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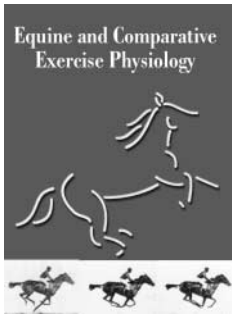

A review of the effects of environmental pollution on the equine respiratory tract: considerations for the 2004 Athens Olympic Games

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Abstract
Chemical pollutants, for example ozone, have been demonstrated to have detrimental effects on the cardiorespiratory system in human beings. Inhalation of pollutants may therefore impact on the performance of athletes at the 2004 Athens Olympic Games. Horses will also be exposed to these pollutants contained within the large volume of air they inhale during exercise. Opportunely, the equine events will be staged outside of Athens and as a result the concentration of atmospheric chemical pollutants is likely to be lower than that experienced by many of the human athletes. The horses' housing environment should also be carefully considered as a source of pollutants, as certain bedding materials can release dust, mould spores and irritants that may induce airway inflammation. In order to achieve the highest level of performance it is essential that both horse and rider be in good health and therefore the influence of environmental pollutants on lung function should to be minimized.

Keywords: performance, lung, ozone, dust

Introduction

Potentially detrimental effects of air pollution on the health and performance of athletes at the 2004 Athens Olympic Games have been raised by the scientific community and highlighted by the media. Airborne pollutants, such as ozone, oxides of sulphur and nitrogen, carbon monoxide, ammonia and airborne particulates, can induce airway inflammation and compromise lung function. After accounting for differences in bodyweight, the maximal minute ventilation of a horse during exercise (approximately 1800 l min^{-1}; 3.6 l min^{-1} kg^{-1} bodyweight) is approximately twice that of a human (approximately 120 l min^{-1}; 1.7 l min^{-1} kg^{-1} bodyweight). Therefore the inhaled dose of pollutants for a given period and intensity of exercise could reasonably be expected to be higher in horses than in humans.

Environment at Athens

Athens is exposed to high levels of pollution, for example from car exhausts. Fortunately the venue for the equestrian events is situated away from the centre of Athens, which may lessen the pollutant burden. Pollutants are concentrated in Athens and its surrounding area due to local geography. Athens is surrounded on the north, east and west by high mountains and by the sea on the south. Onshore breezes prevent the dispersion of pollutants.

Chemical pollutants

Exposure to high concentrations of chemical pollutants has health implications, particularly for sensitive individuals such as human asthmatics, as well as effects on athletic performance. The concentration of iron, which is present in many airborne particulates, has been shown to be higher in the lung-lining fluid of horses from urban environments than in horses from rural areas[1]. Iron (as well as other transition metals) catalyses the formation of hydroxyl radicals from hydrogen peroxide if it is not sequestered in the lung. Hydroxyl radicals are extremely reactive and can result in tissue injury[2]. However, horses from

urban areas did not have increased airway inflammation or bronchoconstriction compared with horses from rural locations[1].

Oxides of sulphur and nitrogen

Relatively little is known regarding the effects of inhalation of chemical irritants on horses. For instance, there are no publications on the detrimental effects of oxides of nitrogen or sulphur on the equine respiratory tract. Sulphur dioxide is a harmful pollutant, but generally has little impact on human lung function or athletic performance because of the low atmospheric concentration. Furthermore, sulfur dioxide has high water solubility, which results in a low dose of this pollutant reaching the distal airways[3]. In healthy humans, nitrogen dioxide exposure can also induce airway inflammation[4]. Nitrogen dioxide does not directly influence lung function but potentiates ozone-induced pulmonary dysfunction[5].

Ozone

One of the most harmful pollutants to human athletes, and the one that has received the most attention, is ozone. Photodecomposition of nitrogen dioxide by sunlight at a wavelength between 295 and 430 nm results in the formation of nitric oxide and atomic oxygen. Ozone is produced from atomic oxygen by reaction with molecular oxygen[6]. Intense sunshine in the summer months results in a high concentration of ozone in Athens. For instance, the ozone concentration in Athens approximated 50 ppb in the summer of 2000[7]. Peak ozone levels are associated with both asthma exacerbations[8] and hospital admissions in the elderly[9].

Ozone induces a number of respiratory responses in humans including neutrophilic airway inflammation, diminished lung function and tissue injury, such as increased airway permeability and epithelial remodelling (reviewed by Mudway and Kelly[6]). Injury may result from the direct action of ozone (oxidative damage) or from ozone-induced airway inflammation. The primary target of ozone is the respiratory tract epithelial lining fluid (ELF). Within ELF the primary targets for ozone are unsaturated fatty acids, proteins and antioxidants, such as ascorbic acid and glutathione[6,10] (Fig. 1). The uptake of ozone by ELF has been suggested to occur by the mechanism of reactive absorption, whereby the rate of ozone uptake is dependent upon the concentration of substrates, such as phospholipids and ascorbic acid, within ELF and the rate of their reaction with ozone[11]. Pryor *et al.*[10,12] proposed that ozone reacts with unsaturated fatty acids present in lipids in ELF and cell membranes forming lipid ozonation products, for example hydrogen peroxide and aldehydes, which function as signal transduction molecules. Lipid ozonation products are small, diffusible and stable molecules, and may cause activation of specific lipases, such as phospholipase $(PL)A_2$, PLC and PLD, which trigger the release of mediators of inflammation from epithelial cells[12]. Therefore lipid ozonation products are able to produce toxic effects distant from the reaction with ozone in a cascade mechanism.

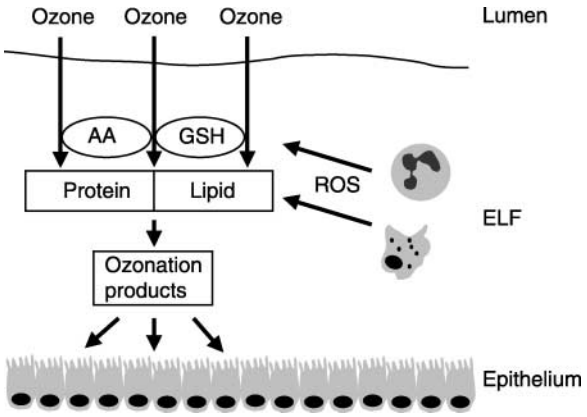

FIG. 1 Ozone interactions in the lung (adapted from Mudway and Kelly[6]). Ozone, which is not buffered by antioxidants (ascorbic acid (AA) and glutathione (GSH)) in the epithelial lining fluid (ELF), reacts with proteins, leading to enzyme inactivation, or with lipids, forming lipid ozonation products. Ozone also induces the infiltration of inflammatory cells into the ELF, which release reactive oxygen species (ROS) on activation

Due to the oxidizing nature of ozone, exposure to this pollutant may compromise pulmonary antioxidant status. Unregulated oxidants have been demonstrated to enhance airway inflammation and promote lung dysfunction and tissue injury in other species including humans and guinea pigs[13,14]. In equine ELF, the major antioxidants are ascorbic acid and glutathione[15]. Inhalation of a relatively high dose of ozone (500 ppb for 12 h) by healthy horses has been demonstrated to induce the oxidation of glutathione in ELF, indicative of oxidative stress[16]. Horses affected by recurrent airway obstruction (RAO: also known as heaves and previously termed equine chronic obstructive pulmonary disease) have a lower concentration of ascorbic acid in ELF[17], which may render them more susceptible to the oxidizing effects of ozone compared with healthy control horses. Interestingly, however, on exposure at rest to 800 ppb for 2 h, there appears to be a sub-population of horses with greater sensitivity to ozone independent of RAO status (Deaton CM, Marlin DJ and Kelly FJ, unpublished results; Fig 2). Similarly, in humans and mice there appear to be responders and non-responders to ozone, which in part is related to genetic factors[18].

Human asthmatic subjects have been demonstrated to have greater decreases in lung function[19] and greater increases in airway inflammation[20,21] than do healthy subjects after ozone exposure, which may reflect ozone-induced up-regulation of neutrophil

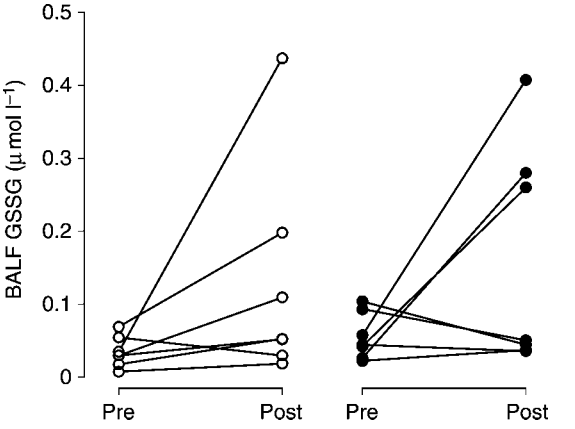

FIG. 2 Individual concentrations of oxidized glutathione (GSSG) in bronchoalveolar lavage fluid (BALF) from seven healthy horses (○) and seven horses affected by recurrent airway obstruction (●) before and after exposure to 800 ppb ozone for 2 h (Deaton CM, Marlin DJ and Kelly FJ, unpublished results)

chemoattractants and T helper type 2 cytokines by epithelial cells[22]. Other studies have found no difference in the degree of airway inflammation or lung dysfunction in asthmatics compared with healthy subjects[23,24]. Ozone (800 ppb for 2 h) exposure at rest did not induce significant airway inflammation in horses (Deaton CM, Marlin DJ and Kelly FJ, unpublished results), which may imply that horses will not be affected by the concentrations of ozone in Athens. However, ozone exposure did induce inflammation in a subset of horses. In humans decrements in lung function are independent of airway inflammation following short-term ozone exposure[25,26]. However, ozone-induced airway inflammation may induce airway remodelling by the release of proteolytic enzymes[27,28]. Furthermore, oxidation of proteins in the ELF by ozone results in the inactivation of enzymes, such as α_1-proteinase inhibitor, and consequently increased elastase release and tissue injury[29].

Two additional factors may also result in ozone compromising equine lung function during the Athens Olympics. Firstly, horses will be exposed to ozone for periods in excess of 2 h and they will be exposed repeatedly. Interestingly, however, repeated ozone exposure of calves and rats resulted in the attenuation of airway inflammation and lung dysfunction compared with that after a single exposure[30,31]. Secondly, the horse studies described above (Mills *et al.*[16]; Deaton CM, Marlin DJ and Kelly FJ, unpublished results) were performed at rest. The dose of ozone delivered to the lungs will be increased by intense and or prolonged exercise at lower intensity during competition and in daily training outside competition. The dose of ozone is equivalent to the product of the concentration of ozone, the exposure time and minute ventilation[32]. Ozone is poorly soluble, therefore increasing the flow rate decreases the time for ozone to diffuse into the nasal ELF and increases the amount of ozone reaching the more sensitive distal airways[33,34]. Therefore, the dose of ozone reaching the small airways will be markedly increased during periods of exercise compared with at rest. A preliminary study[35] in horses reporting the effects of ozone exposure during short-duration high-intensity exercise demonstrated that exposure to either 250 ppb or 800 ppb ozone did not alter maximum oxygen consumption, but did increase heart rate compared with air exposure during exercise. Ozone exposure (250 ppb or 800 ppb) during maximal intensity exercise resulted in tissue injury predominantly to the terminal bronchioles, and exposure to 800 ppb resulted in pulmonary haemorrhage and oedema[35]. In humans, ozone exposure during bicycle exercise reduced maximum oxygen consumption compared with exposure to air[36].

Housing environment

Recurrent airway obstruction

Another major potential source of respiratory pollutants for horses, which is not specific for Athens but warrants comment, is their housing environment. This is of particular relevance to horses affected by RAO. RAO-affected horses are highly sensitive to endotoxin[37] and mould spores, including *Faenia rectivirgula* and *Aspergillus fumigatus*[38], released from poor-quality bedding and hay. Exposure of RAO-affected horses to these pollutants induces airway obstruction, neutrophilic airway inflammation and mucus accumulation, resulting in marked exercise intolerance[39]. The incidence of RAO increases with age and generally affects only horses older than approximately seven years[40]. The true prevalence of RAO is unknown in Northern Europe, but it is thought to be the most common medical condition affecting horses in this region. For example, 54% of sports horses were reported to suffer from RAO in a survey in Switzerland[41]. It is likely that a significant number of horses competing at the Athens Olympics will be affected by RAO. RAO has many similarities to human asthma; however, unlike human asthmatics, horses suffering from RAO are not currently allowed medication for their condition during competition. The induction of clinical signs of RAO can be prevented by housing on a low-dust management system. Wood shavings are the bedding of choice for the majority of competitors from the UK. However, even a low-dust regimen of shavings and silage results in a higher respirable airborne dust concentration than at pasture[42].

Inflammatory airway disease

Airborne pollutants may also play a role in the development of inflammatory airway disease (IAD), where

horses develop airway inflammation without overt clinical signs. Of particular relevance to the Athens Olympics, it has been suggested that hot conditions may exacerbate symptoms of IAD[43]. The presence of IAD is associated with a decrease in athletic performance. Horses with IAD have more severe arterial hypoxaemia during treadmill exercise compared with healthy horses[44]. IAD and the presence of airway fungal material are also risk factors for exercise-induced pulmonary haemorrhage[45], which may have a detrimental effect on athletic performance. Clinically healthy horses are also susceptible to pollutant-induced airway inflammation without the development of airway obstruction in a high-dust environment. Horses may also be exposed to pollutants during their training, as riding surfaces including sand, wood shavings and rubber-based surfaces have been demonstrated to release mould spores and dust[46].

Ammonia

Ammonia is another potential irritant of the respiratory tract and arises from the bacterial metabolism of nitrogen compounds, predominantly urea in urine. It is therefore another pollutant that occurs in poorly managed stables and can induce cough and nasal discharge in horses[47]. The concentration of aerial ammonia has been shown to be higher with horses on sawdust compared with paper bedding[48]; however, in this study there was no evidence of a detrimental effect of ammonia on the respiratory system.

The majority of horses competing at the Athens Olympics are likely to be housed in stables for around 22 hours a day. Preventing the development of airway inflammation and maximizing athletic performance will require the use of minimal-dust management systems. As the horses will be housed in barns, the concentration of airborne pollutants will also depend on the neighbouring stalls' dust load and the efficiency of ventilation. During training and the actual competitions, the concentration of inhaled mould spores and organic dust is likely to be low because of the climate. There is also little agriculture, resulting in a low pollen load. However, the Markopoulo Olympic Equestrian Centre is located close to a marble quarry, which may generate a considerable dust load. Physical irritation of the airways by inert particles has the potential to induce airway inflammation[49].

Summary

In summary, despite the numerous publications on the effects of dust from the housing environment, the effects of chemical pollutants on equine lung function are largely unknown. The concentration and type of pollutants to which horses are exposed will be affected by factors such as time of day of exercise, wind direction and other environmental factors, including sunlight, temperature and cloud cover. Responses between horses to airborne pollutants are likely to be highly variable. Effects of airborne pollutants on airway inflammation and performance, if they occur, are predicted to be sub-clinical and may be attributed to, and impossible to distinguish from, other causes of airway inflammation such as transport, heat or pre-existing airway inflammation. Even if pollutants do not induce clinical signs of lung dysfunction, minor effects may result in a small decrease in performance, which may be enough to alter the medal placings.

Steps that could be taken to reduce the impact of pollutants on horses competing in Athens include:

- Undertake endoscopic examination of the airways well in advance of travel (at least 4–6 weeks prior to departure), as pollutants may exacerbate pre-existing airway inflammation.
- Minimize time of exposure by arriving close to the start of the competition. However, for ozone, repeated exposure may lead to a reduction of airway inflammation. Furthermore, avoidance of pollutants must be balanced against allowing sufficient time for horses to recover from transport and to acclimatize.
- As horses will spend the majority of their time housed whilst at the Games venue, provision of a minimal-dust management system is indicated.
- As treatment for airway inflammation during competition is not permitted, dietary antioxidant supplementation prior to travel and during competition may be beneficial.

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