due to generic polymorphisms of the β_2-adrenoceptor ADRB2.[144] Second, tolerance of the ASM is demonstrated by slow recovery from

bronchoconstriction in response to a β_2-agonist. This has been shown when exercise and methacholine have been used as the provoking agents.[145,146] The greater the bronchoconstriction provoked, the greater the tolerance.[137] Fortunately, both manifestations of tolerance are reversed 72 hours after discontinuation of β_2-agonist therapy.[146] Furthermore, tolerance does not develop to the protective effects when formoterol is taken 3 times weekly,[147] although the effects on recovery from bronchoconstriction are not known with this dosing regimen.

The question that is important to athletes is whether, on balance, the use of β_2-agonists enhances or inhibits the development of AHR and EIB. β_2-Agonists have a wide range of actions at many different sites in the airways.[148] In addition to their well-recognized effects on ASM and mast cells, there is evidence that β_2-agonists reduce MVP.[148-150] Reducing microvascular leakage might reduce airway injury and the development of AHR and EIB in this group.[151] For example, formoterol inhibits the histamine-induced plasma exudation in the lower airways of human subjects,[152] probably by inhibiting endothelial gap formation.[149] There might be additional antiexudative benefits when the β_2-agonists are used in conjunction with inhaled steroids that reduce the inflammatory stimuli for vascular leakage.

On the downside, a reduction in the number of β_2-receptors on mast cells might reduce the threshold for release of mediators in response to osmotic[141,153] and allergic[154] stimuli. Tryptase levels after allergen challenge were enhanced after regular use of β_2-agonists.[155] In patients with rhinitis, but not asthma, there was an increase in sensitivity to hypertonic saline after daily treatment with

β_2-agonists for 4 weeks.[156] In asthmatic subjects 2 weeks' treatment with 800 μg/d salbutamol increased the late response to allergen in association with an increase in eosinophils.[157] Studies have reported an increase in AHR and a decrease in recovery time with daily use of β_2-agonists, and these reports have recently been reviewed.[141,153]

The mechanism for the smooth muscle becoming more sensitive with daily use of β_2-agonists is of interest and could result from an increase in the number of histamine receptors,[158] crosstalk between the relaxing and contractile pathways through Gs protein, or both (Fig 6).[141,159,160] This cross-talk functionally antagonizes β_2-receptor responses *in vitro* and *in vivo*.[161,162]

The frequency of β_2-agonist, either short-acting β-agonists or long-acting β-agonists, use should be audited and the dose minimized if tolerance occurs to reduce the risk of tolerance and tachyphylaxis. The audit could also include confirming that EIB still occurs and that the β_2-agonist is not just being taken before exercise out of habit. The audit should also confirm whether there is a need for combination therapy for control of asthma because a β_2-agonist can be prescribed separately and reserved for use before exercise. Inhaled corticosteroids alone, when taken regularly in an adequate dose, reduce the severity of EIB and the consequent need for a β_2-agonist. When protection from EIB is incomplete, there are also other agents that can be used in conjunction with inhaled steroids to further reduce the frequency of use of a β_2-agonist. These include sodium cromoglycate or nedocromil sodium taken immediately before exercise or an LTRA taken a few hours before exercise. The International Olympic Medical Commission consensus statement on asthma (see this issue of the *Journal*) states that although β_2-agonists remain the most effective bronchodilators, in the future, they might have a less important role in the management of asthma because EIB should be better controlled by use of other therapies. Further understanding of both the positive and negative aspects of regular use of β_2-agonists in elite athletes is needed.

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