Inhalation Toxicology in the Equine Respiratory Tract  

M. S. Davis¹ and W. M. Foster²

¹Department of Physiological Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, USA.
²Division of Pulmonary and Critical Care Medicine, Department of Medicine, Duke University Medical Center, Durham, NC, USA.

Glossary

BALF - Broncho Alveolar Lavage Fluid
VO₂ (V) - Rate of oxygen consumption
ppm - parts per million
COPD - Chronic Obstructive Pulmonary Disease
SiO₂ - Silicone dioxide
ARDS - Acute Respiratory Distress Syndrome
EIB - Exercise Induced Bronchoconstriction
DAC - Dry-Air Challenge

Principles of Inhalation Toxicology

The respiratory tract is a unique interface that is specifically designed to permit the absorption of gaseous nutrients. In contrast to the other nutrient absorption system (the gastrointestinal tract) that optimizes absorption through facilitated diffusion or active transport, the respiratory system does not possess specific gas transporters. Rather, it maintains a high level of exchange systems between the outside environment and the body through constant renewal of the environment in the alveoli. This arrangement comes at a cost: Even when the environment is less than optimal due to pollution or other suspended substances, the respiratory tract is nevertheless obliged to be exposed to these adverse components at a high rate in order to maintain appropriate oxygen absorption and carbon dioxide elimination. It therefore, comes as no surprise that a considerable amount of disease in air-breathing animals is the result of exposure to environmental contaminants. Horses are no exception to this rule. This chapter will describe the principles of inhalation toxicology as it relates to equine husbandry and veterinary medicine, and provide examples of the more commonly recognized syndromes and diseases. In addition, we will use these principles to examine conditions that have a tangential relationship to inhalation toxicology, such as aspiration pneumonia and the effects of cold air on the equine respiratory tract.

The field of toxicology is based on two complementary factors pertaining to the toxin: host interaction: Exposure and Effect. Exposure asks the question "What is the dose, duration, and frequency of exposure of the host to the toxin?" whereas Effect asks "How will the host or tissue respond to the toxin?". The response to any toxin exposure can be assessed using these two factors.

Environment is a critical first step in assessing the risk of a toxin, for the simple reason that if there is to be any risk the toxin must be present in the horse’s environment. For many highly toxic compounds, the assessment remains that simple. However, for less toxic compounds, the amount of toxin is important. The horse’s respiratory tract is capable of a finite amount of resistance and detoxification, and a toxin only results in significant disease if this capability is overwhelmed. Furthermore, in most cases it is the product of the toxin concentration and duration of exposure that defines the overall risk. Most commonly identified toxins at typical levels can be tolerated for limited periods without detectable adverse effects. However, long periods of relatively low levels or unusually high levels for even short periods, can result in disease (Haber’s Law) [1].

The environment of the horse can predict the relative risk posed by many inhaled toxins. Rural areas typically have lower levels of airborne pollution, particularly those derived from internal combustion engines and factories. Certain airborne pollutants have limited geographic distribution, such as silicates in the San Fernando Valley of California (See silicosis). Stabled horses are at greater risk from breakdown products such as methane and ammonia due to their persistent close
proximity to fecal matter. However, the clinician cannot make these types of assumptions universally unless they are intimately familiar with both the history, as well as the ambient environment of the patient. For example primary and secondary air pollutants, e.g., ozone, that are largely by-products of anthropogenic activity and the combustion and burning of fossil fuels can attain high levels during the day in rural areas on the Northeast Atlantic coast of North America due to climatic winds and transport of various pollutants from their urban sources. For more information about source apportionment and monitoring atmospheric pollution, see below.

In the United States, the Environmental Protection Agency (EPA) together with individual state programs continuously monitors concentrations of atmospheric pollutants at sites distributed throughout the country. The results are available real-time and free of charge on the EPA’s website: www.epa.gov/air/data and www.epa.gov/oar/partners.html

The site and magnitude of deposition of a substance within the respiratory tract has a strong relationship to its toxic effect. Deposition is in turn influenced by characteristics of the inhaled toxicant as well as patient factors. Important toxin factors include the size and the reactivity of the toxicant. Larger airborne particles tend to deposit in the proximal respiratory tract, and smaller particles penetrate into the distal respiratory tract. An exception to this rule is aspirated material, which may deposit quite proximally in the trachea but due to the size and volume of material, may then flow distally into the cranioventral lung (See aspiration pneumonia). The reactivity of the substance plays the greatest role in the deposition of highly diffusible substances such as gases. Inert gases will simply distribute in the same volume and proportion as inspired air. However, reactive gases such as ozone and sulfur dioxide will react with the surface of the airway, creating a diffusion gradient flowing from the center of the airway lumen to the wall of the airway [2] (See ozone and other inhaled oxidants). Due to the rapid diffusion of these gases down this gradient, the upper airways effectively "scrub" reactive gases from the inspired air and there is little penetration (and thus minimal effect) in the lung periphery unless extremely high concentrations are inhaled and absorptive defense mechanisms become saturated, or increased depth of breathing, for example during exercise, enhances penetration.

With respect to aerosol and particle exposure, the respiratory pattern (depth of tidal volume and the breathing rate, i.e., airflow velocity) have a strong influence over the size of the airborne particle that deposits, the regional location of the deposition, and the mechanisms (inertial impaction, sedimentation, diffusion) that favor deposition in the respiratory tract [3]. The most important patient factors are the velocity of airflow and the volume of the air inspired. As the velocity increases, peripheral deposition of small diameter aerosols will decrease either due to greater rate of impaction in proximal airways or shorter residence time in the respiratory tract. On the other hand, the rate of exposure increases with increasing tidal volume simply due to the larger amount of air being breathed and the potential for particles to penetrate deeper into the air space and come into contact (sedimentation and diffusion) with a greater surface area. In most situations, both of these changes occur simultaneously, resulting in a complex shift in the deposition of aerosol particles. This relationship has not been clearly defined in horses, but it is probably safe to assume that it will bear qualitative similarity to that described in humans. In the latter subjects, increased minute ventilation results in an increase in the amounts of aerosol deposited in any specific region of the lung, with the greatest proportional change occurring in the large lower airways (trachea and bronchi) [2].

The rate of inactivation or clearance of an inhaled toxicant will affect the type of disease caused by the toxic agent. Diseases produced by substances that are rapidly inactivated and cleared, such as reactive gases, can be expected to resolve rapidly once exposure to the toxicant is eliminated, unless the disease process has resulted in chronic changes to the lung (See ozone and cold air, two examples of inhaled toxicants that lead to chronic airway changes). On the other hand, substances that are very poorly cleared, such as silicates, metals, and oil, result in persistent and frequently progressive diseases due to the constant exposure and deposition of the toxic agent (See silicosis and lipoid pneumonia, two examples of chronic disease caused by persistence of the toxic agent).

Inhaled Toxicants
Ozone and Other Atmospheric Oxidants - Horses appear to be less susceptible to the acute effects of ozone compared to humans or laboratory animals. Horses exposed to 0.5 ppm for 12 hrs resulted in evidence of localized oxidative stress (increased oxidized and reduced glutathione, and increased glutathione redox ratio), but no overt signs of disease [4]. Horses exposed to 0.8 ppm for 2 hrs also did not display any clinical signs of respiratory disease or evidence of inflammation in BALF, despite producing BALF levels of oxidized glutathione and glutathione redox ratio that were twice that produced by 0.5 ppm for 12 hrs [5]. Hyperventilation, such as occurs during exercise, can increase the exposure of the respiratory tract to ozone. In a study by Tyler et al., [6], horses were exposed to 0.25 and 0.8 ppm ozone while exercising up to VO

max, and for 20 min after completion of the exercise sets. No effect on exercise capacity was found. However, the horses that received 0.8 ppm of ozone had scattered histological evidence of mucosal damage and edema in the terminal bronchioles. A small degree of damage to the ciliated cells in terminal bronchioles was seen in the airways of horses exposed to 0.25 ppm ozone. It should
be noted that the effects of these levels of ozone are probably exaggerated compared to the breathing the same concentrations at rest, as the authors found indirect evidence that extraction of the inhaled ozone was virtually 100%, even at the highest inhaled concentrations. Thus, the comparative time-weighted exposure in this study was increased over resting conditions by the same proportional increase as minute ventilation (i.e., nearly 20-fold). Since ozone is a day time air pollutant with only several major urban centers, i.e., Houston and the Los Angeles basin, in the USA having ozone concentrations on a repeated basis exceeding 2- and 3-fold the federal standard, and given that those levels rapidly dissipate after sundown, it is unlikely that ozone is a significant risk factor for the development of respiratory disease in otherwise normal horses. However, the ability of ozone to act either additively or synergistically with other agents or with other pre-existing disease processes, such as described in humans [7], cannot be excluded.

Ammonia - Ammonia is considered an important inhaled toxicant in agriculture due to the persistence of urinary ammonia in poorly ventilated, high-density animal housing. Acute toxicity (1000 ppm for less than 24 hour) causes mucosal damage, impaired ciliary activity, and secondary infections in laboratory animals [8]. However, this level is rarely, if ever, achieved in any situation short of an outright spill of ammonia used for crop nitrogen supplementation. The more likely scenario involves long-term, low level exposure. However, qualitatively similar lesions are found provided the exposure (time X concentration) is sufficient (as would be expected according to Haber’s Law) [9-11]. Exposures as low as 10 ppm for 5 - 7 weeks result in decreased resistance to Pasteurella multocida and increased turbinate atrophy secondary to persistence of P. multocida in the nasal mucosal [12]. Thus, the toxic effect of ammonia seems to be related to its ability to cause mucosal dysfunction, thereby disrupting an important mechanism of innate immunity to other inhaled pathogens. The toxic effects of chronic ammonia exposure do not extend into the lower respiratory tract, suggesting that ammonia shares a tendency with other reactive gases to be chemically scrubbed by the upper airways in all but the most extreme exposure conditions.

Organic Dusts - Organic dusts are the most abundant and important inhaled toxicants for horses that are housed indoors. These dusts consist of microbial spores and by-products, and are found in most materials (bedding, feed, hay, silage) associated with stabled horses. Certain materials have lower amounts of dust than others. For example, dry rolled grains have 30 - 60 fold more respirable dust than whole grains or grains mixed with molasses [13]. Visibly dusty hay had 10 times the respiratory dust as hay judged to be good quality, which in turn had approximately 10 times the respirable dust of processed silage and alfalfa pellets [13]. These findings strongly support the use of processed pelleted feeds to minimize feed as a source of dust. The type of bedding that is clearly superior is less certain. In general, wood shavings have been recommended over straw as low-dust bedding [14]. However, one study that directly compared the dust content of good-quality straw, poor quality straw, and commercial wood shavings (marketed as a low-dust bedding for horses) found the wood shavings to have higher dust concentrations by an order of magnitude over straw [13]. This is not inconsistent with the author’s experience, in that we have measured comparable dust exposures in horses housed on straw and on shavings, provided the farm managers diligently select fresh, clean straw if that is to be the bedding of choice.

An additional factor in the density of organic dusts is the efficiency of barn ventilation. Studies of stable air hygiene have led to recommendations for barn design to improve ventilation, and these improved designs can result in reduced overall dust concentration in the stable environment [15,16]. However, a study by Woods et al., [14] suggested that sampling the stable environment using a static sampler may greatly underestimate the exposure of a horse to inhaled organic dust. In this study, the investigators affixed a vacuum sampler to the individual horses with the intake positioned at the horse's nostrils. A ten-fold increase in the rate of exposure to organic dust was found, and the authors suggested that this was the result of disturbance of the dust sources by the horse's noses during typical behavior. Furthermore, dust exposure due to this type of behavior is probably not affected significantly by barn design, adding to the importance of selecting materials with low inherent dust content. For more information on measuring dust exposure for horses, see below.

Most research and discussion regarding organic dust focuses on the role organic dust plays in the development of clinical exacerbation of allergic airway disease (a.k.a., heaves, COPD, recurrent airway obstruction). However, organic dust is an important toxicant even in horses without specific hypersensitization to one or more of the components of organic dust. Horses exposed to excess organic dust develop mild, often subclinical, lower respiratory inflammation that, while not causing overt respiratory disease, may contribute to poor performance due to interference with optimal respiratory function. Neutrophils in bronchoalveolar lavage fluid are increased in stabled horses compared to horses housed in pasture [17,18], and pulmonary neutrophilia can be elicited in normal horses with environmentally relevant exposures of endotoxin [19] and mold antigens [20]. Furthermore, an increased incidence of lower respiratory disease has been reported in stables with poor ventilation [21,22], reinforcing the importance of minimizing dust exposure, even in horses without hypersensitivity to organic dust.
Silicosis - Pulmonary silicosis is a toxicosis that results from inhalation exposure to silicon dioxide (SiO₂). Once inhaled, SiO₂ is phagocytosed by airway macrophages. However, SiO₂ inhibits and eventually kills the macrophage, resulting in release of lysosomal products into the airway. The long-term result of this process is unremitting granulomatous inflammation and fibrosis. Typical cases (which have been restricted in the literature to regions of California) are presented for chronic weight loss, exercise intolerance, and dyspnea [23]. Radiographic findings are consistent with diffuse miliary or nodular lesions consistent with dispersed granulomas. The diagnosis can be confirmed through demonstration of silica (microscopic crystalline material) from bronchoalveolar lavage, pulmonary biopsies, or necropsy. Treatment is generally unrewarding. In mild cases, glucocorticoids may result in temporary improvement in the clinical signs. However, due to the ability of the silicates to persist in the lung tissue, the trigger of the pulmonary reaction remains even after the horse is removed from the source.

Smoke Inhalation - Smoke inhalation, such as occurs in a stable fire, is a dramatic and acute form of inhalation toxicosis. The inhaled toxicants are likely to be a mixture of gases and particulates produced by combustion, but the exact composition (and thus the potential lesions) will be determined by the specific substances being burned. Still, certain compounds and the resultant injuries are common place. In large laboratory animal models of smoke inhalation there is usually a severe lung injury characterized by carboxyhemoglobin levels of 40 - 50% and acute respiratory failure with airway and alveolar inflammation, epithelial damage, and edema. In addition this is accompanied by a systemic injury response with increased oxygen consumption, increased tissue perfusion, and fluid leakage. The mechanisms of injury are complex and result from the acute exposure to a wide variety of chemicals and combustion products, including aldehydes, heavy metals, and oxidants. Frequently the injury progresses over a 24 hr period, suggesting that any short-lived contaminants in the inhaled smoke are likely to initiate, but do not directly participate in the pathophysiology and amplification of the respiratory injury [24-26]. Carbon monoxide is produced in considerable quantities, and can lead to disease throughout the horse due to interference with blood oxygen transport by hemoglobin. Carbon monoxide has approximately 250 times greater affinity for hemoglobin than oxygen and thus readily displaces oxygen from hemoglobin (carboxyhemoglobin), reducing the blood oxygen content and potentially causing tissue hypoxia. Supplemental oxygen is the treatment of choice, as this will not only ensure oxyhemoglobin saturation but will also speed the displacement of carbon monoxide from carboxyhemoglobin.

Smoke from a stable fire will also contain extensive and heterogenous amounts of particulate matter. All airway surfaces will be exposed to substances of varying toxicity, but in many cases sufficient amounts of smoke are inhaled to make the relative toxicity of the compounds irrelevant. The typical injury is one of mucosal sloughing, cytokine release, and the formation of fibrin-based pseudomembranous casts and generalized airway inflammation and edema. The casts need to be cleared by coughing, since the degree of mucosal damage precludes any effective mucociliary clearance. In one report, severely-affected horses frequently suffered from intermittent airway obstruction due to tracheobronchial casts, ultimately necessitating tracheostomy to facilitate manual removal of the casts [27]. In the author’s experience, tracheostomy is frequently indicated to improve work of breathing when upper airway patency is reduced due to inflammation and copious secretions. However, the benefits of a tracheostomy must be carefully weighed against the detriments, since a tracheostomy will dramatically impair the productivity of coughing and thus can be expected to impair clearance of lower airway debris. Supportive and prophylactic treatments for acute smoke inhalation in horses are directed at improving lung function and preventing shock and secondary sepsis. However, not all of these measures are universally beneficial. Glucocorticoids have
been recommended to help control the inflammatory response. However, studies in humans have shown significant risk of bacterial sepsis in fire victims treated with glucocorticoids. Secondary infection is an important consideration in any patient in which the inherent resistance to infection (mucosal integrity, mucociliary clearance) is compromised. However, there was no decreased risk of infection identified in horses suffering from smoke inhalation when treated with antibiotics, compared to those not receiving empirical antimicrobial prophylaxis. A potential approach for treatment of equine patients with acute smoke exposure is to treat the likely development of oxygen free radical activity (presence in smoke directly and/or released from inflammatory cells in lung airways and parenchyma activated by the smoke injury) [28-30] with an aerosolized antioxidant, i.e., deferoxamine [31,32]. The prognosis for return to normal function is good if the patient promptly responds to treatment. In a report of 5 cases of smoke inhalation in horses, all four patients that survived were ultimately able to resume athletic competition [27].

**Aspiration Pneumonia**

Aspiration pneumonia is perhaps the most dramatic form of inhalation toxicosis. Although markedly different in incidence and circumstances from toxicosis due to pollution, aspiration and pollution-related diseases share a common pathogenic feature of inhaled material overwhelming the innate resistance of the lung in a generic, non-specific manner. For this reason, the pathophysiological processes, secondary complications, and therapeutic principles related to aspiration and/or drowning are quite similar to those for the more common environmental inhalation toxicoses, with specific differences related more to the physical characteristics of the inhaled "toxicant" than any specific or unique pathogenicity of that toxicant. The most common cause of aspiration pneumonia in clinical equine practice is probably esophageal obstruction or "choke" [33,34]. For most horses, the initial stages of esophageal obstruction, whether due to an extraluminal mass next to the esophagus or obstruction due to inadequately masticated feed, do not appear to be particularly distressful. As a result, the horse will continue to eat if allowed, and masticated food will progressively fill the esophagus back to the junction of the esophagus and the pharynx. Subsequent efforts to swallow food or water are unsuccessful in clearing the material from the caudal pharynx, allowing the material to enter the larynx when the swallowing process is completed and the protective seals across the opening of the larynx (the epiglottis and the arytenoids cartilages) relax. Although this can be expected to trigger coughing and thus the majority of the contaminating material may be expelled from the trachea, larynx, and pharynx, the horse is unlikely to completely clear all contaminants from its airways. In a horse that continues to eat and drink despite the persistence of the underlying process (unsuccessful swallowing of ingested food and water), the volume of aspirated material can quickly overwhelm the capacity and/or reduce the efficiency of the mucociliary clearance mechanism, leading to accumulation of material in the trachea and lower airways.

A similar initial pathogenesis occurs secondary to neuroanatomic dysfunction of the pharynx and larynx. Initiation of swallowing occurs when food or water is moved to the pharynx by the action of the tongue and cheek muscles, and is largely a voluntary event [35]. The subsequent activation and coordination of the swallowing reflex is a complicated process involving numerous individual nerves and muscles to ensure that the ingested material is delivered to the esophagus and the entrance to the lower airways (the larynx) is protected from contamination. The pharynx is correctly considered a shared space between the respiratory and gastrointestinal tracts, and success in swallowing is predicated on the pharynx being cleared of ingesta so that subsequent inhalation does not result in solid or liquid becoming entrained in the inspired airflow. Neuromuscular dysfunction of the pharynx and larynx can impair the clearing of ingest from the pharynx, leading to contamination of the inspired air as it travels through the pharynx. Thus, aspiration pneumonia is a possible complication to a variety of diseases causing dysphagia, including lead poisoning, botulism, fracture of the hyoid bone, nigropallidal encephalomalacia, otitis media. In addition, the author has seen occasional cases related to excessive surgical retraction of the arytenoid cartilage during correction of laryngeal hemiplegia.

A third possible scenario for the frank contamination of the lower airways is through iatrogenic introduction of material intended to be administered by gavage. Passage of a stomach tube in horses is by necessity done blindly, and despite various techniques intended to prevent inadvertent passage of the tube into the respiratory tract, occasionally water, mineral oil (paraffin oil), and deworming solutions are pumped into the respiratory tract [36]. In other cases, placement of the stomach tube into the esophagus, but not into the stomach, can cause the material pumped through the tube to reflux up the esophagus and spill into the pharynx. The extent to which these substances are irritating or difficult to clear from the airways will dictate the likelihood of subsequent aspiration pneumonia. For example, inadvertent administration of water or isotonic electrolyte solutions is unlikely to cause a serious illness. In fact, pulmonary administration (with partial recovery) of these types of solutions is routinely and safely performed in the context of bronchoalveolar lavage. With this aspirated material comes the normal microbial flora of the foodstuffs, water, and oropharyngeal cavity. Although some of these may be considered primary pathogens, most of these microbes are opportunists and are not considered capable of infecting the lower respiratory tract without additional compromise. This is accomplished through the inflammatory
Drowning (or more correctly, near drowning, since drowning is by definition fatal) is uncommonly reported in horses [40], and therefore the bulk of information regarding the clinical signs and effective treatments of near-drowning must be extrapolated from the human-oriented literature. Near-drowning is commonly, but erroneously, believed to involve the massive aspiration of water due to submersion. However, initially upon submersion and entry of water into the upper airways, profound laryngospasm occurs, preventing aspiration of water despite attempts to inhale. Laryngospasm can be expected to persist until some time after the horse has lost consciousness due to asphyxia and hypoxemia. Thus, animals that are removed from the water prior to relaxation of the larynx may have aspirated minimal amounts of water and can be treated for simple asphyxia.

Drowning

Drowning (or more correctly, near drowning, since drowning is by definition fatal) is uncommonly reported in horses [40], and therefore the bulk of information regarding the clinical signs and effective treatments of near-drowning must be extrapolated from the human-oriented literature. Near-drowning is commonly, but erroneously, believed to involve the massive aspiration of water due to submersion. However, initially upon submersion and entry of water into the upper airways, profound laryngospasm occurs, preventing aspiration of water despite attempts to inhale. Laryngospasm can be expected to persist until some time after the horse has lost consciousness due to asphyxia and hypoxemia. Thus, animals that are removed from the water prior to relaxation of the larynx may have aspirated minimal amounts of water and can be treated for simple asphyxia.

The principle immediate physiological effect of fresh water aspiration is damage to surfactant, resulting in loss of compliance, atelectasis, and some degree of right-to-left shunt. Due to its hypo-osmolarity, fresh water is immediately and rapidly absorbed into the pulmonary vasculature, making attempts remove water from the airways unproductive and not recommended. Rather, cardiopulmonary support (with positive pressure ventilation) and administration of oxygen are the most appropriate immediate treatments. In human near-drowning patients involving fresh water, voluntary respiration is often rapidly restored and full recovery is the norm. Long-term treatment is dictated in part by the contents of the water that is aspirated. Most human cases of near-drowning involve the relatively sterile water found in swimming pools. However, it can be expected that a substantial number of equine near-drowning cases will involve pond or lake water, and thus it is possible that substantial amounts of microbes will also be aspirated. Thus, immediate institution of broad spectrum antimicrobial treatment is indicated. If, despite these prophylactic treatments, pneumonia develops, treatment protocols and overall...
prognosis can be expected to mimic those for other cases of aspiration pneumonia. Cases of near-drowning of horses in seawater have not been reported, but are certainly possible. A key difference between these cases and cases of fresh water near-drowning is the hypertonicity of the aspirated fluid, which prevents the fluid from readily being absorbed from the airways. Thus, attempts to remove the aspirated water may be fruitful and should be attempted if feasible. Fortunately, seawater appears to have considerably less effect on surfactant compared to fresh water, and if the aspirated fluid is promptly removed, human cases of near-drowning with sea water demonstrate less severe and less persistent alterations in pulmonary function.

Airway Injury by Cold Air

Injury of the respiratory tract by breathing cold, dry air is a unique form of inhalation toxicosis, in that the pathological response is due to the ABSENCE of a substance (vaporized water), rather than the presence of a deleterious agent. During inhalation, the inspired air is passively warmed and humidified due to thermal and vapor pressure gradients between the airway lumen and the airway mucosa, resulting in considerable transfer of heat and water to the inspired air and complementary cooling and desiccation of the airway wall. To raise the temperature of 1 liter of air from 0°C to 37°C requires 0.011 Kcal of heat. However, to fully humidify the same volume of air (assuming an initial relative humidity of 0% and a water temperature of 37°C) requires 0.027 Kcal of heat. In fact, even if the inspired air were fully humidified at 0°C, it would still require 0.023 Kcal/l to evaporate the water necessary for 100% humidity (44 mg of water per liter of air) at body temperature. Exhalation results in partial recovery of the heat and water vapor as the conditioned air passes over relatively cooler and drier mucosal surfaces. Still, the conditioning of ambient air under most conditions results in a net cooling and water loss from at least the most proximal airway surfaces. The efficiency of the airways to recover the heat and water transferred to the inspired air is not high, and it is estimated that approximately 50% of the respiratory heat transferred during inhalation is lost [41]. It is probable that a larger percentage of the water transferred is lost, since the relative humidity of the expired air is typically higher than the inspired air.

Even when a substantial gradient exists (i.e., while breathing subfreezing air), warming and humidification is rapid and nearly complete as the inspired air enters the trachea during resting ventilation[42,43]. However, when inspiratory flow rates increase, such as during exercise, the conditioning process is spread over a larger surface area due to both the additional volume of air inspired as well as the air velocity over the airway mucosa. This results in heat and water gradients (and resultant airway cooling and drying) in airways deep within the lung, and from surfaces that normally do not experience these conditions. The consequences of peripheral airway cooling and desiccation have been extensively studied in humans and laboratory animals, and have recently been addressed in horses. The responses are divided into 3 phases: the acute phase (occurring within minutes of the stimulus), the late phase (occurring hours after the stimulus), and chronic phase (responses that persist, particularly after repeated challenge).

Exercise Induced Bronchoconstriction (EIB) is a syndrome in humans characterized by acute bronchoconstriction after strenuous exercise. It is generally accepted that the key feature of exercise leading to EIB is the increased demands for conditioning the inspired air during hyperventilation. As ventilation increases, adequate respiratory heat and water exchange progressively requires more conducting airway surface to completely warm and humidify the inspired air, resulting in penetration of relatively cool dry air into the peripheral airways [42,44]. The magnitude of airway cooling and subsequent bronchoconstriction correlates with the magnitude of ventilation increase: the greater the increase in ventilation, the more pronounced the airway cooling and more severe the bronchoconstriction. Although EIB tends to be most pronounced in people suffering from asthma, under experimental conditions a milder form of EIB can be demonstrated in normal people after hyperpnea with frigid air [45,46]. These responses appear to be phylogenetically conserved in mammals, as exposure of peripheral airways to unconditioned air results in similar patterns of bronchoconstriction in a variety of experimental models [47], including horses. However, an acute phase response has not been demonstrated to date in horses following exercise. The late phase response is thought to occur 5 - 7 hr after the early phase [48-51] and is preceded by an increase in plasma neutrophil chemotactic activity [48,50]. Most reports concerning humans have reported a 30 - 35% incidence of a late phase response to exercise, though some reports have failed to demonstrate any occurrence [52,53]. A late phase response to peripheral airway cooling and desiccation can be readily demonstrated in dogs following dry-air challenge (DAC). Increased airway resistance and reactivity to histamine characterizes the late phase response in canine peripheral airways is characterized by increased airway resistance, increased reactivity to histamine, and granulocytic inflammation 5 hr after DAC [54]. Interestingly, the development of late phase bronchoconstriction can be blocked by cyclooxygenase inhibition prior to the unconditioned air exposure, even if there is no residual cyclooxygenase inhibition during the late phase.

Recently attention has turned to the possibility that cold air hyperpnea can predispose people to the development of asthma. A number of studies have described an increased incidence of airway hyperreactivity and/or asthma in winter athletes who routinely experience hyperpnea during training under frigid conditions, leading to the term "ski asthma" to describe this
For example, in a 1993 study by Larsson et al., cross-country skiers had significantly higher rates of asthma (56% vs. 3%) and bronchial hyperresponsiveness (79% vs. 10%) than a non-skiing control group of healthy adults [56]. A recent study found an increased incidence of asthma (19.2% vs. 4.2%) and bronchial hyperresponsiveness (34.6% vs. 20.8%) in ice hockey players when compared to athletes performing a similar level of aerobic exercise in milder environmental conditions (basketball players) [60]. Together, these studies suggest that repeated exposure of peripheral airways to cold dry air during strenuous winter exercise may result in chronic airway inflammation. This hypothesis is supported by studies in laboratory dogs in which chronic airway inflammation and remodeling was induced by repeated cooling and drying of airways, similar to that which would be produced by strenuous exercise in cold weather [61-63] (Fig. 1).

![Figure 1. Effect of Repeated DAC on BALF nucleated cell concentrations. Control (white): Unwedged prior to BAL (n = 7); Wedge (hatched): Bronchoscopy only every day for 5 days (n = 6); 2xDAC (cross-hatched): DAC on Days 3 and 4 (n = 6); 4xDAC (black): 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.) - To view this image in full size go to the IVIS website at www.ivis.org. -

The airway circulation may play an important role in maintaining temperature of the airway wall. Studies examining the effects of cooling on the airway vasculature have shown a marked vasodilation when the airways are exposed to cold, dry air [64,65]. These observations have led to the theory that the airway circulation dilates to maintain airway wall temperature and humidity in the face of decreasing air stream temperatures. If airway surface temperatures decrease, secondary effects may impact mucosal and ciliated epithelium of the airway. For example, ciliary activity of airway tissues increases with increased temperature; the exact amount depending on whether temperature is measured locally or is the temperature of the ambient air [66-69]. Acute cessation of bronchial blood flow in large animal model is associated in vivo with decreases in airway mucociliary clearance of surface secretions [70]. In addition, dry air inhalation in canine models significantly impairs removal of secretions from large dependent airways [71].

Compared to humans and laboratory animals, there is little direct evidence of pulmonary disease in horses due to exposure of the lower airways to unconditioned air. However, there is considerable circumstantial evidence that makes this a tenable possibility. Racehorses routinely increase their minute ventilation 20-fold or greater during strenuous exercise (compared to an 8-fold increase in humans), and are often exercising in sub-freezing conditions. Even at relatively moderate conditions (4°C and exercise at only 6 - 8 m/s), we have documented penetration of unconditioned air into the equine lung periphery. Using data derived from a study by Hodgson et al., [72], we estimate that horses exercising under temperate conditions lose approximately 0.05 ml/l of ventilation. This is more than twice the rate of respiratory water loss in strenuously exercising human subjects (0.021 ml/l) [73]. Thus, it is likely that equine peripheral airways are exposed to unconditioned air during strenuous exercise.

Whether or not penetration of unconditioned air into the equine lung periphery leads to airway disease remains to be demonstrated. It is widely appreciated that racehorses have a high incidence of subclinical lower respiratory inflammation, and many causes have been suggested for this inflammation, including stable exposure to aeroallergens and pollution. However, McKane and Rose found that exercise resulted in non-specific airway inflammation after 10 weeks of training compared to non-exercised stablemates [74], suggesting that exercise itself could be a specific cause of airway inflammation. Furthermore, a study of racing Standardbreds found a greater incidence of exercise-induced pulmonary hemorrhage in horses racing in cold weather compared to warmer temperatures [75]. Equine peripheral airways have similar mechanical and morphological responses to experimental exposure to unconditioned air as has been demonstrated in other mammals, including reflex bronchoconstriction (Fig. 2) and mucosal sloughing (Fig. 3). We have also demonstrated increased recovery of bronchial epithelium immediately after cold weather exercise in horses (Fig. 4). Although not definitive, these studies provide support for the idea that hyperventilation, particularly with very cold air, may be an important cause of equine pulmonary pathology and detrimental to athletic performance.

![Figure 2. Reflex bronchoconstriction in equine peripheral airways after local cooling and desiccation by high unidirectional airflow. - To view this image in full size go to the IVIS website at www.ivis.org. -]
Figure 3. Control 7 mm diameter equine airway; B: Equine airway (6 mm diameter) after 500 ml/min room temperature dry air insufflation for 30 min; C & D: Equine airway (6 mm diameter) after 4000 ml/min room temperature dry air insufflation for 5 min; E & F: Equine airway (7 mm diameter) after 8000 ml/min room temperature dry air insufflation for 5 min. To view this image in full size go to the IVIS website at www.ivis.org.

Figure 4. BALF Epithelial cell percentage in horses (n = 6) within 2 hr after routine training in cool weather (~5°C, >90% relative humidity). To view this image in full size go to the IVIS website at www.ivis.org.

References


All rights reserved. This document is available on-line at www.ivis.org. Document No. B0319.0202.