EVALUATION OF BOLDENONE UNDECYLENATE AS AN ANABOLIC AGENT IN HORSES

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Introduction

The parenteral administration of testosterone and other steroids to castrate dogs, rats and intact human males has been reported to increase gains in body weight and nitrogen retention (3, 5, 6) but in addition to the anabolic effects, there were undesirable androgenic side effects. In efforts to find compounds with improved efficiency and reduced side effects, many steroids that possess high anabolic activity and low androgenic activity have been synthesized (1, 7) and some are available as anabolic agents to the medical profession. In veterinary practice an anabolic steroid would be of value in restoring weight losses in animals, particularly horses debilitated from overwork, unthriftiness, or anorexia. The difficulties of conducting adequately controlled studies with such animals have resulted in many reports on restoration of weight loss that are based on clinical impressions rather than on well-designed experimental trials with adequate controls.

The present experiments were conducted to determine the effects in horses of boldenone undecylenate (17 β-hydroxyandrosta-1, 4-dien-3-one, 17-(10-undecenate)),[1] a potent, long-acting anabolic steroid. The parameters studied were weight gain, nitrogen retention, hemoglobin concentration, hematocrit, and blood serum levels of calcium and phosphorus.

Materials and Methods

Tests for Gain in Body Weight

Trial 1

To determine the effect of boldenone undecylenate on weight gain, 10 geldings, weakened by heavy work and in a debilitated condition, were purchased at a local livestock market. Many of the horses had multiple saddle sores and leg lacerations from being transported. Fecal samples obtained before the animals had been placed on test showed the presence of strongyles. All of the horses were treated with thiabendazole[2] as a top dressing on the feed.

Throughout the trial, the horses were allowed medium quality, mixed hay *ad libitum*. A concentrate,[3] prepared especially for horses, was fed individually to each horse at one-half the recommended level (0.50 kg/100 kg BW). The amount of concentrate fed was adjusted after each weighing period.

The horses were randomly assigned to two groups of five horses each. One group was given a single injection of boldenone undecylenate in sesame oil (containing 25 mg/ml) at 0.125 mg/kg BW, IM on Day 1 of Week 1. Two weeks later, a second dose of 0.5 mg/kg BW was administered IM. The other group was given sesame oil IM, at equivalent volumes, as a placebo.

Body weights were recorded on the day of initial treatment and every two weeks thereafter. Prior to each weighing the horses were deprived of water and feed for approximately 20 hours. Blood samples for hemoglobin[4] and hematocrit[5] determinations were collected from each horse five days before the initial treatment and on each weigh day thereafter. All data were subjected to analysis of covariance.

Trial 2

Thirty-three horses, 18 geldings and 15 mares, were purchased at a local livestock market. Data for three of the mares, which were pregnant, were subsequently eliminated. On arrival they were administered a single 10 ml IM injection of procaine penicillin G and

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[1]Equipoise™ – E. R. Squibb & Sons, Inc., P.O. Box 4000, Princeton, New Jersey 08540.

[2]Equizole® – Merck & Co., Inc., Rahway, New Jersey 07065.

[3]Agway, Inc., Buffalo, New York 14240.

[4]Hb-Meter, American Optical Company, Buffalo, New York. 14240.

[5]Microhematocrit method, as cited in Veterinary Hematology. O. W. Schalm, p. 61. Lea & Febiger, Philadelphia, 1961.

dihydrostreptomycin.[6] During an acclimation period of approximately four weeks, the horses were treated for strongyles using thiabendazole in the feed.

The horses in this trial were randomly assigned to three groups according to age, weight and physical appearance. One group served as a control and received two IM injections of sesame oil as a placebo. The second group was given two IM injections of boldenone undecylenate in sesame oil (containing 25 mg/ml) at 0.275 mg/kg BW. The third group was given two injections of the drug in sesame oil (containing 50 mg/ml) at 1.10 mg/kg BW IM. Injections were given on Day 1 of Week 1 of the experiments and again two weeks later. Blood samples were taken from the same two horses (one male and one female in each group) before the injections of drug, on the day of each treatment, and at the end of the study. The sera were analyzed for calcium and phosphorous spectrophotometrically.[7]

During the first two weeks of treatment the horses were fed individually at 1.35 kg/100 kg BW divided into two daily feedings. During the subsequent two weeks, because of improved appetite, the daily ration was increased to 1.50 kg/100 kg BW. The ration consisted of a concentrate[8] and mixed hay of average quality, fed in equal proportions.

Body weights were recorded weekly following overnight deprivation of food and water. Body weight deviations during the treatment period were subjected to analysis of variance to estimate variation. Dunnett's multiple comparison test was used to determine statistical significance of treatment versus control comparisons.

Test for Nitrogen Retention

Four geldings and four fillies, ten to 12 months of age and weighing 184 to 284 kg, were used in this trial. During a 34-day pretreatment adjustment period, the animals were first fed hay and a complete pelleted ration.[9] The hay component of the diet was gradually decreased until the horses had been consuming the complete feed for 22 days before collections were initiated.

Protein intake was calculated according to the National Research Council recommendations (9), so that the diet was neither excessive nor deficient in protein for growing horses. The horses were given 3.8 g of protein/kg BW. Horses were fed twice daily and given water *ad lib.* The urine and feces of the geldings were collected while they were in metabolism stalls (10). Each filly was catheterized with an 80 ml retention catheter. Total daily urine and fecal collections were made. After each was thoroughly mixed, aliquots (urine 10% and feces 20%) were taken for analysis. Nitrogen was determined by Kjeldahl digestion (2) and colorimetric analysis.[10] After urine and feces had been collected, the horses were exercised by walking for 25 minutes each day in a mechanical exerciser.

After the adjustment period, individual control urine and fecal collections were made daily for five days. After the collection on the sixth day, each of the horses was given an IM injection of boldenone undecylenate, 1.10 mg/kg BW. Urinary and fecal nitrogen concentrations were determined individually on the seventh through 13th days and on composited samples on Days 14–15, 19–20, and 24–25. On Day 26 the horses were again injected with boldenone undecylenate in the same manner as described previously. Individual nitrogen concentrations in urine and feces were determined on Days 26 through 31 and composited on Days 34–35, 38–39 and 43–44.

The data were analyzed for statistical significance by the method of least squares analysis of variance.

Results

Tests for Gain in Body Weight

Trial 1

The horses treated with boldenone undecylenate gained an average of 70.0 kg, compared with 53.8 kg for the placebo-treated group (Table I). The difference was not statistically significant and was attributed to variability among the horses. No differences in hemoglobin and hematocrit values were found.

Trial 2

The results of this trial are presented in Table II. When data for both sexes were combined the horses treated with boldenone unde-

[6]Distrycillin® — E. R. Squibb & Sons, Inc., P.O. Box 4000, Princeton, New Jersey 08540.

[7]Clinical manual for the Spectronic 20, Bausch & Lomb, Inc., Rochester, New York 14603.

[8]Trim and Choice (50:50 ratio), Agway, Inc., Buffalo, New York 14240.

[9]Big-um — Ralston Purina Co., St. Louis, Missouri 63155.

[10]Technicon Auto Analyzer — Technicon Instruments Corporation, Tarrytown, New York 10591.

TABLE I

EFFECT OF BOLDENONE UNDECYLENATE[a] ON WEIGHT GAIN IN DEBILITATED HORSES[b]
TRIAL 1

Treatment[c]	Initial Mean Body Weight	Gain In Mean Body Weight (weeks) 2	4	6	8	Total	Final Mean Body Weight
	(kg)	kg					(kg)
Control	371.4±24.5	14.8	17.0	12.8	9.2	53.8±23.7	425.2±23.7
Boldenone undecylenate	349.0±37.1	32.6	12.4	18.8	6.2	70.0±29.4	419.0±29.4

[a]Equipoise™—E. R. Squibb & Sons, Inc., Princeton, New Jersey 08540.
[b]Values are means for five geldings±standard error of the mean
[c]Treatment, 0.125 mg/kg, IM, on day 1 of week 1 and 0.50 mg/kg, IM, 2 weeks later. Drug was in a sesame oil solution containing 25 mg/ml. Controls received equivalent volumes of sesame oil, IM.

TABLE II

EFFECT OF BOLDENONE UNDECYLENATE[a] ON WEIGHT GAIN IN DEBILITATED HORSES[b]
TRIAL 2

Treatment[c]	Dose	Initial Mean Body Weight	Change in Mean Body Weight (Weeks) 1	2	3	4	Total
	mg/kg IM	(kg)	kg				
Castrated Males							
Control	—	410.7±32.8	−0.7	−6.7	6.2	−3.5	−4.7 ± 6.0
Boldenone undecylenate	0.275	429.7±48.8	3.5	−9.5	6.0	−1.9	−1.8 ± 4.7
Boldenone undecylenate	1.10	406.8±35.3	3.5	−3.3	5.5	2.2	7.8 ±12.2
Females							
Control	—	378.7±15.5	1.6	−7.6	4.3	−1.7	−3.3 ± 2.0
Boldenone undecylenate	0.275	415.6±27.0	1.2	−2.4	4.4	0.6	3.8 ± 5.0
Boldenone undecylenate	1.10	407.5±40.6	1.7	−3.7	3.5	3.0	4.5 ± 4.9
Combined Sexes							
Control	—	400.0±32.0	0.1	−7.0	5.6	−2.9	−4.22± 5.0
Boldenone undecylenate	0.275	423.3±41.0	2.4	−6.3	5.3	−0.7	0.73± 5.6
Boldenone undecylenate	1.10	407.1±31.9	2.8	−3.5	4.7	2.5	6.50±10.1[d]

[a]Equipoise™—E. R. Squibb & Sons, Inc., Princeton, New Jersey 08540.
[b]Values are means of 6 geldings and 3, 5 and 4 females per treatment, respectively±standard error of the mean. Unequal distribution of sexes result in slight differences for the combined values.
[c]First injection on day 1 of week 1, followed by a similar injection 2 weeks later. Drug was in a sesame oil solution containing 25 mg/ml and 50 mg/ml for the 0.275 mg/kg and the 1.10 mg/kg groups, respectively. Controls received equivalent volumes of sesame oil, IM.
[d]Significantly different from controls ($p<0.05$).

cylenate, 1.10 mg/kg BW, showed significantly ($p<0.05$) greater gains in body weight than did the horses in the control group. There were no significant differences in results between animals given the higher and lower doses of boldenone undecylenate or between the controls and those given the lower dose of drug.

There were no apparent effects of drug treatment on serum concentrations of calcium or phosphorus.

Test for Nitrogen Retention

Nitrogen balance values are given in Table III. The data show that horses treated with boldenone undecylenate had improved nitrogen retention. The increase in nitrogen retained was statistically significant ($p<0.05$) during the interval between the 12th and 25th days after the first injection when compared with the nitrogen retained during the pretreatment control period. The second injection

TABLE III

EFFECT OF BOLDENONE UNDECYLENATE[a] ON NITROGEN RETENTION IN GROWING HORSES

Days	Means[b]
	(g/day)
Pre-treatment	
1–6	40.67
Post-treatment[c]	
7–11	47.53
12–25	52.33[d]
26–30	44.05
31–44	35.44

[a]Equipoise™-E. R. Squibb & Sons, Inc., Princeton, New Jersey 08540.

[b]Values are the least square means for grams of nitrogen retained by 4 geldings and 4 fillies.

[c]Boldenone undecylenate injections (1.10 mg/kg/BW IM) were given after urinary and fecal collections on days 6 and 25.

[d]Significantly different from the 1–6 day control period ($p < 0.05$).

of boldenone undecylenate did not increase the amount of nitrogen retained.

DISCUSSION

In clinical trials with horses, increased weight gains and better physical performance were observed after injections of boldenone undecylenate (11). A chemical precursor of this drug, methandienone, was used for various ailments in race horses. Animals treated with it showed an improvement in appetite and good progress in their performance (12). The data obtained in the present tests for gains in body weight suggest that boldenone undecylenate increases such gains in debilitated horses. The horses in the first trial were fed *ad lib* but the difficulties of obtaining horses that were uniformly debilitated suggested the technique of feeding individually by body weight in the second trial thereby minimizing variation. Therefore, the magnitude of the changes between the two trials is considered to be due to nutritional intake. This feeding according to body weight no doubt contributed to the generalized loss of weight in Week 2 for Trial 2. As all the animals improved in condition under our management it is likely that their intake was not adequate. Increasing the feed intake (which was possible due to improved health and well-being) permitted the animals to resume their body weight gains. The control animals, however, lacked the stimulatory effects of boldenone undecylenate and lost weight over the entire experimental period.

Androgenic side-effects have been reported for boldenone undecylenate. Stihl (11) in his studies reported that in some cases a temporarily increased libido was observed in geldings. This occurred following two treatments ten days apart. Dontas *et al* (4) also reported increased libido in human females following three treatments. No undesirable side effects were noted in our tests. Nevertheless, androgenic side effects may be expected with overdosing.

Calcium and phosphorus retention are often associated with the administration of anabolic steroids (7). It is important that a drug should not affect calcium and phosphorus in younger animals as mobilization of these minerals may lead to early closure of the epiphyses and thus to stunted growth. Although measurements were made on only two young horses, boldenone undecylenate appeared to have no effect on serum calcium or phosphorus. These results agree with those obtained in humans where administration of boldenone undecylenate to elderly human subjects (4) had no effect on serum phosphorus and, although levels of serum calcium appeared to be reduced, they were within normal physiological limits. Hematocrit values of human subjects were not affected by boldenone undecylenate (4) nor were those of the horses in this study.

Boldenone undecylenate caused a significant increase in nitrogen retention after the first injection, but not after a second injection given 19 days after the first. There are probably two factors responsible for the lack of effect of the second injection. First, the daily consumption of protein was adequate but not in excess, for the average weight gain during the entire test was only 3 kg. It is most likely that under these circumstances a new and higher baseline of nitrogen equilibrium had been established after the first treatment and the amount of dietary protein was not adequate to cause further increase in nitrogen retention after the second injection. Secondly, the catheters caused cystic and urethral irritation in the females toward the end of the trial period as reflected by the presence of blood in the urine, which led to stress. Some negative nitrogen retention was observed such as might be expected during a period of stress (8).

SUMMARY

Boldenone undecylenate, a long-acting anabolic steroid, was tested in horses to study the effect of the drug on weight gains and nitrogen retention.

Two trials were conducted with debilitated horses. In the first trial, the intramuscular (IM) administration of the drug at 0.125 mg/kg body weight (BW) and two weeks later at 0.50 mg/kg BW resulted in an apparent but non-significant increase in weight gains. Hematocrit and hemoglobin concentration were not affected. In a second trial, two IM injections of boldenone undecylenate at 1.10 mg/kg BW two weeks apart resulted in a significant increase in body weight gains.

In a nitrogen retention study, two IM injections of this anabolic steroid 19 days apart at a level of 1.10 mg/kg BW significantly improved nitrogen retention in young horses after the first injection. Nitrogen retention was not significantly increased after the second injection most likely due to a higher baseline of nitrogen retention and stress.

Résumé

Les auteurs ont étudié l'effet de l'undécylénate de boldénone, un stéroïde anabolique à longue action, sur le gain de poids et la rétention de l'azote, chez le cheval.

Ils effectuèrent deux expériences avec des chevaux malades. Dans la première, l'injection intra-musculaire de ce stéroïde, à raison de 0.125 mg/kg et, deux semaines plus tard, à la dose de 0.50 mg/kg, résulta en un gain de poids apparent, mais peu appréciable. Ces injections n'affectèrent pas les valeurs de l'hématocrite ni celles de l'hémoglobine. Au cours de la seconde expérience, deux injections intramusculaires du stéroïde, à raison de 1.10 mg/kg, à deux semaines d'intervalle, produisirent une augmentation appréciable du gain de poids.

Au cours d'une expérience sur la rétention de l'azote, deux injections intra-musculaires de ce stéroïde anabolique, à 19 jours d'intervalle, à raison de 1.10 mg/kg, améliorèrent de façon appréciable la rétention de l'azote, chez les jeunes chevaux, après la première injection. Aucune augmentation significative de la rétention de l'azote ne se produisit après la deuxième injection, vraisemblablement à cause d'un seuil plus élevé de la rétention de l'azote ou d'un "stress".

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