INTRODUCTION

Clenbuterol is the only FDA-approved medication for horses with reversible bronchospasm, administered with a wide dosage range (0.8 to 3.2 μg/kg, q 12 h for up to 30 days). It is delivered as an oral syrup, and bioavailability in horses is excellent (83.9%). This is in contrast to albuterol, which has poor oral absorption in the horse, and should therefore be avoided in favor of clenbuterol if medication per os is desirable. A long-acting β2-adrenoceptor agonist, clenbuterol is administered (Ventipulmin®) to horses for a variety of purposes. Beta-2 adrenergic agonists are a mainstay in the treatment of bronchoconstrictive diseases of both humans and animals, including asthma and COPD in man, and in horses, Recurrent Airway Obstruction (RAO) and Inflammatory Airway Disease (IAD), a disease which affects up to a third of racehorses in training. β2 agonists activate the sympathetic innervation of bronchial smooth muscle resulting in bronchodilation and mucokinesis in the lungs, and causes smooth muscle relaxation in the myometrium, and blood vessels of the liver and kidney. Clenbuterol has been shown to increase dynamic compliance and decrease pulmonary resistance in ponies bronchoconstricted with intravenous histamine. It has a dose-dependent effect, but is not effective in all horses. Normal horses treated with clenbuterol do not demonstrate bronchodilation in response to clenbuterol as there is minimal smooth muscle tone in the small airways. At the commonly administered dose of 0.8μg/kg, only 25% of horses with RAO show a clinical response, which increases up to 75% at the 3.2ug/kg dose. Like horses with RAO, horses with IAD also have a hyperreactivity to inhaled histamine leading to bronchoconstriction. In addition to bronchodilation, clenbuterol has also been shown to have anti-inflammatory effects, speed mucociliary clearance, re-partition fat to muscle, and decrease mucus production by goblet cells.

This lecture will focus largely on the effects clenbuterol, both beneficial and negative, the significance of tachyphylaxis, and make recommendations on its usage in horses.

EFFECTS OF BETA-2 AGONISTS

Bronchodilation and bronchoprotection

In humans, β2 agonists are also widely acknowledged to attenuate bronchoconstriction from both direct and indirect stimuli. Less evaluation of the bronchoprotective effect of clenbuterol has been performed in the horse, although studies in ponies showed no protective effect of intravenous clenbuterol against inhaled histamine, but some effect against intravenous histamine. Likewise, Mazan et al. also found no significant effect on histamine reactivity of 10 days of aerosolized salbutamol in horses with IAD, although based on human data, it is possible that bronchodilator tolerance had already occurred and thus the bronchoprotective window may have been missed.

Anabolic effects

When skeletal myocyte β-2 receptors are stimulated by clenbuterol, there is a direct anabolic effect that causes an increase in muscle mass in humans, food animals, and horses. In addition, clenbuterol induces lipolysis and inhibits adipogenesis associated with alterations in the adipokines leptin and adiponectin in adult horses. This increases muscle-directed protein deposition and reduces total body fat, which is generally known as repartitioning. Clenbuterol causes a switching from type I, oxidative, slow-twitch fibers to a type II, glycolytic, fast-twitch fiber phenotype, even though β2 adrenergic receptors are expressed more on slow-twitch than fast-twitch fibers. This effect is lessened when combined with exercise. Though these changes in fiber type are noted in horses, the contractile properties of isolated muscle fiber remain unchanged when treated with clenbuterol. Clenbuterol is anabolically active in humans and cattle, and in horses although its leptin- and adiponectin-mediated repartitioning effects cause an increase in muscle mass, this potential benefit is offset by a negative ergonomic effect.

We recently performed a pair of double-blinded, placebo-controlled clinical trials which examined the interaction of exercise and chronic low-dose clenbuterol (0.8μg/kg PO q12 for 21 days) administration in both resting (non-working) and working (polo) horses. Our results showed a significant reduction in percent body fat of 8% and 12% in non-working and working horses, respectively that received clenbuterol. There was no evidence of tachyphylaxis of the lipolytic effect by day 21, and horses in the working group returned to their baseline percent body fat within 2 weeks of cessation of clenbuterol treatment. Additionally, there was no decrease in body weight during either trial, suggesting a true repartitioning of fat to muscle rather than simply a loss of adiposity. These results indicate that at even the commonly administered low dose, clenbuterol reduces body fat when compared to a placebo and may have repartitioning effects that are both lipolytic and anabolic in nature.

Effects on performance

In light of its anabolic effects, clenbuterol has been investigated as a therapeutic compound to treat muscle wasting in humans, but due to concerns of abuse, it has been banned in athletes by the International Olympic Committee and it is illegal as a growth promoter in food animals. In horses, however, clenbuterol has long been a legal therapeutic medication in most racing jurisdictions, with published withdrawal times. In a number of treadmill studies it has not been it has not been shown to improve...
performance; in fact any changes noted have been negative ergogenic effects, and may also have deleterious effects on cardiac function in horses.

**Anti-inflammatory effects**

Clenbuterol, like other cyclic AMP inducing drugs, has been shown to have anti-inflammatory properties. In horses with RAO, it protects against the effects of aerosolized endotoxin, hay dust suspension, and Aspergillus fumigatus antigen, showing a significant beneficial effects on lung function, bronchoalveolar lavage neutrophil influx, and pro-inflammatory cytokines and chemokines expression in pulmonary alveolar macrophages. This effect held true even when endotoxin was administered intravenously, where pre-treatment with clenbuterol reduced peak rectal temperature and peak plasma TNFα concentration in adult horses.

**Diagnosis of anhidrosis**

Unlike other mammalian species that sweat in response to cholinergic stimulation, sweating in horses is mediated by β-2 adrenergic receptors. It is likely that anhidrosis, a naturally occurring syndrome of reduced sweat production, is due to gradual failure of the secretory process of the sweat glands apparently caused by desensitization and down-regulation of the β-2 receptors in response to endogenous catecholamines. This process occurs most commonly in horses living in hot, humid climates where there is continual stimulation of sweating, although it is unclear why only some horses are affected. Diagnosis of anhidrosis is usually performed using intradermal β-agonsists, either non-specific (epinephrine) or specific (terbutaline or salbutamol). Normal horses sweat at a 1:1,000,000 concentration of epinephrine, and that failure to sweat at this concentration is diagnostic for hypo- or anhidrosis.

**Tocolytic effects**

Clenbuterol is used as a tocolytic in horses to slow the progression of labor in dystocias, allowing easier manual mutation of the fetus.

**Negative side effects**

Negative side effects of the β2 agonists are those expected by adrenergic activation. They include characterized sweating, tachycardia, agitation by decreases in aerobic capacity, time to fatigue, cardiac function, and VO2max. The use of β2-agonists is nonetheless common in both human and equine athletes with lower airway disease, despite these significant side effects and the rapid occurrence of desensitization or tachyphylaxis to the effects of this class of drug. This is suggested by data from Kearns et alia, who found that at 3.2 μg/kg, side effects including extreme sweating were initially observed but subsided after 10 days, Clenbuterol has been hypothesized to affect thermoregulation, which may be a component to the reduced aerobic capacity of horses receiving high doses for extended periods.

**TACHYPHYLAXIS**

In asthmatic humans as well as other mammalian models of bronchoconstriction, chronic treatment with β2 agonists results in tolerance or tachyphylaxis (desensitization) to the effects of the drug. This can be seen as a positive attribute, in that the negative side-effects of β2 agonist administration such as sweating, tachycardia, and agitation decrease over the first few weeks of use, but also is associated with a decreased efficacy of the bronchodilatory effects of the treatment and an actual worsening of asthma control. Minimal data are available regarding the effect of long-term β2 agonist treatment on bronchodilation in horses, although small studies in horses with RAO and IAD showed conflicting results, with little-to-no effect seen.

The cause of tolerance to β2 agonists appears multifactorial. In the horse, Abraham et al. showed that 12 days of intravenous clenbuterol in adult horses resulted in a reduced density and responsiveness of β2-adrenoceptor on lymphocytes, suggesting that these effects are conserved across species. In the treatment of human asthma, concurrent use of a corticosteroid attenuates the development of tachyphylaxis to bronchodilators and it is now widely recommended that daily β2-agonists should be used only in conjunction with inhaled corticosteroids to prevent rapid desensitization. Although no studies have been performed to evaluate the effect of this drug combination on lung function in horses with IAD/RAO, it has been demonstrated that the decrease in lymphocyte β2-receptor density and function in horses receiving clenbuterol is reversed and prevented by administration of dexamethasone.

In contrast to other mammals, equine sweat glands contain β2 receptors that show rapid desensitization to β2 agonists leading to sub-normal sweat production in vitro. In an experimental setting, there is a gradual decline in sweat production over the course of prolonged epinephrine infusion, and several studies have demonstrated similar findings of apparent desensitization of sweat gland β-2 receptors both in vivo and ex vivo, where cultured equine sweat glands show a rapid and homologous desensitization to epinephrine. However, after 21 days of oral clenbuterol at 0.8 μg/kg twice daily, we found no reduction of sweating using an intradermal epinephrine sweat at concentrations of epinephrine as low as 1:1,000,000, thus suggesting that any reduction in sweating caused by chronic clenbuterol is likely to be sub-clinical.

Using a blinded, randomized cross-over designed trial, we showed clenbuterol at standard dosages (0.8 μg/kg per os every 12 hours) resulted in a decreased bronchoconstrictive response to inhaled histamine compared to horses receiving placebo treatment. Using flowmetric plethysmography and inhaled histamine bronchoprovocation, 5 out of 8 horses (62%) had a ≥100% increase in PC35 (35% percent change in DELTA Aβ100) peaking at day 14 of administration. This degree of efficacy is somewhat higher than previous studies that show 25% of RAO horses demonstrating clinical evidence of bronchodilation at the same dose. This may be due
to superior sensitivity of pulmonary function testing compared to clinical examination for detecting bronchoconstriction, the greater reversibility of bronchoconstriction in horses with IAD compared to those with RAO-induced airway remodeling, or differences in airway secretion volume or composition between the two diseases. Additionally, all horses showing a bronchoprotective effect demonstrated tachyphylaxis by day 21 of treatment; 7/8 horses had PC35 values that were actually lower than baseline, indicating pulmonary function may have worsened during the treatment period. This is consistent with human data that show prolonged administration of β-2 agonists results in worse than baseline lung function. Based on *in vitro* studies in the horse and extensive human clinical data, it is likely that extended use of clenbuterol should be combined with inhaled corticosteroids, although the safety and efficacy of this approach has not been validated in the horse. However, our study suggests that clenbuterol alone is less effective as a bronchodilator after 14 contiguous days of treatment, and courses of administration should not exceed 2 weeks without interruption.