

From The Department of Integrative Medical Biology, Section for Anatomy, Umeå University and The Department for Health Science, Section for Medical Science, Luleå University of Technology, Sweden

## **STRENGTH TRAINING AND ANABOLIC STEROIDS**

**A comparative study of the vastus lateralis, a thigh muscle and the trapezius, a shoulder muscle, of strength-trained athletes**

**Anders Eriksson**



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Cover picture: Cross-sections stained for MyHC IIA of the vastus lateralis muscle from a power lifter (left) and a power lifter who had used anabolic steroids (right).

# **STRENGTH TRAINING AND ANABOLIC STEROIDS**

## **A COMPARATIVE STUDY OF THE VASTUS LATERALIS, A THIGH MUSCLE AND THE TRAPEZIUS, A SHOULDER MUSCLE, OF STRENGTH-TRAINED ATHLETES.**

### **ABSTRACT**

*Anders Eriksson, The Department of Integrative Medical Biology, Section for Anatomy, Umeå University and The Department for Health Science, Section for Medical Science, Luleå University of Technology, Sweden*

Strength training is widely used to increase performance in sports with high physical demands. The use of drugs such as anabolic steroids among athletes is a well-known phenomenon, and the effects of these drugs on physical performance documented.

The studies presented in this thesis focused on the mechanisms of muscle fiber hypertrophy in the vastus lateralis and the trapezius muscles of strength trained elite athletes. The main hypothesis was that the muscle adaptations to strength training and anabolic steroids are muscle specific.

Biopsies were obtained from the trapezius and the vastus lateralis from three groups of elite power lifters. Nine used drugs, ten did not and seven had previously used drugs. Six sedentary males served as controls. The biopsies were frozen and cut in serial cross sections. Histological and immunohistochemical staining techniques were used to analyze muscle fiber morphology and pathology. Fiber type distribution, fiber area, myonuclei number and distribution, satellite cell number and proportion of split fibers were counted and compared for the two muscles within and between the groups.

The main findings were that: a) Muscle fiber hypertrophy by strength training is further increased by anabolic steroids. b) The number of nuclei per muscle fiber is higher in power lifters using anabolic steroids compared to non-steroids using lifters. c) Among power lifters who have withdrawn from anabolic steroid usage and training for several years, the number of myonuclei, both subsarcolemmal and internal, remains high. d) In active power lifters, anabolic steroids have no further effect on the number of satellite cells per fiber. e) Power lifters have a high proportion of split fibers.

High intensity resistance training increases muscle strength and banned substances such as testosterone and anabolic steroids can enhance the training effects. The studies on muscle cell morphology presented in this thesis reveals that anabolic steroids and testosterone increases muscle fiber size and adds more nuclei to the muscle cell.

Based on the morphological appearance of muscle sections from doped and non-doped power lifters, we conclude that testosterone and anabolic steroids enhances the hypertrophic effects of training without adding new features. The addition of myonuclei by training and doping appears to be longer lasting in some muscles than in others. The high proportion of split fibers in power lifter is probably due to high mechanical stress.

The findings and conclusions in this thesis raise questions regarding relevant suspension times for athletes caught with banned substances in the body.

Key words: Strength training, Anabolic steroids, Vastus lateralis, Trapezius, Enzymehistochemistry, Immunohistochemistry, Muscle fiber, Myonuclei, Satellite cell.

## LIST OF PUBLICATIONS

- I Kadi, F., Eriksson, A., Butler-Browne, G.S & Thornell, L-E. Cellular adaptation of the trapezius muscle in strength trained athletes. *Histochemistry and Cell Biology*, 1999, 111: 189-195.
  
- II Kadi, F., Eriksson, A., Holmner, S. & Thornell, L-E. Effects of anabolic steroids on the muscle cells of strength trained athletes. *Medicine & Science in Sports & Exercise*, 1999, 31 (11) : 1528-1534.
  
- III Eriksson, A., Kadi, F., Malm, C. & Thornell, L-E. Skeletal muscle morphology in power-lifters with and without anabolic steroids. *Histochemistry and Cell Biology*, 2005, 124: 167-175.
  
- IV Eriksson, A., Lindström, M., Carlsson, L. & Thornell, L-E. Hypertrophic muscle fibers with clefts and fissures; fiber splitting or abortive regeneration. (Published online 2006-04-20) *Histochemistry and Cell Biology*, 2006
  
- V Eriksson, A., Kadi, F., Tabatabai, P. Bonnerud, P., Thornell, L-E. Anabolic steroids withdrawal in strength trained athletes: How does it affect skeletal muscles. (Manuscript)

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# 1 INTRODUCTION

## 1.1 GENERAL INTRODUCTION

Already during the antiques, we knew that the type of physical exercise that made us stronger also made us able to run faster, jump higher and throw longer. Actually, in all human history, muscle strength has played an important and decisive role for survival and breeding. However, in our ancient history differences in strength between humans were mainly related to inheritable factors and access to protein-rich food. Today, strength training is a complement and an important part of the total training regimen in many sports e.g. track and fields, swimming and ice hockey. Lately, also endurance sports such as cross-country skiing and long distance running have begun to include strength training in their training protocols.

It has been demonstrated that the initial increase in muscle strength is due to neural factors, and that muscle fiber hypertrophy will occur only after several weeks of strength training (Hakkinen *et al.*, 1984; Aagaard, 2006). In the later adaptation phase, muscle protein increases, and the contractile units begin to contribute the most to the changes in performance capabilities (Kraemer *et al.*, 1996). However, the exact mechanisms and cellular events responsible for maintaining and, on demand also improving, muscle strength is not yet fully understood. Furthermore, if different muscles have the same response to strength training is not known and has not yet been investigated.

The use of drugs in sports is today a widespread phenomenon. Already in the 1936 Berlin Olympics, there were rumors that German athletes were given testosterone in order to improve their physical performance (Yesalis, 1993). In 1958, the anabolic steroid Dianabol (methandrostenolone) was released and even if testosterone was known among bodybuilders before that, this was the definite arrival for anabolic steroids in strength intensive sports (Yesalis 1993). The myotrophic effects of anabolic steroids have previously been demonstrated (Bhasin *et al.*, 1996). In this study, the effects were further enhanced when the drug treatment was combined with strength training. However, the mechanisms by which anabolic steroids may increase muscle fiber area and strength were not investigated.

In most sport, associations a caught athlete is nowadays (2006) suspended from all sport activities for 2-4 years. The general apprehension is that anabolic steroids only give effects for a limited time. However, it is not known if any of the effects remain in the muscles for a long time and if long-term effects can be detected.

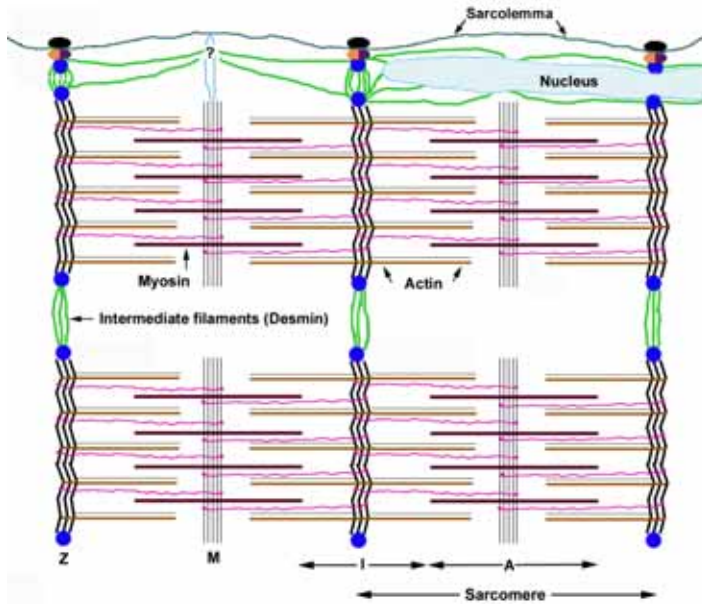
## 1.2 THE HUMAN MUSCULAR SYSTEM

The muscular system in the human body consists of approximately 650 different muscles. Most of them are attached directly or indirectly through tendons to bones, cartilages, ligaments, fascia or a combination of these structures. The tendons transfer the tension from the muscles to the bones and when a muscle shortens, the different skeletal parts come closer to each other. The muscles have different myoarchitecture, meaning that the muscle fibers are arranged in different ways. A fusiform muscle is spindle shaped and has a round thick belly. Pennate muscles are feather like and can be unipennate, bipennate or multipennate. One example of a pennate muscle is the vastus lateralis muscle. Because of the short fiber length compared to the total muscle length in pennate muscles, they accommodate a higher number of fibers per area unit compared to fusiform muscles. By this arrangement, a pennate muscle has great strength but shorter exertion than fusiform muscles. Another myoarchitecture arrangement is parallel fibers associated with an aponeurosis. This is the case in flat muscles such as the trapezius muscle. Thus, there are obvious anatomical differences between the two muscle studied in this thesis.

Muscle contraction is initiated by a nerve signal and controlled by the arrangement of motor units. A motor unit consists of a motor neuron and the muscle fibers it controls. A nerve impulse in a motor neuron in the spinal cord causes all the muscle fibers supplied by that motor unit to contract simultaneously. The number of fibers in a motor unit varies from one or very few in muscles that require high precision, to several hundred in the large thigh and trunk muscles.

Muscle fibers are long, cylindrical and multi-nucleated cells. The contractile elements in a muscle fiber are the myofibrils, the organelles responsible for force production in striated muscles. Each myofibril contains repetitive contractile units called sarcomeres that are arranged along the length of the fiber. Within the sarcomere, myosin and actin are the primary contractile proteins. The entire fiber is surrounded by a basement membrane which when combined with the cell plasma membrane is termed the sarcolemma (Illustration 1).





**Illustration 1.** A sarcomere, the contractile unit of skeletal muscle (by Lena Carlsson by permission)

The sliding filament theory of contraction proposes that a muscle shortens or lengthens because the thick myosin and the thin actin filaments slide past each other without changing length (Huxley & Niedergerke, 1954a, 1954b). In a concentric contraction, the force is generated by the myosin heads, which cyclically bind to actin filaments, rotate in a direction that pulls the Z-discs closer to each other and the whole sarcomere is shortening. Each myosin head consumes one ATP by hydrolysis for each cycle. When the hydrolyzed ATP molecules are replaced by another ATP molecule, the myosin heads detach from the actin filament and the cycle is completed. The maximal force a muscle can produce is the sum of forces from all myosin heads involved in the contraction.

The human skeletal muscle is composed of several different fiber types that can be distinguished by the dominant myosin heavy chain (MyHC) isoform. On the basis of enzyme histochemistry for ATPase activity at different pH and/or by immunohistochemistry, human skeletal muscles can be classified into the main fiber types I, IIA, IIB and IIX).

The myosin molecule consists of two MyHC and four light chains (MyLC). The majority of the muscle fibers in human limb muscles of the MyHC isoforms MyHC I, MyHC IIA and MyHC IIX. Type I fibers express MyHC I, type IIA fibers express MyHC IIA and type IIB fibers express MyHC IIX. Hybrid fibers with a mixture of different

proportions of the MyHCs are of importance for the contractile properties of the muscle (Andersen *et al.*, 1994; Harridge *et al.*, 1996). Type IIAB fibers contains a mixture of MyHC IIA and MyHC IIX and type IIC fibers contains a mixture of MyHC I and MyHC IIA. In reality, most fibers contain a mixture of several myosin isoforms, thus, human skeletal muscle fibers is contains a continuum of fibers, from very slow to very fast.

Type I muscle fibers belong to slow motor units and have slow contraction speed, high endurance capacity and depend mainly on oxidative metabolism. Type IIA, IIAB and IIB fibers belong to fast motor units and are both faster and stronger than type I fibers, but with lower endurance capacity. Type IIA fibers are intermediary and dependent on both the oxidative metabolism and glycolysis while type IIB fibers are the strongest and fastest fiber type, rely mainly on glycolytic metabolism and are not fatigue resistant. MyLCs are present as different isoforms, and together with the MyHCs form different combinations that play a role in regulation of the contractile properties of the muscle fiber (Pette & Staron, 1997).

### **1.3 MUSCULAR ADAPTATION TO STRENGTH TRAINING**

Muscle fiber adaptation to strength training can result in muscle fiber hypertrophy and alteration in fiber type composition. These changes are mediated by different hormones and growth factors, mechanical stretch, intensity in neurosignals and other factors. Because of the complex interaction between these systems the exact mechanisms behind muscle adaptations to strength training are very complicated. Numerous investigations have described the effects of strength training on human skeletal muscles (Tesch, 1988; Staron *et al.*, 1990; Hopp, 1993; Wang *et al.*, 1993; Abernethy *et al.*, 1994; Kraemer *et al.*, 1996; McCall *et al.*, 1996; Green *et al.*, 1999; Kraemer *et al.*, 1999; Hakkinen *et al.*, 2002). In these studies, there is a wide diversity regarding to the number of sets and repetitions used in the training programs as well as differences in the number of training sessions per week. This diversity makes it difficult to form a concise picture describing the effects of strength training. It has been demonstrated that muscular adaptation is highly specific to the types of training programs conducted (Kraemer *et al.*, 1996). After the initial neural adaptation, muscle protein turnover increases and the contractile units begin to contribute to the changes in physical performance capabilities (Hakkinen *et al.*, 1984; Kraemer *et al.*, 1996).

Type I fibers can be stimulated with endurance training but also with strength training by using a low load and a high number of repetitions. This training regimen

generates a low frequency neuronal signal that preferentially stimulates transcription of the genes that produce MyHC I (Schiaffino *et al.*, 1999; Baldwin & Haddad, 2001).

A great load and highest possible moving velocity generates a high frequency neuronal signal and stimulates preferentially transcription of genes for type II MyHCs. Further, the muscle fibers can transform, especially from type IIB fibers into more oxidative types such as type I and type IIA (Jurimae *et al.*, 1996; Pette & Staron, 1997, 2001).

Athletes who need maximal power and/or explosive strength perform their strength training with high loads, few repetitions, often between 1 and 6, or lighter weights but higher moving velocity.

Maximal contractile force of a muscle is related to the muscle fiber area, the total muscle area and the fiber type composition (Bruce *et al.*, 1997; Bamman *et al.*, 2000). Muscle fiber hypertrophy in response to strength training is a well-documented process. It has been demonstrated that strength training increases the amount of myofibrillar proteins by an elevated protein synthesis (MacDougall *et al.*, 1995; Phillips *et al.*, 1997).

#### **1.4 MYONUCLEI AND THEIR RESPONSE TO STRENGTH TRAINING**

In mature skeletal muscle fibers, the myonuclei are located beneath the sarcolemma. Each muscle fiber contains numerous, permanently post-mitotic, myonuclei along the whole fiber length (Stockdale & Holtzer, 1961). This means that an increase in myonuclei number must come from an external source (reviewed by (Allen *et al.*, 1999)).

It is well known that each nucleus supports a certain volume of the cytoplasm with mRNA for protein synthesis. This volume is referred to as a nuclear domain (Cheek, 1985).

Earlier human studies support the idea that the number of myonuclei plays a mechanistic role in muscle fiber hypertrophy (Edgerton & Roy, 1991). In animal studies, it has been shown that the myonuclear number per fiber increase as a response to strength training (Cabric & James, 1983; Edgerton & Roy, 1991; Winchester & Gonyea, 1992; Allen *et al.*, 1995; Roy *et al.*, 1999).

#### **1.5 SATELLITE CELLS AND THEIR RESPONSE TO STRENGTH TRAINING**

Satellite cells (Mauro, 1961) are a population of muscle precursor (stem) cells located between the plasma membrane and the basal lamina. In adult skeletal muscles, the satellite cells can fuse with existing myofibers and contribute to an increased amount of myonuclei and hypertrophy of the muscle fiber.

Different types of stimulation can activate the satellite cells to incorporate into muscle fibers during growth, but the exact mechanisms are still poorly understood.

Several factors are known to be involved in satellite cell activation. HGF is so far the only factor known to activate quiescent satellite cells (Allen *et al.* 1995; Tatsumi *et al.* 1998). Factors known to affect chemotaxis, proliferation and differentiation is e.g. IGF-1, IGF-II, testosterone and growth hormones and (Hawke & Garry, 2001).

Heavy strength training releases growth factors such as IGF-I, IGF-II and testosterone that are all involved in the regulation of the satellite cell population during regeneration (Jennische *et al.*, 1987; Yarasheski *et al.*, 1995; Harridge, 2003; Bird & Tarpinning, 2004). In fact, satellite cell activity is regulated by numerous factors in a temporal and concentration dependent fashion during regeneration (for review see (Allen *et al.*, 1999).

## **1.6 REGENERATIVE RESPONSE TO STRENGTH TRAINING AND SPLIT FIBERS**

In adult skeletal muscles, regeneration normally occurs after muscle damage. For instance, if a muscle fiber ruptures, neighboring satellite cells are incorporated into the damaged fiber and produce new muscle tissue in order to repair the damaged area.

Power lifters perform high intensity strength training and expose their muscles to a very high mechanical stress (Clarkson & Sayers, 1999). In some instances, such stress has been suggested to result in the appearance of split fibers (Schmalbruch, 1976; Tesch, 1988). The mechanism by which muscle fiber splitting is induced is not yet settled. In the literature, five possible mechanisms are considered.

1) Mechanical stress in hypertrophied muscle fibers is suggested to induce cleavage and developing of an invagination of the surface (Swash & Schwartz, 1977).

2) Activation of satellite cells in the absence of muscle fiber necrosis. Such satellite cells might fuse at one or more places with the parent fiber and then mature (James, 1973). Activation of satellite cells has also been reported in experimental denervation (Schultz, 1978) and experimental myotonia (Danon & Carpenter, 1991).

3) If a denervated, angular shaped fiber is reinnervated and subsequently hypertrophy it might assume a split appearance (Chou & Nonaka, 1977).

4) After necrosis, activated satellite cells form myoblasts and later myotubes within a single basal lamina. If the myotubes fail to undergo lateral fusion with one another and only fuse longitudinally and with the surviving stump, the result is split fibers (Schmalbruch, 1976).

5) The fifth possible mechanism involves massed tubules arising from the T system that eventually breaks down and results in formation of vacuoles in the center of the fiber. These vacuoles become lined with basal lamina and connect to the interstitial space by clefts. However, this is only reported in one case and apparently very rare (Carpenter & Karpati, 1992).

Most of the studies on muscle fiber splitting are done on diseased human muscles (Hauser *et al.*, 2000; Al-Ani *et al.*, 2001; Chinnery *et al.*, 2001; Rowinska-Marcinska *et al.*, 2005) or on animals (Gonyea *et al.*, 1977; Ho *et al.*, 1980) and very few are focused on healthy strength training human subjects (Tesch, 1988).

### 1.7 THE VASTUS LATERALIS AND THE TRAPEZIUS MUSCLES

Some muscles are very small and specialized like the eye muscles whereas others are large and strong with capability to move and carry the whole human body weight. The quadriceps is one such large muscle group consisting of four individual muscles named musculus (m) rectus femoris, m. vastus medius, m. vastus intermedius and m. vastus lateralis. This muscle group is localized on the anterior side of the thigh and is involved in walking, running and lifting different loads in the daily life. The most lateral part of the quadriceps muscle, the vastus lateralis muscle, is one of the most studied human skeletal muscles.



**Illustration 2.** Vastus lateralis (left) and trapezius (above). (Jarmo Mietenen, used with permission)

Another large skeletal muscle is the trapezius muscle, localized in the neck and shoulder region. In daily life, the trapezius muscle is involved in arm movement by regulating the scapula by stabilizing and lifting up the shoulders. In myalgia, the painful areas are often located to the trapezius muscle in the neck and shoulder region (Kadi *et al.*, 2000a). A comparison between the vastus lateralis and the trapezius muscles have shown that human neck and limb muscles differ in many aspects. These differences include androgen receptor content, nerve supply, muscle fiber composition, capillary network and mitochondrial organization (Lindman *et al.*, 1990; Lindman *et al.*, 1991; Kadi *et al.*, 2000b). Further, men and women differ significantly in the morphology of the trapezius muscle. While men have a clear variation in fiber type composition and fiber size between different regions of the muscle, none of these variations are seen in the females. In addition, the cross-sectional areas of both type I and type II fibers are considerably smaller in the female muscle (Lindman *et al.* 1990; Lindman *et al.* 1991).

For power athletes, the vastus lateralis muscle is of importance preferentially in the squat event which means that the lifter from an upright position, with the barbell resting across the back of the shoulders, sits or 'squats' down by doing a flexion in the knee and hip-joints to a required depth, and then attempts to return to the original position. Such a lift takes approximately 2-5 seconds and requires a maximal explosive strength. The trapezius muscle is mainly used in a static way to hold up the shoulders in deadlift, where the bar is lifted from the floor to an upright position. This means that during exercise, the two muscles are used in different ways by a power athlete.

## **1.8 ANABOLIC STEROIDS IN SPORTS**

During the last decades, the use of anabolic steroids and other medical substances has become a widespread phenomenon, not only among power athletes, but also in different branches of athletics such as track and field, cycling and ice hockey. Also a large part of the population who simply desire to improve their appearance use anabolic steroids (Buckley *et al.*, 1988; Calfee & Fadale, 2006; Striegel *et al.*, 2006). In 1984 and 1985, the US Olympic committee conducted unannounced drug tests with no punitive actions. Among the tested athletes, approximately 50% tested positive for anabolic steroids. Despite improved and extended drug tests, some athletes always try to find ways to get past these, and new substances keep emerging on the black market (Congeni & Miller, 2002; Foster & Housner, 2004). Because athletes are still (2006) tested positive for testosterone and anabolic steroids, actions taken to eliminate drug abuse from sports has not been completely successful.

Athletes who have taken anabolic steroids report several beneficial effects, including increased strength, less sensitivity to fatigue, faster recovery and better motivation, i.e. important features for a successful athlete (Lukas, 1993). There has been some controversy about the exact mechanisms of testosterone and anabolic steroids.

As seen in Table 1 the results from early human studies of the effects from anabolic steroids were rather heterogeneous. Although many early studies administered very small doses with short duration, many scientists considered the effects as a placebo effect (Hervey *et al.*, 1976; Hickson *et al.*, 1976; Ryan, 1981) (Table 1). Today, athletes often use dosages of anabolic steroids of 100 times or more compared to replacement levels which could mean over 1000 mg per week. Thus, these early studies do not reflect the real situation among athletes today (2006).

During the past few years, both increased muscle strength and size in human subjects have been demonstrated and it has even been reported that anabolic steroids supplementation without strength training will induce hypertrophy in human skeletal muscles (Bhasin *et al.*, 1996; Sinha-Hikim *et al.*, 2002; Herbst & Bhasin, 2004). As seen in Table 1 the results from early human studies of the effects from anabolic steroids were rather heterogeneous. Although many early studies administered very small doses with short duration, many scientists considered the effects as a placebo effect (Hervey *et al.*, 1976; Hickson *et al.*, 1976; Ryan, 1981) (Table 1). Today, athletes often use dosages of anabolic steroids of 100 times or more compared to replacement levels which could mean over 1000 mg per week. Thus, these early studies do not reflect the real situation among athletes today (2006).

During the past few years, both increased muscle strength and size in human subjects have been demonstrated as an effect of anabolic steroids (Bhasin *et al.*, 1996). It has even been reported that anabolic steroids supplementation without strength training will induce hypertrophy in human skeletal muscles (Bhasin *et al.*, 1996; Sinha-Hikim *et al.*, 2002; Herbst & Bhasin, 2004).

**Table 1.** Some of the early studies of relationship between administration of anabolic steroids and muscle strength

Drug/dosage	Weeks (duration)	Increased strength	Reference
Methandrostenolone 100mg/week	6	Yes	(Hervey <i>et al.</i> , 1981)
Methandrostenolone 10mg/day	5	Yes	(Ward, 1973)
Nandrolone 1mg/week	9	No	(Fahey & Brown, 1973)
Methandrostenolone 10mg/day	8	Yes	(Stamford & Moffatt, 1974)
Nandrolone and testosterone 100mg/week	3	No	(Crist <i>et al.</i> , 1983)
Methandrostenolone 20mg/day	16	No	(Fowler <i>et al.</i> , 1965)
Varied self- administration	24	Yes	(Alen <i>et al.</i> , 1984)

## 1.9 REMAINING EFFECTS FROM ANABOLIC STEROID ABUSE

Previously described adverse effects from anabolic steroids involve sudden cardiac death, deteriorated liver function, infertility and, in men, gynecomastia and in women masculinisation (Kennedy & Lawrence, 1993; Nieminen *et al.*, 1996; Hartgens & Kuipers, 2004; Maravelias *et al.*, 2005; Petersson *et al.*, 2006). If anabolic steroids have any adverse effects on the muscle fiber has not been investigated.

An interesting question in the context of anabolic steroid usage is whether the effects induced by these substances are permanent or transient. If there are some beneficial effects and if these are long lasting, it can be an advantage for an athlete to have used these substances in the past. Depending on sport association, the suspension time for caught athletes varies from a few months up to four years. There are no studies so far where long term effects after withdrawal of anabolic steroids has been investigated. The question is relevant to clarify whether or not any beneficial effects for athletic performance remains years after secession of drug abuse.



## 2 AIMS AND QUESTIONS

The purpose of this thesis was to investigate and compare muscle adaptations in two different muscles; the trapezius muscle, a shoulder muscle, and the vastus lateralis muscle, a thigh muscle, in response to heavy strength training with or without the administration of testosterone, anabolic steroids and other substances. Morphological appearance following several years after withdrawal of these substances was also studied.

The main questions in this thesis can be listed as follows:

- I. Are there any differences in muscle fiber type distribution and fiber cross-sectional area between power lifters, power lifters using anabolic steroids and power lifters who previously had used anabolic steroids? Are there differences between the vastus lateralis and the trapezius muscles from the same individuals?
- II. Are there any differences in myonuclei number and distribution between these groups and between the two muscles?
- III. Are there any differences in satellite cell frequency between these groups and between the two muscles?
- IV. Is the very high mechanical stress inflicted upon muscles in power lifters reflected as damaged or pathological muscle fibers, regenerative responses and satellite cell activation?

### 3 METHODS

A total of 32 subjects gave their informed consent to participate in these studies. The Ethical Committee at Umeå University approved this work. Written consent in accordance with the policy statement of the Helsinki declaration of human subjects was obtained from all the subjects.

1. *Six control subjects (C group)*: A total of six sedentary males ( $23.3 \pm 3.1$  years) were used as controls. This group is referred to as the

2. *Male power lifters (P group)*: This group ( $27.7 \pm 7.5$  years) had signed a contract with the local club and the Swedish power lifting federation that committed them never to use any drugs. All of them were frequently tested both in connection to competitions but also unannounced tests connected to training.

3. *Male power-lifters using anabolic steroids (PAS group)*: Nine of the subjects ( $31.4 \pm 3.3$  years) have reported the use of a wide variety of high doses of testosterone and anabolic steroids for a period of  $9.0 \pm 3.3$  years. These nine subjects have been individually interviewed regarding their steroid usage. Testosterone was used in combination to a variety of anabolic steroids (nandrolone, stanozolol, primobolan, oxymetholone, mastoron, proviron and durobolan). At the time for the biopsies, the mean dosage was  $938 \pm 527$  mg of testosterone and anabolic steroids that was self-distributed each week. In addition, three subjects used IGF-1 (mean dosage 40 mg/day) and one subject used growth hormones. Two of them have been caught in regular drug testing. The steroid regimen included both "staging," or simultaneous use of several types at high doses, and "cycling," a drug-free period followed by times when the doses and types of drugs taken were increased to a maximum to anticipate peak performance.

All athletes in the PAS and P groups were highly competitive and participated regularly in Swedish and/or international competitions in power events. They trained regularly four to six times a week, two to three hours per session. The sessions consisted in four to seven sets of exercise and three to twelve repetitions per set.

4. *Seven male power-lifters who previously had used anabolic steroids (PREV group)*: All subjects had withdrawn their administration of anabolic steroids and other substances more than one year ago (mean  $8.1 \pm 3.2$  years). They had previously used anabolic steroids for a period of  $4.5 \pm 0.5$  years. At the time for the muscle biopsies three subjects had stopped all forms of physical exercise and the other four were still performing strength training but with various degree of intensity.

### **3.1 MUSCLE BIOPSIES**

After local anesthesia, biopsies were taken from the upper ventral part of the vastus lateralis muscle and the upper part of the trapezius muscle (descending I) (Lindman *et al.*, 1990). The samples were mounted in Tissue tek, OTC compound (Miles laboratories, Naperville Ill) and quickly frozen in propane cooled in liquid nitrogen and stored at -80°C until analyzed.

### **3.2 ENZYMEHISTOCHEMISTRY AND ROUTINE HISTOLOGY**

Serial, 6-10µm cross-sections were cut at -20° C using a Reichert Jung cryostat (Leica, Nussloch, Germany), then mounted on glass slides and air-dried at room temperature. The sections were stained for the demonstration of myofibrillar ATPase after alkaline (pH 10.4 and 9.4) and acid (pH 4.6 and 4.3) preincubations (Dubowitz, 1985) and the muscle fibers were classified into type I, IIA, IIAB, IIB and IIC according to their staining intensity. For details see (Kadi & Thornell, 1999).

Other sections were stained with hematoxylin-eosin (htx-eosin), nicotinamide adenin dinucleotide tetrazolium reductase (NADH-TR) and Gomori trichrome (GT) (Dubowitz, 1985).

### **3.3 IMMUNOHISTOCHEMISTRY**

Five µm thick cross-sections, serial to those used for enzyme histochemistry, were used for immunohistochemical analysis. Sections were rehydrated in 0.01 M PBS, immersed in 5% non-immune serum and incubated with primary antibodies for 60 min at +37°C or overnight at +4°C. Visualization of bound antibodies was performed with peroxidase-anti-peroxidase (PAP) technique (Sternberger, 1979) using antibodies from Dako (Glostrup, Denmark), or with indirect immunofluorescence using fluorescein (FITC) and rhodamine red-X for green and red fluorescence, respectively (Jackson Immunoresearch Laboratories, West Grove, PA, USA). Double labeling was performed with one monoclonal and one polyclonal antibody, visualized by secondary antibodies coupled to fluorochromes of different wavelengths. Nuclei were identified with 4,6-diamino-2-phenylindole (DAPI). Control sections were treated according to standard protocols except that the primary antibody was exchanged with non-immune serum.

### **3.4 ANTIBODIES**

Myosin heavy chain (MyHC) composition was assessed using well-characterized monoclonal antibodies (mAbs). A4.840 strongly stains type I fibers and moderately stains IIC fibers. N2.261 strongly stains type IIA and IIC fibers, weakly stains type I and IIAB fibers, whereas type IIB fibers are unstained. F1.652 and NCL-MHCn detect embryonic

and fetal MyHC isoforms, respectively. The MyHC Abs are purchased from Developmental Studies Hybridoma Bank, Iowa and Novocastra Laboratories Ltd., UK.

MAB NCL-Merosin against laminin  $\alpha 2$  chain (Aumailley *et al.*, 2005) (Novocastra Laboratories Ltd., UK), mAb 4C7 against laminin  $\alpha 5$ -chain (provided by I Virtanen, Helsinki, Finland) and polyclonal Ab PC128 against human laminin (The Binding Site Ltd., Birmingham, UK) were used for identification of the basement membrane of muscle fibers. MAB NCL-Dys2 against dystrophin was used for detection of the plasma membrane (Novocastra Laboratories Ltd., UK) and the intermediate filament proteins desmin and vimentin were identified with the mAbs D33 and V9 (Dako, Glostrup, Denmark).

Sections were also labeled with the mAb CD56 (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) against the Leu 19 antigen, which is expressed during early stages of muscle fiber formation and in satellite cells (Kadi *et al.*, 1999), visualized by peroxidase (PAP) staining and counterstained with Meyer's hematoxylin. Myonuclei were stained blue whereas a brown rim inside the muscle fiber identified satellite cells.

### **3.5 LIGHT MICROSCOPY AND MORPHOMETRIC ANALYSIS**

An average of 200 fibers were photographed and used for the determination of fiber area, fiber types, myonuclear number, proportion of fibers with internal nuclei and frequency of satellite cells. Fiber area was measured on sections stained with the antibodies against the laminin  $\alpha 2$  chain (5H2). Analysis of the sections regarding fiber area, fiber types and myonuclear number was performed using a light microscope (Zeiss Axiophot) connected to a computerized image analysis system (IBAS; Kontron elektronik). On sections stained for htx-eosin, two randomly chosen areas from each biopsy were scanned to determine the frequency of fibers containing internal myonuclei. The proportion of fibers with internal nuclei was calculated as:  $[\text{number of fibers containing internal nuclei}] / [\text{total number of fibers}] \times 100$ . Satellite cell proportion was calculated as:  $[\text{satellite cell number}] / [\text{myonuclear number} + \text{satellite cell number}] \times 100$ . Because each measured muscle section is  $5\mu\text{m}$  thick, fiber area actually represents a section volume equal to  $(\text{area} \times 5\mu\text{m})$ . In other words, there is a linear relationship between area and volume. Nuclear domain can therefore be calculated as:  $[\text{number of nuclei per fiber}] / [\text{fiber area}]$ .

A split fiber is defined by its content of fissures and clefts within its basal membrane. These fibers were photographed and counted on whole muscle cross sections

(mean 449 fibers / section). The proportion of split fibers was calculated as follows; [number of split fibers] / [total number of myofibers] x 100. These sections were evaluated in a Nikon eclipse E 800 microscope (Nikon Inc., Melville, NY, USA) and a SPOT RT Color camera (Dignostic Instruments Inc., Sterling Heights, MI, USA) was used for image acquisition. Digital images were processed using the Adobe Photoshop software (Adobe Systems Inc., Mountain View, CA, USA).

### **3.6 STATISTICAL ANALYSIS**

Data are presented as means and standard deviations (SD). Because data was normally distributed, the statistical significance of the differences between the two groups was determined using a t-test for unpaired data. The statistical significance of correlations between two parameters was determined by using Fichers r to z test. p-values <0.05 were considered statistically significant.

## 4 RESULTS

### 4.1 FIBER TYPE DISTRIBUTION

#### 4.1.1 PAS and P groups

The mean values for fiber type distribution in both the vastus lateralis and the trapezius muscles were not statistically significant between the P and the PAS groups (Table 2). The predominant fiber types in all groups were type I and type IIA fibers. In general, type IIAB and type IIB fibers were rare. At an individual level, the frequency of type I fibers varied from 28% to 62% and of type IIA fibers from 4% to 68%. Type IIB fibers occurred preferentially in the vastus lateralis muscle in two subjects from the P group.

**Table 2.** Fiber type distribution (mean  $\pm$  SD) for the P, PAS and PREV groups

Fiber type	Vastus lateralis			Trapezius		
	P	PAS	PREV	P	PAS	PREV
Type I	46.4 $\pm$ 8.7	40.0 $\pm$ 12.8	47.1 $\pm$ 9.7	56.0 $\pm$ 10.4	58.7 $\pm$ 6.3	51.4 $\pm$ 14.5
Type IIA	34.7 $\pm$ 19.8	39.8 $\pm$ 16.0	43.8 $\pm$ 7.7	37.2 $\pm$ 9.0	35.6 $\pm$ 9.4	44.2 $\pm$ 19.2
Type IIAB	8.5 $\pm$ 10.8	13.8 $\pm$ 10.9	4.4 $\pm$ 5.6	3.4 $\pm$ 6.5	2.3 $\pm$ 3.2	0.7 $\pm$ 1.2
Type IIB	7.4 $\pm$ 16.1	0.7 $\pm$ 1.6	2.6 $\pm$ 3.0	0.0	0.0	2.9 $\pm$ 5.4
Type IIC	2.5 $\pm$ 2.5	5.0 $\pm$ 2.3	2.1 $\pm$ 1.8	3.5 $\pm$ 7.2	3.5 $\pm$ 4.2	0.9 $\pm$ 1.6

#### 4.1.2 PREV group

No significant difference for the mean values was observed when comparing the PREV group to the PAS and P groups. Individual variations were seen also in the PREV group. In both the vastus lateralis and the trapezius muscles, type I fiber frequency varied from 22% to 63% and type IIA fiber frequency varied from 13% to 78%.

### 4.2 FIBER AREAS

#### 4.2.1 PAS and P groups

Mean fiber areas (all fiber types) of all subjects in the P and the PAS groups are shown in Figure 1 and Tables 3 and 4. The PAS group had significantly larger fiber areas than both the P and the C groups in both muscles ( $p < 0.05$ ).

#### 4.2.2 PREV group

In both the vastus lateralis and the trapezius muscles, the mean fiber area (all fiber types) in the PREV group was significantly smaller than in the PAS group but not smaller

than in the P group (Figure 1 and Table 4). The PREV group had significantly larger fiber areas than the C group in both muscles ( $p < 0.05$ ).

Thus, the ranking order of fiber size in both the vastus lateralis and the trapezius is PAS > P=PREV > C.

**Table 3.** Morphological data from vastus lateralis muscle in the P and PAS groups

Subject (n)	Mean fiber area ( $\mu\text{m}^2$ )	Number of nuclei per fiber	Proportion of fibers with internal nuclei (%)	Number of satellite cells per fiber	Proportion of split fibers (%)
<b>P group</b>					
1	6169	4.9	19.2	0.35	0.8
2	6388	3.7	20.8	0.31	0.3
3	6568	4.6	3.8	0.37	0.1
4	5791	4.4	2.3	0.61	0.3
5	4724	5.2	5.0	0.68	0.0
6	6691	6.3	29.5	0.70	0.2
7	6349	3.7	0.0	0.40	0.0
8	4635	3.8	2.0	0.23	0.9
9	8063	6.4	2.6	0.30	0.8
10	5654	3.6	8.1	0.40	0.4
<b>Mean <math>\pm</math> SD</b>	<b>6103 <math>\pm</math> 995</b>	<b>4.7 <math>\pm</math> 1.0</b>	<b>9.3 <math>\pm</math> 10.1</b>	<b>0.44 <math>\pm</math> 0.17</b>	<b>0.4 <math>\pm</math> 0.3</b>
<b>PAS group</b>					
1	10960	7.7	57.7	0.49	2.0
2	11751	7.6	19.2	1.17	2.1
3	8447	4.8	15.2	0.53	0.6
4	9387	5.3	8.3	0.79	0.3
5	6474	5.1	6.3	0.20	0.9
6	8142	7.0	37.0	0.64	0.8
7	9102	5.8	41.5	0.50	3.0
8	13167	6.1	42.9	0.47	0.4
9	10576	6.8	36.1	0.43	0.6
<b>Mean <math>\pm</math> SD</b>	<b>9778 <math>\pm</math> 2043 *</b>	<b>6.2 <math>\pm</math> 1.1 *</b>	<b>29.3 <math>\pm</math> 17.7 *</b>	<b>0.58 <math>\pm</math> 0.27</b>	<b>1.2 <math>\pm</math> 1.0 *</b>

\* Significant difference from P group

**Table 4.** Morphological data from the trapezius muscle in the P and PAS groups

Subject (n)	Mean fiber area ( $\mu\text{m}^2$ )	Nuclei/fiber (nr per fiber)	Proportion of fibers with internal nuclei (%)	Number of satellite cells per fiber	Proportion of split fibers (%)
<b>P group</b>					
1	4548	4.1	4.0	0.28	2.0
2	4878	4.5	6.0	0.20	0.7
3	8159	5.2	6.0	0.31	8.8
4	5161	4.0	4.0	0.29	0.1
5	8020	3.8	9.0	0.36	0.0
6	7425	4.0	6.0	0.21	3.3
7	5974	4.4	2.0	0.27	0.0
8	8213	4.4	10.0	0.28	2.2
9	8244	5.0	2.0	0.39	0.0
10	7674	4.2	2.0	0.34	0.0
<b>Mean <math>\pm</math> SD</b>	<b>6830 <math>\pm</math> 1516</b>	<b>4.3 <math>\pm</math> 0.4</b>	<b>5.1 <math>\pm</math> 2.8</b>	<b>0.29 <math>\pm</math> 0.06</b>	<b>1.7 <math>\pm</math> 2.8</b>
<b>PAS group</b>					
1	12320	5.5	32.0	0.41	3.4
2	8284	4.7	22.0	0.30	0.4
3	10706	5.2	27.0	0.28	5.2
4	7979	4.8	5.0	0.33	1.3
5	11637	6.3	45.0	0.45	1.3
6	8627	5.2	18.0	0.35	0.0
7	6725	4.5	11.0	0.43	0.5
8	9365	5.1	37.0	0.36	2.0
9	7760	5.2	27.0	0.22	0.2
<b>Mean <math>\pm</math> SD</b>	<b>9267 <math>\pm</math> 1897 *</b>	<b>5.2 <math>\pm</math> 0.5 *</b>	<b>24.9 <math>\pm</math> 12.5 *</b>	<b>0.35 <math>\pm</math> 0.08</b>	<b>1.7 <math>\pm</math> 1.8</b>

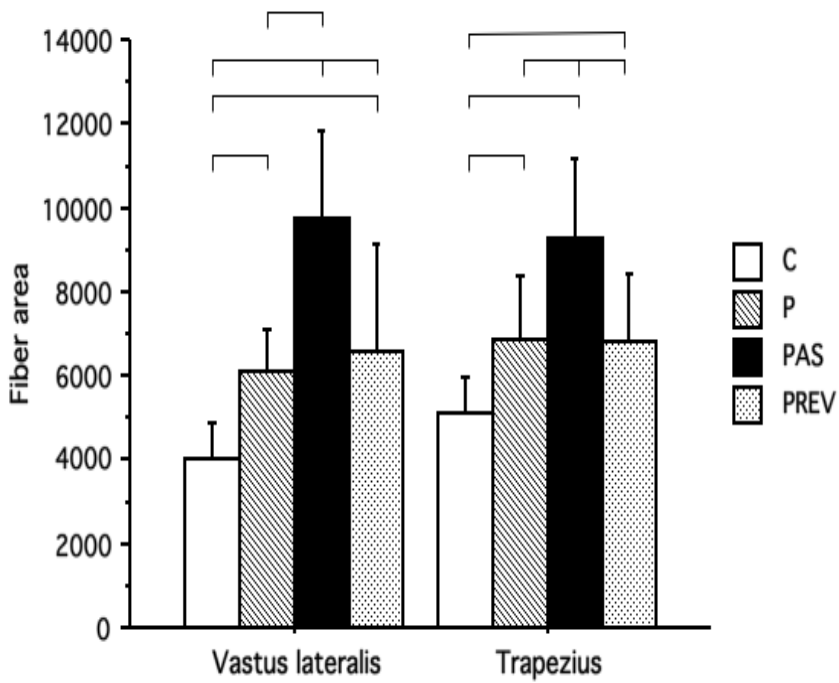
\* Significant difference from P group



**Table 5.** Morphological data from the vastus lateralis and trapezius muscles in the PREV group.

Subject	Mean fiber area	Nuclei/fiber	Proportion of fibers with internal nuclei	Number of satellite cells per fiber	Proportion of split fibers
<b>Vastus lateralis</b>					
1	5501	3.3	4.6	0.22	0.5
2	5227	4.1	10.0	0.25	0.0
3	4782	4.2	30.5	0.15	1.2
4	5099	3.8	7.7	0.10	0.5
5	5688	4.4	10.4	0.19	0.5
6	7716	3.2	9.7	0.16	0.3
7	11888	3.8	7.1	0.16	1.2
<b>Mean ± SD</b>	<b>6558 ± 2539</b>	<b>3.8 ± 0.4 *</b>	<b>11.4 ± 8.7</b>	<b>0.18 ± 0.05</b>	<b>0.6 ± 0.4</b>
<b>Trapezius</b>					
1	5964	7.5	10.0	0.26	0.2
2	8776	8.0	36.4	0.29	2.1
3	6890	7.6	31.0	0.48	0.3
4	4129	4.9	5.5	0.27	0.2
5	7697	7.2	16.7	0.35	1.2
6	6796	5.6	9.3	0.24	0.6
7	5964	8.5	27.0	0.53	1.7
<b>Mean ± SD</b>	<b>6807 ± 1467</b>	<b>7.0 ± 1.3</b>	<b>19.4 ± 12.1</b>	<b>0.35 ± 0.11</b>	<b>0.9 ± 0.8</b>

\* Significant difference from P group

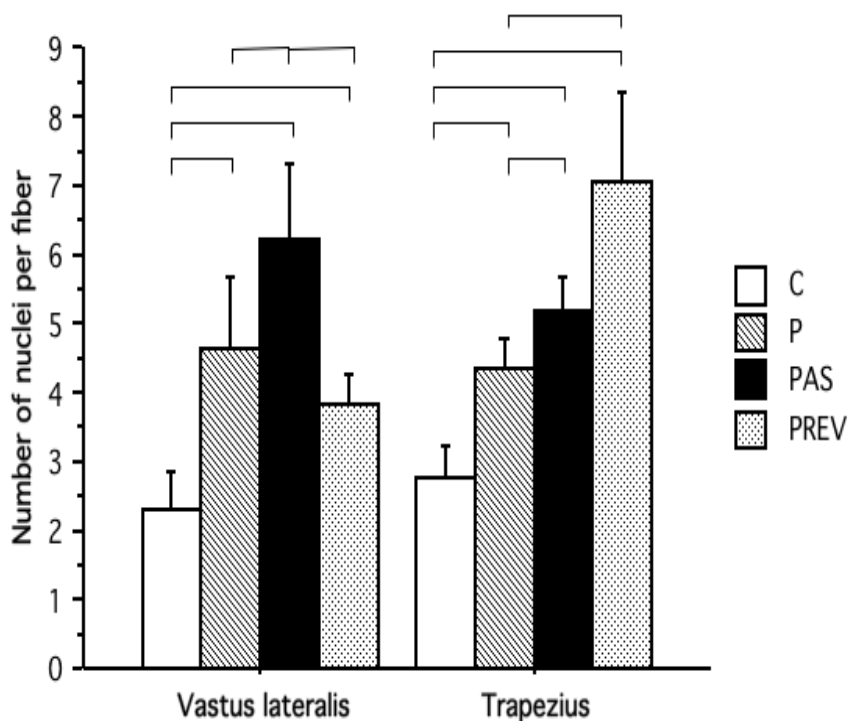


**Figure 1.** Fiber areas ( $\mu\text{m}^2$ ). Within each muscle, the PAS group had the largest mean fiber area except compared to the PREV group in the vastus lateralis. Brackets indicate  $p < 0.05$ .

### 4.3 MYONUCLEI PER FIBER

#### 4.3.1 PAS and P groups

In both the vastus lateralis and the trapezius muscles, the P group had significantly lower number of nuclei per fiber compared to the PAS group, but a higher number than the C group (Figure 2 and Tables 3 and 4).



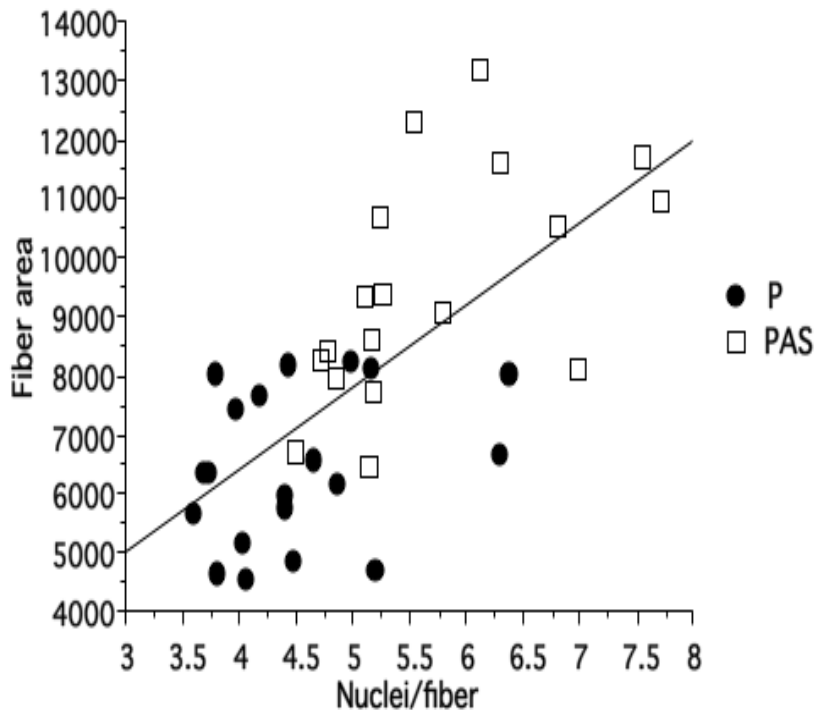
**Figure 2.** Number of nuclei/fiber. The highest number of myonuclei per fiber was found in the PAS group in the vastus lateralis muscle and in the PREV group in the trapezius muscle. Brackets indicate  $p < 0.05$ .

Combining the P and PAS groups, the fiber area was strongly correlated ( $r = 0.91$ ,  $p < 0.0001$ ) to the number of myonuclei per fiber in both muscles (Figure 3).

#### 4.3.2 PREV group

In the vastus lateralis muscle, the PREV group had significantly fewer myonuclei per fiber compared to the PAS group, but higher than the C group (Figure 2 and Table 5). In the trapezius muscle, the PREV group had significantly more myonuclei per fiber than the PAS, P and C groups (Figure 2 and Table 5). The PREV group had significantly more myonuclei per fiber in the trapezius muscle compared to the vastus lateralis muscle (Figure 2 and Table 5).

The ranking order of myonuclei number per fiber for the vastus lateralis muscle is PAS > P = PREV > C and for the trapezius muscle PREV > PAS > P > C (Figure 2 and Tables 3, 4 and 5).



**Figure 3.** A strong correlation was seen between the number of nuclei per fiber and the fiber cross sectional area ( $\mu\text{m}^2$ ).

## 4.4 INTERNAL NUCLEI

### 4.4.1 PAS and P groups

There was a significant correlation ( $r = 0.67$ ;  $p < 0.0001$ ) between the proportion of fibers with internal nuclei and the fiber area in both the vastus lateralis and the trapezius muscles (Figure 4). In the vastus lateralis, the P group had significantly higher proportion of fibers with internal nuclei compared to the C group. In both the vastus lateralis and the trapezius muscles the proportion of fibers containing internal nuclei was significantly higher in the PAS group compared to the P and C groups (Figure 5 and Tables 3 and 4).

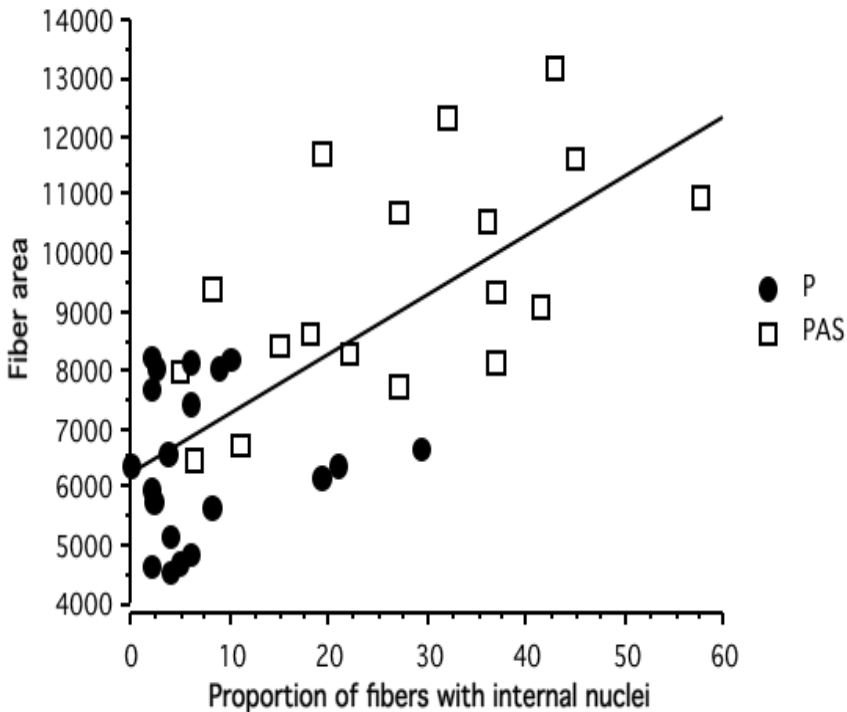


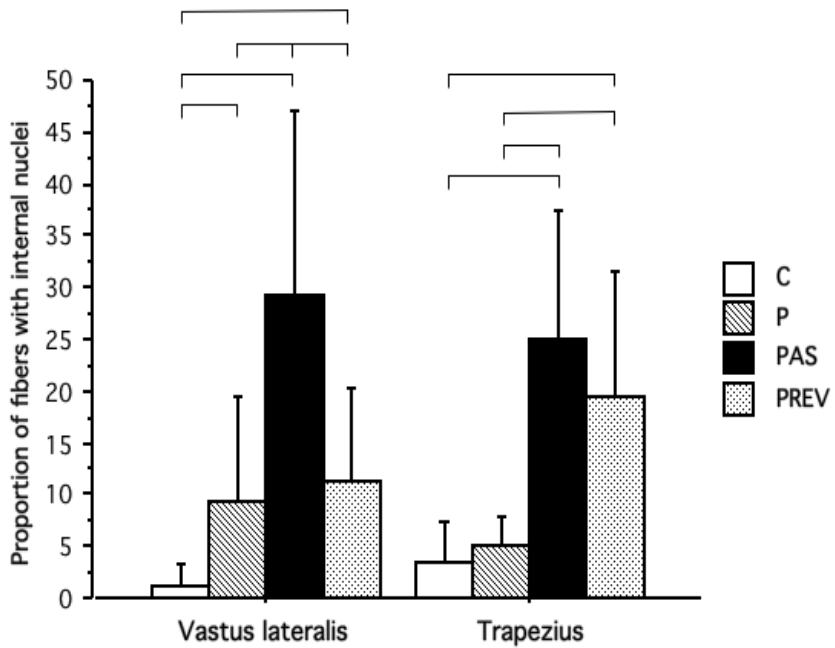
Figure 4. Correlation between the fiber area ( $\mu\text{m}^2$ ) and the proportion of fibers with internal nuclei (%).

### 4.4.2 PREV group

There was no significant difference between the PREV and P groups in vastus lateralis but in trapezius, the PREV group had a significantly higher proportion of fibers with internal nuclei (Figure 5 and Table 5).

The PREV group had a significantly smaller proportion of fibers with internal nuclei compared to the PAS group in the vastus lateralis whereas in the trapezius, there was no such difference (Figure 5 and Table 5).

Thus, the ranking order of proportion of fibers containing internal nuclei in the vastus lateralis is  $PAS > PREV = P > C$  and in the trapezius  $PAS = PREV > P = C$ .



**Figure 5.** Fibers with internal nuclei (%). Brackets indicate  $p < 0.05$ .

## 4.5 NUCLEAR DOMAIN

### 4.5.1 PAS and P groups

In the vastus lateralis muscle there was no significant difference in the size of the mean nuclear domain between the P and the PAS groups (Figure 6). No significant difference was seen between the PAS and the C groups, but the P group had significantly smaller nuclear domains than the C group (Figure 6).

In the trapezius there was no significant difference between the P, PAS and C groups.

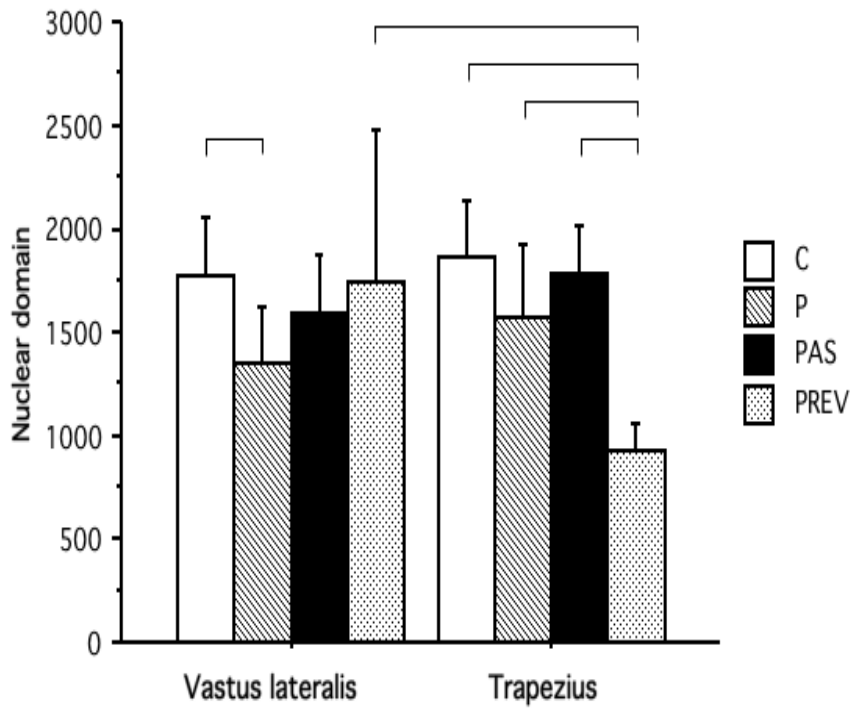
### 4.5.2 PREV group

In vastus lateralis there was no significant difference in the size of the nuclear domains between the PREV, PAS, P and C groups (Figure 6) (Table 3, 4 and 5).

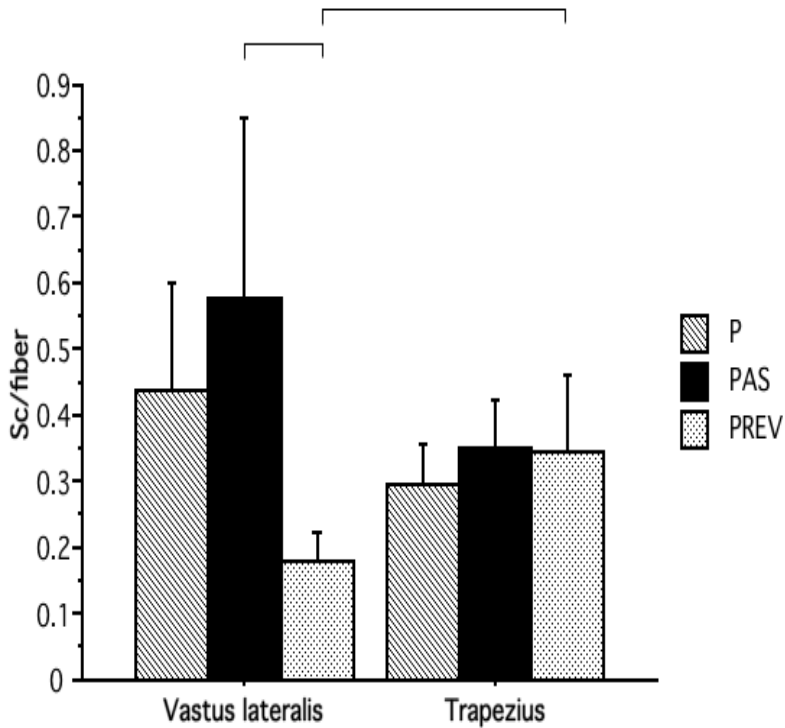
However, in trapezius, the PREV group had smaller nuclear domains than the PAS, P and C groups (Figure 6).

Further, the subjects in the PREV group had smaller nuclear domains in the trapezius compared to the vastus lateralis.

The ranking order for nuclear domain in the vastus lateralis is  $P = PAS = PREV$  and  $C > P$ . The ranking order for nuclear domain in the trapezius is  $PAS = P = C > PREV$ .



**Figure 6.** Nuclear domains as area ( $\mu\text{m}^2$ ) / nuclei. Brackets indicate  $p < 0.05$ .



**Figure 7.** Satellite cells per fiber. Note that in the PREV group, more satellite cells per fiber were found in trapezius compared to vastus lateralis. Sc = satellite cells. Brackets indicate  $p < 0.05$ .

## 4.6 SATELLITE CELLS

### 4.6.1 PAS and P groups

Number of satellite cell per fiber in the P and PAS groups are shown in Figure 7 and Table 3 and 4. No significant difference was seen between the groups in either the vastus lateralis or the trapezius muscles.

### 4.6.2 PREV group

In vastus lateralis, the PREV group had significantly fewer satellite cells per fiber compared to the P and PAS groups. In trapezius, all groups had similar number of satellite cells per fiber (Figure 7, Table 5).

## 4.7 SPLIT FIBERS

### 4.7.1 PAS and P groups

The only significant finding was in the vastus lateralis muscle, where the PAS group had a higher proportion of split fibers compared to the P group (Figure 8 and Table 3). In the C group, one single subject had a few split fibers in the trapezius muscle. In fibers with fissures, the direction of the fissure was often related to an internal myonuclei.

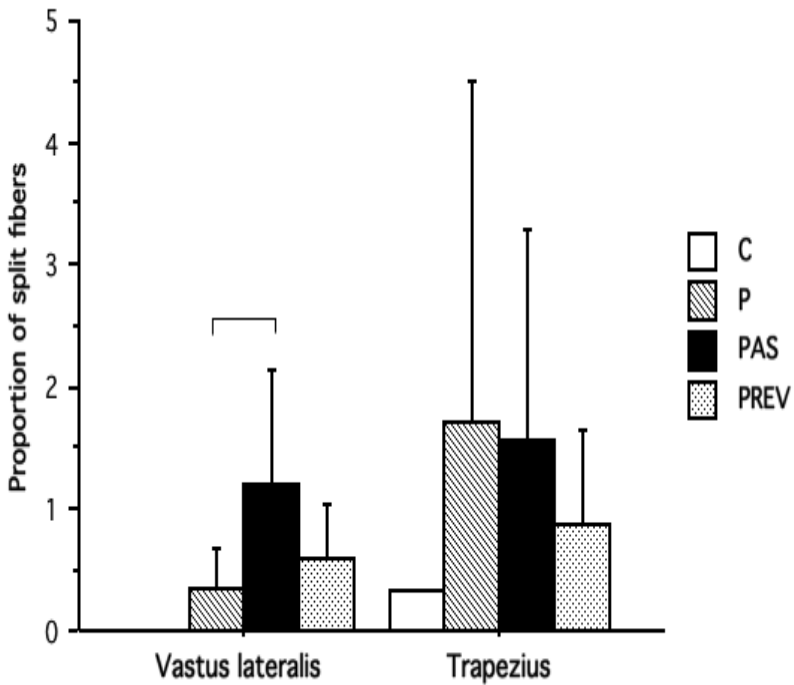


Fissures varied in depth, some partly and some completely dividing the fibers whereas others disappeared i.e. the branched parts merged into one fiber. Some fissures could be followed over a distance up to 200  $\mu\text{m}$ .

Different sections of one split fiber were in general of the same fiber type and showed the same staining pattern for myofibrillar ATP-ases and different MyHC isoforms. However two exceptions were seen, one small part of a branched fiber separated by a basement membrane (not shown), stained positively for Leu 19 whereas in another fiber, the small part stained weaker than the large part with the antibody N2661 against MyHCs I and IIA.

#### 4.7.2 PREV group

In both the examined muscles, no significant difference in proportion of split fibers were observed between the PREV group compared to either the PAS or the P groups (Table 5 and Figure 8).



**Figure 8.** The proportion of split fibers (%) differed between the PAS and P groups in the vastus lateralis muscle. Brackets indicate  $p < 0.05$ .

## 5 DISCUSSION

### 5.1 MAIN RESULTS

- Strength training increases the muscle fiber cross-sectional area, which is further increased by anabolic steroid usage. Among power lifters, there is no effect of anabolic steroids on fiber type distribution.
- The number of nuclei per fiber is higher in both the vastus and trapezius muscles among power lifters using anabolic steroids compared to power lifters not using anabolic steroids.
- Among power lifters who have withdrawn from anabolic steroid usage and training for several years, the number of myonuclei per fiber is higher in the trapezius compared to the vastus lateralis muscle. In addition, in the trapezius this group has a higher number of myonuclei per fiber compared to active elite trained power lifters, even if the active lifters are using anabolic steroids.
- Power lifters using, or having used anabolic steroids display a higher than normal proportion of fibers with internal nuclei.
- In active power lifters, anabolic steroids have no further effect on the number of satellite cells per fiber. After withdrawal from anabolic steroid usage, a lower number of satellite cells per fiber is observed compared to the active athletes in vastus lateralis.
- High mechanical stress inflicted upon muscles results in increased proportion of split fibers, which can be interpreted as disturbed regeneration.

### 5.2 FIBER TYPES AND FIBER CROSS-SECTIONAL AREA

All the strength-trained athletes had a high frequency of type II fibers, mainly type IIA fibers while type IIB fibers were rare in most of the power lifters. The fiber cross sectional area in these athletes was significantly hypertrophied compared to controls.

Type IIB fibers is the fastest and strongest fiber type in humans so theoretically, a person with a high proportion of type IIB fibers should be a successful power lifter. However, this theory is not supported by practical studies (Adams *et al.*, 1993; Hakkinen *et al.*, 1998; Andersen & Aagaard, 2000). On the contrary, a transition from type I to IIA and IIB fibers has been shown in unloaded muscles whereas a transition from type IIB to

IIA and I occurs in muscles exposed to mechanical loading, such as high intensity training (Pette & Staron, 2001).

### **5.2.1 No significant difference in fiber type distribution with training and anabolic steroids but increased amount of small fibers expressing developmental myosin in the PAS group**

Although the skeletal muscles adapt to strength training by fiber type transition, anabolic steroids do not seem to affect the fiber type distribution in skeletal muscles. This finding is also supported by a recent study where the authors showed that supplementation with testosterone did not affect the relative fiber type distribution in human skeletal muscles (Sinha-Hikim *et al.*, 2002).

The individual variation in the studies presented in this thesis was very large in all groups, despite similar training protocols for many years. This may reflect a greater genetic than training influence on fiber type distribution. The power lifter who had the highest percentage of type IIB fibers, with approximately half of the muscle fibers being type IIB, belonged to the P group. Nevertheless, at the time of the biopsy, he had the squat world record in his weight class. It appears that anabolic steroids enhance performance in power lifting not by altering fiber types (to type IIB) but rather by muscle fiber hypertrophy.

Several subjects in the PAS group had a large amount of small fibers expressing fetal myosin (data presented in (Kadi, 2000)). Developing myosin isoforms in adult muscle tissue has been interpreted as signs of hyperplasia (McCormick & Schultz, 1992; McCormick & Thomas, 1992; Antonio & Gonyea, 1993). A significant increase in fibers staining for developing isoforms of myosin, has been demonstrated with strength training (Antonio & Gonyea, 1993; McCall *et al.*, 1996; Kadi & Thornell, 1999; Kadi, 2000). These fibers might reflect newly formed fibers or abortively regenerated fibers. In the latter case, failed innervation can cause degeneration and the new fibers would be of no use for the athletes. It can be speculated that the large number of fibers with developing myosin isoforms is a result of using AS for almost ten years. If this is the case, long-term use of anabolic steroids might lead to formation of small, non-innervated and thus useless fibers.

### **5.2.2 Larger fiber areas with training and enhanced effect with anabolic steroids**

Intensive strength training for many years, results in a significant muscle fiber hypertrophy in human skeletal muscles. This was observed in the P group in this study

and demonstrated repeatedly by others (Tesch, 1988; Staron *et al.*, 1990; Kraemer *et al.*, 2002).

It has been reported that AS supplementation, even without strength training, can induce hypertrophy in human skeletal muscles (Bhasin *et al.*, 1996; Sinha-Hikim *et al.*, 2002). In the study by Sinha-Hikim *et al* the muscle fiber hypertrophy after 20 weeks of AS supplementation (600 mg/week) was 49% in type I fibers and 36% in type II fibers compared to baseline. The dramatic hypertrophic muscle fibers in the PAS group is in accordance with the current conception that testosterone and anabolic steroids increase muscle size by increasing the muscle fiber cross sectional area, for review see (Aagaard, 2004).

Because maximal contraction force of a muscle is related to the total muscle area, the muscle fiber cross sectional area and the fiber types (Bruce *et al.*, 1997; Bamman *et al.*, 2000) the PAS group may have an advantage in physical performance compared to the other groups due to larger muscle cross sectional fiber areas.

### **5.2.3 Relatively large fiber areas in the PREV group**

Despite decreased, or in some cases secession of training for many years, the cross sectional fiber areas in both examined muscles in the PREV group remains comparable to the elite trained and still active power lifters (P group). Possible mechanisms could be related to a high number of residual myonuclei per fiber and is discussed below.

#### **5.3.1 Increased number of myonuclei with training and enhanced effect by anabolic steroids**

The P group had significantly more myonuclei per fiber compared to the C group in both the vastus lateralis and the trapezius muscles, indicating that high intensity strength training can increase the number of myonuclei per fiber.

Each nucleus supports a certain volume, often referred to as a nuclear domain (Cheek, 1985), of the cytoplasm with mRNA for proteins synthesis. It is generally accepted that an increased number of myonuclei per fiber is a prerequisite for a more substantial hypertrophy (Allen *et al.*, 1999; Kadi & Thornell, 2000).

Fiber cross sectional area (cell volume) is proportionally to the number of myonuclei, keeping the nuclear domains constant. This is in accordance with other studies showing that exercise increases the amount of myonuclei in order to keep the myonuclei / cytoplasmic ratio (i.e. nuclear domain) constant (Hikida *et al.*, 2000; Kadi *et al.*, 2005).

In the view of anabolic steroid usage, it has been demonstrated that the number of myonuclei is significantly increased and correlated to the fiber area after 20 weeks of testosterone administration (Sinha-Hikim *et al.*, 2002; Sinha-Hikim *et al.*, 2003). These results support the idea that the number of myonuclei plays a fundamental role in muscle fiber hypertrophy (Edgerton & Roy, 1991; Allen *et al.*, 1999; Kadi, 2000).

In both the vastus lateralis and the trapezius muscles, the significantly higher number of myonuclei in the PAS group, both subsarcolemmal and internal, compared to the P group indirectly demonstrates the enhancing effects of anabolic steroids on the capacity for protein synthesis in the muscle cell.

### **5.3.2 More internal nuclei with training and enhanced effect by anabolic steroids**

Strength training generates not only more myonuclei but also a higher proportion of fibers where the nuclei are centrally located. As with fiber size and myonuclear number, anabolic steroids have an effect also on this variable.

Because both the largest fiber areas and the highest proportion of internal nuclei were found in the PAS group, this supports the idea that the presence of internal nuclei reflects the limited volume of each nuclear domain. The internal nuclei might be needed to support extremely large fibers, preferentially present in the PAS group. In fact, when combining the PAS and the P groups, we demonstrated a significant correlation between the fiber area and the proportion of fibers containing internal nuclei.

Internal nuclei might be a phenomenon of adaptation to intensive strength training because they will reduce the diffusion distances from the nucleus to central parts of very large fibers. Thus, also in this aspect, the use of anabolic steroids can have an enhancing effect on physical performance.

### **5.3.3 Remaining effects after anabolic steroid withdrawal?**

The numbers of myonuclei per fiber in the vastus lateralis muscle was the same in the PREV and P groups and significantly lower than in the PAS group. In the trapezius muscle, the highest number of myonuclei per fiber was found in the PREV group. This group also had the highest proportion of myonuclei with androgen receptor binding sites in vastus lateralis.

In the PREV group, the fibers in the trapezius muscle displayed the highest number of myonuclei per fiber and the smallest nuclear domains compared to all the other groups. In both the P and the PAS groups, a high number of myonuclei was correlated to a large muscle fiber area. If assuming similar muscle fiber cross sectional areas in the PREV group when they were using anabolic steroids as the PAS group, then the fiber cross

sectional areas must have decreased after secession of using these substances. However, the fiber cross sectional area in the PREV group is still comparable to that of active elite trained power lifters who have not use anabolic steroids.

One consequence of this observation is that larger fiber areas necessitate a higher number of myonuclei in order to maintain the size of the nuclear domain. Thereby, the high number of myonuclei per fiber in the trapezius muscle in the PREV group is one possible mechanism for the large fiber areas in this group despite the lack of high intensity training. In relation to physical performance, previous use of anabolic steroids may have advantages for the athlete many years after withdrawal.

Based on published (Kadi *et al.*, 2000b) and unpublished (Eriksson, manuscript) data, one possible mechanism for the remaining large fiber areas (equal to the P group) in vastus lateralis in the PREV group, could be the approximately doubled proportion of myonuclei expressing androgen receptor binding sites compared to the P and PAS groups (Kadi *et al.*, 2000b).

When androgenic hormones bind to androgen receptors, the receptors become activated and mediate the signal into the nucleus where binding to selective genes increase the rate of transcription. Hence, it is possible that a high amount of androgen receptor binding sites in vastus lateralis is one mechanism for preserving large fiber areas in the PREV group, despite the low intensity or even secession strength training in this group.

Interestingly, this would mean that after withdrawal of anabolic steroid usage, the prevention of muscle fiber atrophy is mediated by a high amount of androgen receptor binding sites in the vastus lateralis muscle and by a high amount of myonuclei per fiber in the trapezius muscle.

This indicates that myonuclei, increased in number both by strength training (P group) and the intake of anabolic steroids combined with strength training (PAS group), do not undergo nuclear apoptosis or other forms of atrophy when the demand for proteins synthesis is decreased with decreased training volume.

#### **5.3.4 Internal nuclei in the PREV group**

In the PREV group, internal nuclei were found in 11 % of the fibers in vastus lateralis (similar to P) and in 19 % of the fibers in trapezius (similar to PAS).

It is likely that the PREV group previously had significant larger fiber areas than today, comparable to the PAS group. In both the P and PAS groups, the fiber area was correlated to the proportion of fibers with internal nuclei. This would mean that the high

proportion of fibers with internal nuclei in the PREV group could be a remaining effect from both strength training and the use of anabolic steroids. One possible explanation for the appearance of internal nuclei is focal damage, induced by heavy strength training and the following regenerative attempt (see split fibers below).

## **5.4 SATELLITE CELLS**

Myonuclei in mature muscle fibers are not able to divide, which means that an increase in myonuclei number must come from an external source (reviewed by (Allen *et al.*, 1999)). It is generally accepted that these additional nuclei are derived from satellite cells and/or stem cells (reviewed by (Morgan & Partridge, 2003)).

### **5.4.1 Satellite cell numbers decrease after secession of training**

No difference was seen between the P and PAS groups in proportion of satellite cells in either muscle. The P and PAS groups had a significantly higher proportion of satellite cells in the vastus lateralis muscle compared to the PREV group.

A significant increase in satellite cell number has been observed in young men after supplementation with 300 and 600 mg of testosterone per week for 20 weeks, even without exercise (Sinha-Hikim *et al.*, 2003). This appears to contrast the present findings. However, the PAS group had used testosterone, anabolic steroids and other growth inducing factors for an average of almost ten years. It is likely that the satellite cells have either become resistant to these stimuli or that the satellite cell pool has been diminished.

### **5.4.2 Satellite cells in the PREV group**

All subjects in the PREV and PAS groups reported using, or having used, performance-enhancing substances to increase muscle strength and size. All of these substances have been reported to regulate satellite cells activation and proliferation (Cheema *et al.*, 2005). Thus, both supplementation with testosterone and anabolic steroids as well as high-level resistance training increases the number of satellite cells.

We therefore suggest that the proportion of satellite cells is decreased in the vastus lateralis after a period of decreased training intensity and secession of anabolic steroid administration. One possible explanation could be that satellite cell frequency in the PREV group has returned to normal, several years after reduction in training and termination of anabolic steroid supplementation. Kadi *et al* (Kadi *et al.*, 2004) demonstrated that the number of satellite cells was increased by strength training and decreased after a period of de-training. On the other hand, satellite cell frequency in the trapezius muscle was the same in the PREV as in the P and PAS groups. As in the case of myonuclei per fiber and internal nuclei, the use of anabolic steroids appears to have a

longer lasting effect in trapezius compared to vastus lateralis also on satellite cells. It is also possible that a part of this difference can be explained by the daily life usage.

## **5.5 SPLIT FIBERS**

Fibers with clefts and/or fissures, occupying the area of one fiber are defined as “split fibers”. Several features of split fibers suggest that the splitting is due to defect regeneration. Segmental muscle fiber disruptions and necrosis were present in the P and PAS groups. The split parts of the fibers were often short segments and displayed muscle fiber morphology considered typical for both degenerative and regenerative muscle fibers.

### **5.5.1 Strength training with and without anabolic steroids result in split fibers**

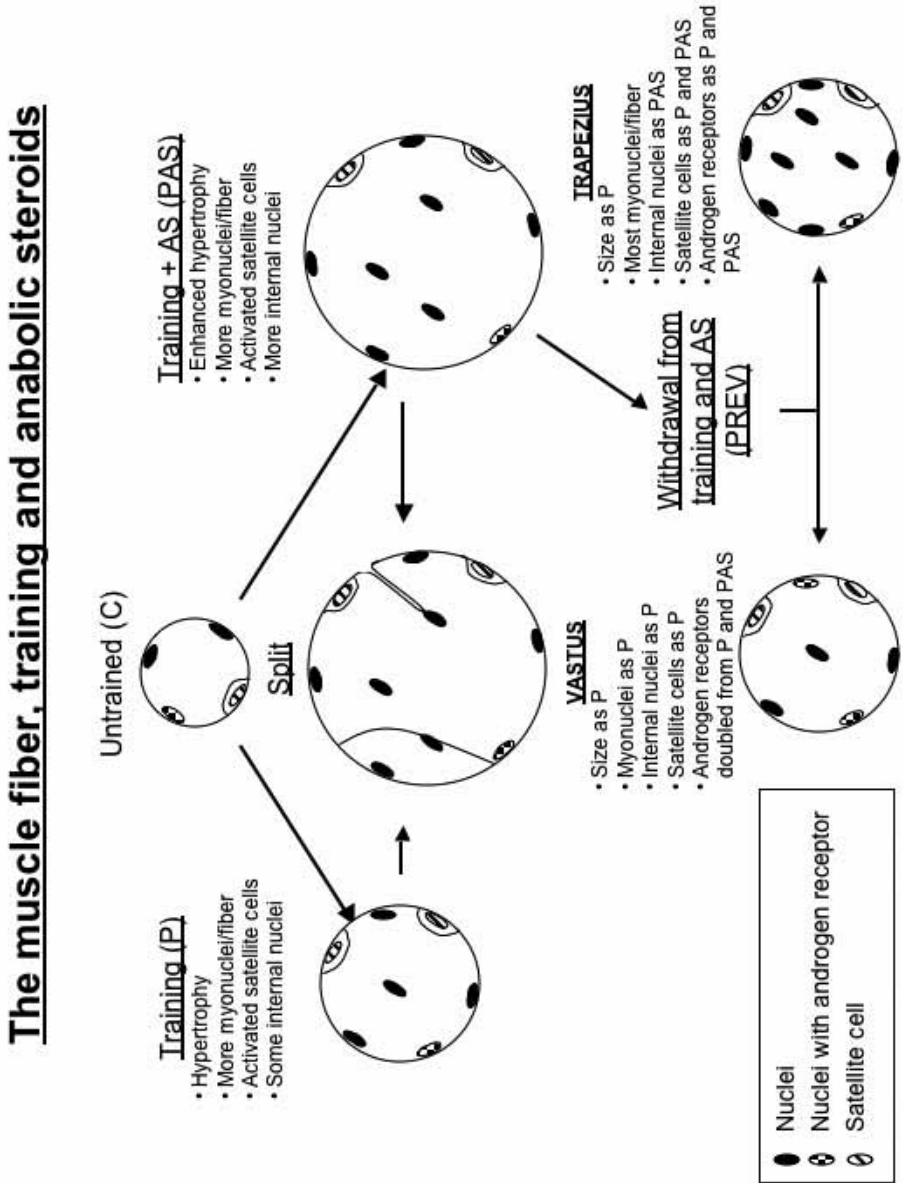
In both the vastus and trapezius muscles from athletes performing heavy strength training (P, PAS and PREV groups) split fibers was more frequently occurring than in controls. The only difference between the groups was that the PAS group had more split fibers than the P group in the vastus lateralis muscle.

We suggest that split fibers in power lifters are caused by a temporal series of events, namely: focal damage → regenerative attempts → satellite cell activation → myotube formation → failed lateral fusion of myotubes. These suggestions are supported by earlier studies demonstrating that heavy resistance training in humans induce split muscle fibers (Larsson & Tesch, 1986; Tesch, 1988) and that split fibers are related to fiber size (Edgerton, 1970; Hall-Craggs, 1970; Hall-Craggs & Lawrence, 1970; Chou & Nonaka, 1977; Swash & Schwartz, 1977).

The failed lateral fusion could be caused by continued high mechanical stress during myotube formation. Another outcome of failed lateral fusion could be internal nuclei. A myonucleus is located under the sarcolemma and if the sarcolemma is invaginated, the nucleus will remain inside that fiber.



5.6 SUMMARY



**Figure 9.** Summary of the effects on the muscle fiber from training, training combined with anabolic steroid usage and withdrawal from high intensity training and anabolic steroid usage.

## 5.7 CONCLUSIONS

High intensity resistance training increases muscle strength and banned substances such as testosterone and anabolic steroids can enhance the training effects. The studies on muscle cell morphology presented in this thesis reveal that anabolic steroids and testosterone increases muscle fiber size and adds more nuclei to the muscle cell.

- The adaptation processes differ between muscles, supporting the concept in the Umeå muscle research group that each muscle is unique.
- Based on the morphological appearance of muscle sections from doped and non-doped power lifters, we conclude that testosterone and anabolic steroids enhances the hypertrophic effects of training without adding new features.
- The addition of myonuclei by training and doping appears to be longer lasting in some muscles than in others. This can be due either to differences in genetic expression or muscle utilization.

Although some of the morphological changes induced by testosterone and anabolic steroids are very long lasting, perhaps life-long, the response is muscle-specific. It is very likely that these changes are beneficial for physical performance.

The findings and conclusions in this thesis raise questions regarding relevant suspension times for athletes caught with banned substances in the body.

## 6 SYNOPSIS

The first time I competed in power lifting was in 1982 in a competition for the northern most part of Sweden. I got a silver medal that I was pleased with, but I was somewhat surprised over some of the other young lifters who were capable of lifting very heavy loads compared to their earlier results. In 1985, I was qualified for the Swedish National Championships for the first time. This was the first time unannounced drug tests were performed and eight lifters were caught (five of them were gold medalists). After that, I realized that doping with mainly testosterone and anabolic steroids was very common in power lifting. Despite the feeling of great unfairness, I continued to perform very heavy and intensive strength training and 1988 I was selected for the Swedish National Team to participate at the European Championships in Oldstad, Germany. At this time, drug testes had become rather common and many lifters were worried about being caught with illegal substances in their bodies. Very surprisingly, I became the new European Champion despite the fact that several other lifters in my weight class had personal bests that widely exceeded my former results. The truth was that this was a fair competition and this was the reason for my victory. After this, new substances have emerged, e.g. water-soluble testosterone, and it is again possible to take these substances without being caught.

Three times in my carrier I have received medals several month after the competition: Two gold medals from the Swedish Championships and one bronze medal from the European Championships because lifters finishing ahead of me were caught in the drug test. This means that three of the greatest moments in my sport carrier was reduced to a letter with a medal and I was deprived the pleasure if receiving the medals at the time of the competition.

These experiences were a large motive for me to start this project in order to find out the differences in muscular adaptations between myself and power lifters who had used anabolic steroids.

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