

# Influence of Anabolic Steroids on the Response to Training of 2 Year Old Horses

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**ABSTRACT** Six fillies and 6 geldings (2 years old) in 3 groups were administered intramuscularly with Control, 0.02 ml arachis oil; Low Dose, 0.55 mg boldenone  $\text{kg}^{-1}$ ; High Dose, 2.2 mg boldenone  $\text{kg}^{-1}$  BW every 3 weeks, and trained intensively for 6 weeks. During standard exercise tests (SET) before and after 1, 2, 4, and 6 weeks of training HR, respiratory and blood lactate (La) responses were measured. There were no interactions between training, or sex, and the boldenone treatment. HR responses to SET with training were limited to an increase in  $V_{200}$  and  $V_{80\%HR_{max}}$  while LA responses as measured by  $V_{LA,4}$  did not change.  $\dot{V}O_2$  responses were essentially unaffected except for  $\dot{V}O_{2max}$ , which increased with training. The results suggest that boldenone, under the conditions of this experiment, had no beneficial effect during training.

*Key words:* Boldenone; exercise; heart rate; lactate; oxygen uptake; horses.

## INTRODUCTION

Anecdotal evidence from many sources suggests that the use of anabolic steroids is widespread in the racehorse population, particularly in the preparation of yearlings for sale and horses for racing. Objective data on the value, or otherwise, of anabolic steroids in the preparation of young horses are limited, as are those on the value of the drugs in the training of normal horses. Administration of anabolic steroids to young stallions was highly detrimental to semen quality, sperm production and output, testis size and serum luteinizing hormone (LH) concentrations.<sup>14</sup> Equivalent findings of small ovaries, fewer oestrous cycles and depressed plasma concentrations of LH, gonadotrophin and progesterone occurred in yearling mares treated for 54 weeks.<sup>5</sup> These changes, plus clitoral enlargement and abnormal sexual behaviour, were found to persist for periods of at least six months in some cases after cessation of the treatment.<sup>15</sup> For both stallions and mares intended for breeding purposes the use of anabolic steroids was contraindicated.

The limited data available indicate that, when administered to healthy horses, anabolic steroids do not increase weight gain, muscle development or athletic performance.<sup>11–13</sup> The purpose of the present study was to measure the effects if any, of boldenone administered every 3 weeks, since 9 months of age, during two year old training.

## MATERIALS AND METHODS

*Experimental design.* Twelve 2 year old horses (6 fillies and 6 colts) treated every 3 weeks with boldenone (17 $\beta$ -hydroxy-androsta-1,4-diene-3-one 10 undecanoate) since 9 months of age were used for this study. The colts were castrated when they were 2 years of age and entered the experiment as geldings. The horses were maintained in the same treatment groups (2 fillies and 2 geldings per group) as they had been when previously administered boldenone: Controls, Low Dose and High Dose steroids. This 3 $\times$ 2 factorial design with two replicates allowed the measurement of training, dose rate and sex effects, and any interac-

tions between these effects and the responses to training. The treatments were administered by intramuscular injection as follows:

Control: 0.02 ml arachis oil  $\text{kg}^{-1}$  every 3 weeks, Low Dose: 0.55 mg boldenone  $\text{kg}^{-1}$  every 3 weeks, High Dose: 2.2 mg boldenone  $\text{kg}^{-1}$  every 3 weeks.

These treatments were continued until the horses had completed the training programme.

The horses were fed a commercial 'working horse ration' containing 9.5 MJ  $\text{kg}^{-1}$  digestible energy and given sufficient food for maintenance, growth and exercise. The amount was increased as the work load increased according to the guidelines of the National Research Council.<sup>6</sup> The horses were weighed weekly on each Monday morning before being fed.

*Training schedule* The horses were acclimatised to treadmill exercise and then submitted to a 2 week conditioning period of walking, trotting and cantering for up to 20 min per day, followed by a 6 week, intensive training programme. The first 2 weeks of training consisted of distance cantering at 5–6  $\text{m s}^{-1}$  on Mondays, Tuesdays, Thursdays and Fridays, with a fast gallop on Wednesdays. The fast gallop was for 1 min at 10  $\text{m s}^{-1}$  in the first week and for two 1 min intervals at that speed in the second week with a 1 min recovery trot between. In subsequent weeks the fast gallops occurred on Tuesdays and Thursdays with distance cantering on Mondays, Wednesdays and Fridays. In week 3 there were two 10  $\text{m s}^{-1}$  gallops on Tuesday and Thursday, after which the number of gallops on these days was increased by 1 per week and the speed by 0.5  $\text{m s}^{-1}$  per week. In the sixth week each horse completed five 1 min gallops at 11.5  $\text{m s}^{-1}$ , with 1 min recoveries between. Distance cantering commenced with 20 min at the start of the first week and was incremented by 1 min per day so that at the end of 6 weeks they were cantering for 40 min per day. All training was with the treadmill set at 6% slope until distance cantering exceeded 30

min duration after which the treadmill was horizontal. Throughout the training period, Saturdays and Sundays were rest days on which the horses had approximately 20 min of light cantering (4–5  $\text{m s}^{-1}$ ) on a level treadmill.

As it was logistically impossible to train and test 12 horses simultaneously, they were divided into 2 groups of 6 which included a filly and a gelding from each treatment group. The first 6 were trained in February and March and the second 6 in May and June.

*Testing schedule* To assess the response to training, the horses were submitted to a standardised exercise test (SET), at 6° slope on the treadmill, before and after the first, second, fourth and sixth weeks of training. This test, conducted on Mondays, was of a continuous incremental type with an initial warm up of a 2 min walk and a 2 min trot, followed by sequential intervals of 1 min each at 7, 8, 9, 10, 11 and 12  $\text{m s}^{-1}$ , or until the horse could not maintain the speed of the treadmill despite humane persuasion.

During the test the horses wore a face mask to collect the expired gases. The fibreglass mask had 2 inlet and 2 outlet ports fitted with valves to ensure a one-way air flow. It was attached closely to the horse's head with a leather sleeve and a band of foam rubber to prevent air leakage. The ports were 80 mm in diameter and were situated as close as possible to the nostrils. Flexible tubing carried the expired air from the outlet ports to a mixing chamber and then on to a flow sensor.<sup>4</sup> Before passing the flow sensor the temperature of the air was measured electronically with an AD590 temperature sensor (Analogue Devices, Norwood, MA) and continuously sampled for  $\text{O}_2$  and  $\text{CO}_2$  analyses (Normocap, Datex Instrument Corp., Helsinki). The output of the sensors was recorded, after appropriate amplification, on computer discs.

From these data, the expiratory minute volume ( $\dot{V}_E$ ), oxygen uptake ( $\dot{V}\text{O}_2$ ),  $\text{CO}_2$  output ( $\dot{V}\text{CO}_2$ ) and respiratory exchange ratio (R) were calculated by formulae 1–3:<sup>3</sup>

## Formulae 1-4

$$\dot{V}_E \text{ STPD} = \frac{\dot{V}_E (1 \text{ min}^{-1}) \text{ ATPS} * (P_B - P_{H_2O})}{760 * (1 + (0.00367 * T))} \times \frac{1000}{\text{BW}} \quad 1$$

(ml min<sup>-1</sup> kg<sup>-1</sup>)

$$\dot{V}O_2 = \dot{V}_E \text{ STPD} * (0.265 * (1 - F_E O_2 - F_E CO_2)) - F_E O_2 \quad 2$$

(ml min<sup>-1</sup> kg<sup>-1</sup>)

$$\dot{V}CO_2 = \dot{V}_E \text{ STPD} * F_E CO_2 \quad 3$$

(ml min<sup>-1</sup> kg<sup>-1</sup>)

$$R = \frac{\dot{V}CO_2}{\dot{V}O_2} \quad 4$$

where ATPS = ambient temperature and pressure saturated, STPD = standard temperature and pressure dry,  $P_B$  = atmospheric pressure,  $P_{H_2O}$  = water vapour pressure,  $F_E CO_2$  and  $F_I CO_2$  = fractional expired and inspired  $CO_2$ ,  $F_E O_2$  and  $F_I O_2$  fractional expired and inspired  $O_2$  concentrations, BW = horse body weight in kg.

Heart rate (HR) was monitored via an elasticised girth strap fitted with brass plate electrodes on the sternum, the left wither and mid-right thoracic wall. Using a proprietary gel for skin contact the electrodes were connected to an electrocardiograph beside the treadmill.

Before each test and during the last 15 s at each speed, HR,  $\dot{V}O_2$ ,  $\dot{V}CO_2$  and  $\dot{V}_E$  were recorded and jugular blood samples collected, via an indwelling catheter, for determination of lactate concentration [LA]. Samples for [LA], also collected at 2, 5 and 15 min after the test, were preserved immediately with fluoride-EDTA and stored on ice until analysed (Monotest Lactat 149 993, Boehringer Mannheim) within 2 to 3 hours of collection. HR and  $\dot{V}O_2$  were considered to have reached maximum values, for this protocol, when they failed to increase with a further increase in treadmill speed or when the horse could no longer maintain the treadmill speed.

*Statistical analyses* The data for each variable obtained during the SET, except [LA], which was first log transformed, were analysed by simple linear regression. These analyses were used to obtain the slope, intercept and the derived variables  $V_{200}$  (the speed producing HR of 200 bpm),  $V_{80\%HR_{max}}$  (the speed producing HR of 80% of the horse's maximum HR) and  $V_{LA4}$  (the speed producing a blood lactate concentration of 4 mmol l<sup>-1</sup>).

These data were then analysed by a split plot Analysis of Variance and partitioned for factorial effects of dose (of steroid), sex, interaction, and any interactions between these effects and the split effect of time (stage of training). Differences between means of significant dose and/or sex, and interaction effects were tested using Scheffé's method<sup>10</sup> for multiple comparisons based on the Analysis of Variance. Interactions between dose and time were of particular interest as these would indicate whether horses were responding differently over time, depending on the treatment received. Differences between means of significant time effects were tested using paired *t*-tests. Differences were not considered significant if  $p > 0.05$ . Results are expressed as mean  $\pm$  SD unless otherwise indicated.

The effects of dose and sex was reparti-

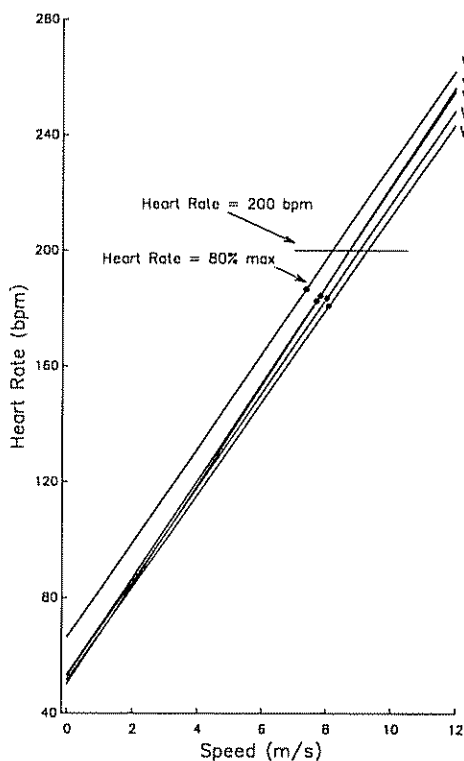


Fig. 1. Mean heart rate responses of 6 fillies and 6 geldings to standard exercise tests (SET) before and after 1, 2, 4 and 6 weeks of training.

tioned into training periods (Feb/March vs May/June), subjected to a split plot Analysis of Variance, and found to be of no significance.

## RESULTS

**Body weight** Mean body weight at the start was  $445 \pm 28.2$  kg, and decreased ( $p < 0.001$ ) by  $30.5 \pm 8.53$  kg after training to  $414.5 \pm 28.3$  kg. This change occurred in the first week, during which the horses sustained a mean loss ( $p < 0.001$ ) of  $26.0 \pm 5.8$  kg to  $419.0 \pm 30.1$  kg. During the remainder of the training programme body weight fluctuated randomly. There were no dose, sex, or dose  $\times$  sex interaction effects on the body weight changes.

**Heart rate** During SET there were no dif-

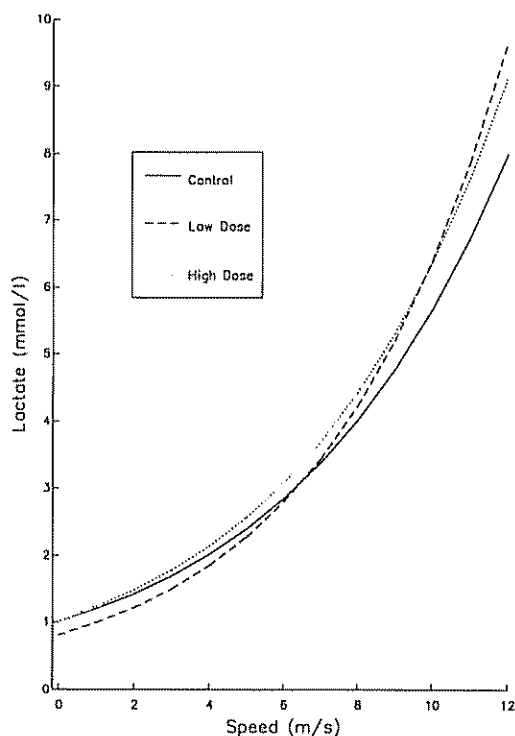


Fig. 2. Mean blood lactate concentration responses measured after 45 s at speed of 4 horses in Control (0.02 ml arachis oil), Low dose (0.55 mg boldenone  $\text{kg}^{-1}$ ) and High dose (2.2 mg boldenone  $\text{kg}^{-1}$ ) groups to standard exercise tests (SET).

ferences in the rate of HR increase due to dose and/or sex. The mean regression intercept before training was greater than that thereafter ( $p < 0.05$ ). During training there was no further change in the intercepts (Fig. 1). Mean maximal heart rate ( $\text{HR}_{\text{max}}$ ) did not change significantly with training and ranged from  $233.2 \pm 6.4$  at the start to  $229.3 \pm 5.0$  after 6 weeks training.

**Lactate** There was no dose and/or sex effects on rates of increase of [LA]. The regression intercepts of [LA] of the Low dose group was significantly ( $p < 0.01$ ) lower than that of either the High dose or Control groups. There was no training effect on the rates of increase of [LA] or the regression intercepts (Fig. 2).

**Oxygen uptake** The control horses had a greater rate of increase in  $\text{VO}_2$  than the Low

Table 1. Mean values ( $\pm$  SD) for  $V_{200}$  and  $V_{80\%HR_{max}}$  ( $m s^{-1}$ ) for 12 horses before and after 1, 2, 4, and 6 weeks of training

|                    | Week of training  |                     |                   |                   |                   |
|--------------------|-------------------|---------------------|-------------------|-------------------|-------------------|
|                    | 0                 | 1                   | 2                 | 4                 | 6                 |
| $V_{200}$          | 8.18 <sup>a</sup> | 8.73 <sup>b</sup>   | 8.71 <sup>b</sup> | 9.25 <sup>c</sup> | 9.02 <sup>c</sup> |
|                    | 0.45              | 0.53                | 0.52              | 0.56              | 0.37              |
| $V_{80\%HR_{max}}$ | 7.35 <sup>a</sup> | 7.80 <sup>b,c</sup> | 7.67 <sup>b</sup> | 8.05 <sup>c</sup> | 7.80 <sup>c</sup> |
|                    | 0.33              | 0.52                | 0.54              | 0.48              | 0.34              |
| <i>n</i> (horses)  | 12                | 12                  | 11                | 12                | 12                |

Means within rows with the same superscript are not significantly different ( $p > 0.05$ ).

dose horses ( $p < 0.05$ ). Neither the rates of increases nor the regression intercepts were affected by training. Mean maximal  $\dot{V}O_{2max}$  values of  $143.5 \pm 15.1$  ml (STPD)  $min^{-1} kg^{-1}$  at the start of training had increased significantly ( $p < 0.001$ ) to  $168.4 \pm 18.6$  ml (STPD)  $min^{-1} kg^{-1}$  after 6 weeks, an increase of 17.4%.

*Carbon dioxide output and respiratory exchange ratio* There were no effects of dose, sex or training, or interactions on the rates of increase or the regression intercepts of either  $\dot{V}CO_2$  or R.

$V_{200}$ ,  $V_{80\%HR_{max}}$  and  $V_{LA4}$  Neither  $V_{200}$ ,  $V_{80\%HR_{max}}$  or  $V_{LA4}$  showed dose and/or sex effects, while  $V_{200}$  and  $V_{80\%HR_{max}}$  showed a training effect (Fig. 1). The mean  $V_{200}$  increased by  $0.5 m s^{-1}$  during the first week of training and later between weeks 2 and 4 (Table 1). The peak  $V_{200}$  reached by all horses after 4 weeks of training was maintained until the end of the experiment. At all stages during training,  $V_{80\%HR_{max}}$  was significantly higher than that before training, increasing by  $0.4 m s^{-1}$  after 1 week of training and not changing significantly thereafter (Table 1).

## DISCUSSION

The results suggest that the administration of the anabolic steroid boldenone was of no benefit to the horses in this study, and the

effects of training were the same irrespective of sex or anabolic treatment.

The drop of some 6.8% of body weight during training with the greater portion occurring during the first week is consistent with observations from an earlier training study (Persson, S.G.B., personal communication.). This is a marked and consistent effect of the training and probably reflects its intensity. When food is unrestricted, losses of body weight are not a feature of training in man<sup>1</sup> or horses<sup>16</sup> unless the training is excessive or prolonged. Weight reduction through loss of body fat as occurs with human endurance training<sup>7</sup> may partly explain the observed changes. The horses were considered to be in good condition after being fed hay and horse pellets for several weeks while in the paddock prior to the training period. It was also likely that the observed weight loss could be partly ascribed to a reduction in large bowel content resulting from reduced dietary fibre, increased peristaltic activity and elevation of muscular tone in the abdominal wall.

The only significant change in the HR responses to SET were a reduction in the regression intercept after the first week and increases in  $V_{200}$  and  $V_{80\%HR_{max}}$ . There was no influence on this response by either sex or steroid treatment. This increase in  $V_{200}$  is consistent with earlier reports of its use as an index of response to training.<sup>8,17,18</sup> While

$V_{200}$  may not be a good measure of a horse's aerobic capacity,<sup>9</sup> %HRmax is highly correlated to % $\dot{V}O_2$ max.<sup>2</sup> While  $\dot{V}O_2$ max was only available before and at the end of training, it did increase significantly as did  $V_{80\%HRmax}$ . That HRmax did not increase with training is consistent with an earlier cited report.<sup>9</sup>

Lactic acid showed typical responses to the incremental work of SET but did not show the expected training response of  $V_{LA4}$  previously described.<sup>17,18</sup>

The effect of boldenone on training responses of  $\dot{V}O_2$  was a reduction of its rate of increase in the Low Dose horses. An earlier study<sup>17</sup> failed to demonstrate a training response in  $\dot{V}O_2$ max while recent work<sup>2</sup> found a significant response in Thoroughbreds after a 12 week training programme. The mean increase in  $\dot{V}O_2$ max of the horses in the present study was of a similar magnitude to that reported elsewhere<sup>2</sup> and represents the major adaptation by the horses to the training programme.

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