STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH
115

Juha Ahtiainen

# Neuromuscular, Hormonal and Molecular Responses to Heavy Resistance Training in Strength Trained Men



JYVÄSKYLÄN YLIOPISTO

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## With Special Reference to Various Resistance Exercise Protocols, Serum Hormones and Gene Expression of Androgen Receptor and Insulin-Like Growth Factor-I

Esitetään Jyväskylän yliopiston liikunta- ja terveystieteiden tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Liikunnan salissa L 304
kesäkuun 21. päivänä 2006 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Sport and Health Sciences of the University of Jyväskylä,
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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2006

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Publishing Unit, University Library of Jyväskylä

Cover pictures by www.sandowmuseum.com

URN:ISBN:9513925711
ISBN 951-39-2571-4 (PDF)

ISBN 951-39-2505-6 (nid.)
ISSN 0356-1070



Jyväskylä University Printing House, Jyväskylä 2006

**Cover pictures and text by www.sandowmuseum.com**

Friederich Wilhelm Mueller alias Eugen Sandow (1867-1925) was perhaps the first modern bodybuilder. He began his career as a sideshow strongman. He could raise a 269 lb. barbell overhead with one arm in a movement he called "The Bent Press". Eugen Sandows friend and mentor was Louis Durlacher, as known as Professor Attila. Attila was the man who invented the hallow barbell, with buckshot filling, so that the weight could be varied. Prior to this lifts were 'anyhows', that is getting the weight up anyhow; muscle was developed but just as a side effect. Attila altered all that, and Sandow was the perfect example, the proof that progressive weight training worked. Sandow trained on the lifts he used in his stage performance, lifts chosen by him and refined so as to put any challenger at a disadvantage. With Attila's new shot loading weights, muscles essential to the lift would be strengthened and resistance could be increased bit by bit. Thus, Sandow was one of the first scientific weightlifters.

Physique was what truly set Eugen Sandow apart from other strongmen. "Muscle Display Performances" made him one of the most famous men of his day. Sandow inspired and motivated millions of people in his day towards better health and increased physical activity. Sandow made it fashionable for a man to have a muscular physique at a time when men were typically in poor physical condition. Sandow also showed that there is no reason a 2000 year old statue should be any more magnificent than a living man.

September 14, 1901 Eugen Sandow organized an event called simply "The Great Competition," the world's first major physique competition. The judges of the contest were the sculptor Sir Charles Lawes, Sandow himself, and the third arbiter was Sir Arthur Conan Doyle, creator of Sherlock Holmes. Each of the lucky victors won an extraordinary prize: a beautifully sculpted statuette of Sandow himself. The third place winner received a statue made of bronze, a silver statue for second, and for William L. Murray of Nottingham, a golden statue was his reward.

The magnificent statue that was awarded to the competitors in this early contest was fated to have a long and distinguished afterlife. Promoters of the 1950 Mr. Universe competition offered a tantalizing trophy; the original bronze Sandow statue that had been awarded to the third-place winner fifty years earlier at the Great Competition. The victor that year was a young American, Steve Reeves. After that the Sandow statue was fated to remain in the shadows for over a quarter century. Today the Mr. Olympia contest is the ultimate prize in professional bodybuilding. Since 1977 the trophy has been a bronze statue of Eugen Sandow, a fitting tribute to the first modern bodybuilder.

# ABSTRACT

Ahtiainen, Juha
Neuromuscular, hormonal and molecular responses to heavy resistance training in strength trained men; with special reference to various resistance exercise protocols, serum hormones and gene expression of androgen receptor and insulin-like growth factor-I
Jyväskylä: University of Jyväskylä, 2006, 119 p.
(Studies in Sport, Physical Education and Health,
ISSN 0356-1070; 115)
ISBN 951-39-2571-4
Finnish summary

The present study was designed to obtain more information on mechanisms leading to muscle hypertrophy by determination of the effects of different heavy resistance exercise protocols on acute and chronic neuromuscular and hormonal responses in previously strength trained young men. The present study also examined gene expression of androgen receptors and insulin-like growth factor I (IGF-I) to further understand the adaptation mechanisms to resistance training. The present results suggest that increased resistance exercise intensity induced by the so-called forced repetitions (FR) exercise system may be beneficial for the development of muscle mass and muscle strength during strength training. However, FR led to increased recovery time after the exercise. The length of the rest periods (2 vs. 5 minutes) between the sets may not play an important role in the magnitude of acute resistance exercise-induced responses and long-term training adaptations. The findings indicate that serum testosterone concentrations may be of importance for training-induced muscle hypertrophy as well as for strength development of the trained muscles. Increased IGF-IEa and MGF mRNA expression due to heavy resistance exercise supports the concept that they may be related to regenerative processes after the exercise and therefore, contribute to training-induced muscle hypertrophy. Because the acute exercise-induced responses and the time needed for recovery may differ considerably between different loading protocols, there is a need to optimize the contents and the frequency of different training sessions in order to create proper resistance training programs to match the individual requirements of trainers. The present findings further suggest that there may be several different ways to create exercise conditions leading to large acute hormonal responses due to hypertophic type of resistance exercises. These results indicate a need to optimise the volume and/or intensity of resistance exercises to meet the level of adaptation of the neuromuscular and endocrine systems in order to further increase muscle mass and strength.

Key words: Resistance exercise, muscular hypertrophy, gene expression, serum hormones, recovery

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## ACKNOWLEDGEMENTS

The work described in this dissertation was carried out by the author in the Department of Biology of Physical Activity at the University of Jyväskylä between January 2001 and June 2006.

I would like to acknowledge all the people I have had a pleasure to collaborate with and who in different ways made this work possible:

- First of all, my deepest gratitude is directed to my supervisor and mentor, Professor Keijo Häkkinen, for providing me opportunity for doctoral studies. During these years he guided me into the field of research of which I am most indebted and grateful for.

- I wish to address my sincere thanks to the referees of my thesis, Professors Stephen Harridge and Per Tesch, for their thorough review, constructive criticism and valuable advice for finishing this work.

- I would like to express my special gratitude to co-authors Arto Pakarinen, Maarit Lehti, Markku Alen, Jyrki Komulainen, and William J Kraemer for their contribution during all stages of this work.

- I gratefully acknowledge all the colleagues, office workers as well as laboratory and technical staff who work at the Department of Biology of Physical Activity for fruitful collaboration. Let this work to be prologue for our future studies.

- I owe my special thanks to all the subjects who volunteered to participate in this study; without their "blood, sweat and tears" this work would never have been done.

- I owe my deepest gratitude to my dear wife Virpi and our beloved son Sampsa, for their love, patience and understanding during my studies over the years.

- Finally, I wish to acknowledge the Ministry of Education, Finland, the Department of Biology of Physical Activity, University of Jyväskylä and LIKES Research Center, who have financially supported this work.

Juha Ahtiainen
Jyväskylä, May 2006

## ORIGINAL PAPERS

This thesis is based on the following original research articles, which will be referred to by their Roman numerals.

I Ahtiainen JP, Pakarinen A, Kraemer WJ, Häkkinen K. Acute hormonal and neuromuscular responses and recovery to forced vs. maximum repetitions multiple resistance exercises. International Journal of Sport Medicine. 2003; 24: 410-418

II Ahtiainen JP, Pakarinen A, Kraemer WJ, Häkkinen K. Acute hormonal responses to heavy resistance exercise in strength athletes and non-athletes. Canadian Journal of Applied Physiology. 2004; 29: 527-543

III Ahtiainen JP, Alen M, Kraemer WJ, Pakarinen A, Häkkinen K. Muscle hypertrophy, hormonal adaptations and strength development during strength training in strength-trained and untrained men. European Journal of Applied Physiology. 2003; 89: 555-563

IV Ahtiainen JP, Pakarinen A, Alen M, Kraemer WJ, Häkkinen K. Short vs. long rest period between the sets in hypertrophic resistance training: Influence on muscle strength, size and hormonal adaptations in trained men. Journal of Strength and Conditioning Research. 2005; 19: 572-582

V Ahtiainen JP, Lehti M, Pakarinen A, Alen M, Kraemer WJ, Komulainen J, Häkkinen K. Expression of IGF-IEa, MGF and androgen receptor mRNA after heavy resistance exercise in strength trained men (Submitted for publication)

VI Ahtiainen JP, Lehti M, Pakarinen A, Alen M, Kraemer WJ, Komulainen J, Häkkinen K. Effects of resistance training on androgen receptor and IGF-I mRNA expression in human skeletal muscle (Submitted for publication)

# CONTENTS

# 1 INTRODUCTION

Muscular strength is important in sport as well as in daily activities. The need for muscular strength runs across a spectrum of people from elite athletes attempting to optimize sports performance to frail elderly trying to perform activities of daily living. An adequately functioning musculoskeletal system (i.e. musculoskeletal fitness) is a key factor for functional capacity and good quality of life (Topp et al. 2004) and an enhanced musculoskeletal fitness is often associated with an improvement in health status (Kell et al 2001, Warburton et al. 2001). Furthermore, if muscle strength is not maintained, musculoskeletal fitness is then compromised which can significantly impact physical health and well-being (Kell et al 2001).

Resistance training is recommended by several health organizations for its potential benefits on health- and performance-related physical fitness (Kraemer et al. 2002b, Topp et al. 2004). Resistance training can improve muscular strength, power and speed, hypertrophy, local muscular endurance, motor performance, balance, and coordination. Thus, participation in regular resistance training elicits a number of favorable responses that contribute to health and may reduce or even prevent a number of functional declines associated with aging (ACSM 1998). The quality and quantity of exercise necessary to elicit important health benefits may differ from that needed to produce significant gains in fitness. In various sports the athletic event will dictate the time commitment to resistance training and form the basis for designing individual workouts to simulate sports-specific movements for optimal transfer of gains made in training to competition (Kraemer et al. 1998a).

Improvements in athletic performance or the extent of the functional and health benefits to be accrued from resistance training depend on factors such as initial performance and health status. Other factors, such as functional capabilities of the individual, age, nutritional status, and behavioral factors (e.g., sleep and health habits) can also affect the adaptations (Descheness and Kraemer 2002). Resistance exercise may functionally be defined as the progressive overload of a skeletal muscle resulting in muscle growth and strength (Close et al. 2005). Progression in resistance training is a dynamic process that requires an exercise prescription process, evaluation of training progress, and careful de-

velopment of target goals. Training programs are highly specific to the types of adaptation. Depending on the specific program design resistance training can enhance strength, power, or local muscular endurance. Optimal adaptation appears to be related to the use of specific resistance training programs to meet individual needs and training objectives. Specific training programs dictate what tissue and how other physiological systems will be affected by the exercise training. The exercise prescription of the specific program design reflecting these targeted program goals includes variables such as choice of exercises, order of exercise, amount of rest used between sets and exercises, number of repetitions and sets used for each exercise, and the intensity of each exercise (Kraemer et al. 1996, Kraemer et al. 2002a, Kraemer and Ratamess 2004).

Long-term resistance training-induced increase in muscle force has been attributed to neural, connective tissue, cellular or excitation-contraction coupling adaptations (Close et al. 2005). Resistance training is a potent stimulus to the endocrine and neuromuscular systems (Descheness and Kraemer 2002). The development of the neuromuscular system appears to be dominated in the early phase of resistance training (Moritani and deVries 1978, Häkkinen and Komi 1983). In the later adaptation phase, muscle protein increases, and the contractile unit begins to contribute the most to the changes in performance capabilities (Komi 1986, Häkkinen 1994a, Kraemer et al. 1996). Strength and muscle mass are increased following resistance training through a series of events that appears to involve increased protein synthesis (Phillips 2000) and the recruitment of satellite cells to support hypertrophy of mature myofibres (Kadi et al. 2005). The endocrine system secretes anabolic hormones, e.g. growth hormone and testosterone, which have influence on resistance training-induced adaptations in skeletal muscle (Sheffield-Moore and Urban 2004). Also growth factors produced locally in worked muscles, such as insulin-like growth factor I (IGF-I), may be an important regulator of these adaptation processes (Goldspink 1999). These functional and physiologic adaptations are similar in nature among men and women at all aged. However, sex and age differences may exist in the absolute magnitude of adaptation to resistance training (Descheness and Kraemer 2002).

The present research was designed to investigate the role of different resistance exercise protocols to acute (studies I and II) and chronic (studies III and IV) training responses of the neuromuscular system and serum concentrations of anabolic and catabolic hormones in previously strength trained young men as well as in untrained young and elderly men. Furthermore, the present studies examined gene expression of androgen receptors and specific growth factors (studies V and VI) to further understand the adaptation mechanisms to resistance training.

# 2 REVIEW OF LITERATURE

## 2.1 Neural and muscular responses to resistance training

### 2.1.1 Resistance training and adaptations in muscular morphology

Muscle strength can be defined as the maximum force generation capacity (Macaluso and De Vito 2004). The neural factors regulate muscle force generation. Increased levels of muscle activation and consequent increase in muscular force are achieved by increases in the firing rate of each motor unit, changes in the pattern of motor unit activation and the recruitment of more motor units (Komi 1986, Häkkinen1994a, Barry and Carson 2004, Kamen 2005). In addition to neural factors the amount of force that an isolated muscle can exert is influenced by factors such as the number and size of muscle fibres, the orientation of fibres with respect to the line of muscle action, and the proportion of myosin heavy and light chain isoforms that are expressed within the muscle fibres (Abernethy et al. 1994). Regular exposure to heavy resistance exercise will result in increases in maximal muscular strength and changes in both neuromuscular function and muscle morphology (Tesch 1988, Harridge et al. 1999, Aagaard 2004, Fry 2004).

It has been well known that systematic resistance training, especially among initially untrained healthy subjects, has a potent effect in promoting increases in size and strength of skeletal muscle. This is true both in men and women. Although women have lower absolute strength than men, the relative increases in strength following a training programme are similar between genders, at least in the beginning of resistance training (Häkkinen and Pakarinen 1994, Staron et al. 1994, Häkkinen et al. 2000a). Adaptation of the human body to prolonged resistance training takes place due to combinations of multiple factors, i.e., mechanical stress, neuromotor control, metabolic demands, and endocrine activities. Neural factors are important for the increases in muscle strength especially in earlier phases of resistance training, while muscular hypertrophy of trained muscles also contribute to strength development during prolonged resistance training (Moritani and deVries 1978,

Häkkinen and Komi 1983, Häkkinen et al. 1985a, Komi 1986, Rutherford and Jones 1986). The increase in the cross-sectional area of trained muscles comes primarily from the increase in size of individual muscle fibers (MacDougall et al. 1977) as a result of increased contractile proteins (Haddad and Adams 2002). In well-trained subjects, as strength athletes, further improvements in strength and training-induced muscle hypertrophy are much more limited than in previously untrained subjects. Strength development and muscle hypertrophy is dependent on the type and intensity of loading as well as volume of the strength training of each individual strength athlete at a given time (Häkkinen 1989).

The extent of these adaptations resulting from the specific resistance training program design variables such as the choice of exercises, order of exercise, amount of rest used between sets and exercises, number of repetitions and sets used for each exercise, and the intensity of each exercise (Abernethy et al. 1994, Kraemer et al. 2002a). One of the basic principles of resistance training is the progressive increase in the training load used. To increase maximal strength, experienced weight trainers need to train with very high loads (e.g. 80-100% of the 1RM). On the other hand, in resistance training aiming mainly for muscle hypertrophy the intensity of the exercises is "only" submaximal (e.g. 60-80% of the 1RM) but multiple repetitions are performed until concentric failure i.e. considerable temporary muscle fatigue occurs (Tesch and Larsson 1982). Traditionally in strength training for muscular hypertrophy various exercises have been performed using a so-called maximum repetition system (i.e. each set is performed to a momentary concentric failure). In order to overload the muscle progressively the training intensity and/or volume and/or frequency should be increased periodically.

The mode of muscle activation pattern dictates how musculoskeletal and other physiological systems will be affected by the resistance training. For the purpose of ultimate training-induced muscle hypertrophy it has been generally recommended to use multiple sets per exercise, moderately a high number of repetitions (e.g. 8-12RM) per sets and short rest periods (i.e. 60-120 sec.) between the sets at a moderate repetition velocity (Kraemer et al. 2002a). However, training protocols emphasizing somewhat higher intensity (load) with longer rest periods between the 8-10RM sets have been recommended in the practical type of strength training publications. Briefly, the basic recommendations in these kinds of high intensity hypertrophic training systems have been that only a few training sets to a momentary concentric failure (i.e. a set until exhaustion) with several minutes recovery time between the sets would be needed per exercise to progressively overload the muscles and to stimulate training induced muscle hypertrophy (Fleck and Kraemer 1997). In practical training the term "intensity" has been used to define the magnitude of the load employed or the rate of work performed (Bosco et al. 2000). In resistance training the intensity can also be modified by special training systems, such as so called "forced repetitions" as defined by Fleck and Kraemer (1997). For muscle hypertrophy it is inappropriate to increase the training

intensity by increasing the magnitude of the load (e.g. load of the 1RM) or the rate of the work performed. To resolve this problem of training programs strength athletes, especially bodybuilders, may increase exercise intensity using different kinds of special exercise systems. One of these systems is a so-called "forced repetitions".

Forced repetitions are a special resistance training system, which strength athletes, especially bodybuilders, use to increase training intensity. Forced repetitions means, that after the trainee has achieved a momentary concentric failure (i.e. a set until exhaustion has been performed), a training partner will assist by lifting or pushing the load just enough to allow the trainee complete three to four additional repetitions. This system "forces" the trainee to continue to produce force, although he or she is already extremely fatigued. It has been speculated that during the sets to exhaustion (i.e. the momentary muscular fatigue) more motor units will be recruited during the exercise leading to a more effective training stimulus than when sets are not performed to exhaustion (Fleck and Kraemer 1997).

#### 2.1.1.1 Muscle hypertrophy

Skeletal muscle fibre hypertrophy is characterized by an expansion of the size and number of myofibrils (MacDougall et al. 1977, Rosenblatt and Woods 1992). In skeletal muscle proteins are constantly and simultaneously being synthesized and degraded (Biolo et al. 1995). Resistance exercise does not induce an acute increase in protein turnover or amino acid oxidation during the exercise (Tarnopolsky et al 1991, Rennie and Tipton 2000) but probably depresses protein synthesis and elevates breakdown acutely (Rennie and Tipton 2000). Repair of damaged proteins and remodelling of structural proteins appears to occur as a result of a resistance exercise stimulus (Biolo et al. 1995). After exercise, muscle protein synthesis rate is stimulated up to 48 h (Chesley et al. 1992, Yarasheski et al. 1993, Biolo et al. 1995, Phillips et al. 1997, 1999) with a concomitant increase in the rate of muscle protein breakdown (Biolo et al. 1995, Phillips et al. 1997, 1999). However, in the absence of feeding net protein balance remains negative (Biolo et al. 1995, Wolfe 2002).

Nutritional intake stimulates muscle protein synthesis to an extent where net protein balance becomes positive (Rennie and Tipton 2000, Wolfe 2002). This feeding- and exercise-induced stimulation of net protein balance results in resistance training to be anabolic (Wolfe 2002, Phillips et al. 2005). Repeated bouts of resistance exercise produce compensatory growth of skeletal muscle which results from chronic increase in the rate of skeletal muscle protein synthesis over the rate of protein degradation with the net result being a deposition of myofibrillar proteins within existing muscle fibers (Phillips 2000, Tipton and Wolfe 2001, Kimball et al. 2002, Hornberger and Esser 2004). Resistance exercise-induced myofibrillar protein turnover is relatively slow (Staron et al. 1994, Hortobagyi et al. 2000) and therefore, repeated exercise stimulus is required for prolonged period (6 to 8 wk) before an outward change in fiber type and/or cross-sectional area of trained muscles are observed

(Staron et al. 1994, McCall et al. 1996, Green et al. 1999). Muscle quality (strength relative to muscle mass) also increases with resistance training possibly for a number of reasons, including increased ability to neurally activate motor units and increased high-energy phosphate availability (Häkkinen and Komi 1983, Hunter et al. 2004). Besides with increases in muscle cross-sectional area resistance training induce changes in muscle architecture including an increase in muscle fascicle pennation angle and decrease in fascicle length in trained muscles (Kawakami et al. 1995, Aagaard et al. 2001, Blazevich et al. 2003). Increases in fiber angle are thought to improve the force-generating capacity of a muscle by allowing a greater muscle mass to attach to a given area of tendon (Kawakami et al. 1993).

#### 2.1.1.2 Changes in muscle phenotype

Human skeletal muscle fibre types can be identified based on the histochemical staining properties of the myosin adenosine triphosphatase (ATPase) enzyme (Staron 1997). Using this terminology, three major fibre types can be identified, types I, IIA and IIX (formerly classificated as IIB). Their functional characteristics are based in large part on the speed of enzyme activity. These fibre types form a continuum, from type I which is the slowest, to IIX which is the fastest (Fry 2004). The resistance training-induced cellular hypertrophy appears to extend to both major fiber types (type I and type II) and subtypes (IIA and IIX), although the magnitude of the increase appears to be fiber type specific (Staron et al. 1994, Green et al. 1999, Fry 2004). There is a genetic predisposition for these respective fibre characteristics but resistance training can induce alterations in the mix of contractile and metabolic proteins present in these cells, e.g. isoform shifts etc. (Staron 1997, Fluck and Hoppeler 2003). In general, there appears to be a conversion of IIX fibres to IIA (Abernethy et al. 1994, Staron et al. 1994, Green et al. 1999, Fry 2004). Phosphagen, glycogen and lipid metabolism and related enzyme adaptations appear to be affected by the modality and duration of resistance training (Tesch et al. 1987, Abernethy et al. 1994).

### 2.1.2 Resistance exercise-induced muscular fatigue

A heavy resistance exercise protocol performed with the progressive overload principle leads to acute responses observed as temporary decreases in maximal force production and electromyography activity of the loaded muscles associated with increases in blood lactate concentrations (e.g. Tesch et al. 1983, Häkkinen et al. 1988c). Therefore, the magnitude of neuromuscular responses can be considered as important indicators of training effects of various heavy resistance exercises. The performance of muscle gradually declines when muscles are used repeatedly at near their maximum force. This muscle fatigue is reflected in reduced force production, reduced shortening velocity and a slower time-course of contraction and relaxation (Allen 2004). Fatigue may be caused by diminished efferent neural command to activated muscles from the

central nervous system (i.e. central fatigue) which inhibits exercise activity before any irreparable damage to muscles and organs occurs. Fatigue may also be caused by factors within the muscle cells (i.e. peripheral fatigue) (St Clair Gibson et al. 2001, Westerblad and Allen 2002). Phosphocreatine (PCr) depletion, intramuscular acidosis and carbohydrate depletion are all potential causes of the fatigue during resistance exercise (Lambert and Flynn 2002). Glycogen store is more rapidly depleted when large amounts of lactic acid are produced anaerobically and muscle performance is severely depressed at low glycogen levels (Allen 2004). Metabolic acidosis during the resistance exercise is caused by an increased reliance on nonmitochondrial ATP turnover. Lactate production is essential for muscle to produce cytosolic NAD+ to support continued ATP regeneration from glycolysis. Lactate also retards acidosis by consuming protons and facilitating proton removal from muscle. However, accumulation of lactate within skeletal muscle or blood directly contributes to intracellular acidosis and is therefore good indirect indicators of increased proton release and decreased cellular pH (Robergs et al. 2004, Lindinger et al. 2005).

Fatigue is associated with increased cytosolic levels of hydrogen ions (H+), Pi, ADP and AMP, while ATP and PCr levels decrease (Dawson et al. 1980, Nagesser et al. 1993). The partial failure of sarcoplasmic reticulum (SR) Ca2+ release is one of the causes of muscle fatigue (Allen 2004). Increased concentration of inorganic phosphate seems to be of major importance for acute fatigue (Westerblad and Allen 2002). Depletion of PCr and the resulting accumulation of ADP may slow SR Ca2+ re-uptake (Steele and Duke 2003). Increased inorganic phosphate (Pi) can affect fatigue development by reducing SR Ca2+ release and also reduces cross-bridge force and the Ca2+ sensitivity of the myofilaments (Cooke and Pate 1985, Allen 2004). Acidification may reduce the force production by a direct effect on the isolated myofibrillar proteins (Fabiato and Fabiato 1978). However, factors other than acidosis are responsible for most of the changes in Ca2+ regulation and deficit of force production that occur during fatigue (Steele and Duke 2003). The fatigue observed during resistance exercise may be mostly caused by reduced Ca2+ release from SR due to ATP-dependent inhibition of the Ca2+ release channels (Westerblad et al. 2000, Steele and Duke 2003).

### 2.1.3 Neural adaptations to resistance training

Neuromuscular performance depends not only by the quantity and quality of the involved muscles, but also by the ability of the nervous system to appropriately activate the muscles. Adaptive changes in the nervous system in response to training are referred to as neural adaptation (Moritani and deVries 1979, Sale 1988, Moritani 1993). Resistance training may cause adaptive changes within the nervous system that allow a trainee to more fully activate prime movers in specific movements (Sale 1988).

Adaptations of the neuromuscular system to resistance training are focused on the development and maintenance of the neuromuscular unit

needed for force production. Resistance training induces adaptations are mediated by supraspinal mechanisms, which include increased excitation (Aagaard et al. 2002b, Griffin and Cafarelli 2005) and changes in the organization of the motor cortex (Barry and Carson 2004). This can influence the manner in which trained muscles are recruited by the CNS during related functional tasks (Carroll et al. 2001). Nervous system adaptation to resistance training may also include descending neural tracts and spinal cord circuitry. Resistance training-induced changes in synaptic efficacy within the motoneuron pool (Semmler and Nordstrom 1998) and neural pathways at the spinal cord may benefit the manner in which muscles are coordinated during related movement tasks (Carroll et al. 2002). Nervous system adaptation to resistance training may also include the motor end plate connections between motoneurons and muscle fibres (Carroll et al. 2001). Increased activity of the myoneural synapse results in morphological changes of the neuromuscular junction which are associated with functional alterations in neuromuscular transmission that enhance neuromuscular transmission (Deschenes et al. 1994). These adaptations can enhance the activation of muscles and are likely to be expressed whenever the motoneuron pool of the trained muscle is activated (Carroll et al. 2001, Barry and Carson 2004).

Early increases in muscle strength due to resistance training are thought to result from neural adaptations and improvements in coordination while later strength increases arise from increased muscle hypertrophy (e.g. Moritani and deVries 1979, Häkkinen and Komi 1983, Komi 1986, Sale 1988, Staron et al. 1994). During the first few weeks of resistance training there is an increase in maximal muscle force output that cannot be accounted for by muscle hypertrophy (Griffin and Cafarelli 2005). Increases in muscular strength due to resistance training may be produced by increased neural drive resulting increases in motor unit discharge rate to agonist muscles (Enoka 1997, Van Cutsem et al. 1998, Patten et al. 2001, Aagaard et al 2002a) and maybe also increases in the recruitment of additional motor units (Barry and Carson 2004). Furthermore, cross-sectional studies suggest that years of resistance training may be associated with increased maximal firing rates (Griffin and Cafarelli 2005). Also synchronization among motor unit firing rate and frequency of doublet firing may increase during resistance training (Enoka 1997, Van Cutsem et al. 1998, Griffin and Cafarelli 2005, Kamen 2005). Neural adaptations to resistance training include reductions in the level of coactivation of the antagonist muscles (Carolan and Cafarelli 1992, Häkkinen et al. 1998, 2000a) and changes in synergistic muscle activation (Rutherford and Jones 1986, Rabita et al. 2000), which could contribute to maximal force generation.

Resistance training may cause adaptive changes within the nervous system that allow a trainee to better coordinate the activation of all relevant muscles, thereby effecting a greater net force in the intended direction of movement (Sale 1988). While resistance training leads to strength increases by increasing the force-generating capacity of individual muscles, it is likely that neural adaptations also comprise changes in the neural activation of muscles,

with modifications occurring in both intramuscular and intermuscular coordination (Rutherford and Jones 1986, Carolan and Cafarelli 1992, Grabiner and Enoka 1995, Häkkinen et al. 1998, 2000a). Some of the adaptations associated with resistance training may be regarded as motor learning, i.e. learning to produce the specific patterns of muscle recruitment that are associated with optimal performance of movement task (Carroll et al. 2001).

## 2.2 Hormonal responses to resistance training

Human skeletal muscle protein undergoes continuous remodelling, which defines the delicate balance between synthesis and breakdown during growth, health, disease and aging (Sheffield-Moore and Urban 2004). Tissue remodelling due to resistance training is a dual process in that catabolism initiates the process during resistance exercise and anabolism predominates in the recovery period leading to growth and repair (Kraemer and Ratamess 2005). Testosterone, growth hormone (GH), insulin and insulin-like growth factor-I (IGF-I) have complex anabolic effects and are important regulators of muscle remodelling processes, whereas glucocorticoids have direct catabolic effects and induce muscle protein loss (Sheffield-Moore and Urban 2004). The stress hormones glucagon, glucocorticoids, and catecholamines cause muscle catabolism when up-regulated together (Fluck and Hoppeler 2003). Anabolic hormones stimulate muscle growth in humans by increasing protein synthesis, by decreasing protein breakdown or both (Phillips et al. 1997, Rooyackers and Nair 1997, Rennie and Tipton 2000).

The net synthesis of protein or protein accretion (i.e. muscle hypertrophy) occurs only when protein synthesis exceeds protein breakdown. Ultimately, hormones are responsible for modulating positive or negative muscle protein balance (Sheffield-Moore and Urban 2004). It has been suggested that increases in anabolic hormones must be maintained for muscle anabolism to occur. Therefore, it seems logical that increases in muscle mass during long-term resistance training could result from hormonal adaptations to training (Consitt et al. 2002). Adaptations to resistance training can entail four general classifications; 1) acute changes during and post-resistance exercise, 2) chronic changes in resting concentrations, 3) chronic changes in the acute response to a resistance exercise stimulus, and 4) changes in receptor content (Kraemer and Ratamess 2005).

During resistance exercise skeletal muscle tissue serves as a repository of protein and free amino acids, in addition to providing precursors for glucose via gluconeogenesis (Sheffield-Moore and Urban 2004). Resistance exercise initiates neuroendocrine responses, including the hypothalamic-pituitary axis and sympathetic nervous system activation, which regulate the utilization of metabolic substrates to meet drastic increase of the energy requirements at muscle level (Leal-Cerro et al. 2003). Thus, catabolic and anabolic hormones are

key regulators of human muscle metabolism during resistance exercise (Consitt et al. 2002, Sheffield-Moore and Urban 2004).

Hormones have also crucial role in muscle regeneration after resistance exercise and therefore changes in hormone levels may have hypertrophic implications (Consitt et al. 2002). It has been suggested that muscle hypertrophy may be due to, at least in part, exercise-induced acute increase in endogenous anabolic hormones which may increase the number of receptor interactions thereby mediating changes in muscle size and neuromuscular function. Since a single hypertrophic type of resistance exercise induces increases in serum hormone concentrations, it is also possible that the magnitude and/or duration of the acute hormone response may change due to prolonged resistance training (Häkkinen et al. 2001). This may be due to adaptation processes in the production and/or clearing mechanisms in the endocrine system (Kraemer et al. 1990).

Resistance exercise acts as a powerful stimulus leading to the acute increases in serum concentrations of several hormones. The nature of this stimulation varies according to the manipulation of the acute programme variables (i.e. intensity (load) of exercise, number of sets and repetitions per set, length of rest periods between sets and muscle mass involved) (e.g. Häkkinen and Pakarinen 1993) and subject characteristics such as age, gender as well as health, nutritional and training status (Häkkinen and Pakarinen 1995, Leal-Cerro et al. 2003, Sheffield-Moore and Urban 2004). The acute decreases in maximal isometric force and EMG activity were rather similar in magnitude after the "hypertrophic" resistance exercise performed with the 10RM protocol for ten sets (Häkkinen 1994b) as compared to the respective acute decreases after the "neural" high loading 1RM protocol of twenty sets (Häkkinen and Pakarinen 1993). In contrast to the 1RM protocol the hypertrophic heavy resistance exercise is known to induce the greatest acute hormone responses when performed by multiple sets per exercise (e.g. 3-5 sets) with short rest periods (e.g. 60-120 sec.) between the sets and with a moderately high number of (e.g. 8-12RM) repetitions per set (e.g. Kraemer et al. 1990, 1991, 1993, Häkkinen and Pakarinen 1993). Therefore, the data of these previous studies suggest that the training mode seems to have a critical influence to the magnitude and/or duration of acute hormonal responses. Previous study of Häkkinen et al. (2001) also suggests that acute hormone responses might have relationship with gains in muscle mass or strength during resistance training. Thus, exercise induced stimulation of the endocrine system may be a trigger for adaptation processes in skeletal muscle cells leading to increases of the contractile proteins.

### 2.2.1 Testosterone

Testosterone is the primary circulating androgen which is synthesized and secreted by testicular Leydig cells under luteinizing hormone stimulation (Mooradian et al. 1987, Evans 2004). Testosterone regulates many physiologic processes in the adult male including muscle protein metabolism, sexual and

cognitive functions, erythropoiesis, plasma lipids, and bone metabolism (Bhasin et al. 1996). In females, the circulating testosterone levels are typically about 10% of those observed in men (Evans 2004). In reproductive tissues, testosterone is converted to dihydrotestosterone (DHT) via 5alfa-reductase. Testosterone is the main androgen in skeletal muscle because of low levels of 5alfa-reductase in muscle (Bhasin et al. 2003)

Testosterone secretion has been shown to be secreted in a circadian manner with the greatest elevations observed early in the morning with less throughout the rest of the waking day (Kraemer et al. 2001b). Circulating androgens are predominately bound to the transport protein sex hormone-binding globulin (SHBG). It is the unbound fraction of testosterone that is biologically active and able to interact with androgen receptors (AR). A change in SHBG concentrations may influence the binding capacity of testosterone and the magnitude of free testosterone available for diffusion across the cell membrane to interact with membrane-bound steroid receptors. The contribution of free testosterone represents the amount of bioactive testosterone, which can act directly with androgen receptors in the target tissue (e.g. skeletal muscle) to mediate changes of the function of muscle cell via enhanced protein synthesis.

During adult life, the average male produces approximately 7 mg of testosterone daily. The normal range of plasma testosterone in males is 300 to 1000 ng/dl but the average value declines by age 80 to approximately 50% of that at age 20 years (Evans 2004). Most aging men show a reduction in circulating serum testosterone concentrations which can be clinically characterized by decreased e.g. muscle strength (Ferrando et al. 2002).

Testosterone is an anabolic hormone that exerts a potent effect on skeletal muscle (Spratt et al. 1988). Testosterone can increase skeletal muscle mass by increasing muscle protein synthesis (Bhasin et al. 2003, Evans 2004). Testosterone can also improve the efficiency of reutilization of amino acids in the muscle and slowing muscle protein degradation due to decreased proteasome peptidase activity (Bhasin et al. 2003).

Testosterone's effects on the muscle might be mediated through an antiglucocorticoid effect. There could be binding competition between androgens and glucocorticoids for the glucocorticoid receptor and/or androgens could down-regulate the expression of the glucocorticoid receptor in the muscle by interfering with glucocorticoid receptor transcriptional activity (Chen et al. 2005). Androgens could also have postreceptor effects on the glucocorticoid pathway (Bhasin et al. 2003).

The anabolic effects of testosterone in muscle may be also mediated by GH and local IGF-I expression (Urban et al. 1995, Mauras et al. 1998, Giustina and Veldhuis 1998, Lewis et al. 2002). Testosterone may increase the sensitivity to IGF-I through up-regulation of the IGF receptor (Thompson et al. 1989). Testosterone may also activate the non-genomic pathways (e.g. Ras/MEK/ERK pathway) by an effects on inositol 1,4,5-trisphosphate (IP3) and increases in intracellular calcium (Estrada et al. 2003). Androgen receptors are expressed in

the mammalian skeletal muscle and in satellite cells (Doumit et al. 1996). Therefore, testosterone may increase skeletal satellite cell proliferation through an androgen receptor-mediated pathway which in turn mediates changes in myonuclear number and muscle fiber hypertrophy (Mulvaney et al. 1988, Joubert and Tobin 1989, 1995, Doumit et al. 1996, Sinha-Hikim et al. 2003). The effect of testosterone on the nervous system may enhance acute force production. Testosterone can interact with receptors on neurons and increase the amount of neurotransmitters released, regenerate nerves, increase cell body size and dendrite length/diameter (Nagaya and Herrera 1995, Brooks et al. 1998)

#### 2.2.1.1 Acute testosterone response to resistance exercise

Resistance exercise has been shown to acutely increase total and free testosterone concentrations in men (Weiss et al. 1983, Häkkinen and Pakarinen 1995). The magnitude of elevation during resistance exercise has been shown to be affected by the muscle mass involved (Hansen et al. 2001), intensity and volume (Kraemer et al. 1990, Häkkinen and Pakarinen 1993, Schwab et al. 1993, Gotshalk et al. 1997, Raastad et al. 2000), nutritional intake (Kraemer et al. 1998d) and training experience (Tremblay et al. 2003). Large muscle-mass exercises with moderate intensity, high volume and relatively short rest intervals have been shown to be potent metabolic stressors (Ratamess et al. 2005) and a higher glycolytic component may be a stimulus for testosterone release (Lu et al. 1997). The response of free testosterone seems to parallel total testosterone (Kraemer and Ratamess 2005). In older men acute testosterone response is lower than that of younger men (Kraemer et al. 1999b). In women no significant changes have been observed following resistance exercise in acute testosterone response (Weiss et al. 1983, Kraemer et al. 1991, 1993, Häkkinen and Pakarinen 1995).

It has been proposed that the exercise-induced acute endocrine responses may reflect some other hormonal regulatory mechanism than those of the regulation of the resting hormonal concentrations (Fry et al. 1991). In resting conditions serum level of testosterone is mainly regulated via a negative feedback loop system involving the anterior pituitary (gonadotrophin-releasing hormone; GnRH), hypothalamus (luteinizing hormone; LH), and testicles; referred to as the hypothalamic-pituitary-testicular axis. Elevations in circulating testosterone concentrations due to heavy resistance exercise have been attributed to plasma volume reductions (Metivier et al. 1980, Kindermann et al. 1982, Weiss et al. 1983, Cadoux-Hudson et al. 1985), reduced hepatic or extra-hepatic clearance rates (Weiss et al. 1983, Cadoux-Hudson et al. 1985) and/or potential increases in testosterone synthesis and/or secretion due to the increased gonadal secretion (Metivier et al. 1980, Cumming et al. 1986), testosterone release by vasodilatation (Meskaitis et al. 1997), direct catecholamine-mediated release of stored testosterone from the testes (Eik-Nes 1969), increases in LH pulsatility or production (Vermeulen et al. 1972, Longcope et al. 1990) and/or a direct (LH-independent) stimulatory effect of

lactate on the secretion of testosterone (Lu et al. 1997, Lin et al. 2001). Catecholamines may increase force production, muscle contraction rate, energy availability, as well as augment the secretion of hormones such as testosterone (Kraemer and Ratamess 2005). An acute bout of resistance exercise has been shown to increase plasma concentrations of epinephrine, norepinephrine and dopamine (Guezennec et al. 1986, Kraemer et al. 1987, 1999, Bush et al. 1999). The elevated exercise-induced symphatic activity may contribute to the augmented acute testosterone response (Jezova and Vigas 1981, Fahrner and Hackney 1998). No LH response during resistance exercise suggesting that acute elevations in serum testosterone concentrations during resistance exercise are due to other regulatory mechanisms e.g. reduced clearance or plasma volume shifts (Häkkinen et al. 1988c). However, the increased serum testosterone levels seen after resistance exercise may contribute to increased protein synthesis (Fluck and Hoppler 2003).

Regardless of the mechanism(s) of exercise-induced increase of serum testosterone concentrations, the skeletal muscle will be exposed to an elevated peripheral testosterone concentration and thus the likelihood of possible interactions with potential muscle cell receptors could increase. Furthermore, the increase of testosterone concentration in serum has been connected to up-regulation of androgen receptors (Doumit et al. 1996). It could be speculated that trained muscle tissue requires - as highly as possible - hormone-hormone receptor interactions to start the recuperation and adaptation processes optimally after the resistance exercises. Therefore, it may be also possible that great heavy resistance exercise-induced hormone responses are physiologically very important for adaptation processes during prolonged resistance training.

#### 2.2.1.2 Chronic testosterone responses to resistance training

The basal serum testosterone concentrations remain usually unaltered or periodical shifts may occur during long-term resistance training in both younger and older men with normal physiological range of testosterone levels (e.g. Häkkinen et al. 1985b, 2000b, Craig et al. 1989, Nicklas et al. 1995). Changes in resting testosterone concentrations during long-term resistance training have been inconsistent or non-existent in men and women (Kraemer and Ratamess 2005). In the study of Kraemer et al. (1992) experienced weightlifters had a greater acute increase in the testosterone response following the heavy resistance exercise than that of unskilled weight trainers. The enhanced acute testosterone response due to the resistance training has been reported (Kraemer et al. 1998c), but other previous studies have not found any significant changes in resistance exercise induced acute testosterone responses due to the long-term resistance training in adult men (Craig et al. 1989, Hickson et al. 1994, McCall et al. 1999a). However, the exercise-induced elevation in total testosterone is attenuated during volume-related overtraining (Häkkinen et al. 1987).

Resting concentrations of testosterone may reflect on substantial changes in the volume and intensity of training (Häkkinen et al. 1987, 1988b, Raastad et al. 2001). The changes in the volume of resistance training have led to changes

in serum total testosterone / cortisol ratio with a concomitant change in serum LH concentrations. Furthermore, the total testosterone / SHBG ratio has been shown to correlate to strength performance in elite weightlifters (Häkkinen et al. 1987) and periodical changes in serum total testosterone concentrations seem to occur during the most intense training periods of prolonged resistance training (Häkkinen et al. 1988c). Circulating levels of testosterone have been shown to correlate with training-induced increases in muscular strength in women (Häkkinen et al. 1990, 1992, 2000b, Häkkinen and Pakarinen 1994) and the capacity to improve strength in older adults involved in a resistance training program (Häkkinen and Pakarinen 1994). It has been suggested that the level of free testosterone may be of importance for trainability (Häkkinen et al. 1985b, Kraemer et al. 1990). Resistance training may also enhance the acute testosterone response to a workout in men (Kraemer et al. 1999a, Tremblay et al. 2003). These results suggest that the periodical adaptative responses in the endogenous hormone balance seem to have an increasing importance for muscle hypertrophy and strength performance during long-term resistance training, especially in strength athletes.

#### 2.2.1.3 Skeletal muscle androgen receptors

Only free testosterone can enter cells in order to effect its biological actions by binding to androgen receptors (AR) to mediate the effects of testosterone upon target tissues. The AR belongs to a superfamily of ligand-dependent transcriptional factors (Truss and Beato 1993, McKenna et al. 1999). Binding of testosterone to the specific AR ligand-binding domain induces a conformational modification of the receptor, followed by the separation of the receptor from cytoplasmic chaperone proteins such as heat shock protein 90 (Hsp90). This allows nuclear translocation, dimerization and binding to androgen response elements of the target genes to regulate gene expression by interacting with the transcription machinery (Freedman 1992, Truss and Beato 1993, Wong et al. 1993).

AR's are expressed in the skeletal muscles while the level of expression varies in different muscle groups (Sar et al. 1990). The AR concentration in skeletal muscle may depend on several factors including muscle fibre type (Deschennes et al. 1994), contractile activity (Inoue 1993, 1994, Bricout et al. 1994) and the concentrations of testosterone (Bricout et al. 1999). Up-regulation of AR mRNA and protein expression by testosterone in skeletal muscle may occur through stabilizing existing receptors or by increasing receptor synthesis by transcriptional and post-transcriptional mechanisms (Mauras et al. 1998, , Sheffield-Moore et al. 1999, Kadi et al. 2000, Carson et al. 2002, Burnstein 2005).

The anabolic effect of testosterone is mediated by ARs in skeletal muscle (Bhasin et al. 2003). Testosterone interaction with the AR creates a milieu of events, ultimately leading to a specific response such as an increase in muscle protein synthesis. Testosterone may have effects on satellite cells through up-regulation of AR levels in satellite cells, which could enhance the sensitivity of satellite cells to testosterone (Chen et al. 2005). Testosterone administration has

been found to increase satellite cell number in humans (Kadi et al. 1999b, Sinha-Hikim et al. 2003). Therefore, AR regulates the transcription of target genes that may control the accumulation of DNA required for muscle growth (Evans 2004). Also non-genomic regulation of AR may occur since cell membrane binding sites for testosterone have been identified on different cells, but not myoblasts. Cell membrane AR may interact or modulate G-protein-coupled receptors inducing rapid rise in the intracellular free Ca2+ concentration as well as regulate the MAPK family of protein kinases (Simoncini and Genazzani 2003).

AR expression on skeletal muscles may be, at least in part, related to the exercise-induced changes in serum testosterone concentrations since androgens are shown to be important regulators of AR mRNA and protein expression through transcriptional and post-transcriptional mechanisms (Yeap et al. 2004). The change in AR content influences the amount of receptor available to interact with testosterone, and therefore, a pathway of change from stabilization, down- to up-regulation of AR may be crucial in mediating the effects of testosterone upon target tissues (Ratamess et al 2005). Animal studies showed that testosterone might induce skeletal muscle cell hypertrophy by enhanced AR expression (Hickson et al. 1983, 1985) followed by increasing muscle protein synthesis (Sheffield-Moore 2000, Bhasin et al. 2001). Acute resistance exercise-induced elevations in circulating testosterone concentrations present a greater likelihood of interaction with receptors. Thus, single resistance exercise as well as long-term resistance training has been shown to up-regulate AR content in humans (Kadi et al. 2000, Bamman et al. 2001). Furthermore, correlation between baseline AR content in the vastus lateralis and 1RM squat, thereby suggesting that AR content, in part, assists in mediating strength changes during resistance training (Ratamess et al. 2005).

The resistance exercise stimulus appears to mediate the magnitude of acute AR modifications since high volume resistance exercise protocol elicited significant down-regulation of AR content compared to single set resistance exercise (Ratamess et al. 2005). Considering that ARs are protein molecules and protein catabolism increases during resistance exercise (Biolo et al. 1995) the AR protein content may initially down-regulate despite elevations in circulating testosterone prior to the up-regulation (Bamman et al. 2001). AR mRNA (Bamman et al. 2001, Willoughby and Taylor 2004) and protein (Willoughby and Taylor 2004) expression has been shown to increase due to resistance exercise. Furthermore, significant relationship between exercise-induced increase in serum total and free testosterone concentration and AR mRNA expression at 48h after the exercise has been observed (Willoughby and Taylor 2004). These results suggest that resistance exercise may increase AR mRNA expression, at least in previously untrained men. Furthermore, long-term resistance training may have effect on AR expression in trained muscles (Kadi et al. 2000, Willoughby and Taylor 2004).

### 2.2.2 Cortisol

Glucocorticoids are released from the adrenal cortex by stimulation of a pituitary hormone ACTH (adrenocorticotropic hormone). Cortisol accounts for approximately 95% of all glucocorticoid activity. About 10% of circulating cortisol is free, while ~15% is bound to albumin and 75% is bound to corticosteroid-binding globulin. The actions of cortisol on muscle are mediated through the glucocorticoid receptor (Chen et al. 1997). Cortisol is a catabolic hormone which among its other functions also takes part to the degradation of proteins from skeletal muscles. Cortisol has greater effects in type II muscle fibres (Crawford et al. 2003). Besides with increases in protein degradation, cortisol stimulates lipolysis in adipose cells and decreases protein synthesis in muscle cells resulting in greater release of lipids and amino acids into circulation. Furthermore, cortisol increases gluconeogenesis. Thus, a prominent role of acute cortisol response is to meet the greater metabolic demands caused by the resistance exercise (Viru et al. 1994). In postexercise recovery period cortisol contributes to maintain sufficient rates of glycogen synthesis, protein turnover and supply of protein synthesis by amino acids (Viru 1996). Amino acid availability is an important regulator of muscle protein metabolism (Biolo et al. 1997). On the other hand, exercise-induced increase in cortisol concentration may suppress gonadotropin release by acting at the level of the pituitary gland, inhibit the secretion of GnRH at the hypothalamic level (MacAdams et al. 1986, Calogero et al.1999, Breen et al. 2004) and/or increased concentration of ACTH may compete with LH of the androgen receptors of Leydig cells (Beitins et al. 1973).

#### 2.2.2.1 Acute cortisol response to resistance exercise

Studies have shown significant elevations in cortisol and ACTH during an acute bout of resistance exercise (e.g. Guezennec et al. 1986, Häkkinen et al. 1988c, Kraemer et al. 1992, 1999). Metabolically demanding resistance exercise protocols high in total work, i.e. high volume, moderate to high intensity with short rest periods, have elicited the greatest acute lactate and cortisol response in men and women at different ages (Kraemer et al. 1987, 1993, Häkkinen and Pakarinen 1993, Williams et al. 2002, Smilious et al. 2003). Specifically, the acute cortisol response has occurred when the overall stress of the exercise protocol has been very high (Häkkinen and Pakarinen 1993, Kraemer et al. 1993) and the response has been linked to the volume of total work or in magnitude to a given heavy-resistance exercise protocol (Kraemer et al. 1987, 1991, 1993, 1995, Gotshalk et al. 1997). These findings suggest that certain threshold should exceed during resistance exercise until the cortisol response occurs (Viru 1992).

#### 2.2.2.2 Chronic cortisol responses to resistance training

Resting cortisol concentrations may generally reflect a long-term training stress. However, resistance training does not appear to produce consistent patterns of

cortisol secretion as no change (Häkkinen et al. 1987, 1988b, 1990, 1992, 2000b, Fry et al. 1994) or reductions (Häkkinen et al. 1985b, Alen et al. 1988, Kraemer et al. 1998c, McCall et al. 1999a, Marx et al. 2001) has been observed. However, long-term resistance training in adult men has had an overall reduction of cortisol responses to exercise stress (Staron et al. 1994, Kraemer et al. 1995, 1999). Also with consistent resistance training down-regulation of the glucocorticoid receptor may occur, thereby reducing the catabolic influence on skeletal muscle tissue (Willoughby et al. 2003). Furthermore, in overtraining conditions the cortisol response has been attenuated due to the increase in the resistance training volume (Fry et al. 1994, 1998). Thus, it appears that the acute cortisol response may reflect metabolic stress whereas the chronic changes (or lack of change) may be involved with tissue homeostasis involving protein metabolism (Kraemer and Ratamess 2005).

A short-term increase in volume and/or intensity (overreaching) may reduce resting concentrations of testosterone (Häkkinen et al. 1988a, Raastad et al. 2001) and elevate corticol levels (Volek et al. 2004). However, similar findings are not observed systematically in other studies (Kraemer and Ratamess 2005). Overtraining, resulting from a chronic large increase in training volume, has been shown to result in elevated cortisol and reductions in resting LH, total and free testosterone concentrations (Fry and Kraemer 1997, Häkkinen and Pakarinen 1991). Detraining periods longer than eight weeks have shown significant reductions in the testosterone/cortisol ratio which correlated highly to strength decrements (Alen et al. 1988, Häkkinen et al. 1985b). The testosterone/cortisol (T/C) ratio and/or free testosterone/cortisol ratio have been suggested to be indicators of the anabolic/catabolic status of skeletal muscle during resistance training (Häkkinen 1989). However, the use of the T/C ratio remains questionable and is at best only an indirect measure of the anabolic/catabolic properties of skeletal muscle (Fry and Kraemer 1997)

### 2.2.3 Growth hormone

The acidophilic cells of the anterior pituitary secrete molecules that make up the family of growth hormone (GH) polypeptides. Pituitary GH encoded by the GH-1 gene is secreted in a pulsatile fashion in 6–12 discreet pulses per day, generally following a circadian rhythm (Godfrey et al. 2003). Many physiologic factors alter pulsatile GH secretion, including age, gender, body composition, sleep, nutrition, exercise and serum concentrations of gonadal steroids, insulin and IGF-I. Among these various factors, the amount of abdominal visceral fat is the most important predictor of the 24- hour integrated GH concentration (Clasey et al. 2001).

GH secretion is regulated by two hypothalamic peptides: GH releasing hormone (GHRH), which stimulates GH synthesis and secretion, and somatostatin, which inhibits GH release without affecting GH synthesis (Giustina and Veldhuis 1998). There are membrane receptors for both GHRH and GHIF (somatostatin) on anterior pituitary cells. These two peptides are in turn influenced by an array of neurotransmitters. There is a tight feedback

control of GH release, involving GH and IGF-I in regulation of GHIF and probably GHRH (Mullis 2005).

Human GH represents a family of proteins rather than a single hormone and over 100 forms of GH have been identified in plasma (Baumann 1991) with apparently different physiological functions (Lewis et al. 2000). In the circulation, GH has a short half-life (20–25 min) and the dominant form of GH is a 22kD protein (Martin 1978). However, approximately 10% of circulating GH is a 20kD protein and there are also various biologically active lower molecular weight fragments of GH and other protein-bound GH and aggregates of GH (Baumann 1991). GH receptors (GHR) are found in many tissues throughout the body including skeletal muscle (Roupas and Heringont 1989, Florini et al. 1996). The regulation of energy metabolism by GH is believed to be mediated by direct interaction of GH with the GHR on target cells. Signal transduction systems that mediate GH action involve GHR dimerisation, activation of Janus kinases, mitogen activated protein kinases and the signal transducers and activators of transcription signalling pathways (Kopchick et al. 1999).

GH has anabolic effects on muscle cell. GH acutely stimulates muscle protein synthesis, decreases rate of glucose use and thereby antagonises the effects of insulin, promotes the release of free fatty acids and glycerol from the adipose tissue, increases circulating free fatty acids and their oxidation in the liver, promote a positive calcium, magnesium and phosphate balance and cause the retention of sodium, potassium and chloride ions (Fryburg and Barret 1993, Dominici and Turyn 2002, Godfrey et al. 2003). In addition, GH stimulates cellular uptake and incorporation of amino acids into protein in several tissues, including skeletal muscle (Hartman et al. 1993). Muscle hypertrophy and an overall increase in lean body mass is one of the outcomes that may be mediated by GH as a response to exercise. In addition to the recognised effects on growth, GH is also believed to affect substrate utilisation during exercise (Godfrey et al. 2003)

Insulin-like growth factor-1 (IGF-I) secreted by hepatic tissue is the primary mediator of many of the responses regulated by GH in tissues throughout the body (Yarasheski 1994) including postnatal development of skeletal muscle (Florini et al. 1996). Despite the significant resistance exercise-induced GH response, much of the stimulus for protein synthesis has been attributed IGF-I (Godfrey et al. 2003). Thus, GH may not alone to increase human skeletal muscle protein and maximum voluntary force but GH and IGF-I in combination produce hypertrophy response (Yarasheski 1994).

GH production and release decreases with age by approximately 14% per decade after the age of 40 years and is decreased in conditions such as obesity (Zadik et al. 1985, Veldhuis et al. 1995, Wideman et al. 2002). Exercise is a potent stimulus of GH release in young adults. Since decreased GH secretion in aging and other conditions such as obesity is associated with many detrimental health effects it can be suggested that the use of regular exercise as a stimulus for GH release may have positive effects on health and well being (Wideman et al. 2002)

#### 2.2.3.1 Acute growth hormone response to resistance exercise

The release of GH is sensitive to many physiological stimuli, including exercise (Roemmich and Rogol 1997, Godfrey et al. 2003). Multiple-set protocols have elicited greater GH responses than single-set protocols (Craig and Kang 1990, Gotshalk et al. 1997). Moderate- to high-intensity, high-volume programmes using short rest periods have shown the greatest acute GH response compared with conventional strength or power training using high loads, low repetitions and long rest intervals in men (VanHelder et al. 1984, Kraemer et al. 1990, 1991, 1993, Häkkinen and Pakarinen 1993, Bosco et al. 2000, Williams et al. 2002, Goto et al. 2003, Smilious et al. 2003). The magnitude appears dependent upon exercise selection and subsequent amount of muscle mass recruited (Kraemer et al. 1992), muscle actions used (i.e. greater response during concentric than eccentric muscle actions) (Durand et al. 2003), intensity (VanHelder et al. 1984, Pyka et al. 1992), volume (Gotshalk et al. 1997), rest intervals between sets (Kraemer et al. 1990, 1991) and training status (e.g. greater acute elevations based on individual strength and the magnitude of total work performed) (Rubin et al. 2005).

Acute resistance exercise can increase GH release in men and women of all age groups (e.g. Kraemer et al. 1990, 1991, 1993, 1999, Pyka et al. 1992, Nicklas et al. 1995, Marcell et al. 1999, Bosco et al. 2000, Nindl et al. 2000, Takarada et al. 2000, Hymer et al. 2001). The serum GH concentration peaks at or slightly after the termination of resistance exercise and returns to baseline levels by approximately within 90 minutes post-exercise (Wideman et al. 2002). GH is an anabolic hormone, and therefore, heavy resistance exercise-induced increased secretion of GH may be important for the process of training-induced muscle hypertrophy (Kraemer et al. 1987). However, the interindividual GH response to acute resistance exercise is highly variable. Dependent on the protocol employed, the average peak GH concentration attained during acute resistance exercise in young men and women ranges between 5–25 µg/L. Similarly, the average peak GH concentration attained during acute aerobic exercise is also between 5–25 µg/L (Wideman et al. 2002).

The GH response to exercise is altered by many factors, including for example sex steroid concentrations, fitness level and the intensity of previous exercise sessions (Veldhuis et al. 1995, Roemmich and Rogol 1997). At the same relative workload the acute GH response to resistance exercise decline with increasing age in both men and women (Pyka et al. 1992, Häkkinen and Pakarinen 1995, Kraemer et al. 1999b, Marcell et al. 1999). Acute responses of serum IGF-I to resistance exercise are somewhat contradictory. A few studies have shown acute elevations in serum IGF-I during and following resistance exercise (Kraemer et al. 1990, 1991, Rubin et al. 2005) whereas some studies have shown no change (Chandler et al. 1994, Kraemer et al. 1995, 1998).

The exercise-induced release of GH involve GHRH release and/or somatostatin withdrawal and possibly, natural GHRP-like ligand release (e.g. ghrelin) or some combination of these mechanisms (Wideman et al. 2002). Although the exact mechanisms of exercise-induced GH response not known,

the best candidates appear to be nitric oxide, lactate and neural stimulation (Godfrey et al. 2003). Nitric oxide (NO) has been identified as an important intra- and intercellular transmitter involved in the control of the hypothalamic-pituitary axis (Pinilla et al. 1999) and it has also been suggested as a mechanism for the release of hormones into the general circulation. Therefore, NO may facilitate GH secretion during resistance exercise (Godfrey et al. 2003). Also sympathetic activity may be an important mediator of the GH response to acute exercise, possibly via activation of central α2-adrenergic neurons (Giustina and Veldhuis 1998). Peak plasma adrenaline and noradrenaline concentrations have been found to precede the peak in serum GH concentrations (Weltman et al. 1997).

Resistance exercise programmes that elicit the greatest GH response also elicit the greatest demand on anaerobic glycolysis and lactate formation as well as acute cortisol response (Roemmich and Rogol 1997, Takarada et al 2000, Kraemer and Ratamess 2005). An increased acidity in the muscle caused by the H+ accumulation produced by anaerobic metabolism during muscle work stimulates metaboreceptors and sends afferent feedback to the central nervous system and hypothalamus leading to increased secretion of GH (Kjaer et al. 1987, Gordon et al. 1994, Gosselink et al. 1998). The isoforms of GH that are measurable in the circulation may be altered by muscle afferent stimulation (McCall et al. 2000).

It may be possible that nervous system have important role in regulating GH secretion during resistance exercise and this regulatory mechanism may be sensitive to specific muscle actions used during resistance training (Kraemer et al. 2001a). With progressive overload motor unit recruitment will increase (Sale 1988). The anterior pituitary is innervated by nerve fibres from central nervous system, e.g. motor cortex (Ju 1999). Therefore, hormonal responses to exercise may be triggered by the central motor command to working muscles (Galbo et al. 1987, Kjaer et al. 1987) and the responses are further modulated by muscle afferent-pituitary axis feedback e.g. from cholinergic pathways and proprio- and metaboreceptors in muscles (Few and Davis 1980, Kjaer et al. 1989, 1992, , Thompson et al. 1993, , McCall et al. 1997, 1999b, Giustina and Veldhuis 1998, Gosselink et al. 1998).

#### 2.2.3.2 Chronic growth hormone responses to resistance training

Long-term resistance training does not appear to systematically affect resting concentrations of GH in younger and older men and women or acute resistance exercise-induced GH response (Consitt et al. 2002, Wideman et al. 2002, Kraemer and Ratamess 2005). However, Häkkinen et al. (2001) showed that the acute GH response became significant and its duration lengthened due to the 21-wk resistance training period in older women.

## 2.3 Cell and molecular responses to resistance training

### 2.3.1 Molecular determinants to skeletal muscle hypertrophy

Understanding the molecular basis of muscle hypertrophy is important to the development of targets for exercise intervention in sports or in muscle wasting conditions such as sarcopenia. Skeletal muscle is a highly plastic tissue that is constantly adapting to changes in loading state (Goldberg 1967, Haddad and Adams 2002). Thus, the tension placed on the muscle plays a critical role in the regulation of its mass (Goldberg et al. 1975, Vandenburgh 1987).

Resistance training stimulates and reinforces cellular and molecular processes that lead to a compensatory hypertrophy response (Haddad and Adams 2002). An increase in fiber size is thought to occur basically via increased gene transcription and protein synthesis rate (Booth and Thomason 1991). Single heavy resistance exercise causes changes in patterns of gene expression in muscle, influence protein synthesis and affect muscle metabolism to produce adaptations in muscle mass and contractility that reflect the tissue's recent loading history (Tidball 2005). Furthermore, alterations in the types and amounts of cellular proteins in myofibers due to long-term resistance training may involve alterations in basal gene expression. This nuclear reprogramming may have an important role in muscle plasticity and may be related to the adaptations in the myosin type, protein turnover, and the cytoplasma-to-myonucleus ratio (Fluck et al. 2005). Muscle growth is optimized by combining exercise and appropriate nutritional strategies, such as amino acid and carbohydrate ingestion (Deldicque et al. 2005). As myofibers undergo hypertrophy in response to resistance exercise, the blood flow dynamics (Degens et al. 1992, McCall et al. 1996) and extracellular matrix (MacDougall et al. 1982, McCormick and Schultz 1994, Moore et al. 2005) adjusts to support the hypertrophy.

It is apparent that there is local as well as systemic control of muscle growth, because exercise-induced skeletal muscle adaptation occurs in exercised muscles not all the muscles of the body (Goldspink and Harridge 2004). Furthermore, alterations in protein synthesis due to mechanical load of skeletal muscle tissue can occur independently of circulating factors such as testosterone, glucocorticoids and growth factors (Palmer et al. 1983, Vandenburgh et al. 1999) suggesting that exercise-induced skeletal muscle adaptation is largely mediated by intrinsic mechanisms of myofiber (Tidball 2005, Goldberg et al. 1975).

Skeletal muscle hypertrophy is regulated by at least three major molecular processes: (1) satellite cell activity; (2) gene transcription; and (3) protein translation (Machida and Booth 2004). Thus, the mechanisms producing a hypertrophic response to exercise include an increased rate of protein synthesis (Goldberg 1968, Goldberg et al. 1975, Vandenburgh 1987), expression growth factors (Goldberg et al. 1975, Vandenburgh 1987, Tipton and Wolfe 2001,

Kimball et al. 2002), and the proliferation of satellite cells, which appears to be necessary to provide additional myonuclei to the enlarging myofibers (Goldberg et al. 1975, Schiaffino et al. 1976, Salleo et al. 1983, Vandenburgh et al. 1991, Allen et al. 1995).

The muscles acutely respond to mechanical load with upregulated expression of mRNAs to the hypertrophic process (Willoughby and Nelson 2002, Bickel et al. 2003, Hameed et al. 2003, Psilander et al. 2003, Willoughby 2004). Hypertrophy process includes a robust increase in the expression of IGF-I mRNA and peptide in the overloaded muscles (DeVol et al. 1990, Adams and Haddad 1996). IGF-I can influence the activity of all hypertrophic mechanisms, including increases in satellite cell proliferation, gene expression (e.g skeletal a-actin) and protein synthesis (Florini et al. 1996, Chakravarthy et al. 2000) (Figure 1). Thus, increased IGF-I expression plays an important role in mediating muscle hypertrophy induced by mechanical loading (Adams and Haddad 1996, Adams and McCue 1998).

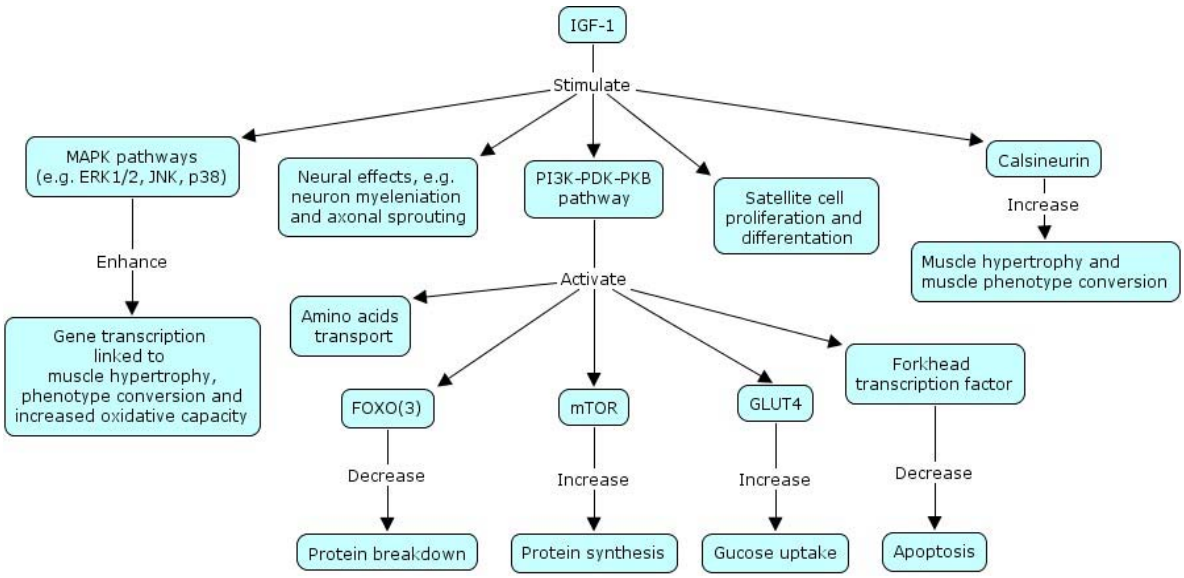


FIGURE 1 Effects of IGF-I on neuromuscular system (Modified from Adams 2002, Kimball et al. 2002, Machida and Booth 2004, Michel et al. 2004, Mourkioti and Rosenthal 2005, Tidball 2005)

### 2.3.2 Resistance exercise-induced myofiber disruption

Mechanical strain to muscle tissue during heavy resistance exercise may produce structural disruptions to contractile elements within the activated muscle fibres leading to muscle soreness and short-term impairment of muscle function after the exercise (Allen 2004). The regenerative phase then restores muscle fibres to their normal condition (Gibala et al. 2000). Depending of the type of exercise protocol, resistance exercise may damage the intracellular and/or extracellular tissue of skeletal muscle ranging from a few macromolecules to large tears in the sarcolemma, basal lamina, and supportive

connective tissue, as well as damage within the contractile and cytoskeletal proteins of the myofiber (Staron et al. 1994, Vierck et al. 2000).

Especially eccentric contractions (i.e. muscle produces force whilst being stretched) causes mechanical damage to the weaker areas of the muscle fibre due to high shearing forces which produce microscopic tears to the plasma membrane (Petrof et al. 1993) and in contractile proteins (McCully and Faulkner 1985, Brooks and Faulkner 2001, Evans 2002). Following repeated eccentric contractions the muscles exhibit an immediate weakness and, over the subsequent days, they remain weak but also become tender, painful and stiff (Newham et al. 1987). These changes can take a week to recover fully (Allen 2004). The changes of sarcomere structure are probably the initiating factor which appears to cause localized membrane tears that subsequently contribute to muscle weakness and damage (Westerblad and Allen 2002, Allen 2004).

Muscle regeneration is characterized by two phases: a degenerative phase and a regenerative phase (Charge and Rudnicki 2004, Mourkioti and Rosenthal 2005). During the degenerative phase of damage, the initial event is necrosis of the muscle fibres which is triggered by disruption of the sarcolemma resulting in increased myofiber permeability (Close et al. 2005) and changes in intracellular calcium homeostasis (Armstrong 1990, Charge and Rudnicki 2004). Disruption of the myofiber integrity is reflected by increased serum levels of muscle proteins, such as creatine kinase, which are usually restricted to the myofiber cytosol. Increased serum creatine kinase is observed after extensive physical exercises (Sorichter et al. 2001).

Muscle degeneration is followed by the activation of a muscle repair process. Revascularization, reinnervation, and reconstitution of the extracellular matrix are essential aspects of the muscle regeneration process (Charge and Rudnicki 2004). The regeneration process is influenced by growth and differentiation factors within the tissue, the degree of injury and the interactions between muscle and the invading inflammatory cells (Mourkioti and Rosenthal 2005). Hyperemia and the release of growth factors and cytokines influence satellite cells in a cascade of regenerative events which ultimately lead to myofiber hypertrophy (Allen et al. 1979, Grounds 1998).

### 2.3.3 Converting mechanical signal to biochemical responses; mechanotransduction

Heavy resistance exercise induces mechanical strain to skeletal muscle tissue (Goldspink 1999). The process of converting mechanical energy into intracellular biological events is termed mechanotransduction (Hornberger and Esser 2004). The lipid membrane can serve as a mechanoreceptor via alterations in the fluidity of the bilayer or if the lipid bilayer is ruptured (Hamill and Martinac 2001, Hornberger and Esser 2004). Mechanical loads influence the activity of ion channels (Tidball 2005,) and produce increases in second-messenger molecules, such as nitric oxide (Franco and Lansman 1990, Tidball et al. 1998), in skeletal muscle membranes. Also G-proteins can be activated which

may induce increase in protein synthesis by signalling through a PI3K-dependent pathway (Hornberger and Esser 2004).

The focal adhesion (FA) and dystrophin–glycoprotein complexes could serve as mechanoreceptors that transmit mechanical information between the extracellular matrix and the cytoskeleton of the cell and convert mechanical information into biochemical signals (Goldspink 2003, Hornberger and Esser 2004). One of the major constituents of the FA is the family of cell surface receptors termed integrins (Schwartz et al. 1995) (Figure 2). The activation of mechanotransduction events is ultimately linked to mechanosensation via integrins and associated kinases (Carson and Wei 2000, Gordon et al. 2001). Resistance exercise-induced mechanical strain (i.e. stretch) to skeletal muscle cells and activation of integrins and dystrophin–glycoprotein complexes induce expression of IGF-I and its splice variants from loaded skeletal muscle (Goldspink and Harridge 2004) leading e.g. to regulation of protein synthesis (Hornberger and Esser 2004). The mechanically induced release and production of IGF-I and the IGF-I splice form MGF potentially representing an important link between contracting skeletal muscles and exercise-related metabolic changes (Goldspink 1999).

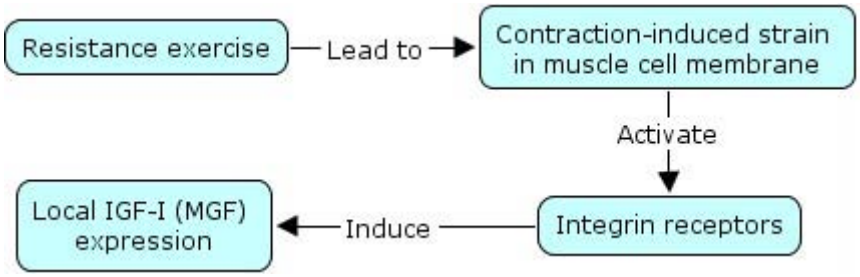


FIGURE 2 Resistance exercise and skeletal muscle IGF-I expression

### 2.3.4 Skeletal muscle IGF-I expression and resistance training

Although GH-induced IGF-I production in the liver is the major source of circulating IGF-I and mediates many GH metabolic effects, it seems that circulating levels of IGF-I are not as important for muscle growth and repair as are the isoforms of IGF-I produced by skeletal muscle, which act in an autocrine/paracrine fashion (Goldspink 1999). Three isoforms of IGF-I that are expressed and released from the overloaded human skeletal muscle cells have been identified (Hill and Goldspink 2003) (Figure 3). IGF-IEa expressed in skeletal muscle is the same as the hepatic endocrine (systemic) type of IGF-I produced by the liver. The other isoform has been called mechano growth factor (MGF or IGF-IEc) as its mRNA was only produced in response to muscular activity. The third isoform is called IGF-IEb and its role in muscle is unknown (Yang et al. 1996, McKoy et al. 1999). MGF isoform only differs from the liver isoforms by the presence of the first 49 base pairs from exon 5 and that it is apparently not glycosylated. Therefore it is expected to be smaller and have

a shorter halflife than the liver IGF-IEa. MGF is thus designed to act in an autocrine/paracrine rather than in a systemic fashion and is probable the end product of mechanotransduction signalling pathways in muscle (Hill and Goldspink 2003).

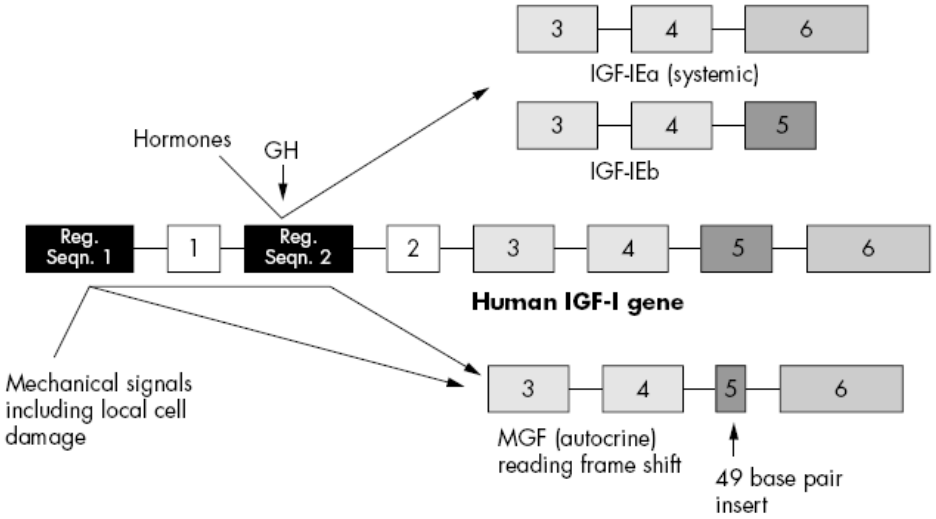


FIGURE 3 The different exons of the insulin-like growth factor gene expressed by muscle and the way they are spliced in response to hormones such growth hormone and to mechanical signals (Goldspink 2003).

IGF-I have autocrine/paracrine functions within muscle cells and its levels are upregulated in skeletal muscle undergoing regeneration (Edwall et al. 1989, Adams 1998, Goldspink 1999, Charge and Rudnicki 2004). There are many different triggers for local IGF1 expression, including androgens (Sheffield-Moore et al. 1999, Ferrando et al. 2002), mechanical load (Bamman et al. 2001) and exercise (Hameed et al. 2003). MGF appears to be particularly sensitive to mechanical signals and to muscle damage (Goldspink 1999, 2003, Bamman et al. 2001, Harridge 2003). Following mechanical stimulation or muscle damage, the IGF-I gene is first spliced to produce MGF and then later to produce the more common IGF-IEa transcript (Hill and Goldspink 2003).

The experimental manipulation of IGF-I levels in muscle in vivo can cause tremendous increases in muscle mass (e.g. Coleman et al. 1995, Musaro et al. 1999, 2001, Barton et al. 2002, Lee et al. 2004,). The anabolic actions of IGF-IEa and MGF are mediated through stimulating protein synthesis and activation, proliferation and differentiation of satellite cells (Adams 2002, Yang and Goldspink 2002, Fiorotto et al. 2003, Harridge 2003). IGF isoforms have the same 5 exons that encode the IGF-I ligand binding domain and processing of pro-peptide yields a mature peptide that is involved in upregulating protein synthesis (Yang and Goldspink 2002). However, carboxyterminal of the MGF peptide may also acts as a separate growth factor in skeletal muscle (Goldspink and Harridge 2004) and stimulate division of satellite cells (Yang and Goldspink, 2002). IGF-I may also improve muscle regeneration via promoting cell survival (Barton et al. 2002), regulate insulin metabolism (Hawke and Garry

2001) and promote reinnervation of motor neurons during muscle repair (Caroni and Grandes 1990, Vergani et al. 1998).

A number of studies by various in vivo and in vitro methods has shown that increased muscle loading can produce elevated expression of IGF-I gene products which could mediate adaptive responses leading to muscle hypertrophy due to an augmentation of muscle protein and DNA content (e.g. DeVol et al. 1990, Yan et al. 1993, Coleman et al. 1995, Adams and Haddad 1996, Adams and McCue 1998, Barton-Davies et al. 1998, Adams et al. 1999, McKoy et al. 1999, Chakravarthy et al. 2000, Bamman et al. 2001, Musaro et al. 2001, Owino et al. 2001, Hameed et al. 2003). Single resistance exercise can induce increases in mRNA expression of IGF-I and its splice variants in human skeletal muscle (Bamman et al. 2001, Hameed et al. 2003, Psilander et al. 2003, Deldicque et al. 2005) In humans long-term resistance training has been shown to increase IGF-I peptide levels (Singh et al. 1999), while IGF-I mRNA expression has bee shown to increase (Hameed et al. 2004) or not to change (Bamman et al. 2003) due to resistance training in elderly subjects. These findings suggest that skeletal muscles may adapt to long-term resistance training by altering IGF-I expression. GH treatment upregulates IGF-I gene expression and when combined with resistance exercise more is spliced towards MGF (Hameed et al. 2004). However, there are a number of reports of GH-independent expression of IGF-I mRNA in skeletal muscle. Thus it seems very clear that IGF-I gene expression can occur in muscle without stimulation by GH (Florini et al. 1996) and the muscle isoforms of IGF-I play a prominent role during tissue remodelling (Hameed et al. 2003).

The ability to produce MGF in response to a mechanical signal decreases with age (Owino et al. 2001, Hameed et al. 2003) possible due to changes in the compliance of muscle with age (Goldspink and Harridge 2004). The reduced ability of older muscles to express MGF may be a result of the age-related decrease in circulating growth hormone levels (Rudman et al. 1981, Goldspink and Harridge 2004) and the amount of the IGF-I primary transcript so there is less to be spliced towards MGF (Goldspink 2003, Hameed et al. 2003).

### 2.3.5 Satellite cell activation and resistance training

Adult skeletal muscle fibers are terminally differentiated and contain several hundred postmototic myonuclei which are therefore unable to undergo mitotic division or directly increase myonuclear number (O'Neill and Stockdale 1972, Chambers and McDermott 1996). Anabolic process is mediated by increases in muscle fibre transcriptional capacity and protein synthesis (Carson 1997), and the activity-dependent regulated assembly of newly-translated proteins into sarcomeres (De Deyne 2000, Torgan and Daniels 2001). Satellite cells are small mononucleated skeletal muscle stem cells outside the sarcolemma and under the basal lamina of the muscle fibre (Mauro 1961, Goldring et al. 2002). Satellite cells fusing with muscle fibres provide the extra nuclei to increase muscle fibre transcriptional capacity during postnatal growth (Moss and Leblond 1970, Schultz and McCormick 1994, Carson 1997, Hill and Goldspink 2003) and they

are also involved in repair and regeneration following local injury of muscle fibres (Grounds 1998, Goldring et al. 2002).

In adult skeletal muscle fibres each myonucleus controls the production of mRNA and protein synthesis over a finite volume of cytoplasm (Hall and Ralston 1989, Pavlath et al. 1989) and relationship between the size of the myofiber and the number of myonuclei present in a given myofiber is maintained, a concept known as the DNA unit or myonuclear domain (Pavlath et al. 1989, McCall et al. 1998). The requirement for additional nuclei to support processes of muscle fibre hypertrophy are acquired via the proliferation, differentiation, and finally the fusion of satellite cells or their progeny with the enlarging or repairing myofibers, providing the new myonuclei needed to produce muscle-specific proteins that increase myofiber size (Schiaffino et al. 1976, Allen et al. 1979, 1995, Rosenblatt and Parry 1992, Rosenblatt et al. 1994, Schultz and McCormick 1994).

Resistance training can result in elevated satellite cell numbers (Kadi et al. 1999a, 2004, Kadi and Thornell 2000, Roth et al. 2001) and induce a process of satellite cell activation, proliferation, chemotaxis, and fusion to existing myofibers to contribute to muscle growth (McCormick and Thomas 1992, Yan et al. 1993, Schultz and McCormick 1994, Adams et al. 1999). The process of satellite cell fusion maintains myonuclear domain size which has been shown to be related to the changes of muscle fiber area due to resistance training and detraining in young and older men and women (McCall et al. 1998, Kadi and Thornell 2000, Roth et al. 2001, Kadi et al. 2004). Contrary to muscle hypertrophy the muscle atrophy results in a reduction in myonuclei number (Grounds 1999, Kadi et al. 2004). Furthermore, sarcopenia has been associated with a decreased number of activated satellite cells which may explain the reduced capacity of age muscle to undergo continuing local cellular repair (Sadeh 1988, Chakravarthy et al. 2000, Carlson et al. 2001, Owino et al. 2001, Harridge 2003).

IGF-I is able to alter myogenic regulatory factors expression and promote both the proliferation and the differentiation/fusion of satellite cells (Florini et al. 1996, Hawke and Garry 2001, Charge and Rudnicki 2004, Machida and Booth 2004, Mourkioti and Rosenthal 2005). Thus, the hypertrophic effects of IGF-I are attributed to providing additional myonuclei in order to maintain the myonucleus to myofiber size ratios of the enlarged myofibers and to increase cytoplasmic-to-DNA volume ratio through increased protein synthesis within existing myofibers (Adams and Haddad 1996, Adams and McCue 1998, Bark et al. 1998, Barton-Davies et al. 1999, Semsarian et al. 1999). Especially MGF is suggested to have effect on initiation of satellite cell proliferation (Hill and Goldspink 2003). Thus, locally produced IGF-I and satellite cells have important role in maintenance of muscle mass, regeneration process and muscle hypertrophy (Adams and Haddad 1996, Florini et al. 1996, Vierck et al. 2000, Hawke and Garry 2001, Adams 2002, Goldspink and Harridge 2004, Machida and Booth 2004).

### 2.3.6 Signal transduction pathways and adaptations to resistance training

Resistance exercise is known to increase skeletal muscle protein synthesis up to 48 h after the completion of resistance exercise (Phillips et al. 1997, 1999, Tipton and Wolfe 1998, Hernandez et al. 2000). Increased protein synthesis immediately after the exercise indicates that existing myonuclei in muscle fibres have the ability to quickly respond to resistance training by enhanced rates of mRNA translation mediated by activation of translation initiation factors. However, long-term changes in protein synthesis are a result of an increase concentration of ribosomes available to translate mRNA which increases the capacity to synthesize protein (Kimball et al. 2002, Goldspink 2003, Bolster et al. 2004, Hornberger and Esser 2004, Kadi et al. 2005). Two major signal transduction pathways have been proposed to induce these adaptations to resistance training: the phosphoinositide-3 kinase (PI3K), protein kinase B (PKB) (or Akt) and the mammalian target of rapamycin (mTOR), i.e. PI3K-PKB-mTOR and the calcineurin/NFAT (nuclear factor of activated T cells) pathways. IGF-I has been shown to activate both these signalling pathways (Musaro and Rosenthal 2002).

Once bound to its receptor, IGF-I (and insulin) activates the intrinsic kinase activity of the receptor leading to its phosphorylation of several substrates, including members of the insulin receptor substrate (IRS) family (Kasuga et al. 1982, White et al. 1985). Phosphorylation of IRS-1 recruits another signalling molecule, the phosphatidylinositol 3-kinase (PI3K) (Backer et al. 1993). PI3K activation is central to a number of important cellular processes, including protection from apoptosis, increased translation, and alteration in intracellular calcium (Adams 2002). One downstream target of PI3K is the serine/threonine protein kinase B (PKB) (Alessi et al. 1996) which activates mTOR (mammalian target of rapamycin). Protein synthesis can be regulated by altering the activation of the translational initiation factors such as 70 kDa ribosomal S6 protein kinase (S6K1/p70S6k), eukaryotic initiation factor 4F (eIF4F) complex and eukaryotic initiation factor 2B (eIF2B) via PI3K-PKB-mTOR pathway (Baar and Esser 1999, Nader and Esser 2001, Nader et al. 2002, Bolster et al. 2003, Hornberger and Esser 2004, Rennie et al. 2004) (Figure 4). Thus, PI3K-PKB-mTOR pathway have important role in the activation of the protein synthetic machinery and it could be involved mainly in skeletal muscle growth (Pallafacchina et al. 2002).

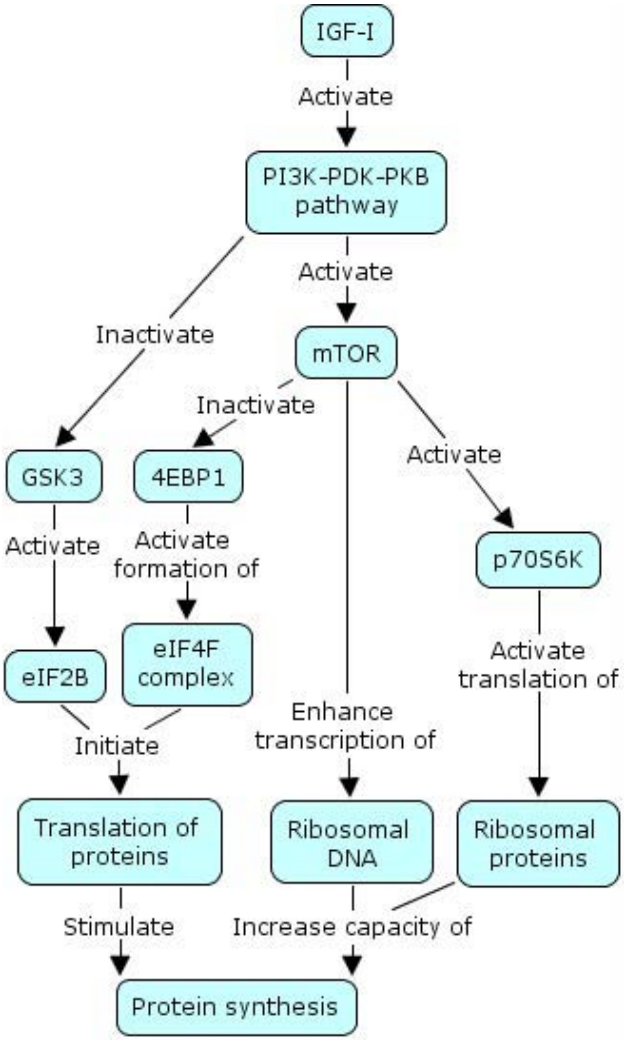


FIGURE 4 IGF-I and transduction pathways leading to increases in protein synthesis. IGF-I and its splice variants activate PI3K-PDK-PKB pathway which activate mTOR protein complex and inactivate GSK3. Consequently this leads to activation of eIF2B, eIF4F and p70S6K proteins which ultimately increase protein synthesis. (PDK, phosphatidylinositol-dependent protein kinase; PKB, protein kinase B; GSK3, glycogen synthase kinase 3; elF2B, eukaryotic initiation factor 2B; mTOR, mammalian target of rapamycin protein kinase; 4EBP1, eIF4E-binding protein 1; p70S6k, 70 kDa ribosomal protein S6 protein kinase) (Modified from Adams 2002, Bolster et al. 2004, Hornberger and Esser 2004, Deldicque et al. 2005)

Calcineurin, a Ca2+-regulated phosphatase, modulates skeletal muscle hypertrophy in response to increased neural activation (Dunn et al. 1999, Musaro et al. 1999, Semsarian et al. 1999, Schulz and Yutzey 2004). Calcineurin is activated by calmodulin that has bound calcium. Therefore, calcineurin activity is essentially controlled by changes in cytosolic calcium concentrations (Tidball 2005). The calcineurin/NFAT pathway controls muscle fibre type by regulating fibre-type-specific genes in skeletal muscle (Chin et al. 1998, Dunn et al. 1999, Delling et al. 2000, Naya et al. 2000, Serrano et al. 2001). Activation of Calcineurin in response to prolonged contractile work is crucial to signalling

the expression of slower more oxidative fibre-specific genes (Chin et al. 1998, Serrano et al. 2001). IGF-I promotes hypertrophy and modulate components of the excitation-contraction coupling mechanism through the induction of calcineurin-mediated signalling and the activation of the GATA2 transcription factor. GATA2 associates with calcineurin and NFAT activating myocyte hypertrophic gene expression program (Musaro et al. 1999, Delling et al. 2000, Michel et al. 2004, Schulz and Yutzey 2004).

Also mitogen-activated protein kinases (MAPK) ERK1/2; p38 MAPK; JNK; ERK5 are activated through external signals, such as growth hormones and cellular stresses (Long et al. 2004). Exercise leads to the activation of at least three MAPK signalling pathways, i.e. ERK1/2, p38 MAPK and JNK which mediate the mitogenic response of skeletal muscle to exercise (Aronson et al. 1997, Boppart et al. 1999, Widegren et al. 2001, Long et al. 2004). IGF-I has been shown to activate satellite cell proliferation through MAPK signalling (Cooligan et al. 1997, Mourkioti and Rosenthal 2005).

# 3 PURPOSE OF THE STUDY

The overall purpose of the present research was to obtain new information on mechanisms leading to muscle hypertrophy by studying of neuromuscular, hormonal and molecular responses to various heavy resistance exercise protocols as well as long-term systematic resistance training.

Detailed purposes of the present studies were as follows:

1) To investigate the role of exercise intensity to acute hormonal and neuromuscular responses during and after various hypertrophic resistance exercises in strength trained men compared to those in untrained men. The primary hypothesis was that greater resistance exercise intensity created by the forced repetitions produces increased acute hormonal and neuromuscular responses. (Original papers I and II)

2) To investigate hormonal adaptations and their relationships to muscle hypertrophy and strength development during long-term systematic resistance training in male strength athletes and non-athletes. Especially, the primary interest was to investigate acute and chronic responses to two hypertrophic heavy resistance protocols performed with the same overall volume of exercise; a higher intensity and longer rest periods between the sets in comparison to that of somewhat lower intensity but shorter rest periods between the sets. The major hypothesis was that changes in serum hormone concentrations during the resistance training are related to training-induced changes in muscle strength and mass. Furthermore, we hypothesized that shorter rest periods between the sets will lead to greater acute hormonal responses which are associated with greater muscle hypertrophy due to long-term resistance training than that of longer rest periods between the sets. (Original papers III and IV)

3) To investigate the effects of hypertrophic heavy resistance exercise on androgen receptor (AR), insulin-like growth factor I Ea (IGF-IEa) and mechano growth factor (MGF) mRNA expression in strength trained men.

Moreover, long-term resistance training induced changes in AR and IGF-I mRNA expression were examined in adult and elderly untrained men and compared to those in strength trained adult men. The main hypothesis was that mRNA expression of AR and IGF-I increases after resistance exercise and changes of AR and IGF-I mRNA expression due to long-term resistance training are related to changes in muscle strength and mass. (Original papers V and VI)

# 4 RESEARCH METHODS

## 4.1 Subjects

The present study included a total of 61 strength trained men (I-VI) as well as 25 younger (II, III and VI) and seven older (VI) previously untrained men volunteered to participate in this study (Table 1). Groups of untrained younger and older men served as controls to present strength trained subjects. None of the strength trained men were competitive strength athletes. Untrained younger and older subjects had no background in regular resistance training. No medication was taken by the subjects that would have been expected to affect physical performance or measured variables. Each subject was informed of the potential risks and discomforts associated with the investigation and all the subjects gave their written informed consent to participate. Medical control revealed that all the elderly subjects (VI) were healthy. The Ethics Committee of the University of Jyväskylä approved the study.

TABLE 1 Physical characteristics of the subjects.

| Original paper | Subject groups | Age (years) | Height (cm) | Weight (kg) | Body fat (%) | Strength training experience (years) |
|---|---|---|---|---|---|---|
| I | SM (n = 16) | 27 ± 4 | 180 ± 6 | 81 ± 9 | 14 ± 4 | Several years |
| II | SM (n = 8) | 27 ± 5 | 177 ± 7 | 86 ± 4 | 14 ± 3 | 9 ± 7 |
| | AM (n = 8) | 26 ± 4 | 183 ± 5 | 79 ± 7 | 13 ± 2 | |
| III | SM (n = 8) | 30 ± 7 | 177 ± 6 | 92 ± 10 | 17 ± 4 | Several years |
| | AM (n = 8) | 34 ± 4 | 177 ± 4 | 86 ± 16 | 19 ± 4 | |
| IV | SM (n = 13) | 29 ± 6 | 181 ± 6 | 84 ± 12 | 15 ± 4 | 7 ± 3 |
| V | SM (n = 8) | 29 ± 7 | 181 ± 5 | 88 ± 12 | 16 ± 3 | 6 ± 3 |
| VI | SM (n = 8) | 29 ± 7 | 183 ± 5 | 88 ± 12 | 16 ± 3 | 6 ± 3 |
| | AM (n = 9) | 42 ± 4 | 178 ± 7 | 83 ± 15 | 19 ± 4 | |
| | OM (n = 7) | 72 ± 3 | 172 ± 7 | 80 ± 10 | 24 ± 4 | |

(Abbreviations: SM = strength trained men, AM = untrained adult men, OM = older men)

## 4.2 Experimental design, measurements and analysis

The experimental designs of the present studies comprised acute heavy resistance exercises and long-term resistance training interventions to study neuromuscular, hormonal and molecular responses to strength training. Acute and chronic responses of resistance trained men were compared to those of untrained adult and older men. Subjects, experimental resistance exercises and long-term resistance training included in each original paper are briefly summarized in table 2.

TABLE 2 Summary of the experimental designs of the present studies.

| Original paper | Subjects | Acute heavy resistance exercises | Long-term resistance training |
|---|---|---|---|
| I | Strength trained men | Maximum vs. forced repetitions | (not included) |
| II | Strength trained men<br>Untrained adult men | Maximum vs. forced repetitions | (not included) |
| III | Strength trained men<br>Untrained adult men | 5 x 10RM leg presses | 6-month progressive resistance training |
| IV | Strength trained men | Shorter vs. longer rest periods between the sets | 3-month short vs. 3-month long rest periods between the sets training |
| V | Strength trained men | 5 x 10RM leg presses and 4 x 10RM squats | (not included) |
| VI | Strength trained men<br>Untrained adult men<br>Older men | (not included) | 6-month progressive resistance training |

Various types of heavy resistance exercises were performed to study acute exercise induced responses and chronic adaptations of the neuromuscular and hormonal systems as well as gene expression. The role of exercise intensity were investigated in studies I and II. Effects of length of the recovery periods between the sets were examined in study IV. The loading protocols and primary measurements performed in studies I-V are summarized in table 3.

TABLE 3 Summary of the loading protocols and measurements during and after the experimental heavy resistance exercises

| Original paper | Loading protocols | Primary measurements during and after the loading sessions |
|---|---|---|
| I | Maximum vs. forced repetition loadings:<br>4 sets of leg press, 2 sets of squats and 2 sets of knee extensions<br>12RM sets<br>Two-minute recovery between the sets<br>Recovery; 3 days after the loadings | Maximal isometric force and EMG<br>Serum total and free testosterone, cortisol and growth hormone concentrations<br>Blood lactate<br>Serum CK activity<br>Subjective muscle soreness<br>Muscle swelling |
| II | Maximum vs. forced repetition loadings:<br>4 sets of squats<br>12RM sets<br>Two-minute recovery between the sets<br>Recovery; 2 days after the loadings | Maximal isometric force and EMG<br>Serum total and free testosterone, cortisol and growth hormone concentrations<br>Blood lactate |
| III | Loading sessions before and after 21 weeks of resistance training period<br>5 sets of leg presses<br>10RM sets<br>Two-minute recovery between the sets | Maximal isometric force<br>Serum total and free testosterone, cortisol and growth hormone concentrations<br>Blood lactate |
| IV | Loading sessions before, after 3 months and after 6 months of resistance training<br><br>Shorter (SR) vs. longer rest periods between the sets (LR)<br>SR = 5 sets of leg presses and 4 sets of squats (10RM) with a 2-minute recovery between the sets<br>LR = 4 sets of leg presses and 3 sets of squats (10RM) with a 5-minute recovery between the sets<br>Recovery; 2 days after the loadings | Maximal isometric force and EMG<br>Serum total and free testosterone, cortisol and growth hormone concentrations<br>Blood lactate |
| V | 5 sets of leg presses and 4 sets of squats (10RM) with a 2-minute recovery between the sets<br>Recovery; 2 days after the loadings | AR, IGF-IEa and MGF mRNA expression<br>Maximal isometric force<br>Serum total and free testosterone concentrations<br>Serum CK activity<br>Subjective muscle soreness<br>Muscle swelling |

### 4.2.1 Familiarization session

The subjects were familiarized with the experimental testing procedures during a control day about 1 week before the actual measurements. Anthropometrical measurements and resistance load verifications for the experimental exercise were also determined for each subject at this time (I-VI). During the control day blood samples were obtained from each subject. One blood sample was drawn in the morning after twelve hours of fasting and approximately eight hours of sleep for the determination of basal serum hormone concentrations (I-VI). Two blood samples were also drawn within ½ h without exercise at the same time of day that each subject would later undertake his heavy resistance loading protocols to determination of normal diurnal variation of serum hormone concentrations (I-IV, V).

### 4.2.2 Experimental resistance exercises

The study design comprised experimental resistance exercises to determine acute exercise-induced neuromuscular, hormonal and molecular responses (I-IV, VI). The loadings were designed to be as similar as possible to be used during the experimental training periods and similar to those in normal strength training of experienced strength athletes for gains in muscle mass and strength. Untrained men served as a control for the strength trained men to investigate long-term adaptations during resistance training.

To minimize acute exercise-induced changes in the measured variables the subjects were asked to refrain from any strenuous activity at least for three days before the experimental loading session. Range of movement was controlled in each exercise. The foot positions and exercise machine settings were identical between the comparable loadings. The duration of the concentric phases of dynamic muscle actions was measured by an electronic goniometer placed on the knee joint

All the sets in every loading protocol were performed with the maximum load possible for the target repetitions. The loads were adjusted during the course of the sessions due to fatigue so that each subject would be able to perform target repetitions at each set. When necessary, the subject was assisted slightly during the last few repetitions of the set to complete the sets. The exact external force produced by the assistant during the concentric phases of the exercises was measured by electromechanical dynamometers when applied. The external force produced by the assistant was analysed and then subtracted from the total volume of the work (loads*sets*reps) to determine actual total work performed by the subject (I, II). The assistant was the same person in all measurements.

Short-term recovery of the loading sessions was examined two or three consecutive days after loadings by measuring maximal isometric force and selected muscle damage markers (I, II, IV, V). The measurements were performed at the corresponding time of the day as the subject's experimental loading session. Basal serum total and free testosterone concentrations were

drawn in the morning after twelve hours of fasting and eight hours of sleep before the exercise as well as first and second mornings after the exercise. Subjects were asked to refrain from any strenuous activity during recovery days after the loadings until all the recovery measurements were performed.

Fluid intake was limited just to moistening the mouth during the loading sessions. Subjects were encouraged to eat similar diets before the loading sessions and throughout the experimental training period, which resulted in the similar caloric and nutrient intakes.

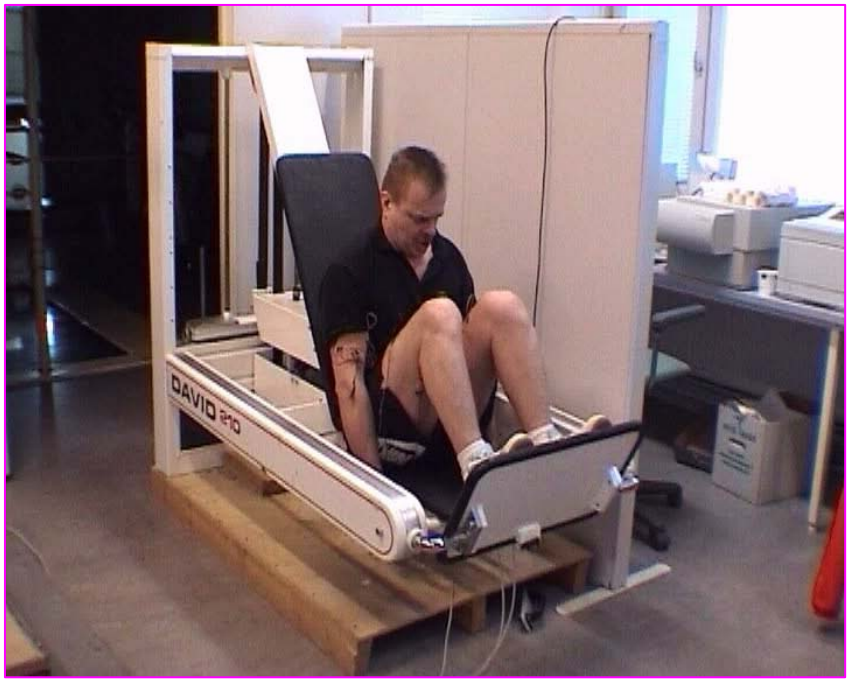


FIGURE 5 Experimental resistance exercise.

#### 4.2.2.1 Experimental loading protocols

Loading protocols (I, II): The experimental design comprised two loading sessions differed by the exercise intensity separated by two weeks performed at the same time of day. The loading protocol in study I included 4 sets of leg press (David 210) 2 sets of squats (Smith machine) and 2 sets of knee extensions (David 200) with a two-minute recovery between the sets and four minutes between the exercises. The loading protocol in study II included 4 sets of squats (Smith machine) with a two-minute recovery between the sets. The first loading session was a so-called maximum repetition (MR) protocol. All the sets were performed with the maximum load possible for 12 repetitions (12RM). The second loading session was a so-called forced repetition (FR) protocol. In FR the loading protocol was same as in MR, but the initial load was assessed higher than in MR so that the subject could lift approximately 8 repetitions by himself and 4 additional reps with assistance. The loadings were planned to be comparable so, that the total volume, as presented by multiplication of load,

sets and repetitions, in both protocols would be as identical as possible. The experimental protocol and loading sessions of study I are presented in figure 6.

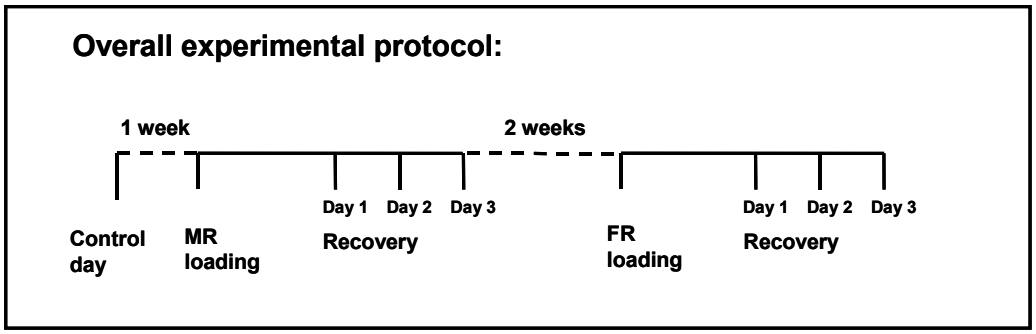


FIGURE 6 Experimental protocol and loading sessions in studies I and II.

Loading protocols (III): The experimental design comprised two acute heavy resistance loading sessions of 5 x 10RM leg presses before and after the 21 weeks resistance training period performed at the same time of day.

Loading protocols (IV): The experimental design comprised two heavy resistance loading sessions differed by the rest periods between the sets within one week: 1) lower intensity with shorter rest periods (2 min) between the sets (SR) and 2) higher intensity with longer rest periods (5 min) between the sets (LR) before the experimental resistance training period as well as after 3 and 6 months of resistance training at the same time of day (Figure 7). The first loading session was a "traditional" type of resistance exercise and included 5 sets of leg presses (David 210) and 4 sets of squats in the Smith-machine with a 2-minute recovery between the sets and 4 minutes between the exercises (SR). The second loading session was a "high intensity" type of resistance exercise. The loadings were planned to be comparable so, that the total volume, as presented by multiplication of load, sets and repetitions, in both protocols would be as identical as possible. The loading protocol was the same as in the first one, but 4 sets of leg presses and 3 sets of squats were done with a 5-minute recovery between the sets and 4 minutes between the exercises (LR). The loads in all sets were approximately 15% higher than in the SR loading. All the sets in both loading protocols were performed with the maximum load possible for 10 repetitions (10RM).

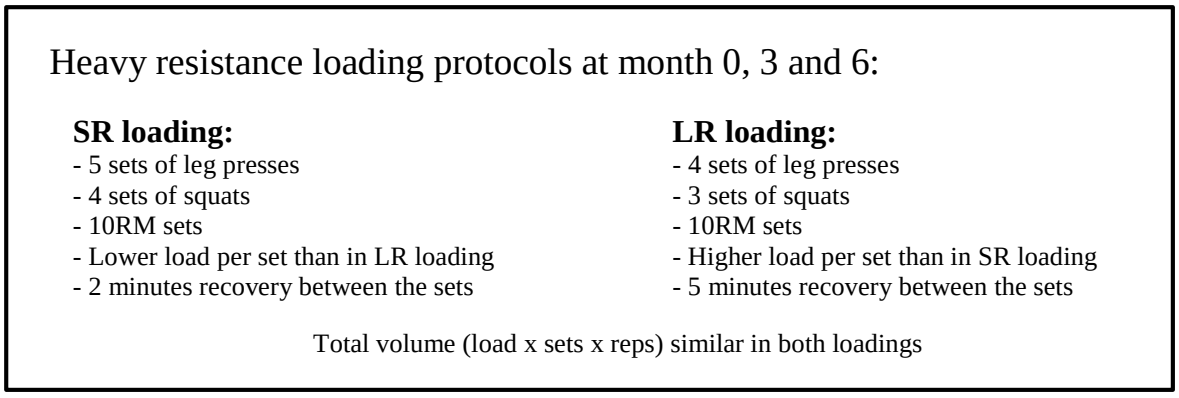


FIGURE 7 Experimental loading sessions in study IV.

Loading protocols (V): The loading protocol comprised five sets of 10 repetition maximum sets of leg presses (David 210) and four sets of squats (Smith machine) with a two-minute recovery between the sets and four minutes between the exercises.

#### 4.2.2.2 Isometric muscle strength measurements (I-VI)

An electromechanical dynamometer was used to measure maximal voluntary isometric force of the bilateral leg extension action at a knee angle of 107° before, and at certain time points during and after the loadings (Figure 8). A minimum of three trials with at least one minute intervals was completed for each subject and the best performance trial with regard to maximal peak force was used for the subsequent statistical analysis. The only exception was during the experimental resistance exercise, when the maximal isometric force was measured immediately (within 10 seconds) after the preceding exercise bout. The force signal was recorded on a computer (486 DX-100) and thereafter digitized and analyzed with a Codas TM computer system (Data Instruments, Inc.). Maximal peak force was defined as the highest value of the force (N) recorded during the bilateral isometric leg extension.

FIGURE 8 Isometric muscle strength measurement for the leg extensors.

#### 4.2.2.3 Muscle activity measurements (I, II, IV)

Electromyographic activity (EMG) was recorded from the agonist muscles vastus lateralis (VL) and vastus medialis (VM) of the right leg during the maximal isometric action. Bipolar surface electrodes (Beckman miniature-sized skin electrodes 650437, Illinois, USA) with 20 mm interelectrode distance were employed. The electrodes were placed longitudinally over the muscle belly. The motor point area was determined by an electrical stimulator (Neuroton 626) (I, II). The positions of the electrodes were marked on the skin by small ink dots to ensure the same electrode positioning in each test during the experimental period (Häkkinen and Komi 1983). EMG signals were recorded telemetrically (Glonner Biomes 2000, Munich, Germany) and stored on magnetic tape (Racall 16, Irvine, USA) and to the computer with CODAS computer system (Dataq Instruments, Inc. Akron, USA). EMG signal was amplified (by a multiplication factor of 200, low-pass cut-off frequency of 360 Hz 3dB-1) and digitized at a sampling frequency of 1000Hz. EMG was full-wave rectified, integrated (iEMG in mV*s) and time normalized. The activity (iEMG) of the VL and VM was averaged and analysed in the maximal force phase (500-1500ms) of the isometric muscle actions (Häkkinen et al. 1985a).

#### 4.2.2.4 Blood collection and analyses (I-VI).

Blood samples were drawn from the antecubital vein via multiple venipunctures for the determination of serum total and free testosterone (I-VI), cortisol (I-IV) and growth hormone (I-IV) concentrations. During the loading session blood samples were drawn before, immediately after the exercises as well as 15 and 30 minutes after the loadings (I-V). Fasting blood samples were obtained for the determination of basal hormone concentrations. Fasting blood samples were obtained after twelve hours of fasting and approximately eight hours of sleep in the morning at 7.30-8.30 (I-VI). All blood samples were obtained at the same body position of the subject. Serum samples for the hormonal analyses were kept frozen at –20°C until assayed. Serum testosterone concentrations were measured by the Chiron Diagnostics ACS:180 automated chemiluminescene system using ACS:180 analyzer (Medfield, MA, USA). The sensitivity of the testosterone assay was 0.42 nmol/l, and the intra-assay coefficient of variation was 6.7%. The concentration of serum free testosterone was measured by radioimmunoassays using kits from Diagnostic Products Corp. (Los Angeles, CA, USA). The sensitivity of the free testosterone assay was 0.52 pmol/l, and the intra-assay coefficient of variation was 3.8%. The assays of serum cortisol were carried out by radioimmunoassays using kits from Farmos Diagnostica (Turku, Finland). The sensitivity of the cortisol assay was 0.05 nmol/l, and the intra-assay coefficient of variation was 4.0%. Concentrations of growth hormone were measured using radioimmunoassay kits from Pharmacia Diagnostics (Uppsala, Sweden). The sensitivity of the GH assay was 0.2 µg/l, and the intra-assay coefficient of variation was 2.5-5%. All the assays were carried out according to the instructions of the manufactures. All samples for each test subject were analysed in the same assay for each hormone (Häkkinen et al. 2000b). Hormone concentrations were not corrected for plasma volume changes since the target tissues sense the actual molar concentrations (Kraemer et al. 1998b). Venous blood samples were also drawn for the determination of serum creatine kinase (CK) activity before as well as during recovery days after the loadings at the same time of day that each subject had done his heavy resistance loading protocols (I, V). Serum CK activity was determined using a Creatine Kinase kit (Roche, Germany). Fingertip blood samples were drawn for the determination of blood lactate (I-IV). Blood lactate concentrations were determined using a Lactate kit (Roche, Germany). Hemoglobin and hematocrit were also determined to estimate changes in plasma volume (I).

#### 4.2.2.5 Muscle biopsy procedure and PCR analysis (V, VI)

Before and 48h after the experimental resistance exercise (V) as well as before and after the 6-month resistance training period (approximately 7 days after the last training session, VI), needle biopsies (a sample size of approximately 50–100 mg) were obtained from the vastus lateralis muscle by the use of the percutaneous needle biopsy technique (Bergström 1962). The latter biopsy was

obtained from the location approximately 0.5 cm lateral to the preceding biopsy at the same depth. Local anaesthetics (2 mL lidocaine–adrenalin, 1%) were administered subcutaneously prior to incision of the skin. The muscle sample was frozen rapidly in isopentane, which was cooled to -160 °C in liquid nitrogen. The samples were stored at -80 °C until the analysis.

**RNA extraction and cDNA synthesis**. Total RNA was isolated from muscle tissue using FastPrep homogenizer and FastPrep Green tubes (Qbiogen, Carlsbad, CA, USA) and TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The RNA concentration was determined photometrically at 260 nm using an OD260 unit equivalent to 40 µg/ml. Five microgram of total RNA was reverse transcribed into cDNA for each muscle sample using the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions.

**Real-time quantitative PCR**. The mRNA content of androgen receptor (AR) (V, VI), IGF-I (design to detect and amplify the IGF-I isoforms IGF-IEa, IGF-IEb and IGF-IEc) (V), IGF-IEa (VI) , MGF (VI) and glyceraldehyde 3-phosphate dehydrogenase (GADPH) (V, VI) was determined using ABI PRISM Sequence Detector 7700 (Applied Biosystems, Foster City, CA, USA) in the University of Jyväskylä. Quantitative PCR was performed in a total reaction volume of 25 µl per sample which contained 12.5 µl SYBR green mix (QuantiTect, Qiagen, Crawley, UK), approximately 7.5 pmol of each forward and reverse primer, and 1 µl cDNA equivalent (made from 0.1 µg RNA). The primers used for real time PCR was designed and/or analysed by Oligo Explorer and Analyzer softwares (Gene Link, Inc) and these were synthesised by Oligomer Ltd. (Helsinki, Finland). The sequences of the primers for each target gene used are given in Table 4. The specificity of the amplified target sequence was confirmed on observing a single rea1ction product of right size on an agarose gel and a single peak on the DNA melting temperature curve determined at the end of the reaction. Each sample was analyzed in triplicate and the mean values were subsequently used for the analysis. The amount of specific mRNA in the sample was measured according to the corresponding gene-specific standard curve created by serial dilutions of pooled samples. The mRNA content of AR and IGF-I were normalized to the content of GADPH, which serve as an endogenous control. In previous studies the GAPDH mRNA levels in human skeletal muscle has not shown to be affected by the endurance or heavy resistance exercise (Psilander et al. 2003, Jemiolo and Trappe 2004, Mahoney et al. 2004). The intra-assay coefficient of variation (CV) was 9.4%, 7.4%, 11.0% and 9.2% for AR, IGF-IEa, MGF and GADPH (V) and 8.3%, 9.5% and 8.0% for AR, IGF-I and GADPH (VI), respectively. In our preliminary studies the threshold cycle (Ct) values were used to calculate the inter-assay CVs. Two separate runs on triplicate samples were performed for GADPH (n = 19), IGF-IEa (n=16), MGF (n=16) and AR (n = 32) with CVs of 5.6%, 2.3%, 2.7% and 4.1%, respectively.

TABLE 4 Primers used in real time PCR in studies V and VI (Hayes et al. 2001, Marcell et al. 2001, Psilander et al. 2003, Hameed et al. 2003)

| Primer name | Sequence (5′ to 3′) | Product size (bp) |
|---|---|---|
| IGF-I forward | GCTTTTGTGATTTCTTGAAGGTGA | 83 |
| IGF-I reverse | GAAGGTGAGCAGGCACAGC | - |
| AR forward | TTGTCCACCGTGTGTCTTCTTCTGC | 225 |
| AR reverse | TGCACTTCCATCCTTGAGCTTGGC | - |
| GADPH forward | GTGATGGGATTTCCATTGAT | 206 |
| GADPH reverse | GGAGTCAACGGATTTGGT | - |
| IGF-IEa forward | ATCTAAGGAGGCTGGAGATGTATTGC | 114 |
| IGF-IEa reverse | TCAAATGTACTTCCTTCTGGGTCTTG | - |
| MGF forward | CGAAGTCTCAGAGAAGGAAAGG | 150 |
| MGF reverse | ACAGGTAACTCGTGCAGAGC | |

#### 4.2.2.6 Measurements during recovery days after the loadings (I, II, IV-VI)

The rate of recovery after the acute loadings was studied at three (I) or two (II, IV-V) consequent days after the loadings. The measurements were done at the corresponding time of the day as the subject's heavy resistance loading protocols. The recordings of maximal isometric force (I, II, IV-V) and concurrent EMG (I, II, IV) evaluated the recovery of the neuromuscular performance after the loadings. Serum CK activity, subjective muscle soreness (DOMS) and muscle swelling were also determined as markers of muscle disruption possibly caused by the resistance exercises (I, V). DOMS was rated on a scale of 0 (= no pain) to 10 (= maximum pain) (I) and from 0 to 5 (V) for the overall muscle soreness of the quadriceps muscles. To examine exercise-induced muscle swelling the thickness of the vastus lateralis was measured at the middle of the thigh with a compound ultrasonic (US) scanner (Aloka SSD-280ls, Tokio, Japan) and a 5-MHz convex transducer. The US measurements were taken twice at each time point and the mean of the two measurements was used in the statistical analysis. Blood samples for the determination of basal hormone concentrations were drawn from each subject after twelve hours of fasting and approximately eight hours of sleep in the first and second mornings after the loadings.

### 4.2.3 Follow-up measurements during experimental resistance training periods

The total duration of the experimental resistance training was 21 weeks (III) or 6 months (IV, VI). The follow-up measurements were repeated during the actual experimental training period at 7-week intervals (i.e. weeks 0, 7, 14 and 21) (III) or three month intervals (0, 3-month and 6-month) (IV) or before and after a 6-month experimental resistance training period (VI) (Table 5).

TABLE 5 Summary of the study designs and measurements during the experimental resistance training periods

| Original paper | Experimental resistance training | Primary follow-up measurements during the resistance training period |
|---|---|---|
| III | 21-week resistance training period<br>Non-athletes (n = 8); 2 exercise session per week<br>Strength athletes (n = 8); 4 exercise session per week | Maximal isometric force<br>Maximal dynamic force<br>Muscle cross-sectional area<br>Serum basal total and free testosterone and cortisol concentrations |
| IV | 6-month resistance training period<br>13 strength trained men<br>3-month training period with short (2 min) rest periods between the sets and 3-month training period with long (5 min) rest periods between the sets in cross-over design<br>4 exercise session per week | Maximal isometric force and EMG<br>Maximal dynamic force<br>Muscle cross-sectional area<br>Serum basal total and free testosterone and cortisol concentrations |
| VI | 6-month resistance training period<br>Untrained adult men (n = 9); 2 exercise session per week<br>Untrained older men (n = 7); 2 exercise session per week<br>Strength trained men (n = 8); 4 exercise session per week | AR and IGF-I mRNA expression<br>Maximal isometric force<br>Muscle cross-sectional area<br>Serum basal total and free testosterone concentrations |

#### 4.2.3.1 Resistance training protocols (III-IV, VI)

Experimental resistance training in study III: Resistance training for non-athletes was carried out 2 times per week. Each training session included two exercises for the leg extensor muscles: the bilateral leg press exercise and the bilateral and/or unilateral knee extension exercise on the David 200 machine. In addition, each training session included four to five exercises for the other main muscle groups of the body. Strength athletes continued training individually as they had used to. The training performed by this group was observed by training diaries. Their resistance training typically included three training days per week. Different body part were trained on different training days with multiple exercises, repetitions were 6-12 per exercises with two to five minutes rest between the sets. Exercises for the leg extensors included typically squat, leg presses and knee extension.

Experimental resistance training in study IV: Experimental training period was 6 months, which comprised two different kinds of 3-month training periods The subjects were randomly divided to two training groups. Group I (n=5) trained the first three months training period with shorter rest between the sets (2 minutes) and multiple sets (i.e. traditional resistance training) followed by a 3-month experimental resistance training period with longer rest between the sets (5 minutes) and fewer sets (i.e. “high intensity” resistance

training) (SR training) and the second training period as higher intensity with longer rest periods between the sets training (LR training). Group II (n=8) performed the experimental training periods using the opposite order. The resistance training sessions were carried out approximately 4 times per week. Different body parts were trained on different training days with multiple exercises and sets with 8-12 repetitions per sets. The training load of the exercises was increased progressively by trying to increase the load for every exercise session. Exercises for the leg extensors were carried out once per week and included typically squat, leg presses and knee extension exercises. The subjects performed their resistance training for every muscle group with the same training protocol according to the training period. The training performed by the subjects was controlled by training diaries and especially leg training was partly supervised. The experimental design of study IV is presented in figure 9.

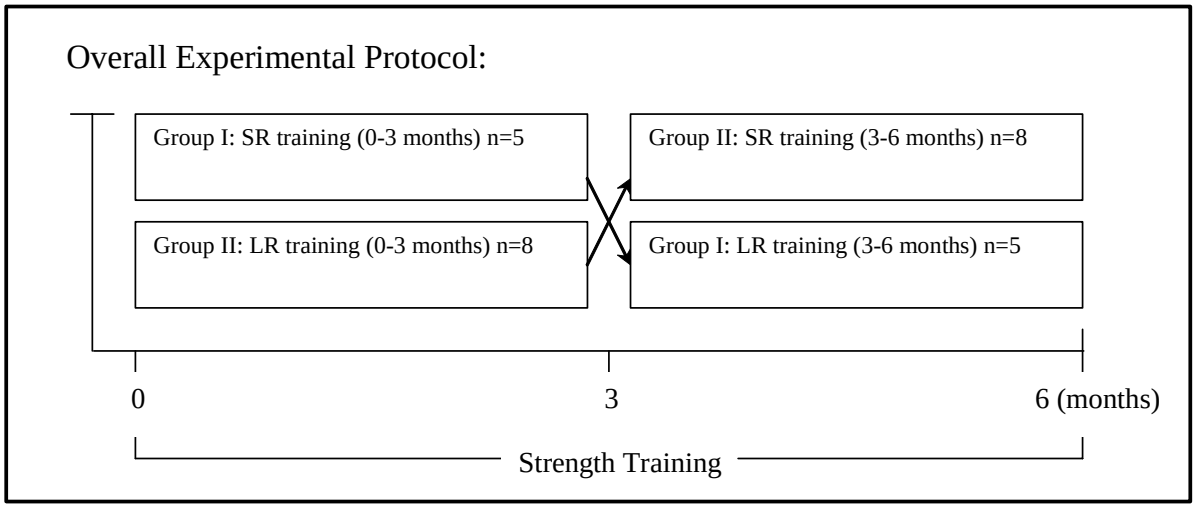


FIGURE 9 Experimental design of study IV.

Experimental resistance training in study VI: Untrained adult (AM) and older men (OM) as well as strength trained men (SM) carried out their resistance training programs designed for their own requirements and starting levels. However, the objectives in all groups were to increase muscle mass and strength extensively throughout the 6-month experimental training period. The supervised resistance training for AM and OM was carried out 2 times per week. Each training session included two exercises for the leg extensor muscles and four to five other exercises for the other main muscle groups of the body. The loads increased progressively throughout the study and a part (20%) of the leg extensor exercises were executing as "explosive"- resistance training. The training performed by SM was partly supervised and controlled by the training diaries. Their resistance training included four training days per week. The training load of the exercises was increased progressively throughout the study by trying to increase the load for every exercise session. Different body parts were trained on different training days with multiple exercises (3-4 sets per

exercise) and repetitions were 6-12 per exercises with two to five minutes rest between the sets. Leg muscles were trained once per week and exercises for the leg extensors included squat, leg press and knee extension.

#### 4.2.3.2 Anthropometry (I-VI)

The percentage of body fat was estimated by measuring skin-fold thickness at four different sites according to Durnin and Rahaman (1967) during the control day as well as after the experimental resistance training period (III-V). The thickness of the m. vastus lateralis was measured by ultrasound (SSD 280ls, Aloka, Japan) from the level of 50% of the thigh length (II).

#### 4.2.3.3 Dynamic muscle strength measurements (III-IV)

A David 210 dynamometer (David Fitness and Medical Ltd. Finland) was used to measure maximal unilateral concentric force production of the leg extensors (hip, knee and ankle extensors) (Häkkinen et al. 1998). The subject was in a seated position so that the hip angle was 110 degrees. On verbal command the subject performed a concentric right leg extension starting from a flexed position of 70 degrees trying to reach a full extension of 180 degrees against the resistance determined by the loads (kg) chosen on the weight stack. In the testing of the maximal load, separate 1 RM (repetition maximum) contractions were performed. After each repetition the load was increased until the subject was unable to extend the legs to the required full extension position. The last acceptable extension with the highest possible load was determined as 1 RM. This dynamic testing action was used in addition to that of the isometric one, since the strength training was also dynamic in nature.

#### 4.2.3.4 Muscle cross-sectional area (III-IV, VI)

The muscle cross-sectional area of the right quadriceps femoris was assessed before and after the experimental training periods using magnetic resonance imaging (MRI) (1.5-Tesla, Gyroscan S15, Philips, Best, The Netherlands) at the Keski-Suomen Magneettikuvaus Ltd., Jyväskylä, Finland. The length of the femur (Lf), taken as the distance from the bottom of the lateral femoral condyle to the lower corner of the femur head, was measured on a coronal plane. Subsequently, fifteen axial scans of the thigh interspaced by a distance of 1/15 Lf were obtained from the level of 1/15 Lf to 15/15 Lf as described previously (Häkkinen et al. 2001). Great care was taken to reproduce the same, individual femur length each time using the appropriate anatomical landmarks. All MR images were then ported to a Macintosh computer for the calculation of muscle CSA. For each axial scan, CSA computation was carried out on the quadriceps femoris as a whole and for the final calculation of the CSA, slices 5/15-12/15 (III) and slices 6/15-11/15 (IV) were used (slice 5 being loser to the knee joint of the thigh). Cross-sectional area (measured as cm2) was determined by tracing manually along the border of the quadriceps femoris. Muscle CSA is

represented as mean of the values from 5/15 to 12/15 Lf (III) and from 6/15 to 11/15 Lf (IV). In strength trained men (VI) the muscle CSA was assessed using MRI as previously described in study IV. Muscle CSA was assessed from untrained adult and older men with a compound ultrasonic scanner (SSD-190 Aloka Fansonic, Tokyo, Japan) and a 5-MHz convex transducer (VI). Two consecutive measurements were taken and then averaged for further analyses. The different methods used to measure muscle CSA were related to the fact that the data were pooled from two separate research projects. However, several previous studies have shown that US and MRI are comparable methods to measure the CSA of thigh muscles (Walton et al. 1997, Bemben 2002). Although MRI may be a more accurate method, the US is also valid and reliable for assessing changes in muscle CSA in response to resistance training (Reeves et al. 2004).

#### 4.2.3.5 Dietary analysis (IV)

Dietary intake was obtained from a food diary and analysed (Nutrica 3.11, Kansaneläkelaitos 1999, Helsinki, Finland) during a three-day period before the heavy resistance loading sessions. Subjects were encouraged to eat similar diets, which resulted in the similar caloric and nutrient intakes throughout the experimental training period.

## 4.3 Statistical methods

Standard statistical methods were used for the calculation of means, standard deviations (SD), standard errors (SE) and Pearson bivariate correlation coefficients (I-VI). The changes in the variables over time from the pre-level were analysed using general linear model (GLM) analysis of variance with repeated measures (SPSS Inc. Chigaco, IL, USA) and utilizing dependent samples of t-tests (I-V) and Wilcoxon signed ranks test (VI) when appropriate. Differences between the experimental groups within each time point were analysed utilizing independent –samples of t-tests (II, III) and Mann-Whitney U test (VI). The $p<0.05$ criterion was used for establishing statistical significance.

# 5 RESULTS

The main findings of the present series of studies are presented below. For more details the original articles (I-VI) should be consulted.

## 5.1 Role of exercise intensity for acute neuromuscular and hormonal responses in strength athletes and non-athletes (I, II)

### 5.1.1 Loads

According to the design of studies I and II, the average load was significantly higher (12-30%, $p<0.001$) in all forced repetition (FR) sets than in maximum repetition (MR) sets (Figure 10). In general, assistance was given for 4.3 to 6.0 repetitions of the 12 repetitions sets in the FR loading. The total volume of the work (loads*sets*reps) was greater in the FR than in the MR loading ($p<0.01$-0.001). However, when the amount of external force produced by the assistant during the concentric phases of the FR repetitions was taken into account, the actual total volume of FR did not differ statistically significantly from that of MR.

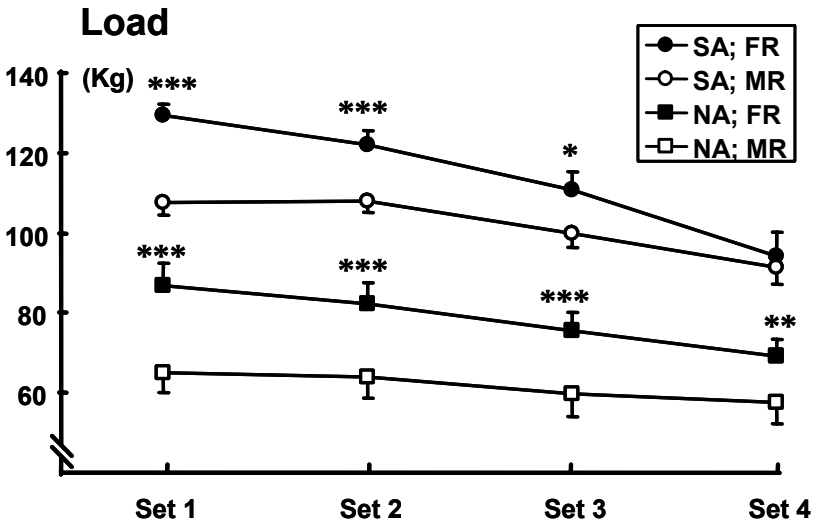


FIGURE 10 The loads in the forced repetitions (FR) and maximum repetitions (MR) squat sets (mean ± SE) in strength athletes (SA) and non-athletes (NA) (II). Statistically significant difference (* = $p < 0.05$, ** = $p < 0.01$ *** = $p < 0.001$) between the MR and FR loadings of the experimental group.

### 5.1.2 Acute neuromuscular responses

#### 5.1.2.1 Isometric force

Significant decreases (32-57%, $p<0.001$) occurred in maximal isometric force in both FR and MR loading protocols (I, II) (Figure 11) in SA and NA (II). The decreases in isomeric force were greater ($p<0.05$-$0.001$) during the FR than during the MR loadings (I, II) in both SA and NA groups (II). The decrease in isometric force remained lowered ($p<0.05$-$0.01$) for 24-72h after the loading as compared to the pre-level.

#### 5.1.2.2 EMG activity

Significant decreases ($p<0.05$-$0.001$) occured in the maximum integrated EMG of the isometric action after both loading protocols in both groups, except in the MR loading in study I (Figure 11). The changes in maximal isometric force and the changes in EMG correlated with each other after the entire FR loading ($r = 0.51$, $p<0.05$) (I). The EMG was significantly lowered ($p<0.05$-$0.01$) in FR compared to MR after the second and fourth leg press sets and after the squat sets (I). There were no statistically significant alterations in EMG activity during the recovery as compared to the pre-level or between the loadings.

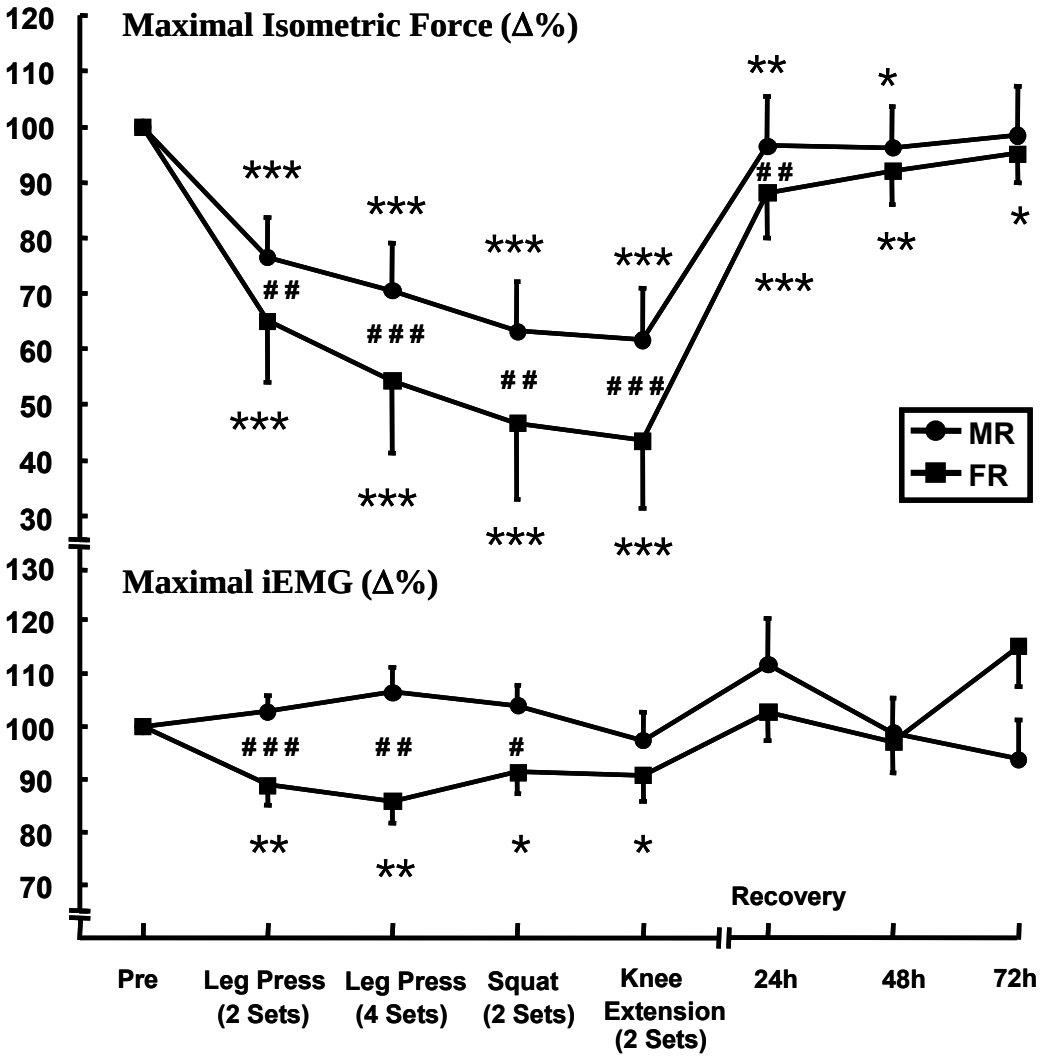


FIGURE 11 Maximal voluntary isometric leg extension force and maximal integrated EMG activity (mean ± SE) in strength trained men during and after the maximum (MR) vs. forced repetiton (FR) loadings (% from pre-loading value). Significantly different (* = $p < 0.05$ ** = $p < 0.01$ *** = $p < 0.001$) from corresponding pre-exercise value. Statistically significant difference (# = $p < 0.05$ ## = $p < 0.01$ ### = $p < 0.001$) between the MR vs. FR loadings. (I)

#### 5.1.2.3 Blood lactate

The blood lactate concentration increased up to 12.7-16.3 mmol/l ($p<0.001$) during the loading protocols. There were no significant differences between the loadings (I, II) or between the study groups (II).

### 5.1.3 Acute hormonal responses

#### 5.1.3.1 Control samples

There were no significant differences in the concentrations of serum hormones examined between the two control blood samples drawn during the control day with no exercise.

#### 5.1.3.2 Exercise samples

Serum testosterone concentrations increased significantly ($p<0.05$-$0.001$) during both loading protocols (I, II). There were no differences observed between the loading protocols, but the increase in serum testosterone concentrations in SA were greater ($p<0.01$) than in NA during both the MR and FR loadings (II) (Figure 12). Serum free testosterone concentrations increased significantly ($p<0.01$-$0.001$) during the loadings with no significant differences between the MR and FR protocols (I, II). The changes in SA during the FR loading were greater ($p<0.05$) than in NA during the MR loading (II). After the MR loading serum testosterone concentrations decreased significantly below the pre-level ($p<0.05$, post 30 minutes) (I) but total and free testosterone response remained increased throughout the recovery of 30 minutes after the FR loading in SA (II). The changes in EMG (post) and the changes in serum free testosterone concentrations correlated with each other in SA during the MR loading ($r = -.93$, $p<0.01$) (II).

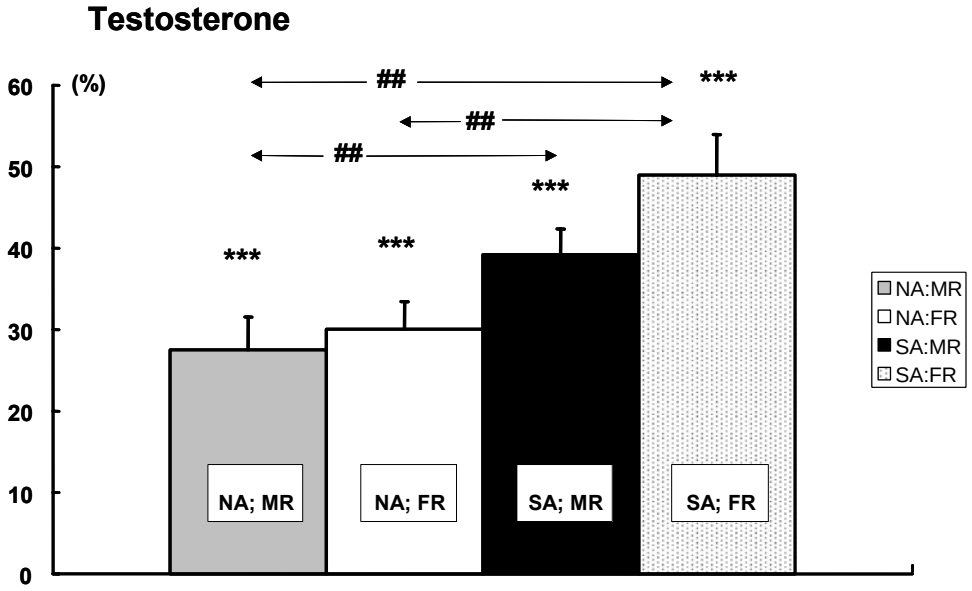


FIGURE 12 Relative changes in serum testosterone concentrations (mean ± SE) before and immediately after the maximum (MR) and forced repetition (FR) loadings in strength athletes (SA) and non-athletes (NA). Statistically significant difference (*** = $p < 0.001$) from the corresponding pre-exercise value. Statistically significant difference (## = $p < 0.01$) between the loading protocols and experimental groups. (II)

Serum cortisol concentrations increased significantly (p<0.01-0.001) during both loading protocols in both groups. The acute responses in cortisol (p<0.05) were larger during the FR than MR loading (I) and during the FR than MR loading in strength athletes (II). The changes in maximal isometric force and the changes in serum cortisol concentrations correlated with each other after the entire FR loading (r = -.55, p<0.05) (I).

Serum GH concentrations increased significantly (p<0.001) during THE MR and FR loadings. The relative changes in GH concentrations were greater (p<0.05-0.01) in FR than in MR (I) (Figure 13). The changes in blood lactate and the changes in serum GH concentrations correlated with each other in both MR (r =0.66, p<0.01) and FR (r =0.55, p<0.05) loadings after the two sets of knee extensions (I). In SA the changes in isometric force (post) and the changes in serum GH concentrations correlated with each other during the FR loading (r =-0.71, p<0.05) (II). The changes in EMG (post) and the changes in serum GH concentrations (post 15min) correlated with each other in NA during the FR loading (r =-0.73, p<0.05) (II).

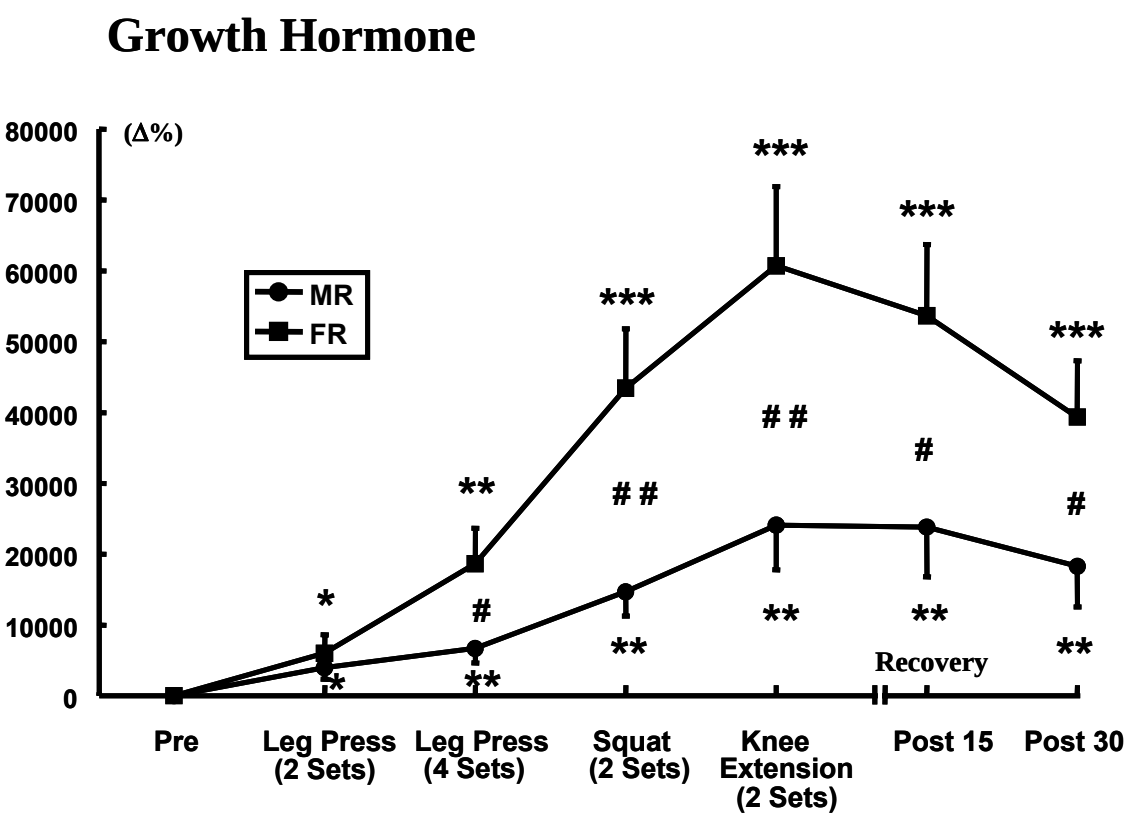


FIGURE 13 The relative changes in serum GH concentrations (mean ± SE) during the MR vs. FR loadings in strength trained men. Significantly different (* = p < 0.05 ** = p < 0.01 *** = p < 0.001) from corresponding pre-exercise value. Statistically significant difference (# = p < 0.05 ## = p < 0.01) between the MR vs. FR loadings. (I)

#### 5.1.3.3 Basal hormone concentrations during recovery

Basal hormone concentrations were unaltered compared to the control day morning values after the loadings (I, II), except for the increased free testosterone values at 48 hours after the MR ($p<0.01$) and FR ($p<0.05$) loading protocols (I).

## 5.2 Neuromuscular and hormonal adaptations to long-term resistance training in strength trained and untrained men (III, IV)

### 5.2.1 Follow-up measurements

#### 5.2.1.1 Maximal isometric force

Before the training period maximal voluntary isomeric leg extension force was greater ($p<0.001$) in SA than in NA. During the 21-week training period significant increases of 21 ±22% ($p<0.05$) and 4 ±10% (ns.) were recorded in the NA and SA groups, respectively (Figure 14) (III).

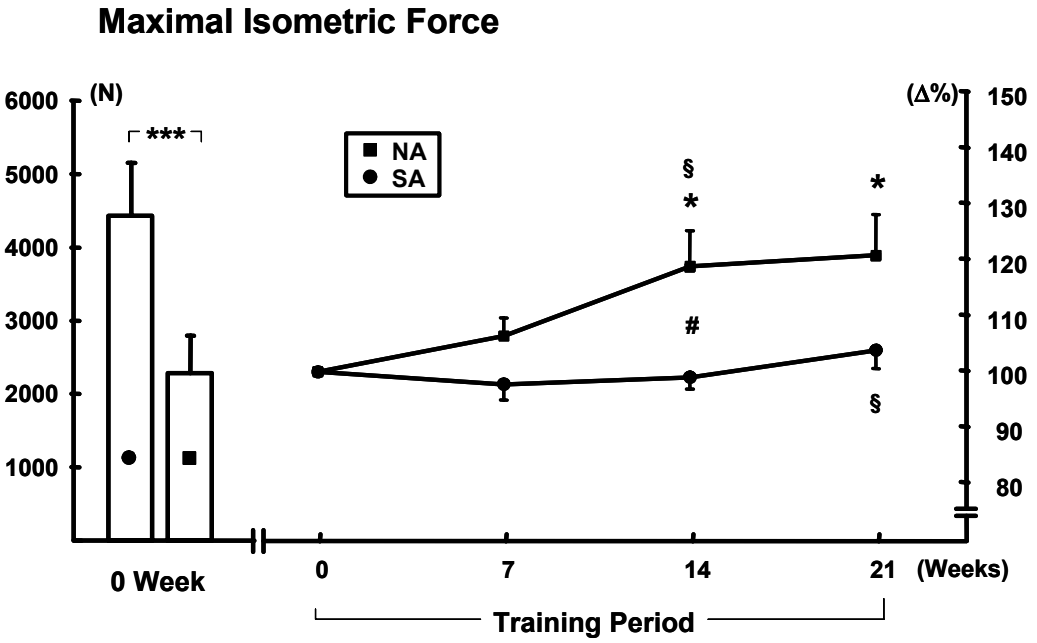


FIGURE 14 The relative changes (mean ± SE) in maximal isometric force during the 21-week resistance training period. Significantly different (* = $p < 0.05$) from the corresponding pre-training value. Statistically significant difference (# = $p < 0.05$) between the groups. Statistically significant difference (§ = $p < 0.05$) from the preceding value. (NA = non-athletes, SA = strength athletes) (III)

During the 3-month short rest (SR) training period maximal isometric force increased by 2.0 ±10.9% (ns.) and during the 3-month long rest (LR) training period by 5.8 ±8.0% ($p<0.05$) with no significant differences between the training protocols (IV) (Figure 15). During the total 6-month training period a significant increase of 6.8 ±8.7% ($p<0.05$) was recorded in maximal isometric leg extension force in the total group of subjects (IV).

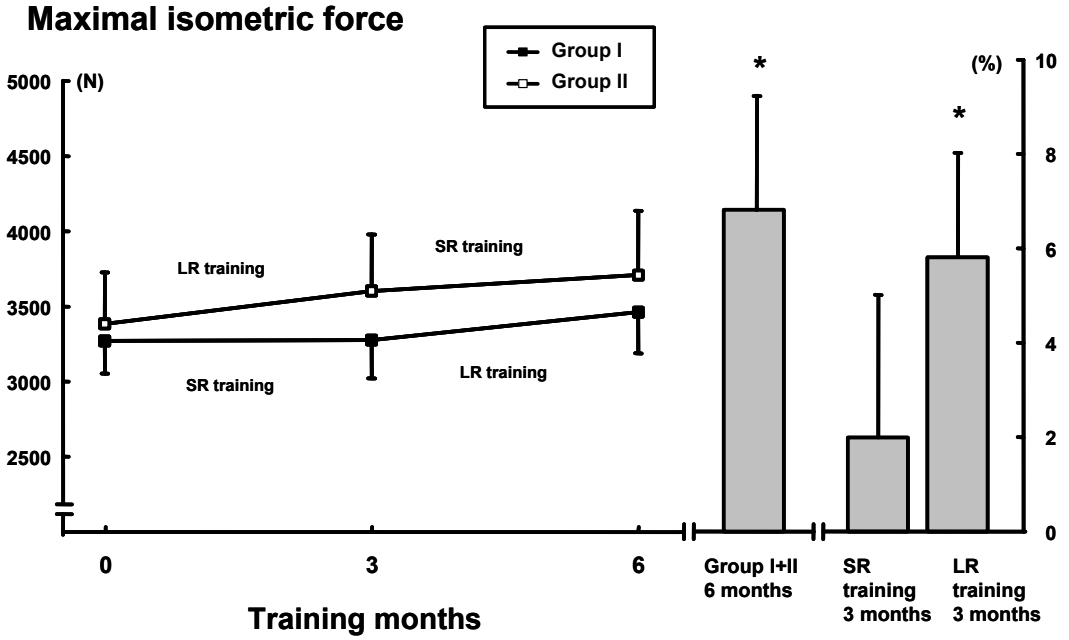


FIGURE 15 Changes (mean ± SE) in maximal isometric force during the experimental six-month resistance training period in both experimental groups and the relative changes (mean ± SD) after the short rest (SR) and long rest (LR) training periods. Significantly different (* = $p < 0.05$) from the corresponding pre-training value. (IV)

### 5.2.1.2 Maximal dynamic force

SA showed larger ($p<0.001$) unilateral right leg extension strength (1RM) than NA before the training. During the training period significant increases of 19% ($p<0.001$) and 7% ($p<0.05$) took place in the 1RM load in the NA and SA groups, respectively (III).

During the 3-month SR training period 1 RM leg extension increased by 8.4 ±13.9% ($p<0.05$) and during the LR by 7.7 ±6.4% ($p<0.001$) with no significant differences between the training protocols (IV). During the total 6-month training period a significant increase of 16.4 ±13.3% ($p<0.01$) took place in the 1RM load in the total group of subjects (IV).

### 5.2.1.3 Muscle cross-sectional area

SA showed larger ($p<0.001$) muscle cross-sectional area (CSA) than NA before the training period. The CSA increased in NA ($p<0.05$-$0.001$) but no increases

occurred in SA during the 21-week training period (Figure 16) (III). Right leg 1RM to muscle CSA ratio increased after the 21-week training period by 8.2 ±6.1% ($p<0.05$) in SA and 10.6 ±6.5% ($p<0.01$) in NA. Maximal isometric force to muscle CSA ratios were greater in SA than in NA before ($p<0.01$) and after ($p<0.001$) the resistance training. In NA the maximal isometric force and right leg 1RM as well as maximal isometric force and muscle CSA correlated with each other ($r = 0.82$, $p<0.05$) and ($r = 0.84$, $p<0.05$), respectively (III).

Muscle CSA increased by 1.8 ±4.7% (ns.) and 1.8 ±3.6% (ns.) during the 3-month SR and LR training periods, respectively (IV). The CSA of the quadriceps femoris increased by 3.5 ±4.3% ($p<0.05$) during the total experimental 6-month training period in the total group of subjects. The relative changes in maximal isometric force and the relative changes in muscle CSA correlated with each other ($r = 0.69$, $p<0.05$) during the experimental 6-month training period in the total group of subjects (IV).

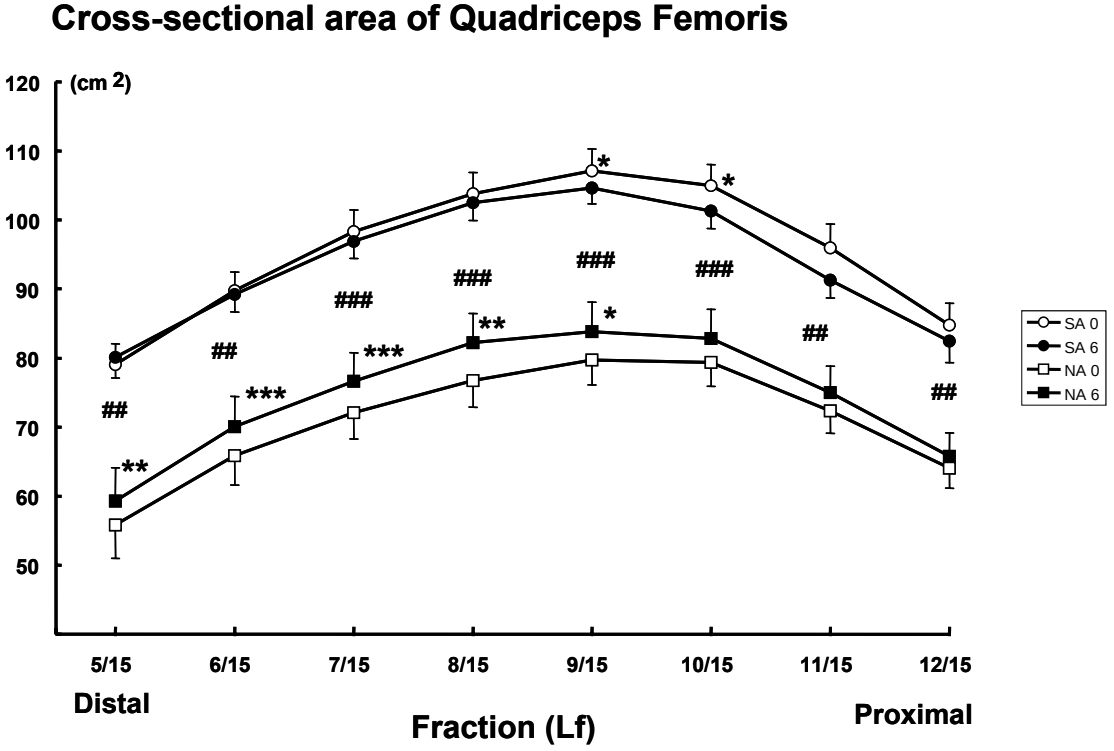


FIGURE 16 A cross-sectional area of the quadriceps femoris (mean ± SE) from 5/15 to 12/15 Lfs before and after the 6-month resistance training period. Significantly different (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$) from the corresponding pre-training value. Statistically significant difference (## = $p < 0.01$, ### = $p < 0.001$) between the groups. (n = 7 in NA and 7 in SA) (NA = non-athletes, SA = strength athletes) (III)

### 5.2.1.4 Basal hormone concentrations

There were no significant changes in serum basal testosterone, free testosterone or cortisol concentrations or total or free testosterone to cortisol ratio during the

total experimental resistance training period (III, IV) in either SA or NA groups (III).

In SA serum basal testosterone and free testosterone concentrations increased ($p<0.01$) throughout the first 14 weeks and decreased ($p<0.01$) during the last training cycle between weeks 14 and 21 (Figure 17) (III). In SA the changes in maximal isometric force after the 21-week training period correlated with the mean (averaged for the 0, 7, 14 and 21 weeks) serum basal total testosterone concentration ($r = 0.84$, $p<0.01$) and total testosterone to cortisol ratio ($r = 0.88$, $p<0.01$). Also in SA the mean (averaged for the 0, 7, 14 and 21 weeks) serum basal free testosterone concentration correlated with maximal isometric values before ($r = 0.76$, $p<0.05$) and after ($r = 0.82$, $p<0.05$) the 21-week training period (III).

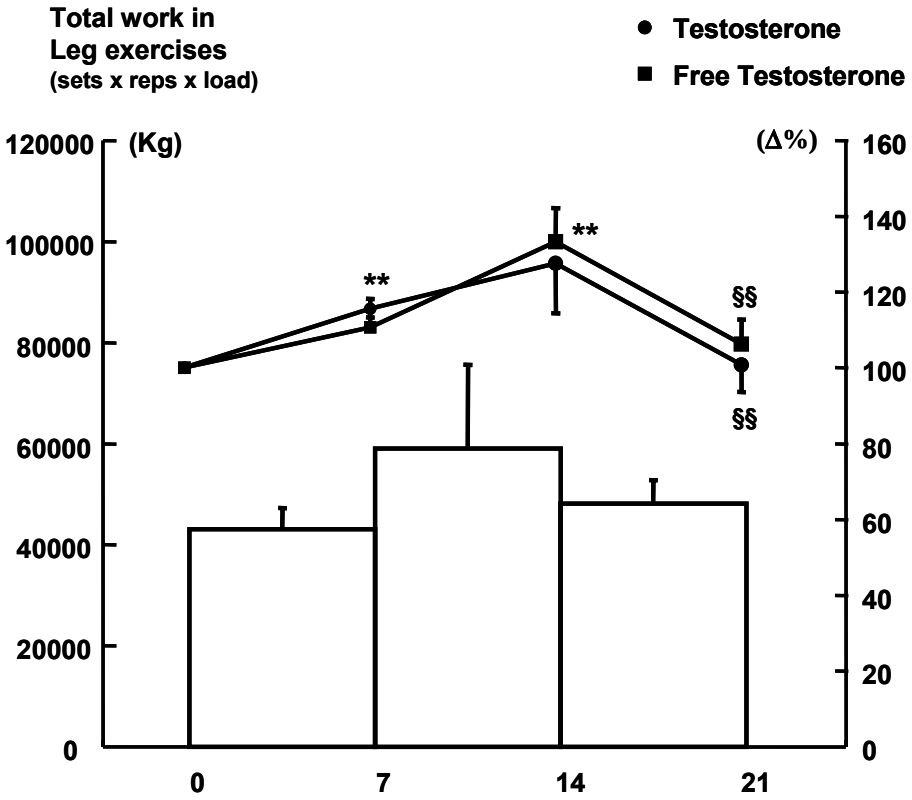


FIGURE 17 The relative changes (mean ± SE) in serum basal testosterone and free testosterone (lines, Δ%) and total work in leg exercises (sets x reps x load) (bars, kg) during the 21-week resistance training period in strength atheletes. Significantly different (** = $p < 0.01$) from corresponding the pre-training value. Statistically significant difference (§§ = $p < 0.01$) from the preceding value. (n = 7) (III)

## 5.3 Heavy resistance loadings before and after experimental resistance training period in strength trained and untrained men

### 5.3.1 Loads and neuromuscular responses

The total volume of the work (loads*sets*reps) in acute heavy resistance exercise increased significantly in NA ($p<0.001$) but not in SA during the training period (III). The total volume of work (loads*sets*reps) was 7.5 ±3.5% ($p<0.001$) greater in the SR than in the LR loading before the training period and increased ($p<0.01$) both in the SR and LR loadings after the six-month resistance training (IV).

Significant acute decreases ($p<0.001$) occured in maximal isometric force due to resistance exercise before and after the experimental resistance training period in both NA and SA groups. No significant differences were observed in the blood lactate concentrations between the experimental groups due to resistance loadings before or after the resistance training period (III).

Significant acute decreases occurred both in SR and LR loadings in the maximal bilateral isometric leg extension force ($p<0.001$) and EMG activity ($p<0.05$) as well as increases in blood lactate concentrations ($p<0.001$) before and after the 6-month experimental traing period. No statistically significant differences were observed between the SR and LR loading sessions (IV).

### 5.3.2 Acute hormonal responses

No significant changes were observed in serum hormone concentrations between the two control blood samples drawn within ½ h without exercise during the control day before the training period (III; IV).

Serum GH (Figure 18), testosterone, free testosterone and cortisol concentrations increased ($p<0.05$-$0.001$) after the loadings both before and after the training period (III). The mean of the acute GH responses (averaged for the 0 and 21 wk) was greater ($p<0.05$, post 15min.) in SA than in NA. The difference in acute total testosterone responses between the heavy resistance loadings before and after training period and changes in muscle CSA correlated with each other ($r = 0.76$, $p<0.05$) in NA (Figure 19) (III).

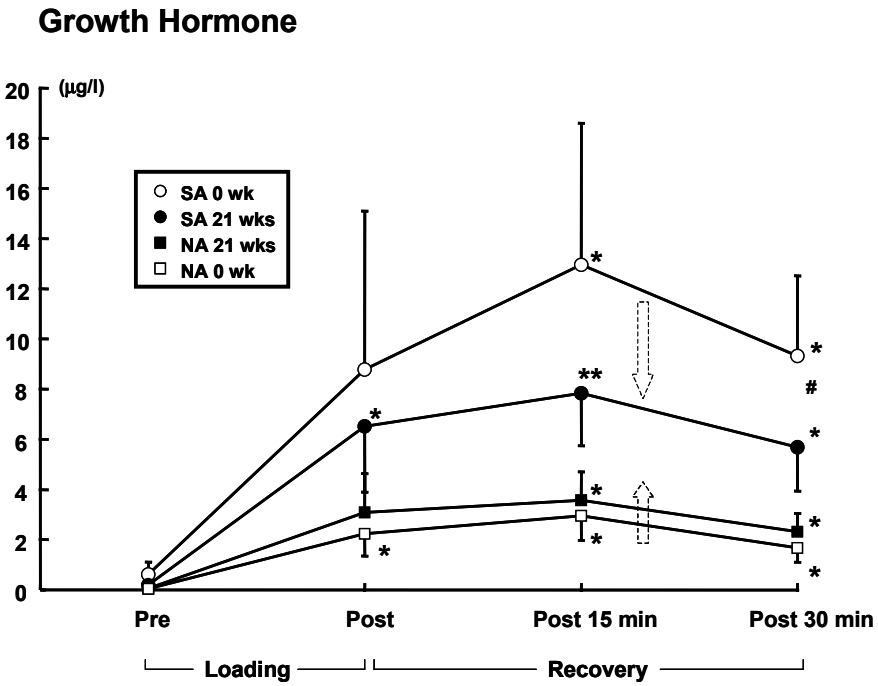


FIGURE 18 Serum GH concentrations (mean ± SE) during the heavy resistance loadings before and after the 21-week resistance training period. Arrows represent the direction of changes in acute GH response due to resistance training. Significantly different (* = $p < 0.05$, ** = $p < 0.01$) from the corresponding pre-exercise value. Statistically significant difference (# = $p < 0.05$) between the groups. (NA = non-athletes, SA = strength athletes) (III)

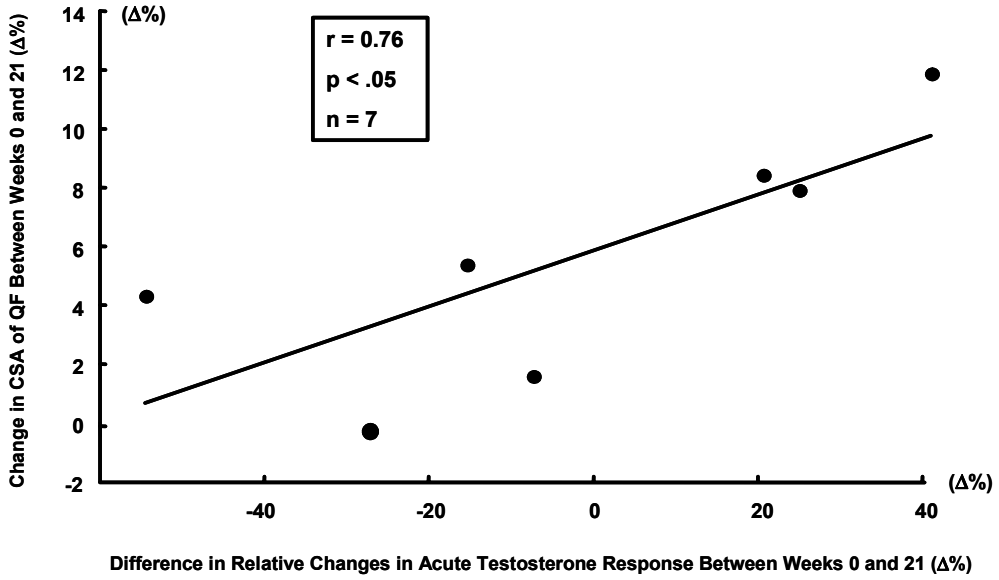


FIGURE 19 A correlation between the relative acute changes in serum testosterone responses between the heavy resistance loadings performed before and after the 21-week resistance training period and the relative changes in CSA of the QF (mean of 5/15 to 12/15) after the 21-week training period in non-athletes ($r = 0.76$, $p<0.05$, $n=7$) (III)

Serum GH (Figure 20), testosterone, free testosterone and cortisol concentrations increased ($p<0.05$-$0.001$) after the loadings both before and after the experimental training period, except for testosterone concentrations in the LR loading at month 6 (IV). No statistically significant differences were observed in the acute hormone responses between the SR and LR loading sessions. The relative changes in the integrated area under the curve (AUC) analysis in acute testosterone and free testosterone responses in the SR loadings during the first 3-month training period and the changes in muscle CSA of the quadriceps femoris correlated with each other ($r = 0.63$, $p<0.05$ and 0.74, $p<0.01$, respectively) in the total group of subjects. A trend towards attenuated hormone responses was observed independently of the training type during the 6-month experimental training period especially in the LR protocol (IV).

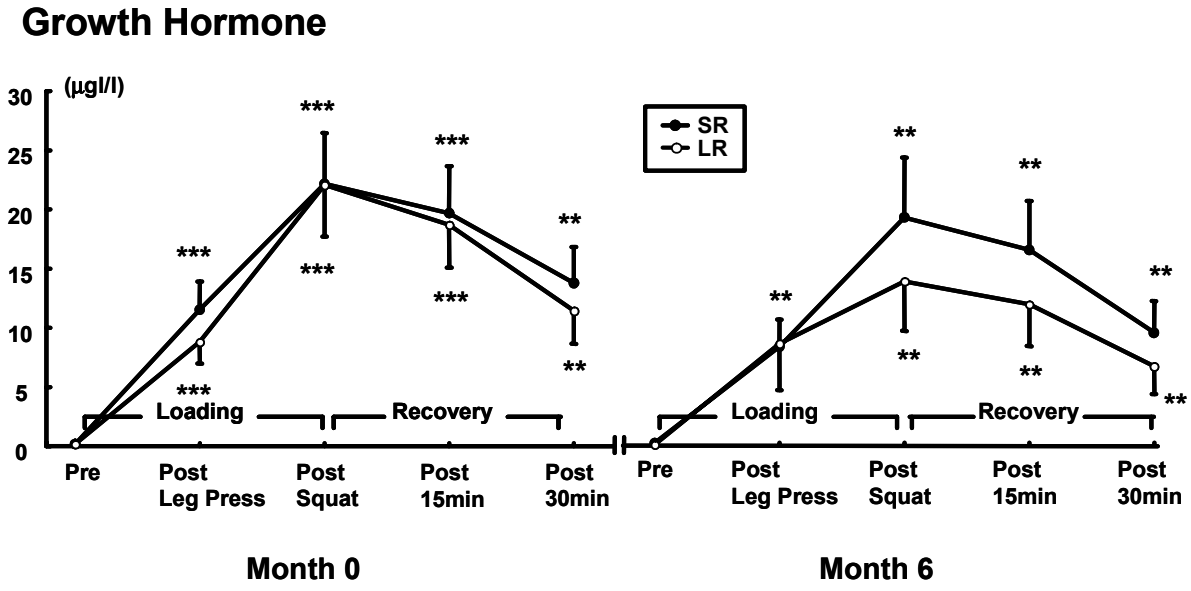


FIGURE 20 Serum GH concentrations (mean ± SE) before, during and after the short (SR) and long rest (LR) periods between the sets heavy resistance loadings before and after the 6-month experimental resistance training period in strength trained men. Significantly different (** = $p < 0.01$, *** = $p < 0.001$) from the corresponding pre-exercise value. (IV)

## 5.4 Androgen receptor and IGF-I mRNA responses to resistance training (V, VI)

### 5.4.1 Resistance exercise and mRNA expression of AR, IGF-IEa and MGF (V)

Maximal bilateral isometric leg extension force decreased during the entire course of the exercise session down to 60 ± 12% (from 3505 ± 568 N to 2087 ± 389 N, $p<0.001$). The maximal isometric force was still 8 ± 10% lowered ($p<0.01$) on the second day after the exercise as compared to the pre-level.

Serum CK-activity increased from 90 ± 34 IU/l up to 238 ± 111 IU/l ($p<0.01$) and 185 ± 91 IU/l ($p<0.05$) at 24h and 48h after the exercise, respectively. Subjective muscle soreness (0 = "no pain" to 5 = "maximum pain") increased from 0.1 ± 0.4 up to 3.3 ± 0.7 ($p<0.001$) and 3.6 ± 0.9 ($p<0.001$) at 24h and 48h after the exercise, respectively. VL thickness increased from 2.9 ± 0.3 cm up to 3.1 ± 0.3 cm ($p<0.001$) and 3.1 ± 0.4 cm ($p<0.01$) at 24h and 48h after the exercise, respectively.

Serum testosterone concentrations increased after the entire course of the exercise from 15.3 ±3.2 nmol/l up to 19.2 ±4.5 nmol/l ($p<0.01$). Serum free testosterone concentrations increased after the entire course of the exercise from 50.6 ±9.1 pmol/l up to 69.4 ±17.9 pmol/l ($p<0.01$). Basal total and free testosterone concentrations remain unaltered compared to the control day values.

The relative change of -33 ± 28% (ns.) was observed in the mean expression of AR mRNA at 48h after the exercise as compared to pre-exercise level (Figure 21). Subject number 6 was excluded from the analysis due to exceptionally high exercise-induced increase in AR mRNA levels (>100 fold) as compared to other subjects. An increased expression of 68 ±11 % ($p<0.001$) and 210 ± 62 % ($p<0.01$) was observed in IGF-IEa (Figure 22) and MGF mRNA expression after the exercise, respectively (Figure 23).

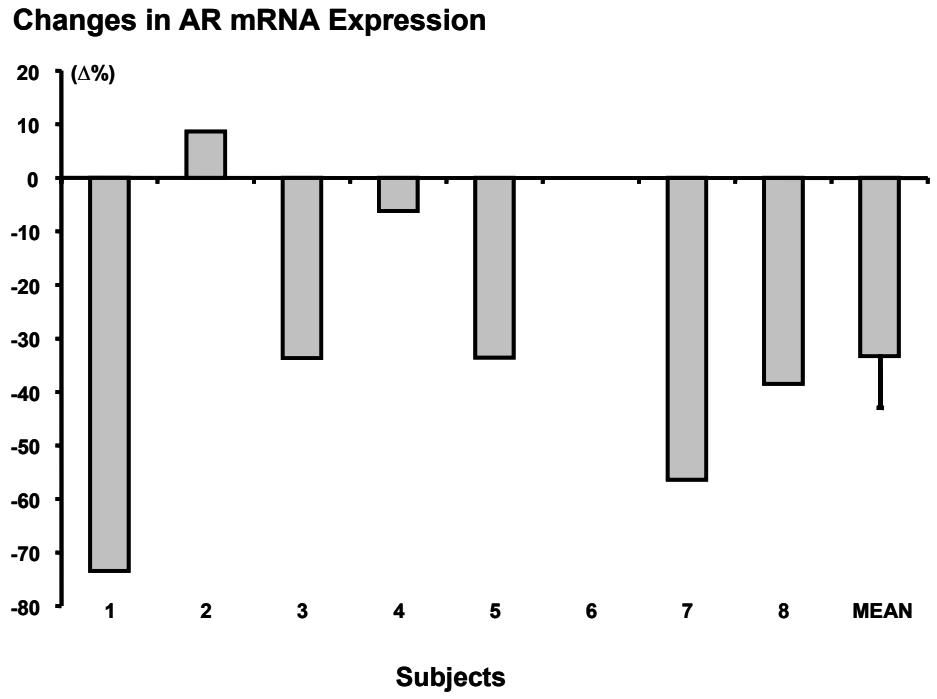


FIGURE 21 Relative changes 48h after the resistance exercise in androgen receptor (AR) mRNA expression of each subject and the total group of strength trained men (mean ± SE) (n = 7). (Subject number 6 was excluded from the data) (V)

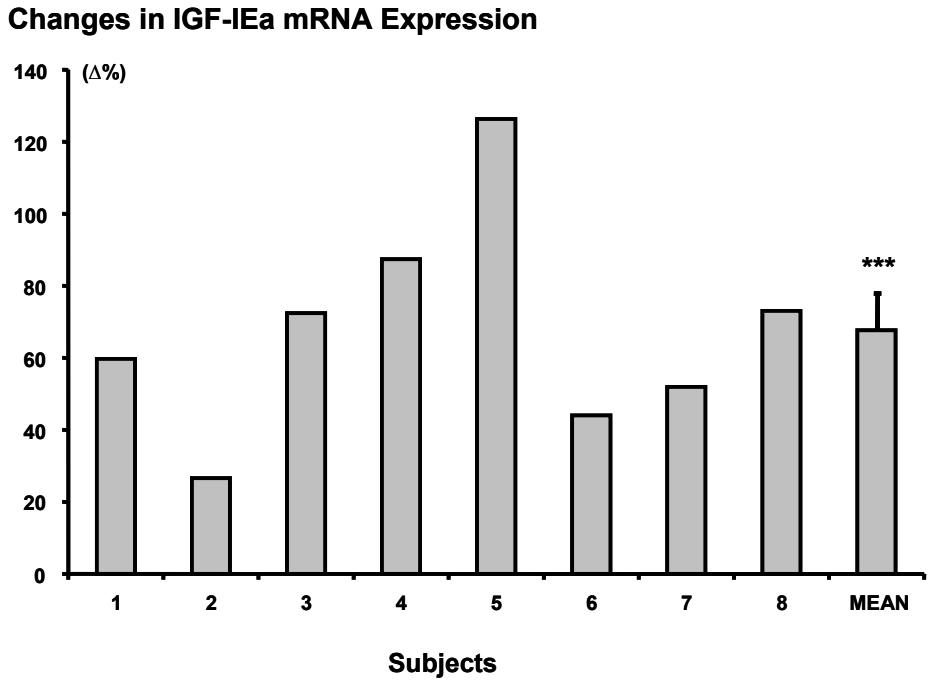


FIGURE 22 Relative changes 48h after the resistance exercise in IGF-IEa mRNA expression of each subject and the total group of strength trained men (mean ± SE). Significantly different (*** = $p < 0.001$) from the corresponding pre-training value. (V)

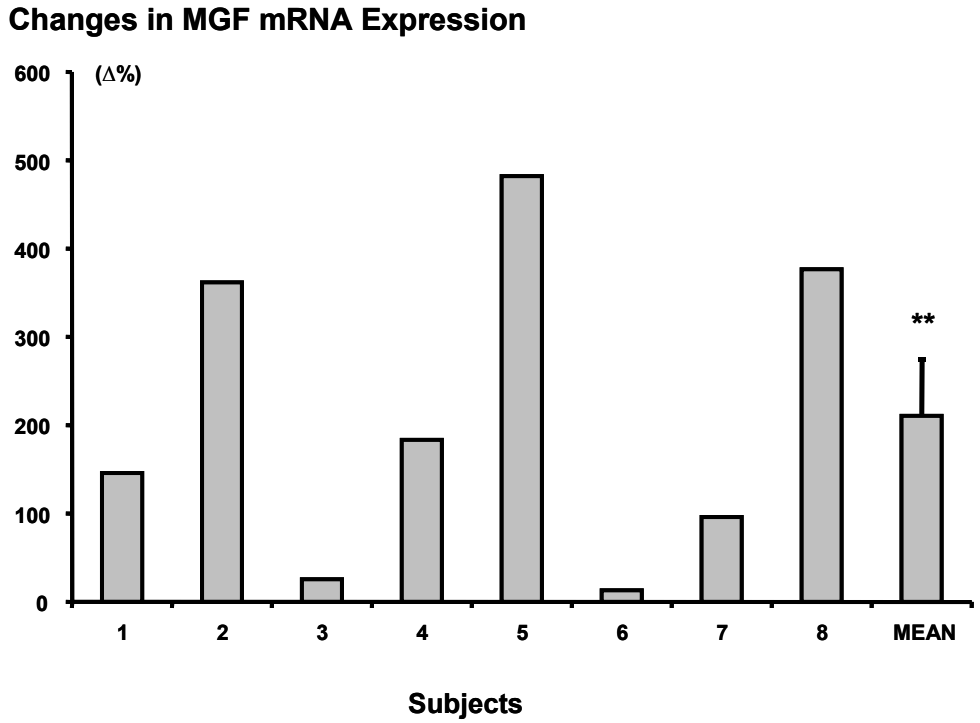


FIGURE 23 Relative changes 48h after the resistance exercise in mechano growth factor (MGF) mRNA expression of each subject and the total group of strength trained men (mean ± SE). Significantly different (** = $p < 0.01$) from the corresponding pre-training value. (V)

### 5.4.2 Effect of long-term resistance training on AR and IGF-I mRNA expression (VI)

No statistically significant changes took place in body mass or body fat percentage in the untrained older men (OM) and adult men (AM) and strength trained men (SM) during the 6-month training period.

Before the training period maximal isomeric force was larger in (SM) than in (AM) ($p<0.001$) and in (OM) ($p<0.001$) and in AM larger than in OM ($p<0.05$) (Figure 24). During the 6-month training period increases of 34.9 ±14.3% ($p<0.01$), 40.4 ±7.9 ($p<0.05$) and 6.8 ±12.3% (ns.) were recorded in the AM, OM and SM groups, respectively.

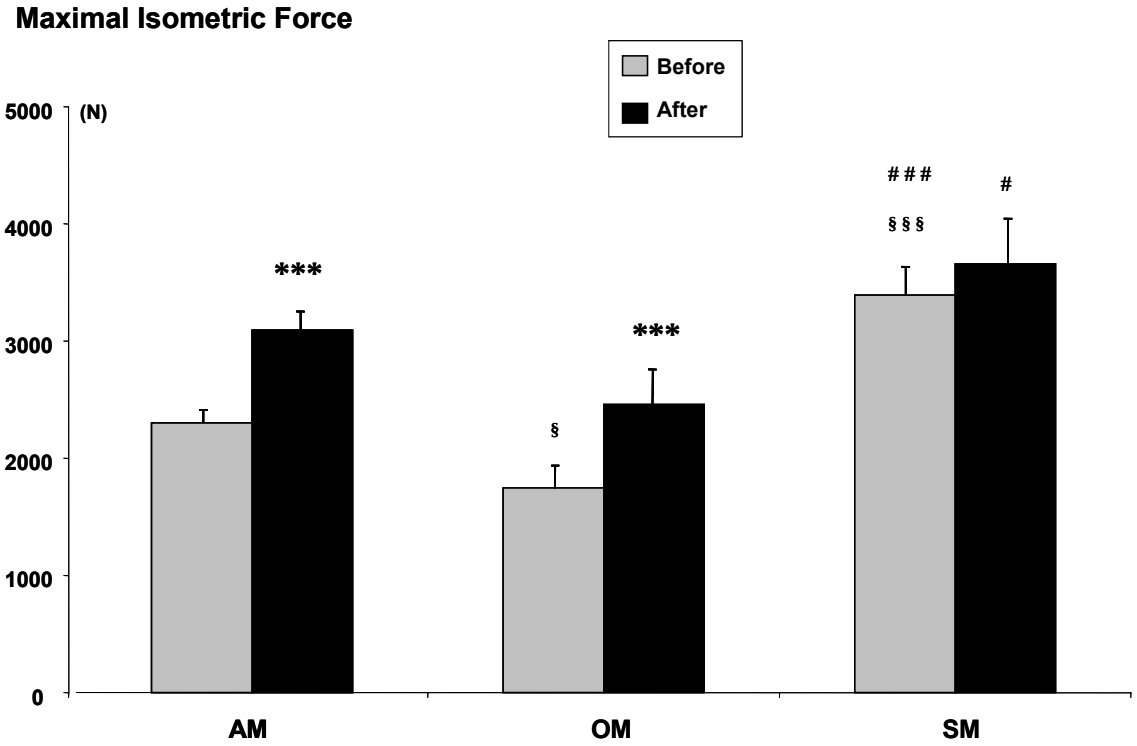


FIGURE 24 Mean (± SE) maximal isometric leg extension force before and after the 6-month strength training period. Statistically significantly different (*** = $p < 0.001$) from the corresponding pre-training value. Statistically significant difference (§ = $p < 0.05$, §§§ = $p < 0.001$) compared to AM. Statistically significant difference (# = $p < 0.05$, ### = $p < 0.001$) compared to OM. (AM = adult men, OM = old men, SM = strength trained men)

SM showed larger CSA values than AM ($p<0.001$) and OM ($p<0.001$) and AM showed larger CSA values than OM ($p<0.05$) before and after the training period. During the 6-month training period increases of 4.3 ±8.0% (ns., $p<0.08$), 3.3 ±7.6 and 1.8 ±3.5% were recorded in the AM, OM and SM, respectively.

Total testosterone concentrations were greater in SM than in AM ($p<0.05$) and OM (ns., $p<0.07$) before the training period as well as after the training period in AM ($p<0.01$) and OM ($p<0.05$). Free testosterone concentrations were greater in SM than in OM before ($p<0.05$) and after ($p<0.05$) the training period.

No significant changes were observed in serum basal testosterone or free testosterone concentrations during the 6-month resistance training period, except for the decreases in free testosterone (-9%, $p<0.05$) in OM. Before and after the experimental training period basal total testosterone concentrations and maximal isometric force (before; $r = 0.45$, $p<0.05$, after; $r = 0.42$, $p<0.05$) as well as basal total testosterone concentrations and muscle CSA (before; $r = 0.52$, $p<0.05$, after; $r = 0.53$, $p<0.01$) correlated with each other in the total group of subjects.

At baseline no significant differences were observed in AR mRNA expression between the experimental groups. Changes of 14.0 ±75.5% (ns.), -19.7 ±75.5% (ns.) and 14.1 ± 139.1% (ns.) were observed in the expression of AR mRNA during the experimental resistance training period in AM, OM and SM, respectively (Figure 25). An increased expression of 2.3 ±1.8 fold ($p<0.05$) and 1.7 ±1.3 fold (ns., $p<0.07$) was observed in IGF-I mRNA levels before the training period in OM as compared to AM and SM, respectively. Changes of 40.4 ±106.4% (ns.), -5.9 ±65.5% (ns.) and 67.0 ± 98.4% (ns., $p<0.09$) were observed in the mean expression of IGF-I mRNA during the experimental resistance training period in AM, OM and SM, respectively (Figure 26).

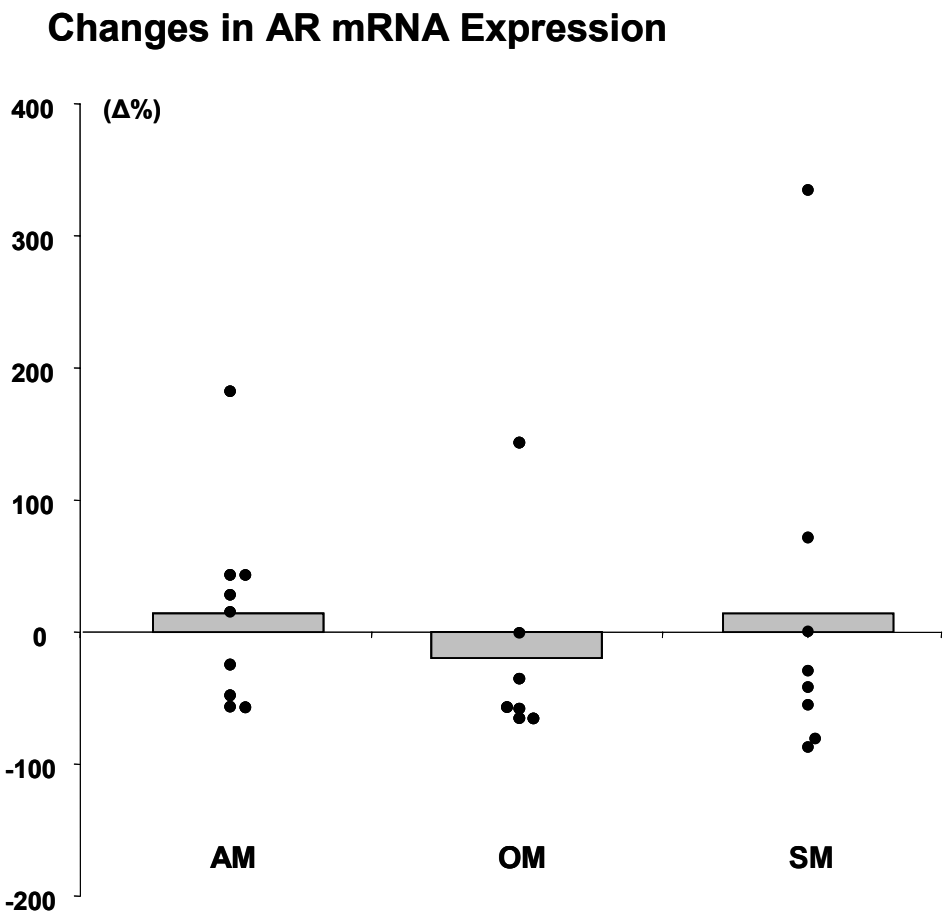


FIGURE 25 Relative changes in androgen receptor mRNA levels during the 6-month resistance training period. Bars represent means and each circle represents an individual sample (AM = adult men, OM = old men, SM = strength trained men). (VI)

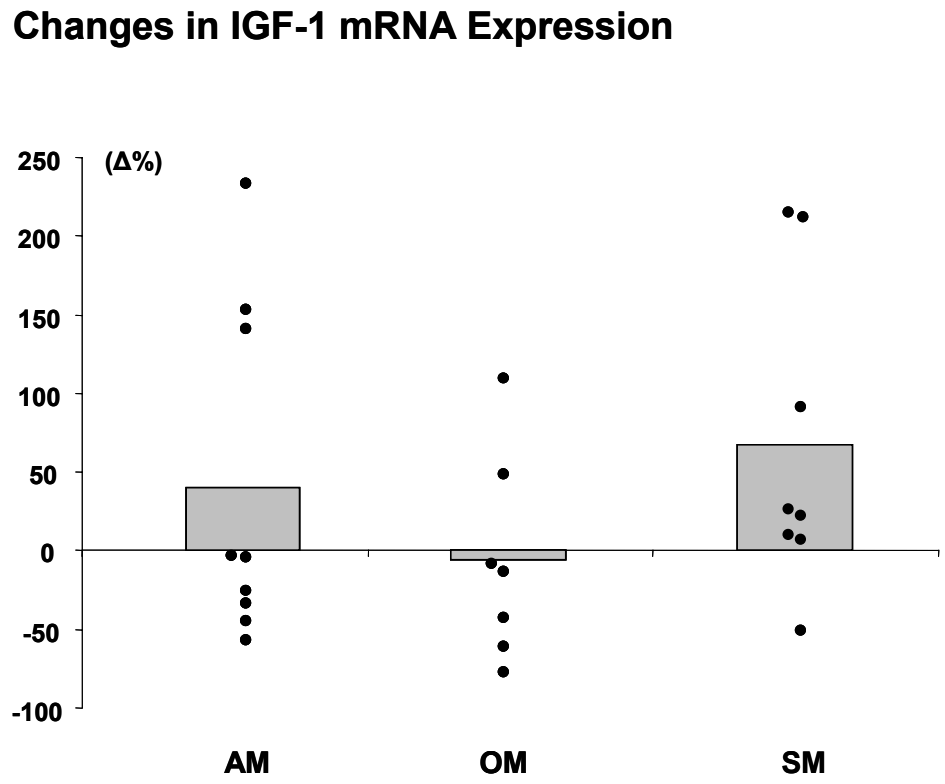


FIGURE 26 Relative changes in insulin-like growth factor 1 mRNA levels during the 6-month resistance training period. Bars represent means and each circle represents an individual sample (AM = adult men, OM = old men, SM = strength trained men). (VI)

For further analysis the subjects were divided to high-responder (HR, n = 12; AM = 5, OM = 4 and SM = 3) and low-responder (LR, n = 11; AM = 4, OM = 3 and SM = 4) groups according to the changes in the cross-sectional area (CSA) of quadriceps femoris (QF) during the experimental resistance training period. In HR the subject's relative change in muscle CSA was greater and in LR lower than the mean change of the respective experimental group. The mean relative change of muscle CSA in HR was 9.1 ± 3.6% ($p<0.01$) and in LR -2.3 ± 3.9% (ns.). The mean relative change of muscle strength in HR was 29.4 ± 13.8% ($p<0.01$) and in LR 28.3 ± 20.9% (ns., $p<0.10$). No statistically significant differences were observed between the HR and LR groups in the before and after values and in the relative changes of AR and IGF-I mRNA expression. The pre-training values of AR and IGF-I mRNA expression or serum T and FT concentrations were not related to the changes in the CSA of QF or maximal isometric force during the resistance training period.

# 6 DISCUSSION

## 6.1 Intensity of resistance exercise and acute responses (I, II)

### 6.1.1 Acute hormonal responses

Heavy resistance exercise is a potent stimulus for acute increases in the concentrations of circulating hormones in young men. These acute increases are highly dependent on the type of resistance exercise protocol i.e. number of sets and repetitions per set, length of rest period between sets and muscle mass involved employed (e.g. Kraemer et al. 1990, Häkkinen and Pakarinen 1993). It is possible that the manipulation of the acute exercise variables may lead to the specific adaptation processes and appropriate training effects. Therefore, studies I and II examined the role of the exercise intensity to acute hormonal and neuromuscular responses and short-term recovery for three days by comparing the responses of the forced repetitions (FR) training protocol to those produced by the traditional maximum repetitions (MR) protocol. Study II further examined responses to the maximum and the forced repetitions resistance protocols in strength athletes versus non-athletes. The loading protocols were designed to be similar as usually used in training for muscle mass and strength development of the leg extensor muscles in athletes such as bodybuilders. The findings of the MR and FR loading protocols are considerd to be comparable since the actual total volume of the work (loads x sets x repetition) performed by the subject himself during the MR and FR loadings did not differ from each other. However, it should be noted that in study I the overall volume of the loading was higher (total 8 sets) than in study II (total 4 sets) which may have some contribution to the present results.

Studies I and II showed that both MR and FR loading protocols led to the great acute hormonal responses. Study II further showed that the heavy resistance exercise-induced acute testosterone responses were greater in strength athletes (SA) than in non-strength athletes (NA). This was true especially when the resistance exercise was performed with the forced repetitions protocol. The FR loading protocol also tended to produce greater

acute free testosterone (II), GH (I, II) and cortisol (I, II) responses in both SA and NA.

#### 6.1.1.1 Serum testosterone responses

In study I serum testosterone and free testosterone concentrations increased significantly during both loading protocols with no significant differences between the loadings. Study II showed that serum testosterone concentrations increased significantly more in SA during the FR loading as compared to the responses in NA during both loading protocols. The testosterone response in SA during the MR loading was also greater than in NA during MR the loading. Furthermore, in the case of serum free testosterone, which represents the amount of bioactive testosterone, the response was greater in SA during the FR loading than in NA during the MR loading. These findings suggest that SA were capable to produce greater testosterone and free testosterone responses, and these responses were further enhanced by the forced repetitions training protocol. These findings are consistent with previous studies, which have indicated a relationship between total work (Kraemer et al. 1990, 1993, Häkkinen and Pakarinen 1993, Gotshalk et al. 1997) and intensity (Jezova et al. 1985, Hickson et al. 1994) of the exercise and the degree of the acute testosterone response.

Some previous studies of acute testosterone responses have showed contradictory results with regard to the training background of subjects. The resistance-training background had no influence on the acute testosterone response produced by the heavy resistance exercise (Fahey et al. 1976). On the other hand, experienced weightlifters showed a greater increase in the testosterone response following the heavy resistance exercise than that of unskilled weight trainers (Kraemer et al. 1992). In the study of Kraemer et al. (1998) the enhanced acute testosterone response due to the short-term resistance training has been reported, while other previous studies have not showed any significant changes in resistance exercise induced acute testosterone responses due to the long-term resistance training (Craig et al. 1989, Hickson et al. 1994, McCall et al. 1999a, Häkkinen et al. 2000b).

In study I serum testosterone concentrations decreased 30 minutes after MR below the pre-level, which is probably caused by the lowered LH response of the testicles due to the exercise-induced increased in serum testosterone concentration. Interestingly, serum total testosterone concentration turned to a downward trend already after the squat exercise, especially in FR. This may be due to smaller activated muscle mass in the knee extension exercise, lowered blood lactate concentration, failure in testosterone production and/or increase in its hepatic clearance. Contrary to total testosterone serum free testosterone increased throughout the entire loading. The concentration of serum free testosterone was also increased on the second morning after the both loadings as compared to the control day value. That is perhaps an indication of a compensation mechanism of the hormonal system against the exercise-induced stress.

#### 6.1.1.2 Serum cortisol responses

The serum cortisol concentrations increased in both loadings (I, II) in both SA and NA groups (II). Furthermore, the exercise-induced cortisol response was greater in FR than in MR (I, II). In study I the exercise response occurred in FR after the four sets of leg press and in MR after the squat, when the threshold for the cortisol response was probably exceeded (Viru 1992). Study II showed that in SA during the FR loading the cortisol response was greater than during the MR loading. This indicates that the cortisol response is also enhanced by resistance exercise intensity besides with magnitude of total work. This result is in line with the endurance exercise studies where the acute cortisol response has been related to the intensity of the exercise (Kuoppasalmi et al. 1980, Kindermann et al. 1982, Farrell et al. 1983).

Exercise-induced cortisol response may be due to glycolytic demands of the exercise, stimulated effect of catecholamines and/or a consequence of neural control of muscle work. The exact mechanism remains unclear, but the greater acute cortisol response during the FR loading may be due to the greater metabolic demands which mediates some of the response via glycolytic and catecholamine stimulatory mechanism (Kraemer et al. 1987, 1993, VanHelder et al. 1986). The exercise response of endocrine action is also triggered by the central motor command and the responses are further supported by positive feedback influences from proprio- and metaboreceptors in muscles (Kjaer 1992). Thus, the neural control of adrenal cortex activity (Holzwarth et al. 1987) and nervous feedback from working muscles (Kjaer et al. 1989) are involved in regular mechanisms of exercise-induced cortisol response. In study I the changes in maximal isometric force and changes in EMG as well as the changes in maximal isometric force and the changes in serum cortisol concentration correlated significantly between each other in FR. This may partly support the hypothesis for the influence of neural control of the muscle work to exercise-induced cortisol response. In study II the decreased EMG activity during the actual FR loading in SA supports also the presumption of the neural control in the acute cortisol response.

#### 6.1.1.3 Serum growth hormone responses

In the present studies I and II serum concentrations of GH increased greatly after both MR and FR loadings, which was true both in SA and NA. In line with the previous study of VanHelder et al. (1984) between exercise intensity and GH response, a trend to the greater GH response was observed after the FR loading (I, II). Exercise-induced increase in serum GH concentrations measured by immunoreactive method (molecular size 22kDa) can be explained mainly by hypoglycemia and stimulatory effect of the motor cortex and the symphatic nervous system to hypothalamus. Further explanation might also be an increased acidity in the muscle caused by anaerobic muscle work, which stimulates metaboreceptors and sends afferent feedback to the central nervous system and hypothalamus leading to increased secretion of GH (Kjaer et al.

1987, Gordon et al. 1994, Gosselink et al. 1998). This is supported by study I showing that serum GH concentrations correlated with blood lactate concentrations in both loadings. However, study II showed no relationships between blood lactate concentrations and changes in serum growth hormone concentrations.

The efferent activity of brain motor center has been connected to the exercise-induced GH response (Few and Davies 1980, Galbo et al. 1987, Kjaer et al. 1987, 1989). In study I the deceased maximal voluntary isometric actions were associated with the decreased EMG activity of the loaded muscles during the FR loading. Therefore, greater relative increase in GH concentrations in FR may indicate the importance of the central motor command to exercise-induced GH response. This phenomenon is also supported by study II because the changes in GH concentrations were related to the changes in EMG in NA after the FR loading and to the changes in isometric force in SA after the FR loading.

### 6.1.2 Acute neuromuscular fatigue

Both MR and FR loading protocols led to remarkable neuromuscular fatigue, similarly in both SA and NA groups. This was observable by the acute decreases in maximal voluntary isometric force associated with high blood lactate concentration (I, II). Only FR led to the decrease in the maximal voluntary EMG of the loaded muscles recorded during the isometric muscle action in study I, while in study II maximal EMG decreased due to both MR and FR loadings.

Intense muscular work used typically during heavy-resistance training leads to a momentary decrease in strength accompanied by decreases in voluntary maximal neural activation of the loaded muscles (Komi and Rusko 1974, Häkkinen 1993, Häkkinen et al. 2000b). As expected, the present heavy resistance exercises produced acute neuromuscular fatigue observed by the decrease in maximal voluntary isometric force (I, II). The load was greater in FR especially at the beginning of the loading session than in MR. However, when the force of the assistance was reduced from the total load, there were no significant difference in the total work that the subjects had performed by themselves between FR and MR (I, II). Nevertheless, study I showed that FR led to a greater decrease in maximal isometric force than MR. Furthermore, decrease in isometric force in study II was greater in SA during the FR loading than in NA during the MR loading. That may be due to a greater accumulation of lactic acid to working muscles during FR and/or the decrease in the ability to activate especially type II motor units by the central nervous system (e.g. Häkkinen 1993). The larger increase in blood lactate concentration and the greater decrease in EMG in FR compared to MR in study I supports this suggestion. It has been demonstrated earlier that the decrease in maximal strength during high-intensity fatiguing resistance training sessions may be associated with the decrease in the maximal voluntary neural activation of the exercised muscles (Häkkinen et al. 1988c, Häkkinen 1993). Therefore, the results of study I suggest that FR protocol may produce greater peripheral and central

fatigue compared to MR where fatigue was caused mostly by the peripheral factors. However, in study II there were no significant differences between the decreases in the maximal EMG activity during the isometric actions or within blood lactate concentrations between the loading protocols or between the experimental groups.

### 6.1.3 Recovery after the exercise

According to the principle of progressive strength training, the next training session should usually take place under the conditions where complete recovery has taken place. Therefore, the magnitude of temporary neuromuscular fatigue and the rate of recovery after intensive heavy-resistance exercise may be an indication of its effectiveness for long-term adaptations of the neuromuscular system. The investigation of the rate of recovery of the neuromuscular and hormonal systems after the heavy resistance exercise may be advantageous to estimate a proper resistance training frequency and/or intensity and/or volume to avoid the overtraining syndrome. Especially the forced repetitions resistance exercise system is supposed to cause muscular soreness (Fleck and Kraemer 1997). Mechanical load due to resistance exercise may cause some level of myofibrillar disruptions to the activated muscles. In study I maximal isometric force, subjective muscle soreness, muscle swelling and CK activity were used to estimate the level of muscle damage caused by the heavy resistance exercise and rate of recovery after the loadings.

Maximal isometric force was significantly more lowered in FR than in MR during the recovery and isometric force remained significantly lowered even on the third day after FR in study I. The results of study II showed that the recovery of the isometric force after the loading protocols in both SA and NA was almost completed during the two recovery days. There were no significant alterations in the maximum iEMG during the recovery period in both studies I and II. In study I the thickness of the vastus lateralis (i.e. muscle swelling) increased during the present loading session and returned to the pre-exercise level on the next day. Although the loading regimens were strenuous, the muscle damage markers as CK –activity and subjective muscle soreness did not increase greatly after the present kinds of high load and low velocity types of dynamic muscle actions. In study II when the overall volume of the experimental exercise session was lower the recovery of the isometric force in both SA and NA was almost completed during the two recovery days. The MR loading was performed first in the experimental design due to practical reasons. In this way it was possible to obtain the true strength level of the subjects at the moment and to create maximal loading for both loading protocols. However, this may not have effect to acute hormonal and neuromuscular responses but the muscle damage markers as CK-activity, subjective muscle soreness and muscle swelling could be diminished on the second experimental loadings via prophylactic effect of the previous loading (Nosaka et al. 1991).

### 6.1.4 Conclusions of studies I and II

In conclusion, the data of studies I and II showed that FR compared to MR loading led to greater stress for the neuromuscular system shown through the larger decrease in isometric force and iEMG of the loaded muscles. Regarding to results of study II this was true both in strength athletes and non-athletes. In study I the exercise-induced differences in isometric force and EMG between MR and FR loading was statistically significant. The FR loading stimulated the hormonal system, especially the secretion of GH, more than MR did (I). The testosterone responses during the FR and MR loading were greater in SA than in NA (II). Furthermore, the free testosterone response was greater in SA during the FR loading than in NA during the MR loading and the cortisol response was greater in SA during the FR loading than during the MR loading.

According to study I the recovery of the isometric force was slower after the FR than MR loading. Because the degree of acute neuromuscular fatigue and the time needed for recovery may differ considerably between the MR and FR loading protocols, there is a need to optimize the contents and the frequency of different training sessions in order to create proper resistance training programs to match the individual requirements of athletes.

The data of studies I and II suggest that the forced repetition exercise system may be beneficial for the development of muscle mass and muscle strength during resistance training especially in strength athletes. On the other hand, the lowered recovery of force production in FR may indicate an increased risk for overtraining if the training frequency is kept too high and/or the overall volume of each training session is too high. However, to what extent the larger acute endogenous hormone responses created by the FR loading protocol are related to training induced muscle hypertrophy and strength development needs further research.

## 6.2 Hormonal and neuromuscular adaptations to long-term resistance training (III, IV)

### 6.2.1 Effect of strength training background on resistance training adaptations (III)

Study III included two experimental groups. As expected, strength athletes (SA) were stronger and had greater muscle mass than their age and height matched controls, a group of non-athletes (NA) with no previous strength training experience. Maximal isometric force and 1RM strength as well as muscle CSA increased during the 21-week resistance training period more in NA than in SA. The difference in the muscle CSA of the quadriceps femoris between the two groups was clear throughout the length of the femur before the experimental training period.

In NA the increase in maximal isometric force was greatest during the first 14 weeks of training, but the 1RM increased gradually throughout the training period. The 1RM increased also significantly in SA but to a lesser degree than in NA. However, for a well-trained strength athlete, even increases of 1-2% in muscle CSA can represent a meaningful difference physiologically (Häkkinen et al. 1988c). The increases observed in the 1RM to muscle CSA ratio during the training indicates that neural adaptations might have occurred during the present training period in both groups. However, greater isometric force to muscle CSA ratio in SA indicates that strength athletes might be able to produce greater maximal voluntary activation of the agonist muscles than NA. In addition to the increased neural drive to the activated muscles, it is also possible that the force to muscle CSA ratio would increase during the training due to architectural changes of leg extensor muscles (Aagaard et al. 2001).

Serum basal testosterone, free testosterone and cortisol concentrations remained unchanged in both groups during the present training period. However, basal testosterone and free testosterone concentrations did increase during the first 14 weeks of the experimental training period in SA with the increase in the volume of the training. Therefore, the decrease in basal testosterone and free testosterone concentrations in SA during the last training cycle from week 14 to 21 may be associated to the decrease observed in the training volume. These findings are in line with previous data by Häkkinen et al. (1987, 1988b) showing serum testosterone concentrations to differ with regard to the volume and/or intensity of the resistance training periods. The correlation observed between serum total testosterone concentrations and the changes in isometric strength suggest that serum basal testosterone concentrations may be an important factor for strength development in strength athletes. However, non-athletes may gain strength and muscle mass in the beginning of the resistance training despite periodical changes in the level of serum testosterone concentration.

Study III also comprised two acute heavy resistance loadings performed before and after the 21-week resistance training period. As expected, the loadings led to remarkable acute decreases in maximal isometric strength, but the magnitudes of the decreases were similar both before and after the training period as well as between the experimental groups. Therefore, the degree of acute neuromuscular fatigue produced by the loading was similar in both experimental groups before and after the resistance training period, when the relative intensity of the loadings was kept the same.

The heavy resistance exercises before and after the training period did induce acute increases in serum testosterone and free testosterone concentrations in both groups. The acute testosterone and free testosterone responses tended to decrease after the experimental training period as observed with the lowered concentrations during the 30-minute recovery period after the exercises. Therefore, it may be necessary to increase the volume of the resistance exercise to achieve similar testosterone and free testosterone responses after the resistance training than before the training period. Interestingly, those NA

subjects who showed increased acute total testosterone response after the resistance training period were also able to gain more muscle CSA than those with the lowered responses. Although the number of subjects was limited, the correlations observed between the changes in acute testosterone response and the changes in muscle CSA suggest that training-induced changes in the endocrine regulation do take place and that changes in acute testosterone responses may be an important factor for training-induced muscle hypertrophy.

The loadings performed before and after the experimental resistance training period did lead in both groups to the great acute increase in serum GH concentrations. The acute GH response induced by the single resistance exercise at week 0 was greater in SA than in NA. After the 21-week training period the acute GH response decreased in SA. The attenuated acute GH response in SA suggests that the exercise stimulus may have changed at week 21, even though it was performed with the same relative load as before the training period or alternatively the training may have changed the type of GH variant being produced leading to a lower concentration of the 22kD isoform (Hymer et al. 2001). It is also possible that it would be necessary to increase the absolute volume of the loading to produce similar GH response after the 21-week training period. However, there was an increased trend in the acute GH response in NA after the resistance training period. This finding is in line with the previous study of Häkkinen et al. (2001) that showed a lengthened duration of the acute GH response due to the 21-wk resistance training period in older women.

In conclusion, the results of study III suggest that serum basal testosterone concentrations and acute increases in serum testosterone concentrations due to a single resistance exercise session may be important factors for training-induced muscle hypertrophy as well as for strength development of the trained muscles. The results also suggest that adaptations in the endocrine system can take place, so that acute hormonal responses may change due to resistance training. These results also suggest that one could try to optimise the volume and/or intensity of resistance exercises especially in previously strength trained athletes to meet the level of adaptation of the neuromuscular and endocrine systems in order to further increase muscle mass and strength.

### 6.2.2 Effect of short and long recovery time between the sets on resistance training adaptations (IV)

In study IV recreationally strength trained young men performed two hypertrophic strength training protocols similar with regard to the total volume but differing in terms of the length of recovery between the sets for the leg muscles; a high intensity with the shorter rest periods between the sets (SR) and somewhat higher intensity with the longer rest periods between the sets (LR). The SR and LR loadings performed before, in the middle and after the experimental training period led to acute decreases in maximal isometric force and EMG activity of the loaded muscles as well as increases in blood lactate concentrations. No differences were observed between the SR and LR loadings.

The significant acute total testosterone, free testosterone, cortisol and growth hormone responses were observable in both SR and LR loading protocols at month 0, 3 and 6. In general, a trend of attenuated acute hormone responses was observed during the 6-month training period. Similar findings have been reported especially in the acute GH and cortisol responses due to prolonged resistance training in previously untrained young men (Kraemer et al. 1999b, Staron et al. 1994). In study IV the decreased acute hormonal responses seemed to be slightly greater in the LR than in the SR loading after the 6-month experimental training period. If the magnitude of acute hormone responses is expected to be crucial for optimal adaptation to resistance training, the results of the present study suggest that the LR exercise protocol in the long term training may create less favourable conditions for the gains in muscle mass. However, there were no statistically significant differences in the development of the muscle mass between the two training protocols. Interestingly, in study IV significant relationships were observed between the increases of the acute testosterone and free testosterone responses and the increase in the muscle CSA of the quadriceps femoris in the SR loadings during the first three-month training period, when both experimental groups were combined. Since testosterone can stimulate protein synthesis after resistance exercise (Tipton and Wolfe 2001), these findings suggest that the increase in acute testosterone response after the heavy resistance loadings due to prolonged resistance training may be an important factor for training induced muscle hypertrophy.

It could be speculated that the hormone system adapted to the present systematic hypertrophic strength training by diminishing the acute hormonal responses. This could be due to the decreased stress response and/or decreased hormone production. It may be necessary e.g. to increase exercise volume to some extent to achieve similar acute hormonal responses after the 6-month resistance training period than in the beginning of the training period. However, it is also possible that subjects of the present study were slightly overreached due to several months of very strenuous resistance training. Actually, our subjects reported some difficulties to carry out intensive resistance exercises as planned in the training program. Therefore, it could be speculated that the attenuated acute hormone responses could be, at least in partly, consequences of too intensive resistance training for too long a training period. However, there were no signs of systematic decrements in maximal muscle strength during the study. Moreover, basal testosterone, free testosterone and cortisol concentrations did not change during the 6-month training period to indicate a possible state of an overtraining condition. However, the exact time course of the decrements in muscle strength and the changes in basal hormone concentrations during long term overtraining are not well known. It is possible that a trend toward a decreased acute hormone response observed in the present subjects may be a preliminary sign of an overtraining condition.

Since no systematic differences were observed in the acute hormonal or neuromuscular responses between the SR and LR loading protocols, the results

indicate that a lower intensity protocol with shorter rest periods between the sets seems to produce similar acute hormonal responses as a protocol performed with a higher intensity with longer rest periods between the sets, when typical hypertrophic sets of 10RM are used. Although only these two protocols were examined, the results suggest that the length of the rest periods between the sets and the number of sets may not have influence on acute exercise responses. This seems to be true at least in young strength trained men, if several sets are performed and if the training intensity of the exercise is kept high as in study IV. In the previous studies of Kraemer et al. (1990, 1991 and 1993) the short rest between the sets (1 min.) elicited greater anabolic hormone responses than that of longer rest periods between the sets (3 min.). Contrary to the our study those studies included recreationally strength trained men as well as women and the experimental loading protocol was carried out with a total of 24 sets of exercises to all muscle groups of the body. Whether the shorter rest periods between the sets (i.e. 60 sec) would produce different acute hormonal responses compared to the exercise protocols used in the present study remains unanswered.

In addition to acute hormonal and neuromuscular responses the present study was also designed to examine long-term hormonal and neuromuscular adaptations to two hypertrophic training protocols differed mainly by the training intensity and length of the rest periods between the sets, while the total volume between the training protocols was as similar as possible. The maximal bilateral isometric leg extension force and the right leg 1RM increased throughout the 6-month experimental resistance training period with no significant differences between training groups I and II or between the training protocols. The quadriceps femoris CSA increased during the first 3-month training period, but no further increase in the CSA was observable during the latter training period from three to six months. When comparing the training periods independently, the present study showed no differences in the changes in the maximal isometric force, right leg 1RM or CSA of the quadriceps femoris between the two training protocols during the 3-month training periods. However, during the SR training maximal isometric force increased only slightly, but during the LR training the increase in maximal isometric force was statistically significant. Although the relative increase in the 1RM strength was similar in magnitude, the increase in the LR training was more systematic than in the SR training. Therefore, the results of the present study suggest that LR training may create more optimal training stimuli for maximal strength development than SR training.

In conclusion, study IV showed that the SR and LR protocols induced similar acute hormonal and neuromuscular responses and that the length of the recovery times (i.e., 2 and 5 minutes) between the sets did not influence the magnitude of these responses. The results of study IV suggest that there may be several different ways to create exercise conditions leading to large acute hormonal responses, at least, when several sets within the hypertophic loading intensity such as the 10RM are performed. The results also showed that long

term training adaptations in muscle strength and mass did not differ between the two hypertrophic strength training protocols examined in the group of young men having already a background in strength training. Nevertheless, the results of the present study further suggest that resistance training induced changes in the acute total and free testosterone responses after the heavy resistance exercise may contribute to muscle hypertrophy of the trained muscles.

## 6.3 Androgen receptor and IGF-I responses to resistance training (V, VI)

Study V was designed to investigate whether the hypertrophic type of heavy resistance exercise induces changes on AR, IGF-IEa and MGF mRNA expression in previously strength trained men. During the exercise bout and 48h recovery period the exercise-induced changes in serum testosterone concentrations as well as muscle damage indicators, serum creatine kinase-activity as well as muscle strength, soreness and swelling were measured. Therefore, the purpose of study V was also to study relationships between exercise-induced changes in gene expression, serum testosterone and selected muscle damage markers. In human skeletal muscle, specific reprogramming of gene expression may occur for the adjustments of muscle tissue to stress, such as exercise (Pilegaard et al. 2003, Schmitt et al. 2003, Wittwer et al. 2004, Coffey et al. 2005, 2006). Furthermore, it could be suggested that resistance training-induced adaptations in skeletal muscle gene expression may differ between younger and older subjects. Therefore, study VI was designed to test the hypothesis that long-term systematic resistance training would induce changes in AR and IGF-I mRNA expression of the trained muscles and these changes could be, at least in part, associated with changes in muscle mass and strength.

### 6.3.1 Acute responses of AR and IGF-I mRNA expression to resistance exercise

The results of study V demonstrate that a single bout of heavy resistance exercise increases skeletal muscle IGF-IEa and MGF but not AR mRNA expression at 48h after the exercise in previously strength trained young men. Muscle biopsy was taken at 48h after the exercise to make the sample time coincide with increased muscle protein synthesis and regenerative phase after an acute bout of heavy resistance exercise (Phillips et al. 1997). IGF-I splice variants IGF-IEa and MGF acts as a separate autocrine growth factors and their expression may be differentially regulated. In animal studies MGF has been found to be expressed earlier than IGF-IEa after the mechanical loading (Haddad and Adams 2002, Hill and Goldspink 2003). The mean IGF-IEa and MGF mRNA expression increased 68% and 211% after the experimental

resistance exercise, respectively. Furthermore, the results were highly individual between the subjects, especially in the magnitude of the MGF mRNA responses. Therefore, it could be speculated that in some subjects MGF expression may be peaked before and already recovered near to pre-exercise level during the 48 hours recovery after the exercise. However, it would be also possible that in some subjects MGF response to resistance exercise is lower as compared to other subjects. The same appears to be true also with IGF-IEa but to a lesser scale. However, the physiological significance of these findings and they significance in adaptation to resistance training of different individuals remains unknown.

Especially the expression of MGF has been demonstrated to be related to the mechanical strain of muscle tissue (Goldspink 2003). The resistance exercise protocol included concentric and eccentric muscle actions and therefore, it could possibly induce disruptions within the activated muscle cells. Maximal isometric muscle force was significantly decreased during the entire 48h recovery period after the exercise. Furthermore, muscle thickness (i.e. muscle swelling) and muscle soreness of the loaded muscles as well as serum CK-activity were significantly increased during the entire recovery period. Therefore, it could be assumed that at least some level of muscle cell disruptions in unspecified location of the exercised muscles has been occurred due to the mechanical strain to muscle tissue during the resistance exercise. However, no relationships were observed between present muscle damage markers and IGF-IEa and MGF mRNA expression. In previous studies the changes in serum testosterone concentrations have been shown to be related in IGF-I mRNA expression in skeletal muscle (Urban et al. 1995, Mauras et al. 1998, Ferrando et al. 2002). However, no relationships were observed between exercise-induced changes or basal levels in serum total or free testosterone concentrations and IFG-IEa and MGF mRNA levels in the present study.

It could be assumed that the endocrine system adapts to long-term resistance training but the mechanisms are not clear. However, changes in AR expression may be crucial mediating the effect of testosterone on muscle tissue. An increase in serum testosterone has shown to induce up-regulation in AR content (Doumit et al. 1996). This finding is supported by the study of Willoughby and Taylor (2004) who investigated the serum testosterone and skeletal muscle AR mRNA responses to three sequential resistance exercises in previously untrained men. They found a correlation between serum testosterone concentration immediately after the resistance exercise and AR mRNA expression at 48h after exercise. In contrast, Bamman et al. (2001) found a significant exercise-induced increase in AR mRNA expression in loaded muscles but no relationships were found between serum testosterone concentration and AR mRNA expression in male subjects. Furthermore, in the cross-sectional study of Marcell et al. (2001) no significant correlations were found between serum testosterone and AR mRNA levels in 27 healthy older men.

Our results showed no significant changes in mean AR mRNA expression due to the resistance exercise and no relationships were observed between testosterone levels and AR mRNA expression. This may be due to the different loading protocol used in the present study compared to other studies. Furthermore, our subjects were already strength trained and their muscles might be already well adapted to resistance exercise and therefore, further increases in AR at mRNA level may not occur. This suggestion is partly supported by the findings of Kadi et al. (2000) who studied the immunohistochemical expression of AR in human vastus lateralis and trapezius muscles, and found that the long-term resistance training and/or self-administration of androgenic-anabolic steroids leads to higher proportion of AR-containing myonuclei in the trapezius muscle. It could be also speculated that the timing of peak AR mRNA response to exercise may have change due to prolonged resistance training in strength trained men compared to previously untrained subjects. Muscle biopsies were taken 48h after the exercise and it is likely that exercise-induced changes in AR mRNA levels, if happened, are already down-regulated to the pre-exercise level or even below that.

In conclusion, study V showed that typical heavy resistance exercise used by strength athletes to increase muscle strength and mass induces significant increases in muscle IGF-IEa and MGF mRNA expression at 48h after the exercise in previously strength trained men. AR mRNA expression of the loaded muscles did not change significantly due to resistance exercise. Although the acute responses in serum testosterone and free testosterone concentrations as well as exercise-induced muscle damage were observed they were not related to the changes in mRNA expressions measured. However, increased IGF-IEa and MGF mRNA expression due to heavy resistance exercise supports the concept that they may be related to regenerative processes after the exercise and therefore, contribute to training-induced muscle hypertrophy.

### 6.3.2 Chronic adaptations of AR and IGF-I mRNA expression to resistance training

Study VI investigated the effects of long-term resistance training on basal AR and IGF-I mRNA expression in skeletal muscle of younger and older men in comparison to adult men with several years experience in resistance training. During ageing muscle strength and mass decline (i.e. sarcopenia) which could be counteract, at least in part, by systematic strength training. Therefore, a group of older men was also included in the study. As expected, a group of strength trained men (SM) had remarkably higher muscle mass and strength values compared to the untrained older men (OM) and adult men (AM). A limited number of studies have been demonstrating changes in gene expression due to resistance training. For example, mRNA expression of myostatin, which is a negative regulator of muscle mass, has been showed to be altered due to long-term resistance training (Roth et al. 2003, Willoughby 2004). Also both AR and IGF-I have regulative roles in changes of muscle mass (Adams 1998, Bhasin et al. 2003). Therefore, it would be assumed that AR and IGF-I expression are

altered in highly trained muscles. However, the present results showed only minor differences between the study groups and statistically insignificant changes in mean AR mRNA expression levels during the 6-month resistance training period. Interestingly, large interindividual differences were observed between the subjects. These results suggest that long-term resistance training may not have systematic effect on resting AR mRNA levels. Furthermore, the changes during the resistance training in AR mRNA expression were not related to the changes in serum total or free testosterone, isometric muscle strength or CSA of the quadriceps femoris muscle. Nevertheless, temporal changes in AR mRNA expression not detected within the present study design should not be excluded.

IGF-I may be the primary mediator of many of the responses regulated by growth hormone in tissues throughout the body and has an important role for the postnatal development of skeletal muscle (Florini et al. 1996, Yarasheski 1994). However, skeletal muscle may respond and adapt to changes in the loading state via mechanisms that appear to be intrinsic to the muscle. One of the mechanisms modulating skeletal muscle adaptation is thought to involve the autocrine and/or paracrine production of IGF-I (Adams 1998). In the previous studies muscle expression of IGF-I expression was not influenced by training (Bamman et al. 2003) or increased expression were observed (Singh et al. 1999, Hameed et al. 2004) in elderly subjects. Welle et al. (2002) found that the mean concentration of IGF-I mRNA in skeletal muscle was ~25% less in older than in younger men. In contrast, we found greater IGF-I mRNA levels in OM than in AM and SM before the experimental training period. It could be speculated that the greater IGF-I expression in OM as compared to AM and SM may be due to compensatory mechanism to sarcopenia. No statistically significant changes were observed in the IGF-I mRNA expression during the resistance training period within or between the experimental groups. Furthermore, no relationships were found between the changes in IGF-I mRNA expression and the changes in muscle mass and strength during the resistance training. Therefore, it could be suggested that the cellular adaptations to exercise training might to be due to the cumulative effects of transient increases in gene transcription after repeated exercise bouts (Fluck et al. 2005).

Large increases were observed in muscle CSA in some subjects, while no changes or even decreases in muscle CSA were observed in some subjects. Therefore, the subjects were divided to high-responder (HR) and low-responder (LR) groups according to the changes in the CSA of quadriceps femoris during the experimental resistance training period. However, no differences were observed between the HR and LR groups in the relative changes of AR and IGF-I mRNA expression during the resistance training. The great increase in maximal isometric force, also in the LR group, during the experimental training period may be explained by neuromuscular adaptations due to resistance training (Häkkinen et al. 2001). The results suggest that the trainability of the individuals, i.e. potent to increase muscle mass due to resistance training, may not be explained by the before the training values of AR and IGF-I mRNA

expression or serum total and free testosterone concentrations, or by their changes during the resistance training.

The SM showed higher serum levels of total and free testosterone concentrations than AM and OM. The results of study VI did not show any relationships between basal levels or training-induced changes in serum total or free testosterone concentrations and IFG-1 mRNA levels. However, the data indicate possible ageing and long-term training-induced effects in testosterone metabolism. The only statistically significant change during the present 6-month experimental resistance training period in serum T and FT concentrations was the decrease in free testosterone in OM. In general, the present results suggest that mean serum concentrations of testosterone in different subject groups do not seem to change systematically during the resistance training lasting for a few moths, although some periodical shifts may take place. Some evidence suggests that endogenous testosterone levels are related to the magnitude of resistance training-induced gain in muscle strength (Häkkinen and Pakarinen 1994, Häkkinen et al. 2000b, Izquierdo et al. 2001) and mass (Häkkinen et al. 2001). We found the significant relationships between serum basal testosterone concentration and muscle strength and CSA before and after the training period in the total group of subjects. These results suggest that serum testosterone concentrations may have some role in maintaining muscle mass and strength during aging as well as long-term resistance training.

In conclusion, the experimental resistance training period did not induce significant changes in the mean levels of AR and IGF-I mRNA expression but large interindividual changes were observed. No relationships were found between the AR and IGF-I expression levels and basal serum total and free testosterone concentrations and muscle mass and strength. Therefore, the data suggest that the resistance training-induced changes in muscle CSA may not be related to the changes in AR or IGF-I mRNA levels in the trained muscles.

# 7 PRIMARY FINDINGS AND CONCLUSIONS

The main findings and conclusions of the present study can be summarised as follows:

**1) Neuromuscular, hormonal and molecular responses to single heavy resistance exercise**

a) Increased resistance exercise intensity induced by the forced repetitions (FR) training protocol led to greater acute hormonal responses and neuromuscular fatigue compared to the traditional maximum repetition (MR) training system. The FR loading stimulated especially the secretion of growth hormone more than MR did. The results also showed that the acute testosterone responses were greater in strength athletes than in non-athletes indicating resistance training-induced adaptations in the endocrine system. Therefore, the present results suggest that the forced repetition exercise system may be beneficial for the development of muscle mass and muscle strength during strength training especially in strength athletes.

b) The recovery of the isometric force was slower after the FR than MR loading. Because the degree of acute neuromuscular fatigue and the time needed for recovery may differ considerably between the MR and FR loading protocols, there is a need to optimize the contents and the frequency of different training sessions in order to create proper resistance training programs to match the individual requirements of athletes.

c) The present study further showed that the short (2min) and long (5min) rest periods between the sets during heavy resistance exercise induced similar acute hormonal and neuromuscular responses. Thus, it could be suggest that there may be several different ways to create exercise conditions leading to large acute hormonal responses, at least when hypertophic type of resistance exercises were performed by young strength trained men.

d) Heavy resistance exercise induced significant increases in IGF-I splice variants IGF-IEa and mechano growth factor (MGF) mRNA expression determinated at 48h after the exercise in skeletal muscle of strength trained men. However, androgen receptor (AR) mRNA expression of the loaded muscles did not alter significantly due to the present resistance exercise. Increased IGF-IEa and MGF mRNA expression due to heavy resistance exercise supports the concept that they may be related to regenerative processes after the exercise and therefore, contribute to training-induced muscle hypertrophy.

**2) Neuromuscular, hormonal and molecular adaptations to long-term resistance training**

a) The present results suggest that adaptations in the endocrine system can take place, so that acute hormonal responses to single heavy resistance exercise may change due to long-term resistance training. The findings further suggest that serum basal testosterone concentrations and resistance training-induced changes in acute testosterone responses to a single resistance exercise session may be important factors for training-induced muscle hypertrophy as well as for strength development of the trained muscles.

b) The length of the recovery times between the sets did not influence the magnitude of long-term training adaptations in muscle strength and mass. Therefore, the length of the rest periods between the sets may not have an important role to the magnitude of acute resistance exercise-induced responses as well as to long-term training adaptations. These results indicate a need to optimise the volume and/or intensity of resistance exercises especially in previously strength trained athletes to meet the level of adaptation of the neuromuscular and endocrine systems in order to further increase muscle mass and strength.

c) The long-term resistance training did not induce significant changes in the mean levels of AR and IGF-I mRNA expression in strength trained men and untrained older and adult men. Interestingly, large differences were observed between the subjects in the changes of AR and IGF-I mRNA expression during the 6-month experimental resistance training period. Nevertheless, relative changes during the training period or before and after values of AR and IGF-I mRNA expression were not related to corresponding values in basal serum total and free testosterone concentrations and muscle mass and strength. Therefore, these results suggest that the resistance training-induced changes in muscle CSA may not be related to the changes in AR or IGF-I mRNA levels in the trained muscles under the present experimental conditions.

# YHTEENVETO

## Neuromuskulaariset, hormonaaliset ja molekulaariset vasteet voimaharjoittelussa voimaurheilijoilla

Tämän tutkimuksen tarkoituksena oli tutkia yksittäisten voimaharjoitusten sekä pitkäkestoisen voimaharjoittelun aiheuttamia vaikutuksia hormoni- ja hermolihasjärjestelmän toimintaan voimaharjoitelleilla miehillä. Voimaharjoituksesta palautumista seurattiin kahtena tai kolmena harjoituksen jälkeisenä päivänä. Erityisesti tutkittiin harjoitusintensiteetin sekä harjoitussarjojen välisen palautusajan vaikutusta harjoitusvasteisiin. Lisäksi tutkittiin luurankolihaksen miessukupuolihormoni (androgeeni) reseptorien sekä insuliinin kaltaisen kasvutekijän (Insulin-like growth factor I, IGF-I) lähetti-RNA:n ilmentymistä yksittäisen voimaharjoituksen ja pitkäkestoisen voimaharjoittelun seurauksena.

Voimaharjoituksen tehoa voidaan lisätä esimerkiksi pakkotoistomenetelmän avulla. Tällöin avustaja avustaa harjoittelijaa suorittamaan muutaman lisätoiston uupumuksen jälkeen. Tässä tutkimuksessa havaittiin pakkotoistoharjoituksen aiheuttavan suuremman kasvuhormonivasteen ja hermo-lihasväsymyksen kuin perinteinen maksimitoistoharjoitusmenetelmä. Lisäksi pakkotoistokuormituksesta palautuminen oli hitaampaa kuin maksimitoistokuormituksesta. Tämän tutkimusten tulosten perusteella pakkotoistojen käyttö voimaharjoituksessa voi lisätä lihasvoiman ja -massan kehittymistä pitkäkestoisen voimaharjoittelun myötä. Tähän viittaa sen aiheuttama suurempi välitön hormonivaste sekä hermolihasjärjestelmän kuormittuminen.

Lisäksi tässä tutkimuksessa havaittiin lyhyen (2 minuuttia) ja pitkän (5 minuuttia) sarjapalautusajan aiheuttavan samankaltaisen välittömän hormoni- ja hermo-lihasjärjestelmän vasteen, kun kokonaistyön määrä (sarjat x toistot x kuorma) pidettiin samana. Tässä tutkimuksessa käytetyillä sarjapalautuksilla (2 ja 5 minuuttia) ei myöskään ollut vaikutusta lihasvoiman ja -massan kasvuun pitkäkestoisen voimaharjoittelun myötä aiemminvoimaharjoitelleilla miehillä. Tutkimuksen tulokset viittaavat testosteronin perustason sekä yksittäisen voimaharjoituksen aiheuttaman testosteronivasteen olevan merkittävä tekijä lihaksen voimantuoton ja massan kasvuun pitkäkestoisen voimaharjoittelun myötä. Yksi tämän tutkimuksen keskeisimmistä löydöistä oli havainto voimaharjoituksen aiheuttaman hormonivasteen muuttumisesta pitkäkestoisen voimaharjoittelun myötä, joka voi olla seurausta hormonijärjestelmän mukautumisesta harjoitteluun. Voimaharjoituksen aiheuttama välitön hormonivaste voi vaimentua pitkäkestoisen systemaattisen voimaharjoittelun myötä. Toisaalta voimaharjoitelleilla henkilöillä havaittiin harjoittelemattomia suurempi välitön hormonivaste.

Yksittäinen voimaharjoitus sai aikaan erityisten IGF-I ilmentymismuotojen IGF-IEa:n ja MGF:n (mechano growth factor) lähetti-RNA:n pitoisuuden kasvun. Androgeeni reseptorien ilmentymiseen ei yksittäisellä voimaharjoituksella ollut tilastollisesti merkitsevää vaikutusta. IGF-IEa:n ja MGF:n lähetti-

RNA pitoisuuden kasvu voimaharjoituksen myötä viittaa niiden merkitykseen lihaksen kuormituksesta palautumisessa ja ne voivat siten edesauttaa lihaksen mukautumista voimaharjoitteluun. Pitkäkestoinen voimaharjoittelu ei muuttanut tilastollisesti merkitsevästi lihaksen androgeenireseptorien tai IGF-I lähetti-RNA pitoisuuksia. Yksilöllinen vaihtelu oli kuitenkin huomattavaa kyseisten geenien ilmentymisessä.

Tutkimuksen perusteella erilaisten voimaharjoitusten aiheuttamat hormonivasteet ja lihasväsymys sekä palautuminen voimaharjoituksesta vaihtelevat, joten harjoitusohjelmassa tulee suunnitella harjoituksen suoritustapa ja harjoituskertojen määrä viikossa vastaamaan urheilijan tai kuntoilijan yksilöllisiä tarpeita. Tuloksista voidaan myös päätellä, ettei voimaharjoittelussa ole ainoastaan yhtä tapaa aikaan saada huomattava välitön hormonivaste vaan tähän voidaan päästä monilla erilaisilla, mutta kuitenkin kuormittavilla voimaharjoitustavoilla. Erityisesti aiemmin voimaharjoitelleilla henkilöillä korostuu harjoitusmäärän ja intensiteetin ohjelmointi lyhyellä ja pitkällä aikavälillä lihasvoiman ja -massan edelleen kehittymisen kannalta.

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