Repeated Hyperventilation Causes Peripheral Airways Inflammation, Hyperreactivity, and Impaired Bronchodilation in Dogs

MICHAEL S. DAVIS and ARTHUR N. FREED

Division of Physiology, Department of Environmental Health Sciences, The Johns Hopkins School of Public Health, Baltimore, Maryland

Winter athletes have an increased incidence of asthma, suggesting that repetitive hyperventilation with cold air may predispose individuals to airways disease. We used a canine model of exercise-induced hyperpnea to examine the effects of repeated hyperventilation with cool, dry air (i.e., dry air challenge [DAC]) on peripheral airway resistance (Rp), reactivity, and inflammation. Specific bronchi were exposed to a single DAC on five consecutive days. Rp and ΔRp to aerosolized histamine, intravenous histamine, or hypocapnia were measured daily. Bronchoalveolar lavage fluid (BALF) was obtained on the fifth day. Rp increased from 0.70 ± 0.08 to 1.13 ± 0.22 cm H_2O/ml/s (n = 25) 24 h after the first DAC, rose to 1.49 ± 0.24 cm H_2O/ml/s by Day 3, and remained elevated throughout the remainder of the protocol. Repeated DAC increased reactivity to hypocapnia and intravenous histamine. Intravenous salbutamol failed to reduce Rp as effectively in challenged airways (111% of Day 1 baseline) as in naive airways (54% of baseline). Repeated DAC caused increased BALF neutrophils, eosinophils, and sulfidopeptide leukotrienes. We conclude that repeated DAC causes peripheral airways inflammation, obstruction, hyperreactivity, and impaired β-agonist-induced relaxation. This suggests that other mechanisms in addition to increased smooth muscle tone may contribute to the development of repetitive hyperventilation–induced bronchial obstruction and hyperreactivity.

Keywords: asthma; β-agonist; bronchoconstriction; hyperreactivity; airway resistance

Numerous studies of human athletes who exercise in cold environments document an increased incidence of airways hyperreactivity in these subjects (1–3). Furthermore, the increased incidence of airways disease seems to be related to the severity of the exercise and the ambient temperature. Athletes who exercise in very cold environments (e.g., cross-country skiers) have a high incidence (∼ 45%) of airways hyperreactivity or asthma (1, 4), whereas athletes who exercise in less frigid conditions (ice hockey players, figure skaters) tend to have a somewhat lower incidence (∼ 30%) (2, 3). Athletes who exercise in a warmer environment at levels comparable to hockey players (e.g., basketball players) do not exhibit an increased incidence of airways hyperreactivity compared with a control population (3). None of these studies has reported a difference in the incidence of atopy between the athletes and control populations, suggesting that repeated hyperventilation with cold dry air predisposes these athletes to airways hyperreactivity.

We have developed a canine model of hyperventilation-induced airways obstruction similar to that described for human athletes. In our model, local insufflation of room temperature air into canine peripheral airways produces the same degree of airway cooling as does hyperventilation with subfreezing air in human peripheral airways (5, 6). Similarities between the human condition and the canine model extend to the timing of hyperventilation-induced bronchoconstriction (HIB) and the attenuation of HIB by numerous pharmacologic agents (5). We recently reported that hyperventilation with cool dry air repeated every other day for 14 d caused bronchial obstruction and eosinophilia in canine peripheral airways (7). The present study was designed to test three specific hypotheses: (*1*) Short-term daily hyperventilation with cool dry air is sufficient for the development of an asthmalike state characterized by airway obstruction, inflammation, and airways hyperreactivity; (*2*) Repetitive hyperventilation-induced peripheral airways obstruction is reversible by a β-adrenergic agonist; and (*3*) Repetitive hyperventilation-induced airways obstruction is associated with leukocyte infiltration and the release of biochemical mediators. In testing these hypotheses, we first hyperventilated canine peripheral airways daily for four consecutive days and documented leukocyte infiltration and changes in baseline peripheral airway resistance (Rp) and reactivity (ΔRp). We then repeated this protocol using salbutamol 24 h after the last dry air challenge (DAC) to document the degree of reversibility in airway obstruction produced by repetitive DAC. Finally, we used correlation analyses to determine relationships between the magnitudes of peripheral airways obstruction observed 24 h after the last DAC, the infiltration of specific leukocytes, and the release of bronchoactive eicosanoids.

METHODS

In all studies, dogs were anesthetized and instrumented on five consecutive days. A sublobar airway was randomly selected on Day 1, wedged with a bronchoscope, and peripheral airway resistance (Rp) was recorded as previously described (8). In protocol A (Table 1) (n = 12), peripheral airway reactivity to hypocapnia, aerosolized histamine, and DAC were measured using established techniques (9). Rp was allowed to recover after each challenge, and a stable baseline Rp was recorded prior to the next challenge. In protocol B (Table 1), a sublobar airway was randomly selected to receive daily DAC as described above (n = 4). However, instead of evaluating reactivity using hypocapnia or aerosolized histamine, reactivity in these airways was evaluated using an intravenous histamine challenge. These protocols were repeated in the same sublobar airways on Days 2 through 5. Data from Protocol A and Protocol B were pooled to determine the effect of repeated hyperventilation on Rp. In protocol C (Table 1), a randomly selected sublobar airway was designated as the control

(*Received in original form March 13, 2000 and in revised form January 23, 2001*)

A. N. Freed wrote this paper in his private capacity. The views expressed in the paper do not necessarily represent the views of NIH, DHHS, nor the government of the United States.

M. S. Davis is currently at the Department of Physiological Sciences, Oklahoma State University, Stillwater, Oklahoma.

Supported by NIH-NHLBI Grants HL09594, HL63186, and HL07534-13.

Correspondence and requests for reprints should be addressed to Arthur N. Freed, Ph.D., Review Branch, Division of Extramural Affairs, National Heart, Lung, and Blood Institute, Two Rockledge Center, Suite 7190, 6701 Rockledge Drive, Bethesda, MD 20892-7924. E-mail: freeda@nhlbi.nih.gov

This article has an online supplement, which is accessible from this issue's table of contents online at www.atsjournals.org

Am J Respir Crit Care Med Vol 164. pp 785–789, 2001
Internet address: www.atsjournals.org

TABLE 1. EXPERIMENTAL DESIGN TO DETERMINE THE EFFECTS OF REPEATED PERIPHERAL AIRWAY HYPERPNEA ON AIRWAY RESISTANCE AND REACTIVITY

Protocols	Day 1	Day 2	Day 3	Day 4	Day 5
A) 4xDAC-aerosol reactivity (n = 12)	Rp, ΔRp, ⇒ DAC	Rp, ΔRp, ⇒ DAC	Rp, ΔRp, ⇒ DAC	Rp, ΔRp, ⇒ DAC	Rp, ΔRp, DAC
B) 4xDAC-IV reactivity (n = 4)	Rp, ΔRp, ⇒ DAC	Rp, ΔRp, ⇒ DAC	Rp, ΔRp, ⇒ DAC	Rp, ΔRp, ⇒ DAC	Rp, ΔRp, DAC
C) Wedge-aerosol reactivity (n = 6)	Rp, ΔRp ⇒	Rp, ΔRp ⇒	Rp, ΔRp ⇒	Rp, ΔRp ⇒	Rp, ΔRp
D) 4xDAC-salbutamol (n = 6)	Rp, DAC ⇒	Rp, DAC ⇒	Rp, DAC ⇒	Rp, DAC ⇒	Rp, Salb, Rp

Definition of abbreviations: DAC = dry air challenge; ΔRp = airway reactivity; Rp = baseline resistance; Salb = intravenous salbutamol; 4xDAC-aerosol reactivity = airway reactivity assessed using hypocapnia and aerosol histamine before daily DAC; 4xDAC-IV reactivity = airway reactivity assessed using intravenous histamine before daily DAC; 4xDAC-salbutamol = airway exposed to 4 daily DACs, and response to intravenous salbutamol measured on Day 5; Wedge-aerosol reactivity = airway reactivity assessed using hypocapnia and aerosol histamine without daily DAC.

(Wedge) airway (n = 6). Baseline Rp and reactivity to hypocapnia and aerosolized histamine were measured as in the 4×DAC airways. However, no DAC was administered to the Wedge airways during this protocol.

To determine the ability of intravenous salbutamol to reverse repeated DAC-induced peripheral airway obstruction, baseline Rp and the response to DAC was measured in a randomly selected sublobar airway (n = 6) on four consecutive days (Protocol D, Table 1). On Day 5, baseline Rp was recorded, and then salbutamol was administered by intravenous infusion. Rp was recorded 5 min after completion of the infusion.

To determine the effect of repeated hyperventilation on airway inflammation, seven dogs were anesthetized and instrumented on 5 consecutive days. In one airway (4 × DAC), Rp was measured before and after DAC. In the second airway, Rp was measured, but the airway was not challenged during the course of the experiment (wedge). This protocol was repeated on Day 2 and Day 3 in the same sublobar airways. In addition, on Day 3 a third sublobar airway was identified (2 × DAC), and Rp was measured before and after DAC. All three airways received their respective treatments on Day 4. Bronchoalveolar lavage (BAL) was done on Day 5 in each of the three principal sublobar airways and a fourth unwedged control airway. The latter BALF served as a control for repeated wedging of the bronchoscope. BAL fluid (BALF) was analyzed for nucleated cell and eicosanoid concentrations using previously published techniques (7).

Rp and reactivity data were analyzed using the Friedman repeated measures analysis of variance (ANOVA). The Dunn's test applied to ranks was used to compare individual treatment means. The Mann-Whitney Rank Sum test was used to compare BALF cellular and biochemical data from control and wedge airways. The Kruskal-Wallis test was used to compare BALF data from wedge, 2 × DAC, and 4 × DAC airways. Statistical significance in all cases was judged at $p < 0.05$. All values are expressed as the mean ± SEM.

RESULTS

All dogs tolerated the protocols well, with no signs of respiratory disease or other effects of repeated anesthesia. Bronchoscopic examination of the principal airways revealed mucus accumulation in challenged airways, whereas wedge control airways appeared grossly normal. For the purposes of presentation and discussion, Day 1 is defined as the first day a specific airway is wedged with a bronchoscope. Thus, because the 2×DAC airway was first wedged on the third day of the protocol, Day 1 for the 2×DAC airways corresponds chronologically with Day 3 for the wedge and 4×DAC airways. Because of interairway variation in the initial (Day 1) Rp values, we express airway resistance and reactivity data graphically as a change from the corresponding Day 1 value. However, the raw data were used for all statistical analyses.

Baseline Rp on Day 1 was 0.88 ± 0.25, 0.70 ± 0.21, and 0.70 ± 0.08 cm H_2O/ml/s for the wedge (n = 13), 2×DAC (n = 7), and 4×DAC (n = 25) lobes, respectively. Baseline Rp in the wedge airway did not change over the course of the protocol ($p > 0.05$), but baseline Rp increased in the 2×DAC and 4×DAC lobes ($p < 0.001$) (Figure 1).

In naive canine airways (n = 6), we found that 11 μg/kg of intravenous salbutamol caused a 46% decrease in the predrug baseline Rp values (presalbutamol Rp = 1.38 ± 0.38 cm H_2O/ml/s, postsalbutamol Rp = 0.70 ± 0.19 cm H_2O/ml/s). In the airways used to examine the effects of intravenous salbutamol on repeated DAC-induced airways obstruction, baseline Rp on Day 1 was 0.63 ± 0.10 cm H_2O/ml/s. Rp increased with repeated DAC ($p = 0.0169$), with Day 4 and Day 5 greater than Day 1 (Figure 2). Intravenous salbutamol caused a 33% decrease in Rp compared with the presalbutamol value ($p = 0.0313$), but was not significantly different from the original baseline Rp on Day 1 ($p = 1.00$).

Reactivity to hypocapnia on Day 1 in the wedge airways, expressed as Rp-CO_2 and ΔRp-CO_2, was 4.06 ± 0.99 and 3.07 ± 0.75 cm H_2O/ml/s respectively. Neither parameter changed significantly with repeated daily bronchoscopy ($p = 0.296$ and $p = 0.056$, respectively). Rp-CO_2 in the 4×DAC airways was 2.88 ± 0.49 cm H_2O/ml/s on Day 1, and increased with repeated DAC ($p < 0.001$) (Figure 3A). Rp-CO_2 on Day 3, Day 4, and Day 5 was significantly greater than that on Day 1. ΔRp-CO_2

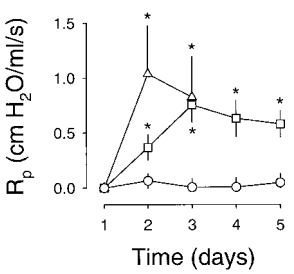

Figure 1. Baseline Rp with repeated DAC. Rp was measured before DAC on each day. 4×DAC (*open squares*) airways received a single DAC daily for four consecutive days (Day 1 Rp = 0.70 ± 0.08 cm H_2O/ml/s, n = 25). 2×DAC (*open triangles*) airways received a single DAC daily for 2 consecutive days (Day 1 Rp = 0.70 ± 0.21 cm H_2O/ml/s, n = 7). Wedge airways (*open circles*) received only bronchoscopy (Day 1 Rp = 0.88 ± 0.25 cm H_2O/ml/s, n = 13). Data are expressed as mean ± SEM change from Day 1. *Significantly different from Day 1, $p < 0.05$.

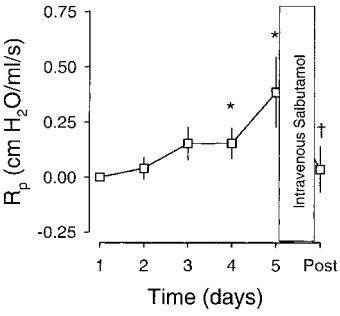

Figure 2. Effect of intravenous salbutamol on repeated DAC-induced airway obstruction. Post = Rp measured 5 min after salbutamol infusion on Day 5. Airways received a single DAC daily for four consecutive days (Days 1–4) (Day 1 Rp = 0.63 ± 0.10 cm H_2O/ml/s, n = 6). Data are expressed as mean ± SEM change from Day 1. *Significantly different from Day 1, †significantly different from Day 5 presalbutamol, $p < 0.05$.

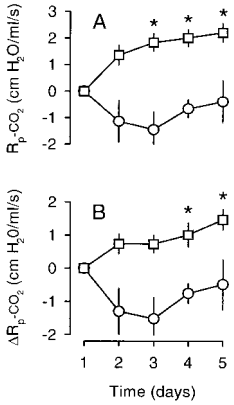

Figure 3. Reactivity to hypocapnia with repeated DAC. Rp-CO_2 = peak airway resistance during hypocapnic challenge; ΔRp-CO_2 = peak increase in airway resistance expressed as change from prechallenge baseline. 4×DAC (*open squares*) airways received a single DAC daily for four consecutive days (Day 1 Rp-CO_2 = 4.06 ± 0.99 cm H_2O/ml/s, ΔRp-CO_2 = 2.14 ± 0.40 cm H_2O/ml/s, n = 12). Reactivity was measured before DAC. Wedge airways (*open circles*) received only bronchoscopy (Day 1 Rp-CO_2 = 4.06 ± 0.99 cm H_2O/ml/s, ΔRp-CO_2 = 3.07 ± 0.75 cm H_2O/ml/s, n = 6). Data are expressed as mean ± SEM change from Day 1. *p < 0.05 compared with Day 1.

in the 4×DAC airways was 2.14 ± 0.40 cm H_2O/ml/s on Day 1, and increased significantly on Days 4 and 5 (p = 0.003) (Figure 3B).

Reactivity to aerosolized histamine on Day 1 in the wedge airways, expressed as Rp_{Hist} and ΔRp_{Hist}, was 2.04 ± 0.85 and 1.05 ± 0.43 cm H_2O/ml/s, respectively. Neither parameter changed significantly with repeated daily bronchoscopy (p = 0.106 and p = 0.078, respectively) (Figure 4). Rp_{Hist} in the 4×DAC airways was 1.48 ± 0.35 cm H_2O/ml/s on Day 1, and increased with repeated DAC (p = 0.00287). Rp_{Hist} values on Day 3, Day 4, and Day 5 were significantly greater than that on Day 1 (Figure 4A). ΔRp_{Hist} in the 4×DAC airways was 0.74 ± 0.24 cm H_2O/ml/s on Day 1, but did not change significantly with repeated DAC (p = 0.515) (Figure 4B). In contrast to aerosolized histamine, repeated DAC significantly increased reactivity to intravenous histamine challenge from 0.56 ± 0.12 cm H_2O/ml/s on Day 1 to 1.42 ± 0.28 cm H_2O/ml/s on Day 5 (Figure 5).

Repeated DAC of peripheral airways resulted in an increase in the peak response to DAC. Rp_{DAC} on Day 1 was 1.40 ± 0.25 cm H_2O/ml/s and increased with repeated challenge (p = 0.00236). Rp_{DAC} was significantly higher than Day 1 on Day 3, Day 4, and Day 5. ΔRp_{DAC} was 0.66 ± 0.17 cm H_2O/ml/s, and did not change over the duration of the protocol.

Repeated bronchoscopy had no significant effects on BALF cell and mediator profiles. However, repeated daily DAC resulted in dose-dependent airway inflammation when compared with repeated bronchoscopy without DAC (Figure 6). Compared with BALF from wedge airways, BALF from 4×DAC (n = 7) airways had increased concentrations of macrophages (p = 0.0246), neutrophils (p = 0.004), and eosinophils (p = 0.0104). BALF from 2×DAC airways (n = 6) had increased concentrations of macrophages (p = 0.0246) and neutrophils (p = 0.004) when compared with BALF from wedge airways. Concentrations of LTB_4, PGD_2, PGE_2, $PGF_{2\alpha}$, and TXB_2 were not increased significantly in airways receiving daily repeated DAC (Figures 7 and 8). However, concentrations of LTC_4, LTD_4, and LTE_4 in 4×DAC and 2×DAC were higher than in BALF from wedge airways (p = 0.0144).

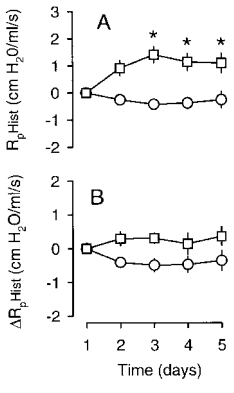

Figure 4. Reactivity to aerosol histamine with repeated DAC. Rp_{Hist} = peak airway resistance after challenge with aerosol histamine; ΔRp_{Hist} = peak increase in airway resistance expressed as change from prechallenge baseline. 4×DAC airways (*open squares*) received a single DAC daily for four consecutive days (Day 1 Rp_{Hist} = 1.48 ± 0.35 cm H_2O/ml/s, ΔRp_{Hist} = 0.74 ± 0.24 cm H_2O/ml/s, n = 12). Reactivity was measured before DAC. Wedge airways (*open circles*) received only bronchoscopy (Day 1 Rp_{Hist} = 2.04 ± 0.85 cm H_2O/ml/s, ΔRp_{Hist} = 1.05 ± 0.43 cm H_2O/ml/s, n = 6). Data are expressed as mean ± SEM change from Day 1. *p < 0.05 compared with Day 1.

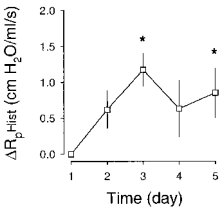

Figure 5. Reactivity to intravenous histamine with repeated DAC. ΔRp_{Hist} = peak increase in airway resistance after infusion with intravenous histamine, expressed as change from prechallenge baseline. Airways received a single DAC daily for four consecutive days (Day 1 ΔRp_{Hist} = 0.56 ± 0.13 cm H_2O/ml/s, n = 4). Data are expressed as mean ± SEM change from Day 1. *p < 0.05 compared with Day 1.

For the purposes of performing correlation analysis, baseline Rp on Day 1 was subtracted from the baseline Rp on subsequent days, and expressed as the change from Day 1 (Rp_{Day5}). Rp_{Day5} was positively correlated with BALF macrophages (Spearman's rank correlation coefficient [r_s] = 0.593, p = 0.0075), neutrophils (r_s = 0.590, p = 0.0079), and LTC_4, LTD_4, and LTE_4 (r_s = 0.507, p = 0.0314). Rp_{Day5} was not significantly correlated with any other BALF parameter.

DISCUSSION

Peripheral airway hyperventilation every 48 h for 2 wk was previously shown to result in obstruction in canine peripheral airways (7). In this study we demonstrate that more frequent hyperventilation challenge results in a more rapid development of airways obstruction (Figure 1), and in peripheral airways that are hyperreactive to hypocapnia (Figure 3) and histamine (Figure 5). We also show for the first time that repeated hyperventilation increases the concentration of sulfidopeptide leukotrienes 24 h after the last DAC (Figure 7), which may contribute to the airway obstruction (Figure 1), as well as the apparent functional antagonism to β_2-agonists that develops (Figure 2). It is important to note that the increase in baseline Rp is unlikely to result from repeated bronchoscopy, because baseline resistance remained remarkably constant in the unchallenged control bronchi. Rather, the persistent airway obstruction appears to be an extension of the DAC-induced late phase (5) that is exacerbated by subsequent repeated challenges.

The increase in baseline Rp is reminiscent of the functional changes that characterize asthma (10), and reflects alterations in airway structure and function that result from repetitive hyperventilation with cool dry air (11). Morphologic changes found 24 h after a single DAC include mucosal damage, bronchovascular leakage, and granulocyte influx in the airway lamina pro-

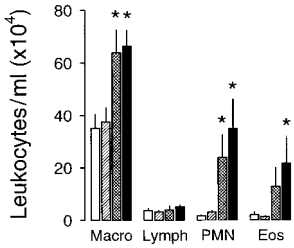

Figure 6. Effect of repeated DAC on BALF nucleated cell concentrations. Control (*open bars*): unwedged before BAL (n = 7); wedge (*hatched bars*): bronchoscopy only every day for 5 d (n = 6); 2×DAC (*cross-hatched bars*): DAC on Days 3 and 4 (n = 6); 4×DAC (*solid bars*): DAC on Days 1–4 (n = 7). BALF obtained from all airways on Day 5. Macro = macrophages; Lymph = lymphocytes; PMN = neutrophils; Eos = eosinophils. Data are expressed as mean ± SEM. *Significantly different from wedge, p < 0.05. (Epithelial cell numbers were negligible in all airways.)

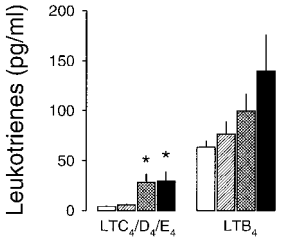

Figure 7. Effect of repeated DAC on BALF leukotriene concentrations. *See* Figure 6 for explanation of bars. BALF obtained from all airways on Day 5. Data are expressed as mean ± SEM. *Significantly different from wedge, $p < 0.05$.

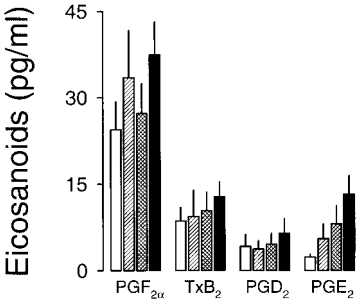

Figure 8. Effect of repeated DAC on BALF prostanoid concentrations. *See* Figure 6 for explanation of bars. BALF obtained from all airways on Day 5. Data are expressed as mean ± SEM.

pria (5). Many of these changes are also present 24 h after repeated DAC (11), and may explain the airway obstruction observed in this study. In addition, the increased concentrations of bronchospastic mediators (Figure 7) and accumulation of intraluminal exudate may contribute to airway narrowing. Although the relative importance of each of these mechanisms is unknown, it is likely that all of them play some role in the development of repeated DAC-induced airway obstruction.

Data from the intravenous salbutamol study provided valuable insights regarding the causes of airway obstruction that occur in response to repeated DAC. In naive canine airways, there is baseline smooth muscle tone that is blocked by intravenous salbutamol, resulting in a 46% decrease in Rp. If repeated DAC-induced airway obstruction was completely reversible by a β_2 agonist, then postsalbutamol airway resistance on Day 5 should have been approximately 0.34 cm H_2O/ml/s (a 46% decrease in the Day 1 baseline). On the other hand, even if repeated DAC-induced airway obstruction was due only to morphologic factors (increased luminal debris or encroachment of the lumen by thickened lamina propria) with no contribution by bronchospasm, the same degree of salbutamol-induced relaxation would still result in a 21% decrease relative to the Day 1 baseline, or 0.50 cm H_2O/ml/s. The fact that salbutamol produced only a 33% reduction in Rp, and resulted in a baseline resistance that was 11% higher rather than 46% lower than that recorded on Day 1, suggests that airways subjected to repeated DAC have impaired smooth muscle responsiveness to β_2-agonists (12). We believe that these data represent the first report of functional antagonism developing in response to repetitive hyperventilation of cold dry air.

Repeated DAC could reduce the response of airway smooth muscle to β-agonists in several ways. First, salbutamol results in smooth muscle relaxation by inhibiting extracellular influx of Ca^{++}. It is possible that the increased concentrations of leukotrienes measured in this study (Figure 7) resulted in functional antagonism through the release of intracellular Ca^{++} stores, thus causing persistent smooth muscle constriction. Second, the bronchial epithelium has been shown to modulate the relaxing properties of adrenergic agents, with the attenuation of relaxation in airways denuded of their epithelium (13). Thus, decreased sensitivity to salbutamol may be related to the persistence of DAC-induced mucosal damage reported in other studies using this model (11). Third, tissue edema outside the airway smooth muscle was shown to limit smooth muscle relaxation in sheep (14). Further investigation is necessary to determine the relative roles of airway smooth muscle and airway wall thickening in repeated DAC-induced airway obstruction.

The reactivity to hypocapnia significantly increased with repeated DAC, thus demonstrating that airways subjected to repeated DAC become hyperreactive (Figure 3). Increased reactivity to hypocapnia presumably results from either changes in the smooth muscle cell that favors pH-induced contraction, thickening of the airway wall, or intraluminal debris that would amplify the effects of smooth muscle constriction (15, 16). We have shown that repeated DAC causes increased lamina propria thickness and increased intraluminal debris (11), so it seems likely that these factors contribute to the hyperreactivity measured in this study. Despite this, we did not measure increased reactivity to aerosolized histamine (Figure 4). The physiologic effect of agonists administered through the airway lumen depends in part on the integrity and permeability of the airway epithelium (17). The washout of dissolved CO_2 in the tissue during the hypocapnic challenge and the subsequent contractile stimulus to the airway smooth muscle is not as dependent upon mucosal permeability as is aerosolized histamine. The stratified squamous epithelium found in repeated DAC bronchi (11) may decrease the penetration of histamine into the airway wall. Additionally, intra-airway mucus (such as that observed in this study) may blunt the reactivity of airways to aerosolized agonists, probably through a barrier effect (18). This suspicion was subsequently confirmed in Protocol B, in which airway reactivity to intravenous histamine challenge on Day 5 was increased approximately 2.5 fold when compared with responsiveness on Day 1 (Figure 5).

The hypothesized mechanisms leading to DAC-induced inflammatory cell infiltration (Figure 6) are based upon the airway drying that occurs during the DAC. Hyperpnea with dry air increases the osmolality of the airway lining fluid and probably the underlying tissues (19). Both mast cells (20) and bronchial epithelial cells (21) have been shown to be osmotically sensitive. Mast cells are known to degranulate in response to DAC (5) and are capable of producing cytokines critical for macrophage and granulocyte influx (22). On the other hand, osmotically stimulated bronchial epithelium produces interleukin-8 (21), an important chemokine for neutrophils (23). Thus, stimulation of mast cells or bronchial epithelial cells during each DAC may initiate the cellular influx observed in this and previous studies.

Leukotrienes may play a prominent role in repeated DAC-induced airway obstruction (Figure 7). The sulfidopeptide leukotrienes are powerful spasmogens of airway smooth muscle (24), and thus have the potential to increase airway resistance through bronchoconstriction. Leukotrienes also may contribute to increased airway resistance by stimulating the secretion of mucus into the airway lumen (24). In addition to a direct role, the sulfidopeptide leukotrienes may play an indirect role in airway obstruction through the potentiation of tachykinin release from airway sensory nerves (25). Thus, the increased BALF concentrations of sulfidopeptide leukotrienes recovered 24 h after repeated DAC (Figure 7), as well as the significant correlation between these concentrations and the change in airway resistance, highlight a probable role for these mediators in the obstruction we observed in this study.

In conclusion, our findings support the hypothesis that repeated hyperventilation of cold dry air, such as that experienced by elite winter athletes, may contribute to the development of

airways disease (1), not just the exacerbation of asthma. Repetitive exposure of canine peripheral airways to unconditioned air results in airway inflammation, obstruction, hyperreactivity, and impaired smooth muscle responsiveness to β-adrenergic drugs. The cause of the airway obstruction is probably multifactorial, including airway exudate, increased airway wall thickening, and increased smooth muscle tone. Repetitive bouts of cooling and drying in the lung periphery results in remodeling of the airway mucosa (11) and these changes may account for the higher incidence of airways hyperreactivity reported in athletes who routinely participate in strenuous winter sports (1).

Acknowledgment: The authors acknowledge the superb technical assistance of Sharron McCulloch and Teresa Myers.

References

1. Larsson K, Ohlsén P, Larsson L, Malmberg P, Rydström P-O, Ulriksen H. High prevalence of asthma in cross country skiers. *Br Med J* 1993;307:1326–1329.
2. Provost-Craig MA, Arbour KS, Sestili DC, Chabalko JJ, Ekinci E. The incidence of exercise-induced bronchospasm in competitive figure skaters. *J Asthma* 1996;33:67–71.
3. Leuppi JD, Kuhn M, Comminot C, Reinhart WH. High prevalence of bronchial hyperresponsiveness and asthma in ice hockey players. *Eur Respir J* 1998;12:13–16.
4. Sue-Chu M, Larsson L, Moen T, Rennard SI, Bjermer L. Bronchoscopy and bronchoalveolar lavage findings in cross-country skiers with and without "ski asthma". *Eur Respir J* 1999;13:626–632.
5. Freed AN. Experimental models of exercise-induced asthma. In: McFadden ER Jr, editor. Exercise-induced asthma. New York: Marcel Dekker; 1999. p. 319–349.
6. McFadden ER Jr, Pichurko BM, Bowman HF, Ingenito E, Burns S, Dowling N, Solway J. Thermal mapping of the airways in humans. *J Appl Physiol* 1985;58:564–570.
7. Davis MS, Freed AN. Repetitive hyperpnea causes peripheral airway obstruction and eosinophilia. *Eur Respir J* 1999;14:57–62.
8. Freed AN, Bromberger-Barnea B, Menkes HA. Dry air–induced constriction in lung periphery: a canine model of exercise-induced asthma. *J Appl Physiol* 1985;59:1986–1990.
9. Freed AN, Stream CE. Airway cooling: stimulus specific modulation of airway responsiveness in the canine lung periphery. *Eur Respir J* 1991; 4:568–574.
10. Lenfant C, Khaltaev N. Global Strategy for Asthma Management and Prevention. NHLBI/WHO Workshop Report. 1–72. Bethesda, MD. NIH-NHLBI, 1996.
11. Davis MS, Freed AN. Repeated dry air hyperventilation causes airway inflammation, edema, and mucosal squamous metaplasia. *Am J Respir Crit Care Med* 1998;157:A672.
12. Lindeman KS, Hirshman CA, Freed AN. Functional antagonism of airway constriction in the canine lung periphery. *J Appl Physiol* 1991; 71:1848–1855.
13. Gao Y, Vanhoutte PM. Epithelium acts as a modulator and a diffusion barrier in the responses of canine airway smooth muscle. *J Appl Physiol* 1994;76:1843–1847.
14. Wagner EM. Effects of edema on small airway narrowing. *J Appl Physiol* 1997;83:784–791.
15. Hogg JC, Paré PD, Moreno R. The effect of submucosal edema on airways resistance. *Am Rev Respir Dis* 1987;135:S54–S56.
16. Yager D, Shore S, Drazen JM. Airway luminal liquid: sources and role as an amplifier of bronchoconstriction. *Am Rev Respir Dis* 1991;143: S52–S54.
17. Mitchell HW, Fisher JT, Sparrow MP. The integrity of the epithelium is a major determinant of the responsiveness of the dog bronchial segment to mucosal provocation. *Pulmon Pharmacol* 1993;6:263–268.
18. King K, Kelly S, Cosio M. Alteration of airway reactivity by mucus. *Respir Physiol* 1985;62:47–59.
19. Freed AN, Davis MS. Hyperventilation with dry air increases airway surface fluid osmolality in canine peripheral airways. *Am J Respir Crit Care Med* 1999;159:1101–1107.
20. Eggelston PA, Kagey-Sobotka A, Lichtenstein LM. A comparison of the osmotic activation of basophils and human lung mast cells. *Am Rev Respir Dis* 1987;135:1043–1048.
21. Hashimoto S, Matsumoto K, Gon Y, Nakayama T, Takeshita I, Horie T. Hyperosmolarity-induced interleukin-8 expression in human bronchial epithelial cells through p38 mitogen-activated protein kinase. *Am J Respir Crit Care Med* 1999;159:634–640.
22. Bradding P, Holgate ST. The mast cell as a source of cytokines in asthma. *Ann NY Acad Sci* 1996;796:272–281.
23. Bittleman DB, Erger RA, Casale TB. Cytokines induce selective granulocyte chemotactic responses. *Inflamm Res* 1996;45:89–95.
24. Lee TH. Mediators of bronchial responsiveness. In: Clark TJH, Godfrey S, Lee TH, editors. Asthma, 3rd ed. London: Chapman & Hall Medical; 1992. p. 162–181.
25. McAlexander MA, Myers AC, Undem BJ. Inhibition of 5-lipoxygenase diminishes neurally evoked tachykinergic contraction of guinea pig isolated airway. *J Pharmacol Exp Ther* 1998;285:602–607.