Effect of Anabolic-Androgenic Steroids on Performance, Body Composition and Skeletal Muscle Characteristics

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Abstract

Under the influence of anabolic androgenic steroids, a progressive high resistance strength trained athlete exhibited improvement in weight-lifting performance, which was in contradiction with muscle biopsy characteristics. The drugs are supposed to act androgenically. In combination with training, they might have deleterious effect on the muscle structure.

Key words: anabolic steroids, testosterone, strength training, skeletal muscle histochemistry.

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Some athletes are supposed to use male hormone testosterone and its derivatives synthetic anabolic-androgenic steroids (AAS) in order to facilitate and/or to improve anabolic processes in organism, especially after they have reached the plateau in process of their training.

AAS mimic the anabolic effects of the natural male hormone testosterone while, at the same time, they have minor androgenic effects. The proposed mechanisms for the actions of AAS in increasing muscle strength are the following: enhancement of protein synthesis in the muscle, blocking of the catabolic effect of glucocorticoids released after exercise and enhancement of aggressive behaviour that promotes greater quantity and quality of training [31].

The main purpose of our research was to detect the supposed anabolic effects of the AAS and to find out how long the muscle strength could be maintained after cessation of hormone drug usage. We followed the changes in the neuromuscular performances, body composition, and histochemical characteristics of the *vastus lateralis* muscle.

Material and Methods

A former decathlete of national level, non-smoker and teetotaler, 47 years old, who is still participating in open and veterans track-and-field meetings and has more than thirty-year experience in weight lifting, participated in the present study, which has been designed in three phases: seven months without hormone drugs, four months with testosterone and AAS and four months without drugs. In all the three phases, progressive high resistance strength training consisted of three various weight-lifting techniques (power squat-lift, bench-press and snatch). Some short sprints, shot-put and discus throws were included periodically.

The training intensity varied between 70-97 percent of the maximum at every training session. The subject performed on average 35-40 contractions at every training session, evaluated by multiple-joint, single repetition with maximum weight technique (1-RM). Starting with 70-75 percent of 1-RM he performed 5 repetitions (reps) per 3-4 sets, for 80-85 percent of 1-RM 5-6 reps per 2-6 sets, for 90 percent of 1-RM three reps and for 95-97 percent of 1-RM 1-2 reps, respectively.

During the phase 2, the subject got the following physician administered AAS: nandrolone-decanoate (19-Nortestosterone-17β-hydroxy-4-oestron-3one, 17-decanoate) (50 mg/injection), Sustanon (3-Oxoandrost-4-17β-yl-propionate; 3-Oxoandrost-4-en-17β-yl-3-phenylpropionate; 3-Oxoandrost-4-en-17β-yl-4-methylpentanoate; 3-Oxoandrost-4-en-17β-yl-decanoate) (250 mg/injection; testosterone-propionate 30 mg; testosterone-phenylpropionate 60 mg; testosterone-isocaproate 60 mg; testosterone-decanoate 100 mg), Winstrol-Depo (17α-methyl 2'H-5α-androst-2-en(3,2-C)-pyrazol-17β-ol) (50 mg/injection) and testosterone-enanthate (3-Oxoandrost-4-17β-yl-4-heptanoate) (250 mg/injection).

The mean value of the dosage for anabolic steroids was 13,6 mg/day, and for testosterone 48,2 mg/day. AAS were injected in the upper part of the gluteal muscle on both sides once per week during the first eight-week period and twice weekly during the second eight-week period.

The subject was continuously under the diet. During phase 1, the mean daily ingest of the food was 4200 kcal,

but 4400 kcal during phase 2 and 3. The mean daily amount of proteins ingested was 2.4 g/kg [17].

Testing of physical characteristics and neuromuscular performance capacity was performed at eight-week intervals.

Muscle samples were obtained from the *vastus lateralis* muscle with the open biopsy technique at the end of each experimental phase and were frozen in liquid nitrogen. Ten µm thick transversal serial sections were cut and proceeded for myofibrillar adenosine triphosphatase (ATPase) at pH 9.4 and after preincubation in acidic media at pH 4.6 and 4.3, respectively [23, 14], NADH-tetrazolium reductase (NADH-TR) [22], menadione-linked alpha-glycero-phosphate dehydrogenase (GPDH) [18], or stained with hematoxyline-eosin.

Percentage and diameter of muscle fibre types were determined by a computer-assisted morphometric method [24]. Muscle fibres were classified into type I, IIA, IIB and IIC [4].

The presence of embryonic and neonatal myosin was confirmed in the third biopsy sample with indirect immunoperoxidase reaction applying monoclonal antibodies BF-45 [29] (1:2.000 in PBS), followed by peroxidase conjugated rabbit anti mouse IgG (Dakopatts, Denmark) (1:40). Peroxidase activity was visualised with diaminobenzidine-tetrahydrochloride. Control sections were incubated without the primary antibody.

Results and Discussion

The results of this research support studies reporting anabolic-androgenic steroid-induced changes in body composition and in neuromuscular performance [1]. All the cited results were obtained at the end of the three experimental phases. Marked gains in fat free weight (56.8, 63.6, 58.7 kg) and body mass (83, 94.1, 89.8 kg), accompanied by changes in body fat in phases 1 (8.8%), 2 (6.8%) and 3 (9.1%) were observed. Results improved in all the three weight-lifting techniques (bench-press, squat-lift and snatch) (fig. 1), and in various jumping tests. Squat jump (SJ) and countermovement jump (CMJ) improved for 18.7% and 13.5% in phase 2, for 12.8% and 8.45% in phase 3. Two months after withdrawal of hormone drugs the gain was even 21.0% and 17.1%, respectively, as compared to the phase 1. The difference between CMJ and SJ decreased for about 21 percents in phases 2 and 3, which points to a possible deleterious effect on muscle elastic properties. A typical strength training does namely not alter this performance trait [1].

It has been proved that in response to weight-lifting exercise the overloaded muscle rapidly increases in weight, which is due primarily to an enlargement of muscle fibres without an increase in their total number [11]. On the other hand Gonyea et al. [12] have proved an increase in fibre number after prolonged weight-lifting exercise.

Nevertheless, biopsy samples exhibited unexpected results. The first biopsy was evidently normal; type I and IIA fibres showed a mosaic distribution pattern, and only

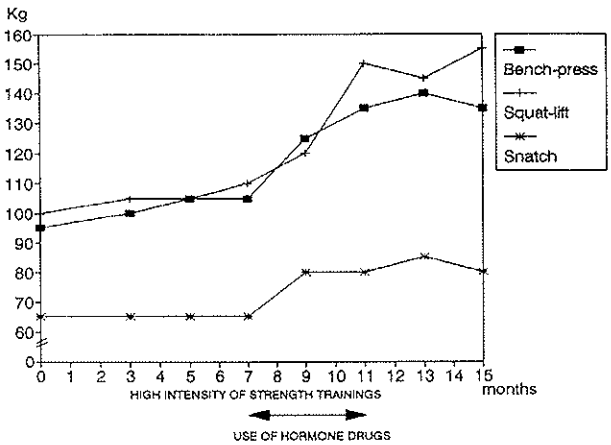

Figure 1: Test of muscle contractile force, measured by 1-RM.

single IIB and IIC fibres were present (fig. 2a, d, g). Preponderance of type I fibres and the diameters which were at upper limits for normal *vastus lateralis* muscle [25] pointed to an arduous long-term excercise, which is known to increase the bulk of type I fibres, both by hypertrophy of fibres and by transformation of fibre types with simultaneous increase in oxidative capacity of the muscle [20, 28].

In the second biopsy sample reduced percentage of type I fibres (55 vs 65%) accompanied by increased percentage of IIA fibres (45 vs 35%) was found. The supposed transformations of type I into IIA fibres most probably resulted from anaerobic training [3]. The process was extremely evident in the atrophic fascicles of the third biopsy sample where some IIB fibres (10%) were present and the percentage of type IIA fibres was increased (50%) due to diminished percentage of type I fibres (13%).

Fibre diameters of the second biopsy were evidently smaller compared to the first sample (28-35 µm vs 60 µm). Type I fibres showed evident central lesions (fig. 2b, e, h, 3 a, c). No differences in diameter between fibre types were noticed in the first and second biopsy samples.

Atrophy of all fibre types and selective damage of type I fibres in the second biopsy, which could have been caused by the cumulative effect of the drugs, by training alone, or by a combination of both effects, do not fit into the anabolic-androgenic steroid-induced hypertrophy model [19, 27]. It is quite possible that above a certain level AAS can have deleterious effect on skeletal muscle tissue. Enlarged intercellular spaces (see fig. 2b) could result from retention of water, caused by hormone drugs.

The third biopsy sample was extremely heterogeneous (fig. 2c, f, i, 3 b, d): whole fascicles of hypertrophic fibres of both types (I and II) existed, in addition to regions, where fascicles were comprised of atrophic fibres of both types. There was an evident increase in the percentage of IIC fibres (13-19%).

In hypertrophic region of the third biopsy sample the diameter of all fibre types was enlarged (62 µm IIC, 68 µm

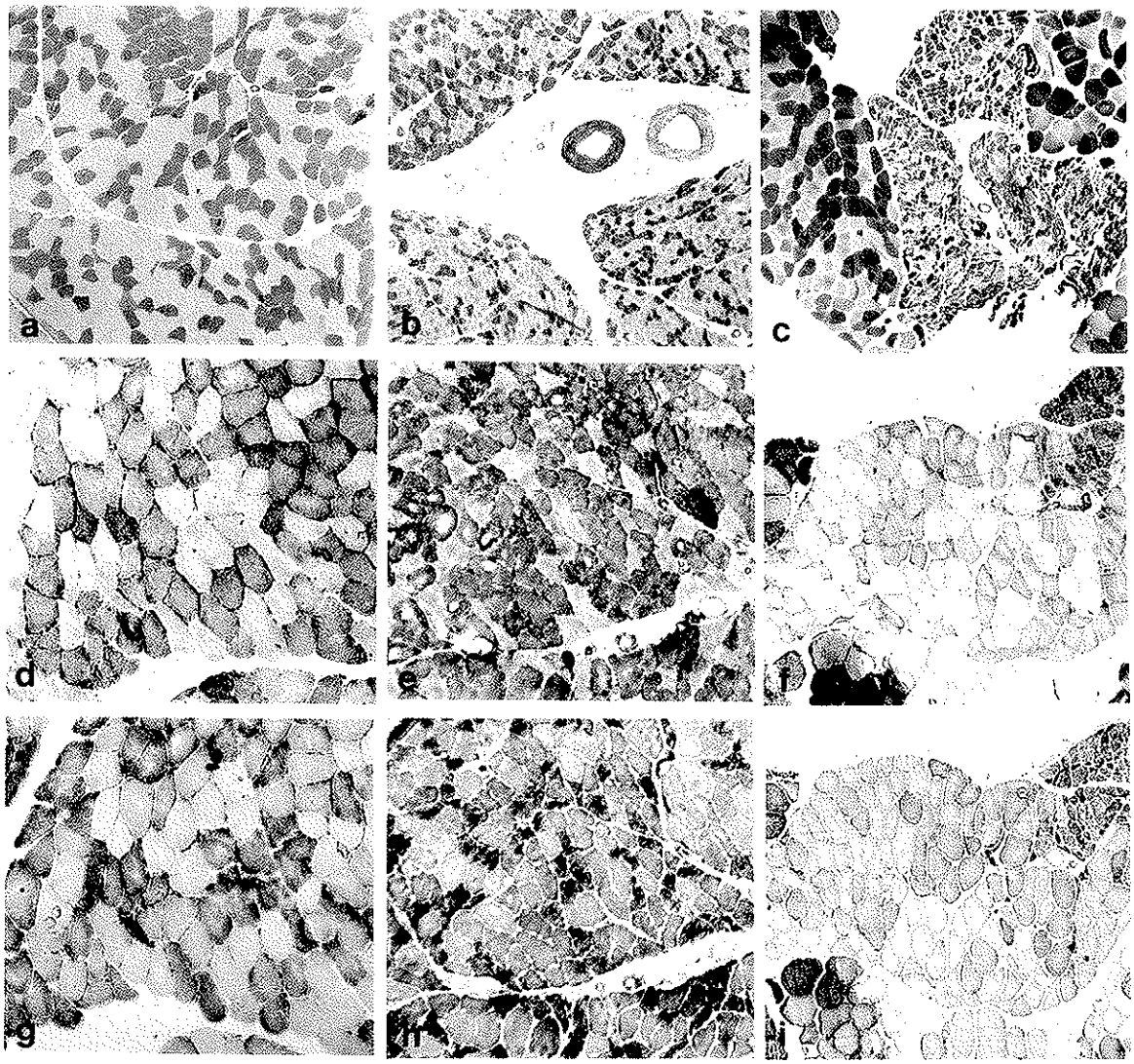

Figure 2. Histochemical characteristics of the three biopsy samples of the vastus lateralis muscle. Myofibrillar adenosine triphosphatase at pH 9.4 of the first (a), second (b) and third sample (c). Magnification 25x. NADH-TR of the first (d), second (e) and third sample (f). Magnification 70x. Menadione-linked alpha-glycerophosphate dehydrogenase of the first (g), second (h) and third (i) sample. Magnification 70x.

I, 88 μm IIA). In the region of atrophic fascicles the diameter of all fibre types was reduced (20-30 μm), absolute values being similar to those of the second biopsy. IIA fibres were the most atrophic fibres.

Gonyea et al. [12] proved an increase in fibre number after prolonged weight-lifting exercise, however, in our material no proofs for hyperplasy have been found. In the second biopsy sample only single fibres possessed centrally located nuclei which can lead to the conclusion that regeneration processes were minimal (Fig 3c). The same holds true for the atrophic region of the third biopsy sample (Fig 3d). The presence of embryonic myosin heavy chains and fibres with centrally located nuclei, as well as the presence of type IIC fibres in the third sample lead to a supposition, that the damage could be reversible. Fascicles composed of hypertrophic fibres probably result from effects of hormone drugs, or else from the regeneration of the damaged muscle.

We are well aware of the biased representativeness of biopsy samples, especially since it has been pointed out that anabolic effects can differ considerably from muscle to muscle and from species to species [27]. The observed damages in muscle tissue could also have been focal, anyhow, it is not excluded that in the second biopsy type I

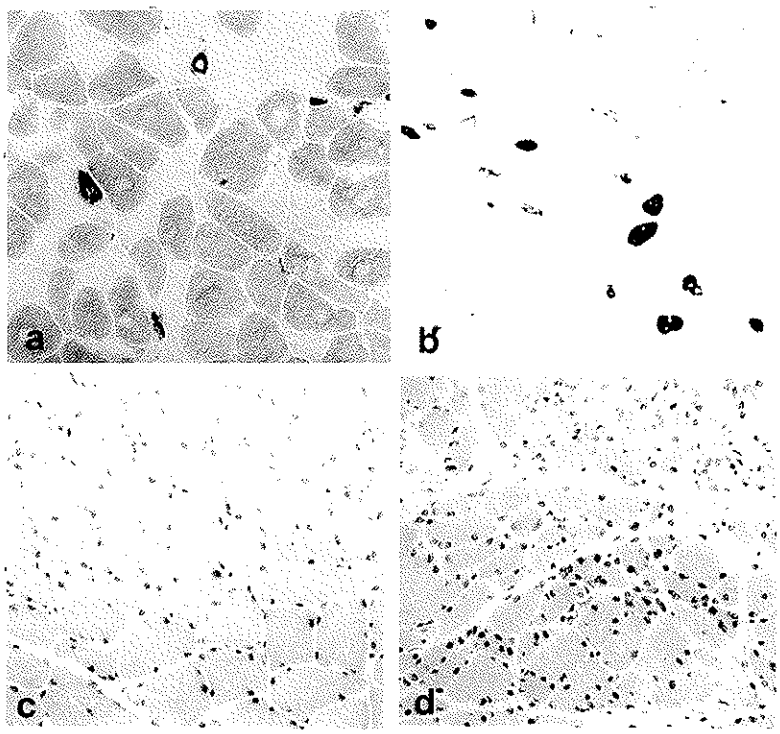

Figure 3. Second biopsy sample: a) myofibrillar adenosine triphosphatase at pH 4.3. Central lesions in type I fibres are clearly seen. Magnification 128x. c) Histological staining with hematoxyline-eosin. Magnification 170x. Third biopsy sample: b) Immunohistochemical demonstration of embryonic myosin. Magnification 100x. d) Hematoxyline-eosin staining. In some fibres central nuclei are clearly seen. Magnification 170x.

fibres could not follow the extreme increases in work load by gradual transformations and were therefore damaged.

Extremely unusual is the finding of atrophic fascicles, composed of both type I and type II fibres. They could have been a consequence of a mechanical damage of a nerve, followed by an atrophy of a whole muscle region which was caused by exaggerated, chronic heavy resistance training. On the other hand they could have just been the persisting atrophic fibres proved in the second biopsy, while other fibres, influenced by AAS, have hypertrophied. It is namely virtually certain that the drugs continued to be released from the depots after administration was stopped, and their effects have therefore overlapped into the third phase of the study.

With respect to present histochemical analyses it must be taken into consideration that AAS are not truly anabolic, but they actually acted androgenically, as it has been proved in chicken [10]. In general the androgenic action makes the athlete more aggressive, which is reflected in his longer training sessions, harder training [5, 6], and the desire to gain better and better results. The possible indirect anabolic action could increase the athlete's appetite, as he needed to eat more. Anyhow, there was no evidence for a direct action on the muscle tissue.

Although, the results reflect the increased motivation that could have been induced by androgenic effects, it is rather difficult to deduce pharmacological effect of the drugs on the muscle. Therefore, it seems reasonable that AAS affected indirectly through androgenic action the quantity and intensity of training sessions and the amount of weight that was lifted in the second phase. Our results are in contradiction with the known positive correlation between muscle cross-sectional area and the maximal strength [32]. Nevertheless, some strength could be gained through tissue expansion due to increase in cellular fluid and general oedema (water retention) [16]. The latter statement can partly be confirmed by enlarged intercellular spaces in the second biopsy sample (see fig. 2b).

In the first and in the second biopsy sample oxidative, oxidative-glycolytic and glycolytic fibres existed. A whole spectrum of NADH-TR and GPDH activities was evident in the hypertrophied regions of the third sample (Fig. 2f, i). On the other hand, atrophic fibres were extremely oxidative-glycolytic.

By qualitative inspection of the biopsy samples an enhancement of oxidative and glycolytic capacity of muscle fibres in the second biopsy has been observed. It was even more evident in the atrophic region of the third biopsy. The

hypertrophic region showed equal or even lower activity of oxidative enzymes, and in single fibres an increased activity of enzymes representing glycolytic metabolism. In female rabbits a significant trend toward lower oxidative potential of muscle fibres was also observed [27].

Though effect of glucocorticoids on skeletal muscles has intensively been studied [13] and steroid myopathy is well known in clinical practise [8], there are only few studies dealing with the effect of anabolic androgenic steroids on human skeletal muscles [1, 2, 15, 19, 21]. Most of them report about hypertrophy of all fibre types. Morphological abnormalities like atrophic fibres, damages of muscle mitochondria and centrally positioned nuclei were also observed [21], however, the authors are not sure, whether the phenomenon is to be ascribed to the effect of AAS alone or to a combined effect of AAS and different pattern of training. The major disadvantage of all the existing investigations is that the effect of AAS is mostly combined with training and no precise information about the dose and sometimes even about the type of AAS is provided.

Though in humans evident effect of AAS on skeletal muscles is expected only in combination with proper exercise [31], myotropic effect of anabolic hormones was observed even without it in rabbit muscles [26].

Controversly, other experiments on animals [7, 8, 30] confirmed the effect of AAS only in combination with training: hypertrophy of slow twitch fibres, a trend towards fast twitch oxidative glycolytic fibres, accompanied by a diminished percentage of fast twitch glycolytic fibres was observed. Occasional disruption of mitochondria was ascribed to the effect of AAS alone [30]. Surprisingly, AAS did not cause any strengthening of muscle contractile capabilities, as proved by in vitro experiments with *extensor digitorum longus* muscle of the rat [8].

Deduced from the persisting improvement in the weight-lifting techniques (Fig. 1), we propose that some athletes might have been tempted for the quick fix in the muscle strength during winter and then use it during summer without the need to take hormonal drugs permanently and to risk their detection.

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