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# Long-Term Chronic Treatment with Stanozolol Lacks Significant Effects on Aggression and Activity in Young and Adult Male Laboratory Mice

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**ABSTRACT. 1. Repeated doses of the anabolic-androgenic steroid stanozolol were assessed for their effects on agonistic behavior, motor activity, and body weight in both young and adult male laboratory mice.**

**2. Stanozolol significantly increased weight gain in young, but not older subjects, especially at the highest doses.**

**3. There were, however, no significant differences in motor activity or in ethologically assessed social behavior (including aggression) in young or adult mice.** GEN PHARMAC 27;2:293–298, 1996.

**KEY WORDS.** Stanozolol, anabolic-androgenic steroids, aggression, activity, body weight, male mice

## INTRODUCTION

Anabolic–androgenic steroid (AAS) abuse is widespread in sport (Lamb, 1984; Tricker *et al.*, 1989; Yesalis *et al.*, 1993) to increase muscle mass, strength, power, and competitiveness and to diminish training fatigue. In recent years, there has been an increasing use by adolescents and young people to enhance physical appearance in countries such as the United States and United Kingdom (Komoroski and Rickert, 1992; Du Rant *et al.*, 1993; Williamson, 1993; Korkia and Stimson, 1994; Morrison, 1994).

These substances have several adverse actions. Physical side effects include increased cardiovascular disease, fluid retention, and hypertension, as well as reduced liver function, testosterone, and gonadotropin levels and spermatogenesis. Other effects such as infectious diseases, specifically hepatitis B and HIV infection, are related to drug adulteration (Nemechek, 1991). Also, several effects on behavior and mental health, including the development of dependency have been described (Wandler, 1994). Among the more important psychological effects are an altered libido, changes in mood, psychotic episodes, and increased irritability and aggressive behavior (Choi *et al.*, 1989; Conacher and Workman, 1989; Moro *et al.*, 1990; Hannan *et al.*, 1991; Dalby, 1992; Choi, 1993; Su *et al.*, 1993; Pope and Katz, 1994). It has been reported that the use of AAS increases subjective perceptions of aggression and irritability (Strauss *et al.*, 1983, 1985; Pope and Katz, 1990). Thus, Choi *et al.* (1990) employing psychometric evaluations, found that AAS users were significantly more hostile and aggressive than nonusers. Lefavi *et al.* (1990) found that steroid users exhibited more frequent episodes of anger, with these episodes being more intense and for longer periods of time, and they were also more likely to show violence and to lack self-control. The increases in aggression include descriptions of violent assault, attempted murder, and, in extreme cases, homicide (Conacher and Workman, 1989; Pope and Katz, 1990). However, most of these studies are descriptions of cases with important methodological problems. To establish the association between AAS and aggression in humans, studies with an appropriate experimental control are required, which presents important difficulties mainly derived from the fact that, in most cases, the users illegally take multiple drugs simultaneously (stacking) and very varied ranges of doses. These reasons have motivated a claim about the need to use animal models to study the effects of these substances on aggressive behavior. Research utilizing appropriate animal models can be critical to understand the causes and consequences of anabolic steroid abuse in humans (Svare, 1990). This approach would permit the extension of the knowledge about the effects of androgens and their derivates on aggression. In fact, recent studies have begun to use different animal models to analyze the effects of specific AAS. Lumia *et al.* (1994) have found that long-term exposure to testosterone propionate potentiates intermale aggression in intact male rats. Their results show that, after a 10-week treatment, the treated animals present more threats and dominant postures and fewer submissive postures than the control group in aggressive encounters in a neutral arena. Using a competitive situation to get pellets, testosterone propionate has been shown to increase dominance in intact male rats that were previously nondominant (Bonson and Winter, 1992; Bonson *et al.*, 1994). In this study, we have analyzed one of the more abused AAS, stanozolol, using an animal model frequently employed to evaluate the effects of psychoactive substances on social interaction in mice.

Stanozolol, a 17-alpha-alkylated androgen, is an anabolic–androgenic steroid much misused in sport. This substance has been categorized as a more anabolic than androgenic steroid (Kochakian, 1993), but it still retains some androgenic activity. Arnold *et al.* (1950) and Potts *et al.* (1960) reported that stanozolol use produced nitrogen retention, weight increment, and anabolic activity. Trabalza and Brunelli (1966) found increases in weight during the course of the treatment and in the following month in children. It has actions similar to those of methandienone after both parenteral and per-oral (PO) administration, being clinically useful in the treatment of vascular diseases, fat disorders, edema, and anorexia.

In clinical use, the recommended adult oral dosage is 5 mg daily and intramuscular injection dosage is 50 mg every 2 or 3 weeks. Athletes use wide variations in dosage, ranging from 6 to 12 mg/day PO for 3–10 weeks (Casner *et al.*, 1971; O'Shea, 1974; Johnson *et al.*, 1975; Strauss *et al.*, 1985) and 50 mg every 2 days intramuscularly (IM) for 6 weeks (Strauss *et al.*, 1985) to 10 mg/day for 4 months with other steroids (Pope and Katz, 1990). Studies of the effects of stanozolol on

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strength have produced contradictory results, showing either no changes (Casner *et al.*, 1971; Johnson *et al.*, 1975) or a positive increase (O'Shea, 1974). The differences in dosages, periods of administration, and subjects could contribute to explain the different findings.

The aim of the present study was to investigate the effects of different doses of stanozolol on agonistic behavior in intact male mice, using an isolation-induced aggression experimental model (Brain *et al.*, 1989). Motor activity was recorded to know its contribution of any changes in aggressive behavior. Additionally, the body weight gain over the period of study was assessed to confirm its anabolic actions. As different physical side effects have been observed according to age in humans and given the increasing use by adolescents, a second experiment was carried out in younger animals to investigate the various possible behavior effects.

## MATERIALS AND METHODS

### *Subjects*

Subjects used in the present studies were male albino mice of the Alderly Park strain, bred and housed under highly controlled conditions in the animal house at the University of Wales, Swansea (Brain and Poole, 1974). Experimental mice were individually housed at 21 days of age in opaque polypropylene cages measuring 30×12×11 cm (North Kent Plastics, Kent, UK).

In the first experiment, 52 56-day-old experimental mice were randomly allocated to each treatment condition; namely, high ($n=13$), moderate ($n=14$), and low ($n=13$) doses and control ($n=12$). In the second experiment, 54 29-day-old experimental mice were randomly allocated to the treatment conditions; namely, high ($n=14$), moderate ($n=14$), and low ($n=12$) doses and control ($n=14$).

The "standard opponents" were housed in groups of five or six in similar cages and were made anosmic by nasal lavage with a 4% zinc sulfate solution 1 and 3 days before testing (Brain *et al.*, 1989). All animals were maintained under a reversed light/dark schedule (white lights on from 23:30–11:30 h GMT) with an ambient temperature of between 18° and 22°C. Food (Pilsbury's Diet, Birmingham, UK) and water were supplied *ad libitum*.

### *Treatments*

Vehicle was made from 1111.1 mg of disodium hydrogen phosphate (May and Baker Ltd., Dagenham, UK), 410.7 mg of sodium chloride (British Drug Houses, Poole, Dorset, UK), and 116.5 mg of Tween-80 (Sigma Chemical Co. Ltd., Poole, Dorset, UK) in 100 ml of distilled water. This was used to produce high (7.0 mg/kg), moderate (0.7 mg/kg), and low (0.07 mg/kg) doses of stanozolol [17 alpha-methyl-17 beta-hydroxyandrostan-(3,2-C)-pyrazol; commercial name Winstrol, Zambon Farmaceutici, Milan, Italy]. The recommended therapeutic amount of drug was used as the moderate dose. The low and high doses were a factor of 10 times lower and higher than the moderate dose, respectively.

In both experiments, subjects were injected intramuscularly (IM) on alternate days over a 21-day period with one of the solutions. This frequency of injection is considerably higher than clinically recommended.

### *Measures*

Twenty-four hours after the last injection, the subject's motor activity was registered for a 10-min period on an Animex activity recorder (A. B. Farad, Sweden). Immediately afterwards, a 10-min encounter between the experimental animal and a "standard opponent" was videotaped in a neutral cage. The behavior was evaluated using the ethopharmacological technique developed by Brain *et al.* (1989). Total duration and frequency of the following behavioral categories were estimated: care of body surface; digging; nonsocial exploration; exploration from a distance; social investigation; threat; attack; avoidance/flee; defensive/submissive; sexual; and immobility (Martínez *et al.*, 1986).

The body weight was determined using a digital balance (Mettler, Model PL 3000) on each day of drug administration.

### *Statistical analysis*

Body weight and motor activity were analyzed by ANOVA with repeated measures. The behavioral categories were separately assessed by nonparametric Kruskal–Wallis tests, because data did not meet the assumptions of ANOVA. Statistical significance was defined as $P<0.05$.

## RESULTS

### *Aggressive behavior*

The total durations, with ranges, allocated to broad behavioral categories for older and younger mice are presented in Tables 1 and 2, respectively. No behavior was recorded in four categories (avoidance/flee, defensive/submissive, sexual, and immobility). In both experiments, the Kruskal–Wallis test did not show significant variance across treatment groups for any behavioral category. Interestingly, in the first experiment (older animals), attack and threat tended to decrease in the treated subjects, in a dose-dependent manner. However, in the second experiment (younger animals) these behavioral changes increased in the moderate- and high-dose groups, the former showing the longest duration (Fig. 1). Data based on frequencies of behavioral elements generated broadly similar conclusions (not shown).

### *Motor activity*

In the older mice, there was a significant decrease in activity due to the effect of time [$F(9,459)=884.487$; $P<0.001$]. Younger subjects showed a progressive decline in activity over the test session, but no significant dose effects were evident when the data were subjected to an analysis of variance.

### *Body weight*

In the older animals ANOVA showed that only time [$F(10,510)=88.957$; $P<0.001$] had a statistically significant effect. However, a statistically significant effect of time [$F(10,500)=173.839$; $P>0.001$] and an interaction between time and dose [$F(30,500)=2.986$; $P<0.001$] on weight gain were found in young mice.

## DISCUSSION

It is well known that androgens are involved in some forms of aggressive behavior in animals, including intermale aggression (Brain and Haug, 1992). Consequently, it might be expected that AAS, as testosterone derivatives, would significantly increase aggression. It has been shown that testosterone propionate increases the dominance of intact male rats (Lumia *et al.*, 1994; Bonson and Winter, 1992; Bonson *et al.*, 1994). However, our results using stanozolol and employing an isolation-induced aggression model in non-castrate male mice, only partially confirmed this prediction. Stanozolol did not significantly alter any behavioral category, although interestingly it appeared to have a greater effect on offensive behaviors (attack and threat) than other elements. Svare (1990) indicated that several variables (gender; dose, duration and route of administration; type of androgen and genotype) modulate the behavioral effects of androgens in animals. Thus, the fact that stanozolol did not significantly augment either the incidence or the

**TABLE 1. Median (with ranges) for times (in seconds) allocated to broad behavioral categories in antagonistic encounters with adult mice treated with different doses (mg/kg) of stanozolol**

| Categories | Control | Treatment | | |
|---|---|---|---|---|
| | | 0.07 mg/kg | 0.7 mg/kg | 7 mg/kg |
| Body care | 46.9 | 58.7 | 58.5 | 57.0 |
| Range | 9.1–112.6 | 16.7–156.6 | 3.8–101.1 | 11.1–94.4 |
| Digging | 19.9 | 40.6 | 20.9 | 21.5 |
| Range | 1.5–78.7 | 2.7–66.8 | 0.9–83.9 | 2.5–68.9 |
| Nonsocial exploration | 205.3 | 215.5 | 204.3 | 236.5 |
| Range | 165.8–248.6 | 158.0–271.8 | 150.9–283.3 | 189.4–297.2 |
| Exploration from a distance | 42.8 | 41.7 | 50.1 | 43.2 |
| Range | 24.6–87.0 | 27.1–123.6 | 28.0–140.9 | 30.7–156.5 |
| Social investigation | 220.2 | 197.4 | 197.0 | 202.1 |
| Range | 87.1–315.8 | 44.0–243.1 | 59.0–320.9 | 37.9–248.6 |
| Threat | 32.7 | 26.8 | 21.6 | 19.7 |
| Range | 0–110.9 | 0–48.3 | 0–104.4 | 0–81.3 |
| Attack | 14 | 12 | 6.5 | 2 |
| Range | 0–55 | 0–32 | 0–42 | 0–44 |

total duration of fighting and threat in treated mice may be explained by different factors. The doses used were comparable to those in the literature, however, the duration of the administration was shorter. Perhaps a longer period of treatment would produce significant effects. The time at which social behavior is assessed may also be important. In the present study, the behavior was assessed 24 h after the last injection, a time interval usually employed for other androgenic steroids, but there is a lack of information about the metabolic pathways and pharmacokinetics of stanozolol. In other studies using long-term treatments, although with very different substances, significantly different behavioral effects have been found depending on the time interval between the last injection and the behavioral tests (Borrás-Valls *et al.*, 1994). However, it is possible that these substances have no important effects on aggression, as they were not detected by the sensitive methods used to evaluate behavior. Similarly, another study carried out by Clark and Barber (1994) concluded that stanozolol had no effect on aggression in castrated male rats. Additionally, none of the studies on steroid abusers analyzed the effect of stanozolol alone on aggression. The subjects did not take this substance in isolation, but rather in combination ("stacking") with other oral and injectable anabolic–androgenic steroids (Burkett and Falduto, 1984; Cohen *et al.*, 1988; Bahrke *et al.*, 1990). Thus, the described effects on humans can be a consequence of the mixed use of several AAS.

Surprisingly, different trends were apparent depending on the age of subjects. Both offensive behaviors (threat and attack) were clearly augmented, in young mice, with increasing doses of stanozolol. In contrast, these behaviors were slightly reduced as the dose was increased in adult mice. These varied effects of stanozolol may be related to

**TABLE 2. Median (with ranges) for times (in seconds) allocated to broad behavioral categories in antagonistic encounters by young mice treated with different doses (mg/kg) of stanozolol**

| Categories | Control | Treatment | | |
|---|---|---|---|---|
| | | 0.07 mg/kg | 0.7 mg/kg | 7 mg/kg |
| Body care | 54.8 | 46.6 | 28.3 | 34.6 |
| Range | 20–155 | 8.4–155.9 | 6.3–84.2 | 9.7–222.3 |
| Digging | 35.6 | 22.9 | 35.4 | 20.3 |
| Range | 0–91.9 | 0–59.1 | 5.9–109.9 | 1.8–45.9 |
| Nonsocial exploration | 183.5 | 210.3 | 200.9 | 195.2 |
| Range | 122.3–347.9 | 105.4–305.8 | 132.9–265.6 | 133.2–275.2 |
| Exploration from a distance | 39.1 | 46.7 | 46.7 | 37.9 |
| Range | 18.7–66.4 | 23.2–54.5 | 23.3–67.2 | 23.1–88.6 |
| Social investigation | 235.7 | 237.2 | 230.1 | 252.9 |
| Range | 114.6–333.3 | 115.6–329.9 | 168.0–300.2 | 14.0–348.1 |
| Threat | 4.2 | 4.8 | 21.3 | 14.0 |
| Range | 0–72.6 | 0–57.7 | 0–39.3 | 0–109.4 |
| Attack | 0.8 | 1.7 | 20.9 | 15.9 |
| Range | 0–63.6 | 0–44.9 | 0–52.2 | 0–108.8 |

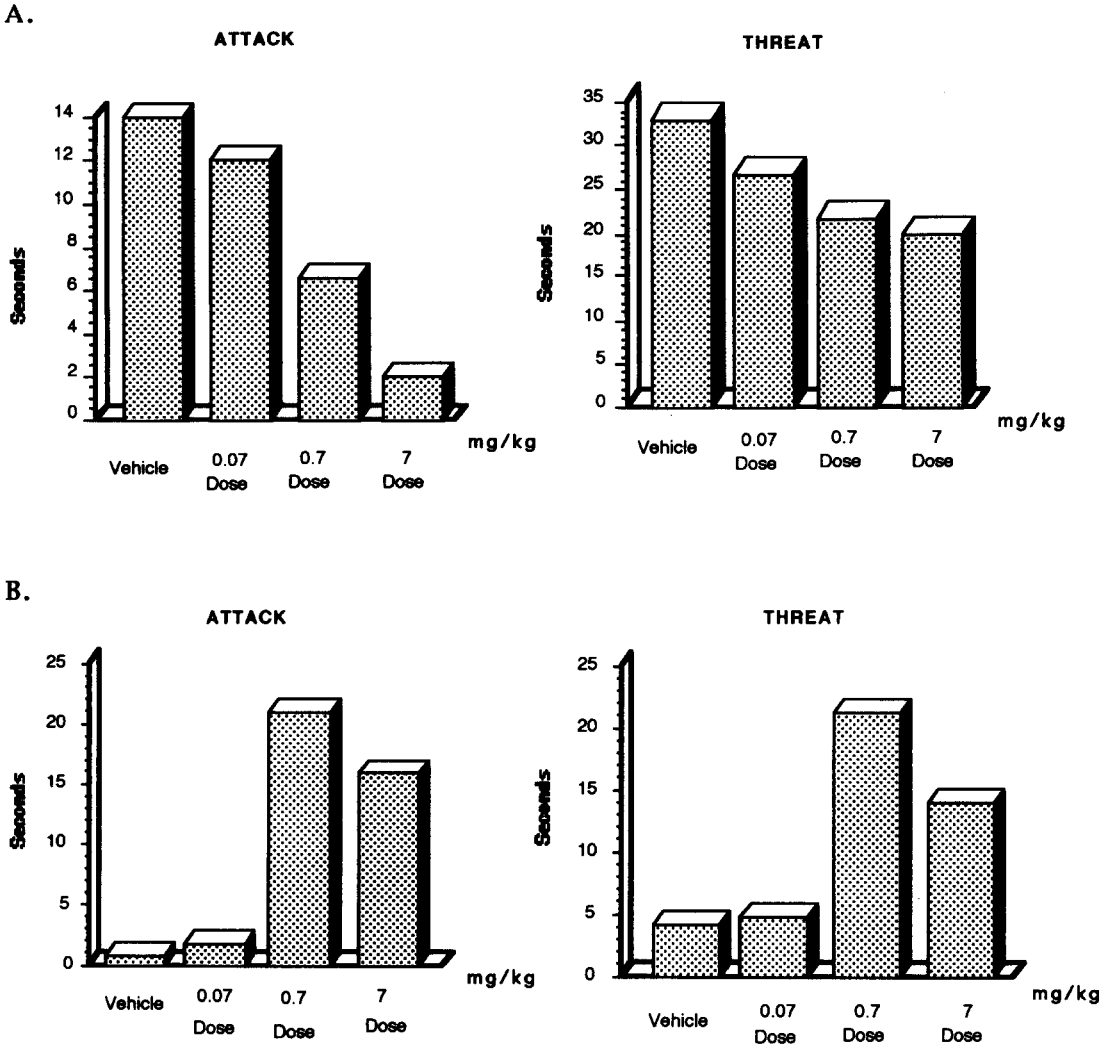


**FIGURE 1. Median values for times allocated to attack and threat categories in an antagonistic encounter by young (A) and older (B) mice treated with different doses (mg/kg) of stanozolol.**

the different endogenous testosterone levels characteristic of these age groups. If control groups for both experiments are compared, the adults clearly exhibit much more aggressive behavior, probably as a consequence of higher endogenous testosterone levels. Small *et al.* (1984) found that a 14-day treatment with stanozolol (10 mg orally per day) decreased serum testosterone levels significantly in male humans as can be seen in our adult subjects. With respect to young animals, as they have lower testosterone levels, two hypotheses can be taken into account. First, the increased aggression could be due to an additive effect of endogenous testosterone plus stanozolol or, second, they could be more sensitive to testosterone derivatives exogenously administered.

In the present experiments, stanozolol did not significantly affect activity. These results are in agreement with those found by Clark and Barber (1994) in castrated rats. The effects on activity have been evaluated using other AAS and, on the whole, results are similar. Thus, Lumia *et al.* (1994), using testosterone propionate and taking the number of approaches to the opponent as a measure of activity, did not find differences between those animals treated and their control ones. However, when testosterone propionate is taken in addition to another substance, results are quite different. Thus, Long *et al.* (1994) found that testosterone propionate decreased the locomotor activity enhanced by cocaine.

On the other hand, the strong anabolic effect of this substance (Kochakian, 1993) was confirmed by the influence of the treatment on body weight gain in young mice. However, it is interesting to note that a similar result was not found in adult mice. Lombardo (1993), in a review of the effects of several AAS on physical parameters, has reported that intact male animals have no body weight gain, whereas in castrated ones the body mass is augmented after treatment. However, recent studies have shown that when castrated adult rats were injected with stanozolol, there were no differences between animals treated and controls (Clark and Barber, 1994). Finally, we might take into account the possible effect of body size on aggression in our experiment, because it has been proven that the greater body size is positively related to aggression. Thus, young mice treated with stanozolol were bigger than their opponents, which could favor increases in offensive behavior. It might be pointed out that the statistically significant effects on body weight and the greater increases in aggressive behavior are found only in young subjects. So, the behavioral effect found in youngsters might be a consequence of the weight gain.

In summary, the data of these experiments did not show that stanozolol, at these doses and with this period of treatment, significantly affected aggression, activity, and body weight (except in young mice). It is possible that stanozolol has an important anabolic effect without having

marked androgenic influences, which supports the hypothesis that this steroid is predominantly anabolic and has relatively modest behavioral influences. There are, however, some potentially interesting influences and trends that require further investigation. Thus, user age appears to be an important variable. This fact has been carefully analyzed due to the increasing abuse by adolescent and young people who could become a special risk population.

## SUMMARY

Anabolic–androgenic steroid (AAS) abuse has been clinically associated with physical and psychological side effects. The present experiment examines the effects of injecting young and adult male mice with different doses of stanozolol, one of the most misused AAS, over a 21-day period. Body weight gain was recorded on each day of injection. Motor activity was registered on an Animex activity recorder after treatment. Social behavior was analyzed by determining the time and frequency allocated to broad behavioral categories in encounters with anosmic male partners.

The results showed that body weight gain was increased in young mice. Motor activity was unaffected. Analysis of behavior suggested a different profile of aggressive behavior in young and adult mice, although those differences were not statistically significant. In older animals it was decreased while in young animals it was increased. In general, the treatment did not significantly affect aggression, activity, and body weight.

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