Athletic exertion stresses all body systems (de Graaf□Roelfsema et al., 2007), increasing inflammatory mediators in all organs, and even suppressing immunity (Cywińska et al., 2012). Stabled racehorses are subjected to high concentrations of fine particulate matter that is able to reach the lower airways, contributing to airway inflammation (Millerick-May et al., 2011). Beyond these immune system changes, intense exercise in horses is associated with a huge increase in the amount of air that is moved in and out of the lungs, far surpassing other athletic species, and further increasing the respiratory particulates inhaled (Hornicke et al., 1987). Inflammatory airway disease is a widespread problem in horses causing impaired gas exchange and poor performance. It can affect as many as 80% of all 2-year-old racehorses and 14% of all age groups of racing horses suffer from IAD (Wood et al., 2005). IAD is the second most common cause of lost training days in racehorses (Wilsher et al., 2006). In addition to environmental management and appropriate antibiotic treatment, a cornerstone of treatment of IAD in racehorses is the FDA approved, safe and effective short term therapy with clenbuterol (Ventipulmin®, Boehringer-Ingelheim) (Couetil et al., 2016). There is no alternative FDA approved therapy to combat this important disease of young racehorses. Unlike mucolytics, like guaifenesin or acetyl-cysteine, clenbuterol does not break up mucus, but rather improves lung function by opening reactive airways, increasing mucus production and improving the mucociliary clearance of particulates in the lungs (Millerick-May et al., 2011). As far back as the initial studies in 1978, this molecule has been shown to improve pulmonary function in horses with inflammatory airway disease (IAD), beyond the effect of other treatments (Sasse and Hajer, 1978). Clenbuterol is a part of the Best Practice therapy for IAD (Couetil et al., 2016).

Clenbuterol, like other β-2 agonists, has been shown to cause a repartitioning effect, wherein the cross-sectional area of the muscle is increased with chronic use (Kearns et al., 2001).

While this effect might "make big muscles," it has unequivocally been shown to be detrimental to aerobic (long distance) performance. A study where high therapeutic levels of clenbuterol were administered to horses twice daily for 8 weeks resulted in a 21% decrease in time to fatigue: the horses just couldn't run as far (Kearns and McKeever, 2002). While it makes muscles big, it hinders the ability of rats to sprint (Duncan et al., 2000) and decreases the anaerobic tolerance of horses (Kearns and McKeever, 2002). Clearly, in racing disciplines where the racing performance depends upon aerobic or long distance exercise, such as is in the Standardbred racing disciplines, chronic clenbuterol usage impairs performance.

Muscle fibers are generally grouped into three types, based on their myosin heavy chain (MHC) type. Type I fibers are slow oxidative fibers that are typically associated with anti-gravity muscles. These are the muscles that fire all the time to keep the animals upright. The concentration of these muscle types is higher in the core of the skeleton, reflecting their role in posture. Type IIa fibers are intermediate fibers that are fast twitch oxidative fibers. They can adapt to either aerobic or anaerobic training and are typically higher in mitochondrial content (the subcellular power-plants that use oxygen and fuel to generate energy) and an enzyme important in the generation of energy, citrate synthase (CS). However, they also have many characteristics related to the anaerobic metabolic pathways used for sprinting and the kick at the end of a race. Anaerobic training will shift the functional characteristics of the IIa fibers towards glycolytic metabolism. Anaerobic training enhances the combined profile and area of the MHC type II fibers towards the type IIx (sometimes called IIb), or fast glycolytic fibers, which have lower mitochondria and CS, and an energy preference of the considerably less efficient, but more powerful non-oxidative (no oxygen is used) glycolytic pathway. As expected, the muscle fiber type distribution between breeds of race horses vary according to the discipline. The table

below, provided courtesy of Dr. Stephanie Valberg, clearly shows that Quarter Horses have considerably higher Type IIx than any other discipline of race horses and Standardbreds have the lowest Type IIx.

TABLE 12–5

**Percent Fiber Type Composition of Various Horse Breeds Based on Immunohistochemical Staining for Contractile Fiber Type**

| Breed | Type 1 | Type 2a | Type 2ax | Type 2x | Musde |
|---|---|---|---|---|---|
| Thoroughbred | 11.7 ± 5.0 | 47.3 ± 5.1 | No data | 41.0 ± 5.1 | gluteal |
| Thoroughbreds | 12.3 ± 4.8 | 43.5 ± 5.5 | 15.6 ± 6.4% | 34.5 ± 4.1 | gluteal |
| Quarter horses | 10.7 ± 1.8 | 32.9 ± 1.5 | No data | 56.4 ± 1.1 | semimembranosus |
| Standardbreds | 17 ± 12.2 | 46 ± 1.4 | 10 ± 1.4 | 27 ± 2.8 | gluteal |
| Andalusians | 41 ± 2 | 35 ± 3 | 15 ± 1 | 7 ± 1 | gluteal |

From Kadisbin K, Essén-Gustavsson B: Myosin heavy chain-based fibre types in red cell hyper- and normovolaemic Standardbred trotters, Equine Vet J 34(Suppl):279, 2002; Rivero JL, Ruz A, Martí-Korff S, et al: Effects of intensity and duration of exercise on muscular responses to training of thoroughbred racehorses, J Appl Physiol 102:1871, 2007; Serrano AL, Quiroz-Rothe E, Rivero JL: Early and long-term changes of equine skeletal muscle in response to endurance training and detraining, Pflugers Arch 441:263, 2000; Yamano S, Eto D, Sugiura T, et al: Effect of growth and training on muscle adaptation in Thoroughbred horses, Am J Vet Res 63:1403, 2002.

Aerobic training, such as that required for Standardbred racing is associated with an increase in Type IIa (Kearns et al., 2002), whereas the increase in muscle cross sectional area seen with clenbuterol administration is occurs in type IIx (fast twitch glycolytic) (Beekley et al., 2003), a phenomenon contrary to the effects of training. In the comparison chart above, compiled by Stephanie Valberg, it is clear that Quarter Horses which race in powerful bursts of speed, have the highest Type IIx, or fast glycolytic fibers and the lowest oxidative fibers. In contrast, Standardbreds exhibit the highest oxidative fiber percentage and lowest Type IIx, or fast glycolytic fibers. Differences among the different racing breeds can be identified using functional measures of oxidative capacity as well. Recent research investigating mitochondrial respiration as a determinant of oxygen utilization has shown that these differences are present even in weanlings (Latham et al., 2019). Mitochondrial density in weanlings was significantly higher in Standardbreds than Quarter Horses, and other differences in oxidative and mitochondrial function were also evident. Clenbuterol, in addition to increasing the cross

sectional area of fast glycolytic Type IIx fibers, also decreases mitochondrial number and enzyme activities (Hoshino et al., 2011). Clearly, any effect of clenbuterol on aerobic performance would be ergolytic.

Kearns et al (2005) unequivocally demonstrated that clenbuterol administration impairs the performance of horses trained in a style similar to that of Standardbred racehorses. Standardbreds, when compared to other racing breeds, and specifically Quarter Horses, have an inherently higher density of mitochondria, a training effect that increases the proportion of Type IIa muscle fibers, as well as increasing mitochondrial density and function. The purported anabolic effects of clenbuterol are limited to increasing the cross-sectional area of Type IIx fibers, decreasing mitochondrial density and enzyme activity, directly antagonistic to the purpose of training and racing a Standardbred. There is no research that has specifically demonstrated ergogenic effects in Quarter Horses specifically trained in a style to that of Quarter Horses. However, it is plausible that the clenbuterol effects may improve the anaerobic glycolytic fiber types. Such assumptions cannot be applied to Standardbreds.

In summary, there is no possibility of performance enhancement with clenbuterol use in Standardbreds, and any use of clenbuterol beyond a short term aid for horses with inflammatory airway disease is associated with decreased performance and adverse health effects.