Regulation of STARS and its downstream targets suggest a novel pathway involved in human skeletal muscle hypertrophy and atrophy

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Skeletal muscle atrophy is a severe consequence of ageing, neurological disorders and chronic disease. Identifying the intracellular signalling pathways controlling changes in skeletal muscle size and function is vital for the future development of potential therapeutic interventions. Striated activator of Rho signalling (STARS), an actin-binding protein, has been implicated in rodent cardiac hypertrophy; however its role in human skeletal muscle has not been determined. This study aimed to establish if STARS, as well as its downstream signalling targets, RhoA, myocardin-related transcription factors A and B (MRTF-A/B) and serum response factor (SRF), were increased and decreased respectively, in human quadriceps muscle biopsies taken after 8 weeks of both hypertrophy-stimulating resistance training and atrophy-stimulating de-training. The mRNA levels of the SRF target genes involved in muscle structure, function and growth, such as α-actin, myosin heavy chain IIa (MHCIIa) and insulin-like growth factor-1 (IGF-1), were also measured. Following resistance training, STARS, MRTF-A, MRTF-B, SRF, α-actin, MHCIIa and IGF-1 mRNA, as well as RhoA and nuclear SRF protein levels were all significantly increased by between 1.25- and 3.6-fold. Following the de-training period all measured targets, except for RhoA, which remained elevated, returned to base-line. Our results show that the STARS signalling pathway is responsive to changes in skeletal muscle loading and appears to play a role in both human skeletal muscle hypertrophy and atrophy.

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Abbreviations IGF-1, insulin-like growth factor-1; MRTF-A and -B, myocardin-related transcription factor-A and -B; MHCIIa, myosin heavy chain isoform IIa; RPLPO, acidic ribosomal phosphoprotein PO; SRF, serum response factor; STARS, striated activator of Rho signalling.

Skeletal muscle is a unique tissue that it is characterized by its strong capacity to alter its size as well as its fibre composition in response to changes in mechanical loading (Fluck & Hoppeler, 2003). A reduction in skeletal muscle size (atrophy), integrity and contractile activity is associated with denervation, neurological disorders, disease, orthopaedic trauma and a reduction in physical activity (Jagoe *et al.* 2002; Glass, 2003). In contrast, an increase in muscle size (hypertrophy) and contractile activity can be stimulated by resistance training (Widrick *et al.* 2002; Leger *et al.* 2006*a*), while electrical stimulation can rescue the loss of muscle mass in spinal cord injured patients (Baldi *et al.* 1998). Changes in skeletal muscle size, structure and function are initiated by extracellular signals (Schiaffino *et al.* 2007). These signals result in the intracellular activation of transcriptional co-activators and transcription factors with a consequent increase in their target genes that encode proteins mediating contractile, metabolic and structural functions of the skeletal muscle. The intracellular signals influencing human skeletal muscle size, structure and function are not well understood and elucidating these pathways will assist with combating the debilitating effects of muscle wasting.

Striated muscle activator of Rho signalling (STARS) (also known as ms1) is a novel actin-binding protein specifically expressed in cardiac and skeletal muscle (Arai *et al.* 2002; Mahadeva *et al.* 2002). In cardiac tissue STARS mRNA is upregulated in response to pressure overload (Arai *et al.* 2002; Mahadeva *et al.*

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2002). Forced overexpression of STARS in mouse heart tissue results in an increased sensitivity to overload resulting in cardiac hypertrophy (Kuwahara *et al.* 2007). It has been shown that STARS increases actin polymerization, partly in collaboration with RhoA (Arai *et al.* 2002). By reducing the cytoplasmic concentration of monomeric G-actin, STARS promotes the nuclear translocation of the serum response factor (SRF) transcriptional co-activators myocardin-related transcription factor-A and -B (MRTF-A and -B), resulting in an increase in SRF-mediated gene transcription (Kuwahara *et al.* 2005).

Several targets downstream of STARS, including RhoA and SRF, have previously been linked with skeletal muscle development and remodelling. Rho is involved in skeletal muscle differentiation (Takano et al. 1998) and regulates the expression of the myogenic transcription factors MyoD and myogenin in myoblasts (Carnac *et al.* 1998; Cai *et al.* 2004). RhoA protein levels in skeletal muscle have been shown to increase and decrease respectively, following functional overload (McClung *et al.* 2003, 2004; Sakuma *et al.* 2003) and hindlimb suspension in rats (McClung *et al.* 2004). RhoA, via increasing actin polymerization, has been shown to increase the nuclear localization of SRF (Liu *et al.* 2003). SRF activity and expression are increased during load-induced hypertrophy in rooster (Fluck *et al.* 1999) and rat (Sakuma *et al.* 2003) skeletal muscle. Gene deletion studies in mice reveal that SRF is required for skeletal muscle growth and maturation (Li *et al.* 2005). Known SRF target genes include the structural protein α-actin (Carson *et al.* 1996), the motor protein myosin heavy chain type IIa (MHCIIa) (Allen *et al.* 2001), and the insulin-like growth factor-1 (IGF-1) (Charvet *et al.* 2006).

STARS signalling through a RhoA/MRTF/SRF pathway may play an important role in skeletal muscle remodelling and therefore may be an interesting target for therapeutic manipulation. However, the regulation of STARS and its downstream signalling targets in human skeletal muscle following hypertrophy and/or atrophy is unknown. Therefore, the aim of the present study was to measure the regulation of STARS, RhoA, MRTF-A/B and SRF, as well as the expression of the SRF target genes, α-actin, MHCIIa and IGF-1. All measurements were made before and after a period of resistance training (hypertrophy) and again after a period of de-training (atrophy) in human skeletal muscle (Leger *et al.* 2006*a*).

Methods

Subjects details

Twenty-five healthy males participated in the study. It was approved by the canton of Valais medical ethics commission (Commission cantonale valaisanne d'éthique médicale; CCVEM) and conformed with the *Declaration of Helsinki*. All participants gave their informed consent and agreed to muscle biopsies and physiological testing. The subjects were physically active but had not participated in a resistance training programme for more than 12 months. The subjects were divided into two resistance training groups (low repetitions or high repetitions) that were matched for age, height, weight, $\dot{V}_{O_2max}$ and for strength and endurance for each of the exercises completed. Physiological characteristics of the subjects are summarized in Table 1.

Table 1. Characteristics of the subjects in the strength and endurance training groups

Parameter	Strength group	Endurance group
Age (years)	36.8 ± 5.5	32.8 ± 2.5
Height (cm)	177 ± 7	180 ± 7
Weight (kg)	80 ± 13	77 ± 13
$\dot{V}_{O_2max}$ (ml kg^{-1} min^{-1})	45.1 ± 6.3	47.7 ± 8.0

Muscle biopsies

Skeletal muscle samples were obtained under local anaesthesia (Rapidocaine, 1% plain) from the belly of the vastus lateralis muscle using a percutaneous needle biopsy technique (Pro-Mag, Medical Device Technologies Inc., Gainsville, FL, USA) . A single incision was made in the skin and three individual muscle samples were taken. The post-training muscle biopsies were taken 48–72 h after the last training session. The muscle samples were immediately frozen in liquid nitrogen and used for RNA and protein extraction. These muscle samples remained from a previously published study (Leger *et al.* 2006*a*).

Measurement of oxygen consumption

$\dot{V}_{O_2max}$ was measured using a Quark B2 metabolic cart (Cosmed, Rome, Italy) while subjects were cycling on an ergometer (Ergoline 900, Sensor Medic, Bitz, Germany). The subjects began cycling at a power of 90 W. The power was increased by 30 W every 3 min until the subject could not maintain a minimal revolution of 75 r.p.m. At the end of each step, lactate concentration was obtained (Lactate Pro, Axon Lab AG, Reichenbach/Stuttgart, Germany). The duration of the test was between 20 and 30 min. Heart rate (Polar) and oxygen consumption were measured continually throughout the test. $\dot{V}_{O_2max}$ was calculated as the highest value averaged over a 30 s period.

Maximal power and endurance tests

The subjects were familiarized with the equipment (Technogym, Gambettola, Italy) and correct lifting technique for the exercises, which included leg press, squat

and leg extension. Maximal strength load was estimated as the load (kg) which corresponded to the estimated hypothetical isometric maximal force determined from the force–velocity curve (Rahmani *et al.* 2001) using a Myotest accelerometer (Myotest SA, Sion, Switzerland). For the leg press the subjects started with a load of 60 kg which was increased by 20 kg per lift until the power–resistance graph demonstrated a decrease in power. For the squat and leg extensions the starting resistance was 25 and 40 kg, respectively, which were increased by 20 and 10 kg respective, per lift. Approximately 3 min rest was provided between each lift. Once the estimated maximal strength load was determined, 50% of this value was calculated for the local muscular endurance test. Following a 10 min rest the subjects completed the maximal number of repetitions possible when lifting 50% of the estimated maximal strength load.

Muscle cross sectional area

Computed tomography (GE Health Care) was used to determine the cross-sectional area (CSA) of the quadriceps. While in the supine position, an image was taken at a distance of 20 cm from the roof of the acetabulum. The CSA was determined by measurement of the surface area of the tissue by two observers (Leger *et al.* 2006*a*).

Training protocols

All subjects completed an 8 week resistance training programme as described previously (Campos *et al.* 2002). The training was performed 2 days per week for the first 4 weeks and 3 days per week for the final 4 weeks. Each session was supervised by a qualified instructor. The subjects were divided into two groups which performed either a LOW number (3–5) or HIGH number (20–28) of repetitions for each exercise, until fatigue. Therefore the resistance for the LOW group was considerably heavier that the resistance used by the HIGH group. The training programme was adapted from previous studies (Anderson & Kearney, 1982; Jackson *et al.* 1990; Campos *et al.* 2002) and designed to be approximately equal in volume (resistance $\times$ repetitions $\times$ sets) with the rest periods between sets adjusted according to the strength–endurance continuum (Fleck & Kraemer, 1997). For example, the LOW group performed three to five repetitions for four sets with 3 min rest between each set, while the HIGH group performed 20–28 repetitions for two sets with 1 min rest between each set (Campos *et al.* 2002). The exercises were performed in the fixed order of leg press, squat and leg extension. The resistance was continually increased during the training programme so that the ranges of repetitions could be respected.

RNA extraction and real time quantitative PCR

RNA from skeletal muscle (approximately 20 mg of muscle) was extracted using a commercially available preparation, peqGOLD Tri-Fast (Peqlab, Germany). Five micrograms of RNA was reverse transcribed to cDNA using Random Hexomer primers and a Stratascript enzyme (Stratagene, The Netherlands; De Bock *et al.* 2005). Real-Time PCR was performed using an MX3000p thermal cycler system and Brilliant® Multiplex QPCR Master Mix (Stratagene) (Leger *et al.* 2006*b*; Vergani *et al.* 2007). The PCR conditions consisted of one denaturing cycle at 90°C for 10 min, followed by 40 cycles consisting of denaturing at 90°C for 30 s and annealing at 60°C for 60 s. To control for any variations due to efficiencies of the reverse transcription and PCR, acidic ribosomal phosphoprotein PO (RPLPO; also known a 36B4) was used as an internal control for all PCR runs and remained stable throughout the training and de-training periods (Leger *et al.* 2008). All PCR runs were performed in triplicate. PCR primer sequences are provided in Table 2.

Protein extraction and Western blotting

Cytosolic and nuclear proteins were extracted from approximately 20 mg of skeletal muscle using the NE-PER kit (Pierce Biotechnology, Rockford, USA) with the addition of 2 μl phosphatase inhibitor cocktails I, II and III and protease inhibitor cocktail (Sigma), as published previously (Leger *et al.* 2006*b*; Doucet *et al.* 2007). The BCA Protein Assay was used to determine protein concentration (Pierce Biotechnology). Electrophoresis and protein transfer were performed as published previously (Leger *et al.* 2006*b*; Doucet *et al.* 2007). Antibodies used for the detection of SRF (sc-335; 1 : 2000) in the nuclear protein fractions and RhoA (sc-119): 1 : 2000) in the cytoplasmic protein fractions were from Santa Cruz Biotechnology, CA, USA. Following incubation at room temperature for 60 min with a horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (Cell Signaling Technology, Inc., Beverly, MA, USA) at a dilution of 1 : 5,000, the membranes were washed for 5 $\times$ 5 min in 0.1% Tween 20–Tris-buffered saline (TBS) and treated for 5 min with a chemiluminescence substrate (ECL-plus, Amersham). All blots were normalized against the tubulin (cytosolic proteins) and lamin A proteins (nuclear proteins) as published previously (Leger *et al.* 2006*b*; Doucet *et al.* 2007).

Statistics

A two-way ANOVA was used to test for the interaction between time and training intensity. Following this a one-way ANOVA with repeated measures was used to test

Table 2. Human Primer sequences, and annealing temperatures used for the real-time PCR

Gene	Sequence 5′–3′	Temp (°C)
STARS	Sense TGG CTG TGG TCA GGA TCA AG Anti CTT TGC AGT TGA GTT TCT CTG TAA ATC T Probe TXS Red-CCC CTT GCC CTC CCA GGT AAA-BHQ2	60
MRTF-A	Sense TCC ACC CCC ACA CTC ATT AAG Anti TCT TGC TGC GCT GTG ACT TC Probe 6-FAM-AAG CCA ACC CAA GTC TGC CAG TG-BHQ1	60
MRTF-B	Sense TCG GAG TCG ACC AGA TCG TT Anti GGG ATG GCT CTG CAA ATG TT Probe TXS Red-TGA ACT TGT CAG GAT GCA CAT TTT A-BHQ2	60
SRF	Sense TAC CAG GTG TCG GAG TCT GAC A Anti GGC AGG TTG GTG ACT GTG AA Probe AGA CCA AGG ACA CAC TGA AGC CGG C	60
α-Actin	Sense GTA GCT ACC CGC CCA GAA ACT Anti AGG CCG GAG CCA TTG TC Probe 6-FAM-ACA ATG TGC GAC GAA GAC GAG ACC AC-BHQ1	60
MHCIIa	Sense GAT GGC ACA GAA GTT GCT GA Anti CTT CTC GTA GAC GGC TTT GG	57
IGF-1	Sense AGG TAT ACT GGA ATC CGATCT CTG A Anti CTT GTT GTT TCC TGC ACT CCC TCT	60
RPLPO	Sense TCT ACA ACC CTG AAG TGC TTG ATA TC Anti GCA GAC AGA CAC TGG CAA CAT T Probe AGG AAA CTC TGC ATT CTC GCT TCC TGG AG	60

for differences between groups. The level of significance was set at $P < 0.05$.

Results

There was no significant effect of training intensity on any of the dependent variables. Therefore the two groups of subjects were pooled for all analyses. As shown previously (Leger *et al.* 2006*a*) the resistance training performed in this study resulted in a 20% and 15% increase in maximal strength, respectively, for the leg extension and squat exercises ($P < 0.01$). Muscular endurance also increased by 30%, 100% and 60%, respectively, for the leg extension, leg press and squat exercises ($P < 0.01$). These functional outcomes were associated with a 10% increase in quadriceps cross-sectional area after training when compared to the pre-training levels ($P < 0.01$). After 8 weeks of de-training a muscle atrophy of 5% was observed when compared with the post-training hypertrophy ($P < 0.05$). Muscle size following de-training was not significantly different from the pre-training size ($P = 0.1$).

Following 8 weeks of resistance training a 3.4-fold increase in STARS mRNA was observed in the post-training group ($P < 0.05$). STARS mRNA returned to basal levels after 8 weeks of detraining (Fig. 1). The increase in STARS mRNA was also paralleled by approximately a 2-fold increase in RhoA protein content following training which remained after the de-training period (Fig. 1).

The mRNA levels for MRTF-A and MRTF-B were increased by 2.5- and 3.6-fold, respectively, following the training period and both returned to basal levels following de-training (Fig. 2.) The skeletal muscle transcription factor SRF showed an increase of 3-fold, at the mRNA level, after 8 weeks of resistance training in the post-training group ($P < 0.005$). Following 8 weeks of detraining, SRF mRNA decreased 6.9-fold from post-training. SRF nuclear protein content was also increased by 1.25-fold post-training and returned to basal levels following the de-training period (Fig. 2).

Following 8 weeks of resistance training, a 2.7-, 2.4- and 2.0-fold increase, respectively, in the SRF target genes, α-actin, MHCIIa and IGF-1, was observed ($P < 0.05$). α-Actin and IGF-1 mRNA returned to basal levels following de-training, while MHCIIa was 65% lower that the pre-training levels (Fig. 3).

Discussion

Identifying the intracellular signalling pathways which control skeletal muscle integrity, growth and function are vital for developing therapeutic strategies aimed at reducing the devastating effects of muscle wasting, as seen in chronic diseases, specific neuromuscular disorders and following musculo-skeletal trauma. At

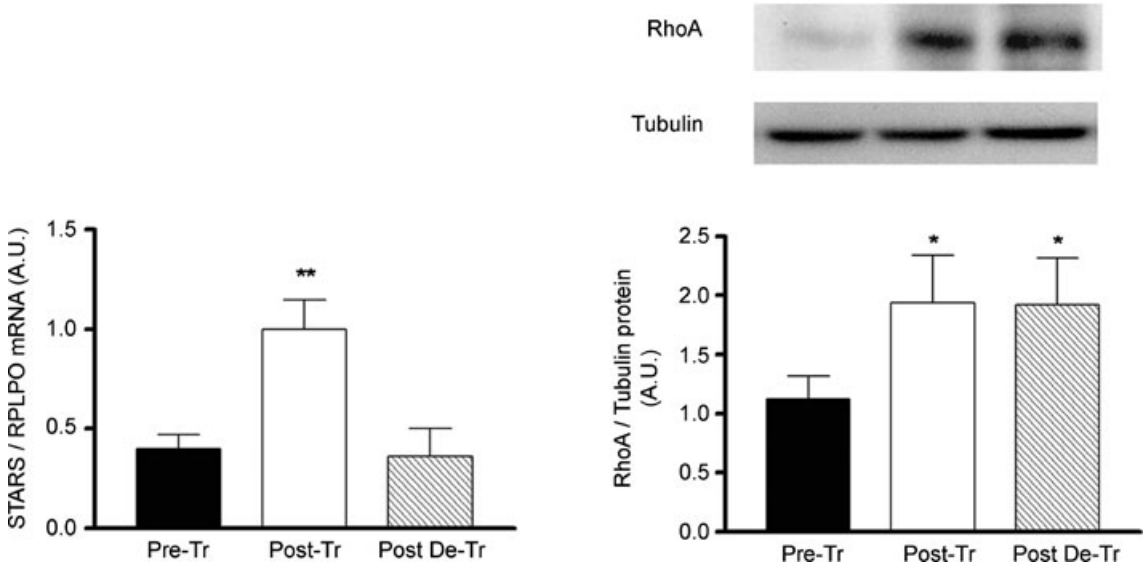

Figure 1. Effect of 8 weeks of resistance training (Post-Tr) and 8 weeks of de-training (Post-DeTr) on STARS mRNA and RhoA protein content
Significantly different from pre-training and 8 weeks post-training levels: $*P < 0.05$; $**P < 0.01$.

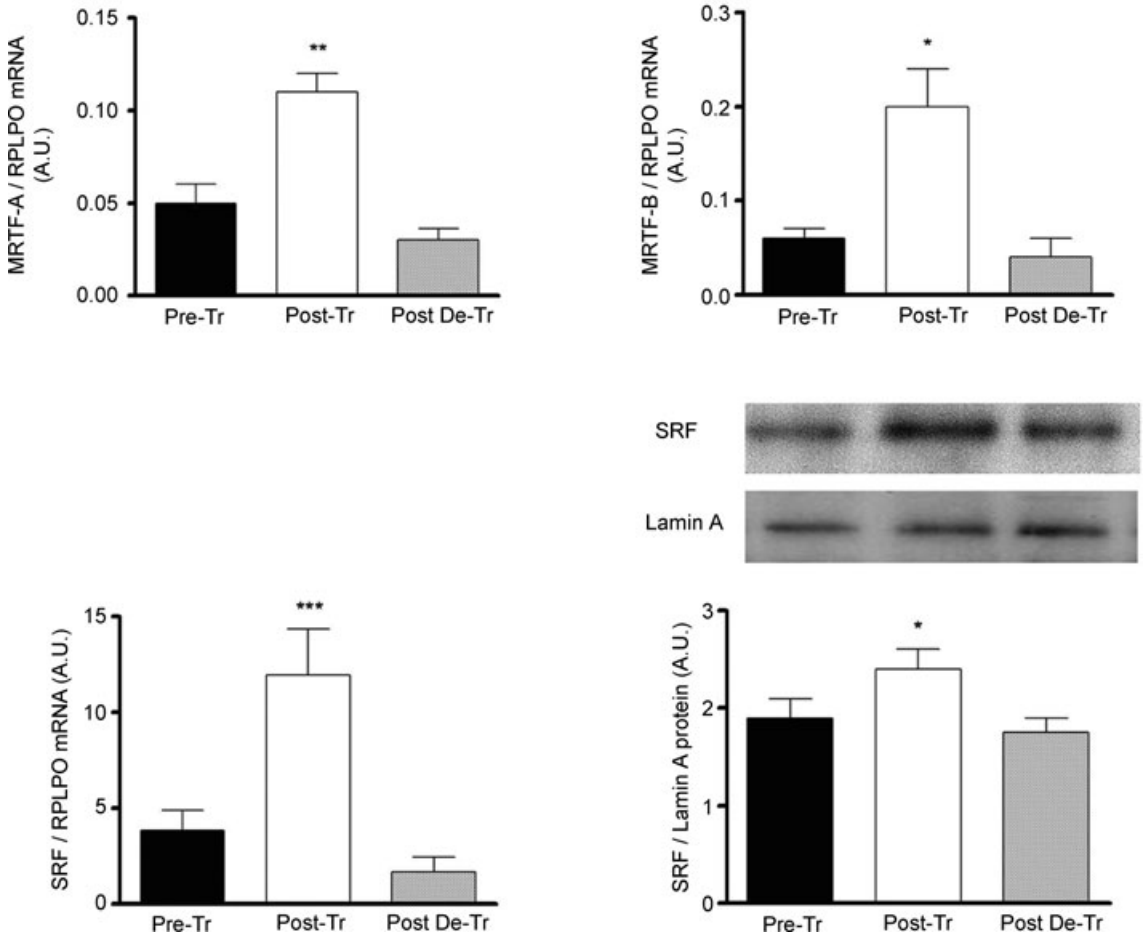

Figure 2. Effect of 8 weeks of resistance training (Post-Tr) and 8 weeks of de-training (Post-DeTr) on MRTF-A, MRTF-B and SRF mRNA expression as well as on SRF protein levels
Significantly different from pre-training and 8 weeks post-training levels: $*P < 0.05$; $**P < 0.01$; $***P < 0.005$.

present, the molecular targets and signalling pathways influencing human muscle hypertrophy and atrophy are not well understood. The results from the present study demonstrate, for the first time in skeletal muscle, the up-regulation of key members of the novel STARS signalling pathway following resistance exercise-induced muscle hypertrophy, as well as their down-regulation following inactivity-induced muscle unloading. Several novel and important findings were observed. Firstly, following 8 weeks of progressive overloading of the upper leg muscles, via resistance training, increases in the mRNA levels of STARS, MRTF-A, MRTF-B and SRF, as well as RhoA protein and nuclear SRF protein were observed. These increases were associated with hypertrophy of the upper leg. Secondly, increases in the SRF target genes, α-actin, MHCIIa and IGF-1, were also observed. Thirdly, these observations were all reversed, except for RhoA, during the period of inactivity-induced muscle unloading. It should be noted that muscle hypertrophy was achieved in both the high weight–low repetition, as well as the in the low weight–high repetition training regimes, the latter being contrary to previous observations in young men (Campos *et al.* 2002). A possible explanation is that the subjects used in the present study were on average 14 years older than those in the study by Campos *et al.* (2002) and may have been significantly less trained. Therefore any type of resistance training may have provided significant functional overload to induce an increase in muscle size.

The adaptation of skeletal muscle to external mechanical stress, such as increased loading or muscle contraction, requires the initial sensing of the stress, followed by the transduction of the stress into signals that will generate the appropriate physiological response. Previous studies have shown that STARS mRNA levels increase in the left ventricle in response to pressure overload caused by aortic banding (Mahadeva *et al.* 2002; Kuwahara *et al.* 2007) and that STARS increases F-actin polymerization, MRTF-A nuclear translocation and consequently, SRF-dependent gene transcription (Kuwahara *et al.* 2005, 2007). Therefore, STARS has been suggested to provide an important link between the transduction of external stress to intracellular signalling which controls genes involved in the maintenance of cytoskeletal integrity and muscle function. In the present study the observed increase in STARS in skeletal muscle, following load-induced skeletal muscle hypertrophy, extends previous findings in cardiac tissue and supports the notion that STARS plays an a role in positively regulating muscle growth.

In addition to STARS, several members of its downstream signalling pathway were also increased in hypertrophied human skeletal muscle following resistance training. For the first time, the present study shows an increase in RhoA protein and SRF mRNA and SRF nuclear protein contents in hypertrophied human skeletal muscle, findings supporting several observations made

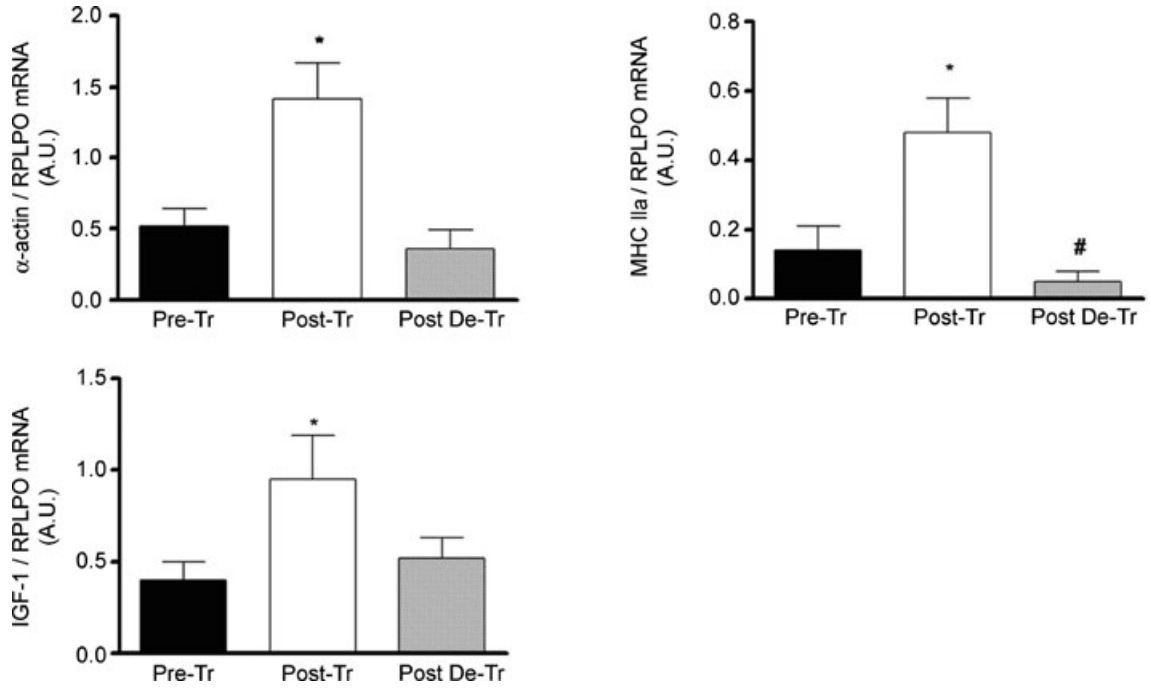

Figure 3. Regulation of α-actin, MHCIIa and IGF-1 mRNA expression following 8 weeks of resistance training (Post-Tr) and 8 weeks of de-training (Post-DeTr)
*Significantly different from pre-training levels: $P < 0.05$; #significantly different from post-training levels: $P < 0.05$.

previously in rat and rooster skeletal muscle. In these studies, RhoA protein and mRNA levels were increased in rat plantaris muscle following 3 and 21 days of functional overload, stimulated by surgical ablation of the distal third of the lateral and medial gastrocnemius muscle (McClung *et al.* 2003). SRF mRNA and nuclear protein content (Fluck *et al.* 1999) as well as SRF activity (Fluck *et al.* 2000) have been shown to increase in rooster anterior latissimus dorsi (ALD) muscle following 7 and 13 days of load-induced hypertrophy. SRF levels were also observed to increase in soleus muscle following 1 day and in plantaris muscle following 8 days of muscle overload-induced stretch (Gordon *et al.* 2001). SRF is a known transcriptional regulator of genes involved in cyto-skeletal structure, growth and contraction (Miano *et al.* 2007) and therefore is seen as an important regulator of muscle integrity, growth and function. In the present study, the increase in nuclear SRF protein levels as well as SRF mRNA was associated with an increase in the SRF target genes, α-actin (Carson *et al.* 1996), MHCIIa (Allen *et al.* 2005), and IGF-1 (Charvet *et al.* 2006). It has previously been shown that SRF binding to its response element sequence on the α-actin promoter is increased in hypertrophied chicken ALD muscles (Carson *et al.* 1996). It should be noted that the parallel changes in nuclear SRF protein content and the SRF target genes, α-actin, MHCIIa, and IGF-1, are associations and no causal relationship can be drawn. It cannot be ruled out that other transcription factors, known to bind to the promoter regions of α-actin, MHCIIa, and IGF-1 in muscle tissue and muscle cells, may have been involved in the regulation of these genes. These include the androgen receptor (Hong *et al.* 2008) and Myc-associated zinc finger protein (MAZ) (Himeda *et al.* 2008) for α-actin, MEF2 and NFAT (Allen *et al.* 2001) for MHCIIa, and STAT5b (Woelfle & Rotwein, 2004) for IGF-1.

It has been well established that SRF transcriptional activity is enhanced by the transcriptional co-activator MRTF-A (Wang *et al.* 2002; Miralles *et al.* 2003; Cen *et al.* 2004) and that STARS overexpression contributes to the nuclear translocation of MRTF-A and MRTF-B (Kuwahara *et al.* 2005, 2007). The increase in MRTF-A and MRTF-B mRNA observed in the present study is the first demonstration that MRTF levels are sensitive to the signals induced by increased loading of skeletal muscle. These results suggest that the MRTFs play a positive role in skeletal muscle adaptation to resistance exercise.

As members of the STARS signalling pathway were increased following a period of increased skeletal muscle loading, it was of interest to determine if unloading, stimulated by reduced physical activity, would reverse these increases. Following 8 weeks of reduced physical activity or de-training, STARS and MRTF-A/B mRNA, as well as SRF mRNA and nuclear protein levels returned to pre-training levels. In parallel, the SRF target genes α-actin, MHCIIa and IGF-1, also returned to pre-training levels following the period of reduced muscle loading. These results are the first to show that, in human skeletal muscle, members of the STARS signalling pathway are negatively influenced by reductions in muscle loading. A recent study observed that STARS, MRTF-A and SRF are lower in the skeletal muscle from sarcopenic 24-month-old mice, when compared with 3-month-old mice (Sakuma *et al.* 2008), suggesting a role for the STARS signalling pathway in age-induced skeletal muscle atrophy. Along similar lines, mice lacking muscle SRF and expressing dominant negative MRTF-A in skeletal muscle presented muscle atrophy, significant fibrosis as well as reduced levels of α-actin mRNA, a phenotype often observed in myopathies and muscle damage. In contrast to the other members of the STARS signalling pathway, RhoA remained elevated following the period of reduced physical activity. This is in contrast to the inactivity model of hindlimb suspension which reduced both muscle mass and RhoA in rat plantaris muscle (McClung *et al.* 2004). However, along similar lines to our observation, RhoA was observed to be elevated in atrophied skeletal muscle of aged, when compared with younger, mice (Sakuma *et al.* 2008). As STARS activation of transcription is only partially attenuated following RhoA inhibition (Arai *et al.* 2002), it appears that STARS signalling can be regulated independently of RhoA.

The regulation of SRF as well as MRTF-A and MRTF-B mRNA in the present study suggests that changes in skeletal muscle loading can regulate a transcriptional programme which may alter the transcription or stability of SRF, MRTF-A and MRTF-B mRNA. This response may, in part, be involved in increasing SRF, MRTF-A and MRTF-B protein levels and therefore provide the muscle with a larger pool of these proteins to be translocated to the nucleus upon stimulation. However, post-translational modifications may also be involved in changes in their protein levels. In the present study MRTF-A and MRTF-B protein levels were not measured; however work by Sakuma *et al.* (2008) has shown that reduced MRTF-A mRNA expression is paralleled by reduced MRTF-A protein levels in both cytoplasmic and nuclear fractions in older when compared with younger mice. It should be noted that while STARS activation has been shown to increase the transcriptional activity of SRF, via MRTF-A nuclear translocation, it has not been established if STARS, and/or MRTF-A, is involved in SRF nuclear translocation. These are areas for future investigation.

In conclusion, our results show that the STARS signalling pathway is up- and downregulated, respectively, in human skeletal muscle hypertrophy and atrophy. During muscle hypertrophy, induced through increased loading via resistance training, STARS and its downstream targets RhoA, MRTF-A/B and SRF were increased, as well as several SRF target genes involved in skeletal muscle

structure, function and growth. The response of these targets was reversed after a period of atrophy-inducing unloading or de-training, when compared to the post-training levels. These findings show that, in human skeletal muscle, STARS signalling is responsive to changes in muscle loading and supports the notion that STARS signalling plays a role in muscle growth and function.

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Author contributions

S.L., M.A.W. and B.L. contributed to the analysis and interpretation of the data, and to revising the manuscript; they approved the final version. A.P.R. was responsible for the conception and design of the study, analysis and interpretation of the data and drafting the article, and approved the final submission. Exercise testing and training were performed at the Clinique Romande de Réadaptation, Sion, Switzerland. The analysis of gene and protein expression was performed at the Institut de recherche en réadaptation-réinsertion, Sion, Switzerland and at the School of Exercise and Nutrition Sciences, Deakin University, Burwood, Australia.

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