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Keywords:	Myokines, IL-15/IL-15Rα axis, strength training, muscle protein synthesis/breakdown

Skeletal muscle IL-15/IL-15Rα and myofibrillar protein synthesis after resistance exercise

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Running head: IL-15/IL-15Rα in Resistance Exercise

ABSTRACT

In vitro and *in vivo* studies described the myokine IL-15 and its receptor IL-15Rα as anabolic/anti-atrophy agents, however the protein expression of IL-15Rα has not been measured in human skeletal muscle and data regarding IL-15 expression remain inconclusive. The purpose of the study was to determine serum and skeletal muscle IL-15 and IL-15Rα responses to resistance exercise session and to analyse their association with myofibrillar protein synthesis (MPS). Fourteen participants performed a bilateral leg resistance exercise composed of 4 sets of leg press and 4 sets of knee extension at 75% 1RM to task failure. Muscle biopsies were obtained at rest, 0, 4 and 24h post-exercise and blood samples at rest, mid-exercise, 0, 0.3, 1, 2, 4 and 24h post-exercise. Serum IL-15 was increased by ~5.3-fold immediately post-exercise, while serum IL-15Rα decreased ~75% over 1h post-exercise ($P<0.001$). Skeletal muscle IL-15Rα mRNA and protein expression were increased at 4h post-exercise by ~2-fold ($P<0.001$) and ~1.3-fold above rest ($P=0.020$), respectively. At 24h post-exercise IL-15 ($P=0.003$) and IL-15Rα mRNAs increased by ~2-fold ($P=0.002$). Myofibrillar fractional synthetic rate between 0-4h was associated with IL-15Rα mRNA at rest ($r=0.662$, $P=0.019$), 4h ($r=0.612$, $P=0.029$) and 24h post-exercise ($r=0.627$, $P=0.029$). Finally, the muscle IL-15Rα protein up-regulation was related to Leg press 1RM ($r=0.688$, $P=0.003$) and total weight lifted ($r=0.628$, $P=0.009$). In conclusion, IL-15/IL-15Rα signalling pathway is activated in skeletal muscle in response to a session of resistance exercise.

Keywords: Myokines, IL-15/IL-15Rα axis, strength training, muscle protein synthesis/breakdown.

INTRODUCTION

Interleukin-15 (IL-15) and its cognate receptor alpha (IL-15Rα) have been implicated in the regulation of anabolic/catabolic balance of human skeletal muscle (Busquets et al., 2005; Furmanczyk & Quinn, 2003; Pistilli et al., 2007; Quinn et al., 2002; Quinn et al., 1995; Riechman et al., 2004). However, most of the evidence is indirect and the protein expression of IL-15Rα has not been determined in human skeletal muscle.

IL-15 is a pleiotropic cytokine member of the 4 alpha-helix bundle family (Grabstein et al., 1994). IL-15 has been shown to stimulate protein accretion and myosin heavy chain (MHC) accumulation in differentiated myocytes (Quinn et al., 1995) and myotubes (Furmanczyk & Quinn, 2003; Quinn et al., 2002), while reducing protein degradation (Quinn et al., 2002). In humans, circulating IL-15 is elevated in response to a single session of resistance exercise in untrained and trained states (Riechman et al., 2004). In agreement with a muscular origin, IL-15 mRNA was increased 2-fold in *vastus lateralis* muscle 24h after a bilateral leg press and knee extension resistance exercise session, although this was not accompanied by a change in circulating or muscular IL-15 protein expression (Nielsen et al., 2007). Therefore, despite the fact that *in vitro* studies indicate a role for skeletal muscle IL-15 in anabolism, studies in humans are inconclusive.

Although part of the effects of IL-15 are mediated by its binding to IL-15Rα (Dubois et al., 2002; Duitman et al., 2008; Sato et al., 2007), this alpha-receptor may also exert functions independent from IL-15 in skeletal muscle. IL-15Rα may have a role in determining the phenotype and fatigability of muscle fibers, and mitochondrial fuel utilization (Loro et al., 2015; O'Connell et al., 2015; O'Connell et al., 2015). In addition, human studies indicate that IL-15Rα may be involved in muscle hypertrophy and strength gains after resistance training (Pistilli et al., 2008; Riechman et al., 2004). In this regard, two single nucleotide polymorphism (SNPs) in exon 7 and 4 of the IL-15Rα could explain part of the variability in the hypertrophy observed after 10 weeks of whole-body resistance training (Riechman et al., 2004), whereas IL-15Rα SNPs, rs2296135 and rs22228059, were positively associated with pre- and post-exercise

isometric strength and muscle volume, respectively, after 12 weeks of resistance training of the flexor-extensor muscles of the elbow (Pistilli et al., 2008).

Despite the potential implication of IL-15 and IL-15Rα in skeletal muscle anabolic/catabolic balance, direct evidence is lacking as no human study has determined whether changes in skeletal muscle IL-15Rα mRNA and protein expression are associated with protein synthesis. Recently, we reported a ~2-fold elevated myofibrillar protein synthesis (MPS) response during the first four hours (0-4h) after a single session of resistance exercise in healthy young males (McKendry et al., 2016), however, skeletal muscle IL-15 and IL-15Rα were not determined. Therefore, the aim of this study was to determine whether circulating and skeletal muscle IL-15 and IL-15Rα might have a role in the regulation of myofibrillar protein synthesis, after a single session of resistance exercise.

We hypothesised that skeletal muscle IL-15 and IL-15Rα expressions would be up-regulated after a single session of resistance exercise and that IL-15 and IL-15Rα expression in skeletal muscle would be associated with myofibrillar protein synthesis.

MATERIALS AND METHODS

Participants

A full description of the methods, study design and participant characteristics, from which part of current data are drawn, has been previously published (McKendry et al., 2016). Volunteers were aged from 18 to 35 years and had been participating in resistance training programmes > 2 days/week during ≥ 1 year prior to start of current study. Subjects' characteristics are presented in Table 1. Prior to study enrolment all procedures were explained to participants who then gave their written informed consent. Ethical approval was obtained through the NHS Black Country Research Ethics Committee (13/WM/0455) in accordance with the latest version (7^{th}) of the Declaration of Helsinki.

Experimental design

All participants reported to the School of Sport, Exercise and Rehabilitation Sciences (SportExR) laboratory on 3 separate occasions. During visit one, participants underwent preliminary assessments of body composition and maximal leg strength. Then, within a period of 8 days, the volunteers returned to the laboratory for the experimental trial, which consisted of a single session of bilateral lower-limb resistance exercise with muscle biopsies obtained at baseline, immediately after (0h), 4 and 24h post-exercise, and blood samples at baseline, mid-exercise, and 0, 0.3, 1, 2, 4 and 24h post-exercise to assess the systemic and skeletal muscle responses of the IL-15/IL-15Rα axis. As part of the original investigation (McKendry et al., 2016), participants were matched in pairs based on anthropometric, strength and training characteristics before to be randomly allocated to either 1-min or 5-min of passive rest between resistance exercise sets. Since no significant differences were observed in circulating or intramuscular IL-15 and IL-15Rα measurements between the 1-min (N = 7) and 5-min groups (N = 7), participants were treated as a single group for the purpose of the present analyses. Of the 16 participants included in the original study, 14 were analysed in the present investigation due to insufficient muscle tissue in the two subjects excluded.

Experimental protocol

A detailed description of the experimental protocol and analytical methods can be found elsewhere (McKendry et al., 2016). Briefly, regional and whole-body composition was determined by dual energy x-ray absorptiometry (Discovery DXA Systems, Hologic Inc., Bedford, MA, USA). Thereafter, one-repetition maximum (1RM) strength during leg press and knee extension was assessed (Cybex VR-3, MA, USA). Approximately seven days later participants reported to the laboratory at 07.00 hours being fasted for 10-12h. Upon arrival, a cannula was inserted into a forearm vein to obtain arterialised blood samples into a tube prepared for serum separation (BD, Oxford, UK). After resting supine in bed for 2.5h, a muscle biopsy was obtained from the *vastus lateralis* of one leg (~120mg of tissue) (Bergstrom, 1975),

under local anaesthesia (1% lidocaine). Skeletal muscle sample was cleaned from any fat or connective tissue before being frozen in liquid nitrogen. Following the muscle biopsy, participants completed a session of bilateral lower-extremity resistance exercise on leg press and knee extension machines. Exercise consisted of four sets of 8-15 repetitions per exercise at 75% of 1RM, each set performed to task failure. At the end of the last repetition, a second muscle biopsy was obtained ~3 cm proximal from the first biopsy through a new incision. Immediately after the second biopsy, the volunteers ingested 25 g of whey protein isolate (MyProtein, Cheshire, UK) dissolved in 400 mL of water. During the next four hours, participants rested supine and then a third muscle biopsy was obtained from a new incision, ~3 cm proximal to the second biopsy. The following morning at 7.00 h, participants returned to the laboratory after a 10-12h overnight fast and a cannula was inserted into a forearm vein to obtain a blood sample followed by the fourth and last biopsy, which was obtained from the *vastus lateralis* of the contralateral leg.

The participants received three standardised meals for consumption the evening prior to the experimental trial, as well as the afternoon and evening after the experimental trial. The diet was composed by ~97 g of CHO (~58%), ~34 g of protein (~20%) and ~37 g of fat (~22%) with an energy content of ~871 kcal per meal. Consumption of ethanol or caffeine was not allowed 24h before the experiments neither during the study.

Blood Analysis

Serum IL-15 and IL-15Rα

After collection, all blood samples were centrifuged for 15 minutes at 1000 *g*, aliquoted and stored at -80 ºC. Two high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits were used to determine the serum concentration of IL-15 and IL-15Rα in duplicates. IL-15 was measured using Human IL-15 Quantikine ELISA kit (R&D Systems, MN, USA) recognizing both natural and recombinant human IL-15 (range: 0.49 – 62.5 pg/mL; intra- and inter-assay

coefficients of variation (CV) were 4.2 and 7.4%, respectively). Serum IL-15Rα was measured using Human IL-15 receptor subunit alpha ELISA kit (Wuhan EIAab Science, Wuhan, China) recognizing both natural and recombinant human IL-15Rα (range: 0.49 – 62.5 pg/mL; intra- and inter-assay CVs were 4.4 and 7.8%, respectively).

Muscle Tissue Analysis

Protein extraction and Western Blot procedures.

Approximately 25-30 mg of muscle tissue was powdered on dry ice using a Cellcrusher^{TM} tissue pulveriser (Cellcrusher limited, Cork, Ireland) and a sucrose lysis buffer was used to prepare the samples for Western Blot as previously described (Philp et al., 2011). Equal amounts of protein (35 μg per sample) were boiled for 5 min in 1 x Laemmli sample buffer, separated on 10% SDS-PAGE gels (Bio-Rad, Copenhagen, Denmark) for 45 min and transferred to polyvinylidene difluoride (PVDF) membranes at constant voltage and 0.4 A for 1.5 h. Subsequently, membranes were incubated overnight with primary antibodies against IL-15 (sc-7889) and IL-15Rα (sc-271366), purchased from Santa Cruz Biotechnology (Dallas, USA). Both antibodies were diluted into BSA-blocking buffer containing 4% bovine serum albumin in Tris-buffered saline with 0.1% Tween 20. Antibody specific labelling was revealed by incubation with an HRP-conjugated goat anti-rabbit (IL-15) or anti-mouse (IL-15Rα) antibodies (1:5000), both diluted in 5% blotto blocking buffer and visualised with ECL Western blotting detection system using a ChemiDoc XRS (Bio-Rad, Copenhagen, Denmark). Imaging and band quantification were performed using the Quantity One 1-D Analysis software (Bio-Rad, Copenhagen, Denmark). Test samples were run together with a control sample from a subject who did not take part in the study. The control sample was loaded in three different lanes and used as an internal control for inter-gel variability. Overall, the mean CVs of the controls were 13.9% (IL-15) and 12.3% (IL-15Rα). Control samples and a total protein staining-technique method (reactive brown) were used to accurate protein quantification for loading control.

RNA Isolation and quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR).

Approximately 15-20 mg of skeletal muscle tissue was used for the RNA isolation. The RNA was extracted by guanine-phenol-chloroform isothiocyanate procedures using TRIzol (Invitrogen, Carlsbad, CA, USA). Then, RNA was recovered from the aqueous phase by precipitation; the amount and purity was measured by optical density at 260/280 nm and 260/230 nm in a NanoDrop ND-100 spectrophotometer (Thermo Fisher Scientific Inc., DE, USA).

Reverse transcription was performed to synthesize cDNA from 200 ng of the total RNA using Oligo dT primers (GE Healthcare Bio-Sicences, Buckinghamshire, UK) and M-MLV reverse transcriptase enzyme (Invitrogen, Carlsbad, CA, USA). The cDNA was amplified using the primers presented in Table 2 (self-designed and tested in skeletal muscle from human donors, data not shown). The qRT-PCR mixture was composed by 5 μL of the inverse transcription product (cDNA) diluted 1:20, 10 μL of iQ SYBR Green Supermix (Bio-Rad, Copenhagen, Denmark) and 1 μL (6 mM) of the primer selected. The final reaction volume (20 μL) was used to perform the qRT-PCR in a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). All samples were subjected to an initial stage of 10 min at 95ºC. The conditions for PCR amplification were as follows: 45 cycles of 95 ºC for 15 s, 60 ºC for 30 s and 72 ºC for 1 minute, for both IL-15 and IL-15Rα. Finally, mRNA expressions of IL-15 and IL-15Rα were determined in triplicates, and normalized using β-actin and glyceraldehyde 3-phosphate-dehydrogenase (GAPDH) as housekeeping genes. β-actin and GAPDH expression remained unchanged.

Myofibrillar and plasma tracer enrichment.

Procedures for muscle myofibrillar protein isolation, plasma-free amino acid extraction and $^{13}C_6$ phenylalanine enrichment, and calculation of myofibrillar fractional synthetic rate (FSR) at rest, 0-4h and 24-28h post-exercise are described in McKendry et al. (2016).

Briefly, a primed continuous infusion of L-[ring-$^{13}C_6$]phenylalanine (prime, 2 μmol kg^{-1}; infusion, 0.05 μmol kg^{-1} min^{-1}; Cambridge Isotope Laboratories, Andover, MA, USA) was implemented during both experimental trial days in conjunction with muscle biopsy and blood sampling. In both experimental days, the infusion was initiated immediately after the drawn of the first blood sample (~7.05 h) and finished when the last muscle biopsy sample of the day was obtained (7.5 h and 5.5 h after the beginning of the infusion on day 1 and 2, respectively). Upon thawing, plasma samples were purified on cation-exchange columns. The amino acids were then converted to their N-tert-butyldimethylsilyl-N-methyltrifluoracetamide (MTBSTFA) derivative. Plasma [$^{13}C_6$]phenylalanine enrichment was determined by gas chromatography–mass spectrometry (GCMS; model 5973; Hewlett Packard, Palo Alto, CA, USA) by monitoring ions 234/240. The myofibrillar protein fraction was extracted and hydrolysed overnight. Constituent amino acids in the myofibrillar fraction were purified on cation-exchange columns. Amino acids in the myofibrillar fraction were then converted to their N-acetyl-n-propyl ester derivative. Plasma [$^{13}C_6$]phenylalanine enrichment was determined by gas chromatography–mass spectrometry (GC-C-IRMS; Delta-plus XP; Thermofinnigan, Hemel Hempstead, UK) by monitoring ions 44/45. Pre-infusion and mean plasma [$^{13}C_6$]phenylalanine enrichment were used as a proxy for basal muscle protein enrichment and to determine an "estimated" intracellular precursor enrichment, respectively. The fractional synthesis rate (FSR) of the myofibrillar protein fraction was calculated from the incorporation of [$^{13}C_6$]phenylalanine into protein using the standard precursor-product model (Wolfe & Chinkes, 2005).

Statistical Analysis

Data collected in the study were analysed using the statistical package SPSS v. 22.0 (SPSS Inc., Chicago, IL, USA), and Graph Prism 6 (GraphPad software, Inc. La Jolla, CA, USA). Firstly, a Shapiro-Wilks was used to test the normality of the data ($P > 0.05$). Subsequently, non-normally distributed variables were logarithmically transformed. Circulating and skeletal muscle expression of IL-15 and IL-15Rα were analysed between groups (1- vs. 5-min rest)

using a two-way, repeated measures ANOVA (time x condition). The area under the curve (AUC) was determined using trapezoid method and compared between groups using a paired Student's t-test. Since no significant differences were observed between the 1- and 5-min recovery, both groups were combined for further analyses.

To determine time effects of the intervention on serum, protein and mRNA levels of IL-15 and IL-15Rα, ANOVA for repeated measures was performed. Tukey HSD correction was used as *post-hoc* test when significant differences were detected. Finally, linear regression analysis was carried out to test the potential associations between skeletal muscle and circulating levels of IL-15 and IL-15Rα, as well as between the former and resistance training variables, body composition and myofibrillar FSR. The effect of size (ES) was calculated as eta squared statistic (η^2) to verify time, condition and between groups differences in systemic and intramuscular IL-15 and IL-15Rα expression. Values are reported as mean ± standard deviation (SD); a $P < 0.05$ was considered statistically significant.

RESULTS

Variables describing the resistance exercise session performed by the subjects are presented in Table 3.

IL-15 response to a single dose of resistance exercise.

Skeletal muscle mRNA and protein expression levels, and serum IL-15 concentrations in response to a single session of resistance exercise are illustrated in Figure 1. A progressive increase in mRNA expression was found following resistance exercise ($P = 0.002$, ES = 0.35), reaching statistical significance at 4h ($P = 0.019$, ES = 0.37) and 24h post-exercise, where a 2-fold elevation above pre-exercise resting values was found ($P = 0.003$, ES = 0.44; Fig. 1A). No significant changes were observed in IL-15 muscle protein expression above pre-exercise resting values ($P = 0.563$, ES = 0.15; Fig. 1B). Serum IL-15 concentration increased significantly above pre-exercise resting values during the post-exercise period ($P = 0.001$, ES =

0.46; Fig. 1C), peaking immediately post-exercise ($P < 0.001$, ES = 0.31), and remaining elevated at 24h post-exercise ($P = 0.001$, ES = 0.42).

IL-15Rα response to a single dose of resistance exercise.

Skeletal muscle mRNA and protein expression levels, and serum IL-15Rα concentration in response to a single session of resistance exercise are illustrated in Figure 2. A significant 2-fold increase in IL-15Rα mRNA expression above pre-exercise resting values was observed at 4h ($P < 0.001$, ES = 0.42) and 24h post-exercise ($P = 0.002$, ES = 0.35; Fig. 2A). In contrast to IL-15, skeletal muscle protein expression of IL-15Rα increased by 1.3-fold ($P = 0.020$, ES = 0.34; Fig. 2B) above pre-exercise resting values at 4h post-exercise, returning to baseline levels 24h post-exercise ($P = 0.036$, ES = 0.32). Despite of the lack of significant differences between inter-set rest period (1- vs. 5-min groups), the 5-min group tended to show an elevated protein and mRNA expression at 4h and post-exercise (~10%; $P = 0.103$, ES = 0.55 and $P = 0.092$, ES = 0.57, respectively; supplementary figure 2). Finally, compared to pre-exercise values, serum IL-15Rα concentration was significantly reduced during the first 60 min following the training session ($P < 0.001$; ES = 0.47; Fig. 2C).

Correlation analysis

IL-15/IL-15Rα and resistance exercise variables.

Leg Press 1RM strength was negatively associated with IL-15 serum concentration ($r = -0.800$, $P = 0.003$) but not with IL-15Rα serum concentration. While the serum IL-15 concentration response to resistance exercise (AUC) was negatively associated with knee extension training volume ($r = -0.637$, $P = 0.042$) and time-under-tension (T-U-T) ($r = -0.718$, $P = 0.019$). Post-exercise (0h), serum IL-15 concentrations were associated with the total volume of knee extension exercise ($r = -0.934$, $P < 0.001$). Furthermore, skeletal muscle protein expression of IL-15Rα at 4h post-exercise was associated positively with 1RM Leg press strength ($r = 0.559$, $P = 0.037$) and total training load ($r = 0.628$, $P = 0.009$).

Skeletal muscle and circulating expressions of IL-15/IL-15Rα

At baseline, serum IL-15 concentration was positively associated with intramuscular protein IL-15 levels (r = 0.649, P = 0.031), but not with mRNA expression. Serum IL-15 immediately post-exercise was associated with pre-exercise levels of skeletal muscle protein expression of IL-15 and IL-15Rα (r = 0.582, P = 0.037; r = -0.599, P = 0.031, respectively). At baseline, IL-15 mRNA was associated with IL-15Rα mRNA (r = 0.592, P = 0.043), as well as immediately (r = 0.791, P = 0.005) and 24h post-exercise (r = 0.653, P = 0.021). Additionally, IL-15 and IL-15Rα mRNA expressions at 24h post-exercise was negatively associated with serum concentration of IL-15 and IL-15Rα (r = -0.620, P = 0.042; r = -0.727, P = 0.005; respectively).

Skeletal muscle IL-15/IL-15Rα expression and myofibrillar protein synthesis.

The myofibrillar fractional synthetic rate (FSR) increased by ~2-fold above resting values from 0-4h post-exercise (McKendry et al., 2016) and was associated with IL-15Rα mRNA levels at baseline (r = 0.662, P = 0.019), 4h (r = 0.612, P = 0.029) and 24h post-exercise (r = 0.627, P = 0.029) (Figure 3). Moreover, the delta changes (Δ), from pre-exercise to 4h post-exercise, of IL-15Rα mRNA expression and FSR showed a tendency to be associated at 4h post-exercise (r = 0.481; P = 0.096). No association was observed between myofibrillar FSR and skeletal muscle IL-15 mRNA or muscle protein expression of either IL-15 or IL-15Rα at any time.

DISCUSSION

The present study demonstrates that the gene and protein expression of IL-15Rα is up-regulated in skeletal muscle after a single session of resistance training. The increase in myofibrillar protein synthesis during 0-4h post-exercise was associated with the expression of IL-15Rα mRNA at 4h, which occurred concomitantly with an increase of skeletal muscle IL-15Rα protein levels, suggesting increased translation of the IL-15Rα gene. These findings indicate

that IL-15Rα could have a role in mediating the increase in myofibrillar protein synthesis observed in skeletal muscle after a single session of resistance training.

Although, in our previous study we demonstrated that myofibrillar protein synthesis rates were greater when high volume, moderate-intensity resistance exercise was performed with long (5-min) compared with short (1-min) inter-set rest duration (McKendry et al., 2016), in the present study, we did not observe significant differences between groups in circulating or intramuscular IL-15 and IL-15Rα expressions, despite skeletal muscle IL-15Rα tended to be elevated in the 5-min compared to the 1-min group (supplementary figure 2). This lack of differences could be interpreted as evidence to refute the association between skeletal muscle IL-15/IL-15Rα and MPS. Nevertheless, the effect sizes and statistical outputs ($P<0.10$) indicate that a potential difference between groups may actually exist. This suggestion is also supported by the fact that the association between IL-15Rα and MPS in the early recovery phase (0-4h post-exercise) was observed in each group separately (1-min group, $r = 0.592$, $P = 0.052$; and 5-min, $r = 0.684$, $P = 0.043$). Therefore, our results provide the framework for future studies to further clarify whether the IL-15/IL-15Rα response to strength training reported here have a physiologically relevant role in human skeletal muscle adaptation to this type of exercise.

The interleukin-15 subunit alpha-receptor (IL-15Rα) is a key subunit receptor of IL-15 that regulates its signalling in several cell types (Budagian et al., 2006; Dubois et al., 2002; Duitman et al., 2008; Sato et al., 2007). In addition to the common receptor-binding functions, IL-15Rα has been shown to function by itself, without the need for IL-15 binding (Loro et al., 2015; O'Connell et al., 2015; Pistilli et al., 2011; Pistilli et al., 2013). Animal experiments have shown that IL-15Rα is necessary to maintain insulin sensitivity, since mice lacking IL-15Rα are hyperglycemic and insulin-resistant, despite increased oxidative capacity and reduced fat mass (Loro et al., 2015). Furthermore, gene-deletion of IL-15Rα in mice is accompanied by enhanced fatigue resistance and a glycolytic-to-oxidative shift in muscle phenotype (O'Connell et al., 2015; Pistilli et al., 2011). It has been demonstrated that strength training promotes a muscle

myosin heavy chain expression shift from IIx to IIa (Andersen et al., 2005; Campos et al., 2002; Pareja-Blanco et al., 2016), increasing fatigue resistance. However, it remains unknown whether IL-15Rα up-regulation contributes to this shift in fiber types (from IIx to IIa) with training in humans. In support of this notion, those participants with a higher IL-15Rα protein expression at 4h post-exercise in our study, performed a greater volume of resistance exercise and had a higher baseline leg press 1RM. Thus, the up-regulation of IL-15Rα could serve as an adaptive response to maintain muscle characteristics associated with force production. Indeed, others have reported that two SNPs in exon 7 and 4 of the IL-15Rα were able to explain a ~11% of the hypertrophy observed after 10 weeks of whole-body resistance exercise in 157 young adults (Riechman et al., 2004). Similarly, another two SNPs, rs2296135 and rs22228059, have been associated with isometric strength and muscle volume before and after 12 weeks of unilateral elbow flexor-extensor resistance exercise (Pistilli et al., 2008).

Overexpression of IL-15 has revealed that the anabolic/anti-atrophic action of this interleukin is associated with decreased skeletal muscle proteolysis (Busquets et al., 2005) and apoptosis through suppression of DNA fragmentation via tumour necrosis factor alpha (TNF-α) signalling (Figueras et al., 2004). Moreover, IL-15Rα mRNA expression is reduced with aging, and may underpin skeletal muscle atrophy in mice (Marzetti et al., 2009). In agreement, we have observed an association between IL-15Rα mRNA and MPS in the early recovery phase following resistance exercise. Interestingly, concomitant with the elevation of MPS and IL-15Rα mRNA expression, an up-regulation of IL-15Rα protein was also found at 4h post-exercise, suggesting that IL-15Rα may have a role in the induction or maintenance of the anabolic stimulus during the early post-exercise recovery phase, potentially counteracting the degree of protein breakdown (Phillips et al., 1997). However, further studies are required to delineate the role of IL-15Rα in exercise-induced muscle remodelling.

In contrast to the response observed in skeletal muscle, serum IL-15Rα was slightly reduced at 60 min post-exercise. The discordance between the circulating and skeletal muscle IL-15Rα response to resistance exercise could imply a counteracting mechanism, by which IL-

15Rα binding of IL-15, in blood or cell membrane, reduces its availability (Rubinstein et al., 2006; Schluns et al., 2005) and potentially allows its reabsorption and subsequent restoration of the intracellular pool of free IL-15.

Although pioneer cell culture studies reported an anabolic effect of IL-15 (Quinn et al., 2002; Quinn et al., 1997; Quinn et al., 1995), this has not been confirmed in humans. Strength training-induced muscle hypertrophy is limited to the trained muscles, implying that the anabolic action of IL-15 in human skeletal muscle cannot be explained by an increase in the circulating fraction of this myokine. In fact, human experiments do not give support to an anabolic action of IL-15 in skeletal muscle (Nielsen et al., 2007; Riechman et al., 2004). However, the physiological relevance of the 24h post-exercise elevation of IL-15 gene expression in response to resistance exercise, as reported in the present and previous studies (Nielsen et al., 2007), although suggestive of a role of IL-15 in exercise-induced skeletal muscle adaptation, remains largely unexplained. The lack of association found between the increase in IL-15 mRNA at 24h post-exercise and MPS does not support a critical anabolic role, but to definitely rule out such effect would require the utilization of IL-15 blockers or antibodies, which cannot be used in humans due to potentially intolerable immunological side effects. Moreover, the anti-atrophic effect of IL-15 in skeletal muscle has not been tested in the present study and cannot be excluded (Busquets et al., 2005; Marzetti et al., 2009).

Interleukin-15 is currently considered as a myokine (Grabstein et al., 1994; Quinn et al., 1995). In agreement, we have observed a positive association between serum concentration of IL-15 and IL-15 protein levels in skeletal muscle, suggesting that muscle may be an important source of IL-15 in the basal state. In the present study, we observed that basal IL-15 protein levels in skeletal muscle were associated with serum concentration immediately post-exercise, also suggesting that the size of the intramuscular pool could determine the magnitude of the increase in serum IL-15 elicited by resistance exercise. Nevertheless, the physiological

relevance of the elevated blood IL-15 concentration in close proximity to the end of exercise remains to be elucidated (Riechman et al., 2004; Tamura et al., 2011).

Interestingly, we found that a lower time-under-tension and a lower amount of total weight lifted during resistance exercise session were associated with higher post-exercise serum IL-15 concentration, which could indicate that a more prolonged muscle activation may attenuate the release of IL-15. Depletion of the intracellular pool or reduced *de novo* synthesis of IL-15 could explain the attenuated release of IL-15 with greater training load, given the short life of IL-15 in plasma (Rubinstein et al., 2006; Stoklasek et al., 2006).

The fact that the increase of circulating IL-15 was not accompanied by an increase in its soluble receptor implies that after resistance training there is more free IL-15, available to act on target tissues (Mortier et al., 2004). IL-15 is a potent pro-inflammatory cytokine that stimulates proliferation, maturation and has protective effects on several immune cells (Budagian et al., 2006). In addition to the immunological effects, IL-15 has anti-adipogenic effects in rodents (Carbo et al., 2001). Therefore, although the physiological role of the systemic elevation of IL-15 in response to strength training remains unknown, immunological and metabolic effects are possible.

In conclusion, the IL-15/IL-15Rα signalling pathway is activated in human skeletal muscle in response to a single session of resistance exercise. Skeletal muscle mRNA levels and protein IL-15Rα expression were elevated four hours after resistance exercise and were positively associated with increased rates of myofibrillar protein synthesis. Therefore, as previously shown in cell culture and *in vivo*, the present investigation lends support to a potentially anabolic effect of IL-15Rα in human skeletal muscle. Moreover, our experimental results indicate that IL-15 and IL-15Rα may play a role in exercise-induced muscle remodelling. Prolonged resistance training studies are necessary to determine the relevance of IL-15Rα in muscle protein synthesis/breakdown, as well as the precise role of circulating and muscular levels of IL-15 and its receptor IL-15Rα in chronic physiological adaptations.

Perspectives

Previous studies have suggested a role of IL-15Rα in muscle phenotypic adaptation to resistance training. The present study confirms the activation of IL-15/IL-15Rα signalling pathway in human skeletal muscle in response to a single session of resistance exercise. It remains to be determined how skeletal muscle contributes to circulating levels of IL-15 and how circulating IL-15 could influence skeletal muscle and adipose tissue mass. Given the important role that IL-15 has in immune responses, the link between physical activity, skeletal muscle IL-15 production and immunity deserves further attention. The fact that IL-15Rα is independently associated with myofibrillar protein synthesis and muscle phenotype implies that the axis IL-15/ IL-15Rα may have an important role in human skeletal muscle remodelling.

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CONFLICT OF INTERESTS

All the authors declare that they have no conflict of interest derived from the outcomes of this study.

AUTHOR CONTRIBUTIONS

APL, JM and LB conceived and designed the experiment. APL, JM and LB collected the data. APL, JM, MMR, DMA, BPK, DV, JB, JALC and LB analysed and interpreted the data. APL, JALC and LB drafted the manuscript and prepared all figures. All authors read and approved the final version of the manuscript.

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FIGURE LEGENDS

Figure 1. IL-15 response to a single session of resistance exercise.

IL-15 mRNA (qRT-PCR) (A) and protein levels (Western blotting) (B) from *vastus lateralis* muscle biopsies, and serum IL-15 levels (ELISA) (C). Values are presented as means ± SD (N = 14). * P<0.05 compared to Pre-exercise. # P<0.05 compared to 0h post-exercise. Data were log-transformed.

Figure 2. IL-15Rα response to a single session of resistance exercise.

IL-15Rα mRNA (qRT-PCR) (A) and protein levels (Western blotting) (B) from *vastus lateralis* muscle biopsies, and serum IL-15Rα levels (ELISA) (C). Values are presented as means ± SD (N = 14). * P<0.05 compared to Pre-exercise. # P<0.05 compared to 0h post-exercise. Φ P<0.05 compared to 24h post-exercise. § P<0.05 differences compared to Mid-exercise. Data were log-transformed.

Figure 3. Relationship between myofibrillar protein synthesis measured as a fractional synthetic rate (FSR) and IL-15Rα mRNA pre- and post-exercise. The association remained significant in Fig. 3B (r = 0.665, P = 0.026) when the lowest FSR values were excluded. a.u., arbitrary units.

Supplementary Figure 1. Skeletal muscle IL-15 response to a single session of resistance exercise by group (1 and 5 min groups).

IL-15 mRNA (qRT-PCR) (A) and protein levels (Western blotting) (B) from *vastus lateralis* muscle biopsies. Values are presented as means ± SD (N = 14). * P<0.05 compared to Pre-exercise. Data were log-transformed. a.u., arbitrary units.

Supplementary Figure 2. Skeletal muscle IL-15Rα response to a single session of resistance exercise by group (1 and 5 min groups).

IL-15Rα mRNA (qRT-PCR) (A) and protein levels (Western blotting) (B) from *vastus lateralis* muscle biopsies. Values are presented as means ± SD (N = 14). * P<0.05 compared to Pre-exercise. # P<0.05 compared to 0h post-exercise. Data were log-transformed. a.u., arbitrary units.

Table 1. Participants' physical characteristics

N	14
Age (yrs)	24.9 ± 4.8
Body mass (kg)	82.2 ± 11.9
BMI (kg/m^2)	25.6 ± 3.1
Whole-body FFM (kg)	66.0 ± 8.8
Legs FFM (kg)	21.5 ± 3.2
Whole-Body FM (kg)	12.9 ± 5.2
Legs FM (kg)	4.5 ± 1.5
Leg Press 1-RM (kg)	268 ± 51
Knee Extension (kg)	169 ± 26
Training experience (yrs)	6 ± 5
Leg training (days/week)	2 ± 1

Values are presented as mean ± SD. BMI, body mass index; FFM, fat free mass; FM, fat mass; 1RM, one-repetition maximum.

Table 2. Primers for qRT-PCR analysis.

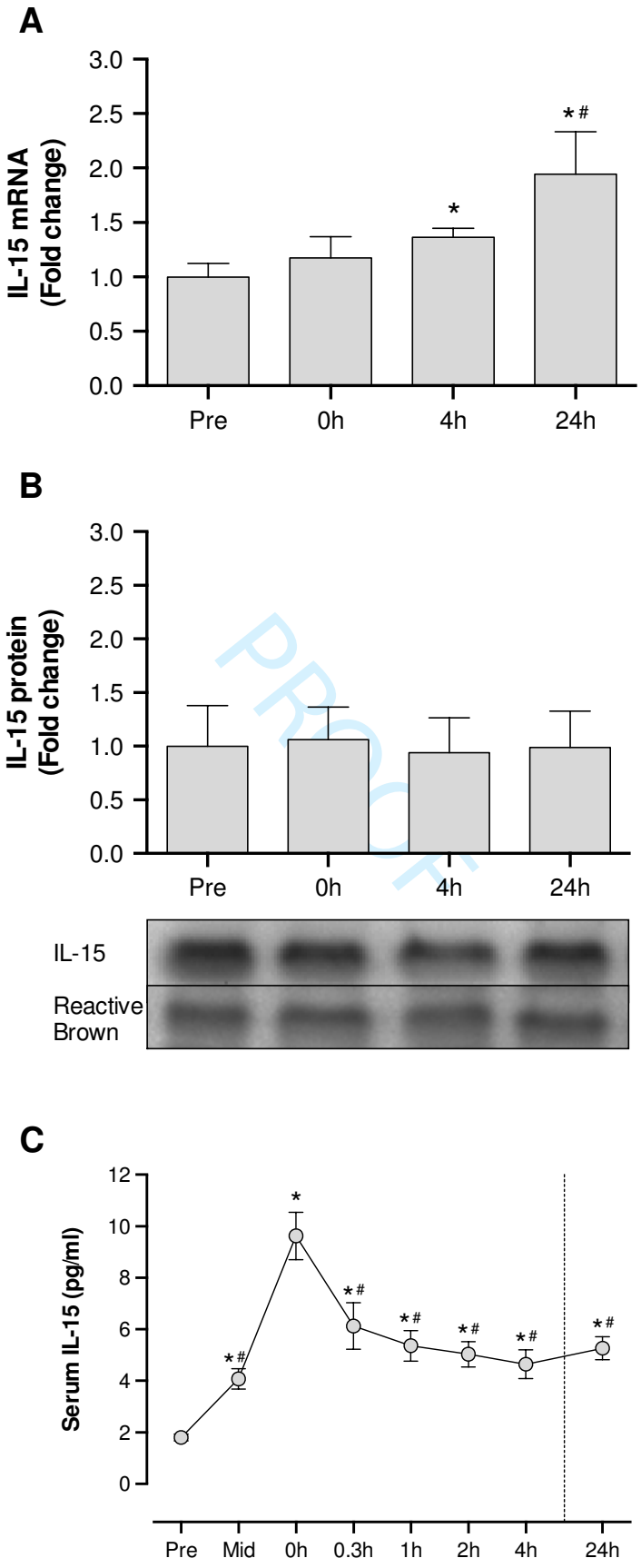
Primer	Sequence	Accession Number	T_m
IL-15	F: 5'-AAAGTGATGTTCACCCCAGTTG	NM_000585.4	60°
	R: 3'-CCTCCAGTTCCTCACATTCTTTG		30s
IL-15Rα	F: 5'-CAGCCGCCAGGTGTGTATC	NM_002189.3	60°
	R: 3'-TTGCCTTGACTTGAGGTAGCA		30s

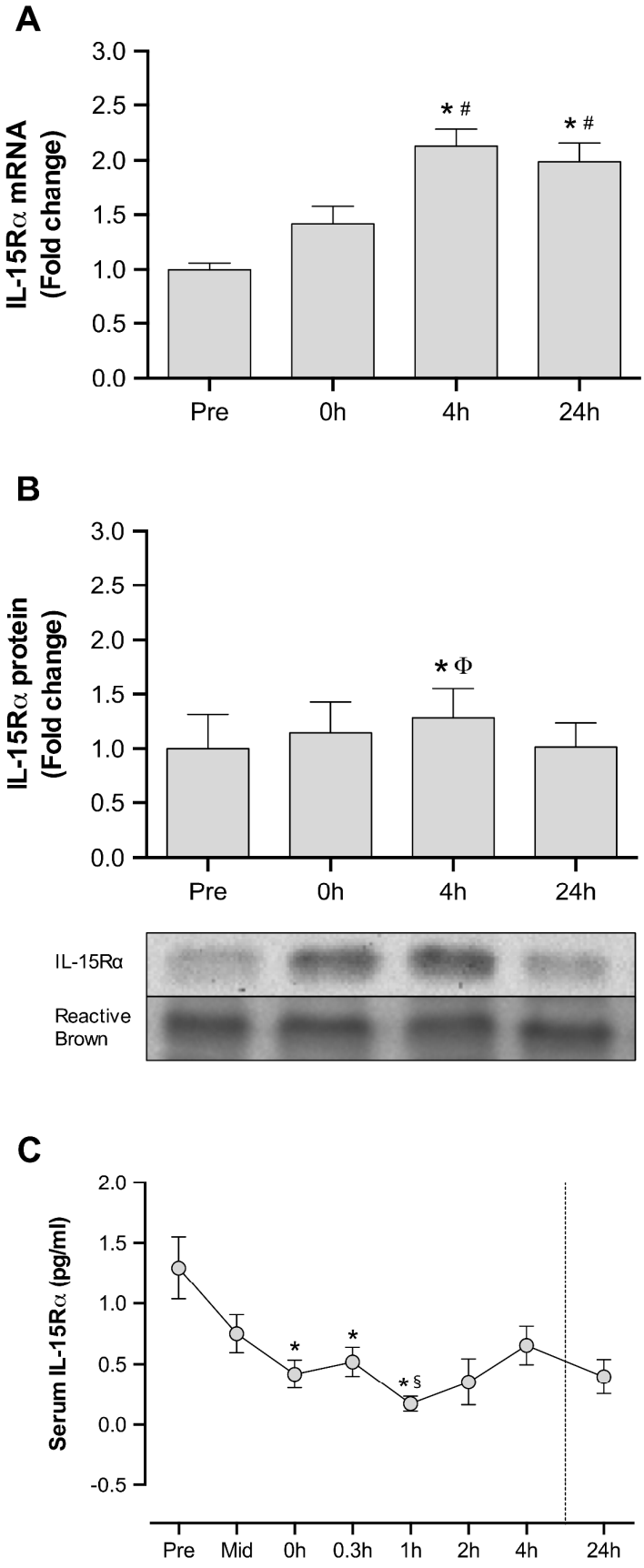
T_m, melting temperature.

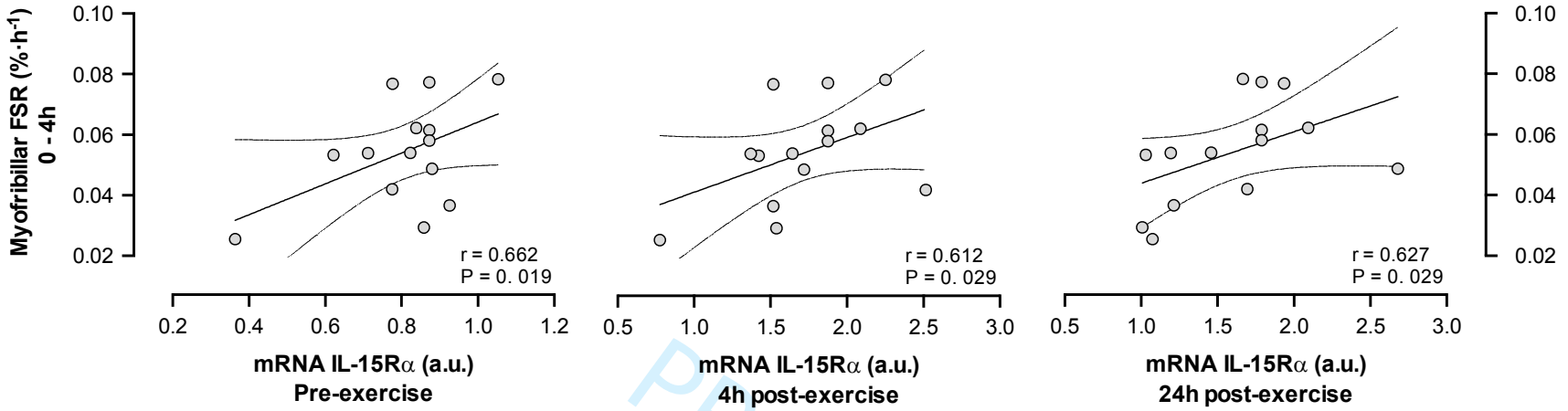
Table 3. Variables describing the resistance session.

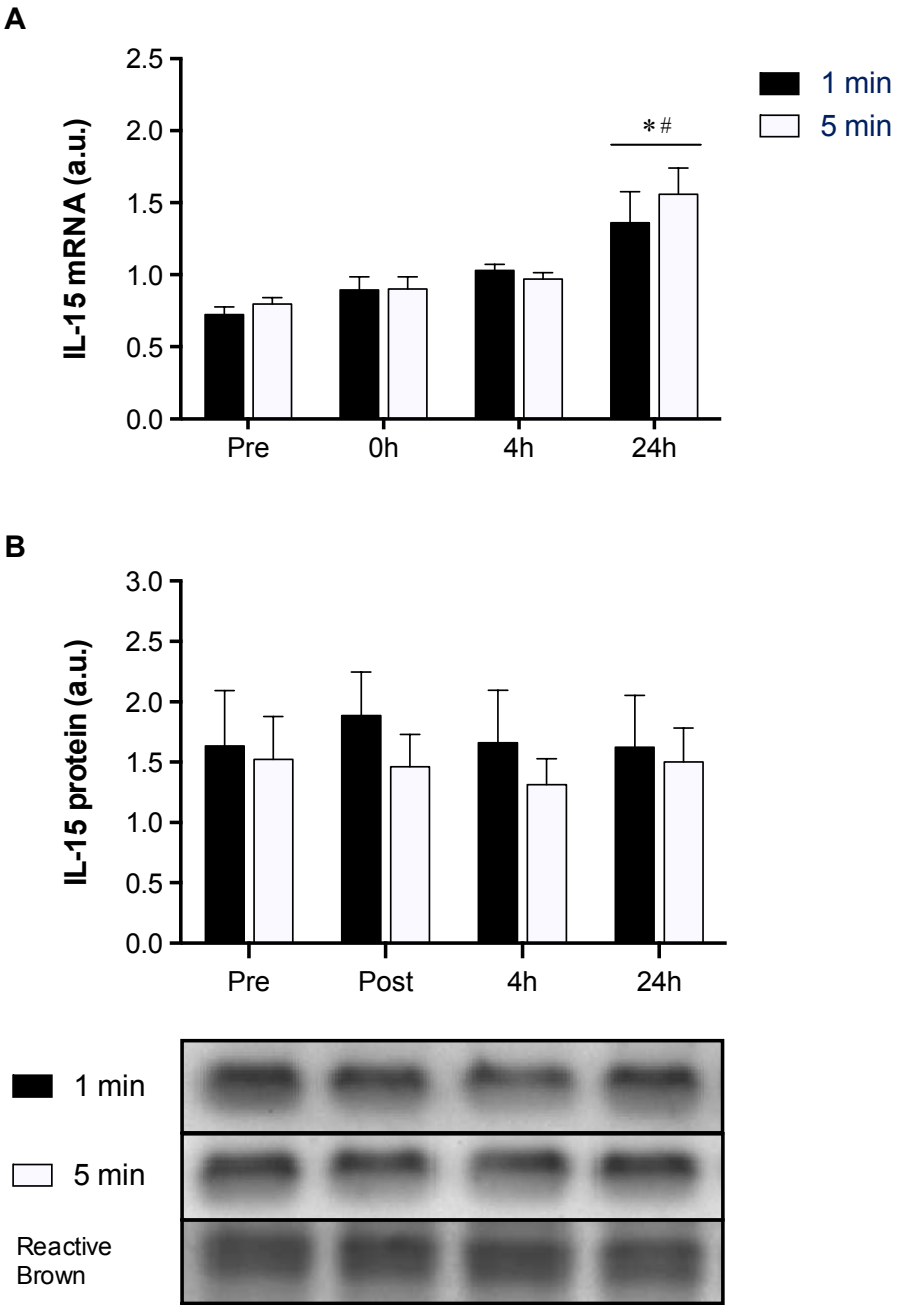
	Leg Press					Knee Extension					Total
Set	1	2	3	4	Total	1	2	3	4	Total	8
Load (kg)	205±40	205±39	205±38	206±37	820±154	123±18	119±19	117±19	114±18	473±73	1293±210
Repetition	13±3	11±2	11±2	10±2	44±6	9±2	9±2	10±2	9±2	37±6	10±1
Volume (kg)	2484±401	2188±296	2260±452	2019±533	8951±1217	1068±276	1119±296	1139±323	1081±352	4407±1127	13358±2026
T-U-T (s)	38.3±11.5	34.4±7.1	33.1±6.2	32.9±5.9	138.7±27.4	23.7±9.5	18.8±3.4	19.2±4.7	18.9±3.1	80.6±17.2	219.4±40.8
RPE (0-10)	9±1	9±1	10±0	10±1	9±0	10±0	10±0	10±0	10±0	10±0	10±0

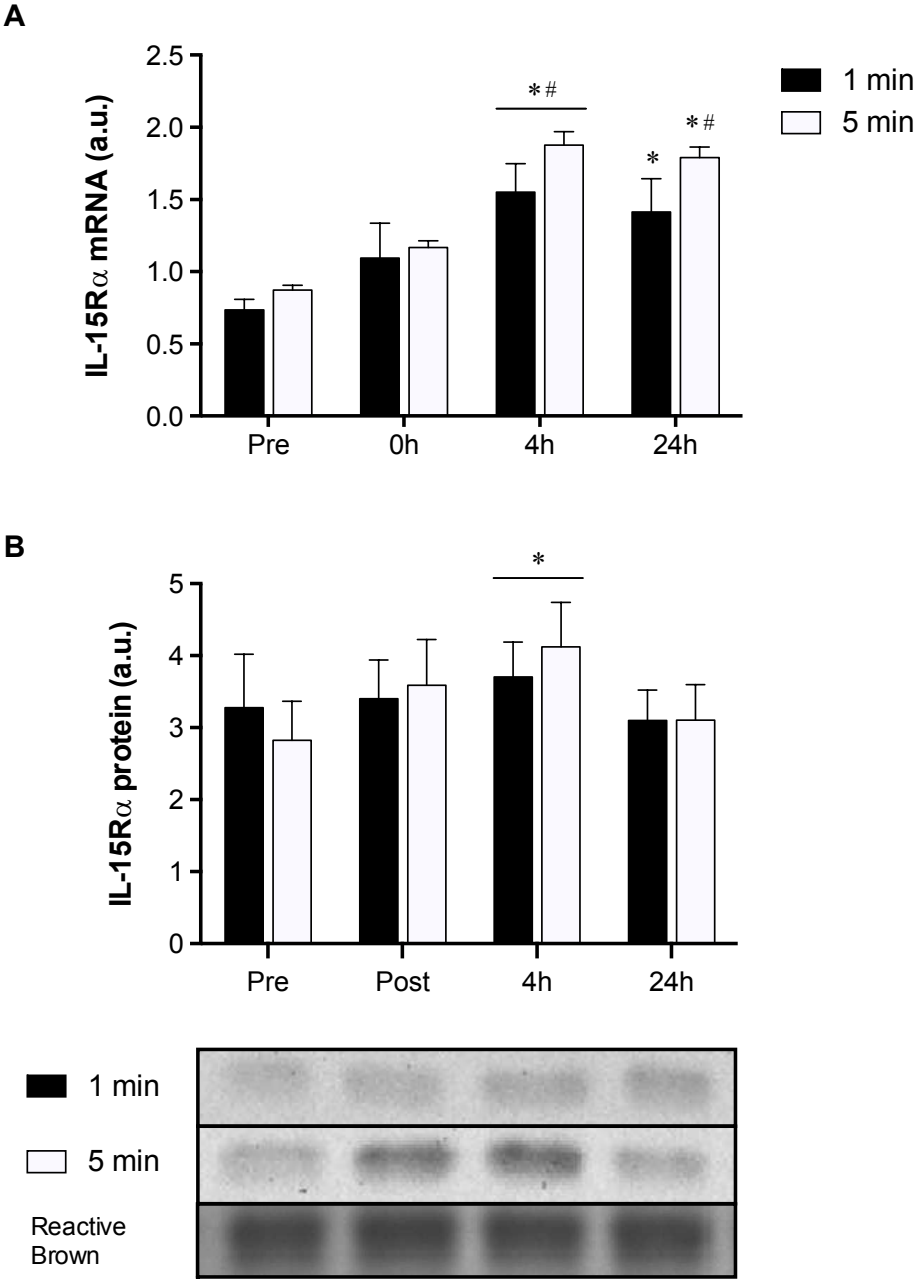
Values are presented as mean ± SD. T-U-T, time-under-tension; RPE, rating of perceived exertion.


A
IL-15 mRNA (Fold change)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*#
*
Pre
0h
4h
24h
B
IL-15 protein (Fold change)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Pre
0h
4h
24h
IL-15
Reactive Brown
C
Serum IL-15 (pg/ml)
12
10
8
6
4
2
0
*
*#
*#
*#
*#
*#
Pre
Mid
0h
0.3h
1h
2h
4h
24h


A
IL-15Rα mRNA (Fold change)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
* #
* #
Pre
0h
4h
24h
B
IL-15Rα protein (Fold change)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
* Φ
Pre
0h
4h
24h
IL-15Rα
Reactive Brown
C
Serum IL-15Rα (pg/ml)
2.0
1.5
1.0
0.5
0.0
-0.5
*
*
* §
Pre
Mid
0h
0.3h
1h
2h
4h
24h


Myofribillar FSR (%·h-1)
0 - 4h
0.10
0.08
0.06
0.04
0.02
r = 0.662
P = 0. 019
0.2
0.4
0.6
0.8
1.0
1.2
mRNA IL-15Rα (a.u.)
Pre-exercise
r = 0.612
P = 0. 029
0.5
1.0
1.5
2.0
2.5
3.0
mRNA IL-15Rα (a.u.)
4h post-exercise
r = 0.627
P = 0. 029
mRNA IL-15Rα (a.u.)
24h post-exercise


A
IL-15 mRNA (a.u.)
2.5
2.0
1.5
1.0
0.5
0.0
Pre
0h
4h
24h
*#
1 min
5 min
B
IL-15 protein (a.u.)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Pre
Post
4h
24h
1 min
5 min
Reactive Brown


A
IL-15Rα mRNA (a.u.)
2.5
2.0
1.5
1.0
0.5
0.0
*#
*#
*
1 min
5 min
Pre
0h
4h
24h
B
IL-15Rα protein (a.u.)
5
4
3
2
1
0
*
Pre
Post
4h
24h
1 min
5 min
Reactive
Brown