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# RECOVERY AFTER HEAVY RESISTANCE EXERCISE AND SKELETAL MUSCLE ANDROGEN RECEPTOR AND INSULIN-LIKE GROWTH FACTOR-I ISOFORM EXPRESSION IN STRENGTH TRAINED MEN

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## ABSTRACT

Ahtiainen, JP, Lehti, M, Hulmi, JJ, Kraemer, WJ, Alen, M, Nyman, K, Selänne, H, Pakarinen, A, Komulainen, A, Kovanen, V, Mero, AA, and Häkkinen, K. Recovery after heavy resistance exercise and skeletal muscle androgen receptor and insulin-like growth factor-I isoform expression in strength trained men. *J Strength Cond Res* 25(3): 767–777, 2011—The effects of heavy resistance exercise on skeletal muscle androgen receptor (AR) protein concentration and mRNAs of AR, insulin-like growth factor-I (IGF)-IEa, and mechano growth factor (MGF) expression were examined from biopsies of vastus lateralis (VL) muscle before and 48 hours after heavy resistance exercise (5 × 10 repetition maximum [RM] leg press and 4 × 10RM squats) in 8 adult strength trained men. The present exercise induced an acute decrease in maximal isometric force and increased serum total testosterone (T) and free testosterone (FT) concentrations. During 2 recovery days, maximal isometric force and subjective perception of physical fitness remained significantly lowered, whereas serum creatine kinase activity, subjective muscle soreness, and muscle swelling (i.e., thickness of VL by ultrasound) were significantly increased compared to pre-exercise values. Subjective perception of physical fitness was followed up to 7 days, and by 6 days postexercise, it was elevated above the pre-exercise level. Basal T and FT concentrations remained unaltered after the

exercise. No statistically significant changes were observed in AR protein or mRNA expression, but IGF-IEa ( $p < 0.05$ ) and MGF ( $p < 0.05$ ) mRNA expression were increased compared to pre-exercise levels. These findings indicate that IGF-IEa and MGF responses may be related to acute regenerative processes in muscle because of exercise and may contribute to muscular adaptation to resistance exercise. Subjective perception of physical fitness suggests that recovery over a pre-exercise level of the present type of heavy resistance exercise can take approximately 6 days.

**KEY WORDS** testosterone, muscle damage, gene expression

## INTRODUCTION

**M**echanical strain on muscle tissue during heavy resistance exercise, depending on the volume and intensity of the loading protocol, may produce structural disruptions to contractile elements within the activated muscle fibers leading to muscle soreness and temporary impairment of muscle function after exercise (11). During the days after resistance exercise, the trained muscles undergo a remodeling process and muscle function recovers to the pre-exercise level, or even beyond that (i.e., supercompensation). With training, the cumulative effects of repeated bouts of heavy resistance exercise produce accumulations of specific proteins eventually leading to increases in muscle mass and strength. Local growth factors expressed by the loaded muscle tissue, such as insulin-like growth factor-I (IGF-I) splice variants IGF-IEa and mechano growth factor (MGF), and systemic hormones, such as testosterone, are part of a signaling network that is involved

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with the regulation of exercise-induced remodeling processes of skeletal muscle (11,12,15,25).

Insulin-like growth factor-I could modulate skeletal muscle adaptation to resistance training by stimulating muscle protein synthesis (7) and activating satellite cells to proliferate and differentiate (2). The IGF-IEa and MGF are IGF-I splice variants that are expressed and released from the overloaded skeletal muscle (14,30,44). It has been suggested that resistance exercise-induced damage or mechanical strain to skeletal muscle cells may increase mRNA expression and availability, especially MGF in muscle cells (12). Accordingly, previous studies with younger and older men and women have shown decreased (33) or unchanged (4,20) IGF-IEa mRNA expression within 6 hours after resistance exercise as compared to pre-exercise levels. At 24 hours after resistance exercise, IGF-IEa mRNA expression has been increased (14), and at 48 hours after resistance exercise increased (31) or not changed (20). Expression of MGF mRNA has been increased (15,42) or not changed (4,20) over the first 6 hours after resistance exercise in comparison to the pre-exercise level. Increases in MGF mRNA expression have also been observed at 24 hours (only in younger men) (15,22) but not at 48 hours (20) after exercise. After eccentric resistance exercise, increases in IGF-IEa mRNA expression has been observed from 2 to 5 and 16 days after exercise and MGF mRNA expression from 6 to 48 hours and 16 days after exercise, compared to pre-exercise values (29,32). Moreover, IGF-IEa protein concentration was increased at 2 and 5 days and MGF at 2 days after eccentric exercise (32). Although the comparison of above-mentioned studies is challenging, because of different loading protocols, biopsy sampling times, and subject characteristics used, these findings indicate that resistance exercise can induce increases in gene expression of IGF-IEa and MGF in human skeletal muscle over several days after exercise. Although the present evidence for a functional implication of these IGF-I isoforms in muscle growth is limited, it has been proposed that they may cause increased myofibrillar protein synthesis and induce satellite cell proliferation and differentiation, followed by fusion of differentiated myoblasts to hypertrophying myofibers (12).

Testosterone is an anabolic hormone, which improves net muscle protein balance by stimulating muscle protein synthesis, decreasing muscle protein degradation, and improving the reuse of amino acids (18). Only about 2% of testosterone within the circulation is unbound and, therefore, free to enter cells to affect its biological actions by binding to androgen receptors (ARs) to mediate the effects of testosterone upon target tissues. After binding to AR, androgen activates AR, and the liganded AR acts as a transcription factor by binding to specific androgen response elements in target gene promoters, causing activation or repression of gene transcription and, subsequently, protein synthesis. The ARs are expressed also in satellite cells (8) and, therefore, testosterone may also activate and increase the number of satellite cells (6). The AR expression on skeletal muscles may

be, at least in part, related to the exercise-induced changes in serum testosterone concentrations because androgens are shown to be important regulators of AR mRNA and protein expression through transcriptional and posttranscriptional mechanisms (45). Testosterone may also have androgen receptor-independent pathways that may work in addition to genomic AR testosterone actions in the development of skeletal muscle hypertrophy (34). However, it could be postulated that the changes in AR content is crucial in mediating the effects of testosterone in skeletal muscle. Only a limited number of studies have been published regarding AR mRNA or protein response to acute resistance exercise in humans. The AR mRNA expression has been increased at 48 hours after heavy resistance exercise (5,20,43) but not at 1 hour (20), 4 hours (26), or at 24 hours (26,36) after exercise. Androgen receptor protein concentration has been shown to decrease (35,41), not change (20,35,38) or increase (24) within 3 hours after heavy resistance exercise and not changed (20) or increased at 48 hours (43) after resistance exercise. These previous results suggest that a single heavy resistance exercise bout may increase AR mRNA and protein expression in exercised skeletal muscle.

To increase muscle mass and strength, several sets of resistance exercises (e.g., leg presses and squats for lower body muscle groups) with moderately short rest periods between the sets are typically performed in strength athletes, such as bodybuilders, for maximal gain in muscle mass and strength (23). This study was designed to investigate the effects of hypertrophic type of heavy resistance exercise on AR, IGF-IEa, and MGF expression in previously strength trained men. During the exercise bout and 48-hour recovery period, exercise-induced changes in serum testosterone concentrations, and muscle cell disruption indicators, such as serum creatine kinase (CK) activity, and muscle strength, soreness, and swelling were measured. Thus, the purpose was also to study relationships between exercise-induced changes in AR, IGF-IEa and MGF expression, serum testosterone, and selected muscle cell disruption markers. Moreover, the subjective recovery after the exercise was followed up to 7 days.

## METHODS

### Experimental Approach to the Problem

The novel aim of this study was to examine whether the IGF-IEa, MGF, and AR expression is induced by the heavy resistance exercise in strength trained men, and whether they are related to the selected recovery markers. For that purpose, experimental heavy resistance exercise session was performed that included exercises for the leg extensor muscles as usually used by strength athletes to increase muscle strength and mass. That type of resistance exercise induces muscle cell disruptions and acute decrease in muscle force production. During the following days after the exercise, loaded muscles recovers to the pre-exercise level, or even beyond. These regenerative processes are thought to be regulated by the androgens and local production of growth factors, such as IGF-I splice

variants. Therefore, muscle biopsies were collected from the vastus lateralis (VL) muscle before and after the exercise, and serum testosterone concentrations, muscle force of the leg extensors, and muscle damage markers were determined during the exercise and recovery days after the exercise.

**Subjects**

Eight recreationally strength trained adult men (mean ± SD; age: 29 ± 7 years; height: 183 ± 5 cm; body mass: 88 ± 12 kg; body fat: 16 ± 3%) who had 6 ± 3 years of experience in continuous strength training aiming to increase muscle mass and strength volunteered to participate in the study. The habitual resistance training programs of the subjects have included 3–4 training sessions per week. Different body parts were trained on different training days using multiple exercises (3–4 sets per exercise) and loading ranges of repetitions between 6 and 12 repetition maximum [RM] with 2–5 minutes rest between the sets. Leg muscles were typically trained once per week, and exercises for the leg extensors included squats, leg presses, and knee extensions.

The subjects were screened by a physician for inclusion in this study including a resting electrocardiogram. Exclusion criteria included cardiovascular and pulmonary diseases, malfunctions of the thyroid gland, diabetes, or any other disease that may have precluded the ability to perform the exercise testing. Exclusion criteria included also medications known to influence the endocrine system, heart rate, and cardiovascular or neuromuscular performance. In accordance with the Declaration of Helsinki all subjects were carefully informed about the possible risks and benefits of the study, and all subjects signed a written consent form before participation in the study. The ethics committee of the University of Jyväskylä approved the study.

**Experimental Design**

This study design and the timetable of the measurements are presented in Figure 1. Heavy resistance exercise was used to investigate exercise-induced responses of IGF-IEa, MGF, and AR mRNA expression, and AR protein concentration in skeletal muscle. Also, serum total testosterone (T) and free testosterone (FT) concentrations, maximal isometric muscle force, and muscle cell disruption indicators of CK enzyme activity, muscle swelling, and subjective muscle soreness were determined. Recovery after the exercise was measured on 2 consecutive days after the experimental heavy resistance exercise at the

corresponding time of the day as the subject’s experimental heavy resistance exercise. Subjective perception of physical fitness was followed up for 7 days after the exercise. The subjects were familiarized with the testing procedures 1 week before the study. Anthropometrical measurements were also determined at this time. Additionally, 1 venous blood sample was drawn in a fasting condition to measure serum basal T and FT concentrations (i.e., control day). To minimize variability in the measures, the subjects were asked to refrain from any strenuous physical activity for at least 3 days before and 2 days after the measurements and maintain a similar activity and dietary behavior pattern throughout the study. Furthermore, all measurements were always performed at the same time of day to exclude the effects of diurnal variations.

**Procedures**

*Experimental Heavy Resistance Exercise.* The experimental heavy resistance exercise (performed between 12 AM and 5 PM) was hypertrophic in nature and comprised 5 sets of 10RM of bilateral leg presses (David 210, David Sports Ltd., Helsinki, Finland) from a knee angle of 60–180° (= knee straight) with a 2-minute recovery between the sets and 4 sets of 10 repetition maximums of squats (Smith machine, HUR, Kokkola, Finland) from a knee angle of 70–180° (=knee straight) with a 2-minute recovery between the sets, and 4 minutes between the exercises. The knee angle of the squats was controlled by an electronic goniometer with a sound signal. The subjects were instructed not to consume any food or beverages for 3 hours before the exercise.

*Anthropometry.* Body mass and height were measured and the percentage of body fat was estimated by measuring skinfold

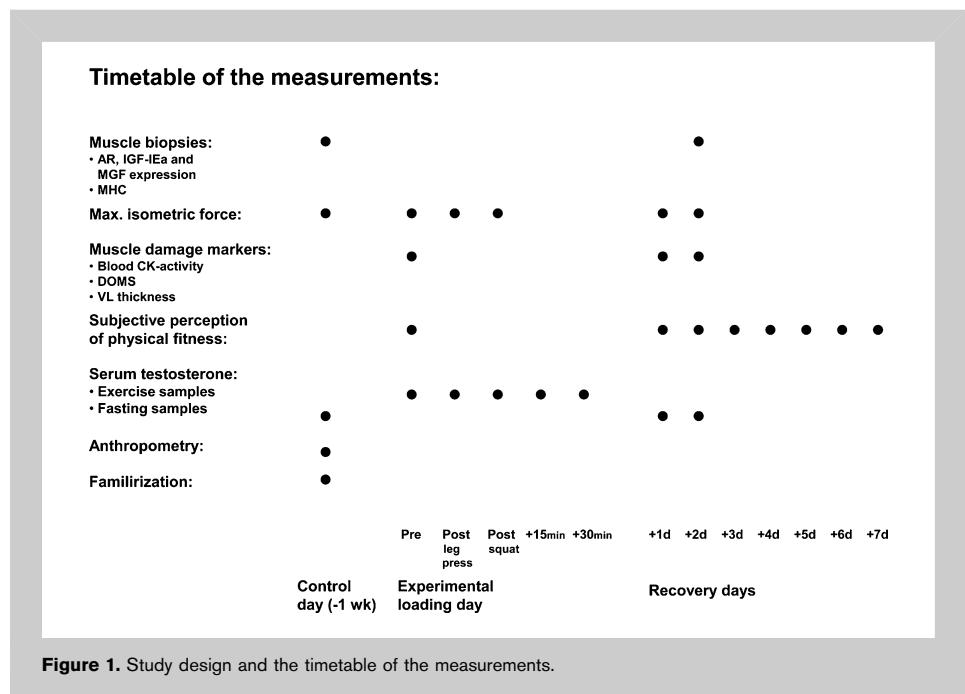


Figure 1. Study design and the timetable of the measurements.

thickness at 4 different sites (biceps, triceps, subscapularis, and suprailiac) (9).

**Muscle Strength Measurements.** Maximal voluntary isometric force of the bilateral leg extension action was measured by an electromechanical dynamometer at a knee angle of 107°. A minimum of 3 trials was completed for each subject and the best performance trial, with regard to maximal peak force, was used for the subsequent statistical analysis. The force signal was recorded on a computer and, thereafter, digitized and analyzed with a Coda TM computer system (Dataq Instruments, Inc. Akron, OH, USA). Maximal peak force was defined as the highest value of the force (N) recorded during the bilateral isometric leg extension. During the experimental exercise session, maximal isometric force was measured before the exercise (Pre), after the leg presses (Post Leg Press) and immediately after the exercise session (Post Squat).

**Blood Collection and Biochemical Analyses.** Blood samples were drawn from an antecubital vein before (Pre), immediately after the leg presses (Post Leg Press), immediately after the whole exercise session (Post Squat), and 15 (Post 15 minutes) and 30 minutes (Post 30 minutes) after the exercise. Fasting blood samples were obtained before the exercise (control day), and on the first and second mornings after the exercise. These samples were taken at 7.30–8.30 AM after 12 hours of fasting and 8 hours of sleep for the determination of basal serum T and FT concentration. Serum samples were kept frozen at  $-80^{\circ}\text{C}$  until assayed. Serum T concentrations were measured by the Chiron Diagnostics ACS:180 automated chemiluminescence system using ACS:180 analyzer (Medfield, MA, USA). The sensitivity of the testosterone assay was  $0.42\text{ nmol}\cdot\text{L}^{-1}$ , and the intraassay coefficient of variation was 6.7%. The concentrations of serum FT were measured by radioimmunoassay using kits from Diagnostic Products Corp. (Los Angeles, CA, USA). The sensitivity of the FT assay was  $0.52\text{ pMol}\cdot\text{L}^{-1}$ , and the intraassay coefficient of variation was 3.8%. All samples for each test subject were analyzed in the same assay. Serum CK activity was determined using a Creatine Kinase kit (CK-NAC MPR2, refno. 1442376, Roche Diagnostic, Mannheim, Germany).

**Recovery after the Loading.** The muscle cell disruption markers and the rate of recovery after the experimental heavy resistance exercise were studied at 24 and 48 hours after the exercise. Serum CK activity, subjective muscle soreness, and muscle swelling were determined as markers of muscle disruption possibly caused by the resistance exercise. Venous blood samples were drawn for determination of serum CK activity. Subjective muscle soreness was determined by a questionnaire and rated on a Likert scale of 0 (= no pain) to 5 (= maximum pain) for the overall muscle soreness of the quadriceps muscles during palpation of the muscle belly and walking up and down stairs, squatting, and sitting down. Muscle swelling was assessed by measuring muscle thickness from the left m.VL at the middle of the femur length with

a compound ultrasonic (US) scanner (SSD-190 Aloka Fanasonic, Tokyo, Japan) and a 5-MHz convex transducer. The scanning head was coated with water-soluble transmission gel to provide acoustic contact without depressing the dermal surface. From the scanned image, the distance between the subcutaneous adipose tissue–muscle interface and intramuscular interface (i.e., aponeurosis) was defined as muscle thickness. At each US measurement, 2 consecutive measurements were taken and then averaged for further analyses. The recordings of maximal isometric force evaluated the recovery of the muscular performance after the exercise. Blood samples for the determination of basal hormone concentrations were drawn from each subject after 12 hours of fasting and approximately 8 hours of sleep on the first and second mornings after the loadings. In addition, subjective perception of physical fitness (i.e., subjectively perceived muscular fatigue and recovery) was followed up to 7 days postexercise, and it was rated on a visual analog scale from 0 to 120 mm (100 mm = subjective estimation of maximal muscular fitness within a week before the experimental resistance exercise).

**Muscle Biopsy Procedure.** Muscle samples were obtained before and 48 hours after the exercise from the middle portion of the right VL muscle by the use of the percutaneous needle biopsy technique. The muscle biopsy after the exercise was obtained approximately 2 cm laterally to the previous biopsy location. The surrounding area was cleaned with an antiseptic solution and local anesthetics (2 ml lidocaine–adrenalin, 1%) were administered subcutaneously before incision of the skin. A needle (5 mm) was inserted into the muscle belly and, with the aid of suction, approximately 100 mg of muscle tissue was extracted. The muscle sample was cleaned of any visible connective and adipose tissue and blood, frozen rapidly in isopentane, which was cooled to  $-160^{\circ}\text{C}$  in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  until the analysis.

**RNA Extraction and cDNA Synthesis.** Muscle samples were homogenized with FastPrep (Bio101 Systems, Carlsbad, CA, USA) tissue homogenizer by using Lysing Matrix D (Q-Biogene, Carlsbad, CA, USA). Total RNA was extracted from 20 to 50 mg of muscle tissue using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The RNA concentration was determined photometrically at 260 nm using an optical density ( $\text{OD}_{260}$ ) unit equivalent to  $40\text{ }\mu\text{g}\cdot\text{ml}^{-1}$ . The muscle total RNA concentration was calculated on the basis of total RNA yield and the weight of the analyzed sample. The data (not shown) revealed that total RNA content of the samples did not change during this study. An  $\text{OD}_{260}/\text{OD}_{280}$  ratio of approximately 2.0 and gel electrophoresis showed that our extraction yielded protein and DNA-free and undegraded RNA, respectively. Three micrograms of total RNA was reverse transcribed to synthesize cDNA according to the manufacturer's instructions in a total volume of 50  $\mu\text{L}$  using a High Capacity cDNA Archive Kit (Part Number 4322171, Applied Biosystems, Foster City, CA, USA).

**TABLE 1.** Primers used in RT-PCR.\*

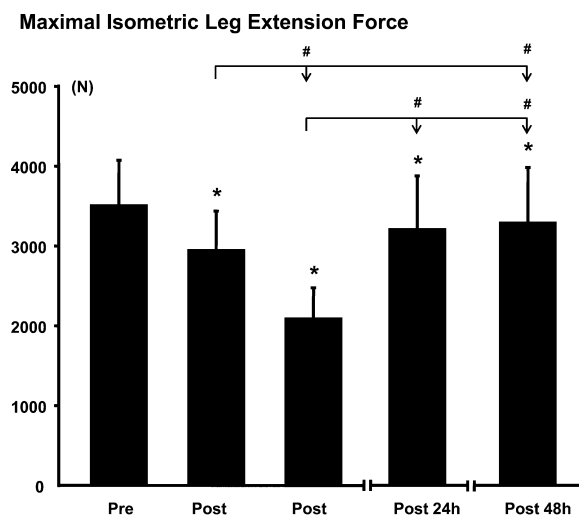
Primer name	Sequence (5' to 3')	Product size (bp)
IGF-IEa forward	ATCTAAGGAGGCTGGAGATGTATTGC	114
IGF-IEa reverse	TCAAATGTA CTTCCTTCTGGGTCTTG	
MGF forward	CGAAGTCTCAGAGAAGGAAAGG	150
MGF reverse	ACAGGTA ACTCGTGCAGAGC	
AR forward	TTGTCCACCGTGTGTCTTCTTCTGC	225
AR reverse	TGCACTTCCATCCTTGAGCTTGGC	
GADPH forward	GTGATGGGATTTCCATTGAT	206
GADPH reverse	GGAGTCAACGGATTGGT	

\*RT-PCR = reverse transcriptase polymerase chain reaction; MGF = mechano growth factor; AR= androgen receptor; GADPH = glyceraldehyde 3-phosphate dehydrogenase; IGF = insulin-like growth factor-I.

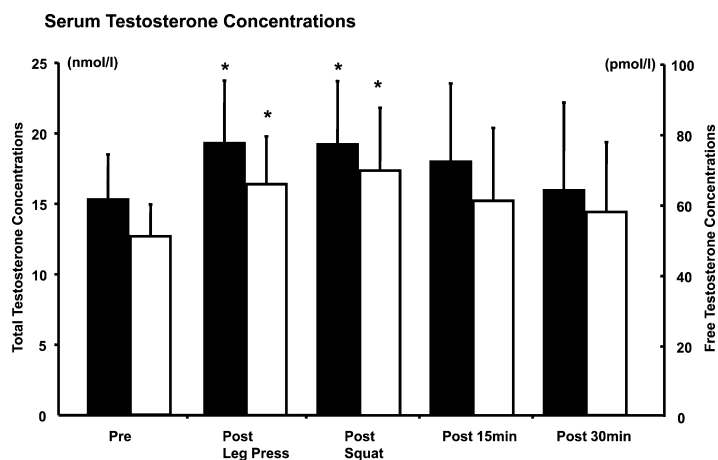
**Real-Time Reverse Transcriptase Polymerase Chain Reaction.** The mRNA expression of AR, IGF-IEa and MGF, and glyceraldehyde 3-phosphate dehydrogenase (GADPH) was determined using ABI 7300 Real-Time quantitative polymerase chain reaction (PCR) System (Applied Biosystems). The PCR for AR, IGF-IEa, and MGF was performed with SYBR green mix (Cat. no. 204143, QuantiTect, Qiagen, Crawley, United Kingdom). The primers used for real-time PCR were designed and analyzed by Oligo Explorer and Analyzer software (Kuopio University, Finland), and these were synthesized by Oligomer Ltd. (Helsinki, Finland). The sequences of the primers and product sizes for IGF-IEa, MGF

(15), AR (27), and GAPDH (17) are given in Table 1. Each PCR reaction mixture included 12.5  $\mu$ L of SYBR Green master mix, 1.5  $\mu$ L of both gene specific primers (100  $\mu$ M, diluted 1:19 to dH<sub>2</sub>O), 8.5  $\mu$ L of dH<sub>2</sub>O, and 1  $\mu$ L of template cDNA (diluted 1:3 to dH<sub>2</sub>O). The PCR cycle parameters for IGF-IEa were 50°C for 2 minutes and 95°C for 15 minutes, and 40 cycles of 94°C for 15 seconds (denaturation), 56°C for 30 seconds (annealing), 72°C for 30 seconds (extension), and 81°C for 15 seconds (data acquisition). The PCR cycle parameters for MGF were 50°C for 2 minutes and 95°C for 15 minutes, and 40 cycles of 94°C for 15 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and 77°C for 15 seconds. The

PCR cycle parameters for AR were 50°C for 2 minutes and 95°C for 15 minutes, and 40 cycles of 94°C for 15 seconds, 57°C for 30 seconds, 72°C for 30 seconds and 81°C for 15 seconds. The PCR cycle parameters for GAPDH were 50°C for 2 minutes and 95°C for 15 minutes, and 40 cycles of 94°C for 15 seconds, 50°C for 30 seconds, 72°C for 30 seconds, and 77.5°C for 15 seconds. The specificity of the amplified target sequence was confirmed on observing a single reaction product of the correct 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.



**Figure 2.** Maximal isometric leg extension force (mean  $\pm$  SD) before, during, and after the experimental heavy resistance exercise. \*Significantly different ( $p < 0.05$ ) from the corresponding pre-exercise value. #Significantly different ( $p < 0.01$ ) from the Post Leg Press or Post Squat values.



**Figure 3.** Serum total (black bars) and free (white bars) testosterone concentrations (mean  $\pm$  SD) during and after the experimental heavy resistance exercise. \*Significantly different ( $p < 0.05$ ) from the corresponding pre-exercise value.

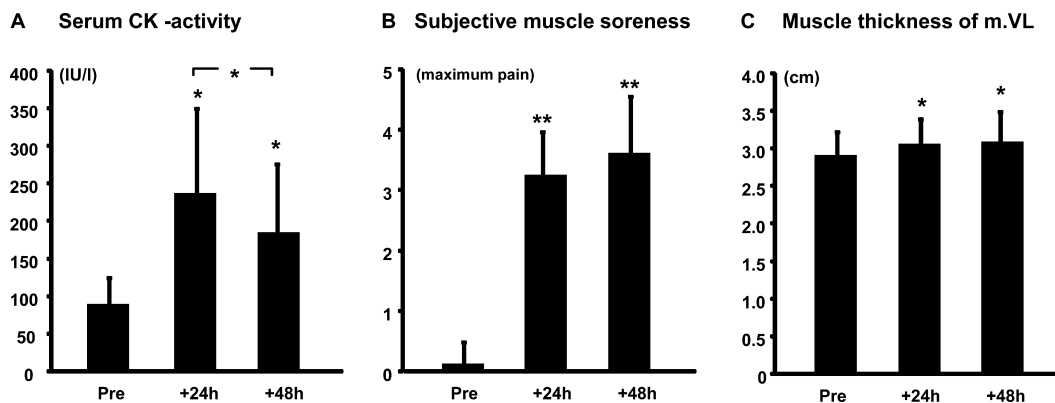
ethylene glycol tetraacetic acid (EGTA), 10 mM ethylenediaminetetraacetic acid (EDTA), 15 mM  $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ , 100 mM  $\beta$ -glycerophosphate, 25 mM NaF, 1 mM  $\text{Na}_3\text{VO}_4$ , 0.5  $\mu\text{g}\cdot\text{mL}^{-1}$  pepstatin, 0.3  $\mu\text{g}\cdot\text{mL}^{-1}$  aprotinin, and 0.5  $\mu\text{g}\cdot\text{mL}^{-1}$  leupeptin, which was added last to the final solution). The homogenate was centrifuged for 15 minutes at  $10,000 \times g$  at  $4^\circ\text{C}$ , and the supernatant was removed for subsequent protein analysis and electrophoresis. The protein concentration of the supernatant was determined using the Lowry-based method (Bio-Rad, Hercules, CA, USA). Each standard (bovine serum albumin [BSA]) and unknown was

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 mRNAs of IGF-IEa and MGF were normalized to a housekeeping gene, GAPDH, which served as an endogenous control to correct for potential variation in RNA loading. GAPDH did not differ between the pre- and postexercise samples in this study (data not shown).

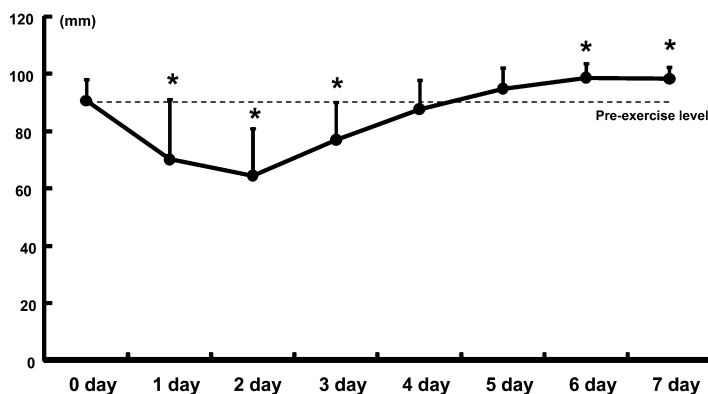
**Western Blot Analysis.** To determine AR protein concentration, each muscle biopsy specimen (~20–30 mg) was homogenized on ice in a 4% (w/v) solution in Mueller lysis buffer (50 mM Hepes, pH 7.4, 0.1% Triton X-100, 4 mM

analyzed for protein concentration by using 5 replicates to increase reliability of the protein quantification.

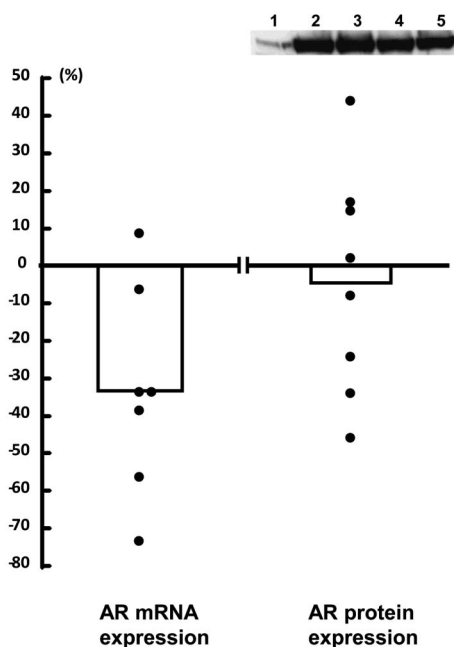
A supernatant volume containing 40  $\mu\text{g}$  of total protein, calculated from the Lowry assay, was incubated for 15 minutes at  $65^\circ\text{C}$  in an equal volume of NuPAGE® (Invitrogen Inc.) lithium dodecyl sulfate (LDS) sample buffer with dithiothreitol (NP0004, NuPAGE®) as a reducer agent. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a 10% polyacrylamide gel (NuPAGE® Novex® Bis-Tris MiniGel, Invitrogen Inc.) and run in a running buffer (1M 3-(N-morpholino) propane sulfonic acid [MOPS], 1M Tris Base, 69.3 mM SDS, and 20.5 mM EDTA) at  $4^\circ\text{C}$  on an ice bed for 2.5 hours at 200 V. Gels



**Figure 4.** Serum creatine kinase (CK) enzyme activity (A), subjective muscle soreness (B), and muscle thickness of vastus lateralis (VL) muscle (C) before (pre), and at 24 and 48 hours after the experimental heavy resistance exercise. \*Significantly different ( $p < 0.05$ , \*\* $p < 0.01$ ) from the corresponding pre-exercise value.

**Subjective perception of physical fitness**

**Figure 5.** Subjective perception of physical fitness (100 mm = subjectively perceived maximal muscular fitness within a week before the experimental resistance exercise) before and days 1–7 after the experimental heavy resistance exercise ( $n = 6$ ). \*Significantly different ( $*p < 0.05$ ) from the corresponding pre-exercise value.

**Changes in AR mRNA and protein expression**

**Figure 6.** Relative changes in androgen receptor (AR) mRNA and protein expression at 48 hours after the experimental heavy resistance exercise when compared to pre-exercise values. Bars represent means, and each circle represents an individual sample. One subject was excluded from the AR mRNA analysis because of unidentified exceptionally high increase in AR mRNA levels ( $>100$ -fold) as compared to other subjects. A representative immunoblot is shown in the upper portion of the figure. Lane 1: positive control; lane 2: subject 1, pre-exercise; lane 3: subject 1, postexercise; lane 4: subject 2, pre-exercise; lane 5: subject 2, postexercise.

were electrophoretically transferred on to a microporous polyvinylidene difluoride (PVDF) membrane (Hybond-P™ 0.45  $\mu$ m, Amersham Biosciences, Piscataway, NJ, USA) at 50 V in a transfer buffer containing 500 mM Bicine, 500 mM Bis-Tris, 20.5 mM EDTA, 0.1% (v/v) antioxidant (NP0005, NuPAGE®), and 20% (v/v) methanol overnight at 4°C. Transferring of proteins was verified by Ponceau S staining. The membrane was blocked with blocking buffer consisting of 1% BSA (A8022, Sigma-Aldrich, St. Louis, MO, USA), 2% Goat Serum (50-062, Zymed Laboratories Inc., San Francisco, CA, USA), and 0.02% Tween® 20 (93773, Fluka

Chemie, Buchs, Switzerland) for 5 hours. Subsequently, the membrane was probed with the polyclonal rabbit antibody against AR (Ab3510, Abcam Inc., Cambridge, United Kingdom) diluted to 1:3,000 overnight at 4°C. After the membrane was washed with Tris buffered saline (TBS), it was incubated for 1 hour with secondary antibody (goat antirabbit, G21234, Molecular Probes, Eugene, OR, USA) diluted to 1:2,000. Then, after the TBS wash, the membrane was incubated with ECL (ECL Plus, Amersham BioSciences) for 5 minutes after which it was exposed to an x-ray film for visualization. Quantification of AR protein was performed by Bio-Rad Molecular Imager (Chemi Doc XRS System) and Quantity One (version 4.6.3) 1-D analysis software (Bio-Rad Laboratories Inc.). From these images, the band intensity (intensity multiplied by area) was determined and used for subsequent analyses. The AR bands were identified with a positive control of AR (T47D Cell Lysate, Santa Cruz BioTechnology, Santa Cruz, CA, USA) and a colored molecular weight standard (Full Range Rainbow™ Molecular Weight Marker, Amersham Biosciences). All samples of each subject were loaded in the same gel.

**Statistical Analyses**

Data are presented as mean values  $\pm$  SD. The Shapiro-Wilk test was used to test that the variables were normally distributed. Because not all of the data were normally distributed, the comparisons between paired data were made using Wilcoxon's signed rank test. To analyze changes over time with multiple variables, a Friedman test was used with post hoc Wilcoxon test. Linear regression analysis (Spearman's product moment) was used to compare association between variables. The level of significance was set at  $p \leq 0.05$ .

## RESULTS

### Maximal Bilateral Isometric Leg Extension Force

No changes were observed in maximal isometric force between control and loading day (data not shown). Maximal isometric force decreased during the entire course of the exercise session down to  $60 \pm 12\%$  (from  $3,505 \pm 568$  N to  $2,087 \pm 389$  N,  $p < 0.05$ ) of pre-exercise level (Figure 2). Maximal isometric force was still  $8 \pm 10\%$  lowered ( $p < 0.05$ ) on the second day of recovery after the exercise as compared to the prelevel.

### Serum Hormone Concentrations

Serum T concentrations increased after the entire course of the exercise from  $15.3 \pm 3.2$  to  $19.2 \pm 4.5$  nmol·L<sup>-1</sup> ( $p < 0.01$ ) (Figure 3). Serum FT concentrations increased after the entire course of the exercise from  $50.6 \pm 9.1$  to  $69.4 \pm 17.9$  pmol·L<sup>-1</sup> ( $p < 0.01$ ) (Figure 3). Basal T concentrations (from  $23.8 \pm 7.4$  to  $23.1 \pm 7.4$  and  $22.2 \pm 9.1$  nmol·L<sup>-1</sup> at pre, +24 and +48 hours, respectively) and FT concentrations (from  $86.0 \pm 17.3$  to  $80.1 \pm 18.9$  and  $75.7 \pm 33.2$  pmol·L<sup>-1</sup> at pre, +24 and +48 hours, respectively) remained unaltered compared to the control day values.

### Muscle Cell Disruption Markers

Serum CK activity increased from  $90 \pm 34$  to  $238 \pm 111$  IU·L<sup>-1</sup> ( $p < 0.05$ ) and  $185 \pm 91$  IU·L<sup>-1</sup> ( $p < 0.05$ ) at 24 and

48 hours after the exercise, respectively (Figure 4). Subjective muscle soreness (0 = “no pain” to 5 = “maximum pain”) increased from  $0.1 \pm 0.4$  to  $3.3 \pm 0.7$  ( $p < 0.01$ ) and  $3.6 \pm 0.9$  ( $p < 0.01$ ) at 24 and 48 hours after the exercise, respectively (Figure 4). The VL thickness increased from  $2.9 \pm 0.3$  to  $3.1 \pm 0.3$  cm ( $p < 0.05$ ) and  $3.1 \pm 0.4$  cm ( $p < 0.05$ ) at 24 and 48 hours after the exercise, respectively (Figure 4). Subjective perception of physical fitness was decreased at days 1–3 ( $p < 0.05$ ) after the exercise and increased at days 6 and 7 ( $p < 0.05$ ) after the exercise when compared to the corresponding pre-exercise values (Figure 5).

### Androgen Receptor mRNA Expression and Protein Concentration

No statistically significant changes were observed in mean AR protein concentration or mRNA expression after the exercise in comparison to the pre-exercise values (Figure 6).

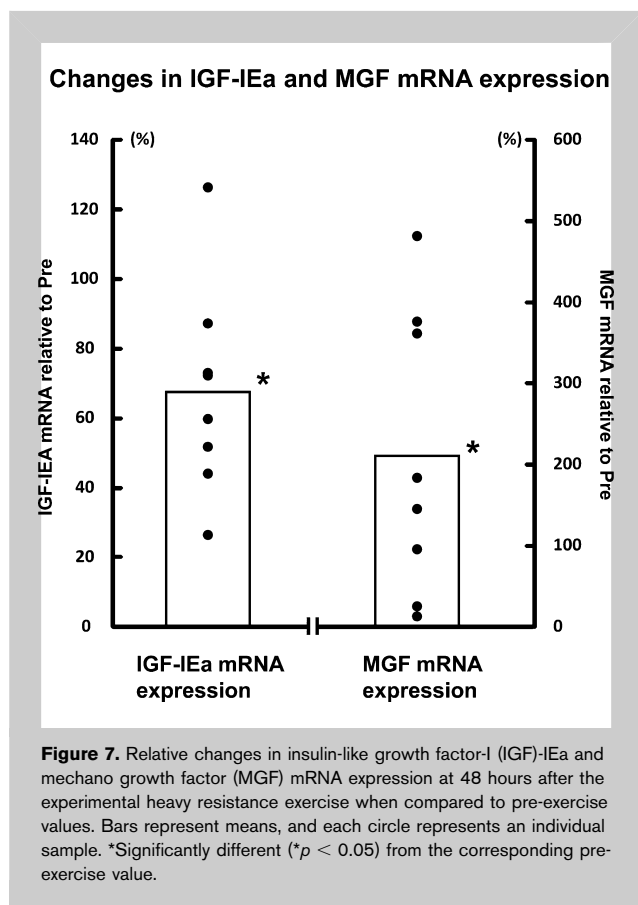
### Insulin-Like Growth Factor-IEa and Mechano Growth Factor mRNA Expression

When compared to the corresponding pre-exercise value, a significant increase (a fold change of  $1.7 \pm 0.3$  from the pre-exercise value,  $p < 0.05$ ) occurred in IGF-IEa mRNA expression at 48 hours postexercise. The MGF mRNA expression also increased (a fold change of  $3.1 \pm 1.8$ ,  $p < 0.05$ ) compared to pre-exercise values (Figure 7). A positive correlation ( $r = 0.74$ ,  $p < 0.05$ ) was found between the changes of IGF-IEa and MGF mRNA expression at 48 hours after the exercise. The changes in MGF mRNA expression correlated with the relative changes of VL thickness at 24 hours postexercise ( $r = 0.80$ ,  $p > 0.05$ ) and also at 48 hours postexercise ( $r = 0.84$ ,  $p > 0.05$ ) when an outlier was excluded from the analysis at 48 hours.

## DISCUSSION

The present heavy resistance exercise induced immediate decreases in isometric muscle strength and increases in serum T and FT concentrations in previously strength trained men. Muscle cell disruption markers, serum CK activity, subjective muscle soreness, and muscle swelling (i.e., thickness of VL muscle) were increased, whereas isometric muscle strength and subjective perception of physical fitness decreased on the first and second days after the exercise. Subjective physical fitness increased over the pre-exercise level at days 6 and 7 after the exercise. Serum basal T and FT remained unaltered over the 2-day recovery period. The main findings of this study were that AR mRNA and protein expression remained unchanged, whereas IGF-IEa and MGF mRNA expressions were increased at 48 hours after the exercise bout when compared to prevalues.

Increases in serum testosterone concentration have shown to induce upregulation in AR mRNA (37) and protein expression (10) in human skeletal muscle. It could be speculated that the exercise-induced changes in serum testosterone concentrations during the exercise bout and basal testosterone levels during the recovery days after the





exercise may be related to AR expression of the loaded muscles. Interestingly, in previous studies, significant relationships have been found (43) and not found (5,20) between the acute resistance exercise-induced increase in serum T and FT concentration and AR mRNA and protein expression in the loaded muscle at 48 hours after the exercise. In this study, no significant relationships were observed between acute or basal changes in serum T and FT concentration and skeletal muscle AR mRNA expression or protein concentration. Furthermore, no statistically significant changes were observed in mean AR mRNA or protein expression at 48 hours after the present exercise bout compared to pre-exercise values. In previous studies, increases of AR mRNA were observed at 48 hours after the heavy resistance exercise (5,20,43), whereas AR protein expression increased (43) or was unchanged (20) at 48 hours after the exercise. In these previous studies, subjects were untrained, whereas the present subjects had already strength trained for several years and their muscles might have already well adapted to resistance exercise training. This suggestion is partly supported by the findings of Kadi et al. (21) who studied the immunohistochemical expression of AR in human trapezius muscles and found that long-term strength training leads to higher proportion of AR-containing myonuclei. Thus, it could be speculated that the training-status can alter a possible AR response to resistance exercise and further changes in muscle AR of well-trained subjects may be attenuated. It is also possible that, in this study, the changes in AR mRNA or protein expression, assuming they occurred, have already come back to pre-exercise levels by 48 hours after the exercise.

Local expression of IGF-I from exercised skeletal muscle is thought to be an important mediator of muscular adaptation to physical training (1,13,19). Specifically, the expression of IGF-I splice variant MGF has been demonstrated to be related to mechanical strain of muscle tissue (30,44). In this study, we did not observe any relationships between the individual changes of IGF-IEa or MGF mRNA expression and other examined variables of exercise-induced muscular strain or damage. The changes in serum testosterone concentrations have been shown to be related to IGF-I mRNA expression in skeletal muscle of young and older men (10,28,31,40). In this study, however, no relationships were observed between the changes of IGF-IEa or MGF mRNA expression at 48 hours after the exercise and acute exercise-induced changes or changes in basal concentrations of serum T and FT. The present finding of increased IGF-IEa and MGF mRNA expression at 48 hours after the exercise compared to pre-exercise values is in agreement with most of the previous studies (15,22,29,32,39) but not all (20). The differences between the present and previous findings are most likely because of the different subject groups and loading protocols used that may affect the timing of peak IGF-IEa and MGF mRNA response to exercise.

Interestingly, the present data showed that the magnitudes of the changes in IGF-IEa and especially in the MGF mRNA were highly individual between the subjects. Furthermore, the positive correlation between IGF-IEa and MGF responses revealed that the greatest changes in both IGF-IEa and MGF mRNA expression were observed in the same subjects. This study also suggests that increases in MGF mRNA levels may be associated with the exercise-induced muscle swelling, which is in line with the suggestion that the expression of MGF is related to mechanical load of the muscle tissue because of exercise. Previous studies suggest that MGF is upregulated earlier after the exercise than IGF-IEa, and they are differentially regulated from each other (16). Thus, it could be speculated that, in some subjects, MGF expression has been peaked earlier and already recovered near pre-exercise levels during the 48-hour recovery after the exercise. It could also be possible that, in some subjects, MGF response to resistance exercise is lower as compared to in other subjects. A similar individual difference in the timing and amplitude of exercise response might be true also with IGF-IEa mRNA expression, but to a lesser scale.

The loading protocol in the present study was hypertrophic in nature, such as used by strength athletes to increase muscle mass and strength. The present exercise bout comprised several sets of 10 repetitions to voluntary exhaustion with relatively short (2-minute) rest periods between the sets. Consequently, acute decreases were observed in muscle strength during and immediately after the exercise. Furthermore, mean blood lactate increased up to  $12.8 \pm 3.2$  mmol·L<sup>-1</sup> ( $p < 0.001$ ) and thickness of VL muscle increased  $15 \pm 7\%$  ( $p < 0.001$ ) immediately after the exercise (unpublished results). The present resistance exercise protocol included concentric and eccentric muscle actions continuing to the temporary concentric failure that potentially induced mechanical strain to contractile elements of skeletal muscle tissue, and consequent disruptions within the activated muscle cells. Accordingly, this study showed exercise-induced decreases in maximal voluntary isometric muscle strength and subjective perception of physical fitness during the 48-hour recovery period after the exercise. Coexistent increases were observed in serum CK activity, subjective muscle soreness, and muscle thickness of VL. These findings indicate that, at least, some level of muscle cell disruptions in unspecified locations of the exercised muscles has occurred because of mechanical strain on muscle tissue during the present heavy resistance exercise. Thus, muscle cell disruption and subsequent regeneration of the loaded muscle cells might explain why we found increased IGF-IEa and MGF mRNA expression at 48 hours after the present exercise bout. Nevertheless, the changes in IGF-IEa and MGF mRNA expression were not related to the changes in muscle cell disruption markers, other than VL thickness, measured in this study. The aim of this study was also to follow the recovery up to 7 days after the exercise. Interestingly, the

subjective perception of physical fitness (Figure 5) showed that the full recovery after the present type of heavy resistance exercise in our strength trained subjects took up to 6 days. We have previously shown that maximal isometric force can be still lowered at 72 hours after very intensive resistance exercise (3). According to our previous and present findings of the recovery profile after the heavy resistance exercise, it could be concluded that the optimal training frequency for the present hypertrophic type of heavy resistance exercise with several 10RM sets of leg presses and squats is approximately as long as 6–7 days.

### PRACTICAL APPLICATIONS

The heavy resistance exercise used in this study in previously strength trained men did not change AR mRNA or protein expression but enhanced gene expression of IGF-IEa and MGF at 48 hours postexercise. The increased skeletal muscle IGF-IEa and MGF mRNA expression after the heavy resistance exercise in this study supports the concept that they may be related to regenerative processes after the resistance exercise, and possibly contribute to training-induced muscular adaptations. The present results showed decreased muscular performance and increased muscle cell disruption markers (serum CK activity, subjective muscle soreness and muscle swelling) up to 48 hours after the exercise. Moreover, subjective perception of physical fitness indicated that total recovery and supercompensation after the exercise protocol used in this study may take up to 6 days. The present findings of the recovery profile after heavy resistance exercise could be taken into account when designing resistance training programs for different subject groups and purposes. Coaches and trainees should allow several days of rest after hypertrophic type of heavy resistance exercise before the next workout for the loaded muscle group(s) is carried out. Coaches and trainees should also consider aims and protocols for, for example, muscle power or endurance training during the recovery days after heavy resistance exercise when maximal muscle force may be still reduced and muscle tissue is in the regenerative phase.

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