Effect of Treatment with an Anabolic Steroid (Nandrolone Decanoate) on Physiological and Morphological Characteristics of Unconditioned and Conditioned Canine Latissimus Dorsi Muscles

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Abstract

Twelve female beagles underwent unilateral electrical stimulation of the latissimus dorsi muscle via the thoracodorsal nerve for 12 weeks with continuous 2 Hz stimulation. Six animals were also treated with an anabolic steroid (steroid group); the other six animals received no steroids (control group). Electrical stimulation and steroid treatment were treated in parallel for 12 weeks. At the the end of the stimulation period, twitch contraction times were measured and a fatigue test was performed with tetanic stimulation at 25 Hz (low), 43 Hz (moderate) and 85 Hz (intense). The burst pattern was 312 msec "on" and 812 msec "off", which resulted in 54 contractions per minute. Over the 12 week period the mean body weight increased by 283 ± 194 g in the steroid group and decreased by 150 ± 72 g in the control group ($p < 0.05$). The muscle weights of the electrically conditioned muscles were about 20% below those of unconditioned muscles; the difference was significant ($p < 0.02$). Change in muscle weight did not appear to be affected by steroid treatment. Electrical conditioning prolonged the twitch contraction time in both groups to similar values. The unconditioned steroid treated muscles showed a longer twitch contraction time than the unconditioned control muscles ($p < 0.03$). The mean peak twitch tension was not statistically different between the unconditioned steroid and the unconditioned control muscles. The greatest muscle fatigue occurred during the most intense tests (85 Hz) in all four groups, but in all cases, the conditioned muscles fatigued at a slower rate than the unconditioned muscles ($p < 0.05$). In addition, both the unconditioned steroid and the conditioned steroid treated muscles fatigued at a slower rate during the two more intense (43, 85 Hz) fatigue tests than their non-steroid treated counterparts. Treatment with anabolic steroids appears to enhance fatigue resistance of canine latissmus dorsi muscle.

Key words: Anabolic steroid, latissimus dorsi, dog, muscle, conditioning, tension, fatigue resistance, histochemistry.

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Reports of the effects of anabolic steroids on skeletal muscle are inconsistent, largely because of variations between muscles, species and experimental conditions. Some studies have indicated that anabolic steroids can influence the histological, histochemical and physiological characteristics of skeletal muscles [7]. Thus, a study by Eggington suggested that anabolic steroids could improve the fatigue resistance of the extensor digitorum longus muscle of the rat [6]. Keul obtained evidence from changes in blood lactate levels during submaximal exercise that anabolic steroids improved the fatigue characteristics of skeletal muscles in weight lifters [12].

Skeletal muscle can be made remarkably fatigue resistant by electrical stimulation of its motor nerve over a period of weeks [13, 15, 23]. This phenomenon, referred to as conditioning, has generated renewed interest in the poten-

tial use of skeletal muscle grafts for cardiac assistance. In this context anabolic steroids might be therapeutically useful in two ways. First, if such treatment, started preoperatively, could induce even a small increase in fatigue resistance, it might enable cardiac assistance to be introduced at an earlier postoperative stage. Second, any myotrophic effect of the steroid might offset the loss of mass that is normally associated with chronic electrical stimulation [13, 16, 23, 24]. The purpose of this study was therefore to investigate the effects of an anabolic steroid on the isometric contractile characteristics, fatigue behavior and fibre type composition of the latissimus dorsi muscle, a muscle that has now been used extensively, both experimentally and clinically, for cardiac assistance [1, 17]. To investigate the possible interaction with indirect electrical stimulation, the experiments were conducted in dogs during conditioning of the latissimus dorsi muscle of one side.

Materials and Methods

Twelve female beagles, aged approximately 1 year and with body weights of 8.7 - 10.8 kg, underwent unilateral electrical stimulation of the latissimus dorsi muscle via the thoracodorsal nerve. Females were used because they have lower circulating testosterone levels and a higher concentration of androgenic receptors than males [3, 4]. Animals were housed individually. Each animal received the same amount of food, based on a balanced diet without protein supplement. Water was available ad libitum. The environment was maintained at a temperature of 20°C and with a daylight period of 12 hours. Six animals were treated with an anabolic steroid (steroid group); the other six animals received no steroids (control group).

Anabolic steroid treatment

The treatment consisted of 12 weekly injections of a long-acting ester of a synthetic testosterone-derivate dissolved in sesame oil (19-nor-17B-hydroxy-3-keto-androst-4-ene, nandrolone decanoate; Decadurabolin, Organon Laboratories). The dose was 4mg/kg on the day electrical stimulation was initiated and 2 mg/kg for the second to twelfth week; this is about twice the recommended therapeutic dose rate for treating anemia of renal disease in humans. The steroid was given by intramuscular injection alternately in the right and left gluteus muscles. To avoid circadian influences all injections were given at the same time of day.

Muscle conditioning

The operation was performed in accordance with the "Guide for the Care and Use of Laboratory Animals". Anesthesia was induced by intravenous thyamylal sodium (2.5%; 11-17.5 mg/kg) and maintained with inhalation of isoflurane (1.5-2%) and 100% oxygen. During surgery a lactate-containing Ringer solution was given intravenously. Bilateral 5-cm skin incisions were made in the mid-axillary line. The proximal thoracodorsal nerve was identified bilaterally and a modified Medtronic pacemaker electrode was placed around the nerve of the right and left latissimus dorsi muscle. One of the two leads was connected to a totally implanted unipolar nerve stimulator (Medtronic, Itrel, Model 7421), which was located posteriorly to the ipsilateral rectus abdominis muscle. The nerve was stimulated at a frequency of 2 Hz, with an amplitude of 0.75 V, continuously for 24 hours a day. The contralateral muscle served as an unstimulated control. In the non-steroid group three of the six animals had their right thoracodorsal nerve stimulated and three their left. Similarly, three in the steroid treated group had the right nerve stimulated and three the left. Prophylactic antibiotic treatment was given on the day of operation (1g cefazolin sodium i.v.) and twice a day for one week (30mg/kg cephalexin).

Physiological assessment

Electrical stimulation and steroid treatment were continued in parallel for 12 weeks. The body weight of the dogs was measured weekly during this period. At the end of the stimulation period a terminal experimental procedure was performed. The animals were anesthetized by intravenous thyamylal sodium (2.5%; 1-17.5% mg/kg) and isoflurane (2-2.5%) inhalation. To maintain adequate hydration, lactated Ringer solution was given intravenously. Arterial blood pressure was monitored through a femoral arterial catheter. Rectal temperature was measured and maintained during the procedure by means of heat lamps and heating blankets. The dog was secured on the operating table by fixing all four limbs with polypropylene lines. The tendon of each latissimus dorsi muscle was detached bilaterally from its insertion on the humerus. A Gore-Tex graft was sutured to the tendon and attached by propylene sutures (#0 Prolene, Ethicon) to Grass strain gauge transducers (FT 10, Grass Instrument Company, Quincy, Mass.).

Muscles were stimulated supramaximally; the voltage was considered supramaximal when a further increase in voltage failed to result in an increase in peak twitch tension. An amplitude of 4-6 volts was determined to be supramaximal. Isometric twitch contractions were measured at different muscle lengths and the length that yielded the greatest twitch amplitude was used both for recording the maximal peak twitch tension and for the subsequent fatigue test. The results were recorded on a Gould ES 1000B recorder fitted with universal amplifiers. Twitch contraction time was calculated from the time of initiation of contraction to the time of peak tension. Fatigue tests were performed successively with tetanic stimulation at 25 Hz (low), 43 Hz (moderate) and 85 Hz (intense). The burst pattern was 312 ms "on" and 812 ms "off", which resulted in 54 contractions per minute. The duration of individual pulses was kept constant at 210 µs. The duration of each test was 20 min, and a rest period of 15 minutes was allowed between the tests. At 5 min intervals during each fatigue test, the peak tension was recorded and the tension-time index, defined as the integrated area under the tension curve generated by a single burst of stimulation, was calculated.

Table 1. Change in body weight of steroid and control group after 12-week treatment period (x ± SEM) (p < 0.05).

	CONTROL GROUP		STEROID GROUP	
	TIME ZERO	12 WEEKS	TIME ZERO	12 WEEKS
	9717 g	9567 g	9600 g	9883 g
WEIGHT CHANGE	-150 ± 72 g		± 283 ± 194 g	
	p = N.S.			

Microscopic analysis

At the completion of fatigue testing the latissimus dorsi muscles were dissected out and weighed. Biopsies were removed from locations distributed equally in the proximo-distal and medio-lateral directions; these samples were then frozen rapidly in liquid nitrogen and stored below -70°C pending further analysis. A sample, from each of the three locations was taken in the same way from each latissimus dorsi muscle (36 samples in all). Transverse 10 cryostat sections were prepared from these samples and stained histologically by the Hematoxylin and Eosin technique for general morphological assessment and histochemically for the demonstration of myofibrillar ATPase (method of Tunell & Hart [26]), for the measurement of fibre composition and size. In the dog this conventional technique demonstrates only two types of fibre, and these were designated Type 1 (light) and Type 2 (dark). Quantitation was performed semi-automatically on a Leitz Diaplan microscope connected via a video link to an IBM PC/AT computer running Imagan 2 (Kompira, Ltd.) image analysis software.

One section from each sample was analyzed. Approximately 200 fibers were typed and traced from at least 2 (normally 3) non-adjacent areas, chosen without regard to fascicular boundaries. Fibre area and the diameter of the circle of equivalent area were computed and stored separately for the two fibre types. The mean values obtained from each section constituted the data set for the 36 samples.

Statistical analysis

Physiological results were evaluated for normality by the Wilk-Shapiro test. RS/1 software (BBN Software Products Corporation) on an IBM-XT computer was used to perform tests of significance: Student t-test was used for unpaired data, and a paired t-test for comparisons involving muscles from the two sides of the same animal. Morphometric data was examined initially by analysis of variance, performed on a mainframe with SAS software (SAS Institute, Inc.) for homogeneity within muscles. Subsequent analysis was carried out by unpaired Student t-test on the pooled data from each muscle.

Results

The dogs tolerated both the steroid treatment and the electrical stimulation of the right or left latissimus dorsi muscle well and were able to move around freely without apparent physical impairment or discomfort. The results of two conditioned muscles of the control group had to be excluded because there were problems with stimulation.

Body weight

Over the 12-week treatment period the mean body weight increased by 283 ± 194 g in the steroid group and decreased by 150 ± 72 g in the control group. The difference in weight change, however, was not statistically significant (see Table 1).

Muscle weight

The muscle weights of the electrically conditioned muscles were about 20% below those of the unconditioned muscles; the difference was significant ($p < 0.02$) (Table 2). This reduction in muscle bulk has been observed previously. It was not affected by steroid treatment: neither conditioned nor unconditioned muscles were significantly different for having been assigned to the steroid or control groups.

Twitch contraction time

Conditioning prolonged the twitch contraction time in both groups to similar values. The unconditioned steroid muscles showed a longer twitch contraction time than the unconditioned control muscles ($p < 0.03$) (Table 3).

Peak twitch tension

The mean peak twitch tension was not statistically different between the unconditioned steroid muscles and the unconditioned control muscles (Figure 1a). The same result was seen when normalizing the peak twitch tension by the muscle weight (Figure 2b). The peak twitch tension and the peak twitch tension/muscle weight ratio were higher in the unconditioned steroid muscles than in the conditioned steroid muscles (p, p) (Figure 1a,b). There was no statistically significant difference between the peak twitch tension of the conditioned steroid muscle and the peak twitch tension of the conditioned control muscles; however the peak twitch tension/muscle weight ratio was lower in the

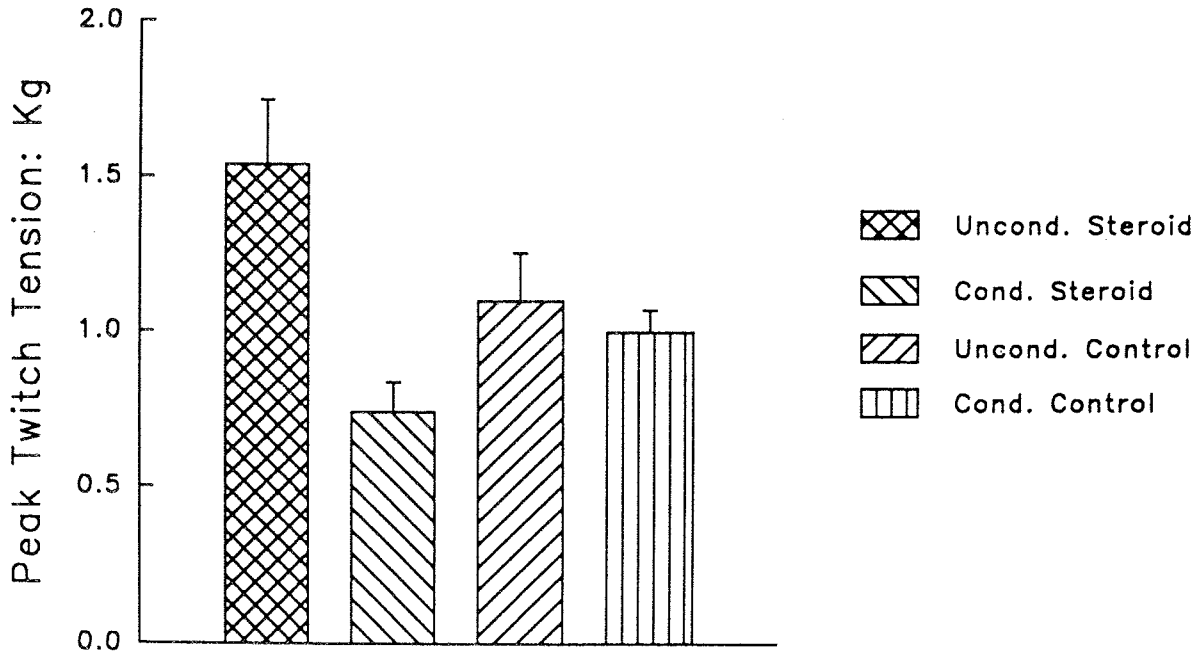

Figure 1a. Peak twitch tension of unconditioned and conditioned latissimus dorsi muscles after 12-week treatment period (x ± SEM).

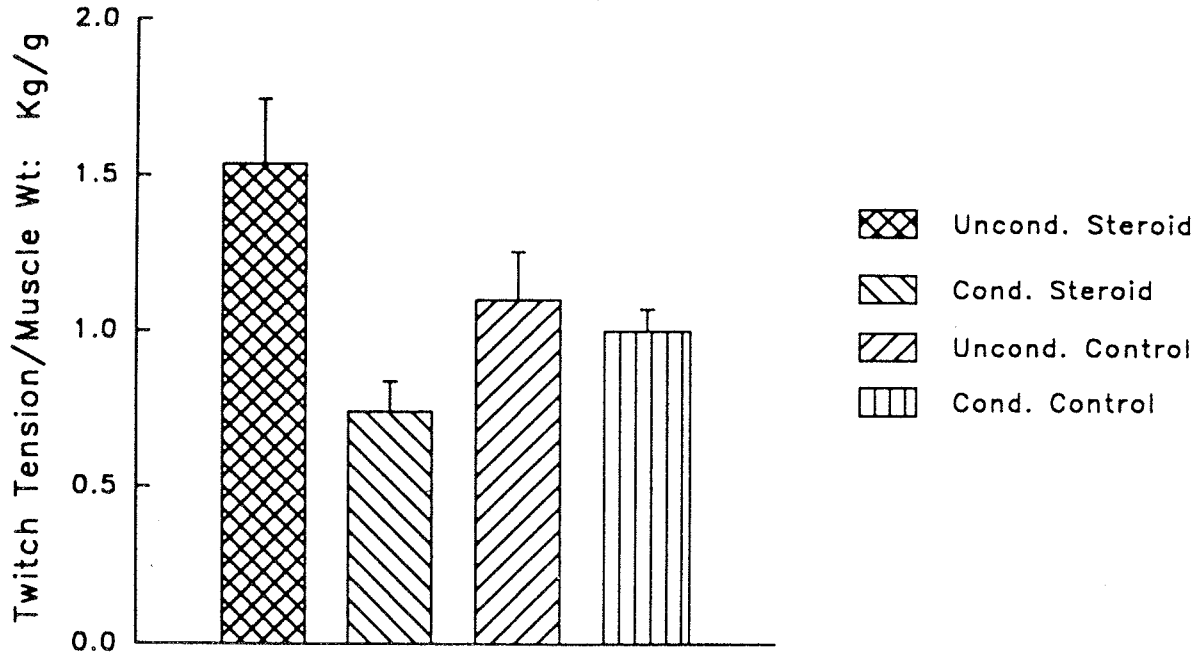

Figure 1b. Peak twitch tension/muscle weight ratio of unconditioned and conditioned latissimus dorsi muscles after 12-week period (x ± SEM) (COND.STEROID/COND.CONTROL: (p < 0.01)

conditioned steroid muscles than in the conditioned control muscles ($p < 0.05$). Conditioning in the control group did not show any statistical difference.

Fatigue test

Fatigue tests were conducted for 20 min at each of three burst frequencies, 25 Hz (low), 43 Hz (moderate) and 84 Hz (intensive). The results are illustrated in Figures 2 (a,b,c), 3 (a,b.c) and 4 (a,b,c), respectively, for percentage

Table 2. Muscle weight of unconditioned and conditioned latissimus dorsi muscles after 12-week treatment period (x ± SEM), N.S. is p > 0.05).

	STEROID GROUP	CONTROL GROUP	
UNCONDITIONED LDM	64.39 ± 4.64	56.14 ± 3.55 g	N.S.
CONDITIONED LDM	49.93 ± 2.15 g	46.17 ± 1.97 g	N.S.
p-Value	< 0.01	< 0.04	

Table 3. Twitch contraction time of unconditioned and conditioned latissimus dorsi muscles after 12-week treatment period (x ± SEM), N.S. is p > 0.05.

	STEROID GROUP	CONTROL GROUP	p-Value
UNCONDITIONED LDM	53 ± 3 ms	42 ± 4 ms	< 0.03
CONDITIONED LDM	83 ± 5 ms	84 ± 7 ms	N.S.

of initial tension-time index, tension-time index, and peak tension. We also analyzed tension-time index per gram of muscle and peak tension per gram of muscle. The graphs for peak tension per gram of muscle and tension-time index per gram of muscle are not shown, but were similar to those for tension-time index and peak twitch tension, respectively, during each of the three different fatigue tests. The results of the fatigue test may be summarized as follows:

1. The greatest muscle fatigue occurred during the most intense test (85 Hz) in all four groups, but in all cases the conditioned muscles fatigued at a slower rate than the unconditioned muscles ($p < 0.05$) (Figure 2a,b,c).
2. During the two more intense (43, 85 Hz) fatigue tests, the muscles in the unconditioned steroid group fatigued less rapidly than the muscles in the unconditioned control group (Figure 2b,c). The differences were statistically significant at the end of the 43 Hz fatigue test ($p < 0.05$) and for the last 10 min of the 85 Hz fatigue test ($p < 0.05$).
3. During the most intense (85 Hz) fatigue test, the conditioned steroid treated muscles fatigued less than the conditioned control muscles. The difference was statistically significant for the last 10 min ($p < 0.05$) (see Figure 2c).
4. The absolute values of the tension-time index and of the peak tension showed only two statistically significant differences among all groups. At the low (25 Hz) fatigue test, the tension-time index and the peak tension were higher in the unconditioned steroid treated group than in the conditioned steroid group ($p < 0.05$) (Figure 3a, 4a). At the more intense (85 Hz) fatigue test the tension-time index and the peak tension were lower in the unconditioned control group than in the conditioned control group ($p < 0.05$) (Figure 3c, 4c).

Morphological analysis

Fibre type areas (and equivalent diameters) and composition were measured in 3 samples from identical locations in each muscle. Preliminary analysis of variance showed that the 3 locations did not differ in terms of these measurements, and they were therefore pooled to provide data sets containing a single value from each muscle.

Conditioned latissimus dorsi muscles showed the anticipated changes in fibre type composition. In the control group the percentage of Type I fibers increased in response to chronic stimulation form 40.1 ± 2.9% to 91.1 ± 9.3% ($p < 0.0001$) (Table 4). In the steroid group, this percentage increased from 37.2 ± 1.8% to 94.2 ± 5.9% ($p < 0.0001$). Neither the fiber type composition nor the degree of transformation was significantly affected by the steroid treatment.

Table 4 also shows the changes in fibre diameter. Type 2 fibers showed a significant reduction in diameter in response to conditioning, but only in the control group ($p < 0.05$). A similar result was obtained by analyzing changes in fiber area (not shown). Type 1 fibers did not differ in size between conditioned and unconditioned muscles. Neither in the conditioned nor in the unconditioned muscles was there any evidence of an effect on steroid

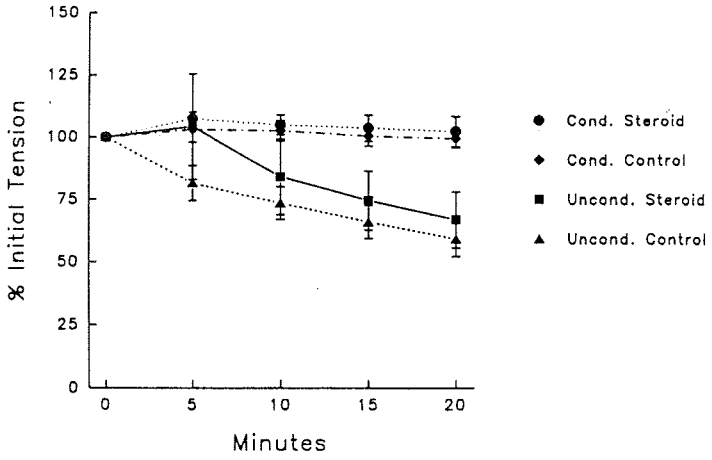

Figure 2a. Fatigue test at 25 Hz (x ± SEM).

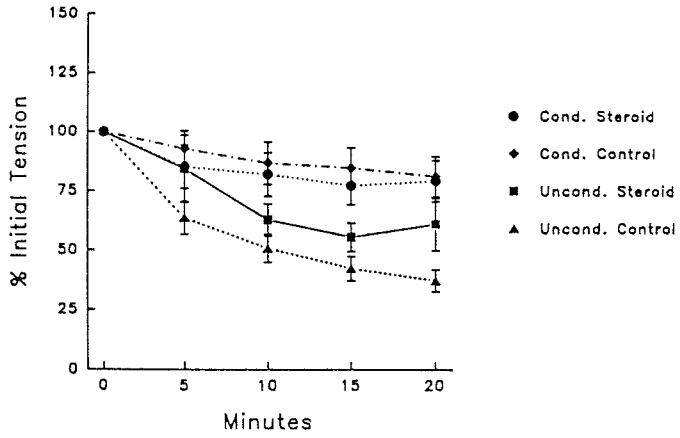

Figure 2b. Fatigue test at 43 Hz (x ± SEM). (UNCOND. STEROID/UNCOND. CONTROL, 20 MIN, P < 0.03).

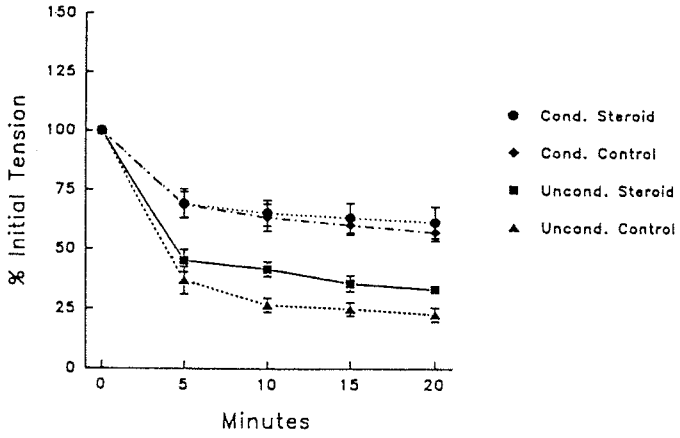

Figure 2c. Fatigue test at 85 Hz (x ± SEM). (UNCOND. STEROID/UNCOND. CONTROL, 10,15,20 MIN, p < 0.05).

Figure 2. Fatigue rates (percentage of initial tension-time index) of unconditioned and conditioned latissimus dorsi muscles after 12-week treatment period versus time.

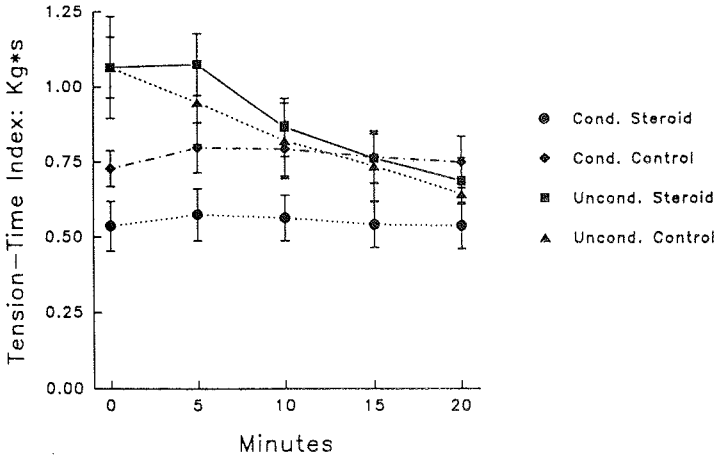

Figure 3a. Fatigue test at 25 Hz (x ± SEM).

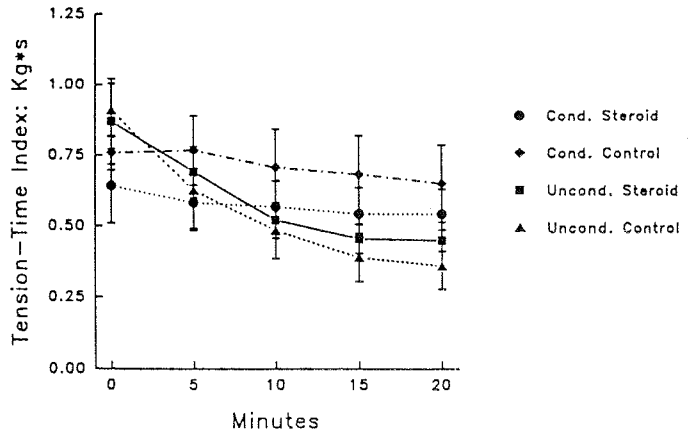

Figure 3b. Fatigue test at 43 Hz (x ± SEM).

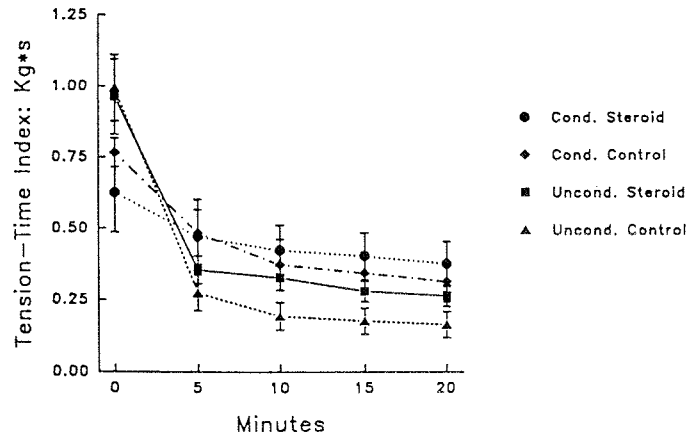

Figure 3c. Fatigue test at 85 Hz (x ± SEM). (UNCOND. STEROID/UNCOND. CONTROL, 10,15 min, p).

Figure 3. Tension-time index of unconditioned and conditioned latissimus doris muscles after 12-week period versus time.

treatment on fibre size; this was the case for both types of fibre. However, in terms of the influence of fibre type composition and size on physiological function, the more relevant measure is the proportion of the muscle cross-sectional area that is contributed by each type of fibre; this measure is included in Table 4. As expected, the area contribution from Type 1 fibers is significantly larger in the conditioned muscles ($p < 0.0001$). Steroid treatment did not affect the size of the relative area contributions of the two types of fibre in the conditioned muscles; in the unconditioned group, however, the area contribution of Type 2 fibers was significantly higher in the steroid-treated group ($p < 0.01$).

Discussion

The mean body weight increased slightly in the animals treated with anabolic steroids. The weight change is more noticeable when comparing it with the control group, where there was a slight decrease in mean body weight. In contrast to some other studies [11, 14, 25] the body weight increase occurred without any protein supplement. Different mechanisms may be responsible for the body weight increase. One reason may be the tendency of anabolic steroids to retain electrolytes and water [2, 9]. Additionally, in high doses anabolic steroids are suggested to undergo metabolic conversion to oestradiol, which itself influences body weight [8]. Also anabolic steroids are thought to have an anticatabolic effect, resulting in an improved amino acid uptake and in an increase in protein synthesis [18]. As shown in our study, the body weight increase caused by the steroid treatment seems not to originate from an increase in muscle bulk. In the unconditioned muscles, neither muscle weight nor fibre size showed any evidence of myotrophic action of the anabolic steroid at the dose level used. In the conditioned muscles, any such effect, even if it were present, is evidently insignificant in comparison to the major changes induced by chronic indirect stimulation. It is known that these include 3-5 fold increases in protein synthesis and degradation [20].

Similar observations have been reported by Salmons [19, 21] who showed that the loss of bulk consequent on chronic stimulation of the rabbit tibialis anterior muscle was not reduced by treatment with nandrolone decanoate. In contrast to the present study, Salmons' experiments did demonstrate a myotrophic effect in the unstimulated muscles [19, 21, 22]. This result was highly muscle-specific. We must be cautious about extrapolating these conclusions to human muscle because of potential species-specific in the effects of anabolic steroids. However, there appears to be no basis for the use of such compounds as a means of preserving bulk in conditioned muscles. Other approaches, including modulation of the stimulation pattern [10], appear to hold more promise in this regard.

*Table 4. Morphological measurements. P values in the table refer to the results of an unpaired Student's t-test between the steroid-treated and control groups of muscles. N.S. is $p > 0.05$. Asterisks are for a similar comparison between the conditioned muscle and the corresponding unconditioned group. ** = $p < 0.0001$; * = $p < 0.05$. Variable names in parentheses in column one are not independent of the variables immediately above them.*

	UNCONDITIONED			CONDITIONED		
	CONTROL	p Value	STEROID	CONTROL	p Value	STEROID
% Type 1 fibres	40.1 ± 2.9 (n = 6)	N.S.	37.3 ± 0.7 (n = 6)	91.1 ± 4.7** (n = 4)	N.S.	94.2 ± 2.4** (n = 6)
(% Type 2 fibres)	59.9 ± 2.9 (n = 6)	N.S.	62.7 ± 0.7 (n = 6)	8.9 ± 4.7** (n =4)	N.S.	5.8 ± 2.4** (n = 6)
Type 1 fibre diameter (μm)	33,6 ± 1.6 (n = 6)	N.S.	30.1 ± 2.7 (n = 6)	31.5 ± 1.2 (n = 4)	N.S.	29.6 ± 10.7 (n = 6)
Type 2 fibre diameter (μm)	41.4 ± 1.5 (n = 6)	N.S.	38.4 ± 3.5 (n = 6)	31.5 ± 4.7* (n = 4)	N.S.	26.7 ± 3.9 (n = 4)
% area contribution : Type 1	32.9 ± 1.7 (n = 6)	< 0.01	26.9 ± 0.8 (n = 6)	90.7 ± 5.1** (n =4)	N.S.	92.0 ± 2.9** (n = 4)
(% area contribution : Type 2)	67.1 ± 1.7 (n = 6)	< 0.01	73.1 ± 0.8 (n = 6)	9.3 ± 5.1** (n = 4)	N.S.	8.0 ± 2.9** (n = 4)

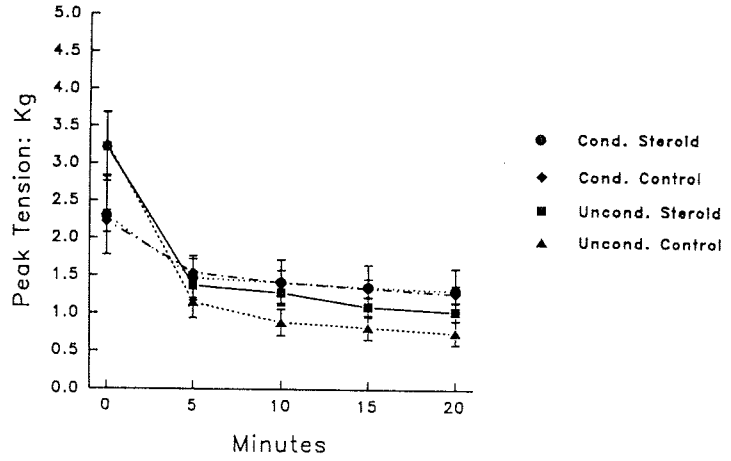

Figure 4a. Fatigue test at 25 Hz (x ± SEM).

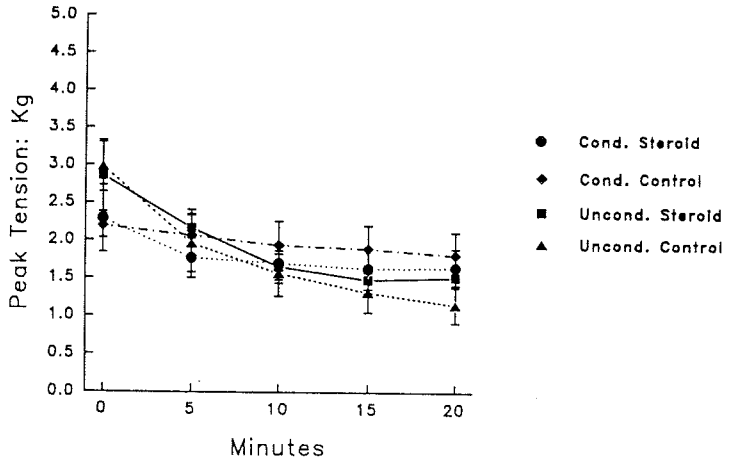

Figure 4b. Fatigue test at 43 Hz (x ± SEM).

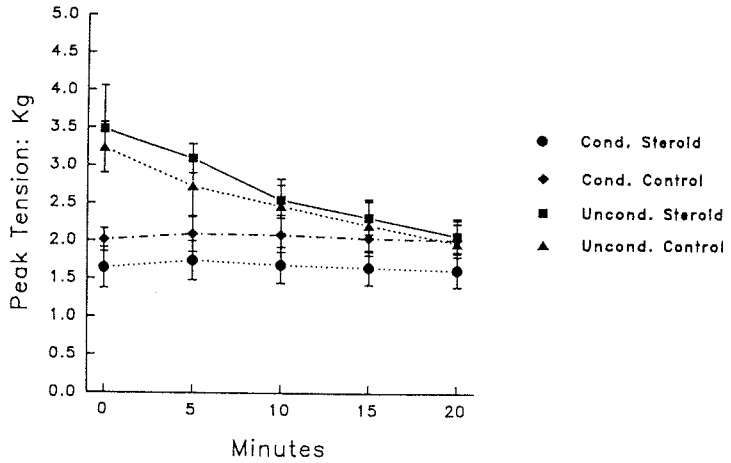

Figure 4c. Fatigue test at 85 Hz (x ± SEM).

Figure 4. Peak tension of unconditioned and conditioned latissimus dorsi muscles after 12-week period versus time.

There appeared to be some effect of anabolic steroids on muscle characteristics in the present study. Our data indicate that the steroid treatment caused a prolongation of the contraction time in the unconditioned muscles. However, we could not show any transformation of fibre types due to anabolic steroid treatment; in fact, anabolic steroids slightly increased the proportion of the cross-sectional area contributed by Type 2 fibers. There was no such ambiguity in the changes in contractile speed induced by conditioning, which were clearly correlated with transformation of muscle fibres from Type 2 to Type 1, as we and others have shown before [13, 15, 23].

Moderate (43 Hz) to intensive (85Hz) fatigue test showed that anabolic steroids improved the fatigue resistance, but not to the same extent as conditioning. At intensive exercise levels, the effects of anabolic steroid and conditioning on fatigue resistance appeared to be additive. There was no evidence of concomitant increase in muscle strength due to anabolic steroid treatment. At first glance the improvement of the fatigue resistance is difficult to explain in terms of the fiber composition, since we could show no transformation from type 2 to Type 1 fibers in the steroid group; indeed, there was some increase in the proportion of the cross-sectional area contributed by Type 2 fibers. However, it must be remembered that typing based on the myofibrillar ATP-ase technique does not reflect metabolic changes that could have an important bearing on fatiguability. Moreover, all fiber types in canine muscle show a high level of oxidative activity. Similar results were reported by Eggington [6], who studied the influence of an anabolic steroid in the extensor digitorum longus muscle of sedentary female rats. He, too, found an improvement in fatigue resistance due to steroid treatment. Eggington described a corresponding increase in the proportion of fast oxidative fibers (Type 2A). In a further study by Eggington [5], the improved endurance due to anabolic steroids was assigned to metabolic changes, reflected in histochemical profile, capillary supply and enzyme activities. Metabolic effects of anabolic steroids have also been described by Keul [12], who found lowered heart rate and blood lactate levels during exercise.

We conclude that treatment with anabolic steroids appears to enhance the fatigue resistance of the canine latissimus dorsi muscle, a predominantly fast-type muscle, possibly through an increase in the capacity for oxidative metabolism.

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