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Pharmacokinetics of boldenone and stanozolol and the results of quantification of anabolic and androgenic steroids in race horses and nonrace horses

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Anabolic steroids (ABS) boldenone (BL; 1.1 mg/kg) and stanozolol (ST; 0.55 mg/kg) were administered i.m. to horses and the plasma samples collected up to 64 days. Anabolic steroids and androgenic steroids (ANS) in plasma were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The limit of detection of all analytes was 25 pg/mL. The median absorption ($t_{1/2\partial}$) and elimination ($t_{1/2e}$) half-lives for BL were 8.5 h and 123.0 h, respectively, and the area under the plasma concentration–time curve (AUC_0^h) was 274.8 ng·h/mL. The median $t_{1/2e}$ for ST was 82.1 h and the AUC_0^h was 700.1 ng·h/mL. Peak mean ($\bar{X} \pm SD$) plasma concentrations (C_{max}) for BL and ST were 1127.8 and 4118.2 pg/mL, respectively. Quantifiable concentrations of ABS and ANS were found in 61.7% of the 988 plasma samples tested from race tracks. In 17.3% of the plasma samples two or more ABS or ANS were quantifiable. Testosterone (TES) concentrations mean ($\bar{X} \pm SE$) in racing and nonracing intact males were 241.3 ± 61.3 and 490.4 ± 35.1 pg/mL, respectively. TES was not quantified in nonracing geldings and female horses, but was in racing females and geldings. Plasma concentrations of endogenous 19-nortestosterone (nandrolone; NA) from racing and nonracing males were 50.2 ± 5.5 and 71.8 ± 4.6 pg/mL, respectively.

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INTRODUCTION

Anabolic steroids (ABS) are synthetic derivatives of the male hormone testosterone (TES) that have been modified to improve their anabolic rather than androgenic actions. The anabolic effects are considered to be those promoting protein synthesis, muscle growth, and erythropoiesis (Mottram & George, 2000). They can exert strong effects on the human body that may be beneficial for athletic performance (Hartgens & Kuipers, 2004). Published information is available on human subjects which suggests improvement in the strength skills following the administration of ABS (Freed *et al.*, 1975; Alen *et al.*, 1984; Elashoff *et al.*, 1991; Kadi *et al.*, 1999; Gonzalez *et al.*, 2000). On the other hand, many have concluded that ABS do not increase muscle size or strength in males with normal gonadal function and have discounted positive results as unduly influenced by biased expectations of athletes, inferior experimental design, poor data analysis, or at best inconclusive results (Elashoff *et al.*, 1991; VanHelder *et al.*, 1991; Kuhn, 2002). In the absence of testing for these agents in some sports, a popular assumption and anecdotal information have led some to consider that many athletes do use ABS to improve athletic performance.

Anabolic steroids have been employed extensively in equine practice over the past 25 years. Their usefulness is largely dependent on subjective opinions, as only minimal studies have been carried out in horses (Snow, 1993).

The purpose of the study was to describe the pharmacokinetics of two commonly used ABS, BL, and ST following i.m. administration. This study also focused on the use of plasma not urine samples for quantifying and confirming the presence of ABS and ANS in competing horses.

METHODS

Experimental animals

The study was approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Two groups of Thoroughbred horses were used. Boldenone (1,4-androstadien-17β-ol-3-one) undecylenate was administered i.m. to seven Thoroughbred horses, six geldings and one female, 7.1 ± 1.9 (SD) years old, and weighing 517.4 ± 50.8 kg. Stanozolol (17-methyl-2′*H*-5(α)-androst-2-eno[3,2-*c*] pyrazol-17(β)-ol) was administered to six Thoroughbred horses, four geldings and two females, 8.5 ± 2.5 (SD) years old, and weighing 567.2 ± 43.9 kg. A single dose of BL as undecylenate ester form (1.1 mg/kg) in sesame oil (Equipoise, Fort Dodge Animal Health, Fort Dodge, IA, USA) and ST (0.55 mg/kg; Red Cross Drugs, Blanchard, OK, USA) were administered i.m.. The stanozolol (ST) formulation was an aqueous suspension containing sodium chloride, polysorbate 80, paraben, water, and sodium carboxymethylcellulose.

Horses were brought into the stall 2 days before the experiment, remained housed for the first 48 h, and then were returned to pasture for the remainder of the study. The horses used were no longer actively racing but were otherwise in good health and were routinely de-wormed and vaccinated.

Collection of administered samples

Prior to placing a 14 French catheter (Angiocath, Becton Dickinson, Sandy, UT, USA) into the jugular vein for collection of blood samples, the area was clipped, washed with sterile water and surgical soap (Chlorhexidine gluconate 4%, Purdue Fredrick Co., Stamford, CT, USA), and rinsed with bacteriocide (Chlorhexidine diacetate, Fort Dodge Health, Fort Dodge, IA, USA) and 70% isopropyl alcohol. The area of catheter placement was infiltrated with a local anesthetic agent (Lidocaine HCl 2%, Vedco, St Joseph, MO, USA). All studies started at 7 AM, blood samples were collected prior to the i.m. administration (0) and at 2, 4, 6, 8, 10, 12, 24, 36, 48, 72, 96, 168, 216, 264, 336, 408, 504, 600, 720, 864, 1032, 1200 and 1536 h (64 days) postadministration. After the initial 48-h sample collection period, samples were collected in pasture by direct venipuncture between 7 and 9 AM. Blood samples were collected in glass tubes containing potassium oxalate (20 mg) and sodium fluoride (25 mg), centrifuged (2500–3000 rpm or 776–1318 ***g***) at 4 °C for 15 min to obtain plasma and 2 mL aliquots of plasma were immediately frozen at −20 °C and within 24 h were stored at −70 °C until analyzed. Each aliquot was used once to eliminate any effects of freezing and thawing on the concentrations of BL and ST.

Collection of race track samples

Races for ABS and ANS testing were selected randomly and represented the blood samples from winning and other horses selected for testing from races at Pennsylvania Thoroughbred and Standardbred race tracks. Blood samples were collected in vacuum tubes (Monoject, Sherwood Medical, St Louis, MO, USA) containing potassium oxalate (20 mg) and sodium fluoride (25 mg). Track samples are refrigerated upon collection, remain refrigerated until shipped overnight. Plasma was then separated from red blood cells by centrifugation at the laboratory. Stability studies showed equine samples were stable for 24 h at ambient temperature and 13 days at 4 °C (Guan *et al.*, 2005).

Nonrace track samples

Samples available from on-going behavioral endocrinology projects and clinical service at The University of Pennsylvania were from stallions, mares, and geldings considered to be free of ABS treatment and were assayed for ANS for comparison with race track samples. These animals included Standardbred, Thoroughbred, warm-bloods, and pony breeds.

Quantification of ABS and ANS in equine plasma

Quantification of ABS and ANS in equine plasma was based on methods previously described by our laboratory (Guan *et al.*, 2005, 2006). A sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was used for detection, quantification, and confirmation of eight major ABS and ANS, boldenone (BL), ST, TES, nandrolone (NA), trenbolone (TRB), normethandrolone (NMH), methandrostenolone (METH), and the designer steroid tetrahydrogestrinone (THG) in equine plasma. The analytes were extracted from equine plasma using methyl *t*-butyl ether (MTBE), separated on a chromatography column (Ace C_8 column, 2.1 × 50 mm, Mac-Mod Analytical, Chad Fords, PA, USA), and detected by tandem mass spectrometry using multiple-reaction monitoring.

Analyses were performed using LC-MS/MS, consisting of a pump, in-line degasser, auto-sampler, and a triple-stage quadrupole mass spectrometer equipped with electrospray ionization probe (Thermo Electron Corporation, San Jose, CA, USA). The limit of quantification of the method was 25 pg/mL for all analytes. The limit of confirmation (LOC) for the ABS and ANS that specifically identify the analytes were the lowest concentration confirmable based on LC retention time and product ion intensity ratio for each analyte. The LOCs will vary based on the structure of the molecule and were 25 pg/mL for BL; 50 pg/mL for NMH, NA, and METH; and 100 pg/mL for TES, THG, TRB, and ST.

Standard Operating Procedures employed by our laboratory for quantification of the analytes meet requirements for accreditation by the American Association for Laboratory Accreditation and ISO 17025 International Guidelines. The method is sensitive, reproducible, and fast for quantification of ABS and ANS in equine plasma.

Pharmacokinetic analysis

Plasma concentration vs. time curve for each horse was analyzed using standard linear compartmental analysis following the i.m.

injection of BL and ST (Boston *et al.*, 1981; Wastney *et al.*, 1999; Stefanovski *et al.*, 2003). A two-compartment absorption and elimination model was used to describe the pharmacokinetics of BL, where k_a and k_e were absorption and elimination rate constant, respectively [Simulation, Analysis and Modeling Software (SAAM), http://www.WinSaam.com]. Stanozolol was fitted to a one-compartment elimination model.

The weights $W(K)$ applied in this study, used the fractional standard deviation (FSD) of the data, and were in the form $W(K) = 1/C \times QO(K)^2$, where $QO(K)$ is the kth observed datum and C was an initially assigned FSD. The weights applied are proportional to the inverse of the QO. The FSD is the SD of the estimate of a parameter divided by the estimate of the parameter. As the fitting process continues the assigned FSD was lowered and the data refitted. Following each fit the FSD was calculated and fitting process repeated until the improvement in the sums of squares of the last iteration was <1%. In the FSD weighting scheme the smaller data points more heavily influence the fit.

The absorption ($t_{1/2\partial}$) and elimination ($t_{1/2e}$) half-lives were calculated as the natural log (base$_2$) divided by k_a and k_e fractional rate constants. The AUC_0^h of BL and ST from 0 to last hour was calculated by the trapezoid rule. Time to maximum plasma concentration (T_{max}) and the maximum plasma concentration (C_{max}) were determined directly from the experimental data.

Statistical analysis

Pharmacokinetic parameter estimates of BL and ST were expressed as median and range and plasma concentrations were expressed as mean ($\bar{X}$) and standard deviation. Nonparametric Wilcoxon and Kruskal–Wallis rank sum test was used for statistical comparisons of pharmacokinetic parameters (Powers, 1990) and ANOVA was used for parametric analysis (JMP Version 4.0, SAS Institute Inc., Cary, NC, USA). Significance was indicated when $P < 0.05$. The ABS and ANS concentrations from track and nontrack samples were expressed as $\bar{X}$ and standard error.

RESULTS

Boldenone

Absorption from the muscle injection site and subsequent decline of the plasma concentration–time curve of BL was described by a two-compartment absorption and elimination model (Fig. 1). Estimates of the pharmacokinetic parameters are shown in Table 1. BL was quantifiable in all seven horses at 30 days with a mean plasma concentration of 58.5 ± 16.5 pg/mL. BL was still quantifiable in three of seven, two of seven, and one of seven horses at days 43, 50 and 64, with mean plasma concentrations of 46.2 ± 10.7, 42.6 ± 3.7 and 27.0 pg/mL, respectively. The mean FSD of the BL estimated fractional rate constants was 0.026 ± 0.018.

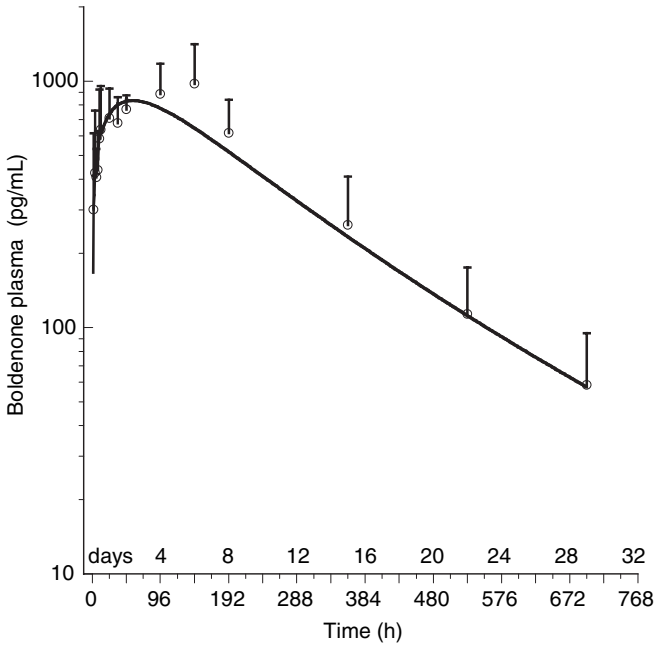

Fig. 1. Plasma concentration of boldenone (BL) following i.m. injection of 1.1 mg/kg. Solid line is the best fit for BL plasma concentration from individual horses. The data points represent the mean and standard deviation of seven horses.

Table 1. Pharmacokinetic parameter estimates [median and range following a single i.m. injection of boldenone (1.1 mg/kg) and stanozolol (0.55 mg/kg)]

Parameter	Median	Range
Boldenone		
k_a (/h)	0.082	0.024–0.715
$t_{1/2\partial}$ (h)	8.48	1.1–28.9
k_e (/h)	0.0056*	0.0026–0.0063
$t_{1/2e}$ (h)	123.0	109.2–265.1
AUC_0^h (ng·h/mL)	318.1†	214.8–438.4
C_{max} (ng/mL)	1027.0‡	679–1704
T_{max} (h)	144	48–192
Stanozolol		
k_e (/h)	0.009*	0.007–0.012
$t_{1/2e}$ (h)	82.1	59.0–99.5
AUC_0^h (ng·h/mL)	700.1†	510.8–900.7
C_{max} (ng/mL)	3796.5‡	3469–5889
T_{max} (h)	168	

k_a, fractional absorption rate constant; k_e, fractional elimination rate constant; $t_{1/2\partial}$, absorption half-life; $t_{1/2e}$, elimination half-life; AUC_0^h, area under the plasma concentration–time curve, 0 to last hour; C_{max}, maximum plasma concentration; T_{max}, time to maximum plasma concentration.
Significant difference: *$P < 0.002$; †$P < 0.001$; ‡$P < 0.001$.

Stanozolol

Absorption from the muscle injection site and subsequent decline of the plasma concentration–time curve of SL was described by a one-compartment elimination model (Fig. 2). Estimates of the pharmacokinetic parameters are shown in Table 1. There was a

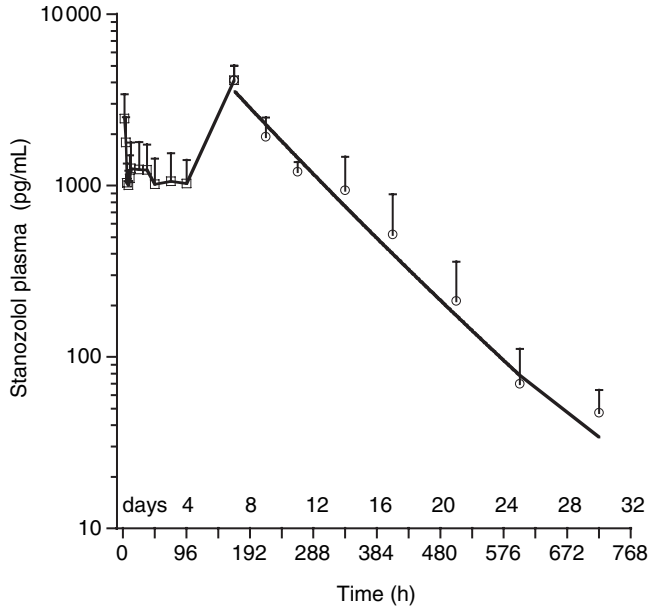

Fig. 2. Plasma concentration of stanozolol (ST) during absorption and elimination phase following i.m. injection of 0.55 mg/kg. Solid line is the best fits for elimination of ST from plasma. Data represents the mean and standard deviation of six horses.

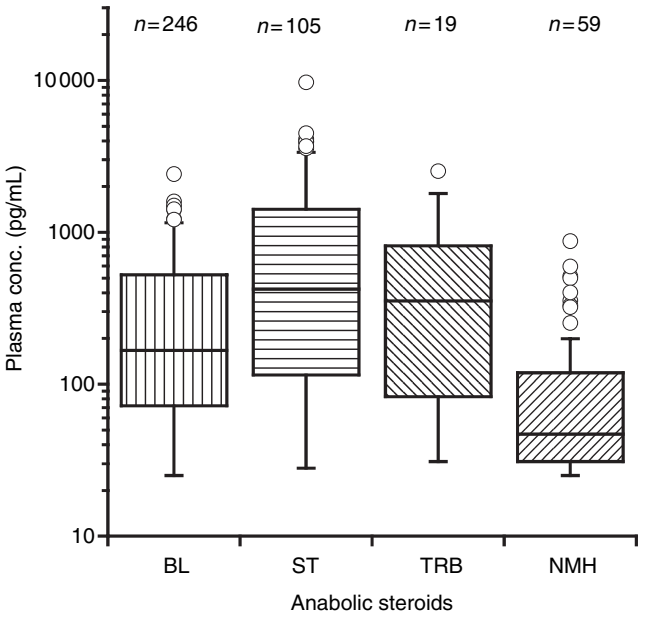

Fig. 3. Box plot of the plasma concentration range of anabolic steroid (ABS); boldenone (BL), stanozolol (ST), trenbolone (TRB), and normethandrolone (NMH). Each box contains 50% of the measured concentrations with the median. The top and bottom of each box mark the limits of ±25% of the variable population. The extending lines are the minimum and maximum concentration. Individual points (open circles) are outliers. The number of ABS quantified in each group is indicated.

rapid absorption of ST from injection site noted at the 2- and 4-h measurement periods with mean plasma concentrations of 2461.7 ± 961.6 and 1788.3 ± 719.6 pg/mL, respectively. There was a subsequent decline in plasma concentrations, between 6 and 96 h. Mean plasma concentration during this period was 1107.9 ± 141.2 pg/mL. Plasma concentrations were at their highest at 168 h (day 7) at 4118.2 ± 889.5, followed by a decline to 65.4 ± 17.3 at 720 h (day 30). Stanozolol was still quantifiable in two horses at 864 h (day 36) and in one horse at 1032 h (day 43). The mean FSD of the ST estimated fractional rate constants was 0.010 ± 0.003.

Track samples

Plasma samples from PA Thoroughbred and Standardbred race tracks were tested for ABS and ANS. Of the 988 plasma samples tested 610 (61.7%) had quantifiable concentrations of an ABS or ANS (Fig. 3). Of these 610 plasma samples 106 samples (17.3%) had two or more quantifiable ABS or ANS. The designer steroid tetrahydrogestrinone and the ABS mibolerone and methandrostenolone were not detected. Fifty-four of the racing horses screened were identified as intact males (Table 2). Testosterone mean plasma (±SE) concentration in racing females and geldings was 912.8 ± 226.5 pg/mL.

Nonrace track horses

Plasma samples (121) for the measurement of TES and NA were collected from 30 nonracing stallions 2 years and older between May 2002 to June 2004 (Table 2). There was a statistically significant ($P < 0.001$) seasonal variation in the plasma concentration of TES. Samples collected in May and June had plasma concentration of 722.9 ± 47.7 pg/mL compared with 225.8 ± 21.2 pg/mL in samples collected in September, December, and January. Nandrolone was not quantified in all plasma samples from the intact males (Table 2). No quantifiable concentrations of TES or NA were present in 47 plasma samples collected from six nonracing geldings and 38 mares.

Table 2. Plasma concentrations of testosterone (TES) and nandrolone (NA) from racing and nonracing intact males ($\bar{X} \pm SD$)

Horses groups	Number of samples	TES (pg/mL)	Number of samples	NA (pg/mL)
Racing	54	241.3 ± 61.3*	37	50.2 ± 5.5†
Nonracing	30	490.4 ± 35.1*	24	71.8 ± 4.6†

Significant difference between horses groups: *$P < 0.001$; †$P < 0.004$.

DISCUSSION

Considerable information is available on the metabolism of ABS and TES in the horse, including detection, identification, and excretion of ABS metabolites in urine (Houghton, 1977; Houghton & Dumasia, 1979, 1980; Dumasia *et al.*, 1983; Houghton *et al.*, 1984, 1986; Weidolf *et al.*, 1988; Teale & Houghton, 1991). Similar information is available from studies in humans (Shimazaki, 1994; Schanzer *et al.*, 1996; Vos *et al.*,

2002; Tseng *et al.*, 2005). However, limited information is available on the pharmacokinetics and plasma concentrations of ABS in the horse and other species. The analytical method that we developed and reported enables quantification of plasma ABS and ANS for use in PK studies and forensic testing.

Radioimmunoassay was used for monitoring plasma concentrations of NA in the dog (Macdougall & Jondorf, 1989). Nandrolone phenylpropionate was detected by radioimmunoassay in both plasma and urine of the horse approximately 12–13 days following a single injection and the detection period was increased to approximately 23–25 days following weekly injections (Chapman *et al.*, 1982).

The results of this study showed an extended period of elimination with $t_{1/2e}$ of 123.0 h and 82.1 h, for BL and ST, respectively. The prolonged elimination and short absorption phase suggest a 'flip-flop model' in which the elimination was determined by the rate of transfer of BL and ST from the injection site to plasma. The longer elimination in the case of BL was related to the undecylenate and sesame oil formulation and its possible slow hydrolysis in muscle tissue. The rapid absorption of ST (Fig. 2) followed by a slower absorption for up to 96 h was an unexpected pattern, but may have related to the aqueous suspension. The ST in solution was absorbed rapidly and the suspended material was absorbed more slowly over time.

In published studies plasma NA concentrations were influenced by different esters and injection sites, with higher and earlier peaks following the administration of phenylpropionate ester, compared with the decanoate ester. From these results, the investigators concluded that vehicle, side chain ester, the injection site, and the volume of NA in oil had an influence on bioavailability (Minto *et al.*, 1997). It is probable that the absorption rate of the esters from the injection depot is the rate-limiting process in the pharmacokinetics of these compounds (van der Vies, 1993). The peak plasma concentrations of ST occurred in all horses at 168 h postadministration and were apparently related to the collection schedule. As a result of the absorption pattern of ST, describing the kinetics of the absorption was difficult and had little value, therefore only the terminal half-life was determined for ST which was significantly shorter than BL.

The benefits of the therapeutic use of ABSs remain questionable and numerous side effects have been reported in the horse (Beroza, 1981). Possible therapeutic value of ABS described in an older veterinary pharmacology textbook include the correction of tissue-depleting process after systemic disease, malnutrition, parasitism, or wasting disease of old age; to speed tissue repair following surgery or trauma; to promote nonspecific stimulation, increase appetite and vigor (McDonald, 1977). A more current veterinary pharmacology textbook does not discuss ABSs from a therapeutic point of view, but only from the regulatory aspects (Sundlof, 2001). Many practicing veterinarians attest to the gains in physical strength, stamina, and mental attitude when used in performance horses. This especially true in horses that have gone off-feed, and have a 'stale' or 'sour' attitude. The apparent improvement in athletic performance may be more of a change in behavior and aggressiveness than any specific effects on the physiologic parameters that affect performance.

Administration of TES or ABS to castrated male horses was based on the perception that castration will lead to reduction in muscle mass and other changes which may be reversed by the administration of ANS or ABS (Blanchard, 1985). However, experimental evidence to substantiate these anecdotal observations was not substantive or at best conflicting.

Nandrolone phenylpropionate given in conjunction with a training program in Thoroughbred geldings did not result in changes in muscle fibers when compared with the control group of horses without the ABS (Nimmo *et al.*, 1982). Measurement of body weight, nitrogen retention, and plasma urea following the administration of NA phenylpropionate failed to demonstrate consistent anabolic effects in resting horses (Snow *et al.*, 1982a). Similarly, in a crossover study no changes in body weight, body measurements, hematology, plasma and urine chemistry, or evidence of improved racing performance in racing horses were found; behavioral changes attributed to the drug were observed for at least 6 weeks after the cessation of treatment (Snow *et al.*, 1982b). Trotters treated with NA showed increased aggression, red blood cell volume, and percentage of type II fibers, but no measurable changes in exercise performance (Hyyppa *et al.*, 1994). A similar observation was reported in 2-year-old trotters in training following the administration of BL (Thornton *et al.*, 1991). Nandrolone administration increased the rate of muscle glycogen repletion after exercise that suggests a shorter recovery period following a race (Hyyppa, 2001). Scientific opinions in this area range from mixed to some improvement in performance in mares and geldings treated with NA or BL (Stihl, 1968; Dawson & Gersten, 1978), to equivocal results (Dietz *et al.*, 1974).

Deleterious effects of ABS administration reported in humans, include sterility, gynecomastia in males, acne, balding, psychological changes, and increased risks of heart disease, liver neoplasia, sudden cardiac death, and a reduction in certain immune responses (Hughes *et al.*, 1995; Sullivan *et al.*, 1998). Impairment in reproductive function in fillies and stallions attributable to the administration of ABS has been suggested (Maher *et al.*, 1983; Blanchard, 1985). Sperm count and mobility, scrotal size, and testicular parenchyma in stallions were affected by administration of exogenous TES (Berndtson *et al.*, 1979; Squires *et al.*, 1981). Adrenal insufficiency was diagnosed in a gelding that had received monthly injections of ST for 8 years. Findings in this horse suggest that ABSs may influence the hypothalamic-pituitary axis in horses (Dowling *et al.*, 1993).

The impetus for testing for ABS and ANS was the common knowledge of the use and the observation made by the commission veterinarians of prerace aggressive behavioral problems in many horses. Behavioral effects following the administration of ABS and ANS have been supported by a number of studies. In female horses large doses of exogenous TES eventually caused total suppression of all reproductive activity and the development of stallion-like behavior and aggression (Turner & Irvine, 1982). Following the administration of ABS to

geldings and mares, the authors observed many aspects of stallion behavior that indicated residual androgenic activity of ABS (Snow *et al.*, 1982a). A number of studies in mares have established that ABSs and TES treatment induce stallion-like behavior characterized by teasing, mounting, and aggressive behavior toward other horses (McDonnell *et al.*, 1986, 1988). Administration of ABS did not result in increase in growth and size of the horse when compared with controls (Maher *et al.*, 1983). Subtle arguments have been made that TES may be beneficial in horses that have been gelded, thereby returning these horses to prior behavior and performance status. This argument has not been substantiated and is certainly not valid for administration to mares.

Testing of blood samples from PA tracks was a survey of the use of ABS and ANS and the concentrations reported do not indicate dose or frequency of administration, but does confirm the rather extensive use of ABS in racehorses in the US. Boldenone, ST, and TES were the three most commonly quantified anabolic–androgenic steroids (ANS). Some of the race track horses that tested positive for TES were intact males and the plasma concentration of TES and NA were significantly lower than those of the nonrace track intact males (Table 2). The lower concentrations of TES and NA in racing intact males may be related to several factors; track survey was conducted (January though April 2004) in the months when the concentrations are expected to be lower, the general lack of breeding stimulus, many of these males had one or more quantifiable ABS, and athletic competition can also influence TES concentrations in the male (Tremblay *et al.*, 2004). Anabolic steroids administered to intact males would contribute to the lower TES concentration.

In nonrace track and race track males 19-nortestosterone (NA) was quantified confirming the observations of others in which plasma samples collected from testicular and jugular veins of intact males demonstrated a testicular secretion of 19-nortestosterone that is synthesized in the testis (Benoit *et al.*, 1985; Dintinger *et al.*, 1989). In mares, 19-norsteroids as intermediates have also been detected in follicular fluid (Shimazaki, 1994).

Nandrolone was not detected in nonrace track geldings or mares, and therefore, its presence in racing geldings or mares was due to exogenous administration of NA. Other possible sources of NA are human nutritional supplements containing 19-norandrostenedione and 19-norandrostenediol that can alter the 19-norsteriod urine profile in the horse (Russell & Maynard, 2004). The impact of the nutritional supplements on circulating biologically active NA and long-term effects are unclear in the horse (Dehennin *et al.*, 2002). Nutritional supplements containing dehydroepiandrosterone and androstenedione are weak androgens. A change in the urine androgen profile of the mare and gelding and transient increase of circulating free TES was reported suggesting possible conversion to more potent TES anabolic/androgenic action by these supplements (Dehennin *et al.*, 2001).

The enzymatic hydrolysis of equine urine samples from the intact male horse resulted in the detection of 19-norandrost-4-ene-3,17-dione and 19-nortestosterone. This may be due to an artifact of the analytical processes and decarboxylation solvolysis procedure on precursors found in urine (Houghton *et al.*, 2004). This process is unlikely to occur in plasma due to the absence of enzymatic hydrolysis and the more gentle procedure used for the extraction of ABS and ANS from plasma.

Basal plasma concentrations of TES in horses with one or two testes ranged from 65 to 1600 pg/mL (Cox *et al.*, 1973) and a relationship between plasma TES concentration and age, breeding season and harem size was suggested (Khalil Ashraf *et al.*, 1998). Our sampling of nonrace track intact males showed a similar range of plasma concentrations of TES.

To date, the United States appears to be the only country with most horse racing jurisdictions ignoring the presence of ABS, compared with European and Asian counterparts that monitor and issue stiff penalties for the use of ABS in equine athletes. Anabolic steroids were added to the list of controlled substances in 1991 under the Anabolic Steroids Control Act. Certain veterinary products fall under this act and have been reclassified as Schedule III drugs. These include BL, mibolerone, ST, TES, and trenbolone and their esters and isomers (Sundlof, 2001). Despite these restrictions ABS are easily obtained.

As a result of the long elimination time, the regulation of ABS would be difficult in parts of the United States where interstate movement of horses from one race track to another is common. Based on this pattern of racing and movement of horses from one state to another, it is important that the control be regionalized or nationalized. The results of this study suggest the withdrawal period may be >6 weeks, a period greater than a minimum of 42 days withdrawal period that was suggested over 25 years ago (Chapman *et al.*, 1982).

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