Effect of chronic clenbuterol administration and exercise training on immune function in horses

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ABSTRACT: Effects of longitudinal exercise training and acute intensive exercise (simulated race test) on immune function have not been reported in horses. Clenbuterol, a β2-adrenergic agonist, is used to manage inflammatory airway disease in horses. This study investigated the interaction of 8 wk of exercise training with or without 12 wk of clenbuterol administration in horses. Twenty-three untrained standardbred mares (10 ± 3 yr, Mean ± SE) were used and divided into four experimental groups. Horses given clenbuterol plus exercise (CLENEX; n = 6) and clenbuterol alone (CLEN; n = 6) received 2.4 μg/kg BW of clenbuterol twice daily (in an average volume of 20 mL) on a schedule of 5 d on and 2 d off for 12 wk. The CLENEX group was also aerobically trained 3 d/wk. Mares given exercise alone (EX; n = 5) were aerobically trained for 3 d/wk, and the control group (CON; n = 6) remained sedentary. Both EX and CON horses were administered similar volumes (approximately 20 mL) of molasses twice daily. A simulated race test (SRT) resulted in an elevation in lymphocyte number postexercise (P < 0.05). There was no significant difference after acute exercise in either monocyte or granulocyte number. Acute exercise resulted in a decrease (P < 0.05) in the percentage of CD4+ and an increase (P < 0.05) in the percentage of CD8+ cells. The SRT resulted in a decreased lymphoproliferative response to pokeweed mitogen (P < 0.05). A SRT had no effect on antibody production in response to equine influenza vaccine. The EX group demonstrated greater cortisol concentrations at rest and at all other time points postexercise after completing the training regimen compared with CLENEX horses (P < 0.05). Preexercise (SRT) peripheral blood monocyte number was lower in CLENEX horses than in other treatment groups (P < 0.05). Clenbuterol and exercise training did not significantly affect post-SRT changes in leukocyte numbers. Exercise training resulted in a decrease (P < 0.05) in the percentage of CD8+ cells post-SRT compared with other groups, but the percentage of CD4+ cells was not altered by either clenbuterol or exercise conditioning. Lymphocyte proliferative response was not affected by clenbuterol or exercise treatment. Horses demonstrated responses to bouts of acute exercise as noted with other species, namely humans and rodents.

Key Words: Adrenergic Agonist, Equine, Exercise, Exertion, Stress


Introduction

Horses subjected to a single bout of intense exercise respond with increased stress-associated hormone (i.e., cortisol) production and substantial alterations in cell-mediated immune responses (Horohov et al., 1999). This coordinated response includes the suppression of the lymphocyte proliferative response, the antibody response to equine influenza virus antigens and an increased capacity to generate lymphokine-activated killer (LAK) cell activity (Keadle et al., 1993). The severity of this effect depends in part on the intensity of exercise and prior conditioning of the subject (Hoffman-Goetz and Pedersen, 1994). We have previously investigated cellular responses and changes in soluble components of the immune system (such as IL-2) after acute physical activity in young and old unfit horses (Horohov et al., 1999; Guirnalda et al., 2001). Exercise caused a significant decrease in lymphoproliferative response and increase in LAK cell activity in younger horses (Horohov et al., 1999).

The effects of longitudinal exercise training (i.e., repeated bouts of acute exercise or conditioning) at moderate intensities and acute high-intensity exercise (simulated race test) on cell-mediated and humoral immune function have not been studied in horses. Clenbuterol is a β2-adrenergic agonist and bronchodilator that can be chronically administered to horses to treat the symp-
toms of inflammatory airway disease (Sasse and Hajer, 1978). Clenbuterol has been shown to have a suppressive effect on ovine humoral antibody response in vivo (Spencer and Oliver, 1996). There is little information on the interaction of chronic exercise training and clenbuterol administration. Therefore, we addressed this question by testing the hypothesis that clenbuterol and exercise training, and the combination of clenbuterol and training would alter cortisol and immune responses to acute exercise.

Materials and Methods

Animals and Drug Administration

Twenty-three untrained standardbred mares (10 ± 3 yr old, approximately 450 kg BW) were used. Mares were unfit (horses were physiologically similar to those reported in Kearns and McKeever, 2002), but accustomed to the laboratory and running on the treadmill before the start of the experiment. During the experiment, the horses were housed as a group on pasture. Mares were fed (as-fed basis) 2 to 4 kg of a commercially available grain pellet and 8 to 12 kg of alfalfa/grass hay to maintain BW. The daily ration was split into two feedings given at 0700 and 1500 with the grain portion of the ration providing approximately 3.09 Mcal/kg of DE (DM basis; 2.73 Mcal/kg as-fed basis) and approximately 18.3% CP (DM basis; 16.3% CP as-fed basis). Horses were given ad libitum access to salt and water and were weighed weekly, as well as before and after each exercise test. The Rutgers University Institutional Animal Care Review Board approved all methods and procedures used.

Horses were divided into four experimental groups. Clenbuterol plus exercise (CLENEX; n = 6) and clenbuterol alone (CLEN; n = 6) mares received 2.4 μg/kg BW of clenbuterol (Ventipulmin, Boehringer Ingelheim, Berkshire, U.K.) twice daily (average volume, 20 mL) on a schedule of 5 d on and 2 d off for 12 wk. Horses in the CLENEX and CLEN groups were maintained on clenbuterol for 12 wk until all exercise tests were completed. The CLENEX group also aerobically trained 3 d/wk for 8 wk. Exercise alone (EX; n = 5) mares aerobically trained for 3 d/wk, and the control group (CON; n = 6) remained sedentary. Both EX and CON horses were administered 20 mL of molasses twice daily for the full 12 wk experiment.

Graded Exercise Test and Simulated Race Test

The experiment presented herein was performed as a separate study testing a distinct hypothesis. However, it was part of a larger collaborative effort involving a series of pretreatment and posttreatment exercise tests (Kearns et al., 2001; Kearns and McKeever, 2002; Sleeper et al. 2002) that are outlined in Table 1. In the present experiment, immunological variables were measured on days that horses performed a simulated race test (SRT). Sampling for hormone analyses was conducted after an uninstrumented graded exercise test (GXT). Tests for each horse were separated by at least 1 wk. During the GXT and SRT, the animals ran on a high-speed horse treadmill (Sato I, Equine Dynamics, Inc., Lexington, KY) at a fixed 6% grade. The GXT started at an initial speed of 4 m/s for 1 min. Speed was then increased to 6 m/s, followed by incremental 1 m/s increases every 60 s until the horse completed the step of the GXT previously shown to elicit maximal oxygen uptake (VO2max) for that individual. The velocity that elicited VO2max was previously measured for each horse in a separate GXT (see Table 1) performed before the exercise tests in the current study (Kearns and McKeever, 2002). During the SRT, horses warmed up for 3 min at 3m/s, followed by 2 min at a speed calculated to elicit 110% VO2max. This was followed by a cool-down period of 3 min at 3m/s.

Pre- and post-SRT blood samples for immunological analyses were collected on d −17 and −16 before the beginning of the experimental treatment and on d 75 and 76 of treatment. Plasma cortisol was measured using RIA (Malinowski et al., 1990) from samples collected on d −10, −9, and −8 before the start of the experiment; on d 82, 83 and 84 during treatment; before exercise; and at 5, 10, 20, 40, 60, and 120 min post-GXT. All SRT and GXT were performed at the same time (between 0800 and 1000) to eliminate the effects of diurnal variation on endocrine and immune variables.

Aerobic Training Program

The exercise program consisted of continuous treadmill running 3 d/wk for 8 wk. Mares ran initially for 15 min/d at a work rate of 50% VO2max (approximately 4 to 6 m/s). After 1 wk, the duration was increased to 20 min/d and was held at this duration for the remainder of the study. Increased duration rather than increased speed was used to increase the training stimulus to prevent development of orthopedic problems (no orthopedic problems were observed at any point in the study). During the exercise training, the treadmill was set at a fixed 6% grade.

Immunological Variables

Peripheral blood leukocyte cell numbers and immune cell subsets (CD4+, CD8+) were measured via flowcytometric analysis (Guirnalda et al., 2001) before and after two SRT. The first SRT was performed before treatment and the second after treatment/training. Lymphocyte proliferative response to stimulation by mitogens (Sigma, St. Louis, MO) including phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM) was determined as previously described (Horohov et al., 1999).

Statistical Analyses

Data were analyzed by analysis of variance for repeated measures using the GLM procedure of SAS (SAS
Table 1. Timeline for testing conducted during the overall set of experiments

<table>
<thead>
<tr>
<th>Day of experiment</th>
<th>Test&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Variables measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>−35 to −31</td>
<td>Pre-GXT 1</td>
<td>Aerobic capacity, markers of performance, and hemodynamics</td>
</tr>
<tr>
<td>−28 to −26</td>
<td>Pre-ECT</td>
<td>Exercise capacity tests</td>
</tr>
<tr>
<td>−17 to −16</td>
<td>Pre-SRT</td>
<td>Immune variables</td>
</tr>
<tr>
<td>−10 to −8</td>
<td>Pre-GXT2</td>
<td>Echocardiographs and endocrine variables</td>
</tr>
<tr>
<td>1 to 56</td>
<td>Exercise training period</td>
<td></td>
</tr>
<tr>
<td>1 to 84</td>
<td>Clenbuterol administration</td>
<td></td>
</tr>
<tr>
<td>60 to 67</td>
<td>Post-GXT 1</td>
<td>Aerobic capacity, markers of performance, and hemodynamics</td>
</tr>
<tr>
<td>68 to 74</td>
<td>Post-ECT</td>
<td>Exercise capacity tests</td>
</tr>
<tr>
<td>75 to 81</td>
<td>Post-SRT</td>
<td>Immune variables</td>
</tr>
<tr>
<td>82 to 84</td>
<td>Post-GXT 2</td>
<td>Echocardiographs and endocrine variables</td>
</tr>
</tbody>
</table>

<sup>a</sup>GXT = graded exercise test; ECT = exercise capacity test; SRT = simulated race test.

Inst., Inc., Cary, NC). Preexercise data were used as a covariate for postexercise data if the group means, measured before acute exercise, before the start of the experiment were different. Post hoc comparison of means was performed using LSD tests, with rejection of the null hypothesis when \( P < 0.05 \). Least squares means ± SEM are reported.

Results

There were no significant group differences among any of the groups for any of the variables measured in the pretreatment SRT and GXT. Therefore, pretreatment data for plasma cortisol concentration, leukocyte number, lymphocyte subset number, and the lymphocyte proliferative response to acute exercise, were pooled (\( n = 23 \)) and analyzed to determine whether there were significant effects of acute exercise on the endocrine and immune variables measured during the experiment.

Effect of Acute Exercise on Plasma Cortisol and Immune Variables

Plasma cortisol concentration before and for 120 min postexercise is shown in Figure 1. Plasma cortisol concentration increased (\( P < 0.05 \)) by 5 min post-GXT and remained elevated for at least 120 min. The SRT resulted in an increase (\( P < 0.05 \)) in lymphocyte number postexercise (Figure 2). There was no significant difference in either monocyte or granulocyte number after acute exercise. Acute exercise resulted in a decrease (\( P < 0.05 \)) in the percentage of CD4+ cells and an increase in the percentage of CD8+ cells (Figure 3). The SRT resulted in decreased (\( P < 0.05 \)) lymphoproliferative response to PWM (Figure 4) but not PHA and ConA.

Effect of Clenbuterol Administration and Exercise Training

All horses displayed a post-GXT increase (\( P < 0.05 \)) in plasma cortisol concentration (Figure 5) in the posttreatment GXT that was similar in duration and magnitude to the response observed in the pretreatment period (Figure 1). Following treatment, however, the EX horses had greater (\( P < 0.05 \)) cortisol concentrations at rest and at all other time points post-GXT compared with CLENEX horses (Figure 5).

The CLENEX group had a lower (\( P < 0.05 \)) monocyte number before exercise (Table 2; data collected in posttreatment period) compared with other groups. Clenbuterol and exercise training did not significantly affect post-SRT changes in leukocyte numbers (Table 2). However, when the effect on peripheral blood cell subsets was examined, the EX group demonstrated a post-SRT decrease (\( P < 0.05 \)) in the percentage of CD8+ cells compared with other groups, but the percentage of CD4+ lymphocytes was not altered in either CLEN or EX groups (Figure 6). Lymphocyte proliferative response was not significantly affected by clenbuterol or exercise treatment.

![Figure 1](image-url) Effect of acute exercise (GXT, graded exercise test) on plasma cortisol concentration in Standardbred mares (\( n = 23 \)). Data are means ± SEM. Asterisks denote pre- and postexercise differences (\( P < 0.05 \)). Resting pre-GXT samples are plotted at 0 min on the graph, with post-GXT samples taken at 5, 10, 20, 40, 60, and 120 min. The GXT resulted in increased plasma cortisol by 5 min postexercise, which remained elevated at 120 min after exercise.
Clenbuterol and immune function

Figure 2. Effect of a simulated race test (SRT) on leukocyte number in standardbred mares (n = 23). Data are means ± SEM. The asterisk denotes an increase in lymphocyte number (P < 0.05) from pre- to postexercise.

Discussion

A frustrating health issue for horse owners is the fact that equine respiratory disease limits the horse’s athletic performance (Sasse and Hajer, 1978). The most common cause of respiratory disease is viral infection (Sasse and Hajer, 1978), and many horses also display allergy-induced respiratory distress (Sasse and Hajer, 1978). The mechanisms responsible for the greater incidence of upper respiratory disease among athletes are not known. However, it has been suggested that high ventilatory flow rates during exercise may adversely affect the mucosal surfaces of the upper respiratory tract, making the individual more susceptible to infection (Peters and Bateman, 1983). In horses, athletic performance or physical exertion has been implicated in microbial infection of the respiratory tract (Anderson et al., 1985; Sweeney et al., 1985). Aspects of nonspecific immunity, an important defense early in infection, may also be affected by exercise (MacKinnon, 1992). Mechanistically, it has been suggested that training-induced

Figure 3. Effect of a simulated race test (SRT) on CD4+ and CD8+ lymphocyte subset. Data are mean percentages of total lymphocytes ± SEM. Asterisks denote a decrease in CD4+ and increase in CD8+ lymphocytes (P < 0.05).

Figure 4. Effect of a simulated race test (SRT) on lymphoproliferative response to mitogens. Data are means ± SEM. Responses to concanavalin A (Con A), phytohemagglutinin (PHA), and pokeweed mitogen (PWM) are shown. The asterisk denotes decreased lymphoproliferative response to PWM only (P < 0.05).

Figure 5. Effect of chronic clenbuterol and exercise training (CLENEX), clenbuterol (CLEN), exercise training (EX), or control (CON) on plasma cortisol concentration after acute exercise (GXT). Resting pre-GXT samples are plotted at 0 min on the graph, with post-GXT samples taken at 5, 10, 20, 40, 60, and 120 min. Pooled SEM = 7.7 (CLEN and CLENEX) and 8.4 (EX and CON). Different letters denote differences between treatment groups. The EX horses demonstrated greater (P < 0.05) cortisol concentrations at rest and at all time points after exercise compared with CLENEX horses. Asterisks denote the increase (P < 0.05) in cortisol post-GXT in all treatment groups.
Table 2. Effect of chronic clenbuterol administration and exercise training on leukocyte number (mean ± SEM) in female Standardbred horses of racing age

<table>
<thead>
<tr>
<th>Item</th>
<th>CLENEX</th>
<th>CLEN</th>
<th>EX</th>
<th>CON</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocytes, 10⁶/mL</td>
<td>Preexercise</td>
<td>0.16 ± 0.02ᵇ</td>
<td>0.22 ± 0.02²</td>
<td>0.21 ± 0.02ᵇᶜ</td>
</tr>
<tr>
<td></td>
<td>Postexercise</td>
<td>0.26 ± 0.03ᵃ</td>
<td>0.29 ± 0.03</td>
<td>0.34 ± 0.04ᵃ</td>
</tr>
<tr>
<td>Granulocytes, 10⁶/mL</td>
<td>Preexercise</td>
<td>3.1 ± 0.3</td>
<td>3.1 ± 0.3</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Postexercise</td>
<td>3.7 ± 0.3</td>
<td>3.3 ± 0.3</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>Lymphocytes, 10⁶/mL</td>
<td>Preexercise</td>
<td>3.5 ± 0.2</td>
<td>3.2 ± 0.2</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Postexercise</td>
<td>4.8 ± 0.3ᵃ</td>
<td>4.6 ± 0.3ᵃ</td>
<td>4.9 ± 0.3ᵃ</td>
</tr>
</tbody>
</table>

ᵃDenotes differences (P < 0.05) due to acute exercise (pre-SRT vs. post-SRT).
ᵇᶜData were collected in the simulated race test (SRT) performed between d 75 and 81 (see Table 1).

CLENEX = chronic clenbuterol and exercise training (n = 6); CLEN = clenbuterol (n = 6); EX = exercise training (n = 5); and CON = control (n = 6).

*b,cTreatment means that do not have a common superscript differ, P < 0.05.

respiratory problems may result from chronic alterations in immune function (Bruin et al., 1994; MacKinnon and Hooper, 2000), both hallmarks of overtraining (Bruin et al., 1994; MacKinnon and Hooper, 2000). To our knowledge, longitudinal studies that have examined both cell-mediated and humoral aspects of immune function with exercise training are nonexistent with regard to equine athletes, and are limited and incomplete for human subjects. Furthermore, no studies of the horse have reported on the effect of an acute SRT on immune function. Thus, the current study presents new and important results, both on the effects of acute exercise similar to a race, as well as on the effects of exercise training on immune function in horses.

Limited data have been reported on the effects of exercise on immune function in the horse (Wong et al., 1992; Horohov et al., 1999). To our knowledge, the current study is the first published report on the effects of a SRT on various immune variables in horses. Our findings agree with previous reports in horses running to exhaustion; a 2-min exercise test at 110% VO₂max resulted in an increase in plasma cortisol concentration and an increase in lymphocyte number (Wong et al., 1992). However, the observation that monocyte and granulocyte numbers were not altered by the SRT was an unexpected finding because it was in contrast to observations made in humans exercised at high intensities (Hoffman-Goetz and Pedersen, 1994). Results from the current study are in agreement with reports in humans (Schaller et al., 1999) that demonstrated that acute exercise causes a decrease in CD4+ T-helper cells and an increase in CD8+ T-cytotoxic/suppressor cells. Such a response may be reflective of the different redistribution of lymphocyte subsets after acute exercise. In a report discussing an acute exercise-induced decrease in CD4⁺:CD8⁺, Fry et al. (1992) attributed the decreased value of the ratio to increases in CD3⁻, CD8⁺ cells (natural killer cells) and not necessarily CD3⁺, CD8⁺ T lymphocytes. Hinton et al. (1997) reported changes in the composition of total lymphocyte cultures, namely increases in both natural killer cells and T-suppressor cells following intensive exercise in humans. The decrease in CD4⁺:CD8⁺ resulted from a decrease in the percentage of CD4⁺ and an increase in the percentage...
of CD8+ cells (Hinton et al., 1997). Studies in other species have attributed a decrease in the ratio to a greater increase in CD8+ cells relative to increases in CD4+ cells. Rowbottom and Green (2000) implicated both catecholamines and cortisol as mediators of an exercise-induced leukocytosis observed during and after exercise.

Green et al. (2002) summarized reports that demonstrated exercise-induced suppression of T-lymphocyte responsiveness to mitogenic stimulation in humans. To investigate the potential occurrence of similar phenomena in equine athletes and to assess the effect of drug treatment on lymphocyte responses, standard mitogens were used that activate immune cell signaling pathways and thus cause activation in the absence of pathogen-derived antigens that are commonly employed to assess lymphocyte immunocompetence (Green et al., 2002). In contrast to research in humans (Green et al., 2002), Con A and PHA responses were not affected by exercise in the horses used in the current study. Interestingly, in the current study, SRT only affected lymphocyte proliferative response to stimulation with PWM. Concanavalin A and PHA, plant-derived lectins that show selectivity for T lymphocytes, were used to examine the ability of polyclonal T cell populations to proliferate on stimulation (Mackinnon, 1992). Such clonal expansion is necessary during the initiation of effective immune responses to pathogens. Pokeweed mitogen was used to examine B lymphocyte function in the presence of T cells and cells associated with innate immunity (Mackinnon, 1992). This method uses standard mitogens to activate immune cell signaling pathways (Mackinnon, 1992). The decrease in the percentage of CD4+ cells may be a factor contributing to the observed decrease in PWM responsiveness observed in this study. Smith et al. (1993) also cited the reversal of CD4+:CD8+ as a source of exercise-induced suppression of PWM responses in humans. Previous reports on the lymphocyte proliferative response to acute exercise in the horse suggest that both duration and intensity of exercise are important in elucidating a change in lymphocyte blastogenesis (Keadle et al., 1993; Horohov et al., 1999). It is possible that in the current study, the lack of a response may have been due to the relatively short duration, but high intensity, of SRT exercise.

Recently, veterinarians have acquired U.S. FDA clearance to use clenbuterol to relieve the symptoms of respiratory disease (Kearns and McKeever, 2002). Clenbuterol, a β2-adrenergic agonist, is a potent selective bronchodilator that initially was used as a drug to treat bronchospasm and to alleviate the symptoms of chronic obstructive pulmonary disease in horses (Sasse and Hajer, 1978). Bronchodilators, such as clenbuterol, relieve airway obstruction associated with inflammatory airway disease and work most effectively when combined with allergen and dust avoidance or corticosteroid administration (Kearns and McKeever, 2002). Clenbuterol has also been shown to improve clinical signs of bronchitis and pneumonia (Sasse and Hajer, 1978) and to increase mucociliary transport rate in both normal horses and horses with chronic obstructive pulmonary disease (Kiely, 1985; Kiely and Jenkins, 1985). However, whereas clenbuterol relieves the symptoms of respiratory disease, it does not improve the horse’s resistance to pulmonary infectious agents such as viruses and bacteria (Kastner et al., 1999). In fact, data from sheep demonstrate that β-adrenergic agonists, such as clenbuterol, may alter immune function in vitro (Spencer and Oliver, 1996). Data on the secondary humoral immune antibody response in vivo suggest that clenbuterol has a suppressive effect on humoral antibody response to infection (Spencer and Oliver, 1996). Those studies focused on chronic administration of clenbuterol. Unfortunately, most investigations of the effects of clenbuterol on cardiorespiratory function have only studied short-term (either one acute dose or following 5.5 d of dosing) administration of the drug, and none has looked at immune function (Rose et al., 1983; Rose and Evans, 1987; Slocombe et al., 1992).

The current study is thus the first to examine the effects of chronic administration of therapeutic doses of clenbuterol on immune function with or without the interaction of exercise training. As expected, all horses displayed a post-GXT increase in plasma cortisol after 8 wk of exercise training and 12 wk of clenbuterol administration. However, the EX horses demonstrated greater cortisol concentrations at rest (before exercise) and at all other time points post-GXT compared with the CLENEX horses. This may have been a response to the anticipatory effect of exercise but also a reflection of overtraining. However, given the relatively low intensity (approximately 50% of maximal aerobic capacity) and duration (20 min/d for 3 d/wk) of the current training protocol, it is unlikely that these horses were overtrained (30 to 60 min of work at 70 to 80% of VO2max or greater, five to six times per week for several months). Data are conflicting in the literature regarding the effect of training on cortisol in horses (McKeever and Gordon, 2004). This is important because glucocorticoids themselves are potent immunosuppressors in humans (Claman, 1987; Munck et al., 1987; Jefferies, 1991) and in horses (Malinowski et al., 1990; Keadle et al., 1993). Several studies have shown that acute exercise causes a stress-induced increase in glucocorticoids, and that the increase is followed by suppression of immune function (Keadle et al., 1993; Horohov et al., 1999). Our data are in contrast with a recent study by Marc et al. (2000), who demonstrated that maximal plasma cortisol response after treadmill exercise was less in trained compared with untrained horses following 10 wk of training. The fact that CLENEX horses did not show greater cortisol concentrations similar to the EX group may be due to the downregulation of the β-adrenergic receptors at the level of the adrenal. Downregulation of β-adrenergic receptors has been shown in rat skeletal muscle following clenbuterol administration (Torgan et al., 1993; Castle et al., 2001).
Functionally, changes in cortisol concentration could potentially be important in the regulation of immune function.

Another important aspect of the current study is the effect of clenbuterol administration and training on immune function. Our results indicated that exercise training and clenbuterol administration alone had no effect on postexercise leukocyte number. However, monocyte number was lower in the CLENEX horses at rest and training resulted in a lower number of CD8+ cells after the SRT in the EX group. The later observation is consistent with studies in humans that have demonstrated a decrease in natural killer cells with training (MacKinnon, 1992; Rowbottom and Green, 2000). A decrease in natural killer cells might account for a drop in CD8+ cells. Clenbuterol administration may have blocked this training-induced decrease in CD8+ cells in the CLENEX group; however, we have no mechanism to explain such an effect.

Results from the current study also demonstrate that acute high-intensity exercise resulted in an increase in plasma cortisol concentration, which remained elevated through 2 h of recovery. Furthermore, this response seems to be exacerbated with training, an adaptive response that may be important physiologically because during exercise, cortisol functions as a metabolic hormone insuring adequate mobilization of energy substrates and the maintenance of blood glucose concentrations (McKeever and Gordon, 2004). Such a response to increase blood glucose concentration has been shown to prevent to onset of central mechanisms of fatigue (Farris et al., 1995). Cortisol also functions to suppress exercise-induced increases in various inflammatory cytokines. Thus, an enhancement of the cortisol response may also have functional significance for the equine athlete during exercise by playing an important postexercise role in the prevention of the inflammation associated with delayed onset muscle soreness (McKeever and Gordon, 2004).

Finally, some trainers and veterinarians have suggested that clenbuterol can be used in a preventative fashion to prevent airway disease (Kearns and McKeever, 2002). However, recent studies have demonstrated that chronic administration of therapeutic doses of clenbuterol has been shown to have negative effects on aerobic capacity, cardiac function, and the ability to tolerate high-intensity exercise (Kearns et al., 2001; Kearns and McKeever, 2002; Sleeper et al., 2002). The SRT used in the current study resulted in decreased lymphoproliferative response to PMW only, which may have important implications for the establishment of humoral immune responses. However, clenbuterol and exercise training did not affect postexercise changes in leukocyte numbers or lymphocyte proliferative response in a positive or negative fashion. These observations may be important when deciding how to manage the health of the equine athlete that trains on a regular basis, and who also may be treated for inflammatory airway disease with clenbuterol.

**Literature Cited**


Clenbuterol and immune function